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Title: MULTISPECIFIC NK ENGAGER PROTEIN

United States Patent Application 20190048093

Kind Code: A1

Abstract: Multispecific proteins that bind and specifically redirect NK cells to lyse a target cell of interest are provided without non-specific activation of NK cells in absence of target cells. The proteins have utility in the treatment of disease, notably cancer or infectious disease.

USA Doctor/Surgeon John Charles Cutler with the United States PUBLIC HEALTH SERVICE is known for his **UNETHICAL and CRIMINAL Acts** in the TUSKEGEE Experiments that **involved INTENTIONALLY "INJECTING"** Black Men with **GONORRAH, SYPHILIS** and **AIDS (HIV)**... here in the United States of America and abroad (i.e. in Guatemala and other Foreign Nations)

THESE ARE WAR CRIMES

YES, Baker Donelson Bearman Caldwell & Berkowitz (**Legal Counsel for the USA**) **WAS INVOLVED** in the Tuskegee Experiments/Tests and **is LEGAL Counsel** for the **United States Public Health Service**.

The USA with CO-Conspirators (as CHINA) are presently responsible for the RELEASE of the alleged PANDEMIC known as COVID-19/CORONAVIRUS here within the United States of America for purposes of "ETHNIC CLEANSING" – i.e. DEPOPULATION, Genocide, Eugenics...

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John Charles Cutler 
Surgeon

John Charles Cutler was a senior surgeon, and the acting chief of the venereal disease program in the United States Public Health Service. After his death, his involvement in several controversial and unethical medical studies of syphilis was revealed, including the Guatemala and the Tuskegee syphilis experiments. [Wikipedia](#)

Born: June 29, 1915, Cleveland, OH
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Spouse: [Eliase Cutler](#) (m. 1943–2003)
Education: Case Western Reserve University
Employer: [United States Public Health Service](#)
Known for: unethical medical experiments regarding syphilitic patients



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Claims: 1. 1-78. (canceled)

79. A multispecific antigen binding protein which promotes the specific lysis of target cells expressing an antigen of interest, which antigen is specifically bound by a therapeutic antibody or therapeutic antigen-binding antibody fragment, wherein said multispecific antigen binding protein comprises: (i) a first antigen binding domain (ABD) which monovalently binds to a human NKp46 polypeptide having the amino acid sequence set forth in SEQ ID NO:1, (ii) a second ABD which comprises a therapeutic antibody or a therapeutic antigen-binding antibody fragment which binds to said antigen of interest expressed by said target cells, and (iii) a CD16A binding polypeptide, wherein: (1) said NKp46-binding ABD comprises a Fab or comprises a variable heavy (V_H) domain and a variable light (V_L) domain separated by a linker ("scFv"); (2) said antigen-binding ABD which comprises said therapeutic antibody or therapeutic antigen-binding antibody fragment is monovalent or bivalent; (3) said CD16A binding polypeptide comprises a human Fc domain polypeptide which binds CD16A; (4) said multispecific antigen binding protein binds to the NKp46 polypeptide monovalently; (5) said multispecific antigen binding protein when administered to a subject directs NKp46-expressing natural killer (NK) cells and CD16A-expressing NK cells to lyse target cells expressing the antigen of interest by a combination of NKp46-mediated signaling and CD16A-mediated antibody-dependent cell-mediated cytotoxicity ("ADCC"); (6) said dimeric Fc domain interposes said first ABD and said second ABD; and (7) said first and second ABD are each connected to said dimeric Fc domain, and one or both of said first and second ABD are connected to the dimeric Fc domain via a flexible polypeptide linker.

80. The multispecific antigen binding protein of claim 79, wherein the therapeutic antibody specifically binds to an antigen expressed by cancer cells.

81. The multispecific antigen binding protein of claim 79, wherein the therapeutic antibody specifically binds to an antigen expressed by an infectious agent.

82. The multispecific antigen binding protein of claim 81, wherein said infectious agent is a virus, parasite, bacterium or another microbe.

83. The multispecific antigen binding protein of claim 79, wherein the therapeutic antigen-binding ABD comprises a Fab or comprises a V_H domain and a V_L domain separated by a linker comprising a linear or cyclic peptide.

84. The multispecific antigen binding protein of claim 79, wherein the NKp46-binding ABD comprises a Fab.

85. The multispecific antigen binding protein of claim 79, wherein the NKp46-binding ABD comprises a V_H domain and a V_L domain separated by a linker comprising a linear or cyclic peptide.

86. The multispecific antigen binding protein of claim 79, wherein said dimeric Fc polypeptide of (3) comprises a modification that enhances CD16A binding relative to the corresponding wild-type Fc region.

87. The multispecific antigen binding protein of claim 79, wherein the administration of said multispecific antigen binding protein to a subject increases the expression of CD137 on the surface of NK cells in said subject.

88. The multispecific antigen binding protein of claim 79, wherein the NKp46-binding ABD is comprised of a V_H domain and a V_L domain, wherein each of the V_H and V_L domains are positioned within a tandem variable region comprising a V_H domain and a V_L domain separated by a



polypeptide linker.

89. The multispecific antigen binding protein of claim 79, wherein the NKp46-binding ABD is a Fab comprised of a V_H domain and a V_L domain, wherein each of the V_H and V_L domains is fused to a human C_{H1} or C_K constant domain.

90. The multispecific antigen binding protein of claim 79, wherein the NKp46-binding ABD is a Fab comprised of (a) a V_H domain fused to a human C_{H1} constant domain and a V_L domain fused to a human C_K constant domain, or (b) a V_H domain fused to a human C_K constant domain and a V_L domain fused to a human C_{H1} constant domain.

91. The multispecific antigen binding protein of claim 79, wherein the therapeutic antigen-binding ABD is a Fab comprised of a V_H domain and a V_L domain, wherein each of the V_H and V_L domains is fused to a human C_{H1} or C_K constant domain.

92. The multispecific antigen binding protein of claim 90, wherein the therapeutic antigen-binding ABD is a Fab comprised of (a) a V_H domain fused to a human C_{H1} constant domain and a V_L domain fused to a human C_K constant domain, or (b) a V_H domain fused to a human C_K constant domain and a V_L domain fused to a human C_{H1} constant domain.

93. The multispecific antigen binding protein of claim 79, wherein either or both the NKp46-binding ABD or the therapeutic antigen-binding ABD is bound to the Fc domain by a flexible polypeptide linker.

94. The multispecific antigen binding protein of claim 79, wherein the monovalent NKp46 ABD comprises V_H and V_L domain polypeptides selected from the group consisting of: (a) the V_H and V_L domains of SEQ ID NOS: 3 and 4 (NKp46-1); (b) the V_H and V_L domains of SEQ ID NOS: 5 and 6 (NKp46-2); (c) the V_H and V_L domains of SEQ ID NOS: 7 and 8 (NKp46-3); (d) the V_H and V_L domains of SEQ ID NOS: 9 and 10 (NKp46-4); (e) the V_H and V_L domains of SEQ ID NOS: 11 and 12 (NKp46-6); and (f) the V_H and V_L domains of SEQ ID NOS: 13 and 14 (NKp46-9).

95. The multispecific antigen binding protein of claim 79, wherein said flexible polypeptide linker is 2-50 amino acids in length.

96. The multispecific antigen binding protein of claim 79, wherein one of the ABDs is connected to the Fc region via a peptide linker which comprises a hinge domain.

97. The multispecific antigen binding protein of claim 91, wherein the C_K constant domain and the human C_{H1} constant domain are each connected to the Fc domain via a hinge domain.

98. The method of claim 92, wherein the C_K constant domain and the human C_{H1} constant domain are each connected to the Fc domain via a hinge domain.

99. The multispecific antigen binding protein of claim 79, wherein said combination of NKp46-mediated signaling and CD16A-mediated ADCC has an additive or synergistic effect on the lysis of target cells expressing the antigen of interest.

100. The multispecific antigen binding protein of claim 80, wherein the therapeutic antigen-binding ABD binds to an antigen expressed by hematological cancer cells.

101. The multispecific antigen binding protein of claim 100, wherein the antigen expressed by said hematological cancer cells comprises CD19 or CD20.

102. The multispecific antigen binding protein of claim 80, wherein the therapeutic antigen-binding ABD binds to an antigen expressed by solid tumor cancer cells.

103. A pharmaceutical composition comprising a pharmaceutically effective amount of the multispecific antigen binding protein of claim 79.

104. A nucleic acid or nucleic acids which separately or in combination encodes for the expression of the multispecific antigen binding protein of claim 80.

105. A vector which comprises nucleic acid or nucleic acids according to claim 104.

106. A recombinant or isolated cell which comprises nucleic acid or nucleic acids according to claim 104 or a vector comprising said nucleic acid or nucleic acids.

107. A method of producing a multispecific antigen binding protein according to claim 79 comprising culturing a cell according which comprises a nucleic acid or nucleic acids which separately or in combination encodes for the expression of said multispecific antigen binding protein wherein culturing is under conditions whereby said multispecific antigen binding protein is expressed by the cell.

108. A method of promoting the specific lysis of target cells expressing an antigen of interest which is specifically bound by a therapeutic antibody or therapeutic antigen-binding antibody fragment in a subject in need thereof, comprising contacting said target cells with an amount of a multispecific antigen binding protein which is sufficient to promote the specific lysis of said target cells expressing said antigen of interest, wherein said multispecific antigen binding protein comprises: (i) a first antigen binding domain (ABD) which monovalently binds to a human NKp46 polypeptide having the amino acid sequence set forth in SEQ ID NO:1, (ii) a second ABD which comprises a therapeutic antibody or a therapeutic antigen-binding antibody fragment which binds to said antigen of interest expressed by target cells, and (iii) a CD16A binding polypeptide, wherein: (1) said NKp46-binding ABD comprises a Fab or comprises a V_H chain domain and a V_L chain domain separated by a linker ("scFv"); (2) said cancer-antigen-binding ABD, which comprises said therapeutic antibody or therapeutic antigen-binding antibody fragment, is monovalent or bivalent; (3) said CD16A binding polypeptide comprises a dimeric human Fc domain polypeptide which binds CD16A; (4) said multispecific antigen binding protein binds to the NKp46 polypeptide monovalently; (5) said multispecific antigen binding protein directs NKp46-expressing NK cells and CD16A-expressing NK cells to lyse target cells expressing the antigen of interest by a combination of NKp46-mediated signaling and CD16A-mediated ADCC; (6) said dimeric Fc domain interposes said first ABD and said second ABD; (7) said first and second ABD are each attached to said dimeric Fc domain, and wherein one or both of said first and second ABD are connected to the Fc domain via a flexible polypeptide linker.

109. The method of claim 108, wherein said combination of NKp46-mediated signaling and CD16A-mediated ADCC has an additive or synergistic effect on the lysis of target cells expressing the antigen of interest.

110. The method of claim 108, wherein said antigen of interest is a cancer antigen.

111. The method of claim 108, wherein said antigen of interest is expressed by an infectious agent.

Description:**CROSS-REFERENCE TO RELATED APPLICATIONS**

This application claims the benefit of U.S. Provisional Application No. 62/271,459 filed Dec. 28, 2015, and is a continuation-in-part of PCT patent application No. PCT/EP2015/064063 filed 23 Jun. 2015; both of which are incorporated herein by reference in their entirety; including any drawings and sequence listings.

REFERENCE TO THE SEQUENCE LISTING

The present application is being filed along with a Sequence Listing in electronic format. The Sequence Listing is provided as a file entitled "NKp46-6_PCT_ST25.txt", created Jun. 23, 2016, which is 355 KB in size. The information in the electronic format of the Sequence Listing is incorporated herein by reference in its entirety.

FIELD OF THE INVENTION

Multispecific proteins that bind and specifically redirect effector cells to lyse a target cell of interest via multiple activating receptors are provided. The proteins have utility in the treatment of disease, notably cancer or infectious disease.

BACKGROUND

Bispecific antibodies binding two different epitopes offer opportunities for increasing specificity, broadening potency, and utilizing novel mechanisms of action that cannot be achieved with a traditional monoclonal antibody. A variety of formats for bispecific antibodies that bind to two targets simultaneously have been reported. Cross-linking two different receptors using a bispecific antibody to inhibit a signaling pathway has shown utility in a number of applications (see, e.g., Jackman, et al., (2010) *J. Biol. Chem.* 285:20850-20859). Bispecific antibodies have also been used to neutralize two different receptors. In other approaches, bispecific antibodies have been used to recruit immune effector cells, where T-cell activation is achieved in proximity to tumor cells by the bispecific antibody which binds receptors simultaneously on the two different cell types (see Baeuerle, P. A., et al, (2009) *Cancer Res* 69(12):4941-4). These antibodies have been referred to as "Bispecific T-cell engager antibodies" (or "BiTE" antibodies). However, in order to fully activate the T-cell, this T-cell and a cluster of BiTEs must interact on the surface of a target cell. Due to the difficulties of finding antibody variable regions which are functional in the BiTE format, to date only a single immune cell receptor (CD3) has been targeted, in the CD19×CD3 specific antibody blinatumamab. Bispecific antibodies developed to date also include those which link the CD3 complex on T cells to a tumor-associated antigen. Also, bispecific antibodies having one arm which binds CD16 (FcγRIIIa) and another which bound to an antigen of interest such as CD19 have been developed (see Kellner et al. (2011) *Cancer Lett.* 303(2): 128-139).

Natural killer (NK) cells are a subpopulation of lymphocytes that are involved in non-conventional immunity. NK cells provide an efficient immunosurveillance mechanism by which undesired cells such as tumor or virally-infected cells can be eliminated. Characteristics and biological properties of NK cells include the expression of surface antigens including CD16, CD56 and/or CD57, the absence of the α/γ or γ/δ TCR complex on the cell surface; the ability to bind to and kill cells that fail to express "self" MHC/HLA antigens by the activation of specific cytolytic enzymes, the ability to kill tumor cells or other diseased cells that express a ligand for NK activating receptors, and the ability to release protein molecules called cytokines that stimulate or inhibit the immune response.

NK cell activity is regulated by a complex mechanism that involves both activating and inhibitory signals. Several distinct NK cell receptors have been identified that play an important role in the NK cell mediated recognition and killing of HLA Class I deficient target cells. One receptor, although not specific to NK cells, is FcγR3a (CD16) which is responsible for NK cell mediated ADCC. NK cells also express a range of other activating and co-activating receptors, including CD137 (4-1BB). Agonist antibodies against anti-4-1BB are in clinical trials in patients with solid tumors, including melanoma, renal carcinoma, and ovarian cancer, and have shown strong activity in different cancer models, including breast cancer, sarcoma, glioma, colon carcinoma, myeloma, and mastocytoma.

Another NK cell receptor is NKp46, a member of the Ig superfamily. NKp46, is specific to NK cells and the cross-linking thereof, induced by specific mAbs, leads to a strong NK cell activation resulting in increased intracellular Ca⁺⁺ levels, the triggering of cytotoxicity, and lymphokine release. International patent publication number WO2005/105858 (Innate Pharma) discloses the use of monospecific full-length IgG anti-NKp46 antibodies that bind Fcγ receptors for treating hematological malignancies that are Fcγ-positive. Fc γ receptors expressed on tumor cells (e.g. B cell malignancies) were proposed to interact with the Fc domain of the anti-NKp46 antibodies which bound NK cells, such that the activated NK cells are brought into close proximity with target cells via the two reactive portions of the antibody (i.e., the antigen-recognizing domain and the Fc domain), thereby enhancing the efficiency of the treatment.

To date, no NK cell-specific bispecific antibodies have been reported. Rather depleting agents that recruit NK cytotoxicity such as anti-tumor antibodies are typically full-length IgG1 antibodies that mediate ADCC via CD16. Despite the existence of a variety of formats for bispecific antibodies, there remains a need in the art for multispecific proteins with new and well-defined mechanisms of action, particularly those that can

provide therapeutic advantages over full-length antibodies.

SUMMARY OF THE INVENTION

The present invention arises from the discovery of functional multi-specific proteins (e.g. a polypeptide, a single chain protein, a multi-chain protein, including but not limited to antibody-based protein formats) that binds NKp46 on NK cells and to an antigen of interest on a target cell, and is capable of redirecting NK cells to lyse a target cell that expresses the antigen of interest, e.g. a cell that contributes to disease. Provided, inter alia, is a multispecific protein comprising a first antigen binding domain and a second antigen binding domain, wherein one of the first or second antigen binding domains binds to a human NKp46 polypeptide and the other binds an antigen of interest, wherein the multispecific protein binds the NKp46 polypeptide monovalently, and wherein the multispecific protein is capable of directing an NKp46-expressing NK cell to lyse a target cell expressing the antigen of interest. Advantageously, in one embodiment, the presence of NK cells and target cells, the multi-specific protein can bind (i) to antigen of interest on target cells and (ii) to NKp46 on NK cells, and, when bound to both antigen of interest on target cells and NKp46, can induce signaling in and/or activation of the NK cells through NKp46 (the protein acts as an NKp46 agonist), thereby promoting activation of NK cells and/or lysis of target cells, notably via the activating signal transmitted by NKp46.

In some embodiment, the multispecific protein comprises at least a portion of a human Fc domain, e.g. an Fc domain that is bound by FcRn.

In certain embodiments, the multispecific antibody is designed to have decreased or substantially lack FcγR binding compared to a conventional full-length human IgG1 antibody. Optionally the multispecific protein has decreased or abolished binding to a human CD16, CD32A, CD32B and/or CD64 polypeptide, compared to a full length wild type human IgG1 antibody. In other embodiments, the multispecific protein is designed to retain substantial FcγR binding, e.g., compared to a conventional full-length human IgG1 antibody. Optionally the multispecific protein binds (e.g. via its Fc domain) to a human CD16, CD32A, CD32B and/or CD64 polypeptide.

The present invention arises in part from the observation that multispecific proteins which bind to an antigen of interest and which are further engineered to bind to NKp46 on NK cells monovalently, and to CD16 or CD16A, exhibit an enhanced capability to promote NK cell-mediated target cell lysis (relative to conventional antibodies). Through comparison of molecules having different functionalities, it was observed that such multispecific proteins elicited the individual and combined effects of these receptors thereby better promoting the lysis of target cells by NK cells. As described in detail herein, various functional multispecific proteins were constructed that bind (a) NKp46 and CD16 on NK cells and (b) to an antigen of interest on a target cell, which are capable of redirecting NK cells to lyse a target cell that expresses the antigen of interest, e.g. a cell that contributes to a disease such as cancer or infection. Moreover, despite binding to NKp46 on NK cells, advantageously these multispecific proteins do not induce lysis of NK cells themselves.

Also provided are novel formats for multispecific Fc proteins that are capable of activating CD16 and NKp46 and which can be used to promote NK-mediated killing of desired target cells. The potency in target cell killing demonstrated by the subject multispecific proteins which bind CD16 and which in addition bind to NKp46 and to an antigen of interest is believed to arise at least in part from an induction and upregulation of the co-activating receptor CD137 which is expressed on the surface NK cells. Particularly, CD137 upregulation occurs on resting NK cells, in the absence of target cells (as well as in the presence of target cells), and without the induction of CD16-mediated lysis of NK cells. NK cells with increased CD137 expression are known to be highly active against target cells (e.g. tumor cells) expressing CD137L (CD137 ligand). Consequently, the instant multispecific proteins which bind CD16 in addition to monovalently binding NKp46 can provide a means to upregulate CD137. Further advantageously, the multispecific protein when binding additionally to an antigen of interest expressed by a target cell, can elicit a multi-pronged recognition of target cells that involves multiple activating receptors expressed on effector cells. Also, despite an ability to cause upregulation of CD137 on NK cells that is comparable to human IgG1 antibodies (in the absence of target cells), surprisingly the multispecific proteins are far more potent in inducing NK cell-mediated lysis of tumor cells than human IgG1 antibodies. This would suggest that the subject multispecific proteins trigger the combined effects of NKp46-, CD16- and/or CD137-mediated activation thereby providing for a synergistic or additive enhancement in the induction of NK cell cytotoxicity by CD16⁺ NKp46⁺ NK cells. Additionally, independently of any contribution of CD137, these multispecific proteins advantageously are able to potentially mobilize both CD16⁺ and CD16⁻ NK cells (all NK cells are NKp46⁺).

Furthermore, despite that the subject multispecific proteins are bound by CD16, unexpectedly they do not induce or increase down-modulation or internalization of the antigen of interest, even when targeting antigens of interest known to be susceptible to down-modulation or internalization

when bound by conventional antibodies (such as full length human IgG1's). Based thereon, the subject multispecific proteins should be well suited for targeting antigens of interest expressed by target cells, e.g., tumor or infected cells, including antigens which are known to be capable of undergoing down-modulation or internalization when bound by conventional antibodies (e.g. antibodies with human IgG1 Fc domains that retain CD16 binding). This is a huge therapeutic benefit since it is known in the art that antigen internalization can substantially impede the ability of conventional human IgG1 antibodies to mediate ADCC against a target cell.

Therefore, in one embodiment, multispecific proteins are provided which bind to an antigen of interest expressed on the surface of a cell monovalently, wherein the protein does not increase or induce down-modulation or intracellular internalization of the antigen of interest.

In another embodiment the invention provides a multispecific protein that comprises an antigen binding domain that binds to a human a NKp46 polypeptide monovalently (e.g., via a single antigen binding domain), and which is capable of binding to human CD16, and which when incubated in soluble form with effector cells expressing a NKp46 polypeptide and CD16 (e.g. human NKp46⁺CD16⁺ NK cells), optionally in the absence and/or presence of target cells, further optionally in the absence of other cells, can cause an increase or induction of CD137 polypeptide expression on the surface of the effector cells (e.g. without inducing detectable lysis of the NK cells). Optionally, the multispecific protein further binds to an antigen of interest.

In another embodiment the invention provides a multispecific protein that comprises (i) a first antigen binding domain ("ABD") that binds to a human NKp46 polypeptide, (ii) an Fc domain that binds to human CD16A, and (iii) a second antigen binding domain that binds to an antigen of interest expressed by a target cell. In one embodiment, the multispecific protein is capable of directing an NKp46-expressing NK cell to lyse a target cell expressing the antigen of interest, wherein said lysis of the target cell is mediated by NKp46-signaling, wherein optionally said lysis of the target cell is mediated by a combination of Nkp46-mediated signaling and CD16-mediated antibody-dependent cell-mediated cytotoxicity ("ADCC").

In another embodiment the invention provides a multispecific antigen binding protein which comprises (i) a monovalent antigen binding polypeptide ("ABD") which binds to a human NKp46 polypeptide (i) an ABD which binds to an antigen of interest, wherein said antigen of interest optionally comprises an antigen expressed by a tumor cell or an infectious agent and (iii) a CD16A binding polypeptide, optionally an Fc polypeptide which Fc polypeptide is optionally modified to enhance CD16A binding relative to the corresponding wild-type Fc polypeptide, wherein the multispecific protein is capable of directing an NKp46-expressing NK cell to lyse a target cell expressing the antigen of interest by NKp46-signaling.

In another embodiment the invention provides an isolated multispecific Fc-protein comprising a first antigen binding domain, a second antigen binding domain, and an Fc domain or portion thereof, which optionally may be modified, that is capable of binding human CD16 or CD16A, wherein one of the first or second antigen binding domains binds to a human NKp46 polypeptide and the other binds an antigen of interest, and wherein the multispecific protein is capable of directing an NKp46-expressing NK cell to lyse a target cell expressing the antigen of interest. In one embodiment, the protein causes lysis of the target cell by enhancing or inducing NKp46-signaling, wherein optionally lysis of the target cell is mediated by a combination of Nkp46 signaling and CD16-mediated antibody-dependent cell-mediated cytotoxicity ("ADCC"). Optionally, the multispecific protein further comprises a third or fourth or more antigen binding domains that each bind to an antigen of interest, e.g., one other than a NKp46 polypeptide, i.e., an antigen of interest expressed by a target cell such as a tumor cell or infectious agent, wherein the antigen of interest is the same or different from the antigen of interest bound by the second antigen binding domain. In one embodiment, the third antigen binding domain binds to the same antigen of interest as the second antigen binding domain, optionally further wherein the third antigen binding domain binds to the same epitope or a different epitope on the antigen of interest as the second antigen binding domain. In one embodiment, the antigen of interest is a cancer antigen. In one embodiment, the antigen of interest is a protein expressed (optionally over-expressed) on the surface of malignant immune cells, e.g. cells involved in a hematological malignancy, leukemia cells, lymphoma cells, a CD19 protein, a CD20 protein, etc. In one embodiment, the protein is used to treat a hematological malignancy, e.g., a leukemia or lymphoma cells. In another embodiment, the antigen of interest is a protein expressed (optionally over-expressed) on the surface of infected cells or by an infectious agent such as virally, bacterially or parasite infected cells.

Optionally, the subject multispecific polypeptides when incubated in soluble form with effector cells expressing NKp46 and CD16 (e.g. human

NKp46⁺CD16⁺ NK cells), optionally in the presence or absence of other cells (e.g. target cells), can elicit an increase or induction of CD137 polypeptide expression on the surface of the NK cells (e.g. without inducing detectable lysis of the NK cells). Optionally, in the presence of target cells expressing the antigen of interest and NKp46⁺CD16⁺ NK cells, the multispecific protein can induce the activation of NK cells and/or lysis of target cells, in particular via the activating signal(s) transmitted by any combination of NKp46, CD16 and/or CD137.

In some embodiments, the multispecific protein binds to NKp46 in monovalent fashion. In one aspect of any embodiment, the multispecific protein comprises an Nkp46-binding ABD which binds to an NKp46 polypeptide monovalently.

In some embodiments in the presence of target cells and NK cells, the multispecific protein is capable of inducing an increase in cell surface CD137, e.g., on NK cells that express NK46 and CD16 (NKp46⁺CD16⁺ NK cells).

In some embodiments in the presence of target cells and NK cells, the multispecific protein is capable of inducing signaling by NK cells through NKp46.

In some embodiments in the presence of target cells and NK cells, the multispecific protein lacks the ability to induce NKp46-mediated signaling or cellular activation of an NK cell (independently of CD16), when incubated with NK cells in the absence of target cells (cells expressing the antigen of interest); for example the multispecific protein lacks the ability to induce NKp46-mediated signaling or NK cell activation when incubated with NK cells in the absence of target cells, when the protein is modified to comprise an Fc domain that does not bind CD16 (e.g. an Fc region containing a N297S substitution); or the multispecific protein lacks the ability to induce NKp46-mediated signaling or NK cell activation when incubated with NKp46⁺CD16⁻ NK cells, in the absence of target cells. In any of the multispecific proteins described herein, such multispecific protein potentially possesses the following characteristics:

(a) capable of inducing human NK cells that express CD16 and NKp46 (NKp46⁺CD16⁺ NK cells) to lyse target cells expressing the antigen of interest, when incubated in the presence of the NK cells and target cells; and

(b) capable of inducing an increase in cell surface CD137 when incubated (e.g. in soluble form) with NK cells that express CD16 and NKp46, optionally in the presence of target cells, or in the absence of target cells.

In another embodiment the invention provides multispecific protein formats adapted for use in an NKp46-based NK cell engager, including antibody-based formats comprising antigen binding domain(s) and/or constant region domain(s) from immunoglobulins capable of forming a dimeric Fc domain that binds to human CD16 (and optionally further binding to FcRn and/or other human Fcγ receptors). By combining the NK-selective expression of NKp46 with multispecific (e.g. bispecific) antibody formats in which the multispecific proteins retain and/or have increased binding to human Fcγ receptor via an Fc domain, the invention provides multispecific antibody formats with favorable pharmacology due to FcRn binding which can direct NK cell cytotoxicity to a target of interest, and which further possess increased ability to lyse target cells via the combined action of activating receptors CD16, NKp46, and in addition optionally CD137.

In another aspect of any embodiment described herein, the multispecific protein can be characterized by a lack of agonist activity for NKp46 when incubated with Fcγ receptor-negative NK cells (e.g. as purified NKp46⁺CD16⁻ NK cells) and in the absence of target cells (e.g. cells expressing the antigen of interest).

In another embodiment the invention provides a method for identifying, testing and/or producing a multispecific protein that binds NKp46 and CD16 on an NK cell and an antigen of interest expressed by a target cell, the method comprising:

(a) assessing whether the multispecific protein is capable of inducing an increase in cell surface CD137 on NK cells when incubated with CD16-expressing NK cells (e.g. NKp46⁺CD16⁺ NK cells); and

(b) assessing whether the multispecific protein has the ability to induce NK cells that express CD16 and NKp46 to lyse target cells, when incubated in the presence of the NK cells and target cells.

Optionally, in any of the foregoing the NK cells are purified NK cells.

In another embodiment the invention provides a method for identifying, testing and/or producing a multispecific protein, the method comprising providing a plurality of multispecific proteins that bind NKp46 and CD16 on an NK cell and an antigen of interest expressed by a target cell:

- (a) assessing whether each multispecific protein has the ability to induce NK cells that express CD16 and NKp46 to lyse target cells, when incubated in the presence of the NK cells and target cells;
- (b) optionally, further assessing each multispecific protein for the ability to induce an increase in cell surface CD137 when incubated with CD16-expressing NK cells (e.g. NKp46⁺CD16⁺ NK cells); and
- (c) selecting a multispecific protein (e.g. for use as a medicament, for further evaluation, for further production, etc.) if the multispecific protein:
 - a. has the ability to induce NK cells that express CD16 and NKp46 to lyse target cells, when incubated in the presence of the NK cells and target cells, and
 - b. optionally, has the ability to induce an increase in cell surface CD137 when incubated with CD16-expressing NK cells.

In another embodiment the invention provides a method for identifying, testing and/or producing a multispecific protein, the method comprising providing a plurality of multispecific proteins that bind NKp46 and CD16 on an NK cell and an antigen of interest expressed by a target cell:

- (a) assessing each multispecific protein to determine whether it is capable of inducing NKp46-mediated signaling or NK cell activation of an NK cell independently of CD16, when incubated with NK cells in the absence of target cells;
- (b) assessing each multispecific protein to determine whether the multispecific protein has the ability to induce NK cells to lyse target cells, when incubated in the presence of the NK cells and target cells;
- (c) optionally, further assessing each multispecific protein to determine whether it is capable of inducing an increase in cell surface CD137 when incubated with CD16-expressing NK cells (e.g. NKp46⁺CD16⁺ NK cells); and
- (d) selecting a multispecific protein (e.g. for use as a medicament, for further evaluation, for further production, etc.) if the multispecific protein:
 - a. lacks the ability to induce NKp46-mediated signaling or NK cell activation in an NK cell independently of CD16, when incubated with NK cells in absence of target cells;
 - b. has the ability to induce NK cells to lyse target cells, when incubated with NK cells and target cells, and
 - c. optionally, has the ability to induce an increase in cell surface CD137 when incubated with CD16-expressing NK cells.

In another embodiment the invention provides a multispecific protein that binds human CD16 (e.g. polypeptide, non-antibody polypeptide, or antibody), comprising: (a) a first antigen binding domain; and (b) a second antigen binding domain, wherein one of the first antigen binding domains binds NKp46 and the other binds to an antigen of interest on a target cell (other than NKp46), wherein the multispecific protein is capable of directing NKp46-expressing NK cells to lyse said target cell. In one embodiment, the protein comprises at least a portion of a human Fc domain, optionally wherein the Fc domain is dimeric, and is bound by human CD16 (and further optionally wherein the Fc domain is also bound by FcRn), and still further optionally wherein the multispecific antibody comprises an Fc domain comprising a modification (compared to a wild-type Fc domain) that results in increased binding to CD16; in one embodiment, the Fc domain is interposed between the two ABDs (one ABD is placed N-terminal and the other is C-terminal to the Fc domain).

In one aspect, the multispecific protein comprises two or more polypeptide chains, i.e. it comprises a multi-chain protein. For example, the multispecific protein or multi-chain protein can be a dimer, trimer or tetramer or may comprise more than 4 polypeptide chains.

An antigen binding domain positioned on a polypeptide chain can itself bind to its target (i.e., NKp46 or an antigen of interest) (e.g. an scFv or single antigen binding domain) or can optionally bind its target when it is in association or together with one or more complementary protein domains (antigen binding domain or domains) positioned on a different polypeptide chain, wherein these polypeptide chains associate to form a multimer (e.g. dimer, trimer, etc.).

In one aspect, the multispecific protein binds an NKp46 polypeptide (e.g. expressed on the surface of a NK cell) in monovalent fashion. In one aspect, the multispecific protein binds the antigen of interest in monovalent fashion.

In another embodiment the invention provides multispecific proteins having a structure in which the freedom of motion (intrachain domain motion) or flexibility of one or more antigen binding domains (ABDs) is increased, e.g. compared to the ABDs of a conventional human IgG antibody. In one embodiment, provided is a multispecific protein comprising a structure that permits the antigen binding site of the first antigen binding domain and the antigen binding site of the second antigen binding domain to be separated by a distance that results in enhanced function, e.g., the ability of the multispecific protein to induce NKp46 signaling and lysis of target cells, e.g., optionally a distance of less than 80 ångström (Å). Multispecific proteins wherein the ABDs possess greater flexibility and/or are separated by an optimized distance may enhance the formation of a lytic NKp46-target synapse, thereby potentiating NKp46-mediated signaling.

In one embodiment, the invention provides multispecific proteins having increased freedom of motion of the antigen binding domains (e.g. compared to the ABDs of a conventional human IgG antibody, e.g., a human IgG1 antibody). One example of such a protein is a monomeric or multimeric Fc domain-containing protein (e.g. a heterodimer or heterotrimer) in which an antigen binding domain (e.g., the ABD that binds NKp46 or the ABD that bind the antigen of interest) is linked or fused to an Fc domain via a flexible linker. The linker can provide flexibility or freedom of motion of one or more ABDs by conferring the ability to bend thereby potentially decreasing the angle between the ABD and the Fc domain (or between the two ABDs) at the linker. Optionally, both antigen binding domains (and optionally more if additional ABDs are present in the multispecific protein) are linked or fused to the Fc domain via a linker, typically a flexible peptide linker. Optionally, other sequences or domains such as constant domains which optionally may be modified to alter (enhance or inhibit) one or more effector functions are placed between the Fc domain and an ABD, e.g. such that the ABD is fused to the Fc domain via a flexible linker and a constant region. The antigen binding domain can for example be comprised of variable region(s), a dAb, a VhH or a non-Ig scaffold. The antigen binding domain may be present in its entirety on a single polypeptide chain or may be formed from the association with a domain present on a separate polypeptide chain. Optionally, the protein with increased freedom of motion permits the protein to adopt a conformation in which the distance between the NKp46 binding site and the antigen of interest binding site is less than that observed in proteins in which both binding domains were Fabs, or less than in full length antibodies.

An ABD can be connected to the Fc domain (or CH2 or CH3 domain thereof) via a flexible linker (optionally via intervening sequences such as constant region domains or portions thereof, e.g. CH1 or Cκ). The linker can be a polypeptide linker, for example peptide linkers comprising a length of at least 5 residues, at least 10 residues, at least 15 residues, at least 20 residues, at least 25 residues, at least 30 residues or more. In other embodiments, the linkers comprises a length of between 2-4 residues, between 2-4 residues, between 2-6 residues, between 2-8 residues, between 2-10 residues, between 2-12 residues, between 2-14 residues, between 2-16 residues, between 2-18 residues, between 2-20 residues, between 2-30 residues, between 10-24 residues, between 10-26 residues, between 10-30 residues, or between 10-50 residues. Optionally a linker comprises an amino acid sequence derived from an antibody constant region, e.g., an N-terminal CH1 or hinge sequence. Optionally a linker comprises the amino acid sequence RTVA. Optionally a linker is a flexible linker predominantly or exclusively comprised of glycine and/or serine residues, e.g., comprising the amino acid sequence GEGTSTGS(G₂S)₂GGAD or the amino acid sequence (G₄S)₃.

In one embodiment, the Fc domain is interposed between the two ABDs (one ABD is placed N-terminal and the other is C-terminal to the Fc domain). The subject multispecific proteins (e.g. dimers, trimers, tetramers) may in some embodiments comprise a domain arrangement as follows, in which domains can be placed on any of the 2, 3 or 4 polypeptide chains, wherein an Fc domain is interposed between the antigen binding domains, and wherein a flexible linker is present between at least one of the ABDs and the Fc domain:

(ABD₁) (Fc domain) (ABD₂).

In another embodiment, the multispecific proteins (e.g. dimers, trimers, tetramers) may comprise a domain arrangement of any of the following in which domains can be placed on any of the 2, 3 or 4 polypeptide chains, wherein the Fc domain is not interposed between ABDs (e.g. the protein has a terminal or distal Fc domain), and wherein a flexible linker is present between at least one of the ABDs and the Fc domain:

(Fc domain) (ABD₁ and ABD₂),

or

(ABD₁ and ABD₂) (Fc domain).

In the above-described domain arrangements, one of ABD₁ and ABD₂ is an antigen binding domain that binds NKp46 and the other is an antigen binding domain that binds an antigen of interest, and wherein the linker is a flexible polypeptide linker. The Fc domain can be a dimeric Fc domain (e.g. that binds human FcRn and/or Fcγ receptors). In one embodiment, each of ABD₁ and ABD₂ are formed from two variable regions present within tandem variable regions, wherein the variable regions that associate to form a particular ABD can be on the same polypeptide chain or on different polypeptide chains. In another embodiment, one of ABD₁ and ABD₂ comprises a tandem variable region and the other comprises a Fab structure.

The invention also identifies specific epitopes on NKp46 which are well suited for targeting with NKp46 binding moieties, including the multispecific polypeptides disclosed herein. For example, bispecific or multispecific proteins which bind to one or more of these Nkp46 epitopes possess advantageous properties, notably high efficacy in causing or directing NK cells to lyse target cells (e.g. via NKp46-mediated signaling). Provided also are CDRs of different anti-NKp46 antibodies suitable for use in the construction of efficient multispecific proteins, e.g., bispecific and trispecific proteins particularly those which potently lyse a target cell of interest, and amino acid and nucleic acid sequences of exemplary multispecific proteins and nucleic acids which encode these proteins.

In one aspect, the protein (and/or the antigen binding domain thereof that binds NKp46) competes for binding to a NKp46 polypeptide with any one or any combination of monoclonal antibodies NKp46-1, -2, -3, -4, -6 or -9 or an Anti-CD19-F5-Anti-NKp46 antibody that comprises such NKp46-1, -2, -3, -4, -6 or -9 bispecific antibodies. In one embodiment, the antigen binding domain that binds NKp46 binds an epitope within (partly or fully within) the D2 (proximal) domain of the NKp46 polypeptide. In one embodiment, the antigen binding domain that binds NKp46 binds an epitope within (partly or fully within) the D1 (distal) domain of the NKp46 polypeptide. In one embodiment, the antigen binding domain that binds NKp46 binds an epitope on an NKp46 polypeptide of SEQ ID NO:1 comprising one, two, three or more residues selected from the residues bound by any one or combination of antibodies NKp46-1, -2, -3, -4, -6 or -9 or an Anti-CD19-F5-Anti-NKp46 antibody that comprises such NKp46-1, -2, -3, -4, -6 or -9 bispecific antibodies. In one embodiment the multispecific protein is capable of binding to human neonatal Fc receptor (FcRn). In one embodiment the multispecific protein has decreased or abolished binding to a human and/or non-human primate (e.g. cynomolgus monkey) Fcγ receptor, e.g., compared to a full length wild type human IgG1 antibody. In one embodiment the multispecific protein is capable of inducing NK-mediated lysis (e.g. as well as or better than a full length human wild-type IgG1 antibody).

In one embodiment of any of the multispecific proteins described herein, the antigen binding domain that binds to an antigen of interest binds to an antigen (e.g. polypeptide) expressed by a target cell which is sought to be lysed by an NK cell. Optionally this antigen is expressed by a cancer cell, or a virally, bacterially or parasite infected cell, an immune cell that contributes to tumor growth or escape (e.g. a tumor-associated monocyte or macrophage), or a cell that contributes to an autoimmunity, an allergic response or inflammatory disease.

In one embodiment, the multispecific protein binds NKp46 in monovalent fashion. In one embodiment, the multispecific protein binds to the antigen of interest in monovalent fashion. In one embodiment, the multispecific protein binds both NKp46 and the antigen of interest in monovalent fashion. In one embodiment, the multispecific protein binds CD16 via a dimeric Fc domain.

In one embodiment, the first antigen binding domain comprises an antibody heavy chain variable domain and a light chain variable domain. Optionally, both said heavy and light chain variable domains are involved in binding interactions with NKp46.

In one embodiment, the second ABD (and optionally third or more ABDs, when present) comprises an antibody heavy chain variable domain and a light chain variable domain. Optionally, both said heavy and light chain variable domains are involved in binding interactions with the antigen bound by the second antigen binding domain. In one embodiment, the second ABD (and optionally third or more ABD, when present) comprises a non-immunoglobulin scaffold.

Optionally, the Fc domain comprises at least a portion of a CH2 domain and at least a portion of a CH3 domain, and when present in a multispecific polypeptide, is part of a dimeric Fc domain.

In one embodiment, the CH2 domain comprises an amino acid modification, compared to a wild-type CH2 domain. In one embodiment, the CH2 modification increases binding (e.g. increases binding affinity) of the bispecific polypeptide to a human CD16 polypeptide relative to a wild-type human Fc region.

In one embodiment, the CH2 domain and/or CH3 domains are naturally occurring (non-engineered) human CH2 and/or CH3 domains. In one embodiment, the multispecific protein comprises an Fc domain that comprises N-linked glycosylation. In one embodiment, the N-linked glycosylation (at residue N297, Kabat EU numbering) comprises glycan structures typical of those found on IgG-class (e.g. IgG1) immunoglobulins produced in mammalian cells (e.g., CHO cells or other rodent cells, non-human primate or human cells).

In one embodiment, the Fc domain comprises modified N-linked glycosylation, e.g. hypofucosylated glycans at N297) which increase binding affinity for a human CD16 polypeptide.

In one embodiment, the Fc-derived polypeptide is a dimer, optionally a homodimer or a heterodimer. In one embodiment, the Fc-derived polypeptide is a heterotrimer. In one embodiment, the Fc-derived polypeptide is a hetero-tetramer.

In one embodiment, heterotrimer proteins are provided comprising two antigen binding domains that are composed of three different polypeptide chains that each comprise at least one V-(CH1/Ck) unit, wherein a first (central) chain comprises two V-(CH1/Ck) units separated by an Fc domain (or portion thereof which optionally binds CD16), and each of the second and third chains comprise one V-(CH1/Ck) unit, wherein one of the V-(CH1/Ck) units of the central chain preferentially undergoes CH1-Ck dimerization with the V-(CH1/Ck) unit of the second chain thereby forming a first antigen binding domain, wherein the other of the V-(CH1/Ck) units of the central chain preferentially undergoes CH1-Ck dimerization with the V-(CH1/Ck) unit of the third chain thereby forming a second antigen binding domain, and wherein the second or third chain further comprise an Fc domain (or portion thereof) placed on the polypeptide chain such that the Fc domain is capable of forming a dimeric Fc domain that binds CD16 together with the Fc domain of the central polypeptide.

In one embodiment, heterotrimer proteins having three antigen binding domains are provided that are composed of three different polypeptide chains that each comprise at least one V-(CH1/Ck) unit, wherein a first (central) chain comprises two V-(CH1/Ck) units and each of the second and third chains comprise one V-(CH1/Ck) unit, wherein one of the V-(CH1/Ck) units of the central chain preferentially undergoes CH1-Ck dimerization with the V-(CH1/Ck) unit of the second chain thereby forming a first antigen binding domain, wherein the other of the V-(CH1/Ck) units of the central chain preferentially undergoes CH1-Ck dimerization with the V-(CH1/Ck) unit of the third chain thereby forming a second antigen binding domain, wherein one of the polypeptide chains further comprises an antigen binding domain (e.g. a tandem variable domain, an scFv) that forms third antigen binding domain, and wherein the first or second chain further comprise an Fc domain (or portion thereof) placed on the polypeptide chain such that the Fc domain is capable of forming a dimeric Fc domain that bind CD16 together with the Fc domain of the central polypeptide.

In one embodiment, the invention provides a heteromultimeric, e.g. heterodimeric, bispecific protein comprising: (a) a first polypeptide chain comprising a first variable region (V), fused to a CH1 or Ck domain, wherein the V-(CH1/Ck) unit is in turn fused to a first terminus (N- or C-terminus) of a human Fc domain (a full Fc domain or a portion thereof); (b) a second polypeptide chain comprising a first variable region (V) fused to a CH1 or Ck domain that is complementary with the CH1 or Ck of the first chain to form a CH1-Ck dimer, wherein the V-(CH1/Ck) unit is fused to at least a human Fc domain (a full Fc domain or a portion thereof), wherein the two first variable regions form an antigen binding domain that binds a first antigen of interest in monovalent fashion, and (c) an antigen binding domain that binds a second antigen (optionally together with a complementary antigen binding domain), and optionally a second CH1 or Ck domain, fused to a second terminus (N- or C-terminus) of the Fc domain of the first polypeptide such that the Fc domain is interposed between the V-(CH1/Ck) unit and the antigen binding domain that binds a second antigen, and wherein the Fc domains (or portions thereof) of the first and second chains of the heteromultimeric protein form a dimeric Fc domain capable of being bound by human CD16 polypeptide, e.g. a dimeric Fc domain comprising N-linked glycosylation at residue N297 (Kabat EU numbering). Optionally the first and second polypeptide chains are bound by non-covalent bonds and optionally further interchain disulfide bonds, e.g. formed between respective CH1 and Ck domains and/or between respective hinge domains. Optionally a V-(CH1/Ck) unit is fused to a human Fc domain directly, or via intervening sequences, e.g. linkers, other protein domain(s), etc. Optionally, one of the antigens is NKp46 and another of the antigens is one expressed on the surface of a cell that is to be lysed by the immune cell, e.g. a cancer or viral or bacterial antigen; optionally further, wherein such antigen present on the surface of a cell that is to be lysed by the immune cell, e.g., NK cell, is a protein that is known to undergo intracellular internalization, notably when bound by an antibody (e.g. a full length antibody of an isotype such as human IgG1 or IgG3 that is bound by CD16).

In one embodiment, the multispecific polypeptide or protein is monoclonal. In one embodiment, the multispecific polypeptide or protein is purified. In one embodiment, the multispecific polypeptide or protein is isolated. In another embodiment the multispecific polypeptide or protein is expressed by a cell, e.g., a human immune cell such as a human NK cell.

In one embodiment of the above heteromultimeric polypeptide or protein, the polypeptide or protein is a heterodimer, wherein the antigen binding domain for a second antigen is an scFv, optionally an scFv that binds NKp46.

In one embodiment of the afore-described heteromultimeric polypeptides or proteins, the heteromultimeric polypeptide or protein is a heterotrimer, comprising an antigen binding domain for a second antigen which comprises or consists of an heavy or light chain variable region, and the heteromultimeric polypeptide or protein further comprises a third polypeptide chain comprising or consisting of a variable region (V) fused to a CH1 or Ck domain that is complementary with the CH1 or Ck of the first chain to form a CH1-Ck dimer wherein the variable region that is the antigen binding domain for a second antigen of the first polypeptide and the variable region of the third chain form an antigen binding domain. The double dimerization yields a trimer. The CH1 or Ck constant region of the third polypeptide is selected to be complementary to the second CH1 or Ck constant region of the first polypeptide chain (but not complementary to the first CH1/Ck of the first polypeptide chain).

In one aspect a heterodimeric polypeptide according to the invention comprises:

- (a) a first polypeptide chain comprising, from N- to C-terminus, a first variable domain (V), a CH1 or Ck constant region, a Fc domain or portion thereof which optionally binds CD16, a second variable domain and third variable domain; and
- (b) a second polypeptide chain comprising, from N- to C-terminus, a first variable domain (V), a CH1 or Ck constant region, an Fc domain or portion thereof, wherein the CH1 or Ck constant region is selected to be complementary to the CH1 or Ck constant region of the first polypeptide chain such that the first and second polypeptides form a CH1-Ck heterodimer in which the first variable domain of the first polypeptide chain and the first variable domain of the second polypeptide form an antigen binding domain that binds the first antigen of interest; and wherein a second variable domain and third variable domain forms an antigen binding domain that binds the second antigen of interest, and wherein the Fc domains (or portions thereof) of the first and second chains of the heteromultimeric protein form a dimeric Fc domain capable of being bound by human CD16 polypeptide, e.g. a dimeric Fc domain comprising N-linked glycosylation at residue N297 (Kabat EU numbering).

In another aspect the invention provides a heterodimeric polypeptide which comprises:

- (a) a first polypeptide chain comprising, from N- to C-terminus, a second variable domain and third variable domain, a Fc domain or portion thereof, a first variable domain (V), and a CH1 or Ck constant region; and
- (b) a second polypeptide chain comprising, from N- to C-terminus, a first variable domain (V), a CH1 or Ck constant region, and a Fc domain or portion thereof, wherein the CH1 or Ck constant region is selected to be complementary to the CH1 or Ck constant region of the first polypeptide chain such that the first and second polypeptides form a CH1-Ck heterodimer in which the first variable domain of the first polypeptide chain and the first variable domain of the second polypeptide form an antigen binding domain that binds to the first antigen of interest; and wherein a second variable domain and third variable domain forms an antigen binding domain that binds to a second antigen of interest, wherein the Fc domains (or portions thereof) of the first and second chains of the heteromultimeric protein form a dimeric Fc domain capable of being bound by human CD16 polypeptide, e.g. a dimeric Fc domain comprising IgG-type N-linked glycosylation at residue N297 (Kabat EU numbering).

In another aspect the invention provides a heterodimeric polypeptide which comprises:

- (a) a first polypeptide chain comprising, from N- to C-terminus, a first variable domain (V) fused to a first CH1 or CKk constant region, an Fc domain or portion thereof, and a second variable domain (V) fused to a second CH1 or Ck constant region;
- (b) a second polypeptide chain comprising, from N- to C-terminus, a variable domain fused to a CH1 or Ck constant region selected to be complementary to the first (but not the second) CH1 or Ck constant region of the first polypeptide chain such that the first and second polypeptides form a CH1-Ck heterodimer, and an Fc domain or portion thereof; and
- (c) a third polypeptide chain comprising, from N- to C-terminus, a variable domain fused to a CH1 or Ck constant region, wherein the CH1 or Ck

constant region is selected to be complementary to the second (but not the first) variable domain and second CH1 or Ck constant region of the first polypeptide chain, wherein the Fc domains (or portions thereof) of the first and second chains of the heteromultimeric protein form a dimeric Fc domain capable of being bound by a human CD16 polypeptide, e.g. a dimeric Fc domain comprising IgG-type N-linked glycosylation at residue N297 (Kabat EU numbering). The first and third polypeptides will therefore form a CH1-Ck heterodimer formed between the CH1 or Ck constant region of the third polypeptide and the second CH1 or Ck constant region of the first polypeptide, but not between the CH1 or Ck constant region of the third polypeptide and the first CH1 or Ck constant region of the first polypeptide. The first, second and third polypeptides form a CH1-Ck heterotrimer, and wherein the first variable domain of the first polypeptide chain and the variable domain of the second polypeptide chain form an antigen binding domain specific for a first antigen of interest, and the second variable domain of the first polypeptide chain and the variable domain on the third polypeptide chain form an antigen binding domain specific for a second antigen of interest.

In another embodiment, the above-described heteromultimeric polypeptides or proteins may comprise one or more additional polypeptide chains.

Any the heteromultimeric polypeptides or proteins described herein may comprise a dimeric Fc domain capable of being bound by human CD16 polypeptide, e.g. a dimeric Fc domain comprising N-linked glycosylation at residue N297 (Kabat EU numbering).

Optionally, the CH1 and/or Ck domain are fused via a hinge region to the Fc domain. Optionally the hinge, CH2 and/or CH3 comprises one or more amino acid modifications which increase binding affinity for human CD16. Optionally the hinge, CH2 and/or CH3 comprises an amino acid modification which increases binding affinity for human FcRn. Optionally the amino acid modifications that increase binding affinity for CD16 may also increase affinity for one or more other human Fcγ receptors. Optionally the hinge, CH2 and/or CH3 comprise an amino acid modification which reduces or substantially abolishes binding to an inhibitory human Fcγ receptor (e.g. CD32B) and/or to an Fcγ receptor other than CD16 (e.g. CD32A and/or CD64). In any embodiment described herein, the CH1 and Ck domains optionally can be of human origin.

In one aspect of any of the embodiments described herein, the bispecific protein binds more strongly or avidly (has a greater binding affinity) for the antigen of interest (e.g. a cancer or viral or other infectious agent antigen) than it binds NKp46. Such antibodies may possess advantageous pharmacological properties. In one aspect of any of the embodiments herein, the polypeptide has a K_d (monovalent binding affinity) to NKp46 of less than 10⁻⁷ M, preferably less than 10⁻⁸ M, or preferably less than 10⁻⁹ M; optionally the polypeptide has a K_d for binding (monovalent binding affinity) to a cancer, viral, bacterial or other antigen that is less than (i.e. has better binding affinity than) the K_d (monovalent binding affinity) to a NKp46 polypeptide. In one aspect of any of the embodiments described herein, the polypeptide has a K_d (monovalent binding affinity) to NKp46 of between 10⁻⁷ M (100 nanomolar) and 10⁻¹⁰ M (0.1 nanomolar) for binding to a NKp46 polypeptide. In one aspect of any of the embodiments disclosed herein, the polypeptide has a K_d (monovalent binding affinity) to NKp46 of between 10⁻⁸ M (10 nanomolar) and 10⁻¹⁰ M (0.1 nanomolar). In one aspect of any of the embodiments herein the multimeric polypeptide has a K_d (monovalent binding affinity) to NKp46 of between 10⁻⁸ M (10 nanomolar) and 10⁻⁹ M (1 nanomolar). Binding can be assessed as in the Examples herein, e.g. by surface plasmon resonance.

In one aspect of any of the embodiments of the invention, the antigen binding domain that binds NKp46 binds to at least one residue on NKp46 corresponding to any of the amino acid residues bound by any one of monoclonal antibodies NKp46-1, -2, -3, -4, -6 or -9 or the Anti-CD19-Anti-NKp46-1, -2, -3, -4, -6 or -9 bispecific antibodies. In one aspect, the antigen binding domain that binds NKp46 binds at least 1, 2, 3, 4 or more amino acids of NKp46 within the epitope bound by any one or combination of monoclonal antibodies NKp46-1, -2, -3, -4, -6 or -9 or the Anti-CD19-Anti-NKp46-1, -2, -3, -4, -6 or -9 bispecific antibodies. In one aspect of any of the embodiments of the invention, the antigen binding domain that binds NKp46 binds to the same epitope on a NKp46 polypeptide as any of monoclonal antibodies NKp46-1, -2, -3, -4, -6 or -9 or to any of the Anti-CD19-Anti-NKp46-1, -2, -3, -4, -6 or -9 bispecific antibodies. In one embodiment, the antigen binding domain that binds NKp46 binds to an epitope on the NKp46 polypeptide of SEQ ID NO:1 wherein the epitope comprises one, two, three or more residues selected from the group of residues bound by any of antibodies NKp46-1, -2, -3, -4, -6 or -9.

In some embodiments, the protein that binds NKp46 exhibits significantly lower binding for a mutant NKp46 polypeptide in which a residue bound by any of antibodies NKp46-1, -2, -3, -4, -6 or -9 is substituted with a different amino acid, compared to a wild-type NKp46 polypeptide of SEQ ID NO: 1.

In one aspect of any of the embodiments of the invention, the protein that binds NKp46 competes for binding to a NKp46 polypeptide with any

one or any combination of monoclonal antibodies NKp46-1, NKp46-2, NKp46-3, NKp46-4, NKp46-6 or NKp46-9, or any of the Anti-CD19-anti-NKp46-1, -2, -3, -4, -6 or -9 bispecific antibodies. In one embodiment, the protein that binds NKp46 competes for binding to an NKp46 polypeptide with an antibody selected from the group consisting of:

- (a) an antibody having respectively a V_H and V_L region of SEQ ID NOS: 3 and 4 (NKp46-1);
- (b) an antibody having respectively a V_H and V_L region of SEQ ID NOS: 5 and 6 (NKp46-2);
- (c) an antibody having respectively a V_H and V_L region of SEQ ID NOS: 7 and 8 (NKp46-3);
- (d) an antibody having respectively a V_H and V_L region of SEQ ID NOS: 9 and 10 (NKp46-4);
- (e) an antibody having respectively a V_H and V_L region of SEQ ID NOS: 11 and 12 (NKp46-6); and
- (f) an antibody having respectively a V_H and V_L region of SEQ ID NOS: 13 and 14 (NKp46-9).

In one embodiment, the invention provides a protein (or nucleic acid encoding such) that specifically binds NKp46 (e.g. a monospecific monoclonal antibody or fragment, a multispecific protein or fragment, a bispecific antibody, etc.) that competes for binding to an NKp46 polypeptide with an antibody selected from the group consisting of:

- (a) an antibody having respectively a V_H and V_L region of SEQ ID NOS: 3 and 4 (NKp46-1);
- (b) an antibody having respectively a V_H and V_L region of SEQ ID NOS: 5 and 6 (NKp46-2);
- (c) (a) an antibody having respectively a V_H and V_L region of SEQ ID NOS: 7 and 8 (NKp46-3);
- (d) (a) an antibody having respectively a V_H and V_L region of SEQ ID NOS: 9 and 10 (NKp46-4);
- (e) an antibody having respectively a V_H and V_L region of SEQ ID NOS: 11 and 12 (NKp46-6); and
- (f) an antibody having respectively a V_H and V_L region of SEQ ID NOS: 13 and 14 (NKp46-9).

In one aspect of any of the embodiments of the invention, the antigen binding domain that binds NKp46 comprises the hypervariable regions of any one of monoclonal antibodies NKp46-1, NKp46-2, NKp46-3, NKp46-4, NKp46-6 or NKp46-9 or a combination of any of the foregoing.

In one aspect of any of the embodiments of the invention, the antigen binding domain that binds NKp46 has a heavy and/or light chain variable region having one, two or three CDRs of the respective heavy and/or light chain of an antibody selected from the group consisting of antibody NKp46-1, NKp46-2, NKp46-3, NKp46-4, NKp46-6 and NKp46-9.

In one embodiment, an isolated multispecific protein that binds NKp46 according to the invention comprises or an antigen binding domain thereof comprises heavy chain CDR1, 2 and 3 and light chain CDR 1, 2 and 3 of any of the antibodies selected from the group consisting of:

- (a) an antibody having respectively a V_H and V_L region of SEQ ID NOS: 3 and 4 (NKp46-1);
- (b) an antibody having respectively a V_H and V_L region of SEQ ID NOS: 5 and 6 (NKp46-2);
- (c) (a) an antibody having respectively a V_H and V_L region of SEQ ID NOS: 7 and 8 (NKp46-3);
- (d) (a) an antibody having respectively a V_H and V_L region of SEQ ID NOS: 9 and 10 (NKp46-4);
- (e) an antibody having respectively a V_H and V_L region of SEQ ID NOS: 11 and 12 (NKp46-6); and
- (f) an antibody having respectively a V_H and V_L region of SEQ ID NOS: 13 and 14 (NKp46-9).

In one embodiment, the invention provides an antibody (e.g. a full length monospecific antibody or a bispecific antibody) or antigen binding domain (which optionally may be humanized, chimerized or affinity matured) that binds NKp46 and comprises:

- (a) (i) a polypeptide chain comprising a CDR 1, 2 and 3 of the heavy chain variable region of NKp46-1 of Table A, and (ii) a polypeptide chain comprising a CDR 1, 2 and 3 of the light chain variable region of NKp46-1 of Table A;
- (b) (i) a polypeptide chain comprising a CDR 1, 2 and 3 of the heavy chain variable region of NKp46-2 of Table A and (ii) a polypeptide chain comprising a CDR 1, 2 and 3 of the light chain variable region of NKp46-2 of Table A;
- (c) (i) a polypeptide chain comprising a CDR 1, 2 and 3 of the heavy chain variable region of NKp46-3 of Table A and (ii) a polypeptide chain comprising a CDR 1, 2 and 3 of the light chain variable region of NKp46-3 of Table A;
- (d) (i) a polypeptide chain comprising a CDR 1, 2 and 3 of the heavy chain variable region of NKp46-4 of Table A and (ii) a polypeptide chain

comprising a CDR 1, 2 and 3 of the light chain variable region of NKp46-4 of Table A;

(e) (i) a polypeptide chain comprising a CDR 1, 2 and 3 of the heavy chain variable region of NKp46-6 of Table A and (ii) a polypeptide chain comprising CDR 1, 2 and 3 of the light chain variable region of NKp46-6 of Table A; or

(f) (i) a polypeptide chain comprising a CDR 1, 2 and 3 of the heavy chain variable region of NKp46-9 of Table A and (ii) a polypeptide chain comprising a CDR 1, 2 and 3 of the light chain variable region of NKp46-9 of Table A.

In one aspect, the invention provides a protein (a monomeric or multimeric protein) that specifically binds NKp46 (e.g. a monospecific monoclonal antibody, a multispecific protein, a bispecific antibody) that binds the same or overlapping epitope on NKp46 as an antibody selected from the group consisting of antibody NKp46-1, NKp46-2, NKp46-3, NKp46-4, NKp46-6 and NKp46-9. The isolated polypeptide may be, for example, a monospecific monoclonal antibody, a multispecific polypeptide or a bispecific antibody.

In one aspect the invention provides an isolated multispecific heterotrimeric protein comprising a first polypeptide chain comprising an amino acid sequence which is at least 50%, 60%, 70%, 80%, 85%, 90%, 95%, 98 or 99% identical to the sequence of a first polypeptide chain of a F5, F13 or T5 protein disclosed herein; a second polypeptide chain comprising an amino acid sequence which is at least 50%, 60%, 70%, 80%, 85%, 90%, 95%, 98 or 99% identical to the sequence of a second polypeptide chain of the respective F5, F13 or T5 protein disclosed herein; and a third polypeptide chain comprising an amino acid sequence which is at least 50%, 60%, 70%, 80%, 85%, 90%, 95%, 98 or 99% identical to the sequence of a third polypeptide chain of a F5, F13 or T5 protein disclosed herein. In one aspect, the protein comprises a dimeric Fc domain capable of being bound by a human CD16 polypeptide, e.g. a dimeric Fc domain comprising N-linked glycosylation at residue N297 (Kabat EU numbering). Optionally any or all of the variable regions or CDRs of the first, second and/or third chains are substituted with different variable regions; optionally any or all of the V-CH1/Ck units of the first, second and/or third chains are substituted with different V-CH1/Ck units. Optionally variable regions, CDRs or V-CH1/Ck units are excluded from the sequences that are considered for computing identity; optionally wherein the anti-NKp46 variable regions, CDRs or V-CH1/Ck units are included for computing identity and the variable regions, CDRs or V-CH1/Ck units for the antigen binding domain that binds the other antigen are excluded from the sequences that are considered for computing identity. In one embodiment of any of the polypeptides described herein, the multispecific polypeptide is capable of directing NKp46-expressing NK cells to lyse a target cell of interest (e.g. a target cell expressing an antigen other than NKp46).

In one aspect of any of the embodiments described herein, the invention provides a recombinant nucleic acid encoding a first polypeptide chain, and/or a second polypeptide chain, and/or a third polypeptide chain and/or a fourth polypeptide. In one aspect of any of the embodiments described herein, the invention provides a recombinant host cell comprising a nucleic acid encoding a first polypeptide chain, and/or a second polypeptide chain and/or a third polypeptide chain, optionally wherein the host cell produces a multimeric or other protein according to the invention with a yield (final productivity or concentration before or after purification) of at least 1, 2, 3 or 4 mg/L. Also provided is a kit or set of nucleic acids comprising a recombinant nucleic acid encoding a first polypeptide chain of the according to the invention, a recombinant nucleic acid encoding a second polypeptide chain according to the invention, and, optionally, a recombinant nucleic acid encoding a third polypeptide chain according to the invention. Also provided are methods of making dimeric, trimeric and tetrameric proteins according to the invention.

Any of the methods can further be characterized as comprising any step described in the application, including notably in the "Detailed Description of the Invention"). The invention further relates to methods of identifying, testing and/or making proteins described herein. The invention further relates to a multispecific protein obtainable by any of present methods. The disclosure further relates to pharmaceutical or diagnostic formulations containing at least one of the multispecific proteins disclosed herein. The disclosure further relates to methods of using the subject multispecific proteins in methods of treatment or diagnosis.

These and additional advantageous aspects and features of the invention may be further described elsewhere herein.

BRIEF DESCRIPTION OF THE FIGURES

FIG. 1 shows that Anti-CD19-F1-Anti-CD3 does not cause T/B cell aggregation in the presence of B221 (CD19) or JURKAT (CD3) cell lines when separate, but it does cause aggregation of cells when both B221 and JURKAT cells are co-incubated.

FIGS. 2A to 2F show different domain arrangements of bispecific anti-NKp46 proteins produced.

FIGS. 3A and 3B respectively demonstrate that bispecific F1 and F2 format proteins having NKp46 binding region based on NKp46-1, NKp46-2, NKp46-3 or NKp46-4 are able to direct resting NK cells to their CD19-positive Daudi tumor target cells, while isotype control antibody did not lead to the elimination of the Daudi cells. Rituximab (RTX) served as the positive control of ADCC, where the maximal response obtained with RTX (at 10 µg/ml in this assay) was 21.6% specific lysis.

FIG. 4A shows that bispecific antibodies having NKp46 and CD19 binding regions in an F2 format protein do not activate resting NK cells in the absence of target cells; by contrast full length anti-NKp46 antibodies as well as positive control alemtuzumab did activate NK cells. FIG. 4B shows that bispecific anti-NKp46xanti-CD19 antibodies (including each of the NKp46-1, NKp46-2, NKp46-3 or NKp46-4 binding domains) activated resting NK cells in presence of Daudi target cells, while full-length anti-CD19 showed at best only very low activation of NK cells and neither full-length anti-NKp46 antibodies nor alemtuzumab elicited a substantial increase in activation beyond what was observed in the presence of NK cells alone. FIG. 4C shows that in the presence of CD19-negative HUT78 cells, none of the bispecific anti-NKp46xanti-CD19 antibodies (including each of the NKp46-1, NKp46-2, NKp46-3 or NKp46-4 variable regions) activated NK cells. However, the full-length anti-NKp46 antibodies and alemtuzumab resulted in detectable activation of NK cells, i.e., at a similar level observed in presence of NK cells alone. Isotype control antibody did not induce activation.

FIGS. 5A and 5B shows that at low effector:target ratios of 1:1 each of the tested bispecific anti-NKp46xanti-CD19 antibodies activated NK cells in the presence of Daudi cells, and that bispecific anti-NKp46xanti-CD19 antibodies were far more potent (better elicited lysis of target cells) than a control anti-CD19 antibody as well as a full-length human IgG1 ADCC inducing antibody.

FIGS. 6A and 6B show that each NKp46xCD19 bispecific protein (Format F3, F5 and F6) induced specific lysis of Daudi (FIG. 6A) or B221 (FIG. 6B) cells by human KHYG-1 CD16-negative hNKp46-positive NK cell line, while rituximab and human IgG1 isotype control (IC) antibodies did not. FIG. 6C shows that a NKp46xKIR3DL2 bispecific protein (Format F6) induced specific lysis of HUT78 tumor cells via NKp46 binding (without CD16 binding) comparably to a conventional IgG1 antibody with the same anti-KIR3DL2 variable regions.

FIG. 7 shows a NKp46xCD19 bispecific protein in F5 format whose Fc domain binds CD16 is far more potent in mediating Daudi target cell lysis than a full-length IgG1 anti-CD19 antibody or a F6 format bispecific protein. The figure also shows that a bispecific anti-CD19 in F6 format whose Fc domain does not bind CD16 was as potent in mediating NK cell lysis of Daudi target cells as the full-length IgG1 anti-CD19 antibody, which is unexpected considering that the control IgG1 anti-CD19 antibody binds CD19 bivalently. At comparable levels of target cell lysis, CD19-F5-NKp46-3 was at least 1000 times more potent than the full-length anti-CD19 IgG1.

FIG. 8 shows the results of cytotoxicity assays using fresh NK cells (Daudi cell in the right hand panel and HUT78 cells in the left hand panel); the CD19-F6-NKp46-3 whose Fc domain does not bind CD16 due to a N297 substitution has as mode of action NKp46 triggering when NK cells encounter the target cell, while the CD19-F5-NKp46-3 bispecific protein demonstrated a far higher potency in mediating cytotoxicity toward Daudi cells. Neither the F5 nor F6 proteins mediated any NK cell cytotoxicity towards HUT78 cells.

FIG. 9 shows the results of flow cytometry staining of NK cell surface markers showed a strong upregulation of CD137 on the surface of NK cells by F5 proteins (Left-most panel: NK cells vs. Daudi; middle panel: NK cells vs. HUT78; right-most panel: NK cells alone). The full-length anti-CD19 IgG1 antibody that binds CD16 also showed CD137 upregulation, but to a far lesser extent than the CD19-F5-NKp46-3 protein. The CD19-F6-NKp46-3 which functions via NKp46 but not CD16 did not show any CD137 upregulation.

FIG. 10 shows the results of cytotoxicity assays which compared the ability of the GA101-F5+-NKp46-1 bispecific protein to a comparison antibody (GA101) containing the same variable regions to lyse Daudi cells. The results therein show that the GA101-F5+-NKp46-1 bispecific protein possesses far higher potency (approximately 10-fold increase in EC₅₀) in mediating cytotoxicity toward Daudi cells than GA101.

DETAILED DESCRIPTION OF THE INVENTION

Definitions

As used in the specification, “a” or “an” may mean one or more. As used in the claim(s), when used in conjunction with the word “comprising”, the words “a” or “an” may mean one or more than one.

Where “comprising” is used, this can optionally be replaced by “consisting essentially of”, or optionally by “consisting of”.

As used herein, the term “antigen binding domain” or “ABD” refers to a domain comprising a three-dimensional structure capable of immunospecifically binding to an epitope. Thus, in one embodiment, said domain can comprise a hypervariable region, optionally a V_H and/or V_L domain of an antibody chain, optionally at least a V_H domain. In another embodiment, the binding domain may comprise at least one complementarity determining region (CDR) of an antibody chain. In another embodiment, the binding domain may comprise a polypeptide domain from a non-immunoglobulin scaffold.

The term “antibody” herein is used in the broadest sense and specifically includes full-length monoclonal antibodies, polyclonal antibodies, multispecific antibodies (e.g., bispecific antibodies), and antibody fragments and derivatives, so long as they exhibit the desired biological activity. Various techniques relevant to the production of antibodies are provided in, e.g., Harlow, et al., *ANTIBODIES: A LABORATORY MANUAL*, Cold Spring Harbor Laboratory Press, Cold Spring Harbor, N.Y., (1988). An “antibody fragment” comprises a portion of a full-length antibody, e.g. antigen-binding or variable regions thereof. Examples of antibody fragments include Fab, Fab', F(ab)₂, F(ab')₂, F(ab)₃, Fv (typically the V_L and V_H domains of a single arm of an antibody), single-chain Fv (scFv), dsFv, Fd fragments (typically the V_H and CH1 domain), and dAb (typically a V_H domain) fragments; V_H , V_L , VhH, and V-NAR domains; minibodies, diabodies, triabodies, tetrabodies, and kappa bodies (see, e.g., Ill et al., *Protein Eng* 1997; 10: 949-57); camel IgG; IgNAR; and multispecific antibody fragments formed from antibody fragments, and one or more isolated CDRs or a functional paratope, where isolated CDRs or antigen-binding residues or polypeptides can be associated or linked together so as to form a functional antibody fragment. Various types of antibody fragments have been described or reviewed in, e.g., Holliger and Hudson, *Nat Biotechnol* 2005; 23, 1126-1136; WO2005040219, and published U.S. Patent Applications 20050238646 and 20020161201.

The term “antibody derivative”, as used herein, comprises a full-length antibody or a fragment of an antibody, e.g. comprising at least antigen-binding or variable regions thereof, wherein one or more of the amino acids are chemically modified, e.g., by alkylation, PEGylation, acylation, ester formation or amide formation or the like. This includes, but is not limited to, PEGylated antibodies, cysteine-PEGylated antibodies, and variants thereof.

The term “hypervariable region” when used herein refers to the amino acid residues of an antibody that are responsible for antigen binding. The hypervariable region generally comprises amino acid residues from a “complementarity-determining region” or “CDR” (e.g. residues 24-34 (L1), 50-56 (L2) and 89-97 (L3) in the light-chain variable domain and 31-35 (H1), 50-65 (H2) and 95-102 (H3) in the heavy-chain variable domain; Kabat et al. 1991) and/or those residues from a “hypervariable loop” (e.g. residues 26-32 (L1), 50-52 (L2) and 91-96 (L3) in the light-chain variable domain and 26-32 (H1), 53-55 (H2) and 96-101 (H3) in the heavy-chain variable domain; Chothia and Lesk, *J. Mol. Biol* 1987; 196:901-917). Typically, the numbering of amino acid residues in this region is performed by the method described in Kabat et al., *supra*. Phrases such as “Kabat position”, “variable domain residue numbering as in Kabat” and “according to Kabat” herein refer to this numbering system for heavy chain variable domains or light chain variable domains. Using the Kabat numbering system, the actual linear amino acid sequence of a peptide may contain fewer or additional amino acids corresponding to a shortening of, or insertion into, a FR or CDR of the variable domain. For example, a heavy chain variable domain may include a single amino acid insert (residue 52a according to Kabat) after residue 52 of CDR H2 and inserted residues (e.g. residues 82a, 82b, and 82c, etc. according to Kabat) after heavy chain FR residue 82. The Kabat numbering of residues may be determined for a given antibody by alignment at regions of homology of the sequence of the antibody with a “standard” Kabat numbered sequence.

By “framework” or “FR” residues as used herein is meant the region of an antibody variable domain exclusive of those regions defined as CDRs. Each antibody variable domain framework can be further subdivided into the contiguous regions separated by the CDRs (FR1, FR2, FR3 and FR4).

By “constant region” as defined herein is meant an antibody-derived constant region that is encoded by one of the light or heavy chain immunoglobulin constant region genes. By “constant light chain” or “light chain constant region” as used herein is meant the region of an antibody encoded by the kappa (C_k) or lambda (C_λ) light chains. The constant light chain typically comprises a single domain, and as defined herein refers to positions 108-214 of C_k , or C_λ , wherein numbering is according to the EU index (Kabat et al., 1991, *Sequences of Proteins of Immunological Interest*, 5th Ed., United States Public Health Service, National Institutes of Health, Bethesda).

By “constant heavy chain” or “heavy chain constant region” as used herein is meant the region of an antibody encoded by the mu, delta, gamma,

alpha, or epsilon genes to define the antibody's isotype as IgM, IgD, IgG, IgA, or IgE, respectively. For full length IgG antibodies, the constant heavy chain, as defined herein, refers to the N-terminus of the CH1 domain to the C-terminus of the CH3 domain, thus comprising positions 118-447, wherein numbering is according to the EU index.

By "Fab" or "Fab region" as used herein is meant the polypeptide that comprises the V_H , CH1, V_L , and C_L immunoglobulin domains. Fab may refer to this region in isolation, or this region in the context of a polypeptide, multispecific polypeptide or ABD, or any other embodiments as outlined herein.

By "single-chain Fv" or "scFv" as used herein are meant antibody fragments comprising the V_H and V_L domains of an antibody, wherein these domains are present in a single polypeptide chain. Generally, the Fv polypeptide further comprises a polypeptide linker between the V_H and V_L domains which enables the scFv to form the desired structure for antigen binding. Methods for producing scFvs are well known in the art. For a review of methods for producing scFvs see Pluckthun in *The Pharmacology of Monoclonal Antibodies*, vol. 113, Rosenberg and Moore eds. Springer-Verlag, New York, pp. 269-315 (1994).

By "Fv" or "Fv fragment" or "Fv region" as used herein is meant a polypeptide that comprises the V_L and V_H domains of a single antibody.

By "Fc" or "Fc region", as used herein is meant the polypeptide comprising the constant region of an antibody excluding the first constant region immunoglobulin domain. Thus Fc refers to the last two constant region immunoglobulin domains of IgA, IgD, and IgG, and the last three constant region immunoglobulin domains of IgE and IgM, and the flexible hinge N-terminal to these domains. For IgA and IgM, Fc may include the J chain. For IgG, Fc comprises immunoglobulin domains Cy2 (CH2) and Cy3 (CH3) and the hinge between Cy1 and Cy2. Although the boundaries of the Fc region may vary, the human IgG heavy chain Fc region is usually defined to comprise residues C226, P230 or A231 to its carboxyl-terminus, wherein the numbering is according to the EU index. Fc may refer to this region in isolation, or this region in the context of an Fc polypeptide, as described below. By "Fc polypeptide" or "Fc-derived polypeptide" as used herein is meant a polypeptide that comprises all or part of an Fc region. Fc polypeptides herein include but are not limited to antibodies, Fc fusions and Fc fragments. Also, Fc regions according to the invention include variants containing at least one modification that alters (enhances or diminishes) an Fc associated effector function. Also, Fc regions according to the invention include chimeric Fc regions comprising different portions or domains of different Fc regions, e.g., derived from antibodies of different isotype or species.

By "variable region" as used herein is meant the region of an antibody that comprises one or more Ig domains substantially encoded by any of the V_L (including V_k (V_k) and V_λ) and/or V_H genes that make up the light chain (including κ and λ) and heavy chain immunoglobulin genetic loci respectively. A light or heavy chain variable region (V_L or V_H) consists of a "framework" or "FR" region interrupted by three hypervariable regions referred to as "complementarity determining regions" or "CDRs". The extent of the framework region and CDRs have been precisely defined, for example as in Kabat (see "Sequences of Proteins of Immunological Interest," E. Kabat et al., U.S. Department of Health and Human Services, (1983)), and as in Chothia. The framework regions of an antibody, that is the combined framework regions of the constituent light and heavy chains, serves to position and align the CDRs, which are primarily responsible for binding to an antigen.

The term "specifically binds to" means that an antibody or polypeptide can bind preferably in a competitive binding assay to the binding partner, e.g. NKp46, as assessed using either recombinant forms of the proteins, epitopes therein, or native proteins present on the surface of isolated target cells. Competitive binding assays and other methods for determining specific binding are further described below and are well known in the art.

When an antibody or polypeptide is said to "compete with" a particular monoclonal antibody (e.g. NKp46-1, -2, -4, -6 or -9 in the context of an anti-NKp46 mono- or bi-specific antibody), it means that the antibody or polypeptide competes with the monoclonal antibody in a binding assay using either recombinant target (e.g. NKp46) molecules or surface expressed target (e.g. NKp46) molecules. For example, if a test antibody reduces the binding of NKp46-1, -2, -4, -6 or -9 to a NKp46 polypeptide or NKp46-expressing cell in a binding assay, the antibody is said to "compete" respectively with NKp46-1, -2, -4, -6 or -9.

The term "affinity", as used herein, means the strength of the binding of an antibody or polypeptide to an epitope. The affinity of an antibody is given by the dissociation constant K_D , defined as $[Ab] \times [Ag] / [Ab-Ag]$, where $[Ab-Ag]$ is the molar concentration of the antibody-antigen complex, $[Ab]$ is the molar concentration of the unbound antibody and $[Ag]$ is the molar concentration of the unbound antigen. The affinity constant K_A is

defined by $1/K_D$. Preferred methods for determining the affinity of mAbs can be found in Harlow, et al., *Antibodies: A Laboratory Manual*, Cold Spring Harbor Laboratory Press, Cold Spring Harbor, N.Y., 1988), Coligan et al., eds., *Current Protocols in Immunology*, Greene Publishing Assoc. and Wiley Interscience, N.Y., (1992, 1993), and Muller, *Meth. Enzymol.* 92:589-601 (1983), which references are entirely incorporated herein by reference. One preferred and standard method well known in the art for determining the affinity of mAbs is the use of surface plasmon resonance (SPR) screening (such as by analysis with a BIAcore™ SPR analytical device).

Within the context of this invention a “determinant” designates a site of interaction or binding on a polypeptide.

The term “epitope” refers to an antigenic determinant, and is the area or region on an antigen to which an antibody or polypeptide binds. A protein epitope may comprise amino acid residues directly involved in the binding as well as amino acid residues which are effectively blocked by the specific antigen binding antibody or peptide, i.e., amino acid residues within the “footprint” of the antibody. It is the simplest form or smallest structural area on a complex antigen molecule that can combine with e.g., an antibody or a receptor. Epitopes can be linear or conformational/structural. The term “linear epitope” is defined as an epitope composed of amino acid residues that are contiguous on the linear sequence of amino acids (primary structure). The term “conformational or structural epitope” is defined as an epitope composed of amino acid residues that are not all contiguous and thus represent separated parts of the linear sequence of amino acids that are brought into proximity to one another by folding of the molecule (secondary, tertiary and/or quaternary structures). A conformational epitope is dependent on the 3-dimensional structure. The term ‘conformational’ is therefore often used interchangeably with ‘structural’. Epitopes may be identified by different methods known in the art including but not limited to alanine scanning, phage display, X-ray crystallography, array-based oligo-peptide scanning or pepscan analysis, site-directed mutagenesis, high throughput mutagenesis mapping, H/D-Ex Mass Spectroscopy, homology modeling, docking, hydrogen-deuterium exchange, among others. (See e.g., Tong et al., Methods and Protocols for prediction of immunogenic epitopes”, *Briefings in Bioinformatics* 8(2):96-108; Gershoni, Jonathan M; Roitburd-Berman, Anna; Siman-Tov, Dror D; Tarnovitski Freund, Natalia; Weiss, Yael (2007). “Epitope Mapping”. *BioDrugs* 21 (3): 145-56; and Flanagan, Nina (May 15, 2011); “Mapping Epitopes with H/D-Ex Mass Spec: ExSAR Expands Repertoire of Technology Platform Beyond Protein Characterization”, *Genetic Engineering & Biotechnology News* 31 (10).

By “amino acid modification” herein is meant an amino acid substitution, insertion, and/or deletion in a polypeptide sequence. An example of amino acid modification herein is a substitution. By “amino acid modification” herein is meant an amino acid substitution, insertion, and/or deletion in a polypeptide sequence. By “amino acid substitution” or “substitution” herein is meant the replacement of an amino acid at a given position in a protein sequence with another amino acid. For example, the substitution Y50W refers to a variant of a parent polypeptide, in which the tyrosine at position 50 is replaced with tryptophan. A “variant” of a polypeptide refers to a polypeptide having an amino acid sequence that is substantially identical to a reference polypeptide, typically a native or “parent” polypeptide. The polypeptide variant may possess one or more amino acid substitutions, deletions, and/or insertions at certain positions within the native amino acid sequence.

“Conservative” amino acid substitutions are those in which an amino acid residue is replaced with an amino acid residue having a side chain with similar physicochemical properties. Families of amino acid residues having similar side chains are known in the art, and include amino acids with basic side chains (e.g., lysine, arginine, histidine), acidic side chains (e.g., aspartic acid, glutamic acid), uncharged polar side chains (e.g., glycine, asparagine, glutamine, serine, threonine, tyrosine, cysteine, tryptophan), nonpolar side chains (e.g., alanine, valine, leucine, isoleucine, proline, phenylalanine, methionine), beta-branched side chains (e.g., threonine, valine, isoleucine) and aromatic side chains (e.g., tyrosine, phenylalanine, tryptophan, histidine).

The term “identity” or “identical”, when used in a relationship between the sequences of two or more polypeptides, refers to the degree of sequence relatedness between polypeptides, as determined by the number of matches between strings of two or more amino acid residues. “Identity” measures the percent of identical matches between the smaller of two or more sequences with gap alignments (if any) addressed by a particular mathematical model or computer program (i.e., “algorithms”). Identity of related polypeptides can be readily calculated by known methods. Such methods include, but are not limited to, those described in *Computational Molecular Biology*, Lesk, A. M., ed., Oxford University Press, New York, 1988; *Biocomputing: Informatics and Genome Projects*, Smith, D. W., ed., Academic Press, New York, 1993; Computer Analysis of Sequence Data, Part 1, Griffin, A. M., and Griffin, H. G., eds., Humana Press, New Jersey, 1994; Sequence Analysis in Molecular Biology, von Heinje, G., Academic Press, 1987; *Sequence Analysis Primer*, Gribskov, M. and Devereux, J., eds., M. Stockton Press, New York, 1991; and Carillo et al., *SIAM J. Applied Math.* 48, 1073 (1988).

Preferred methods for determining identity are designed to give the largest match between the sequences tested. Methods of determining identity are described in publicly available computer programs. Preferred computer program methods for determining identity between two sequences include the GCG program package, including GAP (Devereux et al., *Nucl. Acid. Res.* 12, 387 (1984); Genetics Computer Group, University of Wisconsin, Madison, Wis.), BLASTP, BLASTN, and FASTA (Altschul et al., *J. Mol. Biol.* 215, 403-410 (1990)). The BLASTX program is publicly available from the National Center for Biotechnology Information (NCBI) and other sources (BLAST Manual, Altschul et al. NCB/NLM/NIH Bethesda, Md. 20894; Altschul et al., *supra*). The well-known Smith Waterman algorithm may also be used to determine identity.

An "isolated" molecule is a molecule that is the predominant species in the composition wherein it is found with respect to the class of molecules to which it belongs (i.e., it makes up at least about 50% of the type of molecule in the composition and typically will make up at least about 70%, at least about 80%, at least about 85%, at least about 90%, at least about 95%, or more of the species of molecule, e.g., peptide, in the composition). Commonly, a composition of a polypeptide will exhibit 98%, 98%, or 99% homogeneity for polypeptides in the context of all present peptide species in the composition or at least with respect to substantially active peptide species in the context of proposed use.

In the context herein, "treatment" or "treating" refers to preventing, alleviating, managing, curing or reducing one or more symptoms or clinically relevant manifestations of a disease or disorder, unless contradicted by context. For example, "treatment" of a patient in whom no symptoms or clinically relevant manifestations of a disease or disorder have been identified is preventive or prophylactic therapy, whereas "treatment" of a patient in whom symptoms or clinically relevant manifestations of a disease or disorder have been identified generally does not constitute preventive or prophylactic therapy.

As used herein, the phrase "NK cells" refers to a sub-population of lymphocytes that is involved in non-conventional immunity. NK cells can be identified by virtue of certain characteristics and biological properties, such as the expression of specific surface antigens including CD56 and/or NKP46 for human NK cells, the absence of the alpha/beta or gamma/delta TCR complex on the cell surface, the ability to bind to and kill cells that fail to express "self" MHC/HLA antigens by the activation of specific cytolytic machinery, the ability to kill tumor cells or other diseased cells that express a ligand for NK activating receptors, and the ability to release protein molecules called cytokines that stimulate or inhibit the immune response. Any of these characteristics and activities can be used to identify NK cells, using methods well known in the art. Any subpopulation of NK cells will also be encompassed by the term NK cells. Within the context herein "active" NK cells designate biologically active NK cells, including NK cells having the capacity of lysing target cells or enhancing the immune function of other cells. NK cells can be obtained by various techniques known in the art, such as isolation from blood samples, cytapheresis, tissue or cell collections, etc. Useful protocols for assays involving NK cells can be found in *Natural Killer Cells Protocols* (edited by Campbell K S and Colonna M). Humana Press. pp. 219-238 (2000).

The term "internalization", used interchangeably with "intracellular internalization", refers to the molecular, biochemical and cellular events associated with the process of translocating a molecule from the extracellular surface of a cell to the intracellular surface of a cell. The processes responsible for intracellular internalization of molecules are well-known and can involve, inter alia, the internalization of extracellular molecules (such as hormones, antibodies, and small organic molecules); membrane-associated molecules (such as cell-surface receptors); and complexes of membrane-associated molecules bound to extracellular molecules (for example, a ligand bound to a transmembrane receptor or an antibody bound to a membrane-associated molecule). Thus, "inducing and/or increasing internalization" refer to events wherein intracellular internalization is initiated and/or the rate and/or extent of intracellular internalization is increased.

As used herein, an agent that has "agonist" activity at NKP46 is an agent that can cause or increase "NKP46 signaling". "NKP46 signaling" refers to an ability of an NKP46 polypeptide to activate or transduce an intracellular signaling pathway. Changes in NKP46 signaling activity can be measured, for example, by assays designed to measure changes in NKP46 signaling pathways, e.g. by monitoring phosphorylation of signal transduction components, assays to measure the association of certain signal transduction components with other proteins or intracellular structures, or in the biochemical activity of components such as kinases, or assays designed to measure expression of reporter genes under control of NKP46-sensitive promoters and enhancers, or indirectly by a downstream effect mediated by the NKP46 polypeptide (e.g. activation of specific cytolytic machinery in NK cells). Reporter genes can be naturally occurring genes (e.g. monitoring cytokine production) or they can be genes artificially introduced into a cell. Other genes can be placed under the control of such regulatory elements and thus serve to report the level of NKP46 signaling.

"NKp46" refers to a protein or polypeptide encoded by the Ncr1 gene or by a cDNA prepared from such a gene. Any naturally occurring isoform, allele, ortholog or variant is encompassed by the term NKp46 polypeptide (e.g., an NKp46 polypeptide 90%, 95%, 98% or 99% identical to SEQ ID NO 1, or a contiguous sequence of at least 20, 30, 50, 100 or 200 amino acid residues thereof). The 304 amino acid residue sequence of human NKp46 (isoform a) is shown below:

(SEQ ID NO: 1)

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MSSTLPALLC VGLCLSQRIS AQQQTLPKPF IWAEPHF MVP
KEKQVTICCC GNYGAVEYQL HFEGSLFAVD RPKPPERINK
VKFYIPDMNS RMAGQYSCIY RVGELWSEPS NLLDLVTEM
YDPTLSVHP GPEVISGEKV TFYCRLDTAT SMFLLLKEGR
SSHVQRGYGK VQAEFPLGPV TTAHRGTYRC FGSYNNHAW S
FPSEPVKLLV TGDIENTSLA PEDPTFPADT WGTYLLTTET
GLQKDHALWD HTAQNLLRMG LAFLVLVALV WFLVEDWLSR
KRTRERASRA STWEGRRRLN TQTL.
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SEQ ID NO: 1 corresponds to NCBI accession number NP_004820, the disclosure of which is incorporated herein by reference. The human NKp46 mRNA sequence is described in NCBI accession number NM_004829, the disclosure of which is incorporated herein by reference.

Producing Polypeptides

The antigen binding domains used in the proteins described herein can be readily derived from any of a variety of immunoglobulin or non-immunoglobulin scaffolds, for example affibodies based on the Z-domain of staphylococcal protein A, engineered Kunitz domains, monobodies or adnectins based on the 10th extracellular domain of human fibronectin III, anticalins derived from lipocalins, DARPins (designed ankyrin repeat domains, multimerized LDLR-A module, avimers or cysteine-rich knottin peptides. See, e.g., Gebauer and Skerra (2009) *Current Opinion in Chemical Biology* 13:245-255, the disclosure of which is incorporated herein by reference.

Variable domains are commonly derived from antibodies (immunoglobulin chains), for example in the form of associated V_L and V_H domains found on two polypeptide chains, or a single chain antigen binding domain such as a scFv, a V_H domain, a V_L domain, a dAb, a V-NAR domain or a V_HH domain. In certain advantageous proteins formats disclosed herein that directly enable the use of a wide range of variable regions from Fab or scFv without substantial further requirements for pairing and/or folding, the an antigen binding domain (e.g., ABD₁ and ABD₂) can also be readily derived from antibodies as a Fab or scFv.

Typically, antibodies are initially obtained by immunization of a non-human animal, e.g., a mouse, rat, guinea pig or rabbit, with an immunogen comprising a polypeptide, or a fragment or derivative thereof, typically an immunogenic fragment, for which it is desired to obtain antibodies (e.g. a human polypeptide). The step of immunizing a non-human mammal with an antigen may be carried out in any manner well known in the art for stimulating the production of antibodies in a mouse (see, for example, E. Harlow and D. Lane, *Antibodies: A Laboratory Manual*, Cold Spring Harbor Laboratory Press, Cold Spring Harbor, N.Y. (1988), the entire disclosure of which is herein incorporated by reference). Human antibodies may also be produced by using, for immunization, transgenic animals that have been engineered to express a human antibody repertoire (Jakobovitz et al *Nature* 362 (1993) 255), or by selection of antibody repertoires using phage display methods. For example, a XenoMouse (Abgenix, Fremont, Calif.) can be used for immunization. A XenoMouse is a murine host that has had its immunoglobulin genes replaced by functional human immunoglobulin genes. Thus, antibodies produced by this mouse or in hybridomas made from the B cells of this mouse, are already humanized. The XenoMouse is described in U.S. Pat. No. 6,162,963, which is herein incorporated in its entirety by reference. Antibodies may also be produced by selection of combinatorial libraries of immunoglobulins, as disclosed for instance in (Ward et al. *Nature*, 341 (1989) p. 544, the entire disclosure of which is herein incorporated by reference). Phage display technology (McCafferty et al (1990) *Nature* 348:552-553) can be used to produce antibodies from immunoglobulin variable (V) domain gene repertoires from unimmunized donors. See, e.g., Griffith et al (1993) *EMBO J.* 12:725-734; U.S. Pat. No. 5,565,332; U.S. Pat. No. 5,573,905; U.S. Pat. No. 5,567,610; and U.S. Pat. No. 5,229,275). When combinatorial libraries comprise variable (V) domain gene repertoires of human origin, selection from combinatorial libraries will yield human

antibodies.

Additionally, a wide range of antibodies are available in the scientific and patent literature, including DNA and/or amino acid sequences, or from commercial suppliers. Antibodies will typically be directed to a pre-determined antigen. Examples of antibodies include antibodies that recognize an antigen expressed by a target cell that is to be eliminated, for example a proliferating cell or a cell contributing to a disease pathology. Examples include antibodies that recognize tumor antigens, microbial (e.g. bacterial or parasite) antigens or viral antigens.

Antigen binding domains that bind NKp46 can be derived from the anti-NKp46 antibodies provided herein (see section "CDR Sequences"). Variable regions can be used directly, or can be modified by selecting hypervariable or CDR regions from the NKp46 antibodies and placing them into an appropriate V_L or V_H framework, for example human frameworks. Antigen binding domains that bind NKp46 can also be derived de novo using methods for generating antibodies. Antibodies can be tested for binding to NKp46 polypeptides. In one aspect of any embodiment herein, a polypeptide (e.g. multispecific polypeptide, bispecific or monospecific antibody) that binds to NKp46 will be capable of binding NKp46 expressed on the surface of a cell, e.g. native NKp46 expressed by a NK cell.

Antigen binding domains (ABDs) that bind antigens of interest can be selected based on the desired antigen of interest (e.g. an antigen other than NKp46), and may include for example cancer antigens such as antigens present on tumor cells and/or on immune cells capable of mediating a pro-tumoral effect, e.g. a monocyte or a macrophage, optionally a suppressor T cell, regulatory T cell, or myeloid-derived suppressor cell (for the treatment of cancer); bacterial or viral antigens (for the treatment of infectious disease); or antigens present on pro-inflammatory immune cells, e.g. T cells, neutrophils, macrophages, etc. (for the treatment of inflammatory and/or autoimmune disorder). As used herein, the term "bacterial antigen" includes, but is not limited to, intact, attenuated or killed bacteria, any structural or functional bacterial protein or carbohydrate, or any peptide portion of a bacterial protein of sufficient length (typically about 8 amino acids or longer) to be antigenic. Examples include gram-positive bacterial antigens and gram-negative bacterial antigens. In some embodiments the bacterial antigen is derived from a bacterium selected from the group consisting of *Helicobacter* species, in particular *Helicobacter pylori*; *Borrelia* species, in particular *Borrelia burgdorferi*; *Legionella* species, in particular *Legionella pneumophila*; *Mycobacteria* species, in particular *M. tuberculosis*, *M. avium*, *M. intracellulare*, *M. kansasii*, *M. goodii*; *Staphylococcus* species, in particular *Staphylococcus aureus*; *Neisseria* species, in particular *N. gonorrhoeae*, *N. meningitidis*; *Listeria* species, in particular *Listeria monocytogenes*; *Streptococcus* species, in particular *S. pyogenes*, *S. agalactiae*; *S. faecalis*; *S. bovis*, *S. pneumoniae*; anaerobic *Streptococcus* species; pathogenic *Campylobacter* species; *Enterococcus* species; *Haemophilus* species, in particular *Haemophilus influenzae*; *Bacillus* species, in particular *Bacillus anthracis*; *Corynebacterium* species, in particular *Corynebacterium diphtheriae*; *Erysipelothrix* species, in particular *Erysipelothrix rhusiopathiae*; *Clostridium* species, in particular *C. perfringens*, *C. tetani*; *Enterobacter* species, in particular *Enterobacter aerogenes*, *Klebsiella* species, in particular *Klebsiella 1S pneumoniae*, *Pasteurella* species, in particular *Pasteurella multocida*, *Bacteroides* species; *Fusobacterium* species, in particular *Fusobacterium nucleatum*; *Streptobacillus* species, in particular *Streptobacillus moniliformis*; *Treponema* species, in particular *Treponema pertenue*; *Leptospira*; pathogenic *Escherichia* species; and *Actinomyces* species, in particular *Actinomyces israeli*.

As used herein, the term "viral antigen" includes, but is not limited to, intact, attenuated or killed whole virus, any structural or functional viral protein, or any peptide portion of a viral protein of sufficient length (typically about 8 amino acids or longer) to be antigenic. Sources of a viral antigen include, but are not limited to viruses from the families: Retroviridae (e.g., human immunodeficiency viruses, such as HIV-1 (also referred to as HTLV-III, LAV or HTLV-III/LAV, or HIV-III; and other isolates, such as HIV-LP; Picornaviridae (e.g., polio viruses, hepatitis A virus; enteroviruses, human Coxsackie viruses, rhinoviruses, echoviruses); Calciviridae (e.g., strains that cause gastroenteritis); Togaviridae (e.g., equine encephalitis viruses, rubella viruses); Flaviviridae (e.g., dengue viruses, encephalitis viruses, yellow fever viruses); Coronaviridae (e.g., coronaviruses); Rhabdoviridae (e.g., vesicular stomatitis viruses, rabies viruses); Filoviridae (e.g., Ebola viruses); Paramyxoviridae (e.g., parainfluenza viruses, mumps virus, measles virus, respiratory syncytial virus); Orthomyxoviridae (e.g., influenza viruses); Bunyaviridae (e.g., Hantaan viruses, bunya viruses, phleboviruses and Nairo viruses); Arenaviridae (hemorrhagic fever viruses); Reoviridae (e.g., reoviruses, orbiviruses and rotaviruses); Bornaviridae; Hepadnaviridae (Hepatitis B virus); Parvoviridae (parvoviruses); Papovaviridae (papilloma viruses, polyoma viruses); Adenoviridae (most adenoviruses); Herpesviridae (herpes simplex virus (HSV) 1 and 2, varicella zoster virus, cytomegalovirus (CMV), herpes virus; Poxviridae (variola viruses, vaccinia viruses, pox viruses); and Iridoviridae (e.g., African swine fever virus); and unclassified viruses (e.g., the agent of delta hepatitis (thought to be a defective satellite of hepatitis B virus), Hepatitis C; Norwalk and related viruses, and

astroviruses). Alternatively, a viral antigen may be produced recombinantly.

As used herein, the terms “cancer antigen” and “tumor antigen” are used interchangeably and refer to antigens (other than NKp46 or CD16) that are differentially expressed by cancer cells or are expressed by non-tumoral cells (e.g. immune cells) having a pro-tumoral effect (e.g. an immunosuppressive effect), and can thereby be exploited in order to target cancer cells. Cancer antigens are antigens which can potentially stimulate apparently tumor-specific immune responses. Some of these antigens are encoded, although not necessarily expressed, or expressed at lower levels or less frequently, by normal cells. These antigens can be characterized as those which are normally silent (i.e., not expressed) in normal cells, those that are expressed only at certain stages of differentiation and those that are temporally expressed such as embryonic and fetal antigens. Other cancer antigens are encoded by mutant cellular genes, such as oncogenes (e.g., activated ras oncogene), suppressor genes (e.g., mutant p53), fusion proteins resulting from internal deletions or chromosomal translocations. Still other cancer antigens can be encoded by viral genes such as those carried on RNA and DNA tumor viruses. Still other cancer antigens can be expressed on immune cells capable of contributing to or mediating a pro-tumoral effect, e.g. cell that contributes to immune evasion, a monocyte or a macrophage, optionally a suppressor T cell, regulatory T cell, or myeloid-derived suppressor cell.

The cancer antigens are usually normal cell surface antigens which are either over-expressed or expressed at abnormal times, or are expressed by a targeted population of cells. Ideally the target antigen is expressed only on proliferative cells (e.g., tumor cells) or pro-tumoral cells (e.g. immune cells having an immunosuppressive effect), however this is rarely observed in practice. As a result, target antigens are in many cases selected on the basis of differential expression between proliferative/disease tissue and healthy tissue. Example of cancer antigens include: Receptor Tyrosine Kinase-like Orphan Receptor 1 (ROR1), Crypto, CD4, CD20, CD30, CD19, CD38, CD47, Glycoprotein NMB, CanAg, Her2 (ErbB2/Neu), a Siglec family member, for example CD22 (Siglec2) or CD33 (Siglec3), CD79, CD138, CD171, PSCA, L1-CAM, PSMA (prostate specific membrane antigen), BCMA, CD52, CD56, CD80, CD70, E-selectin, EphB2, Melanotransferrin, Mud 6 and TMEFF2. Examples of cancer antigens also include Immunoglobulin superfamily (IgSF) such as cytokine receptors, Killer-Ig Like Receptor, CD28 family proteins, for example, Killer-Ig Like Receptor 3DL2 (KIR3DL2), B7-H3, B7-H4, B7-H6, PD-L1, IL-6 receptor. Examples also include MAGE, MART-1/Melan-A, gp100, major histocompatibility complex class I-related chain A and B polypeptides (MICA and MICB), adenosine deaminase-binding protein (ADAbp), cyclophilin b, colorectal associated antigen (CRC)-C017-1A/GA733, protein tyrosine kinase 7 (PTK7), receptor protein tyrosine kinase 3 (TYRO-3), nectins (e.g. nectin-4), major histocompatibility complex class I-related chain A and B polypeptides (MICA and MICB), proteins of the UL16-binding protein (ULBP) family, proteins of the retinoic acid early transcript-1 (RAET1) family, carcinoembryonic antigen (CEA) and its immunogenic epitopes CAP-1 and CAP-2, etv6, aml1 prostate specific antigen (PSA), T-cell receptor/CD3-zeta chain, MAGE-family of tumor antigens, GAGE-family of tumor antigens, anti-Müllerian hormone Type II receptor, delta-like ligand 4 (DLL4), DRS, ROR1 (also known as Receptor Tyrosine Kinase-Like Orphan Receptor 1 or NTRKR1 (EC 2.7.10.1), BAGE, RAGE, LAGE-1, NAG, GnT-V, MUM-1, CDK4, MUC family, VEGF, VEGF receptors, Angiopoietin-2, PDGF, TGF- α , EGF, EGF receptor, members of the human EGF-like receptor family, e.g., HER-2/neu, HER-3, HER-4 or a heterodimeric receptor comprised of at least one HER subunit, gastrin releasing peptide receptor antigen, Muc-1, CA125, integrin receptors, $\alpha v \beta 3$ integrins, $\alpha 5 \beta 1$ integrins, $\alpha 1 \text{Ib} \beta 3$ -integrins, PDGF beta receptor, SVE-cadherin, IL-8 receptor, hCG, IL-6 receptor, CSF1R (tumor-associated monocytes and macrophages), α -fetoprotein, E-cadherin, α -catenin, β -catenin and γ -catenin, p120ctn, PRAME, NY-ESO-1, cdc27, adenomatous polyposis coli protein (APC), fodrin, Connexin 37, Ig-idiotypic, p15, gp75, GM2 and GD2 gangliosides, viral products such as human papillomavirus proteins, imp-1, P1A, EBV-encoded nuclear antigen (EBNA)-1, brain glycogen phosphorylase, SSX-1, SSX-2 (HOM-MEL-40), SSX-1, SSX-4, SSX-5, SCP-1 and CT-7, and c-erbB-2, although this is not intended to be exhaustive. In one aspect, the antigen of interest is an antigen (e.g. any one of the antigens listed above) capable of undergoing intracellular internalization, for example when bound by a conventional human IgG1 antibody, either in the presence or absence of Fc γ receptor cells. In one aspect, the antigen of interest is a CD19 or CD20 polypeptide; in one aspect, the multispecific protein comprises a V_H and/or V_L , or a scFv or another ABD that binds CD19 or CD20 comprising an amino acid sequence which is at least 60%, 70%, 80%, 85%, 90% or 95% identical to the sequence of the anti-CD19 or anti-CD20 respective V_H , V_L or scFv described in the Examples herein, or comprises the heavy and light chain CDR1, -2 and -3 of the anti-CD19 or anti-CD20 heavy and light chain variable regions disclosed herein. In one aspect, the multispecific protein competes for binding to a human CD19 or CD20 polypeptide with an antibody, or a F5 or T6 protein, comprising the respective anti-CD19 or anti-CD20 V_H , V_L or scFv disclosed in the Examples.

In one embodiment, the ABD that binds an antigen of interest is derived from (e.g. comprises the hypervariable region of, or comprises one, two, three, four, five or six of the CDRs of) a parental antibody that binds an antigen of interest (e.g. a murine antibody, a human antibody) which, when bound to its antigenic target (the antigen of interest on cells), increases or induces down-modulation or intracellular internalization of the antigen of interest. In one embodiment, the antigen of interest is a cancer antigen, e.g. one of the cancer antigens listed above known to internalize (e.g. Immunoglobulin superfamily (IgSF) members, for example cytokine receptor α or β chains, Killer-Ig Like Receptors, CD28 family proteins, B7-H3, B7-H4, B7-H6, KIR3DL2, PTK7, ROR1, L1-CAM, Siglec family members, EGF receptor and EGF-like receptor family members, EGFR, HER-2, integrins, anti-Müllerian hormone Type II receptor, CSF-1R, and others) In one embodiment, the antigen target is a polypeptide present on an immune cell capable of mediating a pro-tumoral effect, e.g. a monocyte or a macrophage, optionally a suppressor T cell, regulatory T cell, or myeloid-derived suppressor cell.

In one embodiment, the ABD binds to a cancer antigen, a viral antigen, a microbial antigen, or an antigen present on an infected cell (e.g. virally infected) or on a pro-inflammatory immune cell. In one embodiment, said antigen is a polypeptide selectively expressed or overexpressed on a tumor cell, and infected cell or a pro-inflammatory cell. In one embodiment, said antigen is a polypeptide that when inhibited, decreases the proliferation and/or survival of a tumor cell, an infected cell or a pro-inflammatory cell.

The ABDs which are incorporated into the polypeptides can be tested for any desired activity prior to inclusion in a multispecific NKp46-binding protein, for example the ABD can be tested for binding to an antigen of interest.

An ABD derived from an antibody will generally comprise at minimum a hypervariable region sufficient to confer binding activity. It will be appreciated that an ABD may comprise other amino acids or functional domains as may be desired, including but not limited to linker elements (e.g. linker peptides, CH1, C κ or C λ domains, hinges, or fragments thereof). In one example an ABD comprises a scFv, a V_H domain and a V_L domain, or a single domain antibody (nanobody or dAb) such as a V-NAR domain or a V_HH domain. Exemplary antibody formats are further described herein and an ABD can be selected based on the desired format.

In any embodiment, an antigen binding domain can be obtained from a humanized antibody in which residues from a complementary-determining region (CDR) of a human antibody are replaced by residues from a CDR of the original antibody (the parent or donor antibody, e.g. a murine or rat antibody) while maintaining the desired specificity, affinity, and capacity of the original antibody. The CDRs of the parent antibody, some or all of which are encoded by nucleic acids originating in a non-human organism, are grafted in whole or in part into the beta-sheet framework of a human antibody variable region to create an antibody, the specificity of which is determined by the engrafted CDRs. The creation of such antibodies is described in, e.g., WO 92/11018, Jones, 1986, *Nature* 321:522-525, Verhoeven et al., 1988, *Science* 239:1534-1536. An antigen binding domain can thus have non-human hypervariable regions or CDRs and human frameworks region sequences (optionally with back mutations).

Once appropriate antigen binding domains having desired specificity and/or activity are identified, DNA encoding each of the or ABD can be separately placed, in suitable arrangements, in an appropriate expression vector, together with DNA encoding any elements such as CH1, CK, CH2 and CH3 domains or portions thereof and any other optional elements (e.g. DNA encoding a hinge-derived or linker elements) for transfection into an appropriate host. ABDs will be arranged in an expression vector, or in separate vectors as a function of which type of polypeptide is to be produced, so as to produce the Fc-polypeptides having the desired domains operably linked to one another. The host is then used for the recombinant production of the multispecific polypeptide.

For example, a polypeptide fusion product can be produced from a vector in which the first of the two ABD is operably linked (e.g. directly, or via a CH1, C κ or C λ constant region and/or hinge region) to the N-terminus of a CH2 domain, and the CH2 domain is operably linked at its C-terminus to the N-terminus a CH3 domain. The second of the two ABD can be on a second polypeptide chain that forms a dimer, e.g. heterodimer, with the polypeptide comprising the first ABD. The polypeptide may comprise a full length and/or dimeric Fc domain. The multispecific polypeptide can then be produced in an appropriate host cell or by any suitable synthetic process. A host cell chosen for expression of the multispecific polypeptide is an important contributor to the final composition, including, without limitation, the variation in composition of the oligosaccharide moieties decorating the protein in the immunoglobulin CH2 domain. Thus, one aspect of the invention involves the selection of appropriate host cells for use and/or development of a production cell expressing the desired therapeutic protein such

that the multispecific polypeptide retains FcRn and CD16 binding. The host cell may be of mammalian origin or may be selected from COS-1, COS-7, HEK293, BHK21, CHO, BSC-1, Hep G2, 653, SP2/0, 293, HeLa, myeloma, lymphoma, yeast, insect or plant cells, or any derivative, immortalized or transformed cell thereof. The host cell may be any suitable species or organism capable of producing N-linked glycosylated polypeptides, e.g. a mammalian host cell capable of producing human or rodent IgG type N-linked glycosylation.

Multimeric bispecific proteins such as heterodimers, heterotrimers and tetramers (the latter including for example bispecific antibodies with two heavy chains and two light chains) can be produced according to a variety of formats. The multimeric polypeptide will generally comprise a dimeric Fc domain that is capable of binding to human CD16 or CD16A and optionally other Fcγ receptors, e.g., CD16B, CD32A, CD32B and/or CD64). Fc moieties with substantial FcRn and CD16 (CD16A) binding can be obtained through the use of suitable CH2 and/or CH3 domains, as further described herein. In one embodiment, an Fc moiety is derived from a human IgG1 isotype constant region. In one embodiment, an Fc moiety may be obtained by production of the polypeptide in a host cell or by a process that yields N297-linked glycosylation, e.g. a mammalian cell. In one embodiment, an Fc moiety comprises one or more amino acid modifications, e.g. in the CH2 domain, that increases binding to CD16 or CD16A.

In one example, the protein comprises a first and a second polypeptide chain each comprising a variable domain fused to a human Fc domain (comprising a CH3 domain capable of undergoing preferential CH3-CH3 hetero-dimerization), wherein the first and second chain associate via CH3-CH3 dimerization and the protein comprises a dimeric Fc domain. The variable domains of each chain can be part of the same or different antigen binding domains.

One advantageous way of making multimeric polypeptides is through the assembly of different polypeptide chains that each comprise at least one heavy or light chain variable domain fused to a human CH1 or Cκ constant domain (a V-(CH1/Cκ) unit), wherein the protein chains undergo CH1-Cκ dimerization and are bound to one another by non-covalent bonds and optionally further disulfide bonds formed between respective CH1 and Cκ domains. In one embodiment, the invention provides an isolated or purified heterodimeric or heterotrimeric protein that binds to a first and second antigen, wherein the protein comprises at least two or three polypeptide chains, each comprising a V-(CH1/Cκ) unit, whereby the chains are bound to one another by non-covalent bonds and optionally further bound via disulfide bonds between CH1 and Cκ domains, and still further optionally, whereby the chains are bound by non-covalent bonds between the respective variable regions, CH1 and Cκ domains, and CH3 domains of the Fc portion.

In one example, the protein comprises a first and a second polypeptide chain each comprising a variable domain fused to a CH1 or Cκ domain (a V-(CH1/Cκ) unit), in turn fused at its C-terminus to a human Fc domain (comprising a CH3 domain capable of undergoing CH3-CH3 dimerization), wherein the first and second chain associate via CH1-Cκ and CH3-CH3 dimerization and the protein comprises a dimeric Fc domain. The variable domains of each chain can be part of the same or different antigen binding domains.

The variable and constant regions can be selected and configured such that each chain will preferentially associate with its desired complementary partner chain. The resulting multimeric protein will therefore be simple to produce using conventional production methods using recombinant host cells. The choice of which V_H or V_L to associate with a CH1 and Cκ in a unit is based on affinity between the units to be paired so as to drive the formation of the desired multimer. The resulting multimer will be bound by non-covalent bonds between complementary V_H and V_L domains, by non-covalent bonds between complementary CH1 and Cκ domains, and optionally by further disulfide bonding between complementary CH1 and Cκ domains (and optionally further disulfide bonds between complementary hinge domains). V_H—V_L associations are stronger than V_H-V_H or V_L—V_L, consequently, as shown herein, one can place a V_H or a V_L next to either a CH1 or a Cκ, and the resulting V-C unit will partner preferably with its V-C counterpart. For example V_H-Cκ will pair with V_L-CH1 preferentially over V_H-CH1. Additionally, by including an Fc domain, preferred chain pairing is further improved, as the two Fc-containing chains are bound by non-covalent bonds between CH3 domains of the Fc domains. The different V-C combinations, optionally further combined with Fc pairing thereby provides tools to make heteromultimeric proteins.

In one example, the multispecific protein comprises a first and a second polypeptide chain each comprising a variable domain fused to a CH1 or Cκ domain (a V-(CH1/Cκ) unit), in turn fused at its C-terminus to a human Fc domain, wherein the V-(CH1/Cκ) unit of the first chain has undergone CH1-Cκ dimerization with the V-(CH1/Cκ) unit of the second chain thereby forming a first antigen binding domain (ABD₁) and a

dimeric Fc domain, wherein one of the polypeptide chains further comprises an antigen binding domain that forms a second antigen binding domain (ABD₂), and wherein the Fc domain binds to a human CD16 polypeptide. In one embodiment, the Fc domain comprises N-linked glycosylation at residue N297 (Kabat EU numbering). In one example, the protein has a domain arrangement:

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In one example, the protein comprises three polypeptide chains, each comprising a variable domain fused to a CH1 or Cκ domain (a V-(CH1/Cκ) unit), wherein a first (central) chain comprises two V-(CH1/Cκ) units and a human Fc domain interposed between the units, the second chain comprises one V-(CH1/Cκ) unit and a human Fc domain, and the third chain comprises one V-(CH1/Cκ) unit, wherein one of the V-(CH1/Cκ) units of the central chain has undergone CH1-Cκ dimerization with the V-(CH1/Cκ) unit of the second chain thereby forming a first antigen binding domain (ABD₁) and a dimeric Fc domain, and wherein the other of the V-(CH1/Cκ) units of the central chain has undergone CH1-Cκ dimerization with the V-(CH1/Cκ) unit of the third chain thereby forming a second antigen binding domain (ABD₂), and wherein the Fc domain binds to a human CD16 polypeptide. In one embodiment, the Fc domain comprises N-linked glycosylation at residue N297 (Kabat EU numbering). In one example, the protein has a domain arrangement:

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In certain formats, heterodimers are formed in which variable domains adjacent to the CH1 or Cκ domain do not require association with the second chain to form an antigen binding domain. For example, through use of single variable domains, or scFv, each chain will contain a functional ABD. Examples based CH1-Cκ dimerization with single variable exemplary heterodimer molecules can have a domain arrangement:

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wherein V₁ and V₂ are single variable domains (e.g. V_H domain, a V_L domain, a dAb, a V-NAR domain or a V_HH domain), and one of V₁ and V₂ binds NKp46 and the other binds an antigen of interest.

In one embodiment, exemplary heterodimer molecules can have a domain arrangement:

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wherein V_{a-1}, V_{b-1}, V_{a-2} and V_{b-2} are each a V_H domain or a V_L domain, and wherein one of V_{a-1} and V_{b-1} is a V_H and the other is a V_L such that V_{a-1} and V_{b-1} form a first antigen binding domain (ABD), wherein one of V_{a-2} and V_{b-2} is a V_H and the other is a V_L such that V_{a-2} and V_{b-2} form a second antigen binding domain, wherein one of the ABD binds NKp46 and the other binds an antigen of interest. In one variant of the foregoing, any of, or each of the V_{a-1}, V_{b-1}, V_{a-2} and V_{b-2} are a scFv (made up of two variable domains). Each pair of V domains can be separated by a linker peptide (e.g. to form a scFv).

In similar approaches, trimers can be constructed. Exemplary heterotrimer molecules can have the following domain arrangement:

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wherein the first/central chain and the second chain associate by CH3-CH3 dimerization and the first/central chain and the third chain associate by the CH1 or Cκ dimerization, wherein the domains of the first/central chain and the third chain are selected to be complementary to permit the first and third chains to associate by CH1-Cκ dimerization, and wherein V_{a-1}, V_{b-1}, V_{a-2} and V_{b-2} are each a V_H domain or a V_L domain, and wherein one of V_{a-1} and V_{b-1} is a V_H and the other is a V_L such that V_{a-1} and V_{b-1} form a first antigen binding domain (ABD), wherein one of V_{a-2} and V_{b-2} is a V_H and the other is a V_L such that V_{a-2} and V_{b-2} form a second antigen binding domain (e.g. an scFv wherein V_{a-2} and V_{b-2} are separated by a linker), wherein one of the ABD binds NKp46 and the other binds an antigen of interest.

In one embodiment, multimeric proteins are constructed based upon two Fc-containing chains (e.g. chains 1 and 2) to create a dimer via CH3-CH3 dimerization and/or hinge dimerization, and a further chain (e.g. chain 3) comprising a V-C_H/Cκ unit that dimerizes with one of chains 1 or 2. Exemplary molecules can have the following domain arrangement:

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wherein V_{a-1} , V_{b-1} , V_{a-2} and V_{b-2} are each a V_H domain or a V_L domain, and wherein one of V_{a-1} and V_{b-1} is a V_H and the other is a V_L such that V_{a-1} and V_{b-1} form a first antigen binding domain (ABD), wherein one of V_{a-2} and V_{b-2} is a V_H and the other is a V_L such that V_{a-2} and V_{b-2} form a second antigen binding domain.

Exemplary molecules may possess the following domain arrangement:

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wherein V_{a-1} , V_{b-1} , V_{a-2} and V_{b-2} are each a V_H domain or a V_L domain, and wherein one of V_{a-1} and V_{b-1} is a V_H and the other is a V_L such that V_{a-1} and V_{b-1} form a first antigen binding domain (ABD), wherein one of V_{a-2} and V_{b-2} is a V_H and the other is a V_L such that V_{a-2} and V_{b-2} form a second antigen binding domain. The CH1 and Ck are selected such that chain 1 is capable of associating with chain 2 and chain 2 with chain 3. The protein can be configured such that chains 1 and 2 associate via CH3-CH3 dimerization and chains 2 and 3 associate via CH1-Ck dimerization.

Optionally, any of the multispecific proteins of the invention may include, CH3 domains which comprise amino acid substitutions, wherein the CH3 domain interface of the antibody Fc region is mutated to create altered charge polarity across the Fc dimer interface such that co-expression of electrostatically matched Fc chains supports favorable attractive interactions thereby promoting desired Fc heterodimer formation, whereas unfavorable repulsive charge interactions suppress unwanted Fc homodimer formation.

Heterodimeric or heterotrimeric polypeptides with two ABDs and a dimeric Fc domain can optionally be produced as one or more chains that each associate with a central chain, e.g. by CH1-Ck heterodimerization. Such multimers may be composed of a central (first) polypeptide chain comprising two immunoglobulin variable domains that are part of separate antigen binding domains (of different antigen specificities), the one or more other chains that provide the additional and/or complementary variable domains, and an Fc domain placed on the central and/or one or more other chains. In one embodiment, the Fc domain is interposed between the two ABDs in the multimeric protein.

In one example, the first (central) polypeptide chain will provide one variable domain that will, together with a complementary variable domain on a second polypeptide chain, form an antigen binding domain specific for one (e.g. a first) antigen of interest. The first (central) polypeptide chain will also provide a second variable domain (e.g., placed on the opposite end of the interposed Fc domain) that will be paired with a complementary variable domain to form an antigen binding domain specific for another (e.g. a second) antigen of interest; the variable domain that is complementary to the second variable domain can be placed on the central polypeptide (e.g. adjacent to the second variable domain in a tandem variable domain construct such as an scFv), or can be placed on a separate polypeptide chain, notably a third polypeptide chain. The second (and third, if present) polypeptide chains will associate with the central polypeptide chain by CH1-Ck heterodimerization, forming non-covalent bonds and optionally further interchain disulfide bonds between complementary CH1 and Ck domains (and optionally interchain disulfide bonds between hinge regions), with a primary multimeric polypeptide being formed so long as CH/CK and V_H/V_L domains are chosen to give rise to a preferred dimerization configuration that results preferentially in the desired V_H-V_L pairings. Remaining unwanted pairings can remain minimal during production and/or are removed during purification steps. In a trimer, or when polypeptides are constructed for preparation of a trimer, there will generally be one polypeptide chain that comprises a non-naturally occurring VH-CK or VK-CH1 domain arrangement.

Examples of the domain arrangements (N- to C-termini) of central polypeptide chains for use in such heterodimeric proteins include any of the following:

V-(CH1 or Ck)-Fc domain-V-V;

and

V-V-(CH1 or Ck)-Fc domain;

and

Fc domain-V-V;

and

V-V-Fc domain;

and

V-V-Fc domain-V-(CH1 or Ck).

For example, the domain arrangements (N- to C-termini) of central polypeptide chains for use in such heterodimeric proteins can include:

$V_{a-1}-(CH1 \text{ or } Ck)_a$ -Fc domain- V_{a-2} - V_{b-2} ;

and

V_{a-2} - V_{b-2} -Fc domain-(CH1 or Ck)_a

wherein V_{a-1} is a light chain or heavy chain variable domain, and wherein one of V_{a-2} and V_{b-2} is a light chain variable domain and the other is a heavy chain variable domain.

Further examples include:

$V_{a-1}-(CH1 \text{ or } Ck)_a$ -Fc domain V_b ;

and

V_b -Fc domain- $V_{a-1}-(CH1 \text{ or } Ck)_a$

wherein V_b is a single variable domain (e.g. dAb, VhH).

The Fc domain of the central chain may be a full Fc domain (CH2-CH3) or a portion thereof sufficient to confer the desired functionality (e.g. CD16 and optionally FcRn or another Fc receptor binding) when it forms a dimeric Fc with a second chain. A second polypeptide chain will then be configured which will comprise an immunoglobulin variable domain and a CH1 or Ck constant region, e.g., a (CH1 or Ck)_b unit, selected so as to permit CH1-Ck heterodimerization with the central polypeptide chain; the immunoglobulin variable domain will be selected so as to complement the variable domain of the central chain that is adjacent to the CH1 or Ck domain, whereby the complementary variable domains form an antigen binding domain for a first antigen of interest.

For example, a second polypeptide chain can comprise a domain arrangement: $V_{b-1}-(CH1 \text{ or } Ck)_b$ -Fc domain such that the (CH1 or Ck)₂ dimerizes with the (CH1 or Ck), on the central chain, and the V_{b-1} forms an antigen binding domain together with V_{a-1} of the central chain. If the V_{a-1} of the central chain is a light chain variable domain, then V_{b-1} will be a heavy chain variable domain; and if V_{a-1} of the central chain is a heavy chain variable domain, then V_{b-1} will be a light chain variable domain.

The antigen binding domain for the second antigen of interest can then be formed from V_{a-2} and V_{b-2} which are configured as tandem variable domains on the central chain forming the antigen binding domain for the second antigen of interest (e.g. a heavy chain variable domain (V_H) and a light chain (K) variable domain (V_K), for example forming an scFv unit). The antigen binding domain for the second antigen of interest can also alternatively be formed from a single variable domain V_b present on the central chain.

The resulting heterodimer can, for example, have the following configuration (see further Examples of such proteins shown as formats 13 and 14 shown in FIGS. 2D and 2E):

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wherein one of V_{a-1} of the first polypeptide chain and V_{b-1} of the second polypeptide chain is a light chain variable domain and the other is a

heavy chain variable domain, and wherein one of V_{a-2} and V_{b-2} is a light chain variable domain and the other is a heavy chain variable domain.

In one embodiment, the heterodimeric bispecific Fc-derived polypeptide comprises a domain arrangement selected from one of the following, optionally wherein one or both of the hinge domains are replaced by a peptide linker, optionally wherein the Fc domain is fused to anti-NKp46 scFv via a peptide linker):

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Other examples of potential domain arrangements for the heterodimeric polypeptides according to the invention include but are not limited to those shown in the table below:

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Heterotrimeric proteins can for example be formed by using a central (first) polypeptide chain comprising a first variable domain (V) fused to a first CH1 or C κ constant region, a second variable domain (V) fused to a second CH1 or C κ constant region, and an Fc domain or portion thereof interposed between the first and second variable domains (i.e. the Fc domain is interposed between the first and second (V-(CH1/C κ) units. For example, a central polypeptide chain for use in a heterotrimeric protein according to the invention can have the domain arrangements (N- to C-termini) as follows:

V_{a-1} -(CH1 or C κ) $_a$ -Fc domain- V_{a-2} -(CH1 or C κ) $_b$.

A second polypeptide chain can then comprise a domain arrangement (N- to C-termini):

V_{b-1} -(CH1 or C κ) $_c$ -Fc domain

such that the (CH1 or C κ) $_c$ dimerizes with the (CH1 or C κ) $_a$ on the central chain, and the V_{a-1} and V_{b-1} form an antigen binding domain.

A third polypeptide chain can then comprise the following domain arrangement (N- to C-termini):

V_{b-2} -(CH1 or C κ) $_d$,

such that the (CH1 or C κ) $_d$ dimerizes with the (CH1 or C κ) $_b$ unit on the central chain, and the V_{a-2} and V_{b-2} form an antigen binding domain.

An example of a domain configuration of a resulting heterotrimer with a dimeric Fc domain (also shown as formats 5, 6, 7 and 16 in FIGS. 2D and 2E) is shown below:

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Thus, in a trimeric polypeptide according to the invention, the first polypeptide can have two variable domains that each form an antigen binding domain with a variable domain on a separate polypeptide chain (i.e. the variable domain of the second and third chains), the second polypeptide chain has one variable domain, and the third polypeptide has one variable domain.

A trimeric polypeptide according to the invention may further comprise the following:

(a) a first polypeptide chain comprising a first variable domain (V) fused to a first CH1 or C κ constant region, a second variable domain (V) fused to a second CH1 or C κ constant region, and an Fc domain or portion thereof interposed between the first and second variable domains;

(b) a second polypeptide chain comprising a variable domain fused at its C-terminus to a CH1 or C κ constant region selected to be complementary to the first CH1 or C κ constant region of the first polypeptide chain such that the first and second polypeptides form a CH1-C κ heterodimer, and an Fc domain; and

(c) a third polypeptide chain comprising a variable domain fused (e.g. at its C-terminus) to a CH1 or C κ constant region, wherein the variable domain and the constant region are selected to be complementary to the second variable domain and second CH1 or C κ constant region of the first polypeptide chain such that the first and third polypeptides form a CH1-C κ heterodimer bound by non-covalent and optionally further disulfide

bond(s) formed between the CH1 or C κ constant region of the third polypeptide and the second CH1 or C κ constant region of the first polypeptide, but not between the CH1 or C κ constant region of the third polypeptide and the first CH1 or C κ constant region of the first polypeptide

wherein the first, second and third polypeptides form a CH1-C κ heterotrimer, and wherein the first variable domain of the first polypeptide chain and the variable domain of the second polypeptide chain form an antigen binding domain specific for a first antigen of interest, and the second variable domain of the first polypeptide chain and the variable domain on the third polypeptide chain form an antigen binding domain specific for a second antigen of interest.

Examples of potential domain arrangements for such trimeric bispecific polypeptides include but are not limited to those shown below:

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In further examples, heterotrimers can be constructed with three ABDs and a dimeric Fc. One of the ABDs will bind to NKp46, and the other two ABDs can bind to an antigen of interest, wherein the antigen of interest bound by the two ABDs can be the same antigen or a different antigen. Thus, in one aspect of such an embodiment, the multimeric polypeptide can bind the antigen of interest in a bivalent manner (with two ABDs).

Heterotrimeric proteins can for example be formed by using a central (first) polypeptide chain comprising a first variable domain (V) fused to a first CH1 or C κ constant region, a second variable domain (V) fused to a second CH1 or C κ constant region, and an Fc domain or portion thereof interposed between the first and second variable domains (i.e. the Fc domain is interposed between the first and second (V-(CH1/C κ)) units. For example, a central polypeptide chain for use in a heterotrimeric protein can have the domain arrangements (N- to C-termini) as follows:

V_1 -(CH1 or C κ)_a-Fc domain- V_2 -(CH1 or C κ)_b.

A second polypeptide chain can then comprise a domain arrangement (N- to C-termini):

V_1 -(CH1 or C κ)_c,

or

—(CH1 or C κ)_c—Fc domain

such that the (CH1 or C κ)_c dimerizes with the (CH1 or C κ)_a on the central chain, and the V_{a1} and V_{b1} form an antigen binding domain.

A third polypeptide chain can then comprise a domain arrangement (N- to C-termini):

V_2 -(CH1 or C κ)_d-scFv,

such that the (CH1 or C κ)_d dimerizes with the (CH1 or C κ)_b unit on the central chain, and the V_{a1} and V_{b2} form an antigen binding domain.

An example of a configuration of a resulting heterotrimer with a dimeric Fc domain (also shown as formats T5 and T6 in FIG. 2F) has a domain arrangement:

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In any of the polypeptide chains herein, a hinge region will typically be present on a polypeptide chain between a CH1 domain and a CH2 domain of an Fc domain, and/or can be present between a C κ domain and a CH2 domain. A hinge region can optionally be replaced, e.g., by a suitable linker peptide.

In any of the domain arrangements, the Fc domain may comprise a CH2-CH3 unit (a full length CH2 and CH3 domain or a fragment thereof). In heterodimers or heterotrimers comprising two chains with Fc domains (a dimeric Fc domain), the CH3 domain will be capable of CH3-CH3 dimerization (e.g. it will comprise a wild-type CH3 domain).

In some exemplary configurations, the multispecific protein can be a heterodimer, a heterotrimer or a heterotetramers, wherein the polypeptide

chains are engineered for heterodimerization among each other so as to produce the desired protein. In embodiments where the desired chain pairings are not driven by CH1-C κ dimerization, the chains may comprise constant or Fc domains with amino acid modifications (e.g., substitutions) that favor the preferential hetero-dimerization of the two different chains over the homo-dimerization of two identical chains. In some embodiments, a “knob-into-holes” approach is used in which the CH3 domain interface of the antibody Fc region is mutated so that the antibodies preferentially form heterodimers (further including the attached light chains). These mutations create altered charge polarity across the Fc dimer interface such that co-expression of electrostatically matched Fc chains support favorable attractive interactions thereby promoting desired Fc heterodimer formation, whereas unfavorable repulsive charge interactions suppress unwanted Fc homodimer formation. For example one heavy chain comprises a T366W substitution and the second heavy chain comprises a T366S, L368A and Y407V substitution, see, e.g. Ridgway et al (1996) *Protein Eng.*, 9, pp. 617-621; Atwell (1997) *J. Mol. Biol.*, 270, pp. 26-35; and WO2009/089004, the disclosures of which are incorporated herein by reference. In another approach, one heavy chain comprises a F405L substitution and the second heavy chain comprises a K409R substitution, see, e.g., Labrijn et al. (2013) *Proc. Natl. Acad. Sci. U.S.A.*, 110, pp. 5145-5150. In another approach, one heavy chain comprises T350V, L351Y, F405A, and Y407V substitutions and the second heavy chain comprises T350V, T366S, K392L, and T394W substitutions, see, e.g. Von Kreudenstein et al., (2013) mAbs 5:646-654. In another approach, one heavy chain comprises both K409D and K392D substitutions and the second heavy chain comprises both D399K and E356K substitutions, see, e.g. Gunasekaran et al., (2010) *J. Biol. Chem.* 285:19637-19646. In another approach, one heavy chain comprises D221E, P228E and L368E substitutions and the second heavy chain comprises D221R, P228R, and K409R substitutions, see, e.g. Strop et al., (2012) *J. Mol. Biol.* 420: 204-219. In another approach, one heavy chain comprises S364H and F405A substitutions and the second heavy chain comprises Y349T and, T394F substitutions, see, e.g. Moore et al., (2011) mAbs 3: 546-557. In another approach, one heavy chain comprises a H435R substitution and the second heavy chain optionally may or may not comprise a substitution, see, e.g. U.S. Pat. No. 8,586,713. When such hetero-multimeric antibodies have Fc regions derived from a human IgG2 or IgG4, the Fc regions of these antibodies can be engineered to contain amino acid modifications that permit CD16 binding. In some embodiments, the antibody may comprise mammalian antibody-type N-linked glycosylation at residue N297 (Kabat EU numbering).

In some embodiments the invention also comprises a heterodimeric or heterotrimeric protein that comprises an NKp46-binding ABD and an antigen of interest-binding ABD, in which one or both of the ABDs (e.g., a variable region or other antigen binding domain such as a non-immunoglobulin scaffold) is linked to a constant region, e.g., an Fc domain or portion thereof via a linker, e.g., a flexible polypeptide linker. Optionally, the ABD is placed on a single polypeptide chain (e.g. a tandem variable domain, a V_HH or single V domain, a non-immunoglobulin scaffold).

In some embodiments, one or both of the ABDs is comprised in a V_H and V_L domain that associate with one another to form the ABD. In one embodiment, the V_H and V_L that form an ABD are each within a tandem variable region (a V_H and V_L domain separated by a flexible polypeptide linker).

In some embodiments, one of the ABDs is comprised in a Fab or Fab-like structure, in which a variable domain is linked to a CH1 domain and a complementary variable domain is linked to a complementary C κ (or C λ) constant domain, wherein the CH1 and C κ (or C λ) constant domains associate (dimerize). In some embodiments, one of the ABDs is comprised in such a Fab or Fab-like structure and the other ABD is placed on a single polypeptide chain (e.g. a tandem variable domain) and is linked to a constant region, e.g., an Fc domain or portion thereof via a linker, e.g., a flexible polypeptide linker.

