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(54) Title: NOVEL IMMUNOCONJUGATES

(57) Abstract: The present invention generally relates to antigen-specific immunoconjugates for selectively delivering effector moieties that influence cellular activity. More specifically, the invention provides novel immunoconjugates comprising a first antigen binding moiety, an Fc domain and a single effector moiety. In addition, the present invention relates to polynucleotides encoding such immunoconjugates, and vectors and host cells comprising such polynucleotides. The invention further relates to methods for producing the immunoconjugates of the invention, and to methods of using these immunoconjugates in the treatment of disease.

NOVEL IMMUNOCONJUGATES

Field of the Invention

The present invention generally relates to antigen-specific immunoconjugates for selectively delivering effector moieties that influence cellular activity. In addition, the present invention relates to polynucleotides encoding such immunoconjugates, and vectors and host cells comprising such polynucleotides. The invention further relates to methods for producing the immunoconjugates of the invention, and to methods of using these immunoconjugates in the treatment of disease.

Background

The selective destruction of an individual cell or a specific cell type is often desirable in a variety of clinical settings. For example, it is a primary goal of cancer therapy to specifically destroy tumor cells, while leaving healthy cells and tissues intact and undamaged. A multitude of signal transduction pathways in the cell are linked to the cell's survival and/or death. Accordingly, the direct delivery of a pathway factor involved in cell survival or death can be used to contribute to the cell's maintenance or destruction. Similarly, specific factors may be delivered that stimulate immune effector cells in a tumor microenvironment, such as natural killer (NK) cells or cytotoxic T lymphocytes (CTLs), to attack and destroy tumor cells.

Cytokines are cell signaling molecules that participate in regulation of the immune system. When used in cancer therapy, cytokines can act as immunomodulatory agents that have anti-tumor effects and which can increase the immunogenicity of some types of tumors. However, rapid blood clearance and lack of tumor specificity require systemic administration of high doses of the cytokine in order to achieve a concentration of the cytokine at the tumor site sufficient to activate an immune response or have an anti-tumor effect. These high levels of systemic cytokine can lead to severe toxicity and adverse reactions.

For use in therapy, it is therefore desirable to specifically deliver a signal transduction pathway factor, such as a cytokine, to a specific site in vivo (e.g. a tumor or tumor microenvironment in the case of cancer therapy). This can be achieved by conjugating the factor to a targeting moiety, e.g. an antibody or an antibody fragment, specific for the site. Early strategies aimed at

delivering signal transduction pathway factors, such as cytokines, to a specific site *in vivo* included immunoglobulin heavy chains conjugated to various cytokines, including lymphotoxin, tumor necrosis factor- α (TNF- α), interleukin-2 (IL-2), and granulocyte macrophage-colony stimulating factor (GM-CSF) (reviewed e.g. in Lode et al., Pharmacol Ther 80, 277-292 (1998)).

5 Researchers observed that, not only were they able to target cytokines to specific sites *in vivo*, they were also able to take advantage of the fact that monoclonal antibodies have longer serum half-lives than most other proteins. Given the systemic toxicity associated with high doses of certain unconjugated cytokines, e.g. IL-2, the ability of an immunoglobulin-cytokine fusion protein to maximize therapeutically beneficial biological activities at a desired site, e.g. in a
10 tumor, whilst keeping systemic side effects to a minimum at a lower dose led researchers to believe that immunoglobulin-cytokine immunoconjugates were optimal therapeutic agents.

Nevertheless, there are certain disadvantages associated with the immunoglobulin-cytokine immunoconjugates known in the art. For example, these immunoconjugates have at least one cytokine coupled to each of the two immunoglobulin heavy chains, resulting in an
15 immunoconjugate with bivalent target binding and two or more cytokine moieties (reviewed e.g. in Chang et al., Expert Opin Drug Discovery 4, 181-194 (2009), or Ortiz-Sanchez et al., Expert Opin Biol Ther 8, 609-632 (2008)). Figure 1 depicts a conventional immunoglobulin-cytokine immunoconjugate as it is known in the art, where a cytokine is fused to the C-terminus of each of the two antibody heavy chains. Due to the presence of two or more cytokine moieties, such an
20 immunoconjugate has a high avidity to the respective cytokine receptor (for example, picomolar affinity in the case of IL-2), and thus is targeted rather to the immune effector cells expressing the cytokine receptor than to the target antigen of the immunoglobulin (nM affinity) to which the cytokine is linked. Moreover, conventional immunoconjugates are known to be associated with infusion reactions (see e.g. King et al., J Clin Oncol 22, 4463-4473 (2004)), resulting at least
25 partially from activation of cytokine receptors on immune effector cells in peripheral blood by the immunoconjugate's cytokine moieties.

Additionally, via their Fc domain, immunoglobulin-cytokine immunoconjugates can activate complement and interact with Fc receptors. This inherent immunoglobulin feature has been viewed unfavorably because therapeutic immunoconjugates may be targeted to cells expressing
30 Fc receptors rather than the preferred antigen-bearing cells. Moreover, the simultaneous activation of cytokine receptors and Fc receptor signaling pathways leading to cytokine release, especially in combination with the long half-life of immunoglobulin fusion proteins, make their application in a therapeutic setting difficult due to systemic toxicity.

One approach to overcoming this problem is the use of immunoglobulin fragments devoid of an Fc domain, such as scFv or Fab fragments, in immunoconjugates. Examples of immunoglobulin fragment-cytokine immunoconjugates include the scFv-IL-2 immunoconjugate as set forth in PCT publication WO 2001/062298, the scFv-IL-12-scFv immunoconjugate as set forth in PCT publication WO 2006/119897 (wherein each of the two scFv fragments is connected to a subunit of the IL-12 heterodimer that is held together by disulfide bond(s)) or the Fab-IL-2-Fab immunoconjugates as set forth in PCT publication WO 2011/020783. Both the tumor-binding reactivity of the immunoglobulin parent molecule and the functional activity of the cytokine are maintained in most of these types of immunoconjugates, however the half-life of such constructs is considerably shorter than of immunoglobulin fusion proteins.

Therefore there remains a need for immunoconjugates with improved properties, for greater therapeutic effectiveness and a reduction in the number and severity of the side effects of these products (*e.g.*, toxicity, destruction of non-tumor cells, *etc.*).

The present invention provides immunoglobulin-like immunoconjugates that exhibit improved efficacy, high specificity of action, reduced toxicity, and improved half-life and stability in blood relative to known immunoconjugates.

It is to be understood that if any prior art publication is referred to herein, such reference does not constitute an admission that the publication forms a part of the common general knowledge in the art in Australia or any other country.

Summary of the Invention

The present invention is based, in part, on the inventors' recognition that immunoconjugates comprising more than one effector moiety, such as *e.g.* a cytokine, may be targeted to the respective effector moiety receptor rather than the target antigen of the antigen binding moiety of the immunoconjugate.

Therefore, a first aspect provides an immunoconjugate comprising (i) an immunoglobulin molecule comprising a first and a second antigen binding Fab molecule and an Fc domain consisting of two subunits, and (ii) an effector moiety, wherein not more than one effector moiety is present; and wherein said first and said second Fab molecule are directed to CEA and comprise a heavy chain variable region sequence of SEQ ID NO: 191, and a light chain variable region sequence of SEQ ID NO: 189; and said effector moiety is a mutant human

interleukin-2 (IL-2) polypeptide comprising the amino acid substitutions F42A, Y45A, L72G (number relative to the human IL-2 sequence in SEQ ID NO: 2).

A second aspect provides an isolated polynucleotide encoding the immunoconjugate of the first aspect.

- 5 A third aspect provides an expression vector comprising the isolated polynucleotide of the second aspect.

A fourth aspect provides a host cell comprising the isolated polynucleotide of the second aspect or the expression vector of the third aspect.

- 10 A fifth aspect provides a method of producing an immunoconjugate comprising (i) an immunoglobulin molecule comprising a first and a second antigen binding Fab molecule and an Fc domain consisting of two subunits, and (ii) an effector moiety, wherein not more than one effector moiety is present, comprising culturing the host cell of the fourth aspect under conditions suitable for expression of the immunoconjugate and optionally recovering the immunoconjugate.

- 15 A sixth aspect provides an immunoconjugate when produced by the method of the fifth aspect.

A seventh aspect provides a pharmaceutical composition comprising the immunoconjugate of the first or sixth aspect and a pharmaceutically acceptable carrier.

- 20 An eighth aspect provides use of the immunoconjugate of first or sixth aspect, or the pharmaceutical composition of the seventh aspect, in the manufacture of a medicament for treating a cancer expressing CEA in an individual in need thereof.

- 25 A ninth aspect provides a method of treating a cancer expressing CEA in an individual, comprising administering to said individual a therapeutically effective amount of a composition comprising the immunoconjugate of first or sixth aspect in a pharmaceutically acceptable form.

Also disclosed is an immunoconjugate comprising a first antigen binding moiety, an Fc domain consisting of two subunits, and an effector moiety, wherein not more than one effector moiety is present.

- 30 In one embodiment the effector moiety is fused to the amino- or carboxy-terminal amino acid of one of the two subunits of the Fc domain, optionally through a linker peptide. In one embodiment the first antigen binding moiety is fused to the amino-terminal amino acid of one of the two subunits of the Fc domain, optionally through a linker peptide or an immunoglobulin hinge region.

-3b-

In one embodiment the first antigen binding moiety comprises an antigen binding domain of an antibody. In a particular embodiment the first antigen binding moiety is a Fab molecule. In certain embodiments the Fc domain comprises a modification promoting heterodimerization of two non-identical polypeptide chains. In a specific embodiment said modification is a knob-into-hole modification, comprising a knob modification in one of the subunits of the Fc domain and a

5

hole modification in the other one of the two subunits of the Fc domain. In a particular embodiment the effector moiety is fused to the amino- or carboxy-terminal amino acid of the subunit of the Fc domain comprising the knob modification.

In one embodiment the Fc domain is an IgG Fc domain, particularly an IgG₁ Fc domain. In a particular embodiment the Fc domain is human.

In certain embodiments of the invention the Fc domain is engineered to have altered binding to an Fc receptor, specifically altered binding to an Fcγ receptor, and/or altered effector function, specifically altered antibody-dependent cell-mediated cytotoxicity (ADCC).

Although the presence of an Fc domain is essential for prolonging the half-life of the immunoconjugate, the inventors realize that in some situations it will be beneficial to eliminate effector functions associated with engagement of Fc receptors by the Fc domain. Hence, in particular embodiments the altered binding to an Fc receptor and/or effector function is reduced binding and/or effector function. In a specific such embodiment the Fc domain comprises one or more amino acid mutation that reduces the binding of the Fc domain to an Fc receptor, particularly an Fcγ receptor. Preferably, such an amino acid mutation does not reduce binding to FcRn receptors. In one embodiment the Fc domain comprises an amino acid substitution at position P329. In a particular embodiment the Fc domain comprises the amino acid substitutions L234A, L235A and P329G in each of its subunits.

On the other hand, there may be situations where it is desirable to enhance the effector functions of immunoconjugates. Hence, in certain embodiments the Fc domain of the immunoconjugate of the invention is engineered to have altered binding to an Fc receptor, specifically an Fcγ receptor, more specifically an FcγIIIa receptor, and/or altered effector function, wherein the altered binding and/or effector function is increased binding and/or effector function. In one such embodiment the Fc domain is engineered to have an altered oligosaccharide structure, as compared to a non-engineered Fc domain. In a particular such embodiment the Fc domain comprises an increased proportion of non-fucosylated oligosaccharides, as compared to a non-engineered Fc domain. In a more specific embodiment the Fc domain comprises at least 20%, particularly at least 50%, more particularly at least 70% non-fucosylated oligosaccharides. In another specific embodiment the Fc domain comprises an increased proportion of bisected oligosaccharides, as compared to a non-engineered Fc domain. In yet another specific embodiment the Fc domain comprises an increased proportion of bisected, non-fucosylated oligosaccharides, compared to a non-engineered Fc domain. In some embodiments said altered

oligosaccharide structure results from increased β 1,4) -N-acetylglucosaminyltransferase III (GnTIII) activity in a host cell used for expression of the immunoconjugate.

Also disclosed are immunoconjugates that comprise a first and a second antigen binding moiety, an Fc domain consisting of two subunits, and an effector moiety, wherein not more than one effector moiety is present.

In one embodiment the first and the second antigen binding moiety and the Fc domain are part of an immunoglobulin molecule. In certain embodiments the immunoconjugate essentially consists of an immunoglobulin molecule and an effector moiety and optionally one or more linker sequences. In a particular embodiment the immunoglobulin molecule is an IgG class immunoglobulin. In an even more particular embodiment the immunoglobulin is an IgG₁ subclass immunoglobulin. In one embodiment the effector moiety is fused to the carboxy-terminal amino acid of one of the immunoglobulin heavy chains, optionally through a linker peptide.

In a particular embodiment the immunoconjugate of the invention comprises an immunoglobulin molecule comprising two antigen binding moieties and an Fc domain, and an effector moiety fused to the carboxy-terminal amino acid of one of the immunoglobulin heavy chains, wherein not more than one effector moiety is present and wherein the Fc domain is engineered to have reduced binding to an Fc receptor, specifically altered binding to an Fc receptor, and/or reduced effector function.

In certain embodiments said first antigen binding moiety, or said first and said second antigen binding moiety, is directed to an antigen associated with a pathological condition, such as an antigen presented on a tumor cell or in a tumor cell environment, at a site of inflammation, or on a virus-infected cell. In a more specific embodiment said antigen is selected from the group of Fibroblast Activation Protein (FAP), the A1 domain of Tenascin-C (TNC A1), the A2 domain of Tenascin-C (TNC A2), the Extra Domain B of Fibronectin (EDB), Carcinoembryonic Antigen (CEA), and Melanoma-associated Chondroitin Sulfate Proteoglycan (MCSP).

In certain embodiments the effector moiety is a single chain effector moiety. In a particular embodiment the effector moiety is a cytokine. In one embodiment said cytokine is selected from the group of IL-2, IL-7, IL-10, IL-12, IL-15, IFN- α and IFN- γ . In a particular embodiment said cytokine is IL-2. In an even more particular embodiment said cytokine is a mutant IL-2 polypeptide having reduced binding affinity to the α -subunit of the IL-2 receptor. In a specific embodiment said mutant IL-2 polypeptide comprises an amino acid substitution

at one or more positions selected from the positions corresponding to residues 42, 45 and 72 of human IL-2. In another particular embodiment the cytokine is IL-10. In yet another embodiment, the cytokine is IL-15, particularly a mutant IL-15 polypeptide having reduced binding affinity to the α -subunit of the IL-15 receptor. In another embodiment, the cytokine is IFN- α

Also disclosed is an isolated polynucleotide encoding an immunoconjugate of the disclosure or a fragment thereof. Also disclosed is an expression vector comprising the isolated polynucleotide of the disclosure, and a host cell comprising the isolated polynucleotide or the expression vector of the disclosure.

In some embodiments the host cell is a eukaryotic cell, particularly a mammalian cell. In some embodiments, the host cell has been manipulated to express increased levels of one or more polypeptides having β 1,4) -N-acetylglucosaminyltransferase III (GnTIII) activity. In one such embodiment the host cell has been further manipulated to express increased levels of one or more polypeptides having α -mannosidase II (ManII) activity.

Also disclosed is a method of producing the immunoconjugates of the disclosure, comprising the steps of a) culturing the host cell of the disclosure under conditions suitable for the expression of the immunoconjugate and b) recovering the immunoconjugate. Also disclosed is an immunoconjugate produced by the method of the disclosure.

Also disclosed is a pharmaceutical composition comprising an immunoconjugate of the disclosure and a pharmaceutically acceptable carrier.

Also disclosed are methods of using the immunoconjugates and pharmaceutical compositions of the disclosure. Also disclosed is an immunoconjugate or a pharmaceutical composition of the disclosure for use as a medicament. Also disclosed is an immunoconjugate or a pharmaceutical composition according to the disclosure for use in the treatment of a disease in an individual in need thereof. In a specific embodiment the disease is cancer. In other embodiments the disease is an inflammatory disorder. In a particular such embodiment the immunoconjugate comprises an IL-10 effector moiety.

Also disclosed is the use of an immunoconjugate of the disclosure for the manufacture of a medicament for the treatment of a disease in an individual in need thereof; as well as a method of treating a disease in an individual, comprising administering to said individual a therapeutically effective amount of a composition comprising the immunoconjugate according to the disclosure in a pharmaceutically acceptable form. In a specific embodiment

the disease is cancer. In other embodiments the disease is an inflammatory disorder. In a particular such embodiment the immunoconjugate comprises an IL-10 effector moiety.

In any of the above embodiments the individual preferably is a mammal, particularly a human.

- 5 Also disclosed is a conjugate comprising a first Fab molecule which does not specifically bind any antigen, an Fc domain consisting of two subunits, and an effector moiety, wherein not more than one effector moiety is present. In a particular embodiment the first Fab molecule comprises the heavy chain variable region sequence of SEQ ID NO: 299 and the light chain variable region sequence of SEQ ID NO: 297. In one embodiment, the conjugate
- 10 comprises (i) an immunoglobulin molecule, comprising a first and a second Fab molecule which do not specifically bind any antigen and an Fc domain, and (ii) an effector moiety, wherein not more than one effector moiety is present. In one embodiment the immunoglobulin molecule is an IgG class immunoglobulin, particularly an IgG1 subclass immunoglobulin. In a particular embodiment the immunoglobulin molecule comprises the
- 15 heavy chain variable region sequence of SEQ ID NO: 299 and the light chain variable region sequence of SEQ ID NO: 297. Specifically, the heavy chain variable region sequence of SEQ ID NO: 299 and the light chain variable region sequence of SEQ ID NO: 297 are comprised in the first and the second Fab molecule of the immunoglobulin molecule. In one embodiment, the effector moiety is fused to the carboxy-terminal amino acid of one of the
- 20 immunoglobulin heavy chains, optionally through a linker peptide. In some embodiments the Fc domain of the conjugate is engineered to have reduced binding to an Fc receptor, specifically reduced binding to an Fcγ receptor, and/or reduced effector function, specifically reduced ADCC. In a particular embodiment, the Fc domain of the conjugate comprises the amino acid substitutions L234A, L235A and P329G in each of its subunits. In certain
- 25 embodiments the Fc domain of the conjugate comprises a modification promoting heterodimerization of the non-identical polypeptide chains. In a specific embodiment, said modification is a knob-into-hole modification, comprising a knob modification in one of the subunits of the Fc domain and a hole modification in the other one of the two subunits of the Fc domain. In a particular embodiment, the effector moiety is fused to the amino- or carboxy-
- 30 terminal amino acid of the subunit of the Fc domain comprising the knob modification. In one embodiment the effector moiety is a cytokine, particularly IL-2. Additionally, the conjugate can incorporate, alone or in combination, any of the features described herein in

relation to the formats, the Fc domain or the effector moiety of the immunoconjugates of the disclosure.

Also disclosed is an isolated polynucleotide encoding the conjugate of the disclosure or a fragment thereof. In a specific embodiment, the isolated polynucleotide comprises a sequence that is at least about 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99% or 100% identical to the sequence of SEQ ID NO: 298 or SEQ ID NO: 300. Also disclosed is an expression vector comprising the isolated polynucleotide, and a host cell comprising the isolated polynucleotide or the expression vector of the disclosure. Also disclosed is a method of producing the conjugate of the disclosure, comprising the steps of a) culturing the host cell of the disclosure under conditions suitable for the expression of the conjugate and b) recovering the conjugate. Also disclosed is a conjugate produced by the method of the disclosure.

Also disclosed is a pharmaceutical composition comprising the conjugate of the disclosure and a pharmaceutically acceptable carrier. Furthermore, the conjugate can be employed in the methods of use described herein for the immunoconjugates of the disclosure.

Brief Description of the Drawings

FIGURE 1. Schematic representation of typical immunoglobulin-cytokine immunoconjugate as known in the art, with a cytokine (dotted) fused to the C-terminus of each of the two immunoglobulin heavy chains.

FIGURE 2. Schematic representation of novel immunoconjugates according to the invention, comprising not more than one effector moiety (dotted). The effector moiety is fused, optionally via a linker peptide (grey boxes) to the carboxy-terminal (format A and B) or the amino-terminal amino acid (format C) of the Fc domain. The immunoconjugate comprises one (format B and C) or more (typically two, format A) antigen binding moieties, which may be Fab fragments comprising antibody heavy and light chain variable domains (hatched). The Fc domain may comprise a modification promoting heterodimerization of two non-identical polypeptide chains (black dot) and/or a modification altering Fc receptor binding and/or effector function (black star).

FIGURE 3. Purification of FAP-targeted 4G8-based IgG-IL-2 quadruple mutant (qm) immunoconjugate. A) Elution profile of the Protein A affinity chromatography step. B) Elution profile of the size exclusion chromatography step. C) Analytical SDS-PAGE (NuPAGE Novex Bis-Tris Mini Gel, Invitrogen, MOPS running buffer) of the final product.

D) Analytical size exclusion chromatography of the final product on a Superdex 200 column (97% monomer content).

FIGURE 4. Purification of FAP-targeted 28H1-based IgG-IL-2 qm immunoconjugate. A) Elution profile of the Protein A affinity chromatography step. B) Elution profile of the size exclusion chromatography step. C) Analytical SDS-PAGE (reduced: NuPAGE Novex Bis-Tris Mini Gel, Invitrogen, MOPS running buffer; non-reduced: NuPAGE Tris-Acetate, Invitrogen,

Tris-Acetate running buffer) of the final product. D) Analytical size exclusion chromatography of the final product on a Superdex 200 column (100% monomer content).

FIGURE 5. Purification of FAP-targeted 28H1-based IgG-IL-2 qm immunoconjugate from CHO cells. A) Elution profile of the Protein A affinity chromatography step. B) Elution profile of the size exclusion chromatography step. C) Analytical SDS-PAGE (NuPAGE Novex Bis-Tris Mini Gel, Invitrogen, MOPS running buffer) of the final product. D) Analytical size exclusion chromatography of the final product on a Superdex 200 column (100% monomer content).

FIGURE 6. Purification of FAP-targeted 4B9-based IgG-IL-2 qm immunoconjugate. A) Elution profile of the Protein A affinity chromatography step. B) Elution profile of the size exclusion chromatography step. C) Analytical SDS-PAGE (NuPAGE Novex Bis-Tris Mini Gel, Invitrogen, MOPS running buffer) of the final product. D) Analytical size exclusion chromatography of the final product on a Superdex 200 column (100% monomer content).

FIGURE 7. Purification of CEA-targeted CH1A1A 98/99 2F1-based IgG-IL-2 qm immunoconjugate. A) Elution profile of the Protein A affinity chromatography step. B) Elution profile of the size exclusion chromatography step. C) Analytical capillary electrophoresis SDS (Caliper) of the final product. D) Analytical size exclusion chromatography of the final product on a TSKgel G3000 SW XL column (98.8% monomer content).

FIGURE 8. Purification of TNC A2-targeted 2B10-based IgG-IL-2 qm immunoconjugate. A) Elution profile of the Protein A affinity chromatography step. B) Elution profile of the size exclusion chromatography step. C) Analytical capillary electrophoresis SDS (Caliper) of the final product. D) Analytical size exclusion chromatography of the final product on a TSKgel G3000 SW XL column (100% monomer content).

FIGURE 9. Purification of untargeted DP47GS-based IgG-IL-2 qm immunoconjugate. A) Elution profile of the Protein A affinity chromatography step. B) Elution profile of the size exclusion chromatography step. C) Analytical SDS-PAGE (NuPAGE Novex Bis-Tris Mini Gel, Invitrogen, MOPS running buffer) of the final product. D) Analytical size exclusion chromatography of the final product on a Superdex 200 column (100% monomer content).

FIGURE 10. Binding of FAP-targeted 4G8-based IgG-IL-2 qm immunoconjugate to human FAP expressed on stably transfected HEK 293 cells as measured by FACS, compared to the corresponding Fab-IL-2 qm-Fab construct.

FIGURE 11. Interferon (IFN)- γ release on NK92 cells induced by FAP-targeted 4G8-based IgG-IL-2 qm immunoconjugate in solution, compared to the 28H1-based Fab-IL-2 qm-Fab construct.

FIGURE 12. Detection of phosphorylated STAT5 by FACS in different cell types after stimulation for 20 min with FAP-targeted 4G8-based IgG-IL-2 qm immunoconjugate in solution, compared to the 28H1-based Fab-IL-2-Fab and Fab-IL-2 qm-Fab constructs as well as Proleukin.

- 5 A) NK cells ($CD3^+CD56^+$); B) $CD8^+$ T cells ($CD3^+CD8^+$); C) $CD4^+$ T cells ($CD3^+CD4^+CD25^-CD127^+$); D) regulatory T cells ($CD4^+CD25^+FOXP3^+$).

FIGURE 13. Binding of TNC A2-targeted 2B10 IgG-IL-2 qm and corresponding unconjugated IgG to TNC A2-expressing U87MG cells, as measured by FACS.

- FIGURE 14. Induction of NK92 cell proliferation by TNC A2-targeted 2B10 IgG-IL-2 qm, CEA-targeted CH1A1A 98/99 2F1 IgG-IL-2 qm and CH1A1A 98/99 2F1 IgG-IL-2 wt
10 immunoconjugates.

FIGURE 15. Induction of NK92 cell proliferation by FAP-targeted 4B9 IgG-IL-2 qm and 4B9 IgG-IL-2 wt immunoconjugates.

- FIGURE 16. Killing (as measured by LDH release) of CEA-overexpressing A549 tumor cells by PBMCs through ADCC mediated by glycoengineered (ge) and wildtype (wt) CH1A1A IgG-IL-2
15 qm immunoconjugates, compared to unconjugated glycoengineered CH1A1A IgG.

- FIGURE 17. Purification of untargeted DP47GS IgG-IL-2 wt immunoconjugate. A) Elution profile of the Protein A affinity chromatography step. B) Elution profile of the size exclusion chromatography step. C) Analytical SDS-PAGE (NuPAGE Novex Bis-Tris Mini Gel, Invitrogen, MOPS running buffer) of the final product. D) Analytical size exclusion chromatography of the
20 final product on a Superdex 200 column (99.6% monomer content).

- FIGURE 18. Purification of 28H1-based FAP-targeted 28H1 IgG-IL-2 wt immunoconjugate. A) Elution profile of the Protein A affinity chromatography step. B) Elution profile of the size exclusion chromatography step. C) Analytical SDS-PAGE (NuPAGE Novex Bis-Tris Mini Gel, Invitrogen, MOPS running buffer) of the final product. D) Analytical size exclusion
25 chromatography of the final product on a Superdex 200 column (99.6% monomer content).

- FIGURE 19. Purification of CEA-targeted CH1A1A 98/99 2F1-based IgG-IL-2 wt immunoconjugate. A) Elution profile of the Protein A affinity chromatography step. B) Elution profile of the size exclusion chromatography step. C) Analytical capillary electrophoresis SDS (Caliper) of the final product. D) Analytical size exclusion chromatography of the final product
30 on a TSKgel G3000 SW XL column (100% monomer content).

FIGURE 20. Purification of FAP-targeted 4B9-based IgG-IL-2 wt immunoconjugate. A) Elution profile of the combined Protein A affinity and size exclusion chromatography. B) Zoom on the elution profile of the size exclusion chromatography step in A. C) Analytical SDS-PAGE

(NuPAGE Novex Bis-Tris Mini Gel, Invitrogen, MOPS running buffer) of the final product. D) Analytical size exclusion chromatography of the final product on a TSKgel G3000 SW XL column (98.5% monomer content).

FIGURE 21. A) Analytical SDS-PAGE (NuPAGE Novex Bis-Tris Mini Gel (Invitrogen), NuPAGE LDS sample buffer (4x), heated for 10 min at 70°C, MOPS buffer, 160 V, 60 min, MW marker Mark 12, unstained standard (Invitrogen, M) of reduced (1) and non-reduced (2) 2B10 IgG-IL-10M1. B) SPR-based affinity determination (ProteOn XPR36) of 2B10 IgG-IL-10M1 to human TNC A2 fitted globally to a 1:1 interaction model.

(chip: NLC; ligand: TNCA2 (250 RU); analyte: TNCA2 2B10 IgG-IL-10M1 164 kDa; concentration range analyte: 50, 10, 2, 0.4, 0.08, 0 nM; association time: 180s; dissociation time: 600s; flow rate: 50 µl/min; k_{on} 1.80×10^6 1/Ms; k_{off} 9.35×10^{-5} 1/s; K_D : 52 pM). C) SPR-based affinity determination (ProteOn XPR36) of 2B10 IgG-IL-10M1 to human IL-10R1 fitted globally to a 1:1 interaction model (chip: NLC; ligand: IL-10R1 (1600RU); analyte: TNCA2 2B10 IgG-IL-10M1 164 kDa; concentration range analyte: 50, 10, 2, 0.4, 0.08, 0 nM; association time: 180s; dissociation time: 600s; flow rate: 50 µl/min; k_{on} 5.56×10^5 1/Ms; k_{off} 2.89×10^{-4} 1/s; K_D : 520 pM).

FIGURE 22. A) Analytical SDS-PAGE (NuPAGE Novex Bis-Tris Mini Gel (Invitrogen), NuPAGE LDS sample buffer (4x), heated for 10 min at 70°C, MOPS buffer, 160 V, 60 min, MW marker Mark 12, unstained standard (Invitrogen, M) of reduced (1) and non-reduced (2) 4G8 IgG-IL-10M1. B) SPR-based affinity determination (ProteOn XPR36) of 4G8 IgG-IL-10M1 to human FAP fitted globally to a 1:1 interaction model (chip: GLM; ligand: huFAP (500RU); analyte: FAP 4G8 IgG-IL-10M1 164 kDa; concentration range analyte: 10, 2, 0.4, 0.08, 0 nM; association time: 180s; dissociation time: 600s; flow rate: 50 µl/min; k_{on} 6.68×10^5 1/Ms; k_{off} 1.75×10^{-5} 1/s; K_D : 26 pM). C) SPR-based affinity determination (ProteOn XPR36) of 4G8 IgG-IL-10M1 to human IL-10R1 fitted globally to a 1:1 interaction model (chip: NLC; ligand: IL 10R1 (1600RU); analyte: FAP 4G8 IgG-IL-10M1 164 kDa; concentration range analyte: 50, 10, 2, 0.4, 0.08, 0 nM; association time: 180s; dissociation time: 600s; flow rate: 50 µl/min; k_{on} 3.64×10^5 1/Ms; k_{off} 2.96×10^{-4} 1/s; K_D : 815 pM).

FIGURE 23. Purification of FAP-targeted 4B9-based “1+1” IgG-IL-2 qm immunoconjugate. A) Elution profile of the combined Protein A affinity and size exclusion chromatography. B) Analytical SDS-PAGE (NuPAGE Novex Bis-Tris Mini Gel, Invitrogen, MOPS running buffer) of the final product. C) Analytical size exclusion chromatography of the final product on a TSKgel G3000 SW XL column (99.2% monomer content).

FIGURE 24. Purification of FAP-targeted 28H1-based “1+1” IgG-IL-2 qm immunoconjugate. A) Elution profile of the combined Protein A affinity and size exclusion chromatography. B) Analytical SDS-PAGE (NuPAGE Novex Bis-Tris Mini Gel, Invitrogen, MOPS running buffer) of the final product. C) Analytical size exclusion chromatography of the final product on a TSKgel G3000 SW XL column (100% monomer content).

FIGURE 25. Purification of FAP-targeted 4B9-based “1+1” IgG-IL-7 immunoconjugate. A) Elution profile of the combined Protein A affinity and size exclusion chromatography. B) Analytical capillary electrophoresis SDS (Caliper) of the final product. C) Analytical size exclusion chromatography of the final product on a TSKgel G3000 SW XL column (98.6% monomer content).

FIGURE 26. Purification of FAP-targeted 4B9-based “1+1” IgG-IFN- α immunoconjugate. A) Elution profile of the Protein A affinity chromatography step. B) Elution profile of the size exclusion chromatography step. C) Analytical capillary electrophoresis SDS (Caliper) of the final product. D) Analytical size exclusion chromatography of the final product on a TSKgel G3000 SW XL column (92.8% monomer content).

FIGURE 27. Induction of NK92 cell proliferation by FAP-targeted 4B9 “1+1” IgG-IL-2 qm and 28H1 “1+1” IgG-IL-2 wt immunoconjugates, compared to corresponding IgG-IL-2 constructs..

FIGURE 28. Proliferation of PHA-activated (A) CD4 and (B) CD8 T cells induced by 4B9 “1+1” IgG-IL-7 and 4B9 “1+1” IgG-IL-2 qm immunoconjugates, compared to IgG-IL-2 qm and IgG-IL-2 wt constructs.

FIGURE 29. Induction of Daudi cell proliferation by 4B9 “1+1” IgG-IFN- α , compared to Roferon A.

FIGURE 30. Serum concentrations of IL-2 immunoconjugates after a single i.v. administration of FAP-targeted (A) and untargeted (B) IgG-IL-2 constructs comprising either wild-type (wt) or quadruple mutant (qm) IL-2.

FIGURE 31. Tissue distribution of FAP-targeted 28H1 IgG-IL qm compared to unconjugated FAP-targeted 28H1 IgG and 4B9 IgG, as well as untargeted DP47GS IgG, 24 hours after i.v. injection.

FIGURE 32. Binding of 28H1 IgG-IL-2 qm and 28H1 IgG-(IL-2 qm)₂ immunoconjugates to NK92 cells as determined by FACS.

FIGURE 33. Proliferation of NK cells upon incubation with different FAP-targeted 28H1 IL-2 immunoconjugates or Proleukin for 4 (A), 5 (B) or 6 (C) days.

FIGURE 34. Proliferation of CD4 T-cells upon incubation with different FAP-targeted 28H1 IL-2 immunoconjugates or Proleukin for 4 (A), 5 (B) or 6 (C) days.

FIGURE 35. Proliferation of CD8 T-cells upon incubation with different FAP-targeted 28H1 IL-2 immunoconjugates or Proleukin for 4 (A), 5 (B) or 6 (C) days.

5 FIGURE 36. Proliferation of pre-activated CD8 (A) and CD4 (B) T cells after six days incubation with different IL-2 immunoconjugates.

FIGURE 37. Activation induced cell death of CD3⁺ T cells after six days incubation with different IL-2 immunoconjugates and overnight treatment with anti-Fas antibody.

10 FIGURE 38. Serum concentrations of IL-2 immunoconjugates after a single i.v. administration of untargeted DP47GS IgG-IL-2 constructs comprising either wild-type (A) or quadruple mutant IL-2 (B).

15 FIGURE 39. Binding of DP47GS IgG to different antigens. Binding was detected in an ELISA-based assay with the antigens captured on the plate. A human IgG1 antibody which exhibits unspecific binding to almost all of the captured antigens was used as positive control, blank samples did not contain any antibody.

20 FIGURE 40. Binding of DP47GS IgG with or without LALA P329G mutation in the Fc domain to subsets of fresh (A), PHA-L activated (B) and re-stimulated (C) human PBMCs, as determined by FACS analysis. Upper left panel: B cells (in A, B) or CD4⁺ T cells (in C); upper right panel: CD8⁺ T cells; lower left panel: NK cells; lower right panel: CD14⁺ cells (monocytes/neutrophils).

Detailed Description of the Invention

Definitions

25 In the claims which follow and in the description of the invention, except where the context requires otherwise due to express language or necessary implication, the word “comprise” or variations such as “comprises” or “comprising” is used in an inclusive sense, i.e. to specify the presence of the stated features but not to preclude the presence or addition of further features in various embodiments of the invention.

Terms are used herein as generally used in the art, unless otherwise defined in the following.

30 As used herein, the term “conjugate” refers to a fusion polypeptide molecule that includes one effector moiety and a further peptide molecule, particularly an immunoglobulin molecule. As used herein, the term “immunoconjugate” refers to a fusion polypeptide molecule that includes one effector moiety, at least one antigen binding moiety and an Fc domain, provided

that not more than one effector moiety is present. In certain embodiments, the immunoconjugate comprises one effector moiety, two antigen binding moieties, and an Fc domain. Particular immunoconjugates according to the invention essentially consist of one effector moiety, two antigen binding moieties, and an Fc domain, joined by one or more
5 linker sequences. The antigen binding moiety and the effector moiety can be joined to the Fc domain by a variety of

interactions and in a variety of configurations as described herein. In a particular embodiment, the two antigen binding moieties and the Fc domain are joined to each other in a configuration so as to form a full immunoglobulin molecule. An immunoconjugate as referred to herein, is a fusion protein, i.e. the components of the immunoconjugate are linked to each other by peptide-bonds, either directly or through linker peptides.

As used herein, the term "antigen binding moiety" refers to a polypeptide molecule that specifically binds to an antigenic determinant. In one embodiment, an antigen binding moiety is able to direct the entity to which it is attached (e.g. an effector moiety or a second antigen binding moiety) to a target site, for example to a specific type of tumor cell or tumor stroma bearing the antigenic determinant. Antigen binding moieties include antibodies and fragments thereof as further defined herein. Particular antigen binding moieties include an antigen binding domain of an antibody, comprising an antibody heavy chain variable region and an antibody light chain variable region. In certain embodiments, the antigen binding moieties may comprise antibody constant regions as further defined herein and known in the art. Useful heavy chain constant regions include any of the five isotypes: α , δ , ϵ , γ , or μ . Useful light chain constant regions include any of the two isotypes: κ and λ .

As used herein, the term "antigenic determinant" is synonymous with "antigen" and "epitope," and refers to a site (e.g. a contiguous stretch of amino acids or a conformational configuration made up of different regions of non-contiguous amino acids) on a polypeptide macromolecule to which an antigen binding moiety binds, forming an antigen binding moiety-antigen complex. Useful antigenic determinants can be found, for example, on the surfaces of tumor cells, on the surfaces of virus-infected cells, on the surfaces of other diseased cells, free in blood serum, and/or in the extracellular matrix (ECM). In a particular embodiment the antigenic determinant is a human antigen.

By "specifically binds" is meant that the binding is selective for the antigen and can be discriminated from unwanted or non-specific interactions. The ability of an antigen-binding moiety to bind to a specific antigenic determinant can be measured either through an enzyme-linked immunosorbent assay (ELISA) or other techniques familiar to one of skill in the art, e.g. surface plasmon resonance (SPR) technique (analyzed on a BIAcore instrument) (Liljeblad et al., Glyco J 17, 323-329 (2000)), and traditional binding assays (Heeley, Endocr Res 28, 217-229 (2002)). In one embodiment, the extent of binding of an antigen binding moiety to an unrelated protein is less than about 10% of the binding of the antigen binding moiety to the antigen as measured, e.g., by SPR. In certain embodiments, an antigen binding moiety that binds to the

antigen, or an immunoconjugate comprising that antigen binding moiety, has a dissociation constant (K_D) of $\leq 1 \mu\text{M}$, $\leq 100 \text{ nM}$, $\leq 10 \text{ nM}$, $\leq 1 \text{ nM}$, $\leq 0.1 \text{ nM}$, $\leq 0.01 \text{ nM}$, or $\leq 0.001 \text{ nM}$ (e.g. 10^{-8} M or less, e.g. from 10^{-8} M to 10^{-13} M , e.g., from 10^{-9} M to 10^{-13} M).

“Affinity” refers to the strength of the sum total of non-covalent interactions between a single
5 binding site of a molecule (e.g., a receptor) and its binding partner (e.g., a ligand). Unless indicated otherwise, as used herein, “binding affinity” refers to intrinsic binding affinity which reflects a 1:1 interaction between members of a binding pair (e.g., receptor and a ligand). The affinity of a molecule X for its partner Y can generally be represented by the dissociation constant (K_D), which is the ratio of dissociation and association rate constants (k_{off} and k_{on} ,
10 respectively). Thus, equivalent affinities may comprise different rate constants, as long as the ratio of the rate constants remains the same. Affinity can be measured by well established methods known in the art, including those described herein. A particular method for measuring affinity is Surface Plasmon Resonance (SPR).

“Reduced binding”, for example reduced binding to an Fc receptor or to CD25, refers to a
15 decrease in affinity for the respective interaction, as measured for example by SPR. For clarity the term includes also reduction of the affinity to zero (or below the detection limit of the analytic method), i.e. complete abolishment of the interaction. Conversely, “increased binding” refers to an increase in binding affinity for the respective interaction.

As used herein, the terms “first” and “second” with respect to antigen-binding moieties etc., are
20 used for convenience of distinguishing when there is more than one of each type of moiety. Use of these terms is not intended to confer a specific order or orientation of the immunoconjugate unless explicitly so stated.

As used herein, the term “effector moiety” refers to a polypeptide, e.g., a protein or glycoprotein, that influences cellular activity, for example, through signal transduction or other cellular
25 pathways. Accordingly, the effector moiety of the invention can be associated with receptor-mediated signaling that transmits a signal from outside the cell membrane to modulate a response in a cell bearing one or more receptors for the effector moiety. In one embodiment, an effector moiety can elicit a cytotoxic response in cells bearing one or more receptors for the effector moiety. In another embodiment, an effector moiety can elicit a proliferative response in
30 cells bearing one or more receptors for the effector moiety. In another embodiment, an effector moiety can elicit differentiation in cells bearing receptors for the effector moiety. In another embodiment, an effector moiety can alter expression (i.e. upregulate or downregulate) of an endogenous cellular protein in cells bearing receptors for the effector moiety. Non-limiting

examples of effector moieties include cytokines, growth factors, hormones, enzymes, substrates, and cofactors. The effector moiety can be associated with an antigen-binding moiety or an Fc domain in a variety of configurations to form an immunoconjugate.

As used herein, the term "cytokine" refers to a molecule that mediates and/or regulates a biological or cellular function or process (e.g. immunity, inflammation, and hematopoiesis). The term "cytokine" as used herein includes "lymphokines," "chemokines," "monokines," and "interleukins". Examples of useful cytokines include, but are not limited to, GM-CSF, IL-1 α , IL-1 β , IL-2, IL-3, IL-4, IL-5, IL-6, IL-7, IL-8, IL-10, IL-12, IFN- α , IFN- β , IFN- γ , MIP-1 α , MIP-1 β , TGF- β , TNF- α , and TNF- β . Particular cytokines are IL-2, IL-7, IL-10, IL-12, IL-15, IFN- α and IFN- γ . In particular embodiments the cytokine is a human cytokine. The term "cytokine" as used herein is meant to also include cytokine variants comprising one or more amino acid mutations in the amino acid sequences of the corresponding wild-type cytokine, such as for example the IL-2 variants described in Sauv   et al., Proc Natl Acad Sci USA 88, 4636-40 (1991); Hu et al., Blood 101, 4853-4861 (2003) and US Pat. Publ. No. 2003/0124678; Shanafelt et al., Nature Biotechnol 18, 1197-1202 (2000); Heaton et al., Cancer Res 53, 2597-602 (1993) and US Pat. No. 5,229,109; US Pat. Publ. No. 2007/0036752; WO 2008/0034473; WO 2009/061853; or PCT patent application no. PCT/EP2012/051991. Further cytokine variants, for example variants of IL-15, are described herein. In certain embodiments cytokines have been mutated to eliminate glycosylation.

As used herein, the term "single-chain" refers to a molecule comprising amino acid monomers linearly linked by peptide bonds. In one embodiment, the effector moiety is a single-chain effector moiety. Non-limiting examples of single-chain effector moieties include cytokines, growth factors, hormones, enzymes, substrates, and cofactors. When the effector moiety is a cytokine and the cytokine of interest is normally found as a multimer in nature, each subunit of the multimeric cytokine is sequentially encoded by the single-chain of the effector moiety. Accordingly, non-limiting examples of useful single-chain effector moieties include GM-CSF, IL-1 α , IL-1 β , IL-2, IL-3, IL-4, IL-5, IL-6, IL-7, IL-8, IL-10, IL-12, IL-15, IFN- α , IFN- β , IFN- γ , MIP-1 α , MIP-1 β , TGF- β , TNF- α , and TNF- β .

As used herein, the term "control effector moiety" refers to an unconjugated effector moiety. For example, when comparing an IL-2 immunoconjugate as described herein with a control effector moiety, the control effector moiety is free, unconjugated IL-2. Likewise, e.g., when comparing an IL-12 immunoconjugate with a control effector moiety, the control effector moiety is free,

unconjugated IL-12 (e.g. existing as a heterodimeric protein wherein the p40 and p35 subunits share only disulfide bond(s)).

As used herein, the term "effector moiety receptor" refers to a polypeptide molecule capable of binding specifically to an effector moiety. For example, where IL-2 is the effector moiety, the effector moiety receptor that binds to an IL-2 molecule (e.g. an immunoconjugate comprising IL-2) is the IL-2 receptor. Similarly, e.g., where IL-12 is the effector moiety of an immunoconjugate, the effector moiety receptor is the IL-12 receptor. Where an effector moiety specifically binds to more than one receptor, all receptors that specifically bind to the effector moiety are "effector moiety receptors" for that effector moiety.

10 The term "immunoglobulin molecule" refers to a protein having the structure of a naturally occurring antibody. For example, immunoglobulins of the IgG class are heterotetrameric glycoproteins of about 150,000 daltons, composed of two light chains and two heavy chains that are disulfide-bonded. From N- to C-terminus, each heavy chain has a variable region (VH), also called a variable heavy domain or a heavy chain variable domain, followed by three constant domains (CH1, CH2, and CH3), also called a heavy chain constant region. Similarly, from N- to C-terminus, each light chain has a variable region (VL), also called a variable light domain or a light chain variable domain, followed by a constant light (CL) domain, also called a light chain constant region. The heavy chain of an immunoglobulin may be assigned to one of five types, called α (IgA), δ (IgD), ϵ (IgE), γ (IgG), or μ (IgM), some of which may be further divided into subtypes, e.g. γ_1 (IgG₁), γ_2 (IgG₂), γ_3 (IgG₃), γ_4 (IgG₄), α_1 (IgA₁) and α_2 (IgA₂). The light chain of an immunoglobulin may be assigned to one of two types, called kappa (κ) and lambda (λ), based on the amino acid sequence of its constant domain. An immunoglobulin essentially consists of two Fab molecules and an Fc domain, linked via the immunoglobulin hinge region.

25 The term "antibody" herein is used in the broadest sense and encompasses various antibody structures, including but not limited to monoclonal antibodies, polyclonal antibodies, and antibody fragments so long as they exhibit the desired antigen-binding activity.

An "antibody fragment" refers to a molecule other than an intact antibody that comprises a portion of an intact antibody that binds the antigen to which the intact antibody binds. Examples of antibody fragments include but are not limited to Fv, Fab, Fab', Fab'-SH, F(ab')₂, diabodies, linear antibodies, single-chain antibody molecules (e.g. scFv), and single-domain antibodies. For a review of certain antibody fragments, see Hudson et al., Nat Med 9, 129-134 (2003). For a review of scFv fragments, see e.g. Plückthun, in The Pharmacology of Monoclonal Antibodies, vol. 113, Rosenberg and Moore eds., Springer-Verlag, New York, pp. 269-315 (1994); see also

WO 93/16185; and U.S. Patent Nos. 5,571,894 and 5,587,458. For discussion of Fab and F(ab')₂ fragments comprising salvage receptor binding epitope residues and having increased in vivo half-life, see U.S. Patent No. 5,869,046. Diabodies are antibody fragments with two antigen-binding sites that may be bivalent or bispecific. See, for example, EP 404,097; WO 1993/01161; Hudson et al., Nat Med 9, 129-134 (2003); and Hollinger et al., Proc Natl Acad Sci USA 90, 6444-6448 (1993). Triabodies and tetrabodies are also described in Hudson et al., Nat Med 9, 129-134 (2003). Single-domain antibodies are antibody fragments comprising all or a portion of the heavy chain variable domain or all or a portion of the light chain variable domain of an antibody. In certain embodiments, a single-domain antibody is a human single-domain antibody (Domantis, Inc., Waltham, MA; see e.g. U.S. Patent No. 6,248,516 B1). Antibody fragments can be made by various techniques, including but not limited to proteolytic digestion of an intact antibody as well as production by recombinant host cells (e.g. E. coli or phage), as described herein.

The term "antigen binding domain" refers to the part of an antibody that comprises the area which specifically binds to and is complementary to part or all of an antigen. An antigen binding domain may be provided by, for example, one or more antibody variable domains (also called antibody variable regions). Particularly, an antigen binding domain comprises an antibody light chain variable region (VL) and an antibody heavy chain variable region (VH).

The term "variable region" or "variable domain" refers to the domain of an antibody heavy or light chain that is involved in binding the antibody to antigen. The variable domains of the heavy chain and light chain (VH and VL, respectively) of a native antibody generally have similar structures, with each domain comprising four conserved framework regions (FRs) and three hypervariable regions (HVRs). See, e.g., Kindt et al., Kuby Immunology, 6th ed., W.H. Freeman and Co., page 91 (2007). A single VH or VL domain may be sufficient to confer antigen-binding specificity.

The term "hypervariable region" or "HVR", as used herein, refers to each of the regions of an antibody variable domain which are hypervariable in sequence and/or form structurally defined loops ("hypervariable loops"). Generally, native four-chain antibodies comprise six HVRs; three in the VH (H1, H2, H3), and three in the VL (L1, L2, L3). HVRs generally comprise amino acid residues from the hypervariable loops and/or from the complementarity determining regions (CDRs), the latter being of highest sequence variability and/or involved in antigen recognition. With the exception of CDR1 in VH, CDRs generally comprise the amino acid residues that form the hypervariable loops. Hypervariable regions (HVRs) are also referred to as "complementarity

determining regions" (CDRs), and these terms are used herein interchangeably in reference to portions of the variable region that form the antigen binding regions. This particular region has been described by Kabat et al., U.S. Dept. of Health and Human Services, Sequences of Proteins of Immunological Interest (1983) and by Chothia et al., J Mol Biol 196:901-917 (1987), where the definitions include overlapping or subsets of amino acid residues when compared against each other. Nevertheless, application of either definition to refer to a CDR of an antibody or variants thereof is intended to be within the scope of the term as defined and used herein. The appropriate amino acid residues which encompass the CDRs as defined by each of the above cited references are set forth below in Table 1 as a comparison. The exact residue numbers which encompass a particular CDR will vary depending on the sequence and size of the CDR. Those skilled in the art can routinely determine which residues comprise a particular CDR given the variable region amino acid sequence of the antibody.

TABLE 1. CDR Definitions¹

CDR	Kabat	Chothia	AbM²
V _H CDR1	31-35	26-32	26-35
V _H CDR2	50-65	52-58	50-58
V _H CDR3	95-102	95-102	95-102
V _L CDR1	24-34	26-32	24-34
V _L CDR2	50-56	50-52	50-56
V _L CDR3	89-97	91-96	89-97

¹ Numbering of all CDR definitions in Table 1 is according to the numbering conventions set forth by Kabat et al. (see below).

² "AbM" with a lowercase "b" as used in Table 1 refers to the CDRs as defined by Oxford Molecular's "AbM" antibody modeling software.

Kabat et al. also defined a numbering system for variable region sequences that is applicable to any antibody. One of ordinary skill in the art can unambiguously assign this system of "Kabat numbering" to any variable region sequence, without reliance on any experimental data beyond the sequence itself. As used herein, "Kabat numbering" refers to the numbering system set forth by Kabat et al., U.S. Dept. of Health and Human Services, "Sequence of Proteins of Immunological Interest" (1983). Unless otherwise specified, references to the numbering of specific amino acid residue positions in an antibody variable region are according to the Kabat numbering system.

The polypeptide sequences of the sequence listing (i.e., SEQ ID NOs 23, 25, 27, 29, 31, etc.) are not numbered according to the Kabat numbering system. However, it is well within the ordinary

skill of one in the art to convert the numbering of the sequences of the Sequence Listing to Kabat numbering.

"Framework" or "FR" refers to variable domain residues other than hypervariable region (HVR) residues. The FR of a variable domain generally consists of four FR domains: FR1, FR2, FR3, and FR4. Accordingly, the HVR and FR sequences generally appear in the following sequence in VH (or VL): FR1-H1(L1)-FR2-H2(L2)-FR3-H3(L3)-FR4.

The "class" of an antibody or immunoglobulin refers to the type of constant domain or constant region possessed by its heavy chain. There are five major classes of antibodies: IgA, IgD, IgE, IgG, and IgM, and several of these may be further divided into subclasses (isotypes), e.g., IgG₁, IgG₂, IgG₃, IgG₄, IgA₁, and IgA₂. The heavy chain constant domains that correspond to the different classes of immunoglobulins are called α , δ , ϵ , γ , and μ , respectively.

The term "Fc domain" or "Fc region" herein is used to define a C-terminal region of an immunoglobulin heavy chain that contains at least a portion of the constant region. The term includes native sequence Fc regions and variant Fc regions. Although the boundaries of the Fc region of an IgG heavy chain might vary slightly, the human IgG heavy chain Fc region is usually defined to extend from Cys226, or from Pro230, to the carboxyl-terminus of the heavy chain. However, the C-terminal lysine (Lys447) of the Fc region may or may not be present. Unless otherwise specified herein, numbering of amino acid residues in the Fc region or constant region is according to the EU numbering system, also called the EU index, as described in Kabat et al., Sequences of Proteins of Immunological Interest, 5th Ed. Public Health Service, National Institutes of Health, Bethesda, MD, 1991. A "subunit" of an Fc domain as used herein refers to one of the two polypeptides forming the dimeric Fc domain, i.e. a polypeptide comprising C-terminal constant regions of an immunoglobulin heavy chain, capable of stable self-association. For example, a subunit of an IgG Fc domain comprises an IgG CH2 and an IgG CH3 constant domain.

A "modification promoting heterodimerization" is a manipulation of the peptide backbone or the post-translational modifications of a polypeptide that reduces or prevents the association of the polypeptide with an identical polypeptide to form a homodimer. A modification promoting heterodimerization as used herein particularly includes separate modifications made to each of two polypeptides desired to form a dimer, wherein the modifications are complementary to each other so as to promote association of the two polypeptides. For example, a modification promoting heterodimerization may alter the structure or charge of one or both of the polypeptides desired to form a dimer so as to make their association sterically or electrostatically

favorable, respectively. Heterodimerization occurs between two non-identical polypeptides, such as two subunits of an Fc domain wherein further immunoconjugate components fused to each of the subunits (e.g. antigen binding moiety, effector moiety) are not the same. In the immunoconjugates according to the present invention, the modification promoting heterodimerization is in the Fc domain. In some embodiments the modification promoting heterodimerization comprises an amino acid mutation, specifically an amino acid substitution. In a particular embodiment, the modification promoting heterodimerization comprises a separate amino acid mutation, specifically an amino acid substitution, in each of the two subunits of the Fc domain.

- 10 The term “effector functions” refers to those biological activities attributable to the Fc region of an antibody, which vary with the antibody isotype. Examples of antibody effector functions include: C1q binding and complement dependent cytotoxicity (CDC), Fc receptor binding, antibody-dependent cell-mediated cytotoxicity (ADCC), antibody-dependent cellular phagocytosis (ADCP), cytokine secretion, immune complex-mediated antigen uptake by antigen
15 presenting cells, down regulation of cell surface receptors (e.g. B cell receptor), and B cell activation.

- As used herein, the terms “engineer, engineered, engineering”, are considered to include any manipulation of the peptide backbone or the post-translational modifications of a naturally occurring or recombinant polypeptide or fragment thereof. Engineering includes modifications of
20 the amino acid sequence, of the glycosylation pattern, or of the side chain group of individual amino acids, as well as combinations of these approaches. “Engineering”, particularly with the prefix “glyco-”, as well as the term “glycosylation engineering” includes metabolic engineering of the glycosylation machinery of a cell, including genetic manipulations of the oligosaccharide synthesis pathways to achieve altered glycosylation of glycoproteins expressed in cells.
25 Furthermore, glycosylation engineering includes the effects of mutations and cell environment on glycosylation. In one embodiment, the glycosylation engineering is an alteration in glycosyltransferase activity. In a particular embodiment, the engineering results in altered glucosaminyltransferase activity and/or fucosyltransferase activity. Glycosylation engineering can be used to obtain a “host cell having increased GnTIII activity”, a “host cell having increased
30 ManII activity”, or a “host cell having decreased $\alpha(1,6)$ fucosyltransferase activity”.

The term “amino acid mutation” as used herein is meant to encompass amino acid substitutions, deletions, insertions, and modifications. Any combination of substitution, deletion, insertion, and modification can be made to arrive at the final construct, provided that the final construct

possesses the desired characteristics, e.g., reduced binding to an Fc receptor, or reduced binding to CD25. Amino acid sequence deletions and insertions include amino- and/or carboxy-terminal deletions and insertions of amino acids. Particular amino acid mutations are amino acid substitutions. For the purpose of altering e.g. the binding characteristics of an Fc region or a cytokine such as IL-2, non-conservative amino acid substitutions, i.e. replacing one amino acid with another amino acid having different structural and/or chemical properties, are particularly preferred. Amino acid substitutions include replacement by non-naturally occurring amino acids or by naturally occurring amino acid derivatives of the twenty standard amino acids (e.g. 4-hydroxyproline, 3-methylhistidine, ornithine, homoserine, 5-hydroxylysine). Amino acid mutations can be generated using genetic or chemical methods well known in the art. Genetic methods may include site-directed mutagenesis, PCR, gene synthesis and the like. It is contemplated that methods of altering the side chain group of an amino acid by methods other than genetic engineering, such as chemical modification, may also be useful. Various designations may be used herein to indicate the same amino acid mutation. For example, a substitution from proline at position 329 of the Fc domain to glycine can be indicated as 329G, G329, G₃₂₉, P329G, or Pro329Gly.

As used herein, term "polypeptide" refers to a molecule composed of monomers (amino acids) linearly linked by amide bonds (also known as peptide bonds). The term "polypeptide" refers to any chain of two or more amino acids, and does not refer to a specific length of the product. Thus, peptides, dipeptides, tripeptides, oligopeptides, "protein," "amino acid chain," or any other term used to refer to a chain of two or more amino acids, are included within the definition of "polypeptide," and the term "polypeptide" may be used instead of, or interchangeably with any of these terms. The term "polypeptide" is also intended to refer to the products of post-expression modifications of the polypeptide, including without limitation glycosylation, acetylation, phosphorylation, amidation, derivatization by known protecting/blocking groups, proteolytic cleavage, or modification by non-naturally occurring amino acids. A polypeptide may be derived from a natural biological source or produced by recombinant technology, but is not necessarily translated from a designated nucleic acid sequence. It may be generated in any manner, including by chemical synthesis. A polypeptide of the invention may be of a size of about 3 or more, 5 or more, 10 or more, 20 or more, 25 or more, 50 or more, 75 or more, 100 or more, 200 or more, 500 or more, 1,000 or more, or 2,000 or more amino acids. Polypeptides may have a defined three-dimensional structure, although they do not necessarily have such structure. Polypeptides with a defined three-dimensional structure are referred to as folded, and polypeptides which do

not possess a defined three-dimensional structure, but rather can adopt a large number of different conformations, and are referred to as unfolded.

By an "isolated" polypeptide or a variant, or derivative thereof is intended a polypeptide that is not in its natural milieu. No particular level of purification is required. For example, an isolated polypeptide can be removed from its native or natural environment. Recombinantly produced polypeptides and proteins expressed in host cells are considered isolated for the purpose of the invention, as are native or recombinant polypeptides which have been separated, fractionated, or partially or substantially purified by any suitable technique.

"Percent (%) amino acid sequence identity" with respect to a reference polypeptide sequence is defined as the percentage of amino acid residues in a candidate sequence that are identical with the amino acid residues in the reference polypeptide sequence, after aligning the sequences and introducing gaps, if necessary, to achieve the maximum percent sequence identity, and not considering any conservative substitutions as part of the sequence identity. Alignment for purposes of determining percent amino acid sequence identity can be achieved in various ways that are within the skill in the art, for instance, using publicly available computer software such as BLAST, BLAST-2, ALIGN or Megalign (DNASTAR) software. Those skilled in the art can determine appropriate parameters for aligning sequences, including any algorithms needed to achieve maximal alignment over the full length of the sequences being compared. For purposes herein, however, % amino acid sequence identity values are generated using the sequence comparison computer program ALIGN-2. The ALIGN-2 sequence comparison computer program was authored by Genentech, Inc., and the source code has been filed with user documentation in the U.S. Copyright Office, Washington D.C., 20559, where it is registered under U.S. Copyright Registration No. TXU510087. The ALIGN-2 program is publicly available from Genentech, Inc., South San Francisco, California, or may be compiled from the source code. The ALIGN-2 program should be compiled for use on a UNIX operating system, including digital UNIX V4.0D. All sequence comparison parameters are set by the ALIGN-2 program and do not vary. In situations where ALIGN-2 is employed for amino acid sequence comparisons, the % amino acid sequence identity of a given amino acid sequence A to, with, or against a given amino acid sequence B (which can alternatively be phrased as a given amino acid sequence A that has or comprises a certain % amino acid sequence identity to, with, or against a given amino acid sequence B) is calculated as follows:

$$100 \text{ times the fraction } X/Y$$

where X is the number of amino acid residues scored as identical matches by the sequence alignment program ALIGN-2 in that program's alignment of A and B, and where Y is the total number of amino acid residues in B. It will be appreciated that where the length of amino acid sequence A is not equal to the length of amino acid sequence B, the % amino acid sequence identity of A to B will not equal the % amino acid sequence identity of B to A. Unless specifically stated otherwise, all % amino acid sequence identity values used herein are obtained as described in the immediately preceding paragraph using the ALIGN-2 computer program.

The term "polynucleotide" refers to an isolated nucleic acid molecule or construct, e.g. messenger RNA (mRNA), virally-derived RNA, or plasmid DNA (pDNA). A polynucleotide may comprise a conventional phosphodiester bond or a non-conventional bond (e.g. an amide bond, such as found in peptide nucleic acids (PNA)). The term "nucleic acid molecule" refers to any one or more nucleic acid segments, e.g. DNA or RNA fragments, present in a polynucleotide.

By "isolated" nucleic acid molecule or polynucleotide is intended a nucleic acid molecule, DNA or RNA, which has been removed from its native environment. For example, a recombinant polynucleotide encoding a therapeutic polypeptide contained in a vector is considered isolated for the purposes of the present invention. Further examples of an isolated polynucleotide include recombinant polynucleotides maintained in heterologous host cells or purified (partially or substantially) polynucleotides in solution. An isolated polynucleotide includes a polynucleotide molecule contained in cells that ordinarily contain the polynucleotide molecule, but the polynucleotide molecule is present extrachromosomally or at a chromosomal location that is different from its natural chromosomal location. Isolated RNA molecules include *in vivo* or *in vitro* RNA transcripts of the present invention, as well as positive and negative strand forms, and double-stranded forms. Isolated polynucleotides or nucleic acids according to the present invention further include such molecules produced synthetically. In addition, a polynucleotide or a nucleic acid may be or may include a regulatory element such as a promoter, ribosome binding site, or a transcription terminator.

By a nucleic acid or polynucleotide having a nucleotide sequence at least, for example, 95% "identical" to a reference nucleotide sequence of the present invention, it is intended that the nucleotide sequence of the polynucleotide is identical to the reference sequence except that the polynucleotide sequence may include up to five point mutations per each 100 nucleotides of the reference nucleotide sequence. In other words, to obtain a polynucleotide having a nucleotide sequence at least 95% identical to a reference nucleotide sequence, up to 5% of the nucleotides

in the reference sequence may be deleted or substituted with another nucleotide, or a number of nucleotides up to 5% of the total nucleotides in the reference sequence may be inserted into the reference sequence. These alterations of the reference sequence may occur at the 5' or 3' terminal positions of the reference nucleotide sequence or anywhere between those terminal positions, interspersed either individually among residues in the reference sequence or in one or more contiguous groups within the reference sequence. As a practical matter, whether any particular polynucleotide sequence is at least 80%, 85%, 90%, 95%, 96%, 97%, 98% or 99% identical to a nucleotide sequence of the present invention can be determined conventionally using known computer programs, such as the ones discussed above for polypeptides (e.g. ALIGN-2).

The term "expression cassette" refers to a polynucleotide generated recombinantly or synthetically, with a series of specified nucleic acid elements that permit transcription of a particular nucleic acid in a target cell. The recombinant expression cassette can be incorporated into a plasmid, chromosome, mitochondrial DNA, plastid DNA, virus, or nucleic acid fragment. Typically, the recombinant expression cassette portion of an expression vector includes, among other sequences, a nucleic acid sequence to be transcribed and a promoter. In certain embodiments, the expression cassette of the invention comprises polynucleotide sequences that encode immunoconjugates of the invention or fragments thereof.