In some embodiments, one of the ABDs comprises a Fab or Fab-like structure, in which a variable domain is linked to a CH1 domain and a complementary variable domain is linked to a complementary C κ (or C λ) constant domain, wherein the CH1 and C κ (or C λ) constant domains associate to form a heterodimeric protein. For example, a first and second ABD can advantageously comprise or consist of single variable domains (e.g. V_HH domains) having different antigen binding specificities (e.g., V_HH₁ and V_HH₂). Also the V_HH₁ can be fused to a CH1 domain and V_HH₂ can be fused to a C κ or C λ domain. The V₁-C κ (or C λ) chain associates with a V₂-CH1 chain such that a Fab is formed. See, e.g., WO2006/064136 and WO2012/089814 which disclose examples of such antibodies lacking Fc domains, the disclosures of which PCT applications are incorporated herein by reference. The CH1 and/or C κ domains can then be linked to a CH2 domain, optionally via a hinge region (or a linker peptide, e.g., one that has similar functional properties, e.g., dimerization). The CH2 domain(s) is/are then linked to a CH3 domain. The CH2-CH3 domains can thus optionally be embodied as a full-length Fc domain.

In any multispecific protein according to the invention, a hinge region can and generally will be present on a polypeptide chain between a CH1 domain and a CH2 domain, and/or can be present between a C κ domain and a CH2 domain. A hinge region can optionally be replaced for example by a suitable linker peptide, e.g. a flexible polypeptide.

The proteins domains described in the present disclosure can optionally be specified as being from N- to C-termini. Protein arrangements of the disclosure for purposes of illustration are shown from N-terminus (on the left) to C-terminus. Domains can be referred to as fused to one another (e.g. a domain can be said to be fused to the C-terminus of the domain on its left, and/or a domain can be said to be fused to the N-terminus of the domain on its right).

The proteins domains described in the present disclosure can be fused to one another directly or via intervening amino acid sequences. For example, a CH1 or C κ domain can be fused to an Fc domain (or CH2 or CH3 domain thereof) via a linker peptide, optionally a hinge region or a fragment thereof. In another example, a V_H or V κ domain can be fused to a CH3 domain via a linker peptide. V_H and V_L domains linked to another in tandem can and generally will be fused via a linker peptide (e.g. a scFv). V_H and V_L domains linked to an Fc domain will be fused via a linker peptide. Two polypeptide chains will be bound to one another (indicated by “|”), by non-covalent bonds, and optionally can further be attached via interchain disulfide bonds, formed between cysteine residues within complementary CH1 and C κ domains.

Linkers

When ABD(s) or a polypeptide chain(s) comprise(s) a tandem variable region (e.g. scFv), two V domains (e.g. a V_H domain and V_L domains are generally linked together by a linker of sufficient length to enable the ABD to fold in such a way as to permit binding to the antigen for which the ABD is intended to bind. Similarly, when an ABD is linked to a constant domain or Fc domain, the linkage may be via a flexible linker (e.g. polypeptide linker) that permits the ABD to be positioned such that it binds to its target antigen and exhibits the desired functionality, e.g. it possesses a sufficient range of motion relative to the rest of the multispecific protein (the Fc domain and/or other ABD) and thereby mediates NKp46 signalling. Examples of linkers include, for example, linkers derived from antibody hinge regions, an amino sequence RTVA, or linkers comprising glycine and serine residues, e.g., the amino acid sequence GEGTSTGS(G₂S)₂GGAD. In another specific embodiment, the V_H domain and V_L domains of a scFv are linked together by the amino acid sequence (G₄S)₃.

Any of the peptide linkers contained in the subject multispecific proteins may comprise a length of at least 4 residues, at least 5 residues, at least 10 residues, at least 15 residues, at least 20 residues, at least 25 residues, at least 30 residues or more. In other embodiments, the linkers comprise a length of between 2-4 residues, between 2-4 residues, between 2-6 residues, between 2-8 residues, between 2-10 residues, between 2-12 residues, between 2-14 residues, between 2-16 residues, between 2-18 residues, between 2-20 residues, between 2-22 residues, between 2-24 residues, between 2-26 residues, between 2-28 residues, between 2-30 residues, between 2 and 50 residues, or between 10 and 50 residues.

An ABD (e.g. an immunoglobulin variable region) can optionally be linked to a constant domain or Fc domain via a flexible linker (e.g. polypeptide linker) that leads to less structural rigidity or stiffness (e.g. between or amongst the ABD and Fc domain) compared to a conventional (e.g. wild-type full length human IgG) antibody. For example, the multispecific protein may have a structure or a flexible linker between the ABD and constant domain or Fc domain that permits an increased range of domain motion compared to the ABD in a conventional (e.g. wild-type full length human IgG) antibody. In particular, the structure or a flexible linker can be configured to confer on the antigen binding sites greater intrachain domain movement compared to antigen binding sites in a conventional human IgG1 antibody. Rigidity or domain motion/interchain domain movement can be determined, e.g., by computer modeling, electron microscopy, spectroscopy such as Nuclear Magnetic Resonance (NMR), X-ray crystallography, or Sedimentation Velocity Analytical ultracentrifugation (AUC) to measure or compare the radius of gyration of proteins comprising the linker or hinge. A test protein or linker may have lower rigidity relative to a comparator protein if the test protein has a value obtained from one of the tests described in the previous sentence differs from the value of the comparator, e.g., an IgG1 antibody or a hinge, by at least 5%, 10%, 25%, 50%, 75%, or 100%. A person of skill in the art would be able to determine from the tests whether a test protein has at lower rigidity to that of another protein, respectively, by interpreting the results of these tests.

In one embodiment, the multispecific protein may have a structure or a flexible linker between the ABD and constant domain or Fc domain that

permits the Nkp46 ABD and the ABD which binds an antigen of interest to have a spacing between said ABDs comprising less than about 80 angstroms, less than about 60 angstroms or ranges from about 40-60 angstroms.

In one embodiment, the hinge region will be a fragment of a hinge region (e.g. a truncated hinge region without cysteine residues) or may comprise one or more amino acid modifications which remove (e.g. substitute by another amino acid, or delete) a cysteine residue, optionally both cysteine residues in a hinge region. Removing cysteines can be useful to prevent undesired disulfide bond formation, e.g., the formation of disulfide bridges in a monomeric polypeptide.

In one embodiment, a (poly)peptide linker used to link a CH1 or C κ domain to a CH2 or CH3 domain of an Fc domain comprises a fragment of a CH1 domain and/or hinge region. For example, an N-terminal amino acid sequence of CH1 can be fused to a variable domain in order to mimic as closely as possible the natural structure of a wild-type antibody. In one embodiment, the linker comprises an amino acid sequence from a hinge domain or an N-terminal CH1 amino acid. The sequence can be, for example, between 2-4 residues, between 2-4 residues, between 2-6 residues, between 2-8 residues, between 2-10 residues, between 2-12 residues, between 2-14 residues, between 2-16 residues, between 2-18 residues, between 2-20 residues, between 2-22 residues, between 2-24 residues, between 2-26 residues, between 2-28 residues, or between 2-30 residues. In one embodiment the linker comprises or consists of the amino acid sequence RTVA.

In one embodiment, the hinge region (or fragment thereof) is derived from a hinge domain of a human IgG1 antibody. For example a hinge domain may comprise the amino acid sequence: T-H-T-C-S-S-C-P-A-P-E-L-L (one letter code), or an amino acid sequence at least 60%, 70%, 80% or 90% identical thereto, optionally wherein one or both cysteines are deleted or substituted by a different amino acid residue.

In one embodiment, the hinge region (or fragment thereof) is derived from a C μ 2-C μ 3 hinge domain of a human IgM antibody. For example a hinge domain may comprise the amino acid sequence: N-A-S-S-M-C-V-P-S-P-A-P-E-L-L (one letter code), or an amino acid sequence at least 60%, 70%, 80% or 90% identical thereto, optionally wherein one or both cysteines are deleted or substituted by a different amino acid residue.

Polypeptide chains that dimerize and associate with one another via non-covalent bonds may or may not additionally be bound by an interchain disulfide bond formed between respective CH1 and C κ domains, and/or between respective hinge domains on the chains. CH1, C κ and/or hinge domains (or other suitable linking amino acid sequences) can optionally be configured such that interchain disulfide bonds are formed between chains such that the desired pairing of chains is favored and undesired or incorrect disulfide bond formation is avoided. For example, when two polypeptide chains to be paired each possess a CH1 or C κ adjacent to a hinge domain, the polypeptide chains can be configured such that the number of available cysteines for interchain disulfide bond formation between respective CH1/C κ -hinge segments is reduced (or is entirely eliminated). For example, the amino acid sequences of respective CH1, C κ and/or hinge domains can be modified to remove cysteine residues in both the CH1/C κ and the hinge domain of a polypeptide; thereby the CH1 and C κ domains of the two chains that dimerize will associate via non-covalent interaction(s).

In another example, the CH1 or C κ domain adjacent (e.g., N-terminal to) a hinge domain comprises a cysteine capable of interchain disulfide bond formation, and the hinge domain which is placed at the C-terminus of the CH1 or C κ comprises a deletion or substitution of one or both cysteines of the hinge (e.g. Cys 239 and Cys 242, as numbered for human IgG1 hinge according to Kabat). In one embodiment, the hinge region (or fragment thereof) comprise the amino acid sequence: T-H-T-S-P-S-P-A-P-E-L-L (one letter code), or an amino acid sequence at least 60%, 70%, 80% or 90% identical thereto.

In another example, the CH1 or C κ domain adjacent (e.g., N-terminal to) a hinge domain comprises a deletion or substitution at a cysteine residue capable of interchain disulfide bond formation, and the hinge domain placed at the C-terminus of the CH1 or C κ comprises one or both cysteines of the hinge (e.g. Cys 239 and Cys 242, as numbered for human IgG1 hinge according to Kabat). In one embodiment, the hinge region (or fragment thereof) comprises the amino acid sequence: T-H-T-C-S-S-C-P-A-P-E-L-L (one letter code), or an amino acid sequence at least 60%, 70%, 80% or 90% identical thereto.

In another example, a hinge region is derived from an IgM antibody. In such embodiments, the CH1/C κ pairing mimics the C μ 2 domain homodimerization in IgM antibodies. For example, the CH1 or C κ domain adjacent (e.g., N-terminal to) a hinge domain comprises a deletion or substitution at a cysteine capable of interchain disulfide bond formation, and an IgM hinge domain which is placed at the C-terminus of the CH1

or C κ comprises one or both cysteines of the hinge. In one embodiment, the hinge region (or fragment thereof) comprises the amino acid sequence: T-H-T-C-S-S-C-P-A-P-E-L-L (one letter code), or an amino acid sequence at least 60%, 70%, 80% or 90% identical thereto.

Constant Regions

Constant region domains can be derived from any suitable human antibody, including, the constant heavy (CH1) and light (C κ) domains, hinge domains, CH2 and CH3 domains. With respect to heavy chain constant domains, "CH1" generally refers to positions 118-220 according to the EU index as in Kabat.

"CH2" generally refers to positions 237-340 according to the EU index as in Kabat, and "CH3" generally refers to positions 341-447 according to the EU index as in Kabat.

A "hinge" or "hinge region" or "antibody hinge region" herein refers to the flexible polypeptide or linker between the first and second constant domains of an antibody. Structurally, the IgG CH1 domain ends at EU position 220, and the IgG CH2 domain begins at residue EU position 237. Thus for an IgG the hinge generally includes positions 221 (D221 in IgG1) to 236 (G236 in IgG1), wherein the numbering is according to the EU index as in Kabat. References to specific amino acid residues within constant region domains found within the polypeptides shall be, unless otherwise indicated or as otherwise dictated by context, be defined according to Kabat, in the context of an IgG antibody.

CH2 and CH3 domains which may be present in the subject antibodies or multispecific proteins can be derived from any suitable antibody. Such CH2 and CH3 domains can be used as wild-type domains or may serve as the basis for a modified CH2 or CH3 domain. Optionally the CH2 and/or CH3 domain is of human origin or may comprise that of another species (e.g., rodent, rabbit, non-human primate) or may comprise a modified or chimeric CH2 and/or CH3 domain, e.g., one comprising portions or residues from different CH2 or CH3 domains, e.g., from different antibody isotypes or species antibodies.

In embodiments where a multispecific is intended not to bind to human CD16 polypeptide, a CH2 and/or CH3 domain (or Fc domain comprising same) may comprise a modification to decrease or abolish binding to Fc γ RIIIA (CD16). For example, CH2 mutations in a dimeric Fc domain proteins at residue N297 (Kabat numbering) can eliminate CD16 binding. However the person of skill in the art will appreciate that other configurations can be implemented. For example, substitutions into human IgG1 or IgG2 residues at positions 233-236 and IgG4 residues at positions 327, 330 and 331 were shown to greatly reduce binding to Fc γ receptors and thus ADCC and CDC. Furthermore, Idusogie et al. (2000) J. Immunol. 164(8):4178-84 demonstrated that alanine substitution at different positions, including K322, significantly reduced complement activation.

In certain embodiments herein where binding to CD16A is desired, a CH2 and/or CH3 domain (or Fc domain comprising same) may be a wild-type domain or may comprise one or more amino acid modifications (e.g. amino acid substitutions) which increase binding to human CD16 and optionally another receptor such as FcRn. Optionally, the modifications will not substantially decrease or abolish the ability of the Fc-derived polypeptide to bind to neonatal Fc receptor (FcRn), e.g. human FcRn. Typical modifications include modified human IgG1-derived constant regions comprising at least one amino acid modification (e.g. substitution, deletions, insertions), and/or altered types of glycosylation, e.g., hypofucosylation. Such modifications can affect interaction with Fc receptors: Fc γ RI (CD64), Fc γ RII (CD32), and Fc γ RIII (CD16). Fc γ RI (CD64), Fc γ RIIA (CD32A) and Fc γ RIII (CD 16) are activating (i.e., immune system enhancing) receptors while Fc γ RIIB (CD32B) is an inhibiting (i.e., immune system dampening) receptor. A modification may, for example, increase binding of the Fc domain to Fc γ RIIIa on effector (e.g. NK) cells and/or decrease binding to Fc γ RIIB. Examples of modifications are provided in PCT publication no. WO2014/044686, the disclosure of which is incorporated herein by reference. Specific mutations (in IgG1 Fc domains) which affect (enhance) Fc γ RIIIa or FcRn binding are also set forth below.

Iso- Spe- type cies	Modification	Effector Function	Effect of Modification
IgG1 Human	T250Q/M428L	Increased binding to FcRn	Increased half-life

IgG1 Human 1M252Y/S254T/ T256E + H433K/binding to FcRn half-life N434F	Increased	Increased
IgG1 Human E333A	Increased binding to FcγRIIIa	Increased ADCC and CDC
IgG1 Human S239D/I332E or S239D/A330L/ I332E	Increased binding to FcγRIIIa	Increased ADCC
IgG1 Human P257I/Q311	Increased binding to FcRn half-life	Unchanged
IgG1 Human S239D/I332E/ G236A	Increased FcγRIIa/FcγRIIb macrophage ratio	Increased phagocytosis

In some embodiments, the multispecific protein comprises a variant Fc region comprise at least one amino acid modification (for example, possessing 1, 2, 3, 4, 5, 6, 7, 8, 9, or more amino acid modifications) in the CH2 and/or CH3 domain of the Fc region, wherein the modification enhances binding to a human CD16 polypeptide. In other embodiments, the multispecific protein comprises at least one amino acid modification (for example, 1, 2, 3, 4, 5, 6, 7, 8, 9, or more amino acid modifications) in the CH2 domain of the Fc region from amino acids 237-341, or within the lower hinge-CH2 region that comprises residues 231-341. In some embodiments, the multispecific protein comprises at least two amino acid modifications (for example, 2, 3, 4, 5, 6, 7, 8, 9, or more amino acid modifications), wherein at least one of such modifications is within the CH3 region and at least one such modifications is within the CH2 region. Encompassed also are amino acid modifications in the hinge region. In one embodiment, encompassed are amino acid modifications in the CH1 domain, optionally in the upper hinge region that comprises residues 216-230 (Kabat EU numbering). Any suitable functional combination of Fc modifications can be made, for example any combination of the different Fc modifications which are disclosed in any of U.S. Pat. Nos. 7,632,497; 7,521,542; 7,425,619; 7,416,727; 7,371,826; 7,355,008; 7,335,742; 7,332,581; 7,183,387; 7,122,637; 6,821,505 and 6,737,056; and/or in PCT Publications Nos. WO2011/109400; WO 2008/105886; WO 2008/002933; WO 2007/021841; WO 2007/106707; WO 06/088494; WO 05/115452; WO 05/110474; WO 04/1032269; WO 00/42072; WO 06/088494; WO 07/024249; WO 05/047327; WO 04/099249 and WO 04/063351; and/or in Lazar et al. (2006) *Proc. Nat. Acad. Sci. USA* 103(11): 405-410; Presta, L. G. et al. (2002) *Biochem. Soc. Trans.* 30(4):487-490; Shields, R. L. et al. (2002) *J. Biol. Chem.* 26; 277(30):26733-26740 and Shields, R. L. et al. (2001) *J. Biol. Chem.* 276(9):6591-6604).

In some embodiments, the multispecific protein comprises an Fc domain comprising at least one amino acid modification (for example, 1, 2, 3, 4, 5, 6, 7, 8, 9, or more amino acid modifications) relative to a wild-type Fc region, such that the molecule has an enhanced binding affinity for human CD16 relative to the same molecule comprising a wild-type Fc region, optionally wherein the variant Fc region comprises a substitution at any one or more of positions 221, 239, 243, 247, 255, 256, 258, 267, 268, 269, 270, 272, 276, 278, 280, 283, 285, 286, 289, 290, 292, 293, 294, 295, 296, 298, 300, 301, 303, 305, 307, 308, 309, 310, 311, 312, 316, 320, 322, 326, 329, 330, 332, 331, 332, 333, 334, 335, 337, 338, 339, 340, 359, 360, 370, 373, 376, 378, 392, 396, 399, 402, 404, 416, 419, 421, 430, 434, 435, 437, 438 and/or 439 (Kabat EU numbering).

In one embodiment, the multispecific protein comprises an Fc domain comprising at least one amino acid modification (for example, 1, 2, 3, 4, 5, 6, 7, 8, 9, or more amino acid modifications) relative to a wild-type Fc region, such that the molecule has enhanced binding affinity for human CD16 relative to a molecule comprising a wild-type Fc region, optionally wherein the variant Fc region comprises a substitution at any one or more of positions 239, 298, 330, 332, 333 and/or 334 (e.g. S239D, S298A, A330L, I332E, E333A and/or K334A substitutions), optionally wherein the variant Fc region comprises a substitution at residues S239 and I332, e.g. a S239D and I332E substitution (Kabat EU numbering).

In some embodiments, the multispecific protein comprises an Fc domain comprising altered glycosylation patterns that increase binding affinity for human CD16. Such carbohydrate modifications can be accomplished by, for example, by expressing a nucleic acid encoding the multispecific

protein in a host cell with altered glycosylation machinery. Cells with altered glycosylation machinery are known in the art and can be used as host cells in which to express recombinant antibodies to thereby produce an antibody with altered glycosylation. See, for example, Shields, R. L. et al. (2002) *J. Biol. Chem.* 277:26733-26740; Umana et al. (1999) *Nat. Biotech.* 17:176-1, as well as, European Patent No: EP 1,176,195; PCT Publications WO 06/133148; WO 03/035835; WO 99/54342, each of which is incorporated herein by reference in its entirety. In one aspect, the multispecific protein contains one or more hypofucosylated constant regions. Such multispecific protein may comprise an amino acid alteration or may not comprise an amino acid alteration and/or may be expressed or synthesized or treated under conditions that result in hypofucosylation. In one aspect, a multispecific protein composition comprises a multispecific protein described herein, wherein at least 20, 30, 40, 50, 60, 75, 85, 90, 95% or substantially all of the antibody species in the composition have a constant region comprising a core carbohydrate structure (e.g. complex, hybrid and high mannose structures) which lacks fucose. In one embodiment, provided is a multispecific protein composition which is free of N-linked glycans comprising a core carbohydrate structure having fucose. The core carbohydrate will preferably be a sugar chain at Asn297.

Optionally, a multispecific protein comprising a dimeric Fc domain can be characterized by having a binding affinity to a human CD16 polypeptide that is within 1-log of that of a conventional human IgG1 antibody, e.g., as assessed by surface plasmon resonance.

In one embodiment, the multispecific protein comprising a dimeric Fc domain engineered to enhance Fc receptor binding can be characterized by having a binding affinity to a human CD16 polypeptide that is at least 1-log greater than that of a conventional or wild-type human IgG1 antibody, e.g., as assessed by surface plasmon resonance.

Optionally a multispecific protein comprising a dimeric Fc domain can be characterized by a K_d for binding (monovalent) to a human CD16 polypeptide of less than 10^{-5} M (10 μ molar), optionally less than 10^{-6} M (1 μ molar), as assessed by surface plasmon resonance (e.g. as in Example 16, SPR measurements performed on a Biacore T100 apparatus (Biacore GE Healthcare), with bispecific antibodies immobilized on a Sensor Chip CM5 and serial dilutions of soluble CD16 polypeptide injected over the immobilized bispecific antibodies.

CDR Sequences and Epitopes

In some embodiments, the proteins and antibodies herein bind the D1 domain of NKp46, the D2 domain of NKp46, or bind a region spanning the D1 and D2 domains (at the border of the D1 and D2 domains, the D1/D2 junction), of the NKp46 polypeptide of SEQ ID NO: 1. In some embodiments, the multispecific proteins or antibodies according to the invention have an affinity for human NKp46 characterized by a K_D of less than 10^{-5} M, less than 10^{-9} M, or less than 10^{-10} M.

In another embodiment, the inventive antibodies or multispecific proteins bind NKp46 at substantially the same epitope on NKp46 as antibody NKp46-1, NKp46-2, NKp46-3, NKp46-4, NKp46-6 or NKp46-9. In another embodiment, the antibodies at least partially overlaps, or includes at least one residue in the segment or epitope bound by NKp46-1, NKp46-2, NKp46-3, NKp46-4, NKp46-6 or NKp46-9. In one embodiment, all key residues of the epitope are in a segment corresponding to domain D1 or D2. In one embodiment, the antibody or multispecific protein binds a residue present in the D1 domain as well as a residue present in the D2 domain. In one embodiment, the antibodies bind an epitope comprising 1, 2, 3, 4, 5, 6, 7 or more residues in the segment corresponding to domain D1 or D2 of the NKp46 polypeptide of SEQ ID NO: 1. In one embodiment, the antibodies bind domain D1 and further bind an epitope comprising 1, 2, 3, or 4 of the residues R101, V102, E104 and/or L105.

In another embodiment, the antibodies or multispecific proteins bind Nkp46 at the D1/D2 domain junction and bind an epitope comprising or consisting of 1, 2, 3, 4 or 5 of the residues K41, E42, E119, Y121 and/or Y194.

In another embodiment, the antibodies or multispecific proteins bind domain D2 and bind an epitope comprising 1, 2, 3, or 4 of the residues P132, E133, I135, and/or S136.

The Examples section provided infra further describes the construction of a series of mutant human NKp46 polypeptides. In the examples, the binding of anti-NKp46 antibody or multispecific protein to cells transfected with the NKp46 mutants was measured and compared to the ability of anti-NKp46 antibody to bind wild-type NKp46 polypeptide (SEQ ID NO:1). A reduction in binding between an anti-NKp46 antibody or NKp46 binding multispecific protein and a mutant NKp46 polypeptide as described herein means that there is a reduction in binding affinity (e.g., as measured by known methods such FACS testing of cells expressing a particular mutant, or by Biacore testing of binding to mutant polypeptides) and/or a reduction in the total binding capacity of the anti-NKp46 antibody (e.g., as evidenced by a decrease in B_{max} in a plot of anti-NKp46

antibody concentration versus polypeptide concentration). A significant reduction in binding indicates that the mutated residue is directly involved in the binding to the anti-NKp46 antibody to NKp46 or is in close proximity to the binding protein when the anti-NKp46 antibody or NKp46 binding multispecific protein is bound to NKp46. An antibody epitope will thus preferably include such residue and may include additional residues adjacent to such residue.

In some embodiments, a significant reduction in binding means that the binding affinity and/or capacity between an anti-NKp46 antibody or NKp46 binding multispecific protein and a mutant NKp46 polypeptide is reduced by greater than 40%, greater than 50%, greater than 55%, greater than 60%, greater than 65%, greater than 70%, greater than 75%, greater than 80%, greater than 85%, greater than 90% or greater than 95% relative to binding between the antibody and a wild type NKp46 polypeptide (e.g., the polypeptide shown in SEQ ID NO:1). In certain embodiments, binding is reduced below detectable limits. In some embodiments, a significant reduction in binding is evidenced when binding of an anti-NKp46 antibody to a mutant NKp46 polypeptide is less than 50% (e.g., less than 45%, 40%, 35%, 30%, 25%, 20%, 15% or 10%) of the binding observed between the anti-NKp46 antibody and a wild-type NKp46 polypeptide (e.g., the polypeptide shown in SEQ ID NO: 1 (or the extracellular domain thereof)). Such binding measurements can be made using a variety of binding assays known in the art. A specific example of one such assay is described in the Example section.

In some embodiments, anti-NKp46 antibodies or NKp46 binding multispecific proteins are provided that exhibit significantly lower binding for a mutant NKp46 polypeptide in which a residue in a wild-type NKp46 polypeptide (e.g., SEQ ID NO:1) is substituted. In the shorthand notation used here, the format is: Wild type residue: Position in polypeptide: Mutant residue, with the numbering of the residues as indicated in SEQ ID NO: 1.

In some embodiments, an anti-NKp46 antibody binds a wild-type NKp46 polypeptide but has decreased binding to a mutant NKp46 polypeptide having a mutation (e.g., an alanine substitution) any one or more of the residues R101, V102, E104 and/or L105 (with reference to SEQ ID NO:1) compared to binding to the wild-type NKp46).

In some embodiments, an anti-NKp46 antibody or NKp46-binding multispecific protein binds a wild-type NKp46 polypeptide but has decreased binding to a mutant NKp46 polypeptide having a mutation (e.g., an alanine substitution) at one or more of residues K41, E42, E119, Y121 and/or Y194 (with reference to SEQ ID NO:1) compared to binding to the wild-type NKp46).

In some embodiments, an anti-NKp46 antibody or NKp46-binding multispecific protein binds a wild-type NKp46 polypeptide but has decreased binding to a mutant NKp46 polypeptide having a mutation (e.g., an alanine substitution) at one or more of residues P132, E133, I135, and/or S136 (with reference to SEQ ID NO:1) compared to binding to the wild-type NKp46).

The amino acid sequence of the heavy chain variable region of antibodies NKp46-1, NKp46-2, NKp46-3, NKp46-4, NKp46-6 and NKp46-9 are listed herein in Table B (SEQ ID NOS: 3, 5, 7, 9, 11 and 13 respectively), the amino acid sequence of the light chain variable region of antibodies NKp46-1, NKp46-2, NKp46-3, NKp46-4, NKp46-6 and NKp46-9 are also listed herein in Table B (SEQ ID NOS: 4, 6, 8, 10, 12 and 14 respectively).

In a specific embodiment, the invention provides is an antibody, e.g. a full length monospecific antibody, a multispecific or bispecific antibody, including a bispecific monomeric polypeptide, or a NKp46-binding multispecific protein that binds essentially the same epitope or determinant as monoclonal antibody NKp46-1, NKp46-2, NKp46-3, NKp46-4, NKp46-6 or NKp46-9; optionally the antibody comprises a hypervariable region of antibody NKp46-1, NKp46-2, NKp46-3, NKp46-4, NKp46-6 or NKp46-9. In any of the embodiments herein, antibody NKp46-1, NKp46-2, NKp46-3, NKp46-4, NKp46-6 or NKp46-9 can be characterized by its amino acid sequence and/or nucleic acid sequence encoding it. In one embodiment, the antibody comprises the Fab or F(ab')₂ portion of NKp46-1, NKp46-2, NKp46-3, NKp46-4, NKp46-6 or NKp46-9. Also provided is an antibody that comprises the heavy chain variable region of NKp46-1, NKp46-2, NKp46-3, NKp46-4, NKp46-6 or NKp46-9. According to one embodiment, an antibody comprises the three CDRs of the heavy chain variable region of NKp46-1, NKp46-2, NKp46-3, NKp46-4, NKp46-6 or NKp46-9. Also provided is a polypeptide that further comprises one, two or three of the CDRs of the light chain variable region of NKp46-1, NKp46-2, NKp46-3, NKp46-4, NKp46-6 or NKp46-9. Optionally any one or more of said light or heavy chain CDRs may contain one, two, three, four or five or more amino acid modifications (e.g. substitutions, insertions or deletions). Optionally, provided is a multispecific protein or antibody polypeptide where any of the light and/or heavy chain variable regions comprising part or all of an antigen binding region of antibody NKp46-1,

NKp46-2, NKp46-3, NKp46-4, NKp46-6 or NKp46-9 are fused to an immunoglobulin constant region of the human IgG type.

In another aspect, the invention provides a protein, e.g., an antibody, a full length monospecific antibody, a multispecific or a bispecific protein, or a polypeptide chain or fragment thereof, or an NKp46-binding multispecific protein as well as a nucleic acid encoding any of the foregoing, wherein the protein comprises the heavy chain CDRs of NKp46-1, NKp46-2, NKp46-3, NKp46-4, NKp46-6 or NKp46-9, comprising, for the respective antibody: a HCDR1 region comprising an amino acid sequence as set forth in Table A, or a sequence of at least 4, 5, 6, 7, 8, 9 or 10 contiguous amino acids thereof, wherein one or more of these amino acids may be substituted by a different amino acid; a HCDR2 region comprising an amino acid sequence as set forth in Table A, or a sequence of at least 4, 5, 6, 7, 8, 9 or 10 contiguous amino acids thereof, wherein one or more of these amino acids may be substituted by a different amino acid; a HCDR3 region comprising an amino acid sequence as set forth in as set forth in Table A, or a sequence of at least 4, 5, 6, 7, 8, 9 or 10 contiguous amino acids thereof, wherein one or more of these amino acids may be substituted by a different amino acid.

In another aspect, the invention provides a protein, e.g., an antibody, a full length monospecific antibody, a multispecific or a bispecific protein, or a polypeptide chain or fragment thereof, or an NKp46-binding multispecific protein as well as a nucleic acid encoding any of the foregoing, wherein the protein comprises light chain CDRs of NKp46-1, NKp46-2, NKp46-3, NKp46-4, NKp46-6 or NKp46-9, comprising, for the respective antibody: a LCDR1 region comprising an amino acid sequence as set forth in Table A, or a sequence of at least 4, 5, 6, 7, 8, 9 or 10 contiguous amino acids thereof, wherein one or more of these amino acids may be substituted by a different amino acid; a LCDR2 region comprising an amino acid sequence as set forth in Table A, or a sequence of at least 4, 5, 6, 7, 8, 9 or 10 contiguous amino acids thereof, wherein one or more of these amino acids may be substituted by a different amino acid; a LCDR3 region comprising an amino acid sequence as set forth in Table A, or a sequence of at least 4, 5, 6, 7, 8, 9 or 10 contiguous amino acids thereof, wherein one or more of these amino acids may be deleted or substituted by a different amino acid.

In another aspect, the invention provides a multispecific protein or antibody that binds to human NKp46, comprising:

- (a) the heavy chain variable region of NKp46-1, NKp46-2, NKp46-3, NKp46-4, NKp46-6 or NKp46-9 as set forth in Table B, optionally wherein one, two, three or more amino acids may be substituted by a different amino acid;
- (b) the light chain variable region NKp46-1, NKp46-2, NKp46-3, NKp46-4, NKp46-6 or NKp46-9 as set forth in Table B, optionally wherein one, two, three or more amino acids may be substituted by a different amino acid;
- (c) the heavy chain variable region of NKp46-1, NKp46-2, NKp46-3, NKp46-4, NKp46-6 or NKp46-9 as set forth in Table B, optionally wherein one or more of these amino acids may be substituted by a different amino acid; and the respective light chain variable region of NKp46-1, NKp46-2, NKp46-3, NKp46-4, NKp46-6 or NKp46-9 as set forth in Table B, optionally wherein one, two, three or more amino acids may be substituted by a different amino acid;
- (d) the heavy chain CDR 1, 2 and 3 (HCDR1, HCDR2) amino acid sequence of NKp46-1, NKp46-2, NKp46-3, NKp46-4, NKp46-6 or NKp46-9 as shown in Table A, optionally wherein one, two, three or more amino acids in a CDR may be substituted by a different amino acid;
- (e) the light chain CDR 1, 2 and 3 (LCDR1, LCDR2, LCDR3) amino acid sequence of NKp46-1, NKp46-2, NKp46-3, NKp46-4, NKp46-6 or NKp46-9 as shown in Table A, optionally wherein one, two, three or more amino acids in a CDR may be substituted by a different amino acid; or
- (f) the heavy chain CDR 1, 2 and 3 (HCDR1, HCDR2, HCDR3) amino acid sequence of NKp46-1, NKp46-2, NKp46-3, NKp46-4, NKp46-6 or NKp46-9 as shown in Table A, optionally wherein one, two, three or more amino acids in a CDR may be substituted by a different amino acid; and the light chain CDRs 1, 2 and 3 (LCDR1, LCDR2, LCDR3) amino acid sequence of the respective NKp46-1, NKp46-2, NKp46-3, NKp46-4, NKp46-6 or NKp46-9 antibody as shown in Table A, optionally wherein one, two, three or more amino acids in a CDR may be substituted by a different amino acid.

In one embodiment, the aforementioned CDRs are according to Kabat, e.g. as shown in Table A. In one embodiment, the aforementioned CDRs are according to Chothia numbering, e.g. as shown in Table A. In one embodiment, the aforementioned CDRs are according to IMGT numbering, e.g. as shown in Table A.

In another aspect of any of the embodiments herein, any of the CDR1, CDR2 and CDR3 of the heavy and light chains may be characterized by a sequence of at least 4, 5, 6, 7, 8, 9 or 10 contiguous amino acids thereof, and/or as having an amino acid sequence that shares at least 50%, 60%, 70%, 80%, 85%, 90% or 95% sequence identity with the particular CDR or set of CDRs listed in the corresponding SEQ ID NO or Table A.

In another aspect, the invention provides an antibody that competes for NKp46 binding with a monoclonal antibody according to (a) to (f), above.

In another aspect, the invention provides a bispecific antibody comprising an antibody that binds human NKp46 according to (a) to (f), above, or an antibody that competes for binding to NKp46 with any of such antibodies, fused (optionally via intervening amino acid sequences) to a monomeric Fc domain, optionally further fused (optionally via intervening amino acid sequences) to a second antigen binding domain (e.g. a scFv, a V_H domain, a V_L domain, a dAb, a V-NAR domain or a V_HH domain). Optionally the second antigen binding domain will bind a cancer antigen, a viral antigen, a parasitic antigen or a bacterial antigen. The sequences of the CDRs, according to IMGT, Kabat and Chothia definitions systems, are summarized in Table A below. The sequences of the variable chains of the antibodies according to the invention are listed in Table B below. In any embodiment herein, a V_L or V_H sequence can be specified or numbered so as to contain or lack a signal peptide or any part thereof.

TABLE A

mAb	CDR definition	HCDR1		HCDR2		HCDR3	
		SEQ ID	Sequence	SEQ ID	Sequence	SEQ ID	Sequence
NKp46-1	Kabat	15	DYVIN	18	EIYPGSGTNYYNEKFA	21	RGRYGLYAMDY
	Chothia	16	GYTFTDY	19	PGSG	22	GRYGLYAMD
	IMGT	17	GYTFTDYV	20	GYTFTDYVIYPGSGTN	23	ARRGRYGLYA MDY
NKp46-2	Kabat	31	SDYAWN	34	YITYSGSTSYNPSLES	36	GGYYGSSWGV FAY
	Chothia	32	GYSITSDY		YSG	37	GGYGSSWGVFA
	IMGT	33	GYSITSDYA	35	ITYSGST	38	ARGGYGSSW GVFAY
NKp46-3	Kabat	46	EYTMH	49	GISPNI GGTSYNQKFKG	51	RGGSFDY
	Chothia	47	GYTFTEY		PNIG	52	GGSFD
	IMGT	48	GYTFTEYT	50	ISPNI GG T	53	ARRGGSFDY
NKp46-4	Kabat	60	SFTMH	63	YINPSSGYTEYNQKFKD	65	GSSRGFDY
	Chothia	61	GYTFTSF		PSSG	66	SSRGFD
	IMGT	62	GYTFTSFT	64	INPSSGYT	67	VRGSSRGFDY
NKp46-6	Kabat	73	SSWMH	76	HIHPNSGISNYNEKFKG	78	GGRFDD
	Chothia	74	GYTFTSS		PNSG		GRFD
	IMGT	75	GYTFTSSW	77	IHPNSGIS	79	ARGGRFDD
NKp46-9	Kabat	85	SDYAWN	88	YITYSGSTNYNPSLKS	89	CWDYALYAMDC
	Chothia	86	GYSITSDY		YSG	90	WDYALYAMD
	IMGT	87	GYSITSDYA	35	ITYSGST	91	ARCWDYALYA MDC
Bab281	Kabat	97	NYGMN	100	WINTNTGEPTYAEEFKG	102	DYLYYFDY
	Chothia	98	GYTFTNY		TNTG	103	YLYYFD
	IMGT	99	GYTFTNYG	101	INTNTGEP	104	ARDYLYYFDY
	CDR	LCDR1	LCDR2	LCDR3			
mAb	definition	SEQ ID	Sequence	SEQ ID	Sequence	SEQ ID	Sequence

NKp46-1 Kabat	24	RASQDISNYLN	27	YTSRLHS	28	QQGNTRPWT
Chothia	25	SQDISNY		YTS	29	YTSGNTRPW
IMGT	26	QDISNY		YTS	30	YTSQQGNTRP
						WT
NKp46-2 Kabat	39	RVSENIYSYLA	42	NAKTLAE	43	QHHYGTPWT
Chothia	40	SENIYSY		NAK	44	HYGTPW
IMGT	41	ENIYSY		NAK	45	QHHYGTPWT
NKp46-3 Kabat	54	RASQSISDY LH	57	YASQ SIS	58	QNGHSFPLT
Chothia	55	SQSISDY		YAS	59	GHSFPL
IMGT	56	QSISDY		YAS		QNGHSFPLT
NKp46-4 Kabat	68	RASENIYSNLA	70	AATNLAD	71	QHFWGTPRT
Chothia		SENIYSN		AAT	72	FWGTPR
IMGT	69	ENIYSN		AAT		QHFWGTPRT
NKp46-6 Kabat	80	RASQDIGSSLN	81	ATSSLDS	82	LQYASSPWT
Chothia		SQDIGSS		ATS	83	YASSPWT
IMGT		QDIGSS		ATS	84	LQYASSPWT
NKp46-9 Kabat	92	RTSENIYSYLA	93	NAKTLAE	94	QHHYDTPLT
Chothia		SENIYSY		NAK	95	NAKH YDTPL
IMGT		ENIYSY		NAK	96	QHHYDTPLT
Bab281 Kabat	105	KASENVVTVVS	108	GASNRYT	109	GQGYSYPYT
Chothia	106	SENVVTY		GAS	110	GYSYPY
IMGT	107	ENVVTY		GAS	111	GQGYSYPYT

TABLE B

	SEQ ID
Antibody NO	Amino acid sequence
NKp46-1 VH 3	QVQLQQSGPELVKPGASVKMSCKASGYTFTDYVINWGKQRSGQG LEWIGEIYPGSGTNYYNEKFKAKATLTADKSSNIAYMQLSSLTSEDS AVYFCARRGRYGLYAMDYWGQGTSTVTSS
NKp46-1 VL 4	DIQMTQTTSSLSASLGDRVTISCRASQDISNYLNWYQQKPDGTVKL LIYYTSRLHSGVPSRFGSGSGTDYSLTINNLEQEDIATYFCQQGNT RPWTFGGGTKLEIK
NKp46-2 VH 5	EVQLQESGPGLVKPSQSLTCTVTGYSITSDYAWN WIRQFPGNKL EWMGYITYSGSTSYNPSLESRISITRDTSTNQFFLQLNSVTTEDTAT YYCARGGYYGSSWGVFAYWGQGLVTVSA
NKp46-2 VL 6	DIQMTQSPASLSASVGETVITICRVSENIYSYLA WYQQKQKSPQL LVYNAKTLAEGVPSRFGSGSGTQFSLKINS LQPEDFGSYQCQHHY GTPWTFGGGTKLEIK
NKp46-3 VH 7	EVQLQQSGPELVKPGASVKISCKTSGYTFTEYTMHWWKQSHGKSL EWIGGISPNIGGTSYNQKFKGKATLTVDKSSSTAYMELRSLTSEDSA

	VYYCARRGGSFDYWQGTTTLTVSS
NKp46-3 VL 8	DIVMTQSPATLSVTPGDRVSLSCRASQSIDYLHWYQQKSHESPRL LIKYASQSIGIPSRFSGSGSGDFTLSINSVEPEDVGVYYCQNGHS FPLTFGAGTKLELK
NKp46-4 VH 9	QVQLQQSAVELARPGASVKMSCKASGYTFTSFTMHVWKQRPGQG LEWIGYINPSSGYTEYNQKFKDKTTLTADKSSSTAYMQLDSLTSDD SAVYYCVRGSSRGFDYWQGTLVTVSA
NKp46-4 VL 10	DIQMIQSPASLSVSVGETVTITCRASENIYSNLAWFQQKQGKSPQLL VYAATNLADGVPSRFSGSGSGTQYSLKINSLQSEDFGIYYCQHFV GTPRTFGGGTKLEIK
NKp46-6 VH 11	QVQLQQPGSVLVRPGASVKLSCKASGYTFTSSWMHWAKQRPGQ GLEWIGHIHPNSGISNYNEKFKGKATLTVDTSSSTAYVDLSSLTSED SAVYYCARGGRFDDWGAGTTVTVSS
NKp46-6 VL 12	DIQMTQSPSSLSASLGERVSLTCRASQDIGSSSLNLQQEPDGTIKR LIYATSSLDGVPKRFSGSRSGSDYSLTISSESEDFVDYYCLQYAS SPWTFGGGTKLEIK
NKp46-9 VH 13	DVQLQESGPGLVKPSQSLSLTCTVTGYSDYAWNWRQFPGNKL EWMGYITYSGSTNYPNPSLKSRISTRDTSKNQFFLQLNSVTTEDTAT YYCARCWDYALYAMDCWGQGTSTVTVSS
NKp46-9 VL 14	DIQMTQSPASLSASVGETVTITCRTSENIYSYLAWCQQKQGKSPQL LVYNAKTLAEGVPSRFSGSGSGTHFSKINSLQPEDFGIYYCQHYY DTPLTFGAGTKLELK

Also provided, as described in the Examples herein, is a multispecific protein or antibody comprising the amino acid sequences of monomeric bispecific polypeptides which respectively comprise an scFv comprising the heavy and light chain CDR1, 2 and 3 of the respective heavy and light chain variable region listed as SEQ ID NOS: 3-14 of antibodies NKp46-1, NKp46-2, NKp46-3, NKp46-4, NKp46-6 and NKp46-9, a monomeric Fc domain, and an scFv comprising the heavy and light chain CDR1, 2 and 3 of the heavy and light chain variable region of an anti-CD19 antibody, e.g. any of the anti-CD19 antibodies described in the Examples herein.

Once the multispecific protein is produced it can be assessed for biological activity, e.g., antigen binding, ability to elicit target cell lysis and/or specific signaling activities elicited thereby.

In one aspect of any embodiment described herein, the inventive multispecific protein is capable of inducing activation of an NKp46-expressing cell (e.g. an NK cell, a reporter cell) when the protein is incubated in the presence of the NKp46-expressing cell (e.g. purified NK cells) and a target cell that expresses the antigen of interest).

In one aspect of any embodiment described herein, the inventive multispecific protein is capable of inducing an increase of CD137 present on the cell surface of an NKp46- and/or a CD16-expressing cell (e.g. an NK cell, a reporter cell) when the protein is incubated in the presence of the NKp46- and/or a CD16-expressing cell (e.g. purified NK cells), optionally in the absence of target cells.

In one aspect of any embodiment described herein, the inventive multispecific protein is capable of inducing NKp46 signaling in an NKp46-expressing cell (e.g. an NK cell, a reporter cell) when the protein is incubated in the presence of an NKp46-expressing cell (e.g. purified NK cells) and a target cell that expresses the antigen of interest).

Optionally, NK cell activation or signaling is characterized by the increased expression of a cell surface marker of activation, e.g. CD107, CD69,

Sca-1 or Ly-6A/E, KLRG 1, etc.

Activity can be measured for example by bringing target cells and NKp46-expressing cells into contact with one another, in presence of the multispecific polypeptide. In one example, the aggregation of target cells and NK cells is measured. In another example, the multispecific protein may, for example, be assessed for the ability to cause a measurable increase in any property or activity known in the art as associated with NK cell activity, respectively, such as marker of cytotoxicity (CD107) or cytokine production (for example IFN- γ or TNF- α), increases in intracellular free calcium levels, the ability to lyse target cells in a redirected killing assay, etc.

In the presence of target cells (target cells expressing the antigen of interest) and NK cells that express NKp46, the multispecific protein will be capable of causing an increase in a property or activity associated with NK cell activity (e.g. activation of NK cell cytotoxicity, CD107 expression, IFN γ production) in vitro. For example, a multispecific protein according to the invention can be selected based on its ability to increase an NK cell activity by more than about 20%, preferably by least about 30%, at least about 40%, at least about 50%, or more compared to that achieved with the same effector: target cell ratio with the same NK cells and target cells that are not brought into contact with the multispecific protein, as measured by an assay that detects NK cell activity, e.g., an assay which detects the expression of an NK activation marker or which detects NK cell cytotoxicity, e.g., an assay that detects CD107 or CD69 expression, IFN γ production, or a classical in vitro chromium release test of cytotoxicity. Examples of protocols for detecting NK cell activation and cytotoxicity assays are described in the Examples herein, as well as for example, in Pessino et al, *J. Exp. Med.*, 1998, 188 (5): 953-960; Sivori et al, *Eur J Immunol*, 1999, 29:1656-1666; Brando et al, (2005) *J. Leukoc. Biol.* 78:359-371; El-Sherbiny et al, (2007) *Cancer Research* 67(18):8444-9; and Nolte-t Hoen et al, (2007) *Blood* 109:670-673). Optionally, a multispecific protein according to the invention can be selected for or characterized by its ability to have greater ability to induce NK cell activity towards target cells, i.e., lysis of target cells compared to a conventional human IgG1 antibody that binds to the same antigen of interest, as measured by an assay of NK cell activity (e.g. an assay that detects NK cell-mediated lysis of target cells that express the antigen of interest).

As shown herein, a multispecific protein according to the invention which possesses an Fc domain that does not bind CD16, does not, substantially induce NKp46 signaling (and/or NK activation that results therefrom) of NK cells when the protein is not bound to the antigen of interest on target cells (e.g. in the absence of the antigen of interest and/or target cells). Thus, the monovalent NKp46 binding component of the multispecific protein does not itself cause NKp46 signalling. Accordingly, in the case of multispecific proteins possessing an Fc domain that binds CD16, such multispecific protein can be assessed for its ability to elicit NKp46 signaling or NKp46-mediated NK cell activation by testing the effect of this multispecific protein on NKp46 expression, by CD16-negative NK cells. The multispecific protein can optionally be characterized as not substantially causing (or increasing) NKp46 signaling by an NKp46-expressing, CD16-negative cell (e.g. a NKp46⁺CD16⁻ NK cell, a reporter cell) when the multispecific protein is incubated with such NKp46-expressing, CD16-negative cells (e.g., purified NK cells or purified reporter cells) in the absence of target cells.

In one aspect of any embodiment herein, a multispecific protein described herein that binds CD16 can for example be characterized by:

- (a) being capable of inducing NK cells that express CD16 and NKp46 to lyse target cells, when incubated in the presence of the NK cells and target cells; and
- (b) lack of agonist activity at NKp46 when incubated with CD16-negative NK cells, e.g. NKp46-expressing NK cells that do not express CD16, in the absence of target cells. Optionally, the NK cells are purified NK cells.

In one aspect of any embodiment herein, a multispecific protein described herein can for example be characterized by:

- (a) agonist activity at NKp46, when incubated in the presence of NKp46-expressing NK cells and target cells; and
- (b) lack of agonist activity at NKp46 (e.g. when incubated with CD16-negative NK cells, for example CD16-NKp46+NK cells, or when incubated with NK cells and where in the protein comprises (e.g. by modification) an Fc domain that lacks binding to CD16) in the absence of target cells. Optionally, the NK cells are purified NK cells.

Uses of Compounds

In one aspect, provided is the use of any of the compounds defined herein, particularly the inventive multispecific proteins or antibodies and/or

cells which express same for the manufacture of a pharmaceutical preparation for the treatment, prevention or diagnosis of a disease in a mammal in need thereof. Provided also are the use any of the compounds defined above as a medicament or an active component or active substance in a medicament. In a further aspect the invention provides methods for preparing a pharmaceutical composition containing a compound as defined herein, to provide a solid or a liquid formulation for administration orally, topically, or by injection. Such a method or process at least comprises the step of mixing the compound with a pharmaceutically acceptable carrier.

In one aspect, provided is a method to treat, prevent or more generally affect a predefined condition in an individual or to detect a certain condition by using or administering a multispecific protein or antibody described herein, or a (pharmaceutical) composition comprising same.

For example, in one aspect, the invention provides a method of restoring or potentiating the activity of NKp46⁺ NK cells (e.g. NKp46⁺CD16⁺ NK cells) in a patient in need thereof (e.g. a patient having a cancer, or a viral or bacterial infection), comprising the step of administering a multispecific protein described herein to said patient. In one embodiment, the method is directed at increasing the activity of NKp46⁺ lymphocytes (e.g. NKp46⁺CD16⁺ NK cells) in patients having a disease in which increased lymphocyte (e.g. NK cell) activity is beneficial or which is caused or characterized by insufficient NK cell activity, such as a cancer, or a viral or microbial/bacterial infection.

In another aspect, the invention provides a method of restoring or potentiating the activity of NKp46⁺ NK cells (e.g. NKp46⁺CD16⁺ NK cells) in a patient in need thereof (e.g. a patient having a cancer, or a viral, parasite or bacterial infection), comprising the step of contacting cells derived from the patient, e.g., immune cells and optionally target cells expressing an antigen of interest with a multispecific protein according to the invention and reinfusing the multispecific protein treated cells into the patient. In one embodiment, this method is directed at increasing the activity of NKp46⁺ lymphocytes (e.g. NKp46⁺CD16⁺ NK cells) in patients having a disease in which increased lymphocyte (e.g. NK cell) activity is beneficial or which is caused or characterized by insufficient NK cell activity, such as a cancer, or a viral or microbial, e.g., bacterial or parasite infection.

In another embodiment the subject multispecific proteins may be used or administered in combination with immune cells, particularly NK cells, derived from a patient who is to be treated or from a different donor, and these NK cells administered to a patient in need thereof such as a patient having a disease in which increased lymphocyte (e.g. NK cell) activity is beneficial or which is caused or characterized by insufficient NK cell activity, such as a cancer, or a viral or microbial, e.g., bacterial or parasite infection. As NK cells (unlike CAR-T cells) do not express TCRs, these NK cells, even those derived from different donors will not induce a GVHD reaction (see e.g., Glienke et al., "Advantages and applications of CAR-expressing natural killer cells", *Front. Pharmacol.* 6, Art. 21:1-6 (2015); Hermanson and Kaufman, *Front. Immunol.* 6, Art. 195:1-6 (2015))

In one embodiment, the multispecific protein disclosed herein that mediates NK cell activation and/or target cell lysis via multiple activating receptors of effector cells, including NKp46, CD16 and CD137, can be used advantageously for treatment of individuals whose effector cells (e.g. NKp46⁺CD16⁺ NK cells) cells are hypoactive, exhausted or suppressed, for example a patient who has a significant population of effector cells characterized by the expression and/or upregulation of one or multiple inhibitory receptors (e.g. TIM-3, PD1, CD96, TIGIT, etc.).

The multispecific polypeptides described herein can be used to prevent or treat disorders that can be treated with antibodies, such as cancers, solid and non-solid tumors, hematological malignancies, infections such as viral infections, and inflammatory or autoimmune disorders.

In one embodiment, the antigen of interest (the non-NKp46 antigen) is an antigen expressed on the surface of a malignant cell of a type of cancer selected from the group consisting of: carcinoma, including that of the bladder, head and neck, breast, colon, kidney, liver, lung, ovary, prostate, pancreas, stomach, cervix, thyroid and skin, including squamous cell carcinoma; hematopoietic tumors of lymphoid lineage, including leukemia, acute lymphocytic leukemia, acute lymphoblastic leukemia, B-cell lymphoma, T-cell lymphoma, Hodgkin's lymphoma, non-Hodgkin's lymphoma, hairy cell lymphoma and Burkett's lymphoma; hematopoietic tumors of myeloid lineage, including acute and chronic myelogenous leukemias and promyelocytic leukemia; tumors of mesenchymal origin, including fibrosarcoma and rhabdomyosarcoma; other tumors, including neuroblastoma and glioma; tumors of the central and peripheral nervous system, including astrocytoma, neuroblastoma, glioma, and schwannomas; tumors of mesenchymal origin, including fibrosarcoma, rhabdomyosarcoma, and osteosarcoma; and other tumors, including melanoma, xeroderma pigmentosum, keratoacanthoma, seminoma, thyroid follicular cancer and teratocarcinoma, hematopoietic tumors of lymphoid lineage, for example T-cell and B-cell tumors, including but not limited to T-cell disorders such as T-prolymphocytic leukemia (T-PLL),

including of the small cell and cerebriform cell type; large granular lymphocyte leukemia (LGL) preferably of the T-cell type; Sézary syndrome (SS); Adult T-cell leukemia lymphoma (ATLL); a/d T-NHL hepatosplenic lymphoma; peripheral/post-thymic T cell lymphoma (pleomorphic and immunoblastic subtypes); angio immunoblastic T-cell lymphoma; angiocentric (nasal) T-cell lymphoma; anaplastic (Ki 1+) large cell lymphoma; intestinal T-cell lymphoma; T-lymphoblastic; and lymphoma/leukemia (T-Lbly/T-ALL). In one embodiment, the inventive multispecific polypeptides described herein can be used to prevent or treat a cancer selected from the group consisting of: carcinoma, including that of the bladder, head and neck, breast, colon, kidney, liver, lung, ovary, prostate, pancreas, stomach, cervix, thyroid and skin, including squamous cell carcinoma; hematopoietic tumors of lymphoid lineage, including leukemia, acute lymphocytic leukemia, acute lymphoblastic leukemia, B-cell lymphoma, T-cell lymphoma, Hodgkin's lymphoma, non-Hodgkin's lymphoma, hairy cell lymphoma and Burkett's lymphoma; hematopoietic tumors of myeloid lineage, including acute and chronic myelogenous leukemias and promyelocytic leukemia; tumors of mesenchymal origin, including fibrosarcoma and rhabdomyosarcoma; other tumors, including neuroblastoma and glioma; tumors of the central and peripheral nervous system, including astrocytoma, neuroblastoma, glioma, and schwannomas; tumors of mesenchymal origin, including fibrosarcoma, rhabdomyosarcoma, and osteosarcoma; and other tumors, including melanoma, xeroderma pigmentosum, keratoacanthoma, seminoma, thyroid follicular cancer and teratocarcinoma. Other exemplary disorders that can be treated according to the invention include hematopoietic tumors of lymphoid lineage, for example T-cell and B-cell tumors, including but not limited to T-cell disorders such as T-prolymphocytic leukemia (T-PLL), including of the small cell and cerebriform cell type; large granular lymphocyte leukemia (LGL) preferably of the T-cell type; Sezary syndrome (SS); Adult T-cell leukemia lymphoma (ATLL); a/d T-NHL hepatosplenic lymphoma; peripheral/post-thymic T cell lymphoma (pleomorphic and immunoblastic subtypes); angio immunoblastic T-cell lymphoma; angiocentric (nasal) T-cell lymphoma; anaplastic (Ki 1+) large cell lymphoma; intestinal T-cell lymphoma; T-lymphoblastic; and lymphoma/leukaemia (T-Lbly/T-ALL). In one example, the tumor antigen is an antigen expressed on the surface of a lymphoma cell or a leukemia cell, and the multispecific protein is administered to, and/or used for the treatment of, an individual having a lymphoma or a leukemia. Optionally, the tumor antigen is selected from CD19, CD20, CD22, CD30 or CD33.

In one aspect, the methods of treatment comprise administering to an individual a multispecific protein described herein in a therapeutically effective amount, e.g., for the treatment of a disease as disclosed herein, for example any of the cancers identified above. A therapeutically effective amount may be any amount that has a therapeutic effect in a patient having a disease or disorder (or promotes, enhances, and/or induces such an effect in at least a substantial proportion of patients with the disease or disorder and substantially similar characteristics as the patient).

In one embodiment, a multispecific protein according to the invention is used to treat a cancer that is responsive to CD137 activation, e.g. a solid tumor or a hematological cancer, including but not limited to breast cancer, sarcoma, glioma, colon carcinoma, myeloma, mastocytoma, melanoma, renal carcinoma, and ovarian cancer. In one embodiment, the multispecific protein according to the invention is used to treat a CD137L-expressing cancer. As shown herein, the strong efficacy in inducing tumor cell lysis of the multispecific protein is hypothesized to in part mediated by the upregulation of CD137 (4-1BB) on the surface of NK cells. The co-activating CD137 protein can bind and recognize CD137 ligand (CD137L, 4-1BBL) on tumor cells, resulting in enhanced NK cell activation and cytotoxicity of CD137L-expressing cells. CD137L has been found to be expressed on a variety of tumors, and is more commonly expressed by malignant tumors, especially in moderate or low-differentiated tumors (see, e.g., Vinay et al., (2012) *Mol. Cancer Ther.* 11(5):1062-1070). CD137L-expressing cancers include, for example, lung squamous cell carcinoma, nasal cavity squamous cell carcinoma, esophageal squamous cell carcinoma, cervical squamous cell carcinoma, colonic adenocarcinoma, rectal adenocarcinoma, gallbladder adenocarcinoma, pancreatic adenocarcinoma and breast adenocarcinoma.

The multispecific protein according to the invention may be used with or without a prior step of detecting the expression of the antigen of interest on target cells in a biological sample obtained from an individual (e.g. a biological sample comprising cancer cells, cancer tissue or cancer-adjacent tissue). In another embodiment, the disclosure provides a method for the treatment or prevention of a cancer in an individual in need thereof, the method comprising:

- a) detecting cells (e.g. tumor cells) in a sample from the individual that express an antigen of interest, and
- b) upon a determination that cells which express an antigen of interest are comprised in the sample, optionally at a level that is increased compared to a reference level (e.g. corresponding to a healthy individual or an individual not deriving substantial benefit from a protein described

herein), administering to the individual a multispecific protein (e.g. a multispecific protein according to the invention) that binds to an antigen of interest, to NKp46 (e.g., monovalently), and to CD16 (e.g., via its Fc domain). Optionally, the antigen of interest is a cancer antigen (e.g. a cancer antigen disclosed herein), optionally a cancer antigen known to be capable of undergoing intracellular internalization when contacted with a full length human IgG1 antibody that binds specifically thereto.

In one embodiment, the invention provides a method for the treatment or prevention of a cancer in an individual in need thereof, the method comprising:

- a) detecting cells (e.g. tumor cells) in a sample from an individual (or within the tumor and/or within adjacent tissue) that express CD137L (CD137 ligand), and
- b) upon a determination that cells that express CD137L are comprised in the sample (or within the tumor and/or within adjacent tissue), optionally at a level that is increased compared to a reference level (e.g. corresponding to a healthy individual or an individual not deriving substantial benefit from a protein described herein), administering to the individual a multispecific protein (e.g. a protein of the disclosure) that binds to a cancer antigen, to NKp46 (e.g., monovalently), and to CD16. Optionally, the cancer antigen is a cancer antigen disclosed herein), optionally a cancer antigen known to be capable of undergoing intracellular internalization when contacted with a full length human IgG1 antibody that binds specifically thereto.

In one embodiment, the disclosure provides a method for the treatment or prevention of a disease (e.g. a cancer) in an individual in need thereof, the method comprising:

- a) detecting cell surface expression of one or a plurality inhibitory receptors on immune effector cells (e.g. NK cells, T cells) in a sample from the individual (e.g. in circulation or in the tumor environment), and
- b) upon a determination of cell surface expression of one or a plurality inhibitory receptors on immune effector cells, optionally at a level that is increased compared to a reference level (e.g. corresponding to a healthy individual, an individual not suffering from immune exhaustion or suppression, or an individual not deriving substantial benefit from a protein described herein), administering to the individual a multispecific protein (e.g. a multispecific protein according to the invention) that binds to an antigen of interest (e.g. a cancer antigen), to NKp46 (e.g., monovalently), and to CD16. Optionally, the cancer antigen is a cancer antigen disclosed herein), optionally a cancer antigen known to be capable of undergoing intracellular internalization when contacted with a full length human IgG1 antibody that binds specifically thereto.

In one embodiment, a multispecific protein according to the invention may be used as a monotherapy (without other therapeutic agents), or in combined treatments with one or more other therapeutic agents, including agents normally utilized for the particular therapeutic purpose for which the antibody is being administered. The additional therapeutic agent or agents will normally be administered in amounts and treatment regimens typically used for that agent in a monotherapy for the particular disease or condition being treated. Such therapeutic agents when used in the treatment of cancer, include, but are not limited to anti-cancer agents and chemotherapeutic agents; in the treatment of infectious disease, include, but are not limited to anti-viral agents and antibiotics. Such other therapeutic agents may further include other immunomodulatory polypeptides such as Ig-fusion proteins, antibodies, cytokines and the like. In some embodiments the administration of the multispecific protein according to the invention and the other therapeutic agent may elicit an additive or synergistic effect on immunity and/or on therapeutic efficacy.

The multispecific proteins can also be included in kits. The kits may optionally further contain any number of polypeptides and/or other compounds, e.g., 1, 2, 3, 4, or any other number of multispecific proteins according to the invention and/or other compounds. It will be appreciated that this description of the contents of the kits is not limiting in any way. For example, the kit may contain other types of therapeutic compounds. Optionally, the kits also include instructions for using the polypeptides, e.g., detailing the herein-described methods such as in the detection or treatment of specific disease conditions.

The invention also provides pharmaceutical compositions comprising the subject multispecific proteins and optionally other compounds as defined above. A multispecific protein and optionally another compound may be administered in purified form together with a pharmaceutical carrier as a pharmaceutical composition. The form depends on the intended mode of administration and therapeutic or diagnostic application.

The pharmaceutical carrier can be any compatible, nontoxic substance suitable to deliver the compounds to the patient. Pharmaceutically acceptable carriers are well known in the art and include, for example, aqueous solutions such as (sterile) water or physiologically buffered saline or other solvents or vehicles such as glycols, glycerol, oils such as olive oil or injectable organic esters, alcohol, fats, waxes, and inert solids. A pharmaceutically acceptable carrier may further contain physiologically acceptable compounds that act for example to stabilize or to increase the absorption of the compounds. Such physiologically acceptable compounds include, for example, carbohydrates, such as glucose, sucrose or dextrans, antioxidants, such as ascorbic acid or glutathione, chelating agents, low molecular weight proteins or other stabilizers or excipients. One skilled in the art would know that the choice of a pharmaceutically acceptable carrier, including a physiologically acceptable compound, depends, for example, on the route of administration of the composition. Pharmaceutically acceptable adjuvants, buffering agents, dispersing agents, and the like, may also be incorporated into the pharmaceutical compositions. Non-limiting examples of such adjuvants include by way of example inorganic and organic adjuvants such as alum, aluminum phosphate and aluminum hydroxide, squalene, liposomes, lipopolysaccharides, double stranded (ds) RNAs, single stranded (s-s) DNAs, and TLR agonists such as unmethylated CpG's.

Multispecific proteins according to the invention can be administered parenterally. Preparations of the compounds for parenteral administration must be sterile. Sterilization is readily accomplished by filtration through sterile filtration membranes, optionally prior to or following lyophilization and reconstitution. The parenteral route for administration of compounds is in accord with known methods, e.g. injection or infusion by intravenous, intraperitoneal, intramuscular, intraarterial, or intralesional routes. The compounds may be administered continuously by infusion or by bolus injection. A typical composition for intravenous infusion could be made up to contain 100 to 500 ml of sterile 0.9% NaCl or 5% glucose optionally supplemented with a 20% albumin solution and 1 mg to 10 g of the compound, depending on the particular type of compound and its required dosing regimen. Methods for preparing parenterally administrable compositions are well known in the art.

EXAMPLES

Example 1

Generation of Anti-huNKp46 Antibodies

Balb/c mice were immunized with a recombinant human NKp46 extracellular domain recombinant-Fc protein comprising the extracellular domain of the protein of SEQ ID NO: 1. Mice received one primo-immunization with an emulsion of 50 µg NKp46 protein and Complete Freund Adjuvant, intraperitoneally, a 2nd immunization with an emulsion of 50 µg NKp46 protein and Incomplete Freund Adjuvant, intraperitoneally, and finally a boost with 10 µg NKp46 protein, intravenously. Immune spleen cells were fused 3 days after the boost with X63.Ag8.653 immortalized B cells, and cultured in the presence of irradiated spleen cells.

Primary screen: Supernatant (SN) of growing clones were tested in a primary screen by flow cytometry using a cell line expressing the human NKp46 construct at the cell surface. Briefly, for FACS screening, the presence of reactive antibodies in supernatants was revealed by Goat anti-mouse polyclonal antibody (pAb) labeled with PE.

A panel of antibodies that bound NKp46 was selected, produced and their variable regions sequenced and these antibodies and derivatives thereof further evaluated for their activity in the context of a bispecific molecule.

Example 2

Identification of a Bispecific Antibody Format that Binds FcRn but not FcγR for Targeting Effector Cell Receptors

Experiments were conducted with the objective being the development of a new bispecific protein format that places an Fc domain on a polypeptide together with an anti-NKp46 binding domain and an anti-target antigen binding domain. Such bispecific proteins should bind to NKp46 monovalently via its anti-NKp46 binding domain. The monomeric Fc domain should retain at least partial binding to the human neonatal Fc receptor (FcRn), yet not substantially bind human CD16 and/or other human Fcγ receptors. Consequently, such bispecific proteins should not induce Fcγ-mediated (e.g. CD16-mediated) target cell lysis.

Example 2-1 Construction and Binding Analysis of Anti-CD19-IgG1-Fcmono-Anti-CD3

Since no anti-NKp46 bispecific antibody has been produced that could indicate whether such a protein could be functional, CD3 was used as a model antigen in place of NKp46 in order to investigate the possible functionality of a new monovalent bispecific protein format prior to targeting NK cells via NKp46.

A bispecific Fc-based on a scFv specific for tumor antigen CD19 (anti-CD19 scFv) and a scFv specific for activating receptor CD3 on a T cell

(anti-CD3 scFv) was used to assess FcRn binding and CD19-binding functions of a new monomeric bispecific polypeptide format. The domain arrangement of the final polypeptide is referred to as the "F1" format (the star in the CH2 domain indicates an optional N297S mutation, not included in the polypeptide tested here). (See FIG. 2)

A bispecific monomeric Fc-containing polypeptide was constructed based on a scFv specific for the tumor antigen CD19 (anti-CD19 scFv) and a scFv specific for an activating receptor CD3 on a T cell (anti-CD3 scFv). The CH3 domain incorporated the mutations (EU numbering) L351K, T366S, P395V, F405R, T407A and K409Y. The polypeptide has domains arranged as follows: anti-CD19-CH2-CH3-anti-CD3. A DNA sequence coding for a CH3/VH linker peptide having the amino acid sequence STGS was also designed in order to insert a specific Sall restriction site at the CH3-VH junction.

This CH3 domain incorporated the mutations (EU numbering) L351K, T366S, P395V, F405R, T407A and K409Y. The selected CH2 domain was a wild-type CH2. DNA and amino acid sequences for the monomeric CH2-CH3 Fc portion and the anti-CD19 are shown below.

The light chain and heavy chain DNA and amino acid sequences corresponding to the anti-CD19 scFv were as follows:

Sequence	SEQ ID NO
Anti-CD19-V _k DNA	113
Anti-CD19-V _k amino acid	114
Anti-CD19-V _H DNA	115
Anti-CD19-V _H amino acid	116

The DNA sequences for the monomeric CH2-CH3 Fc portion and final bispecific IgG1-Fcmono polypeptide (the last K was removed in that construct) is shown in SEQ ID NO: 117. The amino acid sequence encoded thereby is shown in SEQ ID NO: 2. The Anti-CD19-F1-Anti-CD3 complete sequence (mature protein) is shown in SEQ ID NO: 118.

Cloning and the Production of the Recombinant Proteins

Coding sequences were generated by direct synthesis and/or by PCR. PCR was performed using the PrimeSTAR MAX DNA polymerase (Takara, #R045A) and PCR products were purified from 1% agarose gel using the NucleoSpin gel and PCR clean-up kit (Macherey-Nagel, #740609.250). Once purified the PCR products were quantified prior to the In-Fusion ligation reaction which was performed as described in the manufacturer's protocol (ClonTech, #ST0345). The plasmids were obtained after a miniprep preparation run on an EVO200 (Tecan) using the Nucleospin 96 plasmid kit (Macherey-Nagel, #740625.4). Plasmids were then sequenced for sequence confirmation before to transfecting the CHO cell line.

CHO cells were grown in the CD-CHO medium (Invitrogen) complemented with phenol red and 6 mM GlutaMax. The day before the transfection, cells were counted and seeded at 175,000 cells/ml. For the transfection, cells (200,000 cells/transfection) were prepared as described in the AMAXA SF cell line kit (AMAXA, #V4XC-2032) and nucleofected using the DS137 protocol with the Nucleofector 4D device. All the transfections were performed using 300 ng of verified plasmids. After transfection, cells were seeded into 24 well plates in pre-warmed culture medium. After 24 hours, hygromycin B was added in the culture medium (200 µg/ml). Protein expression was monitored after one week in culture. Cells expressing the proteins were then sub-cloned to obtain the best producers. Sub-cloning was performed using 96 flat-bottom well plates in which the cells are seeded at one cell per well into 200 µl of culture medium complemented with 200 µg/ml of hygromycin B. Cells were left for three weeks before testing the clone's productivity.

Recombinant proteins which contain an IgG1-Fc fragment were purified using Protein-A beads (rProteinA Sepharose fast flow, GE Healthcare). Briefly, cell culture supernatants were concentrated, clarified by centrifugation and injected onto Protein-A columns to capture the recombinant Fc containing proteins. Proteins were eluted at acidic pH (citric acid 0.1M pH 3), and the eluate immediately neutralized using TRIS-HCL pH 8.5 and dialyzed against 1xPBS. Recombinant scFvs which contain a "six his" tag were purified by affinity chromatography using Cobalt resin. Other recombinant scFvs were purified by size exclusion chromatography (SEC).

Example 2-2: Binding Analysis of Anti-CD19-IgG1-Fcmono-Anti-CD3 to B221, JURKAT, HUT78 and CHO Cell Lines

Cells were harvested and stained with the cell supernatant of the anti-CD19-F1-anti-CD3 producing cells during 1 h at 4°C. After two washes in staining buffer (PBS1x/BSA 0.2%/EDTA 2 mM), cells were stained for 30 min at 4°C. with goat anti-human (F c)-PE antibody (IM0550 Beckman Coulter—1/200). After two washes, stainings were conducted on a BD FACS Canto II and analyzed using the FlowJo software.

CD3 and CD19 expression were also controlled by flow cytometry: Cells were harvested and stained in PBS1x/BSA 0.2%/EDTA 2 mM buffer during 30 min at 4°C. using 5 µl of the anti-CD3-APC and 5 µl of the anti-CD19-FITC antibodies. After two washes, stainings were conducted on a BD FACS Canto II and analyzed using the FlowJo software.

The results of these experiments revealed that the Anti-CD19-F1-Anti-CD3 protein binds to CD3 cell lines (HUT78 and JURKAT cell lines) and to the CD19 cell line (B221 cell line) but not to the CHO cell line which was used as a negative control.

Example 2-3

T- and B-Cell Aggregation by Purified Anti-CD19-F1-Anti-CD3

Purified Anti-CD19-F1-Anti-CD3 was tested in a T/B cell aggregation assay to evaluate whether the antibody promotes the aggregation of CD19 and CD3 expressing cells.

The results of this assay are shown in FIG. 1. The top panel shows that Anti-CD19-F1-Anti-CD3 does not cause aggregation in the presence of B221 (CD19) or JURKAT (CD3) cell lines, but it does cause aggregation of cells when both B221 and JURKAT cells are co-incubated, indicating that the bispecific antibody is functional. The lower panel shows the results of the control experiment conducted without antibody.

Example 2-4

Binding of Bispecific Monomeric Fc Polypeptide to FcRn

Affinity Study by Surface Plasmon Resonance (SPR)

Biacore T100 General Procedure and Reagents

SPR measurements were performed on a Biacore T100 apparatus (Biacore GE Healthcare) at 25°C. In all Biacore experiments Acetate Buffer (50 mM Acetate pH5.6, 150 mM NaCl, 0.1% surfactant p20) and HBS-EP+(Biacore GE Healthcare) were used as the running buffer and regeneration buffer respectively. Sensorgrams were analyzed with Biacore T100 Evaluation software. Recombinant mouse FcRn was purchased from R&D Systems.

Immobilization of FcRn

Recombinant FcRn proteins were immobilized covalently to carboxyl groups in the dextran layer on a Sensor Chip CM5. The chip surface was activated with EDC/NHS (N-ethyl-N'-(3-dimethylaminopropyl) carbodiimidehydrochloride and N-hydroxysuccinimide (Biacore GE Healthcare)). FcRn proteins were diluted to 10 µg/ml in coupling buffer (10 mM acetate, pH 5.6) and injected until the appropriate immobilization level was reached (i.e. 2500 RU). Deactivation of the remaining activated groups was performed using 100 mM ethanolamine pH 8 (Biacore GE Healthcare).

Affinity Study

Monovalent affinity study was conducted following the Single Cycle Kinetic (SCK) protocol. Five serial dilutions of soluble analytes (antibodies and bi-specific molecules) ranging from 41.5 to 660 nM were injected over the FcRn (without regeneration) and allowed to dissociate for 10 min before regeneration. For each analyte, the entire sensorgram was fitted using the 1:1 SCK binding model.

Results

Anti-CD19-F1-Anti-CD3 having its CH2-CH3 domains placed between two antigen binding domains, particularly two scFvs, was evaluated to assess whether such bispecific monomeric Fc protein could retain binding to FcRn and possess an improved in vivo half-life compared to conventional bispecific antibodies. The results of these experiments showed that FcRn binding was retained, the model suggesting a 1:1 ratio (1 FcRn for each monomeric Fc) instead of a 2:1 ratio (2 FcRn for each antibody) for a regular or wild-type IgG.

The binding affinity of this multispecific protein was evaluated using SPR, and was compared to a chimeric full length antibody containing intact human IgG1 constant regions. The monomeric Fc retained significant monomeric binding to FcRn (monomeric Fc: affinity of $K_D=194$ nM; full length antibody with bivalent binding: avidity of $K_D=15.4$ nM).

Example 3

Construction of Anti-CD19xAnti-NKp46 Bispecific Monomeric Fc Domain Polypeptides

It was unknown what activating receptors on NK cells would contribute to the lysis of target cells, and moreover since anti-NKp46 antibodies may block NKp46, it was further unknown whether cytotoxicity could be mediated by NKp46. We therefore investigated whether the bispecific protein format could induce NKp46 triggering, and whether it would induce NKp46 agonism in the absence of target cells, which could lead to inappropriate NK activation distant from the target and/or decreased overall activity toward target cells.

A new bispecific protein format was developed as a single chain protein which binds to FcRn but not FcγR. Additionally, multimeric proteins that comprise two or three polypeptide chains, wherein the Fc domain remains monomeric, were developed that are compatible for use with antibody variable regions that do not maintain binding to their target when converted to scFv format. The latter formats can be used conveniently for antibody screening; by incorporating at least one binding region as a F(ab) structure, any anti-target (e.g. anti-tumor) antibody variable region can be directly expressed in a bispecific construct as the F(ab) format within the bispecific protein and tested, irrespective of whether the antibody would retain binding as an scFv, thereby simplifying screening and enhancing the number of antibodies available. These formats in which the Fc domain remains monomeric have the advantage of maintaining maximum conformational flexibility and as shown infra may permit optimal binding to NKp46 or target antigens.

Different constructs were made for use in the preparation of bispecific antibodies using the variable domains from the scFv specific for tumor antigen CD19 described in Example 2-1, and different variable regions from antibodies specific for the NKp46 receptor identified in Example 1. A construct was also made using as the anti-NKp46 the variable regions from a commercially available antibody Bab281 (mIgG1, available commercially from Beckman Coulter, Inc. (Brea, Calif., USA) (see also Pessino et al, *J. Exp. Med.*, 1998, 188 (5): 953-960 and Sivori et al, *Eur J Immunol*, 1999, 29:1656-1666) specific for the NKp46 receptor.

In order for the Fc domain to remain monomeric in single chain polypeptides or in multimers in which only one chain had an Fc domain, CH3-CH3 dimerization was prevented through two different strategies: (1) through the use of CH3 domain incorporating specific mutations (EU numbering), i.e., L351K, T366S, P395V, F405R, T407A and K409Y; or (2) through the use of a tandem CH3 domain in which the tandem CH3 domains are separated by a flexible linker associated with one another, which prevents interchain CH3-CH3 dimerization. The DNA and amino acid sequences for the monomeric CH2-CH3 Fc portion containing the above-identified point mutations were the same as in Example 2-1. The DNA and amino acid sequences for the monomeric CH2-CH3-linker-CH3 Fc portion with tandem CH3 domains are shown in FIGS. 2A-2D.

The light chain and heavy chain DNA and amino acid sequences for the anti-CD19 scFv were also the same as in Example 2-1. Proteins were cloned, produced and purified as in Example 2-1. Shown below are the light chain and heavy chain DNA and amino acid sequences for different anti-NKp46 scFvs.

TABLE 1

Amino acid sequences of different anti-NKp46 scFvs

scFv

anti-NKp46

scFv sequence (VHVK)/-stop

NKp46-1

STGSQVQLQQSGPELVKPGASVKMSCKASGYTFTDYVINWGKQRSGQ
GLEWIGEIYPGSGTNYNEKFKAKATLTADKSSNIAYMQLSSLTSEDSAV
YFCARRGRYGLYAMDYWGQGTSTVTVSSVEGGSGGSGGSGGSGGVDD
IQMTQTTSSLSASLGDRVTISCRASQDISNYLNWYQKPDGTVKLLIYYT
SRLHSGVPSRFSGSGSGTDYSLTINNLEQEDIATYFCQQGNTRPWTFG
GGTKLEIK-(SEQ ID NO: 119)

NKp46-2

STGSEVQLQESGPGLVKPSQSLTCTVTGYSITSDYAWNWRQFPGNK
LEWMGYITYSGSTSYNPSLESISRITRDTSTNQFFLQLNSVTEDTATYY
CARGGYGYSSWGVFAYWGQGLTVTVSAVEGGSGGSGGSGGSGGVDD
DIQMTQSPASLSASVGETVTITCRVSENIYSYLAWYQKQKGKSPQLLVY

	NAKTLAEGVPSRFGSGSGTQFSLKINSLQPEDFGSYQCQHHYGTPTWTFGGGTKLEIK-(SEQ ID NO: 120)
NKp46-3	STGSEVQLQQSGPELVKPGASVKISCKTSGYTFTEYTMHWVKQSHGKSLEWIGGISPNIGGTSYNQKFKGKATLTVDKSSSTAYMELRSLTSEDSAVYYCARRGGSFDYWGGTTLTVSSVEGGSGGSGGSGGSGGVDDIVMTQSPATLSVTPGDRVSLSCRASQSIDYLHWYQQKSHEsprLLIKYASQSIGPSRFGSGSGSDFTLINSVEPEDVGYYCQNGHSFPLTFGAGTKLELK-(SEQ ID NO: 121)
NKp46-4	STGSQVQLQQSAVELARPGASVKMSCKASGYTFTSFTMHWVKQRPGQGLEWIGYINPSSGYTEYNQKFKDKTTLTADKSSSTAYMQLDSLTSDDSAVYYCVRGSSRGFDYWGGTLTVSAVEGGSGGSGGSGGSGGVDDIQMIQSPASLSVSVGETVTITCRASENIYSNLAWFQKQKQKSPQLLVYAATNLADGVPSRFGSGSGTQYSLKINSLQSEDFGIYQCQHFwGTPRTFGGGTKLEIK-(SEQ ID NO: 122)
NKp46-6	STGSQVQLQQPGSVLVRPGASVKLSCKASGYTFTSSWMHWAKQRPGQGLEWIGHIHPNSGISNYNEKFKGKATLTVDTSSSTAYVDLSSLTSEDSAVYYCARGGRFDDWGAGTTVTVSSVEGGSGGSGGSGGSGGVDDIVMTQSPATLSVTPGDRVSLSCRASQSIDYLHWYQQKSHEsprLLIKYASQSIGPSRFGSGSGSDFTLINSVEPEDVGYYCQNGHSFLMYTFGGGTKLEIK-(SEQ ID NO: 123)
NKp46-9	STGSDVQLQESGPGLVKPSQSLSLTCTVTGYSITSDYAWNwIRQFPGNKLEWMGYITYSGSTNYPNPSLKSRIITRDTSKNQFFLQLNSVTTEDTATYYCARCWDYALYAMDCWGQGTSTVTVSSVEGGSGGSGGSGGSGGVDDIQMTQSPASLSASVGETVTITCRTSENIYSYLAWCQKQKQKSPQLLVYNAKTLAEGVPSRFGSGSGTHFSLKINSLQPEDFGIYQCQHHYDTPPLTFGAGTKLELK-(SEQ ID NO: 124)
Bab281	STGSQIQLVQSGPELQKPGETVKISCKASGYTFTNYGMNWVKQAPGKGLKWMGWINTNTGEPTYAEFEKGRFAFSLETSASTAYLQINNlKNEDTATYFCARDYLYYFDYWGGTTLTVSSVEGGSGGSGGSGGSGGVNDIVMTQSPKSMMSVGERVTLTCKASENVVTYVSWYQKPEQSPKLLIYGASNRYTGVPDRFTGSGSATDFTLTISVQAEDLADYHCGQGYSYPYTFGGGTKLEIK-(SEQ ID NO: 125)

TABLE 2A

DNA sequences corresponding to different anti-NKp46 scFvs

scFv anti-NKp46 scFV sequences

NKp46-1	SEQ ID NO: 126
NKp46-2	SEQ ID NO: 127
NKp46-3	SEQ ID NO: 128
NKp46-4	SEQ ID NO: 129

NKp46-6	SEQ ID NO: 130
NKp46-9	SEQ ID NO: 131
Bab281	SEQ ID NO: 132

Format 1 (F1) (Anti-CD19-IgG1-Fcmono-Anti-NKp46 (scFv))

The domain structure of Format 1 (F1) is shown in FIG. 2A. A bispecific Fc-containing polypeptide was constructed based on a scFv specific for the tumor antigen CD19 (anti-CD19 scFv) and an scFv specific for the NKp46 receptor. The polypeptide is a single chain polypeptide having domains arranged (N- to C-termini) as follows: $(V_K-V_H)^{\text{anti-CD19}}\text{-CH2-CH3-(V}_H\text{-V}_K)^{\text{anti-NKp46}}$

A DNA sequence coding for a CH3/VH linker peptide having the amino acid sequence STGS was designed in order to insert a specific Sall restriction site at the CH3-V_H junction. The domain arrangement of the final polypeptide in shown in FIG. 2 (the star in the CH2 domain indicates an optional N297S mutation), where the anti-CD3 scFv is replaced by an anti-NKp46 scFv. The (V_K-V_H) units include a linker between the V_H and V_K domains. Proteins were cloned, produced and purified as in Example 2-1. The amino acid sequences of the bispecific polypeptides (complete sequence (mature protein)) are shown in the corresponding SEQ ID NOS listed in the Table 2B below.

TABLE 2B

Sequence	SEQ ID NO
CD19-F1-NKp46-1	133
CD19-F1-NKp46-2	134
CD19-F1-NKp46-3	135
CD19-F1-NKp46-4	136
CD19-F1-NKp46-6	137
CD19-F1-NKp46-9	138
CD19-F1-Bab281	139

Format 2 (F2): CD19-F2-NKp46-3

The domain structure of F2 polypeptides is shown in FIG. 2A. The DNA and amino acid sequences for the monomeric CH2-CH3 Fc portion were as in Example 2-1 and it similarly contains CH3 domain mutations (the mutations (EU numbering) L351K, T366S, P395V, F405R, T407A and K409Y. The heterodimer is made up of:

(1) a first (central) polypeptide chain having domains arranged as follows (N- to C-termini):

$(V_K-V_H)^{\text{anti-CD19}}\text{-CH2-CH3-V}_H^{\text{anti-NKp46}}\text{-CH1}$

and

(2) a second polypeptide chain having domains arranged as follows (N- to C-termini): $V_K^{\text{anti-NKp46}}\text{-CK}$.

The (V_K-V_H) unit was made up of a V_H domain, a linker and a V_K unit (i.e. an scFv). As with other formats of the inventive bispecific polypeptides, the DNA sequence coded for a CH3/VH linker peptide having the amino acid sequence STGS designed in order to insert a specific Sall restriction site at the CH3-VH junction. Proteins were cloned, produced and purified as in Example 2-1. The amino acid sequences for the first and second chains of the F2 protein are shown in SEQ ID NO: 140 and 141.

Format 3 (F3): CD19-F3-NKp46-3

The domain structure of F3 polypeptides is shown in FIG. 2A. The DNA and amino acid sequences for the CH2-CH3 Fc portion comprised a tandem CH3 domain in which the two CH3 domains on the same polypeptide chain associated with one another, thereby preventing dimerization between different bispecific proteins.

The single chain polypeptide has domains arranged (N- to C-termini) as follows: (V_K-V_H)^{anti-CD19}-CH2-CH3-CH3-(V_H-V_K)^{anti-NKp46}

The (V_K-V_H) units were made up of a V_H domain, a linker and a V_K unit (scFv). Proteins were cloned, produced and purified as in Example 2-1. Bispecific protein was purified from cell culture supernatant by affinity chromatography using prot-A beads and analyzed and purified by SEC. The protein showed a high production yield of 3.4 mg/L and the purified proteins exhibited a simple SEC profile. The amino acid sequence for the F3 protein is shown in SEQ ID NO: 142.

Format 4 (F4): CD19-F4-NKp46-3

The domain structure of F4 polypeptides is shown in FIG. 2A. The DNA and amino acid sequences for the CH2-CH3 Fc portion comprised a tandem CH3 domain as in Format F3, and additionally comprise a N297S mutation which prevents N-linked glycosylation and abolishes FcγR binding. Proteins were cloned, produced and purified as in Example 2-1. Bispecific proteins was purified from cell culture supernatant by affinity chromatography using prot-A beads and analyzed and purified by SEC. The protein showed a good production yield of 1 mg/L and the purified proteins exhibited a simple SEC profile. The amino acid sequence for the F4 protein with NKp46-3 variable domains is shown in SEQ ID NO: 143.

Format 8 (F8)

The domain structure of F8 polypeptides is shown in FIG. 2B. The DNA and amino acid sequences for the monomeric CH2-CH3 Fc portion were as in Format F2 and it similarly contains CH3 domain mutations (the mutations (EU numbering) L351K, T366S, P395V, F405R, T407A and K409Y, as well as a N297S mutation which prevents N-linked glycosylation and moreover abolishes FcγR binding. Three variants of F8 proteins were produced: (a) one wherein the cysteine residues in the hinge region were left intact (wild-type, referred to as F8A), (b) a second wherein the cysteine residues in the hinge region were replaced by serine residues (F8B), and (c) a third including a linker sequence GGGSS replacing residues DKHTCTPPCP in the hinge (F8C). Variants F8B and F8C provided production advantages as these versions avoided the formation of homodimers of the central chain. This heterotrimer is made up of;

(1) a first (central) polypeptide chain having domains arranged as follows (N- to C-termini):

V_H^{anti-CD19}-CH1-CH2-CH3-V_H^{anti-NKp46}-C_K

and

(2) a second polypeptide chain having domains arranged as follows (N- to C-termini): V_K^{anti-NKp46}-CH1

and

(3) a third polypeptide chain having domains arranged as follows (N- to C-termini):

V_K^{anti-CD19}-C_K

Proteins were cloned, produced and purified as in Example 2-1. Bispecific proteins was purified from cell culture supernatant by affinity chromatography using prot-A beads and analyzed and purified by SEC. The protein showed a high production yield of 3.7 mg/L (F8C) and the purified proteins again exhibited a simple SEC profile. The amino acid sequences of the three chains of the F8 protein (C variant) with NKp46-3 variable regions are shown in SEQ ID NOS: 144, 145 and 146.

Format 9 (F9): CD19-F9-NKp46-3

The F9 polypeptide is a trimeric polypeptide having a central polypeptide chain and two polypeptide chains each of which associate with the central chain via CH1-C_K dimerization. The domain structure of the trimeric F9 protein is shown in FIG. 2B, wherein the bonds between the CH1 and C_K domains are interchain disulfide bonds. The two antigen binding domains have a F(ab) structure permitting the use of these antibodies irrespective of whether they remain functional in a scFv format. The DNA and amino acid sequences for the CH2-CH3 Fc portion comprise a tandem CH3 domain as in Format F4 and comprise a CH2 domain comprising a N297S substitution. Three variants of F9 proteins were produced: (a) a first wherein the cysteine residues in the hinge region left intact (wild-type, referred to as F9A), (b) a second wherein the cysteine residues in the hinge region were replaced by serine residues (F9B), and (c) a third containing a linker sequence GGGSS which replaces

residues DKTHTCPPCP in the hinge (F9C). Variants F9B and F9C provided advantages in production by avoiding the formation of homodimers of the central chain. The heterotrimer is made up of:

(1) a first (central) polypeptide chain having domains arranged as follows (N- to C-termini):

$V_{H^{anti-CD19}}-CH1-CH2-CH3-CH3-V_{H^{anti-NKp46}}-C_K$

and

(2) a second polypeptide chain having domains arranged as follows (N- to C-termini): $V_{K^{anti-NKp46}}-CH1$

and

(3) a third polypeptide chain having domains arranged as follows (N- to C-termini):

$V_{K^{anti-CD19}}-C_K$.

Proteins were cloned, produced and purified as in Example 2-1. Bispecific proteins was purified from cell culture supernatant by affinity chromatography using prot-A beads and analyzed and purified by SEC. The protein showed a high production yield of 8.7 mg/L (F9A) and 3.0 mg/L (F9B), and the purified proteins again exhibited a simple SEC profile.

The amino acid sequences of the three chains of the F9 protein variant F9A are shown in the SEQ ID NOS: 147, 148 and 149. The amino acid sequences of the three chains of the F9 protein variant F9B are shown in the SEQ ID NOS: 150, 151 and 152. The amino acid sequences of the three chains of the F9 protein variant F9C are shown in the SEQ ID NOS: 153, 154 and 155.

Format 10 (F10): CD19-F10-NKp46-3

The F10 polypeptide is a dimeric protein having a central polypeptide chain and a second polypeptide chain which associates with the central chain via CH1-Ck dimerization. The domain structure of the dimeric F10 protein is shown in FIG. 2B wherein the bonds between the CH1 and Ck domains are interchain disulfide bonds. One of the two antigen binding domains has a Fab structure, and the other is a scFv. The DNA and amino acid sequences for the CH2-CH3 Fc portion comprise a tandem CH3 domain as shown in Format F4 and comprise a CH2 domain containing a N297S substitution. Three variants of F10 proteins were also produced: (a) a first wherein the cysteine residues in the hinge region were left intact (wild-type, referred to as F10A), (b) a second wherein the cysteine residues in the hinge region were replaced by serine residues (F10B), and (c) a third containing a linker sequence GGGSS replacing residues DKTHTCPPCP in the hinge (F10C). Variants F10B and F10C provided advantages in production as they avoid the formation of homodimers of the central chain. The (V_K-V_H) unit was made up of a V_H domain, a linker and a V_K unit (scFv). The heterodimer is made up of:

(1) a first (central) polypeptide chain having domains arranged as follows (N- to C-termini):

$V_{H^{anti-CD19}}-CH1-CH2-CH3-CH3-(V_{H^{anti-NKp46}}-V_K)^{anti-NKp46}$

and

(2) a second polypeptide chain having domains arranged as follows (N- to C-termini): $V_{K^{anti-CD19}}-C_K$.

These proteins were cloned, produced and purified as in Example 2-1. Bispecific proteins was purified from cell culture supernatant by affinity chromatography using prot-A beads and analyzed and purified by SEC. The protein showed a good production yield of 2 mg/L (F10A) and the purified proteins again exhibited a simple SEC profile. The amino acid sequences of the two chains of the F10A protein variant are shown in SEQ ID NOS: 156 (second chain) and 157 (first chain). The amino acid sequences of the two chains of the F10B protein variant are shown in SEQ ID NOS: 158 (second chain) and 159 (first chain). The amino acid sequences of the two chains of the F10C protein variant are shown in the SEQ ID NOS: 160 (second chain) and 161 (first chain).

Format 11 (F11): CD19-F11-NKp46-3

The domain structure of F11 polypeptides is shown in FIG. 2C. The heterodimeric protein is similar to F10 except that the structures of the

antigen binding domains are reversed. One of the two antigen binding domains has a Fab-like structure, and the other is a scFv. The heterodimer is made up of:

(1) a first (central) polypeptide chain having domains arranged as follows (N- to C-termini):

$(V_K-V_H)^{\text{anti-CD19-CH2-CH3-CH3-VH}^{\text{anti-NKp46-Ck}}$

and

(2) a second polypeptide chain having domains arranged as follows (N- to C-termini): $V_K^{\text{anti-NKp46-CH1}}$.

Proteins were cloned, produced and purified as in Example 2-1. Bispecific proteins was purified from cell culture supernatant by affinity chromatography using prot-A beads and analyzed and purified by SEC. The protein showed a good production yield of 2 mg/L and the purified proteins similarly exhibited a simple SEC profile. The amino acid sequences of the two chains of the F11 protein are shown in SEQ ID NO: 162 (chain 1) and SEQ ID NO: 163 (chain 2).

Format 12 (F12): CD19-F12-NKp46-3

The domain structure of the dimeric F12 polypeptides is shown in FIG. 2C, wherein the bonds between the CH1 and Ck domains are disulfide bonds. The heterodimeric protein is similar to F11 but the CH1 and Ck domains within the F(ab) structure are inversed. The heterodimer is made up of:

(1) a first (central) polypeptide chain having domains arranged as follows (N- to C-termini):

$(V_K-V_H)^{\text{anti-CD19-CH2-CH3-CH3-VH}^{\text{anti-NKp46-CH1}}$

and

(2) a second polypeptide chain having domains arranged as follows (N- to C-termini): $V_K^{\text{anti-NKp46-Ck}}$.

Proteins were cloned, produced and purified as in Example 2-1. Bispecific proteins were purified from the cell culture supernatant by affinity chromatography using prot-A beads and analyzed and purified by SEC. The protein showed a good production yield of 2.8 mg/L and the purified proteins similarly exhibited a simple SEC profile. The amino acid sequences of the two chains of the F12 protein are shown in SEQ ID NO: 164 (chain 1) and SEQ ID NO: 165 (chain 2).

Format 17 (F17): CD19-F17-NKp46-3

The domain structure of the trimeric F17 polypeptides is shown in FIG. 2C, wherein the bonds between the CH1 and Ck domains are disulfide bonds. The heterodimeric protein is similar to F9 but the V_H and V_K domains, and the CH1 and Ck, domains within the C-terminal F(ab) structure are each respectively inversed with their partner. The heterotrimer is made up of:

(1) a first (central) polypeptide chain having domains arranged as follows (N- to C-termini):

$V_H^{\text{anti-CD19-CH1-CH2-CH3-CH3-VK}^{\text{anti-NKp46-CH1}}$

and

(2) a second polypeptide chain having domains arranged as follows (N- to C-termini): $V_H^{\text{anti-NKp46-Ck}}$

and

(3) a third polypeptide chain having domains arranged as follows (N- to C-termini):

$V_K^{\text{anti-CD19-Ck}}$

Additionally, three variants of F17 proteins were produced: (a) a first where the cysteine residues in the hinge region were left intact (wild-type, referred to as F17A), (b) a second wherein the cysteine residues in the hinge region were replaced by serine residues (F10B, and (c) a third

containing a linker sequence GGGSS which replaces residues DKTHTCPPCP in the hinge (F17C). Proteins were cloned, produced and purified as in Example 2-1. The amino acid sequences of the three chains of the F17B protein are shown in SEQ ID NOS: 166, 167 and 168.

Example 4 Bispecific NKp46 Antibody Formats with Dimeric Fc Domains

New protein constructions with dimeric Fc domains were developed that share many of the advantages of the monomeric Fc domain proteins of Example 3 but bind to FcRn with greater affinity. Different protein formats were produced that either had low or substantially lack of binding to FcγR (including CD16) or which had binding to FcγRs (including CD16), e.g. the binding affinity to human CD16 was within 1-log of that of wild-type human IgG1 antibodies, as assessed by SPR (e.g. see methods of Example 16. The different polypeptide formats were tested and compared to investigate the functionality of heterodimeric proteins comprising a central chain with a (V_H-(CH1/Ck)-CH2-CH3-) unit or a (V_K-(CH1 or Ck)-CH2-CH3-) unit. One of both of the CH3 domains are fused, optionally via intervening amino acid sequences or domains, to a variable domain(s) (a single variable domain that associates with a variable domain on a separated polypeptide chain, a tandem variable domain (e.g., an scFv), or a single variable domain that is capable of binding antigen as a single variable domain). The two chains associate by CH1-Ck dimerization to form disulfide linked dimers, or if associated with a third chain, to form trimers.

Different constructs were made for use in the preparation of a bispecific antibody using the variable domains DNA and amino acid sequences derived from the scFv specific for tumor antigen CD19 described in Example 2-1 and different variable regions from antibodies specific for NKp46 identified in Example 1. Proteins were cloned, produced and purified as in Example 2-1. Domains structures are shown in FIGS. 2A-6D.

Format 5 (F5): CD19-F5-NKp46-3

The domain structure of the trimeric F5 polypeptide is shown in FIG. 2D, wherein the interchain bonds between hinge domains (indicated in the figures between CH1/Ck and CH2 domains on a chain) and interchain bonds between the CH1 and Ck domains are interchain disulfide bonds. The heterotrimer is made up of:

(1) a first (central) polypeptide chain having domains arranged as follows (N- to C-termini):

V_H^{anti-CD19}-CH1-CH2-CH3-V_H^{anti-NKp46}-Ck

and

(2) a second polypeptide chain having domains arranged as follows (N- to C-termini): V_K^{anti-CD19}-Ck-CH2-CH3

and

(3) a third polypeptide chain having domains arranged as follows (N- to C-termini):

V_K^{anti-NKp46}-CH1

Proteins were cloned, produced and purified as in Example 2-1. Bispecific proteins was purified from cell culture supernatant by affinity chromatography using prot-A beads and analyzed and purified by SEC. The protein showed a high production yield of 37 mg/L and the purified proteins again exhibited a simple SEC profile. The amino acid sequences of the three polypeptide chains are shown in SEQ ID NOS 169 (second chain), 170 (first chain) and 171 (third chain).

Format 6 (F6): CD19-F6-NKp46-3

The domain structure of heterotrimeric F6 polypeptides is shown in FIG. 2D. The F6 protein is the same as F5, but contains a N297S substitution to avoid N-linked glycosylation. Proteins were cloned, produced and purified as in Example 2-1. Bispecific proteins were purified from cell culture supernatant by affinity chromatography using prot-A beads and analyzed and purified by SEC. The protein showed a high production yield of 12 mg/L and the purified proteins exhibited a simple SEC profile. The amino acid sequences of the three polypeptide chains are shown in SEQ ID NOS: 172 (second chain), 173 (first chain) and 174 (third chain).

Format 7 (F7): CD19-F7-NKp46-3

The domain structure of heterotrimeric F7 polypeptides is shown in FIG. 2D. The F7 protein is the same as F6, except for cysteine to serine substitutions in the CH1 and Ck domains that are linked at their C-termini to Fc domains, in order to prevent formation of a minor population of dimeric species of the central chain with the $V_k^{\text{anti-NKp46}}$ -CH1 chain. Proteins were cloned, produced and purified as in Example 2-1. Bispecific proteins were purified from the cell culture supernatant by affinity chromatography using prot-A beads and analyzed and purified by SEC. The protein showed a high production yield of 11 mg/L and the purified proteins exhibited a simple SEC profile. The amino acid sequences of the three polypeptide chains are shown in SEQ ID NOS: 175 (second chain), 176 (first chain) and 177 (third chain).

Format 13 (F13): CD19-F13-NKp46-3

The domain structure of the dimeric F13 polypeptide is shown in FIG. 2D, wherein the interchain bonds between hinge domains (indicated between CH1/Ck and CH2 domains on a chain) and interchain bonds between the CH1 and Ck domains are interchain disulfide bonds. The heterodimer is made up of:

(1) a first (central) polypeptide chain having domains arranged as follows (N- to C-termini):

$V_H^{\text{anti-CD19}}$ -CH1-CH2-CH3-($V_H V_k$) $^{\text{anti-NKp46}}$

and

(2) a second polypeptide chain having domains arranged as follows (N- to C-termini): $V_k^{\text{anti-CD19}}$ -Ck-CH2-CH3.

The (V_H — V_k) unit was made up of a V_H domain, a linker and a V_k unit (scFv).

Proteins were cloned, produced and purified as in Example 2-1. Bispecific proteins were purified from the cell culture supernatant by affinity chromatography using prot-A beads and analyzed and purified by SEC. The protein showed a high production yield of 6.4 mg/L and the purified proteins exhibited a simple SEC profile. The amino acid sequences of the two polypeptide chains are shown in SEQ ID NOS: 178 (second chain) and 179 (first chain).

Format 14 (F14): CD19-F14-NKp46-3

The domain structure of the dimeric F14 polypeptide is shown in FIG. 2E. The F14 polypeptide is a dimeric polypeptide which shares the structure of the F13 format, but instead of a wild-type Fc domain (CH2-CH3), the F14 bispecific format has CH2 domain mutations N297S to abolish N-linked glycosylation. Proteins were cloned, produced and purified as in Example 2-1. Bispecific proteins were purified from cell culture supernatant by affinity chromatography using prot-A beads and analyzed and purified by SEC. The protein showed a high production yield of 2.4 mg/L and the purified proteins exhibited a simple SEC profile. The amino acid sequences of the two polypeptide chains are shown in SEQ ID NOS: 180 (second chain) and 181 (first chain).

Format 15 (F15): CD19-F15-NKp46-3

The domain structure of the trimeric F15 polypeptides is shown in FIG. 2E. The F15 polypeptide is a dimeric polypeptide which shares the structure of the F6 format, but differs by inversion of the N-terminal V_H -CH1 and V_k -Ck units between the central and second chains. Proteins were cloned, produced and purified as in Example 2-1. Bispecific proteins were purified from the cell culture supernatant by affinity chromatography using prot-A beads and analyzed and purified by SEC. The protein showed a good production yield of 0.9 mg/L and the purified proteins possessed a simple SEC profile. The amino acid sequences of the three polypeptide chains are shown in SEQ ID NOS: 182 (second chain), 183 (first chain) and 184 (third chain).

Format 16 (F16): CD19-F16-NKp46-3

The domain structure of the trimeric F16 polypeptide is shown in FIG. 2E. The F16 polypeptide is a dimeric polypeptide which shares the structure of the F6 format, but differs by inversion of the C-terminal V_H -CK and V_k -CH1 units between the central and second chains. Proteins were cloned, produced and purified as in Example 2-1. The amino acid sequences of the three polypeptide chains are shown in SEQ ID NOS: 185 (second chain), 186 (first chain) and 187 (third chain).

Format T5 (T5)

The domain structure of a trimeric T5 polypeptide is shown in FIG. 2F. The T5 polypeptide is a trimeric polypeptide which shares the structure of the F5 format, but differs by fusion of an scFv unit at the C-terminus of the third chain (the chain lacking the Fc domain). This protein will therefore have two antigen binding domains for antigens of interest, and one for NKp46, and will bind CD16 via its Fc domain. Proteins were cloned, produced and purified as in Example 2-1. The T5 protein had two antigen binding domains that bind human CD20, originating from different antibodies (and binding to different epitopes on CD20). The first anti-CD20 ABD contained the V_H and V_L of the parent antibody GA101 (GAZYVA®, Gazyvaro®, obinutuzumab, Roche Pharmaceuticals). The second anti-CD20 ABD contained the V_H and V_L of the parent antibody rituximab (Rituxan®, Mabthera®, Roche Pharmaceuticals). The third antigen binding domain binds human NKp46. The amino acid sequences of the three chains of the T5 protein are shown below (Rituximab sequences are in bold and underlined, anti-GA101 sequences are underlined, anti-NKp46 sequences are in italics).

GA101-T5-Ritux-NKp46

Polypeptide 1

(SEQ ID NO: 188)

QVQLVQSGAEVKKPGSSVKVSCKASGYAFSYSWINWVRQAPGQGLEWMGR
IFPGDGDTDYNGKFKGRVTITADKSTSTAYMELSSLRSEDTAVYYCARNV
FDGYWLVYWGQGLTVVSASTKGPSVFPLAPSSKSTSGGTAALGCLVKD
YFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVTVVPSSSLGTQTY
ICNVNHKPSNTKVDKRVEPKSCDKHTCPPCPAPELLGGPSVFLFPPKPK
DTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNS
TYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQV
YTLPPSREEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTTPVL
DSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGST
GSQVQLQQPGAELVKPGASVM**SCKASGYTFTSYNMHWVKQTPGRGLEWIG**
AIYPGNGDTSYNQKFKGKATLTADKSSSTAYMQLSSLTSEDS**SAVYYCARS**
TYGGDWYENWVGAGTTVTVSARTVAAPSVFIFPPSDEQLKSGTASV**VCL**
LNNFY**PREAKVQWKVDNALQSGNSQESVTEQDSKDSTYLSSTLTLSKAD**
YEKHKVYACEVTHQGLSSPVTKSFNRGEC-

Polypeptide 2

(SEQ ID NO: 189)

DIVMTQTPLSLPVTPGEPASISCRSSKSLLHNSGITYLYWYLQKPGQSPQ
LLIYQMSNLVSGVPDRFSGSGSGTDFTLKISRVEAEDVGVYYCAQNLELP
YTFGGGTKVEIKRTVAAPSVFIFPPSDEQLKSGTASV**VCL****LNNFY****PREAK**
VQWKVDNALQSGNSQESVTEQDSKDSTYLSSTLTLSKADYEKHKVYACE
VTHQGLSSPVTKSFNRGEC**CDKHTC****PPCPAPELLGGPSVFLFPPKPKDTL**
MISRTPEVTCVVVDVSHEDPEVK**FNWYVDGVEVHNAKTKPREEQYNSTYR**
VVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTL
PPSREEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTTPVLDS
GSFFLYSKLTVDKSRWQQGNV**FSCSV****MHEALHNHYTQKSLSLSPGK-**

Polypeptide 3:

(SEQ ID NO: 190)

QIVLSQSPAILSASPGEKVTMTCRASSSVSYIHWFQQKPGSSPKPWYAT

SNLASGVPVRFSGSGSGTSYSLTISRVEAEDAATYYCQQWTSNPPTEGGG

TKLEIKASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSG

ALTSGVHTFPAVLQSSGLYSLSSVVPSSSLGTQTYICNVNHKPSNTKV

DKRVEPKSCDKTHGGSSSEVQLQQSGPELVKPGASVKISCKTSGYTFTEY

TMHWVKQSHGKSLEWIGGISPNIGGTSYNQKFKGKATLTVDKSSSTAYME

LRSLTSEDSAVYYCARRGGSFDYWGQGTTLTVSSVEGGSGGSGGSGGSGG

VDDIVMTQSPATLSVTPGDRVSLSCRASQSIDYLHWYQQKSHESPRLLI

KYASQSISGIPSRFSGSGSGSDFTLSINSVEPEDVGYYCQNGHSFPLTF

GAGTKLELK-

Format T6 (T6)

The domain structure of the trimeric T6 polypeptide is shown in FIG. 2F. The T6 polypeptide is a trimeric polypeptide which shares the structure of the F6 format, but differs by the fusion of an scFv unit at the C-terminus of the third chain (the chain lacking the Fc domain). This trimeric protein contains two antigen binding domains for antigens of interest, and one for NKp46, and does not bind CD16 via its Fc domain due to the N297 substitution. Proteins were cloned, produced and purified as in Example 2-1. The T6 protein contains two antigen binding domains that bind human CD20. The first anti-CD20 ABD comprises the V_H and V_L of the parent antibody GA101 and the second anti-CD20 ABD comprises the V_H and V_L of rituximab. The amino acid sequences of the three chains of the T6 proteins are shown in SEQ ID NOS: 191, 192 and 193.

Format T98 (T98)

The domain structure of the trimeric T9B polypeptide is shown in FIG. 2F. The T9B polypeptide is a trimeric polypeptide which shares the structure of the F9 format (F9B variant), but differs by the fusion of an scFv unit at the C-terminus of the free CH1 domain (on the third chain). This protein contains two antigen binding domains for antigen of interest, and one for NKp46, but will not bind CD16 via its Fc domain due to the monomeric Fc domain and/or the N297 substitution. Trimeric proteins as above described were cloned, produced and purified as in Example 2-1. The T9B protein had two antigen binding domains that bind human CD20. The first anti-CD20 ABD contained the V_H and V_L of the parent antibody GA101 and the second anti-CD20 ABD contained the V_H and V_L of the parent antibody rituximab. The amino acid sequences of the three chains of the T9B proteins are shown below.

GA101-T9B-Ritux-NKp46

Polypeptide 2:

(SEQ ID NO: 195)

DIVMTQTPLSLPVTPGEPASISCRSSKSLLSNNGITYLYWYLQKPGQSPQ

LLIYQMSNLVSGVPDRFSGSGSGTDFTLKISRVEAEDVGVYYCAQNLELP

YTFGGGTKEIKRTVAAPSVFIFPPSDEQLKSGTASVVCLLNNFYPREAK

VQWKVDNALQSGNSQESVTEQDSKSTYSLSSTLTLSKADYEKHKVYACE

VTHQGLSSPVTKSFNRGEC-

Polypeptide 1:

(SEQ ID NO: 194)

QVQLVQSGAEVKKPGSSVKVCKASGYAFSYSWINWVRQAPGQGLEWMGR

IFPGDGD TDYNGKFKGRVTITADKSTSTAYMELSSLRSEDTAVYYCARNV
 FDGYWL VYWGQGLTVTVSSASTKGPSVFPLAPSSKSTSGGTAALGCLVKD
 YFPEPVT VSWNSGALTSGVHTFPAVLQSSGLYSLSSVTVPSSSLGTQTY
 ICNVNHKPSNTKVDKRVEPKSCDKTHTSPPSPAPELLGGPSVFLFPPKPK
 DTLMSIRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYSS
 TYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQV
 YTLPPSREEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTTPVL
 DSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGGG
 GSGSGGGSGGGSGGQPREPQVYTLPPSREEMTKNQVSLTCLVKGFYPSDI
 AVEWESNGQPENNYKTTTPVLDS DGSFFLYSKLTVDKSRWQQGNVFSCSV
 MHEALHNHYTQKSLSLSPGSTGSQVQLQPGAE LVKPGASVM SCKASGYT
 FTSYNMHWVKQTPGRGLEWIGAIYPGNGDTSYNQKFKGKATLTADKSSST
 AYMQLSSLTSEDSAVYYCARSTYYGGDWYFNVWGAGTTVTVSARTVAAPS
 VFIFPPSDEQLKSGTASVVC LLNNFYPREAKVQWKVDNALQSGNSQESVT
 EQDSKDYSLSSSTLTLSKADYEKHKVYACEVTHQGLSPVTKSFNR
 GEC-

Polypeptide 3:

(SEQ ID NO: 196)

QIVLSQSPAILSASPGEKVTMTCRASSSVSYIHWFQQKPGSSPKPWYAT
 SNLASGVPVRFSGSGSGTSYSLTISRVEAEDAATYYCQQWTSNPPTFGGG
 TKLEIKASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVT VSWNSG
 ALTSGVHTFPAVLQSSGLYSLSSVTVPSSSLGTQTYICNVNHKPSNTKV
 DKRVEPKSCDKTHGGSSSEVQLQQSGPELVKPGASVKISCKTSGYTFTEY
 TMHWVKQSHGKSLEWIGGISPNIGGTSYNQKFKGKATLTVDKSSSTAYME
 LRLSTSEDSAVYYCARRGGSFDYWGGTTLTVSSVEGGSGGSGGSGGGG
 VDDIVMTQSPATLSVTPGDRVSLSCRASQSIDYLHWYQQKSHESPRLLI
 KYASQSIGIPSRFSGSGSGDFTLSINSVEPEDVGYYCQNGHSFPLTF
 GAGTKLELK-

Format T11 (T1): CD19-T11-NKp46-3

The domain structure of the dimeric T11 polypeptide is shown in FIG. 2F. The T11 polypeptide is a trimeric polypeptide which shares the structure of the F11 format, but differs by the fusion of an scFv unit at the C-terminus of the free CH1 domain. This dimeric protein contains two antigen binding domains for antigen of interest, and one for NKp46, and does not bind CD16 via its Fc domain due to the monomeric Fc domain and/or the N297 substitution. Proteins were cloned, produced and purified as in Example 2-1. The T11 protein contains two antigen binding domains that bind human CD20. The first anti-CD20 ABD contained the V_H and V_L of the parent antibody GA101 and the second anti-CD20 ABD contained the V_H and V_L of rituximab. The amino acid sequences of the two chains of the T11 protein are shown below.

GA101-T11-Ritux-NKp46

Polypeptide 1:

(SEQ ID NO: 197)

DIVMTQTPLSLPVTGPGEASISCRSSKSLLSNGITYLYWYLQKPGQSPQ
LLIYQMSNLVSGVPDRFSGSGSGTDFTLKISRVEAEDVGVYYCAQNLELP
YTFGGGTKEIKGGGSGGGGSGGGGSGQVLVQSGAEVKKPGSSVKVSCK
ASGYAFSYSWINWVRQAPGQGLEWMGRIFPGDGD TDYNGKFKGRVTITAD
KSTSTAYMELSSLRSEDTAVYYCARNVFDGYWLVYWGQGT LTVSSASTK
GPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVT VSWNSGALTSGVHTFP
AVLQSSGLYSLSSV VTPSSSLGTQTYICNVNHKPSNTKVDKRVEPKSCD
KTHTSPPSPAPPELLGGPSVFLFPPKPKDTLMISRTP EVTCVVVDVSHEDP
EVKFNWYVDGVEVHNAKTKPREEQYSSTYRVVSVLTVLHQD WLNKGKEYKC
KVSNAKALPAIEKTISKAKGQPREPQVYTLPPSREEMTKNQVSLTCLVKG
FYPSDIAVEWESNGQPENNYKTTTPVLDSGSGFFLYSKLTVDKSRWQQGN
VFSCSVMEALHNHYTQKSLSLSPGGGGSGGGGSGGGGSGQPREPQVYT
LPPSREEMTKNQVSLTCLVKG FYPSDIAVEWESNGQPENNYKTTTPVLDS
DGSFFLYSKLTVDKSRWQQGNVFSCSVMEALHNHYTQKSLSLSPGSTGS
QVQLQQPGAELVKPGASVMSCKASGYTFTSYNMHWVKQTPGRGLEWIGAI
YPGNGDTSYNQKFKGKATLTADKSSSTAYMQLSSLTSEDSAVYYCARSTY
YGGDWYFNWVGAGTTVTVSARTVAAPSVFIFPPSDEQLKSGTASVVCLLN
NFYPREAKVQWKVDNALQSGNSQESVTEQDSKDSTYLSSTLTLSKADYE
KHKVYACEVTHQGLSPVTKSFNRGEC-

Polypeptide 2:

(SEQ ID NO: 198)

QIVLSQSPAILSASPGEKVTMTCRASSSVSYIHW FQQKPGSSPKWIYAT
SNLASGVPVRFSGSGSGTSYSLTISRVEAEDAATYYCQQWTSNPPTFGGG
TKLEIKASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVT VSWNSG
ALTSKVHTFPAVLQSSGLYSLSSV VTPSSSLGTQTYICNVNHKPSNTKV
DKRVEPKSCDKTHGGSSSEVQLQQSGPELVKPGASVKISCKTSGYTFTEY
TMHWVKQSHGKSLEWIGGISPNIGGTSYNQKFKGKATLTVDKSSSTAYME
LRSLTSEDSAVYYCARRGGSFDYWGGTTLTVSSVEGGSGGSGGSGGGGG
VDDIVMTQSPATLSVTPGDRVSLSCRASQSIDY LHWYQQKSHESPRLLI
KYASQSIGIPSRFSGSGSGSDFTLSINSEPEDVGVYYCQNGHSFPLTF
GAGTKLELK-

Example 5

NKp46 Binding Affinity by Bispecific Proteins Detected by Surface Plasmon Resonance (SPR)

Biacore T100 General Procedure and Reagents

SPR measurements were performed on a Biacore T100 apparatus (Biacore GE Healthcare) at 25°C. In all Biacore experiments HBS-EP+ (Biacore GE Healthcare) and NaOH 10 mM served as running buffer and regeneration buffer respectively. Sensorgrams were analyzed with Biacore T100 Evaluation software. Protein-A was purchased from (GE Healthcare). Human NKp46 recombinant proteins were cloned, produced and purified at Innate Pharma.

Immobilization of Protein-A

Protein-A proteins were immobilized covalently to carboxyl groups in the dextran layer on a Sensor Chip CM5. The chip surface was activated with EDC/NHS (N-ethyl-N'-(3-dimethylaminopropyl) carbodiimidehydrochloride and N-hydroxysuccinimide (Biacore GE Healthcare)). Protein-A was diluted to 10 µg/ml in coupling buffer (10 mM acetate, pH 5.6) and injected until the appropriate immobilization level was reached (i.e. 2000 RU). Deactivation of the remaining activated groups was performed using 100 mM ethanolamine pH 8 (Biacore GE Healthcare).

Binding Study

The bispecific proteins were first tested in Format F1 as described in Example 2 having different anti-NKp46 variable regions from NKp46-1, NKp46-2, NKp46-3 or NKp46-4 antibodies. Antibodies were next tested as different formats (F3, F4, F5, F6, F9, F10, F11, F13, F14) having the anti-NKp46 variable regions from the NKp46-3 antibody, and were compared to the NKp46-3 antibody as a full-length human IgG1.

Bispecific proteins at 1 µg/mL were captured onto Protein-A chip and recombinant human NKp46 proteins were injected at 5 µg/mL over captured bispecific antibodies. For blank subtraction, cycles were performed again replacing NKp46 proteins with running buffer.

The Bab281 antibody was separately tested for binding to NKp46 by SPR, and additionally by flow cytometry using a cell line expressing the human NKp46 construct on the cell surface. For FACS screening, the presence of reactive antibodies in the supernatants was detected using Goat anti-mouse polyclonal antibody (pAb) labeled with PE. SPC and FACS results showed that the Bab281 based antibody did not bind the NKp46 cell line or to NKp46-Fc proteins. Bab281 lost the ability to bind to its target when presented in the bispecific format.

Affinity Study

Monovalent affinity study was conducted following a regular Capture-Kinetic protocol as recommended by the manufacturer (Biacore GE Healthcare kinetic wizard). Seven serial dilutions of human NKp46 recombinant proteins, ranging from 6.25 to 400 nM were sequentially injected over the captured Bi-Specific antibodies and allowed to dissociate for 10 min before regeneration. The entire sensorgram sets were fitted using the 1:1 kinetic binding model.

Results

SPR showed that the bispecific polypeptides of format F1 having the NKp46-1, 2, 3 and 4 scFv binding domains bound to NKp46, while the other bispecific polypeptides having the scFv of different anti-NK46 antibodies did not retain NKp46 binding. The binding domains that did not retain binding in monomeric bispecific format initially bound to NKp46 but lost the ability to bind NKp46 upon conversion to the bispecific format. All of the bispecific polypeptides of formats F1, F2 F3, F4, F5, F6, F9, F10, F11, F13, and F14 retained binding to NKp46 when using the NKp46-3 variable regions. Monovalent affinities and kinetic association and dissociation rate constants are shown below in Table 3 below.

TABLE 3

Bispecific mAb	ka (1/Ms)	kd (1/s)	KD (M)
CD19-F1-Bab281	n/a	n/a	n/a
			(loss of binding)
CD19-F1-NKp46-1	1.23E+05	0.001337	1.09E-08
CD19-F1-NKp46-2	1.62E+05	0.001445	8.93E-09
CD19-F1-NKp46-3	7.05E+04	6.44E-04	9.14E-09
CD19-F1-NKp46-4	1.35E+05	6.53E-04	4.85E-09
CD19-F3-NKp46-3	3.905E+5	0.01117	28E-09
CD19-F4-NKp46-3	3.678E+5	0.01100	30E-09
CD19-F5-NKp46-3	7.555E+4	0.00510	67E-09
CD19-F6-NKp46-3	7.934E+4	0.00503	63E-09
CD19-F9A-NKp46-3	2.070E+5	0.00669	32E-09
CD19-F10A-NKp46-3	2.607E+5	0.00754	29E-09
CD19-F11A-NKp46-3	3.388E+5	0.01044	30E-09

CD19-F13-NKp46-3	8.300E+4 0.00565	68E-09
CD19-F14-NKp46-3	8.826E+4 0.00546	62E-09
NKp46-3 IgG1	2.224E+5 0.00433	20E-09

Example 6**Engagement of NK Cells Against Daudi Tumor Target with Fc-Containing NKp46×CD19 Bispecific Protein**

Bispecific antibodies having a monomeric Fc domain and a domain arrangement according to the single chain F1 or dimeric F2 formats described in Example 3, and a NKp46 binding region based on NKp46-1, NKp46-2, NKp46-3 or NKp46-4 were tested for functional ability to direct NK cells to lyse CD19-positive tumor target cells (Daudi, a well characterized B lymphoblast cell line). The F2 proteins additionally included NKp46-9 variable regions which lost the ability to bind NKp46 in the scFv format but which retained the ability to bind NKp46 in the F(ab)-like format of F2.

Briefly, the cytolytic activity of each of (a) resting human NK cells, and (b) human NK cell line KHYG-1 transfected with human NKp46, was assessed in a classical 4-h ^{51}Cr -release assay in U-bottom 96 well plates. Daudi cells were labelled with ^{51}Cr (50 μCi (1.85 MBq)/ 1×10^6 cells), then mixed with KHYG-1 transfected with hNKp46 at an effector/target ratio equal to 50 for KHYG-1, and 10 (for F1 proteins) or 8.8 (for F2 proteins) for resting NK cells, in the presence of monomeric bi-specific antibodies at different concentrations. After brief centrifugation and 4 hours of incubation at 37°C., samples of supernatant were removed and transferred into a LumaPlate (Perkin Elmer Life Sciences, Boston, Mass.), and ^{51}Cr release was measured with a TopCount NXT beta detector (Perkin Elmer Life Sciences, Boston, Mass.). All experimental conditions were analyzed in triplicate, and the percentage of specific lysis was determined as follows: $100 \times (\text{mean cpm experimental release} - \text{mean cpm spontaneous release}) / (\text{mean cpm total release} - \text{mean cpm spontaneous release})$. Percentage of total release is obtained by lysis of target cells with 2% Triton X100 (Sigma) and spontaneous release corresponds to target cells in medium (without effectors or Abs).

Results

In the KHYG-1 hNKp46 NK experimental model, each bi-specific antibody NKp46-1, NKp46-2, NKp46-3, NKp46-4 or NKp46-9 induced specific lysis of Daudi cells by human KHYG-1 hNKp46 NK cell line compared to negative controls (Human IgG1 isotype control (IC) and CD19/CD3 bi-specific antibodies), thereby showing that these antibodies induce Daudi target cell lysis by KHYG-1 hNKp46 through CD19/NKp46 cross-linking.

When resting NK cells were used as effectors, each bi-specific antibody NKp46-1, NKp46-2, NKp46-3, NKp46-4 or NKp46-9 again induced specific lysis of Daudi cells by human NK cells compared to the negative control (Human IgG1 isotype control (IC) antibody), thereby showing that these antibodies induce Daudi target cell lysis by human NK cells through CD19/NKp46 cross-linking. Rituximab (RTX, chimeric IgG1) was used as a positive control of ADCC (Antibody-Dependent Cell Cytotoxicity) by resting human NK cells. The maximal response obtained with RTX (at 10 $\mu\text{g}/\text{ml}$ in this assay) was 21.6% specific lysis illustrating that the inventive bispecific antibodies have high target cell lysis activity. Results for experiments with resting NK cells are shown in FIGS. 3A for the single chain F1 proteins and 3B for the dimeric F2 proteins.

Example 7**Comparison with Full Length Anti-NKp46 mAbs and Depleting Anti-Tumor mAbs: Only NKp46×CD19 Bispecific Proteins Prevent Non-Specific NK Activation**

In these experiments bispecific antibodies possessing a specific bispecific format were produced in order to assess whether such bispecific antibodies can mediate NKp46-mediated NK activation toward cancer target cells without triggering non-specific NK cell activation.

Particularly, NKp46×CD19 bispecific proteins having an arrangement according to the F2 format described in Example 3 with anti-NKp46 variable domains from NKp46-1, NKp46-2, NKp46-3, NKp46-4 or NKp46-9 were compared to:

- (a) full-length monospecific anti-NKp46 antibodies (NKp46-3 as human IgG1), and
- (b) the anti-CD19 antibody as a full-length human IgG1 as ADCC inducing antibody control comparator.

The experiments further included as controls: rituximab, an anti-CD20 ADCC inducing antibody control for a target antigen with high expression levels; an anti-CD52 antibody alemtuzumab, a human IgG1 which binds CD52 target present on both targets and NK cells; and a negative control isotype control therapeutic antibody (a human IgG1 that does not bind a target present on the target cells (HUG1-IC)).

The different proteins were tested in order to assess their relative functional effects on NK cell activation in the presence of CD19-positive tumor target cells (Daudi cells), in the presence of CD19-negative, CD16-positive target cells (HUT78 T-lymphoma cells), and in the absence of target cells.

Briefly, NK activation was tested by assessing CD69 and CD107 expression on NK cells by flow cytometry. The assay was carried out in 96 U well plates in completed RPMI, 150 μ L final/well. Effector cells were fresh NK cells purified from donors. Target cells were Daudi (CD19-positive), HUT78 (CD19-negative) or K562 (NK activation control cell line). In addition to K562 positive control, three conditions were tested, as follows:

- - NK cell alone
 - NK cells vs Daudi (CD19⁺)
 - NK cells vs HUT78 (CD19⁻)

Effector:Target (E:T) ratio was 2.5:1 (50 000 E:20 000 T), with an antibody dilution range starting to 10 μ g/mL with 1/4 dilution (n=8 concentrations). Antibodies, target cells and effector cells were mixed; spun 1 min at 300 g; incubated 4 h at 37° C.; spun 3 min at 500 g; washed twice with Staining Buffer (SB); added 50 μ L of staining Ab mix; incubated 30 min at 300 g; washed twice with SB resuspended pellet with CellFix; stored overnight at 4°C.; and fluorescence was detected with Canto II (HTS).

Results

1. NK Cells Alone

Results of these experiments are shown in FIG. 4A. In the absence of target-antigen expressing cells, none of the bispecific anti-NKp46 \times anti-CD19 antibodies (including each of the NKp46-1, NKp46-2, NKp46-3, NKp46-4 and NKp46-9 variable regions) activated NK cells as assessed by CD69 or CD107 expression. The full-length anti-CD19 also did not activate NK cells. However, the full-length anti-NKp46 antibodies did cause detectable activation of NK cells. Alemtuzumab also induced activation of NK cells, at a very high level. The isotype control antibody did not induce activation.

2. NK Cells Vs Daudi (CD19⁺)

Results of these experiments are shown in FIG. 4B. In the presence of target-antigen expressing cells, each of the bispecific anti-NKp46 \times anti-CD19 antibodies (including each of the NKp46-1, NKp46-2, NKp46-3, NKp46-4 and NKp46-9 binding domains) activated NK cells. The full-length anti-CD19 antibody showed at best only very low activation of NK cells. Neither full-length anti-NKp46 antibodies nor alemtuzumab showed a substantial increase in activation beyond what was observed in presence of NK cells alone. The data in FIG. 4 shows that full-length anti-NKp46 antibodies elicited a similar level of baseline activation as was observed in the presence of NK cells alone. Alemtuzumab also induced the activation of NK cells at a similar level of activation to what was observed in the presence of NK cells alone, and at higher antibody concentrations in this setting (ET 2.5:1) the activation was greater than with the bispecific anti-NKp46 \times anti-CD19 antibody. The isotype control antibody did not induce activation.

3. NK cells vs HUT78 (CD19⁻)

Results of these experiments are shown in FIG. 4C. In the presence of target-antigen-negative HUT78 cells, none of the bispecific anti-NKp46 \times anti-CD19 antibody (including each of the NKp46-1, NKp46-2, NKp46-3, NKp46-4 and NKp46-9 variable regions) activated NK cells. However, the full-length anti-NKp46 antibodies and alemtuzumab caused detectable activation of NK cells at a similar level observed in presence of NK cells alone. Isotype control antibody did not induce activation.

The foregoing results indicate that the inventive bispecific anti-NKp46 proteins are able to activate NK cells in a target-cell specific manner, unlike full-length monospecific anti-NKp46 antibodies and further unlike full-length antibodies of depleting IgG isotypes which also activate NK cells in the absence of target cells. The NK cell activation achieved with anti-NKp46 bispecific proteins remarkably was higher than that observed with full length anti-CD19 IgG1 antibodies. Therefore these bispecific antibodies should elicit less non-specific cytotoxicity and may be more potent when used in therapy.

Example 8**Comparative Efficacy with Depleting Anti-Tumor mAbs: NKp46xCD19 Bispecific Proteins at Low ET Ratio**

These studies aimed to investigate whether bispecific antibodies can mediate NKp46-mediated NK cell activation toward cancer target cells at lower effector: target ratios. The ET ratio used in this Example was 1:1 which is believed to be closer to the setting that would be encountered in vivo than the 2.5:1 ET ratio used in Example 7 or the 10:1 ET ratio of Example 6.

NKp46xCD19 bispecific proteins having an arrangement according to the F2 format described in Example 3 with anti-NKp46 variable domains from NKp46-1, NKp46-2, NKp46-3, NKp46-4 or NKp46-9 were compared to:

- (a) full-length monospecific anti-NKp46 antibodies (NKp46-3 as human IgG1), and
- (b) the anti-CD19 antibody as a full-length human IgG1 as ADCC inducing antibody control comparator.

The experiments further included as controls: rituximab (an anti-CD20 ADCC inducing antibody control for a target antigen with high expression levels); anti-CD52 antibody alemtuzumab (a human IgG1, binds CD52 target present on both targets and NK cells), and negative control isotype control therapeutic antibody (a human IgG1 that does not bind a target present on the target cells (HUG1-IC)). The different proteins were tested for their functional effect on NK cell activation as assessed by CD69 or CD107 expression in the presence of CD19-positive tumor target cells (Daudi cells), in the presence of CD19-negative, CD16-positive target cells (HUT78 T-lymphoma cells), and in the absence of target cells. The experiments were carried out as in Example 7 except that the ET ratio was 1:1.

Results

The results of the above experiments are shown in FIGS. 5 (5A: CD107 and 5B: CD69). In the presence of target-antigen expressing cells, each of the bispecific anti-NKp46xanti-CD19 antibodies (respectively including NKp46-1, NKp46-2, NKp46-3, NKp46-4 or NKp46-9 variable regions) activated NK cells in the presence of Daudi cells.

The activation induced by bispecific anti-NKp46xanti-CD19 antibody in the presence of Daudi cells was far more potent than that elicited by the full-length human IgG1 anti-CD19 antibody. This ADCC inducing antibody had low activity in this setting. Furthermore, in this low E:T ratio setting the activation induced by the bispecific anti-NKp46xanti-CD19 antibody was as potent as the anti-CD20 antibody rituximab, with a difference being observed only at the highest concentrations that were 10 fold higher than concentrations in which differences were observed at the 2.5:1 ET ratio.

In the absence of target cells or in the presence of target antigen-negative HUT78 cells, full-length anti-NKp46 antibodies and alemtuzumab showed a similar level of baseline activation as was observed in the presence of Daudi cells. Anti-NKp46xanti-CD19 antibody did not activate NK cells in presence of HUT78 cells.

The foregoing results indicate that the bispecific anti-NKp46 proteins of the invention are able to activate NK cells in a target-cell specific manner and at lower effector: target ratios and are more effective in mediating NK cell activation than traditional human IgG1 antibodies.

Example 9**NKp46 Mechanism of Action**

NKp46xCD19 bispecific proteins having an arrangement according to the F2, F3, F5 or F6 formats described in Examples 3 or 4 with anti-NKp46 variable domains from NKp46-3 were compared to rituximab (anti-CD20 ADCC inducing antibody), and to a human IgG1 isotype control antibody for their functional ability to direct CD16-/NKp46+NK cell lines to lyse CD19-positive tumor target cells.

Briefly, the cytolytic activity of the CD16-/NKp46⁺ human NK cell line KHYG-1 was assessed in a classical 4-h ⁵¹Cr-release assay in U-bottom 96 well plates. Daudi or B221 cells were labelled with ⁵¹Cr (50 µCi (1.85 MBq)/1×10⁶ cells), then mixed with KHYG-1 at an effector/target ratio equal to 50:1, in the presence of test antibodies at dilution ranges starting from 10⁻⁷ mol/L with ½ dilution (n=8 concentrations).