The term "vector" or "expression vector" is synonymous with "expression construct" and refers to a DNA molecule that is used to introduce and direct the expression of a specific gene to which it is operably associated in a target cell. The term includes the vector as a self-replicating nucleic acid structure as well as the vector incorporated into the genome of a host cell into which it has been introduced. The expression vector of the present invention comprises an expression cassette. Expression vectors allow transcription of large amounts of stable mRNA. Once the expression vector is inside the target cell, the ribonucleic acid molecule or protein that is encoded by the gene is produced by the cellular transcription and/or translation machinery. In one embodiment, the expression vector of the invention comprises an expression cassette that comprises polynucleotide sequences that encode immunoconjugates of the invention or fragments thereof.

The term "artificial" refers to a synthetic, or non-host cell derived composition, e.g. a chemically-synthesized oligonucleotide.

The terms "host cell", "host cell line," and "host cell culture" are used interchangeably and refer to cells into which exogenous nucleic acid has been introduced, including the progeny of such

cells. Host cells include "transformants" and "transformed cells," which include the primary transformed cell and progeny derived therefrom without regard to the number of passages. Progeny may not be completely identical in nucleic acid content to a parent cell, but may contain mutations. Mutant progeny that have the same function or biological activity as screened or
5 selected for in the originally transformed cell are included herein. A host cell is any type of cellular system that can be used to generate the immunoconjugates used for the present invention. In one embodiment, the host cell is engineered to allow the production of an immunoconjugate with modified oligosaccharides in its Fc region. In certain embodiments, the host cells have been manipulated to express increased levels of one or more polypeptides having $\beta(1,4)$ -N-
10 acetylglucosaminyltransferase III (GnTIII) activity. In certain embodiments the host cells have been further manipulated to express increased levels of one or more polypeptides having α -mannosidase II (ManII) activity. Host cells include cultured cells, *e.g.* mammalian cultured cells, such as CHO cells, BHK cells, NS0 cells, SP2/0 cells, YO myeloma cells, P3X63 mouse myeloma cells, PER cells, PER.C6 cells or hybridoma cells, yeast cells, insect cells, and plant
15 cells, to name only a few, but also cells comprised within a transgenic animal, transgenic plant or cultured plant or animal tissue.

As used herein, the term "polypeptide having GnTIII activity" refers to polypeptides that are able to catalyze the addition of a N-acetylglucosamine (GlcNAc) residue in β -1,4 linkage to the β -linked mannoside of the trimannosyl core of N-linked oligosaccharides. This includes fusion
20 polypeptides exhibiting enzymatic activity similar to, but not necessarily identical to, an activity of $\beta(1,4)$ -N-acetylglucosaminyltransferase III, also known as β -1,4-mannosyl-glycoprotein 4-beta-N-acetylglucosaminyl-transferase (EC 2.4.1.144), according to the Nomenclature Committee of the International Union of Biochemistry and Molecular Biology (NC-IUBMB), as measured in a particular biological assay, with or without dose dependency. In the case where
25 dose dependency does exist, it need not be identical to that of GnTIII, but rather substantially similar to the dose-dependency in a given activity as compared to the GnTIII (i.e. the candidate polypeptide will exhibit greater activity or not more than about 25-fold less and, preferably, not more than about ten-fold less activity, and most preferably, not more than about three-fold less activity relative to the GnTIII). In certain embodiments the polypeptide having GnTIII activity is
30 a fusion polypeptide comprising the catalytic domain of GnTIII and the Golgi localization domain of a heterologous Golgi resident polypeptide. Particularly, the Golgi localization domain is the localization domain of mannosidase II or GnTI, most particularly the localization domain of mannosidase II. Alternatively, the Golgi localization domain is selected from the group

consisting of: the localization domain of mannosidase I, the localization domain of GnTII, and the localization domain of α 1,6 core fucosyltransferase. Methods for generating such fusion polypeptides and using them to produce antibodies with increased effector functions are disclosed in WO 2004/065540, U.S. Provisional Pat. Appl. No. 60/495,142 and U.S. Pat. Appl. 5 Publ. No. 2004/0241817, the entire contents of which are expressly incorporated herein by reference.

As used herein, the term "Golgi localization domain" refers to the amino acid sequence of a Golgi resident polypeptide which is responsible for anchoring the polypeptide to a location within the Golgi complex. Generally, localization domains comprise amino terminal "tails" of an 10 enzyme.

As used herein, the term "polypeptide having ManII activity" refers to polypeptides that are able to catalyze the hydrolysis of the terminal 1,3- and 1,6-linked α -D-mannose residues in the branched GlcNAcMan₅GlcNAc₂ mannose intermediate of N-linked oligosaccharides. This includes polypeptides exhibiting enzymatic activity similar to, but not necessarily identical to, an 15 activity of Golgi α -mannosidase II, also known as mannosyl oligosaccharide 1,3-1,6- α -mannosidase II (EC 3.2.1.114), according to the Nomenclature Committee of the International Union of Biochemistry and Molecular Biology (NC-IUBMB).

An "activating Fc receptor" is an Fc receptor that following engagement by an Fc region of an antibody (or immunoconjugate) elicits signaling events that stimulate the receptor-bearing cell to 20 perform effector functions. Activating Fc receptors include Fc γ RIIIa (CD16a), Fc γ RI (CD64), Fc γ RIIa (CD32), and Fc α RI (CD89).

Antibody-dependent cell-mediated cytotoxicity (ADCC) is an immune mechanism leading to the lysis of antibody-coated target cells by immune effector cells. The target cells are cells to which antibodies, immunoconjugates or fragments thereof comprising an Fc region specifically bind, 25 generally via the protein part that is N-terminal to the Fc region. As used herein, the term "increased ADCC" is defined as either an increase in the number of target cells that are lysed in a given time, at a given concentration of immunoconjugate in the medium surrounding the target cells, by the mechanism of ADCC defined above, and/or a reduction in the concentration of immunoconjugate, in the medium surrounding the target cells, required to achieve the lysis of a 30 given number of target cells in a given time, by the mechanism of ADCC. The increase in ADCC is relative to the ADCC mediated by the same immunoconjugate produced by the same type of host cells, using the same standard production, purification, formulation and storage methods (which are known to those skilled in the art), but that has not been engineered. For example the

increase in ADCC mediated by an immunoconjugate produced by host cells engineered to have an altered pattern of glycosylation (e.g. to express the glycosyltransferase, GnTIII, or other glycosyltransferases) by the methods described herein, is relative to the ADCC mediated by the same immunoconjugate produced by the same type of non-engineered host cells.

5 By “immunoconjugate having increased antibody dependent cell-mediated cytotoxicity (ADCC)” is meant an immunoconjugate having increased ADCC as determined by any suitable method known to those of ordinary skill in the art. One accepted *in vitro* ADCC assay is as follows:

- 1) the assay uses target cells that are known to express the target antigen
10 recognized by the antigen binding moiety of the immunoconjugate;
- 2) the assay uses human peripheral blood mononuclear cells (PBMCs), isolated from blood of a randomly chosen healthy donor, as effector cells;
- 3) the assay is carried out according to following protocol:
 - i) the PBMCs are isolated using standard density centrifugation procedures
15 and are suspended at 5×10^6 cells/ml in RPMI cell culture medium;
 - ii) the target cells are grown by standard tissue culture methods, harvested from the exponential growth phase with a viability higher than 90%, washed in RPMI cell culture medium, labeled with 100 micro-Curies of ^{51}Cr , washed twice with cell culture medium, and resuspended in cell culture medium at a density of 10^5 cells/ml;
 - 20 iii) 100 microliters of the final target cell suspension above are transferred to each well of a 96-well microtiter plate;
 - iv) the immunoconjugate is serially-diluted from 4000 ng/ml to 0.04 ng/ml in cell culture medium and 50 microliters of the resulting immunoconjugate solutions are added to the target cells in the 96-well microtiter plate, testing in triplicate various immunoconjugate
25 concentrations covering the whole concentration range above;
 - v) for the maximum release (MR) controls, 3 additional wells in the plate containing the labeled target cells, receive 50 microliters of a 2% (V/V) aqueous solution of non-ionic detergent (Nonidet, Sigma, St. Louis), instead of the immunoconjugate solution (point iv above);
 - 30 vi) for the spontaneous release (SR) controls, 3 additional wells in the plate containing the labeled target cells, receive 50 microliters of RPMI cell culture medium instead of the immunoconjugate solution (point iv above);

vii) the 96-well microtiter plate is then centrifuged at 50 x g for 1 minute and incubated for 1 hour at 4°C;

viii) 50 microliters of the PBMC suspension (point i above) are added to each well to yield an effector:target cell ratio of 25:1 and the plates are placed in an incubator under
5 5% CO₂ atmosphere at 37°C for 4 hours;

ix) the cell-free supernatant from each well is harvested and the experimentally released radioactivity (ER) is quantified using a gamma counter;

x) the percentage of specific lysis is calculated for each immunoconjugate concentration according to the formula $(ER-MR)/(MR-SR) \times 100$, where ER is the average
10 radioactivity quantified (see point ix above) for that immunoconjugate concentration, MR is the average radioactivity quantified (see point ix above) for the MR controls (see point v above), and SR is the average radioactivity quantified (see point ix above) for the SR controls (see point vi above);

4) "increased ADCC" is defined as either an increase in the maximum percentage of
15 specific lysis observed within the immunoconjugate concentration range tested above, and/or a reduction in the concentration of immunoconjugate required to achieve one half of the maximum percentage of specific lysis observed within the immunoconjugate concentration range tested above. The increase in ADCC is relative to the ADCC, measured with the above assay, mediated by the same immunoconjugate, produced by the same type of host cells, using the same standard
20 production, purification, formulation and storage methods, which are known to those skilled in the art, but that has not been engineered.

An "effective amount" of an agent refers to the amount that is necessary to result in a physiological change in the cell or tissue to which it is administered.

A "therapeutically effective amount" of an agent, e.g. a pharmaceutical composition, refers to an
25 amount effective, at dosages and for periods of time necessary, to achieve the desired therapeutic or prophylactic result. A therapeutically effective amount of an agent for example eliminates, decreases, delays, minimizes or prevents adverse effects of a disease.

An "individual" or "subject" is a mammal. Mammals include, but are not limited to, domesticated animals (e.g. cows, sheep, cats, dogs, and horses), primates (e.g. humans and non-
30 human primates such as monkeys), rabbits, and rodents (e.g. mice and rats). Particularly, the individual or subject is a human.

The term "pharmaceutical composition" refers to a preparation which is in such form as to permit the biological activity of an active ingredient contained therein to be effective, and which

contains no additional components which are unacceptably toxic to a subject to which the formulation would be administered.

A “pharmaceutically acceptable carrier” refers to an ingredient in a pharmaceutical composition, other than an active ingredient, which is nontoxic to a subject. A pharmaceutically acceptable
5 carrier includes, but is not limited to, a buffer, excipient, stabilizer, or preservative.

As used herein, “treatment” (and grammatical variations thereof such as “treat” or “treating”) refers to clinical intervention in an attempt to alter the natural course of a disease in the individual being treated, and can be performed either for prophylaxis or during the course of clinical pathology. Desirable effects of treatment include, but are not limited to, preventing
10 occurrence or recurrence of disease, alleviation of symptoms, diminishment of any direct or indirect pathological consequences of the disease, preventing metastasis, decreasing the rate of disease progression, amelioration or palliation of the disease state, and remission or improved prognosis. In some embodiments, immunoconjugates of the invention are used to delay development of a disease or to slow the progression of a disease.

15 The term “package insert” is used to refer to instructions customarily included in commercial packages of therapeutic products, that contain information about the indications, usage, dosage, administration, combination therapy, contraindications and/or warnings concerning the use of such therapeutic products.

Detailed Description of the Embodiments

20 In a first aspect the invention provides an immunoconjugate comprising a first antigen binding moiety, an Fc domain consisting of two subunits, and an effector moiety, wherein not more than one effector moiety is present. The absence of further effector moieties may reduce targeting of the immunoconjugate to sites where the respective effector moiety receptor is presented, thereby improving targeting to and accumulation at sites where the actual target antigen of the
25 immunoconjugate, which is recognized by the antigen binding moiety, is presented. Furthermore, the absence of an avidity effect for the respective effector moiety receptor can reduce activation of effector moiety receptor-positive cells in peripheral blood upon intravenous administration of the immunoconjugate. Furthermore, the serum half-life of immunoconjugates comprising only a single effector moiety appears to be longer as compared to immunoconjugates comprising two or
30 more effector moieties.

Immunoconjugate Formats

The components of the immunoconjugate can be fused to each other in a variety of configurations. Exemplary configurations are depicted in Figure 2. In one embodiment the effector moiety is fused to the amino- or carboxy-terminal amino acid of one of the two subunits of the Fc domain. In one embodiment the effector moiety is fused to the carboxy-terminal amino acid of one of the two subunits of the Fc domain. The effector moiety may be fused to the Fc domain directly or through a linker peptide, comprising one or more amino acids, typically about 2-20 amino acids. Linker peptides are known in the art or are described herein. Suitable, non-immunogenic linker peptides include, for example, $(G_4S)_n$, $(SG_4)_n$ or $G_4(SG_4)_n$ linker peptides. "n" is generally a number between 1 and 10, typically between 2 and 4. Alternatively, where the effector moiety is linked to the N-terminus of an Fc domain subunit, it may be linked via an immunoglobulin hinge region or a portion thereof, with or without an additional linker peptide. Similarly, the first antigen binding moiety can be fused to the amino- or carboxy-terminal amino acid of one of the two subunits of the Fc domain. In one embodiment the first antigen binding moiety is fused to the amino-terminal amino acid of one of the two subunits of the Fc domain. The first antigen binding moiety may be fused to the Fc domain directly or through a linker peptide. In a particular embodiment the first antigen binding moiety is fused to the Fc domain through an immunoglobulin hinge region. In a specific embodiment, the immunoglobulin hinge region is a human IgG₁ hinge region.

In one embodiment the first antigen binding moiety comprises an antigen binding domain of an antibody, comprising an antibody heavy chain variable region and an antibody light chain variable region. In a particular embodiment the first antigen binding moiety is a Fab molecule. In one embodiment the Fab molecule is fused at its heavy or light chain carboxy-terminus to the amino-terminal amino acid of one of the two subunits of the Fc domain. In a particular embodiment the Fab molecule is fused at its heavy chain carboxy-terminus to the amino-terminal amino acid of one of the two subunits of the Fc domain. In a more particular embodiment the Fab molecule is fused to the Fc domain through an immunoglobulin hinge region. In a specific embodiment, the immunoglobulin hinge region is a human IgG₁ hinge region.

In one embodiment the immunoconjugate essentially consists of an antigen binding moiety, an Fc domain consisting of two subunits, an effector moiety, and optionally one or more linker peptides, wherein said antigen binding domain is a Fab molecule and is fused at its heavy chain carboxy-terminus to the amino-terminal amino acid of one of the two subunits of the Fc domain, and wherein said effector moiety is fused either (i) to the amino-terminal amino acid of the other one of the two subunits of the Fc domain, or (ii) to the carboxy-terminal amino acid of one of the

two subunits of the Fc domain. In the latter case, the effector moiety and the first antigen binding moiety may both be fused to the same subunit of the Fc domain, or may each be fused to a different one of the two subunits of the Fc domain.

An immunoconjugate format with a single antigen binding moiety (for example as shown in
5 Figure 2B and 2C) is useful, particularly in cases where internalization of the target antigen is to be expected following binding of a high affinity antigen binding moiety. In such cases, the presence of more than one antigen binding moiety per immunoconjugate may enhance internalization, thereby reducing availability of the target antigen.

In many other cases, however, it will be advantageous to have an immunoconjugate comprising
10 two or more antigen binding moieties and a single effector moiety to optimize targeting to the target antigen versus the effector moiety receptor, and the pharmaceutical window of the immunoconjugate.

Thus, in a particular embodiment the immunoconjugate of the invention comprises a first and a second antigen binding moiety. In one embodiment each of said first and second antigen binding
15 moieties is fused to the amino-terminal amino acid of one of the two subunits of the Fc domain. The first and second antigen binding moieties may be fused to the Fc domain directly or through a linker peptide. In a particular embodiment each of said first and second antigen binding moieties is fused to a subunit of the Fc domain through an immunoglobulin hinge region. In a specific embodiment, the immunoglobulin hinge region is a human IgG₁ hinge region.

In one embodiment each of said first and second antigen binding moieties comprises an antigen
20 binding domain of an antibody, comprising an antibody heavy chain variable region and an antibody light chain variable region. In a particular embodiment each of said first and second antigen binding moieties is a Fab molecule. In one embodiment each of said Fab molecules is fused at its heavy or light chain carboxy-terminus to the amino-terminal amino acid of one of the
25 two subunits of the Fc domain. In a particular embodiment each of said Fab molecules is fused at its heavy chain carboxy-terminus to the amino-terminal amino acid of one of the two subunits of the Fc domain. In a more particular embodiment each of said Fab molecules is fused to a subunit of the Fc domain through an immunoglobulin hinge region. In a specific embodiment, the immunoglobulin hinge region is a human IgG₁ hinge region.

In one embodiment the first and the second antigen binding moiety and the Fc domain are part of
30 an immunoglobulin molecule. In a particular embodiment the immunoglobulin molecule is an IgG class immunoglobulin. In an even more particular embodiment the immunoglobulin is an IgG₁ subclass immunoglobulin. In another particular embodiment the immunoglobulin is a

human immunoglobulin. In other embodiments the immunoglobulin is a chimeric immunoglobulin or a humanized immunoglobulin. In one embodiment the effector moiety is fused to the carboxy-terminal amino acid of one of the immunoglobulin heavy chains. The effector moiety may be fused to the immunoglobulin heavy chain directly or through a linker peptide. In a particular embodiment the immunoconjugate essentially consists of an immunoglobulin molecule, an effector moiety fused to the carboxy-terminal amino acid of one of the immunoglobulin heavy chains, and optionally one or more linker peptides.

In one embodiment the immunoconjugate comprises a polypeptide wherein a Fab heavy chain shares a carboxy-terminal peptide bond with an Fc domain subunit and a polypeptide wherein an Fc domain subunit shares a carboxy-terminal peptide bond with an effector moiety polypeptide. In another embodiment, the immunoconjugate comprises a polypeptide wherein a first Fab heavy chain shares a carboxy-terminal peptide bond with an Fc domain subunit, and a polypeptide wherein a second Fab heavy chain shares a carboxy-terminal peptide bond with an Fc domain subunit, which in turn shares a carboxy-terminal peptide bond with an effector moiety polypeptide. In a further embodiment the immunoconjugate comprises a polypeptide wherein a Fab heavy chain shares a carboxy-terminal peptide bond with an Fc domain subunit and a polypeptide wherein an effector moiety polypeptide shares a carboxy-terminal peptide bond with an Fc domain subunit. In some embodiments the immunoconjugate further comprises a Fab light chain polypeptide. In certain embodiments the polypeptides are covalently linked, e.g., by a disulfide bond.

According to any of the above embodiments, components of the immunoconjugate (e.g. effector moiety, antigen binding moiety, Fc domain) may be linked directly or through various linkers, particularly peptide linkers comprising one or more amino acids, typically about 2-20 amino acids, that are described herein or are known in the art. Suitable, non-immunogenic linker peptides include, for example, $(G_4S)_n$, $(SG_4)_n$ or $G_4(SG_4)_n$ linker peptides, wherein n is generally a number between 1 and 10, typically between 2 and 4.

Fc domain

The Fc domain of the immunoconjugate consists of a pair of polypeptide chains comprising heavy chain domains of an immunoglobulin molecule. For example, the Fc domain of an immunoglobulin G (IgG) molecule is a dimer, each subunit of which comprises the CH2 and CH3 IgG heavy chain constant domains. The two subunits of the Fc domain are capable of stable

association with each other. In one embodiment the immunoconjugate of the invention comprises not more than one Fc domain.

In one embodiment according to the invention the Fc domain of the immunoconjugate is an IgG Fc domain. In a particular embodiment the Fc domain is an IgG₁ Fc domain. In another embodiment, the Fc domain is an IgG₄ Fc domain. In a further particular embodiment the Fc domain is human. An exemplary sequence of a human IgG₁ Fc region is given in SEQ ID NO: 1.

The Fc domain confers to the immunoconjugate a greatly prolonged serum-half life as compared to immunoconjugate formats lacking an Fc domain. Particularly when the immunoconjugate comprises an effector moiety of rather weak activity (but e.g. reduced toxicity), a long half-life might be essential to achieve optimal efficacy in vivo. Moreover, the Fc domain can mediate effector functions, as will be further discussed below..

Fc domain modifications promoting heterodimerization

Immunoconjugates according to the invention comprise only one single effector moiety, fused to one of the two subunits of the Fc domain, thus they comprise two non-identical polypeptide chains. Recombinant co-expression of these polypeptides and subsequent dimerization leads to several possible combinations of the two polypeptides, out of which only heterodimers of the two non-identical polypeptides are useful according to the invention. To improve the yield and purity of immunoconjugates in recombinant production, it can thus be advantageous to introduce in the Fc domain of the immunoconjugate a modification which hinders the formation of homodimers of two identical polypeptides (i.e. two polypeptides comprising an effector moiety, or two polypeptides lacking an effector moiety) and/or promotes the formation of heterodimers of a polypeptide comprising an effector moiety and a polypeptide lacking an effector moiety.

Accordingly, in certain embodiments according to the invention the Fc domain of the immunoconjugate comprises a modification promoting heterodimerization of two non-identical polypeptide chains. The site of most extensive protein-protein interaction between the two polypeptide chains of a human IgG Fc domain is in the CH3 domain of the Fc domain. Thus, in one embodiment said modification is in the CH3 domain of the Fc domain.

In a specific embodiment said modification is a knob-into-hole modification, comprising a knob modification in one of the two subunits of the Fc domain and a hole modification in the other one of the two subunits of the Fc domain.

The knob-into-hole technology is described e.g. in US 5,731,168; US 7,695,936; Ridgway et al., Prot Eng 9, 617-621 (1996) and Carter, J Immunol Meth 248, 7-15 (2001). Generally, the

method involves introducing a protuberance (“knob”) at the interface of a first polypeptide and a corresponding cavity (“hole”) in the interface of a second polypeptide, such that the protuberance can be positioned in the cavity so as to promote heterodimer formation and hinder homodimer formation. Protuberances are constructed by replacing small amino acid side chains from the interface of the first polypeptide with larger side chains (e.g. tyrosine or tryptophan). Compensatory cavities of identical or similar size to the protuberances are created in the interface of the second polypeptide by replacing large amino acid side chains with smaller ones (e.g. alanine or threonine). The protuberance and cavity can be made by altering the nucleic acid encoding the polypeptides, e.g. by site-specific mutagenesis, or by peptide synthesis. In a specific embodiment a knob modification comprises the amino acid substitution T366W in one of the two subunits of the Fc domain, and the hole modification comprises the amino acid substitutions T366S, L368A and Y407V in the other one of the two subunits of the Fc domain. In a further specific embodiment, the subunit of the Fc domain comprising the knob modification additionally comprises the amino acid substitution S354C, and the subunit of the Fc domain comprising the hole modification additionally comprises the amino acid substitution Y349C. Introduction of these two cysteine residues results in formation of a disulfide bridge between the two subunits of the Fc region, further stabilizing the dimer (Carter, J Immunol Methods 248, 7-15 (2001)).

In an alternative embodiment a modification promoting heterodimerization of two non-identical polypeptide chains comprises a modification mediating electrostatic steering effects, e.g. as described in PCT publication WO 2009/089004. Generally, this method involves replacement of one or more amino acid residues at the interface of the two polypeptide chains by charged amino acid residues so that homodimer formation becomes electrostatically unfavorable but heterodimerization electrostatically favorable.

In a particular embodiment the effector moiety is fused to the amino- or carboxy-terminal amino acid of the subunit of the Fc domain comprising the knob modification. Without wishing to be bound by theory, fusion of the effector moiety to the knob-containing subunit of the Fc domain will further minimize the generation of homodimeric immunoconjugates comprising two effector moieties (steric clash of two knob-containing polypeptides).

30 Fc domain modifications altering Fc receptor binding

In certain embodiments of the invention the Fc domain of the immunoconjugate is engineered to have altered binding affinity to an Fc receptor, specifically altered binding affinity to an Fcγ receptor, as compared to a non-engineered Fc domain.

Binding to Fc receptors can be easily determined e.g. by ELISA, or by Surface Plasmon Resonance (SPR) using standard instrumentation such as a BIAcore instrument (GE Healthcare), and Fc receptors such as may be obtained by recombinant expression. A suitable such binding assay is described herein. Alternatively, binding affinity of Fc domains or immunoconjugates comprising an Fc domain for Fc receptors may be evaluated using cell lines known to express particular Fc receptors, such as NK cells expressing FcγIIIa receptor.

In some embodiments the Fc domain of the immunoconjugate is engineered to have altered effector functions, particularly altered ADCC, as compared to a non-engineered Fc domain.

Effector function of an Fc domain, or an immunoconjugate comprising an Fc domain, can be measured by methods known in the art. A suitable assay for measuring ADCC is described herein. Other examples of *in vitro* assays to assess ADCC activity of a molecule of interest are described in U.S. Patent No. 5,500,362; Hellstrom et al. Proc Natl Acad Sci USA 83, 7059-7063 (1986) and Hellstrom et al., Proc Natl Acad Sci USA 82, 1499-1502 (1985); U.S. Patent No. 5,821,337; Bruggemann et al., J Exp Med 166, 1351-1361 (1987). Alternatively, non-radioactive assays methods may be employed (see, for example, ACTI™ non-radioactive cytotoxicity assay for flow cytometry (CellTechnology, Inc. Mountain View, CA); and CytoTox 96® non-radioactive cytotoxicity assay (Promega, Madison, WI)). Useful effector cells for such assays include peripheral blood mononuclear cells (PBMC) and Natural Killer (NK) cells. Alternatively, or additionally, ADCC activity of the molecule of interest may be assessed *in vivo*, e.g. in a animal model such as that disclosed in Clynes et al., Proc Natl Acad Sci USA 95, 652-656 (1998).

In some embodiments binding of the Fc domain to a complement component, specifically to C1q, is altered. Accordingly, in some embodiments wherein the Fc domain is engineered to have altered effector function, said altered effector function includes altered CDC. C1q binding assays may be carried out to determine whether the immunoconjugate is able to bind C1q and hence has CDC activity. See e.g., C1q and C3c binding ELISA in WO 2006/029879 and WO 2005/100402. To assess complement activation, a CDC assay may be performed (see, for example, Gazzano-Santoro et al., J Immunol Methods 202, 163 (1996); Cragg et al., Blood 101, 1045-1052 (2003); and Cragg and Glennie, Blood 103, 2738-2743 (2004)).

30 *a) Decreased Fc receptor binding and/or effector function*

The Fc domain confers to the immunoconjugate favorable pharmacokinetic properties, including a long serum half-life which contributes to good accumulation in the target tissue and a favorable tissue-blood distribution ratio. At the same time it may, however, lead to undesirable targeting of

the immunoconjugate to cells expressing Fc receptors rather than to the preferred antigen-bearing cells. Moreover, the co-activation of Fc receptor signaling pathways may lead to cytokine release which, in combination with the effector moiety and the long half-life of the immunoconjugate, results in excessive activation of cytokine receptors and severe side effects upon systemic administration. In line with this, conventional IgG-IL-2 immunoconjugates have been described to be associated with infusion reactions (see e.g. King et al., J Clin Oncol 22, 4463-4473 (2004)).

Accordingly, in particular embodiments according to the invention the Fc domain of the immunoconjugate is engineered to have reduced binding affinity to an Fc receptor. In one such embodiment the Fc domain comprises one or more amino acid mutation that reduces the binding affinity of the Fc domain to an Fc receptor. Typically, the same one or more amino acid mutation is present in each of the two subunits of the Fc domain. In one embodiment said amino acid mutation reduces the binding affinity of the Fc domain to the Fc receptor by at least 2-fold, at least 5-fold, or at least 10-fold. In embodiments where there is more than one amino acid mutation that reduces the binding affinity of the Fc domain to the Fc receptor, the combination of these amino acid mutations may reduce the binding affinity of the Fc domain to the Fc receptor by at least 10-fold, at least 20-fold, or even at least 50-fold. In one embodiment the immunoconjugate comprising an engineered Fc domain exhibits less than 20%, particularly less than 10%, more particularly less than 5% of the binding affinity to an Fc receptor as compared to an immunoconjugate comprising a non-engineered Fc domain. In one embodiment the Fc receptor is an activating Fc receptor. In a specific embodiment the Fc receptor is an Fc γ receptor, more specifically an Fc γ RIIIa, Fc γ RI or Fc γ RIIa receptor. Preferably, binding to each of these receptors is reduced. In some embodiments binding affinity to a complement component, specifically binding affinity to C1q, is also reduced. In one embodiment binding affinity to neonatal Fc receptor (FcRn) is not reduced. Substantially similar binding to FcRn, i.e. preservation of the binding affinity of the Fc domain to said receptor, is achieved when the Fc domain (or the immunoconjugate comprising said Fc domain) exhibits greater than about 70% of the binding affinity of a non-engineered form of the Fc domain (or the immunoconjugate comprising said non-engineered form of the Fc domain) to FcRn. Fc domains, or immunoconjugates of the invention comprising said Fc domains, may exhibit greater than about 80% and even greater than about 90% of such affinity. In one embodiment the amino acid mutation is an amino acid substitution. In one embodiment the Fc domain comprises an amino acid substitution at position P329. In a more specific embodiment the amino acid substitution is

P329A or P329G, particularly P329G. In one embodiment the Fc domain comprises a further amino acid substitution at a position selected from S228, E233, L234, L235, N297 and P331. In a more specific embodiment the further amino acid substitution is S228P, E233P, L234A, L235A, L235E, N297A, N297D or P331S. In a particular embodiment the Fc domain comprises amino acid substitutions at positions P329, L234 and L235. In a more particular embodiment the Fc domain comprises the amino acid mutations L234A, L235A and P329G (LALA P329G). This combination of amino acid substitutions almost completely abolishes Fc γ receptor binding of a human IgG Fc domain, as described in European patent application no. EP 11160251.2, incorporated herein by reference in its entirety. EP 11160251.2 also describes methods of preparing such mutant Fc domains and methods for determining its properties such as Fc receptor binding or effector functions.

Mutant Fc domains can be prepared by amino acid deletion, substitution, insertion or modification using genetic or chemical methods well known in the art. Genetic methods may include site-specific mutagenesis of the encoding DNA sequence, PCR, gene synthesis, and the like. The correct nucleotide changes can be verified for example by sequencing.

In one embodiment the Fc domain is engineered to have decreased effector function, compared to a non-engineered Fc domain. The decreased effector function can include, but is not limited to, one or more of the following: decreased complement dependent cytotoxicity (CDC), decreased antibody-dependent cell-mediated cytotoxicity (ADCC), decreased antibody-dependent cellular phagocytosis (ADCP), decreased cytokine secretion, decreased immune complex-mediated antigen uptake by antigen-presenting cells, decreased binding to NK cells, decreased binding to macrophages, decreased binding to monocytes, decreased binding to polymorphonuclear cells, decreased direct signaling inducing apoptosis, decreased crosslinking of target-bound antibodies, decreased dendritic cell maturation, or decreased T cell priming.

In one embodiment the decreased effector function is one or more selected from the group of decreased CDC, decreased ADCC, decreased ADCP, and decreased cytokine secretion. In a particular embodiment the decreased effector function is decreased ADCC. In one embodiment the decreased ADCC is less than 20% of the ADCC induced by a non-engineered Fc domain (or an immunoconjugate comprising a non-engineered Fc domain).

In addition to the Fc domains described hereinabove and in European patent application no. EP 11160251.2, Fc domains with reduced Fc receptor binding and/or effector function also include those with substitution of one or more of Fc domain residues 238, 265, 269, 270, 297, 327 and 329 (U.S. Patent No. 6,737,056). Such Fc mutants include Fc mutants with substitutions at two

or more of amino acid positions 265, 269, 270, 297 and 327, including the so-called "DANA" Fc mutant with substitution of residues 265 and 297 to alanine (US Patent No. 7,332,581).

IgG₄ antibodies exhibit reduced binding affinity to Fc receptors and reduced effector functions as compared to IgG₁ antibodies. Hence, in some embodiments the Fc domain of the T cell activating bispecific antigen binding molecules of the invention is an IgG₄ Fc domain, particularly a human IgG₄ Fc domain. In one embodiment the IgG₄ Fc domain comprises amino acid substitutions at position S228, specifically the amino acid substitution S228P. To further reduce its binding affinity to an Fc receptor and/or its effector function, in one embodiment the IgG₄ Fc domain comprises an amino acid substitution at position L235, specifically the amino acid substitution L235E. In another embodiment, the IgG₄ Fc domain comprises an amino acid substitution at position P329, specifically the amino acid substitution P329G. In a particular embodiment, the IgG₄ Fc domain comprises amino acid substitutions at positions S228, L235 and P329, specifically amino acid substitutions S228P, L235E and P329G. Such IgG₄ Fc domain mutants and their Fcγ receptor binding properties are described in European patent application no. EP 11160251.2, incorporated herein by reference in its entirety.

b) Increased Fc receptor binding and/or effector function

Conversely, there may be situations where it is desirable to maintain or even enhance Fc receptor binding and/or effector functions of immunoconjugates, for example when the immunoconjugate is targeted to a highly specific tumor antigen. Hence, in certain embodiments the Fc domain of the immunoconjugates of the invention is engineered to have increased binding affinity to an Fc receptor. Increased binding affinity may be an increase in the binding affinity of the Fc domain to the Fc receptor by at least 2-fold, at least 5-fold, or at least 10-fold. In one embodiment the Fc receptor is an activating Fc receptor. In a specific embodiment the Fc receptor is an Fcγ receptor. In one embodiment the Fc receptor is selected from the group of FcγRIIIa, FcγRI and FcγRIIa. In a particular embodiment the Fc receptor is FcγRIIIa.

In one such embodiment the Fc domain is engineered to have an altered oligosaccharide structure compared to a non-engineered Fc domain. In a particular such embodiment the Fc domain comprises an increased proportion of non-fucosylated oligosaccharides, compared to a non-engineered Fc domain. In a more specific embodiment, at least about 20%, about 25%, about 30%, about 35%, about 40%, about 45%, about 50%, about 55%, about 60%, about 65%, about 70%, about 75%, about 80%, about 85%, about 90%, about 95%, or about 100%, particularly at least about 50%, more particularly at least about 70%, of the N-linked

oligosaccharides in the Fc domain of the immunoconjugate are non-fucosylated. The non-fucosylated oligosaccharides may be of the hybrid or complex type. In another specific embodiment the Fc domain comprises an increased proportion of bisected oligosaccharides, compared to a non-engineered Fc domain. In a more specific embodiment, at least about 10%,
5 about 15%, about 20%, about 25%, about 30%, about 35%, about 40%, about 45%, about 50%, about 55%, about 60%, about 65%, about 70%, about 75%, about 80%, about 85%, about 90%, about 95%, or about 100%, particularly at least about 50%, more particularly at least about 70%, of the N-linked oligosaccharides in the Fc domain of the immunoconjugate are bisected. The bisected oligosaccharides may be of the hybrid or complex type. In yet another specific
10 embodiment the Fc domain comprises an increased proportion of bisected, non-fucosylated oligosaccharides, compared to a non-engineered Fc domain. In a more specific embodiment, at least about 10%, about 15%, about 20%, about 25%, about 30%, about 35%, about 40%, about 45%, about 50%, about 55%, about 60%, about 65%, about 70%, about 75%, about 80%, about 85%, about 90%, about 95%, or about 100%, particularly at least about 15%, more particularly at
15 least about 25%, at least about 35% or at least about 50%, of the N-linked oligosaccharides in the Fc domain of the immunoconjugate are bisected, non-fucosylated. The bisected, non-fucosylated oligosaccharides may be of the hybrid or complex type.

The oligosaccharide structures in the immunoconjugate Fc domain can be analysed by methods well known in the art, e.g. by MALDI TOF mass spectrometry as described in Umana et al., Nat
20 Biotechnol 17, 176-180 (1999) or Ferrara et al., Biotechn Bioeng 93, 851-861 (2006). The percentage of non-fucosylated oligosaccharides is the amount of oligosaccharides lacking fucose residues, relative to all oligosaccharides attached to Asn 297 (e.g. complex, hybrid and high mannose structures) and identified in an N-glycosidase F treated sample by MALDI TOF MS. Asn 297 refers to the asparagine residue located at about position 297 in the Fc domain (EU
25 numbering of Fc region residues); however, Asn297 may also be located about ± 3 amino acids upstream or downstream of position 297, i.e., between positions 294 and 300, due to minor sequence variations in immunoglobulins. The percentage of bisected, or bisected non-fucosylated, oligosaccharides is determined analogously.

Modification of the glycosylation in the Fc domain of the immunoconjugate may result from
30 production of the immunoconjugate in a host cell that has been manipulated to express altered levels of one or more polypeptides having glycosyltransferase activity.

In one embodiment the Fc domain of the immunoconjugate is engineered to have an altered oligosaccharide structure, as compared to a non-engineered Fc domain, by producing the

immunoconjugate in a host cell having altered activity of one or more glycosyltransferase. Glycosyltransferases include for example $\beta(1,4)$ -N-acetylglucosaminyltransferase III (GnTIII), $\beta(1,4)$ -galactosyltransferase (GalT), $\beta(1,2)$ -N-acetylglucosaminyltransferase I (GnTI), $\beta(1,2)$ -N-acetylglucosaminyltransferase II (GnTII) and $\alpha(1,6)$ -fucosyltransferase. In a specific embodiment the Fc domain of the immunoconjugate is engineered to comprise an increased proportion of non-fucosylated oligosaccharides, as compared to a non-engineered Fc domain, by producing the immunoconjugate in a host cell having increased $\beta(1,4)$ -N-acetylglucosaminyltransferase III (GnTIII) activity. In an even more specific embodiment the host cell additionally has increased α -mannosidase II (ManII) activity. The glycoengineering methodology that can be used for glycoengineering immunoconjugates of the present invention has been described in greater detail in Umana et al., Nat Biotechnol 17, 176-180 (1999); Ferrara et al., Biotechn Bioeng 93, 851-861 (2006); WO 99/54342 (U.S. Pat. No. 6,602,684; EP 1071700); WO 2004/065540 (U.S. Pat. Appl. Publ. No. 2004/0241817; EP 1587921), WO 03/011878 (U.S. Pat. Appl. Publ. No. 2003/0175884), the content of each of which is expressly incorporated herein by reference in its entirety.

Generally, any type of cultured cell line, including the cell lines discussed herein, can be used to generate cell lines for the production of immunoconjugates with altered glycosylation pattern. Particular cell lines include CHO cells, BHK cells, NS0 cells, SP2/0 cells, YO myeloma cells, P3X63 mouse myeloma cells, PER cells, PER.C6 cells or hybridoma cells, and other mammalian cells. In certain embodiments, the host cells have been manipulated to express increased levels of one or more polypeptides having $\beta(1,4)$ -N-acetylglucosaminyltransferase III (GnTIII) activity. In certain embodiments the host cells have been further manipulated to express increased levels of one or more polypeptides having α -mannosidase II (ManII) activity. In a specific embodiment, the polypeptide having GnTIII activity is a fusion polypeptide comprising the catalytic domain of GnTIII and the Golgi localization domain of a heterologous Golgi resident polypeptide. Particularly, said Golgi localization domain is the Golgi localization domain of mannosidase II. Methods for generating such fusion polypeptides and using them to produce antibodies with increased effector functions are disclosed in Ferrara et al., Biotechn Bioeng 93, 851-861 (2006) and WO 2004/065540, the entire contents of which are expressly incorporated herein by reference.

The host cells which contain a coding sequence of an immunoconjugate of the invention and/or a coding sequence of a polypeptide having glycosyltransferase activity, and which express the biologically active gene products, may be identified e.g. by DNA-DNA or DNA-RNA

hybridization, the presence or absence of "marker" gene functions, assessing the level of transcription as measured by the expression of the respective mRNA transcripts in the host cell, or detection of the gene product as measured by immunoassay or by its biological activity - methods which are well known in the art. GnTIII or Man II activity can be detected e.g. by
5 employing a lectin which binds to biosynthesis products of GnTIII or ManII, respectively. An example for such a lectin is the E₄-PHA lectin which binds preferentially to oligosaccharides containing bisecting GlcNAc. Biosynthesis products (i.e. specific oligosaccharide structures) of polypeptides having GnTIII or ManII activity can also be detected by mass spectrometric analysis of oligosaccharides released from glycoproteins produced by cells expressing said
10 polypeptides. Alternatively, a functional assay which measures the increased effector function and/or increased Fc receptor binding, mediated by immunoconjugates produced by the cells engineered with the polypeptide having GnTIII or ManII activity may be used.

In another embodiment the Fc domain is engineered to comprise an increased proportion of non-fucosylated oligosaccharides, as compared to a non-engineered Fc domain, by producing the
15 immunoconjugate in a host cell having decreased $\alpha(1,6)$ -fucosyltransferase activity. A host cell having decreased $\alpha(1,6)$ -fucosyltransferase activity may be a cell in which the $\alpha(1,6)$ -fucosyltransferase gene has been disrupted or otherwise deactivated, e.g. knocked out (see Yamane-Ohnuki et al., Biotech Bioeng 87, 614 (2004); Kanda et al., Biotechnol Bioeng 94(4), 680-688 (2006); Niwa et al., J Immunol Methods 306, 151-160 (2006)).

20 Other examples of cell lines capable of producing defucosylated immunoconjugates include Lec13 CHO cells deficient in protein fucosylation (Ripka et al., Arch Biochem Biophys 249, 533-545 (1986); US Pat. Appl. No. US 2003/0157108; and WO 2004/056312, especially at Example 11). The immunoconjugates of the present invention can alternatively be glycoengineered to have reduced fucose residues in the Fc domain according to the techniques
25 disclosed in EP 1 176 195 A1, WO 03/084570, WO 03/085119 and U.S. Pat. Appl. Pub. Nos. 2003/0115614, 2004/093621, 2004/110282, 2004/110704, 2004/132140, US Pat. No. 6,946,292 (Kyowa), e.g. by reducing or abolishing the activity of a GDP-fucose transporter protein in the host cells used for immunoconjugate production.

Glycoengineered immunoconjugates of the invention may also be produced in expression
30 systems that produce modified glycoproteins, such as those taught in WO 2003/056914 (GlycoFi, Inc.) or in WO 2004/057002 and WO 2004/024927 (Greenovation).

In one embodiment the Fc domain of the immunoconjugate is engineered to have increased effector function, compared to a non-engineered Fc domain. The increased effector function can

include, but is not limited to, one or more of the following: increased complement dependent cytotoxicity (CDC), increased antibody-dependent cell-mediated cytotoxicity (ADCC), increased antibody-dependent cellular phagocytosis (ADCP), increased cytokine secretion, increased immune complex-mediated antigen uptake by antigen-presenting cells, increased binding to NK
5 cells, increased binding to macrophages, increased binding to monocytes, increased binding to polymorphonuclear cells, increased direct signaling inducing apoptosis, increased crosslinking of target-bound antibodies, increased dendritic cell maturation, or increased T cell priming.

In one embodiment the increased effector function is one or more selected from the group of increased CDC, increased ADCC, increased ADCP, and increased cytokine secretion. In a
10 particular embodiment the increased effector function is increased ADCC. In one embodiment ADCC induced by an engineered Fc domain (or an immunoconjugate comprising an engineered Fc domain) is a least 2-fold increased as compared to ADCC induced by a non-engineered Fc domain (or an immunoconjugate comprising a non-engineered Fc domain).

15 **Effector Moieties**

The effector moieties for use in the invention are generally polypeptides that influence cellular activity, for example, through signal transduction pathways. Accordingly, the effector moiety of the immunoconjugate useful in the invention can be associated with receptor-mediated signaling that transmits a signal from outside the cell membrane to modulate a response within the cell.

20 For example, an effector moiety of the immunoconjugate can be a cytokine. In particular embodiments the effector moiety is human.

In certain embodiments the effector moiety is a single chain effector moiety. In a particular embodiment the effector moiety is a cytokine. Examples of useful cytokines include, but are not limited to, GM-CSF, IL-1 α , IL-1 β , IL-2, IL-3, IL-4, IL-5, IL-6, IL-7, IL-8, IL-10, IL-12, IL-21,
25 IFN- α , IFN- β , IFN- γ , MIP-1 α , MIP-1 β , TGF- β , TNF- α , and TNF- β . In one embodiment the effector moiety of the immunoconjugate is a cytokine selected from the group of GM-CSF, IL-2, IL-7, IL-8, IL-10, IL-12, IL-15, IL-21, IFN- α , IFN- γ , MIP-1 α , MIP-1 β and TGF- β . In one embodiment the effector moiety of the immunoconjugate is a cytokine selected from the group of IL-2, IL-7, IL-10, IL-12, IL-15, IFN- α , and IFN- γ . In certain embodiments the cytokine
30 effector moiety is mutated to remove N- and/or O-glycosylation sites. Elimination of glycosylation increases homogeneity of the product obtainable in recombinant production.

In a particular embodiment the effector moiety of the immunoconjugate is IL-2. In a specific embodiment, the IL-2 effector moiety can elicit one or more of the cellular responses selected

from the group consisting of: proliferation in an activated T lymphocyte cell, differentiation in an activated T lymphocyte cell, cytotoxic T cell (CTL) activity, proliferation in an activated B cell, differentiation in an activated B cell, proliferation in a natural killer (NK) cell, differentiation in a NK cell, cytokine secretion by an activated T cell or an NK cell, and NK/lymphocyte activated
5 killer (LAK) antitumor cytotoxicity. In another particular embodiment the IL-2 effector moiety is a mutant IL-2 effector moiety having reduced binding affinity to the α -subunit of the IL-2 receptor. Together with the β - and γ -subunits (also known as CD122 and CD132, respectively), the α -subunit (also known as CD25) forms the heterotrimeric high-affinity IL-2 receptor, while the dimeric receptor consisting only of the β - and γ -subunits is termed the intermediate-affinity
10 IL-2 receptor. As described in PCT patent application number PCT/EP2012/051991, which is incorporated herein by reference in its entirety, a mutant IL-2 polypeptide with reduced binding to the α -subunit of the IL-2 receptor has a reduced ability to induce IL-2 signaling in regulatory T cells, induces less activation-induced cell death (AICD) in T cells, and has a reduced toxicity profile in vivo, compared to a wild-type IL-2 polypeptide. The use of such an effector moiety
15 with reduced toxicity is particularly advantageous in an immunoconjugate according to the invention, having a long serum half-life due to the presence of an Fc domain. In one embodiment, the mutant IL-2 effector moiety of the immunoconjugate according to the invention comprises at least one amino acid mutation that reduces or abolishes the affinity of the mutant IL-2 effector moiety to the α -subunit of the IL-2 receptor (CD25) but preserves the affinity of the mutant IL-2
20 effector moiety to the intermediate-affinity IL-2 receptor (consisting of the β - and γ -subunits of the IL-2 receptor), compared to the non-mutated IL-2 effector moiety. In one embodiment the one or more amino acid mutations are amino acid substitutions. In a specific embodiment, the mutant IL-2 effector moiety comprises one, two or three amino acid substitutions at one, two or three position(s) selected from the positions corresponding to residue 42, 45, and 72 of human
25 IL-2. In a more specific embodiment, the mutant IL-2 effector moiety comprises three amino acid substitutions at the positions corresponding to residue 42, 45 and 72 of human IL-2. In an even more specific embodiment, the mutant IL-2 effector moiety is human IL-2 comprising the amino acid substitutions F42A, Y45A and L72G. In one embodiment the mutant IL-2 effector moiety additionally comprises an amino acid mutation at a position corresponding to position 3
30 of human IL-2, which eliminates the O-glycosylation site of IL-2. Particularly, said additional amino acid mutation is an amino acid substitution replacing a threonine residue by an alanine residue. A particular mutant IL-2 effector moiety useful in the invention comprises four amino acid substitutions at positions corresponding to residues 3, 42, 45 and 72 of human IL-2. Specific

amino acid substitutions are T3A, F42A, Y45A and L72G. As demonstrated in PCT patent application number PCT/EP2012/051991 and in the appended Examples, said quadruple mutant IL-2 polypeptide (IL-2 qm) exhibits no detectable binding to CD25, reduced ability to induce apoptosis in T cells, reduced ability to induce IL-2 signaling in T_{reg} cells, and a reduced toxicity profile in vivo. However, it retains ability to activate IL-2 signaling in effector cells, to induce proliferation of effector cells, and to generate IFN- γ as a secondary cytokine by NK cells.

The IL-2 or mutant IL-2 effector moiety according to any of the above embodiments may comprise additional mutations that provide further advantages such as increased expression or stability. For example, the cysteine at position 125 may be replaced with a neutral amino acid such as alanine, to avoid the formation of disulfide-bridged IL-2 dimers. Thus, in certain embodiments the IL-2 or mutant IL-2 effector moiety of the immunoconjugate according to the invention comprises an additional amino acid mutation at a position corresponding to residue 125 of human IL-2. In one embodiment said additional amino acid mutation is the amino acid substitution C125A.

In a specific embodiment the IL-2 effector moiety of the immunoconjugate comprises the polypeptide sequence of SEQ ID NO: 2. In another specific embodiment the IL-2 effector moiety of the immunoconjugate comprises the polypeptide sequence of SEQ ID NO: 3.

In another embodiment the effector moiety of the immunoconjugate is IL-12. In a specific embodiment said IL-12 effector moiety is a single chain IL-12 effector moiety. In an even more specific embodiment the single chain IL-12 effector moiety comprises the polypeptide sequence of SEQ ID NO: 4. In one embodiment, the IL-12 effector moiety can elicit one or more of the cellular responses selected from the group consisting of: proliferation in a NK cell, differentiation in a NK cell, proliferation in a T cell, and differentiation in a T cell.

In another embodiment the effector moiety of the immunoconjugate is IL-10. In a specific embodiment said IL-10 effector moiety is a single chain IL-10 effector moiety. In an even more specific embodiment the single chain IL-10 effector moiety comprises the polypeptide sequence of SEQ ID NO: 5. In another specific embodiment the IL-10 effector moiety is a monomeric IL-10 effector moiety. In a more specific embodiment the monomeric IL-10 effector moiety comprises the polypeptide sequence of SEQ ID NO: 6. In one embodiment, the IL-10 effector moiety can elicit one or more of the cellular responses selected from the group consisting of: inhibition of cytokine secretion, inhibition of antigen presentation by antigen presenting cells, reduction of oxygen radical release, and inhibition of T cell proliferation. An immunoconjugate

according to the invention wherein the effector moiety is IL-10 is particularly useful for downregulation of inflammation, e.g. in the treatment of an inflammatory disorder.

In another embodiment the effector moiety of the immunoconjugate is IL-15. In a specific embodiment said IL-15 effector moiety is a mutant IL-15 effector moiety having reduced
5 binding affinity to the α -subunit of the IL-15 receptor. Without wishing to be bound by theory, a mutant IL-15 polypeptide with reduced binding to to the α -subunit of the IL-15 receptor has a reduced ability to bind to fibroblasts throughout the body, resulting in improved pharmacokinetics and toxicity profile, compared to a wild-type IL-15 polypeptide. The use of an effector moiety with reduced toxicity, such as the described mutant IL-2 and mutant IL-15
10 effector moieties, is particularly advantageous in an immunoconjugate according to the invention, having a long serum half-life due to the presence of an Fc domain. In one embodiment the mutant IL-15 effector moiety of the immunoconjugate according to the invention comprises at least one amino acid mutation that reduces or abolishes the affinity of the mutant IL-15 effector moiety to the α -subunit of the IL-15 receptor but preserves the affinity of the mutant IL-15
15 effector moiety to the intermediate-affinity IL-15/IL-2 receptor (consisting of the β - and γ -subunits of the IL-15/IL-2 receptor), compared to the non-mutated IL-15 effector moiety. In one embodiment the amino acid mutation is an amino acid substitution. In a specific embodiment, the mutant IL-15 effector moiety comprises an amino acid substitution at the position corresponding to residue 53 of human IL-15. In a more specific embodiment, the mutant IL-15
20 effector moiety is human IL-15 comprising the amino acid substitution E53A. In one embodiment the mutant IL-15 effector moiety additionally comprises an amino acid mutation at a position corresponding to position 79 of human IL-15, which eliminates the N-glycosylation site of IL-15. Particularly, said additional amino acid mutation is an amino acid substitution replacing an asparagine residue by an alanine residue. In an even more specific embodiment the
25 IL-15 effector moiety comprises the polypeptide sequence of SEQ ID NO: 7. In one embodiment, the IL-15 effector moiety can elicit one or more of the cellular responses selected from the group consisting of: proliferation in an activated T lymphocyte cell, differentiation in an activated T lymphocyte cell, cytotoxic T cell (CTL) activity, proliferation in an activated B cell, differentiation in an activated B cell, proliferation in a natural killer (NK) cell, differentiation in
30 a NK cell, cytokine secretion by an activated T cell or an NK cell, and NK/lymphocyte activated killer (LAK) antitumor cytotoxicity.

Mutant cytokine molecules useful as effector moieties in the immunoconjugates can be prepared by deletion, substitution, insertion or modification using genetic or chemical methods well

known in the art. Genetic methods may include site-specific mutagenesis of the encoding DNA sequence, PCR, gene synthesis, and the like. The correct nucleotide changes can be verified for example by sequencing. Substitution or insertion may involve natural as well as non-natural amino acid residues. Amino acid modification includes well known methods of chemical
5 modification such as the addition or removal of glycosylation sites or carbohydrate attachments, and the like.

In one embodiment, the effector moiety, particularly a single-chain effector moiety, of the immunoconjugate is GM-CSF. In a specific embodiment, the GM-CSF effector moiety can elicit proliferation and/or differentiation in a granulocyte, a monocyte or a dendritic cell. In one
10 embodiment, the effector moiety, particularly a single-chain effector moiety, of the immunoconjugate is IFN- α . In a specific embodiment, the IFN- α effector moiety can elicit one or more of the cellular responses selected from the group consisting of: inhibiting viral replication in a virus-infected cell, and upregulating the expression of major histocompatibility complex I (MHC I). In another specific embodiment, the IFN- α effector moiety can inhibit
15 proliferation in a tumor cell. In one embodiment the effector moiety, particularly a single-chain effector moiety, of the immunoconjugate is IFN- γ . In a specific embodiment, the IFN- γ effector moiety can elicit one or more of the cellular responses selected from the group of: increased macrophage activity, increased expression of MHC molecules, and increased NK cell activity. In one embodiment the effector moiety, particularly a single-chain effector moiety, of the
20 immunoconjugate is IL-7. In a specific embodiment, the IL-7 effector moiety can elicit proliferation of T and/or B lymphocytes. In one embodiment, the effector moiety, particularly a single-chain effector moiety, of the immunoconjugate is IL-8. In a specific embodiment, the IL-8 effector moiety can elicit chemotaxis in neutrophils. In one embodiment, the effector moiety, particularly a single-chain effector moiety, of the immunoconjugate, is MIP-1 α . In a specific
25 embodiment, the MIP-1 α effector moiety can elicit chemotaxis in monocytes and T lymphocyte cells. In one embodiment, the effector moiety, particularly a single-chain effector moiety, of the immunoconjugate is MIP-1 β . In a specific embodiment, the MIP-1 β effector moiety can elicit chemotaxis in monocytes and T lymphocyte cells. In one embodiment, the effector moiety, particularly a single-chain effector moiety, of the immunoconjugate is TGF- β . In a specific
30 embodiment, the TGF- β effector moiety can elicit one or more of the cellular responses selected from the group consisting of: chemotaxis in monocytes, chemotaxis in macrophages, upregulation of IL-1 expression in activated macrophages, and upregulation of IgA expression in activated B cells.

In one embodiment, the immunoconjugate of the invention binds to an effector moiety receptor with a dissociation constant (K_D) that is at least about 1, 1.5, 2, 2.5, 3, 3.5, 4, 4.5, 5, 5.5, 6, 6.5, 7, 7.5, 8, 8.5, 9, 9.5 or 10 times greater than that for a control effector moiety. In another embodiment, the immunoconjugate binds to an effector moiety receptor with a K_D that is at least
 5 2, 3, 4, 5, 6, 7, 8, 9, or 10 times greater than that for a corresponding immunoconjugate molecule comprising two or more effector moieties. In another embodiment, the immunoconjugate binds to an effector moiety receptor with a dissociation constant K_D that is about 10 times greater than that for a corresponding immunoconjugate molecule comprising two or more effector moieties.

10 Antigen Binding Moieties

The immunoconjugates of the invention comprise at least one antigen binding moiety. In particular embodiments, the immunoconjugates comprises two antigen binding moieties, i.e. a first and a second antigen binding moiety. In one embodiment the immunoconjugate comprises not more than two antigen binding moieties.

15 The antigen binding moiety of the immunoconjugate of the invention is generally a polypeptide molecule that binds to a specific antigenic determinant and is able to direct the entity to which it is attached (e.g. an effector moiety and an Fc domain) to a target site, for example to a specific type of tumor cell or tumor stroma that bears the antigenic determinant. The immunoconjugate can bind to antigenic determinants found, for example, on the surfaces of tumor cells, on the
 20 surfaces of virus-infected cells, on the surfaces of other diseased cells, free in blood serum, and/or in the extracellular matrix (ECM).

In certain embodiments the antigen binding moiety is directed to an antigen associated with a pathological condition, such as an antigen presented on a tumor cell or in a tumor cell environment, at a site of inflammation, or on a virus-infected cell.

25 Non-limiting examples of tumor antigens include MAGE, MART-1/Melan-A, gp100, Dipeptidyl peptidase IV (DPPIV), adenosine deaminase-binding protein (ADAbp), cyclophilin b, Colorectal associated antigen (CRC)-C017-1A/GA733, Carcinoembryonic Antigen (CEA) and its immunogenic epitopes CAP-1 and CAP-2, etv6, aml1, Prostate Specific Antigen (PSA) and its immunogenic epitopes PSA-1, PSA-2, and PSA-3, prostate-specific membrane antigen (PSMA),
 30 MAGE-family of tumor antigens (e.g., MAGE-A1, MAGE-A2, MAGE-A3, MAGE-A4, MAGE-A5, MAGE-A6, MAGE-A7, MAGE-A8, MAGE-A9, MAGE-A10, MAGE-A11, MAGE-A12, MAGE-Xp2 (MAGE-B2), MAGE-Xp3 (MAGE-B3), MAGE-Xp4 (MAGE-B4), MAGE-C1, MAGE-C2, MAGE-C3, MAGE-C4, MAGE-C5), GAGE-family of tumor antigens

(e.g., GAGE-1, GAGE-2, GAGE-3, GAGE-4, GAGE-5, GAGE-6, GAGE-7, GAGE-8, GAGE-9), BAGE, RAGE, LAGE-1, NAG, GnT-V, MUM-1, CDK4, tyrosinase, p53, MUC family, HER2/neu, p21ras, RCAS1, α -fetoprotein, E-cadherin, α -catenin, β -catenin and γ -catenin, p120ctn, gp100 Pmel117, 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 papilloma virus proteins, Smad family of tumor antigens, 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.

Non-limiting examples of viral antigens include influenza virus hemagglutinin, Epstein-Barr virus LMP-1, hepatitis C virus E2 glycoprotein, HIV gp160, and HIV gp120.

Non-limiting examples of ECM antigens include syndecan, heparanase, integrins, osteopontin, link, cadherins, laminin, laminin type EGF, lectin, fibronectin, notch, tenascin, and matrixin.

The immunoconjugates of the invention can bind to the following specific non-limiting examples of cell surface antigens: FAP, Her2, EGFR, IGF-1R, CD22 (B-cell receptor), CD23 (low affinity IgE receptor), CD30 (cytokine receptor), CD33 (myeloid cell surface antigen), CD40 (tumor necrosis factor receptor), IL-6R (IL6 receptor), CD20, MCSP, and PDGF β R (β platelet-derived growth factor receptor). In particular embodiments the antigen is a human antigen.

In certain embodiments the antigen-binding moiety is directed to an antigen presented on a tumor cell or in a tumor cell environment. In other embodiments the antigen binding moiety is directed to an antigen presented at a site of inflammation. In a specific embodiment the antigen-binding moiety is directed to an antigen selected from the group of Fibroblast Activation Protein (FAP), the A1 domain of Tenascin-C (TNC A1), the A2 domain of Tenascin-C (TNC A2), the Extra Domain B of Fibronectin (EDB), Carcinoembryonic Antigen (CEA), and Melanoma-associated Chondroitin Sulfate Proteoglycan (MCSP).

In one embodiment, the immunoconjugate of the invention comprises two or more antigen binding moieties, wherein each of these antigen binding moieties specifically binds to the same antigenic determinant.

The antigen binding moiety can be any type of antibody or fragment thereof that retains specific binding to an antigenic determinant. Antibody fragments include, but are not limited to, V_H fragments, V_L fragments, Fab fragments, $F(ab')_2$ fragments, scFv fragments, Fv fragments, minibodies, diabodies, triabodies, and tetrabodies (see e.g. Hudson and Souriau, Nature Med 9, 129-134 (2003)). In a particular embodiment the antigen binding moiety is a Fab molecule. In one embodiment said Fab molecule is human. In another embodiment said Fab molecule is

humanized. In yet another embodiment said Fab molecule comprises human heavy and light chain constant regions.

In one embodiment the immunoconjugate comprises at least one, typically two or more antigen binding moieties that are specific for the Extra Domain B of fibronectin (EDB). In another
5 embodiment the immunoconjugate comprises at least one, typically two or more antigen binding moieties that can compete with monoclonal antibody L19 for binding to an epitope of EDB. See, e.g., PCT publication WO 2007/128563 A1 (incorporated herein by reference in its entirety).

In yet another embodiment the immunoconjugate comprises a polypeptide sequence wherein a Fab heavy chain derived from the L19 monoclonal antibody shares a carboxy-terminal peptide
10 bond with an Fc domain subunit comprising a knob modification, which in turn shares a carboxy-terminal peptide bond with an IL-2 polypeptide. In a more specific embodiment the immunoconjugate comprises the polypeptide sequence of SEQ ID NO: 215 or a variant thereof that retains functionality. In one embodiment the immunoconjugate comprises a polypeptide sequence wherein a Fab heavy chain derived from the L19 monoclonal antibody shares a
15 carboxy-terminal peptide bond with an Fc domain subunit comprising a hole modification. In a more specific embodiment the immunoconjugate comprises the polypeptide sequence of SEQ ID NO: 213 or a variant thereof that retains functionality. In another embodiment the immunoconjugate comprises a Fab light chain derived from the L19 monoclonal antibody. In a more specific embodiment the immunoconjugate comprises the polypeptide sequence of SEQ ID
20 NO: 217 or a variant thereof that retains functionality. In yet another embodiment the immunoconjugate comprises the polypeptide sequences of SEQ ID NO: 213, SEQ ID NO: 215 and SEQ ID NO: 217, or variants thereof that retain functionality. In another specific embodiment the polypeptides are covalently linked, e.g., by a disulfide bond. In some embodiments the Fc domain subunits each comprise the amino acid substitutions L234A,
25 L235A, and P329G.

In a specific embodiment the immunoconjugate comprises a polypeptide sequence encoded by a polynucleotide sequence that is at least about 80%, 85%, 90%, 95%, 96%, 97%, 98%, or 99% identical to the sequence of SEQ ID NO: 216. In another specific embodiment the immunoconjugate comprises a polypeptide sequence encoded by the polynucleotide sequence of
30 SEQ ID NO: 216. In another specific embodiment the immunoconjugate comprises a polypeptide sequence encoded by a polynucleotide sequence that is at least about 80%, 85%, 90%, 95%, 96%, 97%, 98%, or 99% identical to the sequence of SEQ ID NO: 214. In yet another specific embodiment the immunoconjugate comprises a polypeptide sequence encoded

by the polynucleotide sequence of SEQ ID NO: 214. In another specific embodiment the immunoconjugate comprises a polypeptide sequence encoded by a polynucleotide sequence that is at least about 80%, 85%, 90%, 95%, 96%, 97%, 98%, or 99% identical to the sequence of SEQ ID NO: 218. In yet another specific embodiment the immunoconjugate comprises a polypeptide sequence encoded by the polynucleotide sequence of SEQ ID NO: 218.