After a brief centrifugation and 4 hours of incubation at 37°C., 50 µL of the supernatant were removed and transferred into a LumaPlate (Perkin Elmer Life Sciences, Boston, Mass.), and ⁵¹Cr release was measured with a TopCount NXT beta detector (PerkinElmer Life Sciences, Boston, Mass.). All experimental conditions were analyzed in triplicate, and the percentage of specific lysis was determined as follows: 100x(mean cpm

experimental release–mean cpm spontaneous release)/(mean cpm total release–mean cpm spontaneous release). Percentage of total release is obtained by lysis of target cells with 2% Triton X100 (Sigma) and spontaneous release corresponds to target cells in medium (without effectors or Abs).

Results

The results of the above experiments are shown in FIGS. 6A (KHYG-1 vs Daudi) and 6B (KHYG-1 vs B221). In the KHYG-1 hNKp46 NK experimental model, each NKp46×CD19 bispecific protein (Format F2, F3, F5 and F6) induced specific lysis of Daudi or B221 cells by human KHYG-1 hNKp46 NK cell line, while rituximab and the human IgG1 isotype control (IC) antibodies did not.

Example 10

Anti-KIR3DL2 Bispecific Proteins

Bispecific proteins targeting human KIR3DL2 (KIR3DL2×NKp46 bispecific) were constructed as F6 formats and tested for activity. KIR3DL2 (CD158k; killer cell immunoglobulin like receptor, three Ig domains and long cytoplasmic tail 2) is a disulphide-linked homodimer of three-Ig domain molecules of about 140 kD, described in Pende et al. (1996) J. Exp. Med. 184: 505-518, the disclosure of which is incorporated herein by reference. Several allelic variants have been reported for KIR3DL2 polypeptides, each of these are encompassed by the term KIR3DL2. The amino acid sequence of the mature human KIR3DL2 (allele *002) is shown in Genbank accession no. AAB52520. Briefly, the cytolytic activity of NK cells from Buffy coat from donors was assessed in a classic 4-h ⁵¹Cr-release assay in U-bottom 96 well plates. HUT78 tumor cells (CTCL) that express KIR3DL2 were labelled with ⁵¹Cr, then mixed with NK cells at an effector/target ratio equal to 10:1 (25 000:2500), in the presence of test antibodies at dilution ranges starting from 10 µg/mL (or 100 µg/mL) with 1/10 dilution (n=8). Assays were in cRPMI, 150 µL final/well, in triplicates.

Results are shown in FIG. 6C. Despite its Fc domain not binding to CD16 in this format, the F6 protein structure produced as an NKp46×KIR3DL2 bispecific protein surprisingly exhibited comparable ability to lyse target cells as a known anti-KIR3DL2 human IgG1 antibody that contained the same variable regions and which binds KIR3DL2 bivalently.

Example 11

Effect of Intrachain Domain Motion within Multimeric Proteins

It was theorized by the inventors that the ability of NKp46 bispecific proteins to promote NKp46-mediated lysis of target cells may be affected by the distance between the two antigen binding domains in the bispecific protein which may impact the ability of one or both of the NKp46 antigen binding domain and the antigen binding domain which interacts with an antigen of interest to interact with their respective targets. Also, it was further theorized that NKp46 mediated lysis of target cells may be impacted by the structure of the two antigen binding domains and/or their respective conformation, freedom of motion or flexibility which may be impacted by the structure of the two antigen binding domains as well as the manner by which they are associated with each other, e.g., by a linker peptide and its particular length and chemical composition. Particularly, it was theorized that a lytic NKp46-target cell synapse may vary as a function of the size and structure of the bispecific protein. Therefore, the inventors posited that bispecific proteins wherein the antigen binding domains are in a format whereby the antigen binding domains more closely mimics or approximates the conformation, spacing and flexibility of the antigen binding domains of

This was theorized because conformational flexibility, notably intrachain domain motion or movement, may for example affect the effective distance between NKp46 and antigen-of-interest binding sites, which in turn might have an effect on the NKp46-target cell synapse and the ability of a multimeric bispecific protein to mediate NKp46-mediated signaling and lysis. Based on these suppositions the inventors evaluated the lytic function of multimeric proteins of different bispecific protein formats and which comprise more or less freedom of motion of the antigen binding domains based on the structure of the antigen binding sites and the specific linkers separating these antigen binding sites.

Specifically, different NKp46×tumor antigen bispecific proteins of different formats such as the F3, F4, F9, F10 and F11 format that bound different tumor antigens were evaluated for their relative ability to induce NKp46-mediated lysis of tumor target cells by KHYG-1 NK cells (NKp46×CD16). F5 and F6 bispecific protein formats have distances between the NKp46 binding site and the antigen of interest binding site that are less than that of full-length antibodies. By contrast bispecific proteins targeting human CD19 (CD19×NKp46 bispecific) in F9 format have binding sites that are spaced farther apart, similar to distances in the two binding sites in conventional full-length antibodies. Bispecific proteins

were therefore constructed as F9 formats and compared to F10 and F11 formats. Structurally speaking, format F9, F10 and F11 are very close to one another, however formats F10 and F11 are characterized by one antigen binding domain with a Fab structure and the other antigen binding domain with a tandem variable domain structure (two variable domains separated by a flexible linker). F10 and F11 therefore have greater intrachain domain motion and/or less local steric hindrance, as well as possibly less distance between binding sites than in the F9 proteins.

The cytolytic activity of the CD16-/NKp46+ human NK cell line KHYG-1 was assessed in a classical 4-h ^{51}Cr -release assay in U-bottom 96 well plates. Daudi or B221 cells were labelled with ^{51}Cr (50 μCi (1.85 MBq)/ 1×10^6 cells), then mixed with KHYG-1 at an effector/target ratio equal to 50:1, in the presence of test antibodies at dilution range starting from 10^{-7} mol/L with $\frac{1}{2}$ dilution ($n=8$ concentrations). The results showed that formats F10 and F11 were both more potent than format F9 in inducing Daudi cell lysis by NK cells. As noted above F9 format proteins have distances between the NKp46 binding site and the antigen of interest binding site which is similar to full-length antibodies or about 80 Å, and the F10 and F11 proteins comprise a single chain domain connected to the Fc by a flexible linker and have substantially less than 80 Å between the antigen binding sites (in the case of F10, about 55 Å).

Based thereon we studied the effects of even further shortened distances between the NKp46 and antigen of interest binding domains using other CD19xNKp46 bispecific proteins. In these experiments F3, F4 protein formats were selected for comparison with protein formats F10 and F11. Each of these proteins have distances between antigen binding sites of less than 80 Å, however, F3 and F4 are shorter than F10 and F11, and F3 and F4 have distances between antigen binding sites that are equivalent to F11 but 25 Å less than that of F10. The results of these experiments indicated that the F3, F4, F10 and F11 formats did not significantly differ in their ability to induce Daudi cell lysis by NK cells. These results would suggest that there may be an optimal minimal spacing between the antigen binding domains that improves potency and/or that potency is affected by a combination of the spacing between the antigen binding domains and the flexibility and/or conformation of the antigen binding domains.

Example 12 **Combining NKp46 and CD16 Triggering**

NKp46xCD19 bispecific proteins that bind human CD16 having an arrangement according to the F5 format with anti-NKp46 variable domains from NKp46-3 were compared to the same bispecific antibody in a F6 format (which lacks CD16 binding), and to a human IgG1 isotype anti-CD19 antibody, as well as to a human IgG1 isotype control antibody for functional ability to direct purified NK cells to lyse CD19-positive Daudi tumor target cells.

Briefly, the cytolytic activity of fresh human purified NK cells from EFS Buffy Coat was assessed in a classical 4-h ^{51}Cr -release assay in U-bottom 96 well plates. Daudi or HUT78 cells (negative control cells that do not express CD19) were labelled with ^{51}Cr and then mixed with NK cells at an effector/target ratio equal to 10:1, in the presence of test antibodies at dilution range starting from 10 $\mu\text{g/ml}$ with 1/10 dilution ($n=8$ concentrations).

After brief centrifugation and 4 hours of incubation at 37°C ., 50 μL of supernatant were removed and transferred into a LumaPlate (Perkin Elmer Life Sciences, Boston, Mass.), and ^{51}Cr release was measured with a TopCount NXT beta detector (PerkinElmer Life Sciences, Boston, Mass.). All experimental conditions were analyzed in triplicate, and the percentage of specific lysis was determined as follows: $100 \times (\text{mean cpm experimental release} - \text{mean cpm spontaneous release}) / (\text{mean cpm total release} - \text{mean cpm spontaneous release})$. Percentage of total release is obtained by lysis of target cells with 2% Triton X100 (Sigma) and spontaneous release corresponds to target cells in medium (without effectors or Abs).

The results of these experiments are shown in FIG. 7. The CD19-F6-NKp46 (bispecific protein in F6 format) whose Fc domain does not bind CD16 due to a N297 substitution was as potent in mediating NK cell lysis of Daudi target cells as the full-length IgG1 anti-CD19 antibody. This result is remarkable especially considering that the control IgG1 anti-CD19 antibody binds CD19 bivalently and further since the anti-CD19 antibody is bound by CD16. The F6 protein was also compared to a protein CD19-F5-NKp46 that was identical to the CD19-F6-NKp46 protein with the exception of an asparagine at Kabat residue 297. Surprisingly, despite the strong NK activation mediated by CD16 triggering by the CD19-F5-NKp46 (F5 format protein) whose Fc domain binds CD16, the F5 format was far more potent in mediating Daudi target cell lysis than the full-length IgG1 anti-CD19 antibody or the F6 format bispecific protein. This would suggest that NKp46 can enhance target cell lysis even

when CD16 is triggered. In fact, at comparable levels of target cell lysis, the CD19-F5-NKp46 was at least 1000 times more potent than the full-length anti-CD19 IgG1. These potency results would suggest that the inventive multispecific NKp46 antibodies should be well suited for use in human therapy, e.g., in treating cancer or infectious diseases.

Example 13

Mechanisms of Action of CD16-Binding NKp46×CD19 Bispecific

Lysis of Daudi cells by NKp46×CD19 bispecific F5 and F6 were compared to a conventional human IgG1 antibody. As a control, lysis was also tested on HUT78 cells that lack CD19; positive control for HUT78 cell lysis was an anti-KIR3DL2 of human IgG1 isotype (HUT78 are KIR3DL2-positive). Cytotoxicity assays were carried out as in Example 10. Flow cytometry staining of NK cell surface markers was carried out as in Example 7.

Results for the cytotoxicity assays are shown in FIG. 8 (Daudi cell in the right hand panel and HUT78 cells in the left hand panel). the CD19-F6-NKp46-3 whose Fc domain does not bind CD16 due to a N297 substitution has as mode of action NKp46 triggering when NK cells encounter the target cell, while the CD19-F5-NKp46-3 bispecific protein demonstrated a far higher potency in mediating cytotoxicity toward Daudi cells. Neither the F5 nor the F6 protein mediated any NK cell cytotoxicity towards HUT78 cells.

The results of flow cytometry staining of NK cell surface markers showed a strong upregulation of CD137 on the surface of NK cells by F5 proteins. These results are shown in FIG. 9 (Left-most panel: NK cells vs. Daudi; middle panel: NK cells vs. HUT78; right-most panel: NK cells alone). The CD19-F5-NKp46-3 whose Fc domain binds CD16 demonstrated the highest CD137 upregulation. The full-length anti-CD19 IgG1 antibody that binds CD16 also elicited CD137 upregulation, but to a far lesser extent than CD19-F5-NKp46-3. The CD19-F6-NKp46-3 which functions via NKp46 but not via CD16 did not elicit any detectable CD137 upregulation. It is hypothesized that the remarkable potency of the F5 format may arise from a particularly strong CD137 upregulation on NK cells which may be mediated by the dual targeting of NKp46 and CD16.

Example 14

Fc-Engineered CD16-Binding NKp46×CD20 Bispecific

New bispecific proteins were further constructed in an attempt to generate an agent that could improve on the most potent new generation of Fc enhanced antibodies. In these experiments as the comparison antibody we selected the commercial antibody GA101 (GAZYVA®, Gazyvaro®, obinutuzumab, Roche Pharmaceuticals), which is an Fc-modified human IgG1 antibody having enhanced CD16A binding as a result of hypofucosylated N-linked glycosylation.

NKp46×CD20 bispecific proteins were produced as proteins without CD16 binding (F6 format), with CD16 binding (F5 format), or as Fc-engineered format based on F5 but comprising two amino acid substitutions in the CH2 domain of the heavy chain that increase binding affinity for human CD16A (referred to as "F5+"). In these constructs the anti-CD20 ABDs comprise the V_H and V_L of GA101.

Lysis of Daudi cells by NKp46×CD20 bispecific F5, F5+ and F6 antibodies were compared to the commercial antibody GA101 (GAZYVA®). Cytotoxicity assays were carried out as in Example 10.

Results for the cytotoxicity assays are shown in FIG. 10. As shown therein the GA101-F5+-NKp46-1 bispecific protein demonstrated a far higher potency (approximately 10-fold increase in EC₅₀) in mediating cytotoxicity toward Daudi cells than GA101.

Moreover, when ADCC optimized Fc are used for the bispecific format (F5+) a significant difference was observed between F5+-BS lacking the Nkp46 arm (GA101-F5+-IC; black diamond) and F5+-BS co-engaging CD16+NKp46 (GA101-F5+-NKp46-1; black square) confirming the contribution of NKp46 in GA101-F5+-NKp46-1 activity. Surprisingly, despite the high affinity of GA101-F5+-NKp46-1 for CD16 and the presumable maximum NK-cell mediated lysis, NKp46 nevertheless elicited a substantial further increase in cytotoxic activity. These results would suggest that agents capable of inducing ADCC via CD16, can be improved by further conferring on them the ability to induce NKp46-mediated lysis, and also that the potency of bispecific anti-NKp46 agents can be improved by enhancing affinity for CD16 via Fc engineering.

Example 15

Binding of Different Bispecific Formats to FcRn

The affinity of different antibody formats for human FcRn was studied by Surface Plasmon Resonance (SPR) by immobilizing recombinant FcRn proteins covalently to carboxyl groups in the dextran layer on a Sensor Chip CM5, as described in Example 2-6.

A chimeric full length anti-CD19 antibody having intact human IgG1 constant regions and NKp46xCD19 bispecific proteins having an arrangement according to the F3, F4, F5, F6, F9, F10, F11, F13 or F14 formats described in Examples 3 or 4 with anti-NKp46 variable domains from NKp46-3 (NKp46-2 for F2) were tested; for each analyte, the entire sensorgram was fitted using the steady state or 1:1 SCK binding model.

The results of these experiments are shown in Table 4 below. The bispecific proteins having dimeric Fc domains (formats F5, F6, F13, F14) bound to FcRn with affinity similar to that of the full-length IgG1 antibody. The bispecific proteins with monomeric Fc domains (F3, F4, F9, F10, F11) also displayed binding affinity to FcRn, however with lower affinity than the bispecific proteins having dimeric Fc domains.

TABLE 4

Antibody/Bispecific	SPR method	KD nM
Human IgG1/K Anti-CD19	SCK/Two state reaction	7.8
CD19-F5-NKp46-3	SCK/Two state reaction	2.6
CD19-F6- NKp46-3	SCK/Two state reaction	6.0
CD19-F13- NKp46-3	SCK/Two state reaction	15.2
CD19-F14- NKp46-3	SCK/Two state reaction	14.0
CD19-F3- NKp46-3	Steady State	474.4
CD19-F4- NKp46-3	Steady State	711.7
CD19-F9A- NKp46-3	Steady State	858.5
CD19-F10A- NKp46-3	Steady State	432.8
CD19-F11- NKp46-3	Steady State	595.5

Example 16 Binding to FcγR

Different multimeric Fc proteins were evaluated to assess whether such bispecific monomeric Fc proteins could retain binding to Fcγ receptors.

SPR measurements were performed on a Biacore T100 apparatus (Biacore GE Healthcare) at 25°C. In all Biacore experiments HBS-EP+ (Biacore GE Healthcare) and 10 mM NaOH, 500 mM NaCl served as running buffer and regeneration buffer respectively. Sensorgrams were analyzed with Biacore T100 Evaluation software. Recombinant human FcR's (CD64, CD32a, CD32b, CD16a and CD16b) were cloned, produced and purified.

F5 and F6 bispecific antibodies CD19-F5-NKp46-3 or CD19-F6-NKp46-3 were immobilized covalently to carboxyl groups in the dextran layer on a Sensor Chip CM5. The chip surface was activated with EDC/NHS (N-ethyl-N'-(3-dimethylaminopropyl) carbodiimidehydrochloride and N-hydroxysuccinimide (Biacore GE Healthcare)). Bispecific antibodies were diluted to 10 µg/ml in coupling buffer (10 mM acetate, pH 5.6) and injected until the appropriate immobilization level was reached (i.e. 800 to 900 RU). Deactivation of the remaining activated groups was performed using 100 mM ethanolamine pH 8 (Biacore GE Healthcare).

Monovalent affinity study was assessed following a classical kinetic wizard (as recommended by the manufacturer). Serial dilutions of soluble analytes (FcRs) ranging from 0.7 to 60 nM for CD64 and from 60 to 5000 nM for all the other FcRs were injected over the immobilized bispecific antibodies and allowed to dissociate for 10 min before regeneration. The entire sensorgram sets were fitted using the 1:1 kinetic binding model for CD64 and with the Steady State Affinity model for all the other FcRs.

The results showed that while full length wild type human IgG1 bound to all cynomolgus and human Fcγ receptors, the CD19-F6-NKp46-3 bi-specific antibodies did not bind to any of the receptors. The CD19-F5-NKp46-3, on the other hand, bound to each of the human receptors CD64 (KD=0.7 nM), CD32a (KD=846 nM), CD32b (KD=1850 nM), CD16a (KD=1098 nM) and CD16b (KD=2426 nM). Conventional human anti-IgG1 antibodies have comparable binding to these Fc receptors (KD shown in the table below).

Full length human

CD19-F5-NKp46-3 IgG1 antibody		
Human Fcγ receptor KD (nM)	KD (nM)	
CD64	0.7	0.24
CD32a	846	379
CD32b	1850	1180
CD16a	1098	630
CD16b	2426	2410

Example 17**Epitope Mapping of Anti-NKp46 Antibodies****A. Competition Assays**

Competition Assays were Conducted by Surface Plasmon Resonance (SPR According to the Methods Described Below.

SPR measurements were performed on a Biacore T100 apparatus (Biacore GE Healthcare) at 25°C. In all Biacore experiments HBS-EP+(Biacore GE Healthcare) and NaOH 10 mM NaCl 500 mM served as running buffer and regeneration buffer respectively. Sensorgrams were analyzed with Biacore T100 Evaluation software. Anti-6xHis tag antibody was purchased from QIAGEN. Human 6xHis tagged NKp46 recombinant proteins (NKp46-His) were cloned, produced and purified at Innate Pharma.

Anti-His antibodies were immobilized covalently to carboxyl groups in the dextran layer on a Sensor Chip CM5. The chip surface was activated with EDC/NHS (N-ethyl-N'-(3-dimethylaminopropyl) carbodiimidehydrochloride and N-hydroxysuccinimide (Biacore GE Healthcare)). Protein-A and anti-His antibodies were diluted to 10 µg/ml in coupling buffer (10 mM acetate, pH 5.6) and injected until the appropriate immobilization level was reached (i.e. 2000 to 2500 RU). Deactivation of the remaining activated groups was performed using 100 mM ethanolamine pH 8 (Biacore GE Healthcare).

Parental regular human IgG1 chimeric antibodies having NKp46 binding region corresponding to NKp46-1, NKp46-2, NKp46-3 or NKp46-4 were used for the competition study which has been performed using an Anti-6xHis tag antibody chip. Bispecific antibodies having NKp46 binding region based on NKp46-1, NKp46-2, NKp46-3 or NKp46-4 at 1 µg/mL were captured onto Protein-A chip and recombinant human NKp46 proteins were injected at 5 µg/mL together with a second test bispecific antibody of the NKp46-1, NKp46-2, NKp46-3 or NKp46-4 group.

The results demonstrated that none of NKp46-1, NKp46-2, NKp46-3 or NKp46-4 competed with one another for binding to NKp46. Accordingly these antibodies each bind or interact with a different NKp46 epitope.

B. Binding to NKp46 Mutants

In order to define the epitopes of these anti-NKp46 antibodies, we designed NKp46 mutants defined by one, two or three substitutions of amino acids exposed at the molecular surface over the 2 domains of NKp46. This approach led to the generation of 42 mutants which were transfected in Hek-293T cells, as shown in the table below. The targeted amino acid mutations in Table 5 below are shown both according to the numbering of SEQ ID NO: 1 (also corresponding to the numbering used in Jaron-Mendelson et al. (2012) *J. Immunol.* 88(12):6165-74.

TABLE 5

Substitution		
Mutant (Numbering according to: Jaron-Mendelson and SEQ ID NO 1)		
1	P40A	K43S Q44A
2	K41S	E42A E119A
3	P86A	D87A
4	N89A	R91A
5	K80A	K82A
5bis	E34A	T46A

6	R101A	V102A
7	N52A	Y53A
8	V56A	P75A E76A
9	R77A	I78A
10	S97A	I99A
10bis	Q59A	H61A
11	L66A	V69A
12	E108A	
13	N111A	L112A
14	D114A	
15	T125A	R145S D147A
16	S127A	Y143A
17	H129A	K139A
18	K170A	V172A
19	I135A	S136A
19bis	T182A	R185A
20	R160A	
21	K207A	
22	M152A	R166A
23	N195A	N196A
Stalk1	D213A	I214A T217A
Stalk2	F226A	T233A
Stalk3	L236A	T240A
Supp1	F30A	W32A
Supp2	F62A	F67A
Supp3	E63A	Q95A
Supp4	R71A	K73A
Supp5	Y84A	
Supp6	E104A	L105A
Supp7	Y121A	Y194A
Supp8	P132A	E133A
Supp9	S151A	Y168A
Supp10	S162A	H163A
Supp11	E174A	P176A
Supp12	P179A	H184A
Supp13	R189A	E204A P205A

C. Generation of Mutants

NKp46 mutants were generated by PCR. The sequences amplified were run on agarose gel and purified using the Macherey Nagel PCR Clean-Up Gel Extraction kit. Two or three purified PCR products generated for each mutant were then ligated into an expression vector, with the ClonTech InFusion system. The vectors containing the mutated sequences were prepared as Miniprep and sequenced. After sequencing, the vectors containing the mutated sequences were prepared as Midiprep using the Promega PureYield™ Plasmid Midiprep System. HEK293T cells

were grown in DMEM medium (Invitrogen), transfected with vectors using Invitrogen's Lipofectamine 2000 and incubated at 37°C. in a CO₂ incubator for 24 hours prior to testing for transgene expression.

D. Flow Cytometry Analysis of Anti-NKp46 Binding to the HEK293T Transfected Cells

All the anti-NKp46 antibodies were tested for their binding to each mutant by flow cytometry. A first experiment was performed to identify antibodies that lose their binding to one or several mutants at a particular concentration (10 µg/ml). To confirm the loss of binding, titration of antibodies was done using antibodies for which binding seemed to be affected by the NKp46 mutations (1-0,1-0,01-0,001 µg/ml).

E. Results

Antibody NKp46-1 had decreased binding to the mutant 2 (having a mutation at residues K41, E42 and E119) (numbering in NKp46 wild-type) compared to wild-type NK46. Similarly, NKp46-1 also had decreased binding to the supplementary mutant Supp7 (having a mutation at residues Y121 and Y194).

Antibody NKp46-3 had decreased binding to the mutant 19 (having a mutation at residues I135, and S136). Similarly, NKp46-1 also had decreased binding to the supplementary mutant Supp8 (having a mutation at residues P132 and E133).

Antibody NKp46-4 had decreased binding to the mutant 6 (having a mutation at residues R101, and V102). Similarly, NKp46-1 also had decreased binding to the supplementary mutant Supp6 having a mutation at residues E104 and L105.

Using these methods we identified the epitopes for anti-NKp46 antibodies NKp46-1, NKp46-3 and NKp46-4. We determined that the epitopes of NKp46-4, NKp46-3 and NKp46-1 are on NKp46 D1 domain, D2 domain and D1/D2 junction, respectively. R101, V102, E104 and L105 are essential residues for NKp46-4 binding and defined a part of NKp46-4 epitope. The epitope of NKp46-1 epitope includes K41, E42, E119, Y121 and Y194 residues. The epitope of NKp46-3 includes P132, E133, I135, and S136 residues.

Example 18

Improved Product Profile and Yield of Different Bispecific Formats Compared to Existing Formats

Blinatumomab and two bispecific antibodies having NKp46 and CD19 binding regions based on F1 to F17 formats and NKp46-3, and blinatumomab, respectively were cloned and produced under format 6 (F6), DART™ and BiTE™ formats following the same protocol and using the same expression system. F6, DART™ and BiTE™ bispecific proteins were purified from cell culture supernatant by affinity chromatography using prot-A beads for F6 or Ni-NTA beads for DART™ and BiTE™. Purified proteins were further analyzed and purified by SEC. DART™ and BiTE™ showed a very low production yield compared to F6 and the purified proteins have a very complex SEC profile. DART™ and BiTE™ are barely detectable by SDS-PAGE after Coomassie staining in the expected SEC fractions (3 and 4 for BiTE™ and 4 and 5 for DART™), whereas the F6 format showed a clear and simple SEC and SDS-PAGE profiles with a major peak (fraction 3) containing the multimeric bispecific proteins. The major peak for the F6 format corresponded to about 30% of the total proteins. These results are consistent for those seen with the F1 to F17 proteins (data not shown) indicating that the Fc domain (or Fc-derived domain) present in those formats facilitates the production and improves the quality and solubility of bispecific proteins.

Moreover, the Fc domains present in proteins F1 to F17 have the advantage of being suitable for usage in affinity chromatography without the need for the incorporation of peptide tags. This is desirable as such tags are undesirable in a therapeutic product as they may potentially elicit undesired immunogenicity. By contrast, DART™ and BiTE™ antibodies cannot be purified using protein A, whereas F1 to F17 antibodies are all bound by protein A. Table 6 below shows the productivity of different formats.

TABLE 6

Format	Final			Non Reduced yield
	SEC	Reduced	SDS PAGE « productivity »	
F3	2 peaks	✓	✓	3.4 mg/L
F4	2 peaks	✓	✓	1 mg/L
F5	✓	✓	✓	37 mg/L

F6	✓	✓	✓	12 mg/L
F7	✓	✓	✓	11 mg/L
F8C	✓	✓	✓	3.7 mg/L
F9A	✓	✓	✓	8.7 mg/L
F9B	✓	✓	✓	3.0 mg/L
F10A	✓	✓	✓	2.0 mg/L
F11	✓	✓	✓	2.0 mg/L
F12	✓	✓	✓	2.8 mg/L
F13	✓	✓	✓	6.4 mg/L
F14	✓	✓	✓	2.4 mg/L
F15	✓	✓	✓	0.9 mg/L
BiTe TM	—	—	—	—
DART TM	—	—	—	—

All headings and sub-headings are used herein for convenience only and should not be construed as limiting the invention in any way. Any combination of the above-described elements in all possible variations thereof is encompassed by the invention unless otherwise indicated herein or otherwise clearly contradicted by context. Recitation of ranges of values herein are merely intended to serve as a shorthand method of referring individually to each separate value falling within the range, unless otherwise indicated herein, and each separate value is incorporated into the specification as if it were individually recited herein. Unless otherwise stated, all exact values provided herein are representative of corresponding approximate values (e.g., all exact exemplary values provided with respect to a particular factor or measurement can be considered to also provide a corresponding approximate measurement, modified by "about," where appropriate). All methods described herein can be performed in any suitable order unless otherwise indicated herein or otherwise clearly contradicted by context.

The use of any and all examples, or exemplary language (e.g., "such as") provided herein is intended merely to better illuminate the invention and does not pose a limitation on the scope of the invention unless otherwise indicated. No language in the specification should be construed as indicating any element is essential to the practice of the invention unless as much is explicitly stated.

The description herein of any aspect or embodiment of the invention using terms such as reference to an element or elements is intended to provide support for a similar aspect or embodiment of the invention that "consists of," "consists essentially of" or "substantially comprises" that particular element or elements, unless otherwise stated or clearly contradicted by context (e.g., a composition described herein as comprising a particular element should be understood as also describing a composition consisting of that element, unless otherwise stated or clearly contradicted by context).

This invention includes all modifications and equivalents of the subject matter recited in the aspects or claims presented herein to the maximum extent permitted by applicable law.

All publications and patent applications cited in this specification are herein incorporated by reference in their entireties as if each individual publication or patent application were specifically and individually indicated to be incorporated by reference.

Although the foregoing invention has been described in some detail by way of illustration and example for purposes of clarity of understanding, it will be readily apparent to one of ordinary skill in the art in light of the teachings of this invention that certain changes and modifications may be made thereto without departing from the spirit or scope of the appended claims.

Previous Patent: [ANTIBODY THERAPEUTICS THAT BIND CD38](#)

Next Patent: [REDIRECTING IMMUNE RESPONSES](#)

17 USC § 107 Limitations on Exclusive Rights – FAIR USE

Some pictures have been added for EMPHASIS



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(54) **MULTISPECIFIC NK ENGAGER PROTEIN**

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C07K 16/2803 (2013.01); **C07K 2317/565**
(2013.01); **C07K 2317/526** (2013.01); **C07K**
2317/524 (2013.01); **C07K 2317/522**
(2013.01); **C07K 2317/31** (2013.01); **C07K**
2317/622 (2013.01); **C07K 2317/71** (2013.01);
C07K 2317/732 (2013.01); **C07K 2317/75**
(2013.01); **C07K 2319/30** (2013.01); **C07K**
16/2809 (2013.01)

(21) Appl. No.: **16/173,114**

(22) Filed: **Oct. 29, 2018**

Related U.S. Application Data

(60) Division of application No. 15/190,337, filed on Jun. 23, 2016, now Pat. No. 10,113,003, which is a continuation-in-part of application No. PCT/EP2015/064063, filed on Jun. 23, 2015.

(60) Provisional application No. 62/271,459, filed on Dec. 28, 2015.

(57) **ABSTRACT**

Multispecific proteins that bind and specifically redirect NK cells to lyse a target cell of interest are provided without non-specific activation of NK cells in absence of target cells. The proteins have utility in the treatment of disease, notably cancer or infectious disease.

Specification includes a Sequence Listing.

Figure 1

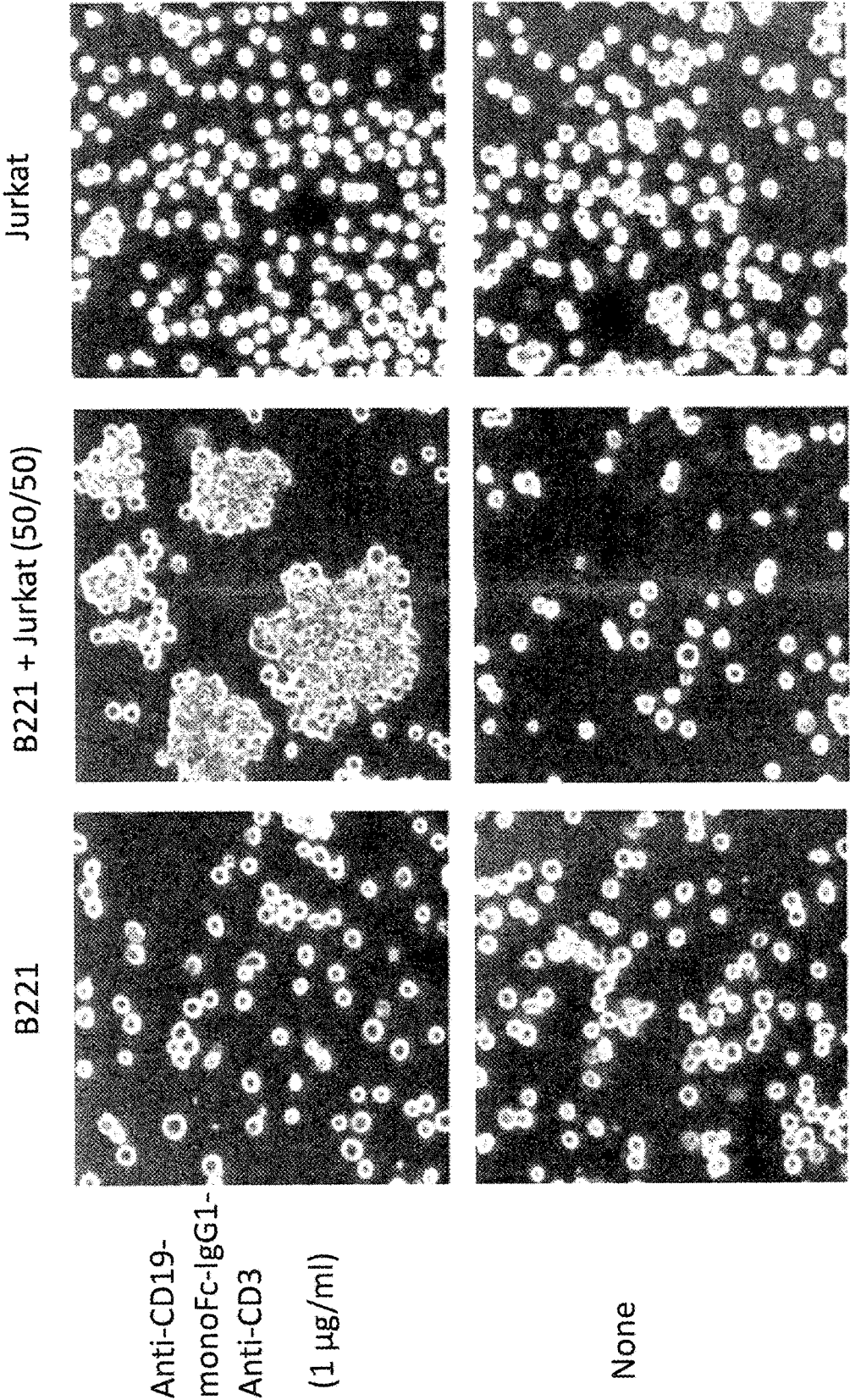


Figure 2A

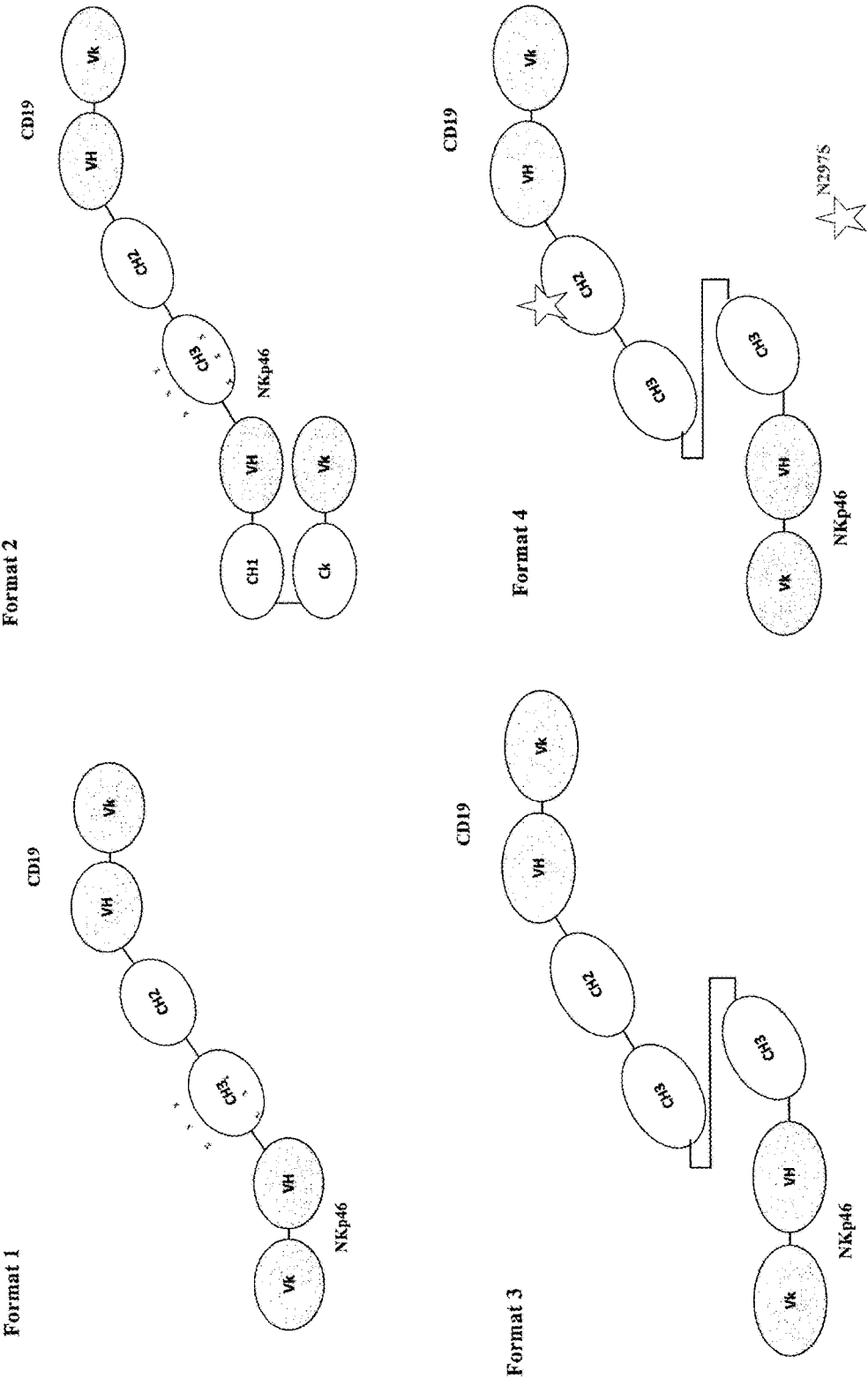
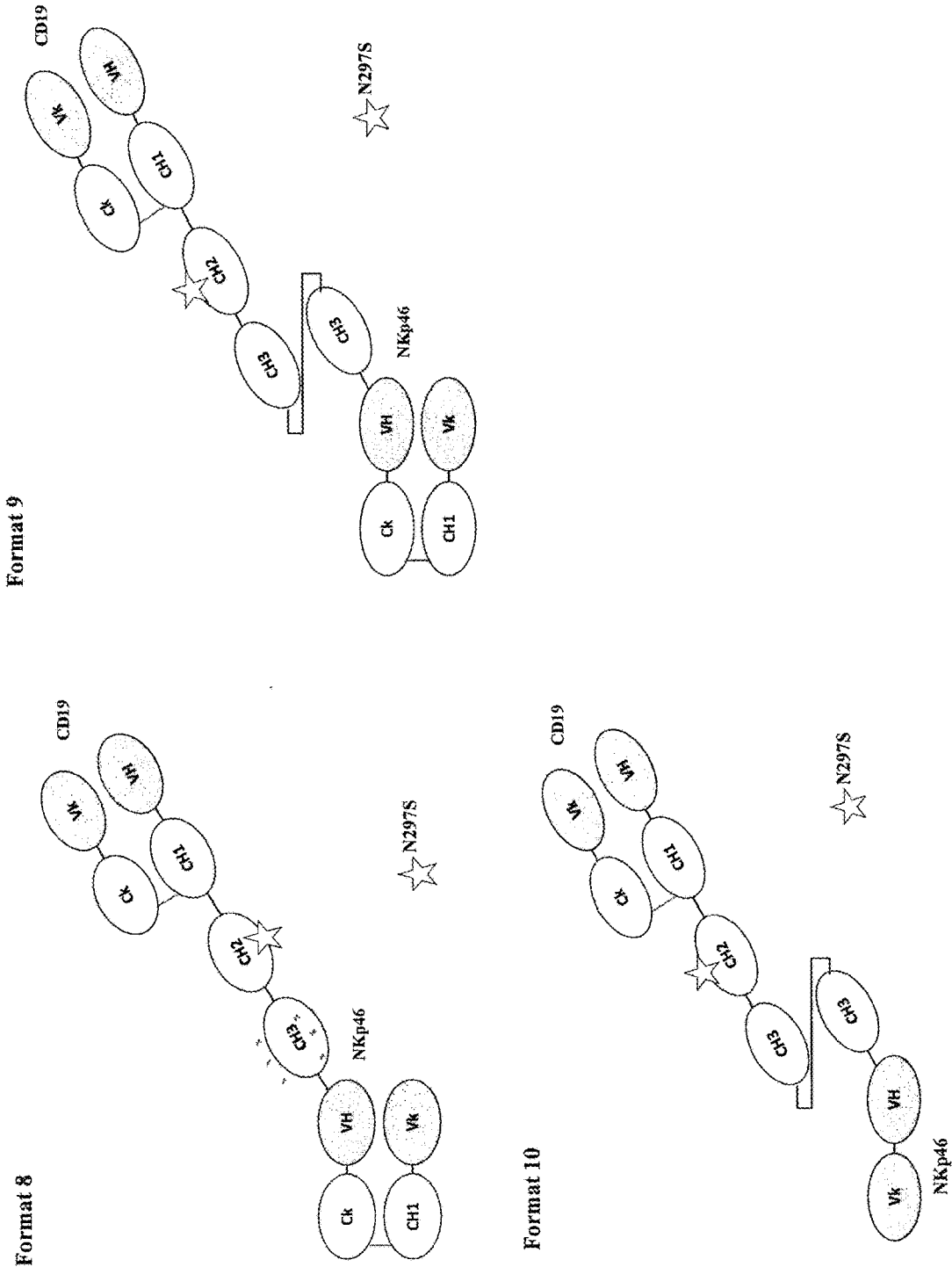


Figure 2B



Format 12

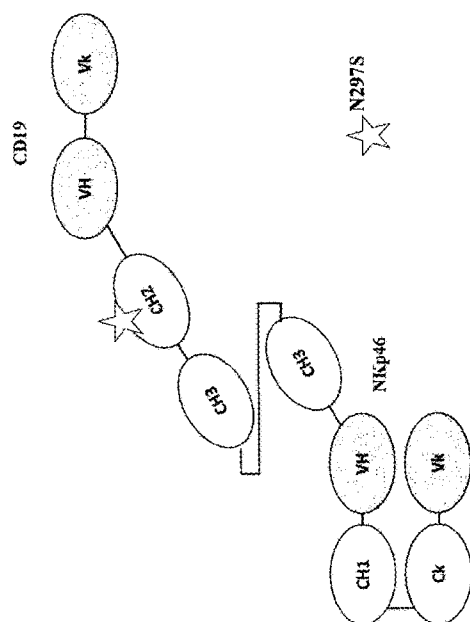
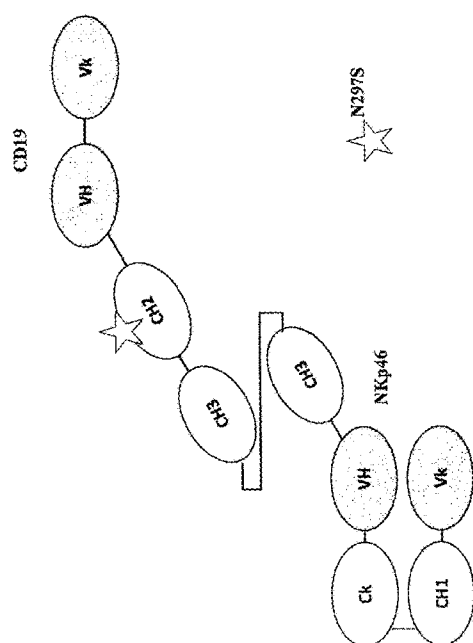
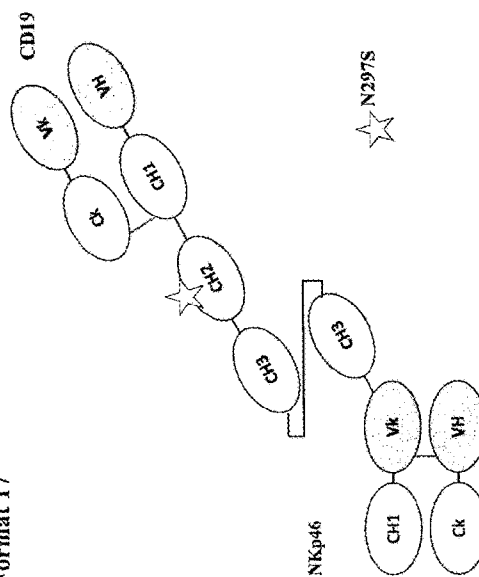


Figure 2C

Format 11



Format 17



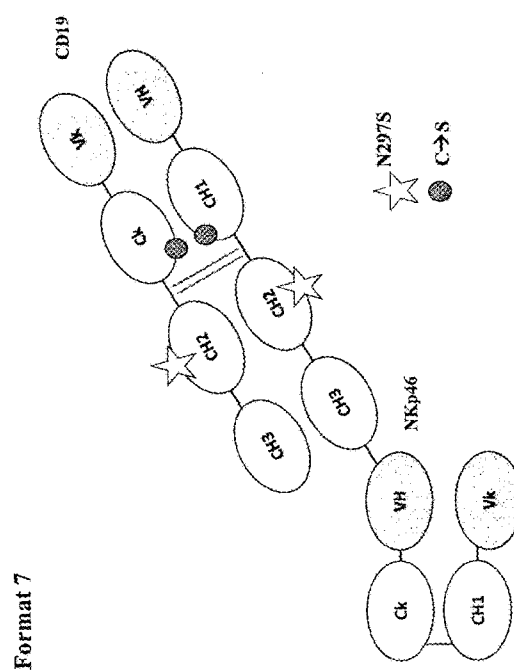
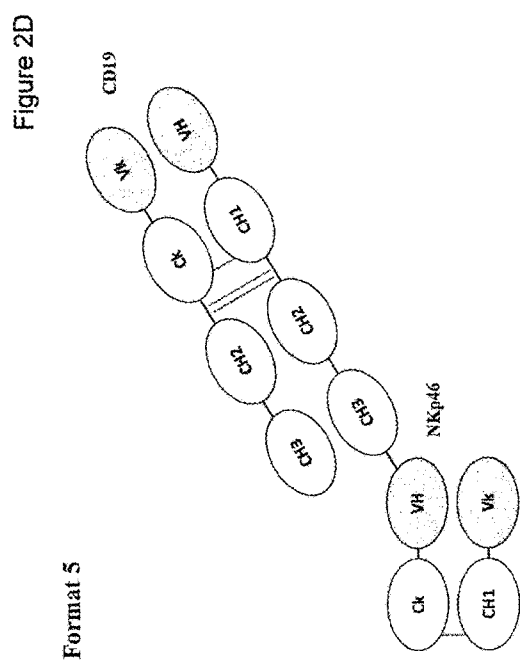
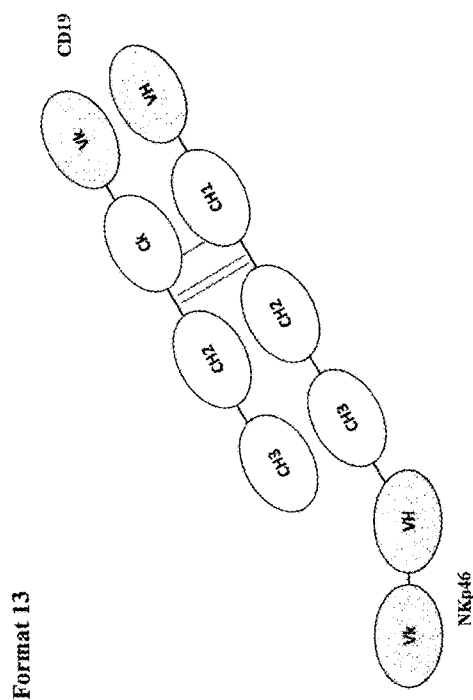
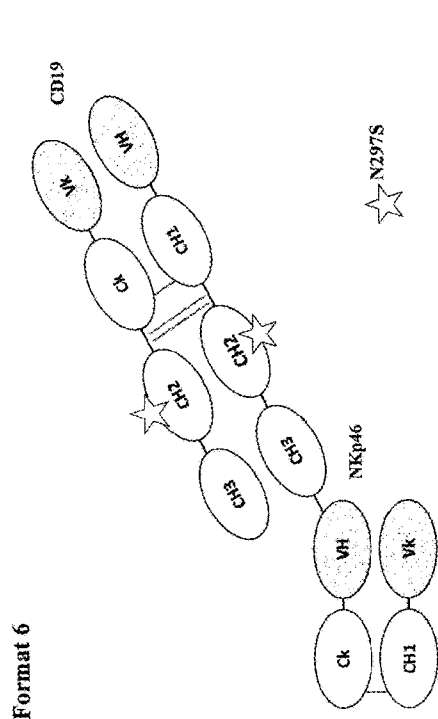
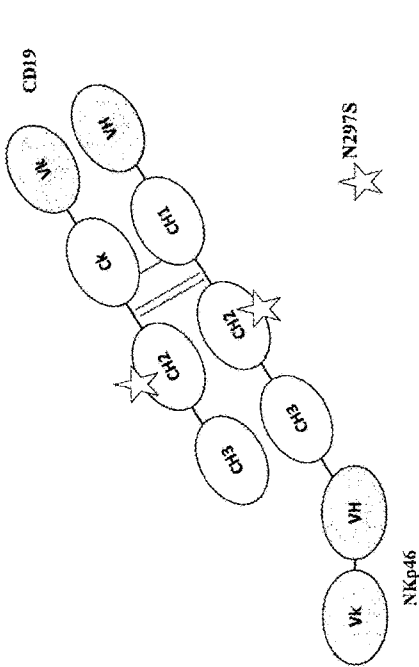
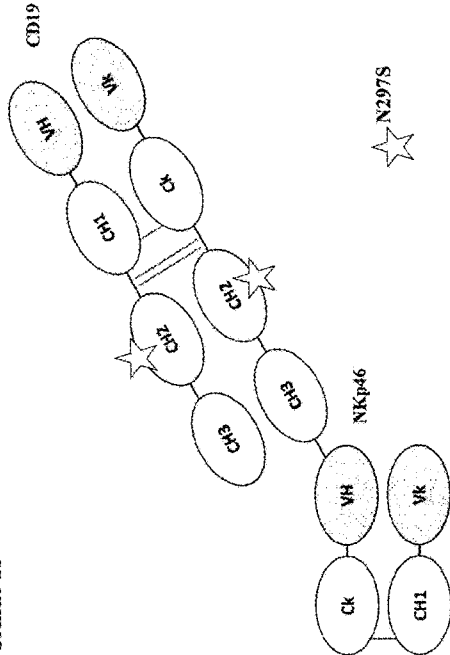


Figure 2E

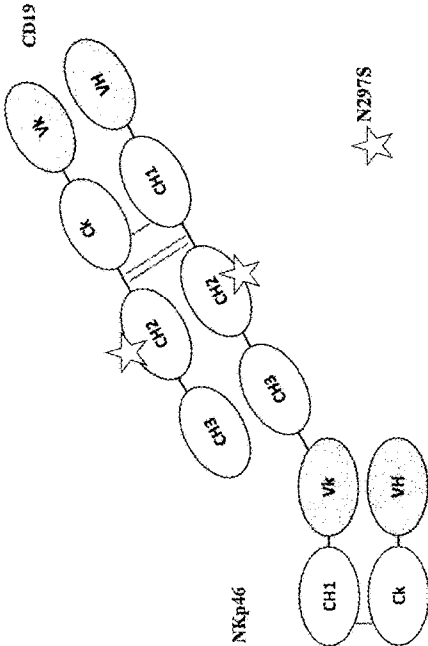
Format 14



Format 15



Format 16



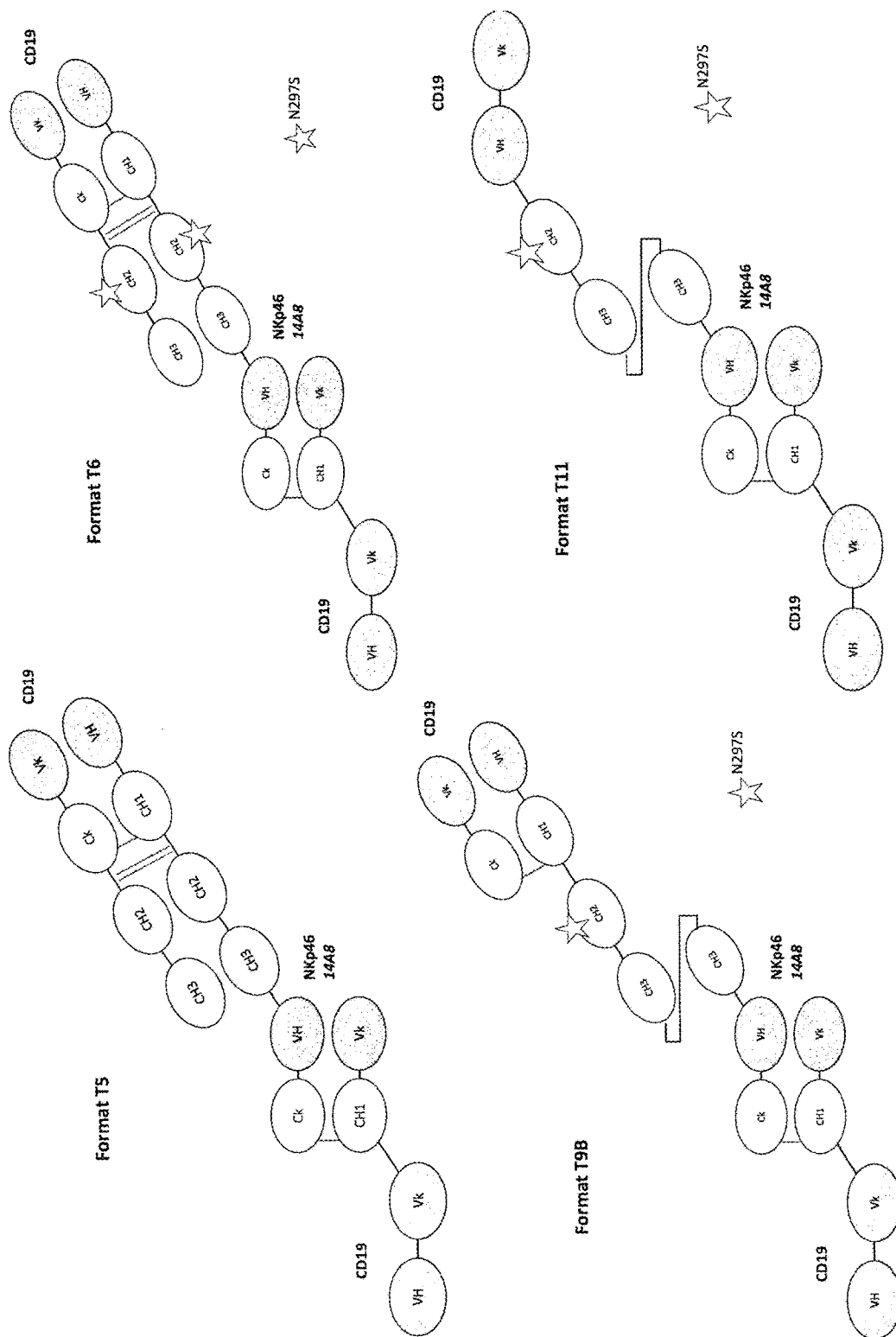


Figure 3A

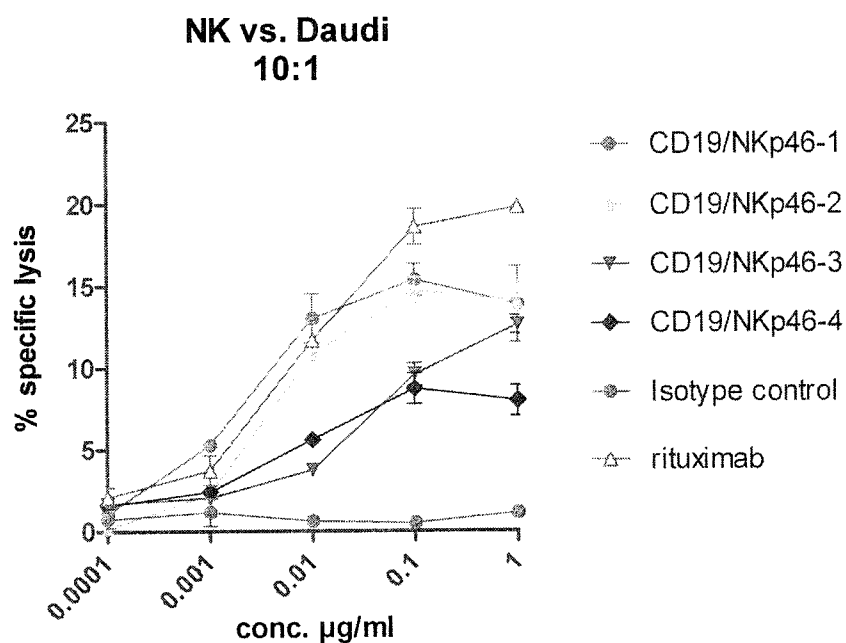


Figure 3B

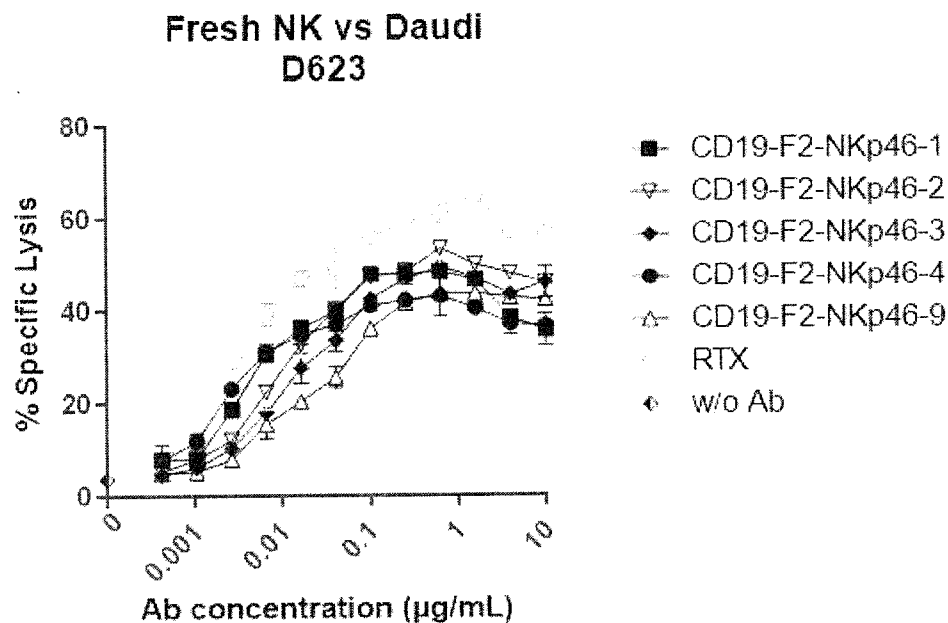


Figure 4A

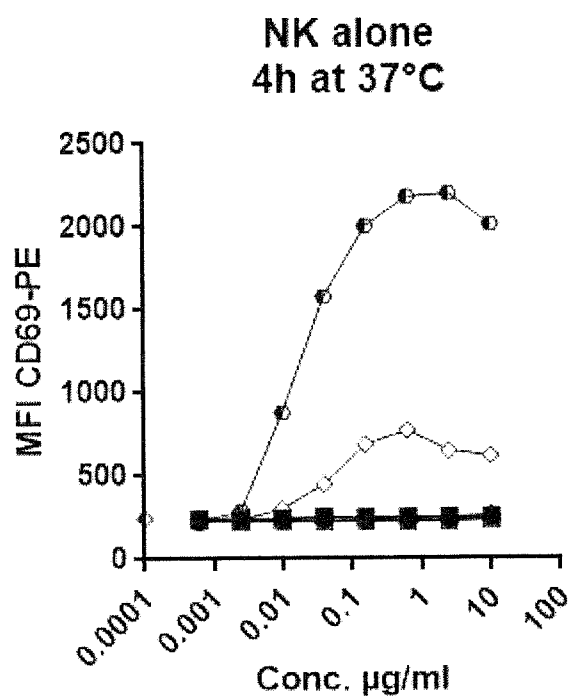
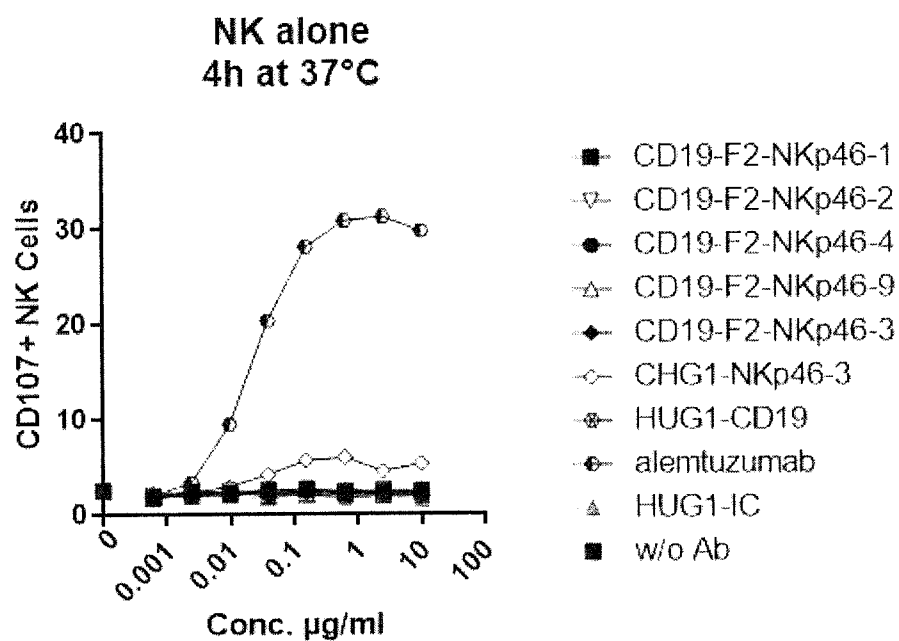


Figure 4B

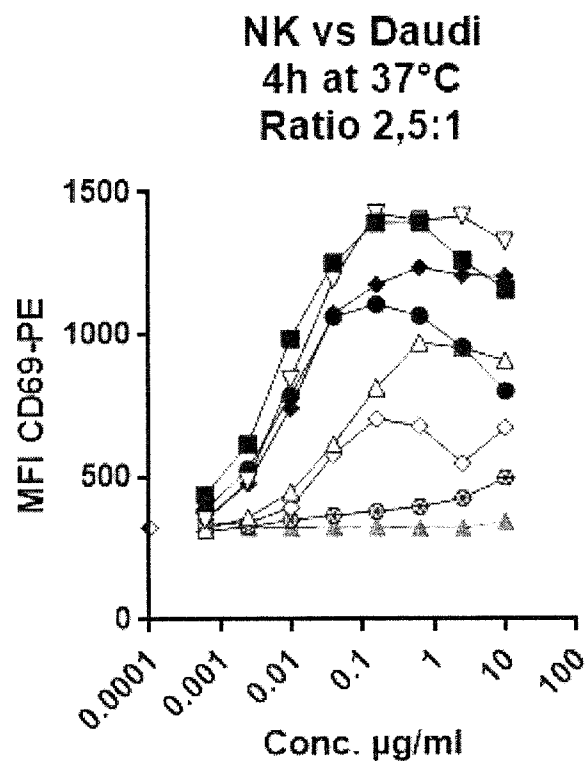
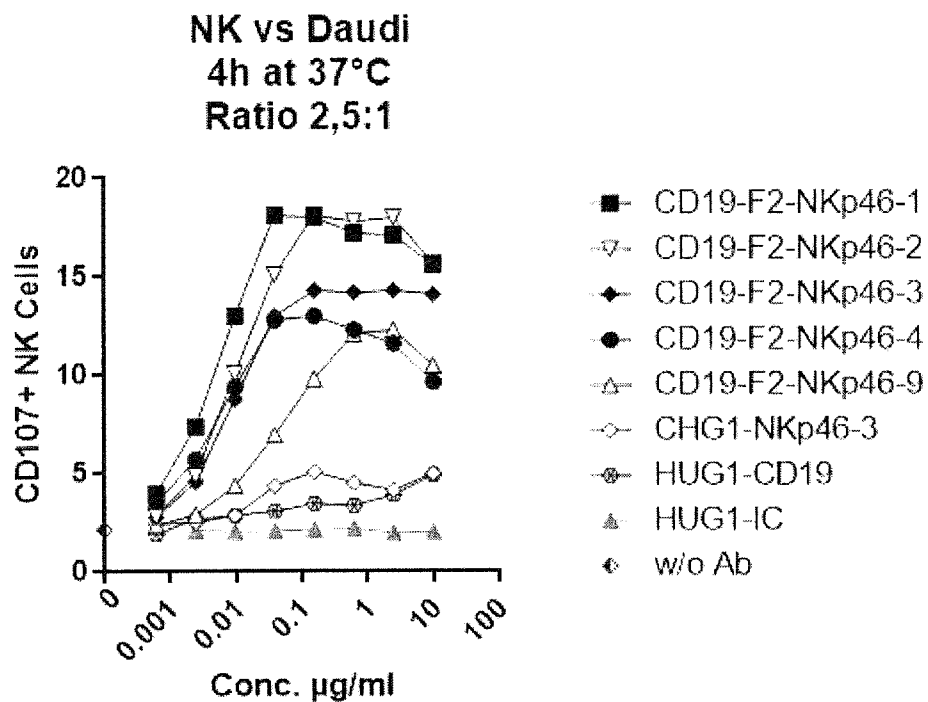


Figure 4C

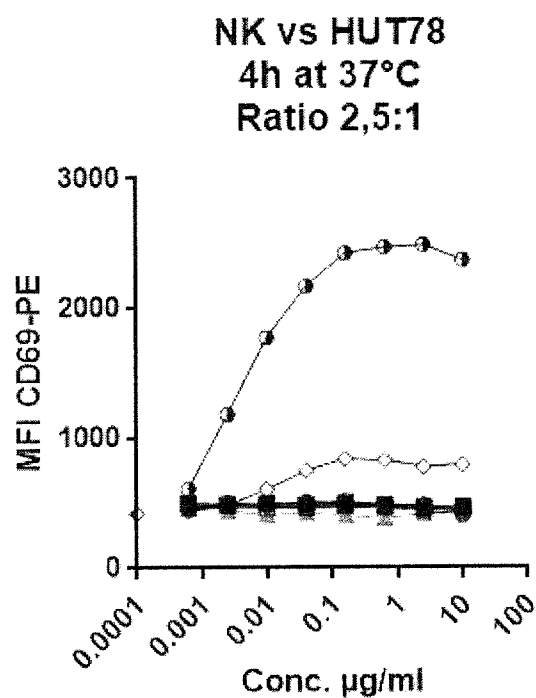
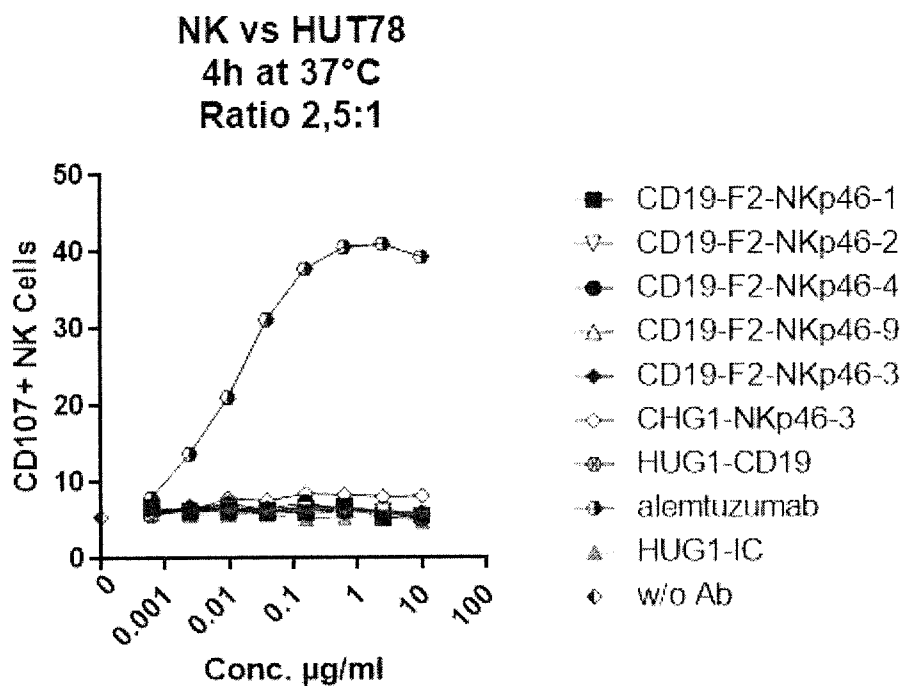


Figure 5A

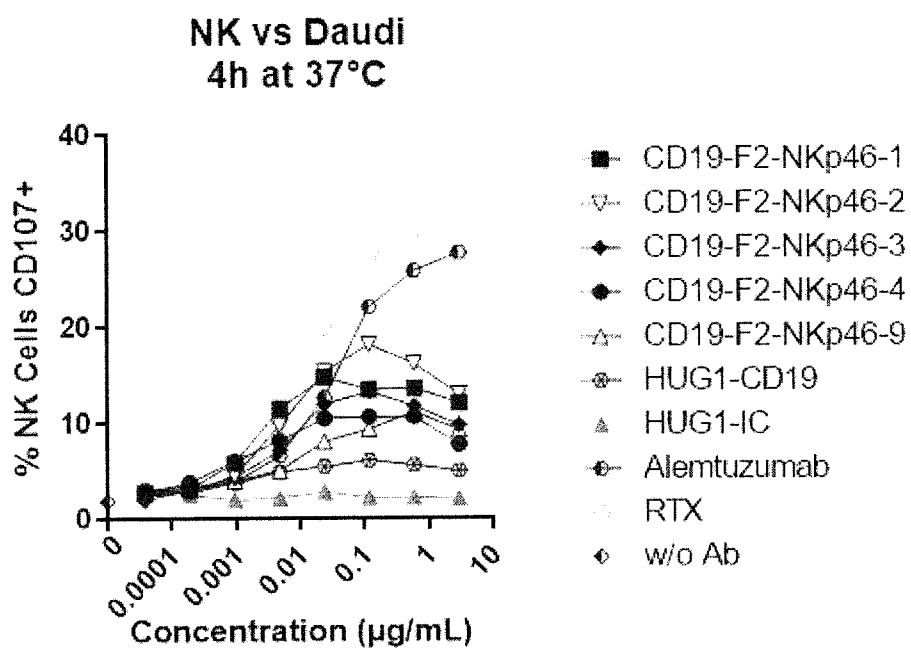


Figure 5B

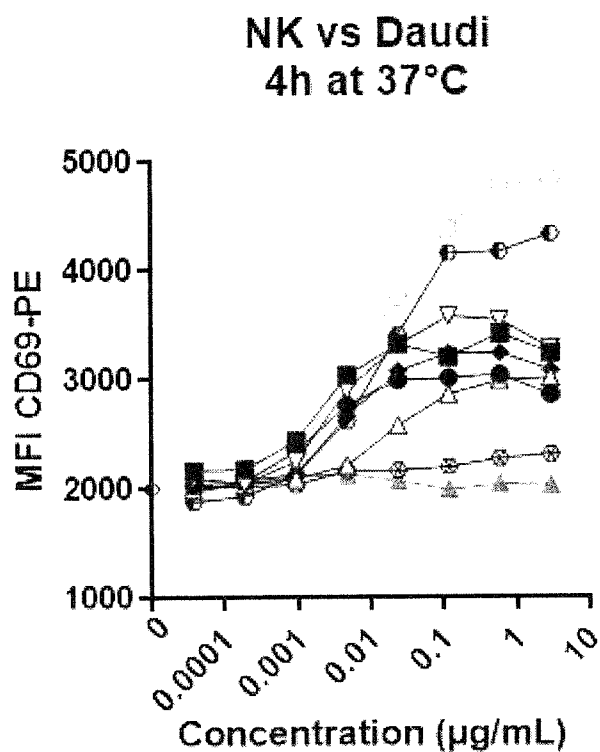


Figure 6A

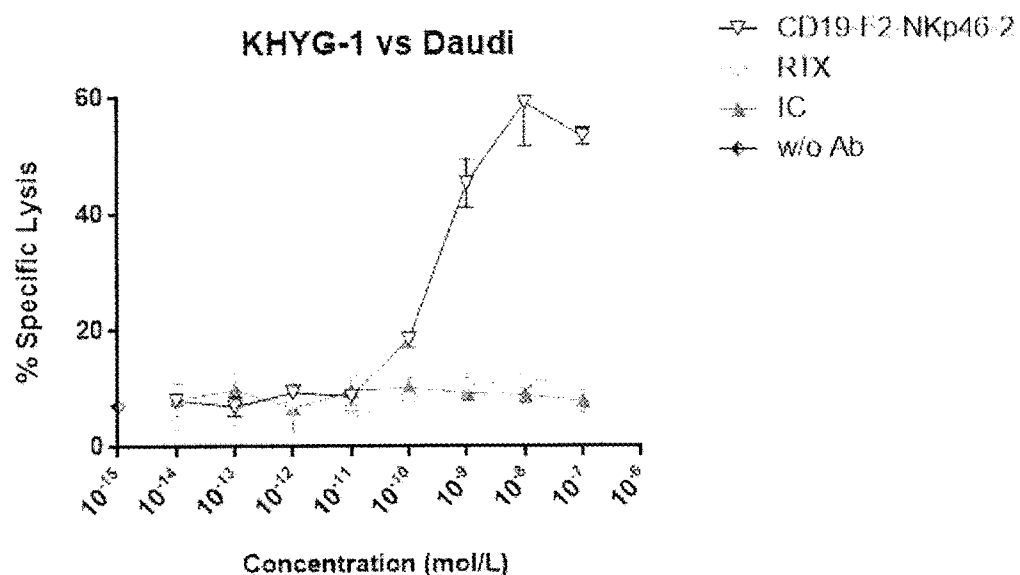


Figure 6B

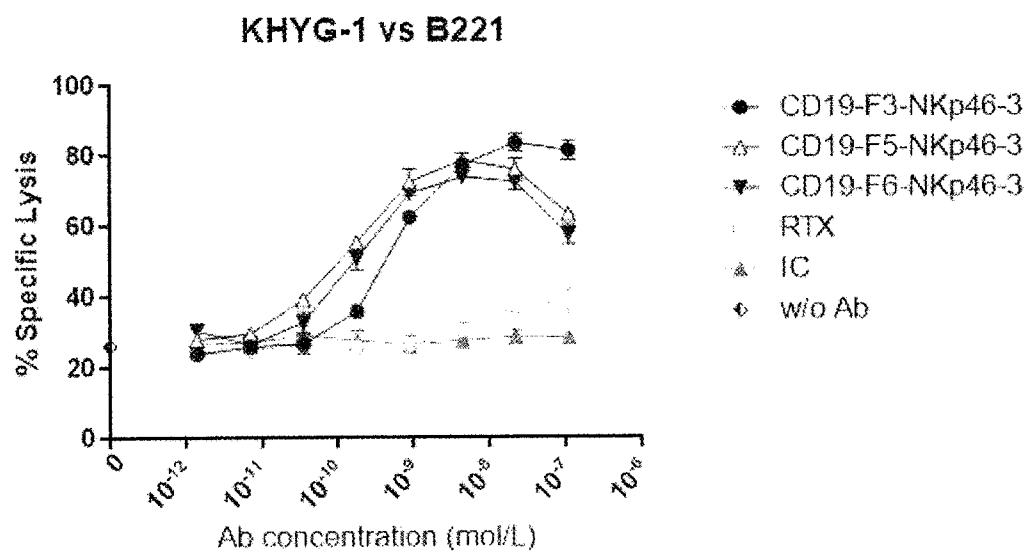


Figure 6C

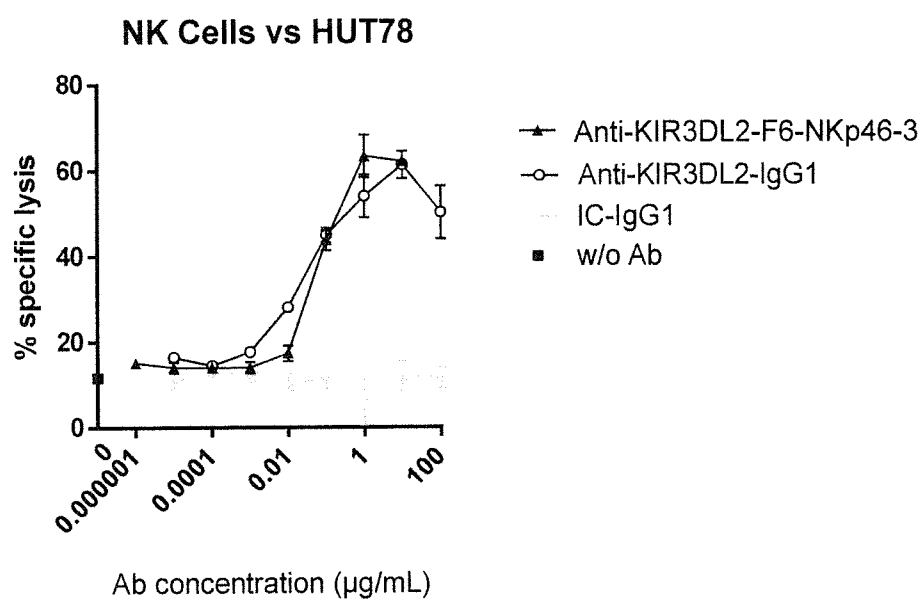


Figure 7

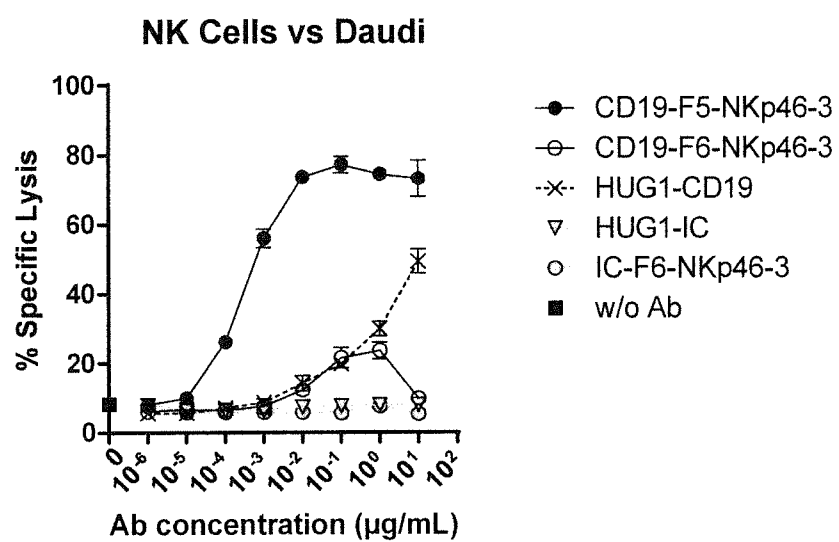


Figure 8

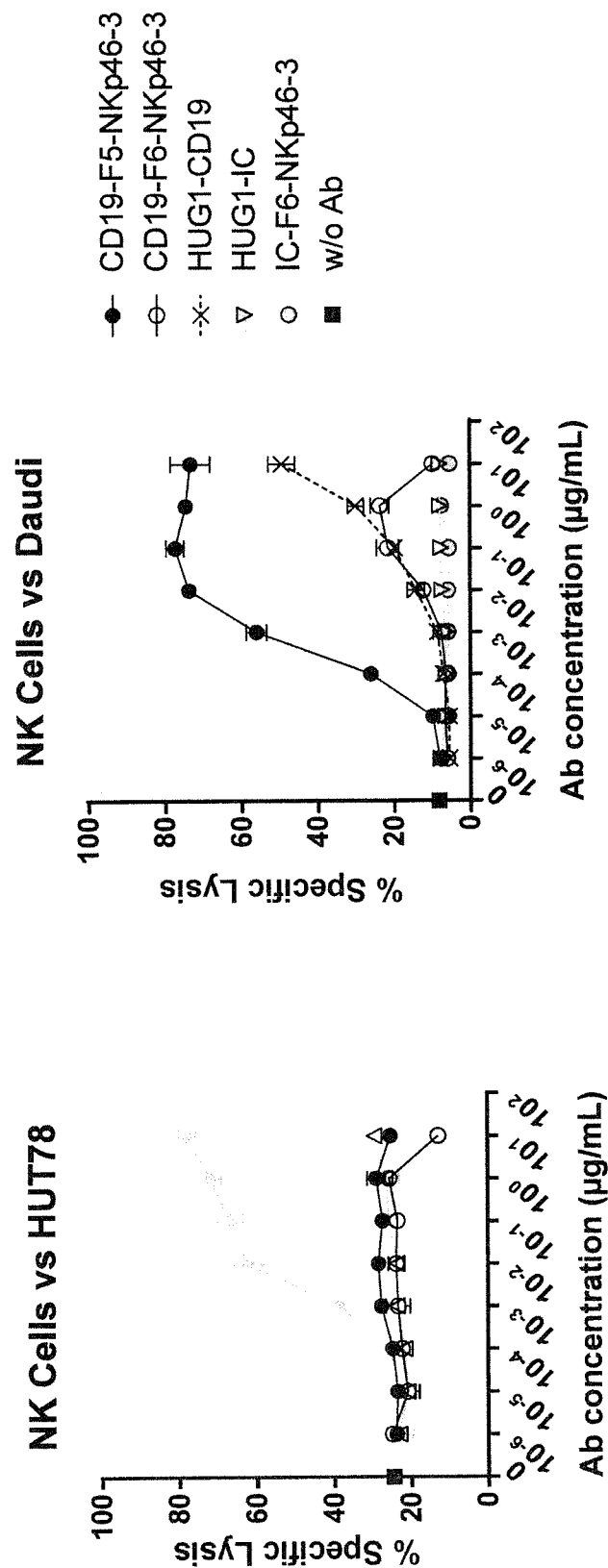
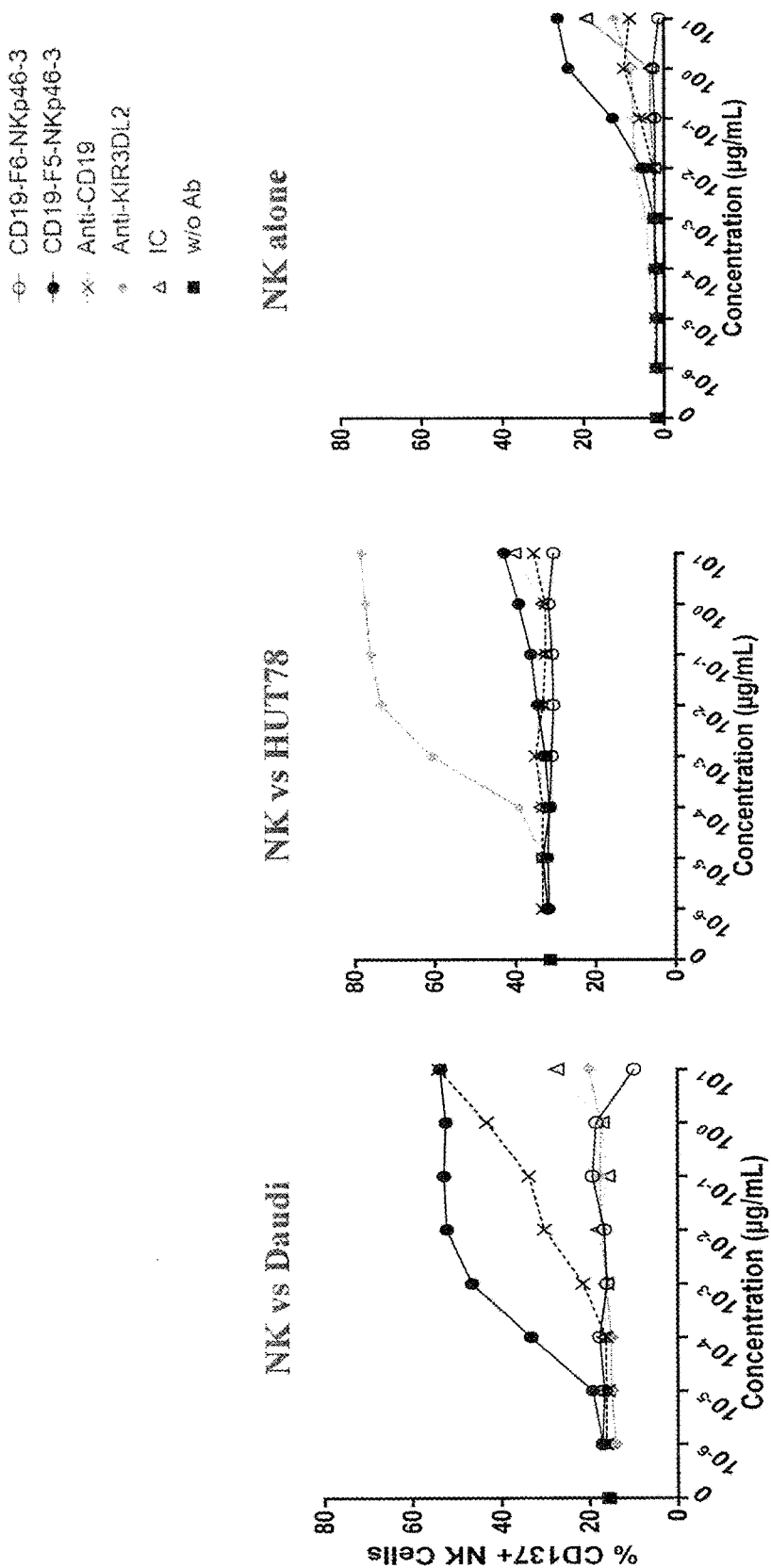


Figure 9



MULTISPECIFIC NK ENGAGER PROTEIN

CROSS-REFERENCE TO RELATED APPLICATIONS

[0001] This application claims the benefit of U.S. Provisional Application No. 62/271,459 filed Dec. 28, 2015, and is a continuation-in-part of PCT patent application No. PCT/EP2015/064063 filed 23 Jun. 2015; both of which are incorporated herein by reference in their entirety; including any drawings and sequence listings.

REFERENCE TO THE SEQUENCE LISTING

[0002] The present application is being filed along with a Sequence Listing in electronic format. The Sequence Listing is provided as a file entitled “NKp46-6_PCT_ST25.txt”, created Jun. 23, 2016, which is 355 KB in size. The information in the electronic format of the Sequence Listing is incorporated herein by reference in its entirety.

FIELD OF THE INVENTION

[0003] Multispecific proteins that bind and specifically redirect effector cells to lyse a target cell of interest via multiple activating receptors are provided. The proteins have utility in the treatment of disease, notably cancer or infectious disease.

BACKGROUND

[0004] Bispecific antibodies binding two different epitopes offer opportunities for increasing specificity, broadening potency, and utilizing novel mechanisms of action that cannot be achieved with a traditional monoclonal antibody. A variety of formats for bispecific antibodies that bind to two targets simultaneously have been reported. Cross-linking two different receptors using a bispecific antibody to inhibit a signaling pathway has shown utility in a number of applications (see, e.g., Jackman, et al., (2010) *J. Biol. Chem.* 285:20850-20859). Bispecific antibodies have also been used to neutralize two different receptors. In other approaches, bispecific antibodies have been used to recruit immune effector cells, where T-cell activation is achieved in proximity to tumor cells by the bispecific antibody which binds receptors simultaneously on the two different cell types (see Baeuerle, P. A., et al, (2009) *Cancer Res* 69(12): 4941-4). These antibodies have been referred to as “Bispecific T-cell engager antibodies” (or “BiTE” antibodies). However, in order to fully activate the T-cell, this T-cell and a cluster of BiTEs must interact on the surface of a target cell. Due to the difficulties of finding antibody variable regions which are functional in the BiTE format, to date only a single immune cell receptor (CD3) has been targeted, in the CD19×CD3 specific antibody blinatumumab. Bispecific antibodies developed to date also include those which link the CD3 complex on T cells to a tumor-associated antigen. Also, bispecific antibodies having one arm which binds CD16 (FcγRIIIa) and another which bound to an antigen of interest such as CD19 have been developed (see Kellner et al. (2011) *Cancer Lett.* 303(2): 128-139).

Natural killer (NK) cells are a subpopulation of lymphocytes that are involved in non-conventional immunity. NK cells provide an efficient immunosurveillance mechanism by which undesired cells such as tumor or virally-infected cells can be eliminated. Characteristics and biological properties of NK cells include the expression of surface antigens

including CD16, CD56 and/or CD57, the absence of the α/γ or γ/δ TCR complex on the cell surface; the ability to bind to and kill cells that fail to express “self” MHC/HLA antigens by the activation of specific cytolytic enzymes, the ability to kill tumor cells or other diseased cells that express a ligand for NK activating receptors, and the ability to release protein molecules called cytokines that stimulate or inhibit the immune response.

[0005] NK cell activity is regulated by a complex mechanism that involves both activating and inhibitory signals. Several distinct NK cell receptors have been identified that play an important role in the NK cell mediated recognition and killing of HLA Class I deficient target cells. One receptor, although not specific to NK cells, is FcγR3a (CD16) which is responsible for NK cell mediated ADCC. NK cells also express a range of other activating and co-activating receptors, including CD137 (4-1BB). Agonist antibodies against anti-4-1BB are in clinical trials in patients with solid tumors, including melanoma, renal carcinoma, and ovarian cancer, and have shown strong activity in different cancer models, including breast cancer, sarcoma, glioma, colon carcinoma, myeloma, and mastocytoma.

[0006] Another NK cell receptor is NKp46, a member of the Ig superfamily. NKp46, is specific to NK cells and the cross-linking thereof, induced by specific mAbs, leads to a strong NK cell activation resulting in increased intracellular Ca^{++} levels, the triggering of cytotoxicity, and lymphokine release. International patent publication number WO2005/105858 (Innate Pharma) discloses the use of monospecific full-length IgG anti-NKp46 antibodies that bind Fcγ receptors for treating hematological malignancies that are Fcγ-positive. Fcγ receptors expressed on tumor cells (e.g. B cell malignancies) were proposed to interact with the Fc domain of the anti-NKp46 antibodies which bound NK cells, such that the activated NK cells are brought into close proximity with target cells via the two reactive portions of the antibody (i.e., the antigen-recognizing domain and the Fc domain), thereby enhancing the efficiency of the treatment.

To date, no NK cell-specific bispecific antibodies have been reported. Rather depleting agents that recruit NK cytotoxicity such as anti-tumor antibodies are typically full-length IgG1 antibodies that mediate ADCC via CD16. Despite the existence of a variety of formats for bispecific antibodies, there remains a need in the art for multispecific proteins with new and well-defined mechanisms of action, particularly those that can provide therapeutic advantages over full-length antibodies.

SUMMARY OF THE INVENTION

[0007] The present invention arises from the discovery of functional multi-specific proteins (e.g. a polypeptide, a single chain protein, a multi-chain protein, including but not limited to antibody-based protein formats) that binds NKp46 on NK cells and to an antigen of interest on a target cell, and is capable of redirecting NK cells to lyse a target cell that expresses the antigen of interest, e.g. a cell that contributes to disease. Provided, inter alia, is a multispecific protein comprising a first antigen binding domain and a second antigen binding domain, wherein one of the first or second antigen binding domains binds to a human NKp46 polypeptide and the other binds an antigen of interest, wherein the multispecific protein binds the NKp46 polypeptide monovalently, and wherein the multispecific protein is capable of directing an NKp46-expressing NK cell to lyse a target cell

expressing the antigen of interest. Advantageously, in one embodiment, the presence of NK cells and target cells, the multi-specific protein can bind (i) to antigen of interest on target cells and (ii) to NKp46 on NK cells, and, when bound to both antigen of interest on target cells and NKp46, can induce signaling in and/or activation of the NK cells through NKp46 (the protein acts as an NKp46 agonist), thereby promoting activation of NK cells and/or lysis of target cells, notably via the activating signal transmitted by NKp46.

[0008] In some embodiment, the multispecific protein comprises at least a portion of a human Fc domain, e.g. an Fc domain that is bound by FcRn.

[0009] In certain embodiments, the multispecific antibody is designed to have decreased or substantially lack FcγR binding compared to a conventional full-length human IgG1 antibody. Optionally the multispecific protein has decreased or abolished binding to a human CD16, CD32A, CD32B and/or CD64 polypeptide, compared to a full length wild type human IgG1 antibody. In other embodiments, the multispecific protein is designed to retain substantial FcγR binding, e.g., compared to a conventional full-length human IgG1 antibody. Optionally the multispecific protein binds (e.g. via its Fc domain) to a human CD16, CD32A, CD32B and/or CD64 polypeptide.

[0010] The present invention arises in part from the observation that multispecific proteins which bind to an antigen of interest and which are further engineered to bind to NKp46 on NK cells monovalently, and to CD16 or CD16A, exhibit an enhanced capability to promote NK cell-mediated target cell lysis (relative to conventional antibodies). Through comparison of molecules having different functionalities, it was observed that such multispecific proteins elicited the individual and combined effects of these receptors thereby better promoting the lysis of target cells by NK cells. As described in detail herein, various functional multispecific proteins were constructed that bind (a) NKp46 and CD16 on NK cells and (b) to an antigen of interest on a target cell, which are capable of redirecting NK cells to lyse a target cell that expresses the antigen of interest, e.g. a cell that contributes to a disease such as cancer or infection. Moreover, despite binding to NKp46 on NK cells, advantageously these multispecific proteins do not induce lysis of NK cells themselves.

[0011] Also provided are novel formats for multispecific Fc proteins that are capable of activating CD16 and NKp46 and which can be used to promote NK-mediated killing of desired target cells. The potency in target cell killing demonstrated by the subject multispecific proteins which bind CD16 and which in addition bind to NKp46 and to an antigen of interest is believed to arise at least in part from an induction and upregulation of the co-activating receptor CD137 which is expressed on the surface NK cells. Particularly, CD137 upregulation occurs on resting NK cells, in the absence of target cells (as well as in the presence of target cells), and without the induction of CD16-mediated lysis of NK cells. NK cells with increased CD137 expression are known to be highly active against target cells (e.g. tumor cells) expressing CD137L (CD137 ligand). Consequently, the instant multispecific proteins which bind CD16 in addition to monovalently binding NKp46 can provide a means to upregulate CD137. Further advantageously, the multispecific protein when binding additionally to an antigen of interest expressed by a target cell, can elicit a multi-pronged recognition of target cells that involves multiple activating

receptors expressed on effector cells. Also, despite an ability to cause upregulation of CD137 on NK cells that is comparable to human IgG1 antibodies (in the absence of target cells), surprisingly the multispecific proteins are far more potent in inducing NK cell-mediated lysis of tumor cells than human IgG1 antibodies. This would suggest that the subject multispecific proteins trigger the combined effects of NKp46-, CD16- and/or CD137-mediated activation thereby providing for a synergistic or additive enhancement in the induction of NK cell cytotoxicity by CD16⁺ NKp46⁺ NK cells. Additionally, independently of any contribution of CD137, these multispecific proteins advantageously are able to potently mobilize both CD16⁺ and CD16⁻ NK cells (all NK cells are NKp46⁺).

[0012] Furthermore, despite that the subject multispecific proteins are bound by CD16, unexpectedly they do not induce or increase down-modulation or internalization of the antigen of interest, even when targeting antigens of interest known to be susceptible to down-modulation or internalization when bound by conventional antibodies (such as full length human IgG1's). Based thereon, the subject multispecific proteins should be well suited for targeting antigens of interest expressed by target cells, e.g., tumor or infected cells, including antigens which are known to be capable of undergoing down-modulation or internalization when bound by conventional antibodies (e.g. antibodies with human IgG1 Fc domains that retain CD16 binding). This is a huge therapeutic benefit since it is known in the art that antigen internalization can substantially impede the ability of conventional human IgG1 antibodies to mediate ADCC against a target cell.

[0013] Therefore, in one embodiment, multispecific proteins are provided which bind to an antigen of interest expressed on the surface of a cell monovalently, wherein the protein does not increase or induce down-modulation or intracellular internalization of the antigen of interest.

[0014] In another embodiment the invention provides a multispecific protein that comprises an antigen binding domain that binds to a human a NKp46 polypeptide monovalently (e.g., via a single antigen binding domain), and which is capable of binding to human CD16, and which when incubated in soluble form with effector cells expressing a NKp46 polypeptide and CD16 (e.g. human NKp46⁺ CD16⁺ NK cells), optionally in the absence and/or presence of target cells, further optionally in the absence of other cells, can cause an increase or induction of CD137 polypeptide expression on the surface of the effector cells (e.g. without inducing detectable lysis of the NK cells). Optionally, the multispecific protein further binds to an antigen of interest.

[0015] In another embodiment the invention provides a multispecific protein that comprises (i) a first antigen binding domain ("ABD") that binds to a human NKp46 polypeptide, (ii) an Fc domain that binds to human CD16A, and (iii) a second antigen binding domain that binds to an antigen of interest expressed by a target cell. In one embodiment, the multispecific protein is capable of directing an NKp46-expressing NK cell to lyse a target cell expressing the antigen of interest, wherein said lysis of the target cell is mediated by NKp46-signaling, wherein optionally said lysis of the target cell is mediated by a combination of NKp46-mediated signaling and CD16-mediated antibody-dependent cell-mediated cytotoxicity ("ADCC").

[0016] In another embodiment the invention provides a multispecific antigen binding protein which comprises (i) a monovalent antigen binding polypeptide (“ABD”) which binds to a human Nkp46 polypeptide (i) an ABD which binds to an antigen of interest, wherein said antigen of interest optionally comprises an antigen expressed by a tumor cell or an infectious agent and (iii) a CD16A binding polypeptide, optionally an Fc polypeptide which Fc polypeptide is optionally modified to enhance CD16A binding relative to the corresponding wild-type Fc polypeptide, wherein the multispecific protein is capable of directing an Nkp46-expressing NK cell to lyse a target cell expressing the antigen of interest by Nkp46-signaling.

[0017] In another embodiment the invention provides an isolated multispecific Fc-protein comprising a first antigen binding domain, a second antigen binding domain, and an Fc domain or portion thereof, which optionally may be modified, that is capable of binding human CD16 or CD16A, wherein one of the first or second antigen binding domains binds to a human Nkp46 polypeptide and the other binds an antigen of interest, and wherein the multispecific protein is capable of directing an Nkp46-expressing NK cell to lyse a target cell expressing the antigen of interest. In one embodiment, the protein causes lysis of the target cell by enhancing or inducing Nkp46-signaling, wherein optionally lysis of the target cell is mediated by a combination of Nkp46 signaling and CD16-mediated antibody-dependent cell-mediated cytotoxicity (“ADCC”). Optionally, the multispecific protein further comprises a third or fourth or more antigen binding domains that each bind to an antigen of interest, e.g., one other than a Nkp46 polypeptide, i.e., an antigen of interest expressed by a target cell such as a tumor cell or infectious agent, wherein the antigen of interest is the same or different from the antigen of interest bound by the second antigen binding domain. In one embodiment, the third antigen binding domain binds to the same antigen of interest as the second antigen binding domain, optionally further wherein the third antigen binding domain binds to the same epitope or a different epitope on the antigen of interest as the second antigen binding domain. In one embodiment, the antigen of interest is a cancer antigen. In one embodiment, the antigen of interest is a protein expressed (optionally over-expressed) on the surface of malignant immune cells, e.g. cells involved in a hematological malignancy, leukemia cells, lymphoma cells, a CD19 protein, a CD20 protein, etc. In one embodiment, the protein is used to treat a hematological malignancy, e.g., a leukemia or lymphoma cells. In another embodiment, the antigen of interest is a protein expressed (optionally over-expressed) on the surface of infected cells or by an infectious agent such as virally, bacterially or parasite infected cells.

[0018] Optionally, the subject multispecific polypeptides when incubated in soluble form with effector cells expressing Nkp46 and CD16 (e.g. human Nkp46⁺CD16⁺ NK cells), optionally in the presence or absence of other cells (e.g. target cells), can elicit an increase or induction of CD137 polypeptide expression on the surface of the NK cells (e.g. without inducing detectable lysis of the NK cells). Optionally, in the presence of target cells expressing the antigen of interest and Nkp46⁺CD16⁺ NK cells, the multispecific protein can induce the activation of NK cells and/or lysis of target cells, in particular via the activating signal(s) transmitted by any combination of Nkp46, CD16 and/or CD137.

[0019] In some embodiments, the multispecific protein binds to Nkp46 in monovalent fashion. In one aspect of any embodiment, the multispecific protein comprises an Nkp46-binding ABD which binds to an Nkp46 polypeptide monovalently.

[0020] In some embodiments in the presence of target cells and NK cells, the multispecific protein is capable of inducing an increase in cell surface CD137, e.g., on NK cells that express Nkp46 and CD16 (Nkp46⁺CD16⁺ NK cells).

[0021] In some embodiments in the presence of target cells and NK cells, the multispecific protein is capable of inducing signaling by NK cells through Nkp46.

[0022] In some embodiments in the presence of target cells and NK cells, the multispecific protein lacks the ability to induce Nkp46-mediated signaling or cellular activation of an NK cell (independently of CD16), when incubated with NK cells in the absence of target cells (cells expressing the antigen of interest); for example the multispecific protein lacks the ability to induce Nkp46-mediated signaling or NK cell activation when incubated with NK cells in the absence of target cells, when the protein is modified to comprise an Fc domain that does not bind CD16 (e.g. an Fc region containing a N297S substitution); or the multispecific protein lacks the ability to induce Nkp46-mediated signaling or NK cell activation when incubated with Nkp46⁺CD16⁻ NK cells, in the absence of target cells. In any of the multispecific proteins described herein, such multispecific protein potentially possesses the following characteristics:

[0023] (a) capable of inducing human NK cells that express CD16 and Nkp46 (Nkp46⁺CD16⁺ NK cells) to lyse target cells expressing the antigen of interest, when incubated in the presence of the NK cells and target cells; and

[0024] (b) capable of inducing an increase in cell surface CD137 when incubated (e.g. in soluble form) with NK cells that express CD16 and Nkp46, optionally in the presence of target cells, or in the absence of target cells.

[0025] In another embodiment the invention provides multispecific protein formats adapted for use in an Nkp46-based NK cell engager, including antibody-based formats comprising antigen binding domain(s) and/or constant region domain(s) from immunoglobulins capable of forming a dimeric Fc domain that binds to human CD16 (and optionally further binding to FcRn and/or other human Fcγ receptors). By combining the NK-selective expression of Nkp46 with multispecific (e.g. bispecific) antibody formats in which the multispecific proteins retain and/or have increased binding to human Fcγ receptor via an Fc domain, the invention provides multispecific antibody formats with favorable pharmacology due to FcRn binding which can direct NK cell cytotoxicity to a target of interest, and which further possess increased ability to lyse target cells via the combined action of activating receptors CD16, Nkp46, and in addition optionally CD137.

[0026] In another aspect of any embodiment described herein, the multispecific protein can be characterized by a lack of agonist activity for Nkp46 when incubated with Fcγ receptor-negative NK cells (e.g. as purified Nkp46⁺CD16⁻ NK cells) and in the absence of target cells (e.g. cells expressing the antigen of interest).

[0027] In another embodiment the invention provides a method for identifying, testing and/or producing a multispecific protein that binds Nkp46 and CD16 on an NK cell and an antigen of interest expressed by a target cell, the method comprising:

[0028] (a) assessing whether the multispecific protein is capable of inducing an increase in cell surface CD137 on NK cells when incubated with CD16-expressing NK cells (e.g. NKp46⁺CD16⁺ NK cells); and

[0029] (b) assessing whether the multispecific protein has the ability to induce NK cells that express CD16 and NKp46 to lyse target cells, when incubated in the presence of the NK cells and target cells.

[0030] Optionally, in any of the foregoing the NK cells are purified NK cells.

[0031] In another embodiment the invention provides a method for identifying, testing and/or producing a multispecific protein, the method comprising providing a plurality of multispecific proteins that bind NKp46 and CD16 on an NK cell and an antigen of interest expressed by a target cell:

[0032] (a) assessing whether each multispecific protein has the ability to induce NK cells that express CD16 and NKp46 to lyse target cells, when incubated in the presence of the NK cells and target cells;

[0033] (b) optionally, further assessing each multispecific protein for the ability to induce an increase in cell surface CD137 when incubated with CD16-expressing NK cells (e.g. NKp46⁺CD16⁺ NK cells); and

[0034] (c) selecting a multispecific protein (e.g. for use as a medicament, for further evaluation, for further production, etc.) if the multispecific protein:

[0035] a. has the ability to induce NK cells that express CD16 and NKp46 to lyse target cells, when incubated in the presence of the NK cells and target cells, and

[0036] b. optionally, has the ability to induce an increase in cell surface CD137 when incubated with CD16-expressing NK cells.

[0037] In another embodiment the invention provides a method for identifying, testing and/or producing a multispecific protein, the method comprising providing a plurality of multispecific proteins that bind NKp46 and CD16 on an NK cell and an antigen of interest expressed by a target cell:

[0038] (a) assessing each multispecific protein to determine whether it is capable of inducing NKp46-mediated signaling or NK cell activation of an NK cell independently of CD16, when incubated with NK cells in the absence of target cells;

[0039] (b) assessing each multispecific protein to determine whether the multispecific protein has the ability to induce NK cells to lyse target cells, when incubated in the presence of the NK cells and target cells;

[0040] (c) optionally, further assessing each multispecific protein to determine whether it is capable of inducing an increase in cell surface CD137 when incubated with CD16-expressing NK cells (e.g. NKp46⁺CD16⁺ NK cells); and

[0041] (d) selecting a multispecific protein (e.g. for use as a medicament, for further evaluation, for further production, etc.) if the multispecific protein:

[0042] a. lacks the ability to induce NKp46-mediated signaling or NK cell activation in an NK cell independently of CD16, when incubated with NK cells in absence of target cells;

[0043] b. has the ability to induce NK cells to lyse target cells, when incubated with NK cells and target cells, and

[0044] c. optionally, has the ability to induce an increase in cell surface CD137 when incubated with CD16-expressing NK cells.

[0045] In another embodiment the invention provides a multispecific protein that binds human CD16 (e.g. polypeptide, non-antibody polypeptide, or antibody), comprising: (a) a first antigen binding domain; and (b) a second antigen binding domain, wherein one of the first antigen binding domains binds NKp46 and the other binds to an antigen of interest on a target cell (other than NKp46), wherein the multispecific protein is capable of directing NKp46-expressing NK cells to lyse said target cell. In one embodiment, the protein comprises at least a portion of a human Fc domain, optionally wherein the Fc domain is dimeric, and is bound by human CD16 (and further optionally wherein the Fc domain is also bound by FcRn), and still further optionally wherein the multispecific antibody comprises an Fc domain comprising a modification (compared to a wild-type Fc domain) that results in increased binding to CD16; in one embodiment, the Fc domain is interposed between the two ABDs (one ABD is placed N-terminal and the other is C-terminal to the Fc domain).

[0046] In one aspect, the multispecific protein comprises two or more polypeptide chains, i.e. it comprises a multi-chain protein. For example, the multispecific protein or multi-chain protein can be a dimer, trimer or tetramer or may comprise more than 4 polypeptide chains.

[0047] An antigen binding domain positioned on a polypeptide chain can itself bind to its target (i.e., NKp46 or an antigen of interest) (e.g. an scFv or single antigen binding domain) or can optionally bind its target when it is in association or together with one or more complementary protein domains (antigen binding domain or domains) positioned on a different polypeptide chain, wherein these polypeptide chains associate to form a multimer (e.g. dimer, trimer, etc.).

[0048] In one aspect, the multispecific protein binds an NKp46 polypeptide (e.g. expressed on the surface of a NK cell) in monovalent fashion. In one aspect, the multispecific protein binds the antigen of interest in monovalent fashion.

[0049] In another embodiment the invention provides multispecific proteins having a structure in which the freedom of motion (intrachain domain motion) or flexibility of one or more antigen binding domains (ABDs) is increased, e.g. compared to the ABDs of a conventional human IgG antibody. In one embodiment, provided is a multispecific protein comprising a structure that permits the antigen binding site of the first antigen binding domain and the antigen binding site of the second antigen binding domain to be separated by a distance that results in enhanced function, e.g., the ability of the multispecific protein to induce NKp46 signaling and lysis of target cells, e.g., optionally a distance of less than 80 ångström (Å). Multispecific proteins wherein the ABDs possess greater flexibility and/or are separated by an optimized distance may enhance the formation of a lytic NKp46-target synapse, thereby potentiating NKp46-mediated signaling.

[0050] In one embodiment, the invention provides multispecific proteins having increased freedom of motion of the antigen binding domains (e.g. compared to the ABDs of a conventional human IgG antibody, e.g., a human IgG1 antibody). One example of such a protein is a monomeric or multimeric Fc domain-containing protein (e.g. a heterodimer or heterotrimer) in which an antigen binding domain

(e.g., the ABD that binds NKp46 or the ABD that bind the antigen of interest) is linked or fused to an Fc domain via a flexible linker. The linker can provide flexibility or freedom of motion of one or more ABDs by conferring the ability to bend thereby potentially decreasing the angle between the ABD and the Fc domain (or between the two ABDs) at the linker. Optionally, both antigen binding domains (and optionally more if additional ABDs are present in the multispecific protein) are linked or fused to the Fc domain via a linker, typically a flexible peptide linker. Optionally, other sequences or domains such as constant domains which optionally may be modified to alter (enhance or inhibit) one or more effector functions are placed between the Fc domain and an ABD, e.g. such that the ABD is fused to the Fc domain via a flexible linker and a constant region. The antigen binding domain can for example be comprised of variable region(s), a dAb, a VhH or a non-Ig scaffold. The antigen binding domain may be present in its entirety on a single polypeptide chain or may be formed from the association with a domain present on a separate polypeptide chain. Optionally, the protein with increased freedom of motion permits the protein to adopt a conformation in which the distance between the NKp46 binding site and the antigen of interest binding site is less than observed in proteins in which both binding domains were Fabs, or less than in full length antibodies.

[0051] An ABD can be connected to the Fc domain (or CH2 or CH3 domain thereof) via a flexible linker (optionally via intervening sequences such as constant region domains or portions thereof, e.g. CH1 or C κ). The linker can be a polypeptide linker, for example peptide linkers comprising a length of at least 5 residues, at least 10 residues, at least 15 residues, at least 20 residues, at least 25 residues, at least 30 residues or more. In other embodiments, the linkers comprises a length of between 2-4 residues, between 2-4 residues, between 2-6 residues, between 2-8 residues, between 2-10 residues, between 2-12 residues, between 2-14 residues, between 2-16 residues, between 2-18 residues, between 2-20 residues, between 2-30 residues, between 10-24 residues, between 10-26 residues, between 10-30 residues, or between 10-50 residues. Optionally a linker comprises an amino acid sequence derived from an antibody constant region, e.g., an N-terminal CH1 or hinge sequence. Optionally a linker comprises the amino acid sequence RTVA. Optionally a linker is a flexible linker predominantly or exclusively comprised of glycine and/or serine residues, e.g., comprising the amino acid sequence GEGTSTGS(G₂S)₂GGAD or the amino acid sequence (G₄S)₃.

[0052] In one embodiment, the Fc domain is interposed between the two ABDs (one ABD is placed N-terminal and the other is C-terminal to the Fc domain). The subject multispecific proteins (e.g. dimers, trimers, tetramers) may in some embodiments comprise a domain arrangement as follows, in which domains can be placed on any of the 2, 3 or 4 polypeptide chains, wherein an Fc domain is interposed between the antigen binding domains, and wherein a flexible linker is present between at least one of the ABDs and the Fc domain:

[0053] (ABD₁) (Fc domain) (ABD₂).

[0054] In another embodiment, the multispecific proteins (e.g. dimers, trimers, tetramers) may comprise a domain arrangement of any of the following in which domains can be placed on any of the 2, 3 or 4 polypeptide chains, wherein the Fc domain is not interposed between ABDs (e.g. the

protein has a terminal or distal Fc domain), and wherein a flexible linker is present between at least one of the ABDs and the Fc domain:

[0055] (Fc domain) (ABD₁ and ABD₂),

[0056] or

[0057] (ABD₁ and ABD₂) (Fc domain).

[0058] In the above-described domain arrangements, one of ABD₁ and ABD₂ is an antigen binding domain that binds NKp46 and the other is an antigen binding domain that binds an antigen of interest, and wherein the linker is a flexible polypeptide linker. The Fc domain can be a dimeric Fc domain (e.g. that binds human FcRn and/or Fc γ receptors). In one embodiment, each of ABD₁ and ABD₂ are formed from two variable regions present within tandem variable regions, wherein the variable regions that associate to form a particular ABD can be on the same polypeptide chain or on different polypeptide chains. In another embodiment, one of ABD₁ and ABD₂ comprises a tandem variable region and the other comprises a Fab structure.

[0059] The invention also identifies specific epitopes on NKp46 which are well suited for targeting with NKp46 binding moieties, including the multispecific polypeptides disclosed herein. For example, bispecific or multispecific proteins which bind to one or more of these NKp46 epitopes possess advantageous properties, notably high efficacy in causing or directing NK cells to lyse target cells (e.g. via NKp46-mediated signaling). Provided also are CDRs of different anti-NKp46 antibodies suitable for use in the construction of efficient multispecific proteins, e.g., bispecific and trispecific proteins particularly those which potentially lyse a target cell of interest, and amino acid and nucleic acid sequences of exemplary multispecific proteins and nucleic acids which encode these proteins.

[0060] In one aspect, the protein (and/or the antigen binding domain thereof that binds NKp46) competes for binding to a NKp46 polypeptide with any one or any combination of monoclonal antibodies NKp46-1, -2, -3, -4, -6 or -9 or an Anti-CD19-F5-Anti-NKp46 antibody that comprises such NKp46-1, -2, -3, -4, -6 or -9 bispecific antibodies. In one embodiment, the antigen binding domain that binds NKp46 binds an epitope within (partly or fully within) the D2 (proximal) domain of the NKp46 polypeptide. In one embodiment, the antigen binding domain that binds NKp46 binds an epitope within (partly or fully within) the D1 (distal) domain of the NKp46 polypeptide. In one embodiment, the antigen binding domain that binds NKp46 binds an epitope on an NKp46 polypeptide of SEQ ID NO:1 comprising one, two, three or more residues selected from the residues bound by any one or combination of antibodies NKp46-1, -2, -3, -4, -6 or -9 or an Anti-CD19-F5-Anti-NKp46 antibody that comprises such NKp46-1, -2, -3, -4, -6 or -9 bispecific antibodies. In one embodiment the multispecific protein is capable of binding to human neonatal Fc receptor (FcRn). In one embodiment the multispecific protein has decreased or abolished binding to a human and/or non-human primate (e.g. cynomolgus monkey) Fc γ receptor, e.g., compared to a full length wild type human IgG1 antibody. In one embodiment the multispecific protein is capable of inducing NK-mediated lysis (e.g. as well as or better than a full length human wild-type IgG1 antibody).

[0061] In one embodiment of any of the multispecific proteins described herein, the antigen binding domain that binds to an antigen of interest binds to an antigen (e.g. polypeptide) expressed by a target cell which is sought to be

lysed by an NK cell. Optionally this antigen is expressed by a cancer cell, or a virally, bacterially or parasite infected cell, an immune cell that contributes to tumor growth or escape (e.g. a tumor-associated monocyte or macrophage), or a cell that contributes to an autoimmunity, an allergic response or inflammatory disease.

[0062] In one embodiment, the multispecific protein binds NKp46 in monovalent fashion. In one embodiment, the multispecific protein binds to the antigen of interest in monovalent fashion. In one embodiment, the multispecific protein binds both NKp46 and the antigen of interest in monovalent fashion. In one embodiment, the multispecific protein binds CD16 via a dimeric Fc domain.

[0063] In one embodiment, the first antigen binding domain comprises an antibody heavy chain variable domain and a light chain variable domain. Optionally, both said heavy and light chain variable domains are involved in binding interactions with NKp46.

[0064] In one embodiment, the second ABD (and optionally third or more ABDs, when present) comprises an antibody heavy chain variable domain and a light chain variable domain. Optionally, both said heavy and light chain variable domains are involved in binding interactions with the antigen bound by the second antigen binding domain. In one embodiment, the second ABD (and optionally third or more ABD, when present) comprises a non-immunoglobulin scaffold.

[0065] Optionally, the Fc domain comprises at least a portion of a CH2 domain and at least a portion of a CH3 domain, and when present in a multispecific polypeptide, is part of a dimeric Fc domain.

[0066] In one embodiment, the CH2 domain comprises an amino acid modification, compared to a wild-type CH2 domain. In one embodiment, the CH2 modification increases binding (e.g. increases binding affinity) of the bispecific polypeptide to a human CD16 polypeptide relative to a wild-type human Fc region.

[0067] In one embodiment, the CH2 domain and/or CH3 domains are naturally occurring (non-engineered) human CH2 and/or CH3 domains. In one embodiment, the multispecific protein comprises an Fc domain that comprises N-linked glycosylation. In one embodiment, the N-linked glycosylation (at residue N297, Kabat EU numbering) comprises glycan structures typical of those found on IgG-class (e.g. IgG1) immunoglobulins produced in mammalian cells (e.g., CHO cells or other rodent cells, non-human primate or human cells).

[0068] In one embodiment, the Fc domain comprises modified N-linked glycosylation, e.g. hypofucosylated glycans at N297) which increase binding affinity for a human CD16 polypeptide.

[0069] In one embodiment, the Fc-derived polypeptide is a dimer, optionally a homodimer or a heterodimer. In one embodiment, the Fc-derived polypeptide is a heterotrimer. In one embodiment, the Fc-derived polypeptide is a heterotetramer.

[0070] In one embodiment, heterotrimer proteins are provided comprising two antigen binding domains that are composed of three different polypeptide chains that each comprise at least one V-(CH1/C κ) unit, wherein a first (central) chain comprises two V-(CH1/C κ) units separated by an Fc domain (or portion thereof which optionally binds CD16), and each of the second and third chains comprise one V-(CH1/C κ) unit, wherein one of the V-(CH1/C κ) units

of the central chain preferentially undergoes CH1-C κ dimerization with the V-(CH1/C κ) unit of the second chain thereby forming a first antigen binding domain, wherein the other of the V-(CH1/C κ) units of the central chain preferentially undergoes CH1-C κ dimerization with the V-(CH1/C κ) unit of the third chain thereby forming a second antigen binding domain, and wherein the second or third chain further comprise an Fc domain (or portion thereof) placed on the polypeptide chain such that the Fc domain is capable of forming a dimeric Fc domain that binds CD16 together with the Fc domain of the central polypeptide.

[0071] In one embodiment, heterotrimer proteins having three antigen binding domains are provided that are composed of three different polypeptide chains that each comprise at least one V-(CH1/C κ) unit, wherein a first (central) chain comprises two V-(CH1/C κ) units and each of the second and third chains comprise one V-(CH1/C κ) unit, wherein one of the V-(CH1/C κ) units of the central chain preferentially undergoes CH1-C κ dimerization with the V-(CH1/C κ) unit of the second chain thereby forming a first antigen binding domain, wherein the other of the V-(CH1/C κ) units of the central chain preferentially undergoes CH1-C κ dimerization with the V-(CH1/C κ) unit of the third chain thereby forming a second antigen binding domain, wherein one of the polypeptide chains further comprises an antigen binding domain (e.g. a tandem variable domain, an scFv) that forms third antigen binding domain, and wherein the first or second chain further comprise an Fc domain (or portion thereof) placed on the polypeptide chain such that the Fc domain is capable of forming a dimeric Fc domain that bind CD16 together with the Fc domain of the central polypeptide.

[0072] In one embodiment, the invention provides a heteromultimeric, e.g. heterodimeric, bispecific protein comprising: (a) a first polypeptide chain comprising a first variable region (V), fused to a CH1 or C κ domain, wherein the V-(CH1/C κ) unit is in turn fused to a first terminus (N- or C-terminus) of a human Fc domain (a full Fc domain or a portion thereof); (b) a second polypeptide chain comprising a first variable region (V) fused to a CH1 or C κ domain that is complementary with the CH1 or C κ of the first chain to form a CH1-C κ dimer, wherein the V-(CH1/C κ) unit is fused to at least a human Fc domain (a full Fc domain or a portion thereof), wherein the two first variable regions form an antigen binding domain that binds a first antigen of interest in monovalent fashion, and (c) an antigen binding domain that binds a second antigen (optionally together with a complementary antigen binding domain), and optionally a second CH1 or C κ domain, fused to a second terminus (N- or C-terminus) of the Fc domain of the first polypeptide such that the Fc domain is interposed between the V-(CH1/C κ) unit and the antigen binding domain that binds a second antigen, and wherein the Fc domains (or portions thereof) of the first and second chains of the heteromultimeric protein form a dimeric Fc domain capable of being bound by human CD16 polypeptide, e.g. a dimeric Fc domain comprising N-linked glycosylation at residue N297 (Kabat EU numbering). Optionally the first and second polypeptide chains are bound by non-covalent bonds and optionally further inter-chain disulfide bonds, e.g. formed between respective CH1 and C κ domains and/or between respective hinge domains. Optionally a V-(CH1/C κ) unit is fused to a human Fc domain directly, or via intervening sequences, e.g. linkers, other protein domain(s), etc. Optionally, one of the antigens

is Nkp46 and another of the antigens is one expressed on the surface of a cell that is to be lysed by the immune cell, e.g. a cancer or viral or bacterial antigen; optionally further, wherein such antigen present on the surface of a cell that is to be lysed by the immune cell, e.g., NK cell, is a protein that is known to undergo intracellular internalization, notably when bound by an antibody (e.g. a full length antibody of an isotype such as human IgG1 or IgG3 that is bound by CD16).

[0073] In one embodiment, the multispecific polypeptide or protein is monoclonal. In one embodiment, the multispecific polypeptide or protein is purified. In one embodiment, the multispecific polypeptide or protein is isolated. In another embodiment the multispecific polypeptide or protein is expressed by a cell, e.g., a human immune cell such as a human NK cell.

[0074] In one embodiment of the above heteromultimeric polypeptide or protein, the polypeptide or protein is a heterodimer, wherein the antigen binding domain for a second antigen is an scFv, optionally an scFv that binds Nkp46.

[0075] In one embodiment of the afore-described heteromultimeric polypeptides or proteins, the heteromultimeric polypeptide or protein is a heterotrimer, comprising an antigen binding domain for a second antigen which comprises or consists of an heavy or light chain variable region, and the heteromultimeric polypeptide or protein further comprises a third polypeptide chain comprising or consisting of a variable region (V) fused to a CH1 or Ck domain that is complementary with the CH1 or Ck of the first chain to form a CH1-Ck dimer wherein the variable region that is the antigen binding domain for a second antigen of the first polypeptide and the variable region of the third chain form an antigen binding domain. The double dimerization yields a trimer. The CH1 or Ck constant region of the third polypeptide is selected to be complementary to the second CH1 or Ck constant region of the first polypeptide chain (but not complementary to the first CH1/Ck of the first polypeptide chain).

[0076] In one aspect a heterodimeric polypeptide according to the invention comprises:

[0077] (a) a first polypeptide chain comprising, from N- to C-terminus, a first variable domain (V), a CH1 or Ck constant region, a Fc domain or portion thereof which optionally binds CD16, a second variable domain and third variable domain; and

[0078] (b) a second polypeptide chain comprising, from N- to C-terminus, a first variable domain (V), a CH1 or Ck constant region, an Fc domain or portion thereof, wherein the CH1 or Ck constant region is selected to be complementary to the CH1 or Ck constant region of the first polypeptide chain such that the first and second polypeptides form a CH1-Ck heterodimer in which the first variable domain of the first polypeptide chain and the first variable domain of the second polypeptide form an antigen binding domain that binds the first antigen of interest; and wherein a second variable domain and third variable domain forms an antigen binding domain that binds the second antigen of interest, and wherein the Fc domains (or portions thereof) of the first and second chains of the heteromultimeric protein form a dimeric Fc domain capable of being bound by human CD16 polypeptide, e.g. a dimeric Fc domain comprising N-linked glycosylation at residue N297 (Kabat EU numbering).

[0079] In another aspect the invention provides a heterodimeric polypeptide which comprises:

[0080] (a) a first polypeptide chain comprising, from N- to C-terminus, a second variable domain and third variable domain, a Fc domain or portion thereof, a first variable domain (V), and a CH1 or Ck constant region; and

[0081] (b) a second polypeptide chain comprising, from N- to C-terminus, a first variable domain (V), a CH1 or Ck constant region, and a Fc domain or portion thereof, wherein the CH1 or Ck constant region is selected to be complementary to the CH1 or Ck constant region of the first polypeptide chain such that the first and second polypeptides form a CH1-Ck heterodimer in which the first variable domain of the first polypeptide chain and the first variable domain of the second polypeptide form an antigen binding domain that binds to the first antigen of interest; and wherein a second variable domain and third variable domain forms an antigen binding domain that binds to a second antigen of interest, wherein the Fc domains (or portions thereof) of the first and second chains of the heteromultimeric protein form a dimeric Fc domain capable of being bound by human CD16 polypeptide, e.g. a dimeric Fc domain comprising IgG-type N-linked glycosylation at residue N297 (Kabat EU numbering).

[0082] In another aspect the invention provides a heterodimeric polypeptide which comprises:

[0083] (a) a first polypeptide chain comprising, from N- to C-terminus, a first variable domain (V) fused to a first CH1 or CKk constant region, an Fc domain or portion thereof, and a second variable domain (V) fused to a second CH1 or Ck constant region;

[0084] (b) a second polypeptide chain comprising, from N- to C-terminus, a variable domain fused to a CH1 or Ck constant region selected to be complementary to the first (but not the second) CH1 or Ck constant region of the first polypeptide chain such that the first and second polypeptides form a CH1-Ck heterodimer, and an Fc domain or portion thereof; and

[0085] (c) a third polypeptide chain comprising, from N- to C-terminus, a variable domain fused to a CH1 or Ck constant region, wherein the CH1 or Ck constant region is selected to be complementary to the second (but not the first) variable domain and second CH1 or Ck constant region of the first polypeptide chain, wherein the Fc domains (or portions thereof) of the first and second chains of the heteromultimeric protein form a dimeric Fc domain capable of being bound by a human CD16 polypeptide, e.g. a dimeric Fc domain comprising IgG-type N-linked glycosylation at residue N297 (Kabat EU numbering). The first and third polypeptides will therefore form a CH1-Ck heterodimer formed between the CH1 or Ck constant region of the third polypeptide and the second CH1 or Ck constant region of the first polypeptide, but not between the CH1 or Ck constant region of the third polypeptide and the first CH1 or Ck constant region of the first polypeptide. The first, second and third polypeptides form a CH1-Ck heterotrimer, and wherein the first variable domain of the first polypeptide chain and the variable domain of the second polypeptide chain form an antigen binding domain specific for a first antigen of interest, and the second variable domain of the first polypeptide chain and the variable domain on the third polypeptide chain form an antigen binding domain specific for a second antigen of interest.

[0086] In another embodiment, the above-described heteromultimeric polypeptides or proteins may comprise one or more additional polypeptide chains.

[0087] Any the heteromultimeric polypeptides or proteins described herein may comprise a dimeric Fc domain capable of being bound by human CD16 polypeptide, e.g. a dimeric Fc domain comprising N-linked glycosylation at residue N297 (Kabat EU numbering).

[0088] Optionally, the CH1 and/or C κ domain are fused via a hinge region to the Fc domain. Optionally the hinge, CH2 and/or CH3 comprises one or more amino acid modifications which increase binding affinity for human CD16. Optionally the hinge, CH2 and/or CH3 comprises an amino acid modification which increases binding affinity for human Fc γ Rn. Optionally the amino acid modifications that increase binding affinity for CD16 may also increase affinity for one or more other human Fc γ receptors. Optionally the hinge, CH2 and/or CH3 comprise an amino acid modification which reduces or substantially abolishes binding to an inhibitory human Fc γ receptor (e.g. CD32B) and/or to an Fc γ receptor other than CD16 (e.g. CD32A and/or CD64). In any embodiment described herein, the CH1 and C κ domains optionally can be of human origin.

In one aspect of any of the embodiments described herein, the bispecific protein binds more strongly or avidly (has a greater binding affinity) for the antigen of interest (e.g. a cancer or viral or other infectious agent antigen) than it binds NKp46. Such antibodies may possess advantageous pharmacological properties. In one aspect of any of the embodiments herein, the polypeptide has a K_d (monovalent binding affinity) to NKp46 of less than 10⁻⁷ M, preferably less than 10⁻⁸ M, or preferably less than 10⁻⁹ M; optionally the polypeptide has a K_d for binding (monovalent binding affinity) to a cancer, viral, bacterial or other antigen that is less than (i.e. has better binding affinity than) the K_d (monovalent binding affinity) to a NKp46 polypeptide. In one aspect of any of the embodiments described herein, the polypeptide has a K_d (monovalent binding affinity) to NKp46 of between 10⁻⁷ M (100 nanomolar) and 10⁻¹⁰ M (0.1 nanomolar) for binding to a NKp46 polypeptide. In one aspect of any of the embodiments disclosed herein, the polypeptide has a K_d (monovalent binding affinity) to NKp46 of between 10⁻⁸ M (10 nanomolar) and 10⁻¹⁰ M (0.1 nanomolar). In one aspect of any of the embodiments herein the multimeric polypeptide has a K_d (monovalent binding affinity) to NKp46 of between 10⁻⁸ M (10 nanomolar) and 10⁻⁹ M (1 nanomolar). Binding can be assessed as in the Examples herein, e.g. by surface plasmon resonance.

[0089] In one aspect of any of the embodiments of the invention, the antigen binding domain that binds NKp46 binds to at least one residue on NKp46 corresponding to any of the amino acid residues bound by any one of monoclonal antibodies NKp46-1, -2, -3, -4, -6 or -9 or the Anti-CD19-Anti-NKp46-1, -2, -3, -4, -6 or -9 bispecific antibodies. In one aspect, the antigen binding domain that binds NKp46 binds at least 1, 2, 3, 4 or more amino acids of NKp46 within the epitope bound by any one or combination of monoclonal antibodies NKp46-1, -2, -3, -4, -6 or -9 or the Anti-CD19-Anti-NKp46-1, -2, -3, -4, -6 or -9 bispecific antibodies. In one aspect of any of the embodiments of the invention, the antigen binding domain that binds NKp46 binds to the same epitope on a NKp46 polypeptide as any of monoclonal antibodies NKp46-1, -2, -3, -4, -6 or -9 or to any of the Anti-CD19-Anti-NKp46-1, -2, -3, -4, -6 or -9 bispecific

antibodies. In one embodiment, the antigen binding domain that binds NKp46 binds to an epitope on the NKp46 polypeptide of SEQ ID NO:1 wherein the epitope comprises one, two, three or more residues selected from the group of residues bound by any of antibodies NKp46-1, -2, -3, -4, -6 or -9.

In some embodiments, the protein that binds NKp46 exhibits significantly lower binding for a mutant NKp46 polypeptide in which a residue bound by any of antibodies NKp46-1, -2, -3, -4, -6 or -9 is substituted with a different amino acid, compared to a wild-type NKp46 polypeptide of SEQ ID NO: 1.

In one aspect of any of the embodiments of the invention, the protein that binds NKp46 competes for binding to a NKp46 polypeptide with any one or any combination of monoclonal antibodies NKp46-1, NKp46-2, NKp46-3, NKp46-4, NKp46-6 or NKp46-9, or any of the Anti-CD19-anti-NKp46-1, -2, -3, -4, -6 or -9 bispecific antibodies. In one embodiment, the protein that binds NKp46 competes for binding to an NKp46 polypeptide with an antibody selected from the group consisting of:

- [0090]** (a) an antibody having respectively a V_H and V_L region of SEQ ID NOS: 3 and 4 (NKp46-1);
- [0091]** (b) an antibody having respectively a V_H and V_L region of SEQ ID NOS: 5 and 6 (NKp46-2);
- [0092]** (c) an antibody having respectively a V_H and V_L region of SEQ ID NOS: 7 and 8 (NKp46-3);
- [0093]** (d) an antibody having respectively a V_H and V_L region of SEQ ID NOS: 9 and 10 (NKp46-4);
- [0094]** (e) an antibody having respectively a V_H and V_L region of SEQ ID NOS:11 and 12 (NKp46-6); and
- [0095]** (f) an antibody having respectively a V_H and V_L region of SEQ ID NOS: 13 and 14 (NKp46-9).

In one embodiment, the invention provides a protein (or nucleic acid encoding such) that specifically binds NKp46 (e.g. a monospecific monoclonal antibody or fragment, a multispecific protein or fragment, a bispecific antibody, etc.) that competes for binding to an NKp46 polypeptide with an antibody selected from the group consisting of:

- [0096]** (a) an antibody having respectively a V_H and V_L region of SEQ ID NOS: 3 and 4 (NKp46-1);
- [0097]** (b) an antibody having respectively a V_H and V_L region of SEQ ID NOS: 5 and 6 (NKp46-2);
- [0098]** (c) (a) an antibody having respectively a V_H and V_L region of SEQ ID NOS: 7 and 8 (NKp46-3);
- [0099]** (d) (a) an antibody having respectively a V_H and V_L region of SEQ ID NOS: 9 and 10 (NKp46-4);
- [0100]** (e) an antibody having respectively a V_H and V_L region of SEQ ID NOS:11 and 12 (NKp46-6); and
- [0101]** (f) an antibody having respectively a V_H and V_L region of SEQ ID NOS: 13 and 14 (NKp46-9).

In one aspect of any of the embodiments of the invention, the antigen binding domain that binds NKp46 comprises the hypervariable regions of any one of monoclonal antibodies NKp46-1, NKp46-2, NKp46-3, NKp46-4, NKp46-6 or NKp46-9 or a combination of any of the foregoing.

In one aspect of any of the embodiments of the invention, the antigen binding domain that binds NKp46 has a heavy and/or light chain variable region having one, two or three CDRs of the respective heavy and/or light chain of an antibody selected from the group consisting of antibody NKp46-1, NKp46-2, NKp46-3, NKp46-4, NKp46-6 and NKp46-9.

In one embodiment, an isolated multispecific protein that binds NKp46 according to the invention comprises or an antigen binding domain thereof comprises heavy chain CDR1, 2 and 3 and light chain CDR 1, 2 and 3 of any of the antibodies selected from the group consisting of:

- [0102]** (a) an antibody having respectively a V_H and V_L region of SEQ ID NOS: 3 and 4 (NKp46-1);
- [0103]** (b) an antibody having respectively a V_H and V_L region of SEQ ID NOS: 5 and 6 (NKp46-2);
- [0104]** (c) (a) an antibody having respectively a V_H and V_L region of SEQ ID NOS: 7 and 8 (NKp46-3);
- [0105]** (d) (a) an antibody having respectively a V_H and V_L region of SEQ ID NOS: 9 and 10 (NKp46-4);
- [0106]** (e) an antibody having respectively a V_H and V_L region of SEQ ID NOS: 11 and 12 (NKp46-6); and
- [0107]** (f) an antibody having respectively a V_H and V_L region of SEQ ID NOS: 13 and 14 (NKp46-9).