In one embodiment the immunoconjugate of the invention comprises at least one, typically two or more antigen binding moieties that are specific for the A1 domain of Tenascin C (TNC-A1). In another embodiment, the immunoconjugate comprises at least one, typically two or more antigen binding moieties that can compete with monoclonal antibody F16 for binding to an epitope of TNC-A1. See, e.g., PCT publication WO 2007/128563 A1 (incorporated herein by reference in its entirety). In one embodiment, the immunoconjugate comprises at least one, typically two or more antigen binding moieties that are specific for the A1 and/or the A4 domain of Tenascin C (TNC-A1 or TNC-A4 or TNC-A1/A4).

In a specific embodiment, the antigen binding moieties of the immunoconjugate comprise a heavy chain variable region sequence that is at least about 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99% or 100% identical to either SEQ ID NO: 33 or SEQ ID NO: 35, or variants thereof that retain functionality. In another specific embodiment, the antigen binding moieties of the immunoconjugate comprise a light chain variable region sequence that is at least about 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99% or 100% identical to either SEQ ID NO: 29 or SEQ ID NO: 31, or variants thereof that retain functionality. In a more specific embodiment, the antigen binding moieties of the immunoconjugate comprise a heavy chain variable region sequence that is at least about 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99% or 100% identical to either SEQ ID NO: 33 or SEQ ID NO: 35 or variants thereof that retain functionality, and a light chain variable region sequence that is at least about 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99% or 100% identical to either SEQ ID NO: 29 or SEQ ID NO: 31 or variants thereof that retain functionality.

In another specific embodiment, the heavy chain variable region sequence of the antigen binding moieties of the immunoconjugate is encoded by a polynucleotide sequence that is at least about 80%, 85%, 90%, 95%, 96%, 97%, 98%, or 99% identical to either SEQ ID NO: 34 or SEQ ID NO: 36. In yet another specific embodiment, the heavy chain variable region sequence of the antigen binding moieties of the immunoconjugate is encoded by the polynucleotide sequence of either SEQ ID NO: 34 or SEQ ID NO: 36. In another specific embodiment, the light chain variable region sequence of the antigen binding moieties of the immunoconjugate is encoded by a polynucleotide sequence that is at least about 80%, 85%, 90%, 95%, 96%, 97%, 98%, or 99%

identical to either SEQ ID NO: 30 or SEQ ID NO: 32. In yet another specific embodiment, the light chain variable region sequence of the antigen binding moieties of the immunoconjugate is encoded by the polynucleotide sequence of either SEQ ID NO: 30 or SEQ ID NO: 32.

- In one embodiment, the immunoconjugate comprises a polypeptide sequence wherein a Fab heavy chain specific for the A1 domain of Tenascin C shares a carboxy-terminal peptide bond with an Fc domain subunit comprising a knob modification, which in turn shares a carboxy-terminal peptide bond with an IL-2 polypeptide. In another embodiment, the immunoconjugate comprises a polypeptide sequence wherein a Fab heavy chain specific for the A1 domain of Tenascin C shares a carboxy-terminal peptide bond with an Fc domain subunit comprising a hole modification. In a more specific embodiment the immunoconjugate comprises both of these polypeptide sequences. In another embodiment, the immunoconjugate further comprises a Fab light chain specific for the A1 domain of Tenascin C. In another specific embodiment, the polypeptides are covalently linked, e.g., by a disulfide bond. In some embodiments the Fc domain subunits each comprise the amino acid substitutions L234A, L235A, and P329G.
- 15 In a particular embodiment, the immunoconjugate comprises at least one, typically two or more antigen binding moieties that are specific for the A2 domain of Tenascin C (TNC-A2). In a specific embodiment, the antigen binding moieties of the immunoconjugate comprise a heavy chain variable region sequence that is at least about 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99% or 100% identical to a sequence selected from the group of SEQ ID NO: 27, SEQ ID NO: 159, SEQ ID NO: 163, SEQ ID NO: 167, SEQ ID NO: 171, SEQ ID NO: 175, SEQ ID NO: 179, SEQ ID NO: 183 and SEQ ID NO: 187, or variants thereof that retain functionality. In another specific embodiment, the antigen binding moieties of the immunoconjugate comprise a light chain variable region sequence that is at least about 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99% or 100% identical to a sequence selected from the group of SEQ ID NO: 23, SEQ ID NO: 25; SEQ ID NO: 157, SEQ ID NO: 161, SEQ ID NO: 165, SEQ ID NO: 169, SEQ ID NO: 173, SEQ ID NO: 177, SEQ ID NO: 181 and SEQ ID NO: 185, or variants thereof that retain functionality.
- 25 In a more specific embodiment, the antigen binding moieties of the immunoconjugate comprise a heavy chain variable region sequence that is at least about 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99% or 100% identical to a sequence selected from the group of SEQ ID NO: 27, SEQ ID NO: 159, SEQ ID NO: 163, SEQ ID NO: 167, SEQ ID NO: 171, SEQ ID NO: 175, SEQ ID NO: 179, SEQ ID NO: 183 and SEQ ID NO: 187, or variants thereof that retain functionality, and a light chain variable region sequence that is at least about 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99% or 100% identical to a sequence selected from the group of SEQ ID NO: 23, SEQ ID NO:
- 30

25; SEQ ID NO: 157, SEQ ID NO: 161, SEQ ID NO: 165, SEQ ID NO: 169, SEQ ID NO: 173, SEQ ID NO: 177, SEQ ID NO: 181 and SEQ ID NO: 185, or variants thereof that retain functionality. In a particular embodiment, the antigen binding moieties of the immunoconjugate comprise the heavy chain variable region sequence of SEQ ID NO: 27 and the light chain
5 variable region sequence of SEQ ID NO: 25.

In another specific embodiment, the heavy chain variable region sequence of the antigen binding moieties of the immunoconjugate is encoded by a polynucleotide sequence that is at least about 80%, 85%, 90%, 95%, 96%, 97%, 98%, or 99% identical to a sequence selected from the group of SEQ ID NO: 28, SEQ ID NO: 160, SEQ ID NO: 164, SEQ ID NO: 168, SEQ ID NO: 172,
10 SEQ ID NO: 176, SEQ ID NO: 180, SEQ ID NO: 184 and SEQ ID NO: 188. In yet another specific embodiment, the heavy chain variable region sequence of the antigen binding moieties of the immunoconjugate is encoded by a polynucleotide sequence selected from the group of SEQ ID NO: 28, SEQ ID NO: 160, SEQ ID NO: 164, SEQ ID NO: 168, SEQ ID NO: 172, SEQ ID NO: 176, SEQ ID NO: 180, SEQ ID NO: 184 and SEQ ID NO: 188. In another specific
15 embodiment, the light chain variable region sequence of the antigen binding moieties of the immunoconjugate is encoded by a polynucleotide sequence that is at least about 80%, 85%, 90%, 95%, 96%, 97%, 98%, or 99% identical to a sequence selected from the group of SEQ ID NO: 24, SEQ ID NO: 26, SEQ ID NO: 158, SEQ ID NO: 162, SEQ ID NO: 166, SEQ ID NO: 170, SEQ ID NO: 174, SEQ ID NO: 178, SEQ ID NO: 182 and SEQ ID NO: 186. In yet another
20 specific embodiment, the light chain variable region sequence of the antigen binding moieties of the immunoconjugate is encoded by a polynucleotide sequence selected from the group of SEQ ID NO: 24, SEQ ID NO: 26, SEQ ID NO: 158, SEQ ID NO: 162, SEQ ID NO: 166, SEQ ID NO: 170, SEQ ID NO: 174, SEQ ID NO: 178, SEQ ID NO: 182 and SEQ ID NO: 186.

In yet another embodiment, the immunoconjugate comprises a polypeptide sequence wherein a
25 Fab heavy chain specific for the A2 domain of Tenascin C shares a carboxy-terminal peptide bond with an Fc domain subunit comprising a hole modification, which in turn shares a carboxy-terminal peptide bond with an IL-10 polypeptide. In a more specific embodiment, the immunoconjugate comprises the polypeptide sequence of SEQ ID NO: 235 or SEQ ID NO: 237, or a variant thereof that retains functionality. In one embodiment the immunoconjugate
30 comprises a polypeptide sequence wherein a Fab heavy chain specific for the A2 domain of Tenascin C shares a carboxy-terminal peptide bond with an Fc domain subunit comprising a knob modification. In a more specific embodiment, the immunoconjugate comprises the polypeptide sequence of SEQ ID NO: 233 or a variant thereof that retains functionality. In

another embodiment, the immunoconjugate comprises a Fab light chain specific for the A2 domain of Tenascin C. In a more specific embodiment, the immunoconjugate comprises the polypeptide sequence of SEQ ID NO: 239 or a variant thereof that retains functionality. In another embodiment, the immunoconjugate comprises the polypeptide sequences of SEQ ID NO: 233, SEQ ID NO: 235 and SEQ ID NO: 239 or variants thereof that retain functionality. In yet another embodiment, the immunoconjugate comprises the polypeptide sequences of SEQ ID NO: 233, SEQ ID NO: 237 and SEQ ID NO: 239 or variants thereof that retain functionality. In another specific embodiment, the polypeptides are covalently linked, e.g., by a disulfide bond. In some embodiments the Fc domain subunits each comprise the amino acid substitutions L234A, L235A, and P329G.

In a specific embodiment, the immunoconjugate comprises a polypeptide sequence encoded by a polynucleotide sequence that is at least about 80%, 85%, 90%, 95%, 96%, 97%, 98%, or 99% identical to the sequence of SEQ ID NO: 236 or SEQ ID NO: 238. In another specific embodiment, the immunoconjugate comprises a polypeptide sequence encoded by the polynucleotide sequence of SEQ ID NO: 236 or SEQ ID NO: 238. In another specific embodiment, the immunoconjugate comprises a polypeptide sequence encoded by a polynucleotide sequence that is at least about 80%, 85%, 90%, 95%, 96%, 97%, 98%, or 99% identical to the sequence of SEQ ID NO: 234. In yet another specific embodiment, the immunoconjugate comprises a polypeptide sequence encoded by the polynucleotide sequence of SEQ ID NO: 234. In another specific embodiment, the immunoconjugate comprises a polypeptide sequence encoded by a polynucleotide sequence that is at least about 80%, 85%, 90%, 95%, 96%, 97%, 98%, or 99% identical to the sequence of SEQ ID NO: 240. In yet another specific embodiment, the immunoconjugate comprises a polypeptide sequence encoded by the polynucleotide sequence of SEQ ID NO: 240.

In yet another embodiment, the immunoconjugate comprises a polypeptide sequence wherein a Fab heavy chain specific for the A2 domain of Tenascin C shares a carboxy-terminal peptide bond with an Fc domain subunit comprising a knob modification, which in turn shares a carboxy-terminal peptide bond with an IL-2 polypeptide. In a more specific embodiment, the immunoconjugate comprises the polypeptide sequence of SEQ ID NO: 285, or a variant thereof that retains functionality. In one embodiment the immunoconjugate comprises a polypeptide sequence wherein a Fab heavy chain specific for the A2 domain of Tenascin C shares a carboxy-terminal peptide bond with an Fc domain subunit comprising a hole modification. In a more specific embodiment, the immunoconjugate comprises the polypeptide sequence of SEQ ID NO:

287, or a variant thereof that retains functionality. In another embodiment, the immunoconjugate comprises a Fab light chain specific for the A2 domain of Tenascin C. In a more specific embodiment, the immunoconjugate comprises the polypeptide sequence of SEQ ID NO: 239 or a variant thereof that retains functionality. In another embodiment, the immunoconjugate
5 comprises the polypeptide sequences of SEQ ID NO: 285, SEQ ID NO: 287 and SEQ ID NO: 239 or variants thereof that retain functionality. In another specific embodiment, the polypeptides are covalently linked, e.g., by a disulfide bond. In some embodiments the Fc domain subunits each comprise the amino acid substitutions L234A, L235A, and P329G.

In a specific embodiment, the immunoconjugate comprises a polypeptide sequence encoded by a
10 polynucleotide sequence that is at least about 80%, 85%, 90%, 95%, 96%, 97%, 98%, or 99% identical to the sequence of SEQ ID NO: 286. In another specific embodiment, the immunoconjugate comprises a polypeptide sequence encoded by the polynucleotide sequence of SEQ ID NO: 286. In another specific embodiment, the immunoconjugate comprises a polypeptide sequence encoded by a polynucleotide sequence that is at least about 80%, 85%,
15 90%, 95%, 96%, 97%, 98%, or 99% identical to the sequence of SEQ ID NO: 288. In yet another specific embodiment, the immunoconjugate comprises a polypeptide sequence encoded by the polynucleotide sequence of SEQ ID NO: 288. In another specific embodiment, the immunoconjugate comprises a polypeptide sequence encoded by a polynucleotide sequence that is at least about 80%, 85%, 90%, 95%, 96%, 97%, 98%, or 99% identical to the sequence of
20 SEQ ID NO: 240. In yet another specific embodiment, the immunoconjugate comprises a polypeptide sequence encoded by the polynucleotide sequence of SEQ ID NO: 240.

In a particular embodiment, the immunoconjugate comprises at least one, typically two or more antigen binding moieties that are specific for the Fibroblast Activated Protein (FAP). In a specific embodiment, the antigen binding moieties of the immunoconjugate comprise a heavy
25 chain variable region sequence that is at least about 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99% or 100% identical to a sequence selected from the group consisting of SEQ ID NO: 41, SEQ ID NO: 45, SEQ ID NO: 47, SEQ ID NO: 51, SEQ ID NO: 55, SEQ ID NO: 59, SEQ ID NO: 63, SEQ ID NO: 67, SEQ ID NO: 71, SEQ ID NO: 75, SEQ ID NO: 79, SEQ ID NO: 83, SEQ ID NO: 87, SEQ ID NO: 91, SEQ ID NO: 95, SEQ ID NO: 99, SEQ ID NO: 103, SEQ ID NO: 107,
30 SEQ ID NO: 111, SEQ ID NO: 115, SEQ ID NO: 119, SEQ ID NO: 123, SEQ ID NO: 127, SEQ ID NO: 131, SEQ ID NO: 135, SEQ ID NO: 139, SEQ ID NO: 143, SEQ ID NO: 147, SEQ ID NO: 151 and SEQ ID NO: 155, or variants thereof that retain functionality. In another specific embodiment, the antigen binding moieties of the immunoconjugate comprise a light chain

variable region sequence that is at least about 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99% or 100% identical to a sequence selected from the group consisting of: SEQ ID NO: 37, SEQ ID NO: 39, SEQ ID NO: 43, SEQ ID NO: 49, SEQ ID NO: 53, SEQ ID NO: 57, SEQ ID NO: 61, SEQ ID NO: 65, SEQ ID NO: 69, SEQ ID NO: 73, SEQ ID NO: 77, SEQ ID NO: 81, SEQ ID NO: 85, SEQ ID NO: 89, SEQ ID NO: 93, SEQ ID NO: 97, SEQ ID NO: 101, SEQ ID NO: 105, SEQ ID NO: 109, SEQ ID NO: 113, SEQ ID NO: 117, SEQ ID NO: 121, SEQ ID NO: 125, SEQ ID NO: 129, SEQ ID NO: 133, SEQ ID NO: 137, SEQ ID NO: 141, SEQ ID NO: 145, SEQ ID NO: 149 and SEQ ID NO: 153, or variants thereof that retain functionality. In a more specific embodiment, the antigen binding moieties of the immunoconjugate comprise a heavy chain variable region sequence that is at least about 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99% or 100% identical to a sequence selected from the group consisting of SEQ ID NO: 41, SEQ ID NO: 45, SEQ ID NO: 47, SEQ ID NO: 51, SEQ ID NO: 55, SEQ ID NO: 59, SEQ ID NO: 63, SEQ ID NO: 67, SEQ ID NO: 71, SEQ ID NO: 75, SEQ ID NO: 79, SEQ ID NO: 83, SEQ ID NO: 87, SEQ ID NO: 91, SEQ ID NO: 95, SEQ ID NO: 99, SEQ ID NO: 103, SEQ ID NO: 107, SEQ ID NO: 111, SEQ ID NO: 115, SEQ ID NO: 119, SEQ ID NO: 123, SEQ ID NO: 127, SEQ ID NO: 131, SEQ ID NO: 135, SEQ ID NO: 139, SEQ ID NO: 143, SEQ ID NO: 147, SEQ ID NO: 151 and SEQ ID NO: 155, or variants thereof that retain functionality, and a light chain variable region sequence that is at least about 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99% or 100% identical to a sequence selected from the group consisting of: SEQ ID NO: 37, SEQ ID NO: 39, SEQ ID NO: 43, SEQ ID NO: 49, SEQ ID NO: 53, SEQ ID NO: 57, SEQ ID NO: 61, SEQ ID NO: 65, SEQ ID NO: 69, SEQ ID NO: 73, SEQ ID NO: 77, SEQ ID NO: 81, SEQ ID NO: 85, SEQ ID NO: 89, SEQ ID NO: 93, SEQ ID NO: 97, SEQ ID NO: 101, SEQ ID NO: 105, SEQ ID NO: 109, SEQ ID NO: 113, SEQ ID NO: 117, SEQ ID NO: 121, SEQ ID NO: 125, SEQ ID NO: 129, SEQ ID NO: 133, SEQ ID NO: 137, SEQ ID NO: 141, SEQ ID NO: 145, SEQ ID NO: 149 and SEQ ID NO: 153, or variants thereof that retain functionality. In a particular embodiment, the antigen binding moieties of the immunoconjugate comprise the heavy chain variable region sequence of SEQ ID NO: 111 and the light chain variable region sequence of SEQ ID NO: 109. In a further particular embodiment, the antigen binding moieties of the immunoconjugate comprise the heavy chain variable region sequence of SEQ ID NO: 143 and the light chain variable region sequence of SEQ ID NO: 141. In yet another particular embodiment, the antigen binding moieties of the immunoconjugate comprise the heavy chain variable region sequence of SEQ ID NO: 51 and the light chain variable region sequence of SEQ ID NO: 49.

In another specific embodiment, the heavy chain variable region sequence of the antigen binding moieties of the immunoconjugate is encoded by a polynucleotide sequence that is at least about 80%, 85%, 90%, 95%, 96%, 97%, 98%, or 99% identical to a sequence selected from the group consisting of: SEQ ID NO: 42, SEQ ID NO: 46, SEQ ID NO: 48, SEQ ID NO: 52, SEQ ID NO: 56, SEQ ID NO: 60, SEQ ID NO: 64, SEQ ID NO: 68, SEQ ID NO: 72, SEQ ID NO: 76, SEQ ID NO: 80, SEQ ID NO: 84, SEQ ID NO: 88, SEQ ID NO: 92, SEQ ID NO: 96, SEQ ID NO: 100, SEQ ID NO: 104, SEQ ID NO: 108, SEQ ID NO: 112, SEQ ID NO: 116, SEQ ID NO: 120, SEQ ID NO: 124, SEQ ID NO: 128, SEQ ID NO: 132, SEQ ID NO: 136, SEQ ID NO: 140, SEQ ID NO: 144, SEQ ID NO: 148, SEQ ID NO: 152, and SEQ ID NO: 156. In yet another specific embodiment, the heavy chain variable region sequence of the antigen binding moieties of the immunoconjugate is encoded by a polynucleotide sequence selected from the group consisting of: SEQ ID NO: 42, SEQ ID NO: 46, SEQ ID NO: 48, SEQ ID NO: 52, SEQ ID NO: 56, SEQ ID NO: 60, SEQ ID NO: 64, SEQ ID NO: 68, SEQ ID NO: 72, SEQ ID NO: 76, SEQ ID NO: 80, SEQ ID NO: 84, SEQ ID NO: 88, SEQ ID NO: 92, SEQ ID NO: 96, SEQ ID NO: 100, SEQ ID NO: 104, SEQ ID NO: 108, SEQ ID NO: 112, SEQ ID NO: 116, SEQ ID NO: 120, SEQ ID NO: 124, SEQ ID NO: 128, SEQ ID NO: 132, SEQ ID NO: 136, SEQ ID NO: 140, SEQ ID NO: 144, SEQ ID NO: 148, SEQ ID NO: 152, and SEQ ID NO: 156. In another specific embodiment, the light chain variable region sequence of the antigen binding moieties of the immunoconjugate is encoded by a polynucleotide sequence that is at least about 80%, 85%, 90%, 95%, 96%, 97%, 98%, or 99% identical to sequence selected from the group consisting of: SEQ ID NO: 38, SEQ ID NO: 40, SEQ ID NO: 44, SEQ ID NO: 50, SEQ ID NO: 54, SEQ ID NO: 58, SEQ ID NO: 62, SEQ ID NO: 66, SEQ ID NO: 70, SEQ ID NO: 74, SEQ ID NO: 78, SEQ ID NO: 82, SEQ ID NO: 86, SEQ ID NO: 90, SEQ ID NO: 94, SEQ ID NO: 98, SEQ ID NO: 102, SEQ ID NO: 106, SEQ ID NO: 110, SEQ ID NO: 114, SEQ ID NO: 118, SEQ ID NO: 122, SEQ ID NO: 126, SEQ ID NO: 130, SEQ ID NO: 134, SEQ ID NO: 138, SEQ ID NO: 142, SEQ ID NO: 146, SEQ ID NO: 150, and SEQ ID NO: 154. In yet another specific embodiment, the light chain variable region sequence of the antigen binding moieties of the immunoconjugate is encoded by a polynucleotide sequence selected from the group consisting of: SEQ ID NO: 38, SEQ ID NO: 40, SEQ ID NO: 44, SEQ ID NO: 50, SEQ ID NO: 54, SEQ ID NO: 58, SEQ ID NO: 62, SEQ ID NO: 66, SEQ ID NO: 70, SEQ ID NO: 74, SEQ ID NO: 78, SEQ ID NO: 82, SEQ ID NO: 86, SEQ ID NO: 90, SEQ ID NO: 94, SEQ ID NO: 98, SEQ ID NO: 102, SEQ ID NO: 106, SEQ ID NO: 110, SEQ ID NO: 114, SEQ ID NO: 118, SEQ ID NO: 122, SEQ ID NO: 126, SEQ ID

NO: 130, SEQ ID NO: 134, SEQ ID NO: 138, SEQ ID NO: 142, SEQ ID NO: 146, SEQ ID NO: 150, and SEQ ID NO: 154.

In one embodiment, the immunoconjugate comprises a polypeptide sequence wherein a Fab heavy chain specific for FAP shares a carboxy-terminal peptide bond with an Fc domain subunit comprising a knob modification, which in turn shares a carboxy-terminal peptide bond with an IL-2 polypeptide. In a more specific embodiment, the immunoconjugate comprises a polypeptide sequence selected from the group of SEQ ID NO: 195, SEQ ID NO: 197, SEQ ID NO: 203, SEQ ID NO: 209, SEQ ID NO: 269, SEQ ID NO: 271 and SEQ ID NO: 273, or variants thereof that retain functionality. In one embodiment, the immunoconjugate comprises a polypeptide sequence wherein a Fab heavy chain specific for FAP shares a carboxy-terminal peptide bond with an Fc domain subunit comprising a knob modification, which in turn shares a carboxy-terminal peptide bond with an IL-15 polypeptide. In a more specific embodiment, the immunoconjugate comprises the polypeptide sequence of SEQ ID NO: 199, or a variant thereof that retains functionality. In one embodiment the immunoconjugate comprises a polypeptide sequence wherein a Fab heavy chain specific for FAP shares a carboxy-terminal peptide bond with an Fc domain subunit comprising a hole modification. In a more specific embodiment, the immunoconjugate comprises a polypeptide sequence selected from the group of SEQ ID NO: 193, SEQ ID NO: 201 and SEQ ID NO: 207, or variants thereof that retain functionality. In another embodiment, the immunoconjugate comprises a Fab light chain specific for FAP. In a more specific embodiment, the immunoconjugate comprises the polypeptide sequence of SEQ ID NO: 205 or SEQ ID NO: 211, or a variant thereof that retains functionality. In another embodiment, the immunoconjugate comprises the polypeptide sequence of SEQ ID NO: 205, the polypeptide sequence of SEQ ID NO: 193, and a polypeptide sequence selected from the group of SEQ ID NO: 195, SEQ ID NO: 197, SEQ ID NO: 199 and SEQ ID NO: 269, or variants thereof that retain functionality. In yet another embodiment, the immunoconjugate comprises the polypeptide sequences of SEQ ID NO: 201, SEQ ID NO: 203 and SEQ ID NO: 205, or variants thereof that retain functionality. In yet another embodiment, the immunoconjugate comprises the polypeptide sequences of SEQ ID NO: 207, SEQ ID NO: 209 and SEQ ID NO: 211, or variants thereof that retain functionality. In yet another embodiment, the immunoconjugate comprises the polypeptide sequences of SEQ ID NO: 205, SEQ ID NO: 193 and SEQ ID NO: 269, or variants thereof that retain functionality. In yet another embodiment, the immunoconjugate comprises the polypeptide sequences of SEQ ID NO: 211, SEQ ID NO: 207 and SEQ ID NO: 271, or variants thereof that retain functionality. In yet another embodiment, the immunoconjugate comprises the

polypeptide sequences of SEQ ID NO: 211, SEQ ID NO: 207 and SEQ ID NO: 273, or variants thereof that retain functionality. In another specific embodiment, the polypeptides are covalently linked, e.g., by a disulfide bond. In some embodiments the Fc domain subunits each comprise the amino acid substitutions L234A, L235A, and P329G.

- 5 In yet another embodiment, the immunoconjugate comprises a polypeptide sequence wherein a Fab heavy chain specific for FAP shares a carboxy-terminal peptide bond with an Fc domain subunit comprising a hole modification, which in turn shares a carboxy-terminal peptide bond with an IL-10 polypeptide. In a more specific embodiment, the immunoconjugate comprises the polypeptide sequence of SEQ ID NO: 243 or SEQ ID NO: 245, or a variant thereof that retains
- 10 functionality. In one embodiment the immunoconjugate comprises a polypeptide sequence wherein a Fab heavy chain specific for FAP shares a carboxy-terminal peptide bond with an Fc domain subunit comprising a knob modification. In a more specific embodiment, the immunoconjugate comprises the polypeptide sequence of SEQ ID NO: 241 or a variant thereof that retains functionality. In another embodiment, the immunoconjugate comprises a Fab light
- 15 chain specific for FAP. In a more specific embodiment, the immunoconjugate comprises the polypeptide sequence of SEQ ID NO: 205 or a variant thereof that retains functionality. In another embodiment, the immunoconjugate comprises the polypeptide sequences of SEQ ID NO: 205, SEQ ID NO: 241 and SEQ ID NO: 243, or variants thereof that retain functionality. In yet another embodiment, the immunoconjugate comprises the polypeptide sequences of SEQ ID
- 20 NO: 205, SEQ ID NO: 241 and SEQ ID NO: 245, or variants thereof that retain functionality. In another specific embodiment, the polypeptides are covalently linked, e.g., by a disulfide bond. In some embodiments the Fc domain subunits each comprise the amino acid substitutions L234A, L235A, and P329G.

- In a specific embodiment, the immunoconjugate comprises a polypeptide sequence encoded by a
- 25 polynucleotide sequence that is at least about 80%, 85%, 90%, 95%, 96%, 97%, 98%, or 99% identical to a sequence selected from the group of SEQ ID NO: 196, SEQ ID NO: 198, SEQ ID NO: 200, SEQ ID NO: 204, SEQ ID NO: 210, SEQ ID NO: 244, SEQ ID NO: 246, SEQ ID NO: 270, SEQ ID NO: 272 and SEQ ID NO: 274. In another specific embodiment, the immunoconjugate comprises a polypeptide sequence encoded by a polynucleotide sequence
- 30 selected from the group of SEQ ID NO: 196, SEQ ID NO: 198, SEQ ID NO: 200, SEQ ID NO: 204, SEQ ID NO: 210, SEQ ID NO: 244, SEQ ID NO: 246, SEQ ID NO: 270, SEQ ID NO: 272 and SEQ ID NO: 274. In another specific embodiment, the immunoconjugate comprises a polypeptide sequence encoded by a polynucleotide sequence that is at least about 80%, 85%,

90%, 95%, 96%, 97%, 98%, or 99% identical to a sequence selected from the group of SEQ ID NO: 194, SEQ ID NO: 202, SEQ ID NO: 208 and SEQ ID NO: 242. In yet another specific embodiment, the immunoconjugate comprises a polypeptide sequence encoded by a polynucleotide sequence selected from the group of SEQ ID NO: 194, SEQ ID NO: 202, SEQ ID NO: 208 and SEQ ID NO: 242. In another specific embodiment, the immunoconjugate comprises a polypeptide sequence encoded by a polynucleotide sequence that is at least about 80%, 85%, 90%, 95%, 96%, 97%, 98%, or 99% identical to the sequence of SEQ ID NO: 206 or SEQ ID NO: 212. In yet another specific embodiment, the immunoconjugate comprises a polypeptide sequence encoded by the polynucleotide sequence of SEQ ID NO: 206 or SEQ ID NO: 212.

In one embodiment, the immunoconjugate comprises at least one, typically two or more antigen binding moieties that are specific for the Carcinoembryonic Antigen (CEA). In a specific embodiment, the antigen binding moieties of the immunoconjugate comprise a heavy chain variable region sequence that is at least about 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99% or 100% identical to the sequence of SEQ ID NO: 191 or SEQ ID NO: 295, or a variant thereof that retains functionality. In another specific embodiment, the antigen binding moieties of the immunoconjugate comprise a light chain variable region sequence that is at least about 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99% or 100% identical to the sequence of SEQ ID NO: 189 or SEQ ID NO: 293, or a variant thereof that retains functionality. In a more specific embodiment, the antigen binding moieties of the immunoconjugate comprise a heavy chain variable region sequence that is at least about 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99% or 100% identical to the sequence of SEQ ID NO: 191, or a variant thereof that retains functionality, and a light chain variable region sequence that is at least about 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99% or 100% identical to the sequence of SEQ ID NO: 189, or a variant thereof that retains functionality. In another specific embodiment, the antigen binding moieties of the immunoconjugate comprise a heavy chain variable region sequence that is at least about 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99% or 100% identical to the sequence of SEQ ID NO: 295, or a variant thereof that retains functionality, and a light chain variable region sequence that is at least about 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99% or 100% identical to the sequence of SEQ ID NO: 293, or a variant thereof that retains functionality.

In another specific embodiment, the heavy chain variable region sequence of the antigen binding moieties of the immunoconjugate is encoded by a polynucleotide sequence that is at least about 80%, 85%, 90%, 95%, 96%, 97%, 98%, or 99% identical to the sequence of SEQ ID NO: 192 or

SEQ ID NO: 296. In yet another specific embodiment, the heavy chain variable region sequence of the antigen binding moieties of the immunoconjugate is encoded by the polynucleotide sequence of SEQ ID NO: 192 or SEQ ID NO: 296. In another specific embodiment, the light chain variable region sequence of the antigen binding moieties of the immunoconjugate is encoded by a polynucleotide sequence that is at least about 80%, 85%, 90%, 95%, 96%, 97%, 98%, or 99% identical to the sequence of SEQ ID NO: 190 or SEQ ID NO: 294. In yet another specific embodiment, the light chain variable region sequence of the antigen binding moieties of the immunoconjugate is encoded by the polynucleotide sequence of SEQ ID NO: 190 or SEQ ID NO: 294.

- 10 In one embodiment, the immunoconjugate comprises a polypeptide sequence wherein a Fab heavy chain specific for CEA shares a carboxy-terminal peptide bond with an Fc domain subunit comprising a knob modification, which in turn shares a carboxy-terminal peptide bond with an IL-2 polypeptide. In a more specific embodiment, the immunoconjugate comprises a polypeptide sequence selected from the group consisting of SEQ ID NO: 229, SEQ ID NO: 275, SEQ ID
- 15 NO: 277 and SEQ ID NO: 279, or a variant thereof that retains functionality. In one embodiment the immunoconjugate comprises a polypeptide sequence wherein a Fab heavy chain specific for CEA shares a carboxy-terminal peptide bond with an Fc domain subunit comprising a hole modification. In a more specific embodiment, the immunoconjugate comprises the polypeptide sequence of SEQ ID NO: 227 or SEQ ID NO: 281, or a variant thereof that retains functionality.
- 20 In another embodiment, the immunoconjugate comprises a Fab light chain specific for CEA. In a more specific embodiment, the immunoconjugate comprises the polypeptide sequence of SEQ ID NO: 231 or SEQ ID NO: 283, or a variant thereof that retains functionality. In another embodiment, the immunoconjugate comprises the polypeptide sequences of SEQ ID NO: 227, SEQ ID NO: 229 and SEQ ID NO: 231, or variants thereof that retain functionality. In another
- 25 embodiment, the immunoconjugate comprises the polypeptide sequences of SEQ ID NO: 275, SEQ ID NO: 281 and SEQ ID NO: 283, or variants thereof that retain functionality. In another embodiment, the immunoconjugate comprises the polypeptide sequences of SEQ ID NO: 277, SEQ ID NO: 281 and SEQ ID NO: 283, or variants thereof that retain functionality. In another embodiment, the immunoconjugate comprises the polypeptide sequences of SEQ ID NO: 279,
- 30 SEQ ID NO: 281 and SEQ ID NO: 283, or variants thereof that retain functionality. In another specific embodiment, the polypeptides are covalently linked, e.g., by a disulfide bond. In some embodiments the Fc domain polypeptide chains comprise the amino acid substitutions L234A, L235A, and P329G.

In a specific embodiment, the immunoconjugate comprises a polypeptide sequence encoded by a polynucleotide sequence that is at least about 80%, 85%, 90%, 95%, 96%, 97%, 98%, or 99% identical to a sequence selected from the group consisting of SEQ ID NO: 230, SEQ ID NO: 276, SEQ ID NO: 278 and SEQ ID NO: 280. In another specific embodiment, the immunoconjugate comprises a polypeptide sequence encoded by the polynucleotide sequence selected from the group consisting of SEQ ID NO: 230, SEQ ID NO: 276, SEQ ID NO: 278 and SEQ ID NO: 280. In another specific embodiment, the immunoconjugate comprises a polypeptide sequence encoded by a polynucleotide sequence that is at least about 80%, 85%, 90%, 95%, 96%, 97%, 98%, or 99% identical to the sequence of SEQ ID NO: 228 or SEQ ID NO: 282. In yet another specific embodiment, the immunoconjugate comprises a polypeptide sequence encoded by the polynucleotide sequence of SEQ ID NO: 228 or SEQ ID NO: 282. In another specific embodiment, the immunoconjugate comprises a polypeptide sequence encoded by a polynucleotide sequence that is at least about 80%, 85%, 90%, 95%, 96%, 97%, 98%, or 99% identical to the sequence of SEQ ID NO: 232 or SEQ ID NO: 284. In yet another specific embodiment, the immunoconjugate comprises a polypeptide sequence encoded by the polynucleotide sequence of SEQ ID NO: 232 or SEQ ID NO: 284.

In some embodiments the immunoconjugate comprises a polypeptide sequence wherein an effector moiety polypeptide shares a carboxy-terminal peptide bond with an Fc domain subunit comprising a knob modification. In a more specific embodiment, the immunoconjugate comprises a polypeptide sequence selected from the group of SEQ ID NO: 247, SEQ ID NO: 249 and SEQ ID NO: 251, or a variant thereof that retains functionality. In one such embodiment the immunoconjugate further comprises a polypeptide sequence wherein a Fab heavy chain specific for FAP shares a carboxy-terminal peptide bond with an Fc domain subunit comprising a hole modification. In a more specific embodiment, the immunoconjugate further comprises a polypeptide sequence selected from the group of SEQ ID NO: 193, SEQ ID NO: 201 and SEQ ID NO: 207, or a variant thereof that retains functionality. In another such embodiment the immunoconjugate further comprises a polypeptide sequence wherein a Fab heavy chain specific for EDB, TNC A1, TNC A2 or CEA shares a carboxy-terminal peptide bond with an Fc domain subunit comprising a hole modification. In some embodiments the Fc domain subunits each comprise the amino acid substitutions L234A, L235A, and P329G. According to any of the above embodiments the immunoconjugate may further comprise a Fab light chain specific for the corresponding antigen.

Immunoconjugates of the invention include those that have sequences that are at least about 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99%, or 100% identical to the sequences set forth in SEQ ID NOs 23, 25, 27, 29, 31, 33, 35, 37, 39, 41, 43, 45, 47, 49, 51, 53, 55, 57, 59, 61, 63, 65, 67, 69, 71, 73, 75, 77, 79, 81, 83, 85, 87, 89, 91, 93, 95, 97, 99, 101, 103, 105, 107, 109, 111, 113, 115, 117, 119, 121, 123, 125, 127, 129, 131, 133, 135, 137, 139, 141, 143, 145, 147, 149, 151, 153, 155, 157, 159, 161, 163, 165, 167, 169, 171, 173, 175, 177, 179, 181, 183, 185, 187, 189, 191, 193, 195, 197, 199, 201, 203, 205, 207, 209, 211, 213, 215, 217, 227, 229, 231, 233, 235, 237, 239, 241, 243, 245, 247, 249, 251, 269, 271, 273, 275, 277, 279, 281, 283, 285 and 287, including functional fragments or variants thereof. The invention also encompasses immunoconjugates comprising these sequences with conservative amino acid substitutions.

Polynucleotides

The invention further provides isolated polynucleotides encoding an immunoconjugate as described herein or a fragment thereof.

Polynucleotides of the invention include those that are at least about 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99%, or 100% identical to the sequences set forth in SEQ ID NOs 24, 26, 28, 30, 32, 34, 36, 38, 40, 42, 44, 46, 48, 50, 52, 54, 56, 58, 60, 62, 64, 66, 68, 70, 72, 74, 76, 78, 80, 82, 84, 86, 88, 90, 92, 94, 96, 98, 100, 102, 104, 106, 108, 110, 112, 114, 116, 118, 120, 122, 124, 126, 128, 130, 132, 134, 136, 138, 140, 142, 144, 146, 148, 150, 152, 154, 156, 158, 160, 162, 164, 166, 168, 170, 172, 174, 176, 178, 180, 182, 184, 186, 188, 190, 192, 194, 196, 198, 200, 202, 204, 206, 208, 210, 212, 214, 216, 218, 228, 230, 232, 234, 236, 238, 240, 242, 244, 246, 248, 250, 252, 270, 272, 274, 276, 278, 280, 282, 284, 286 and 288, including functional fragments or variants thereof.

The polynucleotides encoding immunoconjugates of the invention may be expressed as a single polynucleotide that encodes the entire immunoconjugate or as multiple (e.g., two or more) polynucleotides that are co-expressed. Polypeptides encoded by polynucleotides that are co-expressed may associate through, e.g., disulfide bonds or other means to form a functional immunoconjugate. For example, the light chain portion of an antigen binding moiety may be encoded by a separate polynucleotide from the portion of the immunoconjugate comprising the heavy chain portion of the antigen binding moiety, an Fc domain subunit and optionally the effector moiety. When co-expressed, the heavy chain polypeptides will associate with the light chain polypeptides to form the antigen binding moiety. In another example, the portion of the immunoconjugate comprising the heavy chain portion of a first antigen binding moiety, one of the two Fc domain subunits and the effector moiety could be encoded by a separate

polynucleotide from the portion of the immunoconjugate comprising the heavy chain portion of a second antigen binding moiety and the other of the two Fc domain subunits. When co-expressed, the Fc domain subunits will associate to form the Fc domain.

In one embodiment, an isolated polynucleotide of the invention encodes a fragment of an immunoconjugate comprising a first antigen binding moiety, an Fc domain consisting of two subunits, and a single effector moiety, wherein the antigen binding moiety is an antigen binding domain comprising a heavy chain variable region and a light chain variable region, particularly a Fab molecule. In one embodiment, an isolated polynucleotide of the invention encodes the heavy chain of the first antigen binding moiety, a subunit of the Fc domain, and the effector moiety. In another embodiment, an isolated polynucleotide of the invention encodes the heavy chain of the first antigen binding moiety and a subunit of the Fc domain. In yet another embodiment, an isolated polynucleotide of the invention encodes a subunit of the Fc domain and the effector moiety. In a more specific embodiment the isolated polynucleotide encodes a polypeptide wherein a Fab heavy chain shares a carboxy-terminal peptide bond with an Fc domain subunit. In another specific embodiment the isolated polynucleotide encodes a polypeptide wherein an Fc domain subunit shares a carboxy-terminal peptide bond with an effector moiety polypeptide. In yet another specific embodiment, the isolated polynucleotide encodes a polypeptide wherein a Fab heavy chain shares a carboxy-terminal peptide bond with an Fc domain subunit, which in turn shares a carboxy-terminal peptide bond with an effector moiety polypeptide. In yet another specific embodiment the isolated polynucleotide encodes a polypeptide wherein an effector moiety polypeptide shares a carboxy-terminal peptide bond with an Fc domain subunit.

In another embodiment, the present invention is directed to an isolated polynucleotide encoding an immunoconjugate or fragment thereof, wherein the polynucleotide comprises a sequence that encodes a variable region sequence as shown in SEQ ID NO 23, 25, 27, 29, 31, 33, 35, 37, 39, 41, 43, 45, 47, 49, 51, 53, 55, 57, 59, 61, 63, 65, 67, 69, 71, 73, 75, 77, 79, 81, 83, 85, 87, 89, 91, 93, 95, 97, 99, 101, 103, 105, 107, 109, 111, 113, 115, 117, 119, 121, 123, 125, 127, 129, 131, 133, 135, 137, 139, 141, 143, 145, 147, 149, 151, 153, 155, 157, 159, 161, 163, 165, 167, 169, 171, 173, 175, 177, 179, 181, 183, 185, 187, 189, 191, 293 or 295. In another embodiment, the present invention is directed to an isolated polynucleotide encoding an immunoconjugate or fragment thereof, wherein the polynucleotide comprises a sequence that encodes a polypeptide sequence as shown in SEQ ID NO 193, 195, 197, 199, 201, 203, 205, 207, 209, 211, 213, 215, 217, 227, 229, 231, 233, 235, 237, 239, 241, 243, 245, 247, 249, 251, 269, 271, 273, 275, 277, 279, 281, 283, 285 or 287. In another embodiment, the invention is further directed to an isolated

polynucleotide encoding an immunoconjugate or fragment thereof, wherein the polynucleotide comprises a sequence that is at least about 80%, 85%, 90%, 95%, 96%, 97%, 98%, or 99% identical to a nucleotide sequence shown SEQ ID NO 24, 26, 28, 30, 32, 34, 36, 38, 40, 42, 44, 46, 48, 50, 52, 54, 56, 58, 60, 62, 64, 66, 68, 70, 72, 74, 76, 78, 80, 82, 84, 86, 88, 90, 92, 94, 96, 98, 100, 102, 104, 106, 108, 110, 112, 114, 116, 118, 120, 122, 124, 126, 128, 130, 132, 134, 136, 138, 140, 142, 144, 146, 148, 150, 152, 154, 156, 158, 160, 162, 164, 166, 168, 170, 172, 174, 176, 178, 180, 182, 184, 186, 188, 190, 192, 194, 196, 198, 200, 202, 204, 206, 208, 210, 212, 214, 216, 218, 220, 222, 224, 226, 228, 230, 232, 234, 236, 238, 240, 242, 244, 246, 248, 250, 252, 254, 256, 258, 260, 262, 264, 266, 268, 270, 272, 274, 276, 278, 280, 282, 284, 286 or 288. In another embodiment, the invention is directed to an isolated polynucleotide encoding an immunoconjugate or fragment thereof, wherein the polynucleotide comprises a nucleic acid sequence shown in SEQ ID NO 24, 26, 28, 30, 32, 34, 36, 38, 40, 42, 44, 46, 48, 50, 52, 54, 56, 58, 60, 62, 64, 66, 68, 70, 72, 74, 76, 78, 80, 82, 84, 86, 88, 90, 92, 94, 96, 98, 100, 102, 104, 106, 108, 110, 112, 114, 116, 118, 120, 122, 124, 126, 128, 130, 132, 134, 136, 138, 140, 142, 144, 146, 148, 150, 152, 154, 156, 158, 160, 162, 164, 166, 168, 170, 172, 174, 176, 178, 180, 182, 184, 186, 188, 190, 192, 194, 196, 198, 200, 202, 204, 206, 208, 210, 212, 214, 216, 218, 220, 222, 224, 226, 228, 230, 232, 234, 236, 238, 240, 242, 244, 246, 248, 250, 252, 254, 256, 258, 260, 262, 264, 266, 268, 270, 272, 274, 276, 278, 280, 282, 284, 286 or 288. In another embodiment, the invention is directed to an isolated polynucleotide encoding an immunoconjugate or fragment thereof, wherein the polynucleotide comprises a sequence that encodes a variable region sequence that is at least about 80%, 85%, 90%, 95%, 96%, 97%, 98%, or 99% identical to an amino acid sequence of SEQ ID NO 23, 25, 27, 29, 31, 33, 35, 37, 39, 41, 43, 45, 47, 49, 51, 53, 55, 57, 59, 61, 63, 65, 67, 69, 71, 73, 75, 77, 79, 81, 83, 85, 87, 89, 91, 93, 95, 97, 99, 101, 103, 105, 107, 109, 111, 113, 115, 117, 119, 121, 123, 125, 127, 129, 131, 133, 135, 137, 139, 141, 143, 145, 147, 149, 151, 153, 155, 157, 159, 161, 163, 165, 167, 169, 171, 173, 175, 177, 179, 181, 183, 185, 187, 189, 191, 193, 195, 197, 199, 201, 203, 205, 207, 209, 211, 213, 215, 217, 219, 221, 223, 225, 227, 229, 231, 233, 235, 237, 239, 241, 243, 245, 247, 249, 251, 253, 255, 257, 259, 261, 263, 265, 267, 269, 271, 273, 275, 277, 279, 281, 283, 285 or 287. The invention encompasses an isolated polynucleotide encoding an immunoconjugate or fragment thereof, wherein the polynucleotide comprises a sequence that encodes the variable region sequences of SEQ ID NO 23, 25, 27, 29, 31, 33, 35, 37, 39, 41, 43,

45, 47, 49, 51, 53, 55, 57, 59, 61, 63, 65, 67, 69, 71, 73, 75, 77, 79, 81, 83, 85, 87, 89, 91, 93, 95, 97, 99, 101, 103, 105, 107, 109, 111, 113, 115, 117, 119, 121, 123, 125, 127, 129, 131, 133, 135, 137, 139, 141, 143, 145, 147, 149, 151, 153, 155, 157, 159, 161, 163, 165, 167, 169, 171, 173, 175, 177, 179, 181, 183, 185, 187, 189, 191, 293 or 295 with conservative amino acid
5 substitutions. The invention also encompasses an isolated polynucleotide encoding an immunoconjugate of the invention or fragment thereof, wherein the polynucleotide comprises a sequence that encodes the polypeptide sequences of SEQ ID NO 193, 195, 197, 199, 201, 203, 205, 207, 209, 211, 213, 215, 217, 227, 229, 231, 233, 235, 237, 239, 241, 243, 245, 247, 249, 251, 269, 271, 273, 275, 277, 279, 281, 283, 285 or 287 with conservative amino acid
10 substitutions.

In certain embodiments the polynucleotide or nucleic acid is DNA. In other embodiments, a polynucleotide of the present invention is RNA, for example, in the form of messenger RNA (mRNA). RNA of the present invention may be single stranded or double stranded.

Untargeted Conjugates

15 The invention provides not only immunoconjugates targeted to a specific antigen (e.g. a tumor antigen) but also untargeted conjugates comprising one or more Fab molecules which do not specifically bind to any antigen, particularly not bind to any human antigen. The absence of specific binding of these conjugates to any antigen (i.e. the absence of any binding that can be discriminated from non-specific interaction) can be measured e.g. by ELISA or surface plasmon
20 resonance as described herein. Such conjugates are particularly useful e.g. for enhancing the serum half life of the effector moiety they comprise, as compared to the serum half-life of the unconjugated effector moiety, where targeting to a particular tissue is not desired.

Specifically, the invention provides a conjugate comprising a first Fab molecule which does not specifically bind any antigen, an Fc domain consisting of two subunits, and an effector moiety,
25 wherein not more than one effector moiety is present. More specifically, the invention provides a conjugate comprising a first Fab molecule comprising the heavy chain variable region sequence of SEQ ID NO: 299 and the light chain variable region sequence of SEQ ID NO: 297, an Fc domain consisting of two subunits, and an effector moiety, wherein not more than one effector moiety is present. Like the immunoconjugates of the invention, the conjugates can have a variety
30 of configurations, as described above under "Immunoconjugate Formats" (the antigen binding moiety of the immunoconjugate being replaced by a Fab molecule which does not specifically bind to any antigen, such as a Fab molecule comprising the heavy chain variable region sequence of SEQ ID NO: 299 and the light chain variable region sequence of SEQ ID NO: 297). Likewise,

the features of the Fc domain as well as the effector moiety as described above under “Fc domain” and “Effector moieties” for the immunoconjugates of the invention equally apply, alone or in combination, to the untargeted conjugates of the invention.

In a particular embodiment, the conjugate comprises (i) an immunoglobulin molecule, comprising a first and a second Fab molecule which do not specifically bind any antigen and an Fc domain, and (ii) an effector moiety, wherein not more than one effector moiety is present and wherein the immunoglobulin molecule is a human IgG1 subclass immunoglobulin; the Fc domain comprises a knob modification in one and a hole modification in the other one of its two subunits, and the amino acid substitutions L234A, L235A and P329G in each of its subunits; and the effector moiety is an IL-2 molecule fused to the carboxy-terminal amino acid of one of the immunoglobulin heavy chains, optionally through a linker peptide. In a specific embodiment, the conjugate comprises the heavy chain variable region sequence of SEQ ID NO: 299 and the light chain variable region sequence of SEQ ID NO: 297.

In certain embodiments, the conjugate comprises (i) an immunoglobulin molecule, comprising the heavy chain variable region sequence of SEQ ID NO: 299 and the light chain variable region sequence of SEQ ID NO: 297, and (ii) an effector moiety, wherein not more than one effector moiety is present. In one such embodiment the immunoglobulin molecule is a human IgG1 subclass immunoglobulin. In one such embodiment the Fc domain comprises a knob modification in one and a hole modification in the other one of its two subunits. In a specific such embodiment, the Fc domain comprises the amino acid substitutions L234A, L235A and P329G in each of its subunits. In yet another such embodiment, the effector moiety is an IL-2 molecule fused to the carboxy-terminal amino acid of one of the immunoglobulin heavy chains, optionally through a linker peptide.

In one embodiment the conjugate comprises a polypeptide sequence wherein a Fab heavy chain which does not specifically bind to any antigen shares a carboxy-terminal peptide bond with an Fc domain subunit comprising a knob modification, which in turn shares a carboxy-terminal peptide bond with an IL-2 polypeptide. In a more specific embodiment, the conjugate comprises a polypeptide sequence selected from the group of SEQ ID NO: 221, SEQ ID NO: 223, SEQ ID NO: 289 and SEQ ID NO: 291, or a variant thereof that retains functionality. In one embodiment the conjugate comprises a polypeptide sequence wherein a Fab heavy chain which does not specifically bind to any antigen shares a carboxy-terminal peptide bond with an Fc domain subunit comprising a hole modification. In a more specific embodiment, the conjugate comprises the polypeptide sequence of SEQ ID NO: 219, or a variant thereof that retains functionality. In

another embodiment, the conjugate comprises a Fab light chain which does not specifically bind any antigen. In a more specific embodiment, the conjugate comprises the polypeptide sequence of SEQ ID NO: 225, or a variant thereof that retains functionality. In another embodiment, the conjugate comprises the polypeptide sequences of SEQ ID NO: 219, SEQ ID NO: 221 and SEQ ID NO: 225, or variants thereof that retain functionality. In another embodiment, the conjugate comprises the polypeptide sequences of SEQ ID NO: 219, SEQ ID NO: 223 and SEQ ID NO: 225, or variants thereof that retain functionality. In another embodiment, the conjugate comprises the polypeptide sequences of SEQ ID NO: 219, SEQ ID NO: 289 and SEQ ID NO: 225, or variants thereof that retain functionality. In another embodiment, the conjugate comprises the polypeptide sequences of SEQ ID NO: 219, SEQ ID NO: 291 and SEQ ID NO: 225, or variants thereof that retain functionality. In another specific embodiment, the polypeptides are covalently linked, e.g., by a disulfide bond. In some embodiments the Fc domain polypeptide chains comprise the amino acid substitutions L234A, L235A, and P329G.

In a specific embodiment, the conjugate comprises a polypeptide sequence encoded by a polynucleotide sequence that is at least about 80%, 85%, 90%, 95%, 96%, 97%, 98%, or 99% identical to a sequence selected from the group consisting of SEQ ID NO: 298, SEQ ID NO: 300, SEQ ID NO: 220, SEQ ID NO: 222, SEQ ID NO: 224, SEQ ID NO: 226, SEQ ID NO: 290 and SEQ ID NO: 292. In another specific embodiment, the immunoconjugate comprises a polypeptide sequence encoded by the polynucleotide sequence selected from the group consisting of SEQ ID NO: 298, SEQ ID NO: 300, SEQ ID NO: 220, SEQ ID NO: 222, SEQ ID NO: 224, SEQ ID NO: 226, SEQ ID NO: 290 and SEQ ID NO: 292.

The invention also provides an isolated polynucleotide encoding the conjugate of the invention of a fragment thereof. In a specific embodiment, the isolated polynucleotide comprises a sequence that is at least about 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99% or 100% identical to the a sequence selected from the group of SEQ ID NO: 298, SEQ ID NO: 300, SEQ ID NO: 220, SEQ ID NO: 222, SEQ ID NO: 224, SEQ ID NO: 226, SEQ ID NO: 290 and SEQ ID NO: 292. The invention further provides an expression vector comprising the isolated polynucleotide, and a host cell comprising the isolated polynucleotide or the expression vector of the invention. In another aspect is provided a method of producing the conjugate of the invention, comprising the steps of a) culturing the host cell of the invention under conditions suitable for the expression of the conjugate and b) recovering the conjugate. The invention also encompasses a conjugate produced by the method of the invention. The disclosure provided herein in relating to methods

of producing the immunoconjugates of the invention (see e.g. under "Recombinant Methods") can equally be applied to the conjugates of the invention.

The invention further provides a pharmaceutical composition comprising the conjugate of the invention and a pharmaceutically acceptable carrier. The disclosure provided herein in relating to
5 pharmaceutical compositions of the immunoconjugates of the invention (see e.g. under "Compositions, Formulations, and Routes of Administration") can equally be applied to the conjugates of the invention. Furthermore, the conjugate can be employed in the methods of use described herein for the immunoconjugates of the invention. The disclosure provided herein in relating to methods of using the immunoconjugates of the invention in the treatment of disease
10 (see e.g. under "Therapeutic Methods and Compositions", "Other Agents and Treatments" and "Articles of manufacture") can equally be applied to the conjugates of the invention.

Recombinant Methods

Immunoconjugates of the invention may be obtained, for example, by solid-state peptide
15 synthesis (e.g. Merrifield solid phase synthesis) or recombinant production. For recombinant production one or more polynucleotide encoding the immunoconjugate (fragment), e.g., as described above, is isolated and inserted into one or more vectors for further cloning and/or expression in a host cell. Such polynucleotide may be readily isolated and sequenced using conventional procedures. In one embodiment a vector, preferably an expression vector,
20 comprising one or more of the polynucleotides of the invention is provided. Methods which are well known to those skilled in the art can be used to construct expression vectors containing the coding sequence of an immunoconjugate (fragment) along with appropriate transcriptional/translational control signals. These methods include *in vitro* recombinant DNA techniques, synthetic techniques and *in vivo* recombination/genetic recombination. See, for
25 example, the techniques described in Maniatis et al., MOLECULAR CLONING: A LABORATORY MANUAL, Cold Spring Harbor Laboratory, N.Y. (1989); and Ausubel et al., CURRENT PROTOCOLS IN MOLECULAR BIOLOGY, Greene Publishing Associates and Wiley Interscience, N.Y (1989). The expression vector can be part of a plasmid, virus, or may be a nucleic acid fragment. The expression vector includes an expression cassette into which the polynucleotide
30 encoding the immunoconjugate (fragment) (i.e. the coding region) is cloned in operable association with a promoter and/or other transcription or translation control elements. As used herein, a "coding region" is a portion of nucleic acid which consists of codons translated into amino acids. Although a "stop codon" (TAG, TGA, or TAA) is not translated into an amino acid,

it may be considered to be part of a coding region, if present, but any flanking sequences, for example promoters, ribosome binding sites, transcriptional terminators, introns, 5' and 3' untranslated regions, and the like, are not part of a coding region. Two or more coding regions can be present in a single polynucleotide construct, e.g. on a single vector, or in separate
5 polynucleotide constructs, e.g. on separate (different) vectors. Furthermore, any vector may contain a single coding region, or may comprise two or more coding regions, e.g. a vector of the present invention may encode one or more polypeptides, which are post- or co-translationally separated into the final proteins via proteolytic cleavage. In addition, a vector, polynucleotide, or nucleic acid of the invention may encode heterologous coding regions, either fused or unfused to
10 a polynucleotide encoding the immunoconjugate (fragment) of the invention, or variant or derivative thereof. Heterologous coding regions include without limitation specialized elements or motifs, such as a secretory signal peptide or a heterologous functional domain. An operable association is when a coding region for a gene product, e.g. a polypeptide, is associated with one or more regulatory sequences in such a way as to place expression of the gene product under the
15 influence or control of the regulatory sequence(s). Two DNA fragments (such as a polypeptide coding region and a promoter associated therewith) are "operably associated" if induction of promoter function results in the transcription of mRNA encoding the desired gene product and if the nature of the linkage between the two DNA fragments does not interfere with the ability of the expression regulatory sequences to direct the expression of the gene product or interfere with
20 the ability of the DNA template to be transcribed. Thus, a promoter region would be operably associated with a nucleic acid encoding a polypeptide if the promoter was capable of effecting transcription of that nucleic acid. The promoter may be a cell-specific promoter that directs substantial transcription of the DNA only in predetermined cells. Other transcription control elements, besides a promoter, for example enhancers, operators, repressors, and transcription
25 termination signals, can be operably associated with the polynucleotide to direct cell-specific transcription. Suitable promoters and other transcription control regions are disclosed herein. A variety of transcription control regions are known to those skilled in the art. These include, without limitation, transcription control regions, which function in vertebrate cells, such as, but not limited to, promoter and enhancer segments from cytomegaloviruses (e.g. the immediate
30 early promoter, in conjunction with intron-A), simian virus 40 (e.g. the early promoter), and retroviruses (such as, e.g. Rous sarcoma virus). Other transcription control regions include those derived from vertebrate genes such as actin, heat shock protein, bovine growth hormone and rabbit α -globin, as well as other sequences capable of controlling gene expression in eukaryotic

cells. Additional suitable transcription control regions include tissue-specific promoters and enhancers as well as inducible promoters (e.g. promoters inducible tetracyclins). Similarly, a variety of translation control elements are known to those of ordinary skill in the art. These include, but are not limited to ribosome binding sites, translation initiation and termination
5 codons, and elements derived from viral systems (particularly an internal ribosome entry site, or IRES, also referred to as a CITE sequence). The expression cassette may also include other features such as an origin of replication, and/or chromosome integration elements such as retroviral long terminal repeats (LTRs), or adeno-associated viral (AAV) inverted terminal repeats (ITRs).

- 10 Polynucleotide and nucleic acid coding regions of the present invention may be associated with additional coding regions which encode secretory or signal peptides, which direct the secretion of a polypeptide encoded by a polynucleotide of the present invention. For example, if secretion of the immunoconjugate is desired, DNA encoding a signal sequence may be placed upstream of the nucleic acid encoding an immunoconjugates of the invention or a fragment thereof.
- 15 According to the signal hypothesis, proteins secreted by mammalian cells have a signal peptide or secretory leader sequence which is cleaved from the mature protein once export of the growing protein chain across the rough endoplasmic reticulum has been initiated. Those of ordinary skill in the art are aware that polypeptides secreted by vertebrate cells generally have a signal peptide fused to the N-terminus of the polypeptide, which is cleaved from the translated
20 polypeptide to produce a secreted or "mature" form of the polypeptide. In certain embodiments, the native signal peptide, *e.g.* an immunoglobulin heavy chain or light chain signal peptide is used, or a functional derivative of that sequence that retains the ability to direct the secretion of the polypeptide that is operably associated with it. Alternatively, a heterologous mammalian signal peptide, or a functional derivative thereof, may be used. For example, the wild-type leader
25 sequence may be substituted with the leader sequence of human tissue plasminogen activator (TPA) or mouse β -glucuronidase. Exemplary amino acid and corresponding polynucleotide sequences of secretory signal peptides are shown in SEQ ID NOs 8-16.

DNA encoding a short protein sequence that could be used to facilitate later purification (e.g. a histidine tag) or assist in labeling the immunoconjugate may be included within or at the ends of
30 the immunoconjugate (fragment) encoding polynucleotide.

In a further embodiment, a host cell comprising one or more polynucleotides of the invention is provided. In certain embodiments a host cell comprising one or more vectors of the invention is provided. The polynucleotides and vectors may incorporate any of the features, singly or in

combination, described herein in relation to polynucleotides and vectors, respectively. In one such embodiment a host cell comprises (e.g. has been transformed or transfected with) a vector comprising a polynucleotide that encodes (part of) an immunoconjugate of the invention. As used herein, the term "host cell" refers to any kind of cellular system which can be engineered to

5 generate the immunoconjugates of the invention or fragments thereof. Host cells suitable for replicating and for supporting expression of immunoconjugates are well known in the art. Such cells may be transfected or transduced as appropriate with the particular expression vector and large quantities of vector containing cells can be grown for seeding large scale fermenters to obtain sufficient quantities of the immunoconjugate for clinical applications. Suitable host cells

10 include prokaryotic microorganisms, such as *E. coli*, or various eukaryotic cells, such as Chinese hamster ovary cells (CHO), insect cells, or the like. For example, polypeptides may be produced in bacteria in particular when glycosylation is not needed. After expression, the polypeptide may be isolated from the bacterial cell paste in a soluble fraction and can be further purified. In addition to prokaryotes, eukaryotic microbes such as filamentous fungi or yeast are suitable

15 cloning or expression hosts for polypeptide-encoding vectors, including fungi and yeast strains whose glycosylation pathways have been "humanized", resulting in the production of a polypeptide with a partially or fully human glycosylation pattern. See Gerngross, *Nat Biotech* 22, 1409-1414 (2004), and Li et al., *Nat Biotech* 24, 210-215 (2006). Suitable host cells for the expression of (glycosylated) polypeptides are also derived from multicellular organisms

20 (invertebrates and vertebrates). Examples of invertebrate cells include plant and insect cells. Numerous baculoviral strains have been identified which may be used in conjunction with insect cells, particularly for transfection of *Spodoptera frugiperda* cells. Plant cell cultures can also be utilized as hosts. See e.g. US Patent Nos. 5,959,177, 6,040,498, 6,420,548, 7,125,978, and 6,417,429 (describing PLANTIBODIESTM technology for producing antibodies in transgenic

25 plants). Vertebrate cells may also be used as hosts. For example, mammalian cell lines that are adapted to grow in suspension may be useful. Other examples of useful mammalian host cell lines are monkey kidney CV1 line transformed by SV40 (COS-7); human embryonic kidney line (293 or 293T cells as described, e.g., in Graham et al., *J Gen Virol* 36, 59 (1977)), baby hamster kidney cells (BHK), mouse sertoli cells (TM4 cells as described, e.g., in Mather, *Biol Reprod* 23,

30 243-251 (1980)), monkey kidney cells (CV1), African green monkey kidney cells (VERO-76), human cervical carcinoma cells (HELA), canine kidney cells (MDCK), buffalo rat liver cells (BRL 3A), human lung cells (W138), human liver cells (Hep G2), mouse mammary tumor cells (MMT 060562), TRI cells (as described, e.g., in Mather et al., *Annals N.Y. Acad Sci* 383, 44-68

- (1982)), MRC 5 cells, and FS4 cells. Other useful mammalian host cell lines include Chinese hamster ovary (CHO) cells, including dhfr⁻ CHO cells (Urlaub et al., Proc Natl Acad Sci USA 77, 4216 (1980)); and myeloma cell lines such as YO, NS0, P3X63 and Sp2/0. For a review of certain mammalian host cell lines suitable for protein production, see, e.g., Yazaki and Wu,
- 5 Methods in Molecular Biology, Vol. 248 (B.K.C. Lo, ed., Humana Press, Totowa, NJ), pp. 255-268 (2003). Host cells include cultured cells, e.g., mammalian cultured cells, yeast cells, insect cells, bacterial cells and plant cells, to name only a few, but also cells comprised within a transgenic animal, transgenic plant or cultured plant or animal tissue. In one embodiment, the host cell is a eukaryotic cell, preferably a mammalian cell, such as a Chinese Hamster Ovary
- 10 (CHO) cell, a human embryonic kidney (HEK) cell or a lymphoid cell (e.g., Y0, NS0, Sp20 cell). Standard technologies are known in the art to express foreign genes in these systems. Cells expressing a polypeptide comprising either the heavy or the light chain of an antigen binding domain such as an antibody, may be engineered so as to also express the other of the antibody chains such that the expressed product is an antibody that has both a heavy and a light chain.
- 15 In one embodiment, a method of producing an immunoconjugate according to the invention is provided, wherein the method comprises culturing a host cell comprising a polynucleotide encoding the immunoconjugate, as provided herein, under conditions suitable for expression of the immunoconjugate, and recovering the immunoconjugate from the host cell (or host cell culture medium).
- 20 The components of the immunoconjugate are genetically fused to each other. Immunoconjugates can be designed such that its components are fused directly to each other or indirectly through a linker sequence. The composition and length of the linker may be determined in accordance with methods well known in the art and may be tested for efficacy. Examples of linker sequences between the effector moiety and the Fc domain are found in the sequences shown in SEQ ID NO
- 25 195, 197, 199, 203, 209, 215, 229, 235, 237, 243, 245, 247, 249, 251, 269, 271, 273, 275, 277, 279 and 285. Additional sequences may also be included to incorporate a cleavage site to separate the individual components of the fusion if desired, for example an endopeptidase recognition sequence.
- In certain embodiments the one or more antigen binding moieties of the immunoconjugate
- 30 comprise at least an antibody variable region capable of binding an antigenic determinant. Variable regions can form part of and be derived from naturally or non-naturally occurring antibodies and fragments thereof. Methods to produce polyclonal antibodies and monoclonal antibodies are well known in the art (see e.g. Harlow and Lane, "Antibodies, a laboratory

manual", Cold Spring Harbor Laboratory, 1988). Non-naturally occurring antibodies can be constructed using solid phase-peptide synthesis, can be produced recombinantly (e.g. as described in U.S. patent No. 4,186,567) or can be obtained, for example, by screening combinatorial libraries comprising variable heavy chains and variable light chains (see e.g. U.S. Patent. No. 5,969,108 to McCafferty). Antigen binding moieties and methods for producing the same are also described in detail in PCT publication WO 2011/020783, the entire content of which is incorporated herein by reference.