In one embodiment, the invention provides an antibody (e.g. a full length monospecific antibody or a bispecific antibody) or antigen binding domain (which optionally may be humanized, chimerized or affinity matured) that binds NKp46 and comprises:

- [0108]** (a) (i) a polypeptide chain comprising a CDR 1, 2 and 3 of the heavy chain variable region of NKp46-1 of Table A, and (ii) a polypeptide chain comprising a CDR 1, 2 and 3 of the light chain variable region of NKp46-1 of Table A;
- [0109]** (b) (i) a polypeptide chain comprising a CDR 1, 2 and 3 of the heavy chain variable region of NKp46-2 of Table A and (ii) a polypeptide chain comprising a CDR 1, 2 and 3 of the light chain variable region of NKp46-2 of Table A;
- [0110]** (c) (i) a polypeptide chain comprising a CDR 1, 2 and 3 of the heavy chain variable region of NKp46-3 of Table A and (ii) a polypeptide chain comprising a CDR 1, 2 and 3 of the light chain variable region of NKp46-3 of Table A;
- [0111]** (d) (i) a polypeptide chain comprising a CDR 1, 2 and 3 of the heavy chain variable region of NKp46-4 of Table A and (ii) a polypeptide chain comprising a CDR 1, 2 and 3 of the light chain variable region of NKp46-4 of Table A;
- [0112]** (e) (i) a polypeptide chain comprising a CDR 1, 2 and 3 of the heavy chain variable region of NKp46-6 of Table A and (ii) a polypeptide chain comprising CDR 1, 2 and 3 of the light chain variable region of NKp46-6 of Table A; or
- [0113]** (f) (i) a polypeptide chain comprising a CDR 1, 2 and 3 of the heavy chain variable region of NKp46-9 of Table A and (ii) a polypeptide chain comprising a CDR 1, 2 and 3 of the light chain variable region of NKp46-9 of Table A.

In one aspect, the invention provides a protein (a monomeric or multimeric protein) that specifically binds NKp46 (e.g. a monospecific monoclonal antibody, a multispecific protein, a bispecific antibody) that binds the same or overlapping epitope on NKp46 as an antibody selected from the group consisting of antibody NKp46-1, NKp46-2, NKp46-3, NKp46-4, NKp46-6 and NKp46-9. The isolated polypeptide may be, for example, a monospecific monoclonal antibody, a multispecific polypeptide or a bispecific antibody.

In one aspect the invention provides an isolated multispecific heterotrimeric protein comprising a first polypeptide chain comprising an amino acid sequence which is at least

50%, 60%, 70%, 80%, 85%, 90%, 95%, 98 or 99% identical to the sequence of a first polypeptide chain of a F5, F13 or T5 protein disclosed herein; a second polypeptide chain comprising an amino acid sequence which is at least 50%, 60%, 70%, 80%, 85%, 90%, 95%, 98 or 99% identical to the sequence of a second polypeptide chain of the respective F5, F13 or T5 protein disclosed herein; and a third polypeptide chain comprising an amino acid sequence which is at least 50%, 60%, 70%, 80%, 85%, 90%, 95%, 98 or 99% identical to the sequence of a third polypeptide chain of a F5, F13 or T5 protein disclosed herein. In one aspect, the protein comprises a dimeric Fc domain capable of being bound by a human CD16 polypeptide, e.g. a dimeric Fc domain comprising N-linked glycosylation at residue N297 (Kabat EU numbering). Optionally any or all of the variable regions or CDRs of the first, second and/or third chains are substituted with different variable regions; optionally any or all of the V-CH1/C κ units of the first, second and/or third chains are substituted with different V-CH1/C κ units. Optionally variable regions, CDRs or V-CH1/C κ units are excluded from the sequences that are considered for computing identity; optionally wherein the anti-NKp46 variable regions, CDRs or V-CH1/C κ units are included for computing identity and the variable regions, CDRs or V-CH1/C κ units for the antigen binding domain that binds the other antigen are excluded from the sequences that are considered for computing identity. In one embodiment of any of the polypeptides described herein, the multispecific polypeptide is capable of directing NKp46-expressing NK cells to lyse a target cell of interest (e.g. a target cell expressing an antigen other than NKp46).

In one aspect of any of the embodiments described herein, the invention provides a recombinant nucleic acid encoding a first polypeptide chain, and/or a second polypeptide chain, and/or a third polypeptide chain and/or a fourth polypeptide. In one aspect of any of the embodiments described herein, the invention provides a recombinant host cell comprising a nucleic acid encoding a first polypeptide chain, and/or a second polypeptide chain and/or a third polypeptide chain, optionally wherein the host cell produces a multimeric or other protein according to the invention with a yield (final productivity or concentration before or after purification) of at least 1, 2, 3 or 4 mg/L. Also provided is a kit or set of nucleic acids comprising a recombinant nucleic acid encoding a first polypeptide chain of the according to the invention, a recombinant nucleic acid encoding a second polypeptide chain according to the invention, and, optionally, a recombinant nucleic acid encoding a third polypeptide chain according to the invention. Also provided are methods of making dimeric, trimeric and tetrameric proteins according to the invention.

[0114] Any of the methods can further be characterized as comprising any step described in the application, including notably in the "Detailed Description of the Invention"). The invention further relates to methods of identifying, testing and/or making proteins described herein. The invention further relates to a multispecific protein obtainable by any of present methods. The disclosure further relates to pharmaceutical or diagnostic formulations containing at least one of the multispecific proteins disclosed herein. The disclosure further relates to methods of using the subject multispecific proteins in methods of treatment or diagnosis.

[0115] These and additional advantageous aspects and features of the invention may be further described elsewhere herein.

BRIEF DESCRIPTION OF THE FIGURES

[0116] FIG. 1 shows that Anti-CD19-F1-Anti-CD3 does not cause T/B cell aggregation in the presence of B221 (CD19) or JURKAT (CD3) cell lines when separate, but it does cause aggregation of cells when both B221 and JURKAT cells are co-incubated.

[0117] FIGS. 2A to 2F show different domain arrangements of bispecific anti-NKp46 proteins produced.

[0118] FIGS. 3A and 3B respectively demonstrate that bispecific F1 and F2 format proteins having NKp46 binding region based on NKp46-1, NKp46-2, NKp46-3 or NKp46-4 are able to direct resting NK cells to their CD19-positive Daudi tumor target cells, while isotype control antibody did not lead to the elimination of the Daudi cells. Rituximab (RTX) served as the positive control of ADCC, where the maximal response obtained with RTX (at 10 µg/ml in this assay) was 21.6% specific lysis.

[0119] FIG. 4A shows that bispecific antibodies having NKp46 and CD19 binding regions in an F2 format protein do not activate resting NK cells in the absence of target cells; by contrast full length anti-NKp46 antibodies as well as positive control alemtuzumab did activate NK cells. FIG. 4B shows that bispecific anti-NKp46×anti-CD19 antibodies (including each of the NKp46-1, NKp46-2, NKp46-3 or NKp46-4 binding domains) activated resting NK cells in presence of Daudi target cells, while full-length anti-CD19 showed at best only very low activation of NK cells and neither full-length anti-NKp46 antibodies nor alemtuzumab elicited a substantial increase in activation beyond what was observed in the presence of NK cells alone. FIG. 4C shows that in the presence of CD19-negative HUT78 cells, none of the bispecific anti-NKp46×anti-CD19 antibodies (including each of the NKp46-1, NKp46-2, NKp46-3 or NKp46-4 variable regions) activated NK cells. However, the full-length anti-NKp46 antibodies and alemtuzumab resulted in detectable activation of NK cells, i.e., at a similar level observed in presence of NK cells alone. Isotype control antibody did not induce activation.

[0120] FIGS. 5A and 5B shows that at low effector:target ratios of 1:1 each of the tested bispecific anti-NKp46×anti-CD19 antibodies activated NK cells in the presence of Daudi cells, and that bispecific anti-NKp46×anti-CD19 antibodies were far more potent (better elicited lysis of target cells) than a control anti-CD19 antibody as well as a full-length human IgG1 ADCC inducing antibody.

[0121] FIGS. 6A and 6B show that each NKp46×CD19 bispecific protein (Format F3, F5 and F6) induced specific lysis of Daudi (FIG. 6A) or B221 (FIG. 6B) cells by human KHYG-1 CD16-negative hNKp46-positive NK cell line, while rituximab and human IgG1 isotype control (IC) antibodies did not. FIG. 6C shows that a NKp46×KIR3DL2 bispecific protein (Format F6) induced specific lysis of HUT78 tumor cells via NKp46 binding (without CD16 binding) comparably to a conventional IgG1 antibody with the same anti-KIR3DL2 variable regions.

[0122] FIG. 7 shows a NKp46×CD19 bispecific protein in F5 format whose Fc domain binds CD16 is far more potent in mediating Daudi target cell lysis than a full-length IgG1 anti-CD19 antibody or a F6 format bispecific protein. The figure also shows that a bispecific anti-CD19 in F6 format

whose Fc domain does not bind CD16 was as potent in mediating NK cell lysis of Daudi target cells as the full-length IgG1 anti-CD19 antibody, which is unexpected considering that the control IgG1 anti-CD19 antibody binds CD19 bivalently. At comparable levels of target cell lysis, CD19-F5-NKp46-3 was at least 1000 times more potent than the full-length anti-CD19 IgG1.

[0123] FIG. 8 shows the results of cytotoxicity assays using fresh NK cells (Daudi cell in the right hand panel and HUT78 cells in the left hand panel); the CD19-F6-NKp46-3 whose Fc domain does not bind CD16 due to a N297 substitution has as mode of action NKp46 triggering when NK cells encounter the target cell, while the CD19-F5-NKp46-3 bispecific protein demonstrated a far higher potency in mediating cytotoxicity toward Daudi cells. Neither the F5 nor F6 proteins mediated any NK cell cytotoxicity towards HUT78 cells.

[0124] FIG. 9 shows the results of flow cytometry staining of NK cell surface markers showed a strong upregulation of CD137 on the surface of NK cells by F5 proteins (Left-most panel: NK cells vs. Daudi; middle panel: NK cells vs. HUT78; right-most panel: NK cells alone). The full-length anti-CD19 IgG1 antibody that binds CD16 also showed CD137 upregulation, but to a far lesser extent than the CD19-F5-NKp46-3 protein. The CD19-F6-NKp46-3 which functions via NKp46 but not CD16 did not show any CD137 upregulation.

[0125] FIG. 10 shows the results of cytotoxicity assays which compared the ability of the GA101-F5+ NKp46-1 bispecific protein to a comparison antibody (GA101) containing the same variable regions to lyse Daudi cells. The results therein show that the GA101-F5+ NKp46-1 bispecific protein possesses far higher potency (approximately 10-fold increase in EC₅₀) in mediating cytotoxicity toward Daudi cells than GA101.

DETAILED DESCRIPTION OF THE INVENTION

Definitions

[0126] As used in the specification, “a” or “an” may mean one or more. As used in the claim(s), when used in conjunction with the word “comprising”, the words “a” or “an” may mean one or more than one.

[0127] Where “comprising” is used, this can optionally be replaced by “consisting essentially of”, or optionally by “consisting of”.

[0128] As used herein, the term “antigen binding domain” or “ABD” refers to a domain comprising a three-dimensional structure capable of immunospecifically binding to an epitope. Thus, in one embodiment, said domain can comprise a hypervariable region, optionally a V_H and/or V_L domain of an antibody chain, optionally at least a V_H domain. In another embodiment, the binding domain may comprise at least one complementarity determining region (CDR) of an antibody chain. In another embodiment, the binding domain may comprise a polypeptide domain from a non-immunoglobulin scaffold.

[0129] The term “antibody” herein is used in the broadest sense and specifically includes full-length monoclonal antibodies, polyclonal antibodies, multispecific antibodies (e.g., bispecific antibodies), and antibody fragments and derivatives, so long as they exhibit the desired biological activity. Various techniques relevant to the production of antibodies

are provided in, e.g., Harlow, et al., *ANTIBODIES: A LABORATORY MANUAL*, Cold Spring Harbor Laboratory Press, Cold Spring Harbor, N.Y., (1988). An “antibody fragment” comprises a portion of a full-length antibody, e.g. antigen-binding or variable regions thereof. Examples of antibody fragments include Fab, Fab', F(ab)₂, F(ab')₂, F(ab)₃, Fv (typically the V_L and V_H domains of a single arm of an antibody), single-chain Fv (scFv), dsFv, Fd fragments (typically the V_H and CH1 domain), and dAb (typically a V_H domain) fragments; V_H, V_L, V_HH, and V-NAR domains; minibodies, diabodies, triabodies, tetrabodies, and kappa bodies (see, e.g., Ill et al., *Protein Eng* 1997; 10: 949-57); camel IgG; IgNAR; and multispecific antibody fragments formed from antibody fragments, and one or more isolated CDRs or a functional paratope, where isolated CDRs or antigen-binding residues or polypeptides can be associated or linked together so as to form a functional antibody fragment. Various types of antibody fragments have been described or reviewed in, e.g., Holliger and Hudson, *Nat Biotechnol* 2005; 23, 1126-1136; WO2005040219, and published U.S. Patent Applications 20050238646 and 20020161201.

[0130] The term “antibody derivative”, as used herein, comprises a full-length antibody or a fragment of an antibody, e.g. comprising at least antigen-binding or variable regions thereof, wherein one or more of the amino acids are chemically modified, e.g., by alkylation, PEGylation, acylation, ester formation or amide formation or the like. This includes, but is not limited to, PEGylated antibodies, cysteine-PEGylated antibodies, and variants thereof.

[0131] The term “hypervariable region” when used herein refers to the amino acid residues of an antibody that are responsible for antigen binding. The hypervariable region generally comprises amino acid residues from a “complementarity-determining region” or “CDR” (e.g. residues 24-34 (L1), 50-56 (L2) and 89-97 (L3) in the light-chain variable domain and 31-35 (H1), 50-65 (H2) and 95-102 (H3) in the heavy-chain variable domain; Kabat et al. 1991) and/or those residues from a “hypervariable loop” (e.g. residues 26-32 (L1), 50-52 (L2) and 91-96 (L3) in the light-chain variable domain and 26-32 (H1), 53-55 (H2) and 96-101 (H3) in the heavy-chain variable domain; Chothia and Lesk, *J. Mol. Biol* 1987; 196:901-917). Typically, the numbering of amino acid residues in this region is performed by the method described in Kabat et al., *supra*. Phrases such as “Kabat position”, “variable domain residue numbering as in Kabat” and “according to Kabat” herein refer to this numbering system for heavy chain variable domains or light chain variable domains. Using the Kabat numbering system, the actual linear amino acid sequence of a peptide may contain fewer or additional amino acids corresponding to a shortening of, or insertion into, a FR or CDR of the variable domain. For example, a heavy chain variable domain may include a single amino acid insert (residue 52a according to Kabat) after residue 52 of CDR H2 and inserted residues (e.g. residues 82a, 82b, and 82c, etc. according to Kabat) after heavy chain FR residue 82. The Kabat numbering of residues may be determined for a given antibody by alignment at regions of homology of the sequence of the antibody with a “standard” Kabat numbered sequence.

[0132] By “framework” or “FR” residues as used herein is meant the region of an antibody variable domain exclusive of those regions defined as CDRs. Each antibody variable

domain framework can be further subdivided into the contiguous regions separated by the CDRs (FR1, FR2, FR3 and FR4).

[0133] By “constant region” as defined herein is meant an antibody-derived constant region that is encoded by one of the light or heavy chain immunoglobulin constant region genes. By “constant light chain” or “light chain constant region” as used herein is meant the region of an antibody encoded by the kappa (Cκ) or lambda (Cλ) light chains. The constant light chain typically comprises a single domain, and as defined herein refers to positions 108-214 of Cκ, or Cλ, wherein numbering is according to the EU index (Kabat et al., 1991, *Sequences of Proteins of Immunological Interest*, 5th Ed., United States Public Health Service, National Institutes of Health, Bethesda).

[0134] By “constant heavy chain” or “heavy chain constant region” as used herein is meant the region of an antibody encoded by the mu, delta, gamma, alpha, or epsilon genes to define the antibody's isotype as IgM, IgD, IgG, IgA, or IgE, respectively. For full length IgG antibodies, the constant heavy chain, as defined herein, refers to the N-terminus of the CH1 domain to the C-terminus of the CH3 domain, thus comprising positions 118-447, wherein numbering is according to the EU index.

[0135] By “Fab” or “Fab region” as used herein is meant the polypeptide that comprises the V_H, CH1, V_L, and C_L immunoglobulin domains. Fab may refer to this region in isolation, or this region in the context of a polypeptide, multispecific polypeptide or ABD, or any other embodiment as outlined herein.

[0136] By “single-chain Fv” or “scFv” as used herein are meant antibody fragments comprising the V_H and V_L domains of an antibody, wherein these domains are present in a single polypeptide chain. Generally, the Fv polypeptide further comprises a polypeptide linker between the V_H and V_L domains which enables the scFv to form the desired structure for antigen binding. Methods for producing scFvs are well known in the art. For a review of methods for producing scFvs see Pluckthun in *The Pharmacology of Monoclonal Antibodies*, vol. 113, Rosenberg and Moore eds. Springer-Verlag, New York, pp. 269-315 (1994).

[0137] By “Fv” or “Fv fragment” or “Fv region” as used herein is meant a polypeptide that comprises the V_L and V_H domains of a single antibody.

[0138] By “Fc” or “Fc region”, as used herein is meant the polypeptide comprising the constant region of an antibody excluding the first constant region immunoglobulin domain. Thus Fc refers to the last two constant region immunoglobulin domains of IgA, IgD, and IgG, and the last three constant region immunoglobulin domains of IgE and IgM, and the flexible hinge N-terminal to these domains. For IgA and IgM, Fc may include the J chain. For IgG, Fc comprises immunoglobulin domains Cγ2 (CH2) and Cγ3 (CH3) and the hinge between Cγ1 and Cγ2. Although the boundaries of the Fc region may vary, the human IgG heavy chain Fc region is usually defined to comprise residues C226, P230 or A231 to its carboxyl-terminus, wherein the numbering is according to the EU index. Fc may refer to this region in isolation, or this region in the context of an Fc polypeptide, as described below. By “Fc polypeptide” or “Fc-derived polypeptide” as used herein is meant a polypeptide that comprises all or part of an Fc region. Fc polypeptides herein include but are not limited to antibodies, Fc fusions and Fc fragments. Also, Fc regions according to the invention

include variants containing at least one modification that alters (enhances or diminishes) an Fc associated effector function. Also, Fc regions according to the invention include chimeric Fc regions comprising different portions or domains of different Fc regions, e.g., derived from antibodies of different isotype or species.

[0139] By “variable region” as used herein is meant the region of an antibody that comprises one or more Ig domains substantially encoded by any of the V_L (including V_K (V_K) and V_L) and/or V_H genes that make up the light chain (including κ and λ) and heavy chain immunoglobulin genetic loci respectively. A light or heavy chain variable region (V_L or V_H) consists of a “framework” or “FR” region interrupted by three hypervariable regions referred to as “complementarity determining regions” or “CDRs”. The extent of the framework region and CDRs have been precisely defined, for example as in Kabat (see “Sequences of Proteins of Immunological Interest,” E. Kabat et al., U.S. Department of Health and Human Services, (1983)), and as in Chothia. The framework regions of an antibody, that is the combined framework regions of the constituent light and heavy chains, serves to position and align the CDRs, which are primarily responsible for binding to an antigen.

The term “specifically binds to” means that an antibody or polypeptide can bind preferably in a competitive binding assay to the binding partner, e.g. NKp46, as assessed using either recombinant forms of the proteins, epitopes therein, or native proteins present on the surface of isolated target cells. Competitive binding assays and other methods for determining specific binding are further described below and are well known in the art.

When an antibody or polypeptide is said to “compete with” a particular monoclonal antibody (e.g. NKp46-1, -2, -4, -6 or -9 in the context of an anti-NKp46 mono- or bi-specific antibody), it means that the antibody or polypeptide competes with the monoclonal antibody in a binding assay using either recombinant target (e.g. NKp46) molecules or surface expressed target (e.g. NKp46) molecules. For example, if a test antibody reduces the binding of NKp46-1, -2, -4, -6 or -9 to a NKp46 polypeptide or NKp46-expressing cell in a binding assay, the antibody is said to “compete” respectively with NKp46-1, -2, -4, -6 or -9.

The term “affinity”, as used herein, means the strength of the binding of an antibody or polypeptide to an epitope. The affinity of an antibody is given by the dissociation constant K_D , defined as $[Ab] \times [Ag] / [Ab-Ag]$, where $[Ab-Ag]$ is the molar concentration of the antibody-antigen complex, $[Ab]$ is the molar concentration of the unbound antibody and $[Ag]$ is the molar concentration of the unbound antigen. The affinity constant K_A is defined by $1/K_D$. Preferred methods for determining the affinity of mAbs can be found in Harlow, et al., *Antibodies: A Laboratory Manual*, Cold Spring Harbor Laboratory Press, Cold Spring Harbor, N.Y., (1988), Coligan et al., eds., *Current Protocols in Immunology*, Greene Publishing Assoc. and Wiley Interscience, N.Y., (1992, 1993), and Muller, *Meth. Enzymol.* 92:589-601 (1983), which references are entirely incorporated herein by reference. One preferred and standard method well known in the art for determining the affinity of mAbs is the use of surface plasmon resonance (SPR) screening (such as by analysis with a BIAcore™ SPR analytical device).

Within the context of this invention a “determinant” designates a site of interaction or binding on a polypeptide.

The term “epitope” refers to an antigenic determinant, and is the area or region on an antigen to which an antibody or polypeptide binds. A protein epitope may comprise amino acid residues directly involved in the binding as well as amino acid residues which are effectively blocked by the specific antigen binding antibody or peptide, i.e., amino acid residues within the “footprint” of the antibody. It is the simplest form or smallest structural area on a complex antigen molecule that can combine with e.g., an antibody or a receptor. Epitopes can be linear or conformational/structural. The term “linear epitope” is defined as an epitope composed of amino acid residues that are contiguous on the linear sequence of amino acids (primary structure). The term “conformational or structural epitope” is defined as an epitope composed of amino acid residues that are not all contiguous and thus represent separated parts of the linear sequence of amino acids that are brought into proximity to one another by folding of the molecule (secondary, tertiary and/or quaternary structures). A conformational epitope is dependent on the 3-dimensional structure. The term ‘conformational’ is therefore often used interchangeably with ‘structural’. Epitopes may be identified by different methods known in the art including but not limited to alanine scanning, phage display, X-ray crystallography, array-based oligo-peptide scanning or pepsin analysis, site-directed mutagenesis, high throughput mutagenesis mapping, H/D-Ex Mass Spectroscopy, homology modeling, docking, hydrogen-deuterium exchange, among others. (See e.g., Tong et al., *Methods and Protocols for prediction of immunogenic epitopes*, *Briefings in Bioinformatics* 8(2):96-108; Gershoni, Jonathan M; Roitburd-Berman, Anna; Siman-Tov, Dror D; Tarnovitski Freund, Natalia; Weiss, Yael (2007). “Epitope Mapping”. *BioDrugs* 21 (3): 145-56; and Flanagan, Nina (May 15, 2011); “Mapping Epitopes with H/D-Ex Mass Spec: ExSAR Expands Repertoire of Technology Platform Beyond Protein Characterization”, *Genetic Engineering & Biotechnology News* 31 (10).

[0140] By “amino acid modification” herein is meant an amino acid substitution, insertion, and/or deletion in a polypeptide sequence. An example of amino acid modification herein is a substitution. By “amino acid modification” herein is meant an amino acid substitution, insertion, and/or deletion in a polypeptide sequence. By “amino acid substitution” or “substitution” herein is meant the replacement of an amino acid at a given position in a protein sequence with another amino acid. For example, the substitution Y50W refers to a variant of a parent polypeptide, in which the tyrosine at position 50 is replaced with tryptophan. A “variant” of a polypeptide refers to a polypeptide having an amino acid sequence that is substantially identical to a reference polypeptide, typically a native or “parent” polypeptide. The polypeptide variant may possess one or more amino acid substitutions, deletions, and/or insertions at certain positions within the native amino acid sequence.

[0141] “Conservative” amino acid substitutions are those in which an amino acid residue is replaced with an amino acid residue having a side chain with similar physicochemical properties. Families of amino acid residues having similar side chains are known in the art, and include amino acids with basic side chains (e.g., lysine, arginine, histidine), acidic side chains (e.g., aspartic acid, glutamic acid), uncharged polar side chains (e.g., glycine, asparagine, glutamine, serine, threonine, tyrosine, cysteine, tryptophan), nonpolar side chains (e.g., alanine, valine, leucine, isoleu-

cine, proline, phenylalanine, methionine), beta-branched side chains (e.g., threonine, valine, isoleucine) and aromatic side chains (e.g., tyrosine, phenylalanine, tryptophan, histidine).

[0142] The term “identity” or “identical”, when used in a relationship between the sequences of two or more polypeptides, refers to the degree of sequence relatedness between polypeptides, as determined by the number of matches between strings of two or more amino acid residues. “Identity” measures the percent of identical matches between the smaller of two or more sequences with gap alignments (if any) addressed by a particular mathematical model or computer program (i.e., “algorithms”). Identity of related polypeptides can be readily calculated by known methods. Such methods include, but are not limited to, those described in *Computational Molecular Biology*, Lesk, A. M., ed., Oxford University Press, New York, 1988; *Biocomputing: Informatics and Genome Projects*, Smith, D. W., ed., Academic Press, New York, 1993; *Computer Analysis of Sequence Data*, Part 1, Griffin, A. M., and Griffin, H. G., eds., Humana Press, New Jersey, 1994; *Sequence Analysis in Molecular Biology*, von Heinje, G., Academic Press, 1987; *Sequence Analysis Primer*, Gribskov, M. and Devereux, J., eds., M. Stockton Press, New York, 1991; and Carillo et al., *SIAM J. Applied Math.* 48, 1073 (1988).

Preferred methods for determining identity are designed to give the largest match between the sequences tested. Methods of determining identity are described in publicly available computer programs. Preferred computer program methods for determining identity between two sequences include the GCG program package, including GAP (Devereux et al., *Nucl. Acid. Res.* 12, 387 (1984); Genetics Computer Group, University of Wisconsin, Madison, Wis.), BLASTP, BLASTN, and FASTA (Altschul et al., *J. Mol. Biol.* 215, 403-410 (1990)). The BLASTX program is publicly available from the National Center for Biotechnology Information (NCBI) and other sources (BLAST Manual, Altschul et al. NCB/NLM/NIH Bethesda, Md. 20894; Altschul et al., supra). The well-known Smith Waterman algorithm may also be used to determine identity.

[0143] An “isolated” molecule is a molecule that is the predominant species in the composition wherein it is found with respect to the class of molecules to which it belongs (i.e., it makes up at least about 50% of the type of molecule in the composition and typically will make up at least about 70%, at least about 80%, at least about 85%, at least about 90%, at least about 95%, or more of the species of molecule, e.g., peptide, in the composition). Commonly, a composition of a polypeptide will exhibit 98%, 98%, or 99% homogeneity for polypeptides in the context of all present peptide species in the composition or at least with respect to substantially active peptide species in the context of proposed use.

[0144] In the context herein, “treatment” or “treating” refers to preventing, alleviating, managing, curing or reducing one or more symptoms or clinically relevant manifestations of a disease or disorder, unless contradicted by context. For example, “treatment” of a patient in whom no symptoms or clinically relevant manifestations of a disease or disorder have been identified is preventive or prophylactic therapy, whereas “treatment” of a patient in whom symptoms or clinically relevant manifestations of a disease or disorder have been identified generally does not constitute preventive or prophylactic therapy.

[0145] As used herein, the phrase “NK cells” refers to a sub-population of lymphocytes that is involved in non-conventional immunity. NK cells can be identified by virtue of certain characteristics and biological properties, such as the expression of specific surface antigens including CD56 and/or NKp46 for human NK cells, the absence of the alpha/beta or gamma/delta TCR complex on the cell surface, the ability to bind to and kill cells that fail to express “self” MHC/HLA antigens by the activation of specific cytolytic machinery, the ability to kill tumor cells or other diseased cells that express a ligand for NK activating receptors, and the ability to release protein molecules called cytokines that stimulate or inhibit the immune response. Any of these characteristics and activities can be used to identify NK cells, using methods well known in the art. Any subpopulation of NK cells will also be encompassed by the term NK cells. Within the context herein “active” NK cells designate biologically active NK cells, including NK cells having the capacity of lysing target cells or enhancing the immune function of other cells. NK cells can be obtained by various techniques known in the art, such as isolation from blood samples, cytophoresis, tissue or cell collections, etc. Useful protocols for assays involving NK cells can be found in *Natural Killer Cells Protocols* (edited by Campbell K S and Colonna M). Humana Press. pp. 219-238 (2000).

[0146] The term “internalization”, used interchangeably with “intracellular internalization”, refers to the molecular, biochemical and cellular events associated with the process of translocating a molecule from the extracellular surface of a cell to the intracellular surface of a cell. The processes responsible for intracellular internalization of molecules are well-known and can involve, inter alia, the internalization of extracellular molecules (such as hormones, antibodies, and small organic molecules); membrane-associated molecules (such as cell-surface receptors); and complexes of membrane-associated molecules bound to extracellular molecules (for example, a ligand bound to a transmembrane receptor or an antibody bound to a membrane-associated molecule). Thus, “inducing and/or increasing internalization” refer to events wherein intracellular internalization is initiated and/or the rate and/or extent of intracellular internalization is increased.

As used herein, an agent that has “agonist” activity at NKp46 is an agent that can cause or increase “NKp46 signaling”. “NKp46 signaling” refers to an ability of an NKp46 polypeptide to activate or transduce an intracellular signaling pathway. Changes in NKp46 signaling activity can be measured, for example, by assays designed to measure changes in NKp46 signaling pathways, e.g. by monitoring phosphorylation of signal transduction components, assays to measure the association of certain signal transduction components with other proteins or intracellular structures, or in the biochemical activity of components such as kinases, or assays designed to measure expression of reporter genes under control of NKp46-sensitive promoters and enhancers, or indirectly by a downstream effect mediated by the NKp46 polypeptide (e.g. activation of specific cytolytic machinery in NK cells). Reporter genes can be naturally occurring genes (e.g. monitoring cytokine production) or they can be genes artificially introduced into a cell. Other genes can be placed under the control of such regulatory elements and thus serve to report the level of NKp46 signaling.

[0147] “NKp46” refers to a protein or polypeptide encoded by the Ncr1 gene or by a cDNA prepared from such

a gene. Any naturally occurring isoform, allele, ortholog or variant is encompassed by the term NKp46 polypeptide (e.g., an NKp46 polypeptide 90%, 95%, 98% or 99% identical to SEQ ID NO 1, or a contiguous sequence of at least 20, 30, 50, 100 or 200 amino acid residues thereof). The 304 amino acid residue sequence of human NKp46 (isoform a) is shown below:

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(SEQ ID NO: 1)
MSSTLPALLC VGLCLSQRIS AQQQTLPKPF IWAEPHFMPV
KEKQVTICCG GNYGAVEYQL HFEGSLFAVD RPKPPERINK
VKFYIPDMNS RMAGQYSCIY RVGELWSEPS NLLDLVVTEM
YDTPTLSVHP GPEVISGEKV TFYCRLDTAT SMFLLLKEGR
SSHVQRGYGK VQAEFPLGPV TTAHRGTYRC FGSYNNHAWs
FPSEPVKLLV TGDIENTSLA PEDPTFPADT WGTYLLTTET
GLQKDHALLWD HTAQNLLRMG LAFLVLVALV WFLVEDWLSR
KRTREASRA STWEGRRRLN TQTL.
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[0148] SEQ ID NO: 1 corresponds to NCBI accession number NP_004820, the disclosure of which is incorporated herein by reference. The human NKp46 mRNA sequence is described in NCBI accession number NM_004829, the disclosure of which is incorporated herein by reference.

Producing Polypeptides

[0149] The antigen binding domains used in the proteins described herein can be readily derived from any of a variety of immunoglobulin or non-immunoglobulin scaffolds, for example antibodies based on the Z-domain of staphylococcal protein A, engineered Kunitz domains, monobodies or adnectins based on the 10th extracellular domain of human fibronectin III, anticalins derived from lipocalins, DARPin (designed ankyrin repeat domains, multimerized LDLR-A module, avimers or cysteine-rich knottin peptides. See, e.g., Gebauer and Skerra (2009) *Current Opinion in Chemical Biology* 13:245-255, the disclosure of which is incorporated herein by reference.

[0150] Variable domains are commonly derived from antibodies (immunoglobulin chains), for example in the form of associated V_L and V_H domains found on two polypeptide chains, or a single chain antigen binding domain such as a scFv, a V_H domain, a V_L domain, a dAb, a V-NAR domain or a V_{HH} domain. In certain advantageous protein formats disclosed herein that directly enable the use of a wide range of variable regions from Fab or scFv without substantial further requirements for pairing and/or folding, the antigen binding domain (e.g., ABD₁ and ABD₂) can also be readily derived from antibodies as a Fab or scFv.

[0151] Typically, antibodies are initially obtained by immunization of a non-human animal, e.g., a mouse, rat, guinea pig or rabbit, with an immunogen comprising a polypeptide, or a fragment or derivative thereof, typically an immunogenic fragment, for which it is desired to obtain antibodies (e.g. a human polypeptide). The step of immunizing a non-human mammal with an antigen may be carried out in any manner well known in the art for stimulating the production of antibodies in a mouse (see, for example, E. Harlow and D. Lane, *Antibodies: A Laboratory Manual*, Cold Spring Harbor Laboratory Press, Cold Spring Harbor,

N.Y. (1988), the entire disclosure of which is herein incorporated by reference). Human antibodies may also be produced by using, for immunization, transgenic animals that have been engineered to express a human antibody repertoire (Jakobovitz et al *Nature* 362 (1993) 255), or by selection of antibody repertoires using phage display methods. For example, a XenoMouse (Abgenix, Fremont, Calif.) can be used for immunization. A XenoMouse is a murine host that has had its immunoglobulin genes replaced by functional human immunoglobulin genes. Thus, antibodies produced by this mouse or in hybridomas made from the B cells of this mouse, are already humanized. The XenoMouse is described in U.S. Pat. No. 6,162,963, which is herein incorporated in its entirety by reference. Antibodies may also be produced by selection of combinatorial libraries of immunoglobulins, as disclosed for instance in (Ward et al. *Nature*, 341 (1989) p. 544, the entire disclosure of which is herein incorporated by reference). Phage display technology (McCafferty et al (1990) *Nature* 348:552-553) can be used to produce antibodies from immunoglobulin variable (V) domain gene repertoires from unimmunized donors. See, e.g., Griffith et al (1993) *EMBO J.* 12:725-734; U.S. Pat. No. 5,565,332; U.S. Pat. No. 5,573,905; U.S. Pat. No. 5,567,610; and U.S. Pat. No. 5,229,275). When combinatorial libraries comprise variable (V) domain gene repertoires of human origin, selection from combinatorial libraries will yield human antibodies.

[0152] Additionally, a wide range of antibodies are available in the scientific and patent literature, including DNA and/or amino acid sequences, or from commercial suppliers. Antibodies will typically be directed to a pre-determined antigen. Examples of antibodies include antibodies that recognize an antigen expressed by a target cell that is to be eliminated, for example a proliferating cell or a cell contributing to a disease pathology. Examples include antibodies that recognize tumor antigens, microbial (e.g. bacterial or parasite) antigens or viral antigens.

[0153] Antigen binding domains that bind NKp46 can be derived from the anti-NKp46 antibodies provided herein (see section "CDR Sequences"). Variable regions can be used directly, or can be modified by selecting hypervariable or CDR regions from the NKp46 antibodies and placing them into an appropriate V_L or V_H framework, for example human frameworks. Antigen binding domains that bind NKp46 can also be derived de novo using methods for generating antibodies. Antibodies can be tested for binding to NKp46 polypeptides. In one aspect of any embodiment herein, a polypeptide (e.g. multispecific polypeptide, bispecific or monospecific antibody) that binds to NKp46 will be capable of binding NKp46 expressed on the surface of a cell, e.g. native NKp46 expressed by a NK cell.

[0154] Antigen binding domains (ABDs) that bind antigens of interest can be selected based on the desired antigen of interest (e.g. an antigen other than NKp46), and may include for example cancer antigens such as antigens present on tumor cells and/or on immune cells capable of mediating a pro-tumoral effect, e.g. a monocyte or a macrophage, optionally a suppressor T cell, regulatory T cell, or myeloid-derived suppressor cell (for the treatment of cancer); bacterial or viral antigens (for the treatment of infectious disease); or antigens present on pro-inflammatory immune cells, e.g. T cells, neutrophils, macrophages, etc. (for the treatment of inflammatory and/or autoimmune disorder). As used herein, the term "bacterial antigen" includes, but is not

limited to, intact, attenuated or killed bacteria, any structural or functional bacterial protein or carbohydrate, or any peptide portion of a bacterial protein of sufficient length (typically about 8 amino acids or longer) to be antigenic. Examples include gram-positive bacterial antigens and gram-negative bacterial antigens. In some embodiments the bacterial antigen is derived from a bacterium selected from the group consisting of *Helicobacter* species, in particular *Helicobacter pylori*; *Borrelia* species, in particular *Borrelia burgdorferi*; *Legionella* species, in particular *Legionella pneumophila*; *Mycobacteria* species, in particular *M. tuberculosis*, *M. avium*, *M. intracellulare*, *M. kansasii*, *M. gordonae*; *Staphylococcus* species, in particular *Staphylococcus aureus*; *Neisseria* species, in particular *N. gonorrhoeae*, *N. meningitidis*; *Listeria* species, in particular *Listeria monocytogenes*; *Streptococcus* species, in particular *S. pyogenes*, *S. agalactiae*; *S. faecalis*; *S. bovis*, *S. pneumoniae*; anaerobic *Streptococcus* species; pathogenic *Campylobacter* species; *Enterococcus* species; *Haemophilus* species, in particular *Haemophilus influenzae*; *Bacillus* species, in particular *Bacillus anthracis*; *Corynebacterium* species, in particular *Corynebacterium diphtheriae*; *Erysipelothrix* species, in particular *Erysipelothrix rhusiopathiae*; *Clostridium* species, in particular *C. perfringens*, *C. tetani*; *Enterobacter* species, in particular *Enterobacter aerogenes*, *Klebsiella* species, in particular *Klebsiella* 1S pneumoniae, *Pasteurella* species, in particular *Pasteurella multocida*, *Bacteroides* species; *Fusobacterium* species, in particular *Fusobacterium nucleatum*; *Streptobacillus* species, in particular *Streptobacillus moniliformis*; *Treponema* species, in particular *Treponema pertenue*; *Leptospira*; pathogenic *Escherichia* species; and *Actinomyces* species, in particular *Actinomyces israeli*.

[0155] As used herein, the term “viral antigen” includes, but is not limited to, intact, attenuated or killed whole virus, any structural or functional viral protein, or any peptide portion of a viral protein of sufficient length (typically about 8 amino acids or longer) to be antigenic. Sources of a viral antigen include, but are not limited to viruses from the families: Retroviridae (e.g., human immunodeficiency viruses, such as HIV-1 (also referred to as HTLV-III, LAV or HTLV-III/LAV, or HIV-III); and other isolates, such as HIV-LP; Picornaviridae (e.g., polio viruses, hepatitis A virus; enteroviruses, human Coxsackie viruses, rhinoviruses, echoviruses); Calciviridae (e.g., strains that cause gastroenteritis); Togaviridae (e.g., equine encephalitis viruses, rubella viruses); Flaviviridae (e.g., dengue viruses, encephalitis viruses, yellow fever viruses); Coronaviridae (e.g., coronaviruses); Rhabdoviridae (e.g., vesicular stomatitis viruses, rabies viruses); Filoviridae (e.g., Ebola viruses); Paramyxoviridae (e.g., parainfluenza viruses, mumps virus, measles virus, respiratory syncytial virus); Orthomyxoviridae (e.g., influenza viruses); Bunyaviridae (e.g., Hantaan viruses, bunya viruses, phleboviruses and Nairo viruses); Arenaviridae (hemorrhagic fever viruses); Reoviridae (e.g., reoviruses, orbiviruses and rotaviruses); Bornaviridae; Hepadnaviridae (Hepatitis B virus); Parvoviridae (parvoviruses); Papovaviridae (papilloma viruses, polyoma viruses); Adenoviridae (most adenoviruses); Herpesviridae (herpes simplex virus (HSV) 1 and 2, varicella zoster virus, cytomegalovirus (CMV), herpes virus; Poxviridae (variola viruses, vaccinia viruses, pox viruses); and Iridoviridae (e.g., African swine fever virus); and unclassified viruses (e.g., the agent of delta hepatitis (thought to be a defective

satellite of hepatitis B virus), Hepatitis C; Norwalk and related viruses, and astroviruses). Alternatively, a viral antigen may be produced recombinantly.

[0156] As used herein, the terms “cancer antigen” and “tumor antigen” are used interchangeably and refer to antigens (other than NKp46 or CD16) that are differentially expressed by cancer cells or are expressed by non-tumoral cells (e.g. immune cells) having a pro-tumoral effect (e.g. an immunosuppressive effect), and can thereby be exploited in order to target cancer cells. Cancer antigens are antigens which can potentially stimulate apparently tumor-specific immune responses. Some of these antigens are encoded, although not necessarily expressed, or expressed at lower levels or less frequently, by normal cells. These antigens can be characterized as those which are normally silent (i.e., not expressed) in normal cells, those that are expressed only at certain stages of differentiation and those that are temporally expressed such as embryonic and fetal antigens. Other cancer antigens are encoded by mutant cellular genes, such as oncogenes (e.g., activated ras oncogene), suppressor genes (e.g., mutant p53), fusion proteins resulting from internal deletions or chromosomal translocations. Still other cancer antigens can be encoded by viral genes such as those carried on RNA and DNA tumor viruses. Still other cancer antigens can be expressed on immune cells capable of contributing to or mediating a pro-tumoral effect, e.g. cell that contributes to immune evasion, a monocyte or a macrophage, optionally a suppressor T cell, regulatory T cell, or myeloid-derived suppressor cell.

[0157] The cancer antigens are usually normal cell surface antigens which are either over-expressed or expressed at abnormal times, or are expressed by a targeted population of cells. Ideally the target antigen is expressed only on proliferative cells (e.g., tumor cells) or pro-tumoral cells (e.g. immune cells having an immunosuppressive effect), however this is rarely observed in practice. As a result, target antigens are in many cases selected on the basis of differential expression between proliferative/disease tissue and healthy tissue. Example of cancer antigens include: Receptor Tyrosine Kinase-like Orphan Receptor 1 (ROR1), Crypto, CD4, CD20, CD30, CD19, CD38, CD47, Glycoprotein NMB, CanAg, Her2 (ErbB2/Neu), a Siglec family member, for example CD22 (Siglec2) or CD33 (Siglec3), CD79, CD138, CD171, PSCA, L1-CAM, PSMA (prostate specific membrane antigen), BCMA, CD52, CD56, CD80, CD70, E-selectin, EphB2, Melanotransferrin, Mud 6 and TMEFF2. Examples of cancer antigens also include Immunoglobulin superfamily (IgSF) such as cytokine receptors, Killer-Ig Like Receptor, CD28 family proteins, for example, Killer-Ig Like Receptor 3DL2 (KIR3DL2), B7-H3, B7-H4, B7-H6, PD-L1, IL-6 receptor. Examples also include MAGE, MART-1/Melan-A, gp100, major histocompatibility complex class I-related chain A and B polypeptides (MICA and MICB), adenosine deaminase-binding protein (ADAbp), cyclophilin b, colorectal associated antigen (CRC)-C017-1A/GA733, protein tyrosine kinase 7 (PTK7), receptor protein tyrosine kinase 3 (TYRO-3), nectins (e.g. nectin-4), major histocompatibility complex class I-related chain A and B polypeptides (MICA and MICB), proteins of the UL16-binding protein (ULBP) family, proteins of the retinoic acid early transcript-1 (RAET1) family, carcinoembryonic antigen (CEA) and its immunogenic epitopes CAP-1 and CAP-2, etv6, aml1 prostate specific antigen (PSA), T-cell receptor/CD3-zeta chain, MAGE-family of

tumor antigens, GAGE-family of tumor antigens, anti-Müllerian hormone Type II receptor, delta-like ligand 4 (DLL4), DRS, ROR1 (also known as Receptor Tyrosine Kinase-Like Orphan Receptor 1 or NTRKR1 (EC 2.7.10.1), BAGE, RAGE, LAGE-1, NAG, GnT-V, MUM-1, CDK4, MUC family, VEGF, VEGF receptors, Angiopoietin-2, PDGF, TGF- α , EGF, EGF receptor, members of the human EGF-like receptor family, e.g., HER-2/neu, HER-3, HER-4 or a heterodimeric receptor comprised of at least one HER subunit, gastrin releasing peptide receptor antigen, Muc-1, CA125, integrin receptors, α v β 3 integrins, α 5 β 1 integrins, α IIb β 3-integrins, PDGF beta receptor, SVE-cadherin, IL-8 receptor, hCG, IL-6 receptor, CSF1R (tumor-associated monocytes and macrophages), α -fetoprotein, E-cadherin, α -catenin, β -catenin and γ -catenin, p120ctn, PRAME, NY-ESO-1, cdc27, adenomatous polyposis coli protein (APC), fodrin, Connexin 37, Ig-idiotype, p15, gp75, GM2 and GD2 gangliosides, viral products such as human papillomavirus proteins, imp-1, P1A, EBV-encoded nuclear antigen (EBNA)-1, brain glycogen phosphorylase, SSX-1, SSX-2 (HOM-MEL-40), SSX-1, SSX-4, SSX-5, SCP-1 and CT-7, and c-erbB-2, although this is not intended to be exhaustive. In one aspect, the antigen of interest is an antigen (e.g. any one of the antigens listed above) capable of undergoing intracellular internalization, for example when bound by a conventional human IgG1 antibody, either in the presence of absence of Fc γ receptor cells. In one aspect, the antigen of interest is a CD19 or CD20 polypeptide; in one aspect, the multispecific protein comprises a V_H and/or V_L , or a scFv or another ABD that binds CD19 or CD20 comprising an amino acid sequence which is at least 60%, 70%, 80%, 85%, 90% or 95% identical to the sequence of the anti-CD19 or anti-CD20 respective V_H , V_L or scFv described in the Examples herein, or comprises the heavy and light chain CDR1, -2 and -3 of the anti-CD19 or anti-CD20 heavy and light chain variable regions disclosed herein. In one aspect, the multispecific protein competes for binding to a human CD19 or CD20 polypeptide with an antibody, or a F5 or T6 protein, comprising the respective anti-CD19 or anti-CD20 V_H , V_L or scFv disclosed in the Examples.

[0158] In one embodiment, the ABD that binds an antigen of interest is derived from (e.g. comprises the hypervariable region of, or comprises one, two, three, four, five or six of the CDRs of) a parental antibody that binds an antigen of interest (e.g. a murine antibody, a human antibody) which, when bound to its antigenic target (the antigen of interest on cells), increases or induces down-modulation or intracellular internalization of the antigen of interest. In one embodiment, the antigen of interest is a cancer antigen, e.g. one of the cancer antigens listed above known to internalize (e.g. Immunoglobulin superfamily (IgSF) members, for example cytokine receptor α or β chains, Killer-Ig Like Receptors, CD28 family proteins, B7-H3, B7-H4, B7-H6, KIR3DL2, PTK7, ROR1, L1-CAM, Siglec family members, EGF receptor and EGF-like receptor family members, EGFR, HER-2, integrins, anti-Müllerian hormone Type II receptor, CSF-1R, and others) In one embodiment, the antigen target is a polypeptide present on an immune cell capable of mediating a pro-tumoral effect, e.g. a monocyte or a macrophage, optionally a suppressor T cell, regulatory T cell, or myeloid-derived suppressor cell.

[0159] In one embodiment, the ABD binds to a cancer antigen, a viral antigen, a microbial antigen, or an antigen present on an infected cell (e.g. virally infected) or on a

pro-inflammatory immune cell. In one embodiment, said antigen is a polypeptide selectively expressed or overexpressed on a tumor cell, and infected cell or a pro-inflammatory cell. In one embodiment, said antigen is a polypeptide that when inhibited, decreases the proliferation and/or survival of a tumor cell, an infected cell or a pro-inflammatory cell.

[0160] The ABDs which are incorporated into the polypeptides can be tested for any desired activity prior to inclusion in a multispecific Nkp46-binding protein, for example the ABD can be tested for binding to an antigen of interest.

[0161] An ABD derived from an antibody will generally comprise at minimum a hypervariable region sufficient to confer binding activity. It will be appreciated that an ABD may comprise other amino acids or functional domains as may be desired, including but not limited to linker elements (e.g. linker peptides, CH1, C κ or C λ domains, hinges, or fragments thereof). In one example an ABD comprises a scFv, a V_H domain and a V_L domain, or a single domain antibody (nanobody or dAb) such as a V-NAR domain or a V_{HH} domain. Exemplary antibody formats are further described herein and an ABD can be selected based on the desired format.

In any embodiment, an antigen binding domain can be obtained from a humanized antibody in which residues from a complementary-determining region (CDR) of a human antibody are replaced by residues from a CDR of the original antibody (the parent or donor antibody, e.g. a murine or rat antibody) while maintaining the desired specificity, affinity, and capacity of the original antibody. The CDRs of the parent antibody, some or all of which are encoded by nucleic acids originating in a non-human organism, are grafted in whole or in part into the beta-sheet framework of a human antibody variable region to create an antibody, the specificity of which is determined by the engrafted CDRs. The creation of such antibodies is described in, e.g., WO 92/11018, Jones, 1986, *Nature* 321:522-525, Verhoeven et al., 1988, *Science* 239:1534-1536. An antigen binding domain can thus have non-human hypervariable regions or CDRs and human frameworks region sequences (optionally with back mutations).

[0162] Once appropriate antigen binding domains having desired specificity and/or activity are identified, DNA encoding each of the or ABD can be separately placed, in suitable arrangements, in an appropriate expression vector, together with DNA encoding any elements such as CH1, C κ , CH2 and CH3 domains or portions thereof and any other optional elements (e.g. DNA encoding a hinge-derived or linker elements) for transfection into an appropriate host. ABDs will be arranged in an expression vector, or in separate vectors as a function of which type of polypeptide is to be produced, so as to produce the Fc-polypeptides having the desired domains operably linked to one another. The host is then used for the recombinant production of the multispecific polypeptide.

For example, a polypeptide fusion product can be produced from a vector in which the first of the two ABD is operably linked (e.g. directly, or via a CH1, C κ or C λ constant region and/or hinge region) to the N-terminus of a CH2 domain, and the CH2 domain is operably linked at its C-terminus to the N-terminus a CH3 domain. The second of the two ABD can be on a second polypeptide chain that forms a dimer, e.g.

heterodimer, with the polypeptide comprising the first ABD. The polypeptide may comprise a full length and/or dimeric Fc domain.

The multispecific polypeptide can then be produced in an appropriate host cell or by any suitable synthetic process. A host cell chosen for expression of the multispecific polypeptide is an important contributor to the final composition, including, without limitation, the variation in composition of the oligosaccharide moieties decorating the protein in the immunoglobulin CH2 domain. Thus, one aspect of the invention involves the selection of appropriate host cells for use and/or development of a production cell expressing the desired therapeutic protein such that the multispecific polypeptide retains FcRn and CD16 binding. The host cell may be of mammalian origin or may be selected from COS-1, COS-7, HEK293, BHK21, CHO, BSC-1, Hep G2, 653, SP2/0, 293, HeLa, myeloma, lymphoma, yeast, insect or plant cells, or any derivative, immortalized or transformed cell thereof. The host cell may be any suitable species or organism capable of producing N-linked glycosylated polypeptides, e.g. a mammalian host cell capable of producing human or rodent IgG type N-linked glycosylation.

[0163] Multimeric bispecific proteins such as heterodimers, heterotrimers and tetramers (the latter including for example bispecific antibodies with two heavy chains and two light chains) can be produced according to a variety of formats. The multimeric polypeptide will generally comprise a dimeric Fc domain that is capable of binding to human CD16 or CD16A and optionally other Fcγ receptors, e.g., CD16B, CD32A, CD32B and/or CD64). Fc moieties with substantial FcRn and CD16 (CD16A) binding can be obtained through the use of suitable CH2 and/or CH3 domains, as further described herein. In one embodiment, an Fc moiety is derived from a human IgG1 isotype constant region. In one embodiment, an Fc moiety may be obtained by production of the polypeptide in a host cell or by a process that yields N297-linked glycosylation, e.g. a mammalian cell. In one embodiment, an Fc moiety comprises one or more amino acid modifications, e.g. in the CH2 domain, that increases binding to CD16 or CD16A.

[0164] In one example, the protein comprises a first and a second polypeptide chain each comprising a variable domain fused to a human Fc domain (comprising a CH3 domain capable of undergoing preferential CH3-CH3 hetero-dimerization), wherein the first and second chain associate via CH3-CH3 dimerization and the protein comprises a dimeric Fc domain. The variable domains of each chain can be part of the same or different antigen binding domains.

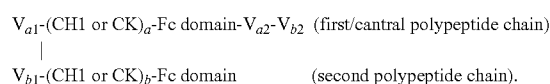
[0165] One advantageous way of making multimeric polypeptides is through the assembly of different polypeptide chains that each comprise at least one heavy or light chain variable domain fused to a human CH1 or Cκ constant domain (a V-(CH1/Cκ) unit), wherein the protein chains undergo CH1-Cκ dimerization and are bound to one another by non-covalent bonds and optionally further disulfide bonds formed between respective CH1 and Cκ domains. In one embodiment, the invention provides an isolated or purified heterodimeric or heterotrimeric protein that binds to a first and second antigen, wherein the protein comprises at least two or three polypeptide chains, each comprising a V-(CH1/Cκ) unit, whereby the chains are bound to one another by non-covalent bonds and optionally further bound via disulfide bonds between CH1 and Cκ domains, and still further optionally, whereby the chains are bound by non-

covalent bonds between the respective variable regions, CH1 and Cκ domains, and CH3 domains of the Fc portion.

[0166] In one example, the protein comprises a first and a second polypeptide chain each comprising a variable domain fused to a CH1 or Cκ domain (a V-(CH1/Cκ) unit), in turn fused at its C-terminus to a human Fc domain (comprising a CH3 domain capable of undergoing CH3-CH3 dimerization), wherein the first and second chain associate via CH1-Cκ and CH3-CH3 dimerization and the protein comprises a dimeric Fc domain. The variable domains of each chain can be part of the same or different antigen binding domains.

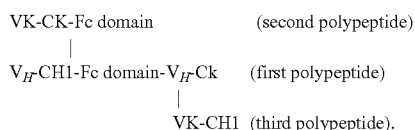
[0167] The variable and constant regions can be selected and configured such that each chain will preferentially associate with its desired complementary partner chain. The resulting multimeric protein will therefore be simple to produce using conventional production methods using recombinant host cells. The choice of which V_H or V_L to associate with a CH1 and Cκ in a unit is based on affinity between the units to be paired so as to drive the formation of the desired multimer. The resulting multimer will be bound by non-covalent bonds between complementary V_H and V_L domains, by non-covalent bonds between complementary CH1 and Cκ domains, and optionally by further disulfide bonding between complementary CH1 and Cκ domains (and optionally further disulfide bonds between complementary hinge domains). V_H-V_L associations are stronger than V_H-V_H or V_L-V_L , consequently, as shown herein, one can place a V_H or a V_L next to either a CH1 or a Cκ, and the resulting V-C unit will partner preferably with its V-C counterpart. For example $V_H-Cκ$ will pair with V_L-CH1 preferentially over V_H-CH1 . Additionally, by including an Fc domain, preferred chain pairing is further improved, as the two Fc-containing chains are bound by non-covalent bonds between CH3 domains of the Fc domains. The different V-C combinations, optionally further combined with Fc pairing thereby provides tools to make heteromultimeric proteins.

[0168] In one example, the multispecific protein comprises a first and a second polypeptide chain each comprising a variable domain fused to a CH1 or Cκ domain (a V-(CH1/Cκ) unit), in turn fused at its C-terminus to a human Fc domain, wherein the V-(CH1/Cκ) unit of the first chain has undergone CH1-Cκ dimerization with the V-(CH1/Cκ) unit of the second chain thereby forming a first antigen binding domain (ABD₁) and a dimeric Fc domain, wherein one of the polypeptide chains further comprises an antigen binding domain that forms a second antigen binding domain (ABD₂), and wherein the Fc domain binds to a human CD16 polypeptide. In one embodiment, the Fc domain comprises N-linked glycosylation at residue N297 (Kabat EU numbering). In one example, the protein has a domain arrangement:

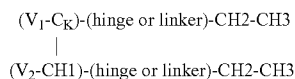


[0169] In one example, the protein comprises three polypeptide chains, each comprising a variable domain fused to a CH1 or Cκ domain (a V-(CH1/Cκ) unit), wherein a first (central) chain comprises two V-(CH1/Cκ) units and a human Fc domain interposed between the units, the second chain comprises one V-(CH1/Cκ) unit and a human Fc

domain, and the third chain comprises one V-(CH1/C κ) unit, wherein one of the V-(CH1/C κ) units of the central chain has undergone CH1-C κ dimerization with the V-(CH1/C κ) unit of the second chain thereby forming a first antigen binding domain (ABD₁) and a dimeric Fc domain, and wherein the other of the V-(CH1/C κ) units of the central chain has undergone CH1-C κ dimerization with the V-(CH1/C κ) unit of the third chain thereby forming a second antigen binding domain (ABD₂), and wherein the Fc domain binds to a human CD16 polypeptide. In one embodiment, the Fc domain comprises N-linked glycosylation at residue N297 (Kabat EU numbering). In one example, the protein has a domain arrangement:

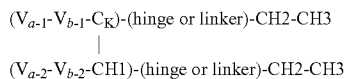


[0170] In certain formats, heterodimers are formed in which variable domains adjacent to the CH1 or C κ domain do not require association with the second chain to form an antigen binding domain. For example, through use of single variable domains, or scFv, each chain will contain a functional ABD. Examples based CH1-C κ dimerization with single variable exemplary heterodimer molecules can have a domain arrangement:



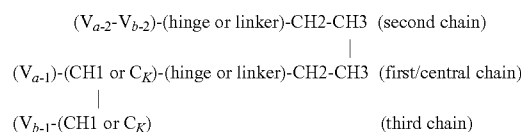
wherein V₁ and V₂ are single variable domains (e.g. V_H domain, a V_L domain, a dAb, a V-NAR domain or a V_HH domain), and one of V₁ and V₂ binds NKp46 and the other binds an antigen of interest.

[0171] In one embodiment, exemplary heterodimer molecules can have a domain arrangement:



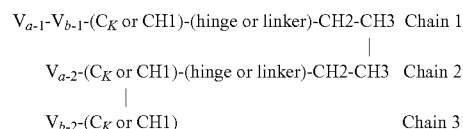
wherein V_{a-1}, V_{b-1}, V_{a-2} and V_{b-2} are each a V_H domain or a V_L domain, and wherein one of V_{a-1} and V_{b-1} is a V_H and the other is a V_L such that V_{a-1} and V_{b-1} form a first antigen binding domain (ABD), wherein one of V_{a-2} and V_{b-2} is a V_H and the other is a V_L such that V_{a-2} and V_{b-2} form a second antigen binding domain, wherein one of the ABD binds NKp46 and the other binds an antigen of interest. In one variant of the foregoing, any of, or each of the V_{a-1}, V_{b-1}, V_{a-2} and V_{b-2} are a scFv (made up of two variable domains). Each pair of V domains can be separated by a linker peptide (e.g. to form a scFv).

[0172] In similar approaches, trimers can be constructed. Exemplary heterotrimer molecules can have the following domain arrangement:



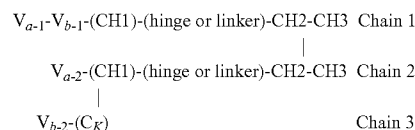
wherein the first/central chain and the second chain associate by CH3-CH3 dimerization and the first/central chain and the third chain associate by the CH1 or C κ dimerization, wherein the domains of the first/central chain and the third chain are selected to be complementary to permit the first and third chains to associate by CH1-C κ dimerization, and wherein V_{a-1}, V_{b-1}, V_{a-2} and V_{b-2} are each a V_H domain or a V_L domain, and wherein one of V_{a-1} and V_{b-1} is a V_H and the other is a V_L such that V_{a-1} and V_{b-1} form a first antigen binding domain (ABD), wherein one of V_{a-2} and V_{b-2} is a V_H and the other is a V_L such that V_{a-2} and V_{b-2} form a second antigen binding domain (e.g. an scFv wherein V_{a-2} and V_{b-2} are separated by a linker), wherein one of the ABD binds NKp46 and the other binds an antigen of interest.

[0173] In one embodiment, multimeric proteins are constructed based upon two Fc-containing chains (e.g. chains 1 and 2) to create a dimer via CH3-CH3 dimerization and/or hinge dimerization, and a further chain (e.g. chain 3) comprising a V-C_H/C κ unit that dimerizes with one of chains 1 or 2. Exemplary molecules can have the following domain arrangement:



wherein V_{a-1}, V_{b-1}, V_{a-2} and V_{b-2} are each a V_H domain or a V_L domain, and wherein one of V_{a-1} and V_{b-1} is a V_H and the other is a V_L such that V_{a-1} and V_{b-1} form a first antigen binding domain (ABD), wherein one of V_{a-2} and V_{b-2} is a V_H and the other is a V_L such that V_{a-2} and V_{b-2} form a second antigen binding domain.

[0174] Exemplary molecules may possess the following domain arrangement:



wherein V_{a-1}, V_{b-1}, V_{a-2} and V_{b-2} are each a V_H domain or a V_L domain, and wherein one of V_{a-1} and V_{b-1} is a V_H and the other is a V_L such that V_{a-1} and V_{b-1} form a first antigen binding domain (ABD), wherein one of V_{a-2} and V_{b-2} is a V_H and the other is a V_L such that V_{a-2} and V_{b-2} form a second antigen binding domain. The CH1 and C κ are selected such that chain 1 is capable of associating with chain 2 and chain 2 with chain 3. The protein can be configured such that chains 1 and 2 associate via CH3-CH3 dimerization and chains 2 and 3 associate via CH1-C κ dimerization.

[0175] Optionally, any of the multispecific proteins of the invention may include, CH3 domains which comprise amino acid substitutions, wherein the CH3 domain interface of the antibody Fc region is mutated to create altered charge polarity across the Fc dimer interface such that co-expression of electrostatically matched Fc chains supports favorable attractive interactions thereby promoting desired Fc heterodimer formation, whereas unfavorable repulsive charge interactions suppress unwanted Fc homodimer formation.

[0176] Heterodimeric or heterotrimeric polypeptides with two ABDs and a dimeric Fc domain can optionally be produced as one or more chains that each associate with a central chain, e.g. by CH1-C κ heterodimerization. Such multimers may be composed of a central (first) polypeptide chain comprising two immunoglobulin variable domains that are part of separate antigen binding domains (of different antigen specificities), the one or more other chains that provide the additional and/or complementary variable domains, and an Fc domain placed on the central and/or one or more other chains. In one embodiment, the Fc domain is interposed between the two ABDs in the multimeric protein.

[0177] In one example, the first (central) polypeptide chain will provide one variable domain that will, together with a complementary variable domain on a second polypeptide chain, form an antigen binding domain specific for one (e.g. a first) antigen of interest. The first (central) polypeptide chain will also provide a second variable domain (e.g., placed on the opposite end of the interposed Fc domain) that will be paired with a complementary variable domain to form an antigen binding domain specific for another (e.g. a second) antigen of interest; the variable domain that is complementary to the second variable domain can be placed on the central polypeptide (e.g. adjacent to the second variable domain in a tandem variable domain construct such as an scFv), or can be placed on a separate polypeptide chain, notably a third polypeptide chain. The second (and third, if present) polypeptide chains will associate with the central polypeptide chain by CH1-C κ heterodimerization, forming non-covalent bonds and optionally further interchain disulfide bonds between complementary CH1 and C κ domains (and optionally interchain disulfide bonds between hinge regions), with a primary multimeric polypeptide being formed so long as CH/CK and V_H/V κ domains are chosen to give rise to a preferred dimerization configuration that results preferentially in the desired V_H-V_L pairings. Remaining unwanted pairings can remain minimal during production and/or are removed during purification steps. In a trimer, or when polypeptides are constructed for preparation of a trimer, there will generally be one polypeptide chain that comprises a non-naturally occurring VH-CK or VK-CH1 domain arrangement.

[0178] Examples of the domain arrangements (N- to C-termini) of central polypeptide chains for use in such heterodimeric proteins include any of the following:

[0179] V-(CH1 or C κ)-Fc domain-V-V;
and

[0180] V-V-(CH1 or C κ)-Fc domain;
and

[0181] Fc domain-V-V;
and

[0182] V-V-Fc domain;
and

[0183] V-V-Fc domain-V-(CH1 or C κ).

[0184] For example, the domain arrangements (N- to C-termini) of central polypeptide chains for use in such heterodimeric proteins can include:

[0185] V_{a-1}-(CH1 or C κ)_a-Fc domain-V_{a-2}-V_{b-2};
and

[0186] V_{a-2}-V_{b-2}-Fc domain-(CH1 or C κ)_a
wherein V_{a-1} is a light chain or heavy chain variable domain, and wherein one of V_{a-2} and V_{b-2} is a light chain variable domain and the other is a heavy chain variable domain.

[0187] Further examples include:

[0188] V_{a-1}-(CH1 or C κ)_a-Fc domain V_b;
and

[0189] V_b-Fc domain-V_{a-1}-(CH1 or C κ)_a

[0190] wherein V_b is a single variable domain (e.g. dAb, VhH).

[0191] The Fc domain of the central chain may be a full Fc domain (CH2-CH3) or a portion thereof sufficient to confer the desired functionality (e.g. CD16 and optionally FcRn or another Fc receptor binding) when it forms a dimeric Fc with a second chain. A second polypeptide chain will then be configured which will comprise an immunoglobulin variable domain and a CH1 or C κ constant region, e.g., a (CH1 or C κ)_b unit, selected so as to permit CH1-C κ heterodimerization with the central polypeptide chain; the immunoglobulin variable domain will be selected so as to complement the variable domain of the central chain that is adjacent to the CH1 or C κ domain, whereby the complementary variable domains form an antigen binding domain for a first antigen of interest.

[0192] For example, a second polypeptide chain can comprise a domain arrangement: V_{b-1}-(CH1 or C κ)_b-Fc domain such that the (CH1 or C κ)₂ dimerizes with the (CH1 or C κ)₁ on the central chain, and the V_{b-1} forms an antigen binding domain together with V_{a-1} of the central chain. If the V_{a-1} of the central chain is a light chain variable domain, then V_{b-1} will be a heavy chain variable domain; and if V_{a-1} of the central chain is a heavy chain variable domain, then V_{b-1} will be a light chain variable domain.

[0193] The antigen binding domain for the second antigen of interest can then be formed from V_{a-2} and V_{b-2} which are configured as tandem variable domains on the central chain forming the antigen binding domain for the second antigen of interest (e.g. a heavy chain variable domain (V_H) and a light chain (K) variable domain (V κ), for example forming an scFv unit). The antigen binding domain for the second antigen of interest can also alternatively be formed from a single variable domain V_b present on the central chain.

[0194] The resulting heterodimer can, for example, have the following configuration (see further Examples of such proteins shown as formats 13 and 14 shown in FIGS. 2D and 2E):

$$\begin{array}{l} V_{a-1}-(CH1 \text{ or } C\kappa)_a\text{-Fc domain-V}_{a-2}\text{-V}_{b-2} \text{ (first/central polypeptide chain)} \\ | \\ V_{b-1}-(CH1 \text{ or } C\kappa)_b\text{-Fc domain} \text{ (second polypeptide chain)} \end{array}$$

[0195] wherein one of V_{a-1} of the first polypeptide chain and V_{b-1} of the second polypeptide chain is a light chain variable domain and the other is a heavy chain variable domain, and wherein one of V_{a-2} and V_{b-2} is a light chain variable domain and the other is a heavy chain variable domain.

[0196] In one embodiment, the heterodimeric bispecific Fc-derived polypeptide comprises a domain arrangement selected from one of the following, optionally wherein one or both of the hinge domains are replaced by a peptide linker, optionally wherein the Fc domain is fused to anti-NKp46 scFv via a peptide linker):

or

(Vk-Ck-hinge)-Fc domain-(anti-NKp46)

|

(V_H-CH1-hinge)-Fc domain

or

(V_H-CH1-hinge)-Fc domain-(anti-NKp46)

|

(Vk-Ck-hinge)-Fc domain

or

(V_H-CH1-hinge)-Fc domain

|

(Vk-CK-hinge)-Fc domain-(anti-NKp46)

[0197] Other examples of potential domain arrangements for the heterodimeric polypeptides according to the invention include but are not limited to those shown in the table below:

V _K -V _H -Fc domain-V _H -(CH1)	V _H -V _K -Fc domain-V _H -(CH1)
Fc domain-V _K -(C _K)	Fc domain-V _K -(C _K)
V _K -V _H -Fc domain-V _K -(CH1)	V _H -V _K -Fc domain-V _K -(CH1)
Fc domain-V _H -(C _K)	Fc domain-V _H -(C _K)
V _H -(CH1)-Fc domain-V _H -V _K	V _H -(CH1)-Fc domain-V _K -V _H
V _K -(C _K)-Fc domain	V _K -(C _K)-Fc domain
V _K -(CH1)-Fc domain-V _H -V _K	V _K -(CH1)-Fc domain-V _K -V _H
V _H -(C _K)-Fc domain	V _H -(C _K)-Fc domain

[0198] Heterotrimeric proteins can for example be formed by using a central (first) polypeptide chain comprising a first variable domain (V) fused to a first CH1 or Cκ constant region, a second variable domain (V) fused to a second CH1 or Cκ constant region, and an Fc domain or portion thereof interposed between the first and second variable domains (i.e. the Fc domain is interposed between the first and second (V-(CH1/Cκ) units. For example, a central polypeptide chain for use in a heterotrimeric protein according to the invention can have the domain arrangements (N- to C-termini) as follows:

[0199] V_{a-1}-(CH1 or Cκ)_a-Fc domain-V_{a-2}-(CH1 or Cκ)_b.

[0200] A second polypeptide chain can then comprise a domain arrangement (N- to C-termini):

[0201] V_{b-1}-(CH1 or Cκ)_c-Fc domain

[0202] such that the (CH1 or Cκ)_c dimerizes with the (CH1 or Cκ)_a on the central chain, and the V_{a-1} and V_{b-1} form an antigen binding domain.

[0203] A third polypeptide chain can then comprise the following domain arrangement (N- to C-termini):

[0204] V_{b-2}-(CH1 or Cκ)_d

[0205] such that the (CH1 or Cκ)_d dimerizes with the (CH1 or Cκ)_b unit on the central chain, and the V_{a-2} and V_{b-2} form an antigen binding domain.

[0206] An example of a domain configuration of a resulting heterotrimer with a dimeric Fc domain (also shown as formats 5, 6, 7 and 16 in FIGS. 2D and 2E) is shown below:

V _{b-1} -(CH1 or Cκ) _c -Fc domain	(second polypeptide)
V _{a-1} -(CH1 or Cκ) _a -Fc domain-V _{a-2} -(CH1 or Cκ) _b	(first polypeptide)
V _{b-2} -(CH1 or Cκ) _d	(third polypeptide)

[0207] Thus, in a trimeric polypeptide according to the invention, the first polypeptide can have two variable domains that each form an antigen binding domain with a variable domain on a separate polypeptide chain (i.e. the variable domain of the second and third chains), the second polypeptide chain has one variable domain, and the third polypeptide has one variable domain.

[0208] A trimeric polypeptide according to the invention may further comprise the following:

[0209] (a) a first polypeptide chain comprising a first variable domain (V) fused to a first CH1 or Cκ constant region, a second variable domain (V) fused to a second CH1 or Cκ constant region, and an Fc domain or portion thereof interposed between the first and second variable domains;

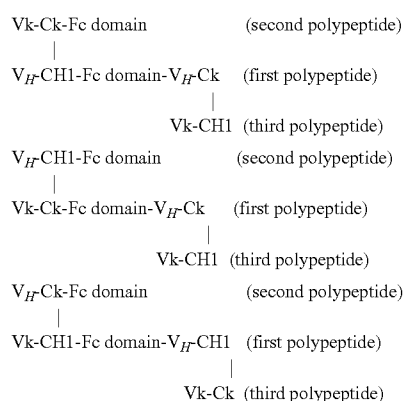
[0210] (b) a second polypeptide chain comprising a variable domain fused at its C-terminus to a CH1 or Cκ constant region selected to be complementary to the first CH1 or Cκ constant region of the first polypeptide chain such that the first and second polypeptides form a CH1-Cκ heterodimer, and an Fc domain; and

[0211] (c) a third polypeptide chain comprising a variable domain fused (e.g. at its C-terminus) to a CH1 or Cκ constant region, wherein the variable domain and the constant region are selected to be complementary to the second variable domain and second CH1 or Cκ constant region of the first polypeptide chain such that the first and third polypeptides form a CH1-Cκ heterodimer bound by non-covalent and optionally further disulfide bond(s) formed between the CH1 or Cκ constant region of the third polypeptide and the second CH1 or Cκ constant region of the first polypeptide, but not between the CH1 or Cκ constant region of the third polypeptide and the first CH1 or Cκ constant region of the first polypeptide

[0212] wherein the first, second and third polypeptides form a CH1-Cκ heterotrimer, and wherein the first variable domain of the first polypeptide chain and the variable domain of the second polypeptide chain form an antigen binding domain specific for a first antigen of interest, and the second variable domain of the first polypeptide chain and the

variable domain on the third polypeptide chain form an antigen binding domain specific for a second antigen of interest.

[0213] Examples of potential domain arrangements for such trimeric bispecific polypeptides include but are not limited to those shown below:



[0214] In further examples, heterotrimers can be constructed with three ABDs and a dimeric Fc. One of the ABDs will bind to NKp46, and the other two ABDs can bind to an antigen of interest, wherein the antigen of interest bound by the two ABDs can be the same antigen or a different antigen. Thus, in one aspect of such an embodiment, the multimeric polypeptide can bind the antigen of interest in a bivalent manner (with two ABDs).

[0215] Heterotrimeric proteins can for example be formed by using a central (first) polypeptide chain comprising a first variable domain (V) fused to a first CH1 or Ck constant region, a second variable domain (V) fused to a second CH1 or Ck constant region, and an Fc domain or portion thereof interposed between the first and second variable domains (i.e. the Fc domain is interposed between the first and second (V-(CH1/Ck)) units. For example, a central polypeptide chain for use in a heterotrimeric protein can have the domain arrangements (N- to C-termini) as follows:

[0216] $V_1-(CH1 \text{ or } Ck)_a\text{-Fc domain-}V_2-(CH1 \text{ or } Ck)_b$.

[0217] A second polypeptide chain can then comprise a domain arrangement (N- to C-termini):

[0218] $V_1-(CH1 \text{ or } Ck)_c$.

[0219] or

[0220] $-(CH1 \text{ or } Ck)_c\text{-Fc domain}$

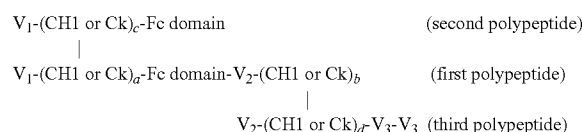
[0221] such that the $(CH1 \text{ or } Ck)_c$ dimerizes with the $(CH1 \text{ or } Ck)_a$ on the central chain, and the V_{a1} and V_{b1} form an antigen binding domain.

[0222] A third polypeptide chain can then comprise a domain arrangement (N- to C-termini):

[0223] $V_2-(CH1 \text{ or } Ck)_d\text{-scFv}$,

[0224] such that the $(CH1 \text{ or } Ck)_d$ dimerizes with the $(CH1 \text{ or } Ck)_b$ unit on the central chain, and the V_{a2} and V_{b2} form an antigen binding domain.

[0225] An example of a configuration of a resulting heterotrimer with a dimeric Fc domain (also shown as formats T5 and T6 in FIG. 2F) has a domain arrangement:



[0226] In any of the polypeptide chains herein, a hinge region will typically be present on a polypeptide chain between a CH1 domain and a CH2 domain of an Fc domain, and/or can be present between a Ck domain and a CH2 domain. A hinge region can optionally be replaced, e.g., by a suitable linker peptide.

[0227] In any of the domain arrangements, the Fc domain may comprise a CH2-CH3 unit (a full length CH2 and CH3 domain or a fragment thereof). In heterodimers or heterotrimers comprising two chains with Fc domains (a dimeric Fc domain), the CH3 domain will be capable of CH3-CH3 dimerization (e.g. it will comprise a wild-type CH3 domain).

[0228] In some exemplary configurations, the multispecific protein can be a heterodimer, a heterotrimer or a heterotetramers, wherein the polypeptide chains are engineered for heterodimerization among each other so as to produce the desired protein. In embodiments where the desired chain pairings are not driven by CH1-Ck dimerization, the chains may comprise constant or Fc domains with amino acid modifications (e.g., substitutions) that favor the preferential hetero-dimerization of the two different chains over the homo-dimerization of two identical chains. In some embodiments, a "knob-into-holes" approach is used in which the CH3 domain interface of the antibody Fc region is mutated so that the antibodies preferentially form heterodimers (further including the attached light chains). These mutations create altered charge polarity across the Fc dimer interface such that co-expression of electrostatically matched Fc chains support favorable attractive interactions thereby promoting desired Fc heterodimer formation, whereas unfavorable repulsive charge interactions suppress unwanted Fc homodimer formation. For example one heavy chain comprises a T366W substitution and the second heavy chain comprises a T366S, L368A and Y407V substitution, see, e.g. Ridgway et al (1996) *Protein Eng.*, 9, pp. 617-621; Atwell (1997) *J. Mol. Biol.*, 270, pp. 26-35; and WO2009/089004, the disclosures of which are incorporated herein by reference. In another approach, one heavy chain comprises a F405L substitution and the second heavy chain comprises a K409R substitution, see, e.g., Labrijn et al. (2013) *Proc. Natl. Acad. Sci. U.S.A.*, 110, pp. 5145-5150. In another approach, one heavy chain comprises T350V, L351Y, F405A, and Y407V substitutions and the second heavy chain comprises T350V, T366S, K392L, and T394W substitutions, see, e.g. Von Kreudenstein et al., (2013) *mAbs* 5:646-654. In another approach, one heavy chain comprises both K409D and K392D substitutions and the second heavy chain comprises both D399K and E356K substitutions, see, e.g. Gunasekaran et al., (2010) *J. Biol. Chem.* 285:19637-19646. In another approach, one heavy chain comprises D221E, P228E and L368E substitutions and the second heavy chain comprises D221R, P228R, and K409R substitutions, see, e.g. Strop et al., (2012) *J. Mol. Biol.* 420: 204-219. In another approach, one heavy chain comprises S364H and F405A substitutions and the second heavy chain comprises Y349T and, T394F substitutions, see, e.g. Moore

et al., (2011) mAbs 3: 546-557. In another approach, one heavy chain comprises a H435R substitution and the second heavy chain optionally may or may not comprise a substitution, see, e.g. U.S. Pat. No. 8,586,713. When such heteromultimeric antibodies have Fc regions derived from a human IgG2 or IgG4, the Fc regions of these antibodies can be engineered to contain amino acid modifications that permit CD16 binding. In some embodiments, the antibody may comprise mammalian antibody-type N-linked glycosylation at residue N297 (Kabat EU numbering).

[0229] In some embodiments the invention also comprises a heterodimeric or heterotrimeric protein that comprises an NKp46-binding ABD and an antigen of interest-binding ABD, in which one or both of the ABDs (e.g., a variable region or other antigen binding domain such as a non-immunoglobulin scaffold) is linked to a constant region, e.g., an Fc domain or portion thereof via a linker, e.g., a flexible polypeptide linker. Optionally, the ABD is placed on a single polypeptide chain (e.g. a tandem variable domain, a V_H or single V domain, a non-immunoglobulin scaffold).

[0230] In some embodiments, one or both of the ABDs is comprised in a V_H and V_L domain that associate with one another to form the ABD. In one embodiment, the V_H and V_L that form an ABD are each within a tandem variable region (a V_H and V_L domain separated by a flexible polypeptide linker).

[0231] In some embodiments, one of the ABDs is comprised in a Fab or Fab-like structure, in which a variable domain is linked to a CH1 domain and a complementary variable domain is linked to a complementary C κ (or C λ) constant domain, wherein the CH1 and C κ (or C λ) constant domains associate (dimerize). In some embodiments, one of the ABDs is comprised in such a Fab or Fab-like structure and the other ABD is placed on a single polypeptide chain (e.g. a tandem variable domain) and is linked to a constant region, e.g., an Fc domain or portion thereof via a linker, e.g., a flexible polypeptide linker.

[0232] In some embodiments, one of the ABDs comprises a Fab or Fab-like structure, in which a variable domain is linked to a CH1 domain and a complementary variable domain is linked to a complementary C κ (or C λ) constant domain, wherein the CH1 and C κ (or C λ) constant domains associate to form a heterodimeric protein. For example, a first and second ABD can advantageously comprise or consist of single variable domains (e.g. VhH domains) having different antigen binding specificities (e.g., VhH₁ and VhH₂). Also the VhH₁ can be fused to a CH1 domain and VhH₂ can be fused to a C κ or C λ domain. The V₁-C κ (or C λ) chain associates with a V₂-CH1 chain such that a Fab is formed. See, e.g., WO2006/064136 and WO2012/089814 which disclose examples of such antibodies lacking Fc domains, the disclosures of which PCT applications are incorporated herein by reference. The CH1 and/or C κ domains can then be linked to a CH2 domain, optionally via a hinge region (or a linker peptide, e.g., one that has similar functional properties, e.g., dimerization). The CH2 domain (s) is/are then linked to a CH3 domain. The CH2-CH3 domains can thus optionally be embodied as a full-length Fc domain.

[0233] In any multispecific protein according to the invention, a hinge region can and generally will be present on a polypeptide chain between a CH1 domain and a CH2 domain, and/or can be present between a C κ domain and a

CH2 domain. A hinge region can optionally be replaced for example by a suitable linker peptide, e.g. a flexible polypeptide.

[0234] The proteins domains described in the present disclosure can optionally be specified as being from N- to C-termini. Protein arrangements of the disclosure for purposes of illustration are shown from N-terminus (on the left) to C-terminus. Domains can be referred to as fused to one another (e.g. a domain can be said to be fused to the C-terminus of the domain on its left, and/or a domain can be said to be fused to the N-terminus of the domain on its right).

[0235] The proteins domains described in the present disclosure can be fused to one another directly or via intervening amino acid sequences. For example, a CH1 or C κ domain can be fused to an Fc domain (or CH2 or CH3 domain thereof) via a linker peptide, optionally a hinge region or a fragment thereof. In another example, a V_H or V_L domain can be fused to a CH3 domain via a linker peptide. V_H and V_L domains linked to another in tandem can and generally will be fused via a linker peptide (e.g. a scFv). V_H and V_L domains linked to an Fc domain will be fused via a linker peptide. Two polypeptide chains will be bound to one another (indicated by “|”), by non-covalent bonds, and optionally can further be attached via interchain disulfide bonds, formed between cysteine residues within complementary CH1 and C κ domains.

[0236] Linkers

[0237] When ABD(s) or a polypeptide chain(s) comprise (s) a tandem variable region (e.g. scFv), two V domains (e.g. a V_H domain and V_L domains are generally linked together by a linker of sufficient length to enable the ABD to fold in such a way as to permit binding to the antigen for which the ABD is intended to bind. Similarly, when an ABD is linked to a constant domain or Fc domain, the linkage may be via a flexible linker (e.g. polypeptide linker) that permits the ABD to be positioned such that it binds to its target antigen and exhibits the desired functionality, e.g. it possesses a sufficient range of motion relative to the rest of the multispecific protein (the Fc domain and/or other ABD) and thereby mediates NKp46 signalling. Examples of linkers include, for example, linkers derived from antibody hinge regions, an amino sequence RTVA, or linkers comprising glycine and serine residues, e.g., the amino acid sequence GEGTSTGS(G₂S)₂GGAD. In another specific embodiment, the V_H domain and V_L domains of a scFv are linked together by the amino acid sequence (G₄S)₃.

[0238] Any of the peptide linkers contained in the subject multispecific proteins may comprise a length of at least 4 residues, at least 5 residues, at least 10 residues, at least 15 residues, at least 20 residues, at least 25 residues, at least 30 residues or more. In other embodiments, the linkers comprise a length of between 2-4 residues, between 2-4 residues, between 2-6 residues, between 2-8 residues, between 2-10 residues, between 2-12 residues, between 2-14 residues, between 2-16 residues, between 2-18 residues, between 2-20 residues, between 2-22 residues, between 2-24 residues, between 2-26 residues, between 2-28 residues, between 2-30 residues, between 2 and 50 residues, or between 10 and 50 residues.

[0239] An ABD (e.g. an immunoglobulin variable region) can optionally be linked to a constant domain or Fc domain via a flexible linker (e.g. polypeptide linker) that leads to less structural rigidity or stiffness (e.g. between or amongst the ABD and Fc domain) compared to a conventional (e.g.

wild-type full length human IgG) antibody. For example, the multispecific protein may have a structure or a flexible linker between the ABD and constant domain or Fc domain that permits an increased range of domain motion compared to the ABD in a conventional (e.g. wild-type full length human IgG) antibody. In particular, the structure or a flexible linker can be configured to confer on the antigen binding sites greater intrachain domain movement compared to antigen binding sites in a conventional human IgG1 antibody. Rigidity or domain motion/interchain domain movement can be determined, e.g., by computer modeling, electron microscopy, spectroscopy such as Nuclear Magnetic Resonance (NMR), X-ray crystallography, or Sedimentation Velocity Analytical ultracentrifugation (AUC) to measure or compare the radius of gyration of proteins comprising the linker or hinge. A test protein or linker may have lower rigidity relative to a comparator protein if the test protein has a value obtained from one of the tests described in the previous sentence differs from the value of the comparator, e.g., an IgG1 antibody or a hinge, by at least 5%, 10%, 25%, 50%, 75%, or 100%. A person of skill in the art would be able to determine from the tests whether a test protein has at lower rigidity to that of another protein, respectively, by interpreting the results of these tests.

[0240] In one embodiment, the multispecific protein may have a structure or a flexible linker between the ABD and constant domain or Fc domain that permits the NKp46 ABD and the ABD which binds an antigen of interest to have a spacing between said ABDs comprising less than about 80 angstroms, less than about 60 angstroms or ranges from about 40-60 angstroms.

[0241] In one embodiment, the hinge region will be a fragment of a hinge region (e.g. a truncated hinge region without cysteine residues) or may comprise one or more amino acid modifications which remove (e.g. substitute by another amino acid, or delete) a cysteine residue, optionally both cysteine residues in a hinge region. Removing cysteines can be useful to prevent undesired disulfide bond formation, e.g., the formation of disulfide bridges in a monomeric polypeptide.

[0242] In one embodiment, a (poly)peptide linker used to link a CH1 or Ck domain to a CH2 or CH3 domain of an Fc domain comprises a fragment of a CH1 domain and/or hinge region. For example, an N-terminal amino acid sequence of CH1 can be fused to a variable domain in order to mimic as closely as possible the natural structure of a wild-type antibody. In one embodiment, the linker comprises an amino acid sequence from a hinge domain or an N-terminal CH1 amino acid. The sequence can be, for example, between 2-4 residues, between 2-4 residues, between 2-6 residues, between 2-8 residues, between 2-10 residues, between 2-12 residues, between 2-14 residues, between 2-16 residues, between 2-18 residues, between 2-20 residues, between 2-22 residues, between 2-24 residues, between 2-26 residues, between 2-28 residues, or between 2-30 residues. In one embodiment the linker comprises or consists of the amino acid sequence RTVA.

[0243] In one embodiment, the hinge region (or fragment thereof) is derived from a hinge domain of a human IgG1 antibody. For example a hinge domain may comprise the amino acid sequence: T-H-T-C-S-S-C-P-A-P-E-L-L (one letter code), or an amino acid sequence at least 60%, 70%,

80% or 90% identical thereto, optionally wherein one or both cysteines are deleted or substituted by a different amino acid residue.

[0244] In one embodiment, the hinge region (or fragment thereof) is derived from a C μ 2-C μ 3 hinge domain of a human IgM antibody. For example a hinge domain may comprise the amino acid sequence: N-A-S-S-M-C-V-P-S-P-A-P-E-L-L (one letter code), or an amino acid sequence at least 60%, 70%, 80% or 90% identical thereto, optionally wherein one or both cysteines are deleted or substituted by a different amino acid residue.

[0245] Polypeptide chains that dimerize and associate with one another via non-covalent bonds may or may not additionally be bound by an interchain disulfide bond formed between respective CH1 and Ck domains, and/or between respective hinge domains on the chains. CH1, Ck and/or hinge domains (or other suitable linking amino acid sequences) can optionally be configured such that interchain disulfide bonds are formed between chains such that the desired pairing of chains is favored and undesired or incorrect disulfide bond formation is avoided. For example, when two polypeptide chains to be paired each possess a CH1 or Ck adjacent to a hinge domain, the polypeptide chains can be configured such that the number of available cysteines for interchain disulfide bond formation between respective CH1/Ck-hinge segments is reduced (or is entirely eliminated). For example, the amino acid sequences of respective CH1, Ck and/or hinge domains can be modified to remove cysteine residues in both the CH1/Ck and the hinge domain of a polypeptide; thereby the CH1 and Ck domains of the two chains that dimerize will associate via non-covalent interaction(s).

[0246] In another example, the CH1 or Ck domain adjacent (e.g., N-terminal to) a hinge domain comprises a cysteine capable of interchain disulfide bond formation, and the hinge domain which is placed at the C-terminus of the CH1 or Ck comprises a deletion or substitution of one or both cysteines of the hinge (e.g. Cys 239 and Cys 242, as numbered for human IgG1 hinge according to Kabat). In one embodiment, the hinge region (or fragment thereof) comprises the amino acid sequence: T-H-T-S-P-P-S-P-A-P-E-L-L (one letter code), or an amino acid sequence at least 60%, 70%, 80% or 90% identical thereto.

[0247] In another example, the CH1 or Ck domain adjacent (e.g., N-terminal to) a hinge domain comprises a deletion or substitution at a cysteine residue capable of interchain disulfide bond formation, and the hinge domain placed at the C-terminus of the CH1 or Ck comprises one or both cysteines of the hinge (e.g. Cys 239 and Cys 242, as numbered for human IgG1 hinge according to Kabat). In one embodiment, the hinge region (or fragment thereof) comprises the amino acid sequence: T-H-T-C-S-S-C-P-A-P-E-L-L (one letter code), or an amino acid sequence at least 60%, 70%, 80% or 90% identical thereto.

[0248] In another example, a hinge region is derived from an IgM antibody. In such embodiments, the CH1/Ck pairing mimics the C μ 2 domain homodimerization in IgM antibodies. For example, the CH1 or Ck domain adjacent (e.g., N-terminal to) a hinge domain comprises a deletion or substitution at a cysteine capable of interchain disulfide bond formation, and an IgM hinge domain which is placed at the C-terminus of the CH1 or Ck comprises one or both cysteines of the hinge. In one embodiment, the hinge region (or fragment thereof) comprises the amino acid sequence:

T-H-T-C-S-S-C-P-A-P-E-L-L (one letter code), or an amino acid sequence at least 60%, 70%, 80% or 90% identical thereto.

[0249] Constant Regions

[0250] Constant region domains can be derived from any suitable human antibody, including, the constant heavy (CH1) and light (Cκ) domains, hinge domains, CH2 and CH3 domains. With respect to heavy chain constant domains, “CH1” generally refers to positions 118-220 according to the EU index as in Kabat.

[0251] “CH2” generally refers to positions 237-340 according to the EU index as in Kabat, and “CH3” generally refers to positions 341-447 according to the EU index as in Kabat.

[0252] A “hinge” or “hinge region” or “antibody hinge region” herein refers to the flexible polypeptide or linker between the first and second constant domains of an antibody. Structurally, the IgG CH1 domain ends at EU position 220, and the IgG CH2 domain begins at residue EU position 237. Thus for an IgG the hinge generally includes positions 221 (D221 in IgG1) to 236 (G236 in IgG1), wherein the numbering is according to the EU index as in Kabat. References to specific amino acid residues within constant region domains found within the polypeptides shall be, unless otherwise indicated or as otherwise dictated by context, be defined according to Kabat, in the context of an IgG antibody.

[0253] CH2 and CH3 domains which may be present in the subject antibodies or multispecific proteins can be derived from any suitable antibody. Such CH2 and CH3 domains can be used as wild-type domains or may serve as the basis for a modified CH2 or CH3 domain. Optionally the CH2 and/or CH3 domain is of human origin or may comprise that of another species (e.g., rodent, rabbit, non-human primate) or may comprise a modified or chimeric CH2 and/or CH3 domain, e.g., one comprising portions or residues from different CH2 or CH3 domains, e.g., from different antibody isotypes or species antibodies.

[0254] In embodiments where a multispecific is intended not to bind to human CD16 polypeptide, a CH2 and/or CH3 domain (or Fc domain comprising same) may comprise a modification to decrease or abolish binding to FcγRIIIA (CD16). For example, CH2 mutations in a dimeric Fc domain proteins at residue N297 (Kabat numbering) can eliminate CD16 binding. However the person of skill in the art will appreciate that other configurations can be implemented. For example, substitutions into human IgG1 or IgG2 residues at positions 233-236 and IgG4 residues at positions 327, 330 and 331 were shown to greatly reduce binding to Fcγ receptors and thus ADCC and CDC. Furthermore, Idusogie et al. (2000) J. Immunol. 164(8):4178-84 demonstrated that alanine substitution at different positions, including K322, significantly reduced complement activation.

[0255] In certain embodiments herein where binding to CD16A is desired, a CH2 and/or CH3 domain (or Fc domain comprising same) may be a wild-type domain or may comprise one or more amino acid modifications (e.g. amino acid substitutions) which increase binding to human CD16 and optionally another receptor such as FcRn. Optionally, the modifications will not substantially decrease or abolish the ability of the Fc-derived polypeptide to bind to neonatal Fc receptor (FcRn), e.g. human FcRn. Typical modifications include modified human IgG1-derived constant regions

comprising at least one amino acid modification (e.g. substitution, deletions, insertions), and/or altered types of glycosylation, e.g., hypofucosylation. Such modifications can affect interaction with Fc receptors: FcγRI (CD64), FcγRII (CD32), and FcγRIII (CD16). FcγRI (CD64), FcγRIIA (CD32A) and FcγRIII (CD 16) are activating (i.e., immune system enhancing) receptors while FcγRIIB (CD32B) is an inhibiting (i.e., immune system dampening) receptor. A modification may, for example, increase binding of the Fc domain to FcγRIIIa on effector (e.g. NK) cells and/or decrease binding to FcγRIIB. Examples of modifications are provided in PCT publication no. WO2014/044686, the disclosure of which is incorporated herein by reference. Specific mutations (in IgG1 Fc domains) which affect (enhance) FcγRIIIa or FcRn binding are also set forth below.

Iso-type	Species	Modification	Effector Function	Effect of Modification
IgG1	Human	T250Q/M428L	Increased binding to FcRn	Increased half-life
IgG1	Human	1M252Y/S254T/T256E + H433K/N434F	Increased binding to FcRn	Increased half-life
IgG1	Human	E333A	Increased binding to FcγRIIIa	Increased ADCC and CDC
IgG1	Human	S239D/I332E or S239D/A330L/I332E	Increased binding to FcγRIIIa	Increased ADCC
IgG1	Human	P257I/Q311	Increased binding to FcRn	Unchanged half-life
IgG1	Human	S239D/I332E/G236A	Increased FcγRIIIa/FcγRIIB ratio	Increased macrophage phagocytosis

In some embodiments, the multispecific protein comprises a variant Fc region comprise at least one amino acid modification (for example, possessing 1, 2, 3, 4, 5, 6, 7, 8, 9, or more amino acid modifications) in the CH2 and/or CH3 domain of the Fc region, wherein the modification enhances binding to a human CD16 polypeptide. In other embodiments, the multispecific protein comprises at least one amino acid modification (for example, 1, 2, 3, 4, 5, 6, 7, 8, 9, or more amino acid modifications) in the CH2 domain of the Fc region from amino acids 237-341, or within the lower hinge-CH2 region that comprises residues 231-341. In some embodiments, the multispecific protein comprises at least two amino acid modifications (for example, 2, 3, 4, 5, 6, 7, 8, 9, or more amino acid modifications), wherein at least one of such modifications is within the CH3 region and at least one such modifications is within the CH2 region. Encompassed also are amino acid modifications in the hinge region. In one embodiment, encompassed are amino acid modifications in the CH1 domain, optionally in the upper hinge region that comprises residues 216-230 (Kabat EU numbering). Any suitable functional combination of Fc modifications can be made, for example any combination of the different Fc modifications which are disclosed in any of U.S. Pat. Nos. 7,632,497; 7,521,542; 7,425,619; 7,416,727; 7,371,826; 7,355,008; 7,335,742; 7,332,581; 7,183,387; 7,122,637; 6,821,505 and 6,737,056; and/or in PCT Publications Nos. WO2011/109400; WO 2008/105886; WO 2008/002933; WO 2007/021841; WO 2007/106707; WO 06/088494; WO 05/115452; WO 05/110474; WO 04/1032269; WO 00/42072; WO 06/088494; WO 07/024249; WO 05/047327; WO 04/099249 and WO

04/063351; and/or in Lazar et al. (2006) *Proc. Nat. Acad. Sci. USA* 103(11): 405-410; Presta, L. G. et al. (2002) *Biochem. Soc. Trans.* 30(4):487-490; Shields, R. L. et al. (2002) *J. Biol. Chem.* 277(30):26733-26740 and Shields, R. L. et al. (2001) *J. Biol. Chem.* 276(9):6591-6604).

In some embodiments, the multispecific protein comprises an Fc domain comprising at least one amino acid modification (for example, 1, 2, 3, 4, 5, 6, 7, 8, 9, or more amino acid modifications) relative to a wild-type Fc region, such that the molecule has an enhanced binding affinity for human CD16 relative to the same molecule comprising a wild-type Fc region, optionally wherein the variant Fc region comprises a substitution at any one or more of positions 221, 239, 243, 247, 255, 256, 258, 267, 268, 269, 270, 272, 276, 278, 280, 283, 285, 286, 289, 290, 292, 293, 294, 295, 296, 298, 300, 301, 303, 305, 307, 308, 309, 310, 311, 312, 316, 320, 322, 326, 329, 330, 332, 331, 332, 333, 334, 335, 337, 338, 339, 340, 359, 360, 370, 373, 376, 378, 392, 396, 399, 402, 404, 416, 419, 421, 430, 434, 435, 437, 438 and/or 439 (Kabat EU numbering).

In one embodiment, the multispecific protein comprises an Fc domain comprising at least one amino acid modification (for example, 1, 2, 3, 4, 5, 6, 7, 8, 9, or more amino acid modifications) relative to a wild-type Fc region, such that the molecule has enhanced binding affinity for human CD16 relative to a molecule comprising a wild-type Fc region, optionally wherein the variant Fc region comprises a substitution at any one or more of positions 239, 298, 330, 332, 333 and/or 334 (e.g. S239D, S298A, A330L, I332E, E333A and/or K334A substitutions), optionally wherein the variant Fc region comprises a substitution at residues S239 and I332, e.g. a S239D and I332E substitution (Kabat EU numbering).

In some embodiments, the multispecific protein comprises an Fc domain comprising altered glycosylation patterns that increase binding affinity for human CD16. Such carbohydrate modifications can be accomplished by, for example, by expressing a nucleic acid encoding the multispecific protein in a host cell with altered glycosylation machinery. Cells with altered glycosylation machinery are known in the art and can be used as host cells in which to express recombinant antibodies to thereby produce an antibody with altered glycosylation. See, for example, Shields, R. L. et al. (2002) *J. Biol. Chem.* 277:26733-26740; Umana et al. (1999) *Nat. Biotech.* 17:176-1, as well as, European Patent No: EP 1,176,195; PCT Publications WO 06/133148; WO 03/035835; WO 99/54342, each of which is incorporated herein by reference in its entirety. In one aspect, the multispecific protein contains one or more hypofucosylated constant regions. Such multispecific protein may comprise an amino acid alteration or may not comprise an amino acid alteration and/or may be expressed or synthesized or treated under conditions that result in hypofucosylation. In one aspect, a multispecific protein composition comprises a multispecific protein described herein, wherein at least 20, 30, 40, 50, 60, 75, 85, 90, 95% or substantially all of the antibody species in the composition have a constant region comprising a core carbohydrate structure (e.g. complex, hybrid and high mannose structures) which lacks fucose. In one embodiment, provided is a multispecific protein composition which is free of N-linked glycans comprising a core carbohydrate structure having fucose. The core carbohydrate will preferably be a sugar chain at Asn297.

Optionally, a multispecific protein comprising a dimeric Fc domain can be characterized by having a binding affinity to a human CD16 polypeptide that is within 1-log of that of a conventional human IgG1 antibody, e.g., as assessed by surface plasmon resonance.

In one embodiment, the multispecific protein comprising a dimeric Fc domain engineered to enhance Fc receptor binding can be characterized by having a binding affinity to a human CD16 polypeptide that is at least 1-log greater than that of a conventional or wild-type human IgG1 antibody, e.g., as assessed by surface plasmon resonance.

Optionally a multispecific protein comprising a dimeric Fc domain can be characterized by a K_D for binding (monovalent) to a human CD16 polypeptide of less than 10^{-5} M (10 μ molar), optionally less than 10^{-6} M (1 μ molar), as assessed by surface plasmon resonance (e.g. as in Example 16, SPR measurements performed on a Biacore T100 apparatus (Biacore GE Healthcare), with bispecific antibodies immobilized on a Sensor Chip CM5 and serial dilutions of soluble CD16 polypeptide injected over the immobilized bispecific antibodies.

CDR Sequences and Epitopes

[0256] In some embodiments, the proteins and antibodies herein bind the D1 domain of NKp46, the D2 domain of NKp46, or bind a region spanning the D1 and D2 domains (at the border of the D1 and D2 domains, the D1/D2 junction), of the NKp46 polypeptide of SEQ ID NO: 1. In some embodiments, the multispecific proteins or antibodies according to the invention have an affinity for human NKp46 characterized by a K_D of less than 10^{-5} M, less than 10^{-9} M, or less than 10^{-10} M.

In another embodiment, the inventive antibodies or multispecific proteins bind NKp46 at substantially the same epitope on NKp46 as antibody NKp46-1, NKp46-2, NKp46-3, NKp46-4, NKp46-6 or NKp46-9. In another embodiment, the antibodies at least partially overlaps, or includes at least one residue in the segment or epitope bound by NKp46-1, NKp46-2, NKp46-3, NKp46-4, NKp46-6 or NKp46-9. In one embodiment, all key residues of the epitope are in a segment corresponding to domain D1 or D2. In one embodiment, the antibody or multispecific protein binds a residue present in the D1 domain as well as a residue present in the D2 domain. In one embodiment, the antibodies bind an epitope comprising 1, 2, 3, 4, 5, 6, 7 or more residues in the segment corresponding to domain D1 or D2 of the NKp46 polypeptide of SEQ ID NO: 1. In one embodiment, the antibodies bind domain D1 and further bind an epitope comprising 1, 2, 3, or 4 of the residues R101, V102, E104 and/or L105.

In another embodiment, the antibodies or multispecific proteins bind NKp46 at the D1/D2 domain junction and bind an epitope comprising or consisting of 1, 2, 3, 4 or 5 of the residues K41, E42, E119, Y121 and/or Y194.

In another embodiment, the antibodies or multispecific proteins bind domain D2 and bind an epitope comprising 1, 2, 3, or 4 of the residues P132, E133, I135, and/or S136.

[0257] The Examples section provided infra further describes the construction of a series of mutant human NKp46 polypeptides. In the examples, the binding of anti-NKp46 antibody or multispecific protein to cells transfected with the NKp46 mutants was measured and compared to the ability of anti-NKp46 antibody to bind wild-type NKp46 polypeptide (SEQ ID NO:1). A reduction in binding between

an anti-NKp46 antibody or NKp46 binding multispecific protein and a mutant NKp46 polypeptide as described herein means that there is a reduction in binding affinity (e.g., as measured by known methods such as FACS testing of cells expressing a particular mutant, or by Biacore testing of binding to mutant polypeptides) and/or a reduction in the total binding capacity of the anti-NKp46 antibody (e.g., as evidenced by a decrease in Bmax in a plot of anti-NKp46 antibody concentration versus polypeptide concentration). A significant reduction in binding indicates that the mutated residue is directly involved in the binding to the anti-NKp46 antibody or is in close proximity to the binding protein when the anti-NKp46 antibody or NKp46 binding multispecific protein is bound to NKp46. An antibody epitope will thus preferably include such residue and may include additional residues adjacent to such residue.

[0258] In some embodiments, a significant reduction in binding means that the binding affinity and/or capacity between an anti-NKp46 antibody or NKp46 binding multispecific protein and a mutant NKp46 polypeptide is reduced by greater than 40%, greater than 50%, greater than 55%, greater than 60%, greater than 65%, greater than 70%, greater than 75%, greater than 80%, greater than 85%, greater than 90% or greater than 95% relative to binding between the antibody and a wild type NKp46 polypeptide (e.g., the polypeptide shown in SEQ ID NO:1). In certain embodiments, binding is reduced below detectable limits. In some embodiments, a significant reduction in binding is evidenced when binding of an anti-NKp46 antibody to a mutant NKp46 polypeptide is less than 50% (e.g., less than 45%, 40%, 35%, 30%, 25%, 20%, 15% or 10%) of the binding observed between the anti-NKp46 antibody and a wild-type NKp46 polypeptide (e.g., the polypeptide shown in SEQ ID NO: 1 (or the extracellular domain thereof)). Such binding measurements can be made using a variety of binding assays known in the art. A specific example of one such assay is described in the Example section.

In some embodiments, anti-NKp46 antibodies or NKp46 binding multispecific proteins are provided that exhibit significantly lower binding for a mutant NKp46 polypeptide in which a residue in a wild-type NKp46 polypeptide (e.g., SEQ ID NO:1) is substituted. In the shorthand notation used here, the format is: Wild type residue: Position in polypeptide: Mutant residue, with the numbering of the residues as indicated in SEQ ID NO: 1.

[0259] In some embodiments, an anti-NKp46 antibody binds a wild-type NKp46 polypeptide but has decreased binding to a mutant NKp46 polypeptide having a mutation (e.g., an alanine substitution) any one or more of the residues R101, V102, E104 and/or L105 (with reference to SEQ ID NO:1) compared to binding to the wild-type NKp46).

[0260] In some embodiments, an anti-NKp46 antibody or NKp46-binding multispecific protein binds a wild-type NKp46 polypeptide but has decreased binding to a mutant NKp46 polypeptide having a mutation (e.g., an alanine substitution) at one or more of residues K41, E42, E119, Y121 and/or Y194 (with reference to SEQ ID NO:1) compared to binding to the wild-type NKp46).

[0261] In some embodiments, an anti-NKp46 antibody or NKp46-binding multispecific protein binds a wild-type NKp46 polypeptide but has decreased binding to a mutant NKp46 polypeptide having a mutation (e.g., an alanine substitution) at one or more of residues P132, E133, I135,

and/or S136 (with reference to SEQ ID NO:1) compared to binding to the wild-type NKp46).

The amino acid sequence of the heavy chain variable region of antibodies NKp46-1, NKp46-2, NKp46-3, NKp46-4, NKp46-6 and NKp46-9 are listed herein in Table B (SEQ ID NOS: 3, 5, 7, 9, 11 and 13 respectively), the amino acid sequence of the light chain variable region of antibodies NKp46-1, NKp46-2, NKp46-3, NKp46-4, NKp46-6 and NKp46-9 are also listed herein in Table B (SEQ ID NOS: 4, 6, 8, 10, 12 and 14 respectively).

In a specific embodiment, the invention provides is an antibody, e.g. a full length monospecific antibody, a multispecific or bispecific antibody, including a bispecific monomeric polypeptide, or a NKp46-binding multispecific protein that binds essentially the same epitope or determinant as monoclonal antibody NKp46-1, NKp46-2, NKp46-3, NKp46-4, NKp46-6 or NKp46-9; optionally the antibody comprises a hypervariable region of antibody NKp46-1, NKp46-2, NKp46-3, NKp46-4, NKp46-6 or NKp46-9. In any of the embodiments herein, antibody NKp46-1, NKp46-2, NKp46-3, NKp46-4, NKp46-6 or NKp46-9 can be characterized by its amino acid sequence and/or nucleic acid sequence encoding it. In one embodiment, the antibody comprises the Fab or F(ab')₂ portion of NKp46-1, NKp46-2, NKp46-3, NKp46-4, NKp46-6 or NKp46-9. Also provided is an antibody that comprises the heavy chain variable region of NKp46-1, NKp46-2, NKp46-3, NKp46-4, NKp46-6 or NKp46-9. According to one embodiment, an antibody comprises the three CDRs of the heavy chain variable region of NKp46-1, NKp46-2, NKp46-3, NKp46-4, NKp46-6 or NKp46-9. Also provided is a polypeptide that further comprises one, two or three of the CDRs of the light chain variable region of NKp46-1, NKp46-2, NKp46-3, NKp46-4, NKp46-6 or NKp46-9. Optionally any one or more of said light or heavy chain CDRs may contain one, two, three, four or five or more amino acid modifications (e.g. substitutions, insertions or deletions). Optionally, provided is a multispecific protein or antibody polypeptide where any of the light and/or heavy chain variable regions comprising part or all of an antigen binding region of antibody NKp46-1, NKp46-2, NKp46-3, NKp46-4, NKp46-6 or NKp46-9 are fused to an immunoglobulin constant region of the human IgG type.

In another aspect, the invention provides a protein, e.g., an antibody, a full length monospecific antibody, a multispecific or a bispecific protein, or a polypeptide chain or fragment thereof, or an NKp46-binding multispecific protein as well as a nucleic acid encoding any of the foregoing, wherein the protein comprises the heavy chain CDRs of NKp46-1, NKp46-2, NKp46-3, NKp46-4, NKp46-6 or NKp46-9, comprising, for the respective antibody: a HCDR1 region comprising an amino acid sequence as set forth in Table A, or a sequence of at least 4, 5, 6, 7, 8, 9 or 10 contiguous amino acids thereof, wherein one or more of these amino acids may be substituted by a different amino acid; a HCDR2 region comprising an amino acid sequence as set forth in Table A, or a sequence of at least 4, 5, 6, 7, 8, 9 or 10 contiguous amino acids thereof, wherein one or more of these amino acids may be substituted by a different amino acid; a HCDR3 region comprising an amino acid sequence as set forth in as set forth in Table A, or a sequence of at least 4, 5, 6, 7, 8, 9 or 10 contiguous amino acids thereof, wherein one or more of these amino acids may be substituted by a different amino acid.

In another aspect, the invention provides a protein, e.g., an antibody, a full length monospecific antibody, a multispecific or a bispecific protein, or a polypeptide chain or fragment thereof, or an NKp46-binding multispecific protein as well as a nucleic acid encoding any of the foregoing, wherein the protein comprises light chain CDRs of NKp46-1, NKp46-2, NKp46-3, NKp46-4, NKp46-6 or NKp46-9, comprising, for the respective antibody: a LCDR1 region comprising an amino acid sequence as set forth in Table A, or a sequence of at least 4, 5, 6, 7, 8, 9 or 10 contiguous amino acids thereof, wherein one or more of these amino acids may be substituted by a different amino acid; a LCDR2 region comprising an amino acid sequence as set forth in Table A, or a sequence of at least 4, 5, 6, 7, 8, 9 or 10 contiguous amino acids thereof, wherein one or more of these amino acids may be substituted by a different amino acid; a LCDR3 region comprising an amino acid sequence as set forth in Table A, or a sequence of at least 4, 5, 6, 7, 8, 9 or 10 contiguous amino acids thereof, wherein one or more of these amino acids may be deleted or substituted by a different amino acid.

[0262] In another aspect, the invention provides a multispecific protein or antibody that binds to human NKp46, comprising:

(a) the heavy chain variable region of NKp46-1, NKp46-2, NKp46-3, NKp46-4, NKp46-6 or NKp46-9 as set forth in Table B, optionally wherein one, two, three or more amino acids may be substituted by a different amino acid;

(b) the light chain variable region NKp46-1, NKp46-2, NKp46-3, NKp46-4, NKp46-6 or NKp46-9 as set forth in Table B, optionally wherein one, two, three or more amino acids may be substituted by a different amino acid;

(c) the heavy chain variable region of NKp46-1, NKp46-2, NKp46-3, NKp46-4, NKp46-6 or NKp46-9 as set forth in Table B, optionally wherein one or more of these amino acids may be substituted by a different amino acid; and the respective light chain variable region of NKp46-1, NKp46-2, NKp46-3, NKp46-4, NKp46-6 or NKp46-9 as set forth in Table B, optionally wherein one, two, three or more amino acids may be substituted by a different amino acid;

(d) the heavy chain CDR 1, 2 and 3 (HCDR1, HCDR2) amino acid sequence of NKp46-1, NKp46-2, NKp46-3, NKp46-4, NKp46-6 or NKp46-9 as shown in Table A, optionally wherein one, two, three or more amino acids in a CDR may be substituted by a different amino acid;

(e) the light chain CDR 1, 2 and 3 (LCDR1, LCDR2, LCDR3) amino acid sequence of NKp46-1, NKp46-2, NKp46-3, NKp46-4, NKp46-6 or NKp46-9 as shown in

Table A, optionally wherein one, two, three or more amino acids in a CDR may be substituted by a different amino acid; or

(f) the heavy chain CDR 1, 2 and 3 (HCDR1, HCDR2, HCDR3) amino acid sequence of NKp46-1, NKp46-2, NKp46-3, NKp46-4, NKp46-6 or NKp46-9 as shown in Table A, optionally wherein one, two, three or more amino acids in a CDR may be substituted by a different amino acid; and the light chain CDRs 1, 2 and 3 (LCDR1, LCDR2, LCDR3) amino acid sequence of the respective NKp46-1, NKp46-2, NKp46-3, NKp46-4, NKp46-6 or NKp46-9 antibody as shown in Table A, optionally wherein one, two, three or more amino acids in a CDR may be substituted by a different amino acid.

In one embodiment, the aforementioned CDRs are according to Kabat, e.g. as shown in Table A. In one embodiment, the aforementioned CDRs are according to Chothia numbering, e.g. as shown in Table A. In one embodiment, the aforementioned CDRs are according to IMGT numbering, e.g. as shown in Table A.

[0263] In another aspect of any of the embodiments herein, any of the CDR1, CDR2 and CDR3 of the heavy and light chains may be characterized by a sequence of at least 4, 5, 6, 7, 8, 9 or 10 contiguous amino acids thereof, and/or as having an amino acid sequence that shares at least 50%, 60%, 70%, 80%, 85%, 90% or 95% sequence identity with the particular CDR or set of CDRs listed in the corresponding SEQ ID NO or Table A.

[0264] In another aspect, the invention provides an antibody that competes for NKp46 binding with a monoclonal antibody according to (a) to (f), above.

In another aspect, the invention provides a bispecific antibody comprising an antibody that binds human NKp46 according to (a) to (f), above, or an antibody that competes for binding to NKp46 with any of such antibodies, fused (optionally via intervening amino acid sequences) to a monomeric Fc domain, optionally further fused (optionally via intervening amino acid sequences) to a second antigen binding domain (e.g. a scFv, a V_H domain, a V_L domain, a dAb, a V-NAR domain or a V_HH domain). Optionally the second antigen binding domain will bind a cancer antigen, a viral antigen, a parasitic antigen or a bacterial antigen. The sequences of the CDRs, according to IMGT, Kabat and Chothia definitions systems, are summarized in Table A below. The sequences of the variable chains of the antibodies according to the invention are listed in Table B below. In any embodiment herein, a V_L or V_H sequence can be specified or numbered so as to contain or lack a signal peptide or any part thereof.

TABLE A

mAb	CDR definition	HCDR1			HCDR2			HCDR3		
		SEQ ID	Sequence		SEQ ID	Sequence		SEQ ID	Sequence	
NKp46-1	Kabat	15	DYVIN		18	EIYPGSGTNYNEKFKA		21	RGRYGLYAMDY	
	Chothia	16	GYTFTDY		19	PGSG		22	GRYGLYAMD	
	IMGT	17	GYTFTDYV		20	GYTFTDYVIYPGSGTN		23	ARRGRYGLYAMDY	
NKp46-2	Kabat	31	SDYAWN		34	YITYSGSTSYNPSLES		36	GGYYGSSWGVFAY	
	Chothia	32	GYSITS DY			YSG		37	GGYYGSSWGVFA	
	IMGT	33	GYSITS DY A		35	ITYSGST		38	ARGGYGSSWGVFAY	

TABLE A-continued

NKp46-3	Kabat	46	EYTMH	49	GISPNIIGGTSYNQKFKG	51	RGGSFYD
	Chothia	47	GYTFTEY		PNIG	52	GGSF
	IMGT	48	GYTFTEYT	50	ISPNIIGGT	53	ARRGGSFYD
NKp46-4	Kabat	60	SFTMH	63	YINPSSGYTEYNQKFKD	65	GSSRGFDY
	Chothia	61	GYTFTSF		PSSG	66	SSRGFD
	IMGT	62	GYTFTSFT	64	INPSSGYT	67	VRGSSRGFDY
NKp46-6	Kabat	73	SSWMH	76	HIHPNSGISNYNEKFKG	78	GGRFDD
	Chothia	74	GYTFTSS		PNSG		GRFD
	IMGT	75	GYTFTSSW	77	IHPNSGIS	79	ARGGRFDD
NKp46-9	Kabat	85	SDYAWN	88	YITYSGSTNYNPSLKS	89	CWDYALYAMDC
	Chothia	86	GYSITSDY		YSG	90	WDYALYAMD
	IMGT	87	GYSITSDYA	35	ITYSGST	91	ARCWDYALYA MDC
Bab281	Kabat	97	NYGMN	100	WINTNTGPTYAEEFKG	102	DYLYYFDY
	Chothia	98	GYTFTNY		TNTG	103	YLYYFD
	IMGT	99	GYTFTNYG	101	INTNTGEP	104	ARDYLYYFDY
<hr/>							
CDR		LCDR1		LCDR2		LCDR3	
mAb	definition	SEQ ID	Sequence	SEQ ID	Sequence	SEQ ID	Sequence
NKp46-1	Kabat	24	RASQDISNYLN	27	YTSRLHS	28	QQGNTRPWT
	Chothia	25	SQDISNY		YTS	29	YTSGNTRPW
	IMGT	26	QDISNY		YTS	30	YTSQQGNTRP WT
NKp46-2	Kabat	39	RVSENIYSYLA	42	NAKTLAE	43	QHHYGTPWT
	Chothia	40	SENIYSY		NAK	44	HYGTPW
	IMGT	41	ENIYSY		NAK	45	QHHYGTPWT
NKp46-3	Kabat	54	RASQISIDYLN	57	YASQIS	58	QNGHSFPLT
	Chothia	55	SQSISDY		YAS	59	GHSFPL
	IMGT	56	QSISDY		YAS		QNGHSFPLT
NKp46-4	Kabat	68	RASENIYSNLA	70	AATNLAD	71	QHFWGTPRT
	Chothia		SENIYSN		AAT	72	FWGTPR
	IMGT	69	ENIYSN		AAT		QHFWGTPRT
NKp46-6	Kabat	80	RASQDIGSSLN	81	ATSSLDS	82	LQYASSPWT
	Chothia		SQDIGSS		ATS	83	YASSPWT
	IMGT		QDIGSS		ATS	84	LQYASSPWT
NKp46-9	Kabat	92	RTSENIYSYLA	93	NAKTLAE	94	QHHYDTPLT
	Chothia		SENIYSY		NAK	95	NAKHYDTPL
	IMGT		ENIYSY		NAK	96	QHHYDTPLT
Bab281	Kabat	105	KASENVVTVYS	108	GASNRYT	109	GQGYSPYT
	Chothia	106	SENVVTV		GAS	110	GYSYPY
	IMGT	107	ENVVTV		GAS	111	GQGYSPYT

TABLE B

Antibody	SEQ ID		Amino acid sequence
	NO		
NKp46-1 VH	3		QVQLQQSGPELVKPGASVKMSCKASGYTFTDYVINWGKQRSGQG LEWIGETIPGSGTNYNEKFKAKATLTADKSNIAVMQLSSLTSEDS AVYFCARRGRYGLYAMDYWGQTSVTVSS
NKp46-1 VL	4		DIQMTQTSSLSASLGDRVTISCRASQDISNYLNWYQKPDGTVKL LIYYTSRLHSGVPSRFSGSGSDYSLTINNLEQEDATYFCQQGNT RPWTFGGGTKLEIK
NKp46-2 VH	5		EVQLQESGPGLVKPSQSLSLTCTVTGYSITSDYAWNWIQFPGNKL EWMGYITYSGSTSYNPSLESRISITRDTSTNQFFLQLNSVTEDTAT YYCARGGYGSSWGVFAYWGQTLVTVSA
NKp46-2 VL	6		DIQMTQSPASLSASVGETVTITCRVSENIYSYLAWYQQKQKGKSPQL LVYNAKTLAEGVPSRFSGSGSDYSLKINSIQPEDFGSYVCQHHY GTPWTFGGGTKLEIK

TABLE B-continued

Antibody	SEQ ID NO	Amino acid sequence
NKp46-3 VH	7	EVQLQQSGPELVKPGASVKISCKTSGYTFTEYTMHWVKQSHGKSL EWIGGISPNIGGTSYNQKFKGKATLTVDKSSSTAYMELRSLTSEDSA VYYCARRGGSFDYWGQGTTLTVSS
NKp46-3 VL	8	DIVMTQSPATLSVTPGDRVSLSCRASQSIDYLHWYQQKSHESPLR LIKYASQISGISPRFSGSGSGSDFTLSINSVEPEDVGVYYCQNGHS FPLTFGAGTKLELK
NKp46-4 VH	9	QVQLQQSAVELARPGASVKMSCKASGYTFTSFTMHVWKQRPQGQ LEWIGYINPSSGYTEYNQKFKDKTTLTADKSSSTAYMQDLSLTSD SAVYYCVRGSSRGFDYWGQGTLLTVSA
NKp46-4 VL	10	DIQMIQSPASLSVSVGETVTITCRASENIYSLNLAWFQQKQKSPQLL VYAATNLADGVPSRFSGSGSGTQYSLKINSLSQSEDFGIYYCQHF WGPRTFGGGTKLEIK
NKp46-6 VH	11	QVQLQQPGSVLVRPGASVKLSCKASGYTFTSSWMHWAKQRPQGQ GLEWIGHIHPNSGISNYNEKFKGKATLTVDTSSSTAYVDLSLTSED SAVYYCARGGRFDDWGAGTTVTVSS
NKp46-6 VL	12	DIQMTQSPSSLSASLGERVSLTCRASQDIGSSSLNWLQPEPDGTIKR LIYATSSLDGVPKRFSGSGSGSDYSLTSSLESEDFVDYVCLQYAS SPWTFGGGKLEIK
NKp46-9 VH	13	DVQLQESGPGLVKPSQSLTCTVTGYSITSDYAWNWIQFPGNKL EWMGYITYSGSTNYPNPSLKRISITRDTSKNQFFLQLNSVTEDTAT YYCARCWDYALYAMDCWGQGTSTVSS
NKp46-9 VL	14	DIQMTQSPASLSASVGETVTITCRTSENIYSYLAWCQQKQKSPQL LVYNAKTLAEGVPSRFSGSGSGTHFSKINSLQPEDFGIYYCQHHY DPLTFGAGTKLELK

[0265] Also provided, as described in the Examples herein, is a multispecific protein or antibody comprising the amino acid sequences of monomeric bispecific polypeptides which respectively comprise an scFv comprising the heavy and light chain CDR1, 2 and 3 of the respective heavy and light chain variable region listed as SEQ ID NOS: 3-14 of antibodies NKp46-1, NKp46-2, NKp46-3, NKp46-4, NKp46-6 and NKp46-9, a monomeric Fc domain, and an scFv comprising the heavy and light chain CDR1, 2 and 3 of the heavy and light chain variable region of an anti-CD19 antibody, e.g. any of the anti-CD19 antibodies described in the Examples herein.

[0266] Once the multispecific protein is produced it can be assessed for biological activity, e.g., antigen binding, ability to elicit target cell lysis and/or specific signaling activities elicited thereby.

[0267] In one aspect of any embodiment described herein, the inventive multispecific protein is capable of inducing activation of an NKp46-expressing cell (e.g. an NK cell, a reporter cell) when the protein is incubated in the presence of the NKp46-expressing cell (e.g. purified NK cells) and a target cell that expresses the antigen of interest).

[0268] In one aspect of any embodiment described herein, the inventive multispecific protein is capable of inducing an increase of CD137 present on the cell surface of an NKp46- and/or a CD16-expressing cell (e.g. an NK cell, a reporter cell) when the protein is incubated in the presence of the NKp46- and/or a CD16-expressing cell (e.g. purified NK cells), optionally in the absence of target cells.

[0269] In one aspect of any embodiment described herein, the inventive multispecific protein is capable of inducing NKp46 signaling in an NKp46-expressing cell (e.g. an NK

cell, a reporter cell) when the protein is incubated in the presence of an NKp46-expressing cell (e.g. purified NK cells) and a target cell that expresses the antigen of interest).

[0270] Optionally, NK cell activation or signaling is characterized by the increased expression of a cell surface marker of activation, e.g. CD107, CD69, Sca-1 or Ly-6A/E, KLRG 1, etc.

[0271] Activity can be measured for example by bringing target cells and NKp46-expressing cells into contact with one another, in presence of the multispecific polypeptide. In one example, the aggregation of target cells and NK cells is measured. In another example, the multispecific protein may, for example, be assessed for the ability to cause a measurable increase in any property or activity known in the art as associated with NK cell activity, respectively, such as marker of cytotoxicity (CD107) or cytokine production (for example IFN- γ or TNF- α), increases in intracellular free calcium levels, the ability to lyse target cells in a redirected killing assay, etc.

[0272] In the presence of target cells (target cells expressing the antigen of interest) and NK cells that express NKp46, the multispecific protein will be capable of causing an increase in a property or activity associated with NK cell activity (e.g. activation of NK cell cytotoxicity, CD107 expression, IFN γ production) in vitro. For example, a multispecific protein according to the invention can be selected based on its ability to increase an NK cell activity by more than about 20%, preferably by least about 30%, at least about 40%, at least about 50%, or more compared to that achieved with the same effector: target cell ratio with the same NK cells and target cells that are not brought into contact with the multispecific protein, as measured by an

assay that detects NK cell activity, e.g., an assay which detects the expression of an NK activation marker or which detects NK cell cytotoxicity, e.g., an assay that detects CD107 or CD69 expression, IFN γ production, or a classical in vitro chromium release test of cytotoxicity. Examples of protocols for detecting NK cell activation and cytotoxicity assays are described in the Examples herein, as well as for example, in Pessino et al, *J. Exp. Med.*, 1998, 188 (5): 953-960; Sivori et al, *Eur J Immunol.*, 1999, 29:1656-1666; Brando et al, (2005) *J. Leukoc. Biol.* 78:359-371; El-Sherbiny et al, (2007) *Cancer Research* 67(18):8444-9; and Nolte-'t Hoen et al, (2007) *Blood* 109:670-673). Optionally, a multispecific protein according to the invention can be selected for or characterized by its ability to have greater ability to induce NK cell activity towards target cells, i.e., lysis of target cells compared to a conventional human IgG1 antibody that binds to the same antigen of interest, as measured by an assay of NK cell activity (e.g. an assay that detects NK cell-mediated lysis of target cells that express the antigen of interest).

[0273] As shown herein, a multispecific protein according to the invention which possesses an Fc domain that does not bind CD16, does not, substantially induce NKp46 signaling (and/or NK activation that results therefrom) of NK cells when the protein is not bound to the antigen of interest on target cells (e.g. in the absence of the antigen of interest and/or target cells). Thus, the monovalent NKp46 binding component of the multispecific protein does not itself cause NKp46 signalling. Accordingly, in the case of multispecific proteins possessing an Fc domain that binds CD16, such multispecific protein can be assessed for its ability to elicit NKp46 signaling or NKp46-mediated NK cell activation by testing the effect of this multispecific protein on NKp46 expression, by CD16-negative NK cells. The multispecific protein can optionally be characterized as not substantially causing (or increasing) NKp46 signaling by an NKp46-expressing, CD16-negative cell (e.g. a NKp46⁺CD16⁻ NK cell, a reporter cell) when the multispecific protein is incubated with such NKp46-expressing, CD16-negative cells (e.g., purified NK cells or purified reporter cells) in the absence of target cells.

[0274] In one aspect of any embodiment herein, a multispecific protein described herein that binds CD16 can for example be characterized by:

[0275] (a) being capable of inducing NK cells that express CD16 and NKp46 to lyse target cells, when incubated in the presence of the NK cells and target cells; and

[0276] (b) lack of agonist activity at NKp46 when incubated with CD16-negative NK cells, e.g. NKp46-expressing NK cells that do not express CD16, in the absence of target cells. Optionally, the NK cells are purified NK cells.

[0277] In one aspect of any embodiment herein, a multispecific protein described herein can for example be characterized by:

[0278] (a) agonist activity at NKp46, when incubated in the presence of NKp46-expressing NK cells and target cells; and

[0279] (b) lack of agonist activity at NKp46 (e.g. when incubated with CD16-negative NK cells, for example CD16-NKp46⁺NK cells, or when incubated with NK cells and where in the protein comprises (e.g. by modification) an Fc domain that lacks binding to CD16) in the absence of target cells. Optionally, the NK cells are purified NK cells.

Uses of Compounds

[0280] In one aspect, provided is the use of any of the compounds defined herein, particularly the inventive multispecific proteins or antibodies and/or cells which express same for the manufacture of a pharmaceutical preparation for the treatment, prevention or diagnosis of a disease in a mammal in need thereof. Provided also are the use any of the compounds defined above as a medicament or an active component or active substance in a medicament. In a further aspect the invention provides methods for preparing a pharmaceutical composition containing a compound as defined herein, to provide a solid or a liquid formulation for administration orally, topically, or by injection. Such a method or process at least comprises the step of mixing the compound with a pharmaceutically acceptable carrier.

[0281] In one aspect, provided is a method to treat, prevent or more generally affect a predefined condition in an individual or to detect a certain condition by using or administering a multispecific protein or antibody described herein, or a (pharmaceutical) composition comprising same.

[0282] For example, in one aspect, the invention provides a method of restoring or potentiating the activity of NKp46⁺ NK cells (e.g. NKp46⁺CD16⁺ NK cells) in a patient in need thereof (e.g. a patient having a cancer, or a viral or bacterial infection), comprising the step of administering a multispecific protein described herein to said patient. In one embodiment, the method is directed at increasing the activity of NKp46⁺ lymphocytes (e.g. NKp46⁺CD16⁺ NK cells) in patients having a disease in which increased lymphocyte (e.g. NK cell) activity is beneficial or which is caused or characterized by insufficient NK cell activity, such as a cancer, or a viral or microbial/bacterial infection.

[0283] In another aspect, the invention provides a method of restoring or potentiating the activity of NKp46⁺ NK cells (e.g. NKp46⁺CD16⁺ NK cells) in a patient in need thereof (e.g. a patient having a cancer, or a viral, parasite or bacterial infection), comprising the step of contacting cells derived from the patient, e.g., immune cells and optionally target cells expressing an antigen of interest with a multispecific protein according to the invention and reinfusing the multispecific protein treated cells into the patient. In one embodiment, this method is directed at increasing the activity of NKp46⁺ lymphocytes (e.g. NKp46⁺CD16⁺ NK cells) in patients having a disease in which increased lymphocyte (e.g. NK cell) activity is beneficial or which is caused or characterized by insufficient NK cell activity, such as a cancer, or a viral or microbial, e.g., bacterial or parasite infection.

[0284] In another embodiment the subject multispecific proteins may be used or administered in combination with immune cells, particularly NK cells, derived from a patient who is to be treated or from a different donor, and these NK cells administered to a patient in need thereof such as a patient having a disease in which increased lymphocyte (e.g. NK cell) activity is beneficial or which is caused or characterized by insufficient NK cell activity, such as a cancer, or a viral or microbial, e.g., bacterial or parasite infection. As NK cells (unlike CAR-T cells) do not express TCRs, these NK cells, even those derived from different donors will not induce a GVHD reaction (see e.g., Glienke et al., "Advantages and applications of CAR-expressing natural killer cells", *Front. Pharmacol.* 6, Art. 21:1-6 (2015); Hermanson and Kaufman, *Front. Immunol.* 6, Art. 195:1-6 (2015))

[0285] In one embodiment, the multispecific protein disclosed herein that mediates NK cell activation and/or target cell lysis via multiple activating receptors of effector cells, including Nkp46, CD16 and CD137, can be used advantageously for treatment of individuals whose effector cells (e.g. Nkp46⁺CD16⁺ NK cells) cells are hypoactive, exhausted or suppressed, for example a patient who has a significant population of effector cells characterized by the expression and/or upregulation of one or multiple inhibitory receptors (e.g. TIM-3, PD1, CD96, TIGIT, etc.).

[0286] The multispecific polypeptides described herein can be used to prevent or treat disorders that can be treated with antibodies, such as cancers, solid and non-solid tumors, hematological malignancies, infections such as viral infections, and inflammatory or autoimmune disorders.