Any animal species of antibody, antibody fragment, antigen binding domain or variable region can be used in the immunoconjugates of the invention. Non-limiting antibodies, antibody fragments, antigen binding domains or variable regions useful in the present invention can be of murine, primate, or human origin. If the immunoconjugate is intended for human use, a chimeric form of antibody may be used wherein the constant regions of the antibody are from a human. A humanized or fully human form of the antibody can also be prepared in accordance with methods well known in the art (see e. g. U.S. Patent No. 5,565,332 to Winter). Humanization may be achieved by various methods including, but not limited to (a) grafting the non-human (e.g., donor antibody) CDRs onto human (e.g. recipient antibody) framework and constant regions with or without retention of critical framework residues (e.g. those that are important for retaining good antigen binding affinity or antibody functions), (b) grafting only the non-human specificity-determining regions (SDRs or a-CDRs; the residues critical for the antibody-antigen interaction) onto human framework and constant regions, or (c) transplanting the entire non-human variable domains, but "cloaking" them with a human-like section by replacement of surface residues. Humanized antibodies and methods of making them are reviewed, e.g., in Almagro and Fransson, *Front Biosci* 13, 1619-1633 (2008), and are further described, e.g., in Riechmann et al., *Nature* 332, 323-329 (1988); Queen et al., *Proc Natl Acad Sci USA* 86, 10029-10033 (1989); US Patent Nos. 5,821,337, 7,527,791, 6,982,321, and 7,087,409; Jones et al., *Nature* 321, 522-525 (1986); Morrison et al., *Proc Natl Acad Sci* 81, 6851-6855 (1984); Morrison and Oi, *Adv Immunol* 44, 65-92 (1988); Verhoeven et al., *Science* 239, 1534-1536 (1988); Padlan, *Molec Immun* 31(3), 169-217 (1994); Kashmiri et al., *Methods* 36, 25-34 (2005) (describing SDR (a-CDR) grafting); Padlan, *Mol Immunol* 28, 489-498 (1991) (describing "resurfacing"); Dall'Acqua et al., *Methods* 36, 43-60 (2005) (describing "FR shuffling"); and Osbourn et al., *Methods* 36, 61-68 (2005) and Klimka et al., *Br J Cancer* 83, 252-260 (2000) (describing the "guided selection" approach to FR shuffling). Human antibodies and human variable regions can be produced using various techniques known in the art. Human antibodies

are described generally in van Dijk and van de Winkel, *Curr Opin Pharmacol* 5, 368-74 (2001) and Lonberg, *Curr Opin Immunol* 20, 450-459 (2008). Human variable regions can form part of and be derived from human monoclonal antibodies made by the hybridoma method (see e.g. *Monoclonal Antibody Production Techniques and Applications*, pp. 51-63 (Marcel Dekker, Inc., New York, 1987)). Human antibodies and human variable regions may also be prepared by administering an immunogen to a transgenic animal that has been modified to produce intact human antibodies or intact antibodies with human variable regions in response to antigenic challenge (see e.g. Lonberg, *Nat Biotech* 23, 1117-1125 (2005). Human antibodies and human variable regions may also be generated by isolating Fv clone variable region sequences selected from human-derived phage display libraries (see e.g., Hoogenboom et al. in *Methods in Molecular Biology* 178, 1-37 (O'Brien et al., ed., Human Press, Totowa, NJ, 2001); and McCafferty et al., *Nature* 348, 552-554; Clackson et al., *Nature* 352, 624-628 (1991)). Phage typically display antibody fragments, either as single-chain Fv (scFv) fragments or as Fab fragments. A detailed description of the preparation of antigen binding moieties for immunoconjugates by phage display can be found in the Examples appended to PCT publication WO 2011/020783.

In certain embodiments, the antigen binding moieties useful in the present invention are engineered to have enhanced binding affinity according to, for example, the methods disclosed in PCT publication WO 2011/020783 (see Examples relating to affinity maturation) or U.S. Pat. Appl. Publ. No. 2004/0132066, the entire contents of which are hereby incorporated by reference. The ability of the immunoconjugate of the invention to bind to a specific antigenic determinant can be measured either through an enzyme-linked immunosorbent assay (ELISA) or other techniques familiar to one of skill in the art, e.g. surface plasmon resonance technique (analyzed on a BIACORE T100 system) (Liljeblad, et al., *Glyco J* 17, 323-329 (2000)), and traditional binding assays (Heeley, *Endocr Res* 28, 217-229 (2002)). Competition assays may be used to identify an antibody, antibody fragment, antigen binding domain or variable domain that competes with a reference antibody for binding to a particular antigen, e.g. an antibody that competes with the L19 antibody for binding to the Extra Domain B of fibronectin (EDB). In certain embodiments, such a competing antibody binds to the same epitope (e.g. a linear or a conformational epitope) that is bound by the reference antibody. Detailed exemplary methods for mapping an epitope to which an antibody binds are provided in Morris (1996) "Epitope Mapping Protocols," in *Methods in Molecular Biology* vol. 66 (Humana Press, Totowa, NJ). In an exemplary competition assay, immobilized antigen (e.g. EDB) is incubated in a solution

comprising a first labeled antibody that binds to the antigen (e.g. L19 antibody) and a second unlabeled antibody that is being tested for its ability to compete with the first antibody for binding to the antigen. The second antibody may be present in a hybridoma supernatant. As a control, immobilized antigen is incubated in a solution comprising the first labeled antibody but
5 not the second unlabeled antibody. After incubation under conditions permissive for binding of the first antibody to the antigen, excess unbound antibody is removed, and the amount of label associated with immobilized antigen is measured. If the amount of label associated with immobilized antigen is substantially reduced in the test sample relative to the control sample, then that indicates that the second antibody is competing with the first antibody for binding to
10 the antigen. See Harlow and Lane (1988) Antibodies: A Laboratory Manual ch.14 (Cold Spring Harbor Laboratory, Cold Spring Harbor, NY).

Immunoconjugates prepared as described herein may be purified by art-known techniques such as high performance liquid chromatography, ion exchange chromatography, gel electrophoresis, affinity chromatography, size exclusion chromatography, and the like. The actual conditions
15 used to purify a particular protein will depend, in part, on factors such as net charge, hydrophobicity, hydrophilicity etc., and will be apparent to those having skill in the art. For affinity chromatography purification an antibody, ligand, receptor or antigen can be used to which the immunoconjugate binds. For example, for affinity chromatography purification of immunoconjugates of the invention, a matrix with protein A or protein G may be used.
20 Sequential Protein A or G affinity chromatography and size exclusion chromatography can be used to isolate an immunoconjugate essentially as described in the Examples. The purity of the immunoconjugate can be determined by any of a variety of well known analytical methods including gel electrophoresis, high pressure liquid chromatography, and the like. For example, the heavy chain fusion proteins expressed as described in the Examples were shown to be intact
25 and properly assembled as demonstrated by reducing SDS-PAGE (see e.g. Figure 4). Three bands were resolved at approximately Mr 25,000, Mr 50,000 and Mr 60,000, corresponding to the predicted molecular weights of the immunoglobulin light chain, heavy chain and heavy chain/effector moiety fusion protein.

30 Assays

Immunoconjugates provided herein may be identified, screened for, or characterized for their physical/chemical properties and/or biological activities by various assays known in the art.

Affinity assays

The affinity of the immunoconjugate for an effector moiety receptor (e.g. IL-10R or various forms of IL-2R), an Fc receptor, or a target antigen, can be determined in accordance with the methods set forth in the Examples by surface plasmon resonance (SPR), using standard instrumentation such as a BIAcore instrument (GE Healthcare), and receptors or target proteins such as may be obtained by recombinant expression. Alternatively, binding of immunoconjugates for different receptors or target antigens may be evaluated using cell lines expressing the particular receptor or target antigen, for example by flow cytometry (FACS). A specific illustrative and exemplary embodiment for measuring binding affinity is described in the following and in the Examples below.

According to one embodiment, K_D is measured by surface plasmon resonance using a BIAcore® T100 machine (GE Healthcare) at 25°C with ligand (e.g. effector moiety receptor, Fc receptor or target antigen) immobilized on CM5 chips. Briefly, carboxymethylated dextran biosensor chips (CM5, GE Healthcare) are activated with N-ethyl-N'-(3-dimethylaminopropyl)-carbodiimide hydrochloride (EDC) and N-hydroxysuccinimide (NHS) according to the supplier's instructions. Recombinant ligand is diluted with 10 mM sodium acetate, pH 5.5, to 0.5-30 µg/ml before injection at a flow rate of 10 µl/minute to achieve approximately 100-5000 response units (RU) of coupled protein. Following the injection of the ligand, 1 M ethanolamine is injected to block unreacted groups. For kinetics measurements, three- to five-fold serial dilutions of immunoconjugate (range between ~0.01 nM to 300 nM) are injected in HBS-EP+ (GE Healthcare, 10 mM HEPES, 150 mM NaCl, 3 mM EDTA, 0.05% Surfactant P20, pH 7.4) at 25°C at a flow rate of approximately 30-50 µl/min. Association rates (k_{on}) and dissociation rates (k_{off}) are calculated using a simple one-to-one Langmuir binding model (BIAcore® T100 Evaluation Software version 1.1.1) by simultaneously fitting the association and dissociation sensorgrams. The equilibrium dissociation constant (K_D) is calculated as the ratio k_{off}/k_{on} . See, e.g., Chen et al., J Mol Biol 293, 865-881 (1999).

Activity assays

Biological activity of the immunoconjugates of the invention can be measured by various assays as described in the Examples. Biological activities may for example include the induction of proliferation of effector moiety receptor-bearing cells, the induction of signaling in effector moiety receptor-bearing cells, the induction of cytokine secretion by effector moiety receptor-bearing cells, and the induction of tumor regression and/or the improvement of survival.

Compositions, Formulations, and Routes of Administration

In a further aspect, the invention provides pharmaceutical compositions comprising any of the immunoconjugates provided herein, e.g., for use in any of the below therapeutic methods. In one
5 embodiment, a pharmaceutical composition comprises any of the immunoconjugates provided herein and a pharmaceutically acceptable carrier. In another embodiment, a pharmaceutical composition comprises any of the immunoconjugates provided herein and at least one additional therapeutic agent, e.g., as described below.

Further provided is a method of producing an immunoconjugate of the invention in a form
10 suitable for administration in vivo, the method comprising (a) obtaining an immunoconjugate according to the invention, and (b) formulating the immunoconjugate with at least one pharmaceutically acceptable carrier, whereby a preparation of immunoconjugate is formulated for administration in vivo.

Pharmaceutical compositions of the present invention comprise a therapeutically effective
15 amount of one or more immunoconjugate dissolved or dispersed in a pharmaceutically acceptable carrier. The phrases "pharmaceutical or pharmacologically acceptable" refers to molecular entities and compositions that are generally non-toxic to recipients at the dosages and concentrations employed, i.e. do not produce an adverse, allergic or other untoward reaction when administered to an animal, such as, for example, a human, as appropriate. The preparation
20 of a pharmaceutical composition that contains at least one immunoconjugate and optionally an additional active ingredient will be known to those of skill in the art in light of the present disclosure, as exemplified by Remington's Pharmaceutical Sciences, 18th Ed. Mack Printing Company, 1990, incorporated herein by reference. Moreover, for animal (e.g., human) administration, it will be understood that preparations should meet sterility, pyrogenicity, general
25 safety and purity standards as required by FDA Office of Biological Standards or corresponding authorities in other countries. Preferred compositions are lyophilized formulations or aqueous solutions. As used herein, "pharmaceutically acceptable carrier" includes any and all solvents, buffers, dispersion media, coatings, surfactants, antioxidants, preservatives (e.g. antibacterial agents, antifungal agents), isotonic agents, absorption delaying agents, salts, preservatives,
30 antioxidants, proteins, drugs, drug stabilizers, polymers, gels, binders, excipients, disintegration agents, lubricants, sweetening agents, flavoring agents, dyes, such like materials and combinations thereof, as would be known to one of ordinary skill in the art (see, for example, Remington's Pharmaceutical Sciences, 18th Ed. Mack Printing Company, 1990, pp. 1289-1329,

incorporated herein by reference). Except insofar as any conventional carrier is incompatible with the active ingredient, its use in the therapeutic or pharmaceutical compositions is contemplated.

The composition may comprise different types of carriers depending on whether it is to be administered in solid, liquid or aerosol form, and whether it need to be sterile for such routes of administration as injection. Immunoconjugates of the present invention (and any additional therapeutic agent) can be administered intravenously, intradermally, intraarterially, intraperitoneally, intralesionally, intracranially, intraarticularly, intraprostatically, intrasplenically, intrarenally, intrapleurally, intratracheally, intranasally, intravitreally, intravaginally, intrarectally, intratumorally, intramuscularly, intraperitoneally, subcutaneously, subconjunctivally, intravesicularly, mucosally, intrapericardially, intraumbilically, intraocularly, orally, topically, locally, by inhalation (e.g. aerosol inhalation), injection, infusion, continuous infusion, localized perfusion bathing target cells directly, via a catheter, via a lavage, in cremes, in lipid compositions (e.g. liposomes), or by other method or any combination of the forgoing as would be known to one of ordinary skill in the art (see, for example, Remington's Pharmaceutical Sciences, 18th Ed. Mack Printing Company, 1990, incorporated herein by reference). Parenteral administration, in particular intravenous injection, is most commonly used for administering polypeptide molecules such as the immunoconjugates of the invention.

Parenteral compositions include those designed for administration by injection, e.g. subcutaneous, intradermal, intralesional, intravenous, intraarterial intramuscular, intrathecal or intraperitoneal injection. For injection, the immunoconjugates of the invention may be formulated in aqueous solutions, preferably in physiologically compatible buffers such as Hanks' solution, Ringer's solution, or physiological saline buffer. The solution may contain formulatory agents such as suspending, stabilizing and/or dispersing agents. Alternatively, the immunoconjugates may be in powder form for constitution with a suitable vehicle, e.g., sterile pyrogen-free water, before use. Sterile injectable solutions are prepared by incorporating the immunoconjugates of the invention in the required amount in the appropriate solvent with various of the other ingredients enumerated below, as required. Sterility may be readily accomplished, e.g., by filtration through sterile filtration membranes. Generally, dispersions are prepared by incorporating the various sterilized active ingredients into a sterile vehicle which contains the basic dispersion medium and/or the other ingredients. In the case of sterile powders for the preparation of sterile injectable solutions, suspensions or emulsion, the preferred methods

of preparation are vacuum-drying or freeze-drying techniques which yield a powder of the active ingredient plus any additional desired ingredient from a previously sterile-filtered liquid medium thereof. The liquid medium should be suitably buffered if necessary and the liquid diluent first rendered isotonic prior to injection with sufficient saline or glucose. The composition must be

5 stable under the conditions of manufacture and storage, and preserved against the contaminating action of microorganisms, such as bacteria and fungi. It will be appreciated that endotoxin contamination should be kept minimally at a safe level, for example, less than 0.5 ng/mg protein. Suitable pharmaceutically acceptable carriers include, but are not limited to: buffers such as phosphate, citrate, and other organic acids; antioxidants including ascorbic acid and methionine;

10 preservatives (such as octadecyldimethylbenzyl ammonium chloride; hexamethonium chloride; benzalkonium chloride; benzethonium chloride; phenol, butyl or benzyl alcohol; alkyl parabens such as methyl or propyl paraben; catechol; resorcinol; cyclohexanol; 3-pentanol; and m-cresol); low molecular weight (less than about 10 residues) polypeptides; proteins, such as serum albumin, gelatin, or immunoglobulins; hydrophilic polymers such as polyvinylpyrrolidone;

15 amino acids such as glycine, glutamine, asparagine, histidine, arginine, or lysine; monosaccharides, disaccharides, and other carbohydrates including glucose, mannose, or dextrans; chelating agents such as EDTA; sugars such as sucrose, mannitol, trehalose or sorbitol; salt-forming counter-ions such as sodium; metal complexes (e.g. Zn-protein complexes); and/or non-ionic surfactants such as polyethylene glycol (PEG). Aqueous injection suspensions may

20 contain compounds which increase the viscosity of the suspension, such as sodium carboxymethyl cellulose, sorbitol, dextran, or the like. Optionally, the suspension may also contain suitable stabilizers or agents which increase the solubility of the compounds to allow for the preparation of highly concentrated solutions. Additionally, suspensions of the active compounds may be prepared as appropriate oily injection suspensions. Suitable lipophilic

25 solvents or vehicles include fatty oils such as sesame oil, or synthetic fatty acid esters, such as ethyl esters or triglycerides, or liposomes.

Active ingredients may be entrapped in microcapsules prepared, for example, by coacervation techniques or by interfacial polymerization, for example, hydroxymethylcellulose or gelatin-microcapsules and poly-(methylmethacrylate) microcapsules, respectively, in colloidal drug

30 delivery systems (for example, liposomes, albumin microspheres, microemulsions, nanoparticles and nanocapsules) or in macroemulsions. Such techniques are disclosed in Remington's Pharmaceutical Sciences (18th Ed. Mack Printing Company, 1990). Sustained-release preparations may be prepared. Suitable examples of sustained-release preparations include

semipermeable matrices of solid hydrophobic polymers containing the polypeptide, which matrices are in the form of shaped articles, e.g. films, or microcapsules. In particular embodiments, prolonged absorption of an injectable composition can be brought about by the use in the compositions of agents delaying absorption, such as, for example, aluminum monostearate, gelatin or combinations thereof.

In addition to the compositions described previously, the immunoconjugates may also be formulated as a depot preparation. Such long acting formulations may be administered by implantation (for example subcutaneously or intramuscularly) or by intramuscular injection. Thus, for example, the immunoconjugates may be formulated with suitable polymeric or hydrophobic materials (for example as an emulsion in an acceptable oil) or ion exchange resins, or as sparingly soluble derivatives, for example, as a sparingly soluble salt.

Pharmaceutical compositions comprising the immunoconjugates of the invention may be manufactured by means of conventional mixing, dissolving, emulsifying, encapsulating, entrapping or lyophilizing processes. Pharmaceutical compositions may be formulated in conventional manner using one or more physiologically acceptable carriers, diluents, excipients or auxiliaries which facilitate processing of the proteins into preparations that can be used pharmaceutically. Proper formulation is dependent upon the route of administration chosen.

The immunoconjugates may be formulated into a composition in a free acid or base, neutral or salt form. Pharmaceutically acceptable salts are salts that substantially retain the biological activity of the free acid or base. These include the acid addition salts, e.g., those formed with the free amino groups of a proteinaceous composition, or which are formed with inorganic acids such as for example, hydrochloric or phosphoric acids, or such organic acids as acetic, oxalic, tartaric or mandelic acid. Salts formed with the free carboxyl groups can also be derived from inorganic bases such as for example, sodium, potassium, ammonium, calcium or ferric hydroxides; or such organic bases as isopropylamine, trimethylamine, histidine or procaine. Pharmaceutical salts tend to be more soluble in aqueous and other protic solvents than are the corresponding free base forms.

Therapeutic Methods and Compositions

Any of the immunoconjugates provided herein may be used in therapeutic methods. Immunoconjugates of the invention can be used as immunotherapeutic agents, for example in the treatment of cancers.

For use in therapeutic methods, immunoconjugates of the invention would be formulated, dosed, and administered in a fashion consistent with good medical practice. Factors for consideration in this context include the particular disorder being treated, the particular mammal being treated, the clinical condition of the individual patient, the cause of the disorder, the site of delivery of the agent, the method of administration, the scheduling of administration, and other factors known to medical practitioners.

In one aspect, immunoconjugates of the invention for use as a medicament are provided. In further aspects, immunoconjugates of the invention for use in treating a disease are provided. In certain embodiments, immunoconjugates of the invention for use in a method of treatment are provided. In one embodiment, the invention provides an immunoconjugate as described herein for use in the treatment of a disease in an individual in need thereof. In certain embodiments, the invention provides an immunoconjugate for use in a method of treating an individual having a disease comprising administering to the individual a therapeutically effective amount of the immunoconjugate. In certain embodiments the disease to be treated is a proliferative disorder. In a particular embodiment the disease is cancer. In other embodiments the disease to be treated is an inflammatory disorder. In certain embodiments the method further comprises administering to the individual a therapeutically effective amount of at least one additional therapeutic agent, e.g., an anti-cancer agent if the disease to be treated is cancer. An "individual" according to any of the above embodiments is a mammal, preferably a human.

In a further aspect, the invention provides for the use of an immunconjugate of the invention in the manufacture or preparation of a medicament for the treatment of a disease in an individual in need thereof. In one embodiment, the medicament is for use in a method of treating a disease comprising administering to an individual having the disease a therapeutically effective amount of the medicament. In certain embodiments the disease to be treated is a proliferative disorder. In a particular embodiment the disease is cancer. In other embodiments the disease to be treated is an inflammatory disorder. In one embodiment, the method further comprises administering to the individual a therapeutically effective amount of at least one additional therapeutic agent, e.g., an anti-cancer agent if the disease to be treated is cancer. An "individual" according to any of the above embodiments may be a mammal, preferably a human.

In a further aspect, the invention provides a method for treating a disease in an individual, comprising administering to said individual a therapeutically effective amount of an immunoconjugate of the invention. In one embodiment a composition is administered to said individual, comprising immunoconjugate of the invention in a pharmaceutically acceptable form.

In certain embodiments the disease to be treated is a proliferative disorder. In a particular embodiment the disease is cancer. In other embodiments the disease to be treated is an inflammatory disorder. In certain embodiments the method further comprises administering to the individual a therapeutically effective amount of at least one additional therapeutic agent, e.g.,
5 an anti-cancer agent if the disease to be treated is cancer. An "individual" according to any of the above embodiments may be a mammal, preferably a human.

In certain embodiments the disease to be treated is a proliferative disorder, particularly cancer. Non-limiting examples of cancers include bladder cancer, brain cancer, head and neck cancer, pancreatic cancer, lung cancer, breast cancer, ovarian cancer, uterine cancer, cervical cancer,
10 endometrial cancer, esophageal cancer, colon cancer, colorectal cancer, rectal cancer, gastric cancer, prostate cancer, blood cancer, skin cancer, squamous cell carcinoma, bone cancer, and kidney cancer. Other cell proliferation disorders that can be treated using an immunoconjugate of the present invention include, but are not limited to neoplasms located in the: abdomen, bone, breast, digestive system, liver, pancreas, peritoneum, endocrine glands (adrenal, parathyroid,
15 pituitary, testicles, ovary, thymus, thyroid), eye, head and neck, nervous system (central and peripheral), lymphatic system, pelvic, skin, soft tissue, spleen, thoracic region, and urogenital system. Also included are pre-cancerous conditions or lesions and cancer metastases. In certain embodiments the cancer is chosen from the group consisting of renal cell cancer, skin cancer, lung cancer, colorectal cancer, breast cancer, brain cancer, head and neck cancer. In some
20 embodiments, particularly where the effector moiety of the immunoconjugate is IL-10, the disease to be treated is an inflammatory disorder. Non-limiting examples of inflammatory disorders include rheumatoid arthritis, psoriasis or Crohn's disease. A skilled artisan readily recognizes that in many cases the immunoconjugates may not provide a cure but may only provide partial benefit. In some embodiments, a physiological change having some benefit is
25 also considered therapeutically beneficial. Thus, in some embodiments, an amount of immunoconjugate that provides a physiological change is considered an "effective amount" or a "therapeutically effective amount". The subject, patient, or individual in need of treatment is typically a mammal, more specifically a human.

The immunoconjugates of the invention are also useful as diagnostic reagents. The binding of an
30 immunoconjugate to an antigenic determinant can be readily detected by using a secondary antibody specific for the effector moiety. In one embodiment, the secondary antibody and the immunoconjugate facilitate the detection of binding of the immunoconjugate to an antigenic determinant located on a cell or tissue surface.

In some embodiments, an effective amount of an immunoconjugate of the invention is administered to a cell. In other embodiments, a therapeutically effective amount of an immunoconjugates of the invention is administered to an individual for the treatment of disease.

For the prevention or treatment of disease, the appropriate dosage of an immunoconjugate of the invention (when used alone or in combination with one or more other additional therapeutic agents) will depend on the type of disease to be treated, the route of administration, the body weight of the patient, the type of immunoconjugate, the severity and course of the disease, whether the immunoconjugate is administered for preventive or therapeutic purposes, previous or concurrent therapeutic interventions, the patient's clinical history and response to the immunoconjugate, and the discretion of the attending physician. The practitioner responsible for administration will, in any event, determine the concentration of active ingredient(s) in a composition and appropriate dose(s) for the individual subject. Various dosing schedules including but not limited to single or multiple administrations over various time-points, bolus administration, and pulse infusion are contemplated herein.

The immunoconjugate is suitably administered to the patient at one time or over a series of treatments. Depending on the type and severity of the disease, about 1 $\mu\text{g/kg}$ to 15 mg/kg (e.g. 0.1 mg/kg – 10 mg/kg) of immunoconjugate can be an initial candidate dosage for administration to the patient, whether, for example, by one or more separate administrations, or by continuous infusion. One typical daily dosage might range from about 1 $\mu\text{g/kg}$ to 100 mg/kg or more, depending on the factors mentioned above. For repeated administrations over several days or longer, depending on the condition, the treatment would generally be sustained until a desired suppression of disease symptoms occurs. One exemplary dosage of the immunoconjugate would be in the range from about 0.005 mg/kg to about 10 mg/kg . In other non-limiting examples, a dose may also comprise from about 1 microgram/kg body weight, about 5 microgram/kg body weight, about 10 microgram/kg body weight, about 50 microgram/kg body weight, about 100 microgram/kg body weight, about 200 microgram/kg body weight, about 350 microgram/kg body weight, about 500 microgram/kg body weight, about 1 milligram/kg body weight, about 5 milligram/kg body weight, about 10 milligram/kg body weight, about 50 milligram/kg body weight, about 100 milligram/kg body weight, about 200 milligram/kg body weight, about 350 milligram/kg body weight, about 500 milligram/kg body weight, to about 1000 mg/kg body weight or more per administration, and any range derivable therein. In non-limiting examples of a derivable range from the numbers listed herein, a range of about 5 mg/kg body weight to about 100 mg/kg body weight, about 5 microgram/kg body weight to about 500 milligram/kg body

weight, etc., can be administered, based on the numbers described above. Thus, one or more doses of about 0.5 mg/kg, 2.0 mg/kg, 5.0 mg/kg or 10 mg/kg (or any combination thereof) may be administered to the patient. Such doses may be administered intermittently, e.g. every week or every three weeks (e.g. such that the patient receives from about two to about twenty, or e.g. about six doses of the immunoconjugate). An initial higher loading dose, followed by one or more lower doses may be administered. However, other dosage regimens may be useful. The progress of this therapy is easily monitored by conventional techniques and assays.

The immunoconjugates of the invention will generally be used in an amount effective to achieve the intended purpose. For use to treat or prevent a disease condition, the immunoconjugates of the invention, or pharmaceutical compositions thereof, are administered or applied in a therapeutically effective amount. Determination of a therapeutically effective amount is well within the capabilities of those skilled in the art, especially in light of the detailed disclosure provided herein.

For systemic administration, a therapeutically effective dose can be estimated initially from *in vitro* assays, such as cell culture assays. A dose can then be formulated in animal models to achieve a circulating concentration range that includes the IC_{50} as determined in cell culture. Such information can be used to more accurately determine useful doses in humans.

Initial dosages can also be estimated from *in vivo* data, e.g., animal models, using techniques that are well known in the art. One having ordinary skill in the art could readily optimize administration to humans based on animal data.

Dosage amount and interval may be adjusted individually to provide plasma levels of the immunoconjugates which are sufficient to maintain therapeutic effect. Usual patient dosages for administration by injection range from about 0.1 to 50 mg/kg/day, typically from about 0.5 to 1 mg/kg/day. Therapeutically effective plasma levels may be achieved by administering multiple doses each day. Levels in plasma may be measured, for example, by HPLC.

In cases of local administration or selective uptake, the effective local concentration of the immunoconjugates may not be related to plasma concentration. One having skill in the art will be able to optimize therapeutically effective local dosages without undue experimentation.

A therapeutically effective dose of the immunoconjugates described herein will generally provide therapeutic benefit without causing substantial toxicity. Toxicity and therapeutic efficacy of an immunoconjugate can be determined by standard pharmaceutical procedures in cell culture or experimental animals. Cell culture assays and animal studies can be used to determine the LD_{50} (the dose lethal to 50% of a population) and the ED_{50} (the dose therapeutically effective in

50% of a population). The dose ratio between toxic and therapeutic effects is the therapeutic index, which can be expressed as the ratio LD_{50}/ED_{50} . Immunoconjugates that exhibit large therapeutic indices are preferred. In one embodiment, the immunoconjugate according to the present invention exhibits a high therapeutic index. The data obtained from cell culture assays and animal studies can be used in formulating a range of dosages suitable for use in humans. The dosage lies preferably within a range of circulating concentrations that include the ED_{50} with little or no toxicity. The dosage may vary within this range depending upon a variety of factors, e.g., the dosage form employed, the route of administration utilized, the condition of the subject, and the like. The exact formulation, route of administration and dosage can be chosen by the individual physician in view of the patient's condition (see, e.g., Fingl et al., 1975, in: The Pharmacological Basis of Therapeutics, Ch. 1, p. 1, incorporated herein by reference in its entirety).

The attending physician for patients treated with immunoconjugates of the invention would know how and when to terminate, interrupt, or adjust administration due to toxicity, organ dysfunction, and the like. Conversely, the attending physician would also know to adjust treatment to higher levels if the clinical response were not adequate (precluding toxicity). The magnitude of an administered dose in the management of the disorder of interest will vary with the severity of the condition to be treated, with the route of administration, and the like. The severity of the condition may, for example, be evaluated, in part, by standard prognostic evaluation methods. Further, the dose and perhaps dose frequency will also vary according to the age, body weight, and response of the individual patient.

Other Agents and Treatments

The immunoconjugates of the invention may be administered in combination with one or more other agents in therapy. For instance, an immunoconjugate of the invention may be co-administered with at least one additional therapeutic agent. The term "therapeutic agent" encompasses any agent administered to treat a symptom or disease in an individual in need of such treatment. Such additional therapeutic agent may comprise any active ingredients suitable for the particular indication being treated, preferably those with complementary activities that do not adversely affect each other. In certain embodiments, an additional therapeutic agent is an immunomodulatory agent, a cytostatic agent, an inhibitor of cell adhesion, a cytotoxic agent, an activator of cell apoptosis, or an agent that increases the sensitivity of cells to apoptotic inducers. In a particular embodiment, the additional therapeutic agent is an anti-cancer agent, for example

a microtubule disruptor, an antimetabolite, a topoisomerase inhibitor, a DNA intercalator, an alkylating agent, a hormonal therapy, a kinase inhibitor, a receptor antagonist, an activator of tumor cell apoptosis, or an antiangiogenic agent.

Such other agents are suitably present in combination in amounts that are effective for the purpose intended. The effective amount of such other agents depends on the amount of immunoconjugate used, the type of disorder or treatment, and other factors discussed above. The immunoconjugates are generally used in the same dosages and with administration routes as described herein, or about from 1 to 99% of the dosages described herein, or in any dosage and by any route that is empirically/clinically determined to be appropriate.

- Such combination therapies noted above encompass combined administration (where two or more therapeutic agents are included in the same or separate compositions), and separate administration, in which case, administration of the immunoconjugate of the invention can occur prior to, simultaneously, and/or following, administration of the additional therapeutic agent and/or adjuvant. Immunoconjugates of the invention can also be used in combination with radiation therapy.

Articles of Manufacture

- In another aspect of the invention, an article of manufacture containing materials useful for the treatment, prevention and/or diagnosis of the disorders described above is provided. The article of manufacture comprises a container and a label or package insert on or associated with the container. Suitable containers include, for example, bottles, vials, syringes, IV solution bags, etc. The containers may be formed from a variety of materials such as glass or plastic. The container holds a composition which is by itself or combined with another composition effective for treating, preventing and/or diagnosing the condition and may have a sterile access port (for example the container may be an intravenous solution bag or a vial having a stopper pierceable by a hypodermic injection needle). At least one active agent in the composition is an immunoconjugate of the invention. The label or package insert indicates that the composition is used for treating the condition of choice. Moreover, the article of manufacture may comprise (a) a first container with a composition contained therein, wherein the composition comprises an immunoconjugate of the invention; and (b) a second container with a composition contained therein, wherein the composition comprises a further cytotoxic or otherwise therapeutic agent. The article of manufacture in this embodiment of the invention may further comprise a package insert indicating that the compositions can be used to treat a particular condition. Alternatively,

or additionally, the article of manufacture may further comprise a second (or third) container comprising a pharmaceutically-acceptable buffer, such as bacteriostatic water for injection (BWFI), phosphate-buffered saline, Ringer's solution and dextrose solution. It may further include other materials desirable from a commercial and user standpoint, including other buffers, diluents, filters, needles, and syringes.

Examples

The following are examples of methods and compositions of the invention. It is understood that various other embodiments may be practiced, given the general description provided above.

5

Example 1

General methods

Recombinant DNA Techniques

Standard methods were used to manipulate DNA as described in Sambrook et al., Molecular cloning: A laboratory manual; Cold Spring Harbor Laboratory Press, Cold Spring Harbor, New York, 1989. The molecular biological reagents were used according to the manufacturer's instructions. General information regarding the nucleotide sequences of human immunoglobulins light and heavy chains is given in: Kabat, E.A. et al., (1991) Sequences of Proteins of Immunological Interest, Fifth Ed., NIH Publication No 91-3242.

DNA Sequencing

15 DNA sequences were determined by double strand sequencing.

Gene Synthesis

Desired gene segments where required were either generated by PCR using appropriate templates or were synthesized by Geneart AG (Regensburg, Germany) from synthetic oligonucleotides and PCR products by automated gene synthesis. In cases where no exact gene sequence was available, oligonucleotide primers were designed based on sequences from closest homologues and the genes were isolated by RT-PCR from RNA originating from the appropriate tissue. The gene segments flanked by singular restriction endonuclease cleavage sites were cloned into standard cloning / sequencing vectors. The plasmid DNA was purified from transformed bacteria and concentration determined by UV spectroscopy. The DNA sequence of the subcloned gene fragments was confirmed by DNA sequencing. Gene segments were designed with suitable restriction sites to allow sub-cloning into the respective expression vectors. All constructs were designed with a 5'-end DNA sequence coding for a leader peptide which targets proteins for secretion in eukaryotic cells. SEQ ID NOs 8-16 give exemplary leader peptides and polynucleotide sequences encoding them.

Preparation of IL-2R $\beta\gamma$ subunit-Fc fusions and IL-2R α subunit Fc fusion

To study IL-2 receptor binding affinity, a tool was generated that allowed for the expression of a heterodimeric IL-2 receptor; the β -subunit of the IL-2 receptor was fused to an Fc molecule that was engineered to heterodimerize (Fc(hole)) (see SEQ ID NOs 17 and 18) using the “knobs-into-holes” technology (Merchant et al., Nat Biotech. 16, 677-681 (1998)). The γ -subunit of the IL-2 receptor was then fused to the Fc(knob) variant (see SEQ ID NOs 19 and 20), which heterodimerized with Fc(hole). This heterodimeric Fc-fusion protein was then used as a substrate for analyzing the IL-2/IL-2 receptor interaction. The IL-2R α -subunit was expressed as monomeric chain with an AcTev cleavage site and an Avi His tag (SEQ ID NOs 21 and 22). The respective IL-2R subunits were transiently expressed in HEK EBNA 293 with serum for the IL-2R $\beta\gamma$ subunit construct and without serum for the α -subunit construct. The IL-2R $\beta\gamma$ subunit construct was purified on protein A (GE Healthcare), followed by size exclusion chromatography (GE Healthcare, Superdex 200). The IL-2R α -subunit was purified via His tag on a NiNTA column (Qiagen) followed by size exclusion chromatography (GE Healthcare, Superdex 75). Amino acid and corresponding nucleotide sequences of various receptor constructs are given in SEQ ID NOs 17-22 and 255-268.

Preparation of immunconjugates

Details about the generation and affinity maturation of antigen binding moieties directed to FAP can be found in the Examples appended to PCT patent application publication no. WO 2012/020006, which is incorporated herein by reference in its entirety. As described therein, various antigen binding domains directed to FAP have been generated by phage display, including the ones designated 4G8, 28H1 and 4B9 used in the following examples. Clone 28H1 is an affinity matured antibody based on parental clone 4G8, while clone 4B9 is an affinity matured antibody based on parental clone 3F2. The antigen binding domain designated 2B10 used herein is directed to the A2 domain of Tenascin C (TNC A2). Details about this and other antigen binding moieties directed against TNC A2 can be found in PCT patent application publication no. WO 2012/020038, which is incorporated herein by reference in its entirety. The antigen binding domain designated L19, directed against the Extra Domain B (EDB) of fibronectin is derived from the L19 antibody described in PCT publication WO 2007/128563. The antigen binding domains designated CH1A1A and CH1A1A 98/99 2F1 used herein are directed to CEA, and are described in more detail in PCT patent application no. PCT/EP2012/053390, which is incorporated herein by reference in its entirety.

The IL-2 quadruple mutant (qm) used as effector moiety in some of the following examples is described in detail in PCT patent application no. PCT/EP2012/051991, which is incorporated herein by reference in its entirety. Briefly, IL-2 qm is characterized by the following mutations:

1. T3A - knockout of predicted O-glycosylation site
- 5 2. F42A - knockout of IL-2/IL-2R α interaction
3. Y45A - knockout of IL-2/IL-2R α interaction
4. L72G - knockout of IL-2/IL-2R α interaction
5. C125A - mutation to avoid disulfide-bridged IL-2 dimers

The T3A mutation was chosen to eliminate the O-glycosylation site and obtain a protein product with higher homogeneity and purity when the IL-2 qm polypeptide or an immunoconjugate comprising it is expressed in eukaryotic cells such as CHO or HEK293 cells. The three mutations F42A, Y45A and L72G were chosen to interfere with the binding to CD25, the α -subunit of the IL-2 receptor. Reduced or abolished CD25 binding results in reduced activation-induced cell death (AICD), lack of preferential activation of regulatory T cells, as well as reduced toxicity (as described in EP 11153964.9).

The DNA sequences were generated by gene synthesis and/or classical molecular biology techniques and subcloned into mammalian expression vectors under the control of an MPSV promoter and upstream of a synthetic polyA site, each vector carrying an EBV OriP sequence. Immunoconjugates as applied in the examples below were produced by co-transfecting exponentially growing HEK293-EBNA cells with the mammalian expression vectors using calcium phosphate-transfection. Alternatively, HEK293 cells growing in suspension were transfected by polyethylenimine (PEI) with the respective expression vectors. Alternatively, stably transfected CHO cell pools or CHO cell clones were used for production in serum-free media. Subsequently, the IgG-cytokine fusion proteins were purified from the supernatant. Briefly, IgG-cytokine fusion proteins were purified by one affinity step with protein A (HiTrap ProtA, GE Healthcare) equilibrated in 20 mM sodium phosphate, 20 mM sodium citrate pH 7.5. After loading of the supernatant, the column was first washed with 20 mM sodium phosphate, 20 mM sodium citrate, pH 7.5 and subsequently washed with 13.3 mM sodium phosphate, 20 mM sodium citrate, 500 mM sodium chloride, pH 5.45. The IgG-cytokine fusion protein was eluted with 20 mM sodium citrate, 100 mM sodium chloride, 100 mM glycine, pH 3. Fractions were neutralized and pooled and purified by size exclusion chromatography (HiLoad 16/60 Superdex 200, GE Healthcare) in final formulation buffer: 25 mM potassium phosphate, 125 mM sodium

chloride, 100 mM glycine pH 6.7. Exemplary detailed purification procedures and results are given for selected constructs below. The protein concentration of purified protein samples was determined by measuring the optical density (OD) at 280 nm, using the molar extinction coefficient calculated on the basis of the amino acid sequence. Purity and molecular weight of immunoconjugates were analyzed by SDS-PAGE in the presence and absence of a reducing agent (5 mM 1,4-dithiothreitol) and stained with Coomassie blue (SimpleBlue™ SafeStain, Invitrogen). The NuPAGE® Pre-Cast gel system (Invitrogen) was used according to the manufacturer's instructions (4-20% Tris-glycine gels or 3-12% Bis-Tris). The aggregate content of immunoconjugate samples was analyzed using a Superdex 200 10/300GL analytical size-exclusion column (GE Healthcare) in 2 mM MOPS, 150 mM NaCl, 0.02% NaN₃, pH 7.3 running buffer at 25°C. The integrity of the amino acid backbone of reduced antibody light and heavy chains can be verified by NanoElectrospray Q-TOF mass spectrometry after removal of N-glycans by enzymatic treatment with Peptide-N Glycosidase F (Roche Molecular Biochemicals). The oligosaccharides attached to the Fc domain of the immunoconjugates are analysed by MALDI TOF-MS as described below. Oligosaccharides are enzymatically released from the immunoconjugates by PNGaseF digestion. The resulting digest solution containing the released oligosaccharides is either prepared directly for MALDI TOF-MS analysis or is further digested with EndoH glycosidase prior to sample preparation for MALDI TOF-MS analysis.

Example 2

FAP-targeted IgG-IL-2 qm fusion proteins were generated based on the FAP-antibodies 4G8, 28H1 and 4B9, wherein one single IL-2 quadruple mutant (qm) was fused to the C-terminus of one heterodimeric heavy chain as shown in Figure 2A. Targeting to the tumor stroma where FAP is selectively expressed is achieved via the bivalent antibody Fab region (avidity effect). Heterodimerization resulting in the presence of a single IL-2 quadruple mutant is achieved by application of the knob-into-hole technology. In order to minimize the generation of homodimeric IgG-cytokine fusions the cytokine was fused to the C-terminus (with deletion of the C-terminal Lys residue) of the knob-containing IgG heavy chain via a (G₄S)₃ or G₄-(SG₄)₂ linker. The antibody-cytokine fusion has IgG-like properties. To reduce FcγR binding/effector function and prevent FcR co-activation, P329G L234A L235A (LALA) mutations were introduced in the Fc domain. The sequences of these immunoconjugates are given SEQ ID NOs 193, 269 and 205 (28H1 with (G₄S)₃ linker), SEQ ID NOs 193, 195 and 205 (28H1 with G₄-(SG₄)₂ linker), SEQ ID NOs 201, 203 and 205 (4G8 with G₄-(SG₄)₂ linker), SEQ ID NOs 207,

209 and 211 (4B9 with $G_4-(SG_4)_2$ linker), SEQ ID NOs 207, 271 and 211 (4B9 with $(G_4S)_3$ linker).

In addition, a CEA-targeted IgG-IL-2 qm fusion protein based on the anti-CEA antibody CH1A1A 98/99 2F1, a control DP47GS non-targeted IgG-IL-2 qm fusion protein wherein the
 5 IgG does not bind to a specified target, as well as a tumor stroma specific 2B10-based IgG-IL-2 qm fusion protein targeted against the A2 domain of tenascin-C were generated. The sequences of these immunoconjugates are given in SEQ ID NOs 275, 281 and 283 (CH1A1A 98/99 2F1 with $G_4-(SG_4)_2$ linker), SEQ ID NOs 277, 281 and 283 (CH1A1A 98/99 2F1 with $(G_4S)_3$ linker), SEQ ID NOs 219, 221 and 225 (DP47GS with $G_4-(SG_4)_2$ linker), SEQ ID NOs 219, 289 and 225
 10 (DP47GS with $(G_4S)_3$ linker), SEQ ID NOs 285, 287 and 239 (2B10 with $(G_4S)_3$ linker). The constructs were generated by transient expression in HEK293 EBNA cells and purified as described above. Figures 3 to 9 show exemplary chromatograms and elution profiles of the purification (A, B) as well as the analytical SDS-PAGE and size exclusion chromatographies of the final purified constructs (C, D). Transient expression yields were 42 mg/L for the 4G8-based,
 15 20 mg/L for the 28H1-based, 10 mg/L for the 4B9-based, 5.3 mg/L for the CH1A1A 98/99 2F1-based, 36.7 mg/L for the 2B10-based and 13.8 mg/L for the DP47GS-based IgG-IL-2 qm immunoconjugate.

In addition a 28H1-based FAP-targeted IgG-IL-15 immunoconjugate is being generated, the sequences of which are given in SEQ ID NOs 193, 199 and 205. In the IL-15 polypeptide
 20 sequence the glutamic acid residue at position 53 is replaced by alanine to reduce binding to the α -subunit of the IL-15 receptor, and the asparagine residue at position 79 is replaced by alanine to abolish glycosylation. The IgG-IL-15 fusion protein is generated by transient expression and purified as described above.

25 FAP binding affinity

The FAP binding activity of the IgG-IL-2 qm immunoconjugates based on 4G8 and 28H1 anti-FAP antibodies were determined by surface plasmon resonance (SPR) on a Biacore machine in comparison to the corresponding unmodified IgG antibodies. Briefly, an anti-His antibody (Penta-His, Qiagen 34660) was immobilized on CM5 chips to capture 10 nM His-tagged human
 30 FAP (20 s). Temperature was 25°C and HBS-EP was used as buffer. Analyte concentration was 50 nM down to 0.05 nM at a flow rate of 50 μ l/min (association: 300 s, dissociation: 900 s, regeneration: 60 s with 10 mM glycine pH 2). Fitting was performed based on a 1:1 binding model, $RI=0$, $R_{max}=local$ (because of capture format). The following table gives the estimated

apparent bivalent affinities (pM avidity) as determined by SPR fitted with 1:1 binding RI=0, Rmax=local.

	Hu FAP
4G8 IgG-IL-2 qm	100 pM
4G8 IgG	50 pM
28H1 IgG-IL-2 qm	175 pM
28H1 IgG	200 pM

- 5 The data show that within the error of the method affinity for human FAP is retained for the 28H1-based immunoconjugate or only slightly decreased for the 4G8-based immunoconjugate as compared to the corresponding unmodified antibodies.

Similarly, the affinity (K_D) of 4B9 IgG-IL-2 qm (16 pM), CH1A1A 98/99 2F1 IgG-IL-2 qm (400 pM), CH1A1A 98/99 2F1 IgG-IL-2 wt (see Example 4; 470 pM) and 2B10 IgG-IL-2 qm (150 pM, vs. 300 pM for unconjugated 2B10 IgG) to human FAP, CEA and TNC A2, respectively,
10 were determined by SPR at 25°C. Cross-reactivity of the 4B9 and 2B10 antibodies to human, murine and cynomolgus FAP or TNC A2, respectively, was also confirmed.

Subsequently, the affinity of the 4G8- and 28H1-based IgG-IL-2 qm immunoconjugates to the IL-2R $\beta\gamma$ heterodimer and the IL-2R α -subunit were determined by surface plasmon resonance
15 (SPR) in direct comparison to the Fab-IL-2 qm-Fab immunoconjugate format described in PCT patent application no. PCT/EP2012/051991. Briefly, the ligands – either the human IL-2R α -subunit or the human IL-2R $\beta\gamma$ heterodimer – were immobilized on a CM5 chip. Subsequently, the 4G8- and 28H1-based IgG-IL-2 qm immunoconjugates or the 4G8- and 28H1-based Fab-IL-2 qm-Fab immunoconjugates for comparison were applied to the chip as analytes at 25°C in
20 HBS-EP buffer in concentrations ranging from 300 nM down to 1.2 nM (1:3 dil.). Flow rate was 30 μ l/min and the following conditions were applied for association: 180s, dissociation: 300 s, and regeneration: 2 x 30 s with 3 M $MgCl_2$ for IL-2R $\beta\gamma$ heterodimer, 10 s with 50 mM NaOH for IL-2R α -subunit. 1:1 binding was applied for fitting (1:1 binding RI $\neq$ 0, Rmax=local for IL-2R $\beta\gamma$, apparent K_D , 1:1 binding RI=0, Rmax=local for IL-2R α). The respective K_D values are
25 given in the table below.

Apparent K_D [nM]	Hu IL-2R $\beta\gamma$	Hu IL-2R α
4G8 IgG-IL-2 qm	5.9	No binding

4G8 Fab-IL-2 qm-Fab	10.4	No binding
28H1 IgG-IL-2 qm	6.2	No binding
28H1 Fab-IL-2 qm-Fab	11.4	No binding

The data show that the 4G8- and 28H1-based IgG-IL-2 qm immunoconjugates bind with at least as good affinity as the Fab-IL-2 qm-Fab immunoconjugates to the IL-2R $\beta\gamma$ heterodimer, whereas they do not bind to the IL-2R α -subunit due to the introduction of the mutations
5 interfering with CD25 binding. Compared to the respective Fab-IL-2 qm-Fab immunoconjugates the affinity of the IgG-IL-2 qm fusion proteins appears to be slightly enhanced within the error of the method.

Similarly, the affinity of further constructs (4B9, DP47GS, 2B10, CH1A1A 98/99 2F1) comprising either IL-2 wt (see Example 4) or IL-2 qm to the IL-2R $\beta\gamma$ heterodimer and the IL-
10 2R α -subunit was determined by SPR at 25°C. For all constructs the apparent K_D for the human IL-2R $\beta\gamma$ heterodimer was between 6 and 12 nM (irrespective of whether the construct comprises IL-2 wt or IL-2 qm), whereas only the constructs comprising IL-2 wt bind to the IL-2R α -subunit at all (K_D for human IL-2R α around 20 nM).

Biological Activity Assays with IgG-cytokine immunoconjugates

15 The biological activity of FAP-targeted 4G8-based IgG-IL-2 qm fusions was investigated in several cellular assays in comparison to commercially available IL-2 (Proleukin, Novartis/Chiron) and/or the Fab-IL-2-Fab immunoconjugates described in EP 11153964.9.

Binding to FAP expressing cells

Binding of FAP-targeted 4G8-based IgG-IL-2 qm immunoconjugate to human FAP expressed on
20 stably transfected HEK293 cells was measured by FACS. Briefly, 250 000 cells per well were incubated with the indicated concentration of the immunoconjugate in a round-bottom 96-well plate, incubated for 30 min at 4°C, and washed once with PBS/0.1 % BSA. Bound immunoconjugate was detected after incubation for 30 min at 4°C with FITC-conjugated AffiniPure F(ab')₂ Fragment goat anti-human F(ab')₂ Specific (Jackson Immuno Research Lab
25 #109-096-097, working solution: 1:20 diluted in PBS/0.1% BSA, freshly prepared) using a FACS CantoII (Software FACS Diva). The results are shown in Figure 10. The data show that the IgG-IL-2 qm immunoconjugate binds to FAP-expressing cells with an EC₅₀ value of 0.9 nM, comparable to that of the corresponding 4G8-based Fab-IL-2 qm-Fab construct (0.7 nM).

IFN- γ release by NK cells (in solution)

Subsequently, FAP-targeted 4G8-based IgG-IL-2 qm immunoconjugate was studied for the induction of IFN- γ release by NK92 cells as induced by activation of IL-2R $\beta\gamma$ signaling. Briefly, IL-2 starved NK92 cells (100 000 cells/well in 96-U-well plate) were incubated with different concentrations of IL-2 immunoconjugate, comprising quadruple mutant IL-2, for 24 h in NK medium (MEM alpha from Invitrogen (#22561-021) supplemented with 10% FCS, 10% horse serum, 0.1 mM 2-mercaptoethanol, 0.2 mM inositol and 0.02 mM folic acid). Supernatants were harvested and the IFN- γ release was analysed using the anti-human IFN- γ ELISA Kit II from Becton Dickinson (#550612). Proleukin (Novartis) and 28H1-based Fab-IL-2 qm-Fab served as positive control for IL-2-mediated activation of the cells. Figure 11 shows that the FAP-targeted 4G8-based IgG-IL-2 qm immunoconjugate is equally efficacious in inducing IFN- γ release as the affinity matured 28H1-based Fab-IL-2 qm-Fab immunoconjugate.

STAT5 phosphorylation assay

In a last set of experiments we studied the effects of the FAP-targeted 4G8-based IgG-IL-2 qm immunoconjugate on the induction of STAT5 phosphorylation compared to the 28H1 based Fab-IL-2-Fab and Fab-IL-2 qm-Fab immunoconjugates as well as Proleukin on human NK cells, CD4⁺ T cells, CD8⁺ T cells and T_{reg} cells from human PBMCs. Briefly, blood from healthy volunteers was taken in heparin-containing syringes and PBMCs were isolated. PBMCs were treated with the indicated immunoconjugates at the indicated concentrations or with Proleukin (Novartis) as control. After 20 min incubation at 37°C, PBMCs were fixed with pre-warmed Cytofix buffer (Becton Dickinson #554655) for 10 min at 37°C, followed by permeabilization with Phosflow Perm Buffer III (Becton Dickinson #558050) for 30 min at 4°C. Cells were washed twice with PBS containing 0.1 % BSA before FACS staining was performed using mixtures of flow cytometry antibodies for detection of different cell populations and phosphorylation of STAT5. Samples were analysed using a FACSCantoII with HTS from Becton Dickinson. NK cells were defined as CD3⁻CD56⁺, CD8 positive T cells were defined as CD3⁺CD8⁺, CD4 positive T cells were defined as CD4⁺CD25⁻CD127⁺ and T_{reg} cells were defined as CD4⁺CD25⁺FoxP3⁺. For NK cells and CD8⁺ T cells that show no or very low CD25 expression (meaning that IL-2R signaling is mediated primarily via the IL-2R $\beta\gamma$ heterodimer) the results show that the 4G8-based IgG-IL-2 qm immunoconjugate was <10-fold less potent in inducing STAT5 phosphorylation than Proleukin, but slightly more potent than 28H1-based Fab-IL-2-Fab and Fab-IL-2 qm-Fab immunoconjugates. On CD4⁺ T cells, that show a rapid up-regulation of CD25 upon stimulation, the 4G8-based IgG-IL-2 qm immunoconjugate was less

potent than the 28H1 Fab-IL-2-Fab immunoconjugate, but slightly more potent than the 28H1 Fab-IL-2 qm-Fab immunoconjugate, and still showed induction of IL-2R signaling at saturating concentrations comparable to Proleukin and 28H1 Fab-IL-2-Fab. This is in contrast to T_{reg} cells where the potency of the 4G8-based IgG-IL-2 qm immunoconjugate was significantly reduced compared to the Fab-IL-2-Fab immunoconjugate due to the high CD25 expression on T_{reg} cells and the low binding affinity of the 4G8-based IgG-IL-2 qm immunoconjugate to CD25. As a consequence of the abolishment of CD25 binding in the 4G8-based IgG-IL-2 qm immunoconjugate, IL-2 signaling in T_{reg} cells is only activated via the IL-2R $\beta\gamma$ heterodimer at concentrations where IL-2R signaling is activated on CD25-negative effector cells through the IL-2R $\beta\gamma$ heterodimer. Taken together the 4G8-based IgG-IL-2 qm immunoconjugate described here is able to activate IL-2R signaling through the IL-2R $\beta\gamma$ heterodimer, but does not result in a preferential stimulation of T_{reg} cells over other effector cells. The results of these experiments are shown in Figure 12.

Binding of 2B10 IgG-IL-2 qm to TNC A2 expressing cells

Binding of TNC A2-targeted 2B10-based IgG-IL-2 qm immunoconjugate to human TNC A2 expressed on U87MG cells was measured by FACS. Briefly, 200 000 cells per well were incubated with the indicated concentration of the immunoconjugate in a round-bottom 96-well plate, incubated for 30 min at 4°C, and washed twice with PBS/0.1 % BSA. Bound immunoconjugate was detected after incubation for 30 min at 4°C with FITC-conjugated AffiniPure F(ab')₂ Fragment goat anti-human IgG Fc γ Specific (Jackson Immuno Research Lab #109-096-098, working solution: 1:20 diluted in PBS/0.1% BSA, freshly prepared) using a FACS CantoII (Software FACS Diva). The results are shown in Figure 13. The data show that the 2B10 IgG-IL-2 qm immunoconjugate binds to TNC A2-expressing U87MG cells equally well as the corresponding unconjugated IgG.

Induction of NK92 cell proliferation by IgG-IL-2 immunoconjugates

2B10 IgG-IL-2 qm, CH1A1A 98/99 2F1 IgG-IL-2 qm, CH1A1A 98/99 2F1 IgG-IL-2 wt, 4B9 IgG-IL-2 qm and 4B9 IgG-IL-2 wt immunoconjugates were tested for their ability to induce proliferation of NK92 cells. For proliferation assays, NK92 cells were starved in IL-2-free medium for 2 hours, 10000 cells/well seeded into a flat-bottom 96-well plate and then incubated for 3 days in a humidified incubator at 37°C, 5% CO₂ in the presence of the IL-2 immunoconjugates (). After 3 days, the ATP content of the cell lysates was measured using the CellTiter-Glo Luminescent Cell Viability Assay from Promega (#G7571/2/3). The percentage of

growth was calculated setting a Proleukin (Novartis) concentration of 1.1 mg/ml to 100 % proliferation and untreated cells without IL-2 stimulus to 0 % proliferation. The results are shown in Figure 14 and 15. The data show that all constructs were able to induce NK92 cell proliferation, with the CH1A1A-based constructs being more active than the 2B10 IgG-IL-2 qm immunoconjugate, and the constructs comprising IL-2 wt being more active than the corresponding constructs with IL-2 qm.

Example 3

In general, the P329G LALA mutations that almost completely abolish FcγR interaction of human IgG₁ antibodies (see European patent application no. EP 11160251.2, incorporated herein by reference in its entirety) are introduced in order to reduce FcγR binding/effector function and thus prevent excessive cytokine release when the respective cytokine receptors are co-activated with FcγR signaling. In specific cases, for example when the antibody is targeting a highly tumor specific antigen, Fc effector functions may be retained by using an unmodified IgG Fc domain or may be even further enhanced via glycoengineering of the IgG Fc domain.

As an example thereof, we generated a CEA-targeted IgG-IL-2 qm immunoconjugate where one single IL-2 quadruple mutant was fused to the C-terminus of one heterodimeric heavy chain via a (SG₄)₃-linker based on the anti-CEA antibody clone CH1A1A. In this immunoconjugate the P329G LALA mutation was not included (see sequences of SEQ ID NOs 227, 229 and 231). The immunoconjugate was expressed and purified as human wildtype IgG- or glycoengineered IgG-IL-2 qm fusion protein as described below.

Preparation of (glycoengineered) IgG-IL-2 qm immunoconjugate

CEA-targeted CH1A1A-based IgG-IL-2 qm immunoconjugate was produced by co-transfecting HEK293-EBNA cells with the mammalian antibody expression vectors. Exponentially growing HEK293-EBNA cells were transfected by the calcium phosphate method. Alternatively, HEK293 cells growing in suspension are transfected by polyethylenimine. For the production of unmodified non-glycoengineered IgG-IL-2 qm immunoconjugate, the cells were transfected only with antibody heavy and light chain expression vectors in a 1:1 ratio (wherein the antibody heavy chain vector is a 1:1 mixture of two vectors: a vector for the heavy chain with the effector moiety, and a vector for the heavy chain without effector moiety).

For the production of the glycoengineered CEA-targeted IgG-IL-2 qm immunoconjugate, the cells were co-transfected with two additional plasmids, one for expression of a GnTIII fusion

polypeptide (a GnT-III expression vector), and one for mannosidase II expression (a Golgi mannosidase II expression vector) at a ratio of 4:4:1:1, respectively. Cells were grown as adherent monolayer cultures in T flasks using DMEM culture medium supplemented with 10% FCS, and were transfected when they are between 50 and 80% confluent. For the transfection of a T150 flask, 15 million cells were seeded 24 hours before transfection in 25 ml DMEM culture medium supplemented with FCS (at 10% v/v final), and cells were placed at 37°C in an incubator with a 5% CO₂ atmosphere overnight. For each T150 flask to be transfected, a solution of DNA, CaCl₂ and water was prepared by mixing 94 µg total plasmid vector DNA divided equally between the light and heavy chain expression vectors, water to a final volume of 469 µl, and 469 µl of a 1M CaCl₂ solution. To this solution, 938 µl of a 50 mM HEPES, 280 mM NaCl, 1.5 mM Na₂HPO₄ solution at pH 7.05 were added, mixed immediately for 10 sec and left to stand at room temperature for 20 sec. The suspension was diluted with 10 ml of DMEM supplemented with 2% FCS, and added to the T150 flask in place of the existing medium. Then additional 13 ml of transfection medium were added. The cells were incubated at 37°C, 5% CO₂ for about 17 to 20 hours, before the medium was replaced with 25 ml DMEM, 10% FCS. The conditioned culture medium was harvested approximately 7 days after the media exchange by centrifugation for 15 min at 210 x g. The solution was sterile filtered (0.22 µm filter) and sodium azide in a final concentration of 0.01% w/v was added, and kept at 4°C.

The secreted wildtype or glycoengineered CEA IgG-IL-2 qm immunoconjugates were purified from cell culture supernatants by affinity chromatography using Protein A affinity chromatography, followed by a size exclusion chromatographic step on a HiLoad Superdex 200 column (GE Healthcare) as described above. Protein concentration, purity, molecular weight, aggregate content and integrity were analysed as described above.

25 **Oligosaccharide structure analysis of (glycoengineered) IgG-IL-2 qm immunoconjugates**

For determination of the relative ratios of fucose-containing and non-fucosylated oligosaccharide structures, released glycans of purified immunoconjugate material are analyzed by MALDI TOF mass spectrometry. The immunoconjugate sample (about 50 µg) is incubated overnight at 37°C with 5 mU N-glycosidase F (QAbio; PNGaseF: E-PNG01) in 2 mM Tris, pH 7.0, in order to release the oligosaccharide from the protein backbone. For deamination of glycans acetic acid to a final concentration of 150 mM is added and incubated for 1h at 37°C. For analysis by MALDI TOF mass spectrometry, 2 µL of the sample are mixed on the MALDI target with 2 µL DHB matrix solution (2, 5-dihydroxybenzoic acid [Bruker Daltonics #201346] dissolved in 50%

ethanol/5 mM NaCl at 4 mg/ml) and analysed with MALDI TOF Mass Spectrometer Autoflex II instrument (Bruker Daltonics). Routinely, 50-300 shots are recorded and summed up to a single experiment. The spectra obtained are evaluated by the flex analysis software (Bruker Daltonics) and masses are determined for the each of the peaks detected. Subsequently, the peaks are
5 assigned to fucose-containing or non-fucosylated carbohydrate structures by comparing the masses calculated and the masses theoretically expected for the respective structures (e.g. complex, hybrid and oligo- or high-mannose, respectively, with and without fucose).