[0287] In one embodiment, the antigen of interest (the non-Nkp46 antigen) is an antigen expressed on the surface of a malignant cell of a type of cancer selected from the group consisting of: carcinoma, including that of the bladder, head and neck, breast, colon, kidney, liver, lung, ovary, prostate, pancreas, stomach, cervix, thyroid and skin, including squamous cell carcinoma; hematopoietic tumors of lymphoid lineage, including leukemia, acute lymphocytic leukemia, acute lymphoblastic leukemia, B-cell lymphoma, T-cell lymphoma, Hodgkin's lymphoma, non-Hodgkin's lymphoma, hairy cell lymphoma and Burkett's lymphoma; hematopoietic tumors of myeloid lineage, including acute and chronic myelogenous leukemias and promyelocytic leukemia; tumors of mesenchymal origin, including fibrosarcoma and rhabdomyosarcoma; other tumors, including neuroblastoma and glioma; tumors of the central and peripheral nervous system, including astrocytoma, neuroblastoma, glioma, and schwannomas; tumors of mesenchymal origin, including fibrosarcoma, rhabdomyosarcoma, and osteosarcoma; and other tumors, including melanoma, xeroderma pigmentosum, keratoacanthoma, seminoma, thyroid follicular cancer and teratocarcinoma, hematopoietic tumors of lymphoid lineage, for example T-cell and B-cell tumors, including but not limited to T-cell disorders such as T-prolymphocytic leukemia (T-PLL), including of the small cell and cerebriform cell type; large granular lymphocyte leukemia (LGL) preferably of the T-cell type; Sézary syndrome (SS); Adult T-cell leukemia lymphoma (ATLL); a/d T-NHL hepatosplenic lymphoma; peripheral/post-thymic T cell lymphoma (pleomorphic and immunoblastic subtypes); angio immunoblastic T-cell lymphoma; angiocentric (nasal) T-cell lymphoma; anaplastic (Ki 1+) large cell lymphoma; intestinal T-cell lymphoma; T-lymphoblastic; and lymphoma/leukemia (T-Lbly/T-ALL). In one embodiment, the inventive multispecific polypeptides described herein can be used to prevent or treat a cancer selected from the group consisting of: carcinoma, including that of the bladder, head and neck, breast, colon, kidney, liver, lung, ovary, prostate, pancreas, stomach, cervix, thyroid and skin, including squamous cell carcinoma; hematopoietic tumors of lymphoid lineage, including leukemia, acute lymphocytic leukemia, acute lymphoblastic leukemia, B-cell lymphoma, T-cell lymphoma, Hodgkin's lymphoma, non-Hodgkin's lymphoma, hairy cell lymphoma and Burkett's lymphoma; hematopoietic tumors of myeloid lineage, including acute and chronic myelogenous leukemias and promyelocytic leukemia; tumors of mesenchymal origin, including fibrosarcoma and rhabdomyosarcoma; other tumors, including neuroblastoma and glioma; tumors of the central and peripheral

nervous system, including astrocytoma, neuroblastoma, glioma, and schwannomas; tumors of mesenchymal origin, including fibrosarcoma, rhabdomyosarcoma, and osteosarcoma; and other tumors, including melanoma, xeroderma pigmentosum, keratoacanthoma, seminoma, thyroid follicular cancer and teratocarcinoma. Other exemplary disorders that can be treated according to the invention include hematopoietic tumors of lymphoid lineage, for example T-cell and B-cell tumors, including but not limited to T-cell disorders such as T-prolymphocytic leukemia (T-PLL), including of the small cell and cerebriform cell type; large granular lymphocyte leukemia (LGL) preferably of the T-cell type; Sézary syndrome (SS); Adult T-cell leukemia lymphoma (ATLL); a/d T-NHL hepatosplenic lymphoma; peripheral/post-thymic T cell lymphoma (pleomorphic and immunoblastic subtypes); angio immunoblastic T-cell lymphoma; angiocentric (nasal) T-cell lymphoma; anaplastic (Ki 1+) large cell lymphoma; intestinal T-cell lymphoma; T-lymphoblastic; and lymphoma/leukaemia (T-Lbly/T-ALL). In one example, the tumor antigen is an antigen expressed on the surface of a lymphoma cell or a leukemia cell, and the multispecific protein is administered to, and/or used for the treatment of, an individual having a lymphoma or a leukemia. Optionally, the tumor antigen is selected from CD19, CD20, CD22, CD30 or CD33.

[0288] In one aspect, the methods of treatment comprise administering to an individual a multispecific protein described herein in a therapeutically effective amount, e.g., for the treatment of a disease as disclosed herein, for example any of the cancers identified above. A therapeutically effective amount may be any amount that has a therapeutic effect in a patient having a disease or disorder (or promotes, enhances, and/or induces such an effect in at least a substantial proportion of patients with the disease or disorder and substantially similar characteristics as the patient).

[0289] In one embodiment, a multispecific protein according to the invention is used to treat a cancer that is responsive to CD137 activation, e.g. a solid tumor or a hematological cancer, including but not limited to breast cancer, sarcoma, glioma, colon carcinoma, myeloma, mastocytoma, melanoma, renal carcinoma, and ovarian cancer. In one embodiment, the multispecific protein according to the invention is used to treat a CD137L-expressing cancer. As shown herein, the strong efficacy in inducing tumor cell lysis of the multispecific protein is hypothesized to in part mediated by the upregulation of CD137 (4-1BB) on the surface of NK cells. The co-activating CD137 protein can bind and recognize CD137 ligand (CD137L, 4-1BBL) on tumor cells, resulting in enhanced NK cell activation and cytotoxicity of CD137L-expressing cells. CD137L has been found to be expressed on a variety of tumors, and is more commonly expressed by malignant tumors, especially in moderate or low-differentiated tumors (see, e.g., Vinay et al., (2012) *Mol. Cancer Ther.* 11(5):1062-1070). CD137L-expressing cancers include, for example, lung squamous cell carcinoma, nasal cavity squamous cell carcinoma, esophageal squamous cell carcinoma, cervical squamous cell carcinoma, colonic adenocarcinoma, rectal adenocarcinoma, gallbladder adenocarcinoma, pancreatic adenocarcinoma and breast adenocarcinoma.

[0290] The multispecific protein according to the invention may be used with or without a prior step of detecting the expression of the antigen of interest on target cells in a

biological sample obtained from an individual (e.g. a biological sample comprising cancer cells, cancer tissue or cancer-adjacent tissue). In another embodiment, the disclosure provides a method for the treatment or prevention of a cancer in an individual in need thereof, the method comprising:

[0291] a) detecting cells (e.g. tumor cells) in a sample from the individual that express an antigen of interest, and

[0292] b) upon a determination that cells which express an antigen of interest are comprised in the sample, optionally at a level that is increased compared to a reference level (e.g. corresponding to a healthy individual or an individual not deriving substantial benefit from a protein described herein), administering to the individual a multispecific protein (e.g. a multispecific protein according to the invention) that binds to an antigen of interest, to NKp46 (e.g., monovalently), and to CD16 (e.g., via its Fc domain). Optionally, the antigen of interest is a cancer antigen (e.g. a cancer antigen disclosed herein), optionally a cancer antigen known to be capable of undergoing intracellular internalization when contacted with a full length human IgG1 antibody that binds specifically thereto.

[0293] In one embodiment, the invention provides a method for the treatment or prevention of a cancer in an individual in need thereof, the method comprising:

[0294] a) detecting cells (e.g. tumor cells) in a sample from an individual (or within the tumor and/or within adjacent tissue) that express CD137L (CD137 ligand), and

[0295] b) upon a determination that cells that express CD137L are comprised in the sample (or within the tumor and/or within adjacent tissue), optionally at a level that is increased compared to a reference level (e.g. corresponding to a healthy individual or an individual not deriving substantial benefit from a protein described herein), administering to the individual a multispecific protein (e.g. a protein of the disclosure) that binds to a cancer antigen, to NKp46 (e.g., monovalently), and to CD16. Optionally, the cancer antigen is a cancer antigen disclosed herein), optionally a cancer antigen known to be capable of undergoing intracellular internalization when contacted with a full length human IgG1 antibody that binds specifically thereto.

[0296] In one embodiment, the disclosure provides a method for the treatment or prevention of a disease (e.g. a cancer) in an individual in need thereof, the method comprising:

[0297] a) detecting cell surface expression of one or a plurality inhibitory receptors on immune effector cells (e.g. NK cells, T cells) in a sample from the individual (e.g. in circulation or in the tumor environment), and

[0298] b) upon a determination of cell surface expression of one or a plurality inhibitory receptors on immune effector cells, optionally at a level that is increased compared to a reference level (e.g. corresponding to a healthy individual, an individual not suffering from immune exhaustion or suppression, or an individual not deriving substantial benefit from a protein described herein), administering to the individual a multispecific protein (e.g. a multispecific protein according to the invention) that binds to an antigen of interest (e.g. a cancer antigen), to NKp46 (e.g., monovalently), and to CD16. Optionally, the cancer antigen is a cancer antigen disclosed herein), optionally a cancer antigen known to be capable of undergoing intracellular internalization when contacted with a full length human IgG1 antibody that binds specifically thereto.

[0299] In one embodiment, a multispecific protein according to the invention may be used as a monotherapy (without other therapeutic agents), or in combined treatments with one or more other therapeutic agents, including agents normally utilized for the particular therapeutic purpose for which the antibody is being administered. The additional therapeutic agent or agents will normally be administered in amounts and treatment regimens typically used for that agent in a monotherapy for the particular disease or condition being treated. Such therapeutic agents when used in the treatment of cancer, include, but are not limited to anti-cancer agents and chemotherapeutic agents; in the treatment of infectious disease, include, but are not limited to anti-viral agents and antibiotics. Such other therapeutic agents may further include other immunomodulatory polypeptides such as Ig-fusion proteins, antibodies, cytokines and the like. In some embodiments the administration of the multispecific protein according to the invention and the other therapeutic agent may elicit an additive or synergistic effect on immunity and/or on therapeutic efficacy.

[0300] The multispecific proteins can also be included in kits. The kits may optionally further contain any number of polypeptides and/or other compounds, e.g., 1, 2, 3, 4, or any other number of multispecific proteins according to the invention and/or other compounds. It will be appreciated that this description of the contents of the kits is not limiting in any way. For example, the kit may contain other types of therapeutic compounds. Optionally, the kits also include instructions for using the polypeptides, e.g., detailing the herein-described methods such as in the detection or treatment of specific disease conditions.

[0301] The invention also provides pharmaceutical compositions comprising the subject multispecific proteins and optionally other compounds as defined above. A multispecific protein and optionally another compound may be administered in purified form together with a pharmaceutical carrier as a pharmaceutical composition. The form depends on the intended mode of administration and therapeutic or diagnostic application. The pharmaceutical carrier can be any compatible, nontoxic substance suitable to deliver the compounds to the patient. Pharmaceutically acceptable carriers are well known in the art and include, for example, aqueous solutions such as (sterile) water or physiologically buffered saline or other solvents or vehicles such as glycols, glycerol, oils such as olive oil or injectable organic esters, alcohol, fats, waxes, and inert solids. A pharmaceutically acceptable carrier may further contain physiologically acceptable compounds that act for example to stabilize or to increase the absorption of the compounds. Such physiologically acceptable compounds include, for example, carbohydrates, such as glucose, sucrose or dextrans, antioxidants, such as ascorbic acid or glutathione, chelating agents, low molecular weight proteins or other stabilizers or excipients. One skilled in the art would know that the choice of a pharmaceutically acceptable carrier, including a physiologically acceptable compound, depends, for example, on the route of administration of the composition. Pharmaceutically acceptable adjuvants, buffering agents, dispersing agents, and the like, may also be incorporated into the pharmaceutical compositions. Non-limiting examples of such adjuvants include by way of example inorganic and organic adjuvants such as alum, aluminum phosphate and aluminum hydroxide, squalene, liposomes, lipopolysaccharides, double

stranded (ds) RNAs, single stranded (s-s) DNAs, and TLR agonists such as unmethylated CpG's.

[0302] Multispecific proteins according to the invention can be administered parenterally. Preparations of the compounds for parenteral administration must be sterile. Sterilization is readily accomplished by filtration through sterile filtration membranes, optionally prior to or following lyophilization and reconstitution. The parenteral route for administration of compounds is in accord with known methods, e.g. injection or infusion by intravenous, intraperitoneal, intramuscular, intraarterial, or intralesional routes. The compounds may be administered continuously by infusion or by bolus injection. A typical composition for intravenous infusion could be made up to contain 100 to 500 ml of sterile 0.9% NaCl or 5% glucose optionally supplemented with a 20% albumin solution and 1 mg to 10 g of the compound, depending on the particular type of compound and its required dosing regimen. Methods for preparing parenterally administrable compositions are well known in the art.

EXAMPLES

Example 1

Generation of Anti-huNKp46 Antibodies

[0303] Balb/c mice were immunized with a recombinant human NKp46 extracellular domain recombinant-Fc protein comprising the extracellular domain of the protein of SEQ ID NO: 1. Mice received one primo-immunization with an emulsion of 50 μ g NKp46 protein and Complete Freund Adjuvant, intraperitoneally, a 2nd immunization with an emulsion of 50 μ g NKp46 protein and Incomplete Freund Adjuvant, intraperitoneally, and finally a boost with 10 μ g NKp46 protein, intravenously. Immune spleen cells were fused 3 days after the boost with X63.Ag8.653 immortalized B cells, and cultured in the presence of irradiated spleen cells.

[0304] Primary screen: Supernatant (SN) of growing clones were tested in a primary screen by flow cytometry using a cell line expressing the human NKp46 construct at the cell surface. Briefly, for FACS screening, the presence of reactive antibodies in supernatants was revealed by Goat anti-mouse polyclonal antibody (pAb) labeled with PE.

[0305] A panel of antibodies that bound NKp46 was selected, produced and their variable regions sequenced and these antibodies and derivatives thereof further evaluated for their activity in the context of a bispecific molecule.

Example 2

Identification of a Bispecific Antibody Format that Binds FcRn but not Fc γ R for Targeting Effector Cell Receptors

[0306] Experiments were conducted with the objective being the development of a new bispecific protein format that places an Fc domain on a polypeptide together with an anti-NKp46 binding domain and an anti-target antigen binding domain. Such bispecific proteins should bind to NKp46 monovalently via its anti-NKp46 binding domain. The monomeric Fc domain should retain at least partial binding to the human neonatal Fc receptor (FcRn), yet not substantially bind human CD16 and/or other human Fc γ receptors.

Consequently, such bispecific proteins should not induce Fc γ -mediated (e.g. CD16-mediated) target cell lysis.

Example 2-1 Construction and Binding Analysis of Anti-CD19-IgG1-Fcmono-Anti-CD3

[0307] Since no anti-NKp46 bispecific antibody has been produced that could indicate whether such a protein could be functional, CD3 was used as a model antigen in place of NKp46 in order to investigate the possible functionality of a new monovalent bispecific protein format prior to targeting NK cells via NKp46.

[0308] A bispecific Fc-based on a scFv specific for tumor antigen CD19 (anti-CD19 scFv) and a scFv specific for activating receptor CD3 on a T cell (anti-CD3 scFv) was used to assess FcRn binding and CD19-binding functions of a new monomeric bispecific polypeptide format. The domain arrangement of the final polypeptide is referred to as the "F1" format (the star in the CH2 domain indicates an optional N297S mutation, not included in the polypeptide tested here). (See FIG. 2)

[0309] A bispecific monomeric Fc-containing polypeptide was constructed based on a scFv specific for the tumor antigen CD19 (anti-CD19 scFv) and a scFv specific for an activating receptor CD3 on a T cell (anti-CD3 scFv). The CH3 domain incorporated the mutations (EU numbering) L351K, T366S, P395V, F405R, T407A and K409Y. The polypeptide has domains arranged as follows: anti-CD19-CH2-CH3-anti-CD3. A DNA sequence coding for a CH3/VH linker peptide having the amino acid sequence STGS was also designed in order to insert a specific Sall restriction site at the CH3-VH junction.

[0310] This CH3 domain incorporated the mutations (EU numbering) L351K, T366S, P395V, F405R, T407A and K409Y. The selected CH2 domain was a wild-type CH2. DNA and amino acid sequences for the monomeric CH2-CH3 Fc portion and the anti-CD19 are shown below.

[0311] The light chain and heavy chain DNA and amino acid sequences corresponding to the anti-CD19 scFv were as follows:

Sequence	SEQ ID NO
Anti-CD19-V κ DNA	113
Anti-CD19-V κ amino acid	114
Anti-CD19-V H DNA	115
Anti-CD19-V H amino acid	116

[0312] The DNA sequences for the monomeric CH2-CH3 Fc portion and final bispecific IgG1-Fcmono polypeptide (the last K was removed in that construct) is shown in SEQ ID NO: 117. The amino acid sequence encoded thereby is shown in SEQ ID NO: 2. The Anti-CD19-F1-Anti-CD3 complete sequence (mature protein) is shown in SEQ ID NO: 118.

Cloning and the Production of the Recombinant Proteins

[0313] Coding sequences were generated by direct synthesis and/or by PCR. PCR was performed using the PrimeSTAR MAX DNA polymerase (Takara, #R045A) and PCR products were purified from 1% agarose gel using the NucleoSpin gel and PCR clean-up kit (Macherey-Nagel, #740609.250). Once purified the PCR products were quantified prior to the In-Fusion ligation reaction which was

performed as described in the manufacturer's protocol (ClonTech, #ST0345). The plasmids were obtained after a miniprep preparation run on an EVO200 (Tecan) using the Nucleospin 96 plasmid kit (Macherey-Nagel, #740625.4). Plasmids were then sequenced for sequence confirmation before to transfecting the CHO cell line.

[0314] CHO cells were grown in the CD-CHO medium (Invitrogen) complemented with phenol red and 6 mM GlutaMax. The day before the transfection, cells were counted and seeded at 175,000 cells/ml. For the transfection, cells (200,000 cells/transfection) were prepared as described in the AMAXA SF cell line kit (AMAXA, #V4XC-2032) and nucleofected using the DS137 protocol with the Nucleofector 4D device. All the transfections were performed using 300 ng of verified plasmids. After transfection, cells were seeded into 24 well plates in pre-warmed culture medium. After 24 hours, hygromycin B was added in the culture medium (200 µg/ml). Protein expression was monitored after one week in culture. Cells expressing the proteins were then sub-cloned to obtain the best producers. Sub-cloning was performed using 96 flat-bottom well plates in which the cells are seeded at one cell per well into 200 µl of culture medium complemented with 200 µg/ml of hygromycin B. Cells were left for three weeks before testing the clone's productivity.

[0315] Recombinant proteins which contain an IgG1-Fc fragment were purified using Protein-A beads (rProteinA Sepharose fast flow, GE Healthcare). Briefly, cell culture supernatants were concentrated, clarified by centrifugation and injected onto Protein-A columns to capture the recombinant Fc containing proteins. Proteins were eluted at acidic pH (citric acid 0.1M pH 3), and the eluate immediately neutralized using TRIS-HCL pH 8.5 and dialyzed against 1xPBS. Recombinant scFvs which contain a "six his" tag were purified by affinity chromatography using Cobalt resin. Other recombinant scFvs were purified by size exclusion chromatography (SEC).

Example 2-2: Binding Analysis of Anti-CD19-IgG1-Fcmono-Anti-CD3 to B221, JURKAT, HUT78 and CHO Cell Lines

[0316] Cells were harvested and stained with the cell supernatant of the anti-CD19-F1-anti-CD3 producing cells during 1 h at 4° C. After two washes in staining buffer (PBS1x/BSA 0.2%/EDTA 2 mM), cells were stained for 30 min at 4° C. with goat anti-human (Fc)-PE antibody (IM0550 Beckman Coulter—1/200). After two washes, stainings were conducted on a BD FACS Canto II and analyzed using the FlowJo software.

[0317] CD3 and CD19 expression were also controlled by flow cytometry: Cells were harvested and stained in PBS1x/BSA 0.2%/EDTA 2 mM buffer during 30 min at 4° C. using 5 µl of the anti-CD3-APC and 5 µl of the anti-CD19-FITC antibodies. After two washes, stainings were conducted on a BD FACS Canto II and analyzed using the FlowJo software.

[0318] The results of these experiments revealed that the Anti-CD19-F1-Anti-CD3 protein binds to CD3 cell lines (HUT78 and JURKAT cell lines) and to the CD19 cell line (B221 cell line) but not to the CHO cell line which was used as a negative control.

Example 2-3

T- and B-Cell Aggregation by Purified Anti-CD19-F1-Anti-CD3

[0319] Purified Anti-CD19-F1-Anti-CD3 was tested in a T/B cell aggregation assay to evaluate whether the antibody promotes the aggregation of CD19 and CD3 expressing cells.

[0320] The results of this assay are shown in FIG. 1. The top panel shows that Anti-CD19-F1-Anti-CD3 does not cause aggregation in the presence of B221 (CD19) or JURKAT (CD3) cell lines, but it does cause aggregation of cells when both B221 and JURKAT cells are co-incubated, indicating that the bispecific antibody is functional. The lower panel shows the results of the control experiment conducted without antibody.

Example 2-4

Binding of Bispecific Monomeric Fc Polypeptide to FcRn

Affinity Study by Surface Plasmon Resonance (SPR)

Biacore T100 General Procedure and Reagents

[0321] SPR measurements were performed on a Biacore T100 apparatus (Biacore GE Healthcare) at 25° C. In all Biacore experiments Acetate Buffer (50 mM Acetate pH5.6, 150 mM NaCl, 0.1% surfactant p20) and HBS-EP+(Biacore GE Healthcare) were used as the running buffer and regeneration buffer respectively. Sensorgrams were analyzed with Biacore T100 Evaluation software. Recombinant mouse FcRn was purchased from R&D Systems.

Immobilization of FcRn

[0322] Recombinant FcRn proteins were immobilized covalently to carboxyl groups in the dextran layer on a Sensor Chip CM5. The chip surface was activated with EDC/NHS (N-ethyl-N'-(3-dimethylaminopropyl) carbodiimidehydrochloride and N-hydroxysuccinimide (Biacore GE Healthcare)). FcRn proteins were diluted to 10 µg/ml in coupling buffer (10 mM acetate, pH 5.6) and injected until the appropriate immobilization level was reached (i.e. 2500 RU). Deactivation of the remaining activated groups was performed using 100 mM ethanolamine pH 8 (Biacore GE Healthcare).

Affinity Study

[0323] Monovalent affinity study was conducted following the Single Cycle Kinetic (SCK) protocol. Five serial dilutions of soluble analytes (antibodies and bi-specific molecules) ranging from 41.5 to 660 nM were injected over the FcRn (without regeneration) and allowed to dissociate for 10 min before regeneration. For each analyte, the entire sensorgram was fitted using the 1:1 SCK binding model.

Results

[0324] Anti-CD19-F1-Anti-CD3 having its CH2-CH3 domains placed between two antigen binding domains, particularly two scFvs, was evaluated to assess whether such bispecific monomeric Fc protein could retain binding to FcRn and possess an improved in vivo half-life compared to

conventional bispecific antibodies. The results of these experiments showed that FcRn binding was retained, the model suggesting a 1:1 ratio (1 FcRn for each monomeric Fc) instead of a 2:1 ratio (2 FcRn for each antibody) for a regular or wild-type IgG.

[0325] The binding affinity of this multispecific protein was evaluated using SPR, and was compared to a chimeric full length antibody containing intact human IgG1 constant regions. The monomeric Fc retained significant monomeric binding to FcRn (monomeric Fc: affinity of KD=194 nM; full length antibody with bivalent binding: avidity of KD=15.4 nM).

Example 3

Construction of Anti-CD19×Anti-NKp46 Bispecific Monomeric Fc Domain Polypeptides

[0326] It was unknown what activating receptors on NK cells would contribute to the lysis of target cells, and moreover since anti-NKp46 antibodies may block NKp46, it was further unknown whether cytotoxicity could be mediated by NKp46. We therefore investigated whether the bispecific protein format could induce NKp46 triggering, and whether it would induce NKp46 agonism in the absence of target cells, which could lead to inappropriate NK activation distant from the target and/or decreased overall activity toward target cells.

[0327] A new bispecific protein format was developed as a single chain protein which binds to FcRn but not FcγR. Additionally, multimeric proteins that comprise two or three polypeptide chains, wherein the Fc domain remains monomeric, were developed that are compatible for use with antibody variable regions that do not maintain binding to their target when converted to scFv format. The latter formats can be used conveniently for antibody screening; by incorporating at least one binding region as a F(ab) structure, any anti-target (e.g. anti-tumor) antibody variable region can be directly expressed in a bispecific construct as the F(ab) format within the bispecific protein and tested, irrespective

of whether the antibody would retain binding as an scFv, thereby simplifying screening and enhancing the number of antibodies available. These formats in which the Fc domain remains monomeric have the advantage of maintaining maximum conformational flexibility and as shown infra may permit optimal binding to NKp46 or target antigens.

[0328] Different constructs were made for use in the preparation of bispecific antibodies using the variable domains from the scFv specific for tumor antigen CD19 described in Example 2-1, and different variable regions from antibodies specific for the NKp46 receptor identified in Example 1. A construct was also made using as the anti-NKp46 the variable regions from a commercially available antibody Bab281 (mIgG1, available commercially from Beckman Coulter, Inc. (Brea, Calif., USA) (see also Pessino et al, *J. Exp. Med.*, 1998, 188 (5): 953-960 and Sivori et al, *Eur J Immunol*, 1999, 29:1656-1666) specific for the NKp46 receptor.

[0329] In order for the Fc domain to remain monomeric in single chain polypeptides or in multimers in which only one chain had an Fc domain, CH3-CH3 dimerization was prevented through two different strategies: (1) through the use of CH3 domain incorporating specific mutations (EU numbering), i.e., L351K, T366S, P395V, F405R, T407A and K409Y; or (2) through the use of a tandem CH3 domain in which the tandem CH3 domains are separated by a flexible linker associated with one another, which prevents inter-chain CH3-CH3 dimerization. The DNA and amino acid sequences for the monomeric CH2-CH3 Fc portion containing the above-identified point mutations were the same as in Example 2-1. The DNA and amino acid sequences for the monomeric CH2-CH3-linker-CH3 Fc portion with tandem CH3 domains are shown in FIGS. 2A-2D.

[0330] The light chain and heavy chain DNA and amino acid sequences for the anti-CD19 scFv were also the same as in Example 2-1. Proteins were cloned, produced and purified as in Example 2-1. Shown below are the light chain and heavy chain DNA and amino acid sequences for different anti-NKp46 scFvs.

TABLE 1

Amino acid sequences of different anti-NKp46 scFvs	
scFv	
anti-NKp46	scFV sequence (VHVK) / -stop
NKp46-1	STGSQVQLQQSGPELVKPGASVKMSCKASGYTFTDYVINWGKQRSGQ GLEWIGEITYPGSGTNYNEKFKAKATLTADKSSNIAYMQLSLTSEDSAV YFCARRGRYGLYAMDYWGQGTSTVTVSSVEGGSGSGSGSGSGGVDD IQMTQTSSLSASLGDRTVISCRAQDISNYLNWYQQKPDGTVKLLIYYT SRLHSGVPSRFSGSGSGTDYSLTINNLEQEDIATYFCQQGNTRPWFTEG GGTKLEIK- (SEQ ID NO: 119)
NKp46-2	STGSEVQLQESGPELVKPSQSLSLTCTVTGYSTSDYAWNWIQFPNGK LEWMGYITYSGSTSYNPSLESRIITRDTSTNQFFLQLNSVTEDTATYY CARGGYGSSWGVFAYWGQGTSLVTVSAVEGGSGSGSGSGSGGVDD DIQMTQSPASLSASVGETVTITCRVSENIYSYLAWYQQKQKSPQLLVY NAKTLAEGVPSRFSGSGSGTQFSLKINSLQPEDFGSYCYQHGYTPWT FEGGTKLEIK- (SEQ ID NO: 120)
NKp46-3	STGSEVQLQQSGPELVKPGASVKISCKTSGYTFTEYTMHWKQSHGKS LEWIGGISPNIGGTSYNQKFKGKATLTVDKSSSTAYMELRSLTSEDSAVY YCARRGGSFQYWGQGTSLVTVSSVEGGSGSGSGSGSGGVDDIVMTQ SPATLSVTPGDRVSLSCRAQSISDYLHWYQQKSHESPRLLIKYASQSSIS GIPSRFSGSGSGSDFTLINSVEPEDVGVIYCYQNGHSFPLTFGAGTKLE LK- (SEQ ID NO: 121)

TABLE 1-continued

Amino acid sequences of different anti-NKp46 scFvs	
scFv	
anti-NKp46	scFV sequence (VHVK) /-stop
NKp46-4	STGSQVQLQQSAVELARPGASVKMSCKASGYTFTSFTMHVWKQRPQG GLEWIGIYINPSSGYTEYNQKFKDKTTLTADKSSSTAYMQLDLSLTSDDSA VYYCVRGSSRGFDYWGQGTLLVTVSAVEGGSGSGSGSGSGGVDDIQ MIQSPASLSVSVGETVTITCRASENIYSNLAWFQQKQKSPQLLVYAATN LADGVPSRFGSGSGTQYSLKINSLQSEDFGIYYCQHFPGTPTFTFGG TKLEIK- (SEQ ID NO: 122)
NKp46-6	STGSQVQLQQPGSVLVRPGASVKLSCKASGYTFTSSWMHWAKQRPQG GLEWIGHIHPNSGISNYNEKFKGKATLTVDTSSTAYVDLSLTSSEDSAV YYCARGGRFDDWGAGTTVTSSVEGGSGSGSGSGSGGVDDIVMTQ SPATLSVTPGDRVSLSCRASQSSIDYLHWYQQKSHESPRLLIKYASQSSIS GIPSRFGSGSGSGDFTLSINSVEPEDVGYYCQNGHSFLMYTFGGGTKL EIK- (SEQ ID NO: 123)
NKp46-9	STGSDVQLQESGPGLVKPSQSLSLTCTVTGYSTSDYAWNWRQFPNGK LEWMGYITYSGSTNYNPSLKSRIITRDTSKNQFFLQLNSVTEDTATYY CARCWDYALYAMDCWGQGTSTVTVSSVEGGSGSGSGSGSGGVDDIQ MTQSPASLSASVGETVTITCRASENIYSYLAWCQQKQKSPQLLVYNAK TLAEGVPSRFGSGSGTHFSKINSLQPEDFGIYYCQHHYDPLTFGAG TKLEIK- (SEQ ID NO: 124)
Bab281	STGSQIQLVQSGPELQKPGETVKISCKASGYTFTNYGMNWVKQAPGKG LKWMGWINTNTGPTYAEFEKGRFAFSLETASTAYLQINNLIKNEEDTAT YFCARDYLYYFDYWGQGTLLTVSSVEGGSGSGSGSGSGGVDDIVMT QSPKMSMSVGERVTLTKASENVVTYVSWYQQKPEQSPKLLIYGASN RYTGVPDRFTGSGSATDFTLTISVQAEDLADYHCGQGYSPYPTFGG TKLEIK- (SEQ ID NO: 125)

TABLE 2A

DNA sequences corresponding to different anti-NKp46 scFvs	
scFv anti-NKp46	scFV sequences
NKp46-1	SEQ ID NO: 126
NKp46-2	SEQ ID NO: 127
NKp46-3	SEQ ID NO: 128
NKp46-4	SEQ ID NO: 129
NKp46-6	SEQ ID NO: 130
NKp46-9	SEQ ID NO: 131
Bab281	SEQ ID NO: 132

TABLE 2B

Sequence	SEQ ID NO
CD19-F1-NKp46-1	133
CD19-F1-NKp46-2	134
CD19-F1-NKp46-3	135
CD19-F1-NKp46-4	136
CD19-F1-NKp46-6	137
CD19-F1-NKp46-9	138
CD19-F1-Bab281	139

Format 1 (F1) (Anti-CD19-IgG1-Fcmono-Anti-NKp46 (scFv))

[0331] The domain structure of Format 1 (F1) is shown in FIG. 2A. A bispecific Fc-containing polypeptide was constructed based on a scFv specific for the tumor antigen CD19 (anti-CD19 scFv) and an scFV specific for the NKp46 receptor. The polypeptide is a single chain polypeptide having domains arranged (N- to C-termini) as follows: $(V_K-V_H)^{anti-CD19}$ -CH2-CH3- $(V_H-V_K)^{anti-NKp46}$

[0332] A DNA sequence coding for a CH3/VH linker peptide having the amino acid sequence STGS was designed in order to insert a specific Sall restriction site at the CH3- V_H junction. The domain arrangement of the final polypeptide is shown in FIG. 2 (the star in the CH2 domain indicates an optional N297S mutation), where the anti-CD3 scFv is replaced by an anti-NKp46 scFv. The (V_K-V_H) units include a linker between the V_H and V_K domains. Proteins were cloned, produced and purified as in Example 2-1. The amino acid sequences of the bispecific polypeptides (complete sequence (mature protein)) are shown in the corresponding SEQ ID NOS listed in the Table 2B below.

Format 2 (F2): CD19-F2-NKp46-3

[0333] The domain structure of F2 polypeptides is shown in FIG. 2A. The DNA and amino acid sequences for the monomeric CH2-CH3 Fc portion were as in Example 2-1 and it similarly contains CH3 domain mutations (the mutations (EU numbering) L351K, T366S, P395V, F405R, T407A and K409Y. The heterodimer is made up of:

[0334] (1) a first (central) polypeptide chain having domains arranged as follows (N- to C-termini):

[0335] $(V_K-V_H)^{anti-CD19}$ -CH2-CH3- $V_H^{anti-NKp46}$ -CH1 and

[0336] (2) a second polypeptide chain having domains arranged as follows (N- to C-termini): $V_K^{anti-NKp46}$ -CK.

[0337] The (V_K-V_H) unit was made up of a V_H domain, a linker and a V_K unit (i.e. an scFv). As with other formats of the inventive bispecific polypeptides, the DNA sequence coded for a CH3/VH linker peptide having the amino acid sequence STGS designed in order to insert a specific Sall restriction site at the CH3-VH junction. Proteins were cloned, produced and purified as in Example 2-1. The amino acid sequences for the first and second chains of the F2 protein are shown in SEQ ID NO: 140 and 141.

Format 3 (F3): CD19-F3-NKp46-3

[0338] The domain structure of F3 polypeptides is shown in FIG. 2A. The DNA and amino acid sequences for the CH2-CH3 Fc portion comprised a tandem CH3 domain in which the two CH3 domains on the same polypeptide chain associated with one another, thereby preventing dimerization between different bispecific proteins.

[0339] The single chain polypeptide has domains arranged (N- to C-termini) as follows: $(V_K-V_H)^{anti-CD19}$ -CH2-CH3-CH3- $(V_H-V_K)^{anti-NKp46}$

[0340] The (V_K-V_H) units were made up of a V_H domain, a linker and a V_K unit (scFv). Proteins were cloned, produced and purified as in Example 2-1. Bispecific protein was purified from cell culture supernatant by affinity chromatography using prot-A beads and analyzed and purified by SEC. The protein showed a high production yield of 3.4 mg/L and the purified proteins exhibited a simple SEC profile. The amino acid sequence for the F3 protein is shown in SEQ ID NO: 142.

Format 4 (F4): CD19-F4-NKp46-3

[0341] The domain structure of F4 polypeptides is shown in FIG. 2A. The DNA and amino acid sequences for the CH2-CH3 Fc portion comprised a tandem CH3 domain as in Format F3, and additionally comprise a N297S mutation which prevents N-linked glycosylation and abolishes FcγR binding. Proteins were cloned, produced and purified as in Example 2-1. Bispecific proteins was purified from cell culture supernatant by affinity chromatography using prot-A beads and analyzed and purified by SEC. The protein showed a good production yield of 1 mg/L and the purified proteins exhibited a simple SEC profile. The amino acid sequence for the F4 protein with NKp46-3 variable domains is shown in SEQ ID NO: 143.

Format 8 (F8)

[0342] The domain structure of F8 polypeptides is shown in FIG. 2B. The DNA and amino acid sequences for the monomeric CH2-CH3 Fc portion were as in Format F2 and it similarly contains CH3 domain mutations (the mutations (EU numbering) L351K, T366S, P395V, F405R, T407A and K409Y, as well as a N297S mutation which prevents N-linked glycosylation and moreover abolishes FcγR binding. Three variants of F8 proteins were produced: (a) one wherein the cysteine residues in the hinge region were left intact (wild-type, referred to as F8A), (b) a second wherein the cysteine residues in the hinge region were replaced by serine residues (F8B), and (c) a third including a linker sequence GGGSS replacing residues DKTHTCPPCP in the hinge (F8C). Variants F8B and F8C provided production advantages as these versions avoided the formation of homodimers of the central chain. This heterotrimer is made up of;

[0343] (1) a first (central) polypeptide chain having domains arranged as follows (N- to C-termini):

[0344] $V_H^{anti-CD19}$ -CH1-CH2-CH3-VH^{anti-NKp46}-C_K and

[0345] (2) a second polypeptide chain having domains arranged as follows (N- to C-termini): $V_K^{anti-NKp46}$ -CH1

and

[0346] (3) a third polypeptide chain having domains arranged as follows (N- to C-termini):

[0347] $V_K^{anti-CD19}$ -C_K

[0348] Proteins were cloned, produced and purified as in Example 2-1. Bispecific proteins was purified from cell culture supernatant by affinity chromatography using prot-A beads and analyzed and purified by SEC. The protein showed a high production yield of 3.7 mg/L (F8C) and the purified proteins again exhibited a simple SEC profile. The amino acid sequences of the three chains of the F8 protein (C variant) with NKp46-3 variable regions are shown in SEQ ID NOS: 144, 145 and 146.

Format 9 (F9): CD19-F9-NKp46-3

[0349] The F9 polypeptide is a trimeric polypeptide having a central polypeptide chain and two polypeptide chains each of which associate with the central chain via CH1-C_K dimerization. The domain structure of the trimeric F9 protein is shown in FIG. 2B, wherein the bonds between the CH1 and C_K domains are interchain disulfide bonds. The two antigen binding domains have a F(ab) structure permitting the use of these antibodies irrespective of whether they remain functional in a scFv format. The DNA and amino acid sequences for the CH2-CH3 Fc portion comprise a tandem CH3 domain as in Format F4 and comprise a CH2 domain comprising a N297S substitution. Three variants of F9 proteins were produced: (a) a first wherein the cysteine residues in the hinge region left intact (wild-type, referred to as F9A), (b) a second wherein the cysteine residues in the hinge region were replaced by serine residues (F9B), and (c) a third containing a linker sequence GGGSS which replaces residues DKTHTCPPCP in the hinge (F9C). Variants F9B and F9C provided advantages in production by avoiding the formation of homodimers of the central chain. The heterotrimer is made up of:

[0350] (1) a first (central) polypeptide chain having domains arranged as follows (N- to C-termini):

[0351] $V_H^{anti-CD19}$ -CH1-CH2-CH3-CH3-V_H^{anti-NKp46}-C_K and

[0352] (2) a second polypeptide chain having domains arranged as follows (N- to C-termini): $V_K^{anti-NKp46}$ -CH1 and

[0353] (3) a third polypeptide chain having domains arranged as follows (N- to C-termini):

[0354] $V_K^{anti-CD19}$ -C_K.

[0355] Proteins were cloned, produced and purified as in Example 2-1. Bispecific proteins was purified from cell culture supernatant by affinity chromatography using prot-A beads and analyzed and purified by SEC. The protein showed a high production yield of 8.7 mg/L (F9A) and 3.0 mg/L (F9B), and the purified proteins again exhibited a simple SEC profile.

[0356] The amino acid sequences of the three chains of the F9 protein variant F9A are shown in the SEQ ID NOS: 147, 148 and 149. The amino acid sequences of the three chains of the F9 protein variant F9B are shown in the SEQ ID NOS: 150, 151 and 152. The amino acid sequences of the three chains of the F9 protein variant F9C are shown in the SEQ ID NOS: 153, 154 and 155.

Format 10 (F10): CD19-F10-NKp46-3

[0357] The F10 polypeptide is a dimeric protein having a central polypeptide chain and a second polypeptide chain which associates with the central chain via CH1-C_K dimerization. The domain structure of the dimeric F10 protein is shown in FIG. 2B wherein the bonds between the

CH1 and C κ domains are interchain disulfide bonds. One of the two antigen binding domains has a Fab structure, and the other is a scFv. The DNA and amino acid sequences for the CH2-CH3 Fc portion comprise a tandem CH3 domain as shown in Format F4 and comprise a CH2 domain containing a N297S substitution. Three variants of F10 proteins were also produced: (a) a first wherein the cysteine residues in the hinge region were left intact (wild-type, referred to as F10A), (b) a second wherein the cysteine residues in the hinge region were replaced by serine residues (F10B), and (c) a third containing a linker sequence GGGSS replacing residues DKTHTCPPCP in the hinge (F10C). Variants F10B and F10C provided advantages in production as they avoid the formation of homodimers of the central chain. The (V κ -V $_H$) unit was made up of a V $_H$ domain, a linker and a V κ unit (scFv). The heterodimer is made up of:

[0358] (1) a first (central) polypeptide chain having domains arranged as follows (N- to C-termini):

[0359] V $_H^{anti-CD19}$ -CH1-CH2-CH3-CH3-(V $_H$ -V κ) $^{anti-NKp46}$

and

[0360] (2) a second polypeptide chain having domains arranged as follows (N- to C-termini): V $\kappa^{anti-CD19}$ -C κ .

[0361] These proteins were cloned, produced and purified as in Example 2-1. Bispecific proteins was purified from cell culture supernatant by affinity chromatography using prot-A beads and analyzed and purified by SEC. The protein showed a good production yield of 2 mg/L (F10A) and the purified proteins again exhibited a simple SEC profile. The amino acid sequences of the two chains of the F10A protein variant are shown in SEQ ID NOS: 156 (second chain) and 157 (first chain). The amino acid sequences of the two chains of the F10B protein variant are shown in SEQ ID NOS: 158 (second chain) and 159 (first chain). The amino acid sequences of the two chains of the F10C protein variant are shown in the SEQ ID NOS: 160 (second chain) and 161 (first chain).

Format 11 (F11): CD19-F11-NKp46-3

[0362] The domain structure of F11 polypeptides is shown in FIG. 2C. The heterodimeric protein is similar to F10 except that the structures of the antigen binding domains are reversed. One of the two antigen binding domains has a Fab-like structure, and the other is a scFv. The heterodimer is made up of:

[0363] (1) a first (central) polypeptide chain having domains arranged as follows (N- to C-termini):

[0364] (V κ -V $_H$) $^{anti-CD19}$ -CH2-CH3-CH3-V $_H^{anti-NKp46}$ -C κ

and

[0365] (2) a second polypeptide chain having domains arranged as follows (N- to C-termini): V $\kappa^{anti-NKp46}$ -CH1.

[0366] Proteins were cloned, produced and purified as in Example 2-1. Bispecific proteins was purified from cell culture supernatant by affinity chromatography using prot-A beads and analyzed and purified by SEC. The protein showed a good production yield of 2 mg/L and the purified proteins similarly exhibited a simple SEC profile. The amino acid sequences of the two chains of the F11 protein are shown in SEQ ID NO: 162 (chain 1) and SEQ ID NO: 163 (chain 2).

Format 12 (F12): CD19-F12-NKp46-3

[0367] The domain structure of the dimeric F12 polypeptides is shown in FIG. 2C, wherein the bonds between the CH1 and C κ domains are disulfide bonds. The heterodimeric protein is similar to F11 but the CH1 and C κ domains within the F(ab) structure are inversed. The heterodimer is made up of:

[0368] (1) a first (central) polypeptide chain having domains arranged as follows (N- to C-termini):

[0369] (V κ -V $_H$) $^{anti-CD19}$ -CH2-CH3-CH3-V $_H^{anti-NKp46}$ -CH1

and

[0370] (2) a second polypeptide chain having domains arranged as follows (N- to C-termini): V $\kappa^{anti-NKp46}$ -C κ .

[0371] Proteins were cloned, produced and purified as in Example 2-1. Bispecific proteins were purified from the cell culture supernatant by affinity chromatography using prot-A beads and analyzed and purified by SEC. The protein showed a good production yield of 2.8 mg/L and the purified proteins similarly exhibited a simple SEC profile. The amino acid sequences of the two chains of the F12 protein are shown in SEQ ID NO: 164 (chain 1) and SEQ ID NO: 165 (chain 2).

Format 17 (F17): CD19-F17-NKp46-3

[0372] The domain structure of the trimeric F17 polypeptides is shown in FIG. 2C, wherein the bonds between the CH1 and C κ domains are disulfide bonds. The heterodimeric protein is similar to F9 but the V $_H$ and V κ domains, and the CH1 and C κ domains within the C-terminal F(ab) structure are each respectively inversed with their partner. The heterotrimer is made up of:

[0373] (1) a first (central) polypeptide chain having domains arranged as follows (N- to C-termini):

[0374] V $_H^{anti-CD19}$ -CH1-CH2-CH3-CH3-V $\kappa^{anti-NKp46}$ -CH1

and

[0375] (2) a second polypeptide chain having domains arranged as follows (N- to C-termini): V $_H^{anti-NKp46}$ -C κ

[0376] (3) a third polypeptide chain having domains arranged as follows (N- to C-termini):

[0377] V $\kappa^{anti-CD19}$ -C κ

[0378] Additionally, three variants of F17 proteins were produced: (a) a first where the cysteine residues in the hinge region were left intact (wild-type, referred to as F17A), (b) a second wherein the cysteine residues in the hinge region were replaced by serine residues (F10B), and (c) a third containing a linker sequence GGGSS which replaces residues DKTHTCPPCP in the hinge (F17C). Proteins were cloned, produced and purified as in Example 2-1. The amino acid sequences of the three chains of the F17B protein are shown in SEQ ID NOS: 166, 167 and 168.

Example 4

Bispecific NKp46 Antibody Formats with Dimeric Fc Domains

[0379] New protein constructions with dimeric Fc domains were developed that share many of the advantages of the monomeric Fc domain proteins of Example 3 but bind to FcRn with greater affinity. Different protein formats were produced that either had low or substantially lack of binding

to FcγR (including CD16) or which had binding to FcγRs (including CD16), e.g. the binding affinity to human CD16 was within 1-log of that of wild-type human IgG1 antibodies, as assessed by SPR (e.g. see methods of Example 16. The different polypeptide formats were tested and compared to investigate the functionality of heterodimeric proteins comprising a central chain with a (V_H-(CH1/Cκ)-CH2-CH3-) unit or a (Vκ-(CH1 or Cκ)-CH2-CH3-) unit. One of both of the CH3 domains are fused, optionally via intervening amino acid sequences or domains, to a variable domain (s) (a single variable domain that associates with a variable domain on a separated polypeptide chain, a tandem variable domain (e.g., an scFv), or a single variable domain that is capable of binding antigen as a single variable domain). The two chains associate by CH1-Cκ dimerization to form disulfide linked dimers, or if associated with a third chain, to form trimers.

[0380] Different constructs were made for use in the preparation of a bispecific antibody using the variable domains DNA and amino acid sequences derived from the scFv specific for tumor antigen CD19 described in Example 2-1 and different variable regions from antibodies specific for NKp46 identified in Example 1. Proteins were cloned, produced and purified as in Example 2-1. Domains structures are shown in FIGS. 2A-6D.

[0381] Format 5 (F5): CD19-F5-NKp46-3

[0382] The domain structure of the trimeric F5 polypeptide is shown in FIG. 2D, wherein the interchain bonds between hinge domains (indicated in the figures between CH1/Cκ and CH2 domains on a chain) and interchain bonds between the CH1 and Cκ domains are interchain disulfide bonds. The heterotrimer is made up of:

[0383] (1) a first (central) polypeptide chain having domains arranged as follows (N- to C-termini):

[0384] V_H^{anti-CD19}-CH1-CH2-CH3-V_H^{anti-NKp46}-Cκ
and

[0385] (2) a second polypeptide chain having domains arranged as follows (N- to C-termini): Vκ^{anti-CD19}-Cκ-CH2-CH3
and

[0386] (3) a third polypeptide chain having domains arranged as follows (N- to C-termini):

[0387] Vκ^{anti-NKp46}-CH1

[0388] Proteins were cloned, produced and purified as in Example 2-1. Bispecific proteins were purified from cell culture supernatant by affinity chromatography using prot-A beads and analyzed and purified by SEC. The protein showed a high production yield of 37 mg/L and the purified proteins again exhibited a simple SEC profile. The amino acid sequences of the three polypeptide chains are shown in SEQ ID NOS 169 (second chain), 170 (first chain) and 171 (third chain).

[0389] Format 6 (F6): CD19-F6-NKp46-3

[0390] The domain structure of heterotrimeric F6 polypeptides is shown in FIG. 2D. The F6 protein is the same as F5, but contains a N297S substitution to avoid N-linked glycosylation. Proteins were cloned, produced and purified as in Example 2-1. Bispecific proteins were purified from cell culture supernatant by affinity chromatography using prot-A beads and analyzed and purified by SEC. The protein showed a high production yield of 12 mg/L and the purified proteins exhibited a simple SEC profile. The amino acid

sequences of the three polypeptide chains are shown in SEQ ID NOS: 172 (second chain), 173 (first chain) and 174 (third chain).

[0391] Format 7 (F7): CD19-F7-NKp46-3

[0392] The domain structure of heterotrimeric F7 polypeptides is shown in FIG. 2D. The F7 protein is the same as F6, except for cysteine to serine substitutions in the CH1 and Cκ domains that are linked at their C-termini to Fc domains, in order to prevent formation of a minor population of dimeric species of the central chain with the Vκ^{anti-NKp46}-CH1 chain. Proteins were cloned, produced and purified as in Example 2-1. Bispecific proteins were purified from the cell culture supernatant by affinity chromatography using prot-A beads and analyzed and purified by SEC. The protein showed a high production yield of 11 mg/L and the purified proteins exhibited a simple SEC profile. The amino acid sequences of the three polypeptide chains are shown in SEQ ID NOS: 175 (second chain), 176 (first chain) and 177 (third chain).

[0393] Format 13 (F13): CD19-F13-NKp46-3

[0394] The domain structure of the dimeric F13 polypeptide is shown in FIG. 2D, wherein the interchain bonds between hinge domains (indicated between CH1/Cκ and CH2 domains on a chain) and interchain bonds between the CH1 and Cκ domains are interchain disulfide bonds. The heterodimer is made up of:

[0395] (1) a first (central) polypeptide chain having domains arranged as follows (N- to C-termini):

[0396] V_H^{anti-CD19}-CH1-CH2-CH3-(V_HVκ)^{anti-NKp46}
and

[0397] (2) a second polypeptide chain having domains arranged as follows (N- to C-termini): Vκ^{anti-CD19}-Cκ-CH2-CH3.

[0398] The (V_H-Vκ) unit was made up of a V_H domain, a linker and a Vκ unit (scFv).

[0399] Proteins were cloned, produced and purified as in Example 2-1. Bispecific proteins were purified from the cell culture supernatant by affinity chromatography using prot-A beads and analyzed and purified by SEC. The protein showed a high production yield of 6.4 mg/L and the purified proteins exhibited a simple SEC profile. The amino acid sequences of the two polypeptide chains are shown in SEQ ID NOS: 178 (second chain) and 179 (first chain).

[0400] Format 14 (F14): CD19-F14-NKp46-3

[0401] The domain structure of the dimeric F14 polypeptide is shown in FIG. 2E. The F14 polypeptide is a dimeric polypeptide which shares the structure of the F13 format, but instead of a wild-type Fc domain (CH2-CH3), the F14 bispecific format has CH2 domain mutations N297S to abolish N-linked glycosylation. Proteins were cloned, produced and purified as in Example 2-1. Bispecific proteins were purified from cell culture supernatant by affinity chromatography using prot-A beads and analyzed and purified by SEC. The protein showed a high production yield of 2.4 mg/L and the purified proteins exhibited a simple SEC profile. The amino acid sequences of the two polypeptide chains are shown in SEQ ID NOS: 180 (second chain) and 181 (first chain).

[0402] Format 15 (F15): CD19-F15-NKp46-3

[0403] The domain structure of the trimeric F15 polypeptides is shown in FIG. 2E. The F15 polypeptide is a dimeric polypeptide which shares the structure of the F6 format, but differs by inversion of the N-terminal V_H-CH1 and Vκ-Cκ units between the central and second chains. Proteins were

cloned, produced and purified as in Example 2-1. Bispecific proteins were purified from the cell culture supernatant by affinity chromatography using prot-A beads and analyzed and purified by SEC. The protein showed a good production yield of 0.9 mg/L and the purified proteins possessed a simple SEC profile. The amino acid sequences of the three polypeptide chains are shown in SEQ ID NOS: 182 (second chain), 183 (first chain) and 184 (third chain).

[0404] Format 16 (F16): CD19-F16-NKp46-3

[0405] The domain structure of the trimeric F16 polypeptide is shown in FIG. 2E. The F16 polypeptide is a dimeric polypeptide which shares the structure of the F6 format, but differs by inversion of the C-terminal V_H -CK and V_K -CH1 units between the central and second chains. Proteins were cloned, produced and purified as in Example 2-1. The amino acid sequences of the three polypeptide chains are shown in SEQ ID NOS: 185 (second chain), 186 (first chain) and 187 (third chain).

[0406] Format T5 (T5)

[0407] The domain structure of a trimeric T5 polypeptide is shown in FIG. 2F. The T5 polypeptide is a trimeric polypeptide which shares the structure of the F5 format, but differs by fusion of an scFv unit at the C-terminus of the third chain (the chain lacking the Fc domain). This protein will therefore have two antigen binding domains for antigens of interest, and one for NKp46, and will bind CD16 via its Fc domain. Proteins were cloned, produced and purified as in Example 2-1. The T5 protein had two antigen binding domains that bind human CD20, originating from different antibodies (and binding to different epitopes on CD20). The first anti-CD20 ABD contained the V_H and V_L of the parent antibody GA101 (GAZYVA®, Gazyvaro®, obinutuzumab, Roche Pharmaceuticals). The second anti-CD20 ABD contained the V_H and V_L of the parent antibody rituximab (Rituxan®, Mabthera®, Roche Pharmaceuticals). The third antigen binding domain binds human NKp46. The amino acid sequences of the three chains of the T5 protein are shown below (Rituximab sequences are in bold and underlined, anti-GA101 sequences are underlined, anti-NKp46 sequences are in italics).

GA101-T5-Ritux-NKp46

[0408]

Polypeptide 1

(SEQ ID NO: 188)

QVQLVQSGAEVKKPGSSVKVSCKASGYAFSYSWINWVRQAPGQGLEWMGR
IFPDGDGTDYNGKFKGRVTITADKSTSTAYMELSSLRSEDTAVYYCARNV
FDGYWLVYWGQGLTVTVSSASTKGPSVFPLAPSSKSTSGGTAALGCLVKD
YFPPEPTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVTVPSSSLGTQTY
ICNVNHKPSNTKVDKRVEPKSCDKTHTCPPCPAPELLGGPSVFLFPPKPK
DTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNS
TYRVSVLTIVLHQDLNGKEYCKVSNKALPAPIEKTISKAKGQPREPQV
YTLPPSREEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTPPVLD
SDSGSFFLYSLKLTVDKSRWQQGNVFCSCVMHEALHNHYTQKSLSLSPGST
GSQVQLQQPGAELVKPGASVMSCKASGYTFTSYNMHWKQTPGRGLEWIG

-continued

AIYPGNGDTSYNQKFKGKATLTADKSSSTAYMQLSSLTSEDSAVYYCARS
TTYGGDWYENVWGAGTTTVTSARTVAAPSVFIFPPSDEQLKSGTASVVC
LNNFYPREAKVQWKVDNALQSGNSQESVTEQDSKDSYSTLSSTLTLSKAD
YEKHKVYACEVTHQGLSSPVTKSFNRGEC-

Polypeptide 2

(SEQ ID NO: 189)

DIVMTQTPLSLPVTPGEPASISCRSSKSLLHNSGITYLYWYLQKPGQSPQ
LLIYQMSNLVSGVPDRFSGSGSGTDFTLKISRVEADGVVYYCAQNLELP
YTFGGGTKEIKRTVAAAPSVFIFPPSDEQLKSGTASVVCLLNNFYPREAK
VQWKVDNALQSGNSQESVTEQDSKDSYSTLSSTLTLSKADYEKHKVYACE
VTHQGLSSPVTKSFNRGECDKTHTCPPCPAPELLGGPSVFLFPPKPKDTL
MISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYR
VVSVLTIVLHQDLNGKEYCKVSNKALPAPIEKTISKAKGQPREPQVYTL
PPSREEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTPPVLDSD
GSFPLYSLKLTVDKSRWQQGNVFCSCVMHEALHNHYTQKSLSLSPGK-

Polypeptide 3:

(SEQ ID NO: 190)

QIVLSQSPAILSASPGKEVMTTCRASSSVSYIHWFPQKPGSSPKPWIIAT
SNLASGVPVRFSGSGSGTSYSLTISRVEAEDAATYYCQQTWTSNPPTEGGG
TKLEIKASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPTVSWNSG
ALTSQGVHTFPAVLQSSGLYSLSSVTVPSSSLGTQTYICNVNHKPSNTKV
DKRVEPKSCDKTHGSSSEVQLQDSGPGLVKPGASVKISCKTSGYTFTEY
TMHWVKQSHGKSLIEWIGGISPNIGGTSYNQKFKGKATLTVDKSSSTAYME
LRLSLTSEDSAVYYCARRGGSDYWGQGTTLTVSSVEGGSGGGGGSGGG
VDDIVMTQSPATLSVTPGDRVSLSCRASQISDYLHWYQKSHESPRLLI
KYASQSIISGIPSRFSGSGSGSDFTLSINSVEPEDVGVVYQCNGHSFPLTF
GAGTKLELK-

[0409] Format T6 (T6)

[0410] The domain structure of the trimeric T6 polypeptide is shown in FIG. 2F. The T6 polypeptide is a trimeric polypeptide which shares the structure of the F6 format, but differs by the fusion of an scFv unit at the C-terminus of the third chain (the chain lacking the Fc domain). This trimeric protein contains two antigen binding domains for antigens of interest, and one for NKp46, and does not bind CD16 via its Fc domain due to the N297 substitution. Proteins were cloned, produced and purified as in Example 2-1. The T6 protein contains two antigen binding domains that bind human CD20. The first anti-CD20 ABD comprises the V_H and V_L of the parent antibody GA101 and the second anti-CD20 ABD comprises the V_H and V_L of rituximab. The amino acid sequences of the three chains of the T6 proteins are shown in SEQ ID NOS: 191, 192 and 193.

[0411] Format T98 (T98)

[0412] The domain structure of the trimeric T9B polypeptide is shown in FIG. 2F. The T9B polypeptide is a trimeric polypeptide which shares the structure of the F9 format (F9B variant), but differs by the fusion of an scFv unit at the C-terminus of the free CH1 domain (on the third chain). This protein contains two antigen binding domains for antigen of

interest, and one for NKp46, but will not bind CD16 via its Fc domain due to the monomeric Fc domain and/or the N297 substitution. Trimeric proteins as above described were cloned, produced and purified as in Example 2-1. The T9B protein had two antigen binding domains that bind human CD20. The first anti-CD20 ABD contained the V_H and V_L of the parent antibody GA101 and the second anti-CD20 ABD contained the V_H and V_L of the parent antibody rituximab. The amino acid sequences of the three chains of the T9B proteins are shown below.

GA101-T9B-Ritux-NKp46

[0413]

Polypeptide 2:

(SEQ ID NO: 195)

DIVMTQTPLSLPVTGPGEPAISCRSSKSLHLSNGITYLYWYLQKPGQSPQ
LLIYQMSNLVSGVPDRFSGSGSGTDFTLKISRVEAEDVGVYYCAQNLELP
YTFGGGTKVEIKRTVAAPS FIFPPSDEQLKSGTASVVCCLNNFYPREAK
VQWKVDNALQSGNSQESVTEQDSKSTYLSSTLTLSKADYEKHKVYACE
VTHQGLSSPVTKSFNRGEC-

Polypeptide 1:

(SEQ ID NO: 194)

QVQLVQSGAEVKKPGSSVKVSKASGYAFSYSWINWVRQAPGQGLEWMGR
IFPGDGD TDYNGKFKGRVTITADKSTSTAYMELSSLRSEDTAVYYCARNV
FDGYWL VYWGQGLTVTVSSASTKGPSVFPLAPSSKSTSGGTAALGCLVKD
YFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVTVPSSSLGTQTY
ICNVNHKPSNTKVDKRVEPKSCDKTHTSPSPAPELLGGPSVFLFPPKPK
DTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYSS
TYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQV
YTLPPSREEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTPPVLD
SDSGSFFLYSKLTVDKSRWQQGNV FSCSVMEALHNHYTQKSLSLSPGGG
GGSGGGSGGGSGQPREPQVYTLPPSREEMTKNQVSLTCLVKGFYPSDI
AVEWESNGQPENNYKTPPVLDSDGSFFLYSKLTVDKSRWQQGNV FSCSV
MEALHNHYTQKSLSLSPGSTGSQVQLQPGAEVLPKPGASVMSCKASGYT
FTSYNMHWVKQTTPGRGLEWIGAIYPGNGDTSYNQKFKGKATLTADKSSST
AYMQLSLTSSEDSAVYYCARSTYYGGDWYFNWVGAGTTVTVSARTVAAPS
VFIFPPSDEQLKSGTASVVCCLNNFYPREAKVQWKVDNALQSGNSQESVT
EQDSKSTYLSSTLTLSKADYEKHKVYACEVTHQGLSSPVTKSFNR
GEC-

Polypeptide 3:

(SEQ ID NO: 196)

QIVLSQSPAILASPGKEKVTMTCRASSSVSYIHWFPQKPGSSPKPWIIYAT
SNLASGVPVRFSGSGSGTSYSLTISRVEAEDAATYYCQWTSNPPTFGGG
TKLEIKASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSG
ALTSGVHTFPAVLQSSGLYSLSSVTVPSSSLGTQTYICNVNHKPSNTKV
DKRVEPKSCDKTHGGSSSEVQLQSGPELVKPGASVKISCKTSGYTFTEY

-continued

TMHWVKQSHGKSLEWIGGISPNIGGTSYNQKFKGKATLTVDKSSSTAYME
LRSLTSEDSAVYYCARRGGSFYWGQGTTLTVSSVEGGSGGSGGSGGSGG
VDDIVMTQSPATLSVTPGDRVSLSCRASQSIDYHLHWYQQKSHSPRLLI
KYASQSI SGIPSRFSGSGSGSDFTLSINSVEPEDVGVYYCQNGHSFPLTF
GAGTKLELK-

[0414] Format T11 (T1): CD19-T11-NKp46-3

[0415] The domain structure of the dimeric T11 polypeptide is shown in FIG. 2F. The T11 polypeptide is a trimeric polypeptide which shares the structure of the F11 format, but differs by the fusion of an scFv unit at the C-terminus of the free CH1 domain. This dimeric protein contains two antigen binding domains for antigen of interest, and one for NKp46, and does not bind CD16 via its Fc domain due to the monomeric Fc domain and/or the N297 substitution. Proteins were cloned, produced and purified as in Example 2-1. The T11 protein contains two antigen binding domains that bind human CD20. The first anti-CD20 ABD contained the V_H and V_L of the parent antibody GA101 and the second anti-CD20 ABD contained the V_H and V_L of rituximab. The amino acid sequences of the two chains of the T11 protein are shown below.

[0416] GA101-T11-Ritux-NKp46

Polypeptide 1:

(SEQ ID NO: 197)

DIVMTQTPLSLPVTGPGEPAISCRSSKSLHLSNGITYLYWYLQKPGQSPQ
LLIYQMSNLVSGVPDRFSGSGSGTDFTLKISRVEAEDVGVYYCAQNLELP
YTFGGGTKVEIKGGGGSGGGSGGGSGQVQLVQSGAEVKKPGSSVKVSK
ASGYAFSYSWINWVRQAPGQGLEWMGRIIFPGDGD TDYNGKFKGRVTITAD
KSTSTAYMELSSLRSEDTAVYYCARNVFDGYWL VYWGQGLTVTVSSASTK
GPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFP
AVLQSSGLYSLSSVTVPSSSLGTQTYICNVNHKPSNTKVDKRVEPKSCD
KTHTSPSPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDP
EVKFNWYVDGVEVHNAKTKPREEQYSS TYRVVSVLTVLHQDWLNGKEYKC
KVSNAKALPAPIEKTISKAKGQPREPQVYTLPPSREEMTKNQVSLTCLVKG
FYPSDIAVEWESNGQPENNYKTPPVLDSDGSFFLYSKLTVDKSRWQQGN
VFSCSVMEALHNHYTQKSLSLSPGGGGSGGGSGGGSGQPREPQVYT
LPPSREEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTPPVLDSD
DGSFFLYSKLTVDKSRWQQGNV FSCSVMEALHNHYTQKSLSLSPGSTGS
QVQLQPGAEVLPKPGASVMSCKASGYTFTSYNMHWVKQTTPGRGLEWIGAI
YPGNGDTSYNQKFKGKATLTADKSSSTAYMQLSLTSSEDSAVYYCARSTY
YGGDWYFNWVGAGTTVTVSARTVAAPS FIFPPSDEQLKSGTASVVCCLN
NFYPREAKVQWKVDNALQSGNSQESVTEQDSKSTYLSSTLTLSKADYE
KHKVYACEVTHQGLSSPVTKSFNRGEC-

-continued

Polypeptide 2: (SEQ ID NO: 198)
QIVLSQSPAILSASPGKEVTMTCRASSSVSYIHWFOQKPGSSPKPWIIYAT
SNLASGVPVRFSGSGSGTSYSLTISRVEADAATYYCQQWTSNPPTFGGG
TKLEIKASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPTVSWNSG
ALTSGVHTFPAVLQSSGLYSLSSVVTVPSSSLGTQTYICNVNHKPSNTKV
DKRVEPKSCDKTHGGSSEVQLQQSGPELVKPGASVKISCKTSGYTFTEY
TMHWVKQSHGKSLIEWIGGISPNIGGTSYNQKFKGKATLTVDKSSSTAYME
LRLSTSEDSAVVYICARRGGSFDYWGQGTTLTVSSVEGGSGGSGGSGGG
VDDIVMTQSPATLSVTPGDRVLSLSCRASQISDYLHWYQQKSHESPRLLI
KYASQISIGIPSRFSGSGSGSDFTLSINSVEPEDVGVYYCQNGHSFPLTF
GAGTKLELK-

Example 5

NKp46 Binding Affinity by Bispecific Proteins
Detected by Surface Plasmon Resonance (SPR)

Biacore T100 General Procedure and Reagents

[0417] SPR measurements were performed on a Biacore T100 apparatus (Biacore GE Healthcare) at 25° C. In all Biacore experiments HBS-EP+(Biacore GE Healthcare) and NaOH 10 mM served as running buffer and regeneration buffer respectively. Sensorgrams were analyzed with Biacore T100 Evaluation software. Protein-A was purchased from (GE Healthcare). Human NKp46 recombinant proteins were cloned, produced and purified at Innate Pharma.

Immobilization of Protein-A

[0418] Protein-A proteins were immobilized covalently to carboxyl groups in the dextran layer on a Sensor Chip CM5. The chip surface was activated with EDC/NHS (N-ethyl-N'-(3-dimethylaminopropyl) carbodiimidehydrochloride and N-hydroxysuccinimide (Biacore GE Healthcare)). Protein-A was diluted to 10 µg/ml in coupling buffer (10 mM acetate, pH 5.6) and injected until the appropriate immobilization level was reached (i.e. 2000 RU). Deactivation of the remaining activated groups was performed using 100 mM ethanolamine pH 8 (Biacore GE Healthcare).

Binding Study

[0419] The bispecific proteins were first tested in Format F1 as described in Example 2 having different anti-NKp46 variable regions from NKp46-1, NKp46-2, NKp46-3 or NKp46-4 antibodies. Antibodies were next tested as different formats (F3, F4, F5, F6, F9, F10, F11, F13, F14) having the anti-NKp46 variable regions from the NKp46-3 antibody, and were compared to the NKp46-3 antibody as a full-length human IgG1.

[0420] Bispecific proteins at 1 µg/mL were captured onto Protein-A chip and recombinant human NKp46 proteins were injected at 5 µg/mL over captured bispecific antibodies. For blank subtraction, cycles were performed again replacing NKp46 proteins with running buffer.

[0421] The Bab281 antibody was separately tested for binding to NKp46 by SPR, and additionally by flow cytometry

using a cell line expressing the human NKp46 construct on the cell surface. For FACS screening, the presence of reactive antibodies in the supernatants was detected using Goat anti-mouse polyclonal antibody (pAb) labeled with PE. SPC and FACS results showed that the Bab281 based antibody did not bind the NKp46 cell line or to NKp46-Fc proteins. Bab281 lost the ability to bind to its target when presented in the bispecific format.

Affinity Study

[0422] Monovalent affinity study was conducted following a regular Capture-Kinetic protocol as recommended by the manufacturer (Biacore GE Healthcare kinetic wizard). Seven serial dilutions of human NKp46 recombinant proteins, ranging from 6.25 to 400 nM were sequentially injected over the captured Bi-Specific antibodies and allowed to dissociate for 10 min before regeneration. The entire sensorgram sets were fitted using the 1:1 kinetic binding model.

Results

[0423] SPR showed that the bispecific polypeptides of format F1 having the NKp46-1, 2, 3 and 4 scFv binding domains bound to NKp46, while the other bispecific polypeptides having the scFv of different anti-NK46 antibodies did not retain NKp46 binding. The binding domains that did not retain binding in monomeric bispecific format initially bound to NKp46 but lost the ability to bindNKp46 upon conversion to the bispecific format. All of the bispecific polypeptides of formats F1, F2 F3, F4, F5, F6, F9, F10, F11, F13, and F14 retained binding to NKp46 when using the NKp46-3 variable regions. Monovalent affinities and kinetic association and dissociation rate constants are shown below in Table 3 below.

TABLE 3

Bispecific mAb	ka (1/Ms)	kd (1/s)	KD (M)
CD19-F1-Bab281	n/a	n/a	n/a (loss of binding)
CD19-F1-NKp46-1	1.23E+05	0.001337	1.09E-08
CD19-F1-NKp46-2	1.62E+05	0.001445	8.93E-09
CD19-F1-NKp46-3	7.05E+04	6.44E-04	9.14E-09
CD19-F1-NKp46-4	1.35E+05	6.53E-04	4.85E-09
CD19-F3-NKp46-3	3.905E+5	0.01117	28E-09
CD19-F4-NKp46-3	3.678E+5	0.01100	30E-09
CD19-F5-NKp46-3	7.555E+4	0.00510	67E-09
CD19-F6-NKp46-3	7.934E+4	0.00503	63E-09
CD19-F9A-NKp46-3	2.070E+5	0.00669	32E-09
CD19-F10A-NKp46-3	2.607E+5	0.00754	29E-09
CD19-F11A-NKp46-3	3.388E+5	0.01044	30E-09
CD19-F13-NKp46-3	8.300E+4	0.00565	68E-09
CD19-F14-NKp46-3	8.826E+4	0.00546	62E-09
NKp46-3 IgG1	2.224E+5	0.00433	20E-09

Example 6

Engagement of NK Cells Against Daudi Tumor
Target with Fc-Containing NKp46×CD19 Bispecific
Protein

[0424] Bispecific antibodies having a monomeric Fc domain and a domain arrangement according to the single chain F1 or dimeric F2 formats described in Example 3, and a NKp46 binding region based on NKp46-1, NKp46-2,

NKp46-3 or NKp46-4 were tested for functional ability to direct NK cells to lyse CD19-positive tumor target cells (Daudi, a well characterized B lymphoblast cell line). The F2 proteins additionally included NKp46-9 variable regions which lost the ability to bind NKp46 in the scFv format but which retained the ability to bind NKp46 in the F(ab)-like format of F2.

[0425] Briefly, the cytolytic activity of each of (a) resting human NK cells, and (b) human NK cell line KHYG-1 transfected with human NKp46, was assessed in a classical 4-h ^{51}Cr -release assay in U-bottom 96 well plates. Daudi cells were labelled with ^{51}Cr (50 μCi (1.85 MBq)/ 1×10^6 cells), then mixed with KHYG-1 transfected with hNKp46 at an effector/target ratio equal to 50 for KHYG-1, and 10 (for F1 proteins) or 8.8 (for F2 proteins) for resting NK cells, in the presence of monomeric bi-specific antibodies at different concentrations. After brief centrifugation and 4 hours of incubation at 37° C., samples of supernatant were removed and transferred into a LumaPlate (Perkin Elmer Life Sciences, Boston, Mass.), and ^{51}Cr release was measured with a TopCount NXT beta detector (Perkin Elmer Life Sciences, Boston, Mass.). All experimental conditions were analyzed in triplicate, and the percentage of specific lysis was determined as follows: $100 \times (\text{mean cpm experimental release} - \text{mean cpm spontaneous release}) / (\text{mean cpm total release} - \text{mean cpm spontaneous release})$. Percentage of total release is obtained by lysis of target cells with 2% Triton X100 (Sigma) and spontaneous release corresponds to target cells in medium (without effectors or Abs).

[0426] Results

[0427] In the KHYG-1 hNKp46 NK experimental model, each bi-specific antibody NKp46-1, NKp46-2, NKp46-3, NKp46-4 or NKp46-9 induced specific lysis of Daudi cells by human KHYG-1 hNKp46 NK cell line compared to negative controls (Human IgG1 isotype control (IC) and CD19/CD3 bi-specific antibodies), thereby showing that these antibodies induce Daudi target cell lysis by KHYG-1 hNKp46 through CD19/NKp46 cross-linking.

[0428] When resting NK cells were used as effectors, each bi-specific antibody NKp46-1, NKp46-2, NKp46-3, NKp46-4 or NKp46-9 again induced specific lysis of Daudi cells by human NK cells compared to the negative control (Human IgG1 isotype control (IC) antibody), thereby showing that these antibodies induce Daudi target cell lysis by human NK cells through CD19/NKp46 cross-linking. Rituximab (RTX, chimeric IgG1) was used as a positive control of ADCC (Antibody-Dependent Cell Cytotoxicity) by resting human NK cells. The maximal response obtained with RTX (at 10 $\mu\text{g}/\text{ml}$ in this assay) was 21.6% specific lysis illustrating that the inventive bispecific antibodies have high target cell lysis activity. Results for experiments with resting NK cells are shown in FIGS. 3A for the single chain F1 proteins and 3B for the dimeric F2 proteins.

Example 7

Comparison with Full Length Anti-NKp46 mAbs and Depleting Anti-Tumor mAbs: Only NKp46 \times CD19 Bispecific Proteins Prevent Non-Specific NK Activation

[0429] In these experiments bispecific antibodies possessing a specific bispecific format were produced in order to assess whether such bispecific antibodies can mediate

NKp46-mediated NK activation toward cancer target cells without triggering non-specific NK cell activation.

[0430] Particularly, NKp46 \times CD19 bispecific proteins having an arrangement according to the F2 format described in Example 3 with anti-NKp46 variable domains from NKp46-1, NKp46-2, NKp46-3, NKp46-4 or NKp46-9 were compared to:

[0431] (a) full-length monospecific anti-NKp46 antibodies (NKp46-3 as human IgG1), and

[0432] (b) the anti-CD19 antibody as a full-length human IgG1 as ADCC inducing antibody control comparator.

[0433] The experiments further included as controls: rituximab, an anti-CD20 ADCC inducing antibody control for a target antigen with high expression levels; an anti-CD52 antibody alemtuzumab, a human IgG1 which binds CD52 target present on both targets and NK cells; and a negative control isotype control therapeutic antibody (a human IgG1 that does not bind a target present on the target cells (HUG1-IC)).

[0434] The different proteins were tested in order to assess their relative functional effects on NK cell activation in the presence of CD19-positive tumor target cells (Daudi cells), in the presence of CD19-negative, CD16-positive target cells (HUT78 T-lymphoma cells), and in the absence of target cells.

[0435] Briefly, NK activation was tested by assessing CD69 and CD107 expression on NK cells by flow cytometry. The assay was carried out in 96 U well plates in completed RPMI, 150 μL final/well. Effector cells were fresh NK cells purified from donors. Target cells were Daudi (CD19-positive), HUT78 (CD19-negative) or K562 (NK activation control cell line). In addition to K562 positive control, three conditions were tested, as follows:

[0436] NK cell alone

[0437] NK cells vs Daudi (CD19 $^{+}$)

[0438] NK cells vs HUT78 (CD19 $^{-}$)

[0439] Effector:Target (E:T) ratio was 2.5:1 (50 000 E:20 000 T), with an antibody dilution range starting to 10 $\mu\text{g}/\text{mL}$ with $1/4$ dilution ($n=8$ concentrations). Antibodies, target cells and effector cells were mixed; spun 1 min at 300 g; incubated 4 h at 37° C.; spun 3 min at 500 g; washed twice with Staining Buffer (SB); added 50 μL of staining Ab mix; incubated 30 min at 300 g; washed twice with SB resuspended pellet with CellFix; stored overnight at 4° C.; and fluorescence was detected with Canto II (HTS).

[0440] Results

[0441] 1. NK Cells Alone

[0442] Results of these experiments are shown in FIG. 4A. In the absence of target-antigen expressing cells, none of the bispecific anti-NKp46 \times anti-CD19 antibodies (including each of the NKp46-1, NKp46-2, NKp46-3, NKp46-4 and NKp46-9 variable regions) activated NK cells as assessed by CD69 or CD107 expression. The full-length anti-CD19 also did not activate NK cells. However, the full-length anti-NKp46 antibodies did cause detectable activation of NK cells. Alemtuzumab also induced activation of NK cells, at a very high level. The isotype control antibody did not induce activation.

[0443] 2. NK Cells Vs Daudi (CD19 $^{+}$)

[0444] Results of these experiments are shown in FIG. 4B. In the presence of target-antigen expressing cells, each of the bispecific anti-NKp46 \times anti-CD19 antibodies (including each of the NKp46-1, NKp46-2, NKp46-3, NKp46-4 and NKp46-9 binding domains) activated NK cells. The full-

length anti-CD19 antibody showed at best only very low activation of NK cells. Neither full-length anti-NKp46 antibodies nor alemtuzumab showed a substantial increase in activation beyond what was observed in presence of NK cells alone. The data in FIG. 4 shows that full-length anti-NKp46 antibodies elicited a similar level of baseline activation as was observed in the presence of NK cells alone. Alemtuzumab also induced the activation of NK cells at a similar level of activation to what was observed in the presence of NK cells alone, and at higher antibody concentrations in this setting (ET 2.5:1) the activation was greater than with the bispecific anti-NKp46×anti-CD19 antibody. The isotype control antibody did not induce activation.

[0445] 3. NK cells vs HUT78 (CD19⁻)

[0446] Results of these experiments are shown in FIG. 4C. In the presence of target-antigen-negative HUT78 cells, none of the bispecific anti-NKp46×anti-CD19 antibody (including each of the NKp46-1, NKp46-2, NKp46-3, NKp46-4 and NKp46-9 variable regions) activated NK cells. However, the full-length anti-NKp46 antibodies and alemtuzumab caused detectable activation of NK cells at a similar level observed in presence of NK cells alone. Isotype control antibody did not induce activation.

[0447] The foregoing results indicate that the inventive bispecific anti-NKp46 proteins are able to activate NK cells in a target-cell specific manner, unlike full-length monospecific anti-NKp46 antibodies and further unlike full-length antibodies of depleting IgG isotypes which also activate NK cells in the absence of target cells. The NK cell activation achieved with anti-NKp46 bispecific proteins remarkably was higher than that observed with full length anti-CD19 IgG1 antibodies. Therefore these bispecific antibodies should elicit less non-specific cytotoxicity and may be more potent when used in therapy.

Example 8

Comparative Efficacy with Depleting Anti-Tumor mAbs: NKp46×CD19 Bispecific Proteins at Low ET Ratio

[0448] These studies aimed to investigate whether bispecific antibodies can mediate NKp46-mediated NK cell activation toward cancer target cells at lower effector: target ratios. The ET ratio used in this Example was 1:1 which is believed to be closer to the setting that would be encountered in vivo than the 2.5:1 ET ratio used in Example 7 or the 10:1 ET ratio of Example 6.

[0449] NKp46×CD19 bispecific proteins having an arrangement according to the F2 format described in Example 3 with anti-NKp46 variable domains from NKp46-1, NKp46-2, NKp46-3, NKp46-4 or NKp46-9 were compared to:

[0450] (a) full-length monospecific anti-NKp46 antibodies (NKp46-3 as human IgG1), and

[0451] (b) the anti-CD19 antibody as a full-length human IgG1 as ADCC inducing antibody control comparator.

[0452] The experiments further included as controls: rituximab (an anti-CD20 ADCC inducing antibody control for a target antigen with high expression levels); anti-CD52 antibody alemtuzumab (a human IgG1, binds CD52 target present on both targets and NK cells), and negative control isotype control therapeutic antibody (a human IgG1 that does not bind a target present on the target cells (HUG1-IC). The different proteins were tested for their functional effect

on NK cell activation as assessed by CD69 or CD107 expression in the presence of CD19-positive tumor target cells (Daudi cells), in the presence of CD19-negative, CD16-positive target cells (HUT78 T-lymphoma cells), and in the absence of target cells. The experiments were carried out as in Example 7 except that the ET ratio was 1:1.

[0453] Results

[0454] The results of the above experiments are shown in FIGS. 5 (5A: CD107 and 5B: CD69). In the presence of target-antigen expressing cells, each of the bispecific anti-NKp46×anti-CD19 antibodies (respectively including NKp46-1, NKp46-2, NKp46-3, NKp46-4 or NKp46-9 variable regions) activated NK cells in the presence of Daudi cells.

[0455] The activation induced by bispecific anti-NKp46×anti-CD19 antibody in the presence of Daudi cells was far more potent than that elicited by the full-length human IgG1 anti-CD19 antibody. This ADCC inducing antibody had low activity in this setting. Furthermore, in this low E:T ratio setting the activation induced by the bispecific anti-NKp46×anti-CD19 antibody was as potent as the anti-CD20 antibody rituximab, with a difference being observed only at the highest concentrations that were 10 fold higher than concentrations in which differences were observed at the 2.5:1 ET ratio.

[0456] In the absence of target cells or in the presence of target antigen-negative HUT78 cells, full-length anti-NKp46 antibodies and alemtuzumab showed a similar level of baseline activation as was observed in the presence of Daudi cells. Anti-NKp46×anti-CD19 antibody did not activate NK cells in presence of HUT78 cells.

[0457] The foregoing results indicate that the bispecific anti-NKp46 proteins of the invention are able to activate NK cells in a target-cell specific manner and at lower effector: target ratios and are more effective in mediating NK cell activation than traditional human IgG1 antibodies.

Example 9

NKp46 Mechanism of Action

[0458] NKp46×CD19 bispecific proteins having an arrangement according to the F2, F3, F5 or F6 formats described in Examples 3 or 4 with anti-NKp46 variable domains from NKp46-3 were compared to rituximab (anti-CD20 ADCC inducing antibody), and to a human IgG1 isotype control antibody for their functional ability to direct CD16-/NKp46+NK cell lines to lyse CD19-positive tumor target cells.

[0459] Briefly, the cytolytic activity of the CD16-/NKp46⁺ human NK cell line KHYG-1 was assessed in a classical 4-h ⁵¹Cr-release assay in U-bottom 96 well plates. Daudi or B221 cells were labelled with ⁵¹Cr (50 μCi (1.85 MBq)/1×10⁶ cells), then mixed with KHYG-1 at an effector/target ratio equal to 50:1, in the presence of test antibodies at dilution ranges starting from 10⁻⁷ mol/L with 1/5 dilution (n=8 concentrations).

[0460] After a brief centrifugation and 4 hours of incubation at 37° C., 50 μL of the supernatant were removed and transferred into a LumaPlate (Perkin Elmer Life Sciences, Boston, Mass.), and ⁵¹Cr release was measured with a TopCount NXT beta detector (PerkinElmer Life Sciences, Boston, Mass.). All experimental conditions were analyzed in triplicate, and the percentage of specific lysis was determined as follows: 100×(mean cpm experimental release-

mean cpm spontaneous release)/(mean cpm total release—mean cpm spontaneous release). Percentage of total release is obtained by lysis of target cells with 2% Triton X100 (Sigma) and spontaneous release corresponds to target cells in medium (without effectors or Abs).

[0461] Results

[0462] The results of the above experiments are shown in FIGS. 6A (KHYG-1 vs Daudi) and 6B (KHYG-1 vs B221). In the KHYG-1 hNKp46 NK experimental model, each NKp46×CD19 bispecific protein (Format F2, F3, F5 and F6) induced specific lysis of Daudi or B221 cells by human KHYG-1 hNKp46 NK cell line, while rituximab and the human IgG1 isotype control (IC) antibodies did not.

Example 10

Anti-KIR3DL2 Bispecific Proteins

[0463] Bispecific proteins targeting human KIR3DL2 (KIR3DL2×NKp46 bispecific) were constructed as F6 formats and tested for activity. KIR3DL2 (CD158k; killer cell immunoglobulin like receptor, three Ig domains and long cytoplasmic tail 2) is a disulphide-linked homodimer of three-Ig domain molecules of about 140 kD, described in Pende et al. (1996) J. Exp. Med. 184: 505-518, the disclosure of which is incorporated herein by reference. Several allelic variants have been reported for KIR3DL2 polypeptides, each of these are encompassed by the term KIR3DL2. The amino acid sequence of the mature human KIR3DL2 (allele *002) is shown in Genbank accession no. AAB52520. Briefly, the cytolytic activity of NK cells from Buffy coat from donors was assessed in a classic 4-h ⁵¹Cr-release assay in U-bottom 96 well plates. HUT78 tumor cells (CTCL) that express KIR3DL2 were labelled with ⁵¹Cr, then mixed with NK cells at an effector/target ratio equal to 10:1 (25 000:2500), in the presence of test antibodies at dilution ranges starting from 10 µg/mL (or 100 µg/mL) with 1/10 dilution (n=8). Assays were in cRPMI, 150 µL final/well, in triplicates.

[0464] Results are shown in FIG. 6C. Despite its Fc domain not binding to CD16 in this format, the F6 protein structure produced as an NKp46×KIR3DL2 bispecific protein surprisingly exhibited comparable ability to lyse target cells as a known anti-KIR3DL2 human IgG1 antibody that contained the same variable regions and which binds KIR3DL2 bivalently.

Example 11

Effect of Intrachain Domain Motion within Multimeric Proteins

[0465] It was theorized by the inventors that the ability of NKp46 bispecific proteins to promote NKp46-mediated lysis of target cells may be affected by the distance between the two antigen binding domains in the bispecific protein which may impact the ability of one or both of the NKp46 antigen binding domain and the antigen binding domain which interacts with an antigen of interest to interact with their respective targets. Also, it was further theorized that NKp46 mediated lysis of target cells may be impacted by the structure of the two antigen binding domains and/or their respective conformation, freedom of motion or flexibility which may be impacted by the structure of the two antigen binding domains as well as the manner by which they are associated with each other, e.g., by a linker peptide and its

particular length and chemical composition. Particularly, it was theorized that a lytic NKp46-target cell synapse may vary as a function of the size and structure of the bispecific protein. Therefore, the inventors posited that bispecific proteins wherein the antigen binding domains are in a format whereby the antigen binding domains more closely mimics or approximates the conformation, spacing and flexibility of the antigen binding domains of

[0466] This was theorized because conformational flexibility, notably intrachain domain motion or movement, may for example affect the effective distance between NKp46 and antigen-of-interest binding sites, which in turn might have an effect on the NKp46-target cell synapse and the ability of a multimeric bispecific protein to mediate NKp46-mediated signaling and lysis. Based on these suppositions the inventors evaluated the lytic function of multimeric proteins of different bispecific protein formats and which comprise more or less freedom of motion of the antigen binding domains based on the structure of the antigen binding sites and the specific linkers separating these antigen binding sites.

[0467] Specifically, different NKp46×tumor antigen bispecific proteins of different formats such as the F3, F4, F9, F10 and F11 format that bound different tumor antigens were evaluated for their relative ability to induce NKp46-mediated lysis of tumor target cells by KHYG-1 NK cells (NKp46×CD16). F5 and F6 bispecific protein formats have distances between the NKp46 binding site and the antigen of interest binding site that are less than that of full-length antibodies. By contrast bispecific proteins targeting human CD19 (CD19×NKp46 bispecific) in F9 format have binding sites that are spaced farther apart, similar to distances in the two binding sites in conventional full-length antibodies. Bispecific proteins were therefore constructed as F9 formats and compared to F10 and F11 formats. Structurally speaking, format F9, F10 and F11 are very close to one another, however formats F10 and F11 are characterized by one antigen binding domain with a Fab structure and the other antigen binding domain with a tandem variable domain structure (two variable domains separated by a flexible linker). F10 and F11 therefore have greater intrachain domain motion and/or less local steric hindrance, as well as possibly less distance between binding sites than in the F9 proteins.

[0468] The cytolytic activity of the CD16-NKp46+ human NK cell line KHYG-1 was assessed in a classical 4-h ⁵¹Cr-release assay in U-bottom 96 well plates. Daudi or B221 cells were labelled with ⁵¹Cr (50 µCi (1.85 MBq)/1×10⁶ cells), then mixed with KHYG-1 at an effector/target ratio equal to 50:1, in the presence of test antibodies at dilution range starting from 10⁻⁷ mol/L with 1/5 dilution (n=8 concentrations). The results showed that formats F10 and F11 were both more potent than format F9 in inducing Daudi cell lysis by NK cells. As noted above F9 format proteins have distances between the NKp46 binding site and the antigen of interest binding site which is similar to full-length antibodies or about 80 Å, and the F10 and F11 proteins comprise a single chain domain connected to the Fc by a flexible linker and have substantially less than 80 Å between the antigen binding sites (in the case of F10, about 55 Å).

[0469] Based thereon we studied the effects of even further shortened distances between the NKp46 and antigen of interest binding domains using other CD19×NKp46 bispecific proteins. In these experiments F3, F4 protein formats

were selected for comparison with protein formats F10 and F11. Each of these proteins have distances between antigen binding sites of less than 80 Å, however, F3 and F4 are shorter than F10 and F11, and F3 and F4 have distances between antigen binding sites that are equivalent to F11 but 25 Å less than that of F10. The results of these experiments indicated that the F3, F4, F10 and F11 formats did not significantly differ in their ability to induce Daudi cell lysis by NK cells. These results would suggest that there may be an optimal minimal spacing between the antigen binding domains that improves potency and/or that potency is affected by a combination of the spacing between the antigen binding domains and the flexibility and/or conformation of the antigen binding domains.

Example 12

Combining NKp46 and CD16 Triggering

[0470] NKp46×CD19 bispecific proteins that bind human CD16 having an arrangement according to the F5 format with anti-NKp46 variable domains from NKp46-3 were compared to the same bispecific antibody in a F6 format (which lacks CD16 binding), and to a human IgG1 isotype anti-CD19 antibody, as well as to a human IgG1 isotype control antibody for functional ability to direct purified NK cells to lyse CD19-positive Daudi tumor target cells.

[0471] Briefly, the cytolytic activity of fresh human purified NK cells from EFS Buffy Coat was assessed in a classical 4-h ⁵¹Cr-release assay in U-bottom 96 well plates. Daudi or HUT78 cells (negative control cells that do not express CD19) were labelled with ⁵¹Cr and then mixed with NK cells at an effector/target ratio equal to 10:1, in the presence of test antibodies at dilution range starting from 10 µg/ml with 1/10 dilution (n=8 concentrations).

[0472] After brief centrifugation and 4 hours of incubation at 37° C., 50 µL of supernatant were removed and transferred into a LumaPlate (Perkin Elmer Life Sciences, Boston, Mass.), and ⁵¹Cr release was measured with a TopCount NXT beta detector (PerkinElmer Life Sciences, Boston, Mass.). All experimental conditions were analyzed in triplicate, and the percentage of specific lysis was determined as follows: 100×(mean cpm experimental release—mean cpm spontaneous release)/(mean cpm total release—mean cpm spontaneous release). Percentage of total release is obtained by lysis of target cells with 2% Triton X100 (Sigma) and spontaneous release corresponds to target cells in medium (without effectors or Abs).

[0473] The results of these experiments are shown in FIG. 7. The CD19-F6-NKp46 (bispecific protein in F6 format) whose Fc domain does not bind CD16 due to a N297 substitution was as potent in mediating NK cell lysis of Daudi target cells as the full-length IgG1 anti-CD19 antibody. This result is remarkable especially considering that the control IgG1 anti-CD19 antibody binds CD19 bivalently and further since the anti-CD19 antibody is bound by CD16. The F6 protein was also compared to a protein CD19-F5-NKp46 that was identical to the CD19-F6-NKp46 protein with the exception of an asparagine at Kabat residue 297. Surprisingly, despite the strong NK activation mediated by CD16 triggering by the CD19-F5-NKp46 (F5 format protein) whose Fc domain binds CD16, the F5 format was far more potent in mediating Daudi target cell lysis than the full-length IgG1 anti-CD19 antibody or the F6 format bispecific protein. This would suggest that NKp46 can enhance

target cell lysis even when CD16 is triggered. In fact, at comparable levels of target cell lysis, the CD19-F5-NKp46 was at least 1000 times more potent than the full-length anti-CD19 IgG1. These potency results would suggest that the inventive multispecific NKp46 antibodies should be well suited for use in human therapy, e.g., in treating cancer or infectious diseases.

Example 13

Mechanisms of Action of CD16-Binding NKp46×CD19 Bispecific

[0474] Lysis of Daudi cells by NKp46×CD19 bispecific F5 and F6 were compared to a conventional human IgG1 antibody. As a control, lysis was also tested on HUT78 cells that lack CD19; positive control for HUT78 cell lysis was an anti-KIR3DL2 of human IgG1 isotype (HUT78 are KIR3DL2-positive). Cytotoxicity assays were carried out as in Example 10. Flow cytometry staining of NK cell surface markers was carried out as in Example 7.

[0475] Results for the cytotoxicity assays are shown in FIG. 8 (Daudi cell in the right hand panel and HUT78 cells in the left hand panel). the CD19-F6-NKp46-3 whose Fc domain does not bind CD16 due to a N297 substitution has as mode of action NKp46 triggering when NK cells encounter the target cell, while the CD19-F5-NKp46-3 bispecific protein demonstrated a far higher potency in mediating cytotoxicity toward Daudi cells. Neither the F5 nor the F6 protein mediated any NK cell cytotoxicity towards HUT78 cells.

[0476] The results of flow cytometry staining of NK cell surface markers showed a strong upregulation of CD137 on the surface of NK cells by F5 proteins. These results are shown in FIG. 9 (Left-most panel: NK cells vs. Daudi; middle panel: NK cells vs. HUT78; right-most panel: NK cells alone). The CD19-F5-NKp46-3 whose Fc domain binds CD16 demonstrated the highest CD137 upregulation. The full-length anti-CD19 IgG1 antibody that binds CD16 also elicited CD137 upregulation, but to a far lesser extent than CD19-F5-NKp46-3. The CD19-F6-NKp46-3 which functions via NKp46 but not via CD16 did not elicit any detectable CD137 upregulation. It is hypothesized that the remarkable potency of the F5 format may arise from a particularly strong CD137 upregulation on NK cells which may be mediated by the dual targeting of NKp46 and CD16.

Example 14

Fc-Engineered CD16-Binding NKp46×CD20 Bispecific

[0477] New bispecific proteins were further constructed in an attempt to generate an agent that could improve on the most potent new generation of Fc enhanced antibodies. In these experiments as the comparison antibody we selected the commercial antibody GA101 (GAZYVA®, Gazyvaro®, obinutuzumab, Roche Pharmaceuticals), which is an Fc-modified human IgG1 antibody having enhanced CD16A binding as a result of hypofucosylated N-linked glycosylation.

[0478] NKp46×CD20 bispecific proteins were produced as proteins without CD16 binding (F6 format), with CD16 binding (F5 format), or as Fc-engineered format based on F5 but comprising two amino acid substitutions in the CH2 domain of the heavy chain that increase binding affinity for

human CD16A (referred to as "F5+"). In these constructs the anti-CD20 ABDs comprise the V_H and V_L of GA101.

[0479] Lysis of Daudi cells by NKp46×CD20 bispecific F5, F5+ and F6 antibodies were compared to the commercial antibody GA101 (GAZYVA®). Cytotoxicity assays were carried out as in Example 10.

[0480] Results for the cytotoxicity assays are shown in FIG. 10. As shown therein the GA101-F5+-NKp46-1 bispecific protein demonstrated a far higher potency (approximately 10-fold increase in EC_{50}) in mediating cytotoxicity toward Daudi cells than GA101.

[0481] Moreover, when ADCC optimized Fc are used for the bispecific format (F5+) a significant difference was observed between F5+-BS lacking the Nkp46 arm (GA101-F5+-IC; black diamond) and F5+-BS co-engaging CD16+ NKp46 (GA101-F5+-NKp46-1; black square) confirming the contribution of NKp46 in GA101-F5+-NKp46-1 activity. Surprisingly, despite the high affinity of GA101-F5+-NKp46-1 for CD16 and the presumable maximum NK-cell mediated lysis, NKp46 nevertheless elicited a substantial further increase in cytotoxic activity. These results would suggest that agents capable of inducing ADCC via CD16, can be improved by further conferring on them the ability to induce NKp46-mediated lysis, and also that the potency of bispecific anti-NKp46 agents can be improved by enhancing affinity for CD16 via Fc engineering.

Example 15

Binding of Different Bispecific Formats to FcRn

[0482] The affinity of different antibody formats for human FcRn was studied by Surface Plasmon Resonance (SPR) by immobilizing recombinant FcRn proteins covalently to carboxyl groups in the dextran layer on a Sensor Chip CM5, as described in Example 2-6.

[0483] A chimeric full length anti-CD19 antibody having intact human IgG1 constant regions and NKp46×CD19 bispecific proteins having an arrangement according to the F3, F4, F5, F6, F9, F10, F11, F13 or F14 formats described in Examples 3 or 4 with anti-NKp46 variable domains from NKp46-3 (NKp46-2 for F2) were tested; for each analyte, the entire sensorgram was fitted using the steady state or 1:1 SCK binding model.

[0484] The results of these experiments are shown in Table 4 below. The bispecific proteins having dimeric Fc domains (formats F5, F6, F13, F14) bound to FcRn with affinity similar to that of the full-length IgG1 antibody. The bispecific proteins with monomeric Fc domains (F3, F4, F9, F10, F11) also displayed binding affinity to FcRn, however with lower affinity than the bispecific proteins having dimeric Fc domains.

TABLE 4

Antibody/Bispecific	SPR method	KD nM
Human IgG1/K Anti-CD19	SCK/Two state reaction	7.8
CD19-F5-NKp46-3	SCK/Two state reaction	2.6
CD19-F6- NKp46-3	SCK/Two state reaction	6.0
CD19-F13- NKp46-3	SCK/Two state reaction	15.2
CD19-F14- NKp46-3	SCK/Two state reaction	14.0
CD19-F3- NKp46-3	Steady State	474.4
CD19-F4- NKp46-3	Steady State	711.7

TABLE 4-continued

Antibody/Bispecific	SPR method	KD nM
CD19-F9A- NKp46-3	Steady State	858.5
CD19-F10A- NKp46-3	Steady State	432.8
CD19-F11- NKp46-3	Steady State	595.5

Example 16

Binding to FcγR

[0485] Different multimeric Fc proteins were evaluated to assess whether such bispecific monomeric Fc proteins could retain binding to Fcγ receptors.

[0486] SPR measurements were performed on a Biacore T100 apparatus (Biacore GE Healthcare) at 25° C. In all Biacore experiments HBS-EP+(Biacore GE Healthcare) and 10 mM NaOH, 500 mM NaCl served as running buffer and regeneration buffer respectively. Sensorgrams were analyzed with Biacore T100 Evaluation software. Recombinant human FcR's (CD64, CD32a, CD32b, CD16a and CD16b) were cloned, produced and purified.

[0487] F5 and F6 bispecific antibodies CD19-F5-NKp46-3 or CD19-F6-NKp46-3 were immobilized covalently to carboxyl groups in the dextran layer on a Sensor Chip CM5. The chip surface was activated with EDC/NHS (N-ethyl-N'-(3-dimethylaminopropyl) carbodiimidehydrochloride and N-hydroxysuccinimide (Biacore GE Healthcare)). Bispecific antibodies were diluted to 10 µg/ml in coupling buffer (10 mM acetate, pH 5.6) and injected until the appropriate immobilization level was reached (i.e. 800 to 900 RU). Deactivation of the remaining activated groups was performed using 100 mM ethanolamine pH 8 (Biacore GE Healthcare).

[0488] Monovalent affinity study was assessed following a classical kinetic wizard (as recommended by the manufacturer). Serial dilutions of soluble analytes (FcRs) ranging from 0.7 to 60 nM for CD64 and from 60 to 5000 nM for all the other FcRs were injected over the immobilized bispecific antibodies and allowed to dissociate for 10 min before regeneration. The entire sensorgram sets were fitted using the 1:1 kinetic binding model for CD64 and with the Steady State Affinity model for all the other FcRs.

[0489] The results showed that while full length wild type human IgG1 bound to all cynomolgus and human Fcγ receptors, the CD19-F6-NKp46-3 bi-specific antibodies did not bind to any of the receptors. The CD19-F5-NKp46-3, on the other hand, bound to each of the human receptors CD64 (KD=0.7 nM), CD32a (KD=846 nM), CD32b (KD=1850 nM), CD16a (KD=1098 nM) and CD16b (KD=2426 nM). Conventional human anti-IgG1 antibodies have comparable binding to these Fc receptors (KD shown in the table below).

Human Fcγ receptor	CD19-F5-NKp46-3 KD (nM)	Full length human IgG1 antibody KD (nM)
CD64	0.7	0.24
CD32a	846	379
CD32b	1850	1180
CD16a	1098	630
CD16b	2426	2410

Example 17

Epitope Mapping of Anti-NKp46 Antibodies

A. Competition Assays

[0490] Competition Assays were Conducted by Surface Plasmon Resonance (SPR According to the Methods Described Below.

[0491] SPR measurements were performed on a Biacore T100 apparatus (Biacore GE Healthcare) at 25° C. In all Biacore experiments HBS-EP+(Biacore GE Healthcare) and NaOH 10 mM NaCl 500 mM served as running buffer and regeneration buffer respectively. Sensorgrams were analyzed with Biacore T100 Evaluation software. Anti-6×His tag antibody was purchased from QIAGEN. Human 6×His tagged NKp46 recombinant proteins (NKp46-His) were cloned, produced and purified at Innate Pharma.

[0492] Anti-His antibodies were immobilized covalently to carboxyl groups in the dextran layer on a Sensor Chip CM5. The chip surface was activated with EDC/NHS (N-ethyl-N'-(3-dimethylaminopropyl) carbodiimidehydrochloride and N-hydroxysuccinimide (Biacore GE Healthcare)). Protein-A and anti-His antibodies were diluted to 10 µg/ml in coupling buffer (10 mM acetate, pH 5.6) and injected until the appropriate immobilization level was reached (i.e. 2000 to 2500 RU). Deactivation of the remaining activated groups was performed using 100 mM ethanolamine pH 8 (Biacore GE Healthcare).

Parental regular human IgG1 chimeric antibodies having NKp46 binding region corresponding to NKp46-1, NKp46-2, NKp46-3 or NKp46-4 were used for the competition study which has been performed using an Anti-6×His tag antibody chip. Bispecific antibodies having NKp46 binding region based on NKp46-1, NKp46-2, NKp46-3 or NKp46-4 at 1 µg/mL were captured onto Protein-A chip and recombinant human NKp46 proteins were injected at 5 µg/mL together with a second test bispecific antibody of the NKp46-1, NKp46-2, NKp46-3 or NKp46-4 group.

[0493] The results demonstrated that none of NKp46-1, NKp46-2, NKp46-3 or NKp46-4 competed with one another for binding to NKp46. Accordingly these antibodies each bind or interact with a different NKp46 epitope.

B. Binding to NKp46 Mutants

[0494] In order to define the epitopes of these anti-NKp46 antibodies, we designed NKp46 mutants defined by one, two or three substitutions of amino acids exposed at the molecular surface over the 2 domains of NKp46. This approach led to the generation of 42 mutants which were transfected in HEK-293T cells, as shown in the table below. The targeted amino acid mutations in Table 5 below are shown both according to the numbering of SEQ ID NO: 1 (also corresponding to the numbering used in Jaron-Mendelson et al. (2012) *J. Immunol.* 88(12):6165-74.

TABLE 5

Mutant	Substitution		
	(Numbering according to: Jaron-Mendelson and SEQ ID NO 1)		
1	P40A	K43S	Q44A
2	K41S	E42A	E119A
3	P86A	D87A	
4	N89A	R91A	

TABLE 5-continued

Mutant	Substitution		
	(Numbering according to: Jaron-Mendelson and SEQ ID NO 1)		
5	K80A	K82A	
5bis	E34A	T46A	
6	R101A	V102A	
7	N52A	Y53A	
8	V56A	P75A	E76A
9	R77A	I78A	
10	S97A	I99A	
10bis	Q59A	H61A	
11	L66A	V69A	
12	E108A		
13	N111A	L112A	
14	D114A		
15	T125A	R145S	D147A
16	S127A	Y143A	
17	H129A	K139A	
18	K170A	V172A	
19	I135A	S136A	
19bis	T182A	R185A	
20	R160A		
21	K207A		
22	M152A	R166A	
23	N195A	N196A	
Stalk1	D213A	I214A	T217A
Stalk2	F226A	T233A	
Stalk3	L236A	T240A	
Supp1	F30A	W32A	
Supp2	F62A	F67A	
Supp3	E63A	Q95A	
Supp4	R71A	K73A	
Supp5	Y84A		
Supp6	E104A	L105A	
Supp7	Y121A	Y194A	
Supp8	P132A	E133A	
Supp9	S151A	Y168A	
Supp10	S162A	H163A	
Supp11	E174A	P176A	
Supp12	P179A	H184A	
Supp13	R189A	E204A	P205A

C. Generation of Mutants

[0495] NKp46 mutants were generated by PCR. The sequences amplified were run on agarose gel and purified using the Macherey Nagel PCR Clean-Up Gel Extraction kit. Two or three purified PCR products generated for each mutant were then ligated into an expression vector, with the ClonTech InFusion system. The vectors containing the mutated sequences were prepared as Miniprep and sequenced. After sequencing, the vectors containing the mutated sequences were prepared as Midiprep using the Promega PureYield™ Plasmid Midiprep System. HEK293T cells were grown in DMEM medium (Invitrogen), transfected with vectors using Invitrogen's Lipofectamine 2000 and incubated at 37° C. in a CO₂ incubator for 24 hours prior to testing for transgene expression.

D. Flow Cytometry Analysis of Anti-NKp46 Binding to the HEK293T Transfected Cells

[0496] All the anti-NKp46 antibodies were tested for their binding to each mutant by flow cytometry. A first experiment was performed to identify antibodies that lose their binding to one or several mutants at a particular concentration (10 µg/ml). To confirm the loss of binding, titration of antibodies was done using antibodies for which binding seemed to be affected by the NKp46 mutations (1-0,1-0,01-0,001 µg/ml).

E. Results

[0497] Antibody NKp46-1 had decreased binding to the mutant 2 (having a mutation at residues K41, E42 and E119) (numbering in NKp46 wild-type) compared to wild-type NK46. Similarly, NKp46-1 also had decreased binding to the supplementary mutant Supp7 (having a mutation at residues Y121 and Y194).

[0498] Antibody NKp46-3 had decreased binding to the mutant 19 (having a mutation at residues I135, and S136). Similarly, NKp46-1 also had decreased binding to the supplementary mutant Supp8 (having a mutation at residues P132 and E133).

[0499] Antibody NKp46-4 had decreased binding to the mutant 6 (having a mutation at residues R101, and V102). Similarly, NKp46-1 also had decreased binding to the supplementary mutant Supp6 having a mutation at residues E104 and L105.

[0500] Using these methods we identified the epitopes for anti-NKp46 antibodies NKp46-1, NKp46-3 and NKp46-4. We determined that the epitopes of NKp46-4, NKp46-3 and NKp46-1 are on NKp46 D1 domain, D2 domain and D1/D2 junction, respectively. R101, V102, E104 and L105 are essential residues for NKp46-4 binding and defined a part of NKp46-4 epitope. The epitope of NKp46-1 epitope includes K41, E42, E119, Y121 and Y194 residues. The epitope of NKp46-3 includes P132, E133, I135, and S136 residues.

Example 18

Improved Product Profile and Yield of Different Bispecific Formats Compared to Existing Formats

[0501] Blinatumomab and two bispecific antibodies having NKp46 and CD19 binding regions based on F1 to F17 formats and NKp46-3, and blinatumomab, respectively were cloned and produced under format 6 (F6), DART™ and BiTe™ formats following the same protocol and using the same expression system. F6, DART™ and BiTe™ bispecific proteins were purified from cell culture supernatant by affinity chromatography using prot-A beads for F6 or Ni-NTA beads for DART™ and BiTe™. Purified proteins were further analyzed and purified by SEC. DART™ and BiTe™ showed a very low production yield compared to F6 and the purified proteins have a very complex SEC profile. DART™ and BiTe™ are barely detectable by SDS-PAGE after Coomassie staining in the expected SEC fractions (3 and 4 for BiTe™ and 4 and 5 for DART™), whereas the F6 format showed a clear and simple SEC and SDS-PAGE profiles with a major peak (fraction 3) containing the multimeric bispecific proteins. The major peak for the F6 format corresponded to about 30% of the total proteins. These results are consistent for those seen with the F1 to F17 proteins (data not shown) indicating that the Fc domain (or Fc-derived domain) present in those formats facilitates the production and improves the quality and solubility of bispecific proteins.

[0502] Moreover, the Fc domains present in proteins F1 to F17 have the advantage of being suitable for usage in affinity chromatography without the need for the incorporation of peptide tags. This is desirable as such tags are undesirable in a therapeutic product as they may potentially elicit undesired immunogenicity. By contrast, DART™ and BiTe™ antibodies cannot be purified using protein A, whereas F1 to F17

antibodies are all bound by protein A. Table 6 below shows the productivity of different formats.

TABLE 6

Format	SDS PAGE			Final « productivity »
	SEC	Reduced	Non Reduced	yield
F3	2 peaks	✓	✓	3.4 mg/L
F4	2 peaks	✓	✓	1 mg/L
F5	✓	✓	✓	37 mg/L
F6	✓	✓	✓	12 mg/L
F7	✓	✓	✓	11 mg/L
F8C	✓	✓	✓	3.7 mg/L
F9A	✓	✓	✓	8.7 mg/L
F9B	✓	✓	✓	3.0 mg/L
F10A	✓	✓	✓	2.0 mg/L
F11	✓	✓	✓	2.0 mg/L
F12	✓	✓	✓	2.8 mg/L
F13	✓	✓	✓	6.4 mg/L
F14	✓	✓	✓	2.4 mg/L
F15	✓	✓	✓	0.9 mg/L
BiTe™	—	—	—	—
DART™	—	—	—	—

All headings and sub-headings are used herein for convenience only and should not be construed as limiting the invention in any way. Any combination of the above-described elements in all possible variations thereof is encompassed by the invention unless otherwise indicated herein or otherwise clearly contradicted by context. Recitation of ranges of values herein are merely intended to serve as a shorthand method of referring individually to each separate value falling within the range, unless otherwise indicated herein, and each separate value is incorporated into the specification as if it were individually recited herein. Unless otherwise stated, all exact values provided herein are representative of corresponding approximate values (e.g., all exact exemplary values provided with respect to a particular factor or measurement can be considered to also provide a corresponding approximate measurement, modified by “about,” where appropriate). All methods described herein can be performed in any suitable order unless otherwise indicated herein or otherwise clearly contradicted by context.

[0503] The use of any and all examples, or exemplary language (e.g., “such as”) provided herein is intended merely to better illuminate the invention and does not pose a limitation on the scope of the invention unless otherwise indicated. No language in the specification should be construed as indicating any element is essential to the practice of the invention unless as much is explicitly stated.

The description herein of any aspect or embodiment of the invention using terms such as reference to an element or elements is intended to provide support for a similar aspect or embodiment of the invention that “consists of,” “consists essentially of” or “substantially comprises” that particular element or elements, unless otherwise stated or clearly contradicted by context (e.g., a composition described herein as comprising a particular element should be understood as also describing a composition consisting of that element, unless otherwise stated or clearly contradicted by context).

[0504] This invention includes all modifications and equivalents of the subject matter recited in the aspects or

claims presented herein to the maximum extent permitted by applicable law.

[0505] All publications and patent applications cited in this specification are herein incorporated by reference in their entireties as if each individual publication or patent application were specifically and individually indicated to be incorporated by reference.

[0506] Although the foregoing invention has been described in some detail by way of illustration and example for purposes of clarity of understanding, it will be readily apparent to one of ordinary skill in the art in light of the teachings of this invention that certain changes and modifications may be made thereto without departing from the spirit or scope of the appended claims.

SEQUENCE LISTING

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<210> SEQ ID NO 1

<211> LENGTH: 304

<212> TYPE: PRT

<213> ORGANISM: homo sapiens

<400> SEQUENCE: 1

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      20      25      30

Ala Glu Pro His Phe Met Val Pro Lys Glu Lys Gln Val Thr Ile Cys
      35      40      45

Cys Gln Gly Asn Tyr Gly Ala Val Glu Tyr Gln Leu His Phe Glu Gly
      50      55      60

Ser Leu Phe Ala Val Asp Arg Pro Lys Pro Pro Glu Arg Ile Asn Lys
      65      70      75      80

Val Lys Phe Tyr Ile Pro Asp Met Asn Ser Arg Met Ala Gly Gln Tyr
      85      90      95

Ser Cys Ile Tyr Arg Val Gly Glu Leu Trp Ser Glu Pro Ser Asn Leu
      100     105     110

Leu Asp Leu Val Val Thr Glu Met Tyr Asp Thr Pro Thr Leu Ser Val
      115     120     125

His Pro Gly Pro Glu Val Ile Ser Gly Glu Lys Val Thr Phe Tyr Cys
      130     135     140

Arg Leu Asp Thr Ala Thr Ser Met Phe Leu Leu Leu Lys Glu Gly Arg
      145     150     155     160

Ser Ser His Val Gln Arg Gly Tyr Gly Lys Val Gln Ala Glu Phe Pro
      165     170     175

Leu Gly Pro Val Thr Thr Ala His Arg Gly Thr Tyr Arg Cys Phe Gly
      180     185     190

Ser Tyr Asn Asn His Ala Trp Ser Phe Pro Ser Glu Pro Val Lys Leu
      195     200     205

Leu Val Thr Gly Asp Ile Glu Asn Thr Ser Leu Ala Pro Glu Asp Pro
      210     215     220

Thr Phe Pro Ala Asp Thr Trp Gly Thr Tyr Leu Leu Thr Thr Glu Thr
      225     230     235     240

Gly Leu Gln Lys Asp His Ala Leu Trp Asp His Thr Ala Gln Asn Leu
      245     250     255

Leu Arg Met Gly Leu Ala Phe Leu Val Leu Val Ala Leu Val Trp Phe
      260     265     270

Leu Val Glu Asp Trp Leu Ser Arg Lys Arg Thr Arg Glu Arg Ala Ser
      275     280     285

Arg Ala Ser Thr Trp Glu Gly Arg Arg Arg Leu Asn Thr Gln Thr Leu
      290     295     300

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<210> SEQ ID NO 2
<211> LENGTH: 216
<212> TYPE: PRT
<213> ORGANISM: homo sapiens

<400> SEQUENCE: 2
Ala Pro Glu Leu Leu Gly Gly Pro Ser Val Phe Leu Phe Pro Pro Lys
1          5          10          15
Pro Lys Asp Thr Leu Met Ile Ser Arg Thr Pro Glu Val Thr Cys Val
20          25          30
Val Val Asp Val Ser His Glu Asp Pro Glu Val Lys Phe Asn Trp Tyr
35          40          45
Val Asp Gly Val Glu Val His Asn Ala Lys Thr Lys Pro Arg Glu Glu
50          55          60
Gln Tyr Asn Ser Thr Tyr Arg Val Val Ser Val Leu Thr Val Leu His
65          70          75          80
Gln Asp Trp Leu Asn Gly Lys Glu Tyr Lys Cys Lys Val Ser Asn Lys
85          90          95
Ala Leu Pro Ala Pro Ile Glu Lys Thr Ile Ser Lys Ala Lys Gly Gln
100         105         110
Pro Arg Glu Pro Gln Val Tyr Thr Leu Pro Pro Ser Arg Glu Glu Met
115         120         125
Thr Lys Asn Gln Val Ser Leu Thr Cys Leu Val Lys Gly Phe Tyr Pro
130         135         140
Ser Asp Ile Ala Val Glu Trp Glu Ser Asn Gly Gln Pro Glu Asn Asn
145         150         155         160
Tyr Lys Thr Thr Pro Pro Val Leu Asp Ser Asp Gly Ser Phe Phe Leu
165         170         175
Thr Ser Lys Leu Thr Val Asp Lys Ser Arg Trp Gln Gln Gly Asn Val
180         185         190
Phe Ser Cys Ser Val Met His Glu Ala Leu His Asn His Tyr Thr Gln
195         200         205
Lys Ser Leu Ser Leu Ser Pro Gly
210         215

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<210> SEQ ID NO 3
<211> LENGTH: 120
<212> TYPE: PRT
<213> ORGANISM: Mus musculus

<400> SEQUENCE: 3
Gln Val Gln Leu Gln Gln Ser Gly Pro Glu Leu Val Lys Pro Gly Ala
1          5          10          15
Ser Val Lys Met Ser Cys Lys Ala Ser Gly Tyr Thr Phe Thr Asp Tyr
20          25          30
Val Ile Asn Trp Gly Lys Gln Arg Ser Gly Gln Gly Leu Glu Trp Ile
35          40          45
Gly Glu Ile Tyr Pro Gly Ser Gly Thr Asn Tyr Tyr Asn Glu Lys Phe
50          55          60
Lys Ala Lys Ala Thr Leu Thr Ala Asp Lys Ser Ser Asn Ile Ala Tyr
65          70          75          80
Met Gln Leu Ser Ser Leu Thr Ser Glu Asp Ser Ala Val Tyr Phe Cys
85          90          95

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Ala Arg Arg Gly Arg Tyr Gly Leu Tyr Ala Met Asp Tyr Trp Gly Gln
100 105 110

Gly Thr Ser Val Thr Val Ser Ser
115 120

<210> SEQ ID NO 4
<211> LENGTH: 107
<212> TYPE: PRT
<213> ORGANISM: Mus musculus

<400> SEQUENCE: 4

Asp Ile Gln Met Thr Gln Thr Thr Ser Ser Leu Ser Ala Ser Leu Gly
1 5 10 15

Asp Arg Val Thr Ile Ser Cys Arg Ala Ser Gln Asp Ile Ser Asn Tyr
20 25 30

Leu Asn Trp Tyr Gln Gln Lys Pro Asp Gly Thr Val Lys Leu Leu Ile
35 40 45

Tyr Tyr Thr Ser Arg Leu His Ser Gly Val Pro Ser Arg Phe Ser Gly
50 55 60

Ser Gly Ser Gly Thr Asp Tyr Ser Leu Thr Ile Asn Asn Leu Glu Gln
65 70 75 80

Glu Asp Ile Ala Thr Tyr Phe Cys Gln Gln Gly Asn Thr Arg Pro Trp
85 90 95

Thr Phe Gly Gly Gly Thr Lys Leu Glu Ile Lys
100 105

<210> SEQ ID NO 5
<211> LENGTH: 122
<212> TYPE: PRT
<213> ORGANISM: Mus musculus

<400> SEQUENCE: 5

Glu Val Gln Leu Gln Glu Ser Gly Pro Gly Leu Val Lys Pro Ser Gln
1 5 10 15

Ser Leu Ser Leu Thr Cys Thr Val Thr Gly Tyr Ser Ile Thr Ser Asp
20 25 30

Tyr Ala Trp Asn Trp Ile Arg Gln Phe Pro Gly Asn Lys Leu Glu Trp
35 40 45

Met Gly Tyr Ile Thr Tyr Ser Gly Ser Thr Ser Tyr Asn Pro Ser Leu
50 55 60

Glu Ser Arg Ile Ser Ile Thr Arg Asp Thr Ser Thr Asn Gln Phe Phe
65 70 75 80

Leu Gln Leu Asn Ser Val Thr Thr Glu Asp Thr Ala Thr Tyr Tyr Cys
85 90 95

Ala Arg Gly Gly Tyr Tyr Gly Ser Ser Trp Gly Val Phe Ala Tyr Trp
100 105 110

Gly Gln Gly Thr Leu Val Thr Val Ser Ala
115 120

<210> SEQ ID NO 6
<211> LENGTH: 107
<212> TYPE: PRT
<213> ORGANISM: Mus musculus

<400> SEQUENCE: 6

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Asp Ile Gln Met Thr Gln Ser Pro Ala Ser Leu Ser Ala Ser Val Gly
 1 5 10 15
 Glu Thr Val Thr Ile Thr Cys Arg Val Ser Glu Asn Ile Tyr Ser Tyr
 20 25 30
 Leu Ala Trp Tyr Gln Gln Lys Gln Gly Lys Ser Pro Gln Leu Leu Val
 35 40 45
 Tyr Asn Ala Lys Thr Leu Ala Glu Gly Val Pro Ser Arg Phe Ser Gly
 50 55 60
 Ser Gly Ser Gly Thr Gln Phe Ser Leu Lys Ile Asn Ser Leu Gln Pro
 65 70 75 80
 Glu Asp Phe Gly Ser Tyr Tyr Cys Gln His His Tyr Gly Thr Pro Trp
 85 90 95
 Thr Phe Gly Gly Gly Thr Lys Leu Glu Ile Lys
 100 105

<210> SEQ ID NO 7
 <211> LENGTH: 116
 <212> TYPE: PRT
 <213> ORGANISM: Mus musculus

<400> SEQUENCE: 7

Glu Val Gln Leu Gln Gln Ser Gly Pro Glu Leu Val Lys Pro Gly Ala
 1 5 10 15
 Ser Val Lys Ile Ser Cys Lys Thr Ser Gly Tyr Thr Phe Thr Glu Tyr
 20 25 30
 Thr Met His Trp Val Lys Gln Ser His Gly Lys Ser Leu Glu Trp Ile
 35 40 45
 Gly Gly Ile Ser Pro Asn Ile Gly Gly Thr Ser Tyr Asn Gln Lys Phe
 50 55 60
 Lys Gly Lys Ala Thr Leu Thr Val Asp Lys Ser Ser Ser Thr Ala Tyr
 65 70 75 80
 Met Glu Leu Arg Ser Leu Thr Ser Glu Asp Ser Ala Val Tyr Tyr Cys
 85 90 95
 Ala Arg Arg Gly Gly Ser Phe Asp Tyr Trp Gly Gln Gly Thr Thr Leu
 100 105 110
 Thr Val Ser Ser
 115

<210> SEQ ID NO 8
 <211> LENGTH: 107
 <212> TYPE: PRT
 <213> ORGANISM: Mus musculus

<400> SEQUENCE: 8

Asp Ile Val Met Thr Gln Ser Pro Ala Thr Leu Ser Val Thr Pro Gly
 1 5 10 15
 Asp Arg Val Ser Leu Ser Cys Arg Ala Ser Gln Ser Ile Ser Asp Tyr
 20 25 30
 Leu His Trp Tyr Gln Gln Lys Ser His Glu Ser Pro Arg Leu Leu Ile
 35 40 45
 Lys Tyr Ala Ser Gln Ser Ile Ser Gly Ile Pro Ser Arg Phe Ser Gly
 50 55 60
 Ser Gly Ser Gly Ser Asp Phe Thr Leu Ser Ile Asn Ser Val Glu Pro
 65 70 75 80

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Glu Asp Val Gly Val Tyr Tyr Cys Gln Asn Gly His Ser Phe Pro Leu
85 90 95

Thr Phe Gly Ala Gly Thr Lys Leu Glu Leu Lys
100 105

<210> SEQ ID NO 9
<211> LENGTH: 117
<212> TYPE: PRT
<213> ORGANISM: Mus musculus

<400> SEQUENCE: 9

Gln Val Gln Leu Gln Gln Ser Ala Val Glu Leu Ala Arg Pro Gly Ala
1 5 10 15
Ser Val Lys Met Ser Cys Lys Ala Ser Gly Tyr Thr Phe Thr Ser Phe
20 25 30
Thr Met His Trp Val Lys Gln Arg Pro Gly Gln Gly Leu Glu Trp Ile
35 40 45
Gly Tyr Ile Asn Pro Ser Ser Gly Tyr Thr Glu Tyr Asn Gln Lys Phe
50 55 60
Lys Asp Lys Thr Thr Leu Thr Ala Asp Lys Ser Ser Ser Thr Ala Tyr
65 70 75 80
Met Gln Leu Asp Ser Leu Thr Ser Asp Asp Ser Ala Val Tyr Tyr Cys
85 90 95
Val Arg Gly Ser Ser Arg Gly Phe Asp Tyr Trp Gly Gln Gly Thr Leu
100 105 110
Val Thr Val Ser Ala
115

<210> SEQ ID NO 10
<211> LENGTH: 107
<212> TYPE: PRT
<213> ORGANISM: Mus musculus

<400> SEQUENCE: 10

Asp Ile Gln Met Ile Gln Ser Pro Ala Ser Leu Ser Val Ser Val Gly
1 5 10 15
Glu Thr Val Thr Ile Thr Cys Arg Ala Ser Glu Asn Ile Tyr Ser Asn
20 25 30
Leu Ala Trp Phe Gln Gln Lys Gln Gly Lys Ser Pro Gln Leu Leu Val
35 40 45
Tyr Ala Ala Thr Asn Leu Ala Asp Gly Val Pro Ser Arg Phe Ser Gly
50 55 60
Ser Gly Ser Gly Thr Gln Tyr Ser Leu Lys Ile Asn Ser Leu Gln Ser
65 70 75 80
Glu Asp Phe Gly Ile Tyr Tyr Cys Gln His Phe Trp Gly Thr Pro Arg
85 90 95
Thr Phe Gly Gly Gly Thr Lys Leu Glu Ile Lys
100 105

<210> SEQ ID NO 11
<211> LENGTH: 115
<212> TYPE: PRT
<213> ORGANISM: Mus musculus

<400> SEQUENCE: 11

Gln Val Gln Leu Gln Gln Pro Gly Ser Val Leu Val Arg Pro Gly Ala

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1	5	10	15
Ser Val Lys Leu Ser Cys Lys Ala Ser Gly Tyr Thr Phe Thr Ser Ser	20	25	30
Trp Met His Trp Ala Lys Gln Arg Pro Gly Gln Gly Leu Glu Trp Ile	35	40	45
Gly His Ile His Pro Asn Ser Gly Ile Ser Asn Tyr Asn Glu Lys Phe	50	55	60
Lys Gly Lys Ala Thr Leu Thr Val Asp Thr Ser Ser Ser Thr Ala Tyr	65	70	75
Val Asp Leu Ser Ser Leu Thr Ser Glu Asp Ser Ala Val Tyr Tyr Cys	85	90	95
Ala Arg Gly Gly Arg Phe Asp Asp Trp Gly Ala Gly Thr Thr Val Thr	100	105	110
Val Ser Ser	115		

<210> SEQ ID NO 12
 <211> LENGTH: 108
 <212> TYPE: PRT
 <213> ORGANISM: Mus musculus

<400> SEQUENCE: 12

Asp Ile Val Met Thr Gln Ser Pro Ala Thr Leu Ser Val Thr Pro Gly	1	5	10	15
Asp Arg Val Ser Leu Ser Cys Arg Ala Ser Gln Ser Ile Ser Asp Tyr	20	25	30	
Leu His Trp Tyr Gln Gln Lys Ser His Glu Ser Pro Arg Leu Leu Ile	35	40	45	
Lys Tyr Ala Ser Gln Ser Ile Ser Gly Ile Pro Ser Arg Phe Ser Gly	50	55	60	
Ser Gly Ser Gly Ser Asp Phe Thr Leu Ser Ile Asn Ser Val Glu Pro	65	70	75	80
Glu Asp Val Gly Val Tyr Tyr Cys Gln Asn Gly His Ser Phe Leu Met	85	90	95	
Tyr Thr Phe Gly Gly Gly Thr Lys Leu Glu Ile Lys	100	105		

<210> SEQ ID NO 13
 <211> LENGTH: 120
 <212> TYPE: PRT
 <213> ORGANISM: Mus musculus

<400> SEQUENCE: 13

Asp Val Gln Leu Gln Glu Ser Gly Pro Gly Leu Val Lys Pro Ser Gln	1	5	10	15
Ser Leu Ser Leu Thr Cys Thr Val Thr Gly Tyr Ser Ile Thr Ser Asp	20	25	30	
Tyr Ala Trp Asn Trp Ile Arg Gln Phe Pro Gly Asn Lys Leu Glu Trp	35	40	45	
Met Gly Tyr Ile Thr Tyr Ser Gly Ser Thr Asn Tyr Asn Pro Ser Leu	50	55	60	
Lys Ser Arg Ile Ser Ile Thr Arg Asp Thr Ser Lys Asn Gln Phe Phe	65	70	75	80
Leu Gln Leu Asn Ser Val Thr Thr Glu Asp Thr Ala Thr Tyr Tyr Cys				

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	85							90						95	
Ala	Arg	Cys	Trp	Asp	Tyr	Ala	Leu	Tyr	Ala	Met	Asp	Cys	Trp	Gly	Gln
			100					105					110		
Gly	Thr	Ser	Val	Thr	Val	Ser	Ser								
			115				120								

<210> SEQ ID NO 14
<211> LENGTH: 107
<212> TYPE: PRT
<213> ORGANISM: Mus musculus

<400> SEQUENCE: 14

Asp	Ile	Gln	Met	Thr	Gln	Ser	Pro	Ala	Ser	Leu	Ser	Ala	Ser	Val	Gly
1				5					10					15	
Glu	Thr	Val	Thr	Ile	Thr	Cys	Arg	Thr	Ser	Glu	Asn	Ile	Tyr	Ser	Tyr
			20					25					30		
Leu	Ala	Trp	Cys	Gln	Gln	Lys	Gln	Gly	Lys	Ser	Pro	Gln	Leu	Leu	Val
			35				40					45			
Tyr	Asn	Ala	Lys	Thr	Leu	Ala	Glu	Gly	Val	Pro	Ser	Arg	Phe	Ser	Gly
	50					55				60					
Ser	Gly	Ser	Gly	Thr	His	Phe	Ser	Leu	Lys	Ile	Asn	Ser	Leu	Gln	Pro
65					70					75				80	
Glu	Asp	Phe	Gly	Ile	Tyr	Tyr	Cys	Gln	His	His	Tyr	Asp	Thr	Pro	Leu
				85					90					95	
Thr	Phe	Gly	Ala	Gly	Thr	Lys	Leu	Glu	Leu	Lys					
			100					105							

<210> SEQ ID NO 15
<211> LENGTH: 5
<212> TYPE: PRT
<213> ORGANISM: mus musculus

<400> SEQUENCE: 15

Asp	Tyr	Val	Ile	Asn
1				5

<210> SEQ ID NO 16
<211> LENGTH: 7
<212> TYPE: PRT
<213> ORGANISM: mus musculus

<400> SEQUENCE: 16

Gly	Tyr	Thr	Phe	Thr	Asp	Tyr
1				5		

<210> SEQ ID NO 17
<211> LENGTH: 8
<212> TYPE: PRT
<213> ORGANISM: mus musculus

<400> SEQUENCE: 17

Gly	Tyr	Thr	Phe	Thr	Asp	Tyr	Val
1				5			

<210> SEQ ID NO 18
<211> LENGTH: 17
<212> TYPE: PRT
<213> ORGANISM: mus musculus

-continued

<400> SEQUENCE: 18

Glu Ile Tyr Pro Gly Ser Gly Thr Asn Tyr Tyr Asn Glu Lys Phe Lys
1 5 10 15

Ala

<210> SEQ ID NO 19

<211> LENGTH: 4

<212> TYPE: PRT

<213> ORGANISM: mus musculus

<400> SEQUENCE: 19

Pro Gly Ser Gly

1

<210> SEQ ID NO 20

<211> LENGTH: 16

<212> TYPE: PRT

<213> ORGANISM: mus musculus

<400> SEQUENCE: 20

Gly Tyr Thr Phe Thr Asp Tyr Val Ile Tyr Pro Gly Ser Gly Thr Asn
1 5 10 15

<210> SEQ ID NO 21

<211> LENGTH: 11

<212> TYPE: PRT

<213> ORGANISM: mus musculus

<400> SEQUENCE: 21

Arg Gly Arg Tyr Gly Leu Tyr Ala Met Asp Tyr
1 5 10

<210> SEQ ID NO 22

<211> LENGTH: 9

<212> TYPE: PRT

<213> ORGANISM: mus musculus

<400> SEQUENCE: 22

Gly Arg Tyr Gly Leu Tyr Ala Met Asp
1 5

<210> SEQ ID NO 23

<211> LENGTH: 13

<212> TYPE: PRT

<213> ORGANISM: mus musculus

<400> SEQUENCE: 23

Ala Arg Arg Gly Arg Tyr Gly Leu Tyr Ala Met Asp Tyr
1 5 10

<210> SEQ ID NO 24

<211> LENGTH: 11

<212> TYPE: PRT

<213> ORGANISM: mus musculus

<400> SEQUENCE: 24

Arg Ala Ser Gln Asp Ile Ser Asn Tyr Leu Asn
1 5 10

<210> SEQ ID NO 25

<211> LENGTH: 7

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<212> TYPE: PRT
<213> ORGANISM: Mus musculus

<400> SEQUENCE: 25

Ser Gln Asp Ile Ser Asn Tyr
1 5

<210> SEQ ID NO 26
<211> LENGTH: 6
<212> TYPE: PRT
<213> ORGANISM: mus musculus

<400> SEQUENCE: 26

Gln Asp Ile Ser Asn Tyr
1 5

<210> SEQ ID NO 27
<211> LENGTH: 7
<212> TYPE: PRT
<213> ORGANISM: Mus musculus

<400> SEQUENCE: 27

Tyr Thr Ser Arg Leu His Ser
1 5

<210> SEQ ID NO 28
<211> LENGTH: 9
<212> TYPE: PRT
<213> ORGANISM: mus musculus

<400> SEQUENCE: 28

Gln Gln Gly Asn Thr Arg Pro Trp Thr
1 5

<210> SEQ ID NO 29
<211> LENGTH: 9
<212> TYPE: PRT
<213> ORGANISM: Mus musculus

<400> SEQUENCE: 29

Tyr Thr Ser Gly Asn Thr Arg Pro Trp
1 5

<210> SEQ ID NO 30
<211> LENGTH: 12
<212> TYPE: PRT
<213> ORGANISM: Mus musculus

<400> SEQUENCE: 30

Tyr Thr Ser Gln Gln Gly Asn Thr Arg Pro Trp Thr
1 5 10

<210> SEQ ID NO 31
<211> LENGTH: 6
<212> TYPE: PRT
<213> ORGANISM: Mus musculus

<400> SEQUENCE: 31

Ser Asp Tyr Ala Trp Asn
1 5

<210> SEQ ID NO 32

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<211> LENGTH: 8
<212> TYPE: PRT
<213> ORGANISM: Mus musculus