For determination of the ratio of hybrid structures, the antibody samples are digested with N-glycosidase F and Endo-glycosidase H [QAbio; EndoH: E-EH02] concomitantly. N-glycosidase
10 F releases all N-linked glycan structures (complex, hybrid and oligo- and high mannose structures) from the protein backbone and the Endo-glycosidase H cleaves all the hybrid type glycans additionally between the two N-acetylglucosamine (GlcNAc) residues at the reducing end of the glycan. This digest is subsequently treated and analysed by MALDI TOF mass spectrometry in the same way as described above for the N-glycosidase F digested sample. By
15 comparing the pattern from the N-glycosidase F digest and the combined N-glycosidase F / Endo H digest, the degree of reduction of the signals of a specific carbohydrate structure is used to estimate the relative content of hybrid structures. The relative amount of each carbohydrate structure is calculated from the ratio of the peak height of an individual structure and the sum of the peak heights of all oligosaccharides detected. The amount of fucose is the percentage of
20 fucose-containing structures related to all carbohydrate structures identified in the N-glycosidase F treated sample (e.g. complex, hybrid and oligo- and high-mannose structures). The degree of non-fucosylation is the percentage of structures lacking fucose relative to all carbohydrates identified in the N-glycosidase F treated sample (e.g. complex, hybrid and oligo- and high-mannose structures).

25

Antibody-dependent cell-mediated cytotoxicity assay

The wildtype and glycoengineered CEA-targeted CH1A1A IgG-IL-2 qm immunoconjugates were compared in ADCC assays for their potential to mediate antibody mediated cellular cytotoxicity. Briefly, CEA-overexpressing A549 human tumor cells as target cells were collected,
30 washed and resuspended in culture medium, stained with freshly prepared Calcein AM (Molecular Probes) at 37°C for 30 min, washed three times, counted and diluted to 300 000 cells/ml. This suspension was transferred to a round-bottom 96-well plate (30000 cells/well), the respective immunoconjugate dilution was added and incubated for 10 min to allow the binding

of the tested immunoconjugate to the cells prior to contact with effector cells. Effector to target ratio was 25 to 1 for freshly isolated PBMCs. Co-incubation was performed for 4 hours. Two different read-out systems were used: the release of lactate dehydrogenase (LDH) into supernatant after disintegration of the attacked cells, and the retention of Calcein in the remaining living cells. LDH from co-culture supernatant was collected and analyzed with a LDH detection Kit (Roche Applied Science). Substrate conversion by the LDH enzyme was measured with an ELISA absorbance reader (SoftMaxPro software, reference wavelengths: 490 nm versus 650 nm). Residual Calcein in living cells was analyzed in a fluorescence reader (Wallac VICTOR3 1420 Multilabel COUNTER (Perkin Elmer)) after removing the rest of supernatant from pelletized cells, one washing step in PBS prior to lysis, and fixation of the cells by borate buffer (50 mM borate, 0.1% Triton).

Figure 16 shows the result based on LDH detection. A similar result was obtained based on the calcein retention (not shown). Both the constructs were able to mediate ADCC, the glycoengineered construct being similarly active as the corresponding glycoengineered unconjugated IgG. As expected, the non-glycoengineered construct showed reduced activity as compared to the glycoengineered construct.

Example 4

FAP-targeted 28H1- or 4B9-based, CEA-targeted CH1A1A 98/99 2F1-based and non-targeted DP47GS-based IgG-IL-2 immunoconjugates were generated wherein one single wildtype IL-2 polypeptide is fused to the C-terminus of one heterodimeric heavy chain. Heterodimerization resulting in an immunoconjugate with a single IL-2 moiety was achieved by application of the knob-into-hole technology. In order to minimize the generation of homodimeric IgG-IL-2 fusions proteins the cytokine was fused to the knob-containing heavy chain (with deletion of the C-terminal Lys residue) via a $G_4-(SG_4)_2$ or a $(G_4S)_3$ linker. The sequences of these immunoconjugates are given in SEQ ID NOs 193, 197 and 205 (28H1 with $G_4-(SG_4)_2$ linker) SEQ ID NOs 207, 273 and 211 (4B9 with $(G_4S)_3$ linker), SEQ ID NOs 277, 279 and 283 (CH1A1A 98/99 2F1 with $(G_4S)_3$ linker), SEQ ID NOs 219, 223 and 225 (DP47GS with $G_4-(SG_4)_2$ linker), SEQ ID NOs 219, 293 and 225 (DP47GS with $(G_4S)_3$ linker). The antibody-cytokine fusion has IgG-like properties. To reduce Fc γ R binding/effector function and prevent FcR co-activation, P329G LALA mutations were introduced in the Fc domain. Both constructs were purified according to the methods described above. Final purification was done by size exclusion chromatography (HiLoad 26/60 Superdex 200, GE Healthcare) in the final formulation

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buffer 20 mM histidine, 140 mM sodium chloride pH 6. Figures 17 to 20 show the respective chromatograms and elution profiles of the purification (A, B) as well as the analytical SDS-PAGE and size exclusion chromatographies of the final purified constructs (C, D). Yield was 15.6 mg/L for the untargeted DP47GS IgG-IL-2 immunoconjugate, 26.7 mg/ml for the 28H1
5 IgG-IL-2 immunoconjugate, 4.6 mg/L for the CH1A1A 98/99 2F1 IgG-IL-2 immunoconjugate and 11 mg/L for the 4B9 IgG-IL-2 immunoconjugate.

Subsequently, their binding properties to FAP, respectively lack of binding, as well as binding to IL-2R $\beta\gamma$ and IL-2R α chain were determined by SPR as described above (see Example 2). Cellular activity on immune effector cell populations and in vivo pharmacodynamic effects were
10 also studied.

Example 5

FAP-targeted 4G8-based as well as TNC A2-targeted 2B10-based IgG-IL-10 immunoconjugates were constructed by fusing two different IL-10 cytokine formats to the C-terminus of the heavy chain of the heterodimeric IgG comprising a hole modification: either a single-chain IL-10
15 wherein a (G₄S)₄ 20-mer linker was inserted between two IL-10 molecules, or an engineered monomeric IL-10 (Josephson et al., J Biol Chem 275, 13552-7 (2000)). Both molecules were fused via a (G₄S)₃ 15-mer linker to the C-terminus of the heavy chain comprising a hole modification, with deletion of the C-terminal Lys residue. Heterodimerization resulting in only one heavy chain carrying an IL-10 moiety was achieved by application of the knob-into-hole
20 technology. The IgG-cytokine fusion has IgG-like properties. To reduce Fc γ R binding/effector function and prevent FcR co-activation, P329G LALA mutations were introduced in the Fc domain of the immunoconjugate. The sequences of the respective constructs are given in SEQ ID NOs 233, 235 and 239 (2B10 with scIL-10), SEQ ID NOs 233, 237 and 239 (2B10 with monomeric IL-10 "IL-10M1"), SEQ ID NOs 241, 243 and 205 (4G8 with scIL-10), SEQ ID
25 NOs 241, 245 and 205 (4G8 with IL-10M1). All these immunoconjugates were purified according to the methods described above. Subsequently, their binding properties to FAP or TNC A2, respectively, as well as their affinities to human IL-10R1 were determined by SPR using the ProteOn XPR36 biosensor. Briefly, the targets FAP or TNC A2 as well as human IL-10R1 were immobilized in vertical orientation on the sensorchip surface (FAP by standard amine
30 coupling, TNC A2 and human IL-10R1 (both biotinylated via a C-terminal avi-tag) by neutravidin-capture). Subsequently, the IgG-IL-10 immunoconjugates were injected in six different concentrations, including a zero-concentration, as analytes in horizontal orientation.

After double-referencing, the sensorgrams were fit to a 1:1 interaction model to determine kinetic rate constants and affinities. The results from analytical SDS PAGE analysis and SPR-based affinity determinations to target antigens as well as IL-10 receptor are shown in Figures 21 and 22. The data show that the immunoconjugates bind to TNC A2 or FAP with K_D values of 52 or 26 pM, respectively, while K_D values for IL-10 receptor are 520 and 815 pM.

Example 6

According to the methods described above, IgG-cytokine fusion proteins were generated and expressed consisting of one single 28H1-based or 4B9-based Fab region directed to FAP fused to the N-terminus of an Fc domain subunit comprising a hole modification, while the second Fab region of the IgG heavy chain with the knob modification was replaced by a cytokine moiety via a $(G_4S)_n$ linker ($n=1$). See Figure 2C for a schematic representation of this immunoconjugate format (also referred to as “1+1” format). Cytokine moieties used were the IL-2 quadruple mutant described above and in PCT patent application no. PCT/EP2012/051991 (see SEQ ID NO: 3), IL-7 and IFN- α . Corresponding sequences of the fusion polypeptides comprising the cytokine moiety, fused to the N-terminus of an Fc domain subunit comprising a knob modification via a linker peptide, are given in SEQ ID NOs 247 (comprising quadruple mutant IL-2), 249 (comprising IL-7), and 251 (comprising IFN- α). In these constructs, targeting of the immunoconjugate is achieved via the high affinity monovalent Fab region. This format may be recommended in cases where internalization of the antigen may be reduced using a monovalent binder. The immunoconjugates were produced, purified and analysed as described above. For constructs comprising IL-2 qm or IL-7, protein A affinity chromatography and size exclusion chromatography were combined in a single run. 20 mM histidine, 140 mM NaCl pH 6.0 was used as size exclusion chromatography and final formulation buffer. Figures 23-26 show the elution profiles and chromatograms of the purifications as well as the analytical SDS-PAGE and size exclusion chromatograms of the final purified constructs. The yields were 11 mg/L for the 4B9 “1+1” IgG-IL-2 qm, 43 mg/L for the 28H1 “1+1” IgG-IL-2 qm, 20.5 mg/L for the 4B9 “1+1” IgG-IL-7 and 10.5 mg/L for the 4B9 “1+1” IgG-IFN- α constructs.

The ability of “1+1” constructs comprising IL-2 qm to induce NK cell proliferation, compared to IgG-IL-2 qm immunoconjugates, was tested. NK-92 cells were starved for 2 h before seeding 10000 cells/well into 96-well-black-flat-clear bottom plates. The immunoconjugates were titrated onto the seeded NK-92 cells. After 72 h the ATP content was measured to determine the number of viable cells using the “CellTiter-Glo Luminescent Cell Viability Assay” Kit (Promega)

according to the manufacturer's instructions. Figure 27 shows that the "1+1" constructs are able to induce proliferation of NK-92 cells, being slightly less active than the corresponding IgG-IL-2 qm constructs.

The 4B9-based "1+1" constructs comprising IL-2 qm or IL-7 were tested for their ability to induce T cell proliferation, compared to IgG-IL-2 immunoconjugates. Peripheral blood mononuclear cells (PBMC) were prepared using Histopaque-1077 (Sigma Diagnostics Inc., St. Louis, MO, USA). In brief, blood from buffy coats was diluted 5:1 with calcium- and magnesium-free PBS, and layered on Histopaque-1077. The gradient was centrifuged at 450 x g for 30 min at room temperature (RT) without breaks. The interphase containing the PBMCs was collected and washed three times with PBS (350 x g followed by 300 x g for 10 min at RT). PBMCs were pre-stimulated with 1 µg/ml PHA-M (Sigma Aldrich #L8902) overnight, before they were labeled with 100 nM CFSE (carboxyfluorescein succinimidyl ester) for 15 min at 37°C. Cells were washed with 20 ml medium before recovering the labeled PBMCs for 30 min at 37°C. The cells were washed, counted, and 100000 cells were seeded into 96-well-U-bottom plates. The immunoconjugates were titrated onto the seeded cells for an incubation time of 6 days. Thereafter, cells were washed, stained for appropriate cell surface markers, and analyzed by FACS using a BD FACSCantoII. CD4 T cells were defined as CD3⁺/CD8⁻, and CD8 T cells as CD3⁺/CD8⁺.

Figure 28 shows that the "1+1" constructs comprising either IL-2 qm or IL-7 are able to induce proliferation of PHA-activated CD4 (A) and CD8 T cells (B). As for NK cells, the "1+1" construct comprising IL-2 qm is slightly less active than an IgG-IL-2 qm construct.

The 4B9-based "1+1" construct comprising IFN-α was tested for its ability to inhibit Daudi cell proliferation, in comparison to Roferon A (Roche). Briefly, Daudi cells were labeled with 100 nM CFSE and seeded into a 96-well U-bottom plate (50'000 cells/well). The molecules were added at the indicated concentrations, followed by incubation for 3 days at 37°C. Proliferation was measured by analyzing the CFSE dilution, excluding dead cells from analysis by use of life/dead stain.

Figure 29 shows that the construct was able to inhibit proliferation of Daudi cells, at least as potently as Roferon A.

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Example 7

A single dose pharmacokinetics (PK) study was performed in tumor-free immunocompetent 129 mice for FAP-targeted IgG-IL2 immunoconjugates comprising either wild type or quadruple

mutant IL-2, and untargeted IgG-IL-2 immunoconjugates comprising either wild type or quadruple mutant IL-2.

Female 129 mice (Harlan, United Kingdom), aged 8-9 weeks at the start of the experiment, were maintained under specific-pathogen-free conditions with daily cycles of 12 h light / 12 h darkness according to committed guidelines (GV-Solas; Felasa; TierschG). The experimental study protocol was reviewed and approved by local government (P 2008016). After arrival, animals were maintained for one week to get accustomed to the new environment and for observation. Continuous health monitoring was carried out on a regular basis.

Mice were injected i.v. once with FAP-targeted 28H1 IgG-IL2 wt (2.5 mg/kg) or 28H1 IgG-IL2 qm (5 mg/kg), or untargeted DP47GS IgG-IL2 wt (5 mg/kg) or DP47GS IgG-IL2 qm (5 mg/kg). All mice were injected i.v. with 200 µl of the appropriate solution. To obtain the proper amount of immunoconjugate per 200 µl, the stock solutions were diluted with PBS as necessary.

Mice were bled at 1, 8, 24, 48, 72, 96 h; and every 2 days thereafter for 3 weeks. Sera were extracted and stored at -20°C until ELISA analysis. Immunoconjugate concentrations in serum were determined using an ELISA for quantification of the IL-2-immunoconjugate antibody (Roche-Penzberg). Absorption was measured using a measuring wavelength of 405 nm and a reference wavelength of 492 nm (VersaMax tunable microplate reader, Molecular Devices).

Figure 30 shows the pharmacokinetics of these IL-2 immunoconjugates. Both the FAP-targeted (A) and untargeted (B) IgG-IL2 qm constructs have a longer serum half-life (approx. 30 h) than the corresponding IgG-IL-2 wt constructs (approx. 15 h). Of note, although the experimental conditions are not directly comparable, the serum half-life of the IL-2 immunoconjugates of the invention appears to be longer than the serum half-life of art-known “2+2” IgG-IL-2 immunoconjugates (see Figure 1) as reported e.g. in Gillies et al., Clin Cancer Res 8, 210-216 (2002).

Compound	Dose	Formulation buffer	Concentration (mg/mL)
28H1-IgG-IL2 wt	2.5 mg/kg	20 mM Histidine, 140 mM NaCl, pH 6.0	3.84 (= stock solution)
28H1-IgG-IL2 qm	5 mg/kg	20 mM Histidine, 140 mM NaCl, pH 6.0	2.42 (= stock solution)
DP47GS-IgG-IL2wt	5 mg/kg	20 mM Histidine, 140 mM NaCl, pH 6.0	3.74 (= stock solution)
DP47GS-IgG-IL2QM	5 mg/kg	20 mM Histidine, 140 mM NaCl, pH 6.0	5.87 (= stock solution)

Example 8

A biodistribution study was performed to assess tumor targeting of the immunoconjugates of the invention. FAP-targeted 28H1-based IgG-IL-2 qm was compared to FAP-targeted unconjugated 28H1 IgG and 4B9 IgG, and untargeted DP47GS IgG. Furthermore, a SPECT/CT imaging study was performed with 4B9 IgG-IL-2 qm, compared to DP47GS IgG-IL-2 qm, 4B9 IgG and DP47GS IgG.

DTPA conjugation and ¹¹¹In labeling

- 10 Solutions of 28H1 IgG-IL-2 qm, 28H1 IgG₁, 4B9 IgG-IL-2 qm, 4B9 IgG₁ and DP47 IgG₁ were dialysed against phosphate buffered saline (PBS, 15 mM). Two mg of the constructs (5 mg/ml) were conjugated with isothiocyanatobenzyl-diethylenetriaminepentaacetic acid (ITC-DTPA, Macrocyclus, Dallas, TX) in 0.1 M NaHCO₃, pH 8.2, under strict metal-free conditions, by incubation with a 5-fold molar excess of ITC-DTPA for one hour at room temperature (RT).
- 15 Unconjugated ITC-DTPA was removed by dialysis against 0.1 M 2-(N-morpholino)ethanesulfonic acid (MES) buffer, pH 5.5.

The purified conjugates were radiolabeled by incubation with ¹¹¹In (Covidien BV, Petten, The Netherlands) in 0.1 M MES buffer, pH 5.5 containing 0.05% bovine serum albumin (BSA) and 0.05% Tween-80, at RT, under strict metal-free conditions for 30 min. After radiolabeling

20 ethylenediaminetetraacetic acid (EDTA) was added to a final concentration of 5 mM to chelate the unbound ¹¹¹In. The ¹¹¹In labeled products were purified by gel filtration on disposable G25M columns (PD10, Amersham Biosciences, Uppsala, Sweden). Radiochemical purity of purified ¹¹¹In labeled constructs were determined by instant thin-layer chromatography (ITLC) on TEC

Control chromatography strips (Biodex, Shirley, NY), using 0.1 M citrate buffer, pH 6.0, as the mobile phase. The specific activity of the ^{111}In -labeled preparations was 0.6-4.6 MBq/ μg .

Lindmo assay

The immunoreactive fraction of ^{111}In labeled antibody preparations was determined as described previously (Lindmo et al. (1984) J Immunol Methods 72, 77-89). Briefly, a serial dilution series of human embryonic kidney (HEK) cells transfected with fibroblast activation protein (FAP) cDNA (HEK-FAP cells) were incubated with 200 Bq of the ^{111}In -labeled construct at 37°C for 1 hour. A duplicate of the lowest cell concentration was incubated in the presence of an excess of non-labeled construct to correct for non-specific binding. After incubation, the cells were washed, spun down and cell associated radioactivity was determined in the cell pellet in a gamma-counter (Wallac Wizzard 3'' 1480 automatic γ -counter, Pharmacia LKB). The immunoreactive fraction of the preparations ranged between 75-94%.

Animals

Female BALB/c nude mice (8-9 weeks, +/- 20 g) were purchased from Janvier and housed in the Central Animal Facility of the Radboud University Nijmegen Medical Centre under standard conditions with 5 animals in individually ventilated cages with *ad lib.* access to food and water. After one week acclimatization the animals were inoculated s.c. with 10×10^6 HEK-FAP cells in matrigel (1:3) in the left flank and optionally with 5×10^6 HEK-293 cells in matrigel (1:3) in the right flank. Xenograft growth was monitored by caliper measurement (volume = $(4/3 \cdot \pi) \cdot (1/2 \cdot \text{length}) \cdot (1/2 \cdot \text{width}) \cdot (1/2 \cdot \text{height})$). When xenografts reached a volume of 100 mm³, mice were injected i.v. with the ^{111}In -labeled constructs.

Biodistribution (28H1 IgG-IL-2 qm, 28H1 IgG₁, 4B9 IgG₁ and DP47GS IgG₁)

^{111}In -labeled constructs (5 MBq, 150 μg , 200 μl) were injected i.v. via the tail vein. Twenty-four hours after injection the animals were euthanized by suffocation in CO₂/O₂ atmosphere. Blood, muscle, xenograft, lung, spleen, pancreas, kidney, stomach (empty), duodenum (empty) and liver were collected, weighed and radioactivity was determined in a gamma-counter (Wallac Wizzard). Standards of the injected dose (1%) were counted simultaneously and tissue uptake was calculated as % of the injected dose per gram tissue (%ID/g).

SPECT-CT analysis (4B9 IgG-IL-2 qm, 4B9 IgG₁, DP47GS IgG-IL-2 qm and DP47GS IgG₁)

¹¹¹In-labeled 4B9-IgG-IL-2 qm, 4B9-IgG₁, DP47GS-IgG-IL-2 qm and DP47GS-IgG₁ were injected i.v. (20 MBq, 50, 150, 300 µg, 200 µl). At 4, 24, 72 and 144 hours after injection the animals were anesthetized with isoflurane/O₂ and scanned for 30 to 60 min in a U-SPECT II microSPECT/CT camera (MILabs, Utrecht, The Netherlands) equipped with a 1.0 mm mouse collimator. Computed tomography (CT) was performed directly after SPECT. Both SPECT (voxel size of 0.4 mm) and CT scans were reconstructed with MILabs software and SPECT and CT scans were co-registered to determine exact location of radio-signal. 3D images were created using Siemens Inveon Research Workplace software.

Figure 31 shows that there is no significant difference between tissue distribution and tumor targeting of 28H1 and 4B9 IgG₁ and 28H1 IgG-IL-2 qm at 24 hours (hence the cytokine does not significantly alter the tissue distribution and tumor targeting properties of the immunoconjugates), and that tumor-to-blood ratios for the FAP-targeted constructs are significantly greater than for the non-targeted DP47GS control IgG.

These results were confirmed in SPECT/CT imaging for the 4B9 IgG-IL-2 qm immunoconjugate (data not shown). 4B9 IgG-IL-2 qm localized in the FAP-positive HEK-FAP but not in the FAP-negative HEK-293 control tumors, while the untargeted DP47GS immunoconjugate did not localize in either tumor. Unlike with the unconjugated IgGs, a weak uptake of 4B9 IgG-IL-2 qm was observed also in the spleen.

Example 9

Binding of 28H1-based IgG-IL-2 qm and a 28H1-based IgG-(IL-2 qm)₂ (i.e. a “2+2” format immunoconjugate as depicted in Figure 1; sequences are shown in SEQ ID NOs 253 and 205) to NK 92 cells was compared. 200000 NK92 cells per well were seeded in a 96-well plate. The immunoconjugates were titrated onto the NK92 cells and incubated for 30 min at 4°C to allow binding. The cells were washed twice with PBS containing 0.1% BSA to remove unbound constructs. For detection of the immunoconjugates a FITC-labeled anti-human Fc-specific antibody was added for 30 min at 4°C. The cells were again washed twice with PBS containing 0.1 % BSA and analyzed by FACS using a BD FACSCantoII.

As illustrated in Figure 32, the “2+2” immunoconjugate shows better binding to NK 92 cells than the corresponding “2+1” construct.

Example 10

Induction of human PBMC proliferation by IL-2 immunoconjugates

Peripheral blood mononuclear cells (PBMC) were prepared using Histopaque-1077 (Sigma Diagnostics Inc., St. Louis, MO, USA). In brief, venous blood from healthy volunteers was drawn into heparinized syringes. The blood was diluted 2:1 with calcium- and magnesium-free PBS, and layered on Histopaque-1077. The gradient was centrifuged at 450 x g for 30 min at room temperature (RT) without breaks. The interphase containing the PBMCs was collected and washed three times with PBS (350 x g followed by 300 x g for 10 min at RT).

Subsequently, PBMCs were labeled with 40 nM CFSE (carboxyfluorescein succinimidyl ester) for 15 min at 37°C. Cells were washed with 20 ml medium before recovering the labeled PBMCs for 30 min at 37°C. The cells were washed, counted, and 100000 cells were seeded into 96-well-U-bottom plates. Pre-diluted Proleukin (commercially available wild-type IL-2) or IL2-immunoconjugates were titrated onto the seeded cells which were incubated for the indicated time points. After 4-6 days, cells were washed, stained for appropriate cell surface markers, and analyzed by FACS using a BD FACSCantoII. NK cells were defined as CD3⁻/CD56⁺, CD4 T cells as CD3⁺/CD8⁻, and CD8 T cells as CD3⁺/CD8⁺.

Figure 33 shows proliferation of NK cells after incubation with different FAP-targeted 28H1 IL-2 immunoconjugates for 4 (A), 5 (B) or 6 (C) days. All tested constructs induced NK cell proliferation in a concentration-dependent manner. Proleukin was more efficacious than the immunoconjugates at lower concentrations, this difference no longer existed at higher concentrations, however. At earlier time points (day 4), the IgG-IL2 constructs appeared slightly more potent than the Fab-IL2-Fab constructs. At later time points (day 6), all constructs had comparable efficacy, with the Fab-IL2 qm-Fab construct being least potent at the low concentrations.

Figure 34 shows proliferation of CD4 T-cells after incubation with different FAP-targeted 28H1 IL-2 immunoconjugates for 4 (A), 5 (B) or 6 (C) days. All tested constructs induced CD4 T cell proliferation in a concentration-dependent manner. Proleukin had a higher activity than the immunoconjugates, and the immunoconjugates comprising wild-type IL-2 were slightly more potent than the ones comprising quadruple mutant IL-2. As for the NK cells, the Fab-IL2 qm-Fab construct had the lowest activity. Most likely the proliferating CD4 T cells are partly regulatory T cells, at least for the wild-type IL-2 constructs.

Figure 35 shows proliferation of CD8 T-cells after incubation with different FAP-targeted 28H1 IL-2 immunoconjugates for 4 (A), 5 (B) or 6 (C) days. All tested constructs induced CD8 T cell proliferation in a concentration-dependent manner. Proleukin had a higher activity than the

immunoconjugates, and the immunoconjugates comprising wild-type IL-2 were slightly more potent than the ones comprising quadruple mutant IL-2. As for the NK and CD4 T cells, the Fab-IL2 qm-Fab construct had the lowest activity.

5

Example 11

Proliferation and activation induced cell death of IL-2 activated PBMCs

Freshly isolated PBMCs from healthy donors were pre-activated overnight with PHA-M at 1 µg/ml in RPMI1640 with 10% FCS and 1% Glutamine. After pre-activation PBMCs were harvested, labeled with 40 nM CFSE in PBS, and seeded in 96-well plates at 100 000 cells/well.

- 10 Pre-activated PBMCs were stimulated with different concentrations of IL-2 immunoconjugates (4B9 IgG-IL-2 wt, 4B9 IgG-IL-2 qm, 4B9 Fab-IL-2 wt-Fab, and 4B9 Fab-IL-2 qm-Fab). After six days of IL-2 treatment PBMCs were treated with 0.5 µg/ml activating anti-Fas antibody overnight. Proliferation of CD4 (CD3⁺CD8⁻) and CD8 (CD3⁺CD8⁺) T cells was analyzed after six days by CFSE dilution. The percentage of living T cells after anti-Fas treatment was
- 15 determined by gating on CD3⁺ Annexin V negative living cells.

- As shown in Figure 36, all constructs induced proliferation of pre-activated T cells. At low concentrations the constructs comprising wild-type IL-2 wt were more active than the IL-2 qm-comprising constructs. IgG-IL-2 wt, Fab-IL-2 wt-Fab and Proleukin had similar activity. Fab-IL-2 qm-Fab was slightly less active than IgG-IL-2 qm. The constructs comprising wild-type IL-2
- 20 were more active on CD4 T cells than on CD8 T cells, most probably because of the activation of regulatory T cells. The constructs comprising quadruple mutant IL-2 were similarly active on CD8 and CD4 T cells.

- As shown in Figure 37, T cells stimulated with high concentrations of wild-type IL-2 are more sensitive to anti-Fas induced apoptosis than T cells treated with quadruple mutant IL-2.

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Example 12

- The untargeted DP47GS construct (see SEQ ID NO: 299 and 297 for VH and VL sequences, respectively) was further characterized. As described above, conjugates of DP47GS IgG with wild-type or quadruple mutant IL-2 were made. These constructs showed similar binding to IL-
- 30 2R and induction of immune cell (e.g. NK cell, CD8⁺ cell and CD4⁺ cell) proliferation in vitro as corresponding targeted constructs (data not shown). In contrast to immunoconjugates targeting a tumor antigen, however, they did not accumulate in tumor tissue (see Example 8).

A further pharmacokinetic study (in addition to the one shown in Example 7) was performed with the untargeted DP47GS IgG-IL-2 constructs comprising either wild-type or quadruple mutant IL-2. Male C57BL/6J mice (n = 6 per group) were injected i.v. with 0.3, 1, 3 or 10 mg/kg DP47GS IgG-IL-2 wt or DP47GS IgG-IL-2 qm construct. The injection volume was 1 ml for all mice. Blood samples were taken at 2, 4, 8, 24, 48, 72, 96 and 168 hours after injection (from 3 mice at each time point) and stored at -20°C until analysis. The constructs were quantified in the serum samples by ELISA, using anti-Fab antibodies for capturing and detection of the constructs. All samples and calibration standards were diluted 1:25 in mouse serum (obtained from Bioreclamation) prior to the analysis. Briefly, streptavidin-coated 96 well plates (Roche) were washed three times for 10 sec with PBS/0.05% Tween 20, before incubation with 100 µl/well (0.5 µg/ml) biotinylated anti-human Fab antibody (M-1.7.10; Roche Diagnostics) for 1 hour at room temperature. After washing the plate again three times with PBS/0.05% Tween 20, 50 µl/well of the serum samples or calibration standards and 50 µl/well PBS/0.5% BSA were added to give a final sample dilution of 1:50. Samples were incubated for 1 hour at room temperature, followed by washing the plate again three times with PBS/0.05% Tween 20. Next, the plate was incubated with 100 µl/well (0.5 µg/ml) digoxigenin-labeled anti-human Fab antibody (M-1.19.31; Roche Diagnostics) for 1 hour at room temperature, washed, incubated with 100 µl/well anti-digoxigenin POD (Roche Diagnostics Cat# 11633716001) for 1 hour at room temperature, and washed again. Finally, 100 µl/well TMB peroxidase substrate (Roche Diagnostics Cat# 11484281001) was added for about 5 minutes, before the substrate reaction was stopped with 50 µl/well 2N HCl. The plate was read within 2 minutes after stopping the reaction at 450 nm with a reference wavelength of 650 nm.

The result of this study is shown in Figure 38. Both constructs showed long serum half life, with the construct comprising quadruple mutant IL-2 (B) being even longer lived than the one comprising wild-type IL-2 (A).

In addition, the lack of binding of DP47GS IgG to various proteins as well as human cells (PBMCs) was confirmed.

The binding specificity (or lack of such) of the DP47GS antibody was assessed in an ELISA-based test system with a panel of different unrelated antigens. The test was performed on 384 well MaxiSorp™ microtiter plates (Thermo Scientific Nunc, Cat# 460372). After each incubation step the plates were washed three times with PBS/0.05% Tween-20. First, the different antigens, diluted in PBS, were coated on plates overnight at 6°C. The test concentrations and detailed information for the used antigens are listed in the table below.

Antigen	Source	Supplier	Cat#	Test concentration [µg/ml]
Histons	calf thymus	Roche Diagnostics	10223565601	2
BSA Fraction V	bovine	Roche Diagnostics	10735108001	2
Insulin	human	Roche Diagnostics	11376497001	2
Cardiolipin	bovine	Sigma-Aldrich	C1649	2
Heparin	porcine	Sigma-Aldrich	H9902	2
CD40 (hFc)	human	Sino Biological	1077-H03H	1
Parathyroid hormone aa 1-34 (PTH) (biotinylated)	human	AnaSpec	20690	0.5
dsDNA	calf thymus	Sigma-Aldrich	D4522	0.16
Hemocyanin	keyhole limpet	Sigma-Aldrich	H7017	0.22
Actin beta 2	human	Cytoskeleton	APHL99	0.67
Streptavidin	Streptomyces avidinii	Roche Diagnostics	11721674001	1
Gelatin	bovine	Roche Diagnostics	11111965001	2% blocking buffer
E. coli lysate	E. coli	inhouse	-	diluted 1:600

Thereafter, the wells were blocked with 2% gelatin in water for 1 hour at room temperature (RT). The DP47GS antibody (1 µg/ml in PBS) was incubated with the panel of captured antigens for 1.5 hours at RT. Bound antibody was detected using anti-human IgG antibody-HRP conjugate (GE Healthcare, Cat# 9330V; diluted 1:1000 in PBS with 0.2% Tween-20 and 0.5% gelatin). After 1 hour incubation the plates were washed 6 times with PBS/0.05% Tween-20 and developed with freshly prepared BM blue POD substrate solution (BM blue: 3,3'-5,5'-tetramethylbenzidine, Roche Diagnostics, Cat# 11484281001) for 30 minutes at RT. Absorbance was measured at 370 nm. The blank value was defined without addition of antibody. An inhouse human IgG₁ antibody which exhibits unspecific binding to almost all of the captured antigens served as positive control.

The result of this experiment is shown in Figure 39. The DP47GS antibody showed no binding to any of the captured antigens. The detected signals were in the range of the control samples without antibody.

Finally, the binding of the DP47GS antibody to human PBMCs was assessed. Since in the course of a typical immune response the combination of cell surface-presented proteins changes dramatically, binding was tested on PBMCs directly after isolation from healthy adults as well as after in vitro activation with two different stimuli.

Human PBMCs were isolated by Ficoll density gradient centrifugation from buffy coats or from heparinized fresh blood from healthy volunteers using Histopaque 1077 (Sigma-Aldrich, Germany). PBMCs were either directly subjected to binding assays (fresh PBMCs) or cultured and stimulated further. PBMCs were cultured at a cell density of 2×10^6 cells/ml in T cell medium consisting of RPMI 1640 (Gibco) supplemented with 10% (v/v) heat-inactivated FBS (PAA Laboratories), 1 mM sodium pyruvate (Sigma-Aldrich), 1% (v/v) L-alanyl-L-glutamine (Biochrom) and 10 nM β -mercaptoethanol (Sigma-Aldrich) at 37°C. For in vitro stimulation, Proleukin (200 U/ml, Novartis) and phytohaemagglutinin (PHA-L; 2 μ g/mL, Sigma-Aldrich) were added during six days of cultivation (PHA-L activated PBMC). For in vitro re-stimulation, 6-well cell culture plates were coated with mouse anti-human CD3 (clone KT3, 1 μ g/ml) and mouse anti-human CD28 antibodies (clone 28.2, 2 μ g/ml, both from eBioscience) and PHA-L activated PBMC were added for additional 24 hours (re-stimulated PBMC). Binding of DP47GS antibody (with or without the L234A L235A (LALA) P329G mutation in the Fc domain) to cell surface proteins was monitored for a five-fold serial dilution series (highest concentration 200 nM) using a goat anti-human IgG Fc-specific secondary antibody conjugated to fluorescein isothiocyanate (FITC) (Jackson Laboratories) and flow cytometric analysis. All assays were performed at 4°C to prevent internalization of surface proteins. Incubation of primary and secondary antibody was for 2 hours and for 1 hour, respectively. To allow simultaneous typing of leukocytes, combinations of fluorochrome-labeled mouse anti-human CD14, CD15, CD4, CD19 (all Biolegend), NKp46, CD3, CD56, CD8 (all BD Pharmingen) were added to the secondary antibody. Propidium iodide (1 μ g/ml) was added directly before measurement on a FACSCantoII device running FACS Diva software (both BD Bioscience) to exclude permeable dead cells. Propidium iodide negative living cells were gated for T cells (CD14⁻CD3⁺CD4⁺/CD8⁺), B cells (CD14⁻CD19⁺), NK Cells (CD14⁻NKp46⁺/CD56⁺) or monocytes/neutrophils (CD3⁻CD56⁻CD14⁺/CD15⁺). The median FITC fluorescence of the various leukocyte types was

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determined as indicator for bound primary antibody and blotted against the primary antibody concentration using Prism4 (GraphPad Software).

As shown in Figure 40, the DP47GS IgG antibody without Fc mutation showed binding only to Fc γ receptor bearing cells, e.g. NK cells and monocytes/neutrophils. No binding of DP47GS
5 (LALA P329G) was detected on human PBMCs, regardless of their activation status. The LALA P329G mutation in the Fc domain completely abolished binding also to Fc γ receptor bearing cells.

* * *

10 Although the foregoing invention has been described in some detail by way of illustration and example for purposes of clarity of understanding, the descriptions and examples should not be construed as limiting the scope of the invention. The disclosures of all patent and scientific literature cited herein are expressly incorporated in their entirety by reference.

Claims

1. An immunoconjugate comprising (i) an immunoglobulin molecule comprising a first and a second antigen binding Fab molecule and an Fc domain consisting of two subunits, and (ii) an effector moiety, wherein not more than one effector moiety is present; and wherein said first and said second Fab molecule are directed to CEA and comprise a heavy chain variable region sequence of SEQ ID NO: 191, and a light chain variable region sequence of SEQ ID NO: 189; and said effector moiety is a mutant human interleukin-2 (IL-2) polypeptide comprising the amino acid substitutions F42A, Y45A, L72G (number relative to the human IL-2 sequence in SEQ ID NO: 2).
2. The immunoconjugate of claim 1, wherein said effector moiety is fused to the carboxy-terminal amino acid of one of said two subunits of the Fc domain, optionally through a linker peptide.
3. The immunoconjugate of claim 1 or claim 2, wherein said Fc domain comprises a modification promoting heterodimerization of two non-identical polypeptide chains.
4. The immunoconjugate of claim 3, wherein said modification is a knob-into-hole modification, comprising a knob modification in one of the subunits of the Fc domain and a hole modification in the other one of the two subunits of the Fc domain.
5. The immunoconjugate of claim 4, wherein said knob modification comprises the amino acid substitution T366W, and said hole modification comprises the amino acid substitutions T366S, L368A and Y407V (EU numbering).
6. The immunoconjugate of claim 5, wherein the subunit of the Fc domain comprising the knob modification additionally comprises the amino acid substitution S354C, and the subunit of the Fc domain comprising the hole modification additionally comprises the amino acid substitution Y349C (EU numbering).
7. The immunoconjugate of any one of claims 4 to 6, wherein said effector moiety is fused to the carboxy-terminal amino acid of the subunit of the Fc domain comprising the knob modification.

8. The immunoconjugate of any one of claims 1 to 7, wherein said Fc domain is an IgG Fc domain.
9. The immunoconjugate of any one of claims 1 to 8, wherein said Fc domain is an IgG1 Fc domain.
10. The immunoconjugate of any one of claims 1 to 9, wherein said Fc domain is engineered to have altered binding to an Fc receptor and/or altered effector function.
11. The immunoconjugate of claim 10, wherein said Fc receptor is an Fcγ receptor.
12. The immunoconjugate of claim 10, wherein said effector function is ADCC.
13. The immunoconjugate of claim 10, wherein said altered binding and/or effector function is reduced binding and/or effector function.
14. The immunoconjugate of claim 13, wherein said Fc domain comprises one or more amino acid mutation that reduces the binding of the Fc domain to an Fc receptor, optionally an Fcγ receptor.
15. The immunoconjugate of claim 14, wherein said amino acid mutation is an amino acid substitution at position P329 (EU numbering).
16. The immunoconjugate of claim 14 or claim 15, wherein the Fc domain comprises the amino acid substitutions L234A, L235A and P329G (EU numbering) in each of its subunits.
17. The immunoconjugate of claim 10, wherein said altered binding and/or effector function is increased binding and/or effector function.
18. The immunoconjugate of claim 17, wherein said Fc domain is engineered to have an altered oligosaccharide structure, as compared to a non-engineered Fc domain.
19. The immunoconjugate of claim 18, wherein said Fc domain comprises an increased proportion of non-fucosylated oligosaccharides, as compared to a non-engineered Fc domain.
20. The immunoconjugate of any one of claims 1 to 19, wherein said immunoglobulin molecule is an IgG class immunoglobulin.
21. The immunoconjugate of any one of claims 1 to 20, wherein said immunoglobulin molecule is a IgG1 class immunoglobulin.

22. The immunoconjugate of any one of claims 1 to 21, wherein said effector moiety is fused to the carboxy-terminal amino acid of one of the immunoglobulin heavy chains, optionally through a linker peptide.
23. The immunoconjugate of any one of claims 1 to 22, wherein said mutant human interleukin-2 (IL-2) polypeptide further comprises the amino acid substitution T3A.
24. The immunoconjugate of any one of claims 1 to 23, wherein said mutant human interleukin-2 (IL-2) polypeptide further comprises the amino acid substitution C125A.
25. The immunoconjugate of any one of claims 1 to 24, wherein said mutant human interleukin-2 (IL-2) polypeptide comprises the polypeptide sequence of SEQ ID NO: 3.
26. The immunoconjugate of any one of claims 1 to 25, comprising the polypeptide sequences of SEQ ID NO: 277, SEQ ID NO: 281 and SEQ ID NO: 283.
27. An isolated polynucleotide encoding the immunoconjugate of any one of claims 1 to 26.
28. An expression vector comprising the isolated polynucleotide of claim 27.
29. A host cell comprising the isolated polynucleotide of claim 27 or the expression vector of claim 28.
30. The host cell of claim 29, wherein said host cell has been manipulated to express increased levels of one or more polypeptides having β 1,4) -N-acetylglucosaminyltransferase III (GnTIII) activity.
31. A method of producing an immunoconjugate comprising (i) an immunoglobulin molecule comprising a first and a second antigen binding Fab molecule and an Fc domain consisting of two subunits, and (ii) an effector moiety, wherein not more than one effector moiety is present, comprising culturing the host cell of claim 29 or claim 30 under conditions suitable for expression of the immunoconjugate and optionally recovering the immunoconjugate.
32. An immunoconjugate when produced by the method of claim 31.
33. A pharmaceutical composition comprising the immunoconjugate of any one of claims 1 to 26 or claim 32 and a pharmaceutically acceptable carrier.

34. Use of the immunoconjugate of any one of claims 1 to 26 or claim 32, or the pharmaceutical composition of claim 33, in the manufacture of a medicament for treating a cancer expressing CEA in an individual in need thereof.

35. A method of treating a cancer expressing CEA in an individual, comprising administering to said individual a therapeutically effective amount of a composition comprising the immunoconjugate of any one of claims 1 to 26 or claim 32 in a pharmaceutically acceptable form.

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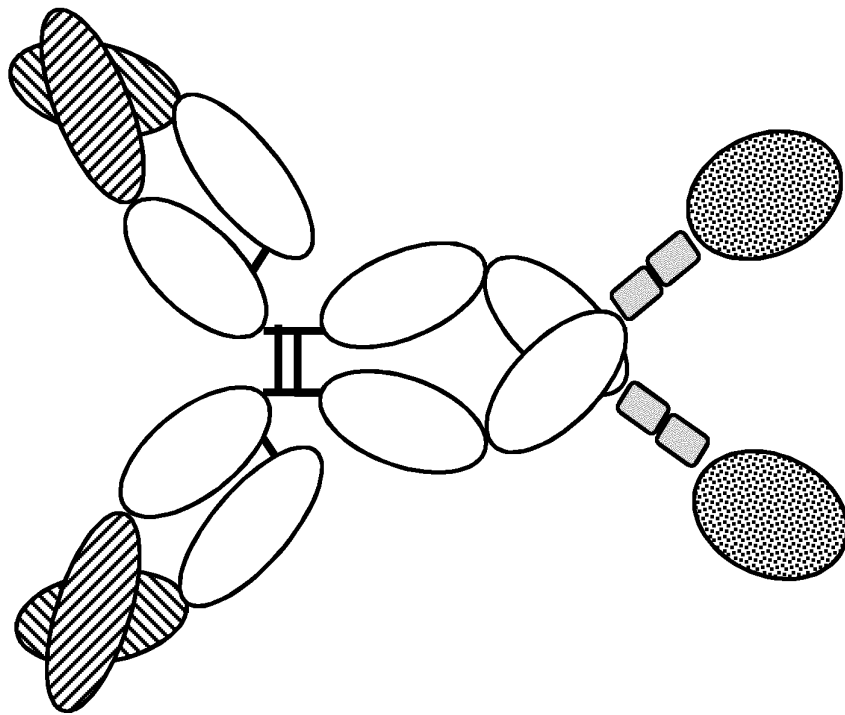
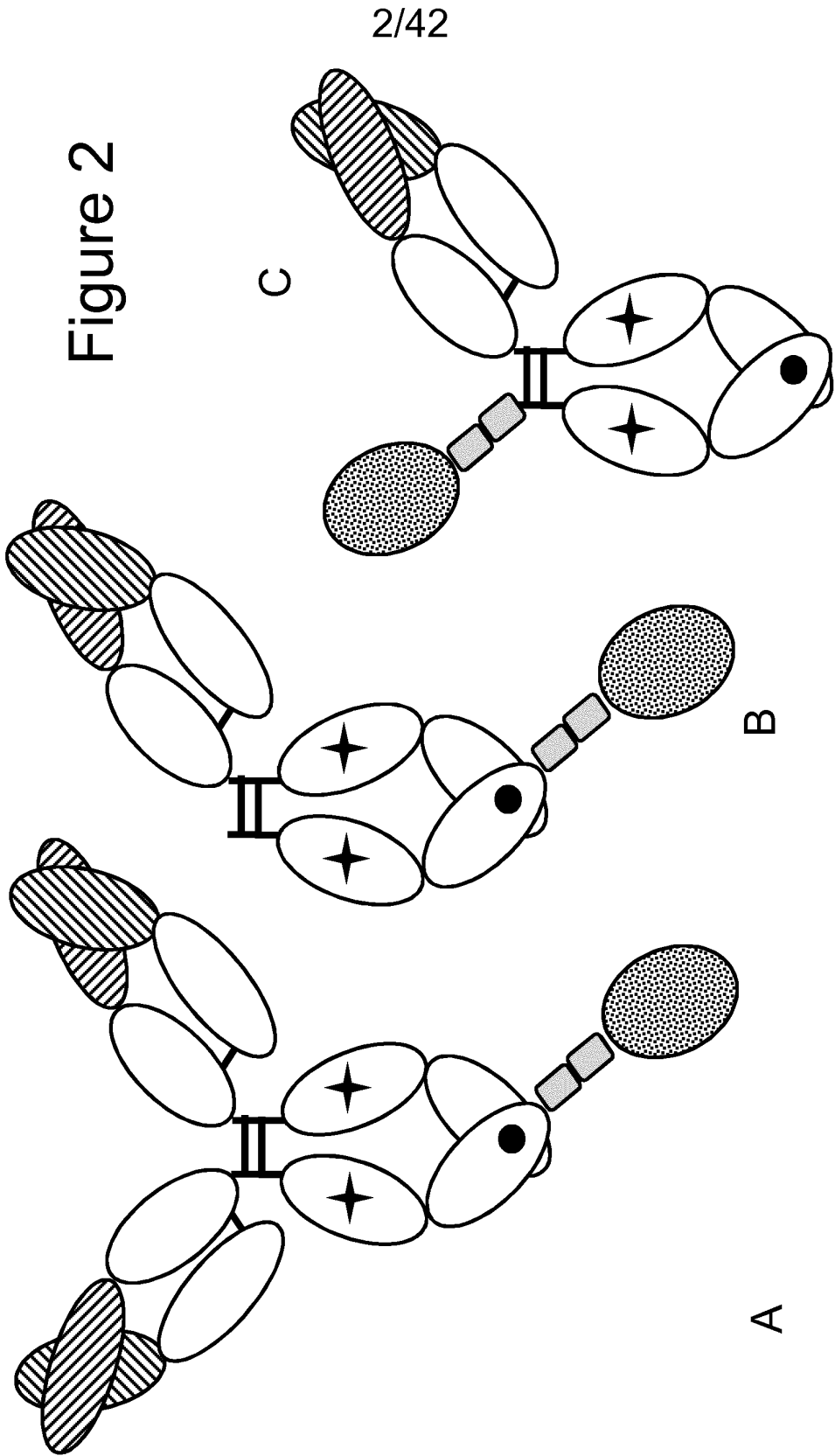
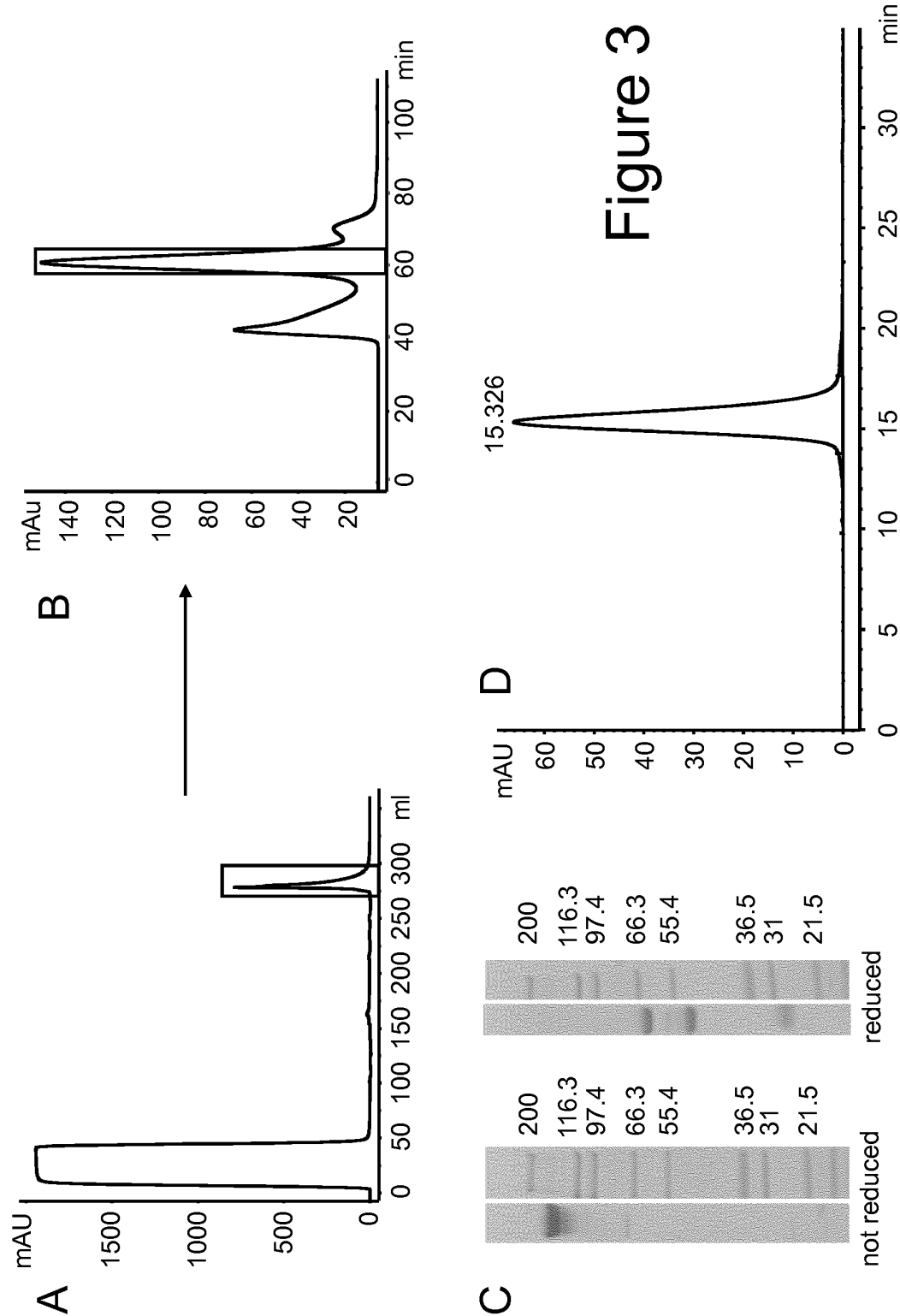
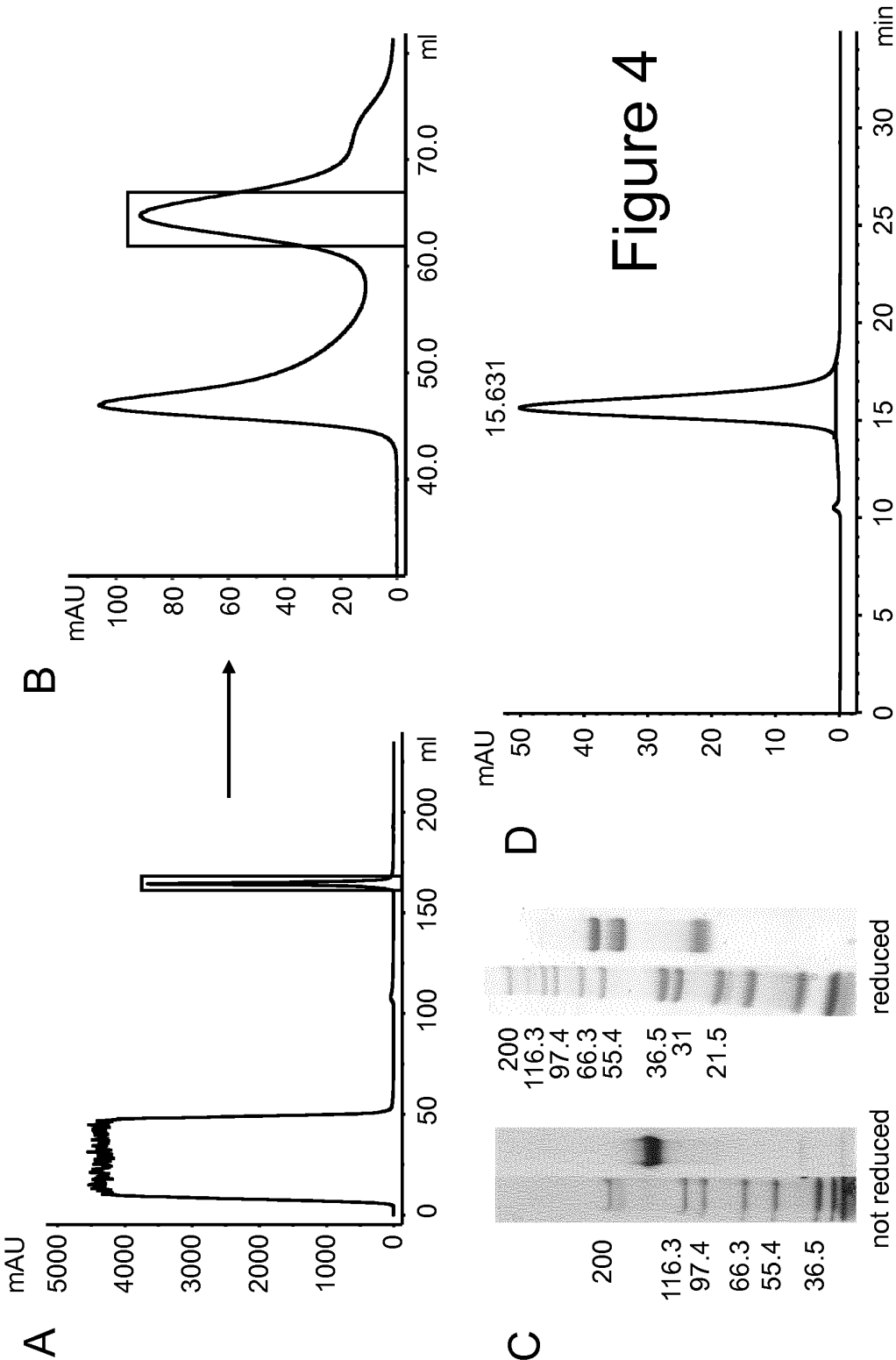
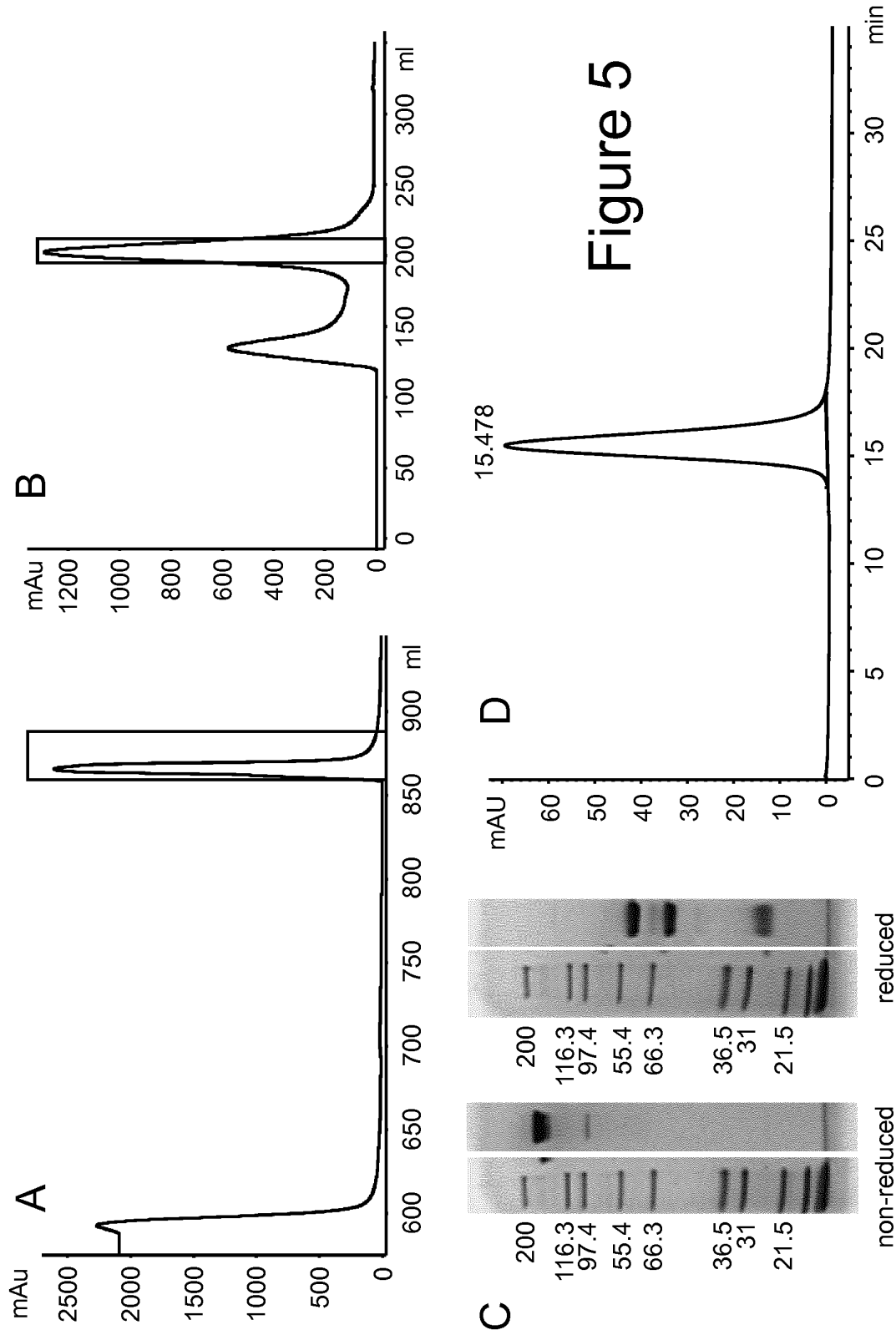


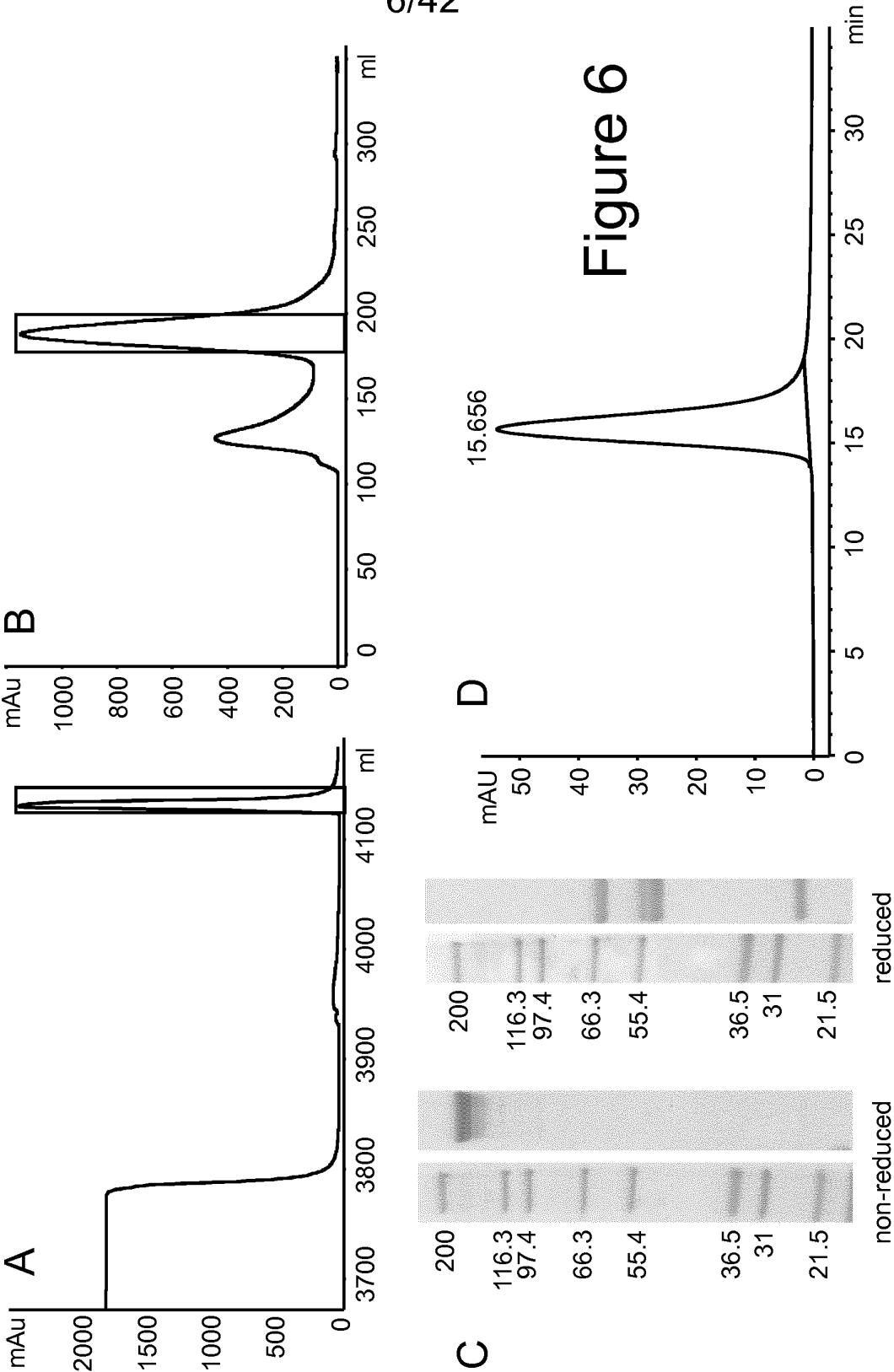
Figure 1











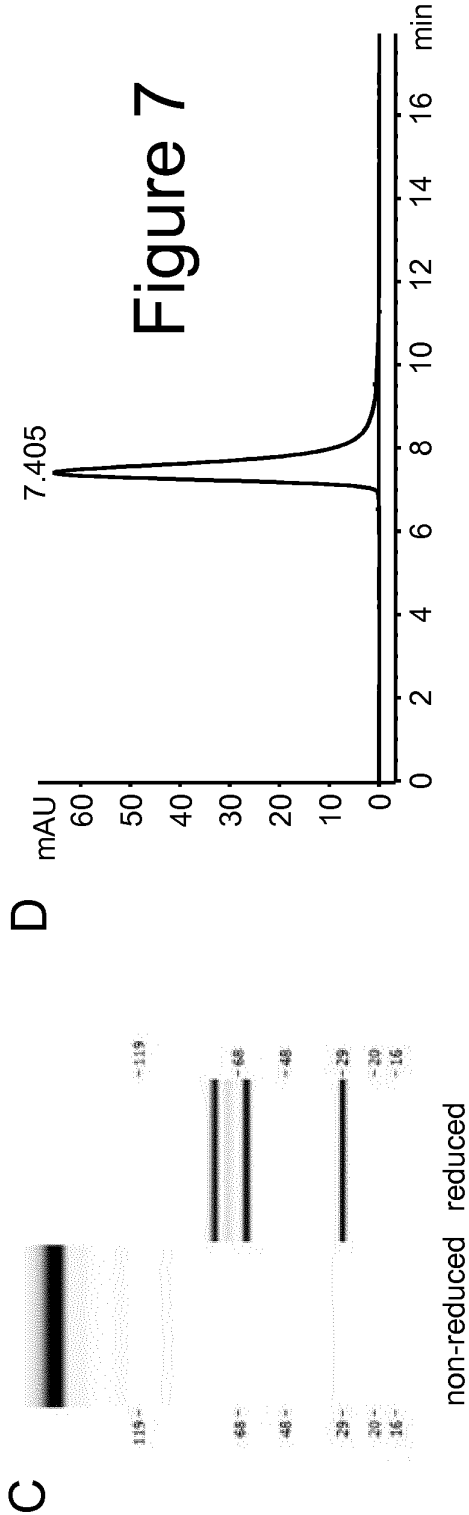
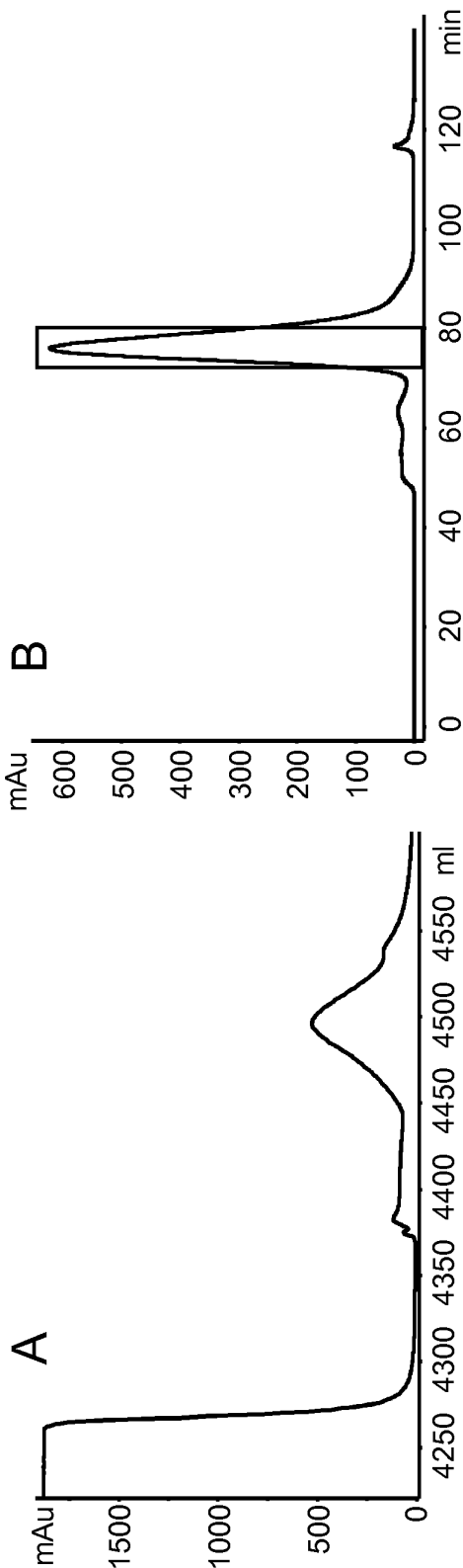


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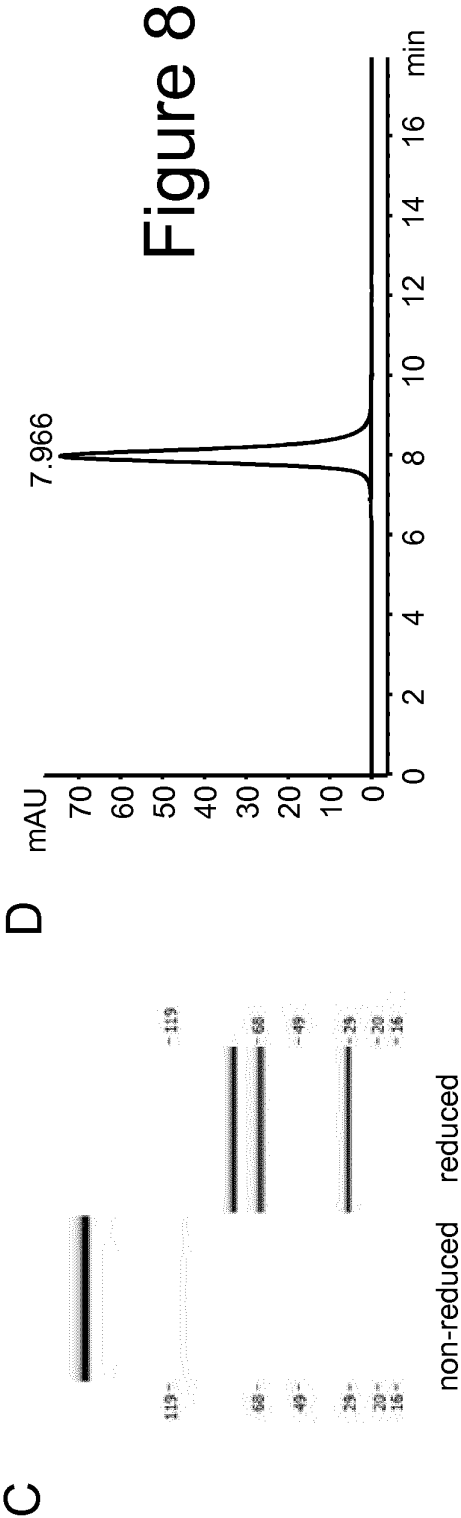
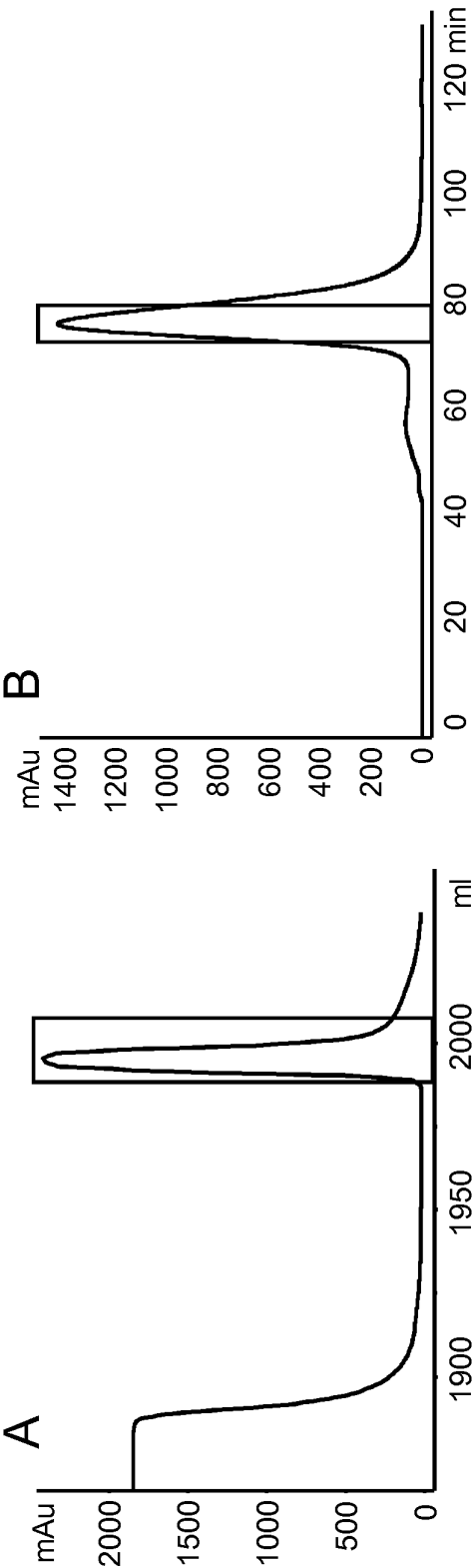
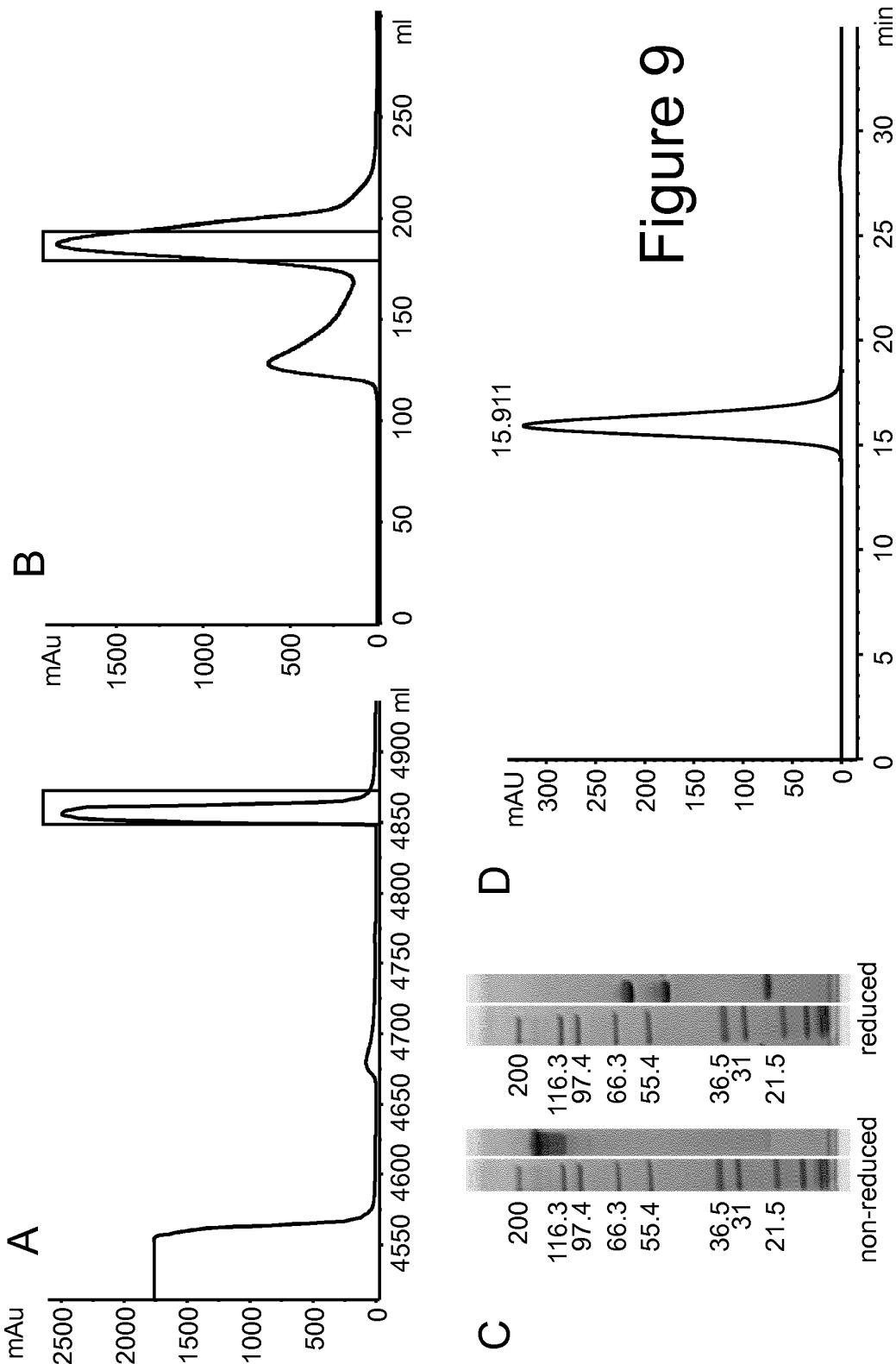
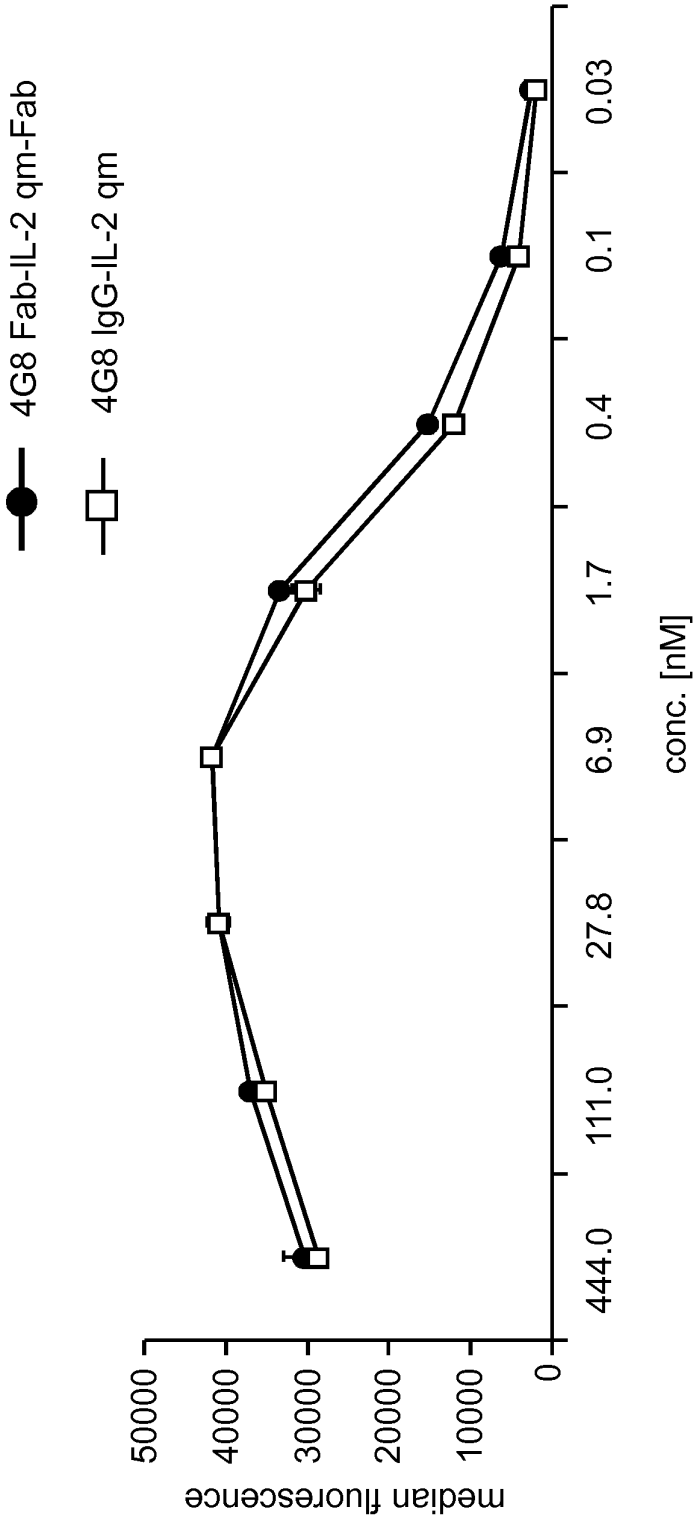


Figure 8



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Figure 10



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Figure 11

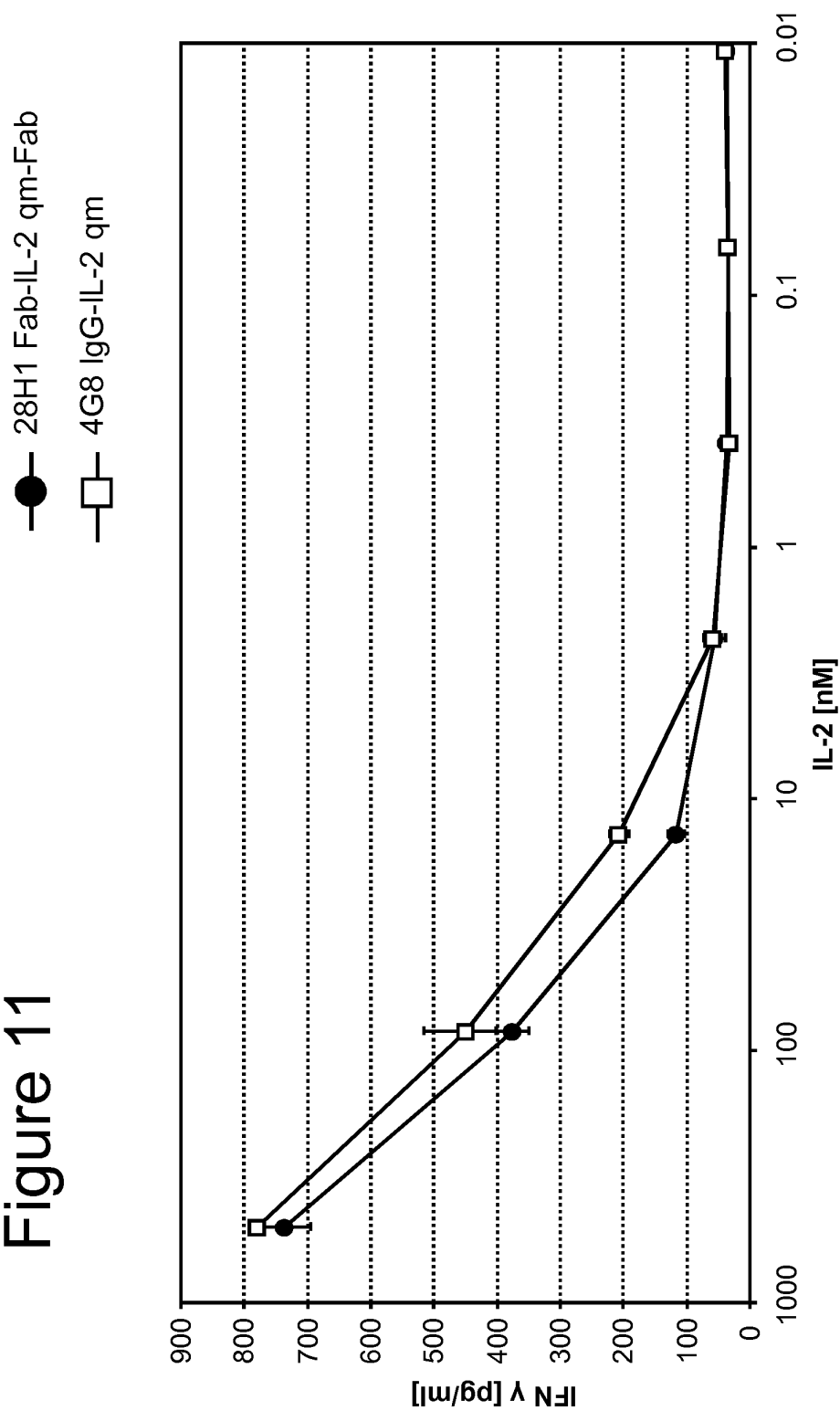


Figure 12

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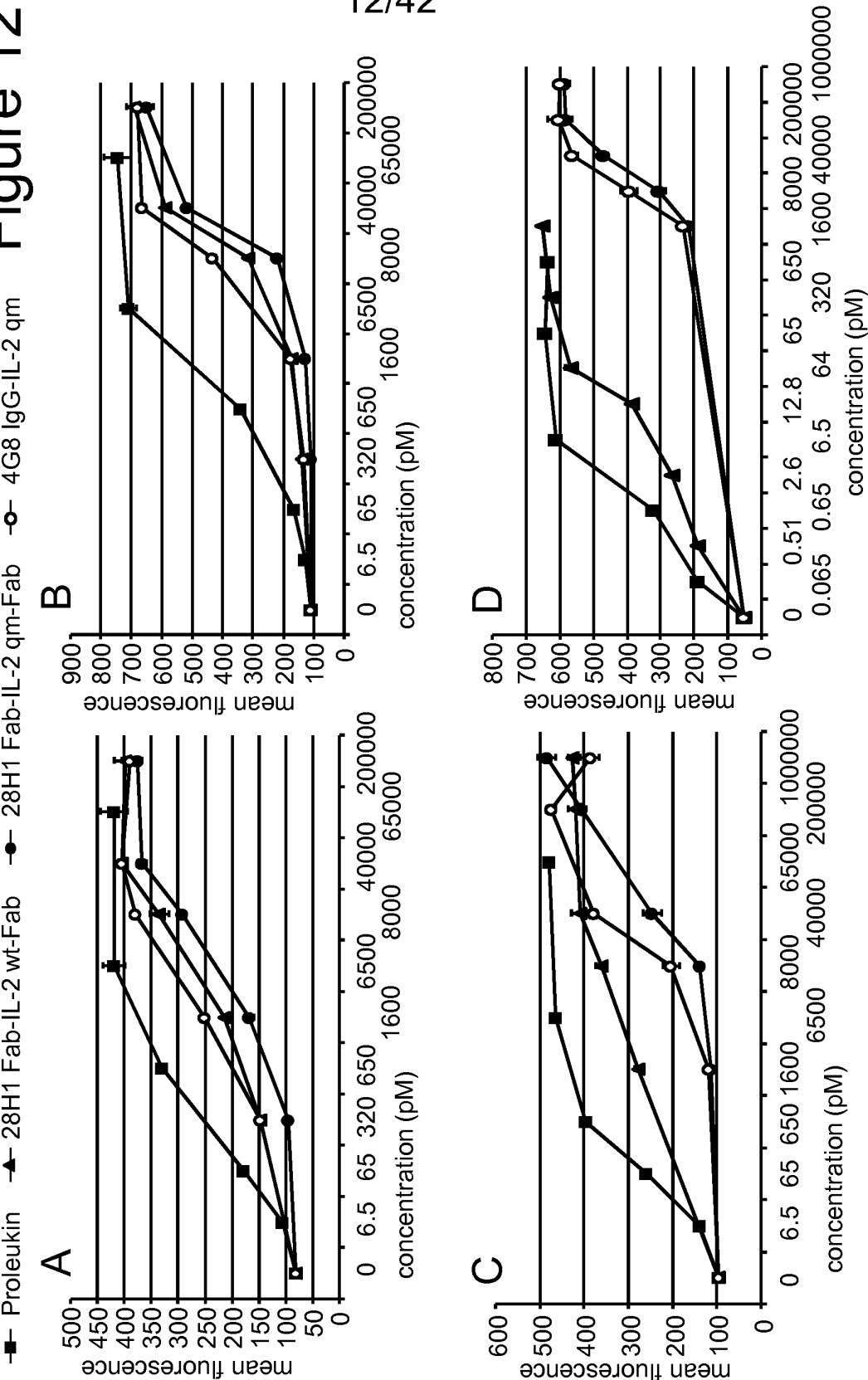
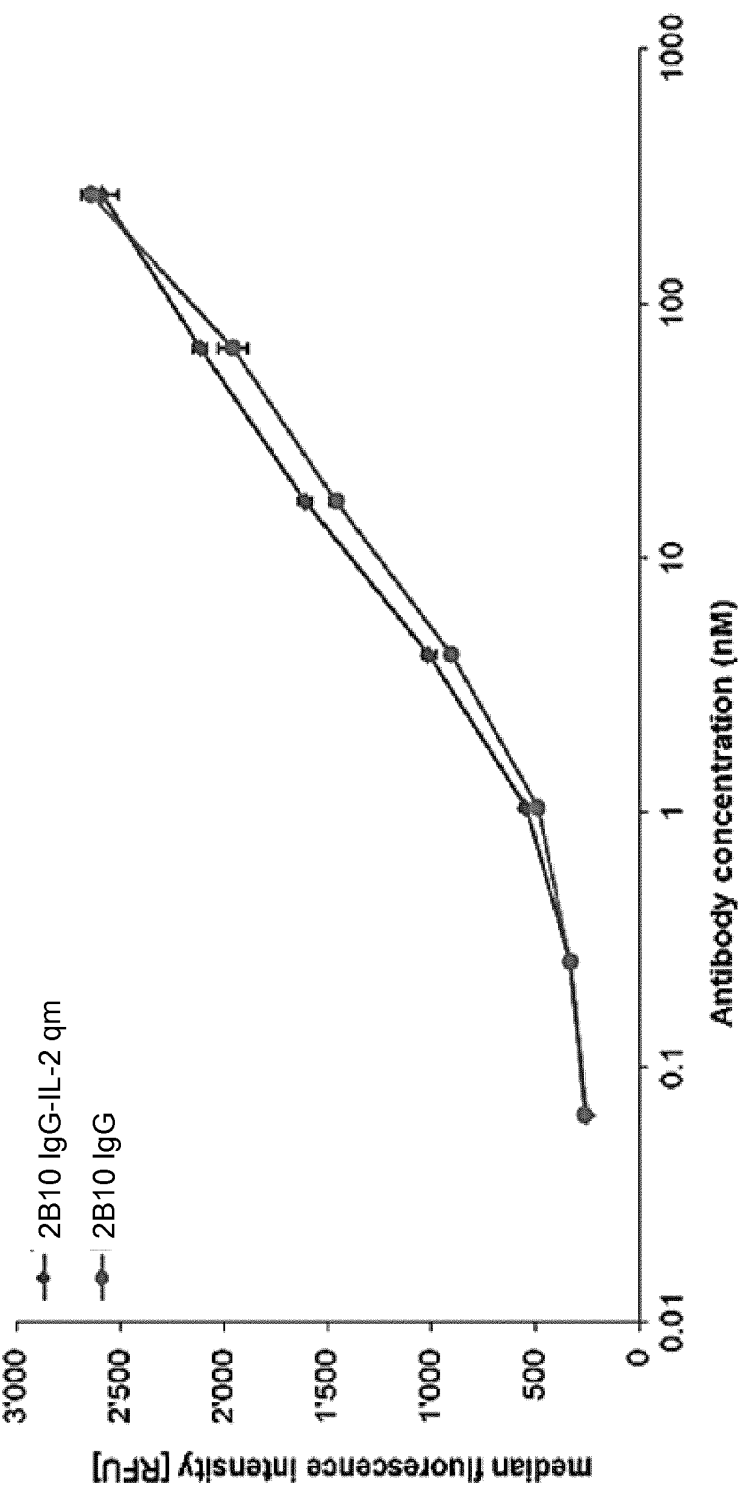


Figure 13



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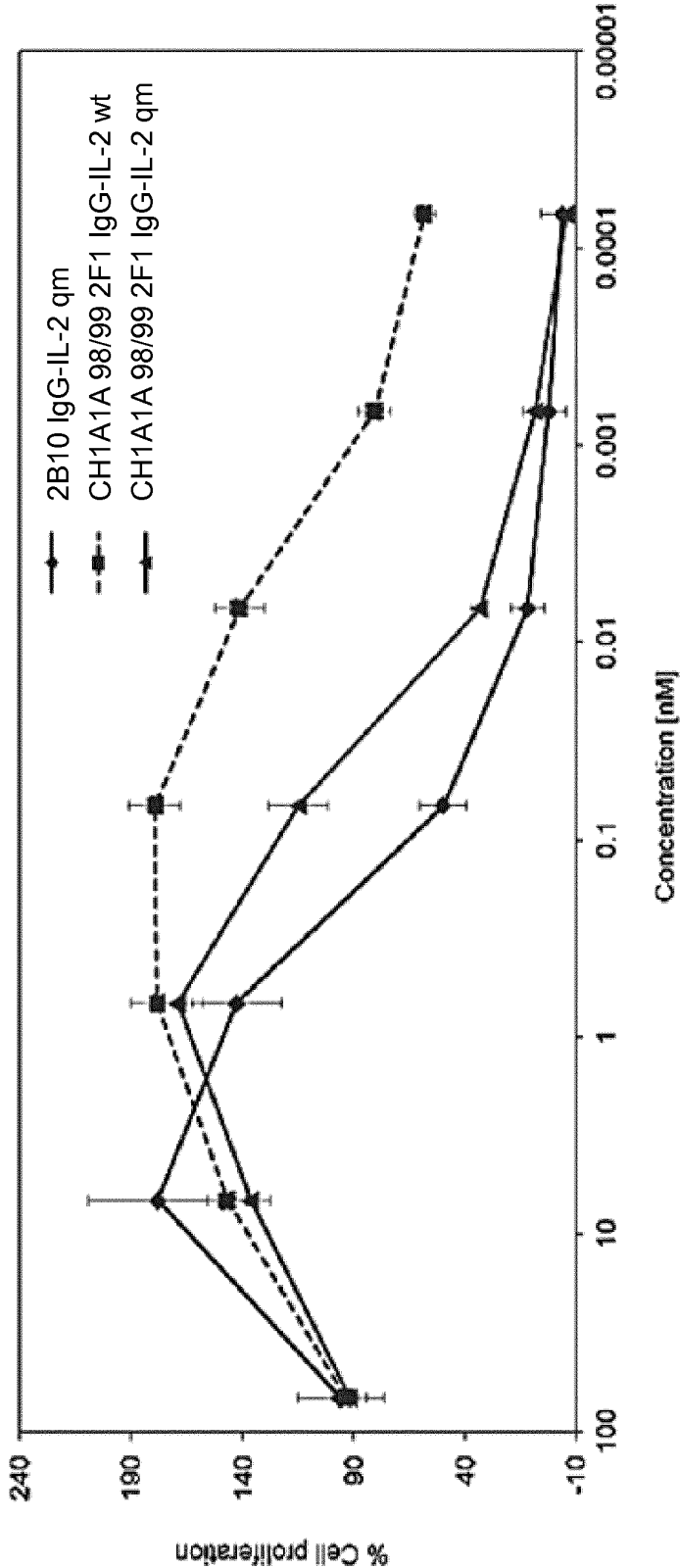


Figure 14

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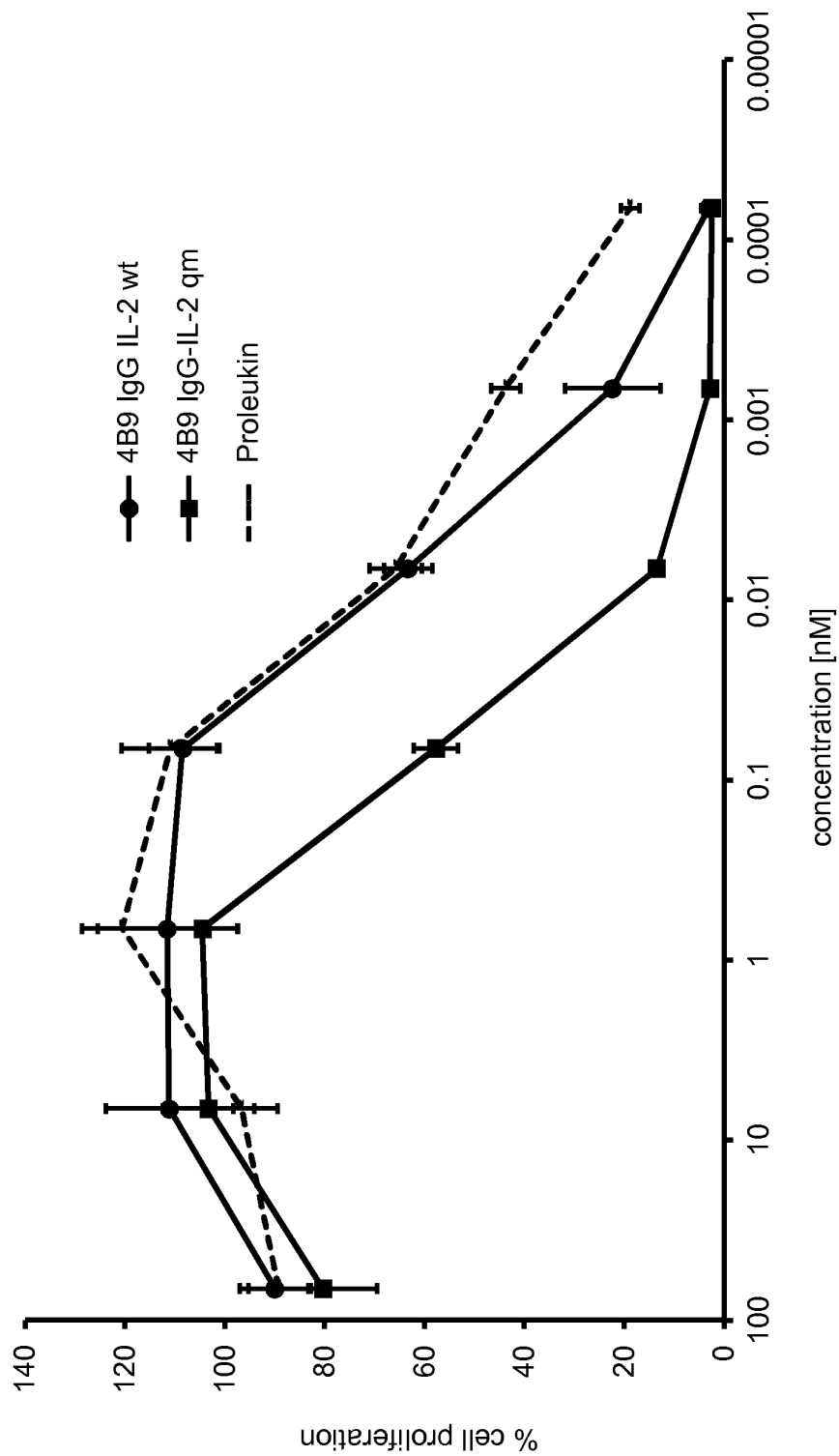


Figure 15

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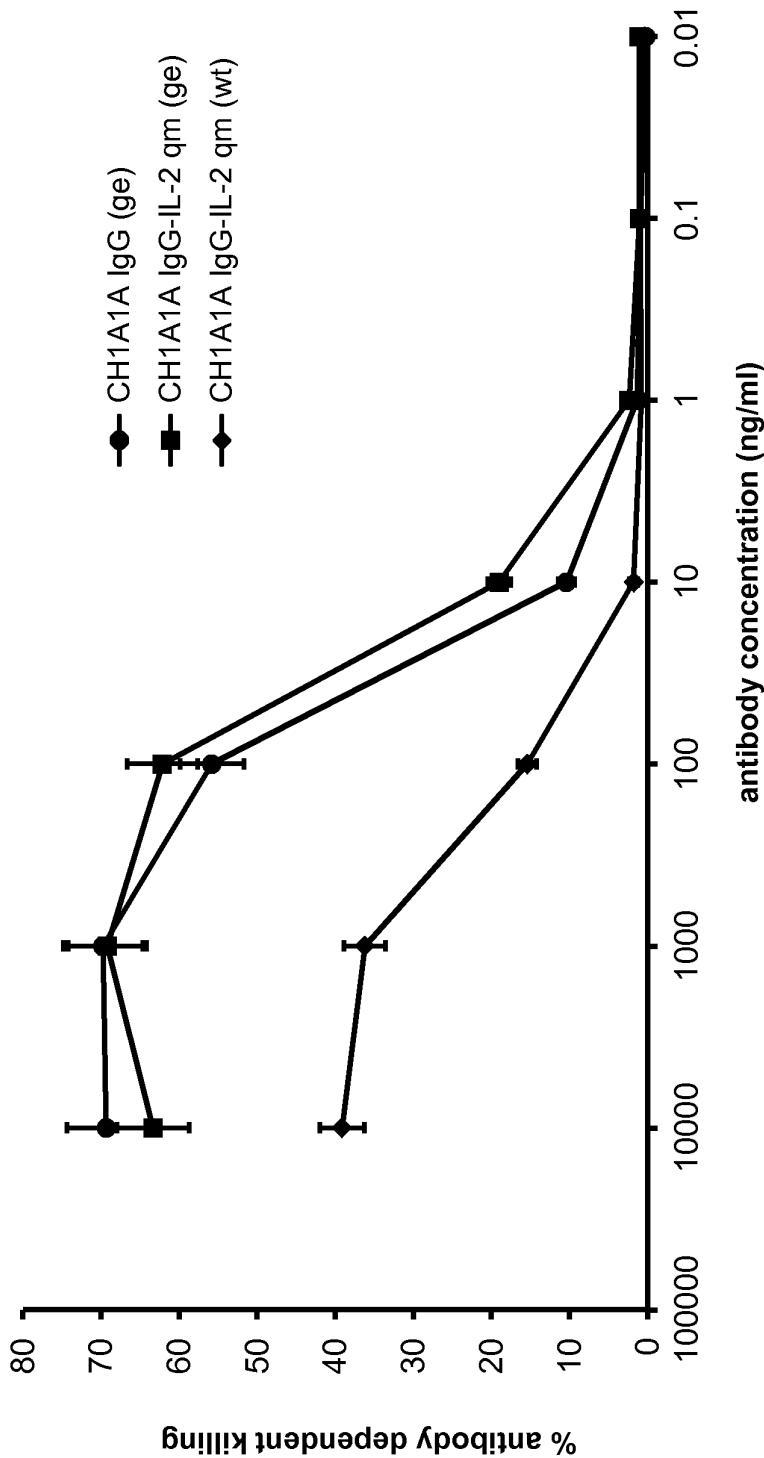
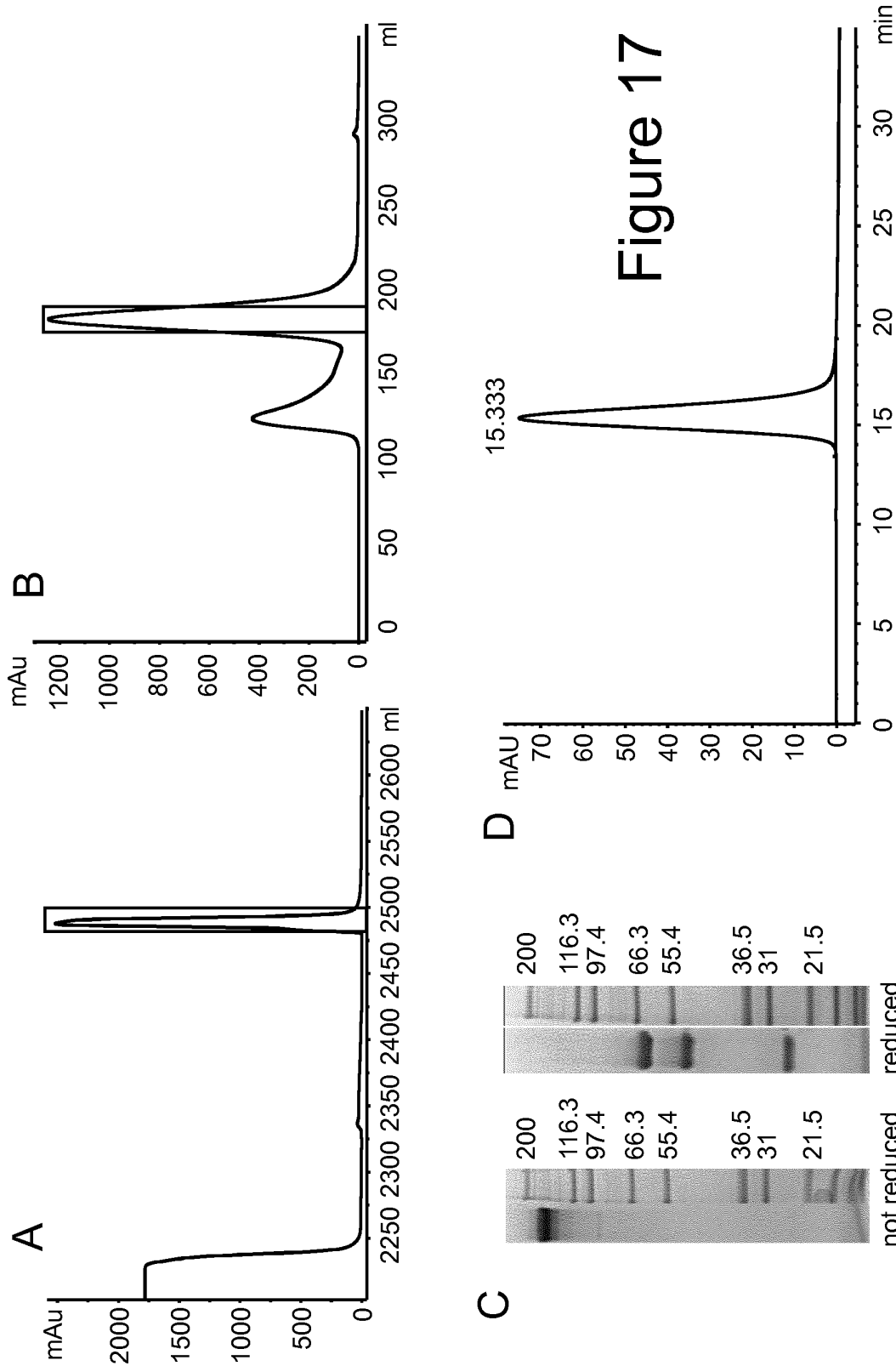
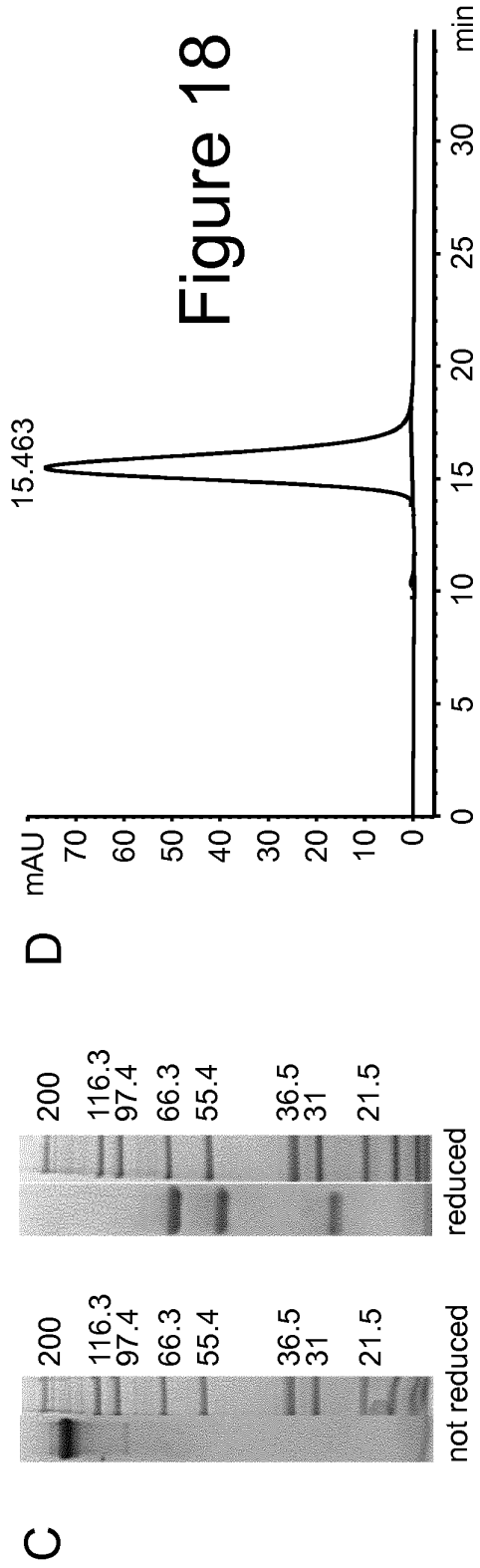
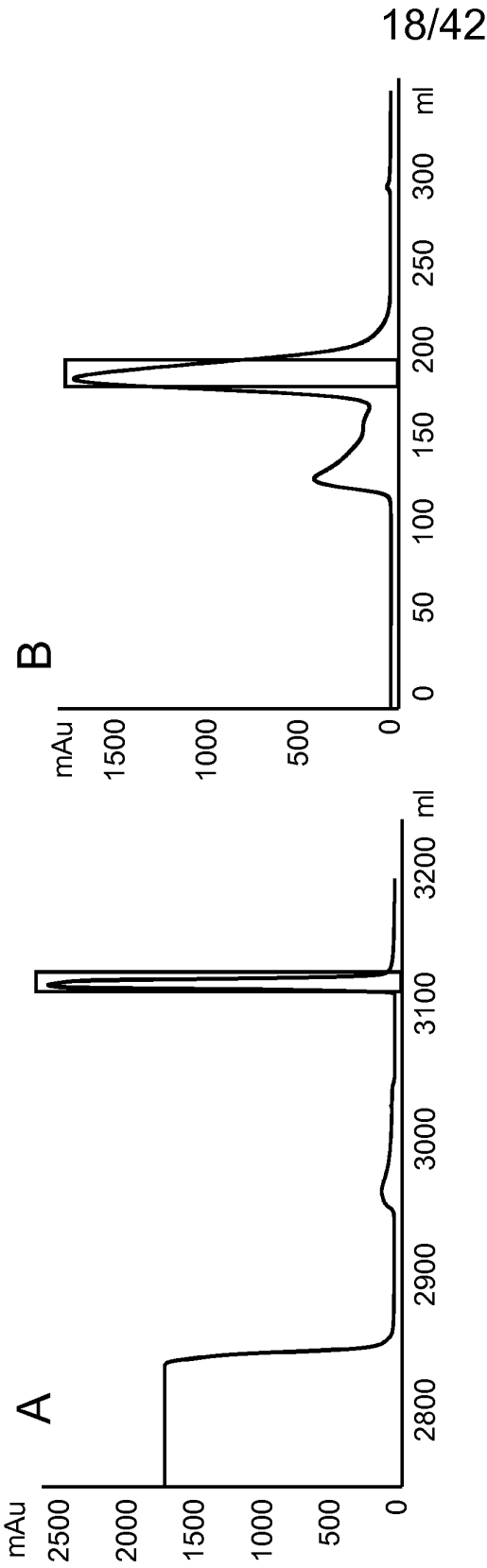
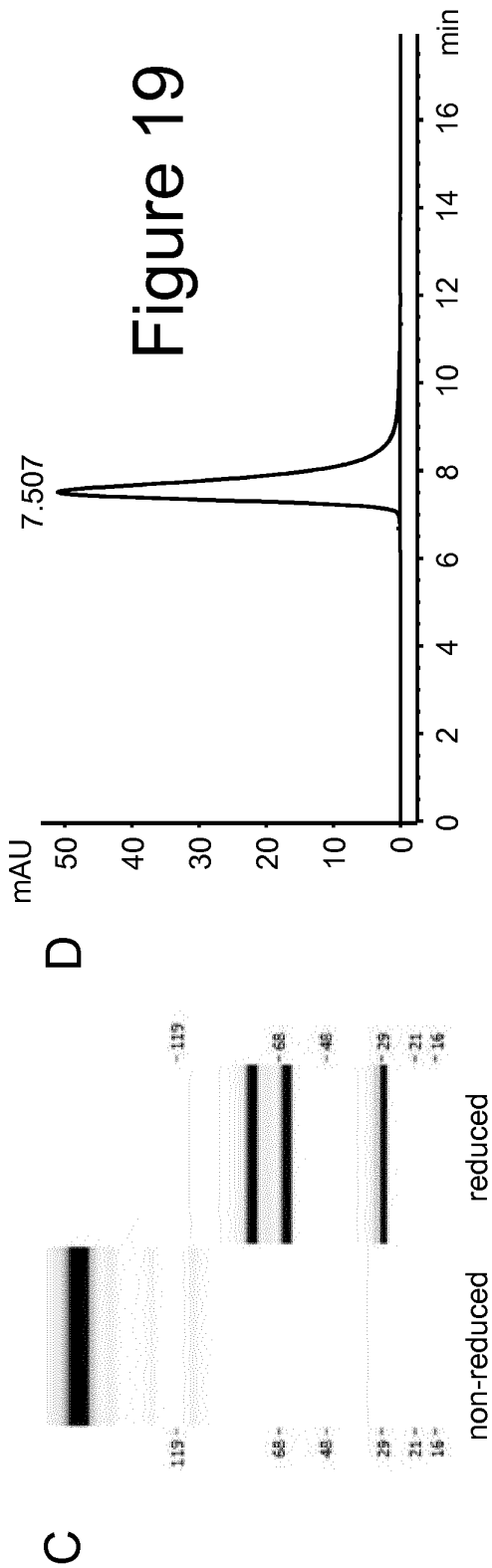
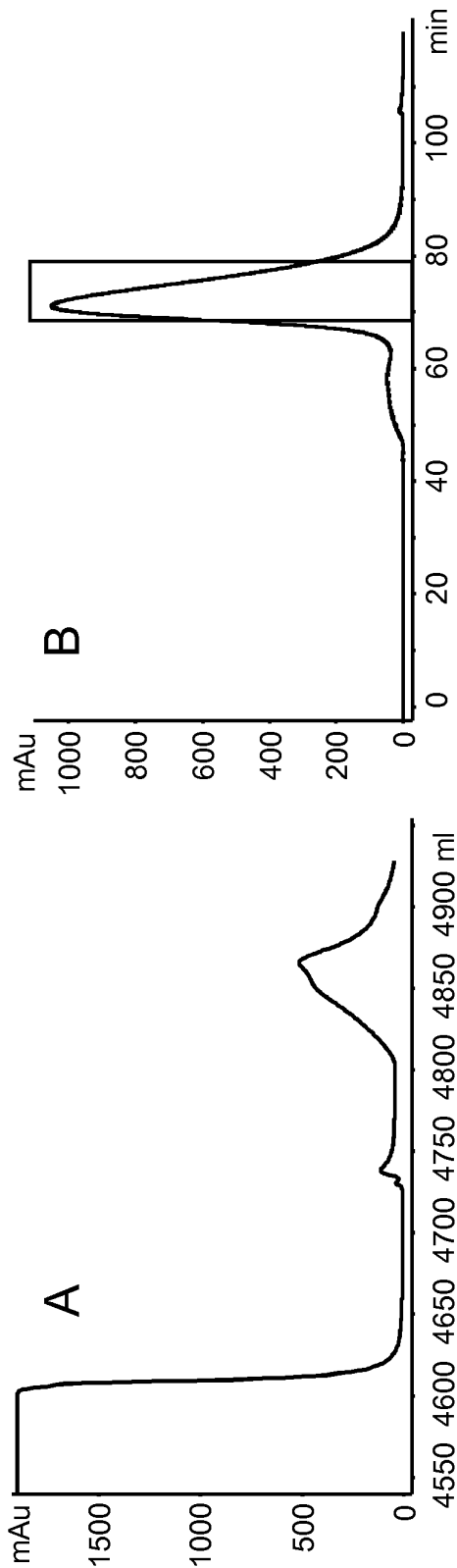


Figure 16







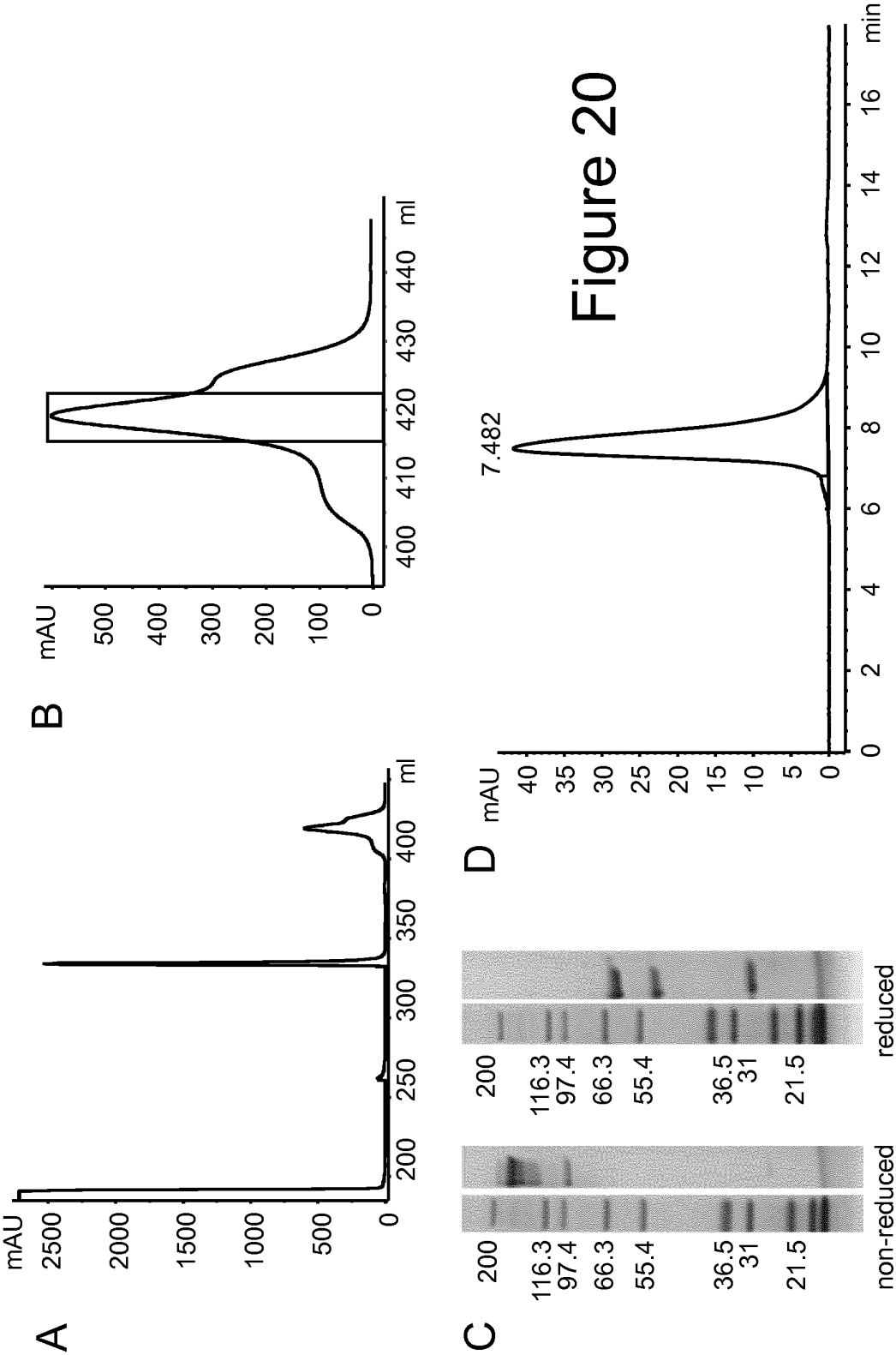
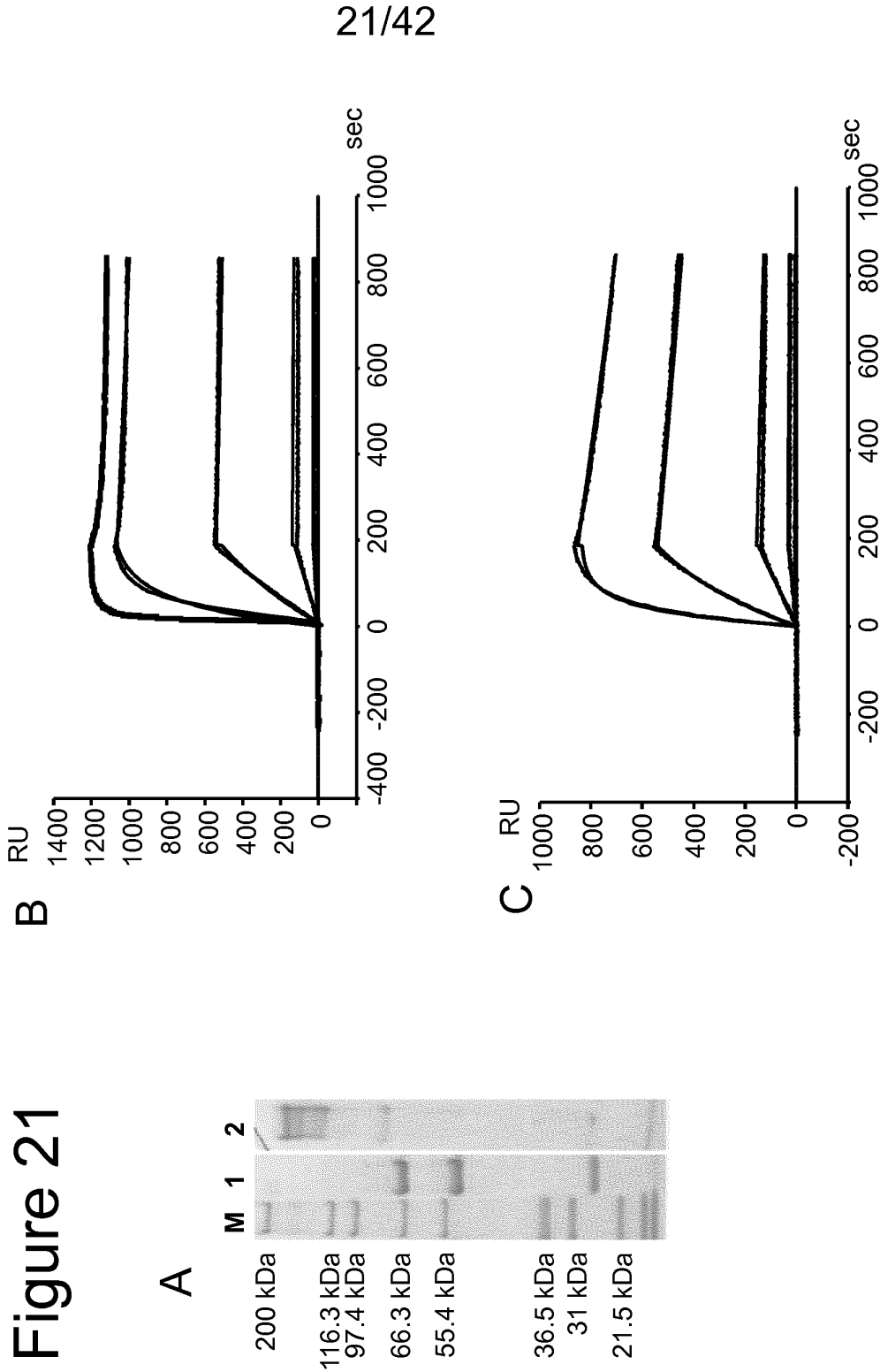


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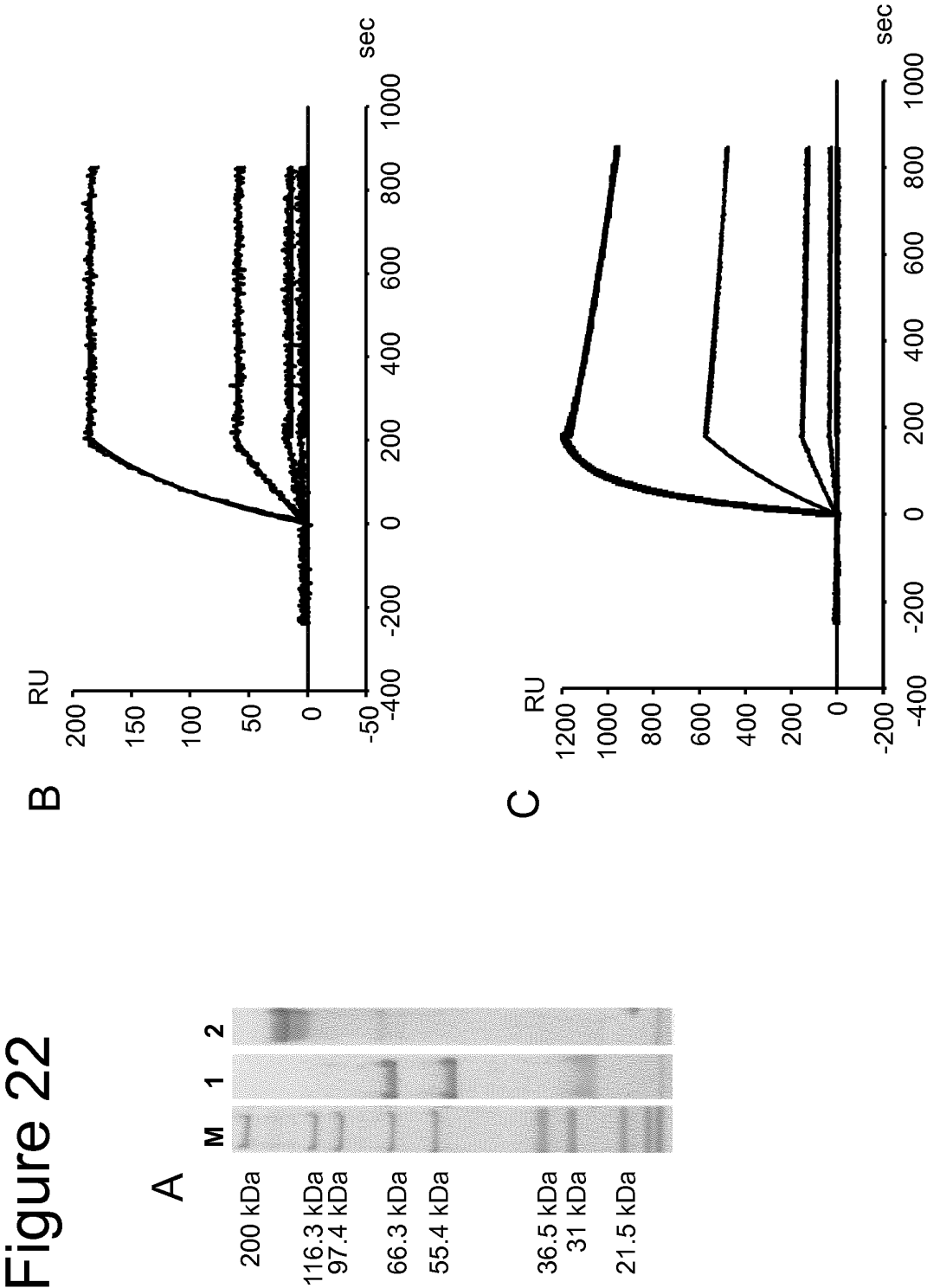


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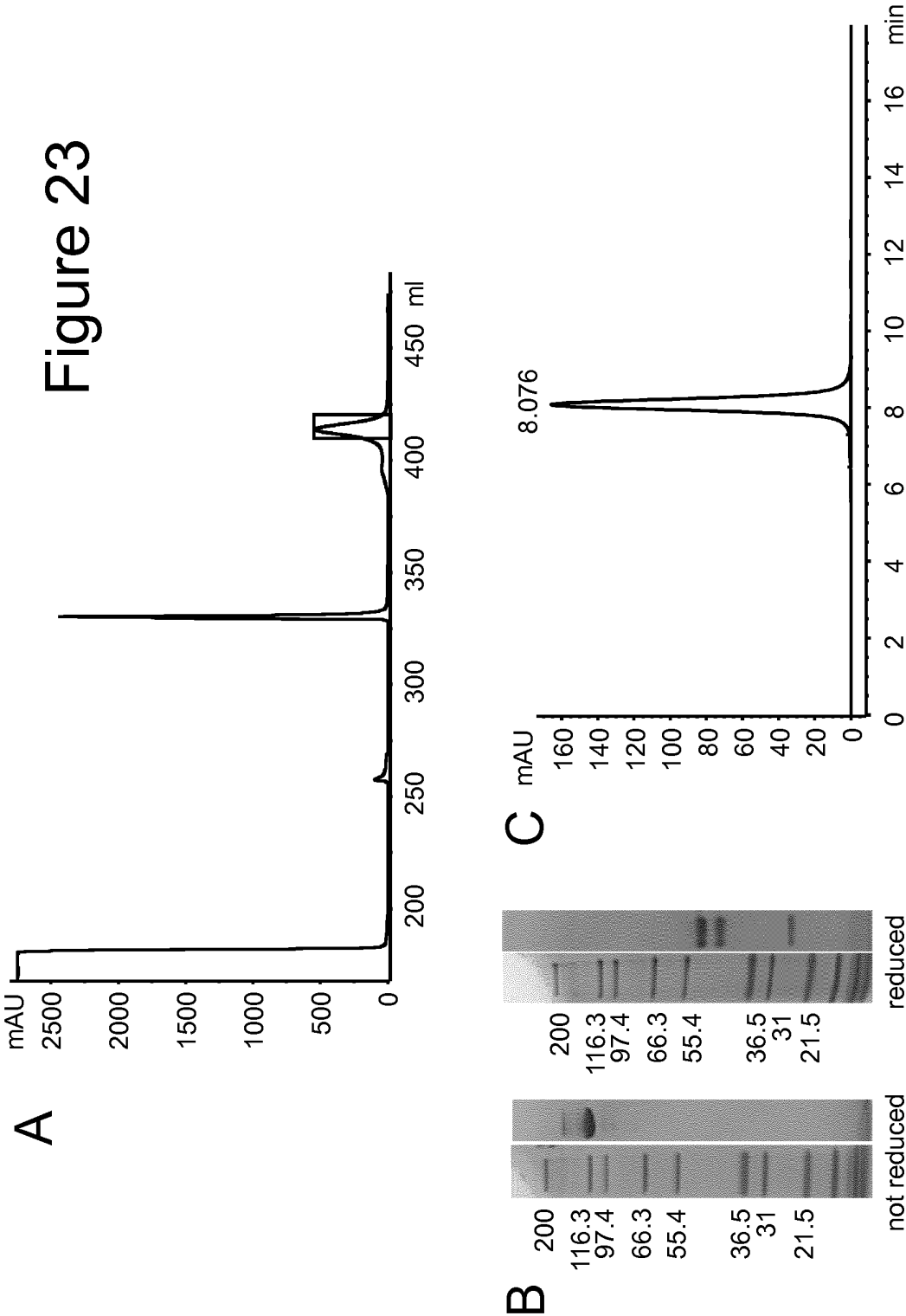