<400> SEQUENCE: 32

Gly Tyr Ser Ile Thr Ser Asp Tyr
1 5

<210> SEQ ID NO 33
<211> LENGTH: 9
<212> TYPE: PRT
<213> ORGANISM: Mus musculus

<400> SEQUENCE: 33

Gly Tyr Ser Ile Thr Ser Asp Tyr Ala
1 5

<210> SEQ ID NO 34
<211> LENGTH: 16
<212> TYPE: PRT
<213> ORGANISM: Mus musculus

<400> SEQUENCE: 34

Tyr Ile Thr Tyr Ser Gly Ser Thr Ser Tyr Asn Pro Ser Leu Glu Ser
1 5 10 15

<210> SEQ ID NO 35
<211> LENGTH: 7
<212> TYPE: PRT
<213> ORGANISM: Mus musculus

<400> SEQUENCE: 35

Ile Thr Tyr Ser Gly Ser Thr
1 5

<210> SEQ ID NO 36
<211> LENGTH: 13
<212> TYPE: PRT
<213> ORGANISM: Mus musculus

<400> SEQUENCE: 36

Gly Gly Tyr Tyr Gly Ser Ser Trp Gly Val Phe Ala Tyr
1 5 10

<210> SEQ ID NO 37
<211> LENGTH: 11
<212> TYPE: PRT
<213> ORGANISM: Mus musculus

<400> SEQUENCE: 37

Gly Tyr Tyr Gly Ser Ser Trp Gly Val Phe Ala
1 5 10

<210> SEQ ID NO 38
<211> LENGTH: 15
<212> TYPE: PRT
<213> ORGANISM: Mus musculus

<400> SEQUENCE: 38

Ala Arg Gly Gly Tyr Tyr Gly Ser Ser Trp Gly Val Phe Ala Tyr
1 5 10 15

-continued

<210> SEQ ID NO 39
<211> LENGTH: 11
<212> TYPE: PRT
<213> ORGANISM: Mus musculus

<400> SEQUENCE: 39

Arg Val Ser Glu Asn Ile Tyr Ser Tyr Leu Ala
1 5 10

<210> SEQ ID NO 40
<211> LENGTH: 7
<212> TYPE: PRT
<213> ORGANISM: Mus musculus

<400> SEQUENCE: 40

Ser Glu Asn Ile Tyr Ser Tyr
1 5

<210> SEQ ID NO 41
<211> LENGTH: 6
<212> TYPE: PRT
<213> ORGANISM: Mus musculus

<400> SEQUENCE: 41

Glu Asn Ile Tyr Ser Tyr
1 5

<210> SEQ ID NO 42
<211> LENGTH: 7
<212> TYPE: PRT
<213> ORGANISM: Mus musculus

<400> SEQUENCE: 42

Asn Ala Lys Thr Leu Ala Glu
1 5

<210> SEQ ID NO 43
<211> LENGTH: 9
<212> TYPE: PRT
<213> ORGANISM: Mus musculus

<400> SEQUENCE: 43

Gln His His Tyr Gly Thr Pro Trp Thr
1 5

<210> SEQ ID NO 44
<211> LENGTH: 6
<212> TYPE: PRT
<213> ORGANISM: Mus musculus

<400> SEQUENCE: 44

His Tyr Gly Thr Pro Trp
1 5

<210> SEQ ID NO 45
<211> LENGTH: 9
<212> TYPE: PRT
<213> ORGANISM: mus musculus

<400> SEQUENCE: 45

Gln His His Tyr Gly Thr Pro Trp Thr
1 5

-continued

<210> SEQ ID NO 46
<211> LENGTH: 5
<212> TYPE: PRT
<213> ORGANISM: Mus musculus

<400> SEQUENCE: 46

Glu Tyr Thr Met His
1 5

<210> SEQ ID NO 47
<211> LENGTH: 7
<212> TYPE: PRT
<213> ORGANISM: Mus musculus

<400> SEQUENCE: 47

Gly Tyr Thr Phe Thr Glu Tyr
1 5

<210> SEQ ID NO 48
<211> LENGTH: 8
<212> TYPE: PRT
<213> ORGANISM: Mus musculus

<400> SEQUENCE: 48

Gly Tyr Thr Phe Thr Glu Tyr Thr
1 5

<210> SEQ ID NO 49
<211> LENGTH: 17
<212> TYPE: PRT
<213> ORGANISM: Mus musculus

<400> SEQUENCE: 49

Gly Ile Ser Pro Asn Ile Gly Gly Thr Ser Tyr Asn Gln Lys Phe Lys
1 5 10 15

Gly

<210> SEQ ID NO 50
<211> LENGTH: 8
<212> TYPE: PRT
<213> ORGANISM: Mus musculus

<400> SEQUENCE: 50

Ile Ser Pro Asn Ile Gly Gly Thr
1 5

<210> SEQ ID NO 51
<211> LENGTH: 7
<212> TYPE: PRT
<213> ORGANISM: Mus musculus

<400> SEQUENCE: 51

Arg Gly Gly Ser Phe Asp Tyr
1 5

<210> SEQ ID NO 52
<211> LENGTH: 5
<212> TYPE: PRT
<213> ORGANISM: Mus musculus

<400> SEQUENCE: 52

-continued

Gly Gly Ser Phe Asp
1 5

<210> SEQ ID NO 53
<211> LENGTH: 9
<212> TYPE: PRT
<213> ORGANISM: Mus musculus

<400> SEQUENCE: 53

Ala Arg Arg Gly Gly Ser Phe Asp Tyr
1 5

<210> SEQ ID NO 54
<211> LENGTH: 11
<212> TYPE: PRT
<213> ORGANISM: Mus musculus

<400> SEQUENCE: 54

Arg Ala Ser Gln Ser Ile Ser Asp Tyr Leu His
1 5 10

<210> SEQ ID NO 55
<211> LENGTH: 7
<212> TYPE: PRT
<213> ORGANISM: Mus musculus

<400> SEQUENCE: 55

Ser Gln Ser Ile Ser Asp Tyr
1 5

<210> SEQ ID NO 56
<211> LENGTH: 6
<212> TYPE: PRT
<213> ORGANISM: Mus musculus

<400> SEQUENCE: 56

Gln Ser Ile Ser Asp Tyr
1 5

<210> SEQ ID NO 57
<211> LENGTH: 7
<212> TYPE: PRT
<213> ORGANISM: Mus musculus

<400> SEQUENCE: 57

Tyr Ala Ser Gln Ser Ile Ser
1 5

<210> SEQ ID NO 58
<211> LENGTH: 9
<212> TYPE: PRT
<213> ORGANISM: Mus musculus

<400> SEQUENCE: 58

Gln Asn Gly His Ser Phe Pro Leu Thr
1 5

<210> SEQ ID NO 59
<211> LENGTH: 6
<212> TYPE: PRT
<213> ORGANISM: Mus musculus

<400> SEQUENCE: 59

-continued

Gly His Ser Phe Pro Leu
1 5

<210> SEQ ID NO 60
<211> LENGTH: 5
<212> TYPE: PRT
<213> ORGANISM: Mus musculus

<400> SEQUENCE: 60

Ser Phe Thr Met His
1 5

<210> SEQ ID NO 61
<211> LENGTH: 7
<212> TYPE: PRT
<213> ORGANISM: Mus musculus

<400> SEQUENCE: 61

Gly Tyr Thr Phe Thr Ser Phe
1 5

<210> SEQ ID NO 62
<211> LENGTH: 8
<212> TYPE: PRT
<213> ORGANISM: Mus musculus

<400> SEQUENCE: 62

Gly Tyr Thr Phe Thr Ser Phe Thr
1 5

<210> SEQ ID NO 63
<211> LENGTH: 17
<212> TYPE: PRT
<213> ORGANISM: Mus musculus

<400> SEQUENCE: 63

Tyr Ile Asn Pro Ser Ser Gly Tyr Thr Glu Tyr Asn Gln Lys Phe Lys
1 5 10 15

Asp

<210> SEQ ID NO 64
<211> LENGTH: 8
<212> TYPE: PRT
<213> ORGANISM: Mus musculus

<400> SEQUENCE: 64

Ile Asn Pro Ser Ser Gly Tyr Thr
1 5

<210> SEQ ID NO 65
<211> LENGTH: 8
<212> TYPE: PRT
<213> ORGANISM: Mus musculus

<400> SEQUENCE: 65

Gly Ser Ser Arg Gly Phe Asp Tyr
1 5

<210> SEQ ID NO 66
<211> LENGTH: 6
<212> TYPE: PRT

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<213> ORGANISM: Mus musculus

<400> SEQUENCE: 66

Ser Ser Arg Gly Phe Asp
1 5

<210> SEQ ID NO 67

<211> LENGTH: 10

<212> TYPE: PRT

<213> ORGANISM: Mus musculus

<400> SEQUENCE: 67

Val Arg Gly Ser Ser Arg Gly Phe Asp Tyr
1 5 10

<210> SEQ ID NO 68

<211> LENGTH: 11

<212> TYPE: PRT

<213> ORGANISM: Mus musculus

<400> SEQUENCE: 68

Arg Ala Ser Glu Asn Ile Tyr Ser Asn Leu Ala
1 5 10

<210> SEQ ID NO 69

<211> LENGTH: 6

<212> TYPE: PRT

<213> ORGANISM: Mus musculur

<400> SEQUENCE: 69

Glu Asn Ile Tyr Ser Asn
1 5

<210> SEQ ID NO 70

<211> LENGTH: 7

<212> TYPE: PRT

<213> ORGANISM: mus musculur

<400> SEQUENCE: 70

Ala Ala Thr Asn Leu Ala Asp
1 5

<210> SEQ ID NO 71

<211> LENGTH: 9

<212> TYPE: PRT

<213> ORGANISM: Mus musculus

<400> SEQUENCE: 71

Gln His Phe Trp Gly Thr Pro Arg Thr
1 5

<210> SEQ ID NO 72

<211> LENGTH: 6

<212> TYPE: PRT

<213> ORGANISM: Mus musculus

<400> SEQUENCE: 72

Phe Trp Gly Thr Pro Arg
1 5

<210> SEQ ID NO 73

<211> LENGTH: 5

-continued

<212> TYPE: PRT
<213> ORGANISM: Mus musculus

<400> SEQUENCE: 73

Ser Ser Trp Met His
1 5

<210> SEQ ID NO 74
<211> LENGTH: 7
<212> TYPE: PRT
<213> ORGANISM: Mus musculus

<400> SEQUENCE: 74

Gly Tyr Thr Phe Thr Ser Ser
1 5

<210> SEQ ID NO 75
<211> LENGTH: 8
<212> TYPE: PRT
<213> ORGANISM: Mus musculus

<400> SEQUENCE: 75

Gly Tyr Thr Phe Thr Ser Ser Trp
1 5

<210> SEQ ID NO 76
<211> LENGTH: 17
<212> TYPE: PRT
<213> ORGANISM: Mus musculus

<400> SEQUENCE: 76

His Ile His Pro Asn Ser Gly Ile Ser Asn Tyr Asn Glu Lys Phe Lys
1 5 10 15

Gly

<210> SEQ ID NO 77
<211> LENGTH: 8
<212> TYPE: PRT
<213> ORGANISM: Mus musculus

<400> SEQUENCE: 77

Ile His Pro Asn Ser Gly Ile Ser
1 5

<210> SEQ ID NO 78
<211> LENGTH: 6
<212> TYPE: PRT
<213> ORGANISM: Mus musculus

<400> SEQUENCE: 78

Gly Gly Arg Phe Asp Asp
1 5

<210> SEQ ID NO 79
<211> LENGTH: 8
<212> TYPE: PRT
<213> ORGANISM: Mus musculus

<400> SEQUENCE: 79

Ala Arg Gly Gly Arg Phe Asp Asp
1 5

-continued

<210> SEQ ID NO 80
<211> LENGTH: 11
<212> TYPE: PRT
<213> ORGANISM: Mus musculus

<400> SEQUENCE: 80

Arg Ala Ser Gln Asp Ile Gly Ser Ser Leu Asn
1 5 10

<210> SEQ ID NO 81
<211> LENGTH: 7
<212> TYPE: PRT
<213> ORGANISM: Mus musculus

<400> SEQUENCE: 81

Ala Thr Ser Ser Leu Asp Ser
1 5

<210> SEQ ID NO 82
<211> LENGTH: 9
<212> TYPE: PRT
<213> ORGANISM: Mus musculus

<400> SEQUENCE: 82

Leu Gln Tyr Ala Ser Ser Pro Trp Thr
1 5

<210> SEQ ID NO 83
<211> LENGTH: 7
<212> TYPE: PRT
<213> ORGANISM: Mus musculus

<400> SEQUENCE: 83

Tyr Ala Ser Ser Pro Trp Thr
1 5

<210> SEQ ID NO 84
<211> LENGTH: 9
<212> TYPE: PRT
<213> ORGANISM: Mus musculus

<400> SEQUENCE: 84

Leu Gln Tyr Ala Ser Ser Pro Trp Thr
1 5

<210> SEQ ID NO 85
<211> LENGTH: 6
<212> TYPE: PRT
<213> ORGANISM: mus musculus

<400> SEQUENCE: 85

Ser Asp Tyr Ala Trp Asn
1 5

<210> SEQ ID NO 86
<211> LENGTH: 8
<212> TYPE: PRT
<213> ORGANISM: Mus musculus

<400> SEQUENCE: 86

Gly Tyr Ser Ile Thr Ser Asp Tyr
1 5

-continued

<210> SEQ ID NO 87
<211> LENGTH: 9
<212> TYPE: PRT
<213> ORGANISM: Mus musculus

<400> SEQUENCE: 87

Gly Tyr Ser Ile Thr Ser Asp Tyr Ala
1 5

<210> SEQ ID NO 88
<211> LENGTH: 16
<212> TYPE: PRT
<213> ORGANISM: Mus musculus

<400> SEQUENCE: 88

Tyr Ile Thr Tyr Ser Gly Ser Thr Asn Tyr Asn Pro Ser Leu Lys Ser
1 5 10 15

<210> SEQ ID NO 89
<211> LENGTH: 11
<212> TYPE: PRT
<213> ORGANISM: Mus musculus

<400> SEQUENCE: 89

Cys Trp Asp Tyr Ala Leu Tyr Ala Met Asp Cys
1 5 10

<210> SEQ ID NO 90
<211> LENGTH: 9
<212> TYPE: PRT
<213> ORGANISM: mus musculus

<400> SEQUENCE: 90

Trp Asp Tyr Ala Leu Tyr Ala Met Asp
1 5

<210> SEQ ID NO 91
<211> LENGTH: 13
<212> TYPE: PRT
<213> ORGANISM: Mus musculus

<400> SEQUENCE: 91

Ala Arg Cys Trp Asp Tyr Ala Leu Tyr Ala Met Asp Cys
1 5 10

<210> SEQ ID NO 92
<211> LENGTH: 11
<212> TYPE: PRT
<213> ORGANISM: Mus musculus

<400> SEQUENCE: 92

Arg Thr Ser Glu Asn Ile Tyr Ser Tyr Leu Ala
1 5 10

<210> SEQ ID NO 93
<211> LENGTH: 7
<212> TYPE: PRT
<213> ORGANISM: Mus musculus

<400> SEQUENCE: 93

Asn Ala Lys Thr Leu Ala Glu

-continued

1 5

<210> SEQ ID NO 94
<211> LENGTH: 9
<212> TYPE: PRT
<213> ORGANISM: Mus musculus

<400> SEQUENCE: 94

Gln His His Tyr Asp Thr Pro Leu Thr
1 5

<210> SEQ ID NO 95
<211> LENGTH: 9
<212> TYPE: PRT
<213> ORGANISM: mus musculus

<400> SEQUENCE: 95

Asn Ala Lys His Tyr Asp Thr Pro Leu
1 5

<210> SEQ ID NO 96
<211> LENGTH: 9
<212> TYPE: PRT
<213> ORGANISM: Mus musculus

<400> SEQUENCE: 96

Gln His His Tyr Asp Thr Pro Leu Thr
1 5

<210> SEQ ID NO 97
<211> LENGTH: 5
<212> TYPE: PRT
<213> ORGANISM: Mus musculus

<400> SEQUENCE: 97

Asn Tyr Gly Met Asn
1 5

<210> SEQ ID NO 98
<211> LENGTH: 7
<212> TYPE: PRT
<213> ORGANISM: mus musculus

<400> SEQUENCE: 98

Gly Tyr Thr Phe Thr Asn Tyr
1 5

<210> SEQ ID NO 99
<211> LENGTH: 8
<212> TYPE: PRT
<213> ORGANISM: mus musculus

<400> SEQUENCE: 99

Gly Tyr Thr Phe Thr Asn Tyr Gly
1 5

<210> SEQ ID NO 100
<211> LENGTH: 17
<212> TYPE: PRT
<213> ORGANISM: mus musculus

<400> SEQUENCE: 100

-continued

Trp	Ile	Asn	Thr	Asn	Thr	Gly	Glu	Pro	Thr	Tyr	Ala	Glu	Glu	Phe	Lys
1				5					10					15	

Gly

<210> SEQ ID NO 101
<211> LENGTH: 8
<212> TYPE: PRT
<213> ORGANISM: Mus musculus

<400> SEQUENCE: 101

Ile	Asn	Thr	Asn	Thr	Gly	Glu	Pro
1			5				

<210> SEQ ID NO 102
<211> LENGTH: 8
<212> TYPE: PRT
<213> ORGANISM: Mus musculus

<400> SEQUENCE: 102

Asp	Tyr	Leu	Tyr	Tyr	Phe	Asp	Tyr
1			5				

<210> SEQ ID NO 103
<211> LENGTH: 6
<212> TYPE: PRT
<213> ORGANISM: mus musculus

<400> SEQUENCE: 103

Tyr	Leu	Tyr	Tyr	Phe	Asp
1			5		

<210> SEQ ID NO 104
<211> LENGTH: 10
<212> TYPE: PRT
<213> ORGANISM: Mus musculus

<400> SEQUENCE: 104

Ala	Arg	Asp	Tyr	Leu	Tyr	Tyr	Phe	Asp	Tyr
1				5					10

<210> SEQ ID NO 105
<211> LENGTH: 11
<212> TYPE: PRT
<213> ORGANISM: Mus musculus

<400> SEQUENCE: 105

Lys	Ala	Ser	Glu	Asn	Val	Val	Thr	Tyr	Val	Ser
1				5					10	

<210> SEQ ID NO 106
<211> LENGTH: 7
<212> TYPE: PRT
<213> ORGANISM: Mus musculus

<400> SEQUENCE: 106

Ser	Glu	Asn	Val	Val	Thr	Tyr
1			5			

<210> SEQ ID NO 107
<211> LENGTH: 6
<212> TYPE: PRT
<213> ORGANISM: Mus musculus

-continued

<400> SEQUENCE: 107

Glu Asn Val Val Thr Tyr
1 5

<210> SEQ ID NO 108

<211> LENGTH: 7

<212> TYPE: PRT

<213> ORGANISM: Mus musculus

<400> SEQUENCE: 108

Gly Ala Ser Asn Arg Tyr Thr
1 5

<210> SEQ ID NO 109

<211> LENGTH: 9

<212> TYPE: PRT

<213> ORGANISM: mus musculus

<400> SEQUENCE: 109

Gly Gln Gly Tyr Ser Tyr Pro Tyr Thr
1 5

<210> SEQ ID NO 110

<211> LENGTH: 6

<212> TYPE: PRT

<213> ORGANISM: mus musculus

<400> SEQUENCE: 110

Gly Tyr Ser Tyr Pro Tyr
1 5

<210> SEQ ID NO 111

<211> LENGTH: 9

<212> TYPE: PRT

<213> ORGANISM: mus musculus

<400> SEQUENCE: 111

Gly Gln Gly Tyr Ser Tyr Pro Tyr Thr
1 5

<210> SEQ ID NO 112

<211> LENGTH: 227

<212> TYPE: PRT

<213> ORGANISM: homo sapiens

<400> SEQUENCE: 112

Gly Gln Pro Arg Glu Pro Gln Val Tyr Thr Leu Pro Pro Ser Arg Glu
1 5 10 15Glu Met Thr Lys Asn Gln Val Ser Leu Thr Cys Leu Val Lys Gly Phe
20 25 30Tyr Pro Ser Asp Ile Ala Val Glu Trp Glu Ser Asn Gly Gln Pro Glu
35 40 45Asn Asn Tyr Lys Thr Thr Pro Pro Val Leu Asp Ser Asp Gly Ser Phe
50 55 60Phe Leu Tyr Ser Lys Leu Thr Val Asp Lys Ser Arg Trp Gln Gln Gly
65 70 75 80Asn Val Phe Ser Cys Ser Val Met His Glu Ala Leu His Asn His Tyr
85 90 95

-continued

Thr Gln Lys Ser Leu Ser Leu Ser Pro Gly Gly Gly Gly Ser Gly
 100 105 110
 Gly Gly Gly Ser Gly Gly Gly Gly Ser Gly Gln Pro Arg Glu Pro Gln
 115 120 125
 Val Tyr Thr Leu Pro Pro Ser Arg Glu Glu Met Thr Lys Asn Gln Val
 130 135 140
 Ser Leu Thr Cys Leu Val Lys Gly Phe Tyr Pro Ser Asp Ile Ala Val
 145 150 155 160
 Glu Trp Glu Ser Asn Gly Gln Pro Glu Asn Asn Tyr Lys Thr Thr Pro
 165 170 175
 Pro Val Leu Asp Ser Asp Gly Ser Phe Phe Leu Tyr Ser Lys Leu Thr
 180 185 190
 Val Asp Lys Ser Arg Trp Gln Gln Gly Asn Val Phe Ser Cys Ser Val
 195 200 205
 Met His Glu Ala Leu His Asn His Tyr Thr Gln Lys Ser Leu Ser Leu
 210 215 220
 Ser Pro Gly
 225

<210> SEQ ID NO 113
 <211> LENGTH: 333
 <212> TYPE: DNA
 <213> ORGANISM: mus musculus

<400> SEQUENCE: 113

```

gacattcagc tgacccaatc tccagcttct ttggtgtgt ctctagggca gagggccacc      60
atctcctgca aggccagcca aagtgttgat tatgatggtg atagttatntt gaactggtac    120
caacagatac caggacagcc acccaaaactc ctcatctatg atgcatccaa tctagtatct    180
gggattccac ccaggtttag tggcagtggtg tctgggacag acttcaccct caacatccat    240
cctgtggaga aggtggatgc tgcaacctat cactgtcagc aaagtactga ggacccttgg    300
acgttcggtg gaggcaccaa gctggaaatc aaa                                  333
  
```

<210> SEQ ID NO 114
 <211> LENGTH: 111
 <212> TYPE: PRT
 <213> ORGANISM: mus musculus

<400> SEQUENCE: 114

Asp Ile Gln Leu Thr Gln Ser Pro Ala Ser Leu Ala Val Ser Leu Gly
 1 5 10 15
 Gln Arg Ala Thr Ile Ser Cys Lys Ala Ser Gln Ser Val Asp Tyr Asp
 20 25 30
 Gly Asp Ser Tyr Leu Asn Trp Tyr Gln Gln Ile Pro Gly Gln Pro Pro
 35 40 45
 Lys Leu Leu Ile Tyr Asp Ala Ser Asn Leu Val Ser Gly Ile Pro Pro
 50 55 60
 Arg Phe Ser Gly Ser Gly Ser Gly Thr Asp Phe Thr Leu Asn Ile His
 65 70 75 80
 Pro Val Glu Lys Val Asp Ala Ala Thr Tyr His Cys Gln Gln Ser Thr
 85 90 95
 Glu Asp Pro Trp Thr Phe Gly Gly Gly Thr Lys Leu Glu Ile Lys
 100 105 110

-continued

<210> SEQ ID NO 115
 <211> LENGTH: 372
 <212> TYPE: DNA
 <213> ORGANISM: mus musculus

<400> SEQUENCE: 115

```
cagggttcagc tgcagcagtc tggggctgag ctggtgcggc ctgggtcctc agtgaagatt    60
tctcgcaaag catctggcta cgcattcagt agctactgga tgaactgggt gaagcagagg    120
cctggacagg gtcttgagtg gattggacag atttggcctg gagatggtga tactaactac    180
aacggaaagt tcaagggcaa ggccacactg actgcagacg aatcctccag cacagcctac    240
atgcagctca gcagcctggc ctctgaggac tctgcggtct atttctgtgc aagacgagaa    300
acgaccactg tcgggcgtta ttactatgct atggactact ggggtcaagg aaccacagtc    360
accgtctcct ca                                                    372
```

<210> SEQ ID NO 116
 <211> LENGTH: 124
 <212> TYPE: PRT
 <213> ORGANISM: Mus musculus

<400> SEQUENCE: 116

```
Gln Val Gln Leu Gln Gln Ser Gly Ala Glu Leu Val Arg Pro Gly Ser
1          5          10          15
Ser Val Lys Ile Ser Cys Lys Ala Ser Gly Tyr Ala Phe Ser Ser Tyr
20        25        30
Trp Met Asn Trp Val Lys Gln Arg Pro Gly Gln Gly Leu Glu Trp Ile
35        40        45
Gly Gln Ile Trp Pro Gly Asp Gly Asp Thr Asn Tyr Asn Gly Lys Phe
50        55        60
Lys Gly Lys Ala Thr Leu Thr Ala Asp Glu Ser Ser Ser Thr Ala Tyr
65        70        75        80
Met Gln Leu Ser Ser Leu Ala Ser Glu Asp Ser Ala Val Tyr Phe Cys
85        90        95
Ala Arg Arg Glu Thr Thr Thr Val Gly Arg Tyr Tyr Tyr Ala Met Asp
100       105       110
Tyr Trp Gly Gln Gly Thr Thr Val Thr Val Ser Ser
115       120
```

<210> SEQ ID NO 117
 <211> LENGTH: 648
 <212> TYPE: DNA
 <213> ORGANISM: homo sapiens

<400> SEQUENCE: 117

```
gcacctgaac tctctggggg accgtcagtc ttcctcttcc ccccaaaacc caaggacacc    60
ctcatgatct cccggacccc tgaggtcaca tgcgtggtgg tggacgtgag ccacgaagac    120
cctgaggtca agttcaactg gtacgtggac ggcgtggagg tgcataatgc caagacaaag    180
ccgcggggagg agcagtacaa cagcacgtac cgtgtggtca gcgtcctcac cgtcctgcac    240
caggactggc tgaatggcaa ggagtacaag tgcaaggtct ccaacaaagc cctcccagcc    300
cccatcgaga aaaccatctc caaagccaaa gggcagcccc gagaaccaca ggtgtacacc    360
aagcccccac cccgggagga gatgaccaag aaccaggtca gcctgtctctg cctgggtcaaa    420
```

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```

ggcttctatc ccagcgacat cgccgtggag tgggagagca atgggcagcc ggagaacaac   480
tacaagacca cggttcccggt gctggactcc gacggctcct tccgcctcgc tagctacctc   540
accgtggaca agagcaggtg gcagcagggg aacgtcttct catgctccgt gatgcatgag   600
gctctgcaca accactacac gcagaagagc ctctccctgt ccccgggg                 648

```

```

<210> SEQ ID NO 118
<211> LENGTH: 718
<212> TYPE: PRT
<213> ORGANISM: Artificial
<220> FEATURE:
<223> OTHER INFORMATION: Chimeric homo sapiens mus musculus

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<400> SEQUENCE: 118

```

```

Asp Ile Gln Leu Thr Gln Ser Pro Ala Ser Leu Ala Val Ser Leu Gly
1           5           10          15
Gln Arg Ala Thr Ile Ser Cys Lys Ala Ser Gln Ser Val Asp Tyr Asp
20          25          30
Gly Asp Ser Tyr Leu Asn Trp Tyr Gln Gln Ile Pro Gly Gln Pro Pro
35          40          45
Lys Leu Leu Ile Tyr Asp Ala Ser Asn Leu Val Ser Gly Ile Pro Pro
50          55          60
Arg Phe Ser Gly Ser Gly Ser Gly Thr Asp Phe Thr Leu Asn Ile His
65          70          75          80
Pro Val Glu Lys Val Asp Ala Ala Thr Tyr His Cys Gln Gln Ser Thr
85          90          95
Glu Asp Pro Trp Thr Phe Gly Gly Gly Thr Lys Leu Glu Ile Lys Gly
100         105         110
Gly Gly Gly Ser Gly Gly Gly Gly Ser Gly Gly Gly Gly Ser Gln Val
115         120         125
Gln Leu Gln Gln Ser Gly Ala Glu Leu Val Arg Pro Gly Ser Ser Val
130         135         140
Lys Ile Ser Cys Lys Ala Ser Gly Tyr Ala Phe Ser Ser Tyr Trp Met
145         150         155         160
Asn Trp Val Lys Gln Arg Pro Gly Gln Gly Leu Glu Trp Ile Gly Gln
165         170         175
Ile Trp Pro Gly Asp Gly Asp Thr Asn Tyr Asn Gly Lys Phe Lys Gly
180         185         190
Lys Ala Thr Leu Thr Ala Asp Glu Ser Ser Ser Thr Ala Tyr Met Gln
195         200         205
Leu Ser Ser Leu Ala Ser Glu Asp Ser Ala Val Tyr Phe Cys Ala Arg
210         215         220
Arg Glu Thr Thr Thr Val Gly Arg Tyr Tyr Tyr Ala Met Asp Tyr Trp
225         230         235         240
Gly Gln Gly Thr Thr Val Thr Val Ser Ser Gly Gly Gly Ser Ser Ala
245         250         255
Pro Glu Leu Leu Gly Gly Pro Ser Val Phe Leu Phe Pro Pro Lys Pro
260         265         270
Lys Asp Thr Leu Met Ile Ser Arg Thr Pro Glu Val Thr Cys Val Val
275         280         285
Val Asp Val Ser His Glu Asp Pro Glu Val Lys Phe Asn Trp Tyr Val
290         295         300
Asp Gly Val Glu Val His Asn Ala Lys Thr Lys Pro Arg Glu Glu Gln

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305					310						315				320
Tyr	Asn	Ser	Thr	Tyr	Arg	Val	Val	Ser	Val	Leu	Thr	Val	Leu	His	Gln
				325					330					335	
Asp	Trp	Leu	Asn	Gly	Lys	Glu	Tyr	Lys	Cys	Lys	Val	Ser	Asn	Lys	Ala
		340						345					350		
Leu	Pro	Ala	Pro	Ile	Glu	Lys	Thr	Ile	Ser	Lys	Ala	Lys	Gly	Gln	Pro
	355						360					365			
Arg	Glu	Pro	Gln	Val	Tyr	Thr	Lys	Pro	Pro	Ser	Arg	Glu	Glu	Met	Thr
	370					375					380				
Lys	Asn	Gln	Val	Ser	Leu	Ser	Cys	Leu	Val	Lys	Gly	Phe	Tyr	Pro	Ser
385					390					395					400
Asp	Ile	Ala	Val	Glu	Trp	Glu	Ser	Asn	Gly	Gln	Pro	Glu	Asn	Asn	Tyr
		405						410						415	
Lys	Thr	Thr	Val	Pro	Val	Leu	Asp	Ser	Asp	Gly	Ser	Phe	Arg	Leu	Ala
		420						425					430		
Ser	Tyr	Leu	Thr	Val	Asp	Lys	Ser	Arg	Trp	Gln	Gln	Gly	Asn	Val	Phe
	435						440					445			
Ser	Cys	Ser	Val	Met	His	Glu	Ala	Leu	His	Asn	His	Tyr	Thr	Gln	Lys
	450					455					460				
Ser	Leu	Ser	Leu	Ser	Pro	Gly	Ser	Thr	Gly	Ser	Asp	Ile	Lys	Leu	Gln
465					470					475					480
Gln	Ser	Gly	Ala	Glu	Leu	Ala	Arg	Pro	Gly	Ala	Ser	Val	Lys	Met	Ser
			485						490					495	
Cys	Lys	Thr	Ser	Gly	Tyr	Thr	Phe	Thr	Arg	Tyr	Thr	Met	His	Trp	Val
		500						505					510		
Lys	Gln	Arg	Pro	Gly	Gln	Gly	Leu	Glu	Trp	Ile	Gly	Tyr	Ile	Asn	Pro
	515						520					525			
Ser	Arg	Gly	Tyr	Thr	Asn	Tyr	Asn	Gln	Lys	Phe	Lys	Asp	Lys	Ala	Thr
	530					535					540				
Leu	Thr	Thr	Asp	Lys	Ser	Ser	Ser	Thr	Ala	Tyr	Met	Gln	Leu	Ser	Ser
545					550					555					560
Leu	Thr	Ser	Glu	Asp	Ser	Ala	Val	Tyr	Tyr	Cys	Ala	Arg	Tyr	Tyr	Asp
			565						570					575	
Asp	His	Tyr	Cys	Leu	Asp	Tyr	Trp	Gly	Gln	Gly	Thr	Thr	Leu	Thr	Val
		580						585					590		
Ser	Ser	Val	Glu	Gly	Gly	Ser	Gly	Gly	Ser	Gly	Gly	Ser	Gly	Gly	Ser
		595				600						605			
Gly	Gly	Val	Asp	Asp	Ile	Gln	Leu	Thr	Gln	Ser	Pro	Ala	Ile	Met	Ser
	610					615					620				
Ala	Ser	Pro	Gly	Glu	Lys	Val	Thr	Met	Thr	Cys	Arg	Ala	Ser	Ser	Ser
625					630					635					640
Val	Ser	Tyr	Met	Asn	Trp	Tyr	Gln	Gln	Lys	Ser	Gly	Thr	Ser	Pro	Lys
			645						650					655	
Arg	Trp	Ile	Tyr	Asp	Thr	Ser	Lys	Val	Ala	Ser	Gly	Val	Pro	Tyr	Arg
		660						665					670		
Phe	Ser	Gly	Ser	Gly	Ser	Gly	Thr	Ser	Tyr	Ser	Leu	Thr	Ile	Ser	Ser
		675					680					685			
Met	Glu	Ala	Glu	Asp	Ala	Ala	Thr	Tyr	Tyr	Cys	Gln	Gln	Trp	Ser	Ser
	690					695					700				
Asn	Pro	Leu	Thr	Phe	Gly	Ala	Gly	Thr	Lys	Leu	Glu	Leu	Lys		
705					710					715					

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<210> SEQ ID NO 119

<211> LENGTH: 249

<212> TYPE: PRT

<213> ORGANISM: mus musculus

<400> SEQUENCE: 119

Ser Thr Gly Ser Gln Val Gln Leu Gln Gln Ser Gly Pro Glu Leu Val
1 5 10 15

Lys Pro Gly Ala Ser Val Lys Met Ser Cys Lys Ala Ser Gly Tyr Thr
20 25 30

Phe Thr Asp Tyr Val Ile Asn Trp Gly Lys Gln Arg Ser Gly Gln Gly
35 40 45

Leu Glu Trp Ile Gly Glu Ile Tyr Pro Gly Ser Gly Thr Asn Tyr Tyr
50 55 60

Asn Glu Lys Phe Lys Ala Lys Ala Thr Leu Thr Ala Asp Lys Ser Ser
65 70 75 80

Asn Ile Ala Tyr Met Gln Leu Ser Ser Leu Thr Ser Glu Asp Ser Ala
85 90 95

Val Tyr Phe Cys Ala Arg Arg Gly Arg Tyr Gly Leu Tyr Ala Met Asp
100 105 110

Tyr Trp Gly Gln Gly Thr Ser Val Thr Val Ser Ser Val Glu Gly Gly
115 120 125

Ser Gly Gly Ser Gly Gly Ser Gly Gly Val Asp Asp Ile
130 135 140

Gln Met Thr Gln Thr Thr Ser Ser Leu Ser Ala Ser Leu Gly Asp Arg
145 150 155 160

Val Thr Ile Ser Cys Arg Ala Ser Gln Asp Ile Ser Asn Tyr Leu Asn
165 170 175

Trp Tyr Gln Gln Lys Pro Asp Gly Thr Val Lys Leu Leu Ile Tyr Tyr
180 185 190

Thr Ser Arg Leu His Ser Gly Val Pro Ser Arg Phe Ser Gly Ser Gly
195 200 205

Ser Gly Thr Asp Tyr Ser Leu Thr Ile Asn Asn Leu Glu Gln Glu Asp
210 215 220

Ile Ala Thr Tyr Phe Cys Gln Gln Gly Asn Thr Arg Pro Trp Thr Phe
225 230 235 240

Gly Gly Gly Thr Lys Leu Glu Ile Lys
245

<210> SEQ ID NO 120

<211> LENGTH: 251

<212> TYPE: PRT

<213> ORGANISM: mus musculus

<400> SEQUENCE: 120

Ser Thr Gly Ser Glu Val Gln Leu Gln Glu Ser Gly Pro Gly Leu Val
1 5 10 15

Lys Pro Ser Gln Ser Leu Ser Leu Thr Cys Thr Val Thr Gly Tyr Ser
20 25 30

Ile Thr Ser Asp Tyr Ala Trp Asn Trp Ile Arg Gln Phe Pro Gly Asn
35 40 45

Lys Leu Glu Trp Met Gly Tyr Ile Thr Tyr Ser Gly Ser Thr Ser Tyr
50 55 60

-continued

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Asn Pro Ser Leu Glu Ser Arg Ile Ser Ile Thr Arg Asp Thr Ser Thr
65              70              75              80

Asn Gln Phe Phe Leu Gln Leu Asn Ser Val Thr Thr Glu Asp Thr Ala
            85              90              95

Thr Tyr Tyr Cys Ala Arg Gly Gly Tyr Tyr Gly Ser Ser Trp Gly Val
            100              105              110

Phe Ala Tyr Trp Gly Gln Gly Thr Leu Val Thr Val Ser Ala Val Glu
            115              120              125

Gly Gly Ser Gly Gly Ser Gly Gly Ser Gly Gly Ser Gly Gly Val Asp
            130              135              140

Asp Ile Gln Met Thr Gln Ser Pro Ala Ser Leu Ser Ala Ser Val Gly
145              150              155              160

Glu Thr Val Thr Ile Thr Cys Arg Val Ser Glu Asn Ile Tyr Ser Tyr
            165              170              175

Leu Ala Trp Tyr Gln Gln Lys Gln Gly Lys Ser Pro Gln Leu Leu Val
            180              185              190

Tyr Asn Ala Lys Thr Leu Ala Glu Gly Val Pro Ser Arg Phe Ser Gly
            195              200              205

Ser Gly Ser Gly Thr Gln Phe Ser Leu Lys Ile Asn Ser Leu Gln Pro
            210              215              220

Glu Asp Phe Gly Ser Tyr Tyr Cys Gln His His Tyr Gly Thr Pro Trp
225              230              235              240

Thr Phe Gly Gly Gly Thr Lys Leu Glu Ile Lys
            245              250

```

<210> SEQ ID NO 121

<211> LENGTH: 245

<212> TYPE: PRT

<213> ORGANISM: mus musculus

<400> SEQUENCE: 121

```

Ser Thr Gly Ser Glu Val Gln Leu Gln Gln Ser Gly Pro Glu Leu Val
1              5              10              15

Lys Pro Gly Ala Ser Val Lys Ile Ser Cys Lys Thr Ser Gly Tyr Thr
            20              25              30

Phe Thr Glu Tyr Thr Met His Trp Val Lys Gln Ser His Gly Lys Ser
            35              40              45

Leu Glu Trp Ile Gly Gly Ile Ser Pro Asn Ile Gly Gly Thr Ser Tyr
            50              55              60

Asn Gln Lys Phe Lys Gly Lys Ala Thr Leu Thr Val Asp Lys Ser Ser
65              70              75              80

Ser Thr Ala Tyr Met Glu Leu Arg Ser Leu Thr Ser Glu Asp Ser Ala
            85              90              95

Val Tyr Tyr Cys Ala Arg Arg Gly Gly Ser Phe Asp Tyr Trp Gly Gln
            100              105              110

Gly Thr Thr Leu Thr Val Ser Ser Val Glu Gly Gly Ser Gly Gly Ser
            115              120              125

Gly Gly Ser Gly Gly Ser Gly Gly Val Asp Asp Ile Val Met Thr Gln
            130              135              140

Ser Pro Ala Thr Leu Ser Val Thr Pro Gly Asp Arg Val Ser Leu Ser
145              150              155              160

Cys Arg Ala Ser Gln Ser Ile Ser Asp Tyr Leu His Trp Tyr Gln Gln

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165					170					175					
Lys	Ser	His	Glu	Ser	Pro	Arg	Leu	Leu	Ile	Lys	Tyr	Ala	Ser	Gln	Ser
			180					185					190		
Ile	Ser	Gly	Ile	Pro	Ser	Arg	Phe	Ser	Gly	Ser	Gly	Ser	Gly	Ser	Asp
		195					200					205			
Phe	Thr	Leu	Ser	Ile	Asn	Ser	Val	Glu	Pro	Glu	Asp	Val	Gly	Val	Tyr
		210					215					220			
Tyr	Cys	Gln	Asn	Gly	His	Ser	Phe	Pro	Leu	Thr	Phe	Gly	Ala	Gly	Thr
		225					230					235			240
Lys	Leu	Glu	Leu	Lys											
			245												

<210> SEQ ID NO 122

<211> LENGTH: 246

<212> TYPE: PRT

<213> ORGANISM: Mus musculus

<400> SEQUENCE: 122

Ser	Thr	Gly	Ser	Gln	Val	Gln	Leu	Gln	Gln	Ser	Ala	Val	Glu	Leu	Ala
1				5					10					15	
Arg	Pro	Gly	Ala	Ser	Val	Lys	Met	Ser	Cys	Lys	Ala	Ser	Gly	Tyr	Thr
		20						25					30		
Phe	Thr	Ser	Phe	Thr	Met	His	Trp	Val	Lys	Gln	Arg	Pro	Gly	Gln	Gly
		35					40					45			
Leu	Glu	Trp	Ile	Gly	Tyr	Ile	Asn	Pro	Ser	Ser	Gly	Tyr	Thr	Glu	Tyr
		50				55						60			
Asn	Gln	Lys	Phe	Lys	Asp	Lys	Thr	Thr	Leu	Thr	Ala	Asp	Lys	Ser	Ser
		65			70							75			80
Ser	Thr	Ala	Tyr	Met	Gln	Leu	Asp	Ser	Leu	Thr	Ser	Asp	Asp	Ser	Ala
			85						90					95	
Val	Tyr	Tyr	Cys	Val	Arg	Gly	Ser	Ser	Arg	Gly	Phe	Asp	Tyr	Trp	Gly
			100					105					110		
Gln	Gly	Thr	Leu	Val	Thr	Val	Ser	Ala	Val	Glu	Gly	Gly	Ser	Gly	Gly
		115					120						125		
Ser	Gly	Gly	Ser	Gly	Gly	Ser	Gly	Gly	Val	Asp	Asp	Ile	Gln	Met	Ile
		130				135						140			
Gln	Ser	Pro	Ala	Ser	Leu	Ser	Val	Ser	Val	Gly	Glu	Thr	Val	Thr	Ile
		145			150							155			160
Thr	Cys	Arg	Ala	Ser	Glu	Asn	Ile	Tyr	Ser	Asn	Leu	Ala	Trp	Phe	Gln
			165						170					175	
Gln	Lys	Gln	Gly	Lys	Ser	Pro	Gln	Leu	Leu	Val	Tyr	Ala	Ala	Thr	Asn
		180						185					190		
Leu	Ala	Asp	Gly	Val	Pro	Ser	Arg	Phe	Ser	Gly	Ser	Gly	Ser	Gly	Thr
		195					200					205			
Gln	Tyr	Ser	Leu	Lys	Ile	Asn	Ser	Leu	Gln	Ser	Glu	Asp	Phe	Gly	Ile
		210				215						220			
Tyr	Tyr	Cys	Gln	His	Phe	Trp	Gly	Thr	Pro	Arg	Thr	Phe	Gly	Gly	Gly
		225			230						235				240
Thr	Lys	Leu	Glu	Ile	Lys										
			245												

<210> SEQ ID NO 123

<211> LENGTH: 245

-continued

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<212> TYPE: PRT
<213> ORGANISM: Mus musculus

<400> SEQUENCE: 123

Ser Thr Gly Ser Gln Val Gln Leu Gln Gln Pro Gly Ser Val Leu Val
1      5      10      15
Arg Pro Gly Ala Ser Val Lys Leu Ser Cys Lys Ala Ser Gly Tyr Thr
      20      25      30
Phe Thr Ser Ser Trp Met His Trp Ala Lys Gln Arg Pro Gly Gln Gly
      35      40      45
Leu Glu Trp Ile Gly His Ile His Pro Asn Ser Gly Ile Ser Asn Tyr
      50      55      60
Asn Glu Lys Phe Lys Gly Lys Ala Thr Leu Thr Val Asp Thr Ser Ser
65      70      75      80
Ser Thr Ala Tyr Val Asp Leu Ser Ser Leu Thr Ser Glu Asp Ser Ala
      85      90      95
Val Tyr Tyr Cys Ala Arg Gly Gly Arg Phe Asp Asp Trp Gly Ala Gly
      100     105     110
Thr Thr Val Thr Val Ser Ser Val Glu Gly Gly Ser Gly Gly Ser Gly
      115     120     125
Gly Ser Gly Gly Ser Gly Gly Val Asp Asp Ile Val Met Thr Gln Ser
      130     135     140
Pro Ala Thr Leu Ser Val Thr Pro Gly Asp Arg Val Ser Leu Ser Cys
145     150     155     160
Arg Ala Ser Gln Ser Ile Ser Asp Tyr Leu His Trp Tyr Gln Gln Lys
      165     170     175
Ser His Glu Ser Pro Arg Leu Leu Ile Lys Tyr Ala Ser Gln Ser Ile
      180     185     190
Ser Gly Ile Pro Ser Arg Phe Ser Gly Ser Gly Ser Gly Ser Asp Phe
      195     200     205
Thr Leu Ser Ile Asn Ser Val Glu Pro Glu Asp Val Gly Val Tyr Tyr
      210     215     220
Cys Gln Asn Gly His Ser Phe Leu Met Tyr Thr Phe Gly Gly Gly Thr
225     230     235     240
Lys Leu Glu Ile Lys
      245

```

```

<210> SEQ ID NO 124
<211> LENGTH: 249
<212> TYPE: PRT
<213> ORGANISM: Mus musculus

<400> SEQUENCE: 124

```

```

Ser Thr Gly Ser Asp Val Gln Leu Gln Glu Ser Gly Pro Gly Leu Val
1      5      10      15
Lys Pro Ser Gln Ser Leu Ser Leu Thr Cys Thr Val Thr Gly Tyr Ser
      20      25      30
Ile Thr Ser Asp Tyr Ala Trp Asn Trp Ile Arg Gln Phe Pro Gly Asn
      35      40      45
Lys Leu Glu Trp Met Gly Tyr Ile Thr Tyr Ser Gly Ser Thr Asn Tyr
      50      55      60
Asn Pro Ser Leu Lys Ser Arg Ile Ser Ile Thr Arg Asp Thr Ser Lys
65      70      75      80

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Asn Gln Phe Phe Leu Gln Leu Asn Ser Val Thr Thr Glu Asp Thr Ala
      85                               90          95
Thr Tyr Tyr Cys Ala Arg Cys Trp Asp Tyr Ala Leu Tyr Ala Met Asp
      100                               105          110
Cys Trp Gly Gln Gly Thr Ser Val Thr Val Ser Ser Val Glu Gly Gly
      115                               120          125
Ser Gly Gly Ser Gly Gly Ser Gly Gly Ser Gly Gly Val Asp Asp Ile
      130                               135          140
Gln Met Thr Gln Ser Pro Ala Ser Leu Ser Ala Ser Val Gly Glu Thr
      145                               150          155          160
Val Thr Ile Thr Cys Arg Thr Ser Glu Asn Ile Tyr Ser Tyr Leu Ala
      165                               170          175
Trp Cys Gln Gln Lys Gln Gly Lys Ser Pro Gln Leu Leu Val Tyr Asn
      180                               185          190
Ala Lys Thr Leu Ala Glu Gly Val Pro Ser Arg Phe Ser Gly Ser Gly
      195                               200          205
Ser Gly Thr His Phe Ser Leu Lys Ile Asn Ser Leu Gln Pro Glu Asp
      210                               215          220
Phe Gly Ile Tyr Tyr Cys Gln His His Tyr Asp Thr Pro Leu Thr Phe
      225                               230          235          240
Gly Ala Gly Thr Lys Leu Glu Leu Lys
      245

```

<210> SEQ ID NO 125

<211> LENGTH: 246

<212> TYPE: PRT

<213> ORGANISM: Mus musculus

<400> SEQUENCE: 125

```

Ser Thr Gly Ser Gln Ile Gln Leu Val Gln Ser Gly Pro Glu Leu Gln
1      5      10      15
Lys Pro Gly Glu Thr Val Lys Ile Ser Cys Lys Ala Ser Gly Tyr Thr
20     25     30
Phe Thr Asn Tyr Gly Met Asn Trp Val Lys Gln Ala Pro Gly Lys Gly
35     40     45
Leu Lys Trp Met Gly Trp Ile Asn Thr Asn Thr Gly Glu Pro Thr Tyr
50     55     60
Ala Glu Glu Phe Lys Gly Arg Phe Ala Phe Ser Leu Glu Thr Ser Ala
65     70     75     80
Ser Thr Ala Tyr Leu Gln Ile Asn Asn Leu Lys Asn Glu Asp Thr Ala
85     90     95
Thr Tyr Phe Cys Ala Arg Asp Tyr Leu Tyr Tyr Phe Asp Tyr Trp Gly
100    105    110
Gln Gly Thr Thr Leu Thr Val Ser Ser Val Glu Gly Gly Ser Gly Gly
115    120    125
Ser Gly Gly Ser Gly Gly Ser Gly Gly Val Asp Asn Ile Val Met Thr
130    135    140
Gln Ser Pro Lys Ser Met Ser Met Ser Val Gly Glu Arg Val Thr Leu
145    150    155    160
Thr Cys Lys Ala Ser Glu Asn Val Val Thr Tyr Val Ser Trp Tyr Gln
165    170    175
Gln Lys Pro Glu Gln Ser Pro Lys Leu Leu Ile Tyr Gly Ala Ser Asn
180    185    190

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Arg Tyr Thr Gly Val Pro Asp Arg Phe Thr Gly Ser Gly Ser Ala Thr
 195 200 205

Asp Phe Thr Leu Thr Ile Ser Ser Val Gln Ala Glu Asp Leu Ala Asp
 210 215 220

Tyr His Cys Gly Gln Gly Tyr Ser Tyr Pro Tyr Thr Phe Gly Gly Gly
 225 230 235 240

Thr Lys Leu Glu Ile Lys
 245

<210> SEQ ID NO 126
 <211> LENGTH: 751
 <212> TYPE: DNA
 <213> ORGANISM: mus musculus

<400> SEQUENCE: 126

```

gtcgactgga agccaggtac agctgcagca gtctggccct gaactcgtca aaccaggagc   60
ttcctgtaag atgtcctgca aggcttcagg gtacacgttt accgactatg tgatcaattg   120
gggtaagcag cgctctgggc aaggcttgga gtggattggc gagatctatc ctgggagtgg   180
gaccaactat tacaacgaga agttcaaggc caaagccact ttgactgcag acaagagctc   240
aaacattgcc tacatgcaac tgagctccct gacatcagag gattctgctg tgtacttctg   300
tgcacgtaga ggtcggtacg gtctgtatgc catggactat tggggccaag gcacttccgt   360
gacagtcagc tctgtggaag gaggaagtgg cggttcagga ggtagcggag ggtccggagg   420
agtggatgac attcagatga cacagaccac ttctagcctc tccgcatccc ttggggatag   480
ggtcaccatc agttgtaggg ctagccagga catttccaat tacctgaact ggtatcagca   540
gaaacccgat ggcacagtta agcttctgat ctactacaca agcagactgc actcaggggt   600
tccatctcgg tttagtggaa gtggtctctg taccgactat tccctgacca tcaacaatct   660
ggaacaggaa gatatcgcca cctacttctg ccaacagggc aatactcgac cctggacatt   720
tggtggcggc acgaaactcg agataaaata a                               751

```

<210> SEQ ID NO 127
 <211> LENGTH: 757
 <212> TYPE: DNA
 <213> ORGANISM: Mus musculus

<400> SEQUENCE: 127

```

gtcgactgga tccgaggtac agttgcagga gagtgggcct ggactggtca aaccctccca   60
atctctgagc ttgacatgca cagtcacagg ctacagcatc acctccgact acgcttgga   120
ttggattcga cagtttcccg gcaacaagct ggaatggatg ggctacatca cctatagtgg   180
tagcacttcc tataatccct cacttgagag cgggatttcc atcactaggg atacgagcac   240
caaccagttc ttctgcagc tgaatagcgt caccaccgaa gatactgcca cctattactg   300
cgcaagaggc gggtactatg gcagttcatg ggggttatc gcctattggg gacaggggac   360
acttgtgaca gtgtctgctg ttgaagggtg atccggcgga tcaggaggga gtggtggcag   420
tggaggtgtt gacgacattc agatgaccca atcccctgct tctctctcag cctctgtggg   480
agagactgtg accataacct gtcgtgtag cgagaacatc tactcctate tcgcttggtg   540
tcagcagaaa caggggaaat ccccaact gctcgtgtac aatgccaaga ctctggcaga   600
aggagtgcca agccgctttt cgggtctctg gtctgggaca cagttctcac tgaagatcaa   660

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ctctttgcaa cctgaggatt ttggctctta ctactgtcag catcactatg gcacaccatg 720

gacgttttgg ggccgcacta agctggagat taagtaa 757

<210> SEQ ID NO 128

<211> LENGTH: 739

<212> TYPE: DNA

<213> ORGANISM: Mus musculus

<400> SEQUENCE: 128

gtcgactggg tccgaagtgc aactgcaaca gtctggccct gagctggtea aaccgggtgc 60

ttcagtgaag atctcctgca agacatccgg ctacaccttc actgagtaca ccatgcactg 120

ggtaaacacag tctcacggta agagcctgga gtggataggc ggaatttcac ccaacattgg 180

agggacctcc tataaccaga agttcaaggg caaagccacc cttacagttg acaagagcag 240

ttcaactgcc tacatggaac tgcgctcatt gacctccgag gattcagccg tgtattactg 300

cgctagaagg ggaggatcct tcgattattg gggccaaggc actacgctta ccgtgagcag 360

cgttgaagggt gggtctggcg gctctgggtg aagtggaggg agtggcgggg tagacgacat 420

cgtagtgact cagagtcacg caactctgtc cgttacacct ggagatcgag tgtctctgag 480

ttgtcgtgca agccagtcta tctctgacta tctgcaactg tatcagcaga agagccatga 540

gtcacctagg ctggtgatca agtacgcctc tcagtcattt agcgggatcc catcccggtt 600

tagtggctct ggctccggta gtgacttcac actcagcatc aatagcgctg aaccagagga 660

tgtaggggtg tactactgtc agaatgggca ttcctttccc ctcacatttg gagctggtag 720

caaaactcgag ctgaaataa 739

<210> SEQ ID NO 129

<211> LENGTH: 742

<212> TYPE: DNA

<213> ORGANISM: Mus musculus

<400> SEQUENCE: 129

gtcgactggc tcccaagtac agcttcagca gtctgccgtc gaacttgctc gaccaggagc 60

ttcagtgaag atgagctgca aagcctctgg ttacaccttc acgtccttta ccatgcattg 120

ggtagagcag cgtcctggcc aaggcctgga gtggattggc tacatcaatc cctccagcgg 180

gtataccgag tacaaccaga agttcaagga caaaacaacc ctgactgccg ataagtcaag 240

tagcacagcc tatatgcagc tggattccct gacatcagac gatagcgctg tgtattactg 300

cgtagggggc tctagcagag ggttcgacta ttgggggtcaa ggcacactgg tcacgggttag 360

tgccgttgaa ggaggctctg gaggcagtgg aggttctgga gggtcaggcg gtgtggatga 420

cattcagatg attcagagtc ccgctagctt gagtgtgaag gtcggtgaga cagtgacct 480

cacttgctgc gcatccgaaa acatctactc caatctcgca tggttccagc agaaacaggg 540

caaatcacc ccaattgctg tgtatgccgc aactaatctg gctgatgggtg tgccttcag 600

gttagcggg tctgatctg ggactcagta ctccctgaag atcaactccc tccagtctga 660

ggacttcggg atctattact gtcagcactt ttgggggaact ccacggacct ttggaggcgg 720

gacaaaactg gagataaagt aa 742

<210> SEQ ID NO 130

<211> LENGTH: 696

-continued

<212> TYPE: DNA

<213> ORGANISM: Mus musculus

<400> SEQUENCE: 130

```

ctggtgaggc caggtgcac tgtgaagctg tcatgcaaag catccgggta caggttcacc    60
tcttcatgga tgcattgggc caaacagcgt ccaggccagg gccttgagtg gattggacac    120
attcacccca atagcggcat atccaactac aacgagaagt tcaagggcaa agccacactg    180
acagtggata cttccagctc tacagcctat gtggacctta gtagcttgac cagtgaggat    240
tctgccgtat actactgtgc tagagggtggg cggtttgacg attgggtgac tgggaccaca    300
gtcaccgtga gcagtgtcga aggtggatca ggtggatctg gaggtcagg cggttctggc    360
ggtgttgacg acatcgtgat gactcaaagc cctgtactc tctctgtcac acccgagat    420
agggtaaagc tcagtgtcgc agcaagccag tcaatcagcg actatctgca ctggtatcag    480
cagaagtccc atgaatcccc acgcttgctc atcaagtagc ccagtcagtc catcagtggc    540
attccttccc ggttttctgg gtctggatcc gggtcagact tcactctgag cattaactcc    600
gtcgaacccc aggatgttgg cgtgtattat tgccagaatg gacattcctt cctgatgtac    660
acctttggcg gagggaccaa actggagatc aagtaa                                696

```

<210> SEQ ID NO 131

<211> LENGTH: 751

<212> TYPE: DNA

<213> ORGANISM: Mus musculus

<400> SEQUENCE: 131

```

gtcgactggg tctgatgtgc agttgcagga gtcaggacct gggcttggtc agccaagcca    60
gagcctcagt ctcacttgca ctgtcacagg ctatagcatc acatccgact atgcttgga    120
ttggattagg cagtttctcg gcaataagct ggaatggatg gggtagatca cctattccgg    180
cagtaccaac tacaatcccc gcttgaaatc tcggatttcc ataacacgcg atactagcaa    240
gaaccagttc ttccttcage tgaactctgt gacaacagag gataccgcta cgtactattg    300
cgccagatgc tgggattacg ccctgtatgc catggactgt tggggcaag gtaccagcgt    360
tactgtgtct agcgtcgaag gcggaagtgg cggctcagga gggtcaggag gctcaggcgg    420
agtggatgac attcagatga cccaatctcc cgcattccctg tccgcacag taggggagac    480
agtgaaccatt acctgtcgta cttccgagaa catctactcc tatcttgctt ggtgtcaaca    540
gaaacagggg aaaagtccac agctgctggg gtataacgcc aagaccttgg cagaagggtg    600
tcccagtcga ttctctgggt ccggatccgg tacacacttc agcctgaaga tcaattctct    660
gcaaccagag gactttggaa tctactactg ccagcatcac tacgacactc cctgacgtt    720
tggcgctggg accaagctcg aactgaaata a                                751

```

<210> SEQ ID NO 132

<211> LENGTH: 742

<212> TYPE: DNA

<213> ORGANISM: Mus musculus

<400> SEQUENCE: 132

```

gtcgactggt agccagatag agctggtaca gtcaggacca gagctgcaga aacctggaga    60
gacagtgaag atcagctgca aggctagcgg gtacaccttc acgaattacg ggatgaactg    120
ggtcaagcag gctccaggca aagggtgtaa gtggatgggc tggattaaca ccaatactgg    180

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ggaaccaacc tatgccgagg aattcaaggg gagatttgcc tttccctcg aaaccagcgc 240
ctcaaccgcc tatctccaga tcaacaacct gaagaatgag gataccgcta cctacttctg 300
tgcaaggggac tacctctact acttcgacta ttggggccaa ggtacgactc ttacagtctc 360
tagtggtgag ggaggggagt gaggttctgg aggctctggt ggctctggag gcgttgacaa 420
catcgtgatg actcagtctc ccaaaagcat gagtatgagt gtgggtgaac gagttacctt 480
gacatgcaaa gcctccgaga atgtcgtgac atacgtgtcc tggatatcagc agaaaccgca 540
gcaatccctc aagctcgtga tctatggcgc tagcaatcgc tatactggcg tacctgatcg 600
gttcacagga tcaggctcag ccaactgactt tactcttacc atttcctccg tgcaggcaga 660
agatttggca gattaccact gtgggcaagg ttactcttat ccctatacat ttggaggcgg 720
cacaagctg gagattaagt aa 742

```

```

<210> SEQ ID NO 133
<211> LENGTH: 720
<212> TYPE: PRT
<213> ORGANISM: Artificial
<220> FEATURE:
<223> OTHER INFORMATION: Chimeric homo sapiens mus musculus

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<400> SEQUENCE: 133

```

```

Asp Ile Gln Leu Thr Gln Ser Pro Ala Ser Leu Ala Val Ser Leu Gly
1           5           10          15
Gln Arg Ala Thr Ile Ser Cys Lys Ala Ser Gln Ser Val Asp Tyr Asp
20          25          30
Gly Asp Ser Tyr Leu Asn Trp Tyr Gln Gln Ile Pro Gly Gln Pro Pro
35          40          45
Lys Leu Leu Ile Tyr Asp Ala Ser Asn Leu Val Ser Gly Ile Pro Pro
50          55          60
Arg Phe Ser Gly Ser Gly Ser Gly Thr Asp Phe Thr Leu Asn Ile His
65          70          75          80
Pro Val Glu Lys Val Asp Ala Ala Thr Tyr His Cys Gln Gln Ser Thr
85          90          95
Glu Asp Pro Trp Thr Phe Gly Gly Gly Thr Lys Leu Glu Ile Lys Gly
100         105         110
Gly Gly Gly Ser Gly Gly Gly Gly Ser Gly Gly Gly Gly Ser Gln Val
115         120         125
Gln Leu Gln Gln Ser Gly Ala Glu Leu Val Arg Pro Gly Ser Ser Val
130         135         140
Lys Ile Ser Cys Lys Ala Ser Gly Tyr Ala Phe Ser Ser Tyr Trp Met
145         150         155         160
Asn Trp Val Lys Gln Arg Pro Gly Gln Gly Leu Glu Trp Ile Gly Gln
165         170         175
Ile Trp Pro Gly Asp Gly Asp Thr Asn Tyr Asn Gly Lys Phe Lys Gly
180         185         190
Lys Ala Thr Leu Thr Ala Asp Glu Ser Ser Ser Thr Ala Tyr Met Gln
195         200         205
Leu Ser Ser Leu Ala Ser Glu Asp Ser Ala Val Tyr Phe Cys Ala Arg
210         215         220
Arg Glu Thr Thr Thr Val Gly Arg Tyr Tyr Tyr Ala Met Asp Tyr Trp
225         230         235         240

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Gly	Gln	Gly	Thr	Thr	Val	Thr	Val	Ser	Ser	Gly	Gly	Gly	Ser	Ser	Ala	
				245					250					255		
Pro	Glu	Leu	Leu	Gly	Gly	Pro	Ser	Val	Phe	Leu	Phe	Pro	Pro	Lys	Pro	
			260					265					270			
Lys	Asp	Thr	Leu	Met	Ile	Ser	Arg	Thr	Pro	Glu	Val	Thr	Cys	Val	Val	
	275						280					285				
Val	Asp	Val	Ser	His	Glu	Asp	Pro	Glu	Val	Lys	Phe	Asn	Trp	Tyr	Val	
	290					295					300					
Asp	Gly	Val	Glu	Val	His	Asn	Ala	Lys	Thr	Lys	Pro	Arg	Glu	Glu	Gln	
305					310					315					320	
Tyr	Asn	Ser	Thr	Tyr	Arg	Val	Val	Ser	Val	Leu	Thr	Val	Leu	His	Gln	
			325						330					335		
Asp	Trp	Leu	Asn	Gly	Lys	Glu	Tyr	Lys	Cys	Lys	Val	Ser	Asn	Lys	Ala	
		340						345					350			
Leu	Pro	Ala	Pro	Ile	Glu	Lys	Thr	Ile	Ser	Lys	Ala	Lys	Gly	Gln	Pro	
		355					360					365				
Arg	Glu	Pro	Gln	Val	Tyr	Thr	Lys	Pro	Pro	Ser	Arg	Glu	Glu	Met	Thr	
	370					375					380					
Lys	Asn	Gln	Val	Ser	Leu	Ser	Cys	Leu	Val	Lys	Gly	Phe	Tyr	Pro	Ser	
385					390					395				400		
Asp	Ile	Ala	Val	Glu	Trp	Glu	Ser	Asn	Gly	Gln	Pro	Glu	Asn	Asn	Tyr	
			405						410					415		
Lys	Thr	Thr	Val	Pro	Val	Leu	Asp	Ser	Asp	Gly	Ser	Phe	Arg	Leu	Ala	
			420					425					430			
Ser	Tyr	Leu	Thr	Val	Asp	Lys	Ser	Arg	Trp	Gln	Gln	Gly	Asn	Val	Phe	
		435					440					445				
Ser	Cys	Ser	Val	Met	His	Glu	Ala	Leu	His	Asn	His	Tyr	Thr	Gln	Lys	
	450					455					460					
Ser	Leu	Ser	Leu	Ser	Pro	Gly	Ser	Thr	Gly	Ser	Gln	Val	Gln	Leu	Gln	
465					470					475					480	
Gln	Ser	Gly	Pro	Glu	Leu	Val	Lys	Pro	Gly	Ala	Ser	Val	Lys	Met	Ser	
			485						490					495		
Cys	Lys	Ala	Ser	Gly	Tyr	Thr	Phe	Thr	Asp	Tyr	Val	Ile	Asn	Trp	Gly	
		500						505					510			
Lys	Gln	Arg	Ser	Gly	Gln	Gly	Leu	Glu	Trp	Ile	Gly	Glu	Ile	Tyr	Pro	
	515						520					525				
Gly	Ser	Gly	Thr	Asn	Tyr	Tyr	Asn	Glu	Lys	Phe	Lys	Ala	Lys	Ala	Thr	
	530					535					540					
Leu	Thr	Ala	Asp	Lys	Ser	Ser	Asn	Ile	Ala	Tyr	Met	Gln	Leu	Ser	Ser	
545					550					555					560	
Leu	Thr	Ser	Glu	Asp	Ser	Ala	Val	Tyr	Phe	Cys	Ala	Arg	Arg	Gly	Arg	
			565						570					575		
Tyr	Gly	Leu	Tyr	Ala	Met	Asp	Tyr	Trp	Gly	Gln	Gly	Thr	Ser	Val	Thr	
		580					585						590			
Val	Ser	Ser	Val	Glu	Gly	Gly	Ser	Gly	Gly	Ser	Gly	Gly	Ser	Gly	Gly	
		595					600					605				
Ser	Gly	Gly	Val	Asp	Asp	Ile	Gln	Met	Thr	Gln	Thr	Thr	Ser	Ser	Leu	
	610					615					620					
Ser	Ala	Ser	Leu	Gly	Asp	Arg	Val	Thr	Ile	Ser	Cys	Arg	Ala	Ser	Gln	
625					630					635					640	
Asp	Ile	Ser	Asn	Tyr	Leu	Asn	Trp	Tyr	Gln	Gln	Lys	Pro	Asp	Gly	Thr	

645										650					655				
Val	Lys	Leu	Leu	Ile	Tyr	Tyr	Thr	Ser	Arg	Leu	His	Ser	Gly	Val	Pro				
			660				665						670						
Ser	Arg	Phe	Ser	Gly	Ser	Gly	Ser	Gly	Thr	Asp	Tyr	Ser	Leu	Thr	Ile				
			675				680						685						
Asn	Asn	Leu	Glu	Gln	Glu	Asp	Ile	Ala	Thr	Tyr	Phe	Cys	Gln	Gln	Gly				
			690				695						700						
Asn	Thr	Arg	Pro	Trp	Thr	Phe	Gly	Gly	Gly	Thr	Lys	Leu	Glu	Ile	Lys				
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<223> OTHER INFORMATION: Chimeric homo sapiens mus musculus																			
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1				5				10						15					
Gln	Arg	Ala	Thr	Ile	Ser	Cys	Lys	Ala	Ser	Gln	Ser	Val	Asp	Tyr	Asp				
			20				25						30						
Gly	Asp	Ser	Tyr	Leu	Asn	Trp	Tyr	Gln	Gln	Ile	Pro	Gly	Gln	Pro	Pro				
			35				40						45						
Lys	Leu	Leu	Ile	Tyr	Asp	Ala	Ser	Asn	Leu	Val	Ser	Gly	Ile	Pro	Pro				
			50				55						60						
Arg	Phe	Ser	Gly	Ser	Gly	Ser	Gly	Thr	Asp	Phe	Thr	Leu	Asn	Ile	His				
			65				70						75	80					
Pro	Val	Glu	Lys	Val	Asp	Ala	Ala	Thr	Tyr	His	Cys	Gln	Gln	Ser	Thr				
			85				90						95						
Glu	Asp	Pro	Trp	Thr	Phe	Gly	Gly	Gly	Thr	Lys	Leu	Glu	Ile	Lys	Gly				
			100				105						110						
Gly	Gly	Gly	Ser	Gly	Gly	Gly	Gly	Ser	Gly	Gly	Gly	Gly	Ser	Gln	Val				
			115				120						125						
Gln	Leu	Gln	Gln	Ser	Gly	Ala	Glu	Leu	Val	Arg	Pro	Gly	Ser	Ser	Val				
			130				135						140						
Lys	Ile	Ser	Cys	Lys	Ala	Ser	Gly	Tyr	Ala	Phe	Ser	Ser	Tyr	Trp	Met				
			145				150						155	160					
Asn	Trp	Val	Lys	Gln	Arg	Pro	Gly	Gln	Gly	Leu	Glu	Trp	Ile	Gly	Gln				
			165				170						175						
Ile	Trp	Pro	Gly	Asp	Gly	Asp	Thr	Asn	Tyr	Asn	Gly	Lys	Phe	Lys	Gly				
			180				185						190						
Lys	Ala	Thr	Leu	Thr	Ala	Asp	Glu	Ser	Ser	Ser	Thr	Ala	Tyr	Met	Gln				
			195				200						205						
Leu	Ser	Ser	Leu	Ala	Ser	Glu	Asp	Ser	Ala	Val	Tyr	Phe	Cys	Ala	Arg				
			210				215						220						
Arg	Glu	Thr	Thr	Thr	Val	Gly	Arg	Tyr	Tyr	Tyr	Ala	Met	Asp	Tyr	Trp				
			225				230						235	240					
Gly	Gln	Gly	Thr	Thr	Val	Thr	Val	Ser	Ser	Gly	Gly	Gly	Ser	Ser	Ala				
			245				250						255						
Pro	Glu	Leu	Leu	Gly	Gly	Pro	Ser	Val	Phe	Leu	Phe	Pro	Pro	Lys	Pro				
			260				265						270						
Lys	Asp	Thr	Leu	Met	Ile	Ser	Arg	Thr	Pro	Glu	Val	Thr	Cys	Val	Val				

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275					280					285					
Val	Asp	Val	Ser	His	Glu	Asp	Pro	Glu	Val	Lys	Phe	Asn	Trp	Tyr	Val
	290					295					300				
Asp	Gly	Val	Glu	Val	His	Asn	Ala	Lys	Thr	Lys	Pro	Arg	Glu	Glu	Gln
305					310					315					320
Tyr	Asn	Ser	Thr	Tyr	Arg	Val	Val	Ser	Val	Leu	Thr	Val	Leu	His	Gln
				325					330					335	
Asp	Trp	Leu	Asn	Gly	Lys	Glu	Tyr	Lys	Cys	Lys	Val	Ser	Asn	Lys	Ala
		340						345					350		
Leu	Pro	Ala	Pro	Ile	Glu	Lys	Thr	Ile	Ser	Lys	Ala	Lys	Gly	Gln	Pro
	355						360					365			
Arg	Glu	Pro	Gln	Val	Tyr	Thr	Lys	Pro	Pro	Ser	Arg	Glu	Glu	Met	Thr
	370					375					380				
Lys	Asn	Gln	Val	Ser	Leu	Ser	Cys	Leu	Val	Lys	Gly	Phe	Tyr	Pro	Ser
385					390					395					400
Asp	Ile	Ala	Val	Glu	Trp	Glu	Ser	Asn	Gly	Gln	Pro	Glu	Asn	Asn	Tyr
		405							410					415	
Lys	Thr	Thr	Val	Pro	Val	Leu	Asp	Ser	Asp	Gly	Ser	Phe	Arg	Leu	Ala
		420						425					430		
Ser	Tyr	Leu	Thr	Val	Asp	Lys	Ser	Arg	Trp	Gln	Gln	Gly	Asn	Val	Phe
	435						440					445			
Ser	Cys	Ser	Val	Met	His	Glu	Ala	Leu	His	Asn	His	Tyr	Thr	Gln	Lys
	450					455					460				
Ser	Leu	Ser	Leu	Ser	Pro	Gly	Ser	Thr	Gly	Ser	Glu	Val	Gln	Leu	Gln
465					470					475					480
Glu	Ser	Gly	Pro	Gly	Leu	Val	Lys	Pro	Ser	Gln	Ser	Leu	Ser	Leu	Thr
			485						490					495	
Cys	Thr	Val	Thr	Gly	Tyr	Ser	Ile	Thr	Ser	Asp	Tyr	Ala	Trp	Asn	Trp
		500						505					510		
Ile	Arg	Gln	Phe	Pro	Gly	Asn	Lys	Leu	Glu	Trp	Met	Gly	Tyr	Ile	Thr
	515						520					525			
Tyr	Ser	Gly	Ser	Thr	Ser	Tyr	Asn	Pro	Ser	Leu	Glu	Ser	Arg	Ile	Ser
	530					535					540				
Ile	Thr	Arg	Asp	Thr	Ser	Thr	Asn	Gln	Phe	Phe	Leu	Gln	Leu	Asn	Ser
545					550					555					560
Val	Thr	Thr	Glu	Asp	Thr	Ala	Thr	Tyr	Tyr	Cys	Ala	Arg	Gly	Gly	Tyr
			565						570					575	
Tyr	Gly	Ser	Ser	Trp	Gly	Val	Phe	Ala	Tyr	Trp	Gly	Gln	Gly	Thr	Leu
		580						585					590		
Val	Thr	Val	Ser	Ala	Val	Glu	Gly	Gly	Ser	Gly	Gly	Ser	Gly	Gly	Ser
	595						600					605			
Gly	Gly	Ser	Gly	Gly	Val	Asp	Asp	Ile	Gln	Met	Thr	Gln	Ser	Pro	Ala
	610					615					620				
Ser	Leu	Ser	Ala	Ser	Val	Gly	Glu	Thr	Val	Thr	Ile	Thr	Cys	Arg	Val
625					630					635					640
Ser	Glu	Asn	Ile	Tyr	Ser	Tyr	Leu	Ala	Trp	Tyr	Gln	Gln	Lys	Gln	Gly
			645						650					655	
Lys	Ser	Pro	Gln	Leu	Leu	Val	Tyr	Asn	Ala	Lys	Thr	Leu	Ala	Glu	Gly
		660						665					670		
Val	Pro	Ser	Arg	Phe	Ser	Gly	Ser	Gly	Ser	Gly	Thr	Gln	Phe	Ser	Leu
	675						680					685			

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Lys Ile Asn Ser Leu Gln Pro Glu Asp Phe Gly Ser Tyr Tyr Cys Gln
 690 695 700
 His His Tyr Gly Thr Pro Trp Thr Phe Gly Gly Gly Thr Lys Leu Glu
 705 710 715 720
 Ile Lys
 <210> SEQ ID NO 135
 <211> LENGTH: 716
 <212> TYPE: PRT
 <213> ORGANISM: Artificial
 <220> FEATURE:
 <223> OTHER INFORMATION: Chimeric homo sapiens mus musculus
 <400> SEQUENCE: 135
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 Gln Arg Ala Thr Ile Ser Cys Lys Ala Ser Gln Ser Val Asp Tyr Asp
 20 25 30
 Gly Asp Ser Tyr Leu Asn Trp Tyr Gln Gln Ile Pro Gly Gln Pro Pro
 35 40 45
 Lys Leu Leu Ile Tyr Asp Ala Ser Asn Leu Val Ser Gly Ile Pro Pro
 50 55 60
 Arg Phe Ser Gly Ser Gly Ser Gly Thr Asp Phe Thr Leu Asn Ile His
 65 70 75 80
 Pro Val Glu Lys Val Asp Ala Ala Thr Tyr His Cys Gln Gln Ser Thr
 85 90 95
 Glu Asp Pro Trp Thr Phe Gly Gly Gly Thr Lys Leu Glu Ile Lys Gly
 100 105 110
 Gly Gly Gly Ser Gly Gly Gly Gly Ser Gly Gly Gly Ser Gln Val
 115 120 125
 Gln Leu Gln Gln Ser Gly Ala Glu Leu Val Arg Pro Gly Ser Ser Val
 130 135 140
 Lys Ile Ser Cys Lys Ala Ser Gly Tyr Ala Phe Ser Ser Tyr Trp Met
 145 150 155 160
 Asn Trp Val Lys Gln Arg Pro Gly Gln Gly Leu Glu Trp Ile Gly Gln
 165 170 175
 Ile Trp Pro Gly Asp Gly Asp Thr Asn Tyr Asn Gly Lys Phe Lys Gly
 180 185 190
 Lys Ala Thr Leu Thr Ala Asp Glu Ser Ser Ser Thr Ala Tyr Met Gln
 195 200 205
 Leu Ser Ser Leu Ala Ser Glu Asp Ser Ala Val Tyr Phe Cys Ala Arg
 210 215 220
 Arg Glu Thr Thr Thr Val Gly Arg Tyr Tyr Tyr Ala Met Asp Tyr Trp
 225 230 235 240
 Gly Gln Gly Thr Thr Val Thr Val Ser Ser Gly Gly Gly Ser Ser Ala
 245 250 255
 Pro Glu Leu Leu Gly Gly Pro Ser Val Phe Leu Phe Pro Pro Lys Pro
 260 265 270
 Lys Asp Thr Leu Met Ile Ser Arg Thr Pro Glu Val Thr Cys Val Val
 275 280 285
 Val Asp Val Ser His Glu Asp Pro Glu Val Lys Phe Asn Trp Tyr Val
 290 295 300

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Asp	Gly	Val	Glu	Val	His	Asn	Ala	Lys	Thr	Lys	Pro	Arg	Glu	Glu	Gln	305	310	315	320
Tyr	Asn	Ser	Thr	Tyr	Arg	Val	Val	Ser	Val	Leu	Thr	Val	Leu	His	Gln	325	330	335	
Asp	Trp	Leu	Asn	Gly	Lys	Glu	Tyr	Lys	Cys	Lys	Val	Ser	Asn	Lys	Ala	340	345	350	
Leu	Pro	Ala	Pro	Ile	Glu	Lys	Thr	Ile	Ser	Lys	Ala	Lys	Gly	Gln	Pro	355	360	365	
Arg	Glu	Pro	Gln	Val	Tyr	Thr	Lys	Pro	Pro	Ser	Arg	Glu	Glu	Met	Thr	370	375	380	
Lys	Asn	Gln	Val	Ser	Leu	Ser	Cys	Leu	Val	Lys	Gly	Phe	Tyr	Pro	Ser	385	390	395	400
Asp	Ile	Ala	Val	Glu	Trp	Glu	Ser	Asn	Gly	Gln	Pro	Glu	Asn	Asn	Tyr	405	410	415	
Lys	Thr	Thr	Val	Pro	Val	Leu	Asp	Ser	Asp	Gly	Ser	Phe	Arg	Leu	Ala	420	425	430	
Ser	Tyr	Leu	Thr	Val	Asp	Lys	Ser	Arg	Trp	Gln	Gln	Gly	Asn	Val	Phe	435	440	445	
Ser	Cys	Ser	Val	Met	His	Glu	Ala	Leu	His	Asn	His	Tyr	Thr	Gln	Lys	450	455	460	
Ser	Leu	Ser	Leu	Ser	Pro	Gly	Ser	Thr	Gly	Ser	Glu	Val	Gln	Leu	Gln	465	470	475	480
Gln	Ser	Gly	Pro	Glu	Leu	Val	Lys	Pro	Gly	Ala	Ser	Val	Lys	Ile	Ser	485	490	495	
Cys	Lys	Thr	Ser	Gly	Tyr	Thr	Phe	Thr	Glu	Tyr	Thr	Met	His	Trp	Val	500	505	510	
Lys	Gln	Ser	His	Gly	Lys	Ser	Leu	Glu	Trp	Ile	Gly	Gly	Ile	Ser	Pro	515	520	525	
Asn	Ile	Gly	Gly	Thr	Ser	Tyr	Asn	Gln	Lys	Phe	Lys	Gly	Lys	Ala	Thr	530	535	540	
Leu	Thr	Val	Asp	Lys	Ser	Ser	Ser	Thr	Ala	Tyr	Met	Glu	Leu	Arg	Ser	545	550	555	560
Leu	Thr	Ser	Glu	Asp	Ser	Ala	Val	Tyr	Tyr	Cys	Ala	Arg	Arg	Gly	Gly	565	570	575	
Ser	Phe	Asp	Tyr	Trp	Gly	Gln	Gly	Thr	Thr	Leu	Thr	Val	Ser	Ser	Val	580	585	590	
Glu	Gly	Gly	Ser	Gly	Gly	Ser	Gly	Gly	Ser	Gly	Gly	Ser	Gly	Gly	Val	595	600	605	
Asp	Asp	Ile	Val	Met	Thr	Gln	Ser	Pro	Ala	Thr	Leu	Ser	Val	Thr	Pro	610	615	620	
Gly	Asp	Arg	Val	Ser	Leu	Ser	Cys	Arg	Ala	Ser	Gln	Ser	Ile	Ser	Asp	625	630	635	640
Tyr	Leu	His	Trp	Tyr	Gln	Gln	Lys	Ser	His	Glu	Ser	Pro	Arg	Leu	Leu	645	650	655	
Ile	Lys	Tyr	Ala	Ser	Gln	Ser	Ile	Ser	Gly	Ile	Pro	Ser	Arg	Phe	Ser	660	665	670	
Gly	Ser	Gly	Ser	Gly	Ser	Asp	Phe	Thr	Leu	Ser	Ile	Asn	Ser	Val	Glu	675	680	685	
Pro	Glu	Asp	Val	Gly	Val	Tyr	Tyr	Cys	Gln	Asn	Gly	His	Ser	Phe	Pro	690	695	700	
Leu	Thr	Phe	Gly	Ala	Gly	Thr	Lys	Leu	Glu	Leu	Lys								

705	710								715							
<210> SEQ ID NO 136																
<211> LENGTH: 717																
<212> TYPE: PRT																
<213> ORGANISM: Artificial																
<220> FEATURE:																
<223> OTHER INFORMATION: Chimeric homo sapiens mus musculus																
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				5					10					15		
Gln	Arg	Ala	Thr	Ile	Ser	Cys	Lys	Ala	Ser	Gln	Ser	Val	Asp	Tyr	Asp	
				20					25					30		
Gly	Asp	Ser	Tyr	Leu	Asn	Trp	Tyr	Gln	Gln	Ile	Pro	Gly	Gln	Pro	Pro	
				35					40					45		
Lys	Leu	Leu	Ile	Tyr	Asp	Ala	Ser	Asn	Leu	Val	Ser	Gly	Ile	Pro	Pro	
				50					55					60		
Arg	Phe	Ser	Gly	Ser	Gly	Ser	Gly	Thr	Asp	Phe	Thr	Leu	Asn	Ile	His	
				65					70					75		
Pro	Val	Glu	Lys	Val	Asp	Ala	Ala	Thr	Tyr	His	Cys	Gln	Gln	Ser	Thr	
				85					90					95		
Glu	Asp	Pro	Trp	Thr	Phe	Gly	Gly	Gly	Thr	Lys	Leu	Glu	Ile	Lys	Gly	
				100					105					110		
Gly	Gly	Gly	Ser	Gly	Gly	Gly	Gly	Ser	Gly	Gly	Gly	Gly	Ser	Gln	Val	
				115					120					125		
Gln	Leu	Gln	Gln	Ser	Gly	Ala	Glu	Leu	Val	Arg	Pro	Gly	Ser	Ser	Val	
				130					135					140		
Lys	Ile	Ser	Cys	Lys	Ala	Ser	Gly	Tyr	Ala	Phe	Ser	Ser	Tyr	Trp	Met	
				145					150					155		
Asn	Trp	Val	Lys	Gln	Arg	Pro	Gly	Gln	Gly	Leu	Glu	Trp	Ile	Gly	Gln	
				165					170					175		
Ile	Trp	Pro	Gly	Asp	Gly	Asp	Thr	Asn	Tyr	Asn	Gly	Lys	Phe	Lys	Gly	
				180					185					190		
Lys	Ala	Thr	Leu	Thr	Ala	Asp	Glu	Ser	Ser	Ser	Thr	Ala	Tyr	Met	Gln	
				195					200					205		
Leu	Ser	Ser	Leu	Ala	Ser	Glu	Asp	Ser	Ala	Val	Tyr	Phe	Cys	Ala	Arg	
				210					215					220		
Arg	Glu	Thr	Thr	Thr	Val	Gly	Arg	Tyr	Tyr	Tyr	Ala	Met	Asp	Tyr	Trp	
				225					230					235		
Gly	Gln	Gly	Thr	Thr	Val	Thr	Val	Ser	Ser	Gly	Gly	Gly	Ser	Ser	Ala	
				245					250					255		
Pro	Glu	Leu	Leu	Gly	Gly	Pro	Ser	Val	Phe	Leu	Phe	Pro	Pro	Lys	Pro	
				260					265					270		
Lys	Asp	Thr	Leu	Met	Ile	Ser	Arg	Thr	Pro	Glu	Val	Thr	Cys	Val	Val	
				275					280					285		
Val	Asp	Val	Ser	His	Glu	Asp	Pro	Glu	Val	Lys	Phe	Asn	Trp	Tyr	Val	
				290					295					300		
Asp	Gly	Val	Glu	Val	His	Asn	Ala	Lys	Thr	Lys	Pro	Arg	Glu	Glu	Gln	
				305					310					315		
Tyr	Asn	Ser	Thr	Tyr	Arg	Val	Val	Ser	Val	Leu	Thr	Val	Leu	His	Gln	
				325					330					335		
Asp	Trp	Leu	Asn	Gly	Lys	Glu	Tyr	Lys	Cys	Lys	Val	Ser	Asn	Lys	Ala	

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340				345				350							
Leu	Pro	Ala	Pro	Ile	Glu	Lys	Thr	Ile	Ser	Lys	Ala	Lys	Gly	Gln	Pro
	355						360					365			
Arg	Glu	Pro	Gln	Val	Tyr	Thr	Lys	Pro	Pro	Ser	Arg	Glu	Glu	Met	Thr
	370						375					380			
Lys	Asn	Gln	Val	Ser	Leu	Ser	Cys	Leu	Val	Lys	Gly	Phe	Tyr	Pro	Ser
	385				390					395					400
Asp	Ile	Ala	Val	Glu	Trp	Glu	Ser	Asn	Gly	Gln	Pro	Glu	Asn	Asn	Tyr
			405						410						415
Lys	Thr	Thr	Val	Pro	Val	Leu	Asp	Ser	Asp	Gly	Ser	Phe	Arg	Leu	Ala
			420						425				430		
Ser	Tyr	Leu	Thr	Val	Asp	Lys	Ser	Arg	Trp	Gln	Gln	Gly	Asn	Val	Phe
		435					440					445			
Ser	Cys	Ser	Val	Met	His	Glu	Ala	Leu	His	Asn	His	Tyr	Thr	Gln	Lys
	450					455					460				
Ser	Leu	Ser	Leu	Ser	Pro	Gly	Ser	Thr	Gly	Ser	Gln	Val	Gln	Leu	Gln
	465				470					475					480
Gln	Ser	Ala	Val	Glu	Leu	Ala	Arg	Pro	Gly	Ala	Ser	Val	Lys	Met	Ser
			485						490						495
Cys	Lys	Ala	Ser	Gly	Tyr	Thr	Phe	Thr	Ser	Phe	Thr	Met	His	Trp	Val
		500						505					510		
Lys	Gln	Arg	Pro	Gly	Gln	Gly	Leu	Glu	Trp	Ile	Gly	Tyr	Ile	Asn	Pro
		515					520					525			
Ser	Ser	Gly	Tyr	Thr	Glu	Tyr	Asn	Gln	Lys	Phe	Lys	Asp	Lys	Thr	Thr
	530						535					540			
Leu	Thr	Ala	Asp	Lys	Ser	Ser	Ser	Thr	Ala	Tyr	Met	Gln	Leu	Asp	Ser
	545				550					555					560
Leu	Thr	Ser	Asp	Asp	Ser	Ala	Val	Tyr	Tyr	Cys	Val	Arg	Gly	Ser	Ser
			565						570						575
Arg	Gly	Phe	Asp	Tyr	Trp	Gly	Gln	Gly	Thr	Leu	Val	Thr	Val	Ser	Ala
		580					585						590		
Val	Glu	Gly	Gly	Ser	Gly	Gly	Ser	Gly	Gly	Ser	Gly	Gly	Ser	Gly	Gly
	595						600					605			
Val	Asp	Asp	Ile	Gln	Met	Ile	Gln	Ser	Pro	Ala	Ser	Leu	Ser	Val	Ser
	610					615					620				
Val	Gly	Glu	Thr	Val	Thr	Ile	Thr	Cys	Arg	Ala	Ser	Glu	Asn	Ile	Tyr
	625				630					635					640
Ser	Asn	Leu	Ala	Trp	Phe	Gln	Gln	Lys	Gln	Gly	Lys	Ser	Pro	Gln	Leu
			645						650						655
Leu	Val	Tyr	Ala	Ala	Thr	Asn	Leu	Ala	Asp	Gly	Val	Pro	Ser	Arg	Phe
		660					665						670		
Ser	Gly	Ser	Gly	Ser	Gly	Thr	Gln	Tyr	Ser	Leu	Lys	Ile	Asn	Ser	Leu
		675					680					685			
Gln	Ser	Glu	Asp	Phe	Gly	Ile	Tyr	Tyr	Cys	Gln	His	Phe	Trp	Gly	Thr
	690					695					700				
Pro	Arg	Thr	Phe	Gly	Gly	Gly	Thr	Lys	Leu	Glu	Ile	Lys			
	705					710				715					

<210> SEQ ID NO 137

<211> LENGTH: 716

<212> TYPE: PRT

<213> ORGANISM: Artificial

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<220> FEATURE:

<223> OTHER INFORMATION: Chimeric homo sapiens mus musculus

<400> SEQUENCE: 137

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                20           25           30
Gly Asp Ser Tyr Leu Asn Trp Tyr Gln Gln Ile Pro Gly Gln Pro Pro
          35           40           45
Lys Leu Leu Ile Tyr Asp Ala Ser Asn Leu Val Ser Gly Ile Pro Pro
          50           55           60
Arg Phe Ser Gly Ser Gly Ser Gly Thr Asp Phe Thr Leu Asn Ile His
65           70           75           80
Pro Val Glu Lys Val Asp Ala Ala Thr Tyr His Cys Gln Gln Ser Thr
          85           90           95
Glu Asp Pro Trp Thr Phe Gly Gly Gly Thr Lys Leu Glu Ile Lys Gly
          100          105          110
Gly Gly Gly Ser Gly Gly Gly Gly Ser Gly Gly Gly Gly Ser Gln Val
          115          120          125
Gln Leu Gln Gln Ser Gly Ala Glu Leu Val Arg Pro Gly Ser Ser Val
          130          135          140
Lys Ile Ser Cys Lys Ala Ser Gly Tyr Ala Phe Ser Ser Tyr Trp Met
145          150          155          160
Asn Trp Val Lys Gln Arg Pro Gly Gln Gly Leu Glu Trp Ile Gly Gln
          165          170          175
Ile Trp Pro Gly Asp Gly Asp Thr Asn Tyr Asn Gly Lys Phe Lys Gly
          180          185          190
Lys Ala Thr Leu Thr Ala Asp Glu Ser Ser Ser Thr Ala Tyr Met Gln
          195          200          205
Leu Ser Ser Leu Ala Ser Glu Asp Ser Ala Val Tyr Phe Cys Ala Arg
          210          215          220
Arg Glu Thr Thr Thr Val Gly Arg Tyr Tyr Tyr Ala Met Asp Tyr Trp
225          230          235          240
Gly Gln Gly Thr Thr Val Thr Val Ser Ser Gly Gly Gly Ser Ser Ala
          245          250          255
Pro Glu Leu Leu Gly Gly Pro Ser Val Phe Leu Phe Pro Pro Lys Pro
          260          265          270
Lys Asp Thr Leu Met Ile Ser Arg Thr Pro Glu Val Thr Cys Val Val
          275          280          285
Val Asp Val Ser His Glu Asp Pro Glu Val Lys Phe Asn Trp Tyr Val
          290          295          300
Asp Gly Val Glu Val His Asn Ala Lys Thr Lys Pro Arg Glu Glu Gln
305          310          315          320
Tyr Asn Ser Thr Tyr Arg Val Val Ser Val Leu Thr Val Leu His Gln
          325          330          335
Asp Trp Leu Asn Gly Lys Glu Tyr Lys Cys Lys Val Ser Asn Lys Ala
          340          345          350
Leu Pro Ala Pro Ile Glu Lys Thr Ile Ser Lys Ala Lys Gly Gln Pro
          355          360          365
Arg Glu Pro Gln Val Tyr Thr Lys Pro Pro Ser Arg Glu Glu Met Thr
          370          375          380

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Lys Asn Gln Val Ser Leu Ser Cys Leu Val Lys Gly Phe Tyr Pro Ser
 385 390 395 400
 Asp Ile Ala Val Glu Trp Glu Ser Asn Gly Gln Pro Glu Asn Asn Tyr
 405 410 415
 Lys Thr Thr Val Pro Val Leu Asp Ser Asp Gly Ser Phe Arg Leu Ala
 420 425 430
 Ser Tyr Leu Thr Val Asp Lys Ser Arg Trp Gln Gln Gly Asn Val Phe
 435 440 445
 Ser Cys Ser Val Met His Glu Ala Leu His Asn His Tyr Thr Gln Lys
 450 455 460
 Ser Leu Ser Leu Ser Pro Gly Ser Thr Gly Ser Gln Val Gln Leu Gln
 465 470 475 480
 Gln Pro Gly Ser Val Leu Val Arg Pro Gly Ala Ser Val Lys Leu Ser
 485 490 495
 Cys Lys Ala Ser Gly Tyr Thr Phe Thr Ser Ser Trp Met His Trp Ala
 500 505 510
 Lys Gln Arg Pro Gly Gln Gly Leu Glu Trp Ile Gly His Ile His Pro
 515 520 525
 Asn Ser Gly Ile Ser Asn Tyr Asn Glu Lys Phe Lys Gly Lys Ala Thr
 530 535 540
 Leu Thr Val Asp Thr Ser Ser Ser Thr Ala Tyr Val Asp Leu Ser Ser
 545 550 555 560
 Leu Thr Ser Glu Asp Ser Ala Val Tyr Tyr Cys Ala Arg Gly Gly Arg
 565 570 575
 Phe Asp Asp Trp Gly Ala Gly Thr Thr Val Thr Val Ser Ser Val Glu
 580 585 590
 Gly Gly Ser Gly Gly Ser Gly Gly Ser Gly Gly Ser Gly Gly Val Asp
 595 600 605
 Asp Ile Val Met Thr Gln Ser Pro Ala Thr Leu Ser Val Thr Pro Gly
 610 615 620
 Asp Arg Val Ser Leu Ser Cys Arg Ala Ser Gln Ser Ile Ser Asp Tyr
 625 630 635 640
 Leu His Trp Tyr Gln Gln Lys Ser His Glu Ser Pro Arg Leu Leu Ile
 645 650 655
 Lys Tyr Ala Ser Gln Ser Ile Ser Gly Ile Pro Ser Arg Phe Ser Gly
 660 665 670
 Ser Gly Ser Gly Ser Asp Phe Thr Leu Ser Ile Asn Ser Val Glu Pro
 675 680 685
 Glu Asp Val Gly Val Tyr Tyr Cys Gln Asn Gly His Ser Phe Leu Met
 690 695 700
 Tyr Thr Phe Gly Gly Gly Thr Lys Leu Glu Ile Lys
 705 710 715

<210> SEQ ID NO 138

<211> LENGTH: 720

<212> TYPE: PRT

<213> ORGANISM: Artificial

<220> FEATURE:

<223> OTHER INFORMATION: Chimeric homo sapiens mus musculus

<400> SEQUENCE: 138

Asp Ile Gln Leu Thr Gln Ser Pro Ala Ser Leu Ala Val Ser Leu Gly
 1 5 10 15

Gln	Arg	Ala	Thr	Ile	Ser	Cys	Lys	Ala	Ser	Gln	Ser	Val	Asp	Tyr	Asp
			20					25					30		
Gly	Asp	Ser	Tyr	Leu	Asn	Trp	Tyr	Gln	Gln	Ile	Pro	Gly	Gln	Pro	Pro
		35					40					45			
Lys	Leu	Leu	Ile	Tyr	Asp	Ala	Ser	Asn	Leu	Val	Ser	Gly	Ile	Pro	Pro
		50				55					60				
Arg	Phe	Ser	Gly	Ser	Gly	Ser	Gly	Thr	Asp	Phe	Thr	Leu	Asn	Ile	His
					70					75					80
Pro	Val	Glu	Lys	Val	Asp	Ala	Ala	Thr	Tyr	His	Cys	Gln	Gln	Ser	Thr
				85					90					95	
Glu	Asp	Pro	Trp	Thr	Phe	Gly	Gly	Gly	Thr	Lys	Leu	Glu	Ile	Lys	Gly
			100					105					110		
Gly	Gly	Gly	Ser	Gly	Gly	Gly	Gly	Ser	Gly	Gly	Gly	Gly	Ser	Gln	Val
		115					120					125			
Gln	Leu	Gln	Gln	Ser	Gly	Ala	Glu	Leu	Val	Arg	Pro	Gly	Ser	Ser	Val
		130				135					140				
Lys	Ile	Ser	Cys	Lys	Ala	Ser	Gly	Tyr	Ala	Phe	Ser	Ser	Tyr	Trp	Met
					150					155					160
Asn	Trp	Val	Lys	Gln	Arg	Pro	Gly	Gln	Gly	Leu	Glu	Trp	Ile	Gly	Gln
				165					170					175	
Ile	Trp	Pro	Gly	Asp	Gly	Asp	Thr	Asn	Tyr	Asn	Gly	Lys	Phe	Lys	Gly
			180					185					190		
Lys	Ala	Thr	Leu	Thr	Ala	Asp	Glu	Ser	Ser	Ser	Thr	Ala	Tyr	Met	Gln
							200					205			
Leu	Ser	Ser	Leu	Ala	Ser	Glu	Asp	Ser	Ala	Val	Tyr	Phe	Cys	Ala	Arg
		210				215					220				
Arg	Glu	Thr	Thr	Thr	Val	Gly	Arg	Tyr	Tyr	Tyr	Ala	Met	Asp	Tyr	Trp
					230					235					240
Gly	Gln	Gly	Thr	Thr	Val	Thr	Val	Ser	Ser	Gly	Gly	Gly	Ser	Ser	Ala
				245					250					255	
Pro	Glu	Leu	Leu	Gly	Gly	Pro	Ser	Val	Phe	Leu	Phe	Pro	Pro	Lys	Pro
			260					265					270		
Lys	Asp	Thr	Leu	Met	Ile	Ser	Arg	Thr	Pro	Glu	Val	Thr	Cys	Val	Val
			275				280					285			
Val	Asp	Val	Ser	His	Glu	Asp	Pro	Glu	Val	Lys	Phe	Asn	Trp	Tyr	Val
		290				295					300				
Asp	Gly	Val	Glu	Val	His	Asn	Ala	Lys	Thr	Lys	Pro	Arg	Glu	Glu	Gln
					310					315					320
Tyr	Asn	Ser	Thr	Tyr	Arg	Val	Val	Ser	Val	Leu	Thr	Val	Leu	His	Gln
				325					330					335	
Asp	Trp	Leu	Asn	Gly	Lys	Glu	Tyr	Lys	Cys	Lys	Val	Ser	Asn	Lys	Ala
			340					345					350		
Leu	Pro	Ala	Pro	Ile	Glu	Lys	Thr	Ile	Ser	Lys	Ala	Lys	Gly	Gln	Pro
			355				360					365			
Arg	Glu	Pro	Gln	Val	Tyr	Thr	Lys	Pro	Pro	Ser	Arg	G			

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Lys Thr Thr Val Pro Val Leu Asp Ser Asp Gly Ser Phe Arg Leu Ala
 420 425 430
 Ser Tyr Leu Thr Val Asp Lys Ser Arg Trp Gln Gln Gly Asn Val Phe
 435 440 445
 Ser Cys Ser Val Met His Glu Ala Leu His Asn His Tyr Thr Gln Lys
 450 455 460
 Ser Leu Ser Leu Ser Pro Gly Ser Thr Gly Ser Asp Val Gln Leu Gln
 465 470 475 480
 Glu Ser Gly Pro Gly Leu Val Lys Pro Ser Gln Ser Leu Ser Leu Thr
 485 490 495
 Cys Thr Val Thr Gly Tyr Ser Ile Thr Ser Asp Tyr Ala Trp Asn Trp
 500 505 510
 Ile Arg Gln Phe Pro Gly Asn Lys Leu Glu Trp Met Gly Tyr Ile Thr
 515 520 525
 Tyr Ser Gly Ser Thr Asn Tyr Asn Pro Ser Leu Lys Ser Arg Ile Ser
 530 535 540
 Ile Thr Arg Asp Thr Ser Lys Asn Gln Phe Phe Leu Gln Leu Asn Ser
 545 550 555 560
 Val Thr Thr Glu Asp Thr Ala Thr Tyr Tyr Cys Ala Arg Cys Trp Asp
 565 570 575
 Tyr Ala Leu Tyr Ala Met Asp Cys Trp Gly Gln Gly Thr Ser Val Thr
 580 585 590
 Val Ser Ser Val Glu Gly Gly Ser Gly Gly Ser Gly Gly Ser Gly Gly
 595 600 605
 Ser Gly Gly Val Asp Asp Ile Gln Met Thr Gln Ser Pro Ala Ser Leu
 610 615 620
 Ser Ala Ser Val Gly Glu Thr Val Thr Ile Thr Cys Arg Thr Ser Glu
 625 630 635 640
 Asn Ile Tyr Ser Tyr Leu Ala Trp Cys Gln Gln Lys Gln Gly Lys Ser
 645 650 655
 Pro Gln Leu Leu Val Tyr Asn Ala Lys Thr Leu Ala Glu Gly Val Pro
 660 665 670
 Ser Arg Phe Ser Gly Ser Gly Ser Gly Thr His Phe Ser Leu Lys Ile
 675 680 685
 Asn Ser Leu Gln Pro Glu Asp Phe Gly Ile Tyr Tyr Cys Gln His His
 690 695 700
 Tyr Asp Thr Pro Leu Thr Phe Gly Ala Gly Thr Lys Leu Glu Leu Lys
 705 710 715 720

<210> SEQ ID NO 139

<211> LENGTH: 717

<212> TYPE: PRT

<213> ORGANISM: Artificial

<220> FEATURE:

<223> OTHER INFORMATION: Chimeric homo sapiens mus musculus

<400> SEQUENCE: 139

Asp Ile Gln Leu Thr Gln Ser Pro Ala Ser Leu Ala Val Ser Leu Gly
 1 5 10 15
 Gln Arg Ala Thr Ile Ser Cys Lys Ala Ser Gln Ser Val Asp Tyr Asp
 20 25 30
 Gly Asp Ser Tyr Leu Asn Trp Tyr Gln Gln Ile Pro Gly Gln Pro Pro
 35 40 45

Lys 50	Leu	Ile	Tyr	Asp	Ala 55	Ser	Asn	Leu	Val	Ser 60	Gly	Ile	Pro	Pro	
Arg 65	Phe	Ser	Gly	Ser	Gly 70	Ser	Gly	Thr	Asp	Phe 75	Thr	Leu	Asn	Ile	His 80
Pro	Val	Glu	Lys	Val 85	Asp	Ala	Ala	Thr	Tyr 90	His	Cys	Gln	Gln	Ser 95	Thr
Glu	Asp	Pro	Trp	Thr 100	Phe	Gly	Gly	Gly 105	Thr	Lys	Leu	Glu	Ile 110	Lys	Gly
Gly	Gly	Gly	Ser	Gly	Gly	Gly	Gly	Ser 120	Gly	Gly	Gly	Gly	Ser	Gln	Val
Gln	Leu	Gln	Gln	Ser	Gly	Ala 135	Glu	Leu	Val	Arg 140	Pro	Gly	Ser	Ser	Val
Lys 145	Ile	Ser	Cys	Lys	Ala 150	Ser	Gly	Tyr	Ala	Phe 155	Ser	Ser	Tyr	Trp	Met 160
Asn	Trp	Val	Lys	Gln 165	Arg	Pro	Gly	Gln	Gly 170	Leu	Glu	Trp	Ile	Gly 175	Gln
Ile	Trp	Pro	Gly	Asp 180	Gly	Asp	Thr	Asn	Tyr 185	Asn	Gly	Lys	Phe 190	Lys	Gly
Lys	Ala	Thr	Leu	Thr 195	Ala	Asp	Glu	Ser	Ser	Ser	Thr	Ala 205	Tyr	Met	Gln
Leu	Ser 210	Ser	Leu	Ala	Ser	Glu 215	Asp	Ser	Ala	Val	Tyr 220	Phe	Cys	Ala	Arg
Arg 225	Glu	Thr	Thr	Thr	Val 230	Gly	Arg	Tyr	Tyr	Tyr 235	Ala	Met	Asp	Tyr	Trp 240
Gly	Gln	Gly	Thr	Thr 245	Val	Thr	Val	Ser	Ser	Gly 250	Gly	Gly	Ser	Ser 255	Ala
Pro	Glu	Leu	Leu	Gly 260	Gly	Pro	Ser	Val	Phe	Leu	Phe	Pro	Pro 270	Lys	Pro
Lys	Asp	Thr 275	Leu	Met	Ile	Ser	Arg 280	Thr	Pro	Glu	Val	Thr 285	Cys	Val	Val
Val	Asp 290	Val	Ser	His	Glu	Asp 295	Pro	Glu	Val	Lys	Phe	Asn	Trp	Tyr	Val
Asp 305	Gly	Val	Glu	Val	His 310	Asn	Ala	Lys	Thr	Lys 315	Pro	Arg	Glu	Glu	Gln
Tyr	Asn	Ser	Thr	Tyr 325	Arg	Val	Val	Ser	Val	Leu	Thr	Val	Leu	His 335	Gln
Asp	Trp	Leu	Asn	Gly 340	Lys	Glu	Tyr	Lys 345	Cys	Lys	Val	Ser	Asn 350	Lys	Ala
Leu	Pro	Ala 355	Pro	Ile	Glu	Lys	Thr 360	Ile	Ser	Lys	Ala	Lys 365	Gly	Gln	Pro
Arg	Glu 370	Pro	Gln	Val	Tyr	Thr 375	Lys	Pro	Pro	Ser	Arg	Glu	Glu	Met	Thr
Lys 385	Asn	Gln	Val	Ser	Leu 390	Ser	Cys	Leu	Val	Lys 395	Gly	Phe	Tyr	Pro	Ser
Asp	Ile	Ala	Val	Glu 405	Trp	Glu	Ser	Asn	Gly 410	Gln	Pro	Glu	Asn	Asn	Tyr
Lys	Thr	Thr	Val	Pro	Val	Leu	Asp 425	Ser	Asp	Gly	Ser	Phe	Arg	Leu	Ala
Ser	Tyr	Leu	Thr	Val	Asp	Lys	Ser 440	Arg	Trp	Gln	Gln	Gly	Asn	Val	Phe
Ser	Cys	Ser	Val	Met	His	Glu	Ala	Leu	His	Asn	His	Tyr	Thr	Gln	Lys

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450	455	460
Ser Leu Ser Leu Ser Pro Gly Ser Thr Gly Ser Gln Ile Gln Leu Val		
465	470	475 480
Gln Ser Gly Pro Glu Leu Gln Lys Pro Gly Glu Thr Val Lys Ile Ser		
	485	490 495
Cys Lys Ala Ser Gly Tyr Thr Phe Thr Asn Tyr Gly Met Asn Trp Val		
	500	505 510
Lys Gln Ala Pro Gly Lys Gly Leu Lys Trp Met Gly Trp Ile Asn Thr		
	515	520 525
Asn Thr Gly Glu Pro Thr Tyr Ala Glu Glu Phe Lys Gly Arg Phe Ala		
	530	535 540
Phe Ser Leu Glu Thr Ser Ala Ser Thr Ala Tyr Leu Gln Ile Asn Asn		
	545	550 555 560
Leu Lys Asn Glu Asp Thr Ala Thr Tyr Phe Cys Ala Arg Asp Tyr Leu		
	565	570 575
Tyr Tyr Phe Asp Tyr Trp Gly Gln Gly Thr Thr Leu Thr Val Ser Ser		
	580	585 590
Val Glu Gly Gly Ser Gly Gly Ser Gly Gly Ser Gly Gly Ser Gly Gly		
	595	600 605
Val Asp Asn Ile Val Met Thr Gln Ser Pro Lys Ser Met Ser Met Ser		
	610	615 620
Val Gly Glu Arg Val Thr Leu Thr Cys Lys Ala Ser Glu Asn Val Val		
	625	630 635 640
Thr Tyr Val Ser Trp Tyr Gln Gln Lys Pro Glu Gln Ser Pro Lys Leu		
	645	650 655
Leu Ile Tyr Gly Ala Ser Asn Arg Tyr Thr Gly Val Pro Asp Arg Phe		
	660	665 670
Thr Gly Ser Gly Ser Ala Thr Asp Phe Thr Leu Thr Ile Ser Ser Val		
	675	680 685
Gln Ala Glu Asp Leu Ala Asp Tyr His Cys Gly Gln Gly Tyr Ser Tyr		
	690	695 700
Pro Tyr Thr Phe Gly Gly Gly Thr Lys Leu Glu Ile Lys		
	705	710 715

<210> SEQ ID NO 140

<211> LENGTH: 698

<212> TYPE: PRT

<213> ORGANISM: Artificial

<220> FEATURE:

<223> OTHER INFORMATION: Chimeric homo sapiens mus musculus

<400> SEQUENCE: 140

Asp Ile Gln Leu Thr Gln Ser Pro Ala Ser Leu Ala Val Ser Leu Gly
1 5 10 15
Gln Arg Ala Thr Ile Ser Cys Lys Ala Ser Gln Ser Val Asp Tyr Asp
20 25 30
Gly Asp Ser Tyr Leu Asn Trp Tyr Gln Gln Ile Pro Gly Gln Pro Pro
35 40 45
Lys Leu Leu Ile Tyr Asp Ala Ser Asn Leu Val Ser Gly Ile Pro Pro
50 55 60
Arg Phe Ser Gly Ser Gly Ser Gly Thr Asp Phe Thr Leu Asn Ile His
65 70 75 80
Pro Val Glu Lys Val Asp Ala Ala Thr Tyr His Cys Gln Gln Ser Thr

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85								90								95							
Glu	Asp	Pro	Trp	Thr	Phe	Gly	Gly	Gly	Thr	Lys	Leu	Glu	Ile	Lys	Gly								
			100							105				110									
Gly	Gly	Gly	Ser	Gly	Gly	Gly	Gly	Ser	Gly	Gly	Gly	Gly	Ser	Gln	Val								
			115							120				125									
Gln	Leu	Gln	Gln	Ser	Gly	Ala	Glu	Leu	Val	Arg	Pro	Gly	Ser	Ser	Val								
			130				135						140										
Lys	Ile	Ser	Cys	Lys	Ala	Ser	Gly	Tyr	Ala	Phe	Ser	Ser	Tyr	Trp	Met								
			145				150						155										
Asn	Trp	Val	Lys	Gln	Arg	Pro	Gly	Gln	Gly	Leu	Glu	Trp	Ile	Gly	Gln								
			165							170			175										
Ile	Trp	Pro	Gly	Asp	Gly	Asp	Thr	Asn	Tyr	Asn	Gly	Lys	Phe	Lys	Gly								
			180										190										
Lys	Ala	Thr	Leu	Thr	Ala	Asp	Glu	Ser	Ser	Ser	Thr	Ala	Tyr	Met	Gln								
			195				200						205										
Leu	Ser	Ser	Leu	Ala	Ser	Glu	Asp	Ser	Ala	Val	Tyr	Phe	Cys	Ala	Arg								
			210				215						220										
Arg	Glu	Thr	Thr	Thr	Val	Gly	Arg	Tyr	Tyr	Tyr	Ala	Met	Asp	Tyr	Trp								
			225				230						235										
Gly	Gln	Gly	Thr	Thr	Val	Thr	Val	Ser	Ser	Gly	Gly	Gly	Ser	Ser	Ala								
			245							250			255										
Pro	Glu	Leu	Leu	Gly	Gly	Pro	Ser	Val	Phe	Leu	Phe	Pro	Pro	Lys	Pro								
			260							265			270										
Lys	Asp	Thr	Leu	Met	Ile	Ser	Arg	Thr	Pro	Glu	Val	Thr	Cys	Val	Val								
			275				280						285										
Val	Asp	Val	Ser	His	Glu	Asp	Pro	Glu	Val	Lys	Phe	Asn	Trp	Tyr	Val								
			290				295						300										
Asp	Gly	Val	Glu	Val	His	Asn	Ala	Lys	Thr	Lys	Pro	Arg	Glu	Glu	Gln								
			305							315			320										
Tyr	Asn	Ser	Thr	Tyr	Arg	Val	Val	Ser	Val	Leu	Thr	Val	Leu	His	Gln								
			325							330			335										
Asp	Trp	Leu	Asn	Gly	Lys	Glu	Tyr	Lys	Cys	Lys	Val	Ser	Asn	Lys	Ala								
			340							345			350										
Leu	Pro	Ala	Pro	Ile	Glu	Lys	Thr	Ile	Ser	Lys	Ala	Lys	Gly	Gln	Pro								
			355				360						365										
Arg	Glu	Pro	Gln	Val	Tyr	Thr	Lys	Pro	Pro	Ser	Arg	Glu	Glu	Met	Thr								
			370				375						380										
Lys	Asn	Gln	Val	Ser	Leu	Ser	Cys	Leu	Val	Lys	Gly	Phe	Tyr	Pro	Ser								
			385				390						395										
Asp	Ile	Ala	Val	Glu	Trp	Glu	Ser	Asn	Gly	Gln	Pro	Glu	Asn	Asn	Tyr								
			405							410			415										
Lys	Thr	Thr	Val	Pro	Val	Leu	Asp	Ser	Asp	Gly	Ser	Phe	Arg	Leu	Ala								
			420							425			430										
Ser	Tyr	Leu	Thr	Val	Asp	Lys	Ser	Arg	Trp	Gln	Gln	Gly	Asn	Val	Phe								
			435				440						445										
Ser	Cys	Ser	Val	Met	His	Glu	Ala	Leu	His	Asn	His	Tyr	Thr	Gln	Lys								
			450				455						460										
Ser	Leu	Ser	Leu	Ser	Pro	Gly	Ser	Thr	Gly	Ser	Glu	Val	Gln	Leu	Gln								
			465				470						475										
Gln	Ser	Gly	Pro	Glu	Leu	Val	Lys	Pro	Gly	Ala	Ser	Val	Lys	Ile	Ser								
			485							490			495										

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Cys Lys Thr Ser Gly Tyr Thr Phe Thr Glu Tyr Thr Met His Trp Val
 500 505 510
 Lys Gln Ser His Gly Lys Ser Leu Glu Trp Ile Gly Gly Ile Ser Pro
 515 520 525
 Asn Ile Gly Gly Thr Ser Tyr Asn Gln Lys Phe Lys Gly Lys Ala Thr
 530 535 540
 Leu Thr Val Asp Lys Ser Ser Ser Thr Ala Tyr Met Glu Leu Arg Ser
 545 550 555 560
 Leu Thr Ser Glu Asp Ser Ala Val Tyr Tyr Cys Ala Arg Arg Gly Gly
 565 570 575
 Ser Phe Asp Tyr Trp Gly Gln Gly Thr Thr Leu Thr Val Ser Ser Ala
 580 585 590
 Ser Thr Lys Gly Pro Ser Val Phe Pro Leu Ala Pro Ser Ser Lys Ser
 595 600 605
 Thr Ser Gly Gly Thr Ala Ala Leu Gly Cys Leu Val Lys Asp Tyr Phe
 610 615 620
 Pro Glu Pro Val Thr Val Ser Trp Asn Ser Gly Ala Leu Thr Ser Gly
 625 630 635 640
 Val His Thr Phe Pro Ala Val Leu Gln Ser Ser Gly Leu Tyr Ser Leu
 645 650 655
 Ser Ser Val Val Thr Val Pro Ser Ser Ser Leu Gly Thr Gln Thr Tyr
 660 665 670
 Ile Cys Asn Val Asn His Lys Pro Ser Asn Thr Lys Val Asp Lys Arg
 675 680 685
 Val Glu Pro Lys Ser Cys Asp Lys Thr His
 690 695

<210> SEQ ID NO 141
 <211> LENGTH: 214
 <212> TYPE: PRT
 <213> ORGANISM: Artificial
 <220> FEATURE:
 <223> OTHER INFORMATION: Chimeric homo sapiens mus musculus

<400> SEQUENCE: 141

Asp Ile Val Met Thr Gln Ser Pro Ala Thr Leu Ser Val Thr Pro Gly
 1 5 10 15
 Asp Arg Val Ser Leu Ser Cys Arg Ala Ser Gln Ser Ile Ser Asp Tyr
 20 25 30
 Leu His Trp Tyr Gln Gln Lys Ser His Glu Ser Pro Arg Leu Leu Ile
 35 40 45
 Lys Tyr Ala Ser Gln Ser Ile Ser Gly Ile Pro Ser Arg Phe Ser Gly
 50 55 60
 Ser Gly Ser Gly Ser Asp Phe Thr Leu Ser Ile Asn Ser Val Glu Pro
 65 70 75 80
 Glu Asp Val Gly Val Tyr Tyr Cys Gln Asn Gly His Ser Phe Pro Leu
 85 90 95
 Thr Phe Gly Ala Gly Thr Lys Leu Glu Leu Lys Arg Thr Val Ala Ala
 100 105 110
 Pro Ser Val Phe Ile Phe Pro Pro Ser Asp Glu Gln Leu Lys Ser Gly
 115 120 125
 Thr Ala Ser Val Val Cys Leu Leu Asn Asn Phe Tyr Pro Arg Glu Ala
 130 135 140

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Lys Val Gln Trp Lys Val Asp Asn Ala Leu Gln Ser Gly Asn Ser Gln
 145 150 155 160

Glu Ser Val Thr Glu Gln Asp Ser Lys Asp Ser Thr Tyr Ser Leu Ser
 165 170 175

Ser Thr Leu Thr Leu Ser Lys Ala Asp Tyr Glu Lys His Lys Val Tyr
 180 185 190

Ala Cys Glu Val Thr His Gln Gly Leu Ser Ser Pro Val Thr Lys Ser
 195 200 205

Phe Asn Arg Gly Glu Cys
 210

<210> SEQ ID NO 142
 <211> LENGTH: 837
 <212> TYPE: PRT
 <213> ORGANISM: Artificial
 <220> FEATURE:
 <223> OTHER INFORMATION: Chimeric homo sapiens mus musculus

<400> SEQUENCE: 142

Asp Ile Gln Leu Thr Gln Ser Pro Ala Ser Leu Ala Val Ser Leu Gly
 1 5 10 15

Gln Arg Ala Thr Ile Ser Cys Lys Ala Ser Gln Ser Val Asp Tyr Asp
 20 25 30

Gly Asp Ser Tyr Leu Asn Trp Tyr Gln Gln Ile Pro Gly Gln Pro Pro
 35 40 45

Lys Leu Leu Ile Tyr Asp Ala Ser Asn Leu Val Ser Gly Ile Pro Pro
 50 55 60

Arg Phe Ser Gly Ser Gly Ser Gly Thr Asp Phe Thr Leu Asn Ile His
 65 70 75 80

Pro Val Glu Lys Val Asp Ala Ala Thr Tyr His Cys Gln Gln Ser Thr
 85 90 95

Glu Asp Pro Trp Thr Phe Gly Gly Gly Thr Lys Leu Glu Ile Lys Gly
 100 105 110

Gly Gly Gly Ser Gly Gly Gly Gly Ser Gly Gly Gly Gly Ser Gln Val
 115 120 125

Gln Leu Gln Gln Ser Gly Ala Glu Leu Val Arg Pro Gly Ser Ser Val
 130 135 140

Lys Ile Ser Cys Lys Ala Ser Gly Tyr Ala Phe Ser Ser Tyr Trp Met
 145 150 155 160

Asn Trp Val Lys Gln Arg Pro Gly Gln Gly Leu Glu Trp Ile Gly Gln
 165 170 175

Ile Trp Pro Gly Asp Gly Asp Thr Asn Tyr Asn Gly Lys Phe Lys Gly
 180 185 190

Lys Ala Thr Leu Thr Ala Asp Glu Ser Ser Ser Thr Ala Tyr Met Gln
 195 200 205

Leu Ser Ser Leu Ala Ser Glu Asp Ser Ala Val Tyr Phe Cys Ala Arg
 210 215 220

Arg Glu Thr Thr Thr Val Gly Arg Tyr Tyr Tyr Ala Met Asp Tyr Trp
 225 230 235 240

Gly Gln Gly Thr Thr Val Thr Val Ser Ser Gly Gly Gly Ser Ser Ala
 245 250 255

Pro Glu Leu Leu Gly Gly Pro Ser Val Phe Leu Phe Pro Pro Lys Pro
 260 265 270

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Lys	Asp	Thr	Leu	Met	Ile	Ser	Arg	Thr	Pro	Glu	Val	Thr	Cys	Val	Val	275	280	285
Val	Asp	Val	Ser	His	Glu	Asp	Pro	Glu	Val	Lys	Phe	Asn	Trp	Tyr	Val	290	295	300
Asp	Gly	Val	Glu	Val	His	Asn	Ala	Lys	Thr	Lys	Pro	Arg	Glu	Glu	Gln	305	310	315
Tyr	Asn	Ser	Thr	Tyr	Arg	Val	Val	Ser	Val	Leu	Thr	Val	Leu	His	Gln	325	330	335
Asp	Trp	Leu	Asn	Gly	Lys	Glu	Tyr	Lys	Cys	Lys	Val	Ser	Asn	Lys	Ala	340	345	350
Leu	Pro	Ala	Pro	Ile	Glu	Lys	Thr	Ile	Ser	Lys	Ala	Lys	Gly	Gln	Pro	355	360	365
Arg	Glu	Pro	Gln	Val	Tyr	Thr	Leu	Pro	Pro	Ser	Arg	Glu	Glu	Met	Thr	370	375	380
Lys	Asn	Gln	Val	Ser	Leu	Thr	Cys	Leu	Val	Lys	Gly	Phe	Tyr	Pro	Ser	385	390	395
Asp	Ile	Ala	Val	Glu	Trp	Glu	Ser	Asn	Gly	Gln	Pro	Glu	Asn	Asn	Tyr	405	410	415
Lys	Thr	Thr	Pro	Pro	Val	Leu	Asp	Ser	Asp	Gly	Ser	Phe	Phe	Leu	Tyr	420	425	430
Ser	Lys	Leu	Thr	Val	Asp	Lys	Ser	Arg	Trp	Gln	Gln	Gly	Asn	Val	Phe	435	440	445
Ser	Cys	Ser	Val	Met	His	Glu	Ala	Leu	His	Asn	His	Tyr	Thr	Gln	Lys	450	455	460
Ser	Leu	Ser	Leu	Ser	Pro	Gly	Gly	Gly	Gly	Gly	Ser	Gly	Gly	Gly	Gly	465	470	475
Ser	Gly	Gly	Gly	Gly	Ser	Gly	Gln	Pro	Arg	Glu	Pro	Gln	Val	Tyr	Thr	485	490	495
Leu	Pro	Pro	Ser	Arg	Glu	Glu	Met	Thr	Lys	Asn	Gln	Val	Ser	Leu	Thr	500	505	510
Cys	Leu	Val	Lys	Gly	Phe	Tyr	Pro	Ser	Asp	Ile	Ala	Val	Glu	Trp	Glu	515	520	525
Ser	Asn	Gly	Gln	Pro	Glu	Asn	Asn	Tyr	Lys	Thr	Thr	Pro	Pro	Val	Leu	530	535	540
Asp	Ser	Asp	Gly	Ser	Phe	Phe	Leu	Tyr	Ser	Lys	Leu	Thr	Val	Asp	Lys	545	550	555
Ser	Arg	Trp	Gln	Gln	Gly	Asn	Val	Phe	Ser	Cys	Ser	Val	Met	His	Glu	565	570	575
Ala	Leu	His	Asn	His	Tyr	Thr	Gln	Lys	Ser	Leu	Ser	Leu	Ser	Pro	Gly	580	585	590
Ser	Thr	Gly	Ser	Glu	Val	Gln	Leu	Gln	Gln	Ser	Gly	Pro	Glu	Leu	Val	595	600	605
Lys	Pro	Gly	Ala	Ser	Val	Lys	Ile	Ser	Cys	Lys	Thr	Ser	Gly	Tyr	Thr	610	615	620
Phe	Thr	Glu	Tyr	Thr	Met	His	Trp	Val	Lys	Gln	Ser	His	Gly	Lys	Ser	625	630	635
Leu	Glu	Trp	Ile	Gly	Gly	Ile	Ser	Pro	Asn	Ile	Gly	Gly	Thr	Ser	Tyr	645	650	655
Asn	Gln	Lys	Phe	Lys	Gly	Lys	Ala	Thr	Leu	Thr	Val	Asp	Lys	Ser	Ser	660	665	670

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Ser Thr Ala Tyr Met Glu Leu Arg Ser Leu Thr Ser Glu Asp Ser Ala
   675                               680                               685

Val Tyr Tyr Cys Ala Arg Arg Gly Gly Ser Phe Asp Tyr Trp Gly Gln
   690                               695                               700

Gly Thr Thr Leu Thr Val Ser Ser Val Glu Gly Gly Ser Gly Gly Ser
  705                               710                               715                               720

Gly Gly Ser Gly Gly Ser Gly Gly Val Asp Asp Ile Val Met Thr Gln
                               725                               730                               735

Ser Pro Ala Thr Leu Ser Val Thr Pro Gly Asp Arg Val Ser Leu Ser
   740                               745                               750

Cys Arg Ala Ser Gln Ser Ile Ser Asp Tyr Leu His Trp Tyr Gln Gln
   755                               760                               765

Lys Ser His Glu Ser Pro Arg Leu Leu Ile Lys Tyr Ala Ser Gln Ser
   770                               775                               780

Ile Ser Gly Ile Pro Ser Arg Phe Ser Gly Ser Gly Ser Gly Ser Asp
  785                               790                               795                               800

Phe Thr Leu Ser Ile Asn Ser Val Glu Pro Glu Asp Val Gly Val Tyr
   805                               810                               815

Tyr Cys Gln Asn Gly His Ser Phe Pro Leu Thr Phe Gly Ala Gly Thr
   820                               825                               830

Lys Leu Glu Leu Lys
   835

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<210> SEQ ID NO 143
<211> LENGTH: 837
<212> TYPE: PRT
<213> ORGANISM: Artificial
<220> FEATURE:
<223> OTHER INFORMATION: Chimeric homo sapiens mus musculus

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<400> SEQUENCE: 143

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Asp Ile Gln Leu Thr Gln Ser Pro Ala Ser Leu Ala Val Ser Leu Gly
  1                               5                               10                               15

Gln Arg Ala Thr Ile Ser Cys Lys Ala Ser Gln Ser Val Asp Tyr Asp
   20                               25                               30

Gly Asp Ser Tyr Leu Asn Trp Tyr Gln Gln Ile Pro Gly Gln Pro Pro
   35                               40                               45

Lys Leu Leu Ile Tyr Asp Ala Ser Asn Leu Val Ser Gly Ile Pro Pro
   50                               55                               60

Arg Phe Ser Gly Ser Gly Ser Gly Thr Asp Phe Thr Leu Asn Ile His
  65                               70                               75                               80

Pro Val Glu Lys Val Asp Ala Ala Thr Tyr His Cys Gln Gln Ser Thr
   85                               90                               95

Glu Asp Pro Trp Thr Phe Gly Gly Gly Thr Lys Leu Glu Ile Lys Gly
  100                               105                               110

Gly Gly Gly Ser Gly Gly Gly Gly Ser Gly Gly Gly Gly Ser Gln Val
  115                               120                               125

Gln Leu Gln Gln Ser Gly Ala Glu Leu Val Arg Pro Gly Ser Ser Val
  130                               135                               140

Lys Ile Ser Cys Lys Ala Ser Gly Tyr Ala Phe Ser Ser Tyr Trp Met
  145                               150                               155                               160

Asn Trp Val Lys Gln Arg Pro Gly Gln Gly Leu Glu Trp Ile Gly Gln
  165                               170                               175

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Ile	Trp	Pro	Gly	Asp	Gly	Asp	Thr	Asn	Tyr	Asn	Gly	Lys	Phe	Lys	Gly	180	185	190
Lys	Ala	Thr	Leu	Thr	Ala	Asp	Glu	Ser	Ser	Ser	Thr	Ala	Tyr	Met	Gln	195	200	205
Leu	Ser	Ser	Leu	Ala	Ser	Glu	Asp	Ser	Ala	Val	Tyr	Phe	Cys	Ala	Arg	210	215	220
Arg	Glu	Thr	Thr	Thr	Val	Gly	Arg	Tyr	Tyr	Tyr	Ala	Met	Asp	Tyr	Trp	225	230	235
Gly	Gln	Gly	Thr	Thr	Val	Thr	Val	Ser	Ser	Gly	Gly	Gly	Ser	Ser	Ala	245	250	255
Pro	Glu	Leu	Leu	Gly	Gly	Pro	Ser	Val	Phe	Leu	Phe	Pro	Pro	Lys	Pro	260	265	270
Lys	Asp	Thr	Leu	Met	Ile	Ser	Arg	Thr	Pro	Glu	Val	Thr	Cys	Val	Val	275	280	285
Val	Asp	Val	Ser	His	Glu	Asp	Pro	Glu	Val	Lys	Phe	Asn	Trp	Tyr	Val	290	295	300
Asp	Gly	Val	Glu	Val	His	Asn	Ala	Lys	Thr	Lys	Pro	Arg	Glu	Glu	Gln	305	310	315
Tyr	Ser	Ser	Thr	Tyr	Arg	Val	Val	Ser	Val	Leu	Thr	Val	Leu	His	Gln	325	330	335
Asp	Trp	Leu	Asn	Gly	Lys	Glu	Tyr	Lys	Cys	Lys	Val	Ser	Asn	Lys	Ala	340	345	350
Leu	Pro	Ala	Pro	Ile	Glu	Lys	Thr	Ile	Ser	Lys	Ala	Lys	Gly	Gln	Pro	355	360	365
Arg	Glu	Pro	Gln	Val	Tyr	Thr	Leu	Pro	Pro	Ser	Arg	Glu	Glu	Met	Thr	370	375	380
Lys	Asn	Gln	Val	Ser	Leu	Thr	Cys	Leu	Val	Lys	Gly	Phe	Tyr	Pro	Ser	385	390	395
Asp	Ile	Ala	Val	Glu	Trp	Glu	Ser	Asn	Gly	Gln	Pro	Glu	Asn	Asn	Tyr	405	410	415
Lys	Thr	Thr	Pro	Pro	Val	Leu	Asp	Ser	Asp	Gly	Ser	Phe	Phe	Leu	Tyr	420	425	430
Ser	Lys	Leu	Thr	Val	Asp	Lys	Ser	Arg	Trp	Gln	Gln	Gly	Asn	Val	Phe	435	440	445
Ser	Cys	Ser	Val	Met	His	Glu	Ala	Leu	His	Asn	His	Tyr	Thr	Gln	Lys	450	455	460
Ser	Leu	Ser	Leu	Ser	Pro	Gly	Gly	Gly	Gly	Gly	Ser	Gly	Gly	Gly	Gly	465	470	475
Ser	Gly	Gly	Gly	Gly	Ser	Gly	Gln	Pro	Arg	Glu	Pro	Gln	Val	Tyr	Thr	485	490	495
Leu	Pro	Pro	Ser	Arg	Glu	Glu	Met	Thr	Lys	Asn	Gln	Val	Ser	Leu	Thr	500	505	510
Cys	Leu	Val	Lys	Gly	Phe	Tyr	Pro	Ser	Asp	Ile	Ala	Val	Glu	Trp	Glu	515	520	525
Ser	Asn	Gly	Gln	Pro	Glu	Asn	Asn	Tyr	Lys	Thr	Thr	Pro	Pro	Val	Leu	530	535	540
Asp	Ser	Asp	Gly	Ser	Phe	Phe	Leu	Tyr	Ser	Lys	Leu	Thr	Val	Asp	Lys	545	550	555
Ser	Arg	Trp	Gln	Gln	Gly	Asn	Val	Phe	Ser	Cys	Ser	Val	Met	His	Glu	565	570	575
Ala	Leu	His	Asn	His	Tyr	Thr	Gln	Lys	Ser	Leu	Ser	Leu	Ser	Pro	Gly			