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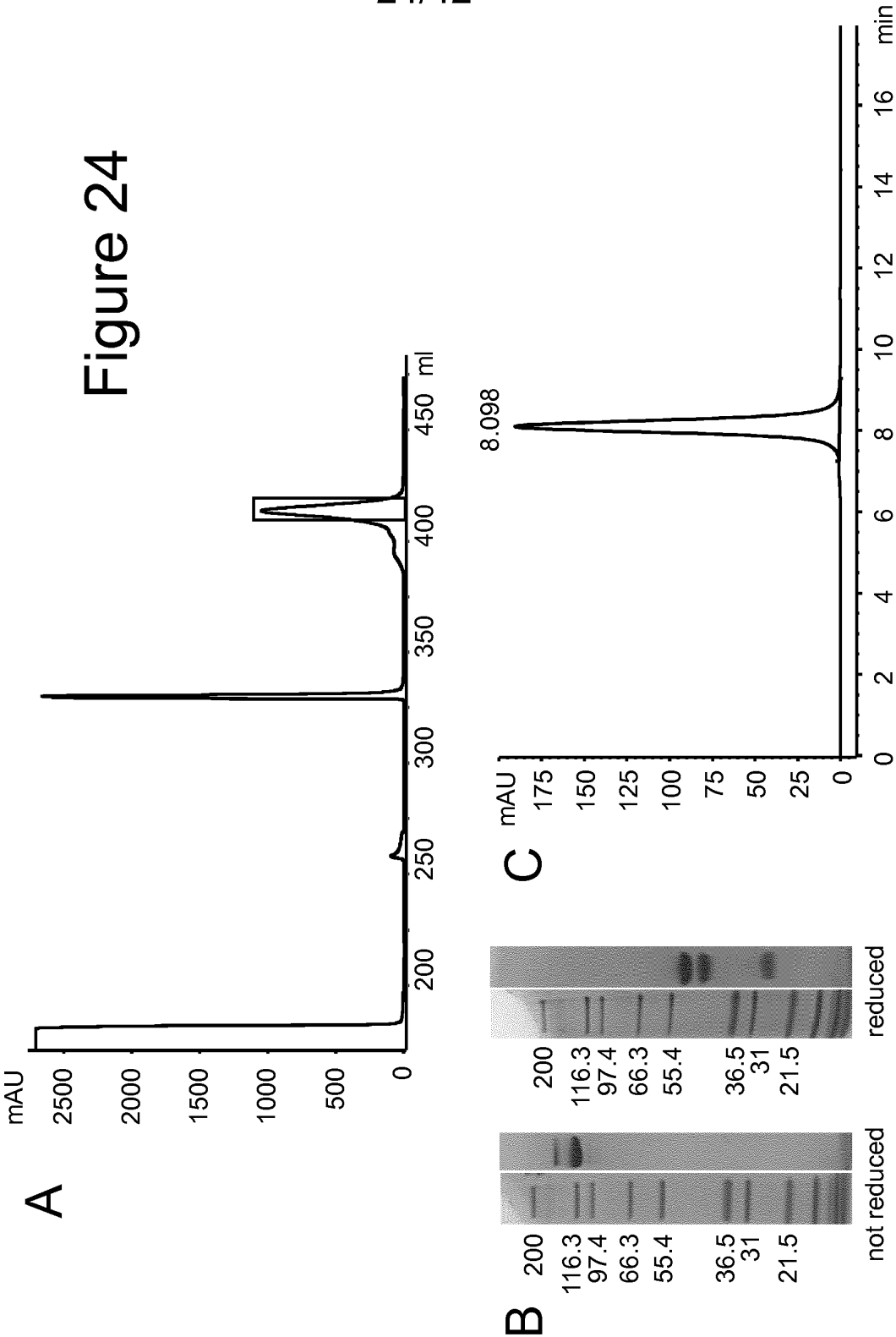
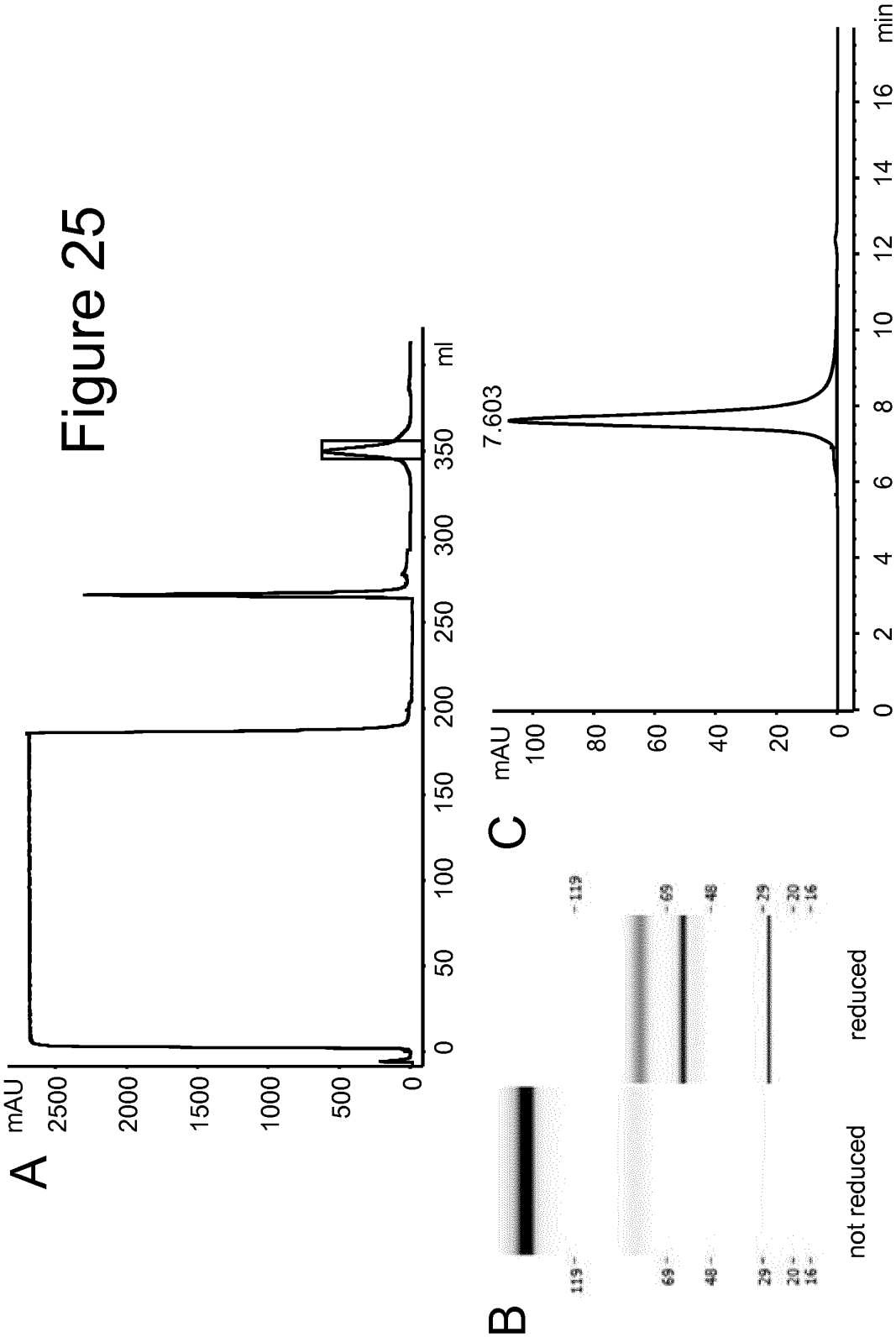


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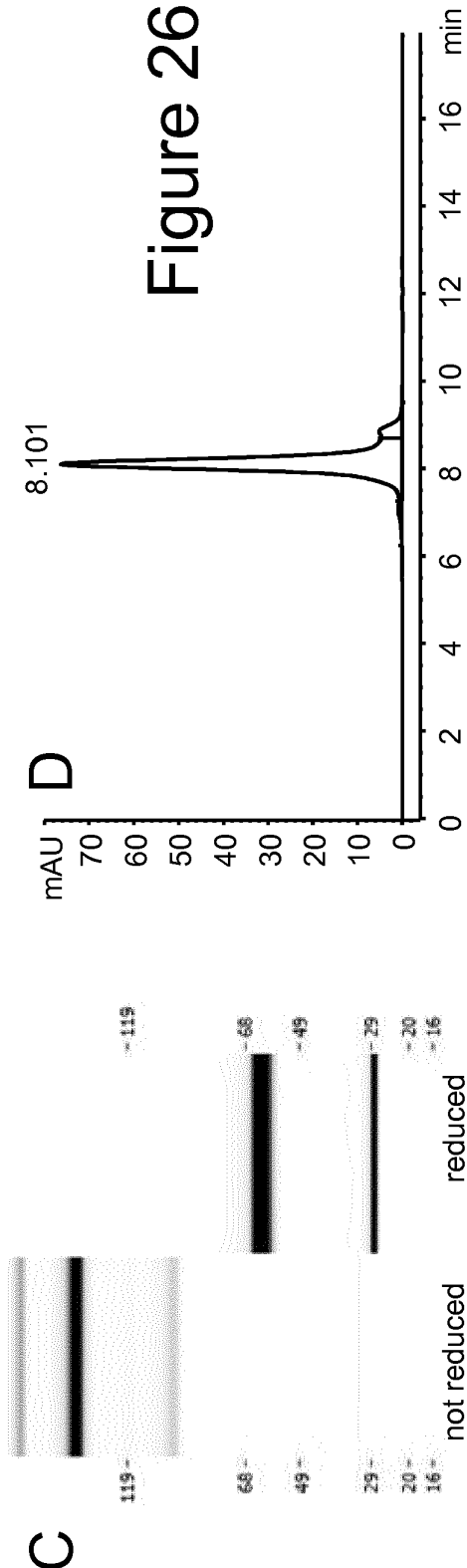
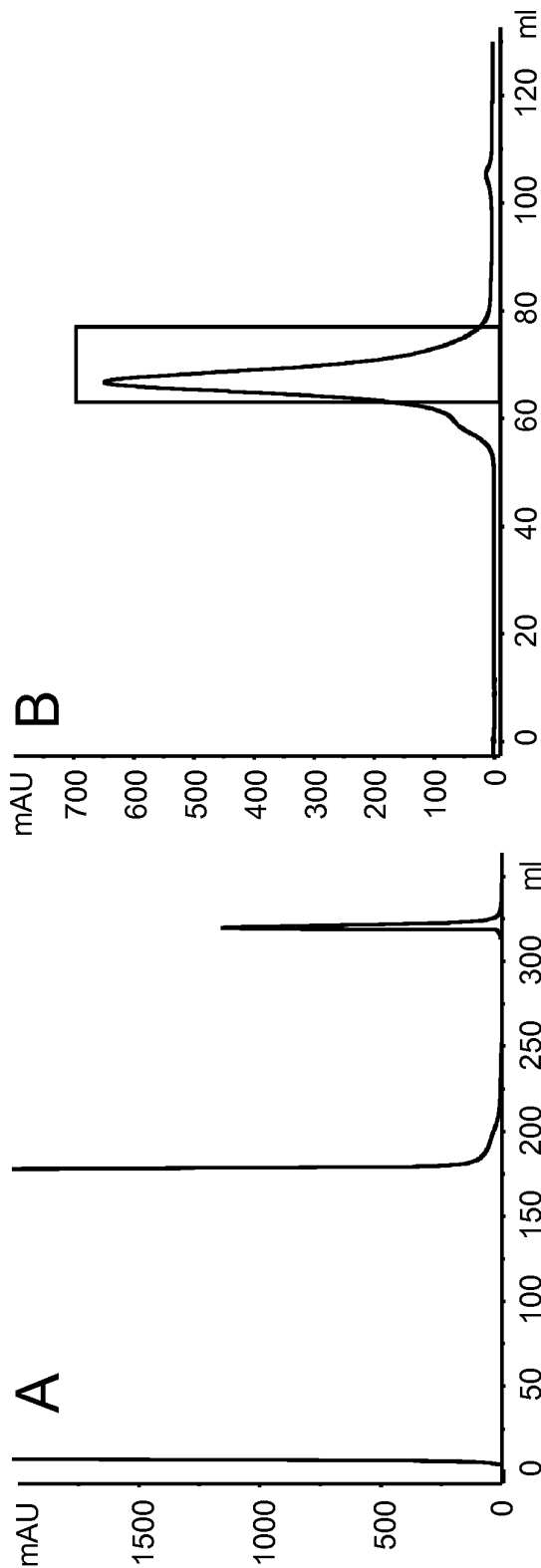


Figure 26

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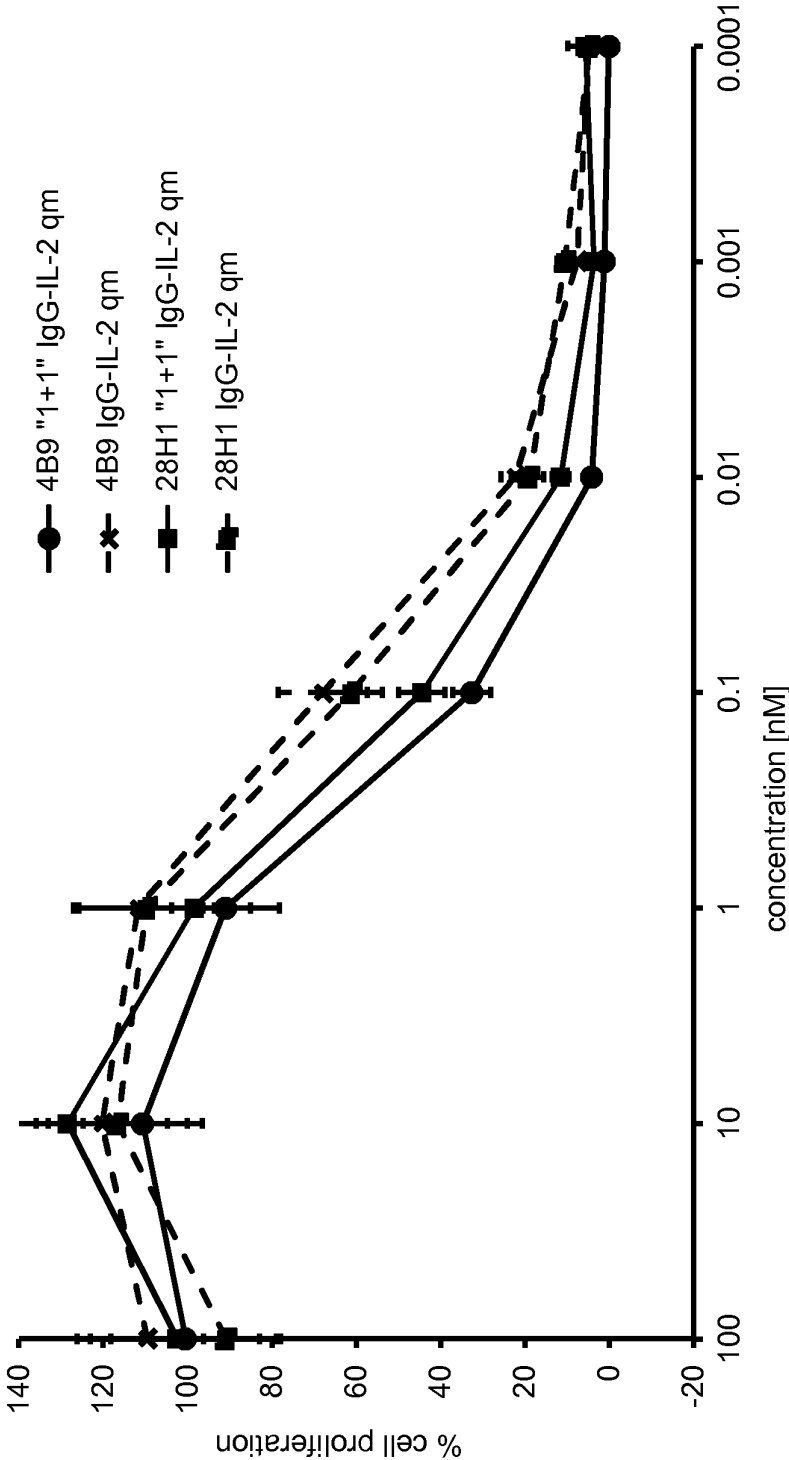


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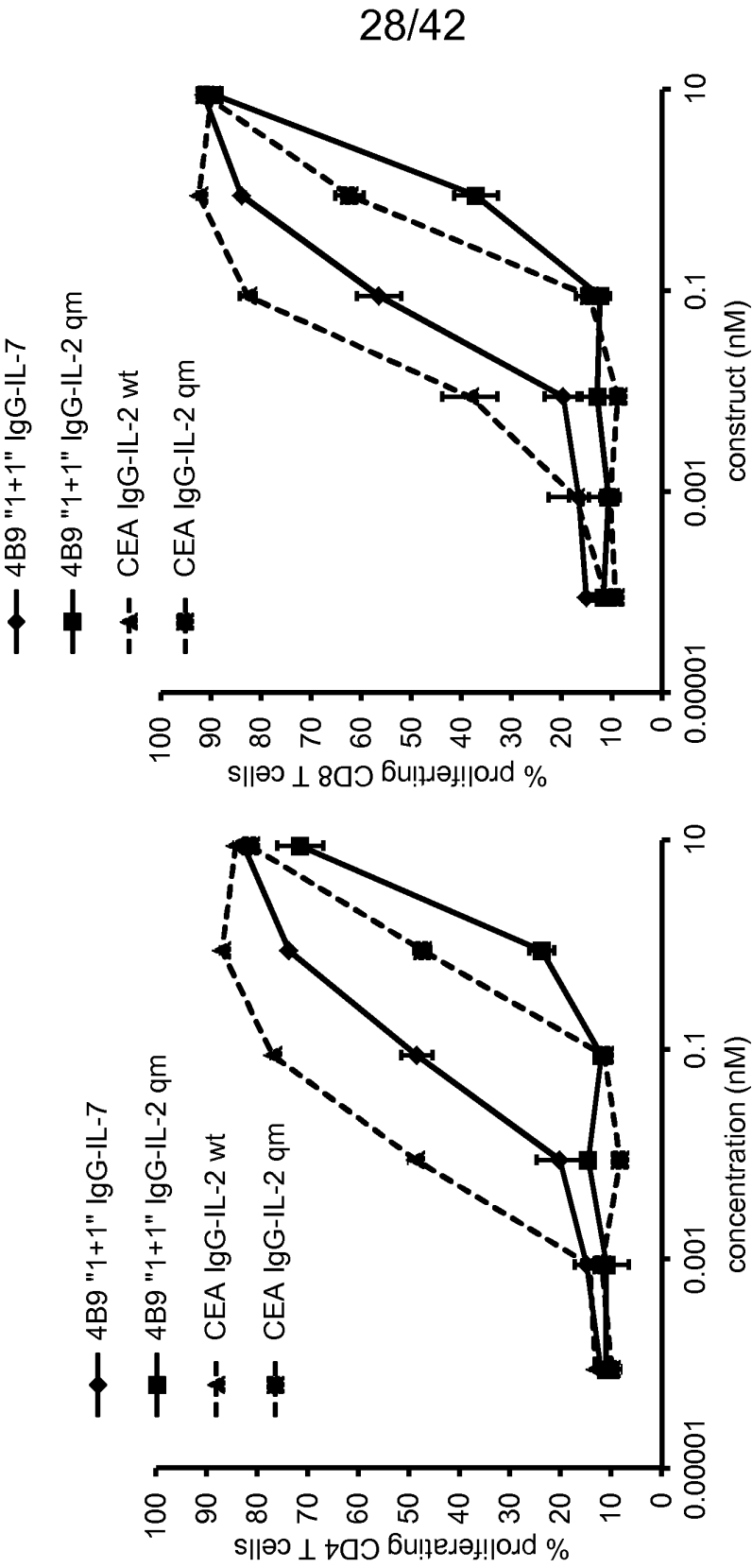


Figure 28

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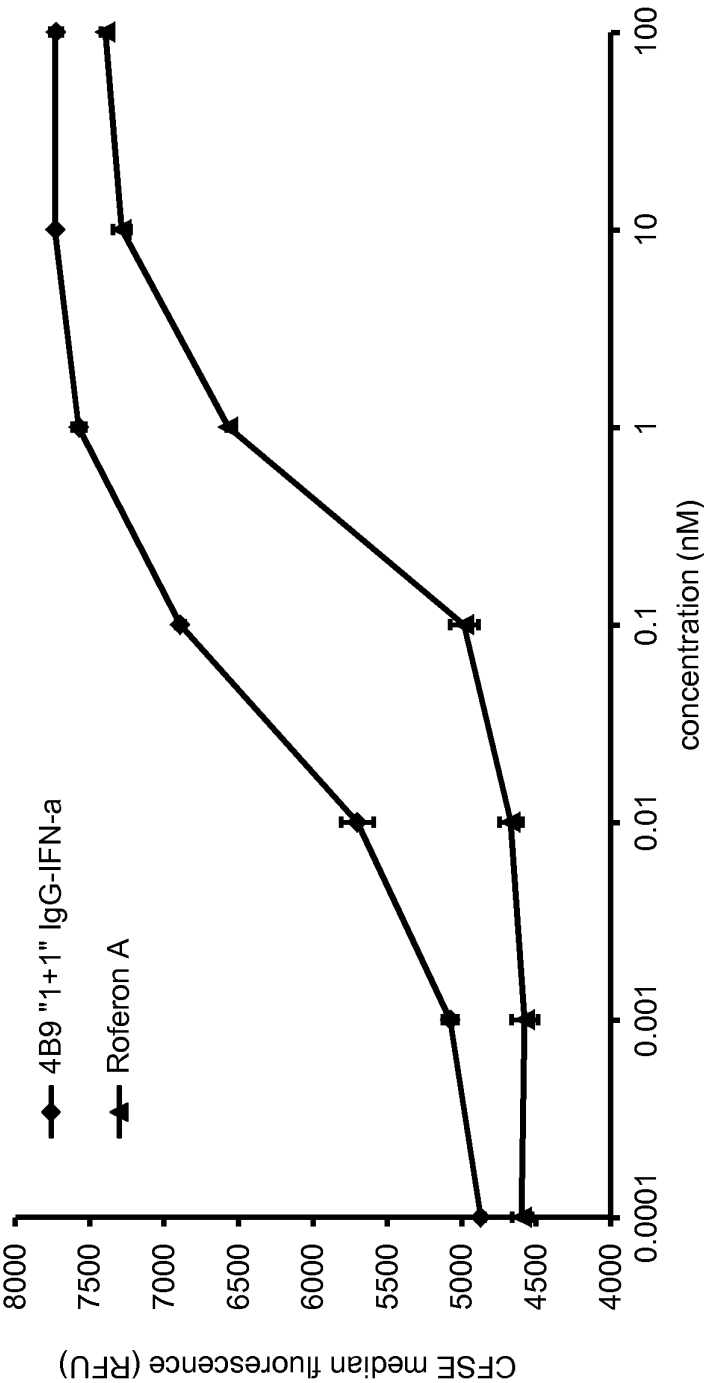


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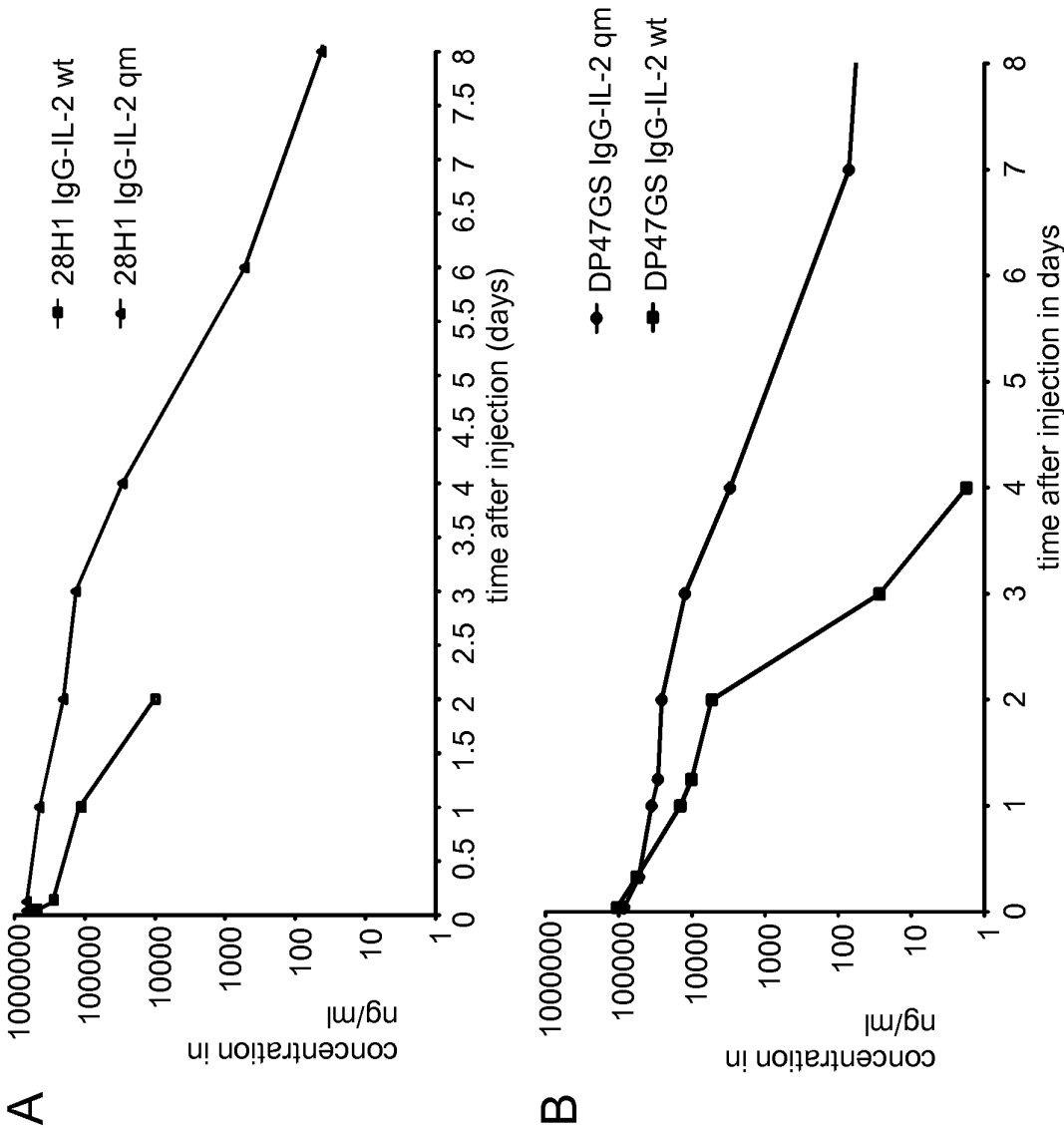


Figure 30

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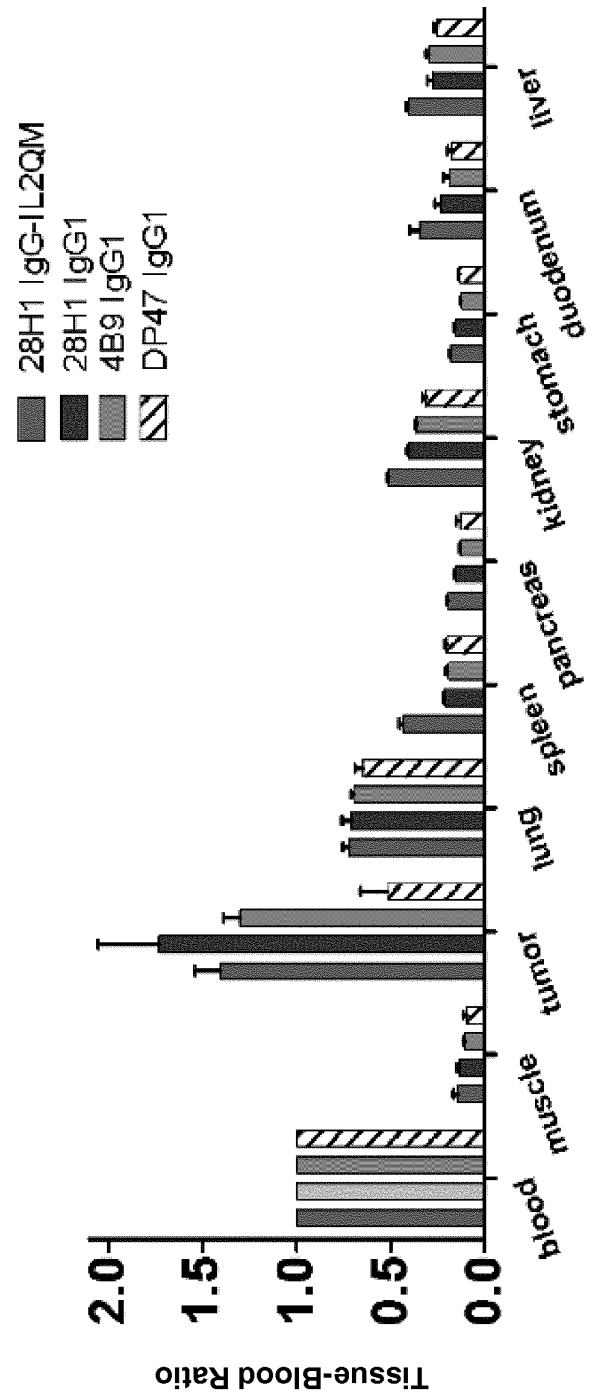


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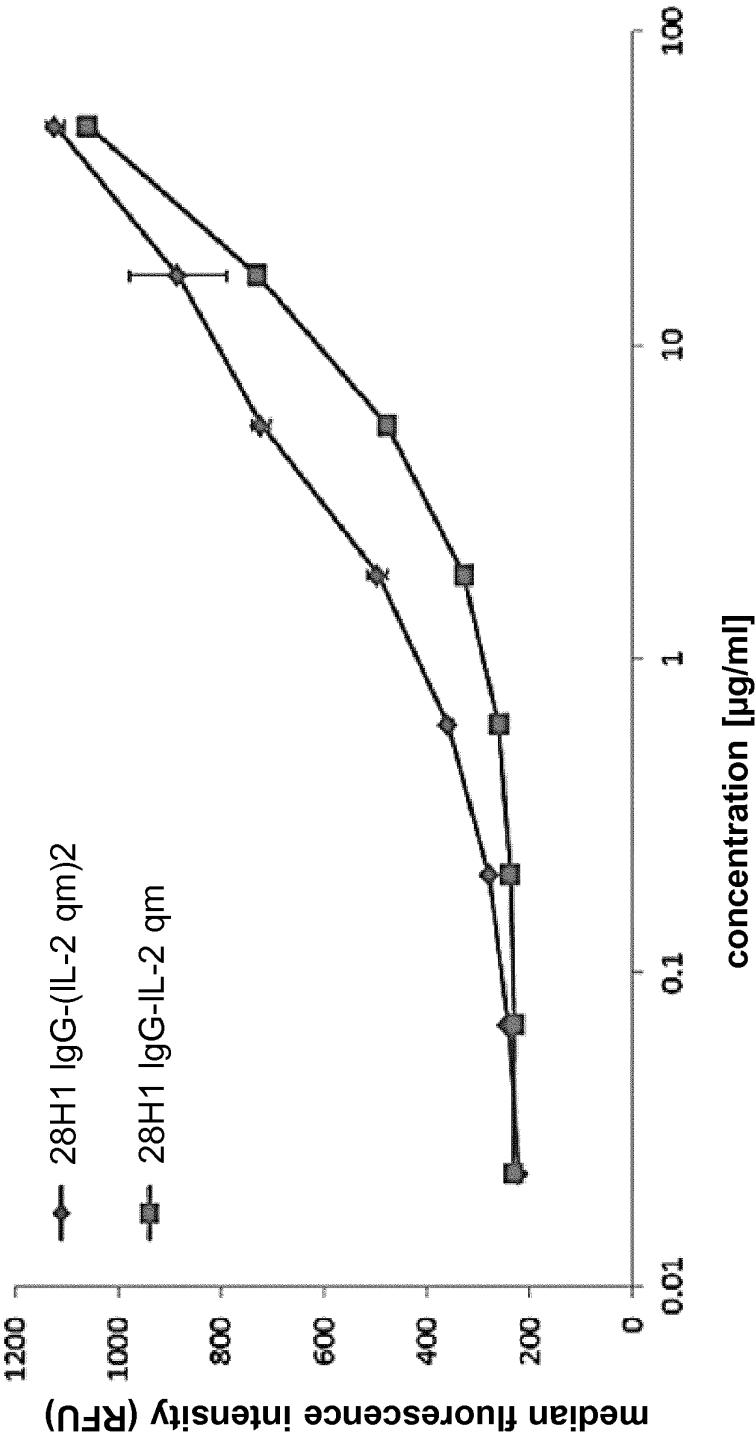


Figure 32

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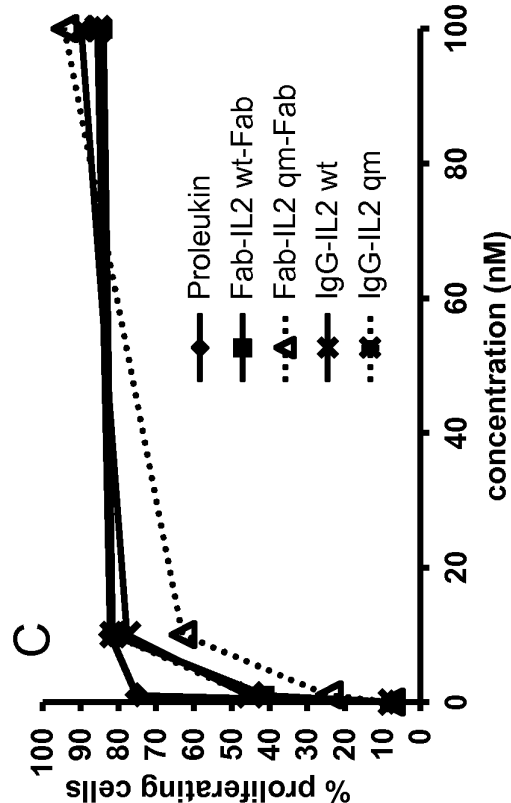
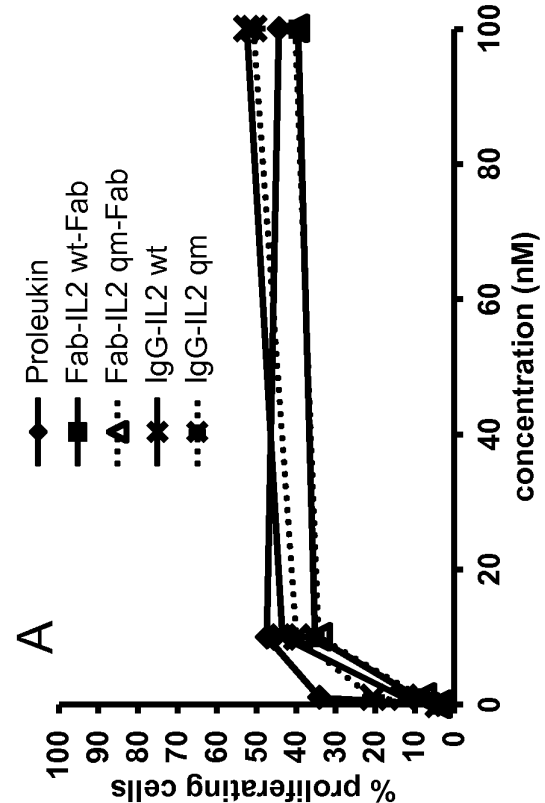
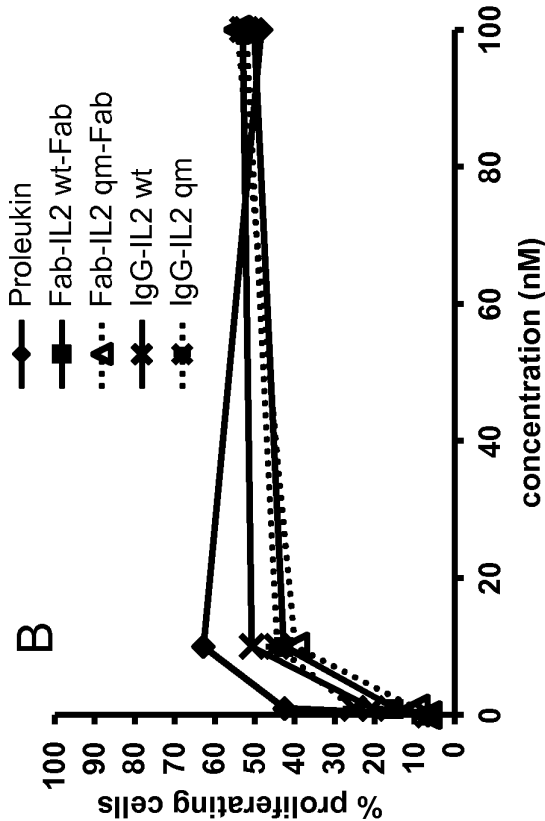


Figure 33

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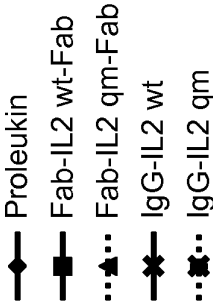
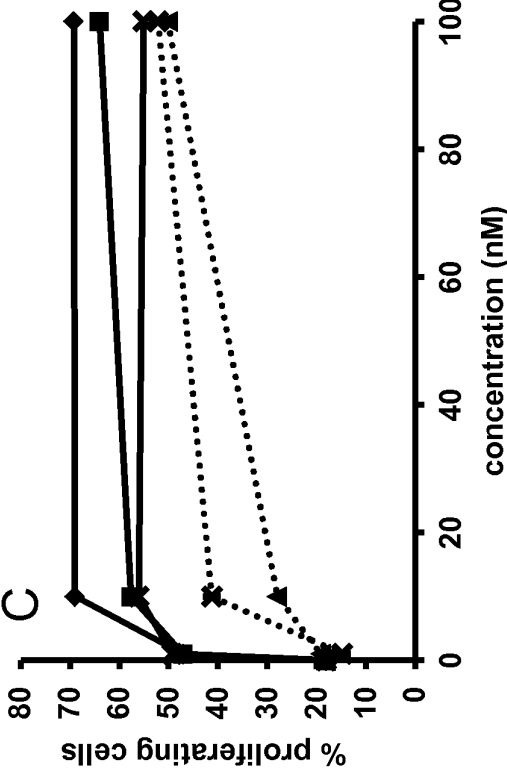
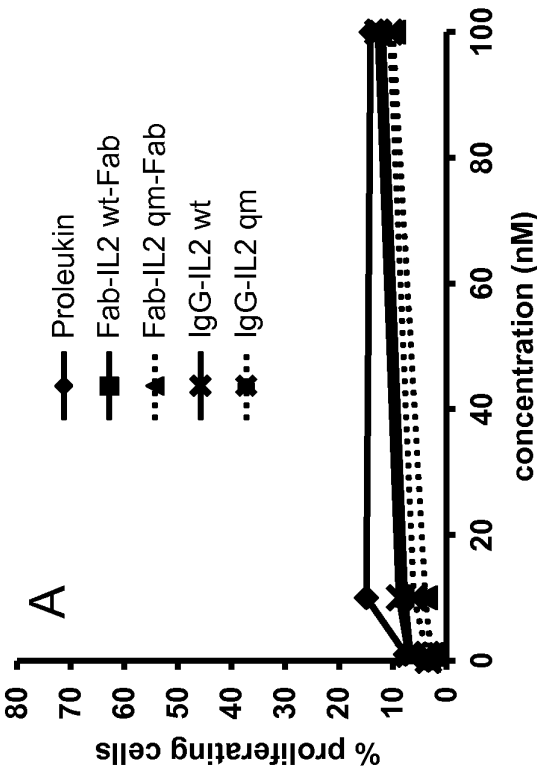
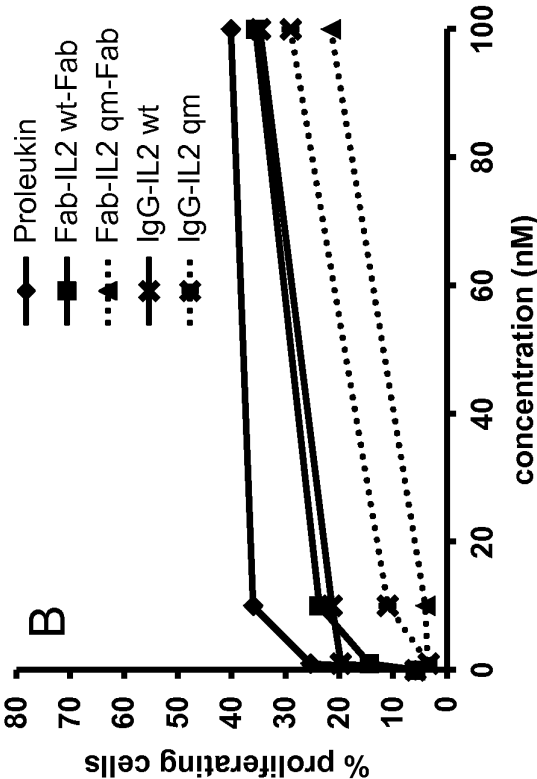


Figure 34

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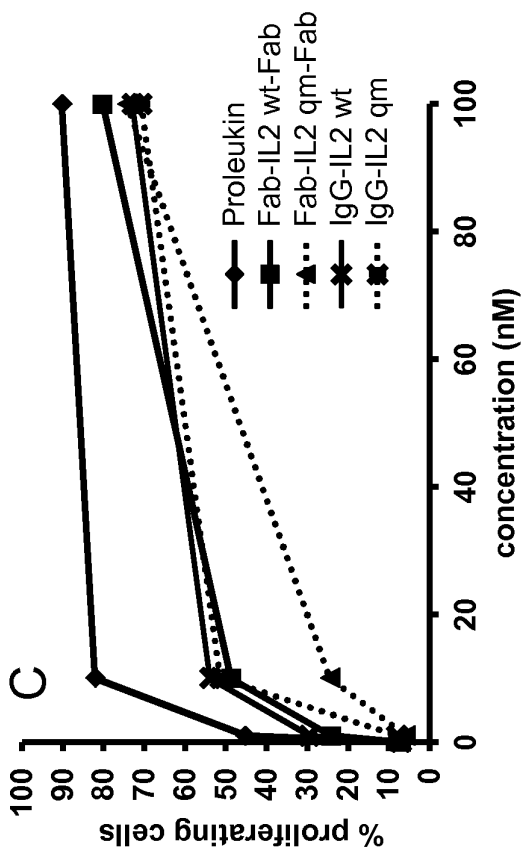
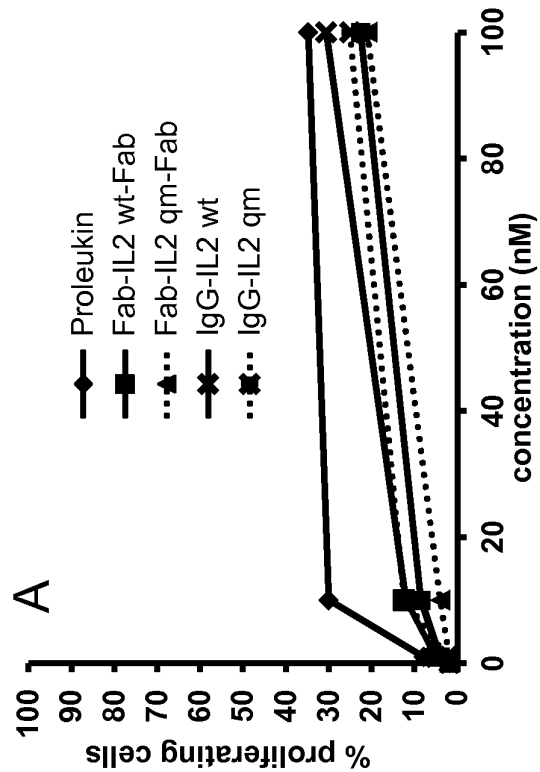
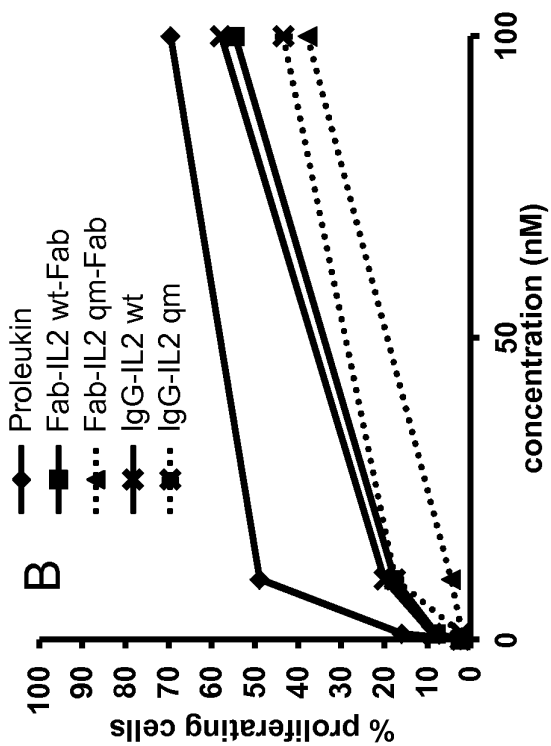


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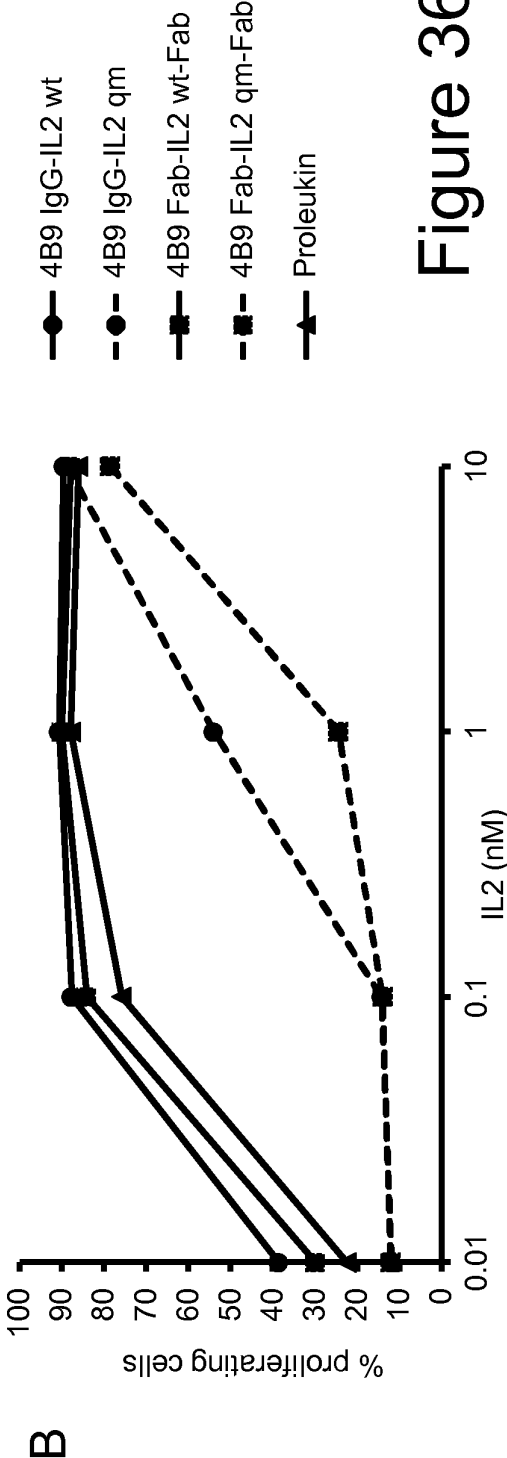
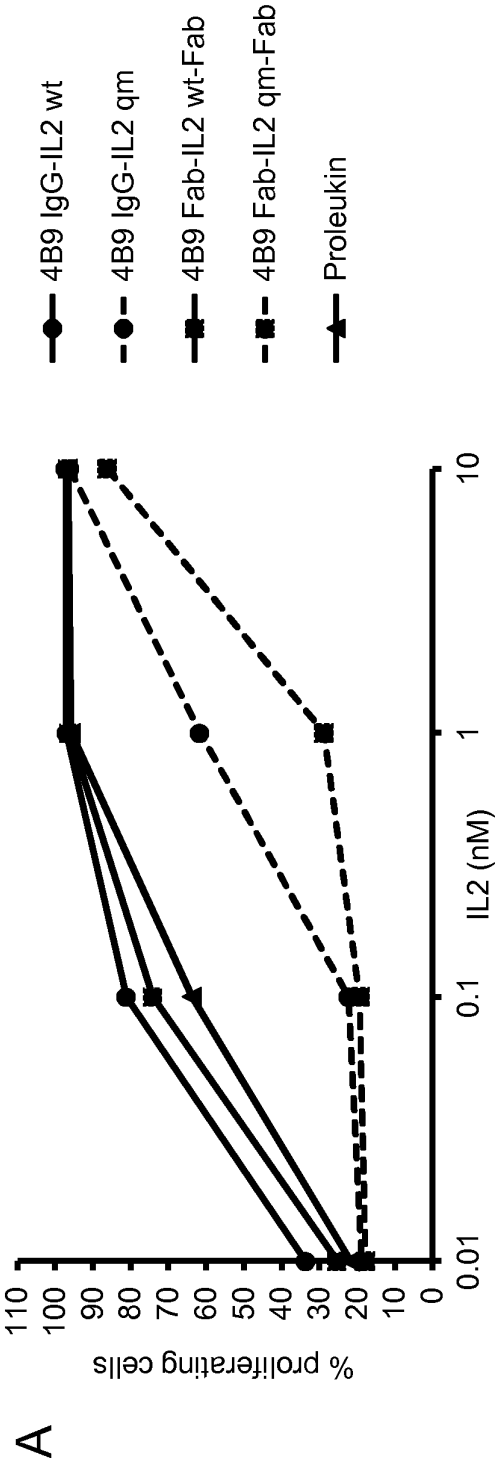


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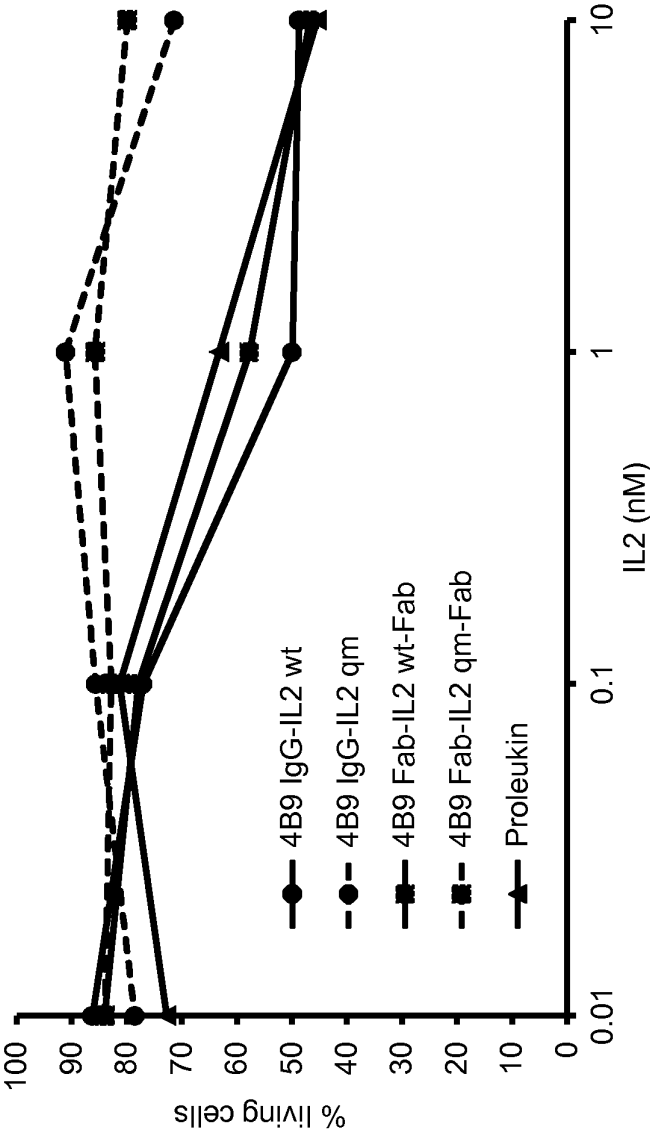


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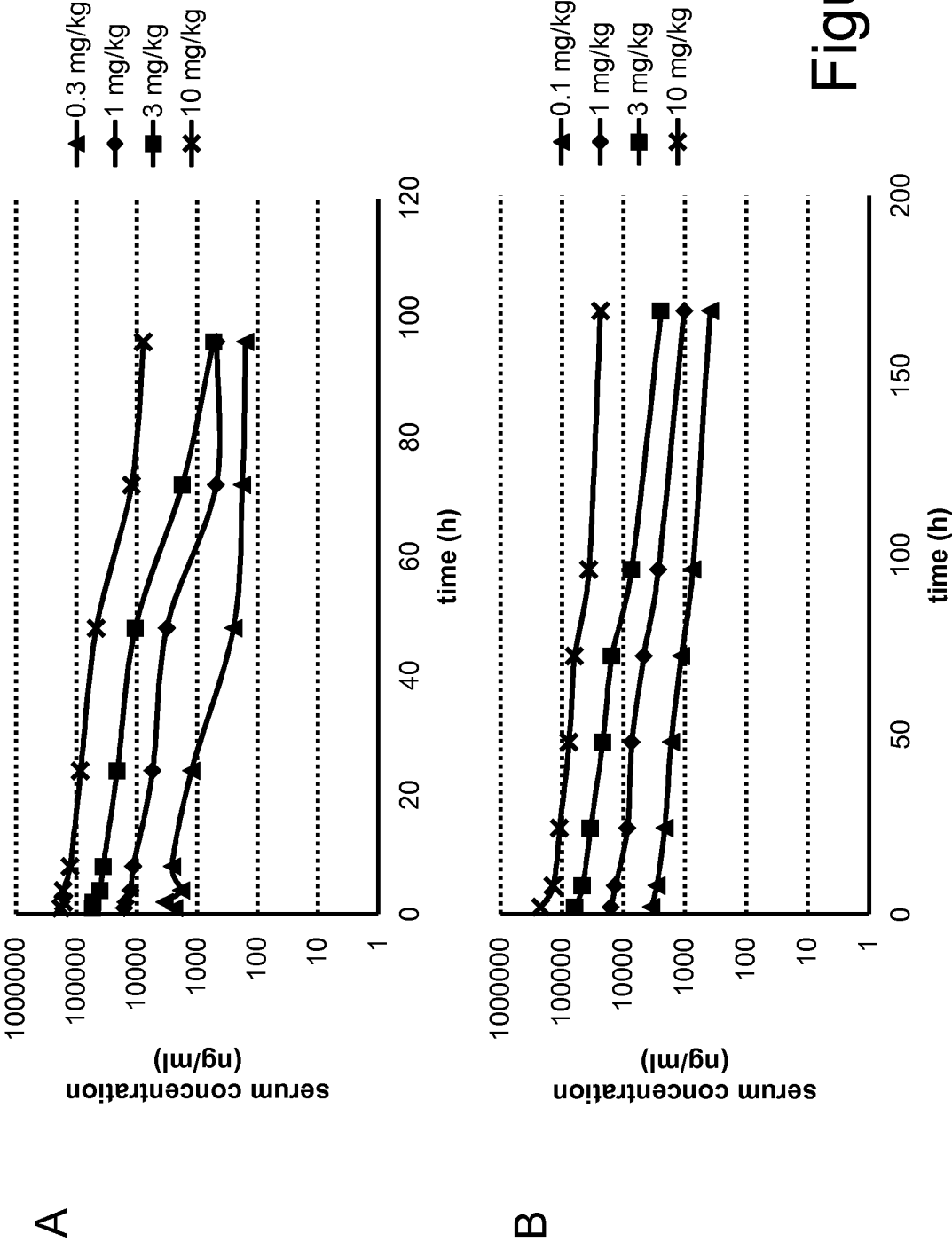


Figure 38

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Figure 39

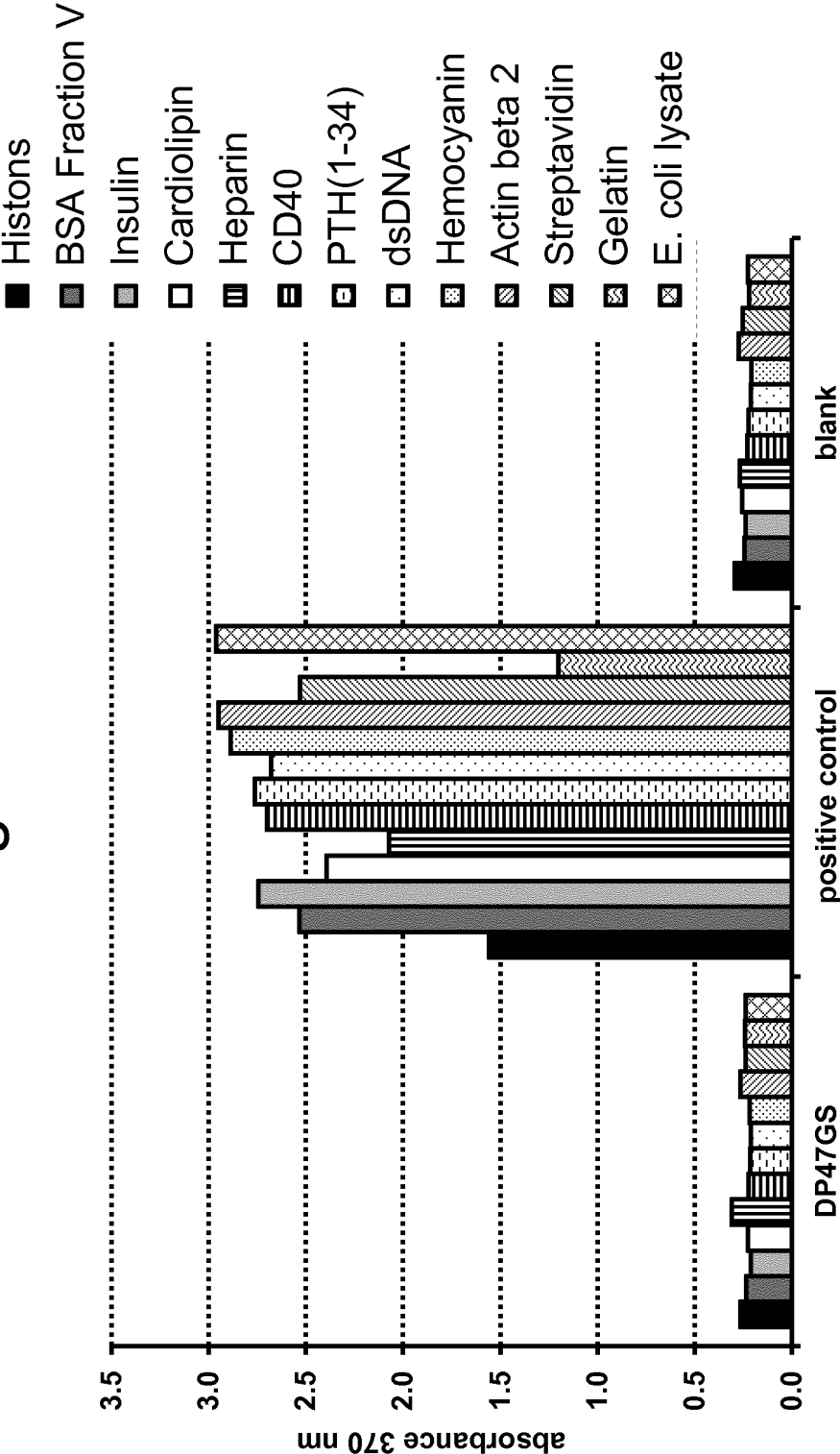
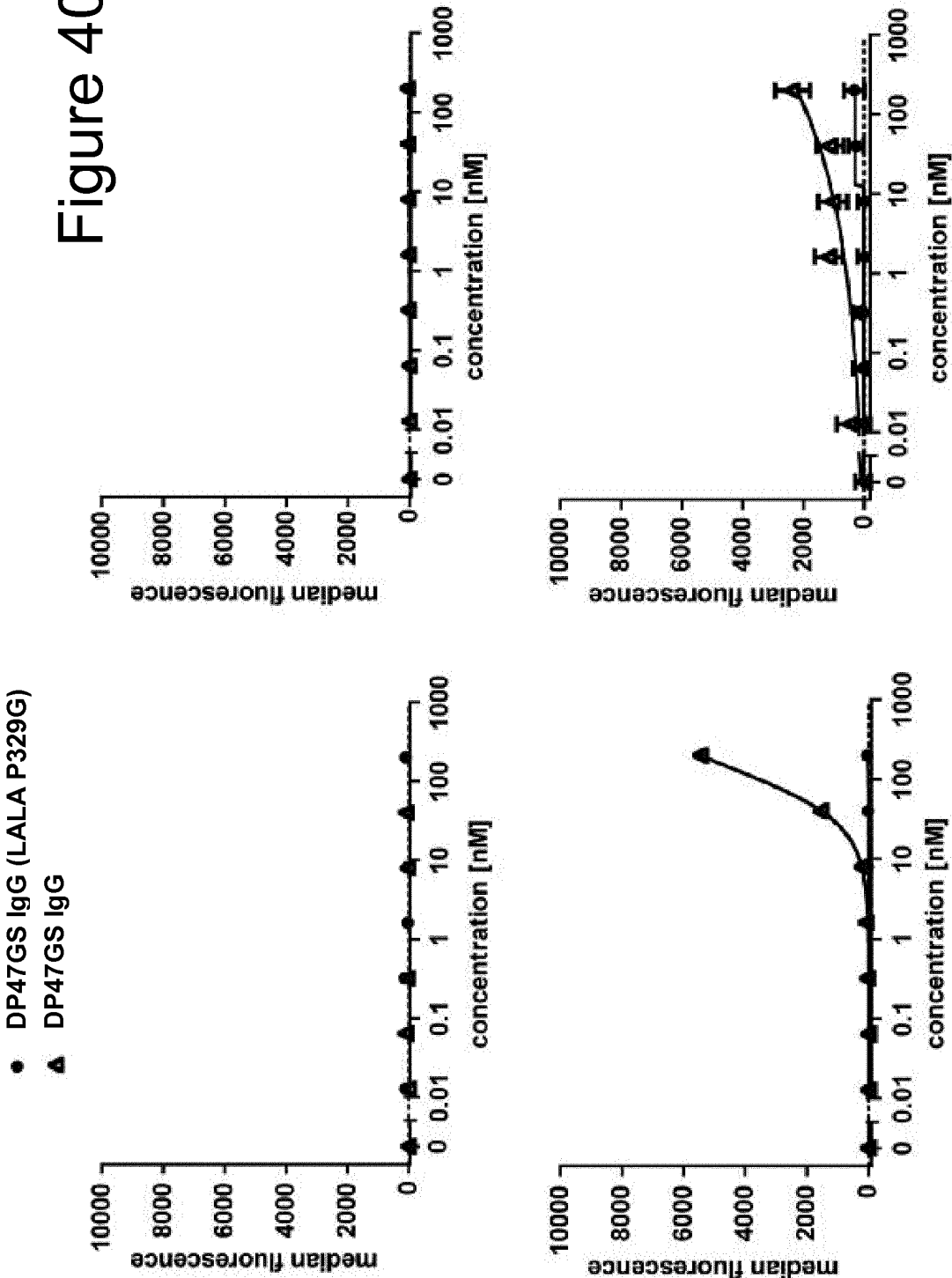
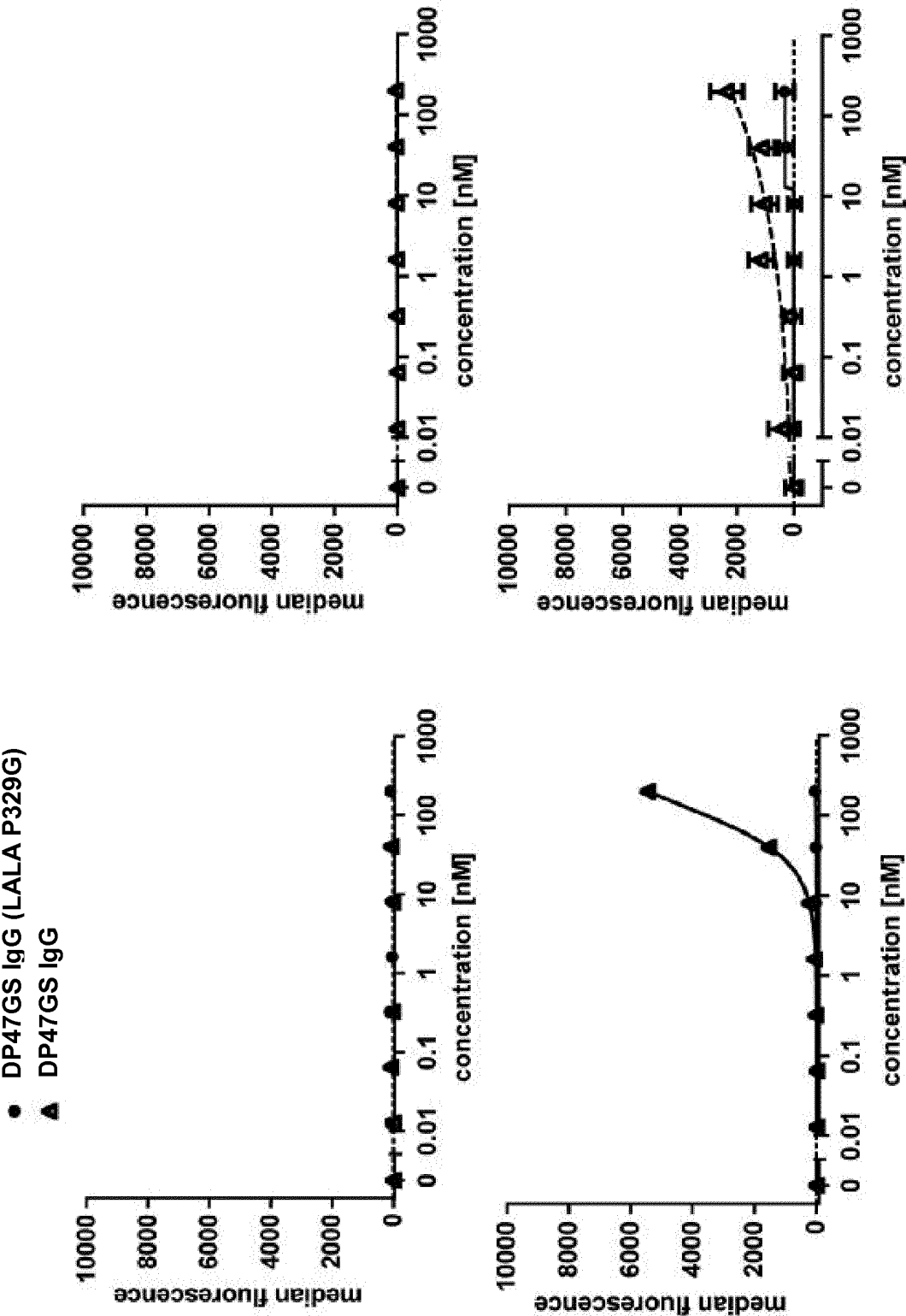
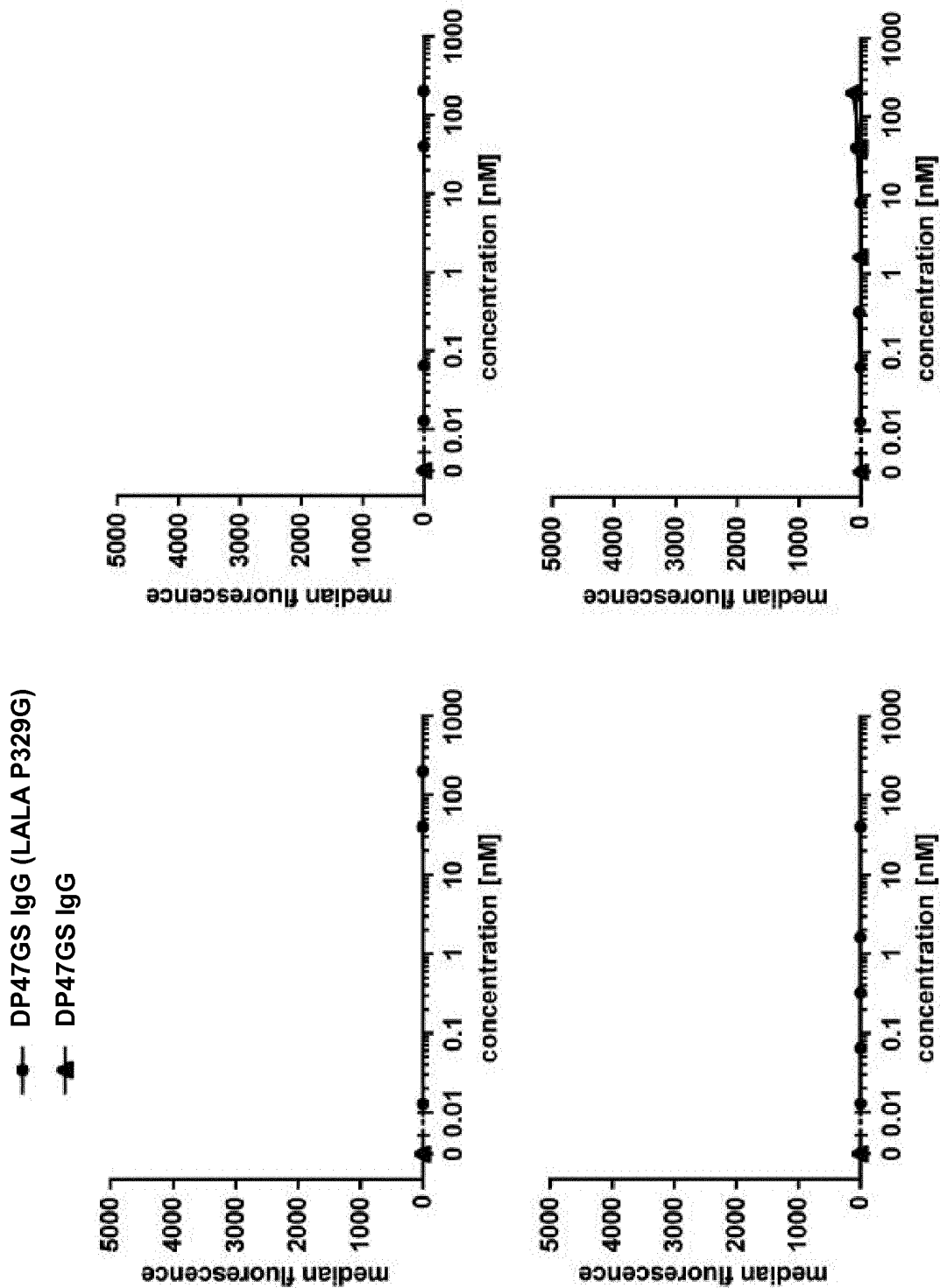