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580					585					590						
Ser	Thr	Gly	Ser	Glu	Val	Gln	Leu	Gln	Gln	Ser	Gly	Pro	Glu	Leu	Val	
595					600					605						
Lys	Pro	Gly	Ala	Ser	Val	Lys	Ile	Ser	Cys	Lys	Thr	Ser	Gly	Tyr	Thr	
610					615					620						
Phe	Thr	Glu	Tyr	Thr	Met	His	Trp	Val	Lys	Gln	Ser	His	Gly	Lys	Ser	
625					630					635					640	
Leu	Glu	Trp	Ile	Gly	Gly	Ile	Ser	Pro	Asn	Ile	Gly	Gly	Thr	Ser	Tyr	
645					650					655						
Asn	Gln	Lys	Phe	Lys	Gly	Lys	Ala	Thr	Leu	Thr	Val	Asp	Lys	Ser	Ser	
660					665					670						
Ser	Thr	Ala	Tyr	Met	Glu	Leu	Arg	Ser	Leu	Thr	Ser	Glu	Asp	Ser	Ala	
675					680					685						
Val	Tyr	Tyr	Cys	Ala	Arg	Arg	Gly	Gly	Ser	Phe	Asp	Tyr	Trp	Gly	Gln	
690					695					700						
Gly	Thr	Thr	Leu	Thr	Val	Ser	Ser	Val	Glu	Gly	Gly	Ser	Gly	Gly	Ser	
705					710					715					720	
Gly	Gly	Ser	Gly	Gly	Ser	Gly	Gly	Val	Asp	Asp	Ile	Val	Met	Thr	Gln	
725					730					735						
Ser	Pro	Ala	Thr	Leu	Ser	Val	Thr	Pro	Gly	Asp	Arg	Val	Ser	Leu	Ser	
740					745					750						
Cys	Arg	Ala	Ser	Gln	Ser	Ile	Ser	Asp	Tyr	Leu	His	Trp	Tyr	Gln	Gln	
755					760					765						
Lys	Ser	His	Glu	Ser	Pro	Arg	Leu	Leu	Ile	Lys	Tyr	Ala	Ser	Gln	Ser	
770					775					780						
Ile	Ser	Gly	Ile	Pro	Ser	Arg	Phe	Ser	Gly	Ser	Gly	Ser	Gly	Ser	Asp	
785					790					795					800	
Phe	Thr	Leu	Ser	Ile	Asn	Ser	Val	Glu	Pro	Glu	Asp	Val	Gly	Val	Tyr	
805					810					815						
Tyr	Cys	Gln	Asn	Gly	His	Ser	Phe	Pro	Leu	Thr	Phe	Gly	Ala	Gly	Thr	
820					825					830						
Lys	Leu	Glu	Leu	Lys												
835																

<210> SEQ ID NO 144

<211> LENGTH: 218

<212> TYPE: PRT

<213> ORGANISM: Artificial

<220> FEATURE:

<223> OTHER INFORMATION: Chimeric homo sapiens mus musculus

<400> SEQUENCE: 144

Asp	Ile	Gln	Leu	Thr	Gln	Ser	Pro	Ala	Ser	Leu	Ala	Val	Ser	Leu	Gly
1	5				10				15						
Gln	Arg	Ala	Thr	Ile	Ser	Cys	Lys	Ala	Ser	Gln	Ser	Val	Asp	Tyr	Asp
20				25				30							
Gly	Asp	Ser	Tyr	Leu	Asn	Trp	Tyr	Gln	Gln	Ile	Pro	Gly	Gln	Pro	Pro
35				40				45							
Lys	Leu	Leu	Ile	Tyr	Asp	Ala	Ser	Asn	Leu	Val	Ser	Gly	Ile	Pro	Pro
50				55				60							
Arg	Phe	Ser	Gly	Ser	Gly	Ser	Gly	Thr	Asp	Phe	Thr	Leu	Asn	Ile	His
65				70				75				80			
Pro	Val	Glu	Lys	Val	Asp	Ala	Ala	Thr	Tyr	His	Cys	Gln	Gln	Ser	Thr

-continued

85					90					95					
Glu	Asp	Pro	Trp	Thr	Phe	Gly	Gly	Gly	Thr	Lys	Leu	Glu	Ile	Lys	Arg
			100					105					110		
Thr	Val	Ala	Ala	Pro	Ser	Val	Phe	Ile	Phe	Pro	Pro	Ser	Asp	Glu	Gln
			115					120					125		
Leu	Lys	Ser	Gly	Thr	Ala	Ser	Val	Val	Cys	Leu	Leu	Asn	Asn	Phe	Tyr
			130					135					140		
Pro	Arg	Glu	Ala	Lys	Val	Gln	Trp	Lys	Val	Asp	Asn	Ala	Leu	Gln	Ser
			145					150					155		160
Gly	Asn	Ser	Gln	Glu	Ser	Val	Thr	Glu	Gln	Asp	Ser	Lys	Asp	Ser	Thr
			165					170					175		
Tyr	Ser	Leu	Ser	Ser	Thr	Leu	Thr	Leu	Ser	Lys	Ala	Asp	Tyr	Glu	Lys
			180					185					190		
His	Lys	Val	Tyr	Ala	Cys	Glu	Val	Thr	His	Gln	Gly	Leu	Ser	Ser	Pro
			195					200					205		
Val	Thr	Lys	Ser	Phe	Asn	Arg	Gly	Glu	Cys						
			210					215							

<210> SEQ ID NO 145

<211> LENGTH: 675

<212> TYPE: PRT

<213> ORGANISM: Artificial

<220> FEATURE:

<223> OTHER INFORMATION: Chimeric homo sapiens mus musculus

<400> SEQUENCE: 145

Gln	Val	Gln	Leu	Gln	Gln	Ser	Gly	Ala	Glu	Leu	Val	Arg	Pro	Gly	Ser
1				5					10					15	
Ser	Val	Lys	Ile	Ser	Cys	Lys	Ala	Ser	Gly	Tyr	Ala	Phe	Ser	Ser	Tyr
			20					25					30		
Trp	Met	Asn	Trp	Val	Lys	Gln	Arg	Pro	Gly	Gln	Gly	Leu	Glu	Trp	Ile
			35				40					45			
Gly	Gln	Ile	Trp	Pro	Gly	Asp	Gly	Asp	Thr	Asn	Tyr	Asn	Gly	Lys	Phe
			50				55					60			
Lys	Gly	Lys	Ala	Thr	Leu	Thr	Ala	Asp	Glu	Ser	Ser	Ser	Thr	Ala	Tyr
			65				70			75				80	
Met	Gln	Leu	Ser	Ser	Leu	Ala	Ser	Glu	Asp	Ser	Ala	Val	Tyr	Phe	Cys
				85					90					95	
Ala	Arg	Arg	Glu	Thr	Thr	Thr	Val	Gly	Arg	Tyr	Tyr	Tyr	Ala	Met	Asp
			100					105					110		
Tyr	Trp	Gly	Gln	Gly	Thr	Thr	Val	Thr	Val	Ser	Ser	Ala	Ser	Thr	Lys
			115				120					125			
Gly	Pro	Ser	Val	Phe	Pro	Leu	Ala	Pro	Ser	Ser	Lys	Ser	Thr	Ser	Gly
			130				135					140			
Gly	Thr	Ala	Ala	Leu	Gly	Cys	Leu	Val	Lys	Asp	Tyr	Phe	Pro	Glu	Pro
			145				150			155				160	
Val	Thr	Val	Ser	Trp	Asn	Ser	Gly	Ala	Leu	Thr	Ser	Gly	Val	His	Thr
				165					170					175	
Phe	Pro	Ala	Val	Leu	Gln	Ser	Ser	Gly	Leu	Tyr	Ser	Leu	Ser	Ser	Val
			180					185					190		
Val	Thr	Val	Pro	Ser	Ser	Ser	Leu	Gly	Thr	Gln	Thr	Tyr	Ile	Cys	Asn
			195				200					205			
Val	Asn	His	Lys	Pro	Ser	Asn	Thr	Lys	Val	Asp	Lys	Arg	Val	Glu	Pro

-continued

210	215	220
Lys Ser Cys Gly Gly Gly Ser Ser Ala Pro Glu Leu Leu Gly Gly Pro		
225	230	235 240
Ser Val Phe Leu Phe Pro Pro Lys Pro Lys Asp Thr Leu Met Ile Ser		
	245	250 255
Arg Thr Pro Glu Val Thr Cys Val Val Val Asp Val Ser His Glu Asp		
	260	265 270
Pro Glu Val Lys Phe Asn Trp Tyr Val Asp Gly Val Glu Val His Asn		
	275	280 285
Ala Lys Thr Lys Pro Arg Glu Glu Gln Tyr Ser Ser Thr Tyr Arg Val		
	290	295 300
Val Ser Val Leu Thr Val Leu His Gln Asp Trp Leu Asn Gly Lys Glu		
305	310	315 320
Tyr Lys Cys Lys Val Ser Asn Lys Ala Leu Pro Ala Pro Ile Glu Lys		
	325	330 335
Thr Ile Ser Lys Ala Lys Gly Gln Pro Arg Glu Pro Gln Val Tyr Thr		
	340	345 350
Lys Pro Pro Ser Arg Glu Glu Met Thr Lys Asn Gln Val Ser Leu Ser		
	355	360 365
Cys Leu Val Lys Gly Phe Tyr Pro Ser Asp Ile Ala Val Glu Trp Glu		
	370	375 380
Ser Asn Gly Gln Pro Glu Asn Asn Tyr Lys Thr Thr Val Pro Val Leu		
385	390	395 400
Asp Ser Asp Gly Ser Phe Arg Leu Ala Ser Tyr Leu Thr Val Asp Lys		
	405	410 415
Ser Arg Trp Gln Gln Gly Asn Val Phe Ser Cys Ser Val Met His Glu		
	420	425 430
Ala Leu His Asn His Tyr Thr Gln Lys Ser Leu Ser Leu Ser Pro Gly		
	435	440 445
Ser Thr Gly Ser Glu Val Gln Leu Gln Gln Ser Gly Pro Glu Leu Val		
	450	455 460
Lys Pro Gly Ala Ser Val Lys Ile Ser Cys Lys Thr Ser Gly Tyr Thr		
465	470	475 480
Phe Thr Glu Tyr Thr Met His Trp Val Lys Gln Ser His Gly Lys Ser		
	485	490 495
Leu Glu Trp Ile Gly Gly Ile Ser Pro Asn Ile Gly Gly Thr Ser Tyr		
	500	505 510
Asn Gln Lys Phe Lys Gly Lys Ala Thr Leu Thr Val Asp Lys Ser Ser		
	515	520 525
Ser Thr Ala Tyr Met Glu Leu Arg Ser Leu Thr Ser Glu Asp Ser Ala		
	530	535 540
Val Tyr Tyr Cys Ala Arg Arg Gly Gly Ser Phe Asp Tyr Trp Gly Gln		
545	550	555 560
Gly Thr Thr Leu Thr Val Ser Ser Arg Thr Val Ala Ala Pro Ser Val		
	565	570 575
Phe Ile Phe Pro Pro Ser Asp Glu Gln Leu Lys Ser Gly Thr Ala Ser		
	580	585 590
Val Val Cys Leu Leu Asn Asn Phe Tyr Pro Arg Glu Ala Lys Val Gln		
	595	600 605
Trp Lys Val Asp Asn Ala Leu Gln Ser Gly Asn Ser Gln Glu Ser Val		
	610	615 620

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Thr Glu Gln Asp Ser Lys Asp Ser Thr Tyr Ser Leu Ser Ser Thr Leu
625 630 635 640

Thr Leu Ser Lys Ala Asp Tyr Glu Lys His Lys Val Tyr Ala Cys Glu
645 650 655

Val Thr His Gln Gly Leu Ser Ser Pro Val Thr Lys Ser Phe Asn Arg
660 665 670

Gly Glu Cys
675

<210> SEQ ID NO 146
 <211> LENGTH: 214
 <212> TYPE: PRT
 <213> ORGANISM: Artificial
 <220> FEATURE:
 <223> OTHER INFORMATION: Chimeric homo sapiens mus musculus

<400> SEQUENCE: 146

Asp Ile Val Met Thr Gln Ser Pro Ala Thr Leu Ser Val Thr Pro Gly
1 5 10 15

Asp Arg Val Ser Leu Ser Cys Arg Ala Ser Gln Ser Ile Ser Asp Tyr
20 25 30

Leu His Trp Tyr Gln Gln Lys Ser His Glu Ser Pro Arg Leu Leu Ile
35 40 45

Lys Tyr Ala Ser Gln Ser Ile Ser Gly Ile Pro Ser Arg Phe Ser Gly
50 55 60

Ser Gly Ser Gly Ser Asp Phe Thr Leu Ser Ile Asn Ser Val Glu Pro
65 70 75 80

Glu Asp Val Gly Val Tyr Tyr Cys Gln Asn Gly His Ser Phe Pro Leu
85 90 95

Thr Phe Gly Ala Gly Thr Lys Leu Glu Leu Lys Ala Ser Thr Lys Gly
100 105 110

Pro Ser Val Phe Pro Leu Ala Pro Ser Ser Lys Ser Thr Ser Gly Gly
115 120 125

Thr Ala Ala Leu Gly Cys Leu Val Lys Asp Tyr Phe Pro Glu Pro Val
130 135 140

Thr Val Ser Trp Asn Ser Gly Ala Leu Thr Ser Gly Val His Thr Phe
145 150 155 160

Pro Ala Val Leu Gln Ser Ser Gly Leu Tyr Ser Leu Ser Ser Val Val
165 170 175

Thr Val Pro Ser Ser Ser Leu Gly Thr Gln Thr Tyr Ile Cys Asn Val
180 185 190

Asn His Lys Pro Ser Asn Thr Lys Val Asp Lys Arg Val Glu Pro Lys
195 200 205

Ser Cys Asp Lys Thr His
210

<210> SEQ ID NO 147
 <211> LENGTH: 218
 <212> TYPE: PRT
 <213> ORGANISM: Artificial
 <220> FEATURE:
 <223> OTHER INFORMATION: Chimeric homo sapiens mus musculus

<400> SEQUENCE: 147

Asp Ile Gln Leu Thr Gln Ser Pro Ala Ser Leu Ala Val Ser Leu Gly

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1	5	10	15
Gln Arg Ala Thr Ile Ser Cys Lys Ala Ser Gln Ser Val Asp Tyr Asp	20	25	30
Gly Asp Ser Tyr Leu Asn Trp Tyr Gln Gln Ile Pro Gly Gln Pro Pro	35	40	45
Lys Leu Leu Ile Tyr Asp Ala Ser Asn Leu Val Ser Gly Ile Pro Pro	50	55	60
Arg Phe Ser Gly Ser Gly Ser Gly Thr Asp Phe Thr Leu Asn Ile His	65	70	75
Pro Val Glu Lys Val Asp Ala Ala Thr Tyr His Cys Gln Gln Ser Thr	85	90	95
Glu Asp Pro Trp Thr Phe Gly Gly Gly Thr Lys Leu Glu Ile Lys Arg	100	105	110
Thr Val Ala Ala Pro Ser Val Phe Ile Phe Pro Pro Ser Asp Glu Gln	115	120	125
Leu Lys Ser Gly Thr Ala Ser Val Val Cys Leu Leu Asn Asn Phe Tyr	130	135	140
Pro Arg Glu Ala Lys Val Gln Trp Lys Val Asp Asn Ala Leu Gln Ser	145	150	155
Gly Asn Ser Gln Glu Ser Val Thr Glu Gln Asp Ser Lys Asp Ser Thr	165	170	175
Tyr Ser Leu Ser Ser Thr Leu Thr Leu Ser Lys Ala Asp Tyr Glu Lys	180	185	190
His Lys Val Tyr Ala Cys Glu Val Thr His Gln Gly Leu Ser Ser Pro	195	200	205
Val Thr Lys Ser Phe Asn Arg Gly Glu Cys	210	215	

<210> SEQ ID NO 148

<211> LENGTH: 801

<212> TYPE: PRT

<213> ORGANISM: Artificial

<220> FEATURE:

<223> OTHER INFORMATION: Chimeric homo sapiens mus musculus

<400> SEQUENCE: 148

Gln Val Gln Leu Gln Gln Ser Gly Ala Glu Leu Val Arg Pro Gly Ser	1	5	10	15
Ser Val Lys Ile Ser Cys Lys Ala Ser Gly Tyr Ala Phe Ser Ser Tyr	20	25	30	
Trp Met Asn Trp Val Lys Gln Arg Pro Gly Gln Gly Leu Glu Trp Ile	35	40	45	
Gly Gln Ile Trp Pro Gly Asp Gly Asp Thr Asn Tyr Asn Gly Lys Phe	50	55	60	
Lys Gly Lys Ala Thr Leu Thr Ala Asp Glu Ser Ser Ser Thr Ala Tyr	65	70	75	80
Met Gln Leu Ser Ser Leu Ala Ser Glu Asp Ser Ala Val Tyr Phe Cys	85	90	95	
Ala Arg Arg Glu Thr Thr Thr Val Gly Arg Tyr Tyr Tyr Ala Met Asp	100	105	110	
Tyr Trp Gly Gln Gly Thr Thr Val Thr Val Ser Ser Ala Ser Thr Lys	115	120	125	
Gly Pro Ser Val Phe Pro Leu Ala Pro Ser Ser Lys Ser Thr Ser Gly				

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130					135					140					
Gly 145	Thr	Ala	Ala	Leu	Gly 150	Cys	Leu	Val	Lys	Asp 155	Tyr	Phe	Pro	Glu	Pro 160
Val	Thr	Val	Ser	Trp 165	Asn	Ser	Gly	Ala	Leu	Thr 170	Ser	Gly	Val	His	Thr 175
Phe	Pro	Ala	Val	Leu	Gln	Ser	Ser	Gly 185	Leu	Tyr	Ser	Leu	Ser	Ser	Val 190
Val	Thr	Val	Pro	Ser	Ser	Ser	Leu	Gly	Thr	Gln	Thr	Tyr 205	Ile	Cys	Asn
Val	Asn	His	Lys	Pro	Ser	Asn 215	Thr	Lys	Val	Asp	Lys 220	Arg	Val	Glu	Pro
Lys 225	Ser	Cys	Asp	Lys	Thr 230	His	Thr	Cys	Pro	Pro 235	Cys	Pro	Ala	Pro	Glu 240
Leu	Leu	Gly	Gly	Pro 245	Ser	Val	Phe	Leu	Phe 250	Pro	Pro	Lys	Pro	Lys	Asp 255
Thr	Leu	Met	Ile 260	Ser	Arg	Thr	Pro	Glu 265	Val	Thr	Cys	Val	Val 270	Val	Asp
Val	Ser	His	Glu	Asp	Pro	Glu 280	Val	Lys	Phe	Asn	Trp	Tyr 285	Val	Asp	Gly
Val	Glu 290	Val	His	Asn	Ala	Lys 295	Thr	Lys	Pro	Arg	Glu 300	Glu	Gln	Tyr	Ser
Ser 305	Thr	Tyr	Arg	Val	Val 310	Ser	Val	Leu	Thr	Val 315	Leu	His	Gln	Asp	Trp 320
Leu	Asn	Gly	Lys	Glu 325	Tyr	Lys	Cys	Lys	Val 330	Ser	Asn	Lys	Ala	Leu	Pro 335
Ala	Pro	Ile	Glu	Lys	Thr	Ile	Ser	Lys 345	Ala	Lys	Gly	Gln	Pro 350	Arg	Glu
Pro	Gln 355	Val	Tyr	Thr	Leu	Pro	Pro 360	Ser	Arg	Glu	Glu	Met 365	Thr	Lys	Asn
Gln 370	Val	Ser	Leu	Thr	Cys	Leu 375	Val	Lys	Gly	Phe 380	Tyr	Pro	Ser	Asp	Ile
Ala 385	Val	Glu	Trp	Glu	Ser 390	Asn	Gly	Gln	Pro	Glu 395	Asn	Asn	Tyr	Lys	Thr 400
Thr	Pro	Pro	Val	Leu 405	Asp	Ser	Asp	Gly	Ser 410	Phe	Phe	Leu	Tyr	Ser	Lys 415
Leu	Thr	Val	Asp 420	Lys	Ser	Arg	Trp	Gln 425	Gln	Gly	Asn	Val	Phe 430	Ser	Cys
Ser	Val	Met 435	His	Glu	Ala	Leu	His 440	Asn	His	Tyr	Thr	Gln 445	Lys	Ser	Leu
Ser 450	Leu	Ser	Pro	Gly	Gly 455	Gly	Gly	Gly	Ser	Gly 460	Gly	Gly	Gly	Ser	Gly
Gly 465	Gly	Gly	Ser	Gly	Gln 470	Pro	Arg	Glu	Pro	Gln 475	Val	Tyr	Thr	Leu	Pro 480
Pro	Ser	Arg	Glu	Glu	Met 485	Thr	Lys	Asn	Gln 490	Val	Ser	Leu	Thr	Cys	Leu 495
Val	Lys	Gly	Phe 500	Tyr	Pro	Ser	Asp	Ile 505	Ala	Val	Glu	Trp	Glu 510	Ser	Asn
Gly	Gln 515	Pro	Glu	Asn	Asn	Tyr	Lys 520	Thr	Thr	Pro	Pro	Val 525	Leu	Asp	Ser
Asp 530	Gly	Ser	Phe	Phe	Leu	Tyr 535	Ser	Lys	Leu	Thr	Val 540	Asp	Lys	Ser	Arg

-continued

Trp Gln Gln Gly Asn Val Phe Ser Cys Ser Val Met His Glu Ala Leu
 545 550 555 560
 His Asn His Tyr Thr Gln Lys Ser Leu Ser Leu Ser Pro Gly Ser Thr
 565 570 575
 Gly Ser Glu Val Gln Leu Gln Gln Ser Gly Pro Glu Leu Val Lys Pro
 580 585 590
 Gly Ala Ser Val Lys Ile Ser Cys Lys Thr Ser Gly Tyr Thr Phe Thr
 595 600 605
 Glu Tyr Thr Met His Trp Val Lys Gln Ser His Gly Lys Ser Leu Glu
 610 615 620
 Trp Ile Gly Gly Ile Ser Pro Asn Ile Gly Gly Thr Ser Tyr Asn Gln
 625 630 635 640
 Lys Phe Lys Gly Lys Ala Thr Leu Thr Val Asp Lys Ser Ser Ser Thr
 645 650 655
 Ala Tyr Met Glu Leu Arg Ser Leu Thr Ser Glu Asp Ser Ala Val Tyr
 660 665 670
 Tyr Cys Ala Arg Arg Gly Gly Ser Phe Asp Tyr Trp Gly Gln Gly Thr
 675 680 685
 Thr Leu Thr Val Ser Ser Arg Thr Val Ala Ala Pro Ser Val Phe Ile
 690 695 700
 Phe Pro Pro Ser Asp Glu Gln Leu Lys Ser Gly Thr Ala Ser Val Val
 705 710 715 720
 Cys Leu Leu Asn Asn Phe Tyr Pro Arg Glu Ala Lys Val Gln Trp Lys
 725 730 735
 Val Asp Asn Ala Leu Gln Ser Gly Asn Ser Gln Glu Ser Val Thr Glu
 740 745 750
 Gln Asp Ser Lys Asp Ser Thr Tyr Ser Leu Ser Ser Thr Leu Thr Leu
 755 760 765
 Ser Lys Ala Asp Tyr Glu Lys His Lys Val Tyr Ala Cys Glu Val Thr
 770 775 780
 His Gln Gly Leu Ser Ser Pro Val Thr Lys Ser Phe Asn Arg Gly Glu
 785 790 795 800
 Cys

<210> SEQ ID NO 149
 <211> LENGTH: 214
 <212> TYPE: PRT
 <213> ORGANISM: Artificial
 <220> FEATURE:
 <223> OTHER INFORMATION: Chimeric homo sapiens mus musculus

<400> SEQUENCE: 149

Asp Ile Val Met Thr Gln Ser Pro Ala Thr Leu Ser Val Thr Pro Gly
 1 5 10 15
 Asp Arg Val Ser Leu Ser Cys Arg Ala Ser Gln Ser Ile Ser Asp Tyr
 20 25 30
 Leu His Trp Tyr Gln Gln Lys Ser His Glu Ser Pro Arg Leu Leu Ile
 35 40 45
 Lys Tyr Ala Ser Gln Ser Ile Ser Gly Ile Pro Ser Arg Phe Ser Gly
 50 55 60
 Ser Gly Ser Gly Ser Asp Phe Thr Leu Ser Ile Asn Ser Val Glu Pro
 65 70 75 80

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<210> SEQ ID NO 150
<211> LENGTH: 218
<212> TYPE: PRT
<213> ORGANISM: Artificial
<220> FEATURE:
<223> OTHER INFORMATION: Chimeric homo sapiens mus musculus

<400> SEQUENCE: 150

Asp Ile Gln Leu Thr Gln Ser Pro Ala Ser Leu Ala Val Ser Leu Gly
1          5          10          15
Gln Arg Ala Thr Ile Ser Cys Lys Ala Ser Gln Ser Val Asp Tyr Asp
20          25          30
Gly Asp Ser Tyr Leu Asn Trp Tyr Gln Gln Ile Pro Gly Gln Pro Pro
35          40          45
Lys Leu Leu Ile Tyr Asp Ala Ser Asn Leu Val Ser Gly Ile Pro Pro
50          55          60
Arg Phe Ser Gly Ser Gly Ser Gly Thr Asp Phe Thr Leu Asn Ile His
65          70          75          80
Pro Val Glu Lys Val Asp Ala Ala Thr Tyr His Cys Gln Gln Ser Thr
85          90          95
Glu Asp Pro Trp Thr Phe Gly Gly Gly Thr Lys Leu Glu Ile Lys Arg
100         105         110
Thr Val Ala Ala Pro Ser Val Phe Ile Phe Pro Pro Ser Asp Glu Gln
115         120         125
Leu Lys Ser Gly Thr Ala Ser Val Val Cys Leu Leu Asn Asn Phe Tyr
130         135         140
Pro Arg Glu Ala Lys Val Gln Trp Lys Val Asp Asn Ala Leu Gln Ser
145         150         155         160
Gly Asn Ser Gln Glu Ser Val Thr Glu Gln Asp Ser Lys Asp Ser Thr
165         170         175
Tyr Ser Leu Ser Ser Thr Leu Thr Leu Ser Lys Ala Asp Tyr Glu Lys
180         185         190
His Lys Val Tyr Ala Cys Glu Val Thr His Gln Gly Leu Ser Ser Pro
195         200         205

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Val Thr Lys Ser Phe Asn Arg Gly Glu Cys
 210 215

<210> SEQ ID NO 151
 <211> LENGTH: 801
 <212> TYPE: PRT
 <213> ORGANISM: Artificial
 <220> FEATURE:
 <223> OTHER INFORMATION: Chimeric homo sapiens mus musculus

<400> SEQUENCE: 151

Gln Val Gln Leu Gln Gln Ser Gly Ala Glu Leu Val Arg Pro Gly Ser
 1 5 10 15

Ser Val Lys Ile Ser Cys Lys Ala Ser Gly Tyr Ala Phe Ser Ser Tyr
 20 25 30

Trp Met Asn Trp Val Lys Gln Arg Pro Gly Gln Gly Leu Glu Trp Ile
 35 40 45

Gly Gln Ile Trp Pro Gly Asp Gly Asp Thr Asn Tyr Asn Gly Lys Phe
 50 55 60

Lys Gly Lys Ala Thr Leu Thr Ala Asp Glu Ser Ser Ser Thr Ala Tyr
 65 70 75 80

Met Gln Leu Ser Ser Leu Ala Ser Glu Asp Ser Ala Val Tyr Phe Cys
 85 90 95

Ala Arg Arg Glu Thr Thr Thr Val Gly Arg Tyr Tyr Tyr Ala Met Asp
 100 105 110

Tyr Trp Gly Gln Gly Thr Thr Val Thr Val Ser Ser Ala Ser Thr Lys
 115 120 125

Gly Pro Ser Val Phe Pro Leu Ala Pro Ser Ser Lys Ser Thr Ser Gly
 130 135 140

Gly Thr Ala Ala Leu Gly Cys Leu Val Lys Asp Tyr Phe Pro Glu Pro
 145 150 155 160

Val Thr Val Ser Trp Asn Ser Gly Ala Leu Thr Ser Gly Val His Thr
 165 170 175

Phe Pro Ala Val Leu Gln Ser Ser Gly Leu Tyr Ser Leu Ser Ser Val
 180 185 190

Val Thr Val Pro Ser Ser Ser Leu Gly Thr Gln Thr Tyr Ile Cys Asn
 195 200 205

Val Asn His Lys Pro Ser Asn Thr Lys Val Asp Lys Arg Val Glu Pro
 210 215 220

Lys Ser Cys Asp Lys Thr His Thr Cys Pro Pro Cys Pro Ala Pro Glu
 225 230 235 240

Leu Leu Gly Gly Pro Ser Val Phe Leu Phe Pro Pro Lys Pro Lys Asp
 245 250 255

Thr Leu Met Ile Ser Arg Thr Pro Glu Val Thr Cys Val Val Val Asp
 260 265 270

Val Ser His Glu Asp Pro Glu Val Lys Phe Asn Trp Tyr Val Asp Gly
 275 280 285

Val Glu Val His Asn Ala Lys Thr Lys Pro Arg Glu Glu Gln Tyr Ser
 290 295 300

Ser Thr Tyr Arg Val Val Ser Val Leu Thr Val Leu His Gln Asp Trp
 305 310 315 320

Leu Asn Gly Lys Glu Tyr Lys Cys Lys Val Ser Asn Lys Ala Leu Pro
 325 330 335

Ala 340	Pro	Ile	Glu 340	Lys	Thr	Ile	Ser	Lys 345	Ala	Lys	Gly	Gln	Pro 350	Arg	Glu
Pro 355	Gln	Val	Tyr	Thr	Leu	Pro	Pro 360	Ser	Arg	Glu	Glu	Met 365	Thr	Lys	Asn
Gln 370	Val	Ser	Leu	Thr	Cys	Leu	Val 375	Lys	Gly	Phe	Tyr	Pro 380	Ser	Asp	Ile
Ala 385	Val	Glu	Trp	Glu	Ser	Asn	Gly 390	Gln	Pro	Glu	Asn	Asn 395	Tyr	Lys	Thr
Thr 400	Pro	Pro	Val	Leu	Asp	Ser	Asp 405	Gly	Ser	Phe	Phe	Leu 410	Tyr	Ser	Lys
Leu 415	Thr	Val	Asp	Lys	Ser	Arg	Trp 420	Gln	Gln	Gly	Asn	Val 425	Phe	Ser	Cys
Ser 430	Val	Met	His	Glu	Ala	Leu	His 435	Asn	His	Tyr	Thr	Gln 440	Lys	Ser	Leu
Ser 445	Leu	Ser	Pro	Gly	Gly	Gly	Gly 450	Gly	Ser	Gly	Gly	Gly 455	Gly	Ser	Gly
Gly 460	Gly	Gly	Ser	Gly	Gln	Pro	Arg 465	Glu	Pro	Gln	Val	Tyr 470	Thr	Leu	Pro
Pro 475	Ser	Arg	Glu	Glu	Met	Thr	Lys 480	Asn	Gln	Val	Ser	Leu 485	Thr	Cys	Leu
Val 490	Lys	Gly	Phe	Tyr	Pro	Ser	Asp 495	Ile	Ala	Val	Glu	Trp 500	Glu	Ser	Asn
Gly 505	Gln	Pro	Glu	Asn	Asn	Tyr	Lys 510	Thr	Thr	Pro	Pro	Val 515	Leu	Asp	Ser
Asp 520	Gly	Ser	Phe	Phe	Leu	Tyr	Ser 525	Lys	Leu	Thr	Val	Asp 530	Lys	Ser	Arg
Trp 535	Gln	Gln	Gly	Asn	Val	Phe	Ser 540	Cys	Ser	Val	Met	His 545	Glu	Ala	Leu
His 550	Asn	His	Tyr	Thr	Gln	Lys	Ser 555	Leu	Ser	Leu	Ser	Pro 560	Gly	Ser	Thr
Gly 565	Ser	Glu	Val	Gln	Leu	Gln	Gln 570	Ser	Gly	Pro	Glu	Leu 575	Val	Lys	Pro
Gly 580	Ala	Ser	Val	Lys	Ile	Ser	Cys 585	Lys	Thr	Ser	Gly	Tyr 590	Thr	Phe	Thr
Glu 595	Tyr	Thr	Met	His	Trp	Val	Lys 600	Gln	Ser	His	Gly	Lys 605	Ser	Leu	Glu
Trp 610	Ile	Gly	Gly	Ile	Ser	Pro	Asn 615	Ile	Gly	Gly	Thr	Ser 620	Tyr	Asn	Gln
Lys 625	Phe	Lys	Gly	Lys	Ala	Thr	Leu 630	Thr	Val	Asp	Lys	Ser 635	Ser	Ser	Thr
Ala 640	Tyr	Met	Glu	Leu	Arg	Ser	Leu 645	Thr	Ser	Glu	Asp	Ser 650	Ala	Val	Tyr
Tyr 655	Cys	Ala	Arg	Arg	Gly	Gly	Ser 660	Phe	Asp	Tyr	Trp	Gly 665	Gln	Gly	Thr
Thr 670	Leu	Thr	Val	Ser	Ser	Arg	Thr 675	Val	Ala	Ala	Pro	Ser 680	Val	Phe	Ile
Phe 685	Pro	Pro	Ser	Asp	Glu	Gln	Leu 690	Lys	Ser	Gly	Thr	Ala 695	Ser	Val	Val
Cys 700	Leu	Leu	Asn	Asn	Phe	Tyr	Pro 705	Arg	Glu	Ala	Lys	Val 710	Gln	Trp	Lys
Val 715	Asp	Asn	Ala	Leu	Gln	Ser	Gly 720	Asn	Ser	Gln	Glu	Ser 725	Val	Thr	Glu

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740	745	750
Gln Asp Ser Lys Asp Ser Thr Tyr Ser Leu Ser Ser Thr Leu Thr Leu		
755	760	765
Ser Lys Ala Asp Tyr Glu Lys His Lys Val Tyr Ala Cys Glu Val Thr		
770	775	780
His Gln Gly Leu Ser Ser Pro Val Thr Lys Ser Phe Asn Arg Gly Glu		
785	790	795
		800

Cys

<210> SEQ ID NO 152
 <211> LENGTH: 214
 <212> TYPE: PRT
 <213> ORGANISM: Artificial
 <220> FEATURE:
 <223> OTHER INFORMATION: Chimeric homo sapiens mus musculus

<400> SEQUENCE: 152

Asp Ile Val Met Thr Gln Ser Pro Ala Thr Leu Ser Val Thr Pro Gly		
1	5	10
Asp Arg Val Ser Leu Ser Cys Arg Ala Ser Gln Ser Ile Ser Asp Tyr		
20	25	30
Leu His Trp Tyr Gln Gln Lys Ser His Glu Ser Pro Arg Leu Leu Ile		
35	40	45
Lys Tyr Ala Ser Gln Ser Ile Ser Gly Ile Pro Ser Arg Phe Ser Gly		
50	55	60
Ser Gly Ser Gly Ser Asp Phe Thr Leu Ser Ile Asn Ser Val Glu Pro		
65	70	75
Glu Asp Val Gly Val Tyr Tyr Cys Gln Asn Gly His Ser Phe Pro Leu		
85	90	95
Thr Phe Gly Ala Gly Thr Lys Leu Glu Leu Lys Ala Ser Thr Lys Gly		
100	105	110
Pro Ser Val Phe Pro Leu Ala Pro Ser Ser Lys Ser Thr Ser Gly Gly		
115	120	125
Thr Ala Ala Leu Gly Cys Leu Val Lys Asp Tyr Phe Pro Glu Pro Val		
130	135	140
Thr Val Ser Trp Asn Ser Gly Ala Leu Thr Ser Gly Val His Thr Phe		
145	150	155
Pro Ala Val Leu Gln Ser Ser Gly Leu Tyr Ser Leu Ser Ser Val Val		
165	170	175
Thr Val Pro Ser Ser Ser Leu Gly Thr Gln Thr Tyr Ile Cys Asn Val		
180	185	190
Asn His Lys Pro Ser Asn Thr Lys Val Asp Lys Arg Val Glu Pro Lys		
195	200	205
Ser Cys Asp Lys Thr His		
210		

<210> SEQ ID NO 153
 <211> LENGTH: 218
 <212> TYPE: PRT
 <213> ORGANISM: Artificial
 <220> FEATURE:
 <223> OTHER INFORMATION: Chimeric homo sapiens mus musculus

<400> SEQUENCE: 153

Asp Ile Gln Leu Thr Gln Ser Pro Ala Ser Leu Ala Val Ser Leu Gly

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1	5	10	15
Gln Arg Ala Thr Ile Ser Cys Lys Ala Ser Gln Ser Val Asp Tyr Asp	20	25	30
Gly Asp Ser Tyr Leu Asn Trp Tyr Gln Gln Ile Pro Gly Gln Pro Pro	35	40	45
Lys Leu Leu Ile Tyr Asp Ala Ser Asn Leu Val Ser Gly Ile Pro Pro	50	55	60
Arg Phe Ser Gly Ser Gly Ser Gly Thr Asp Phe Thr Leu Asn Ile His	65	70	75
Pro Val Glu Lys Val Asp Ala Ala Thr Tyr His Cys Gln Gln Ser Thr	85	90	95
Glu Asp Pro Trp Thr Phe Gly Gly Gly Thr Lys Leu Glu Ile Lys Arg	100	105	110
Thr Val Ala Ala Pro Ser Val Phe Ile Phe Pro Pro Ser Asp Glu Gln	115	120	125
Leu Lys Ser Gly Thr Ala Ser Val Val Cys Leu Leu Asn Asn Phe Tyr	130	135	140
Pro Arg Glu Ala Lys Val Gln Trp Lys Val Asp Asn Ala Leu Gln Ser	145	150	155
Gly Asn Ser Gln Glu Ser Val Thr Glu Gln Asp Ser Lys Asp Ser Thr	165	170	175
Tyr Ser Leu Ser Ser Thr Leu Thr Leu Ser Lys Ala Asp Tyr Glu Lys	180	185	190
His Lys Val Tyr Ala Cys Glu Val Thr His Gln Gly Leu Ser Ser Pro	195	200	205
Val Thr Lys Ser Phe Asn Arg Gly Glu Cys	210	215	

<210> SEQ ID NO 154

<211> LENGTH: 796

<212> TYPE: PRT

<213> ORGANISM: Artificial

<220> FEATURE:

<223> OTHER INFORMATION: Chimeric homo sapiens mus musculus

<400> SEQUENCE: 154

Gln Val Gln Leu Gln Gln Ser Gly Ala Glu Leu Val Arg Pro Gly Ser	1	5	10	15
Ser Val Lys Ile Ser Cys Lys Ala Ser Gly Tyr Ala Phe Ser Ser Tyr	20	25	30	
Trp Met Asn Trp Val Lys Gln Arg Pro Gly Gln Gly Leu Glu Trp Ile	35	40	45	
Gly Gln Ile Trp Pro Gly Asp Gly Asp Thr Asn Tyr Asn Gly Lys Phe	50	55	60	
Lys Gly Lys Ala Thr Leu Thr Ala Asp Glu Ser Ser Ser Thr Ala Tyr	65	70	75	80
Met Gln Leu Ser Ser Leu Ala Ser Glu Asp Ser Ala Val Tyr Phe Cys	85	90	95	
Ala Arg Arg Glu Thr Thr Thr Val Gly Arg Tyr Tyr Tyr Ala Met Asp	100	105	110	
Tyr Trp Gly Gln Gly Thr Thr Val Thr Val Ser Ser Ala Ser Thr Lys	115	120	125	
Gly Pro Ser Val Phe Pro Leu Ala Pro Ser Ser Lys Ser Thr Ser Gly				

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130					135					140					
Gly 145	Thr	Ala	Ala	Leu	Gly 150	Cys	Leu	Val	Lys	Asp 155	Tyr	Phe	Pro	Glu	Pro 160
Val	Thr	Val	Ser	Trp 165	Asn	Ser	Gly	Ala	Leu 170	Thr	Ser	Gly	Val	His 175	Thr
Phe	Pro	Ala	Val	Leu 180	Gln	Ser	Ser	Gly 185	Leu	Tyr	Ser	Leu	Ser	Ser	Val
Val	Thr	Val	Pro	Ser 195	Ser	Ser	Leu 200	Gly	Thr	Gln	Thr	Tyr 205	Ile	Cys	Asn
Val	Asn 210	His	Lys	Pro	Ser	Asn 215	Thr	Lys	Val	Asp	Lys 220	Arg	Val	Glu	Pro
Lys 225	Ser	Cys	Gly	Gly	Gly 230	Ser	Ser	Ala	Pro	Glu 235	Leu	Leu	Gly	Gly	Pro 240
Ser	Val	Phe	Leu	Phe 245	Pro	Pro	Lys	Pro	Lys 250	Asp	Thr	Leu	Met	Ile 255	Ser
Arg	Thr	Pro	Glu 260	Val	Thr	Cys	Val	Val 265	Val	Asp	Val	Ser	His 270	Glu	Asp
Pro	Glu 275	Val	Lys	Phe	Asn	Trp	Tyr 280	Val	Asp	Gly	Val	Glu 285	Val	His	Asn
Ala	Lys 290	Thr	Lys	Pro	Arg	Glu 295	Glu	Gln	Tyr	Ser	Ser 300	Thr	Tyr	Arg	Val
Val 305	Ser	Val	Leu	Thr	Val 310	Leu	His	Gln	Asp	Trp 315	Leu	Asn	Gly	Lys	Glu 320
Tyr	Lys	Cys	Lys 325	Val	Ser	Asn	Lys	Ala	Leu 330	Pro	Ala	Pro	Ile	Glu 335	Lys
Thr	Ile	Ser	Lys 340	Ala	Lys	Gly	Gln	Pro 345	Arg	Glu	Pro	Gln 350	Val	Tyr	Thr
Leu	Pro	Pro 355	Ser	Arg	Glu	Glu 360	Met	Thr	Lys	Asn	Gln 365	Val	Ser	Leu	Thr
Cys 370	Leu	Val	Lys	Gly	Phe 375	Tyr	Pro	Ser	Asp	Ile 380	Ala	Val	Glu	Trp	Glu
Ser 385	Asn	Gly	Gln	Pro	Glu 390	Asn	Asn	Tyr	Lys	Thr 395	Thr	Pro	Pro	Val	Leu 400
Asp	Ser	Asp	Gly 405	Ser	Phe	Phe	Leu	Tyr	Ser 410	Lys	Leu	Thr	Val	Asp 415	Lys
Ser	Arg	Trp	Gln 420	Gln	Gly	Asn	Val	Phe 425	Ser	Cys	Ser	Val 430	Met	His	Glu
Ala	Leu	His 435	Asn	His	Tyr	Thr	Gln 440	Lys	Ser	Leu	Ser	Leu 445	Ser	Pro	Gly
Gly 450	Gly	Gly	Gly	Ser	Gly 455	Gly	Gly	Gly	Ser	Gly 460	Gly	Gly	Gly	Ser	Gly
Gln 465	Pro	Arg	Glu	Pro	Gln 470	Val	Tyr	Thr	Leu	Pro 475	Pro	Ser	Arg	Glu	Glu 480
Met	Thr	Lys	Asn 485	Gln	Val	Ser	Leu	Thr	Cys 490	Leu	Val	Lys	Gly	Phe 495	Tyr
Pro	Ser	Asp	Ile 500	Ala	Val	Glu	Trp	Glu 505	Ser	Asn	Gly	Gln 510	Pro	Glu	Asn
Asn	Tyr	Lys 515	Thr	Thr	Pro	Pro	Val 520	Leu	Asp	Ser	Asp	Gly 525	Ser	Phe	Phe
Leu 530	Tyr	Ser	Lys	Leu	Thr 535	Val	Asp	Lys	Ser	Arg	Trp 540	Gln	Gln	Gly	Asn

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Val Phe Ser Cys Ser	Val Met His Glu Ala	Leu His Asn His Tyr Thr
545	550	555 560
Gln Lys Ser Leu Ser	Leu Ser Pro Gly Ser Thr	Gly Ser Glu Val Gln
	565	570 575
Leu Gln Gln Ser Gly	Pro Glu Leu Val Lys Pro	Gly Ala Ser Val Lys
	580	585 590
Ile Ser Cys Lys Thr	Ser Gly Tyr Thr Phe Thr	Glu Tyr Thr Met His
	595	600 605
Trp Val Lys Gln Ser	His Gly Lys Ser Leu Glu Trp	Ile Gly Gly Ile
	610	615 620
Ser Pro Asn Ile Gly	Gly Thr Ser Tyr Asn Gln Lys	Phe Lys Gly Lys
625	630	635 640
Ala Thr Leu Thr Val	Asp Lys Ser Ser Ser Thr	Ala Tyr Met Glu Leu
	645	650 655
Arg Ser Leu Thr Ser	Glu Asp Ser Ala Val Tyr Tyr	Cys Ala Arg Arg
	660	665 670
Gly Gly Ser Phe Asp	Tyr Trp Gly Gln Gly Thr Thr	Leu Thr Val Ser
	675	680 685
Ser Arg Thr Val Ala	Ala Pro Ser Val Phe Ile Phe	Pro Pro Ser Asp
	690	695 700
Glu Gln Leu Lys Ser	Gly Thr Ala Ser Val Val	Cys Leu Leu Asn Asn
705	710	715 720
Phe Tyr Pro Arg Glu	Ala Lys Val Gln Trp Lys Val	Asp Asn Ala Leu
	725	730 735
Gln Ser Gly Asn Ser	Gln Glu Ser Val Thr Glu	Gln Asp Ser Lys Asp
	740	745 750
Ser Thr Tyr Ser Leu	Ser Ser Thr Leu Thr Leu Ser	Lys Ala Asp Tyr
	755	760 765
Glu Lys His Lys Val	Tyr Ala Cys Glu Val Thr His	Gln Gly Leu Ser
	770	775 780
Ser Pro Val Thr Lys	Ser Phe Asn Arg Gly Glu Cys	
785	790	795

<210> SEQ ID NO 155

<211> LENGTH: 214

<212> TYPE: PRT

<213> ORGANISM: Artificial

<220> FEATURE:

<223> OTHER INFORMATION: Chimeric homo sapiens mus musculus

<400> SEQUENCE: 155

Asp Ile Val Met Thr	Gln Ser Pro Ala Thr	Leu Ser Val Thr Pro Gly
1	5	10 15
Asp Arg Val Ser Leu	Ser Cys Arg Ala Ser	Gln Ser Ile Ser Asp Tyr
	20	25 30
Leu His Trp Tyr Gln	Gln Lys Ser His Glu Ser	Pro Arg Leu Leu Ile
	35	40 45
Lys Tyr Ala Ser Gln	Ser Ile Ser Gly Ile Pro	Ser Arg Phe Ser Gly
	50	55 60
Ser Gly Ser Gly Ser	Asp Phe Thr Leu Ser	Ile Asn Ser Val Glu Pro
65	70	75 80
Glu Asp Val Gly Val	Tyr Tyr Cys Gln Asn	Gly His Ser Phe Pro Leu
	85	90 95

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Thr Phe Gly Ala Gly Thr Lys Leu Glu Leu Lys Ala Ser Thr Lys Gly
 100 105 110

Pro Ser Val Phe Pro Leu Ala Pro Ser Ser Lys Ser Thr Ser Gly Gly
 115 120 125

Thr Ala Ala Leu Gly Cys Leu Val Lys Asp Tyr Phe Pro Glu Pro Val
 130 135 140

Thr Val Ser Trp Asn Ser Gly Ala Leu Thr Ser Gly Val His Thr Phe
 145 150 155 160

Pro Ala Val Leu Gln Ser Ser Gly Leu Tyr Ser Leu Ser Ser Val Val
 165 170 175

Thr Val Pro Ser Ser Ser Leu Gly Thr Gln Thr Tyr Ile Cys Asn Val
 180 185 190

Asn His Lys Pro Ser Asn Thr Lys Val Asp Lys Arg Val Glu Pro Lys
 195 200 205

Ser Cys Asp Lys Thr His
 210

<210> SEQ ID NO 156
 <211> LENGTH: 218
 <212> TYPE: PRT
 <213> ORGANISM: Artificial
 <220> FEATURE:
 <223> OTHER INFORMATION: Chimeric homo sapiens mus musculus

<400> SEQUENCE: 156

Asp Ile Gln Leu Thr Gln Ser Pro Ala Ser Leu Ala Val Ser Leu Gly
 1 5 10 15

Gln Arg Ala Thr Ile Ser Cys Lys Ala Ser Gln Ser Val Asp Tyr Asp
 20 25 30

Gly Asp Ser Tyr Leu Asn Trp Tyr Gln Gln Ile Pro Gly Gln Pro Pro
 35 40 45

Lys Leu Leu Ile Tyr Asp Ala Ser Asn Leu Val Ser Gly Ile Pro Pro
 50 55 60

Arg Phe Ser Gly Ser Gly Ser Gly Thr Asp Phe Thr Leu Asn Ile His
 65 70 75 80

Pro Val Glu Lys Val Asp Ala Ala Thr Tyr His Cys Gln Gln Ser Thr
 85 90 95

Glu Asp Pro Trp Thr Phe Gly Gly Gly Thr Lys Leu Glu Ile Lys Arg
 100 105 110

Thr Val Ala Ala Pro Ser Val Phe Ile Phe Pro Pro Ser Asp Glu Gln
 115 120 125

Leu Lys Ser Gly Thr Ala Ser Val Val Cys Leu Leu Asn Asn Phe Tyr
 130 135 140

Pro Arg Glu Ala Lys Val Gln Trp Lys Val Asp Asn Ala Leu Gln Ser
 145 150 155 160

Gly Asn Ser Gln Glu Ser Val Thr Glu Gln Asp Ser Lys Asp Ser Thr
 165 170 175

Tyr Ser Leu Ser Ser Thr Leu Thr Leu Ser Lys Ala Asp Tyr Glu Lys
 180 185 190

His Lys Val Tyr Ala Cys Glu Val Thr His Gln Gly Leu Ser Ser Pro
 195 200 205

Val Thr Lys Ser Phe Asn Arg Gly Glu Cys
 210 215

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<210> SEQ ID NO 157
<211> LENGTH: 819
<212> TYPE: PRT
<213> ORGANISM: Artificial
<220> FEATURE:
<223> OTHER INFORMATION: Chimeric homo sapiens mus musculus

<400> SEQUENCE: 157

Gln Val Gln Leu Gln Gln Ser Gly Ala Glu Leu Val Arg Pro Gly Ser
1      5      10      15
Ser Val Lys Ile Ser Cys Lys Ala Ser Gly Tyr Ala Phe Ser Ser Tyr
20     25     30
Trp Met Asn Trp Val Lys Gln Arg Pro Gly Gln Gly Leu Glu Trp Ile
35     40     45
Gly Gln Ile Trp Pro Gly Asp Gly Asp Thr Asn Tyr Asn Gly Lys Phe
50     55     60
Lys Gly Lys Ala Thr Leu Thr Ala Asp Glu Ser Ser Ser Thr Ala Tyr
65     70     75     80
Met Gln Leu Ser Ser Leu Ala Ser Glu Asp Ser Ala Val Tyr Phe Cys
85     90     95
Ala Arg Arg Glu Thr Thr Thr Val Gly Arg Tyr Tyr Tyr Ala Met Asp
100    105    110
Tyr Trp Gly Gln Gly Thr Thr Val Thr Val Ser Ser Ala Ser Thr Lys
115    120    125
Gly Pro Ser Val Phe Pro Leu Ala Pro Ser Ser Lys Ser Thr Ser Gly
130    135    140
Gly Thr Ala Ala Leu Gly Cys Leu Val Lys Asp Tyr Phe Pro Glu Pro
145    150    155    160
Val Thr Val Ser Trp Asn Ser Gly Ala Leu Thr Ser Gly Val His Thr
165    170    175
Phe Pro Ala Val Leu Gln Ser Ser Gly Leu Tyr Ser Leu Ser Ser Val
180    185    190
Val Thr Val Pro Ser Ser Ser Leu Gly Thr Gln Thr Tyr Ile Cys Asn
195    200    205
Val Asn His Lys Pro Ser Asn Thr Lys Val Asp Lys Arg Val Glu Pro
210    215    220
Lys Ser Cys Asp Lys Thr His Thr Cys Pro Pro Cys Pro Ala Pro Glu
225    230    235    240
Leu Leu Gly Gly Pro Ser Val Phe Leu Phe Pro Pro Lys Pro Lys Asp
245    250    255
Thr Leu Met Ile Ser Arg Thr Pro Glu Val Thr Cys Val Val Val Asp
260    265    270
Val Ser His Glu Asp Pro Glu Val Lys Phe Asn Trp Tyr Val Asp Gly
275    280    285
Val Glu Val His Asn Ala Lys Thr Lys Pro Arg Glu Glu Gln Tyr Ser
290    295    300
Ser Thr Tyr Arg Val Val Ser Val Leu Thr Val Leu His Gln Asp Trp
305    310    315    320
Leu Asn Gly Lys Glu Tyr Lys Cys Lys Val Ser Asn Lys Ala Leu Pro
325    330    335
Ala Pro Ile Glu Lys Thr Ile Ser Lys Ala Lys Gly Gln Pro Arg Glu
340    345    350

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Pro	Gln	Val	Tyr	Thr	Leu	Pro	Pro	Ser	Arg	Glu	Glu	Met	Thr	Lys	Asn	
	355						360					365				
Gln	Val	Ser	Leu	Thr	Cys	Leu	Val	Lys	Gly	Phe	Tyr	Pro	Ser	Asp	Ile	
	370					375					380					
Ala	Val	Glu	Trp	Glu	Ser	Asn	Gly	Gln	Pro	Glu	Asn	Asn	Tyr	Lys	Thr	
385					390					395					400	
Thr	Pro	Pro	Val	Leu	Asp	Ser	Asp	Gly	Ser	Phe	Phe	Leu	Tyr	Ser	Lys	
			405					410						415		
Leu	Thr	Val	Asp	Lys	Ser	Arg	Trp	Gln	Gln	Gly	Asn	Val	Phe	Ser	Cys	
		420						425					430			
Ser	Val	Met	His	Glu	Ala	Leu	His	Asn	His	Tyr	Thr	Gln	Lys	Ser	Leu	
	435						440					445				
Ser	Leu	Ser	Pro	Gly	Gly	Gly	Gly	Gly	Ser	Gly	Gly	Gly	Gly	Ser	Gly	
	450				455					460						
Gly	Gly	Gly	Ser	Gly	Gln	Pro	Arg	Glu	Pro	Gln	Val	Tyr	Thr	Leu	Pro	
465					470					475					480	
Pro	Ser	Arg	Glu	Glu	Met	Thr	Lys	Asn	Gln	Val	Ser	Leu	Thr	Cys	Leu	
			485					490						495		
Val	Lys	Gly	Phe	Tyr	Pro	Ser	Asp	Ile	Ala	Val	Glu	Trp	Glu	Ser	Asn	
		500						505					510			
Gly	Gln	Pro	Glu	Asn	Asn	Tyr	Lys	Thr	Thr	Pro	Pro	Val	Leu	Asp	Ser	
	515						520					525				
Asp	Gly	Ser	Phe	Phe	Leu	Tyr	Ser	Lys	Leu	Thr	Val	Asp	Lys	Ser	Arg	
	530					535					540					
Trp	Gln	Gln	Gly	Asn	Val	Phe	Ser	Cys	Ser	Val	Met	His	Glu	Ala	Leu	
545				550						555					560	
His	Asn	His	Tyr	Thr	Gln	Lys	Ser	Leu	Ser	Leu	Ser	Pro	Gly	Ser	Thr	
			565					570						575		
Gly	Ser	Glu	Val	Gln	Leu	Gln	Gln	Ser	Gly	Pro	Glu	Leu	Val	Lys	Pro	
		580						585					590			
Gly	Ala	Ser	Val	Lys	Ile	Ser	Cys	Lys	Thr	Ser	Gly	Tyr	Thr	Phe	Thr	
	595						600					605				
Glu	Tyr	Thr	Met	His	Trp	Val	Lys	Gln	Ser	His	Gly	Lys	Ser	Leu	Glu	
	610					615					620					
Trp	Ile	Gly	Gly	Ile	Ser	Pro	Asn	Ile	Gly	Gly	Thr	Ser	Tyr	Asn	Gln	
625				630					635						640	
Lys	Phe	Lys	Gly	Lys	Ala	Thr	Leu	Thr	Val	Asp	Lys	Ser	Ser	Ser	Thr	
			645					650						655		
Ala	Tyr	Met	Glu	Leu	Arg	Ser	Leu	Thr	Ser	Glu	Asp	Ser	Ala	Val	Tyr	
	660						665						670			
Tyr	Cys	Ala	Arg	Arg	Gly	Gly	Ser	Phe	Asp	Tyr	Trp	Gly	Gln	Gly	Thr	
	675						680					685				
Thr	Leu	Thr	Val	Ser	Ser	Val	Glu	Gly	Gly	Ser	Gly	Gly	Ser	Gly	Gly	
	690					695					700					
Ser	Gly	Gly	Ser	Gly	Gly	Val	Asp	Asp	Ile	Val	Met	Thr	Gln	Ser	Pro	
705					710					715					720	
Ala	Thr	Leu	Ser	Val	Thr	Pro	Gly	Asp	Arg	Val	Ser	Leu	Ser	Cys	Arg	
			725						730					735		
Ala	Ser	Gln	Ser	Ile	Ser	Asp	Tyr	Leu	His	Trp	Tyr	Gln	Gln	Lys	Ser	
	740							745						750		

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His Glu Ser Pro Arg Leu Leu Ile Lys Tyr Ala Ser Gln Ser Ile Ser
755 760 765

Gly Ile Pro Ser Arg Phe Ser Gly Ser Gly Ser Gly Ser Asp Phe Thr
770 775 780

Leu Ser Ile Asn Ser Val Glu Pro Glu Asp Val Gly Val Tyr Tyr Cys
785 790 795 800

Gln Asn Gly His Ser Phe Pro Leu Thr Phe Gly Ala Gly Thr Lys Leu
805 810 815

Glu Leu Lys

<210> SEQ ID NO 158
 <211> LENGTH: 218
 <212> TYPE: PRT
 <213> ORGANISM: Artificial
 <220> FEATURE:
 <223> OTHER INFORMATION: Chimeric homo sapiens mus musculus

<400> SEQUENCE: 158

Asp Ile Gln Leu Thr Gln Ser Pro Ala Ser Leu Ala Val Ser Leu Gly
1 5 10 15

Gln Arg Ala Thr Ile Ser Cys Lys Ala Ser Gln Ser Val Asp Tyr Asp
20 25 30

Gly Asp Ser Tyr Leu Asn Trp Tyr Gln Gln Ile Pro Gly Gln Pro Pro
35 40 45

Lys Leu Leu Ile Tyr Asp Ala Ser Asn Leu Val Ser Gly Ile Pro Pro
50 55 60

Arg Phe Ser Gly Ser Gly Ser Gly Thr Asp Phe Thr Leu Asn Ile His
65 70 75 80

Pro Val Glu Lys Val Asp Ala Ala Thr Tyr His Cys Gln Gln Ser Thr
85 90 95

Glu Asp Pro Trp Thr Phe Gly Gly Gly Thr Lys Leu Glu Ile Lys Arg
100 105 110

Thr Val Ala Ala Pro Ser Val Phe Ile Phe Pro Pro Ser Asp Glu Gln
115 120 125

Leu Lys Ser Gly Thr Ala Ser Val Val Cys Leu Leu Asn Asn Phe Tyr
130 135 140

Pro Arg Glu Ala Lys Val Gln Trp Lys Val Asp Asn Ala Leu Gln Ser
145 150 155 160

Gly Asn Ser Gln Glu Ser Val Thr Glu Gln Asp Ser Lys Asp Ser Thr
165 170 175

Tyr Ser Leu Ser Ser Thr Leu Thr Leu Ser Lys Ala Asp Tyr Glu Lys
180 185 190

His Lys Val Tyr Ala Cys Glu Val Thr His Gln Gly Leu Ser Ser Pro
195 200 205

Val Thr Lys Ser Phe Asn Arg Gly Glu Cys
210 215

<210> SEQ ID NO 159
 <211> LENGTH: 819
 <212> TYPE: PRT
 <213> ORGANISM: Artificial
 <220> FEATURE:
 <223> OTHER INFORMATION: Chimeric homo sapiens mus musculus

<400> SEQUENCE: 159

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Gln	Val	Gln	Leu	Gln	Gln	Ser	Gly	Ala	Glu	Leu	Val	Arg	Pro	Gly	Ser	1	5	10	15
Ser	Val	Lys	Ile	Ser	Cys	Lys	Ala	Ser	Gly	Tyr	Ala	Phe	Ser	Ser	Tyr	20	25	30	
Trp	Met	Asn	Trp	Val	Lys	Gln	Arg	Pro	Gly	Gln	Gly	Leu	Glu	Trp	Ile	35	40	45	
Gly	Gln	Ile	Trp	Pro	Gly	Asp	Gly	Asp	Thr	Asn	Tyr	Asn	Gly	Lys	Phe	50	55	60	
Lys	Gly	Lys	Ala	Thr	Leu	Thr	Ala	Asp	Glu	Ser	Ser	Ser	Thr	Ala	Tyr	65	70	75	80
Met	Gln	Leu	Ser	Ser	Leu	Ala	Ser	Glu	Asp	Ser	Ala	Val	Tyr	Phe	Cys	85	90	95	
Ala	Arg	Arg	Glu	Thr	Thr	Thr	Val	Gly	Arg	Tyr	Tyr	Tyr	Ala	Met	Asp	100	105	110	
Tyr	Trp	Gly	Gln	Gly	Thr	Thr	Val	Thr	Val	Ser	Ser	Ala	Ser	Thr	Lys	115	120	125	
Gly	Pro	Ser	Val	Phe	Pro	Leu	Ala	Pro	Ser	Ser	Lys	Ser	Thr	Ser	Gly	130	135	140	
Gly	Thr	Ala	Ala	Leu	Gly	Cys	Leu	Val	Lys	Asp	Tyr	Phe	Pro	Glu	Pro	145	150	155	160
Val	Thr	Val	Ser	Trp	Asn	Ser	Gly	Ala	Leu	Thr	Ser	Gly	Val	His	Thr	165	170	175	
Phe	Pro	Ala	Val	Leu	Gln	Ser	Ser	Gly	Leu	Tyr	Ser	Leu	Ser	Ser	Val	180	185	190	
Val	Thr	Val	Pro	Ser	Ser	Ser	Leu	Gly	Thr	Gln	Thr	Tyr	Ile	Cys	Asn	195	200	205	
Val	Asn	His	Lys	Pro	Ser	Asn	Thr	Lys	Val	Asp	Lys	Arg	Val	Glu	Pro	210	215	220	
Lys	Ser	Cys	Asp	Lys	Thr	His	Thr	Ser	Pro	Pro	Ser	Pro	Ala	Pro	Glu	225	230	235	240
Leu	Leu	Gly	Gly	Pro	Ser	Val	Phe	Leu	Phe	Pro	Pro	Lys	Pro	Lys	Asp	245	250	255	
Thr	Leu	Met	Ile	Ser	Arg	Thr	Pro	Glu	Val	Thr	Cys	Val	Val	Val	Asp	260	265	270	
Val	Ser	His	Glu	Asp	Pro	Glu	Val	Lys	Phe	Asn	Trp	Tyr	Val	Asp	Gly	275	280	285	
Val	Glu	Val	His	Asn	Ala	Lys	Thr	Lys	Pro	Arg	Glu	Glu	Gln	Tyr	Ser	290	295	300	
Ser	Thr	Tyr	Arg	Val	Val	Ser	Val	Leu	Thr	Val	Leu	His	Gln	Asp	Trp	305	310	315	320
Leu	Asn	Gly	Lys	Glu	Tyr	Lys	Cys	Lys	Val	Ser	Asn	Lys	Ala	Leu	Pro	325	330	335	
Ala	Pro	Ile	Glu	Lys	Thr	Ile	Ser	Lys	Ala	Lys	Gly	Gln	Pro	Arg	Glu	340	345	350	
Pro	Gln	Val	Tyr	Thr	Leu	Pro	Pro	Ser	Arg	Glu	Glu	Met	Thr	Lys	Asn	355	360	365	
Gln	Val	Ser	Leu	Thr	Cys	Leu	Val	Lys	Gly	Phe	Tyr	Pro	Ser	Asp	Ile	370	375	380	
Ala	Val	Glu	Trp	Glu	Ser	Asn	Gly	Gln	Pro	Glu	Asn	Asn	Tyr	Lys	Thr	385	390	395	400
Thr	Pro	Pro	Val	Leu	Asp	Ser	Asp	Gly	Ser	Phe	Phe	Leu	Tyr	Ser	Lys				

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405								410					415				
Leu	Thr	Val	Asp	Lys	Ser	Arg	Trp	Gln	Gln	Gly	Asn	Val	Phe	Ser	Cys		
			420				425						430				
Ser	Val	Met	His	Glu	Ala	Leu	His	Asn	His	Tyr	Thr	Gln	Lys	Ser	Leu		
		435				440					445						
Ser	Leu	Ser	Pro	Gly	Gly	Gly	Gly	Ser	Gly	Gly	Gly	Gly	Gly	Ser	Gly		
		450				455					460						
Gly	Gly	Gly	Ser	Gly	Gln	Pro	Arg	Glu	Pro	Gln	Val	Tyr	Thr	Leu	Pro		
465				470						475			480				
Pro	Ser	Arg	Glu	Glu	Met	Thr	Lys	Asn	Gln	Val	Ser	Leu	Thr	Cys	Leu		
			485						490			495					
Val	Lys	Gly	Phe	Tyr	Pro	Ser	Asp	Ile	Ala	Val	Glu	Trp	Glu	Ser	Asn		
			500						505			510					
Gly	Gln	Pro	Glu	Asn	Asn	Tyr	Lys	Thr	Thr	Pro	Pro	Val	Leu	Asp	Ser		
		515					520					525					
Asp	Gly	Ser	Phe	Phe	Leu	Tyr	Ser	Lys	Leu	Thr	Val	Asp	Lys	Ser	Arg		
		530					535					540					
Trp	Gln	Gln	Gly	Asn	Val	Phe	Ser	Cys	Ser	Val	Met	His	Glu	Ala	Leu		
545				550						555			560				
His	Asn	His	Tyr	Thr	Gln	Lys	Ser	Leu	Ser	Leu	Ser	Pro	Gly	Ser	Thr		
			565						570			575					
Gly	Ser	Glu	Val	Gln	Leu	Gln	Gln	Ser	Gly	Pro	Glu	Leu	Val	Lys	Pro		
			580						585			590					
Gly	Ala	Ser	Val	Lys	Ile	Ser	Cys	Lys	Thr	Ser	Gly	Tyr	Thr	Phe	Thr		
		595					600					605					
Glu	Tyr	Thr	Met	His	Trp	Val	Lys	Gln	Ser	His	Gly	Lys	Ser	Leu	Glu		
		610					615					620					
Trp	Ile	Gly	Gly	Ile	Ser	Pro	Asn	Ile	Gly	Gly	Thr	Ser	Tyr	Asn	Gln		
625				630						635			640				
Lys	Phe	Lys	Gly	Lys	Ala	Thr	Leu	Thr	Val	Asp	Lys	Ser	Ser	Ser	Thr		
			645						650			655					
Ala	Tyr	Met	Glu	Leu	Arg	Ser	Leu	Thr	Ser	Glu	Asp	Ser	Ala	Val	Tyr		
			660						665			670					
Tyr	Cys	Ala	Arg	Arg	Gly	Gly	Ser	Phe	Asp	Tyr	Trp	Gly	Gln	Gly	Thr		
		675					680					685					
Thr	Leu	Thr	Val	Ser	Ser	Val	Glu	Gly	Gly	Ser	Gly	Gly	Ser	Gly	Gly		
		690					695					700					
Ser	Gly	Gly	Ser	Gly	Gly	Val	Asp	Asp	Ile	Val	Met	Thr	Gln	Ser	Pro		
705				710						715			720				
Ala	Thr	Leu	Ser	Val	Thr	Pro	Gly	Asp	Arg	Val	Ser	Leu	Ser	Cys	Arg		
			725						730			735					
Ala	Ser	Gln	Ser	Ile	Ser	Asp	Tyr	Leu	His	Trp	Tyr	Gln	Gln	Lys	Ser		
			740						745			750					
His	Glu	Ser	Pro	Arg	Leu	Leu	Ile	Lys	Tyr	Ala	Ser	Gln	Ser	Ile	Ser		
		755					760					765					
Gly	Ile	Pro	Ser	Arg	Phe	Ser	Gly	Ser	Gly	Ser	Gly	Ser	Asp	Phe	Thr		
		770					775					780					
Leu	Ser	Ile	Asn	Ser	Val	Glu	Pro	Glu	Asp	Val	Gly	Val	Tyr	Tyr	Cys		
785				790						795			800				
Gln	Asn	Gly	His	Ser	Phe	Pro	Leu	Thr	Phe	Gly	Ala	Gly	Thr	Lys	Leu		
			805						810			815					

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Glu Leu Lys

<210> SEQ ID NO 160
<211> LENGTH: 218
<212> TYPE: PRT
<213> ORGANISM: Artificial
<220> FEATURE:
<223> OTHER INFORMATION: Chimeric homo sapiens mus musculus

<400> SEQUENCE: 160

Asp Ile Gln Leu Thr Gln Ser Pro Ala Ser Leu Ala Val Ser Leu Gly
1 5 10 15
Gln Arg Ala Thr Ile Ser Cys Lys Ala Ser Gln Ser Val Asp Tyr Asp
20 25 30
Gly Asp Ser Tyr Leu Asn Trp Tyr Gln Gln Ile Pro Gly Gln Pro Pro
35 40 45
Lys Leu Leu Ile Tyr Asp Ala Ser Asn Leu Val Ser Gly Ile Pro Pro
50 55 60
Arg Phe Ser Gly Ser Gly Ser Gly Thr Asp Phe Thr Leu Asn Ile His
65 70 75 80
Pro Val Glu Lys Val Asp Ala Ala Thr Tyr His Cys Gln Gln Ser Thr
85 90 95
Glu Asp Pro Trp Thr Phe Gly Gly Gly Thr Lys Leu Glu Ile Lys Arg
100 105 110
Thr Val Ala Ala Pro Ser Val Phe Ile Phe Pro Pro Ser Asp Glu Gln
115 120 125
Leu Lys Ser Gly Thr Ala Ser Val Val Cys Leu Leu Asn Asn Phe Tyr
130 135 140
Pro Arg Glu Ala Lys Val Gln Trp Lys Val Asp Asn Ala Leu Gln Ser
145 150 155 160
Gly Asn Ser Gln Glu Ser Val Thr Glu Gln Asp Ser Lys Asp Ser Thr
165 170 175
Tyr Ser Leu Ser Ser Thr Leu Thr Leu Ser Lys Ala Asp Tyr Glu Lys
180 185 190
His Lys Val Tyr Ala Cys Glu Val Thr His Gln Gly Leu Ser Ser Pro
195 200 205
Val Thr Lys Ser Phe Asn Arg Gly Glu Cys
210 215

<210> SEQ ID NO 161
<211> LENGTH: 815
<212> TYPE: PRT
<213> ORGANISM: Artificial
<220> FEATURE:
<223> OTHER INFORMATION: Chimeric homo sapiens mus musculus

<400> SEQUENCE: 161

Gln Val Gln Leu Gln Gln Ser Gly Ala Glu Leu Val Arg Pro Gly Ser
1 5 10 15
Ser Val Lys Ile Ser Cys Lys Ala Ser Gly Tyr Ala Phe Ser Ser Tyr
20 25 30
Trp Met Asn Trp Val Lys Gln Arg Pro Gly Gln Gly Leu Glu Trp Ile
35 40 45
Gly Gln Ile Trp Pro Gly Asp Gly Asp Thr Asn Tyr Asn Gly Lys Phe
50 55 60

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Lys	Gly	Lys	Ala	Thr	Leu	Thr	Ala	Asp	Glu	Ser	Ser	Ser	Thr	Ala	Tyr	65	70	75	80
Met	Gln	Leu	Ser	Ser	Leu	Ala	Ser	Glu	Asp	Ser	Ala	Val	Tyr	Phe	Cys	85	90	95	
Ala	Arg	Arg	Glu	Thr	Thr	Thr	Val	Gly	Arg	Tyr	Tyr	Tyr	Ala	Met	Asp	100	105	110	
Tyr	Trp	Gly	Gln	Gly	Thr	Thr	Val	Thr	Val	Ser	Ser	Ala	Ser	Thr	Lys	115	120	125	
Gly	Pro	Ser	Val	Phe	Pro	Leu	Ala	Pro	Ser	Ser	Lys	Ser	Thr	Ser	Gly	130	135	140	
Gly	Thr	Ala	Ala	Leu	Gly	Cys	Leu	Val	Lys	Asp	Tyr	Phe	Pro	Glu	Pro	145	150	155	160
Val	Thr	Val	Ser	Trp	Asn	Ser	Gly	Ala	Leu	Thr	Ser	Gly	Val	His	Thr	165	170	175	
Phe	Pro	Ala	Val	Leu	Gln	Ser	Ser	Gly	Leu	Tyr	Ser	Leu	Ser	Ser	Val	180	185	190	
Val	Thr	Val	Pro	Ser	Ser	Ser	Leu	Gly	Thr	Gln	Thr	Tyr	Ile	Cys	Asn	195	200	205	
Val	Asn	His	Lys	Pro	Ser	Asn	Thr	Lys	Val	Asp	Lys	Arg	Val	Glu	Pro	210	215	220	
Lys	Ser	Cys	Gly	Gly	Gly	Ser	Ser	Pro	Ala	Pro	Glu	Leu	Leu	Gly	Gly	225	230	235	240
Pro	Ser	Val	Phe	Leu	Phe	Pro	Pro	Lys	Pro	Lys	Asp	Thr	Leu	Met	Ile	245	250	255	
Ser	Arg	Thr	Pro	Glu	Val	Thr	Cys	Val	Val	Val	Asp	Val	Ser	His	Glu	260	265	270	
Asp	Pro	Glu	Val	Lys	Phe	Asn	Trp	Tyr	Val	Asp	Gly	Val	Glu	Val	His	275	280	285	
Asn	Ala	Lys	Thr	Lys	Pro	Arg	Glu	Glu	Gln	Tyr	Ser	Ser	Thr	Tyr	Arg	290	295	300	
Val	Val	Ser	Val	Leu	Thr	Val	Leu	His	Gln	Asp	Trp	Leu	Asn	Gly	Lys	305	310	315	320
Glu	Tyr	Lys	Cys	Lys	Val	Ser	Asn	Lys	Ala	Leu	Pro	Ala	Pro	Ile	Glu	325	330	335	
Lys	Thr	Ile	Ser	Lys	Ala	Lys	Gly	Gln	Pro	Arg	Glu	Pro	Gln	Val	Tyr	340	345	350	
Thr	Leu	Pro	Pro	Ser	Arg	Glu	Glu	Met	Thr	Lys	Asn	Gln	Val	Ser	Leu	355	360	365	
Thr	Cys	Leu	Val	Lys	Gly	Phe	Tyr	Pro	Ser	Asp	Ile	Ala	Val	Glu	Trp	370	375	380	
Glu	Ser	Asn	Gly	Gln	Pro	Glu	Asn	Asn	Tyr	Lys	Thr	Thr	Pro	Pro	Val	385	390	395	400
Leu	Asp	Ser	Asp	Gly	Ser	Phe	Phe	Leu	Tyr	Ser	Lys	Leu	Thr	Val	Asp	405	410	415	
Lys	Ser	Arg	Trp	Gln	Gln	Gly	Asn	Val	Phe	Ser	Cys	Ser	Val	Met	His	420	425	430	
Glu	Ala	Leu	His	Asn	His	Tyr	Thr	Gln	Lys	Ser	Leu	Ser	Leu	Ser	Pro	435	440	445	
Gly	Gly	Gly	Gly	Gly	Ser	Gly	Gly	Gly	Gly	Ser	Gly	Gly	Gly	Gly	Ser	450	455	460	

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Gly	Gln	Pro	Arg	Glu	Pro	Gln	Val	Tyr	Thr	Leu	Pro	Pro	Ser	Arg	Glu	465	470	475	480
Glu	Met	Thr	Lys	Asn	Gln	Val	Ser	Leu	Thr	Cys	Leu	Val	Lys	Gly	Phe	485	490	495	
Tyr	Pro	Ser	Asp	Ile	Ala	Val	Glu	Trp	Glu	Ser	Asn	Gly	Gln	Pro	Glu	500	505	510	
Asn	Asn	Tyr	Lys	Thr	Thr	Pro	Pro	Val	Leu	Asp	Ser	Asp	Gly	Ser	Phe	515	520	525	
Phe	Leu	Tyr	Ser	Lys	Leu	Thr	Val	Asp	Lys	Ser	Arg	Trp	Gln	Gln	Gly	530	535	540	
Asn	Val	Phe	Ser	Cys	Ser	Val	Met	His	Glu	Ala	Leu	His	Asn	His	Tyr	545	550	555	560
Thr	Gln	Lys	Ser	Leu	Ser	Leu	Ser	Pro	Gly	Ser	Thr	Gly	Ser	Glu	Val	565	570	575	
Gln	Leu	Gln	Gln	Ser	Gly	Pro	Glu	Leu	Val	Lys	Pro	Gly	Ala	Ser	Val	580	585	590	
Lys	Ile	Ser	Cys	Lys	Thr	Ser	Gly	Tyr	Thr	Phe	Thr	Glu	Tyr	Thr	Met	595	600	605	
His	Trp	Val	Lys	Gln	Ser	His	Gly	Lys	Ser	Leu	Glu	Trp	Ile	Gly	Gly	610	615	620	
Ile	Ser	Pro	Asn	Ile	Gly	Gly	Thr	Ser	Tyr	Asn	Gln	Lys	Phe	Lys	Gly	625	630	635	640
Lys	Ala	Thr	Leu	Thr	Val	Asp	Lys	Ser	Ser	Ser	Thr	Ala	Tyr	Met	Glu	645	650	655	
Leu	Arg	Ser	Leu	Thr	Ser	Glu	Asp	Ser	Ala	Val	Tyr	Tyr	Cys	Ala	Arg	660	665	670	
Arg	Gly	Gly	Ser	Phe	Asp	Tyr	Trp	Gly	Gln	Gly	Thr	Thr	Leu	Thr	Val	675	680	685	
Ser	Ser	Val	Glu	Gly	Gly	Ser	Gly	Gly	Ser	Gly	Gly	Ser	Gly	Gly	Ser	690	695	700	
Gly	Gly	Val	Asp	Asp	Ile	Val	Met	Thr	Gln	Ser	Pro	Ala	Thr	Leu	Ser	705	710	715	720
Val	Thr	Pro	Gly	Asp	Arg	Val	Ser	Leu	Ser	Cys	Arg	Ala	Ser	Gln	Ser	725	730	735	
Ile	Ser	Asp	Tyr	Leu	His	Trp	Tyr	Gln	Gln	Lys	Ser	His	Glu	Ser	Pro	740	745	750	
Arg	Leu	Leu	Ile	Lys	Tyr	Ala	Ser	Gln	Ser	Ile	Ser	Gly	Ile	Pro	Ser	755	760	765	
Arg	Phe	Ser	Gly	Ser	Gly	Ser	Gly	Ser	Asp	Phe	Thr	Leu	Ser	Ile	Asn	770	775	780	
Ser	Val	Glu	Pro	Glu	Asp	Val	Gly	Val	Tyr	Tyr	Cys	Gln	Asn	Gly	His	785	790	795	800
Ser	Phe	Pro	Leu	Thr	Phe	Gly	Ala	Gly	Thr	Lys	Leu	Glu	Leu	Lys		805	810	815	

<210> SEQ ID NO 162

<211> LENGTH: 819

<212> TYPE: PRT

<213> ORGANISM: Artificial

<220> FEATURE:

<223> OTHER INFORMATION: Chimeric homo sapiens mus musculus

<400> SEQUENCE: 162

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Asp	Ile	Gln	Leu	Thr	Gln	Ser	Pro	Ala	Ser	Leu	Ala	Val	Ser	Leu	Gly	1	5	10	15
Gln	Arg	Ala	Thr	Ile	Ser	Cys	Lys	Ala	Ser	Gln	Ser	Val	Asp	Tyr	Asp	20	25	30	
Gly	Asp	Ser	Tyr	Leu	Asn	Trp	Tyr	Gln	Gln	Ile	Pro	Gly	Gln	Pro	Pro	35	40	45	
Lys	Leu	Leu	Ile	Tyr	Asp	Ala	Ser	Asn	Leu	Val	Ser	Gly	Ile	Pro	Pro	50	55	60	
Arg	Phe	Ser	Gly	Ser	Gly	Ser	Gly	Thr	Asp	Phe	Thr	Leu	Asn	Ile	His	65	70	75	80
Pro	Val	Glu	Lys	Val	Asp	Ala	Ala	Thr	Tyr	His	Cys	Gln	Gln	Ser	Thr	85	90	95	
Glu	Asp	Pro	Trp	Thr	Phe	Gly	Gly	Gly	Thr	Lys	Leu	Glu	Ile	Lys	Gly	100	105	110	
Gly	Gly	Gly	Ser	Gly	Gly	Gly	Gly	Ser	Gly	Gly	Gly	Gly	Ser	Gln	Val	115	120	125	
Gln	Leu	Gln	Gln	Ser	Gly	Ala	Glu	Leu	Val	Arg	Pro	Gly	Ser	Ser	Val	130	135	140	
Lys	Ile	Ser	Cys	Lys	Ala	Ser	Gly	Tyr	Ala	Phe	Ser	Ser	Tyr	Trp	Met	145	150	155	160
Asn	Trp	Val	Lys	Gln	Arg	Pro	Gly	Gln	Gly	Leu	Glu	Trp	Ile	Gly	Gln	165	170	175	
Ile	Trp	Pro	Gly	Asp	Gly	Asp	Thr	Asn	Tyr	Asn	Gly	Lys	Phe	Lys	Gly	180	185	190	
Lys	Ala	Thr	Leu	Thr	Ala	Asp	Glu	Ser	Ser	Ser	Thr	Ala	Tyr	Met	Gln	195	200	205	
Leu	Ser	Ser	Leu	Ala	Ser	Glu	Asp	Ser	Ala	Val	Tyr	Phe	Cys	Ala	Arg	210	215	220	
Arg	Glu	Thr	Thr	Thr	Val	Gly	Arg	Tyr	Tyr	Tyr	Ala	Met	Asp	Tyr	Trp	225	230	235	240
Gly	Gln	Gly	Thr	Thr	Val	Thr	Val	Ser	Ser	Gly	Gly	Gly	Ser	Ser	Ala	245	250	255	
Pro	Glu	Leu	Leu	Gly	Gly	Pro	Ser	Val	Phe	Leu	Phe	Pro	Pro	Lys	Pro	260	265	270	
Lys	Asp	Thr	Leu	Met	Ile	Ser	Arg	Thr	Pro	Glu	Val	Thr	Cys	Val	Val	275	280	285	
Val	Asp	Val	Ser	His	Glu	Asp	Pro	Glu	Val	Lys	Phe	Asn	Trp	Tyr	Val	290	295	300	
Asp	Gly	Val	Glu	Val	His	Asn	Ala	Lys	Thr	Lys	Pro	Arg	Glu	Glu	Gln	305	310	315	320
Tyr	Ser	Ser	Thr	Tyr	Arg	Val	Val	Ser	Val	Leu	Thr	Val	Leu	His	Gln	325	330	335	
Asp	Trp	Leu	Asn	Gly	Lys	Glu	Tyr	Lys	Cys	Lys	Val	Ser	Asn	Lys	Ala	340	345	350	
Leu	Pro	Ala	Pro	Ile	Glu	Lys	Thr	Ile	Ser	Lys	Ala	Lys	Gly	Gln	Pro	355	360	365	
Arg	Glu	Pro	Gln	Val	Tyr	Thr	Leu	Pro	Pro	Ser	Arg	Glu	Glu	Met	Thr	370	375	380	
Lys	Asn	Gln	Val	Ser	Leu	Thr	Cys	Leu	Val	Lys	Gly	Phe	Tyr	Pro	Ser	385	390	395	400
Asp	Ile	Ala	Val	Glu	Trp	Glu	Ser	Asn	Gly	Gln	Pro	Glu	Asn	Asn	Tyr				

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405								410					415				
Lys	Thr	Thr	Pro	Pro	Val	Leu	Asp	Ser	Asp	Gly	Ser	Phe	Phe	Leu	Tyr		
			420					425					430				
Ser	Lys	Leu	Thr	Val	Asp	Lys	Ser	Arg	Trp	Gln	Gln	Gly	Asn	Val	Phe		
			435					440					445				
Ser	Cys	Ser	Val	Met	His	Glu	Ala	Leu	His	Asn	His	Tyr	Thr	Gln	Lys		
			450		455						460						
Ser	Leu	Ser	Leu	Ser	Pro	Gly	Gly	Gly	Gly	Gly	Ser	Gly	Gly	Gly	Gly		
465						470					475		480				
Ser	Gly	Gly	Gly	Gly	Ser	Gly	Gln	Pro	Arg	Glu	Pro	Gln	Val	Tyr	Thr		
			485					490						495			
Leu	Pro	Pro	Ser	Arg	Glu	Glu	Met	Thr	Lys	Asn	Gln	Val	Ser	Leu	Thr		
			500					505					510				
Cys	Leu	Val	Lys	Gly	Phe	Tyr	Pro	Ser	Asp	Ile	Ala	Val	Glu	Trp	Glu		
			515					520					525				
Ser	Asn	Gly	Gln	Pro	Glu	Asn	Asn	Tyr	Lys	Thr	Thr	Pro	Pro	Val	Leu		
530						535					540						
Asp	Ser	Asp	Gly	Ser	Phe	Phe	Leu	Tyr	Ser	Lys	Leu	Thr	Val	Asp	Lys		
545						550					555		560				
Ser	Arg	Trp	Gln	Gln	Gly	Asn	Val	Phe	Ser	Cys	Ser	Val	Met	His	Glu		
			565					570						575			
Ala	Leu	His	Asn	His	Tyr	Thr	Gln	Lys	Ser	Leu	Ser	Leu	Ser	Pro	Gly		
			580					585					590				
Ser	Thr	Gly	Ser	Glu	Val	Gln	Leu	Gln	Gln	Ser	Gly	Pro	Glu	Leu	Val		
			595					600					605				
Lys	Pro	Gly	Ala	Ser	Val	Lys	Ile	Ser	Cys	Lys	Thr	Ser	Gly	Tyr	Thr		
610						615					620						
Phe	Thr	Glu	Tyr	Thr	Met	His	Trp	Val	Lys	Gln	Ser	His	Gly	Lys	Ser		
625						630					635		640				
Leu	Glu	Trp	Ile	Gly	Gly	Ile	Ser	Pro	Asn	Ile	Gly	Gly	Thr	Ser	Tyr		
			645					650						655			
Asn	Gln	Lys	Phe	Lys	Gly	Lys	Ala	Thr	Leu	Thr	Val	Asp	Lys	Ser	Ser		
			660					665					670				
Ser	Thr	Ala	Tyr	Met	Glu	Leu	Arg	Ser	Leu	Thr	Ser	Glu	Asp	Ser	Ala		
			675					680					685				
Val	Tyr	Tyr	Cys	Ala	Arg	Arg	Gly	Gly	Ser	Phe	Asp	Tyr	Trp	Gly	Gln		
690						695					700						
Gly	Thr	Thr	Leu	Thr	Val	Ser	Ser	Arg	Thr	Val	Ala	Ala	Pro	Ser	Val		
705						710					715		720				
Phe	Ile	Phe	Pro	Pro	Ser	Asp	Glu	Gln	Leu	Lys	Ser	Gly	Thr	Ala	Ser		
			725					730						735			
Val	Val	Cys	Leu	Leu	Asn	Asn	Phe	Tyr	Pro	Arg	Glu	Ala	Lys	Val	Gln		
			740					745					750				
Trp	Lys	Val	Asp	Asn	Ala	Leu	Gln	Ser	Gly	Asn	Ser	Gln	Glu	Ser	Val		
			755					760					765				
Thr	Glu	Gln	Asp	Ser	Lys	Asp	Ser	Thr	Tyr	Ser	Leu	Ser	Ser	Thr	Leu		
770						775					780						
Thr	Leu	Ser	Lys	Ala	Asp	Tyr	Glu	Lys	His	Lys	Val	Tyr	Ala	Cys	Glu		
785						790					795		800				
Val	Thr	His	Gln	Gly	Leu	Ser	Ser	Pro	Val	Thr	Lys	Ser	Phe	Asn	Arg		
			805					810						815			

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Gly Glu Cys

<210> SEQ ID NO 163
<211> LENGTH: 214
<212> TYPE: PRT
<213> ORGANISM: Artificial
<220> FEATURE:
<223> OTHER INFORMATION: Chimeric homo sapiens mus musculus

<400> SEQUENCE: 163

Asp Ile Val Met Thr Gln Ser Pro Ala Thr Leu Ser Val Thr Pro Gly
1 5 10 15
Asp Arg Val Ser Leu Ser Cys Arg Ala Ser Gln Ser Ile Ser Asp Tyr
20 25 30
Leu His Trp Tyr Gln Gln Lys Ser His Glu Ser Pro Arg Leu Leu Ile
35 40 45
Lys Tyr Ala Ser Gln Ser Ile Ser Gly Ile Pro Ser Arg Phe Ser Gly
50 55 60
Ser Gly Ser Gly Ser Asp Phe Thr Leu Ser Ile Asn Ser Val Glu Pro
65 70 75 80
Glu Asp Val Gly Val Tyr Tyr Cys Gln Asn Gly His Ser Phe Pro Leu
85 90 95
Thr Phe Gly Ala Gly Thr Lys Leu Glu Leu Lys Ala Ser Thr Lys Gly
100 105 110
Pro Ser Val Phe Pro Leu Ala Pro Ser Ser Lys Ser Thr Ser Gly Gly
115 120 125
Thr Ala Ala Leu Gly Cys Leu Val Lys Asp Tyr Phe Pro Glu Pro Val
130 135 140
Thr Val Ser Trp Asn Ser Gly Ala Leu Thr Ser Gly Val His Thr Phe
145 150 155 160
Pro Ala Val Leu Gln Ser Ser Gly Leu Tyr Ser Leu Ser Ser Val Val
165 170 175
Thr Val Pro Ser Ser Ser Leu Gly Thr Gln Thr Tyr Ile Cys Asn Val
180 185 190
Asn His Lys Pro Ser Asn Thr Lys Val Asp Lys Arg Val Glu Pro Lys
195 200 205
Ser Cys Asp Lys Thr His
210

<210> SEQ ID NO 164
<211> LENGTH: 819
<212> TYPE: PRT
<213> ORGANISM: Artificial
<220> FEATURE:
<223> OTHER INFORMATION: Chimeric homo sapiens mus musculus

<400> SEQUENCE: 164

Asp Ile Gln Leu Thr Gln Ser Pro Ala Ser Leu Ala Val Ser Leu Gly
1 5 10 15
Gln Arg Ala Thr Ile Ser Cys Lys Ala Ser Gln Ser Val Asp Tyr Asp
20 25 30
Gly Asp Ser Tyr Leu Asn Trp Tyr Gln Gln Ile Pro Gly Gln Pro Pro
35 40 45
Lys Leu Leu Ile Tyr Asp Ala Ser Asn Leu Val Ser Gly Ile Pro Pro
50 55 60

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Arg	Phe	Ser	Gly	Ser	Gly	Ser	Gly	Thr	Asp	Phe	Thr	Leu	Asn	Ile	His	65	70	75	80
Pro	Val	Glu	Lys	Val	Asp	Ala	Ala	Thr	Tyr	His	Cys	Gln	Gln	Ser	Thr	85	90	95	
Glu	Asp	Pro	Trp	Thr	Phe	Gly	Gly	Gly	Thr	Lys	Leu	Glu	Ile	Lys	Gly	100	105	110	
Gly	Gly	Gly	Ser	Gly	Gly	Gly	Gly	Ser	Gly	Gly	Gly	Gly	Ser	Gln	Val	115	120	125	
Gln	Leu	Gln	Gln	Ser	Gly	Ala	Glu	Leu	Val	Arg	Pro	Gly	Ser	Ser	Val	130	135	140	
Lys	Ile	Ser	Cys	Lys	Ala	Ser	Gly	Tyr	Ala	Phe	Ser	Ser	Tyr	Trp	Met	145	150	155	160
Asn	Trp	Val	Lys	Gln	Arg	Pro	Gly	Gln	Gly	Leu	Glu	Trp	Ile	Gly	Gln	165	170	175	
Ile	Trp	Pro	Gly	Asp	Gly	Asp	Thr	Asn	Tyr	Asn	Gly	Lys	Phe	Lys	Gly	180	185	190	
Lys	Ala	Thr	Leu	Thr	Ala	Asp	Glu	Ser	Ser	Ser	Thr	Ala	Tyr	Met	Gln	195	200	205	
Leu	Ser	Ser	Leu	Ala	Ser	Glu	Asp	Ser	Ala	Val	Tyr	Phe	Cys	Ala	Arg	210	215	220	
Arg	Glu	Thr	Thr	Thr	Val	Gly	Arg	Tyr	Tyr	Tyr	Ala	Met	Asp	Tyr	Trp	225	230	235	240
Gly	Gln	Gly	Thr	Thr	Val	Thr	Val	Ser	Ser	Gly	Gly	Gly	Ser	Ser	Ala	245	250	255	
Pro	Glu	Leu	Leu	Gly	Gly	Pro	Ser	Val	Phe	Leu	Phe	Pro	Pro	Lys	Pro	260	265	270	
Lys	Asp	Thr	Leu	Met	Ile	Ser	Arg	Thr	Pro	Glu	Val	Thr	Cys	Val	Val	275	280	285	
Val	Asp	Val	Ser	His	Glu	Asp	Pro	Glu	Val	Lys	Phe	Asn	Trp	Tyr	Val	290	295	300	
Asp	Gly	Val	Glu	Val	His	Asn	Ala	Lys	Thr	Lys	Pro	Arg	Glu	Glu	Gln	305	310	315	320
Tyr	Ser	Ser	Thr	Tyr	Arg	Val	Val	Ser	Val	Leu	Thr	Val	Leu	His	Gln	325	330	335	
Asp	Trp	Leu	Asn	Gly	Lys	Glu	Tyr	Lys	Cys	Lys	Val	Ser	Asn	Lys	Ala	340	345	350	
Leu	Pro	Ala	Pro	Ile	Glu	Lys	Thr	Ile	Ser	Lys	Ala	Lys	Gly	Gln	Pro	355	360	365	
Arg	Glu	Pro	Gln	Val	Tyr	Thr	Leu	Pro	Pro	Ser	Arg	Glu	Glu	Met	Thr	370	375	380	
Lys	Asn	Gln	Val	Ser	Leu	Thr	Cys	Leu	Val	Lys	Gly	Phe	Tyr	Pro	Ser	385	390	395	400
Asp	Ile	Ala	Val	Glu	Trp	Glu	Ser	Asn	Gly	Gln	Pro	Glu	Asn	Asn	Tyr	405	410	415	
Lys	Thr	Thr	Pro	Pro	Val	Leu	Asp	Ser	Asp	Gly	Ser	Phe	Phe	Leu	Tyr	420	425	430	
Ser	Lys	Leu	Thr	Val	Asp	Lys	Ser	Arg	Trp	Gln	Gln	Gly	Asn	Val	Phe	435	440	445	
Ser	Cys	Ser	Val	Met	His	Glu	Ala	Leu	His	Asn	His	Tyr	Thr	Gln	Lys	450	455	460	

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Ser	Leu	Ser	Leu	Ser	Pro	Gly	Gly	Gly	Gly	Gly	Ser	Gly	Gly	Gly	Gly	465	470	475	480
Ser	Gly	Gly	Gly	Gly	Ser	Gly	Gln	Pro	Arg	Glu	Pro	Gln	Val	Tyr	Thr	485	490	495	
Leu	Pro	Pro	Ser	Arg	Glu	Glu	Met	Thr	Lys	Asn	Gln	Val	Ser	Leu	Thr	500	505	510	
Cys	Leu	Val	Lys	Gly	Phe	Tyr	Pro	Ser	Asp	Ile	Ala	Val	Glu	Trp	Glu	515	520	525	
Ser	Asn	Gly	Gln	Pro	Glu	Asn	Asn	Tyr	Lys	Thr	Thr	Pro	Pro	Val	Leu	530	535	540	
Asp	Ser	Asp	Gly	Ser	Phe	Phe	Leu	Tyr	Ser	Lys	Leu	Thr	Val	Asp	Lys	545	550	555	560
Ser	Arg	Trp	Gln	Gln	Gly	Asn	Val	Phe	Ser	Cys	Ser	Val	Met	His	Glu	565	570	575	
Ala	Leu	His	Asn	His	Tyr	Thr	Gln	Lys	Ser	Leu	Ser	Leu	Ser	Pro	Gly	580	585	590	
Ser	Thr	Gly	Ser	Glu	Val	Gln	Leu	Gln	Gln	Ser	Gly	Pro	Glu	Leu	Val	595	600	605	
Lys	Pro	Gly	Ala	Ser	Val	Lys	Ile	Ser	Cys	Lys	Thr	Ser	Gly	Tyr	Thr	610	615	620	
Phe	Thr	Glu	Tyr	Thr	Met	His	Trp	Val	Lys	Gln	Ser	His	Gly	Lys	Ser	625	630	635	640
Leu	Glu	Trp	Ile	Gly	Gly	Ile	Ser	Pro	Asn	Ile	Gly	Gly	Thr	Ser	Tyr	645	650	655	
Asn	Gln	Lys	Phe	Lys	Gly	Lys	Ala	Thr	Leu	Thr	Val	Asp	Lys	Ser	Ser	660	665	670	
Ser	Thr	Ala	Tyr	Met	Glu	Leu	Arg	Ser	Leu	Thr	Ser	Glu	Asp	Ser	Ala	675	680	685	
Val	Tyr	Tyr	Cys	Ala	Arg	Arg	Gly	Gly	Ser	Phe	Asp	Tyr	Trp	Gly	Gln	690	695	700	
Gly	Thr	Thr	Leu	Thr	Val	Ser	Ser	Ala	Ser	Thr	Lys	Gly	Pro	Ser	Val	705	710	715	720
Phe	Pro	Leu	Ala	Pro	Ser	Ser	Lys	Ser	Thr	Ser	Gly	Gly	Thr	Ala	Ala	725	730	735	
Leu	Gly	Cys	Leu	Val	Lys	Asp	Tyr	Phe	Pro	Glu	Pro	Val	Thr	Val	Ser	740	745	750	
Trp	Asn	Ser	Gly	Ala	Leu	Thr	Ser	Gly	Val	His	Thr	Phe	Pro	Ala	Val	755	760	765	
Leu	Gln	Ser	Ser	Gly	Leu	Tyr	Ser	Leu	Ser	Ser	Val	Val	Thr	Val	Pro	770	775	780	
Ser	Ser	Ser	Leu	Gly	Thr	Gln	Thr	Tyr	Ile	Cys	Asn	Val	Asn	His	Lys	785	790	795	800
Pro	Ser	Asn	Thr	Lys	Val	Asp	Lys	Arg	Val	Glu	Pro	Lys	Ser	Cys	Asp	805	810	815	
Lys	Thr	His																	

<210> SEQ ID NO 165

<211> LENGTH: 214

<212> TYPE: PRT

<213> ORGANISM: Artificial

<220> FEATURE:

<223> OTHER INFORMATION: Chimeric homo sapiens mus musculus

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<400> SEQUENCE: 165

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Asp Ile Val Met Thr Gln Ser Pro Ala Thr Leu Ser Val Thr Pro Gly
1      5      10      15
Asp Arg Val Ser Leu Ser Cys Arg Ala Ser Gln Ser Ile Ser Asp Tyr
20     25     30
Leu His Trp Tyr Gln Gln Lys Ser His Glu Ser Pro Arg Leu Leu Ile
35     40     45
Lys Tyr Ala Ser Gln Ser Ile Ser Gly Ile Pro Ser Arg Phe Ser Gly
50     55     60
Ser Gly Ser Gly Ser Asp Phe Thr Leu Ser Ile Asn Ser Val Glu Pro
65     70     75     80
Glu Asp Val Gly Val Tyr Tyr Cys Gln Asn Gly His Ser Phe Pro Leu
85     90     95
Thr Phe Gly Ala Gly Thr Lys Leu Glu Leu Lys Arg Thr Val Ala Ala
100    105    110
Pro Ser Val Phe Ile Phe Pro Pro Ser Asp Glu Gln Leu Lys Ser Gly
115    120    125
Thr Ala Ser Val Val Cys Leu Leu Asn Asn Phe Tyr Pro Arg Glu Ala
130    135    140
Lys Val Gln Trp Lys Val Asp Asn Ala Leu Gln Ser Gly Asn Ser Gln
145    150    155    160
Glu Ser Val Thr Glu Gln Asp Ser Lys Asp Ser Thr Tyr Ser Leu Ser
165    170    175
Ser Thr Leu Thr Leu Ser Lys Ala Asp Tyr Glu Lys His Lys Val Tyr
180    185    190
Ala Cys Glu Val Thr His Gln Gly Leu Ser Ser Pro Val Thr Lys Ser
195    200    205
Phe Asn Arg Gly Glu Cys
210

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<210> SEQ ID NO 166

<211> LENGTH: 218

<212> TYPE: PRT

<213> ORGANISM: Artificial

<220> FEATURE:

<223> OTHER INFORMATION: Chimeric homo sapiens mus musculus

<400> SEQUENCE: 166

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Asp Ile Gln Leu Thr Gln Ser Pro Ala Ser Leu Ala Val Ser Leu Gly
1      5      10      15
Gln Arg Ala Thr Ile Ser Cys Lys Ala Ser Gln Ser Val Asp Tyr Asp
20     25     30
Gly Asp Ser Tyr Leu Asn Trp Tyr Gln Gln Ile Pro Gly Gln Pro Pro
35     40     45
Lys Leu Leu Ile Tyr Asp Ala Ser Asn Leu Val Ser Gly Ile Pro Pro
50     55     60
Arg Phe Ser Gly Ser Gly Ser Gly Thr Asp Phe Thr Leu Asn Ile His
65     70     75     80
Pro Val Glu Lys Val Asp Ala Ala Thr Tyr His Cys Gln Gln Ser Thr
85     90     95
Glu Asp Pro Trp Thr Phe Gly Gly Gly Thr Lys Leu Glu Ile Lys Arg
100    105    110
Thr Val Ala Ala Pro Ser Val Phe Ile Phe Pro Pro Ser Asp Glu Gln

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115	120	125
Leu Lys Ser Gly Thr Ala Ser Val Val Cys Leu Leu Asn Asn Phe Tyr		
130	135	140
Pro Arg Glu Ala Lys Val Gln Trp Lys Val Asp Asn Ala Leu Gln Ser		
145	150	155
Gly Asn Ser Gln Glu Ser Val Thr Glu Gln Asp Ser Lys Asp Ser Thr		
	165	170
		175
Tyr Ser Leu Ser Ser Thr Leu Thr Leu Ser Lys Ala Asp Tyr Glu Lys		
	180	185
		190
His Lys Val Tyr Ala Cys Glu Val Thr His Gln Gly Leu Ser Ser Pro		
	195	200
		205
Val Thr Lys Ser Phe Asn Arg Gly Glu Cys		
	210	215

<210> SEQ ID NO 167
 <211> LENGTH: 792
 <212> TYPE: PRT
 <213> ORGANISM: Artificial
 <220> FEATURE:
 <223> OTHER INFORMATION: Chimeric homo sapiens mus musculus

<400> SEQUENCE: 167

Gln Val Gln Leu Gln Gln Ser Gly Ala Glu Leu Val Arg Pro Gly Ser
1 5 10 15
Ser Val Lys Ile Ser Cys Lys Ala Ser Gly Tyr Ala Phe Ser Ser Tyr
20 25 30
Trp Met Asn Trp Val Lys Gln Arg Pro Gly Gln Gly Leu Glu Trp Ile
35 40 45
Gly Gln Ile Trp Pro Gly Asp Gly Asp Thr Asn Tyr Asn Gly Lys Phe
50 55 60
Lys Gly Lys Ala Thr Leu Thr Ala Asp Glu Ser Ser Ser Thr Ala Tyr
65 70 75 80
Met Gln Leu Ser Ser Leu Ala Ser Glu Asp Ser Ala Val Tyr Phe Cys
85 90 95
Ala Arg Arg Glu Thr Thr Thr Val Gly Arg Tyr Tyr Tyr Ala Met Asp
100 105 110
Tyr Trp Gly Gln Gly Thr Thr Val Thr Val Ser Ser Ala Ser Thr Lys
115 120 125
Gly Pro Ser Val Phe Pro Leu Ala Pro Ser Ser Lys Ser Thr Ser Gly
130 135 140
Gly Thr Ala Ala Leu Gly Cys Leu Val Lys Asp Tyr Phe Pro Glu Pro
145 150 155 160
Val Thr Val Ser Trp Asn Ser Gly Ala Leu Thr Ser Gly Val His Thr
165 170 175
Phe Pro Ala Val Leu Gln Ser Ser Gly Leu Tyr Ser Leu Ser Ser Val
180 185 190
Val Thr Val Pro Ser Ser Ser Leu Gly Thr Gln Thr Tyr Ile Cys Asn
195 200 205
Val Asn His Lys Pro Ser Asn Thr Lys Val Asp Lys Arg Val Glu Pro
210 215 220
Lys Ser Cys Asp Lys Thr His Thr Ser Pro Pro Ser Pro Ala Pro Glu
225 230 235 240
Leu Leu Gly Gly Pro Ser Val Phe Leu Phe Pro Pro Lys Pro Lys Asp

-continued

245						250						255				
Thr	Leu	Met	Ile	Ser	Arg	Thr	Pro	Glu	Val	Thr	Cys	Val	Val	Val	Asp	
			260						265			270				
Val	Ser	His	Glu	Asp	Pro	Glu	Val	Lys	Phe	Asn	Trp	Tyr	Val	Asp	Gly	
			275						280			285				
Val	Glu	Val	His	Asn	Ala	Lys	Thr	Lys	Pro	Arg	Glu	Glu	Gln	Tyr	Ser	
			290			295						300				
Ser	Thr	Tyr	Arg	Val	Val	Ser	Val	Leu	Thr	Val	Leu	His	Gln	Asp	Trp	
305						310			315			320				
Leu	Asn	Gly	Lys	Glu	Tyr	Lys	Cys	Lys	Val	Ser	Asn	Lys	Ala	Leu	Pro	
			325						330			335				
Ala	Pro	Ile	Glu	Lys	Thr	Ile	Ser	Lys	Ala	Lys	Gly	Gln	Pro	Arg	Glu	
			340						345			350				
Pro	Gln	Val	Tyr	Thr	Leu	Pro	Pro	Ser	Arg	Glu	Glu	Met	Thr	Lys	Asn	
			355			360						365				
Gln	Val	Ser	Leu	Thr	Cys	Leu	Val	Lys	Gly	Phe	Tyr	Pro	Ser	Asp	Ile	
370						375						380				
Ala	Val	Glu	Trp	Glu	Ser	Asn	Gly	Gln	Pro	Glu	Asn	Asn	Tyr	Lys	Thr	
385						390			395			400				
Thr	Pro	Pro	Val	Leu	Asp	Ser	Asp	Gly	Ser	Phe	Phe	Leu	Tyr	Ser	Lys	
			405						410			415				
Leu	Thr	Val	Asp	Lys	Ser	Arg	Trp	Gln	Gln	Gly	Asn	Val	Phe	Ser	Cys	
			420						425			430				
Ser	Val	Met	His	Glu	Ala	Leu	His	Asn	His	Tyr	Thr	Gln	Lys	Ser	Leu	
			435			440						445				
Ser	Leu	Ser	Pro	Gly	Gly	Gly	Gly	Gly	Ser	Gly	Gly	Gly	Gly	Ser	Gly	
450						455						460				
Gly	Gly	Gly	Ser	Gly	Gln	Pro	Arg	Glu	Pro	Gln	Val	Tyr	Thr	Leu	Pro	
465						470			475			480				
Pro	Ser	Arg	Glu	Glu	Met	Thr	Lys	Asn	Gln	Val	Ser	Leu	Thr	Cys	Leu	
			485						490			495				
Val	Lys	Gly	Phe	Tyr	Pro	Ser	Asp	Ile	Ala	Val	Glu	Trp	Glu	Ser	Asn	
			500						505			510				
Gly	Gln	Pro	Glu	Asn	Asn	Tyr	Lys	Thr	Thr	Pro	Pro	Val	Leu	Asp	Ser	
			515			520						525				
Asp	Gly	Ser	Phe	Phe	Leu	Tyr	Ser	Lys	Leu	Thr	Val	Asp	Lys	Ser	Arg	
530						535						540				
Trp	Gln	Gln	Gly	Asn	Val	Phe	Ser	Cys	Ser	Val	Met	His	Glu	Ala	Leu	
545						550			555			560				
His	Asn	His	Tyr	Thr	Gln	Lys	Ser	Leu	Ser	Leu	Ser	Pro	Gly	Ser	Thr	
			565						570			575				
Gly	Ser	Asp	Ile	Val	Met	Thr	Gln	Ser	Pro	Ala	Thr	Leu	Ser	Val	Thr	
			580						585			590				
Pro	Gly	Asp	Arg	Val	Ser	Leu	Ser	Cys	Arg	Ala	Ser	Gln	Ser	Ile	Ser	
			595			600						605				
Asp	Tyr	Leu	His	Trp	Tyr	Gln	Gln	Lys	Ser	His	Glu	Ser	Pro	Arg	Leu	
610						615						620				
Leu	Ile	Lys	Tyr	Ala	Ser	Gln	Ser	Ile	Ser	Gly	Ile	Pro	Ser	Arg	Phe	
625						630			635			640				
Ser	Gly	Ser	Gly	Ser	Gly	Ser	Asp	Phe	Thr	Leu	Ser	Ile	Asn	Ser	Val	
			645						650			655				

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Glu Pro Glu Asp Val Gly Val Tyr Tyr Cys Gln Asn Gly His Ser Phe
 660 665 670
 Pro Leu Thr Phe Gly Ala Gly Thr Lys Leu Glu Leu Lys Ala Ser Thr
 675 680 685
 Lys Gly Pro Ser Val Phe Pro Leu Ala Pro Ser Ser Lys Ser Thr Ser
 690 695 700
 Gly Gly Thr Ala Ala Leu Gly Cys Leu Val Lys Asp Tyr Phe Pro Glu
 705 710 715 720
 Pro Val Thr Val Ser Trp Asn Ser Gly Ala Leu Thr Ser Gly Val His
 725 730 735
 Thr Phe Pro Ala Val Leu Gln Ser Ser Gly Leu Tyr Ser Leu Ser Ser
 740 745 750
 Val Val Thr Val Pro Ser Ser Ser Leu Gly Thr Gln Thr Tyr Ile Cys
 755 760 765
 Asn Val Asn His Lys Pro Ser Asn Thr Lys Val Asp Lys Arg Val Glu
 770 775 780
 Pro Lys Ser Cys Asp Lys Thr His
 785 790

<210> SEQ ID NO 168
 <211> LENGTH: 223
 <212> TYPE: PRT
 <213> ORGANISM: Artificial
 <220> FEATURE:
 <223> OTHER INFORMATION: Chimeric homo sapiens mus musculus

<400> SEQUENCE: 168

Glu Val Gln Leu Gln Gln Ser Gly Pro Glu Leu Val Lys Pro Gly Ala
 1 5 10 15
 Ser Val Lys Ile Ser Cys Lys Thr Ser Gly Tyr Thr Phe Thr Glu Tyr
 20 25 30
 Thr Met His Trp Val Lys Gln Ser His Gly Lys Ser Leu Glu Trp Ile
 35 40 45
 Gly Gly Ile Ser Pro Asn Ile Gly Gly Thr Ser Tyr Asn Gln Lys Phe
 50 55 60
 Lys Gly Lys Ala Thr Leu Thr Val Asp Lys Ser Ser Ser Thr Ala Tyr
 65 70 75 80
 Met Glu Leu Arg Ser Leu Thr Ser Glu Asp Ser Ala Val Tyr Tyr Cys
 85 90 95
 Ala Arg Arg Gly Gly Ser Phe Asp Tyr Trp Gly Gln Gly Thr Thr Leu
 100 105 110
 Thr Val Ser Ser Arg Thr Val Ala Ala Pro Ser Val Phe Ile Phe Pro
 115 120 125
 Pro Ser Asp Glu Gln Leu Lys Ser Gly Thr Ala Ser Val Val Cys Leu
 130 135 140
 Leu Asn Asn Phe Tyr Pro Arg Glu Ala Lys Val Gln Trp Lys Val Asp
 145 150 155 160
 Asn Ala Leu Gln Ser Gly Asn Ser Gln Glu Ser Val Thr Glu Gln Asp
 165 170 175
 Ser Lys Asp Ser Thr Tyr Ser Leu Ser Ser Thr Leu Thr Leu Ser Lys
 180 185 190
 Ala Asp Tyr Glu Lys His Lys Val Tyr Ala Cys Glu Val Thr His Gln
 195 200 205

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Gly Leu Ser Ser Pro Val Thr Lys Ser Phe Asn Arg Gly Glu Cys
 210 215 220

<210> SEQ ID NO 169
 <211> LENGTH: 445
 <212> TYPE: PRT
 <213> ORGANISM: Artificial
 <220> FEATURE:
 <223> OTHER INFORMATION: Chimeric homo sapiens mus musculus

<400> SEQUENCE: 169

Asp Ile Gln Leu Thr Gln Ser Pro Ala Ser Leu Ala Val Ser Leu Gly
 1 5 10 15
 Gln Arg Ala Thr Ile Ser Cys Lys Ala Ser Gln Ser Val Asp Tyr Asp
 20 25 30
 Gly Asp Ser Tyr Leu Asn Trp Tyr Gln Gln Ile Pro Gly Gln Pro Pro
 35 40 45
 Lys Leu Leu Ile Tyr Asp Ala Ser Asn Leu Val Ser Gly Ile Pro Pro
 50 55 60
 Arg Phe Ser Gly Ser Gly Ser Gly Thr Asp Phe Thr Leu Asn Ile His
 65 70 75 80
 Pro Val Glu Lys Val Asp Ala Ala Thr Tyr His Cys Gln Gln Ser Thr
 85 90 95
 Glu Asp Pro Trp Thr Phe Gly Gly Gly Thr Lys Leu Glu Ile Lys Arg
 100 105 110
 Thr Val Ala Ala Pro Ser Val Phe Ile Phe Pro Pro Ser Asp Glu Gln
 115 120 125
 Leu Lys Ser Gly Thr Ala Ser Val Val Cys Leu Leu Asn Asn Phe Tyr
 130 135 140
 Pro Arg Glu Ala Lys Val Gln Trp Lys Val Asp Asn Ala Leu Gln Ser
 145 150 155 160
 Gly Asn Ser Gln Glu Ser Val Thr Glu Gln Asp Ser Lys Asp Ser Thr
 165 170 175
 Tyr Ser Leu Ser Ser Thr Leu Thr Leu Ser Lys Ala Asp Tyr Glu Lys
 180 185 190
 His Lys Val Tyr Ala Cys Glu Val Thr His Gln Gly Leu Ser Ser Pro
 195 200 205
 Val Thr Lys Ser Phe Asn Arg Gly Glu Cys Asp Lys Thr His Thr Cys
 210 215 220
 Pro Pro Cys Pro Ala Pro Glu Leu Leu Gly Gly Pro Ser Val Phe Leu
 225 230 235 240
 Phe Pro Pro Lys Pro Lys Asp Thr Leu Met Ile Ser Arg Thr Pro Glu
 245 250 255
 Val Thr Cys Val Val Val Asp Val Ser His Glu Asp Pro Glu Val Lys
 260 265 270
 Phe Asn Trp Tyr Val Asp Gly Val Glu Val His Asn Ala Lys Thr Lys
 275 280 285
 Pro Arg Glu Glu Gln Tyr Asn Ser Thr Tyr Arg Val Val Ser Val Leu
 290 295 300
 Thr Val Leu His Gln Asp Trp Leu Asn Gly Lys Glu Tyr Lys Cys Lys
 305 310 315 320
 Val Ser Asn Lys Ala Leu Pro Ala Pro Ile Glu Lys Thr Ile Ser Lys
 325 330 335

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Ala Lys Gly Gln Pro Arg Glu Pro Gln Val Tyr Thr Leu Pro Pro Ser
 340 345 350

Arg Glu Glu Met Thr Lys Asn Gln Val Ser Leu Thr Cys Leu Val Lys
 355 360 365

Gly Phe Tyr Pro Ser Asp Ile Ala Val Glu Trp Glu Ser Asn Gly Gln
 370 375 380

Pro Glu Asn Asn Tyr Lys Thr Thr Pro Pro Val Leu Asp Ser Asp Gly
 385 390 395 400

Ser Phe Phe Leu Tyr Ser Lys Leu Thr Val Asp Lys Ser Arg Trp Gln
 405 410 415

Gln Gly Asn Val Phe Ser Cys Ser Val Met His Glu Ala Leu His Asn
 420 425 430

His Tyr Thr Gln Lys Ser Leu Ser Leu Ser Pro Gly Lys
 435 440 445

<210> SEQ ID NO 170

<211> LENGTH: 680

<212> TYPE: PRT

<213> ORGANISM: Artificial

<220> FEATURE:

<223> OTHER INFORMATION: Chimeric homo sapiens mus musculus

<400> SEQUENCE: 170

Gln Val Gln Leu Gln Gln Ser Gly Ala Glu Leu Val Arg Pro Gly Ser
 1 5 10 15

Ser Val Lys Ile Ser Cys Lys Ala Ser Gly Tyr Ala Phe Ser Ser Tyr
 20 25 30

Trp Met Asn Trp Val Lys Gln Arg Pro Gly Gln Gly Leu Glu Trp Ile
 35 40 45

Gly Gln Ile Trp Pro Gly Asp Gly Asp Thr Asn Tyr Asn Gly Lys Phe
 50 55 60

Lys Gly Lys Ala Thr Leu Thr Ala Asp Glu Ser Ser Ser Thr Ala Tyr
 65 70 75 80

Met Gln Leu Ser Ser Leu Ala Ser Glu Asp Ser Ala Val Tyr Phe Cys
 85 90 95

Ala Arg Arg Glu Thr Thr Thr Val Gly Arg Tyr Tyr Tyr Ala Met Asp
 100 105 110

Tyr Trp Gly Gln Gly Thr Thr Val Thr Val Ser Ser Ala Ser Thr Lys
 115 120 125

Gly Pro Ser Val Phe Pro Leu Ala Pro Ser Ser Lys Ser Thr Ser Gly
 130 135 140

Gly Thr Ala Ala Leu Gly Cys Leu Val Lys Asp Tyr Phe Pro Glu Pro
 145 150 155 160

Val Thr Val Ser Trp Asn Ser Gly Ala Leu Thr Ser Gly Val His Thr
 165 170 175

Phe Pro Ala Val Leu Gln Ser Ser Gly Leu Tyr Ser Leu Ser Ser Val
 180 185 190

Val Thr Val Pro Ser Ser Ser Leu Gly Thr Gln Thr Tyr Ile Cys Asn
 195 200 205

Val Asn His Lys Pro Ser Asn Thr Lys Val Asp Lys Arg Val Glu Pro
 210 215 220

Lys Ser Cys Asp Lys Thr His Thr Cys Pro Pro Cys Pro Ala Pro Glu
 225 230 235 240

Leu	Leu	Gly	Gly	Pro	Ser	Val	Phe	Leu	Phe	Pro	Pro	Lys	Pro	Lys	Asp
				245					250					255	
Thr	Leu	Met	Ile	Ser	Arg	Thr	Pro	Glu	Val	Thr	Cys	Val	Val	Val	Asp
			260					265					270		
Val	Ser	His	Glu	Asp	Pro	Glu	Val	Lys	Phe	Asn	Trp	Tyr	Val	Asp	Gly
		275					280					285			
Val	Glu	Val	His	Asn	Ala	Lys	Thr	Lys	Pro	Arg	Glu	Glu	Gln	Tyr	Asn
	290					295				300					
Ser	Thr	Tyr	Arg	Val	Val	Ser	Val	Leu	Thr	Val	Leu	His	Gln	Asp	Trp
305					310					315					320
Leu	Asn	Gly	Lys	Glu	Tyr	Lys	Cys	Lys	Val	Ser	Asn	Lys	Ala	Leu	Pro
				325					330					335	
Ala	Pro	Ile	Glu	Lys	Thr	Ile	Ser	Lys	Ala	Lys	Gly	Gln	Pro	Arg	Glu
			340					345					350		
Pro	Gln	Val	Tyr	Thr	Leu	Pro	Pro	Ser	Arg	Glu	Glu	Met	Thr	Lys	Asn
		355					360					365			
Gln	Val	Ser	Leu	Thr	Cys	Leu	Val	Lys	Gly	Phe	Tyr	Pro	Ser	Asp	Ile
	370					375					380				
Ala	Val	Glu	Trp	Glu	Ser	Asn	Gly	Gln	Pro	Glu	Asn	Asn	Tyr	Lys	Thr
385					390					395					400
Thr	Pro	Pro	Val	Leu	Asp	Ser	Asp	Gly	Ser	Phe	Phe	Leu	Tyr	Ser	Lys
			405						410					415	
Leu	Thr	Val	Asp	Lys	Ser	Arg	Trp	Gln	Gln	Gly	Asn	Val	Phe	Ser	Cys
			420					425				430			
Ser	Val	Met	His	Glu	Ala	Leu	His	Asn	His	Tyr	Thr	Gln	Lys	Ser	Leu
	435						440					445			
Ser	Leu	Ser	Pro	Gly	Ser	Thr	Gly	Ser	Glu	Val	Gln	Leu	Gln	Gln	Ser
	450					455					460				
Gly	Pro	Glu	Leu	Val	Lys	Pro	Gly	Ala	Ser	Val	Lys	Ile	Ser	Cys	Lys
465					470					475					480
Thr	Ser	Gly	Tyr	Thr	Phe	Thr	Glu	Tyr	Thr	Met	His	Trp	Val	Lys	Gln
			485						490					495	
Ser	His	Gly	Lys	Ser	Leu	Glu	Trp	Ile	Gly	Gly	Ile	Ser	Pro	Asn	Ile
			500					505					510		
Gly	Gly	Thr	Ser	Tyr	Asn	Gln	Lys	Phe	Lys	Gly	Lys	Ala	Thr	Leu	Thr
		515					520					525			
Val	Asp	Lys	Ser	Ser	Ser	Thr	Ala	Tyr	Met	Glu	Leu	Arg	Ser	Leu	Thr
	530					535					540				
Ser	Glu	Asp	Ser	Ala	Val	Tyr	Tyr	Cys	Ala	Arg	Arg	Gly	Gly	Ser	Phe
545					550					555					560
Asp	Tyr	Trp	Gly	Gln	Gly	Thr	Thr	Leu	Thr	Val	Ser	Ser	Arg	Thr	Val
			565						570					575	
Ala	Ala	Pro	Ser	Val	Phe	Ile	Phe	Pro	Pro	Ser	Asp	Glu	Gln	Leu	Lys
			580					585					590		
Ser	Gly	Thr	Ala	Ser	Val	Val	C								

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Leu Ser Ser Thr Leu Thr Leu Ser Lys Ala Asp Tyr Glu Lys His Lys
645 650 655

Val Tyr Ala Cys Glu Val Thr His Gln Gly Leu Ser Ser Pro Val Thr
660 665 670

Lys Ser Phe Asn Arg Gly Glu Cys
675 680

<210> SEQ ID NO 171
 <211> LENGTH: 214
 <212> TYPE: PRT
 <213> ORGANISM: Artificial
 <220> FEATURE:
 <223> OTHER INFORMATION: Chimeric homo sapiens mus musculus

<400> SEQUENCE: 171

Asp Ile Val Met Thr Gln Ser Pro Ala Thr Leu Ser Val Thr Pro Gly
1 5 10 15

Asp Arg Val Ser Leu Ser Cys Arg Ala Ser Gln Ser Ile Ser Asp Tyr
20 25 30

Leu His Trp Tyr Gln Gln Lys Ser His Glu Ser Pro Arg Leu Leu Ile
35 40 45

Lys Tyr Ala Ser Gln Ser Ile Ser Gly Ile Pro Ser Arg Phe Ser Gly
50 55 60

Ser Gly Ser Gly Ser Asp Phe Thr Leu Ser Ile Asn Ser Val Glu Pro
65 70 75 80

Glu Asp Val Gly Val Tyr Tyr Cys Gln Asn Gly His Ser Phe Pro Leu
85 90 95

Thr Phe Gly Ala Gly Thr Lys Leu Glu Leu Lys Ala Ser Thr Lys Gly
100 105 110

Pro Ser Val Phe Pro Leu Ala Pro Ser Ser Lys Ser Thr Ser Gly Gly
115 120 125

Thr Ala Ala Leu Gly Cys Leu Val Lys Asp Tyr Phe Pro Glu Pro Val
130 135 140

Thr Val Ser Trp Asn Ser Gly Ala Leu Thr Ser Gly Val His Thr Phe
145 150 155 160

Pro Ala Val Leu Gln Ser Ser Gly Leu Tyr Ser Leu Ser Ser Val Val
165 170 175

Thr Val Pro Ser Ser Ser Leu Gly Thr Gln Thr Tyr Ile Cys Asn Val
180 185 190

Asn His Lys Pro Ser Asn Thr Lys Val Asp Lys Arg Val Glu Pro Lys
195 200 205

Ser Cys Asp Lys Thr His
210

<210> SEQ ID NO 172
 <211> LENGTH: 445
 <212> TYPE: PRT
 <213> ORGANISM: Artificial
 <220> FEATURE:
 <223> OTHER INFORMATION: Chimeric homo sapiens mus musculus

<400> SEQUENCE: 172

Asp Ile Gln Leu Thr Gln Ser Pro Ala Ser Leu Ala Val Ser Leu Gly
1 5 10 15

Gln Arg Ala Thr Ile Ser Cys Lys Ala Ser Gln Ser Val Asp Tyr Asp
20 25 30

Gly 35	Asp	Ser	Tyr	Leu	Asn	Trp	Tyr 40	Gln	Gln	Ile	Pro	Gly 45	Gln	Pro	Pro
Lys 50	Leu	Leu	Ile	Tyr	Asp	Ala 55	Ser	Asn	Leu	Val	Ser 60	Gly	Ile	Pro	Pro
Arg 65	Phe	Ser	Gly	Ser	Gly 70	Ser	Gly	Thr	Asp	Phe 75	Thr	Leu	Asn	Ile	His 80
Pro	Val	Glu	Lys	Val 85	Asp	Ala	Ala	Thr	Tyr 90	His	Cys	Gln	Gln	Ser 95	Thr
Glu	Asp	Pro	Trp 100	Thr	Phe	Gly	Gly	Gly 105	Thr	Lys	Leu	Glu	Ile 110	Lys	Arg
Thr	Val	Ala 115	Ala	Pro	Ser	Val	Phe 120	Ile	Phe	Pro	Pro	Ser 125	Asp	Glu	Gln
Leu	Lys 130	Ser	Gly	Thr	Ala	Ser 135	Val	Val	Cys	Leu	Leu 140	Asn	Asn	Phe	Tyr
Pro 145	Arg	Glu	Ala	Lys	Val 150	Gln	Trp	Lys	Val	Asp 155	Asn	Ala	Leu	Gln	Ser 160
Gly	Asn	Ser	Gln	Glu 165	Ser	Val	Thr	Glu	Gln 170	Asp	Ser	Lys	Asp	Ser 175	Thr
Tyr	Ser	Leu	Ser	Ser 180	Thr	Leu	Thr	Leu 185	Ser	Lys	Ala	Asp	Tyr 190	Glu	Lys
His	Lys 195	Val	Tyr	Ala	Cys	Glu	Val 200	Thr	His	Gln	Gly	Leu 205	Ser	Ser	Pro
Val	Thr 210	Lys	Ser	Phe	Asn	Arg 215	Gly	Glu	Cys	Asp 220	Lys	Thr	His	Thr	Cys
Pro 225	Pro	Cys	Pro	Ala	Pro 230	Glu	Leu	Leu	Gly	Gly 235	Pro	Ser	Val	Phe	Leu 240
Phe	Pro	Pro	Lys	Pro 245	Lys	Asp	Thr	Leu	Met	Ile 250	Ser	Arg	Thr	Pro 255	Glu
Val	Thr	Cys	Val 260	Val	Val	Asp	Val	Ser 265	His	Glu	Asp	Pro	Glu	Val 270	Lys
Phe	Asn 275	Trp	Tyr	Val	Asp	Gly	Val 280	Glu	Val	His	Asn	Ala 285	Lys	Thr	Lys
Pro	Arg 290	Glu	Glu	Gln	Tyr	Ser 295	Ser	Thr	Tyr	Arg	Val 300	Val	Ser	Val	Leu
Thr 305	Val	Leu	His	Gln	Asp 310	Trp	Leu	Asn	Gly	Lys 315	Glu	Tyr	Lys	Cys	Lys 320
Val	Ser	Asn	Lys	Ala 325	Leu	Pro	Ala	Pro	Ile	Glu	Lys	Thr	Ile	Ser 335	Lys
Ala	Lys	Gly	Gln 340	Pro	Arg	Glu	Pro	Gln 345	Val	Tyr	Thr	Leu	Pro 350	Pro	Ser
Arg	Glu	Glu	Met 355	Thr	Lys	Asn	Gln 360	Val	Ser	Leu	Thr	Cys	Leu 365	Val	Lys
Gly	Phe 370	Tyr	Pro	Ser	Asp	Ile 375	Ala	Val	Glu	Trp	Glu 380	Ser	Asn	Gly	Gln
Pro 385	Glu	Asn	Asn	Tyr	Lys 390	Thr	Thr	Pro	Pro	Val 395	Leu	Asp	Ser	Asp	Gly 400
Ser	Phe	Phe	Leu 405	Tyr	Ser	Lys	Leu	Thr	Val 410	Asp	Lys	Ser	Arg	Trp 415	Gln
Gln	Gly	Asn	Val 420	Phe	Ser	Cys	Ser	Val 425	Met	His	Glu	Ala	Leu 430	His	Asn

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His Tyr Thr Gln Lys Ser Leu Ser Leu Ser Pro Gly Lys
 435 440 445

<210> SEQ ID NO 173
 <211> LENGTH: 680
 <212> TYPE: PRT
 <213> ORGANISM: Artificial
 <220> FEATURE:
 <223> OTHER INFORMATION: Chimeric homo sapiens mus musculus

<400> SEQUENCE: 173

Gln Val Gln Leu Gln Gln Ser Gly Ala Glu Leu Val Arg Pro Gly Ser
 1 5 10 15
 Ser Val Lys Ile Ser Cys Lys Ala Ser Gly Tyr Ala Phe Ser Ser Tyr
 20 25 30
 Trp Met Asn Trp Val Lys Gln Arg Pro Gly Gln Gly Leu Glu Trp Ile
 35 40 45
 Gly Gln Ile Trp Pro Gly Asp Gly Asp Thr Asn Tyr Asn Gly Lys Phe
 50 55 60
 Lys Gly Lys Ala Thr Leu Thr Ala Asp Glu Ser Ser Ser Thr Ala Tyr
 65 70 75 80
 Met Gln Leu Ser Ser Leu Ala Ser Glu Asp Ser Ala Val Tyr Phe Cys
 85 90 95
 Ala Arg Arg Glu Thr Thr Thr Val Gly Arg Tyr Tyr Tyr Ala Met Asp
 100 105 110
 Tyr Trp Gly Gln Gly Thr Thr Val Thr Val Ser Ser Ala Ser Thr Lys
 115 120 125
 Gly Pro Ser Val Phe Pro Leu Ala Pro Ser Ser Lys Ser Thr Ser Gly
 130 135 140
 Gly Thr Ala Ala Leu Gly Cys Leu Val Lys Asp Tyr Phe Pro Glu Pro
 145 150 155 160
 Val Thr Val Ser Trp Asn Ser Gly Ala Leu Thr Ser Gly Val His Thr
 165 170 175
 Phe Pro Ala Val Leu Gln Ser Ser Gly Leu Tyr Ser Leu Ser Ser Val
 180 185 190
 Val Thr Val Pro Ser Ser Ser Leu Gly Thr Gln Thr Tyr Ile Cys Asn
 195 200 205
 Val Asn His Lys Pro Ser Asn Thr Lys Val Asp Lys Arg Val Glu Pro
 210 215 220
 Lys Ser Cys Asp Lys Thr His Thr Cys Pro Pro Cys Pro Ala Pro Glu
 225 230 235 240
 Leu Leu Gly Gly Pro Ser Val Phe Leu Phe Pro Pro Lys Pro Lys Asp
 245 250 255
 Thr Leu Met Ile Ser Arg Thr Pro Glu Val Thr Cys Val Val Val Asp
 260 265 270
 Val Ser His Glu Asp Pro Glu Val Lys Phe Asn Trp Tyr Val Asp Gly
 275 280 285
 Val Glu Val His Asn Ala Lys Thr Lys Pro Arg Glu Glu Gln Tyr Ser
 290 295 300
 Ser Thr Tyr Arg Val Val Ser Val Leu Thr Val Leu His Gln Asp Trp
 305 310 315 320
 Leu Asn Gly Lys Glu Tyr Lys Cys Lys Val Ser Asn Lys Ala Leu Pro
 325 330 335

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Ala	Pro	Ile	Glu	Lys	Thr	Ile	Ser	Lys	Ala	Lys	Gly	Gln	Pro	Arg	Glu		
			340					345					350				
Pro	Gln	Val	Tyr	Thr	Leu	Pro	Pro	Ser	Arg	Glu	Glu	Met	Thr	Lys	Asn		
		355					360					365					
Gln	Val	Ser	Leu	Thr	Cys	Leu	Val	Lys	Gly	Phe	Tyr	Pro	Ser	Asp	Ile		
	370					375					380						
Ala	Val	Glu	Trp	Glu	Ser	Asn	Gly	Gln	Pro	Glu	Asn	Asn	Tyr	Lys	Thr		
385					390					395					400		
Thr	Pro	Pro	Val	Leu	Asp	Ser	Asp	Gly	Ser	Phe	Phe	Leu	Tyr	Ser	Lys		
			405						410					415			
Leu	Thr	Val	Asp	Lys	Ser	Arg	Trp	Gln	Gln	Gly	Asn	Val	Phe	Ser	Cys		
		420						425					430				
Ser	Val	Met	His	Glu	Ala	Leu	His	Asn	His	Tyr	Thr	Gln	Lys	Ser	Leu		
	435						440					445					
Ser	Leu	Ser	Pro	Gly	Ser	Thr	Gly	Ser	Glu	Val	Gln	Leu	Gln	Gln	Ser		
	450					455					460						
Gly	Pro	Glu	Leu	Val	Lys	Pro	Gly	Ala	Ser	Val	Lys	Ile	Ser	Cys	Lys		
465					470					475					480		
Thr	Ser	Gly	Tyr	Thr	Phe	Thr	Glu	Tyr	Thr	Met	His	Trp	Val	Lys	Gln		
			485						490					495			
Ser	His	Gly	Lys	Ser	Leu	Glu	Trp	Ile	Gly	Gly	Ile	Ser	Pro	Asn	Ile		
		500						505					510				
Gly	Gly	Thr	Ser	Tyr	Asn	Gln	Lys	Phe	Lys	Gly	Lys	Ala	Thr	Leu	Thr		
	515					520						525					
Val	Asp	Lys	Ser	Ser	Ser	Thr	Ala	Tyr	Met	Glu	Leu	Arg	Ser	Leu	Thr		
	530					535					540						
Ser	Glu	Asp	Ser	Ala	Val	Tyr	Tyr	Cys	Ala	Arg	Arg	Gly	Gly	Ser	Phe		
545					550					555					560		
Asp	Tyr	Trp	Gly	Gln	Gly	Thr	Thr	Leu	Thr	Val	Ser	Ser	Arg	Thr	Val		
			565						570					575			
Ala	Ala	Pro	Ser	Val	Phe	Ile	Phe	Pro	Pro	Ser	Asp	Glu	Gln	Leu	Lys		
		580						585					590				
Ser	Gly	Thr	Ala	Ser	Val	Val	Cys	Leu	Leu	Asn	Asn	Phe	Tyr	Pro	Arg		
	595						600					605					
Glu	Ala	Lys	Val	Gln	Trp	Lys	Val	Asp	Asn	Ala	Leu	Gln	Ser	Gly	Asn		
	610					615					620						
Ser	Gln	Glu	Ser	Val	Thr	Glu	Gln	Asp	Ser	Lys	Asp	Ser	Thr	Tyr	Ser		
625					630					635					640		
Leu	Ser	Ser	Thr	Leu	Thr	Leu	Ser	Lys	Ala	Asp	Tyr	Glu	Lys	His	Lys		
			645						650					655			
Val	Tyr	Ala	Cys	Glu	Val	Thr	His	Gln	Gly	Leu	Ser	Ser	Pro	Val	Thr		
		660						665					670				
Lys	Ser	Phe	Asn	Arg	Gly	Glu	Cys										
		675					680										

<210> SEQ ID NO 174

<211> LENGTH: 214

<212> TYPE: PRT

<213> ORGANISM: Artificial

<220> FEATURE:

<223> OTHER INFORMATION: Chimeric homo sapiens mus musculus

<400> SEQUENCE: 174

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Asp Ile Val Met Thr Gln Ser Pro Ala Thr Leu Ser Val Thr Pro Gly
1           5           10           15

Asp Arg Val Ser Leu Ser Cys Arg Ala Ser Gln Ser Ile Ser Asp Tyr
           20           25           30

Leu His Trp Tyr Gln Gln Lys Ser His Glu Ser Pro Arg Leu Leu Ile
           35           40           45

Lys Tyr Ala Ser Gln Ser Ile Ser Gly Ile Pro Ser Arg Phe Ser Gly
           50           55           60

Ser Gly Ser Gly Ser Asp Phe Thr Leu Ser Ile Asn Ser Val Glu Pro
65           70           75           80

Glu Asp Val Gly Val Tyr Tyr Cys Gln Asn Gly His Ser Phe Pro Leu
           85           90           95

Thr Phe Gly Ala Gly Thr Lys Leu Glu Leu Lys Ala Ser Thr Lys Gly
           100          105          110

Pro Ser Val Phe Pro Leu Ala Pro Ser Ser Lys Ser Thr Ser Gly Gly
           115          120          125

Thr Ala Ala Leu Gly Cys Leu Val Lys Asp Tyr Phe Pro Glu Pro Val
           130          135          140

Thr Val Ser Trp Asn Ser Gly Ala Leu Thr Ser Gly Val His Thr Phe
145          150          155          160

Pro Ala Val Leu Gln Ser Ser Gly Leu Tyr Ser Leu Ser Ser Val Val
           165          170          175

Thr Val Pro Ser Ser Ser Leu Gly Thr Gln Thr Tyr Ile Cys Asn Val
           180          185          190

Asn His Lys Pro Ser Asn Thr Lys Val Asp Lys Arg Val Glu Pro Lys
           195          200          205

Ser Cys Asp Lys Thr His
           210

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<210> SEQ ID NO 175
<211> LENGTH: 445
<212> TYPE: PRT
<213> ORGANISM: Artificial
<220> FEATURE:
<223> OTHER INFORMATION: Chimeric homo sapiens mus musculus

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<400> SEQUENCE: 175

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Asp Ile Gln Leu Thr Gln Ser Pro Ala Ser Leu Ala Val Ser Leu Gly
1           5           10           15

Gln Arg Ala Thr Ile Ser Cys Lys Ala Ser Gln Ser Val Asp Tyr Asp
           20           25           30

Gly Asp Ser Tyr Leu Asn Trp Tyr Gln Gln Ile Pro Gly Gln Pro Pro
           35           40           45

Lys Leu Leu Ile Tyr Asp Ala Ser Asn Leu Val Ser Gly Ile Pro Pro
           50           55           60

Arg Phe Ser Gly Ser Gly Ser Gly Thr Asp Phe Thr Leu Asn Ile His
65           70           75           80

Pro Val Glu Lys Val Asp Ala Ala Thr Tyr His Cys Gln Gln Ser Thr
           85           90           95

Glu Asp Pro Trp Thr Phe Gly Gly Gly Thr Lys Leu Glu Ile Lys Arg
           100          105          110

Thr Val Ala Ala Pro Ser Val Phe Ile Phe Pro Pro Ser Asp Glu Gln
           115          120          125

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Leu Lys Ser Gly Thr Ala Ser Val Val Cys Leu Leu Asn Asn Phe Tyr
 130          135          140

Pro Arg Glu Ala Lys Val Gln Trp Lys Val Asp Asn Ala Leu Gln Ser
145          150          155          160

Gly Asn Ser Gln Glu Ser Val Thr Glu Gln Asp Ser Lys Asp Ser Thr
          165          170          175

Tyr Ser Leu Ser Ser Thr Leu Thr Leu Ser Lys Ala Asp Tyr Glu Lys
          180          185          190

His Lys Val Tyr Ala Cys Glu Val Thr His Gln Gly Leu Ser Ser Pro
          195          200          205

Val Thr Lys Ser Phe Asn Arg Gly Glu Ser Asp Lys Thr His Thr Cys
          210          215          220

Pro Pro Cys Pro Ala Pro Glu Leu Leu Gly Gly Pro Ser Val Phe Leu
225          230          235          240

Phe Pro Pro Lys Pro Lys Asp Thr Leu Met Ile Ser Arg Thr Pro Glu
          245          250          255

Val Thr Cys Val Val Val Asp Val Ser His Glu Asp Pro Glu Val Lys
          260          265          270

Phe Asn Trp Tyr Val Asp Gly Val Glu Val His Asn Ala Lys Thr Lys
          275          280          285

Pro Arg Glu Glu Gln Tyr Ser Ser Thr Tyr Arg Val Val Ser Val Leu
          290          295          300

Thr Val Leu His Gln Asp Trp Leu Asn Gly Lys Glu Tyr Lys Cys Lys
305          310          315          320

Val Ser Asn Lys Ala Leu Pro Ala Pro Ile Glu Lys Thr Ile Ser Lys
          325          330          335

Ala Lys Gly Gln Pro Arg Glu Pro Gln Val Tyr Thr Leu Pro Pro Ser
          340          345          350

Arg Glu Glu Met Thr Lys Asn Gln Val Ser Leu Thr Cys Leu Val Lys
          355          360          365

Gly Phe Tyr Pro Ser Asp Ile Ala Val Glu Trp Glu Ser Asn Gly Gln
          370          375          380

Pro Glu Asn Asn Tyr Lys Thr Thr Pro Pro Val Leu Asp Ser Asp Gly
385          390          395          400

Ser Phe Phe Leu Tyr Ser Lys Leu Thr Val Asp Lys Ser Arg Trp Gln
          405          410          415

Gln Gly Asn Val Phe Ser Cys Ser Val Met His Glu Ala Leu His Asn
          420          425          430

His Tyr Thr Gln Lys Ser Leu Ser Leu Ser Pro Gly Lys
          435          440          445

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<210> SEQ ID NO 176
<211> LENGTH: 680
<212> TYPE: PRT
<213> ORGANISM: Artificial
<220> FEATURE:
<223> OTHER INFORMATION: Chimeric homo sapiens mus musculus

<400> SEQUENCE: 176

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Gln Val Gln Leu Gln Gln Ser Gly Ala Glu Leu Val Arg Pro Gly Ser
 1          5          10          15

Ser Val Lys Ile Ser Cys Lys Ala Ser Gly Tyr Ala Phe Ser Ser Tyr
          20          25          30

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Trp	Met	Asn	Trp	Val	Lys	Gln	Arg	Pro	Gly	Gln	Gly	Leu	Glu	Trp	Ile	
	35						40					45				
Gly	Gln	Ile	Trp	Pro	Gly	Asp	Gly	Asp	Thr	Asn	Tyr	Asn	Gly	Lys	Phe	
	50				55					60						
Lys	Gly	Lys	Ala	Thr	Leu	Thr	Ala	Asp	Glu	Ser	Ser	Ser	Thr	Ala	Tyr	
	65				70				75					80		
Met	Gln	Leu	Ser	Ser	Leu	Ala	Ser	Glu	Asp	Ser	Ala	Val	Tyr	Phe	Cys	
			85					90						95		
Ala	Arg	Arg	Glu	Thr	Thr	Thr	Val	Gly	Arg	Tyr	Tyr	Tyr	Ala	Met	Asp	
			100					105					110			
Tyr	Trp	Gly	Gln	Gly	Thr	Thr	Val	Thr	Val	Ser	Ser	Ala	Ser	Thr	Lys	
		115					120					125				
Gly	Pro	Ser	Val	Phe	Pro	Leu	Ala	Pro	Ser	Ser	Lys	Ser	Thr	Ser	Gly	
	130					135					140					
Gly	Thr	Ala	Ala	Leu	Gly	Cys	Leu	Val	Lys	Asp	Tyr	Phe	Pro	Glu	Pro	
	145				150				155					160		
Val	Thr	Val	Ser	Trp	Asn	Ser	Gly	Ala	Leu	Thr	Ser	Gly	Val	His	Thr	
			165					170						175		
Phe	Pro	Ala	Val	Leu	Gln	Ser	Ser	Gly	Leu	Tyr	Ser	Leu	Ser	Ser	Val	
			180					185					190			
Val	Thr	Val	Pro	Ser	Ser	Ser	Leu	Gly	Thr	Gln	Thr	Tyr	Ile	Cys	Asn	
			195				200					205				
Val	Asn	His	Lys	Pro	Ser	Asn	Thr	Lys	Val	Asp	Lys	Arg	Val	Glu	Pro	
	210					215					220					
Lys	Ser	Ser	Asp	Lys	Thr	His	Thr	Cys	Pro	Pro	Cys	Pro	Ala	Pro	Glu	
	225				230					235				240		
Leu	Leu	Gly	Gly	Pro	Ser	Val	Phe	Leu	Phe	Pro	Pro	Lys	Pro	Lys	Asp	
				245				250						255		
Thr	Leu	Met	Ile	Ser	Arg	Thr	Pro	Glu	Val	Thr	Cys	Val	Val	Val	Asp	
			260					265					270			
Val	Ser	His	Glu	Asp	Pro	Glu	Val	Lys	Phe	Asn	Trp	Tyr	Val	Asp	Gly	
		275					280					285				
Val	Glu	Val	His	Asn	Ala	Lys	Thr	Lys	Pro	Arg	Glu	Glu	Gln	Tyr	Ser	
	290					295					300					
Ser	Thr	Tyr	Arg	Val	Val	Ser	Val	Leu	Thr	Val	Leu	His	Gln	Asp	Trp	
	305				310					315				320		
Leu	Asn	Gly	Lys	Glu	Tyr	Lys	Cys	Lys	Val	Ser	Asn	Lys	Ala	Leu	Pro	
				325					330					335		
Ala	Pro	Ile	Glu	Lys	Thr	Ile	Ser	Lys	Ala	Lys	Gly	Gln	Pro	Arg	Glu	
			340					345					350			
Pro	Gln	Val	Tyr	Thr	Leu	Pro	Pro	Ser	Arg	Glu	Glu	Met	Thr	Lys	Asn	
		355					360					365				
Gln	Val	Ser	Leu	Thr	Cys	Leu	Val	Lys	Gly	Phe	Tyr	Pro	Ser	Asp	Ile	
	370					375					380					
Ala	Val	Glu	Trp	Glu	Ser	Asn	Gly	Gln	Pro	Glu	Asn	Asn	Tyr	Lys	Thr	
	385				390					395				400		
Thr	Pro	Pro	Val	Leu	Asp	Ser	Asp	Gly	Ser	Phe	Phe	Leu	Tyr	Ser	Lys	
			405					410					415			
Leu	Thr	Val	Asp	Lys	Ser	Arg	Trp	Gln	Gln	Gly	Asn	Val	Phe	Ser	Cys	
		420						425					430			
Ser	Val	Met	His	Glu	Ala	Leu	His	Asn	His	Tyr	Thr	Gln	Lys	Ser	Leu	

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435					440					445					
Ser	Leu	Ser	Pro	Gly	Ser	Thr	Gly	Ser	Glu	Val	Gln	Leu	Gln	Gln	Ser
450					455						460				
Gly	Pro	Glu	Leu	Val	Lys	Pro	Gly	Ala	Ser	Val	Lys	Ile	Ser	Cys	Lys
465					470						475				480
Thr	Ser	Gly	Tyr	Thr	Phe	Thr	Glu	Tyr	Thr	Met	His	Trp	Val	Lys	Gln
				485					490					495	
Ser	His	Gly	Lys	Ser	Leu	Glu	Trp	Ile	Gly	Gly	Ile	Ser	Pro	Asn	Ile
			500					505					510		
Gly	Gly	Thr	Ser	Tyr	Asn	Gln	Lys	Phe	Lys	Gly	Lys	Ala	Thr	Leu	Thr
		515					520					525			
Val	Asp	Lys	Ser	Ser	Ser	Thr	Ala	Tyr	Met	Glu	Leu	Arg	Ser	Leu	Thr
	530					535						540			
Ser	Glu	Asp	Ser	Ala	Val	Tyr	Tyr	Cys	Ala	Arg	Arg	Gly	Gly	Ser	Phe
545					550					555					560
Asp	Tyr	Trp	Gly	Gln	Gly	Thr	Thr	Leu	Thr	Val	Ser	Ser	Arg	Thr	Val
			565					570						575	
Ala	Ala	Pro	Ser	Val	Phe	Ile	Phe	Pro	Pro	Ser	Asp	Glu	Gln	Leu	Lys
			580					585					590		
Ser	Gly	Thr	Ala	Ser	Val	Val	Cys	Leu	Leu	Asn	Asn	Phe	Tyr	Pro	Arg
	595						600					605			
Glu	Ala	Lys	Val	Gln	Trp	Lys	Val	Asp	Asn	Ala	Leu	Gln	Ser	Gly	Asn
	610					615						620			
Ser	Gln	Glu	Ser	Val	Thr	Glu	Gln	Asp	Ser	Lys	Asp	Ser	Thr	Tyr	Ser
625					630					635					640
Leu	Ser	Ser	Thr	Leu	Thr	Leu	Ser	Lys	Ala	Asp	Tyr	Glu	Lys	His	Lys
			645					650						655	
Val	Tyr	Ala	Cys	Glu	Val	Thr	His	Gln	Gly	Leu	Ser	Ser	Pro	Val	Thr
			660					665					670		
Lys	Ser	Phe	Asn	Arg	Gly	Glu	Cys								
	675					680									

<210> SEQ ID NO 177

<211> LENGTH: 214

<212> TYPE: PRT

<213> ORGANISM: Artificial

<220> FEATURE:

<223> OTHER INFORMATION: Chimeric homo sapiens mus musculus

<400> SEQUENCE: 177

Asp	Ile	Val	Met	Thr	Gln	Ser	Pro	Ala	Thr	Leu	Ser	Val	Thr	Pro	Gly
1				5					10					15	
Asp	Arg	Val	Ser	Leu	Ser	Cys	Arg	Ala	Ser	Gln	Ser	Ile	Ser	Asp	Tyr
		20						25					30		
Leu	His	Trp	Tyr	Gln	Gln	Lys	Ser	His	Glu	Ser	Pro	Arg	Leu	Leu	Ile
		35					40					45			
Lys	Tyr	Ala	Ser	Gln	Ser	Ile	Ser	Gly	Ile	Pro	Ser	Arg	Phe	Ser	Gly
	50					55				60					
Ser	Gly	Ser	Gly	Ser	Asp	Phe	Thr	Leu	Ser	Ile	Asn	Ser	Val	Glu	Pro
65					70				75					80	
Glu	Asp	Val	Gly	Val	Tyr	Tyr	Cys	Gln	Asn	Gly	His	Ser	Phe	Pro	Leu
			85					90						95	
Thr	Phe	Gly	Ala	Gly	Thr	Lys	Leu	Glu	Leu	Lys	Ala	Ser	Thr	Lys	Gly

-continued

100					105					110					
Pro	Ser	Val	Phe	Pro	Leu	Ala	Pro	Ser	Ser	Lys	Ser	Thr	Ser	Gly	Gly
115							120					125			
Thr	Ala	Ala	Leu	Gly	Cys	Leu	Val	Lys	Asp	Tyr	Phe	Pro	Glu	Pro	Val
130						135					140				
Thr	Val	Ser	Trp	Asn	Ser	Gly	Ala	Leu	Thr	Ser	Gly	Val	His	Thr	Phe
145					150					155					160
Pro	Ala	Val	Leu	Gln	Ser	Ser	Gly	Leu	Tyr	Ser	Leu	Ser	Ser	Val	Val
			165						170					175	
Thr	Val	Pro	Ser	Ser	Ser	Leu	Gly	Thr	Gln	Thr	Tyr	Ile	Cys	Asn	Val
		180						185					190		
Asn	His	Lys	Pro	Ser	Asn	Thr	Lys	Val	Asp	Lys	Arg	Val	Glu	Pro	Lys
		195					200					205			
Ser	Cys	Asp	Lys	Thr	His										
		210													

<210> SEQ ID NO 178

<211> LENGTH: 445

<212> TYPE: PRT

<213> ORGANISM: Artificial

<220> FEATURE:

<223> OTHER INFORMATION: Chimeric homo sapiens mus musculus

<400> SEQUENCE: 178

Asp	Ile	Gln	Leu	Thr	Gln	Ser	Pro	Ala	Ser	Leu	Ala	Val	Ser	Leu	Gly
1				5					10					15	
Gln	Arg	Ala	Thr	Ile	Ser	Cys	Lys	Ala	Ser	Gln	Ser	Val	Asp	Tyr	Asp
		20					25						30		
Gly	Asp	Ser	Tyr	Leu	Asn	Trp	Tyr	Gln	Gln	Ile	Pro	Gly	Gln	Pro	Pro
	35						40					45			
Lys	Leu	Leu	Ile	Tyr	Asp	Ala	Ser	Asn	Leu	Val	Ser	Gly	Ile	Pro	Pro
	50					55					60				
Arg	Phe	Ser	Gly	Ser	Gly	Ser	Gly	Thr	Asp	Phe	Thr	Leu	Asn	Ile	His
65					70					75					80
Pro	Val	Glu	Lys	Val	Asp	Ala	Ala	Thr	Tyr	His	Cys	Gln	Gln	Ser	Thr
			85						90					95	
Glu	Asp	Pro	Trp	Thr	Phe	Gly	Gly	Gly	Thr	Lys	Leu	Glu	Ile	Lys	Arg
	100							105					110		
Thr	Val	Ala	Ala	Pro	Ser	Val	Phe	Ile	Phe	Pro	Pro	Ser	Asp	Glu	Gln
	115						120					125			
Leu	Lys	Ser	Gly	Thr	Ala	Ser	Val	Val	Cys	Leu	Leu	Asn	Asn	Phe	Tyr
	130					135						140			
Pro	Arg	Glu	Ala	Lys	Val	Gln	Trp	Lys	Val	Asp	Asn	Ala	Leu	Gln	Ser
145				150						155					160
Gly	Asn	Ser	Gln	Glu	Ser	Val	Thr	Glu	Gln	Asp	Ser	Lys	Asp	Ser	Thr
		165						170						175	
Tyr	Ser	Leu	Ser	Ser	Thr	Leu	Thr	Leu	Ser	Lys	Ala	Asp	Tyr	Glu	Lys
	180							185					190		
His	Lys	Val	Tyr	Ala	Cys	Glu	Val	Thr	His	Gln	Gly	Leu	Ser	Ser	Pro
	195						200					205			
Val	Thr	Lys	Ser	Phe	Asn	Arg	Gly	Glu	Cys	Asp	Lys	Thr	His	Thr	Cys
	210					215					220				
Pro	Pro	Cys	Pro	Ala	Pro	Glu	Leu	Leu	Gly	Gly	Pro	Ser	Val	Phe	Leu

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225		230		235		240									
Phe	Pro	Pro	Lys	Pro	Lys	Asp	Thr	Leu	Met	Ile	Ser	Arg	Thr	Pro	Glu
			245						250					255	
Val	Thr	Cys	Val	Val	Val	Asp	Val	Ser	His	Glu	Asp	Pro	Glu	Val	Lys
			260					265					270		
Phe	Asn	Trp	Tyr	Val	Asp	Gly	Val	Glu	Val	His	Asn	Ala	Lys	Thr	Lys
		275				280						285			
Pro	Arg	Glu	Glu	Gln	Tyr	Asn	Ser	Thr	Tyr	Arg	Val	Val	Ser	Val	Leu
	290					295					300				
Thr	Val	Leu	His	Gln	Asp	Trp	Leu	Asn	Gly	Lys	Glu	Tyr	Lys	Cys	Lys
305				310						315					320
Val	Ser	Asn	Lys	Ala	Leu	Pro	Ala	Pro	Ile	Glu	Lys	Thr	Ile	Ser	Lys
			325						330						335
Ala	Lys	Gly	Gln	Pro	Arg	Glu	Pro	Gln	Val	Tyr	Thr	Leu	Pro	Pro	Ser
			340					345					350		
Arg	Glu	Glu	Met	Thr	Lys	Asn	Gln	Val	Ser	Leu	Thr	Cys	Leu	Val	Lys
	355					360						365			
Gly	Phe	Tyr	Pro	Ser	Asp	Ile	Ala	Val	Glu	Trp	Glu	Ser	Asn	Gly	Gln
	370					375					380				
Pro	Glu	Asn	Asn	Tyr	Lys	Thr	Thr	Pro	Pro	Val	Leu	Asp	Ser	Asp	Gly
385					390					395					400
Ser	Phe	Phe	Leu	Tyr	Ser	Lys	Leu	Thr	Val	Asp	Lys	Ser	Arg	Trp	Gln
			405						410					415	
Gln	Gly	Asn	Val	Phe	Ser	Cys	Ser	Val	Met	His	Glu	Ala	Leu	His	Asn
		420						425					430		
His	Tyr	Thr	Gln	Lys	Ser	Leu	Ser	Leu	Ser	Pro	Gly	Lys			
	435					440						445			

<210> SEQ ID NO 179

<211> LENGTH: 698

<212> TYPE: PRT

<213> ORGANISM: Artificial

<220> FEATURE:

<223> OTHER INFORMATION: Chimeric homo sapiens mus musculus

<400> SEQUENCE: 179

Gln	Val	Gln	Leu	Gln	Gln	Ser	Gly	Ala	Glu	Leu	Val	Arg	Pro	Gly	Ser
1			5						10					15	
Ser	Val	Lys	Ile	Ser	Cys	Lys	Ala	Ser	Gly	Tyr	Ala	Phe	Ser	Ser	Tyr
		20						25					30		
Trp	Met	Asn	Trp	Val	Lys	Gln	Arg	Pro	Gly	Gln	Gly	Leu	Glu	Trp	Ile
	35					40						45			
Gly	Gln	Ile	Trp	Pro	Gly	Asp	Gly	Asp	Thr	Asn	Tyr	Asn	Gly	Lys	Phe
	50					55					60				
Lys	Gly	Lys	Ala	Thr	Leu	Thr	Ala	Asp	Glu	Ser	Ser	Ser	Thr	Ala	Tyr
65					70				75					80	
Met	Gln	Leu	Ser	Ser	Leu	Ala	Ser	Glu	Asp	Ser	Ala	Val	Tyr	Phe	Cys
			85						90					95	
Ala	Arg	Arg	Glu	Thr	Thr	Thr	Val	Gly	Arg	Tyr	Tyr	Tyr	Ala	Met	Asp
			100					105					110		
Tyr	Trp	Gly	Gln	Gly	Thr	Thr	Val	Thr	Val	Ser	Ser	Ala	Ser	Thr	Lys
		115					120					125			
Gly	Pro	Ser	Val	Phe	Pro	Leu	Ala	Pro	Ser	Ser	Lys	Ser	Thr	Ser	Gly

-continued

130					135					140					
Gly 145	Thr	Ala	Ala	Leu	Gly 150	Cys	Leu	Val	Lys	Asp 155	Tyr	Phe	Pro	Glu	Pro 160
Val	Thr	Val	Ser	Trp 165	Asn	Ser	Gly	Ala	Leu	Thr 170	Ser	Gly	Val	His	Thr 175
Phe	Pro	Ala	Val	Leu	Gln	Ser	Ser	Gly 185	Leu	Tyr	Ser	Leu	Ser	Ser	Val
Val	Thr	Val	Pro	Ser	Ser	Ser	Leu	Gly	Thr	Gln	Thr	Tyr 205	Ile	Cys	Asn
Val	Asn	His	Lys	Pro	Ser	Asn 215	Thr	Lys	Val	Asp	Lys 220	Arg	Val	Glu	Pro
Lys 225	Ser	Cys	Asp	Lys	Thr 230	His	Thr	Cys	Pro	Pro 235	Cys	Pro	Ala	Pro	Glu 240
Leu	Leu	Gly	Gly	Pro 245	Ser	Val	Phe	Leu	Phe 250	Pro	Pro	Lys	Pro	Lys	Asp 255
Thr	Leu	Met	Ile 260	Ser	Arg	Thr	Pro	Glu 265	Val	Thr	Cys	Val	Val 270	Val	Asp
Val	Ser	His	Glu	Asp	Pro	Glu 280	Val	Lys	Phe	Asn	Trp	Tyr 285	Val	Asp	Gly
Val	Glu 290	Val	His	Asn	Ala	Lys 295	Thr	Lys	Pro	Arg	Glu 300	Glu	Gln	Tyr	Asn
Ser 305	Thr	Tyr	Arg	Val	Val 310	Ser	Val	Leu	Thr	Val 315	Leu	His	Gln	Asp	Trp 320
Leu	Asn	Gly	Lys	Glu 325	Tyr	Lys	Cys	Lys	Val 330	Ser	Asn	Lys	Ala	Leu	Pro 335
Ala	Pro	Ile	Glu	Lys	Thr	Ile	Ser	Lys 345	Ala	Lys	Gly	Gln	Pro 350	Arg	Glu
Pro	Gln 355	Val	Tyr	Thr	Leu	Pro	Pro 360	Ser	Arg	Glu	Glu	Met 365	Thr	Lys	Asn
Gln 370	Val	Ser	Leu	Thr	Cys	Leu 375	Val	Lys	Gly	Phe 380	Tyr	Pro	Ser	Asp	Ile
Ala 385	Val	Glu	Trp	Glu	Ser 390	Asn	Gly	Gln	Pro	Glu 395	Asn	Asn	Tyr	Lys	Thr 400
Thr	Pro	Pro	Val	Leu 405	Asp	Ser	Asp	Gly	Ser 410	Phe	Phe	Leu	Tyr	Ser	Lys 415
Leu	Thr	Val	Asp 420	Lys	Ser	Arg	Trp	Gln 425	Gln	Gly	Asn	Val	Phe 430	Ser	Cys
Ser	Val	Met 435	His	Glu	Ala	Leu	His 440	Asn	His	Tyr	Thr	Gln 445	Lys	Ser	Leu
Ser 450	Leu	Ser	Pro	Gly	Ser	Thr 455	Gly	Ser	Glu	Val 460	Gln	Leu	Gln	Gln	Ser
Gly 465	Pro	Glu	Leu	Val	Lys 470	Pro	Gly	Ala	Ser	Val 475	Lys	Ile	Ser	Cys	Lys 480
Thr	Ser	Gly	Tyr	Thr 485	Phe	Thr	Glu	Tyr	Thr 490	Met	His	Trp	Val	Lys	Gln 495
Ser	His	Gly	Lys 500	Ser	Leu	Glu	Trp	Ile 505	Gly	Gly	Ile	Ser	Pro 510	Asn	Ile
Gly	Gly	Thr	Ser	Tyr	Asn	Gln	Lys 520	Phe	Lys	Gly	Lys	Ala 525	Thr	Leu	Thr
Val 530	Asp	Lys	Ser	Ser	Ser	Thr 535	Ala	Tyr	Met	Glu 540	Leu	Arg	Ser	Leu	Thr

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Ser Glu Asp Ser Ala Val Tyr Tyr Cys Ala Arg Arg Gly Gly Ser Phe
 545 550 555 560
 Asp Tyr Trp Gly Gln Gly Thr Thr Leu Thr Val Ser Ser Val Glu Gly
 565 570 575
 Gly Ser Gly Gly Ser Gly Gly Ser Gly Gly Ser Gly Val Asp Asp
 580 585 590
 Ile Val Met Thr Gln Ser Pro Ala Thr Leu Ser Val Thr Pro Gly Asp
 595 600 605
 Arg Val Ser Leu Ser Cys Arg Ala Ser Gln Ser Ile Ser Asp Tyr Leu
 610 615 620
 His Trp Tyr Gln Gln Lys Ser His Glu Ser Pro Arg Leu Leu Ile Lys
 625 630 635 640
 Tyr Ala Ser Gln Ser Ile Ser Gly Ile Pro Ser Arg Phe Ser Gly Ser
 645 650 655
 Gly Ser Gly Ser Asp Phe Thr Leu Ser Ile Asn Ser Val Glu Pro Glu
 660 665 670
 Asp Val Gly Val Tyr Tyr Cys Gln Asn Gly His Ser Phe Pro Leu Thr
 675 680 685
 Phe Gly Ala Gly Thr Lys Leu Glu Leu Lys
 690 695

<210> SEQ ID NO 180
 <211> LENGTH: 445
 <212> TYPE: PRT
 <213> ORGANISM: Artificial
 <220> FEATURE:
 <223> OTHER INFORMATION: Chimeric homo sapiens mus musculus

<400> SEQUENCE: 180

Asp Ile Gln Leu Thr Gln Ser Pro Ala Ser Leu Ala Val Ser Leu Gly
 1 5 10 15
 Gln Arg Ala Thr Ile Ser Cys Lys Ala Ser Gln Ser Val Asp Tyr Asp
 20 25 30
 Gly Asp Ser Tyr Leu Asn Trp Tyr Gln Gln Ile Pro Gly Gln Pro Pro
 35 40 45
 Lys Leu Leu Ile Tyr Asp Ala Ser Asn Leu Val Ser Gly Ile Pro Pro
 50 55 60
 Arg Phe Ser Gly Ser Gly Ser Gly Thr Asp Phe Thr Leu Asn Ile His
 65 70 75 80
 Pro Val Glu Lys Val Asp Ala Ala Thr Tyr His Cys Gln Gln Ser Thr
 85 90 95
 Glu Asp Pro Trp Thr Phe Gly Gly Gly Thr Lys Leu Glu Ile Lys Arg
 100 105 110
 Thr Val Ala Ala Pro Ser Val Phe Ile Phe Pro Pro Ser Asp Glu Gln
 115 120 125
 Leu Lys Ser Gly Thr Ala Ser Val Val Cys Leu Leu Asn Asn Phe Tyr
 130 135 140
 Pro Arg Glu Ala Lys Val Gln Trp Lys Val Asp Asn Ala Leu Gln Ser
 145 150 155 160
 Gly Asn Ser Gln Glu Ser Val Thr Glu Gln Asp Ser Lys Asp Ser Thr
 165 170 175
 Tyr Ser Leu Ser Ser Thr Leu Thr Leu Ser Lys Ala Asp Tyr Glu Lys
 180 185 190

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His Lys Val Tyr Ala Cys Glu Val Thr His Gln Gly Leu Ser Ser Pro
 195 200 205
 Val Thr Lys Ser Phe Asn Arg Gly Glu Cys Asp Lys Thr His Thr Cys
 210 215 220
 Pro Pro Cys Pro Ala Pro Glu Leu Leu Gly Gly Pro Ser Val Phe Leu
 225 230 235 240
 Phe Pro Pro Lys Pro Lys Asp Thr Leu Met Ile Ser Arg Thr Pro Glu
 245 250 255
 Val Thr Cys Val Val Val Asp Val Ser His Glu Asp Pro Glu Val Lys
 260 265 270
 Phe Asn Trp Tyr Val Asp Gly Val Glu Val His Asn Ala Lys Thr Lys
 275 280 285
 Pro Arg Glu Glu Gln Tyr Ser Ser Thr Tyr Arg Val Val Ser Val Leu
 290 295 300
 Thr Val Leu His Gln Asp Trp Leu Asn Gly Lys Glu Tyr Lys Cys Lys
 305 310 315 320
 Val Ser Asn Lys Ala Leu Pro Ala Pro Ile Glu Lys Thr Ile Ser Lys
 325 330 335
 Ala Lys Gly Gln Pro Arg Glu Pro Gln Val Tyr Thr Leu Pro Pro Ser
 340 345 350
 Arg Glu Glu Met Thr Lys Asn Gln Val Ser Leu Thr Cys Leu Val Lys
 355 360 365
 Gly Phe Tyr Pro Ser Asp Ile Ala Val Glu Trp Glu Ser Asn Gly Gln
 370 375 380
 Pro Glu Asn Asn Tyr Lys Thr Thr Pro Pro Val Leu Asp Ser Asp Gly
 385 390 395 400
 Ser Phe Phe Leu Tyr Ser Lys Leu Thr Val Asp Lys Ser Arg Trp Gln
 405 410 415
 Gln Gly Asn Val Phe Ser Cys Ser Val Met His Glu Ala Leu His Asn
 420 425 430
 His Tyr Thr Gln Lys Ser Leu Ser Leu Ser Pro Gly Lys
 435 440 445

<210> SEQ ID NO 181

<211> LENGTH: 698

<212> TYPE: PRT

<213> ORGANISM: Artificial

<220> FEATURE:

<223> OTHER INFORMATION: Chimeric homo sapiens mus musculus

<400> SEQUENCE: 181

Gln Val Gln Leu Gln Gln Ser Gly Ala Glu Leu Val Arg Pro Gly Ser
 1 5 10 15
 Ser Val Lys Ile Ser Cys Lys Ala Ser Gly Tyr Ala Phe Ser Ser Tyr
 20 25 30
 Trp Met Asn Trp Val Lys Gln Arg Pro Gly Gln Gly Leu Glu Trp Ile
 35 40 45
 Gly Gln Ile Trp Pro Gly Asp Gly Asp Thr Asn Tyr Asn Gly Lys Phe
 50 55 60
 Lys Gly Lys Ala Thr Leu Thr Ala Asp Glu Ser Ser Ser Thr Ala Tyr
 65 70 75 80
 Met Gln Leu Ser Ser Leu Ala Ser Glu Asp Ser Ala Val Tyr Phe Cys
 85 90 95

Ala	Arg	Arg	Glu 100	Thr	Thr	Thr	Val	Gly 105	Arg	Tyr	Tyr	Tyr	Ala 110	Met	Asp	
Tyr	Trp	Gly 115	Gln	Gly	Thr	Thr	Val 120	Thr	Val	Ser	Ser	Ala 125	Ser	Thr	Lys	
Gly	Pro	Ser	Val	Phe	Pro	Leu 135	Ala	Pro	Ser	Ser	Lys 140	Ser	Thr	Ser	Gly	
Gly 145	Thr	Ala	Ala	Leu	Gly 150	Cys	Leu	Val	Lys	Asp 155	Tyr	Phe	Pro	Glu	Pro 160	
Val	Thr	Val	Ser	Trp 165	Asn	Ser	Gly	Ala	Leu 170	Thr	Ser	Gly	Val	His	Thr 175	
Phe	Pro	Ala	Val 180	Leu	Gln	Ser	Ser	Gly 185	Leu	Tyr	Ser	Leu	Ser	Ser	Val 190	
Val	Thr	Val	Pro 195	Ser	Ser	Ser	Leu 200	Gly	Thr	Gln	Thr	Tyr 205	Ile	Cys	Asn 210	
Val	Asn 210	His	Lys	Pro	Ser	Asn 215	Thr	Lys	Val	Asp 220	Lys	Arg	Val	Glu	Pro 225	
Lys 225	Ser	Cys	Asp	Lys	Thr 230	His	Thr	Cys	Pro	Pro 235	Cys	Pro	Ala	Pro	Glu 240	
Leu	Leu	Gly	Gly 245	Pro	Ser	Val	Phe	Leu	Phe 250	Pro	Pro	Lys	Pro	Lys	Asp 255	
Thr	Leu	Met	Ile 260	Ser	Arg	Thr	Pro	Glu 265	Val	Thr	Cys	Val 270	Val	Val	Asp	
Val	Ser	His 275	Glu	Asp	Pro	Glu	Val 280	Lys	Phe	Asn	Trp	Tyr 285	Val	Asp	Gly 290	
Val 290	Glu	Val	His	Asn	Ala	Lys 295	Thr	Lys	Pro	Arg 300	Glu	Glu	Gln	Tyr	Ser 305	
Ser 305	Thr	Tyr	Arg	Val	Val 310	Ser	Val	Leu	Thr	Val 315	Leu	His	Gln	Asp	Trp 320	
Leu	Asn	Gly	Lys 325	Glu	Tyr	Lys	Cys	Lys	Val 330	Ser	Asn	Lys	Ala	Leu	Pro 335	
Ala	Pro	Ile	Glu 340	Lys	Thr	Ile	Ser	Lys 345	Ala	Lys	Gly	Gln 350	Pro	Arg	Glu 355	
Pro	Gln	Val	Tyr 355	Thr	Leu	Pro	Pro	Ser 360	Arg	Glu	Glu	Met 365	Thr	Lys	Asn 370	
Gln 370	Val	Ser	Leu	Thr	Cys 375	Leu	Val	Lys	Gly	Phe 380	Tyr	Pro	Ser	Asp	Ile 385	
Ala 385	Val	Glu	Trp	Glu	Ser 390	Asn	Gly	Gln	Pro	Glu 395	Asn	Asn	Tyr	Lys	Thr 400	
Thr	Pro	Pro	Val 405	Leu	Asp	Ser	Asp	Gly	Ser 410	Phe	Phe	Leu 415	Tyr	Ser	Lys 420	
Leu	Thr	Val	Asp 420	Lys	Ser	Arg	Trp	Gln 425	Gln	Gly	Asn	Val 430	Phe	Ser	Cys 435	
Ser	Val	Met	His 435	Glu	Ala	Leu	His	Asn 440	His	Tyr	Thr	Gln 445	Lys	Ser	Leu 450	
Ser 450	Leu	Ser	Pro	Gly	Ser	Thr	Gly	Ser	Glu	Val 460	Gln	Leu	Gln	Gln	Ser 465	
Gly 465	Pro	Glu	Leu	Val	Lys 470	Pro	Gly	Ala	Ser	Val 475	Lys	Ile	Ser	Cys	Lys 480	
Thr	Ser	Gly	Tyr 485	Thr	Phe	Thr	Glu	Tyr 490	Thr	Met	His	Trp 495	Val	Lys	Gln 500	

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Ser His Gly Lys Ser Leu Glu Trp Ile Gly Gly Ile Ser Pro Asn Ile
      500                               505                510

Gly Gly Thr Ser Tyr Asn Gln Lys Phe Lys Gly Lys Ala Thr Leu Thr
      515                               520                525

Val Asp Lys Ser Ser Ser Thr Ala Tyr Met Glu Leu Arg Ser Leu Thr
      530                               535                540

Ser Glu Asp Ser Ala Val Tyr Tyr Cys Ala Arg Arg Gly Gly Ser Phe
      545                               550                555                560

Asp Tyr Trp Gly Gln Gly Thr Thr Leu Thr Val Ser Ser Val Glu Gly
      565                               570                575

Gly Ser Gly Gly Ser Gly Gly Ser Gly Gly Ser Gly Gly Val Asp Asp
      580                               585                590

Ile Val Met Thr Gln Ser Pro Ala Thr Leu Ser Val Thr Pro Gly Asp
      595                               600                605

Arg Val Ser Leu Ser Cys Arg Ala Ser Gln Ser Ile Ser Asp Tyr Leu
      610                               615                620

His Trp Tyr Gln Gln Lys Ser His Glu Ser Pro Arg Leu Leu Ile Lys
      625                               630                635                640

Tyr Ala Ser Gln Ser Ile Ser Gly Ile Pro Ser Arg Phe Ser Gly Ser
      645                               650                655

Gly Ser Gly Ser Asp Phe Thr Leu Ser Ile Asn Ser Val Glu Pro Glu
      660                               665                670

Asp Val Gly Val Tyr Tyr Cys Gln Asn Gly His Ser Phe Pro Leu Thr
      675                               680                685

Phe Gly Ala Gly Thr Lys Leu Glu Leu Lys
      690                               695

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<210> SEQ ID NO 182
<211> LENGTH: 454
<212> TYPE: PRT
<213> ORGANISM: Artificial
<220> FEATURE:
<223> OTHER INFORMATION: Chimeric homo sapiens mus musculus

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<400> SEQUENCE: 182

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Gln Val Gln Leu Gln Gln Ser Gly Ala Glu Leu Val Arg Pro Gly Ser
1          5          10          15

Ser Val Lys Ile Ser Cys Lys Ala Ser Gly Tyr Ala Phe Ser Ser Tyr
      20          25          30

Trp Met Asn Trp Val Lys Gln Arg Pro Gly Gln Gly Leu Glu Trp Ile
      35          40          45

Gly Gln Ile Trp Pro Gly Asp Gly Asp Thr Asn Tyr Asn Gly Lys Phe
      50          55          60

Lys Gly Lys Ala Thr Leu Thr Ala Asp Glu Ser Ser Ser Thr Ala Tyr
      65          70          75          80

Met Gln Leu Ser Ser Leu Ala Ser Glu Asp Ser Ala Val Tyr Phe Cys
      85          90          95

Ala Arg Arg Glu Thr Thr Thr Val Gly Arg Tyr Tyr Tyr Ala Met Asp
      100         105         110

Tyr Trp Gly Gln Gly Thr Thr Val Thr Val Ser Ser Ala Ser Thr Lys
      115         120         125

Gly Pro Ser Val Phe Pro Leu Ala Pro Ser Ser Lys Ser Thr Ser Gly
      130         135         140

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Gly	Thr	Ala	Ala	Leu	Gly	Cys	Leu	Val	Lys	Asp	Tyr	Phe	Pro	Glu	Pro	145	150	155	160
Val	Thr	Val	Ser	Trp	Asn	Ser	Gly	Ala	Leu	Thr	Ser	Gly	Val	His	Thr	165	170	175	
Phe	Pro	Ala	Val	Leu	Gln	Ser	Ser	Gly	Leu	Tyr	Ser	Leu	Ser	Ser	Val	180	185	190	
Val	Thr	Val	Pro	Ser	Ser	Ser	Leu	Gly	Thr	Gln	Thr	Tyr	Ile	Cys	Asn	195	200	205	
Val	Asn	His	Lys	Pro	Ser	Asn	Thr	Lys	Val	Asp	Lys	Arg	Val	Glu	Pro	210	215	220	
Lys	Ser	Cys	Asp	Lys	Thr	His	Thr	Cys	Pro	Pro	Cys	Pro	Ala	Pro	Glu	225	230	235	240
Leu	Leu	Gly	Gly	Pro	Ser	Val	Phe	Leu	Phe	Pro	Pro	Lys	Pro	Lys	Asp	245	250	255	
Thr	Leu	Met	Ile	Ser	Arg	Thr	Pro	Glu	Val	Thr	Cys	Val	Val	Val	Asp	260	265	270	
Val	Ser	His	Glu	Asp	Pro	Glu	Val	Lys	Phe	Asn	Trp	Tyr	Val	Asp	Gly	275	280	285	
Val	Glu	Val	His	Asn	Ala	Lys	Thr	Lys	Pro	Arg	Glu	Glu	Gln	Tyr	Ser	290	295	300	
Ser	Thr	Tyr	Arg	Val	Val	Ser	Val	Leu	Thr	Val	Leu	His	Gln	Asp	Trp	305	310	315	320
Leu	Asn	Gly	Lys	Glu	Tyr	Lys	Cys	Lys	Val	Ser	Asn	Lys	Ala	Leu	Pro	325	330	335	
Ala	Pro	Ile	Glu	Lys	Thr	Ile	Ser	Lys	Ala	Lys	Gly	Gln	Pro	Arg	Glu	340	345	350	
Pro	Gln	Val	Tyr	Thr	Leu	Pro	Pro	Ser	Arg	Glu	Glu	Met	Thr	Lys	Asn	355	360	365	
Gln	Val	Ser	Leu	Thr	Cys	Leu	Val	Lys	Gly	Phe	Tyr	Pro	Ser	Asp	Ile	370	375	380	
Ala	Val	Glu	Trp	Glu	Ser	Asn	Gly	Gln	Pro	Glu	Asn	Asn	Tyr	Lys	Thr	385	390	395	400
Thr	Pro	Pro	Val	Leu	Asp	Ser	Asp	Gly	Ser	Phe	Phe	Leu	Tyr	Ser	Lys	405	410	415	
Leu	Thr	Val	Asp	Lys	Ser	Arg	Trp	Gln	Gln	Gly	Asn	Val	Phe	Ser	Cys	420	425	430	
Ser	Val	Met	His	Glu	Ala	Leu	His	Asn	His	Tyr	Thr	Gln	Lys	Ser	Leu	435	440	445	
Ser	Leu	Ser	Pro	Gly	Lys											450			

<210> SEQ ID NO 183
 <211> LENGTH: 670
 <212> TYPE: PRT
 <213> ORGANISM: Artificial
 <220> FEATURE:
 <223> OTHER INFORMATION: Chimeric homo sapiens mus musculus

<400> SEQUENCE: 183

Asp	Ile	Gln	Leu	Thr	Gln	Ser	Pro	Ala	Ser	Leu	Ala	Val	Ser	Leu	Gly	1	5	10	15
Gln	Arg	Ala	Thr	Ile	Ser	Cys	Lys	Ala	Ser	Gln	Ser	Val	Asp	Tyr	Asp	20	25	30	

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Gly	Asp	Ser	Tyr	Leu	Asn	Trp	Tyr	Gln	Gln	Ile	Pro	Gly	Gln	Pro	Pro	35	40	45
Lys	Leu	Leu	Ile	Tyr	Asp	Ala	Ser	Asn	Leu	Val	Ser	Gly	Ile	Pro	Pro	50	55	60
Arg	Phe	Ser	Gly	Ser	Gly	Ser	Gly	Thr	Asp	Phe	Thr	Leu	Asn	Ile	His	65	70	75
Pro	Val	Glu	Lys	Val	Asp	Ala	Ala	Thr	Tyr	His	Cys	Gln	Gln	Ser	Thr	85	90	95
Glu	Asp	Pro	Trp	Thr	Phe	Gly	Gly	Gly	Thr	Lys	Leu	Glu	Ile	Lys	Arg	100	105	110
Thr	Val	Ala	Ala	Pro	Ser	Val	Phe	Ile	Phe	Pro	Pro	Ser	Asp	Glu	Gln	115	120	125
Leu	Lys	Ser	Gly	Thr	Ala	Ser	Val	Val	Cys	Leu	Leu	Asn	Asn	Phe	Tyr	130	135	140
Pro	Arg	Glu	Ala	Lys	Val	Gln	Trp	Lys	Val	Asp	Asn	Ala	Leu	Gln	Ser	145	150	155
Gly	Asn	Ser	Gln	Glu	Ser	Val	Thr	Glu	Gln	Asp	Ser	Lys	Asp	Ser	Thr	165	170	175
Tyr	Ser	Leu	Ser	Ser	Thr	Leu	Thr	Leu	Ser	Lys	Ala	Asp	Tyr	Glu	Lys	180	185	190
His	Lys	Val	Tyr	Ala	Cys	Glu	Val	Thr	His	Gln	Gly	Leu	Ser	Ser	Pro	195	200	205
Val	Thr	Lys	Ser	Phe	Asn	Arg	Gly	Glu	Cys	Asp	Lys	Thr	His	Thr	Cys	210	215	220
Pro	Pro	Cys	Pro	Ala	Pro	Glu	Leu	Leu	Gly	Gly	Pro	Ser	Val	Phe	Leu	225	230	235
Phe	Pro	Pro	Lys	Pro	Lys	Asp	Thr	Leu	Met	Ile	Ser	Arg	Thr	Pro	Glu	245	250	255
Val	Thr	Cys	Val	Val	Val	Asp	Val	Ser	His	Glu	Asp	Pro	Glu	Val	Lys	260	265	270
Phe	Asn	Trp	Tyr	Val	Asp	Gly	Val	Glu	Val	His	Asn	Ala	Lys	Thr	Lys	275	280	285
Pro	Arg	Glu	Glu	Gln	Ser	Ser	Thr	Tyr	Arg	Val	Val	Ser	Val	Leu	Thr	290	295	300
Val	Leu	His	Gln	Asp	Trp	Leu	Asn	Gly	Lys	Glu	Tyr	Lys	Cys	Lys	Val	305	310	315
Ser	Asn	Lys	Ala	Leu	Pro	Ala	Pro	Ile	Glu	Lys	Thr	Ile	Ser	Lys	Ala	325	330	335
Lys	Gly	Gln	Pro	Arg	Glu	Pro	Gln	Val	Tyr	Thr	Leu	Pro	Pro	Ser	Arg	340	345	350
Glu	Glu	Met	Thr	Lys	Asn	Gln	Val	Ser	Leu	Thr	Cys	Leu	Val	Lys	Gly	355	360	365
Phe	Tyr	Pro	Ser	Asp	Ile	Ala	Val	Glu	Trp	Glu	Ser	Asn	Gly	Gln	Pro	370	375	380
Glu	Asn	Asn	Tyr	Lys	Thr	Thr	Pro	Pro	Val	Leu	Asp	Ser	Asp	Gly	Ser	385	390	395
Phe	Phe	Leu	Tyr	Ser	Lys	Leu	Thr	Val	Asp	Lys	Ser	Arg	Trp	Gln	Gln	405	410	415
Gly	Asn	Val	Phe	Ser	Cys	Ser	Val	Met	His	Glu	Ala	Leu	His	Asn	His	420	425	430
Tyr	Thr	Gln	Lys	Ser	Leu	Ser	Leu	Ser	Pro	Gly	Ser	Thr	Gly	Ser	Glu			

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435	440	445
Val Gln Leu Gln Gln Ser Gly Pro Glu Leu Val Lys Pro Gly Ala Ser		
450	455	460
Val Lys Ile Ser Cys Lys Thr Ser Gly Tyr Thr Phe Thr Glu Tyr Thr		
465	470	475
Met His Trp Val Lys Gln Ser His Gly Lys Ser Leu Glu Trp Ile Gly		
	485	490
Gly Ile Ser Pro Asn Ile Gly Gly Thr Ser Tyr Asn Gln Lys Phe Lys		
	500	505
Gly Lys Ala Thr Leu Thr Val Asp Lys Ser Ser Ser Thr Ala Tyr Met		
	515	520
Glu Leu Arg Ser Leu Thr Ser Glu Asp Ser Ala Val Tyr Tyr Cys Ala		
	530	535
Arg Arg Gly Gly Ser Phe Asp Tyr Trp Gly Gln Gly Thr Thr Leu Thr		
545	550	555
Val Ser Ser Arg Thr Val Ala Ala Pro Ser Val Phe Ile Phe Pro Pro		
	565	570
Ser Asp Glu Gln Leu Lys Ser Gly Thr Ala Ser Val Val Cys Leu Leu		
	580	585
Asn Asn Phe Tyr Pro Arg Glu Ala Lys Val Gln Trp Lys Val Asp Asn		
	595	600
Ala Leu Gln Ser Gly Asn Ser Gln Glu Ser Val Thr Glu Gln Asp Ser		
	610	615
Lys Asp Ser Thr Tyr Ser Leu Ser Ser Thr Leu Thr Leu Ser Lys Ala		
625	630	635
Asp Tyr Glu Lys His Lys Val Tyr Ala Cys Glu Val Thr His Gln Gly		
	645	650
Leu Ser Ser Pro Val Thr Lys Ser Phe Asn Arg Gly Glu Cys		
	660	665
		670

<210> SEQ ID NO 184

<211> LENGTH: 214

<212> TYPE: PRT

<213> ORGANISM: Artificial

<220> FEATURE:

<223> OTHER INFORMATION: Chimeric homo sapiens mus musculus

<400> SEQUENCE: 184

Asp Ile Val Met Thr Gln Ser Pro Ala Thr Leu Ser Val Thr Pro Gly		
1	5	10
Asp Arg Val Ser Leu Ser Cys Arg Ala Ser Gln Ser Ile Ser Asp Tyr		
	20	25
Leu His Trp Tyr Gln Gln Lys Ser His Glu Ser Pro Arg Leu Leu Ile		
	35	40
Lys Tyr Ala Ser Gln Ser Ile Ser Gly Ile Pro Ser Arg Phe Ser Gly		
	50	55
Ser Gly Ser Gly Ser Asp Phe Thr Leu Ser Ile Asn Ser Val Glu Pro		
65	70	75
Glu Asp Val Gly Val Tyr Tyr Cys Gln Asn Gly His Ser Phe Pro Leu		
	85	90
Thr Phe Gly Ala Gly Thr Lys Leu Glu Leu Lys Ala Ser Thr Lys Gly		
	100	105
Pro Ser Val Phe Pro Leu Ala Pro Ser Ser Lys Ser Thr Ser Gly Gly		
		110

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115	120	125
Thr Ala Ala Leu Gly Cys Leu Val Lys Asp Tyr Phe Pro Glu Pro Val		
130	135	140
Thr Val Ser Trp Asn Ser Gly Ala Leu Thr Ser Gly Val His Thr Phe		
145	150	155
Pro Ala Val Leu Gln Ser Ser Gly Leu Tyr Ser Leu Ser Ser Val Val		
	165	170
		175
Thr Val Pro Ser Ser Ser Leu Gly Thr Gln Thr Tyr Ile Cys Asn Val		
	180	185
		190
Asn His Lys Pro Ser Asn Thr Lys Val Asp Lys Arg Val Glu Pro Lys		
	195	200
		205
Ser Cys Asp Lys Thr His		
210		
<210> SEQ ID NO 185		
<211> LENGTH: 445		
<212> TYPE: PRT		
<213> ORGANISM: Artificial		
<220> FEATURE:		
<223> OTHER INFORMATION: Chimeric homo sapiens mus musculus		
<400> SEQUENCE: 185		
Asp Ile Gln Leu Thr Gln Ser Pro Ala Ser Leu Ala Val Ser Leu Gly		
1	5	10
		15
Gln Arg Ala Thr Ile Ser Cys Lys Ala Ser Gln Ser Val Asp Tyr Asp		
	20	25
		30
Gly Asp Ser Tyr Leu Asn Trp Tyr Gln Gln Ile Pro Gly Gln Pro Pro		
	35	40
		45
Lys Leu Leu Ile Tyr Asp Ala Ser Asn Leu Val Ser Gly Ile Pro Pro		
	50	55
		60
Arg Phe Ser Gly Ser Gly Ser Gly Thr Asp Phe Thr Leu Asn Ile His		
	65	70
		75
Pro Val Glu Lys Val Asp Ala Ala Thr Tyr His Cys Gln Gln Ser Thr		
	85	90
		95
Glu Asp Pro Trp Thr Phe Gly Gly Gly Thr Lys Leu Glu Ile Lys Arg		
	100	105
		110
Thr Val Ala Ala Pro Ser Val Phe Ile Phe Pro Pro Ser Asp Glu Gln		
	115	120
		125
Leu Lys Ser Gly Thr Ala Ser Val Val Cys Leu Leu Asn Asn Phe Tyr		
	130	135
		140
Pro Arg Glu Ala Lys Val Gln Trp Lys Val Asp Asn Ala Leu Gln Ser		
	145	150
		155
Gly Asn Ser Gln Glu Ser Val Thr Glu Gln Asp Ser Lys Asp Ser Thr		
	165	170
		175
Tyr Ser Leu Ser Ser Thr Leu Thr Leu Ser Lys Ala Asp Tyr Glu Lys		
	180	185
		190
His Lys Val Tyr Ala Cys Glu Val Thr His Gln Gly Leu Ser Ser Pro		
	195	200
		205
Val Thr Lys Ser Phe Asn Arg Gly Glu Cys Asp Lys Thr His Thr Cys		
	210	215
		220
Pro Pro Cys Pro Ala Pro Glu Leu Leu Gly Gly Pro Ser Val Phe Leu		
	225	230
		235
		240
Phe Pro Pro Lys Pro Lys Asp Thr Leu Met Ile Ser Arg Thr Pro Glu		

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245					250					255					
Val	Thr	Cys	Val	Val	Val	Asp	Val	Ser	His	Glu	Asp	Pro	Glu	Val	Lys
			260					265					270		
Phe	Asn	Trp	Tyr	Val	Asp	Gly	Val	Glu	Val	His	Asn	Ala	Lys	Thr	Lys
			275				280					285			
Pro	Arg	Glu	Glu	Gln	Tyr	Ser	Ser	Thr	Tyr	Arg	Val	Val	Ser	Val	Leu
	290					295					300				
Thr	Val	Leu	His	Gln	Asp	Trp	Leu	Asn	Gly	Lys	Glu	Tyr	Lys	Cys	Lys
305				310						315				320	
Val	Ser	Asn	Lys	Ala	Leu	Pro	Ala	Pro	Ile	Glu	Lys	Thr	Ile	Ser	Lys
			325					330						335	
Ala	Lys	Gly	Gln	Pro	Arg	Glu	Pro	Gln	Val	Tyr	Thr	Leu	Pro	Pro	Ser
			340					345					350		
Arg	Glu	Glu	Met	Thr	Lys	Asn	Gln	Val	Ser	Leu	Thr	Cys	Leu	Val	Lys
	355					360						365			
Gly	Phe	Tyr	Pro	Ser	Asp	Ile	Ala	Val	Glu	Trp	Glu	Ser	Asn	Gly	Gln
	370				375						380				
Pro	Glu	Asn	Asn	Tyr	Lys	Thr	Thr	Pro	Pro	Val	Leu	Asp	Ser	Asp	Gly
385				390						395				400	
Ser	Phe	Phe	Leu	Tyr	Ser	Lys	Leu	Thr	Val	Asp	Lys	Ser	Arg	Trp	Gln
			405					410					415		
Gln	Gly	Asn	Val	Phe	Ser	Cys	Ser	Val	Met	His	Glu	Ala	Leu	His	Asn
		420						425					430		
His	Tyr	Thr	Gln	Lys	Ser	Leu	Ser	Leu	Ser	Pro	Gly	Lys			
	435					440						445			

<210> SEQ ID NO 186

<211> LENGTH: 671

<212> TYPE: PRT

<213> ORGANISM: Artificial

<220> FEATURE:

<223> OTHER INFORMATION: Chimeric homo sapiens mus musculus

<400> SEQUENCE: 186

Gln	Val	Gln	Leu	Gln	Gln	Ser	Gly	Ala	Glu	Leu	Val	Arg	Pro	Gly	Ser
1			5						10					15	
Ser	Val	Lys	Ile	Ser	Cys	Lys	Ala	Ser	Gly	Tyr	Ala	Phe	Ser	Ser	Tyr
		20					25					30			
Trp	Met	Asn	Trp	Val	Lys	Gln	Arg	Pro	Gly	Gln	Gly	Leu	Glu	Trp	Ile
	35					40						45			
Gly	Gln	Ile	Trp	Pro	Gly	Asp	Gly	Asp	Thr	Asn	Tyr	Asn	Gly	Lys	Phe
	50				55					60					
Lys	Gly	Lys	Ala	Thr	Leu	Thr	Ala	Asp	Glu	Ser	Ser	Ser	Thr	Ala	Tyr
65				70					75					80	
Met	Gln	Leu	Ser	Ser	Leu	Ala	Ser	Glu	Asp	Ser	Ala	Val	Tyr	Phe	Cys
			85					90						95	
Ala	Arg	Arg	Glu	Thr	Thr	Thr	Val	Gly	Arg	Tyr	Tyr	Tyr	Ala	Met	Asp
	100						105						110		
Tyr	Trp	Gly	Gln	Gly	Thr	Thr	Val	Thr	Val	Ser	Ser	Ala	Ser	Thr	Lys
	115					120						125			
Gly	Pro	Ser	Val	Phe	Pro	Leu	Ala	Pro	Ser	Ser	Lys	Ser	Thr	Ser	Gly
	130				135						140				
Gly	Thr	Ala	Ala	Leu	Gly	Cys	Leu	Val	Lys	Asp	Tyr	Phe	Pro	Glu	Pro

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145					150					155					160
Val	Thr	Val	Ser	Trp	Asn	Ser	Gly	Ala	Leu	Thr	Ser	Gly	Val	His	Thr
				165					170					175	
Phe	Pro	Ala	Val	Leu	Gln	Ser	Ser	Gly	Leu	Tyr	Ser	Leu	Ser	Ser	Val
			180					185					190		
Val	Thr	Val	Pro	Ser	Ser	Ser	Leu	Gly	Thr	Gln	Thr	Tyr	Ile	Cys	Asn
		195					200					205			
Val	Asn	His	Lys	Pro	Ser	Asn	Thr	Lys	Val	Asp	Lys	Arg	Val	Glu	Pro
	210					215				220					
Lys	Ser	Cys	Asp	Lys	Thr	His	Thr	Cys	Pro	Pro	Cys	Pro	Ala	Pro	Glu
225					230					235					240
Leu	Leu	Gly	Gly	Pro	Ser	Val	Phe	Leu	Phe	Pro	Pro	Lys	Pro	Lys	Asp
				245					250					255	
Thr	Leu	Met	Ile	Ser	Arg	Thr	Pro	Glu	Val	Thr	Cys	Val	Val	Val	Asp
		260						265					270		
Val	Ser	His	Glu	Asp	Pro	Glu	Val	Lys	Phe	Asn	Trp	Tyr	Val	Asp	Gly
		275					280					285			
Val	Glu	Val	His	Asn	Ala	Lys	Thr	Lys	Pro	Arg	Glu	Glu	Gln	Tyr	Ser
	290					295					300				
Ser	Thr	Tyr	Arg	Val	Val	Ser	Val	Leu	Thr	Val	Leu	His	Gln	Asp	Trp
305					310					315					320
Leu	Asn	Gly	Lys	Glu	Tyr	Lys	Cys	Lys	Val	Ser	Asn	Lys	Ala	Leu	Pro
			325						330					335	
Ala	Pro	Ile	Glu	Lys	Thr	Ile	Ser	Lys	Ala	Lys	Gly	Gln	Pro	Arg	Glu
		340						345					350		
Pro	Gln	Val	Tyr	Thr	Leu	Pro	Pro	Ser	Arg	Glu	Glu	Met	Thr	Lys	Asn
		355					360					365			
Gln	Val	Ser	Leu	Thr	Cys	Leu	Val	Lys	Gly	Phe	Tyr	Pro	Ser	Asp	Ile
	370					375					380				
Ala	Val	Glu	Trp	Glu	Ser	Asn	Gly	Gln	Pro	Glu	Asn	Asn	Tyr	Lys	Thr
385					390					395					400
Thr	Pro	Pro	Val	Leu	Asp	Ser	Asp	Gly	Ser	Phe	Phe	Leu	Tyr	Ser	Lys
			405					410						415	
Leu	Thr	Val	Asp	Lys	Ser	Arg	Trp	Gln	Gln	Gly	Asn	Val	Phe	Ser	Cys
		420						425					430		
Ser	Val	Met	His	Glu	Ala	Leu	His	Asn	His	Tyr	Thr	Gln	Lys	Ser	Leu
	435						440					445			
Ser	Leu	Ser	Pro	Gly	Ser	Thr	Gly	Ser	Asp	Ile	Val	Met	Thr	Gln	Ser
	450					455					460				
Pro	Ala	Thr	Leu	Ser	Val	Thr	Pro	Gly	Asp	Arg	Val	Ser	Leu	Ser	Cys
465					470					475					480
Arg	Ala	Ser	Gln	Ser	Ile	Ser	Asp	Tyr	Leu	His	Trp	Tyr	Gln	Gln	Lys
			485					490						495	
Ser	His	Glu	Ser	Pro	Arg	Leu	Leu	Ile	Lys	Tyr	Ala	Ser	Gln	Ser	Ile
		500						505					510		
Ser	Gly	Ile	Pro	Ser	Arg	Phe	Ser	Gly	Ser	Gly	Ser	Gly	Ser	Asp	Phe
		515					520					525			
Thr	Leu	Ser	Ile	Asn	Ser	Val	Glu	Pro	Glu	Asp	Val	Gly	Val	Tyr	Tyr
	530					535					540				
Cys	Gln	Asn	Gly	His	Ser	Phe	Pro	Leu	Thr	Phe	Gly	Ala	Gly	Thr	Lys
545					550					555					560

<400> SEQUENCE: 187

<210> SEQ ID NO 188

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<211> LENGTH: 680
<212> TYPE: PRT
<213> ORGANISM: Artificial
<220> FEATURE:
<223> OTHER INFORMATION: Human-mouse chimeric

<400> SEQUENCE: 188

Gln Val Gln Leu Val Gln Ser Gly Ala Glu Val Lys Lys Pro Gly Ser
1      5      10      15
Ser Val Lys Val Ser Cys Lys Ala Ser Gly Tyr Ala Phe Ser Tyr Ser
20      25      30
Trp Ile Asn Trp Val Arg Gln Ala Pro Gly Gln Gly Leu Glu Trp Met
35      40      45
Gly Arg Ile Phe Pro Gly Asp Gly Asp Thr Asp Tyr Asn Gly Lys Phe
50      55      60
Lys Gly Arg Val Thr Ile Thr Ala Asp Lys Ser Thr Ser Thr Ala Tyr
65      70      75      80
Met Glu Leu Ser Ser Leu Arg Ser Glu Asp Thr Ala Val Tyr Tyr Cys
85      90      95
Ala Arg Asn Val Phe Asp Gly Tyr Trp Leu Val Tyr Trp Gly Gln Gly
100     105     110
Thr Leu Val Thr Val Ser Ser Ala Ser Thr Lys Gly Pro Ser Val Phe
115     120     125
Pro Leu Ala Pro Ser Ser Lys Ser Thr Ser Gly Gly Thr Ala Ala Leu
130     135     140
Gly Cys Leu Val Lys Asp Tyr Phe Pro Glu Pro Val Thr Val Ser Trp
145     150     155     160
Asn Ser Gly Ala Leu Thr Ser Gly Val His Thr Phe Pro Ala Val Leu
165     170     175
Gln Ser Ser Gly Leu Tyr Ser Leu Ser Ser Val Val Thr Val Pro Ser
180     185     190
Ser Ser Leu Gly Thr Gln Thr Tyr Ile Cys Asn Val Asn His Lys Pro
195     200     205
Ser Asn Thr Lys Val Asp Lys Arg Val Glu Pro Lys Ser Cys Asp Lys
210     215     220
Thr His Thr Cys Pro Pro Cys Pro Ala Pro Glu Leu Leu Gly Gly Pro
225     230     235     240
Ser Val Phe Leu Phe Pro Pro Lys Pro Lys Asp Thr Leu Met Ile Ser
245     250     255
Arg Thr Pro Glu Val Thr Cys Val Val Val Asp Val Ser His Glu Asp
260     265     270
Pro Glu Val Lys Phe Asn Trp Tyr Val Asp Gly Val Glu Val His Asn
275     280     285
Ala Lys Thr Lys Pro Arg Glu Glu Gln Tyr Asn Ser Thr Tyr Arg Val
290     295     300
Val Ser Val Leu Thr Val Leu His Gln Asp Trp Leu Asn Gly Lys Glu
305     310     315     320
Tyr Lys Cys Lys Val Ser Asn Lys Ala Leu Pro Ala Pro Ile Glu Lys
325     330     335
Thr Ile Ser Lys Ala Lys Gly Gln Pro Arg Glu Pro Gln Val Tyr Thr
340     345     350
Leu Pro Pro Ser Arg Glu Glu Met Thr Lys Asn Gln Val Ser Leu Thr
355     360     365

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Cys Leu Val Lys Gly Phe Tyr Pro Ser Asp Ile Ala Val Glu Trp Glu
 370 375 380
 Ser Asn Gly Gln Pro Glu Asn Asn Tyr Lys Thr Thr Pro Pro Val Leu
 385 390 395 400
 Asp Ser Asp Gly Ser Phe Phe Leu Tyr Ser Lys Leu Thr Val Asp Lys
 405 410 415
 Ser Arg Trp Gln Gln Gly Asn Val Phe Ser Cys Ser Val Met His Glu
 420 425 430
 Ala Leu His Asn His Tyr Thr Gln Lys Ser Leu Ser Leu Ser Pro Gly
 435 440 445
 Ser Thr Gly Ser Gln Val Gln Leu Gln Gln Pro Gly Ala Glu Leu Val
 450 455 460
 Lys Pro Gly Ala Ser Val Lys Met Ser Cys Lys Ala Ser Gly Tyr Thr
 465 470 475 480
 Phe Thr Ser Tyr Asn Met His Trp Val Lys Gln Thr Pro Gly Arg Gly
 485 490 495
 Leu Glu Trp Ile Gly Ala Ile Tyr Pro Gly Asn Gly Asp Thr Ser Tyr
 500 505 510
 Asn Gln Lys Phe Lys Gly Lys Ala Thr Leu Thr Ala Asp Lys Ser Ser
 515 520 525
 Ser Thr Ala Tyr Met Gln Leu Ser Ser Leu Thr Ser Glu Asp Ser Ala
 530 535 540
 Val Tyr Tyr Cys Ala Arg Ser Thr Tyr Tyr Gly Gly Asp Trp Tyr Phe
 545 550 555 560
 Asn Val Trp Gly Ala Gly Thr Thr Val Thr Val Ser Ala Arg Thr Val
 565 570 575
 Ala Ala Pro Ser Val Phe Ile Phe Pro Pro Ser Asp Glu Gln Leu Lys
 580 585 590
 Ser Gly Thr Ala Ser Val Val Cys Leu Leu Asn Asn Phe Tyr Pro Arg
 595 600 605
 Glu Ala Lys Val Gln Trp Lys Val Asp Asn Ala Leu Gln Ser Gly Asn
 610 615 620
 Ser Gln Glu Ser Val Thr Glu Gln Asp Ser Lys Asp Ser Thr Tyr Ser
 625 630 635 640
 Leu Ser Ser Thr Leu Thr Leu Ser Lys Ala Asp Tyr Glu Lys His Lys
 645 650 655
 Val Tyr Ala Cys Glu Val Thr His Gln Gly Leu Ser Ser Pro Val Thr
 660 665 670
 Lys Ser Phe Asn Arg Gly Glu Cys
 675 680

<210> SEQ ID NO 189

<211> LENGTH: 446

<212> TYPE: PRT

<213> ORGANISM: Artificial

<220> FEATURE:

<223> OTHER INFORMATION: Human-mouse chimeric

<400> SEQUENCE: 189

Asp Ile Val Met Thr Gln Thr Pro Leu Ser Leu Pro Val Thr Pro Gly
 1 5 10 15
 Glu Pro Ala Ser Ile Ser Cys Arg Ser Ser Lys Ser Leu Leu His Ser
 20 25 30

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Asn	Gly	Ile	Thr	Tyr	Leu	Tyr	Trp	Tyr	Leu	Gln	Lys	Pro	Gly	Gln	Ser	35	40	45
Pro	Gln	Leu	Leu	Ile	Tyr	Gln	Met	Ser	Asn	Leu	Val	Ser	Gly	Val	Pro	50	55	60
Asp	Arg	Phe	Ser	Gly	Ser	Gly	Ser	Gly	Thr	Asp	Phe	Thr	Leu	Lys	Ile	65	70	75
Ser	Arg	Val	Glu	Ala	Glu	Asp	Val	Gly	Val	Tyr	Tyr	Cys	Ala	Gln	Asn	85	90	95
Leu	Glu	Leu	Pro	Tyr	Thr	Phe	Gly	Gly	Gly	Thr	Lys	Val	Glu	Ile	Lys	100	105	110
Arg	Thr	Val	Ala	Ala	Pro	Ser	Val	Phe	Ile	Phe	Pro	Pro	Ser	Asp	Glu	115	120	125
Gln	Leu	Lys	Ser	Gly	Thr	Ala	Ser	Val	Val	Cys	Leu	Leu	Asn	Asn	Phe	130	135	140
Tyr	Pro	Arg	Glu	Ala	Lys	Val	Gln	Trp	Lys	Val	Asp	Asn	Ala	Leu	Gln	145	150	155
Ser	Gly	Asn	Ser	Gln	Glu	Ser	Val	Thr	Glu	Gln	Asp	Ser	Lys	Asp	Ser	165	170	175
Thr	Tyr	Ser	Leu	Ser	Ser	Thr	Leu	Thr	Leu	Ser	Lys	Ala	Asp	Tyr	Glu	180	185	190
Lys	His	Lys	Val	Tyr	Ala	Cys	Glu	Val	Thr	His	Gln	Gly	Leu	Ser	Ser	195	200	205
Pro	Val	Thr	Lys	Ser	Phe	Asn	Arg	Gly	Glu	Cys	Asp	Lys	Thr	His	Thr	210	215	220
Cys	Pro	Pro	Cys	Pro	Ala	Pro	Glu	Leu	Leu	Gly	Gly	Pro	Ser	Val	Phe	225	230	235
Leu	Phe	Pro	Pro	Lys	Pro	Lys	Asp	Thr	Leu	Met	Ile	Ser	Arg	Thr	Pro	245	250	255
Glu	Val	Thr	Cys	Val	Val	Val	Asp	Val	Ser	His	Glu	Asp	Pro	Glu	Val	260	265	270
Lys	Phe	Asn	Trp	Tyr	Val	Asp	Gly	Val	Glu	Val	His	Asn	Ala	Lys	Thr	275	280	285
Lys	Pro	Arg	Glu	Glu	Gln	Tyr	Asn	Ser	Thr	Tyr	Arg	Val	Val	Ser	Val	290	295	300
Leu	Thr	Val	Leu	His	Gln	Asp	Trp	Leu	Asn	Gly	Lys	Glu	Tyr	Lys	Cys	305	310	315
Lys	Val	Ser	Asn	Lys	Ala	Leu	Pro	Ala	Pro	Ile	Glu	Lys	Thr	Ile	Ser	325	330	335
Lys	Ala	Lys	Gly	Gln	Pro	Arg	Glu	Pro	Gln	Val	Tyr	Thr	Leu	Pro	Pro	340	345	350
Ser	Arg	Glu	Glu	Met	Thr	Lys	Asn	Gln	Val	Ser	Leu	Thr	Cys	Leu	Val	355	360	365
Lys	Gly	Phe	Tyr	Pro	Ser	Asp	Ile	Ala	Val	Glu	Trp	Glu	Ser	Asn	Gly	370	375	380
Gln	Pro	Glu	Asn	Asn	Tyr	Lys	Thr	Thr	Pro	Pro	Val	Leu	Asp	Ser	Asp	385	390	395
Gly	Ser	Phe	Phe	Leu	Tyr	Ser	Lys	Leu	Thr	Val	Asp	Lys	Ser	Arg	Trp	405	410	415
Gln	Gln	Gly	Asn	Val	Phe	Ser	Cys	Ser	Val	Met	His	Glu	Ala	Leu	His	420	425	430

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Asn	His	Tyr	Thr	Gln	Lys	Ser	Leu	Ser	Leu	Ser	Pro	Gly	Lys
		435					440					445	

<210> SEQ ID NO 190
 <211> LENGTH: 459
 <212> TYPE: PRT
 <213> ORGANISM: Artificial
 <220> FEATURE:
 <223> OTHER INFORMATION: Human-mouse chimeric

<400> SEQUENCE: 190

Gln	Ile	Val	Leu	Ser	Gln	Ser	Pro	Ala	Ile	Leu	Ser	Ala	Ser	Pro	Gly
1				5					10					15	
Glu	Lys	Val	Thr	Met	Thr	Cys	Arg	Ala	Ser	Ser	Ser	Val	Ser	Tyr	Ile
			20					25					30		
His	Trp	Phe	Gln	Gln	Lys	Pro	Gly	Ser	Ser	Pro	Lys	Pro	Trp	Ile	Tyr
		35					40					45			
Ala	Thr	Ser	Asn	Leu	Ala	Ser	Gly	Val	Pro	Val	Arg	Phe	Ser	Gly	Ser
		50				55					60				
Gly	Ser	Gly	Thr	Ser	Tyr	Ser	Leu	Thr	Ile	Ser	Arg	Val	Glu	Ala	Glu
65					70				75					80	
Asp	Ala	Ala	Thr	Tyr	Tyr	Cys	Gln	Gln	Trp	Thr	Ser	Asn	Pro	Pro	Thr
				85					90					95	
Phe	Gly	Gly	Gly	Thr	Lys	Leu	Glu	Ile	Lys	Ala	Ser	Thr	Lys	Gly	Pro
			100					105					110		
Ser	Val	Phe	Pro	Leu	Ala	Pro	Ser	Ser	Lys	Ser	Thr	Ser	Gly	Gly	Thr
		115					120					125			
Ala	Ala	Leu	Gly	Cys	Leu	Val	Lys	Asp	Tyr	Phe	Pro	Glu	Pro	Val	Thr
		130				135					140				
Val	Ser	Trp	Asn	Ser	Gly	Ala	Leu	Thr	Ser	Gly	Val	His	Thr	Phe	Pro
145					150					155					160
Ala	Val	Leu	Gln	Ser	Ser	Gly	Leu	Tyr	Ser	Leu	Ser	Ser	Val	Val	Thr
				165					170					175	
Val	Pro	Ser	Ser	Ser	Leu	Gly	Thr	Gln	Thr	Tyr	Ile	Cys	Asn	Val	Asn
				180				185					190		
His	Lys	Pro	Ser	Asn	Thr	Lys	Val	Asp	Lys	Arg	Val	Glu	Pro	Lys	Ser
		195					200					205			
Cys	Asp	Lys	Thr	His	Gly	Gly	Ser	Ser	Ser	Glu	Val	Gln	Leu	Gln	Gln
	210					215					220				
Ser	Gly	Pro	Glu	Leu	Val	Lys	Pro	Gly	Ala	Ser	Val	Lys	Ile	Ser	Cys
225					230				235					240	
Lys	Thr	Ser	Gly	Tyr	Thr	Phe	Thr	Glu	Tyr	Thr	Met	His	Trp	Val	Lys
			245					250						255	
Gln	Ser	His	Gly	Lys	Ser	Leu	Glu	Trp	Ile	Gly	Gly	Ile	Ser	Pro	Asn
			260					265					270		
Ile	Gly	Gly	Thr	Ser	Tyr	Asn	Gln	Lys	Phe	Lys	Gly	Lys	Ala	Thr	Leu
		275					280					285			
Thr	Val	Asp	Lys	Ser	Ser	Ser	Thr	Ala	Tyr	Met	Glu	Leu	Arg	Ser	Leu
		290				295					300				
Thr	Ser	Glu	Asp	Ser	Ala	Val	Tyr	Tyr	Cys	Ala	Arg	Arg	Gly	Gly	Ser
305					310					315					320
Phe	Asp	Tyr	Trp	Gly	Gln	Gly	Thr	Thr	Leu	Thr	Val	Ser	Ser	Val	Glu
				325					330						335

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Gly Gly Ser Gly Gly Ser Gly Gly Ser Gly Gly Ser Gly Gly Val Asp
 340 345 350
 Asp Ile Val Met Thr Gln Ser Pro Ala Thr Leu Ser Val Thr Pro Gly
 355 360 365
 Asp Arg Val Ser Leu Ser Cys Arg Ala Ser Gln Ser Ile Ser Asp Tyr
 370 375 380
 Leu His Trp Tyr Gln Gln Lys Ser His Glu Ser Pro Arg Leu Leu Ile
 385 390 395 400
 Lys Tyr Ala Ser Gln Ser Ile Ser Gly Ile Pro Ser Arg Phe Ser Gly
 405 410 415
 Ser Gly Ser Gly Ser Asp Phe Thr Leu Ser Ile Asn Ser Val Glu Pro
 420 425 430
 Glu Asp Val Gly Val Tyr Tyr Cys Gln Asn Gly His Ser Phe Pro Leu
 435 440 445
 Thr Phe Gly Ala Gly Thr Lys Leu Glu Leu Lys
 450 455

<210> SEQ ID NO 191
 <211> LENGTH: 446
 <212> TYPE: PRT
 <213> ORGANISM: Artificial
 <220> FEATURE:
 <223> OTHER INFORMATION: Human-mouse chimeric

<400> SEQUENCE: 191

Asp Ile Val Met Thr Gln Thr Pro Leu Ser Leu Pro Val Thr Pro Gly
 1 5 10 15
 Glu Pro Ala Ser Ile Ser Cys Arg Ser Ser Lys Ser Leu Leu His Ser
 20 25 30
 Asn Gly Ile Thr Tyr Leu Tyr Trp Tyr Leu Gln Lys Pro Gly Gln Ser
 35 40 45
 Pro Gln Leu Leu Ile Tyr Gln Met Ser Asn Leu Val Ser Gly Val Pro
 50 55 60
 Asp Arg Phe Ser Gly Ser Gly Ser Gly Thr Asp Phe Thr Leu Lys Ile
 65 70 75 80
 Ser Arg Val Glu Ala Glu Asp Val Gly Val Tyr Tyr Cys Ala Gln Asn
 85 90 95
 Leu Glu Leu Pro Tyr Thr Phe Gly Gly Gly Thr Lys Val Glu Ile Lys
 100 105 110
 Arg Thr Val Ala Ala Pro Ser Val Phe Ile Phe Pro Pro Ser Asp Glu
 115 120 125
 Gln Leu Lys Ser Gly Thr Ala Ser Val Val Cys Leu Leu Asn Asn Phe
 130 135 140
 Tyr Pro Arg Glu Ala Lys Val Gln Trp Lys Val Asp Asn Ala Leu Gln
 145 150 155 160
 Ser Gly Asn Ser Gln Glu Ser Val Thr Glu Gln Asp Ser Lys Asp Ser
 165 170 175
 Thr Tyr Ser Leu Ser Ser Thr Leu Thr Leu Ser Lys Ala Asp Tyr Glu
 180 185 190
 Lys His Lys Val Tyr Ala Cys Glu Val Thr His Gln Gly Leu Ser Ser
 195 200 205
 Pro Val Thr Lys Ser Phe Asn Arg Gly Glu Cys Asp Lys Thr His Thr
 210 215 220

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Cys	Pro	Pro	Cys	Pro	Ala	Pro	Glu	Leu	Leu	Gly	Gly	Pro	Ser	Val	Phe	225	230	235	240
Leu	Phe	Pro	Pro	Lys	Pro	Lys	Asp	Thr	Leu	Met	Ile	Ser	Arg	Thr	Pro	245	250	255	
Glu	Val	Thr	Cys	Val	Val	Val	Asp	Val	Ser	His	Glu	Asp	Pro	Glu	Val	260	265	270	
Lys	Phe	Asn	Trp	Tyr	Val	Asp	Gly	Val	Glu	Val	His	Asn	Ala	Lys	Thr	275	280	285	
Lys	Pro	Arg	Glu	Glu	Gln	Tyr	Ser	Ser	Thr	Tyr	Arg	Val	Val	Ser	Val	290	295	300	
Leu	Thr	Val	Leu	His	Gln	Asp	Trp	Leu	Asn	Gly	Lys	Glu	Tyr	Lys	Cys	305	310	315	320
Lys	Val	Ser	Asn	Lys	Ala	Leu	Pro	Ala	Pro	Ile	Glu	Lys	Thr	Ile	Ser	325	330	335	
Lys	Ala	Lys	Gly	Gln	Pro	Arg	Glu	Pro	Gln	Val	Tyr	Thr	Leu	Pro	Pro	340	345	350	
Ser	Arg	Glu	Glu	Met	Thr	Lys	Asn	Gln	Val	Ser	Leu	Thr	Cys	Leu	Val	355	360	365	
Lys	Gly	Phe	Tyr	Pro	Ser	Asp	Ile	Ala	Val	Glu	Trp	Glu	Ser	Asn	Gly	370	375	380	
Gln	Pro	Glu	Asn	Asn	Tyr	Lys	Thr	Thr	Pro	Pro	Val	Leu	Asp	Ser	Asp	385	390	395	400
Gly	Ser	Phe	Phe	Leu	Tyr	Ser	Lys	Leu	Thr	Val	Asp	Lys	Ser	Arg	Trp	405	410	415	
Gln	Gln	Gly	Asn	Val	Phe	Ser	Cys	Ser	Val	Met	His	Glu	Ala	Leu	His	420	425	430	
Asn	His	Tyr	Thr	Gln	Lys	Ser	Leu	Ser	Leu	Ser	Pro	Gly	Lys			435	440	445	

<210> SEQ ID NO 192

<211> LENGTH: 680

<212> TYPE: PRT

<213> ORGANISM: Artificial

<220> FEATURE:

<223> OTHER INFORMATION: Human mouse chimeric

<400> SEQUENCE: 192

Gln	Val	Gln	Leu	Val	Gln	Ser	Gly	Ala	Glu	Val	Lys	Lys	Pro	Gly	Ser	1	5	10	15
Ser	Val	Lys	Val	Ser	Cys	Lys	Ala	Ser	Gly	Tyr	Ala	Phe	Ser	Tyr	Ser	20	25	30	
Trp	Ile	Asn	Trp	Val	Arg	Gln	Ala	Pro	Gly	Gln	Gly	Leu	Glu	Trp	Met	35	40	45	
Gly	Arg	Ile	Phe	Pro	Gly	Asp	Gly	Asp	Thr	Asp	Tyr	Asn	Gly	Lys	Phe	50	55	60	
Lys	Gly	Arg	Val	Thr	Ile	Thr	Ala	Asp	Lys	Ser	Thr	Ser	Thr	Ala	Tyr	65	70	75	80
Met	Glu	Leu	Ser	Ser	Leu	Arg	Ser	Glu	Asp	Thr	Ala	Val	Tyr	Tyr	Cys	85	90	95	
Ala	Arg	Asn	Val	Phe	Asp	Gly	Tyr	Trp	Leu	Val	Tyr	Trp	Gly	Gln	Gly	100	105	110	
Thr	Leu	Val	Thr	Val	Ser	Ser	Ala	Ser	Thr	Lys	Gly	Pro	Ser	Val	Phe	115	120	125	

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Pro	Leu	Ala	Pro	Ser	Ser	Lys	Ser	Thr	Ser	Gly	Gly	Thr	Ala	Ala	Leu
130						135					140				
Gly	Cys	Leu	Val	Lys	Asp	Tyr	Phe	Pro	Glu	Pro	Val	Thr	Val	Ser	Trp
145				150						155				160	
Asn	Ser	Gly	Ala	Leu	Thr	Ser	Gly	Val	His	Thr	Phe	Pro	Ala	Val	Leu
				165					170					175	
Gln	Ser	Ser	Gly	Leu	Tyr	Ser	Leu	Ser	Ser	Val	Val	Thr	Val	Pro	Ser
			180					185					190		
Ser	Ser	Leu	Gly	Thr	Gln	Thr	Tyr	Ile	Cys	Asn	Val	Asn	His	Lys	Pro
		195					200					205			
Ser	Asn	Thr	Lys	Val	Asp	Lys	Arg	Val	Glu	Pro	Lys	Ser	Cys	Asp	Lys
	210					215					220				
Thr	His	Thr	Cys	Pro	Pro	Cys	Pro	Ala	Pro	Glu	Leu	Leu	Gly	Gly	Pro
225					230					235					240
Ser	Val	Phe	Leu	Phe	Pro	Pro	Lys	Pro	Lys	Asp	Thr	Leu	Met	Ile	Ser
				245					250					255	
Arg	Thr	Pro	Glu	Val	Thr	Cys	Val	Val	Val	Asp	Val	Ser	His	Glu	Asp
			260					265					270		
Pro	Glu	Val	Lys	Phe	Asn	Trp	Tyr	Val	Asp	Gly	Val	Glu	Val	His	Asn
	275						280					285			
Ala	Lys	Thr	Lys	Pro	Arg	Glu	Glu	Gln	Tyr	Ser	Ser	Thr	Tyr	Arg	Val
	290					295					300				
Val	Ser	Val	Leu	Thr	Val	Leu	His	Gln	Asp	Trp	Leu	Asn	Gly	Lys	Glu
305					310					315					320
Tyr	Lys	Cys	Lys	Val	Ser	Asn	Lys	Ala	Leu	Pro	Ala	Pro	Ile	Glu	Lys
			325						330					335	
Thr	Ile	Ser	Lys	Ala	Lys	Gly	Gln	Pro	Arg	Glu	Pro	Gln	Val	Tyr	Thr
		340						345					350		
Leu	Pro	Pro	Ser	Arg	Glu	Glu	Met	Thr	Lys	Asn	Gln	Val	Ser	Leu	Thr
		355					360					365			
Cys	Leu	Val	Lys	Gly	Phe	Tyr	Pro	Ser	Asp	Ile	Ala	Val	Glu	Trp	Glu
	370					375					380				
Ser	Asn	Gly	Gln	Pro	Glu	Asn	Asn	Tyr	Lys	Thr	Thr	Pro	Pro	Val	Leu
385					390					395					400
Asp	Ser	Asp	Gly	Ser	Phe	Phe	Leu	Tyr	Ser	Lys	Leu	Thr	Val	Asp	Lys
			405						410					415	
Ser	Arg	Trp	Gln	Gln	Gly	Asn	Val	Phe	Ser	Cys	Ser	Val	Met	His	Glu
			420					425					430		
Ala	Leu	His	Asn	His	Tyr	Thr	Gln	Lys	Ser	Leu	Ser	Leu	Ser	Pro	Gly
		435					440					445			
Ser	Thr	Gly	Ser	Gln	Val	Gln	Leu	Gln	Gln	Pro	Gly	Ala	Glu	Leu	Val
	450					455					460				
Lys	Pro	Gly	Ala	Ser	Val	Lys	Met	Ser	Cys	Lys	Ala	Ser	Gly	Tyr	Thr
465					470					475					480
Phe	Thr	Ser	Tyr	Asn	Met	His	Trp	Val	Lys	Gln	Thr	Pro	Gly	Arg	Gly
			485						490					495	
Leu	Glu	Trp	Ile	Gly	Ala	Ile	Tyr	Pro	Gly	Asn	Gly	Asp	Thr	Ser	Tyr
		500						505					510		
Asn	Gln	Lys	Phe	Lys	Gly	Lys	Ala	Thr	Leu	Thr	Ala	Asp	Lys	Ser	Ser
		515					520					525			
Ser	Thr	Ala	Tyr	Met	Gln	Leu	Ser	Ser	Leu	Thr	Ser	Glu	Asp	Ser	Ala

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530	535	540
Val Tyr Tyr Cys Ala Arg Ser Thr Tyr Tyr Gly Gly Asp Trp Tyr Phe 545 550 555 560		
Asn Val Trp Gly Ala Gly Thr Thr Val Thr Val Ser Ala Arg Thr Val 565 570 575		
Ala Ala Pro Ser Val Phe Ile Phe Pro Pro Ser Asp Glu Gln Leu Lys 580 585 590		
Ser Gly Thr Ala Ser Val Val Cys Leu Leu Asn Asn Phe Tyr Pro Arg 595 600 605		
Glu Ala Lys Val Gln Trp Lys Val Asp Asn Ala Leu Gln Ser Gly Asn 610 615 620		
Ser Gln Glu Ser Val Thr Glu Gln Asp Ser Lys Asp Ser Thr Tyr Ser 625 630 635 640		
Leu Ser Ser Thr Leu Thr Leu Ser Lys Ala Asp Tyr Glu Lys His Lys 645 650 655		
Val Tyr Ala Cys Glu Val Thr His Gln Gly Leu Ser Ser Pro Val Thr 660 665 670		
Lys Ser Phe Asn Arg Gly Glu Cys 675 680		
<210> SEQ ID NO 193		
<211> LENGTH: 459		
<212> TYPE: PRT		
<213> ORGANISM: Artificial		
<220> FEATURE:		
<223> OTHER INFORMATION: Human mouse chimeric		
<400> SEQUENCE: 193		
Gln Ile Val Leu Ser Gln Ser Pro Ala Ile Leu Ser Ala Ser Pro Gly 1 5 10 15		
Glu Lys Val Thr Met Thr Cys Arg Ala Ser Ser Ser Val Ser Tyr Ile 20 25 30		
His Trp Phe Gln Gln Lys Pro Gly Ser Ser Pro Lys Pro Trp Ile Tyr 35 40 45		
Ala Thr Ser Asn Leu Ala Ser Gly Val Pro Val Arg Phe Ser Gly Ser 50 55 60		
Gly Ser Gly Thr Ser Tyr Ser Leu Thr Ile Ser Arg Val Glu Ala Glu 65 70 75 80		
Asp Ala Ala Thr Tyr Tyr Cys Gln Gln Trp Thr Ser Asn Pro Pro Thr 85 90 95		
Phe Gly Gly Gly Thr Lys Leu Glu Ile Lys Ala Ser Thr Lys Gly Pro 100 105 110		
Ser Val Phe Pro Leu Ala Pro Ser Ser Lys Ser Thr Ser Gly Gly Thr 115 120 125		
Ala Ala Leu Gly Cys Leu Val Lys Asp Tyr Phe Pro Glu Pro Val Thr 130 135 140		
Val Ser Trp Asn Ser Gly Ala Leu Thr Ser Gly Val His Thr Phe Pro 145 150 155 160		
Ala Val Leu Gln Ser Ser Gly Leu Tyr Ser Leu Ser Ser Val Val Thr 165 170 175		
Val Pro Ser Ser Ser Leu Gly Thr Gln Thr Tyr Ile Cys Asn Val Asn 180 185 190		
His Lys Pro Ser Asn Thr Lys Val Asp Lys Arg Val Glu Pro Lys Ser		

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195					200					205				
Cys	Asp	Lys	Thr	His	Gly	Gly	Ser	Ser	Ser	Glu	Val	Gln	Leu	Gln
210					215					220				
Ser	Gly	Pro	Glu	Leu	Val	Lys	Pro	Gly	Ala	Ser	Val	Lys	Ile	Ser
225					230					235				240
Lys	Thr	Ser	Gly	Tyr	Thr	Phe	Thr	Glu	Tyr	Thr	Met	His	Trp	Val
			245						250				255	
Gln	Ser	His	Gly	Lys	Ser	Leu	Glu	Trp	Ile	Gly	Gly	Ile	Ser	Pro
			260					265					270	Asn
Ile	Gly	Gly	Thr	Ser	Tyr	Asn	Gln	Lys	Phe	Lys	Gly	Lys	Ala	Thr
	275					280							285	Leu
Thr	Val	Asp	Lys	Ser	Ser	Ser	Thr	Ala	Tyr	Met	Glu	Leu	Arg	Ser
	290					295					300			Leu
Thr	Ser	Glu	Asp	Ser	Ala	Val	Tyr	Tyr	Cys	Ala	Arg	Arg	Gly	Ser
305					310					315				320
Phe	Asp	Tyr	Trp	Gly	Gln	Gly	Thr	Thr	Leu	Thr	Val	Ser	Ser	Val
			325						330					335
Gly	Gly	Ser	Gly	Gly	Ser	Gly	Gly	Ser	Gly	Gly	Ser	Gly	Val	Asp
			340					345					350	
Asp	Ile	Val	Met	Thr	Gln	Ser	Pro	Ala	Thr	Leu	Ser	Val	Thr	Pro
	355						360					365		Gly
Asp	Arg	Val	Ser	Leu	Ser	Cys	Arg	Ala	Ser	Gln	Ser	Ile	Ser	Asp
	370					375						380		Tyr
Leu	His	Trp	Tyr	Gln	Gln	Lys	Ser	His	Glu	Ser	Pro	Arg	Leu	Ile
385						390					395			400
Lys	Tyr	Ala	Ser	Gln	Ser	Ile	Ser	Gly	Ile	Pro	Ser	Arg	Phe	Ser
			405						410					415
Ser	Gly	Ser	Gly	Ser	Asp	Phe	Thr	Leu	Ser	Ile	Asn	Ser	Val	Glu
			420					425					430	Pro
Glu	Asp	Val	Gly	Val	Tyr	Tyr	Cys	Gln	Asn	Gly	His	Ser	Phe	Pro
	435						440					445		Leu
Thr	Phe	Gly	Ala	Gly	Thr	Lys	Leu	Glu	Leu	Lys				
	450					455								

<210> SEQ ID NO 194

<211> LENGTH: 801

<212> TYPE: PRT

<213> ORGANISM: Artificial

<220> FEATURE:

<223> OTHER INFORMATION: Human-mouse chimeric

<400> SEQUENCE: 194

Gln	Val	Gln	Leu	Val	Gln	Ser	Gly	Ala	Glu	Val	Lys	Lys	Pro	Gly	Ser
1				5					10					15	
Ser	Val	Lys	Val	Ser	Cys	Lys	Ala	Ser	Gly	Tyr	Ala	Phe	Ser	Tyr	Ser
			20					25					30		
Trp	Ile	Asn	Trp	Val	Arg	Gln	Ala	Pro	Gly	Gln	Gly	Leu	Glu	Trp	Met
	35					40						45			
Gly	Arg	Ile	Phe	Pro	Gly	Asp	Gly	Asp	Thr	Asp	Tyr	Asn	Gly	Lys	Phe
	50					55				60					
Lys	Gly	Arg	Val	Thr	Ile	Thr	Ala	Asp	Lys	Ser	Thr	Ser	Thr	Ala	Tyr
65					70				75					80	
Met	Glu	Leu	Ser	Ser	Leu	Arg	Ser	Glu	Asp	Thr	Ala	Val	Tyr	Tyr	Cys

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85								90					95				
Ala	Arg	Asn	Val	Phe	Asp	Gly	Tyr	Trp	Leu	Val	Tyr	Trp	Gly	Gln	Gly		
100								105	110								
Thr	Leu	Val	Thr	Val	Ser	Ser	Ala	Ser	Thr	Lys	Gly	Pro	Ser	Val	Phe		
115								120	125								
Pro	Leu	Ala	Pro	Ser	Ser	Lys	Ser	Thr	Ser	Gly	Gly	Thr	Ala	Ala	Leu		
130								135	140								
Gly	Cys	Leu	Val	Lys	Asp	Tyr	Phe	Pro	Glu	Pro	Val	Thr	Val	Ser	Trp		
145	150								155							160	
Asn	Ser	Gly	Ala	Leu	Thr	Ser	Gly	Val	His	Thr	Phe	Pro	Ala	Val	Leu		
165								170	175								
Gln	Ser	Ser	Gly	Leu	Tyr	Ser	Leu	Ser	Ser	Val	Val	Thr	Val	Pro	Ser		
180								185	190								
Ser	Ser	Leu	Gly	Thr	Gln	Thr	Tyr	Ile	Cys	Asn	Val	Asn	His	Lys	Pro		
195								200	205								
Ser	Asn	Thr	Lys	Val	Asp	Lys	Arg	Val	Glu	Pro	Lys	Ser	Cys	Asp	Lys		
210								215	220								
Thr	His	Thr	Ser	Pro	Pro	Ser	Pro	Ala	Pro	Glu	Leu	Leu	Gly	Gly	Pro		
225	230								235							240	
Ser	Val	Phe	Leu	Phe	Pro	Pro	Lys	Pro	Lys	Asp	Thr	Leu	Met	Ile	Ser		
245								250	255								
Arg	Thr	Pro	Glu	Val	Thr	Cys	Val	Val	Val	Asp	Val	Ser	His	Glu	Asp		
260								265	270								
Pro	Glu	Val	Lys	Phe	Asn	Trp	Tyr	Val	Asp	Gly	Val	Glu	Val	His	Asn		
275								280	285								
Ala	Lys	Thr	Lys	Pro	Arg	Glu	Glu	Gln	Tyr	Ser	Ser	Thr	Tyr	Arg	Val		
290								295	300								
Val	Ser	Val	Leu	Thr	Val	Leu	His	Gln	Asp	Trp	Leu	Asn	Gly	Lys	Glu		
305	310								315							320	
Tyr	Lys	Cys	Lys	Val	Ser	Asn	Lys	Ala	Leu	Pro	Ala	Pro	Ile	Glu	Lys		
325								330	335								
Thr	Ile	Ser	Lys	Ala	Lys	Gly	Gln	Pro	Arg	Glu	Pro	Gln	Val	Tyr	Thr		
340								345	350								
Leu	Pro	Pro	Ser	Arg	Glu	Glu	Met	Thr	Lys	Asn	Gln	Val	Ser	Leu	Thr		
355								360	365								
Cys	Leu	Val	Lys	Gly	Phe	Tyr	Pro	Ser	Asp	Ile	Ala	Val	Glu	Trp	Glu		
370								375	380								
Ser	Asn	Gly	Gln	Pro	Glu	Asn	Asn	Tyr	Lys	Thr	Thr	Pro	Pro	Val	Leu		
385	390								395							400	
Asp	Ser	Asp	Gly	Ser	Phe	Phe	Leu	Tyr	Ser	Lys	Leu	Thr	Val	Asp	Lys		
405								410	415								
Ser	Arg	Trp	Gln	Gln	Gly	Asn	Val	Phe	Ser	Cys	Ser	Val	Met	His	Glu		
420								425	430								
Ala	Leu	His	Asn	His	Tyr	Thr	Gln	Lys	Ser	Leu	Ser	Leu	Ser	Pro	Gly		
435								440	445								
Gly	Gly	Gly	Gly	Ser	Gly	Gly	Gly	Gly	Ser	Gly	Gly	Gly	Gly	Ser	Gly		
450								455	460								
Gln	Pro	Arg	Glu	Pro	Gln	Val	Tyr	Thr	Leu	Pro	Pro	Ser	Arg	Glu	Glu		
465	470								475							480	
Met	Thr	Lys	Asn	Gln	Val	Ser	Leu	Thr	Cys	Leu	Val	Lys	Gly	Phe	Tyr		
485								490	495								

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Pro Ser Asp Ile Ala Val Glu Trp Glu Ser Asn Gly Gln Pro Glu Asn
      500                      505                      510
Asn Tyr Lys Thr Thr Pro Pro Val Leu Asp Ser Asp Gly Ser Phe Phe
      515                      520                      525
Leu Tyr Ser Lys Leu Thr Val Asp Lys Ser Arg Trp Gln Gln Gly Asn
      530                      535                      540
Val Phe Ser Cys Ser Val Met His Glu Ala Leu His Asn His Tyr Thr
      545                      550                      555                      560
Gln Lys Ser Leu Ser Leu Ser Pro Gly Ser Thr Gly Ser Gln Val Gln
      565                      570                      575
Leu Gln Gln Pro Gly Ala Glu Leu Val Lys Pro Gly Ala Ser Val Lys
      580                      585                      590
Met Ser Cys Lys Ala Ser Gly Tyr Thr Phe Thr Ser Tyr Asn Met His
      595                      600                      605
Trp Val Lys Gln Thr Pro Gly Arg Gly Leu Glu Trp Ile Gly Ala Ile
      610                      615                      620
Tyr Pro Gly Asn Gly Asp Thr Ser Tyr Asn Gln Lys Phe Lys Gly Lys
      625                      630                      635                      640
Ala Thr Leu Thr Ala Asp Lys Ser Ser Ser Thr Ala Tyr Met Gln Leu
      645                      650                      655
Ser Ser Leu Thr Ser Glu Asp Ser Ala Val Tyr Tyr Cys Ala Arg Ser
      660                      665                      670
Thr Tyr Tyr Gly Gly Asp Trp Tyr Phe Asn Val Trp Gly Ala Gly Thr
      675                      680                      685
Thr Val Thr Val Ser Ala Arg Thr Val Ala Ala Pro Ser Val Phe Ile
      690                      695                      700
Phe Pro Pro Ser Asp Glu Gln Leu Lys Ser Gly Thr Ala Ser Val Val
      705                      710                      715                      720
Cys Leu Leu Asn Asn Phe Tyr Pro Arg Glu Ala Lys Val Gln Trp Lys
      725                      730                      735
Val Asp Asn Ala Leu Gln Ser Gly Asn Ser Gln Glu Ser Val Thr Glu
      740                      745                      750
Gln Asp Ser Lys Asp Ser Thr Tyr Ser Leu Ser Ser Thr Leu Thr Leu
      755                      760                      765
Ser Lys Ala Asp Tyr Glu Lys His Lys Val Tyr Ala Cys Glu Val Thr
      770                      775                      780
His Gln Gly Leu Ser Ser Pro Val Thr Lys Ser Phe Asn Arg Gly Glu
      785                      790                      795                      800
Cys

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<210> SEQ ID NO 195
<211> LENGTH: 219
<212> TYPE: PRT
<213> ORGANISM: Artificial
<220> FEATURE:
<223> OTHER INFORMATION: Human-mouse chimeric

<400> SEQUENCE: 195

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Asp Ile Val Met Thr Gln Thr Pro Leu Ser Leu Pro Val Thr Pro Gly
1           5           10           15
Glu Pro Ala Ser Ile Ser Cys Arg Ser Ser Lys Ser Leu Leu His Ser
20           25           30

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Asn Gly Ile Thr Tyr Leu Tyr Trp Tyr Leu Gln Lys Pro Gly Gln Ser
   35                               40                               45

Pro Gln Leu Leu Ile Tyr Gln Met Ser Asn Leu Val Ser Gly Val Pro
   50                               55                               60

Asp Arg Phe Ser Gly Ser Gly Ser Gly Thr Asp Phe Thr Leu Lys Ile
   65                               70                               75                               80

Ser Arg Val Glu Ala Glu Asp Val Gly Val Tyr Tyr Cys Ala Gln Asn
           85                               90                               95

Leu Glu Leu Pro Tyr Thr Phe Gly Gly Gly Thr Lys Val Glu Ile Lys
           100                               105                               110

Arg Thr Val Ala Ala Pro Ser Val Phe Ile Phe Pro Pro Ser Asp Glu
           115                               120                               125

Gln Leu Lys Ser Gly Thr Ala Ser Val Val Cys Leu Leu Asn Asn Phe
           130                               135                               140

Tyr Pro Arg Glu Ala Lys Val Gln Trp Lys Val Asp Asn Ala Leu Gln
           145                               150                               155                               160

Ser Gly Asn Ser Gln Glu Ser Val Thr Glu Gln Asp Ser Lys Asp Ser
           165                               170                               175

Thr Tyr Ser Leu Ser Ser Thr Leu Thr Leu Ser Lys Ala Asp Tyr Glu
           180                               185                               190

Lys His Lys Val Tyr Ala Cys Glu Val Thr His Gln Gly Leu Ser Ser
           195                               200                               205

Pro Val Thr Lys Ser Phe Asn Arg Gly Glu Cys
           210                               215

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<210> SEQ ID NO 196
<211> LENGTH: 459
<212> TYPE: PRT
<213> ORGANISM: Artificial
<220> FEATURE:
<223> OTHER INFORMATION: Human - mouse chimeric

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<400> SEQUENCE: 196

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```

Gln Ile Val Leu Ser Gln Ser Pro Ala Ile Leu Ser Ala Ser Pro Gly
1           5           10           15

Glu Lys Val Thr Met Thr Cys Arg Ala Ser Ser Ser Val Ser Tyr Ile
           20           25           30

His Trp Phe Gln Gln Lys Pro Gly Ser Ser Pro Lys Pro Trp Ile Tyr
           35           40           45

Ala Thr Ser Asn Leu Ala Ser Gly Val Pro Val Arg Phe Ser Gly Ser
           50           55           60

Gly Ser Gly Thr Ser Tyr Ser Leu Thr Ile Ser Arg Val Glu Ala Glu
           65           70           75           80

Asp Ala Ala Thr Tyr Tyr Cys Gln Gln Trp Thr Ser Asn Pro Pro Thr
           85           90           95

Phe Gly Gly Gly Thr Lys Leu Glu Ile Lys Ala Ser Thr Lys Gly Pro
           100          105          110

Ser Val Phe Pro Leu Ala Pro Ser Ser Lys Ser Thr Ser Gly Gly Thr
           115          120          125

Ala Ala Leu Gly Cys Leu Val Lys Asp Tyr Phe Pro Glu Pro Val Thr
           130          135          140

Val Ser Trp Asn Ser Gly Ala Leu Thr Ser Gly Val His Thr Phe Pro
           145          150          155          160

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Ala	Val	Leu	Gln	Ser	Ser	Gly	Leu	Tyr	Ser	Leu	Ser	Ser	Val	Val	Thr	
				165					170						175	
Val	Pro	Ser	Ser	Ser	Leu	Gly	Thr	Gln	Thr	Tyr	Ile	Cys	Asn	Val	Asn	
			180					185					190			
His	Lys	Pro	Ser	Asn	Thr	Lys	Val	Asp	Lys	Arg	Val	Glu	Pro	Lys	Ser	
		195					200					205				
Cys	Asp	Lys	Thr	His	Gly	Gly	Ser	Ser	Ser	Glu	Val	Gln	Leu	Gln	Gln	
	210					215					220					
Ser	Gly	Pro	Glu	Leu	Val	Lys	Pro	Gly	Ala	Ser	Val	Lys	Ile	Ser	Cys	
	225				230					235					240	
Lys	Thr	Ser	Gly	Tyr	Thr	Phe	Thr	Glu	Tyr	Thr	Met	His	Trp	Val	Lys	
			245						250					255		
Gln	Ser	His	Gly	Lys	Ser	Leu	Glu	Trp	Ile	Gly	Gly	Ile	Ser	Pro	Asn	
			260					265					270			
Ile	Gly	Gly	Thr	Ser	Tyr	Asn	Gln	Lys	Phe	Lys	Gly	Lys	Ala	Thr	Leu	
	275						280					285				
Thr	Val	Asp	Lys	Ser	Ser	Ser	Thr	Ala	Tyr	Met	Glu	Leu	Arg	Ser	Leu	
	290					295					300					
Thr	Ser	Glu	Asp	Ser	Ala	Val	Tyr	Tyr	Cys	Ala	Arg	Arg	Gly	Gly	Ser	
	305				310					315					320	
Phe	Asp	Tyr	Trp	Gly	Gln	Gly	Thr	Thr	Leu	Thr	Val	Ser	Ser	Val	Glu	
			325						330					335		
Gly	Gly	Ser	Gly	Gly	Ser	Gly	Gly	Ser	Gly	Gly	Ser	Gly	Gly	Val	Asp	
		340						345					350			
Asp	Ile	Val	Met	Thr	Gln	Ser	Pro	Ala	Thr	Leu	Ser	Val	Thr	Pro	Gly	
	355						360					365				
Asp	Arg	Val	Ser	Leu	Ser	Cys	Arg	Ala	Ser	Gln	Ser	Ile	Ser	Asp	Tyr	
	370					375					380					
Leu	His	Trp	Tyr	Gln	Gln	Lys	Ser	His	Glu	Ser	Pro	Arg	Leu	Leu	Ile	
	385				390					395					400	
Lys	Tyr	Ala	Ser	Gln	Ser	Ile	Ser	Gly	Ile	Pro	Ser	Arg	Phe	Ser	Gly	
			405						410					415		
Ser	Gly	Ser	Gly	Ser	Asp	Phe	Thr	Leu	Ser	Ile	Asn	Ser	Val	Glu	Pro	
		420					425						430			
Glu	Asp	Val	Gly	Val	Tyr	Tyr	Cys	Gln	Asn	Gly	His	Ser	Phe	Pro	Leu	
		435					440					445				
Thr	Phe	Gly	Ala	Gly	Thr	Lys	Leu	Glu	Leu	Lys						
	450					455										

<210> SEQ ID NO 197

<211> LENGTH: 928

<212> TYPE: PRT

<213> ORGANISM: Artificial

<220> FEATURE:

<223> OTHER INFORMATION: Human-mouse chimeric

<400> SEQUENCE: 197

Asp	Ile	Val	Met	Thr	Gln	Thr	Pro	Leu	Ser	Leu	Pro	Val	Thr	Pro	Gly	
1				5					10					15		
Glu	Pro	Ala	Ser	Ile	Ser	Cys	Arg	Ser	Ser	Lys	Ser	Leu	Leu	His	Ser	
		20					25					30				
Asn	Gly	Ile	Thr	Tyr	Leu	Tyr	Trp	Tyr	Leu	Gln	Lys	Pro	Gly	Gln	Ser	
	35						40					45				

-continued

Pro	Gln	Leu	Leu	Ile	Tyr	Gln	Met	Ser	Asn	Leu	Val	Ser	Gly	Val	Pro
50						55					60				
Asp	Arg	Phe	Ser	Gly	Ser	Gly	Ser	Gly	Thr	Asp	Phe	Thr	Leu	Lys	Ile
65					70					75					80
Ser	Arg	Val	Glu	Ala	Glu	Asp	Val	Gly	Val	Tyr	Tyr	Cys	Ala	Gln	Asn
			85						90					95	
Leu	Glu	Leu	Pro	Tyr	Thr	Phe	Gly	Gly	Gly	Thr	Lys	Val	Glu	Ile	Lys
			100						105					110	
Gly	Gly	Gly	Gly	Ser	Gly	Gly	Gly	Gly	Ser	Gly	Gly	Gly	Gly	Ser	Gln
			115						120					125	
Val	Gln	Leu	Val	Gln	Ser	Gly	Ala	Glu	Val	Lys	Lys	Pro	Gly	Ser	Ser
			130						135					140	
Val	Lys	Val	Ser	Cys	Lys	Ala	Ser	Gly	Tyr	Ala	Phe	Ser	Tyr	Ser	Trp
			145						150					155	160
Ile	Asn	Trp	Val	Arg	Gln	Ala	Pro	Gly	Gln	Gly	Leu	Glu	Trp	Met	Gly
				165					170					175	
Arg	Ile	Phe	Pro	Gly	Asp	Gly	Asp	Thr	Asp	Tyr	Asn	Gly	Lys	Phe	Lys
			180						185					190	
Gly	Arg	Val	Thr	Ile	Thr	Ala	Asp	Lys	Ser	Thr	Ser	Thr	Ala	Tyr	Met
			195						200					205	
Glu	Leu	Ser	Ser	Leu	Arg	Ser	Glu	Asp	Thr	Ala	Val	Tyr	Tyr	Cys	Ala
			210						215					220	
Arg	Asn	Val	Phe	Asp	Gly	Tyr	Trp	Leu	Val	Tyr	Trp	Gly	Gln	Gly	Thr
			225						230					235	240
Leu	Val	Thr	Val	Ser	Ser	Ala	Ser	Thr	Lys	Gly	Pro	Ser	Val	Phe	Pro
				245					250					255	
Leu	Ala	Pro	Ser	Ser	Lys	Ser	Thr	Ser	Gly	Gly	Thr	Ala	Ala	Leu	Gly
			260						265					270	
Cys	Leu	Val	Lys	Asp	Tyr	Phe	Pro	Glu	Pro	Val	Thr	Val	Ser	Trp	Asn
			275						280					285	
Ser	Gly	Ala	Leu	Thr	Ser	Gly	Val	His	Thr	Phe	Pro	Ala	Val	Leu	Gln
			290						295					300	
Ser	Ser	Gly	Leu	Tyr	Ser	Leu	Ser	Ser	Val	Val	Thr	Val	Pro	Ser	Ser
			305						310					315	320
Ser	Leu	Gly	Thr	Gln	Thr	Tyr	Ile	Cys	Asn	Val	Asn	His	Lys	Pro	Ser
				325					330					335	
Asn	Thr	Lys	Val	Asp	Lys	Arg	Val	Glu	Pro	Lys	Ser	Cys	Asp	Lys	Thr
			340						345					350	
His	Thr	Ser	Pro	Pro	Ser	Pro	Ala	Pro	Glu	Leu	Leu	Gly	Gly	Pro	Ser
			355						360					365	
Val	Phe	Leu	Phe	Pro	Pro	Lys	Pro	Lys	Asp	Thr	Leu	Met	Ile	Ser	Arg
			370						375					380	
Thr	Pro	Glu	Val	Thr	Cys	Val	Val	Val	Asp	Val	Ser	His	Glu	Asp	Pro
				385					390					395	400
Glu	Val	Lys	Phe	Asn	Trp	Tyr	Val	Asp	Gly	Val	Glu	Val	His	Asn	Ala
				405					410					415	
Lys	Thr	Lys	Pro	Arg	Glu	Glu	Gln	Tyr	Ser	Ser	Thr	Tyr	Arg	Val	Val
			420						425					430	
Ser	Val	Leu	Thr	Val	Leu	His	Gln	Asp	Trp	Leu	Asn	Gly	Lys	Glu	Tyr
			435						440					445	
Lys	Cys	Lys	Val	Ser	Asn	Lys	Ala	Leu	Pro	Ala	Pro	Ile	Glu	Lys	Thr

-continued

450	455	460
Ile Ser Lys Ala Lys Gly Gln Pro Arg Glu Pro Gln Val Tyr Thr Leu		
465	470	475 480
Pro Pro Ser Arg Glu Glu Met Thr Lys Asn Gln Val Ser Leu Thr Cys		
	485	490 495
Leu Val Lys Gly Phe Tyr Pro Ser Asp Ile Ala Val Glu Trp Glu Ser		
	500	505 510
Asn Gly Gln Pro Glu Asn Asn Tyr Lys Thr Thr Pro Pro Val Leu Asp		
	515	520 525
Ser Asp Gly Ser Phe Phe Leu Tyr Ser Lys Leu Thr Val Asp Lys Ser		
	530	535 540
Arg Trp Gln Gln Gly Asn Val Phe Ser Cys Ser Val Met His Glu Ala		
545	550	555 560
Leu His Asn His Tyr Thr Gln Lys Ser Leu Ser Leu Ser Pro Gly Gly		
	565	570 575
Gly Gly Gly Ser Gly Gly Gly Gly Ser Gly Gly Gly Ser Gly Gln		
	580	585 590
Pro Arg Glu Pro Gln Val Tyr Thr Leu Pro Pro Ser Arg Glu Glu Met		
	595	600 605
Thr Lys Asn Gln Val Ser Leu Thr Cys Leu Val Lys Gly Phe Tyr Pro		
	610	615 620
Ser Asp Ile Ala Val Glu Trp Glu Ser Asn Gly Gln Pro Glu Asn Asn		
625	630	635 640
Tyr Lys Thr Thr Pro Pro Val Leu Asp Ser Asp Gly Ser Phe Phe Leu		
	645	650 655
Tyr Ser Lys Leu Thr Val Asp Lys Ser Arg Trp Gln Gln Gly Asn Val		
	660	665 670
Phe Ser Cys Ser Val Met His Glu Ala Leu His Asn His Tyr Thr Gln		
	675	680 685
Lys Ser Leu Ser Leu Ser Pro Gly Ser Thr Gly Ser Gln Val Gln Leu		
	690	695 700
Gln Gln Pro Gly Ala Glu Leu Val Lys Pro Gly Ala Ser Val Lys Met		
705	710	715 720
Ser Cys Lys Ala Ser Gly Tyr Thr Phe Thr Ser Tyr Asn Met His Trp		
	725	730 735
Val Lys Gln Thr Pro Gly Arg Gly Leu Glu Trp Ile Gly Ala Ile Tyr		
	740	745 750
Pro Gly Asn Gly Asp Thr Ser Tyr Asn Gln Lys Phe Lys Gly Lys Ala		
	755	760 765
Thr Leu Thr Ala Asp Lys Ser Ser Ser Thr Ala Tyr Met Gln Leu Ser		
	770	775 780
Ser Leu Thr Ser Glu Asp Ser Ala Val Tyr Tyr Cys Ala Arg Ser Thr		
785	790	795 800
Tyr Tyr Gly Gly Asp Trp Tyr Phe Asn Val Trp Gly Ala Gly Thr Thr		
	805	810 815
Val Thr Val Ser Ala Arg Thr Val Ala Ala Pro Ser Val Phe Ile Phe		
	820	825 830
Pro Pro Ser Asp Glu Gln Leu Lys Ser Gly Thr Ala Ser Val Val Cys		
	835	840 845
Leu Leu Asn Asn Phe Tyr Pro Arg Glu Ala Lys Val Gln Trp Lys Val		
	850	855 860

-continued

Asp Asn Ala Leu Gln Ser Gly Asn Ser Gln Glu Ser Val Thr Glu Gln
865 870 875 880

Asp Ser Lys Asp Ser Thr Tyr Ser Leu Ser Ser Thr Leu Thr Leu Ser
885 890 895

Lys Ala Asp Tyr Glu Lys His Lys Val Tyr Ala Cys Glu Val Thr His
900 905 910

Gln Gly Leu Ser Ser Pro Val Thr Lys Ser Phe Asn Arg Gly Glu Cys
915 920 925

<210> SEQ ID NO 198

<211> LENGTH: 459

<212> TYPE: PRT

<213> ORGANISM: Artificial

<220> FEATURE:

<223> OTHER INFORMATION: Human - mouse chimeric

<400> SEQUENCE: 198

Gln Ile Val Leu Ser Gln Ser Pro Ala Ile Leu Ser Ala Ser Pro Gly
1 5 10 15

Glu Lys Val Thr Met Thr Cys Arg Ala Ser Ser Ser Val Ser Tyr Ile
20 25 30

His Trp Phe Gln Gln Lys Pro Gly Ser Ser Pro Lys Pro Trp Ile Tyr
35 40 45

Ala Thr Ser Asn Leu Ala Ser Gly Val Pro Val Arg Phe Ser Gly Ser
50 55 60

Gly Ser Gly Thr Ser Tyr Ser Leu Thr Ile Ser Arg Val Glu Ala Glu
65 70 75 80

Asp Ala Ala Thr Tyr Tyr Cys Gln Gln Trp Thr Ser Asn Pro Pro Thr
85 90 95

Phe Gly Gly Gly Thr Lys Leu Glu Ile Lys Ala Ser Thr Lys Gly Pro
100 105 110

Ser Val Phe Pro Leu Ala Pro Ser Lys Ser Thr Ser Gly Gly Thr
115 120 125

Ala Ala Leu Gly Cys Leu Val Lys Asp Tyr Phe Pro Glu Pro Val Thr
130 135 140

Val Ser Trp Asn Ser Gly Ala Leu Thr Ser Gly Val His Thr Phe Pro
145 150 155 160

Ala Val Leu Gln Ser Ser Gly Leu Tyr Ser Leu Ser Ser Val Val Thr
165 170 175

Val Pro Ser Ser Ser Leu Gly Thr Gln Thr Tyr Ile Cys Asn Val Asn
180 185 190

His Lys Pro Ser Asn Thr Lys Val Asp Lys Arg Val Glu Pro Lys Ser
195 200 205

Cys Asp Lys Thr His Gly Gly Ser Ser Ser Glu Val Gln Leu Gln Gln
210 215 220

Ser Gly Pro Glu Leu Val Lys Pro Gly Ala Ser Val Lys Ile Ser Cys
225 230 235 240

Lys Thr Ser Gly Tyr Thr Phe Thr Glu Tyr Thr Met His Trp Val Lys
245 250 255

Gln Ser His Gly Lys Ser Leu Glu Trp Ile Gly Gly Ile Ser Pro Asn
260 265 270

Ile Gly Gly Thr Ser Tyr Asn Gln Lys Phe Lys Gly Lys Ala Thr Leu
275 280 285

-continued

Thr	Val	Asp	Lys	Ser	Ser	Ser	Thr	Ala	Tyr	Met	Glu	Leu	Arg	Ser	Leu
	290					295					300				
Thr	Ser	Glu	Asp	Ser	Ala	Val	Tyr	Tyr	Cys	Ala	Arg	Arg	Gly	Gly	Ser
305					310					315					320
Phe	Asp	Tyr	Trp	Gly	Gln	Gly	Thr	Thr	Leu	Thr	Val	Ser	Ser	Val	Glu
				325					330					335	
Gly	Gly	Ser	Gly	Gly	Ser	Gly	Gly	Ser	Gly	Gly	Ser	Gly	Gly	Val	Asp
		340					345						350		
Asp	Ile	Val	Met	Thr	Gln	Ser	Pro	Ala	Thr	Leu	Ser	Val	Thr	Pro	Gly
		355					360					365			
Asp	Arg	Val	Ser	Leu	Ser	Cys	Arg	Ala	Ser	Gln	Ser	Ile	Ser	Asp	Tyr
	370					375					380				
Leu	His	Trp	Tyr	Gln	Gln	Lys	Ser	His	Glu	Ser	Pro	Arg	Leu	Leu	Ile
385					390					395					400
Lys	Tyr	Ala	Ser	Gln	Ser	Ile	Ser	Gly	Ile	Pro	Ser	Arg	Phe	Ser	Gly
				405					410					415	
Ser	Gly	Ser	Gly	Ser	Asp	Phe	Thr	Leu	Ser	Ile	Asn	Ser	Val	Glu	Pro
			420					425					430		
Glu	Asp	Val	Gly	Val	Tyr	Tyr	Cys	Gln	Asn	Gly	His	Ser	Phe	Pro	Leu
		435					440					445			
Thr	Phe	Gly	Ala	Gly	Thr	Lys	Leu	Glu	Leu	Lys					
	450					455									

1-78. (canceled)

79. A multispecific antigen binding protein which promotes the specific lysis of target cells expressing an antigen of interest, which antigen is specifically bound by a therapeutic antibody or therapeutic antigen-binding antibody fragment, wherein said multispecific antigen binding protein comprises:

- (i) a first antigen binding domain (ABD) which monovalently binds to a human NKp46 polypeptide having the amino acid sequence set forth in SEQ ID NO:1,
- (ii) a second ABD which comprises a therapeutic antibody or a therapeutic antigen-binding antibody fragment which binds to said antigen of interest expressed by said target cells, and
- (iii) a CD16A binding polypeptide,

wherein:

- (1) said NKp46-binding ABD comprises a Fab or comprises a variable heavy (V_H) domain and a variable light (V_L) domain separated by a linker ("scFv");
- (2) said antigen-binding ABD which comprises said therapeutic antibody or therapeutic antigen-binding antibody fragment is monovalent or bivalent;
- (3) said CD16A binding polypeptide comprises a human Fc domain polypeptide which binds CD16A;
- (4) said multispecific antigen binding protein binds to the NKp46 polypeptide monovalently;
- (5) said multispecific antigen binding protein when administered to a subject directs NKp46-expressing natural killer (NK) cells and CD16A-expressing NK cells to lyse target cells expressing the antigen of interest by a combination of NKp46-mediated signaling and CD16A-mediated antibody-dependent cell-mediated cytotoxicity ("ADCC");

(6) said dimeric Fc domain interposes said first ABD and said second ABD; and

(7) said first and second ABD are each connected to said dimeric Fc domain, and one or both of said first and second ABD are connected to the dimeric Fc domain via a flexible polypeptide linker.

80. The multispecific antigen binding protein of claim **79**, wherein the therapeutic antibody specifically binds to an antigen expressed by cancer cells.

81. The multispecific antigen binding protein of claim **79**, wherein the therapeutic antibody specifically binds to an antigen expressed by an infectious agent.

82. The multispecific antigen binding protein of claim **81**, wherein said infectious agent is a virus, parasite, bacterium or another microbe.

83. The multispecific antigen binding protein of claim **79**, wherein the therapeutic antigen-binding ABD comprises a Fab or comprises a V_H domain and a V_L domain separated by a linker comprising a linear or cyclic peptide.

84. The multispecific antigen binding protein of claim **79**, wherein the NKp46-binding ABD comprises a Fab.

85. The multispecific antigen binding protein of claim **79**, wherein the NKp46-binding ABD comprises a V_H domain and a V_L domain separated by a linker comprising a linear or cyclic peptide.

86. The multispecific antigen binding protein of claim **79**, wherein said dimeric Fc polypeptide of (3) comprises a modification that enhances CD16A binding relative to the corresponding wild-type Fc region.

87. The multispecific antigen binding protein of claim **79**, wherein the administration of said multispecific antigen binding protein to a subject increases the expression of CD137 on the surface of NK cells in said subject.

88. The multispecific antigen binding protein of claim **79**, wherein the NKp46-binding ABD is comprised of a V_H domain and a V_L domain, wherein each of the V_H and V_L domains are positioned within a tandem variable region comprising a V_H domain and a V_L domain separated by a polypeptide linker.

89. The multispecific antigen binding protein of claim **79**, wherein the NKp46-binding ABD is a Fab comprised of a V_H domain and a V_L domain, wherein each of the V_H and V_L domains is fused to a human C_{H1} or C_K constant domain.

90. The multispecific antigen binding protein of claim **79**, wherein the NKp46-binding ABD is a Fab comprised of (a) a V_H domain fused to a human C_{H1} constant domain and a V_L domain fused to a human C_K constant domain, or (b) a V_H domain fused to a human C_K constant domain and a V_L domain fused to a human C_{H1} constant domain.

91. The multispecific antigen binding protein of claim **79**, wherein the therapeutic antigen-binding ABD is a Fab comprised of a V_H domain and a V_L domain, wherein each of the V_H and V_L domains is fused to a human C_{H1} or C_K constant domain.

92. The multispecific antigen binding protein of claim **90**, wherein the therapeutic antigen-binding ABD is a Fab comprised of (a) a V_H domain fused to a human C_{H1} constant domain and a V_L domain fused to a human C_K constant domain, or (b) a V_H domain fused to a human C_K constant domain and a V_L domain fused to a human C_{H1} constant domain.

93. The multispecific antigen binding protein of claim **79**, wherein either or both the NKp46-binding ABD or the therapeutic antigen-binding ABD is bound to the Fc domain by a flexible polypeptide linker.

94. The multispecific antigen binding protein of claim **79**, wherein the monovalent NKp46 ABD comprises V_H and V_L domain polypeptides selected from the group consisting of:

- (a) the V_H and V_L domains of SEQ ID NOS: 3 and 4 (NKp46-1);
- (b) the V_H and V_L domains of SEQ ID NOS: 5 and 6 (NKp46-2);
- (c) the V_H and V_L domains of SEQ ID NOS: 7 and 8 (NKp46-3);
- (d) the V_H and V_L domains of SEQ ID NOS: 9 and 10 (NKp46-4);
- (e) the V_H and V_L domains of SEQ ID NOS: 11 and 12 (NKp46-6); and
- (f) the V_H and V_L domains of SEQ ID NOS: 13 and 14 (NKp46-9).

95. The multispecific antigen binding protein of claim **79**, wherein said flexible polypeptide linker is 2-50 amino acids in length.

96. The multispecific antigen binding protein of claim **79**, wherein one of the ABDs is connected to the Fc region via a peptide linker which comprises a hinge domain.

97. The multispecific antigen binding protein of claim **91**, wherein the C_K constant domain and the human C_{H1} constant domain are each connected to the Fc domain via a hinge domain.

98. The method of claim **92**, wherein the C_K constant domain and the human C_{H1} constant domain are each connected to the Fc domain via a hinge domain.

99. The multispecific antigen binding protein of claim **79**, wherein said combination of NKp46-mediated signaling and

CD16A-mediated ADCC has an additive or synergistic effect on the lysis of target cells expressing the antigen of interest.

100. The multispecific antigen binding protein of claim **80**, wherein the therapeutic antigen-binding ABD binds to an antigen expressed by hematological cancer cells.

101. The multispecific antigen binding protein of claim **100**, wherein the antigen expressed by said hematological cancer cells comprises CD19 or CD20.

102. The multispecific antigen binding protein of claim **80**, wherein the therapeutic antigen-binding ABD binds to an antigen expressed by solid tumor cancer cells.

103. A pharmaceutical composition comprising a pharmaceutically effective amount of the multispecific antigen binding protein of claim **79**.

104. A nucleic acid or nucleic acids which separately or in combination encodes for the expression of the multispecific antigen binding protein of claim **80**.

105. A vector which comprises nucleic acid or nucleic acids according to claim **104**.

106. A recombinant or isolated cell which comprises nucleic acid or nucleic acids according to claim **104** or a vector comprising said nucleic acid or nucleic acids.

107. A method of producing a multispecific antigen binding protein according to claim **79** comprising culturing a cell according which comprises a nucleic acid or nucleic acids which separately or in combination encodes for the expression of said multispecific antigen binding protein wherein culturing is under conditions whereby said multispecific antigen binding protein is expressed by the cell.

108. A method of promoting the specific lysis of target cells expressing an antigen of interest which is specifically bound by a therapeutic antibody or therapeutic antigen-binding antibody fragment in a subject in need thereof, comprising contacting said target cells with an amount of a multispecific antigen binding protein which is sufficient to promote the specific lysis of said target cells expressing said antigen of interest, wherein said multispecific antigen binding protein comprises:

- (i) a first antigen binding domain (ABD) which monovalently binds to a human NKp46 polypeptide having the amino acid sequence set forth in SEQ ID NO:1,
- (ii) a second ABD which comprises a therapeutic antibody or a therapeutic antigen-binding antibody fragment which binds to said antigen of interest expressed by target cells, and
- (iii) a CD16A binding polypeptide,

wherein:

- (1) said NKp46-binding ABD comprises a Fab or comprises a V_H chain domain and a V_L chain domain separated by a linker ("scFv");
- (2) said cancer-antigen-binding ABD, which comprises said therapeutic antibody or therapeutic antigen-binding antibody fragment, is monovalent or bivalent;
- (3) said CD16A binding polypeptide comprises a dimeric human Fc domain polypeptide which binds CD16A;
- (4) said multispecific antigen binding protein binds to the NKp46 polypeptide monovalently;
- (5) said multispecific antigen binding protein directs NKp46-expressing NK cells and CD16A-expressing NK cells to lyse target cells expressing the antigen of interest by a combination of NKp46-mediated signaling and CD16A-mediated ADCC;

(6) said dimeric Fc domain interposes said first ABD and said second ABD;

(7) said first and second ABD are each attached to said dimeric Fc domain, and wherein one or both of said first and second ABD are connected to the Fc domain via a flexible polypeptide linker.

109. The method of claim **108**, wherein said combination of NKp46-mediated signaling and CD16A-mediated ADCC has an additive or synergistic effect on the lysis of target cells expressing the antigen of interest.

110. The method of claim **108**, wherein said antigen of interest is a cancer antigen.

111. The method of claim **108**, wherein said antigen of interest is expressed by an infectious agent.

* * * * *