Figure 40A





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Thr Ser Phe Ile Thr Asn Gly Ser Cys Leu Ala Ser Arg Lys Thr Ser
405 410 415

Phe Met Met Ala Leu Cys Leu Ser Ser Ile Tyr Glu Asp Leu Lys Met
420 425 430

Tyr Gln Val Glu Phe Lys Thr Met Asn Ala Lys Leu Leu Met Asp Pro
435 440 445

Lys Arg Gln Ile Phe Leu Asp Gln Asn Met Leu Ala Val Ile Asp Glu
450 455 460

Leu Met Gln Ala Leu Asn Phe Asn Ser Glu Thr Val Pro Gln Lys Ser
465 470 475 480

Ser Leu Glu Glu Pro Asp Phe Tyr Lys Thr Lys Ile Lys Leu Cys Ile
485 490 495

Leu Leu His Ala Phe Arg Ile Arg Ala Val Thr Ile Asp Arg Val Met
500 505 510

Ser Tyr Leu Asn Ala Ser
515

<210> 5
<211> 340
<212> PRT
<213> Artificial Sequence

<220>
<223> Single chain human IL-10

<400> 5

Ser Pro Gly Gln Gly Thr Gln Ser Glu Asn Ser Cys Thr His Phe Pro
1 5 10 15

Gly Asn Leu Pro Asn Met Leu Arg Asp Leu Arg Asp Ala Phe Ser Arg
20 25 30

Val Lys Thr Phe Phe Gln Met Lys Asp Gln Leu Asp Asn Leu Leu Leu
35 40 45

Lys Glu Ser Leu Leu Glu Asp Phe Lys Gly Tyr Leu Gly Cys Gln Ala
50 55 60

eolf-seql.txt

Leu Ser Glu Met Ile Gln Phe Tyr Leu Glu Glu Val Met Pro Gln Ala
 65 70 75 80
 Glu Asn Gln Asp Pro Asp Ile Lys Ala His Val Asn Ser Leu Gly Glu
 85 90 95
 Asn Leu Lys Thr Leu Arg Leu Arg Leu Arg Arg Cys His Arg Phe Leu
 100 105 110
 Pro Cys Glu Asn Lys Ser Lys Ala Val Glu Gln Val Lys Asn Ala Phe
 115 120 125
 Asn Lys Leu Gln Glu Lys Gly Ile Tyr Lys Ala Met Ser Glu Phe Asp
 130 135 140
 Ile Phe Ile Asn Tyr Ile Glu Ala Tyr Met Thr Met Lys Ile Arg Asn
 145 150 155 160
 Gly Gly Gly Gly Ser Gly Gly Gly Gly Ser Gly Gly Gly Gly Ser Gly
 165 170 175
 Gly Gly Gly Ser Ser Pro Gly Gln Gly Thr Gln Ser Glu Asn Ser Cys
 180 185 190
 Thr His Phe Pro Gly Asn Leu Pro Asn Met Leu Arg Asp Leu Arg Asp
 195 200 205
 Ala Phe Ser Arg Val Lys Thr Phe Phe Gln Met Lys Asp Gln Leu Asp
 210 215 220
 Asn Leu Leu Leu Lys Glu Ser Leu Leu Glu Asp Phe Lys Gly Tyr Leu
 225 230 235 240
 Gly Cys Gln Ala Leu Ser Glu Met Ile Gln Phe Tyr Leu Glu Glu Val
 245 250 255
 Met Pro Gln Ala Glu Asn Gln Asp Pro Asp Ile Lys Ala His Val Asn
 260 265 270
 Ser Leu Gly Glu Asn Leu Lys Thr Leu Arg Leu Arg Leu Arg Arg Cys
 275 280 285
 His Arg Phe Leu Pro Cys Glu Asn Lys Ser Lys Ala Val Glu Gln Val
 290 295 300
 Lys Asn Ala Phe Asn Lys Leu Gln Glu Lys Gly Ile Tyr Lys Ala Met
 305 310 315 320
 Ser Glu Phe Asp Ile Phe Ile Asn Tyr Ile Glu Ala Tyr Met Thr Met
 325 330 335

eolf-seql.txt

Lys Ile Arg Asn
340

<210> 6
<211> 166
<212> PRT
<213> Artificial Sequence

<220>
<223> Human IL-10 monomer (IL-10M1)

<400> 6

Ser Pro Gly Gln Gly Thr Gln Ser Glu Asn Ser Cys Thr His Phe Pro
1 5 10 15

Gly Asn Leu Pro Asn Met Leu Arg Asp Leu Arg Asp Ala Phe Ser Arg
20 25 30

Val Lys Thr Phe Phe Gln Met Lys Asp Gln Leu Asp Asn Leu Leu Leu
35 40 45

Lys Glu Ser Leu Leu Glu Asp Phe Lys Gly Tyr Leu Gly Cys Gln Ala
50 55 60

Leu Ser Glu Met Ile Gln Phe Tyr Leu Glu Glu Val Met Pro Gln Ala
65 70 75 80

Glu Asn Gln Asp Pro Asp Ile Lys Ala His Val Asn Ser Leu Gly Glu
85 90 95

Asn Leu Lys Thr Leu Arg Leu Arg Leu Arg Arg Cys His Arg Phe Leu
100 105 110

Pro Cys Glu Asn Gly Gly Gly Ser Gly Gly Lys Ser Lys Ala Val Glu
115 120 125

Gln Val Lys Asn Ala Phe Asn Lys Leu Gln Glu Lys Gly Ile Tyr Lys
130 135 140

Ala Met Ser Glu Phe Asp Ile Phe Ile Asn Tyr Ile Glu Ala Tyr Met
145 150 155 160

Thr Met Lys Ile Arg Asn
165

<210> 7
<211> 114
<212> PRT
<213> Artificial Sequence

<220>
<223> Mutant human IL-15 (E53A, N79A)

eolf-seql.txt

<400> 7

Asn Trp Val Asn Val Ile Ser Asp Leu Lys Lys Ile Glu Asp Leu Ile
1 5 10 15

Gln Ser Met His Ile Asp Ala Thr Leu Tyr Thr Glu Ser Asp Val His
20 25 30

Pro Ser Cys Lys Val Thr Ala Met Lys Cys Phe Leu Leu Glu Leu Gln
35 40 45

Val Ile Ser Leu Ala Ser Gly Asp Ala Ser Ile His Asp Thr Val Glu
50 55 60

Asn Leu Ile Ile Leu Ala Asn Asn Ser Leu Ser Ser Asn Gly Ala Val
65 70 75 80

Thr Glu Ser Gly Cys Lys Glu Cys Glu Glu Leu Glu Glu Lys Asn Ile
85 90 95

Lys Glu Phe Leu Gln Ser Phe Val His Ile Val Gln Met Phe Ile Asn
100 105 110

Thr Ser

<210> 8

<211> 19

<212> PRT

<213> Artificial Sequence

<220>

<223> leader sequence

<400> 8

Met Asp Trp Thr Trp Arg Ile Leu Phe Leu Val Ala Ala Ala Thr Gly
1 5 10 15

Ala His Ser

<210> 9

<211> 57

<212> DNA

<213> Artificial Sequence

<220>

<223> leader sequence

<400> 9

atggactgga cctggagaat cctcttcttg gtggcagcag ccacaggagc ccactcc

57

<210> 10

<211> 57

<212> DNA

<213> Artificial Sequence

eolf-seql.txt

<220>
 <223> leader sequence
 <400> 10
 atggactgga cctggaggat cctcttcttg gtggcagcag ccacaggagc ccactcc 57

<210> 11
 <211> 22
 <212> PRT
 <213> Artificial Sequence

<220>
 <223> leader sequence

<400> 11
 Met Asp Met Arg Val Pro Ala Gln Leu Leu Gly Leu Leu Leu Leu Trp
 1 5 10 15

Phe Pro Gly Ala Arg Cys
 20

<210> 12
 <211> 66
 <212> DNA
 <213> Artificial Sequence

<220>
 <223> leader sequence

<400> 12
 atggacatga ggggtccccgc tcagctcctg ggctctctgc tgctctgggtt cccaggtgcc 60
 aggtgt 66

<210> 13
 <211> 19
 <212> PRT
 <213> Artificial Sequence

<220>
 <223> leader sequence

<400> 13
 Met Gly Trp Ser Cys Ile Ile Leu Phe Leu Val Ala Thr Ala Thr Gly
 1 5 10 15

Val His Ser

<210> 14
 <211> 57
 <212> DNA
 <213> Artificial Sequence

<220>
 <223> leader sequence

<400> 14
 atgggatgga gctgtatcat cctcttcttg gtagcaacag ctaccgggtgt gcattcc 57

eolf-seql.txt

<210> 15
 <211> 57
 <212> DNA
 <213> Artificial Sequence

<220>
 <223> leader sequence

<400> 15
 atgggctggg cctgcatcat cctgtttctg gtggctaccg ccactggagt gcattcc 57

<210> 16
 <211> 57
 <212> DNA
 <213> Artificial Sequence

<220>
 <223> leader sequence

<400> 16
 atgggctggg cctgcatcat cctgtttctg gtcgccacag ccaccggcgt gcactct 57

<210> 17
 <211> 466
 <212> PRT
 <213> Artificial Sequence

<220>
 <223> Human IL-2R-beta-Fc(hole) fusion protein

<400> 17

Met Asp Met Arg Val Pro Ala Gln Leu Leu Gly Leu Leu Leu Leu Trp
 1 5 10 15

Phe Pro Gly Ala Arg Cys Ala Val Asn Gly Thr Ser Gln Phe Thr Cys
 20 25 30

Phe Tyr Asn Ser Arg Ala Asn Ile Ser Cys Val Trp Ser Gln Asp Gly
 35 40 45

Ala Leu Gln Asp Thr Ser Cys Gln Val His Ala Trp Pro Asp Arg Arg
 50 55 60

Arg Trp Asn Gln Thr Cys Glu Leu Leu Pro Val Ser Gln Ala Ser Trp
 65 70 75 80

Ala Cys Asn Leu Ile Leu Gly Ala Pro Asp Ser Gln Lys Leu Thr Thr
 85 90 95

Val Asp Ile Val Thr Leu Arg Val Leu Cys Arg Glu Gly Val Arg Trp
 100 105 110

Arg Val Met Ala Ile Gln Asp Phe Lys Pro Phe Glu Asn Leu Arg Leu
 115 120 125

eolf-seql.txt

Met Ala Pro Ile Ser Leu Gln Val Val His Val Glu Thr His Arg Cys
130 135 140

Asn Ile Ser Trp Glu Ile Ser Gln Ala Ser His Tyr Phe Glu Arg His
145 150 155 160

Leu Glu Phe Glu Ala Arg Thr Leu Ser Pro Gly His Thr Trp Glu Glu
165 170 175

Ala Pro Leu Leu Thr Leu Lys Gln Lys Gln Glu Trp Ile Cys Leu Glu
180 185 190

Thr Leu Thr Pro Asp Thr Gln Tyr Glu Phe Gln Val Arg Val Lys Pro
195 200 205

Leu Gln Gly Glu Phe Thr Thr Trp Ser Pro Trp Ser Gln Pro Leu Ala
210 215 220

Phe Arg Thr Lys Pro Ala Ala Leu Gly Lys Asp Thr Gly Ala Gln Asp
225 230 235 240

Lys Thr His Thr Cys Pro Pro Cys Pro Ala Pro Glu Leu Leu Gly Gly
245 250 255

Pro Ser Val Phe Leu Phe Pro Pro Lys Pro Lys Asp Thr Leu Met Ile
260 265 270

Ser Arg Thr Pro Glu Val Thr Cys Val Val Val Asp Val Ser His Glu
275 280 285

Asp Pro Glu Val Lys Phe Asn Trp Tyr Val Asp Gly Val Glu Val His
290 295 300

Asn Ala Lys Thr Lys Pro Arg Glu Glu Gln Tyr Asn Ser Thr Tyr Arg
305 310 315 320

Val Val Ser Val Leu Thr Val Leu His Gln Asp Trp Leu Asn Gly Lys
325 330 335

Glu Tyr Lys Cys Lys Val Ser Asn Lys Ala Leu Pro Ala Pro Ile Glu
340 345 350

Lys Thr Ile Ser Lys Ala Lys Gly Gln Pro Arg Glu Pro Gln Val Cys
355 360 365

Thr Leu Pro Pro Ser Arg Asp Glu Leu Thr Lys Asn Gln Val Ser Leu
370 375 380

Ser Cys Ala Val Lys Gly Phe Tyr Pro Ser Asp Ile Ala Val Glu Trp
385 390 395 400

eolf-seql.txt

Glu Ser Asn Gly Gln Pro Glu Asn Asn Tyr Lys Thr Thr Pro Pro Val
405 410 415

Leu Asp Ser Asp Gly Ser Phe Phe Leu Val Ser Lys Leu Thr Val Asp
420 425 430

Lys Ser Arg Trp Gln Gln Gly Asn Val Phe Ser Cys Ser Val Met His
435 440 445

Glu Ala Leu His Asn His Tyr Thr Gln Lys Ser Leu Ser Leu Ser Pro
450 455 460

Gly Lys
465

<210> 18
<211> 1401
<212> DNA
<213> Artificial Sequence

<220>
<223> Human IL-2R-beta-Fc(hole) fusion protein

<400> 18
atggacatga ggggtccccgc tcagctcctg ggcctcctgc tgctctgggtt cccaggtgcc 60
aggtgtgcgg tgaatggcac ttcccagttc acatgcttct acaactcgag agccaacatc 120
tcctgtgtct ggagccaaga tggggctctg caggacactt cctgccaagt ccatgcctgg 180
ccggacagac ggcgggtggaa ccaaacctgt gagctgctcc ccgtgagtca agcatcctgg 240
gcctgcaacc tgatcctcgg agccccagat tctcagaaac tgaccacagt tgacatcgtc 300
accctgaggg tgctgtgccg tgaggggggtg cgatggaggg tgatggccat ccaggacttc 360
aagccctttg agaaccttcg cctgatggcc cccatctccc tccaagttgt ccacgtggag 420
acccacagat gcaacataag ctgggaaatc tccaagcct cccactactt tgaaagacac 480
ctggagtctg agggccggac gctgtcccca ggccacacct gggaggaggc ccccctgctg 540
actctcaagc agaagcagga atggatctgc ctggagacgc tcacccaga caccagtat 600
gagtttcagg tgcgggtcaa gcctctgcaa ggcgagttca cgacctggag cccctggagc 660
cagcccctgg ccttcagaac aaagcctgca gcccttgga aggacaccgg agctcaggac 720
aaaactcaca catgcccacc gtgcccagca cctgaactcc tgggggggacc gtcagtcttc 780
ctcttcccc caaaaccaa ggacaccctc atgatctccc ggaccctga ggtcacatgc 840
gtggtggtgg acgtgagcca cgaagaccct gaggtcaagt tcaactggta cgtggacggc 900
gtggaggtgc ataatgcaa gacaaagccg cgggaggagc agtacaacag cacgtaccgt 960
gtggtcagcg tcctcaccgt cctgcaccag gactggctga atggcaagga gtacaagtgc 1020
aaggtctcca acaaagccct cccagcccc atcgagaaaa ccatctcaa agccaaaggg 1080
cagccccgag aaccacaggt gtgcaccctg ccccatccc gggatgagct gaccaagaac 1140
caggtcagcc tctcgtgcgc agtcaaaggc ttctatccca gcgacatcgc cgtggagtgg 1200

eolf-seql.txt

gagagcaatg ggcagccgga gaacaactac aagaccacgc ctcccgctgct ggactccgac 1260
 ggctccttct tcctcgtgag caagctcacc gtggacaaga gcagggtggca gcaggggaac 1320
 gtcttctcat gctccgtgat gcatgaggct ctgcacaacc actacacgca gaagagcctc 1380
 tccctgtctc cgggtaaata a 1401

<210> 19
 <211> 492
 <212> PRT
 <213> Artificial Sequence

<220>
 <223> Human IL-2R-gamma-Fc(knob) fusion protein

<400> 19

Met Leu Lys Pro Ser Leu Pro Phe Thr Ser Leu Leu Phe Leu Gln Leu
 1 5 10 15

Pro Leu Leu Gly Val Gly Leu Asn Thr Thr Ile Leu Thr Pro Asn Gly
 20 25 30

Asn Glu Asp Thr Thr Ala Asp Phe Phe Leu Thr Thr Met Pro Thr Asp
 35 40 45

Ser Leu Ser Val Ser Thr Leu Pro Leu Pro Glu Val Gln Cys Phe Val
 50 55 60

Phe Asn Val Glu Tyr Met Asn Cys Thr Trp Asn Ser Ser Ser Glu Pro
 65 70 75 80

Gln Pro Thr Asn Leu Thr Leu His Tyr Trp Tyr Lys Asn Ser Asp Asn
 85 90 95

Asp Lys Val Gln Lys Cys Ser His Tyr Leu Phe Ser Glu Glu Ile Thr
 100 105 110

Ser Gly Cys Gln Leu Gln Lys Lys Glu Ile His Leu Tyr Gln Thr Phe
 115 120 125

Val Val Gln Leu Gln Asp Pro Arg Glu Pro Arg Arg Gln Ala Thr Gln
 130 135 140

Met Leu Lys Leu Gln Asn Leu Val Ile Pro Trp Ala Pro Glu Asn Leu
 145 150 155 160

Thr Leu His Lys Leu Ser Glu Ser Gln Leu Glu Leu Asn Trp Asn Asn
 165 170 175

Arg Phe Leu Asn His Cys Leu Glu His Leu Val Gln Tyr Arg Thr Asp
 180 185 190

eolf-seql.txt

Trp Asp His Ser Trp Thr Glu Gln Ser Val Asp Tyr Arg His Lys Phe
195 200 205

Ser Leu Pro Ser Val Asp Gly Gln Lys Arg Tyr Thr Phe Arg Val Arg
210 215 220

Ser Arg Phe Asn Pro Leu Cys Gly Ser Ala Gln His Trp Ser Glu Trp
225 230 235 240

Ser His Pro Ile His Trp Gly Ser Asn Thr Ser Lys Glu Asn Pro Phe
245 250 255

Leu Phe Ala Leu Glu Ala Gly Ala Gln Asp Lys Thr His Thr Cys Pro
260 265 270

Pro Cys Pro Ala Pro Glu Leu Leu Gly Gly Pro Ser Val Phe Leu Phe
275 280 285

Pro Pro Lys Pro Lys Asp Thr Leu Met Ile Ser Arg Thr Pro Glu Val
290 295 300

Thr Cys Val Val Val Asp Val Ser His Glu Asp Pro Glu Val Lys Phe
305 310 315 320

Asn Trp Tyr Val Asp Gly Val Glu Val His Asn Ala Lys Thr Lys Pro
325 330 335

Arg Glu Glu Gln Tyr Asn Ser Thr Tyr Arg Val Val Ser Val Leu Thr
340 345 350

Val Leu His Gln Asp Trp Leu Asn Gly Lys Glu Tyr Lys Cys Lys Val
355 360 365

Ser Asn Lys Ala Leu Pro Ala Pro Ile Glu Lys Thr Ile Ser Lys Ala
370 375 380

Lys Gly Gln Pro Arg Glu Pro Gln Val Tyr Thr Leu Pro Pro Cys Arg
385 390 395 400

Asp Glu Leu Thr Lys Asn Gln Val Ser Leu Trp Cys Leu Val Lys Gly
405 410 415

Phe Tyr Pro Ser Asp Ile Ala Val Glu Trp Glu Ser Asn Gly Gln Pro
420 425 430

Glu Asn Asn Tyr Lys Thr Thr Pro Pro Val Leu Asp Ser Asp Gly Ser
435 440 445

Phe Phe Leu Tyr Ser Lys Leu Thr Val Asp Lys Ser Arg Trp Gln Gln
450 455 460

eolf-seql.txt

Gly Asn Val Phe Ser Cys Ser Val Met His Glu Ala Leu His Asn His
465 470 475 480

Tyr Thr Gln Lys Ser Leu Ser Leu Ser Pro Gly Lys
485 490

<210> 20
<211> 1479
<212> DNA
<213> Artificial Sequence

<220>
<223> Human IL-2R-gamma-Fc(knob) fusion protein

<400> 20
atgttgaagc catcattacc attcacatcc ctcttattcc tgcagctgcc cctgctggga 60
gtggggctga acacgacaat tctgacgcc aatgggaatg aagacaccac agctgatttc 120
ttcctgacca ctatgccac tgactccctc agtggtttcca ctctgcccct cccagagggtt 180
cagtgttttg tgttcaatgt cgagtacatg aattgcactt ggaacagcag ctctgagccc 240
cagcctacca acctcactct gcattattgg tacaagaact cggataatga taaagtccag 300
aagtgcagcc actatctatt ctctgaagaa atcacttctg gctgtcagtt gcaaaaaaag 360
gagatccacc tctacaaac atttgttgtt cagctccagg acccacggga acccaggaga 420
caggccacac agatgctaaa actgcagaat ctggtgatcc cctgggctcc agagaaccta 480
acacttcaca aactgagtga atcccagcta gaactgaact ggaacaacag attcttgaac 540
cactgttttg agcacttggg gcagtaccgg actgactggg accacagctg gactgaacaa 600
tcagtggatt atagacataa gttctccttg cctagtgtgg atgggcagaa acgctacacg 660
tttcgtgttc ggagccgctt taaccactc tgtggaagtg ctcagcattg gagtgaatgg 720
agccacccaa tccactgggg gagcaatact tcaaaagaga atcctttcct gtttgcattg 780
gaagccggag ctcaggacaa aactcacaca tgcccaccgt gccagcacc tgaactcctg 840
gggggaccgt cagtcttctt cttccccca aaaccaagg acaccctcat gatctcccgg 900
accctgagg tcacatgcgt ggtggtggac gtgagccacg aagaccctga ggtcaagttc 960
aactggtacg tggacggcgt ggaggtgcat aatgccaaga caaagccgcg ggaggagcag 1020
tacaacagca cgtaccgtgt ggtcagcgtc ctcaccgtcc tgcaccagga ctggctgaat 1080
ggcaaggagt acaagtgcaa ggtctccaac aaagccctcc cagcccccat cgagaaaacc 1140
atctccaaag ccaaagggca gccccgagaa ccacaggtgt acaccctgcc cccatgccgg 1200
gatgagctga ccaagaacca ggtcagcctg tggtgcctgg tcaaaggctt ctatcccagc 1260
gacatcgccg tggagtggga gagcaatggg cagccggaga acaactacaa gaccacgcct 1320
cccgtgctgg actccgacgg ctcttcttct ctctacagca agctcaccgt ggacaagagc 1380
agggtggcagc aggggaacgt cttctcatgc tccgtgatgc atgaggctct gcacaaccac 1440
tacacgcaga agagcctctc cctgtctccg ggtaaata 1479

eolf-seql.txt

<210> 21
 <211> 219
 <212> PRT
 <213> Artificial Sequence

<220>
 <223> Human IL-2R alpha subunit + Avi-tag + His-tag

<400> 21

Met Gly Trp Ser Cys Ile Ile Leu Phe Leu Val Ala Thr Ala Thr Gly
 1 5 10 15

Val His Ser Glu Leu Cys Asp Asp Asp Pro Pro Glu Ile Pro His Ala
 20 25 30

Thr Phe Lys Ala Met Ala Tyr Lys Glu Gly Thr Met Leu Asn Cys Glu
 35 40 45

Cys Lys Arg Gly Phe Arg Arg Ile Lys Ser Gly Ser Leu Tyr Met Leu
 50 55 60

Cys Thr Gly Asn Ser Ser His Ser Ser Trp Asp Asn Gln Cys Gln Cys
 65 70 75 80

Thr Ser Ser Ala Thr Arg Asn Thr Thr Lys Gln Val Thr Pro Gln Pro
 85 90 95

Glu Glu Gln Lys Glu Arg Lys Thr Thr Glu Met Gln Ser Pro Met Gln
 100 105 110

Pro Val Asp Gln Ala Ser Leu Pro Gly His Cys Arg Glu Pro Pro Pro
 115 120 125

Trp Glu Asn Glu Ala Thr Glu Arg Ile Tyr His Phe Val Val Gly Gln
 130 135 140

Met Val Tyr Tyr Gln Cys Val Gln Gly Tyr Arg Ala Leu His Arg Gly
 145 150 155 160

Pro Ala Glu Ser Val Cys Lys Met Thr His Gly Lys Thr Arg Trp Thr
 165 170 175

Gln Pro Gln Leu Ile Cys Thr Gly Val Asp Glu Gln Leu Tyr Phe Gln
 180 185 190

Gly Gly Ser Gly Leu Asn Asp Ile Phe Glu Ala Gln Lys Ile Glu Trp
 195 200 205

His Glu Ala Arg Ala His His His His His His
 210 215

<210> 22
 <211> 660

eolf-seql.txt

<212> DNA
<213> Artificial Sequence

<220>
<223> Human IL-2R alpha subunit + Avi-tag + His-tag

<400> 22
atgggatgga gctgtatcat cctcttcttg gtagcaacag ctaccggtgt gcattccgag 60
ctctgtgacg atgacccgcc agagatccca cacgccacat tcaaagccat ggcctacaag 120
gaaggaacca tgttgaactg tgaatgcaag agaggtttcc gcagaataaa aagcgggtca 180
ctctatatgc tctgtacagg aaactctagc cactcgtcct gggacaacca atgtcaatgc 240
acaagctctg ccactcggaa cacaacgaaa caagtgcac ctcaacctga agaacagaaa 300
gaaaggaaaa ccacagaaat gcaaagtcca atgcagccag tggaccaagc gagccttcca 360
ggtcactgca gggaacctcc accatgggaa aatgaagcca cagagagaat ttatcatttc 420
gtgggtggggc agatggttta ttatcagtgc gtccagggat acagggctct acacagaggt 480
cctgctgaga gcgtctgcaa aatgaccac gggaagacaa ggtggacca gcccagctc 540
atatgcacag gtgtcgacga acagttatat tttcagggcg gctcaggcct gaacgacatc 600
ttcgaggccc agaagatcga gtggcacgag gctcgagctc accaccatca ccactactga 660

<210> 23
<211> 107
<212> PRT
<213> Artificial Sequence

<220>
<223> 2B10; VL

<400> 23
Asp Ile Gln Met Thr Gln Ser Pro Ser Ser Leu Ser Ala Ser Val Gly
1 5 10 15
Asp Arg Val Thr Ile Thr Cys Arg Ala Ser Gln Gly Ile Arg Asn Asp
20 25 30
Leu Gly Trp Tyr Gln Gln Lys Pro Gly Lys Ala Pro Lys Arg Leu Ile
35 40 45
Tyr Ala Ala Ser Ser Leu Gln Ser Gly Val Pro Ser Arg Phe Ser Gly
50 55 60
Gly Gly Ser Gly Thr Glu Phe Thr Leu Thr Ile Ser Ser Leu Gln Pro
65 70 75 80
Glu Asp Phe Ala Thr Tyr Tyr Cys Leu Gln Asn Gly Leu Gln Pro Ala
85 90 95
Thr Phe Gly Gln Gly Thr Lys Val Glu Ile Lys
100 105

eolf-seql.txt

<210> 24
 <211> 321
 <212> DNA
 <213> Artificial Sequence

<220>
 <223> 2B10; VL

<400> 24
 gatatccaga tgaccagtc tccatcctcc ctgtctgcat ctgtcggaga ccgggtcacc 60
 atcacctgcc gggcaagtca gggcattaga aatgatttag gctggtacca gcagaagcca 120
 gggaaagccc ctaagcgcct gatctatgct gcatccagtt tgcagagtgg cgtcccatca 180
 aggttcagcg gcggtggatc cgggacagag ttcactctca ccatcagcag cttgcagcct 240
 gaagattttg ccacctatta ctgcttgag aatgggtctgc agcccgcgac gtttggccag 300
 ggcaccaaag tcgagatcaa g 321

<210> 25
 <211> 107
 <212> PRT
 <213> Artificial Sequence

<220>
 <223> 2B10(GS); VL

<400> 25
 Asp Ile Gln Met Thr Gln Ser Pro Ser Ser Leu Ser Ala Ser Val Gly
 1 5 10 15
 Asp Arg Val Thr Ile Thr Cys Arg Ala Ser Gln Gly Ile Arg Asn Asp
 20 25 30
 Leu Gly Trp Tyr Gln Gln Lys Pro Gly Lys Ala Pro Lys Arg Leu Ile
 35 40 45
 Tyr Ala Ala Ser Ser Leu Gln Ser Gly Val Pro Ser Arg Phe Ser Gly
 50 55 60
 Ser Gly Ser Gly Thr Glu Phe Thr Leu Thr Ile Ser Ser Leu Gln Pro
 65 70 75 80
 Glu Asp Phe Ala Thr Tyr Tyr Cys Leu Gln Asn Gly Leu Gln Pro Ala
 85 90 95
 Thr Phe Gly Gln Gly Thr Lys Val Glu Ile Lys
 100 105

<210> 26
 <211> 321
 <212> DNA
 <213> Artificial Sequence

<220>
 <223> 2B10(GS); VL

eolf-seql.txt

<400> 26
gatatccaga tgaccagtc tccatcctcc ctgtctgcat ctgtcggaga ccgggtcacc 60
atcacctgcc gggcaagtca gggcattaga aatgatttag gctggtacca gcagaagcca 120
gggaaagccc ctaagcgcct gatctatgct gcatccagtt tgcagagtgg cgtcccatca 180
aggttcagcg gcagtggatc cgggacagag ttcactctca ccatcagcag cttgcagcct 240
gaagattttg ccacctatta ctgcttgag aatgggtctgc agcccgcgac gtttggccag 300
ggcaccaaag tcgagatcaa g 321

<210> 27
<211> 121
<212> PRT
<213> Artificial Sequence

<220>
<223> 2B10; VH

<400> 27

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 Gly Thr Phe Ser Ser Tyr
20 25 30
Ala Ile Ser Trp Val Arg Gln Ala Pro Gly Gln Gly Leu Glu Trp Met
35 40 45
Gly Gly Ile Ile Pro Ile Phe Gly Thr Ala Asn Tyr Ala Gln Lys Phe
50 55 60
Gln 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 Leu Tyr Gly Tyr Ala Tyr Tyr Gly Ala Phe Asp Tyr Trp Gly
100 105 110
Gln Gly Thr Thr Val Thr Val Ser Ser
115 120

<210> 28
<211> 363
<212> DNA
<213> Artificial Sequence

<220>
<223> 2B10; VH

<400> 28
caggtgcaat tggtgcagtc tggggctgag gtgaagaagc ctgggtcctc ggtgaaggtc 60
tcctgcaagg cctccggagg cacattcagc agctacgcta taagctgggt gcgacaggcc 120

eolf-seql.txt

cctggacaag ggctcgagt gatgggaggg atcatcccta tctttggtac agcaaactac 180
gcacagaagt tccagggcag ggtcaccatt actgcagaca aatccacgag cacagcctac 240
atggagctga gcagcctgag atctgaggac accgccgtgt attactgtgc gagactgtac 300
ggttacgctt actacggtgc ttttgactac tggggccaag ggaccaccgt gaccgtctcc 360
tca 363

<210> 29
<211> 108
<212> PRT
<213> Artificial Sequence

<220>
<223> 2F11; VL

<400> 29

Glu Ile Val Leu Thr Gln Ser Pro Gly Thr Leu Ser Leu Ser Pro Gly
1 5 10 15

Glu Arg Ala Thr Leu Ser Cys Arg Ala Ser Gln Ser Val Ser Ser Ser
20 25 30

Tyr Leu Ala Trp Tyr Gln Gln Lys Pro Gly Gln Ala Pro Arg Leu Leu
35 40 45

Ile Tyr Gly Ala Ser Ser Arg Ala Thr Gly Val Pro Asp Arg Phe Ser
50 55 60

Gly Ser Gly Ser Gly Thr Asp Phe Thr Leu Thr Ile Ser Arg Leu Glu
65 70 75 80

Pro Glu Asp Phe Ala Val Tyr Tyr Cys Gln Gln Gly Gln Tyr Thr Pro
85 90 95

Pro Thr Phe Gly Gln Gly Thr Lys Val Glu Ile Lys
100 105

<210> 30
<211> 324
<212> DNA
<213> Artificial Sequence

<220>
<223> 2F11; VL

<400> 30

gaaatcgtgt taacgcagtc tccaggcacc ctgtctttgt ctccagggga aagagccacc 60
ctctcttgca gggccagtca gagggttagc agcagctact tagcctggta ccagcagaaa 120
cctggccagg ctcccaggct cctcatctat ggagcatcca gcagggccac tggcgtccca 180
gacaggttca gtggcagtgg atccgggaca gacttcactc tcaccatcag cagactggag 240
cctgaagatt ttgcagtgtta ttactgtcag cagggtcagt atactcccc caggttcggc 300

caggggacca aagtggaaat caaa

<210> 31
 <211> 108
 <212> PRT
 <213> Artificial Sequence

<220>
 <223> 2F11(VI); VL

<400> 31

Glu Ile Val Leu Thr Gln Ser Pro Gly Thr Leu Ser Leu Ser Pro Gly
 1 5 10 15

Glu Arg Ala Thr Leu Ser Cys Arg Ala Ser Gln Ser Val Ser Ser Ser
 20 25 30

Tyr Leu Ala Trp Tyr Gln Gln Lys Pro Gly Gln Ala Pro Arg Leu Leu
 35 40 45

Ile Tyr Gly Ala Ser Ser Arg Ala Thr Gly Ile Pro Asp Arg Phe Ser
 50 55 60

Gly Ser Gly Ser Gly Thr Asp Phe Thr Leu Thr Ile Ser Arg Leu Glu
 65 70 75 80

Pro Glu Asp Phe Ala Val Tyr Tyr Cys Gln Gln Gly Gln Tyr Thr Pro
 85 90 95

Pro Thr Phe Gly Gln Gly Thr Lys Val Glu Ile Lys
 100 105

<210> 32
 <211> 324
 <212> DNA
 <213> Artificial Sequence

<220>
 <223> 2F11(VI); VL

<400> 32

gaaatcgtgt taacgcagtc tccaggcacc ctgtctttgt ctccagggga aagagccacc 60
 ctctcttgca gggccagtca gagtgttagc agcagctact tagcctggta ccagcagaaa 120
 cctggccagg ctcccaggct cctcatctat ggagcatcca gcagggccac tggcatccca 180
 gacaggttca gtggcagtg atccgggaca gacttcactc tcaccatcag cagactggag 240
 cctgaagatt ttgcagtgt ttactgtcag cagggtcagt atactcccc caggttcggc 300
 caggggacca aagtggaaat caaa 324

<210> 33
 <211> 117
 <212> PRT
 <213> Artificial Sequence

eolf-seql.txt

<220>

<223> 2F11; VH

<400> 33

Glu Val Gln Leu Leu Glu Ser Gly Gly Gly Leu Val Gln Pro Gly Gly
1 5 10 15

Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Phe Thr Phe Ser Ser Tyr
20 25 30

Ala Met Ser Trp Val Arg Gln Ala Pro Gly Lys Gly Leu Glu Trp Val
35 40 45

Ser Ala Ile Ser Gly Ser Gly Gly Ser Thr Tyr Tyr Ala Asp Ser Val
50 55 60

Lys Gly Arg Phe Thr Ile Ser Arg Asp Asn Ser Lys Asn Thr Leu Tyr
65 70 75 80

Leu Gln Met Asn Ser Leu Arg Ala Glu Asp Met Ala Val Tyr Tyr Cys
85 90 95

Ala Lys Trp Arg Trp Met Met Phe Asp Tyr Trp Gly Gln Gly Thr Leu
100 105 110

Val Thr Val Ser Ser
115

<210> 34

<211> 351

<212> DNA

<213> Artificial Sequence

<220>

<223> 2F11; VH

<400> 34

gaggtgcaat tgttggagtc tgggggaggc ttggtacagc ctgggggggtc cctgagactc 60

tcctgtgcag cctccggatt caccttttagc agttatgccca tgagctgggt ccgccaggct 120

ccaggaagg ggctggagtg ggtctcagct attagtggta gtggtggtag cacatactac 180

gcagactccg tgaagggccg gttcaccatc tccagagaca attccaagaa cacgctgtat 240

ctgcagatga acagcctgag agccgaggac atggccgtat attactgtgc gaaatggaga 300

tggatgatgt ttgactactg gggccaagga accctgggtca ccgtctcgag t 351

<210> 35

<211> 117

<212> PRT

<213> Artificial Sequence

<220>

<223> 2F11(MT); VH

eolf-seql.txt

<400> 35

Glu Val Gln Leu Leu Glu Ser Gly Gly Gly Leu Val Gln Pro Gly Gly
1 5 10 15

Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Phe Thr Phe Ser Ser Tyr
20 25 30

Ala Met Ser Trp Val Arg Gln Ala Pro Gly Lys Gly Leu Glu Trp Val
35 40 45

Ser Ala Ile Ser Gly Ser Gly Gly Ser Thr Tyr Tyr Ala Asp Ser Val
50 55 60

Lys Gly Arg Phe Thr Ile Ser Arg Asp Asn Ser Lys Asn Thr Leu Tyr
65 70 75 80

Leu Gln Met Asn Ser Leu Arg Ala Glu Asp Thr Ala Val Tyr Tyr Cys
85 90 95

Ala Lys Trp Arg Trp Met Met Phe Asp Tyr Trp Gly Gln Gly Thr Leu
100 105 110

Val Thr Val Ser Ser
115

<210> 36

<211> 351

<212> DNA

<213> Artificial Sequence

<220>

<223> 2F11(MT); VH

<400> 36

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tcctgtgcag cctccggatt cacctttagc agttatgcca tgagctgggt ccgccaggct	120
ccaggaagg ggctggagtg ggtctcagct attagtggta gtggtggtag cacatactac	180
gcagactccg tgaagggccg gttcaccatc tccagagaca attccaagaa cacgctgtat	240
ctgcagatga acagcctgag agccgaggac accgccgtat attactgtgc gaaatggaga	300
tggatgatgt ttgactactg gggccaagga accctggtca ccgtctcgag t	351

<210> 37

<211> 108

<212> PRT

<213> Artificial Sequence

<220>

<223> 3F2; VL

<400> 37

Glu Ile Val Leu Thr Gln Ser Pro Gly Thr Leu Ser Leu Tyr Pro Gly
1 5 10 15

eolf-seql.txt

Glu Arg Ala Thr Leu Ser Cys Arg Ala Ser Gln Ser Val Thr Ser Ser
20 25 30

Tyr Leu Ala Trp Tyr Gln Gln Lys Pro Gly Gln Ala Pro Arg Leu Leu
35 40 45

Ile Asn Val Gly Ser Arg Arg Ala Thr Gly Ile Pro Asp Arg Phe Ser
50 55 60

Gly Ser Gly Ser Gly Thr Asp Phe Thr Leu Thr Ile Ser Arg Leu Glu
65 70 75 80

Pro Glu Asp Phe Ala Val Tyr Tyr Cys Gln Gln Gly Ile Met Leu Pro
85 90 95

Pro Thr Phe Gly Gln Gly Thr Lys Val Glu Ile Lys
100 105

<210> 38
<211> 324
<212> DNA
<213> Artificial Sequence

<220>
<223> 3F2; VL

<400> 38
gaaatcgtgt taacgcagtc tccaggcacc ctgtctttgt atccagggga aagagccacc 60
ctctcttgca gggccagtca gagtgttacc agtagctact tagcctggta ccagcagaaa 120
cctggccagg ctcccaggct cctcatcaat gtgggctccc gtagggccac tggcatccca 180
gacaggttca gtggcagtgg atccgggaca gacttcactc tcaccatcag cagactggag 240
cctgaagatt ttgcagtgtg ttactgtcag cagggtatta tgcttcccc gacgttcggc 300
caggggacca aagtggaaat caaa 324

<210> 39
<211> 108
<212> PRT
<213> Artificial Sequence

<220>
<223> 3F2(YS); VL

<400> 39

Glu Ile Val Leu Thr Gln Ser Pro Gly Thr Leu Ser Leu Ser Pro Gly
1 5 10 15

Glu Arg Ala Thr Leu Ser Cys Arg Ala Ser Gln Ser Val Thr Ser Ser
20 25 30

Tyr Leu Ala Trp Tyr Gln Gln Lys Pro Gly Gln Ala Pro Arg Leu Leu
35 40 45

eolf-seql.txt

Ile Asn Val Gly Ser Arg Arg Ala Thr Gly Ile Pro Asp Arg Phe Ser
50 55 60

Gly Ser Gly Ser Gly Thr Asp Phe Thr Leu Thr Ile Ser Arg Leu Glu
65 70 75 80

Pro Glu Asp Phe Ala Val Tyr Tyr Cys Gln Gln Gly Ile Met Leu Pro
85 90 95

Pro Thr Phe Gly Gln Gly Thr Lys Val Glu Ile Lys
100 105

<210> 40
<211> 324
<212> DNA
<213> Artificial Sequence

<220>
<223> 3F2(YS); VL

<400> 40
gaaatcgtgt taacgcagtc tccaggcacc ctgtctttgt ctccagggga aagagccacc 60
ctctcttgca gggccagtca gagtgttacc agtagctact tagcctggta ccagcagaaa 120
cctggccagg ctcccaggct cctcatcaat gtgggctccc gtagggccac tggcatccca 180
gacaggttca gtggcagtgg atccgggaca gacttcactc tcaccatcag cagactggag 240
cctgaagatt ttgcagtgtg ttactgtcag caggggtatta tgcttcccc gacgttcggc 300
caggggacca aagtggaaat caaa 324

<210> 41
<211> 117
<212> PRT
<213> Artificial Sequence

<220>
<223> 3F2; VH

<400> 41

Glu Val Gln Leu Leu Glu Ser Gly Gly Gly Leu Val Gln Pro Gly Gly
1 5 10 15

Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Phe Thr Phe Ser Ser Tyr
20 25 30

Ala Met Ser Trp Val Arg Gln Ala Pro Gly Lys Gly Leu Glu Trp Val
35 40 45

Ser Ala Ile Ser Gly Ser Gly Gly Ser Thr Tyr Tyr Ala Asp Ser Val
50 55 60

Lys Gly Arg Phe Thr Ile Ser Arg Asp Asn Ser Lys Asn Thr Leu Tyr
65 70 75 80

eolf-seql.txt

Leu Gln Met Asn Ser Leu Arg Ala Glu Asp Thr Ala Val Tyr Tyr Cys
85 90 95

Ala Lys Gly Trp Phe Gly Gly Phe Asn Tyr Trp Gly Gln Gly Thr Leu
100 105 110

Val Thr Val Ser Ser
115

<210> 42
<211> 351
<212> DNA
<213> Artificial Sequence

<220>
<223> 3F2; VH

<400> 42
gagggtgcaat tggttgagtc tgggggaggc ttggtacagc ctgggggggct cctgagactc 60
tcctgtgcag cctccgatt cacctttagc agttatgcca tgagctgggt ccgccaggct 120
ccaggaagg ggctggagt ggtctcagct attagtggta gtggtggttag cacatactac 180
gcagactccg tgaagggccg gttcaccatc tccagagaca attccaagaa cacgctgtat 240
ctgcagatga acagcctgag agccgaggac acggccgtat attactgtgc gaaaggggtg 300
tttgggtggtt ttaactactg gggccaagga accctgggtca ccgtctcgag t 351

<210> 43
<211> 108
<212> PRT
<213> Artificial Sequence

<220>
<223> 3D9, VL

<400> 43

Glu Ile Val Leu Thr Gln Ser Pro Gly Thr Leu Ser Leu Ser Pro Gly
1 5 10 15

Glu Arg Ala Thr Leu Ser Cys Arg Ala Ser Gln Ser Val Ser Ser Ser
20 25 30

Tyr Leu Ala Trp Tyr Gln Gln Lys Pro Gly Gln Ala Pro Arg Leu Leu
35 40 45

Ile Tyr Gly Ala Ser Ser Arg Ala Thr Gly Ile Pro Asp Arg Phe Ser
50 55 60

Gly Ser Gly Ser Gly Thr Asp Phe Thr Leu Thr Ile Ser Arg Leu Glu
65 70 75 80

Pro Glu Asp Phe Ala Val Tyr Tyr Cys Gln Gln Gly Gln Leu Ile Pro
85 90 95

eolf-seql.txt

Pro Thr Phe Gly Gln Gly Thr Lys Val Glu Ile Lys
100 105

<210> 44
<211> 324
<212> DNA
<213> Artificial Sequence

<220>
<223> 3D9, VL

<400> 44
gaaatcgtgt taacgcagtc tccaggcacc ctgtctttgt ctccagggga aagagccacc 60
ctctcttgca gggccagtca gagtgttagc agcagctact tagcctggta ccagcagaaa 120
cctggccagg ctcccaggct cctcatctat ggagcatcca gcagggccac tggcatccca 180
gacaggttca gtggcagtgg atccgggaca gacttcactc tcaccatcag cagactggag 240
cctgaagatt ttgcagtgtg ttactgtcag cagggtcagc ttattccccc tacgttcggc 300
caggggacca aagtggaaat caaa 324

<210> 45
<211> 117
<212> PRT
<213> Artificial Sequence

<220>
<223> 3D9, VH

<400> 45

Glu Val Gln Leu Leu Glu Ser Gly Gly Gly Leu Val Gln Pro Gly Gly
1 5 10 15

Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Phe Thr Phe Ser Ser Tyr
20 25 30

Ala Met Ser Trp Val Arg Gln Thr Pro Gly Lys Gly Leu Glu Trp Val
35 40 45

Ser Ala Ile Gly Val Ser Thr Gly Ser Thr Tyr Tyr Ala Asp Ser Val
50 55 60

Lys Gly Arg Phe Thr Ile Ser Arg Asp Asn Ser Lys Asn Thr Leu Tyr
65 70 75 80

Leu Gln Met Asn Ser Leu Arg Ala Glu Asp Thr Ala Val Tyr Tyr Cys
85 90 95

Ala Lys Gly Trp Leu Gly Pro Phe Asp Tyr Trp Gly Gln Gly Thr Leu
100 105 110

Val Thr Val Ser Ser
115

eolf-seql.txt

<210> 46
 <211> 351
 <212> DNA
 <213> Artificial Sequence

<220>
 <223> 3D9, VH

<400> 46
 gaggtgcaat tggtggagtc tgggggaggc ttggtacagc ctgggggggtc cctgagactc 60
 tcctgtgcag cctccggatt caccttttagc agttatgcta tgagctgggt ccgccagact 120
 ccaggggaagg ggctggagtg ggtctcagct attggtgtta gtactggtag cacatactac 180
 gcagactccg tgaagggccg gttcaccatc tccagagaca attccaagaa cacgctgtat 240
 ctgcagatga acagcctgag agccgaggac acggccgtat attactgtgc gaaagggttg 300
 ctgggtcctt ttgactactg gggccaagga accctggtca ccgtctcgag t 351

<210> 47
 <211> 117
 <212> PRT
 <213> Artificial Sequence

<220>
 <223> 3D9(TA); VH

<400> 47
 Glu Val Gln Leu Leu Glu Ser Gly Gly Gly Leu Val Gln Pro Gly Gly
 1 5 10 15
 Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Phe Thr Phe Ser Ser Tyr
 20 25 30
 Ala Met Ser Trp Val Arg Gln Ala Pro Gly Lys Gly Leu Glu Trp Val
 35 40 45
 Ser Ala Ile Gly Val Ser Thr Gly Ser Thr Tyr Tyr Ala Asp Ser Val
 50 55 60
 Lys Gly Arg Phe Thr Ile Ser Arg Asp Asn Ser Lys Asn Thr Leu Tyr
 65 70 75 80
 Leu Gln Met Asn Ser Leu Arg Ala Glu Asp Thr Ala Val Tyr Tyr Cys
 85 90 95
 Ala Lys Gly Trp Leu Gly Pro Phe Asp Tyr Trp Gly Gln Gly Thr Leu
 100 105 110
 Val Thr Val Ser Ser
 115

<210> 48
 <211> 351

eolf-seql.txt

<212> DNA
<213> Artificial Sequence

<220>
<223> 3D9(TA); VH

<400> 48
gaggtgcaat tggttgagtc tgggggaggc ttggtacagc ctgggggggtc cctgagactc 60
tcctgtgcag cctccggatt cacctttagc agttatgcta tgagctgggt ccgccaggct 120
ccagggaagg ggctggagtg ggtctcagct attggtgtta gtactggtag cacatactac 180
gcagactccg tgaagggccg gttcaccatc tccagagaca attccaagaa cacgctgtat 240
ctgcagatga acagcctgag agccgaggac acggccgtat attactgtgc gaaagggttg 300
ctgggtcctt ttgactactg gggccaagga accctggtca ccgtctcgag t 351

<210> 49
<211> 108
<212> PRT
<213> Artificial Sequence

<220>
<223> 4G8; VL

<400> 49

Glu Ile Val Leu Thr Gln Ser Pro Gly Thr Leu Ser Leu Ser Pro Gly
1 5 10 15

Glu Arg Ala Thr Leu Ser Cys Arg Ala Ser Gln Ser Val Ser Arg Ser
20 25 30

Tyr Leu Ala Trp Tyr Gln Gln Lys Pro Gly Gln Ala Pro Arg Leu Leu
35 40 45

Ile Ile Gly Ala Ser Thr Arg Ala Thr Gly Ile Pro Asp Arg Phe Ser
50 55 60

Gly Ser Gly Ser Gly Thr Asp Phe Thr Leu Thr Ile Ser Arg Leu Glu
65 70 75 80

Pro Glu Asp Phe Ala Val Tyr Tyr Cys Gln Gln Gly Gln Val Ile Pro
85 90 95

Pro Thr Phe Gly Gln Gly Thr Lys Val Glu Ile Lys
100 105

<210> 50
<211> 324
<212> DNA
<213> Artificial Sequence

<220>
<223> 4G8; VL

<400> 50
gaaatcgtgt taacgcagtc tccaggcacc ctgtctttgt ctccagggga aagagccacc 60

eolf-seql.txt

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ctctcttgca gggccagtca gagtgttagc cgcagctact tagcctggta ccagcagaaa      120
cctggccagg ctcccaggct cctcatcatt ggggcctcca ccagggccac tggcatccca      180
gacaggttca gtggcagtgg atccgggacg gacttcactc tcaccatcag cagactggag      240
cctgaagatt ttgcagtgtg ttactgtcag cagggtcagg ttattccccc tacgttcggc      300
caggggacca aagtggaaat caaa                                             324
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<210> 51
 <211> 117
 <212> PRT
 <213> Artificial Sequence

<220>
 <223> 4G8; VH

<400> 51

Glu Val Gln Leu Leu Glu Ser Gly Gly Gly Leu Val Gln Pro Gly Gly
 1 5 10 15

Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Phe Thr Phe Ser Ser Tyr
 20 25 30

Ala Met Ser Trp Val Arg Gln Ala Pro Gly Lys Gly Leu Glu Trp Val
 35 40 45

Ser Ala Ile Ser Gly Ser Gly Gly Ser Thr Tyr Tyr Ala Asp Ser Val
 50 55 60

Lys Gly Arg Phe Thr Ile Ser Arg Asp Asn Ser Lys Asn Thr Leu Tyr
 65 70 75 80

Leu Gln Met Asn Ser Leu Arg Ala Glu Asp Thr Ala Val Tyr Tyr Cys
 85 90 95

Ala Lys Gly Trp Leu Gly Asn Phe Asp Tyr Trp Gly Gln Gly Thr Leu
 100 105 110

Val Thr Val Ser Ser
 115

<210> 52
 <211> 351
 <212> DNA
 <213> Artificial Sequence

<220>
 <223> 4G8; VH

<400> 52

gaggtgcaat tggtggagtc tgggggaggc ttggtacagc ctgggggggtc cctgagactc 60

tcctgtgcag cctccgatt caccttttagc agttatgcca tgagctgggt ccgccaggct 120

ccaggggaagg ggctggagtg ggtctcagct attagtggta gtggtggttag cacatactac 180

eolf-seql.txt

gcagactccg tgaagggccg gttcaccatc tccagagaca attccaagaa cacgctgtat 240
 ctgcagatga acagcctgag agccgaggac acggccgtat attactgtgc gaaaggggtgg 300
 ctgggtaatt ttgactactg gggccaagga accctgggtca ccgtctcgag t 351

<210> 53
 <211> 108
 <212> PRT
 <213> Artificial Sequence

<220>
 <223> 4B3; VL

<400> 53

Glu Ile Val Leu Thr Gln Ser Pro Gly Thr Leu Ser Leu Ser Pro Gly
 1 5 10 15

Glu Arg Ala Thr Leu Ser Cys Arg Ala Ser Gln Ser Val Ser Ser Asn
 20 25 30

Tyr Leu Ala Trp Tyr Gln Gln Lys Pro Gly Gln Ala Pro Arg Leu Leu
 35 40 45

Ile Tyr Gly Ala Tyr Ile Arg Ala Thr Gly Ile Pro Asp Arg Phe Ser
 50 55 60

Gly Ser Gly Ser Gly Thr Asp Phe Thr Leu Thr Ile Ser Arg Leu Glu
 65 70 75 80

Pro Glu Asp Phe Ala Val Tyr Tyr Cys Gln Gln Gly Gln Val Ile Pro
 85 90 95

Pro Thr Phe Gly Gln Gly Thr Lys Val Glu Ile Lys
 100 105

<210> 54
 <211> 324
 <212> DNA
 <213> Artificial Sequence

<220>
 <223> 4B3; VL

<400> 54

gaaatcgtgt taacgcagtc tccaggcacc ctgtctttgt ctccagggga aagagccacc 60
 ctctcttgca gggccagtc gagtgtagc agcaattact tagcctggta ccagcagaaa 120
 cctggccagg ctcccaggct cctcatctat ggcgcctaca tcagggccac tggcatccca 180
 gacaggttca gtggcagtg atccgggaca gacttcactc tcaccatcag cagactggag 240
 cctgaagatt ttgcagtgt ttactgtcag cagggtcagg ttattccccc tacgttcggc 300
 caggggacca aagtggaaat caaa 324

eolf-seql.txt

<210> 55
 <211> 117
 <212> PRT
 <213> Artificial Sequence

<220>
 <223> 4B3; VH

<400> 55

Glu Val Gln Leu Leu Glu Ser Gly Gly Gly Leu Val Gln Pro Gly Gly
 1 5 10 15

Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Phe Thr Phe Ser Ser Tyr
 20 25 30

Ala Met Ser Trp Val Arg Gln Ala Pro Gly Lys Gly Leu Glu Trp Val
 35 40 45

Ser Ala Ile Ser Gly Ser Gly Gly Ser Thr Tyr Tyr Ala Asp Ser Val
 50 55 60

Lys Gly Arg Phe Thr Ile Ser Arg Asp Asn Ser Lys Asn Thr Leu Tyr
 65 70 75 80

Leu Gln Met Asn Ser Leu Arg Ala Glu Asp Thr Ala Val Tyr Tyr Cys
 85 90 95

Ala Lys Gly Trp Leu Gly Asn Phe Asp Tyr Trp Gly Gln Gly Thr Leu
 100 105 110

Val Thr Val Ser Ser
 115

<210> 56
 <211> 351
 <212> DNA
 <213> Artificial Sequence

<220>
 <223> 4B3; VH

<400> 56

gaggtgcaat tggtggagtc tgggggaggc ttggtacagc ctgggggggtc cctgagactc 60
 tcctgtgcag cctccggatt caccttttagc agttatgcca tgagctgggt ccgccaggct 120
 ccagggaagg ggctggagtg ggtctcagct attagtggtg gtggtggttag cacatactac 180
 gcagactccg tgaagggccg gttcaccatc tccagagaca attccaagaa cacgctgtat 240
 ctgcagatga acagcctgag agccgaggac acggccgtat attactgtgc gaaaggggtg 300
 ctgggtaatt ttgactactg gggccaagga accctggtca ccgtctcgag t 351

<210> 57
 <211> 108
 <212> PRT
 <213> Artificial Sequence

eolf-seql.txt

<220>

<223> 4D6; VL

<400> 57

Glu Ile Val Leu Thr Gln Ser Pro Gly Thr Leu Ser Leu Ser Pro Gly
1 5 10 15

Glu Arg Ala Thr Leu Ser Cys Arg Ala Ser Gln Ser Val Ser Ser Asn
20 25 30

Tyr Leu Ala Trp Tyr Gln Gln Lys Pro Gly Gln Ala Pro Arg Leu Leu
35 40 45

Ile Gln Gly Ala Ser Ser Arg Ala Thr Gly Ile Pro Asp Arg Phe Ser
50 55 60

Gly Ser Gly Ser Gly Thr Asp Phe Thr Leu Thr Ile Ser Arg Leu Glu
65 70 75 80

Pro Glu Asp Phe Ala Val Tyr Tyr Cys Gln Gln Gly Gln Val Ile Pro
85 90 95

Pro Thr Phe Gly Gln Gly Thr Lys Val Glu Ile Lys
100 105

<210> 58

<211> 324

<212> DNA

<213> Artificial Sequence

<220>

<223> 4D6; VL

<400> 58

gaaatcgtgt taacgcagtc tccaggcacc ctgtctttgt ctccagggga aagagccacc 60
ctctcttgca gggccagtca gagtgttagc agcaactact tagcctggta ccagcagaaa 120
cctggccagg ctcccaggct cctcatccag ggcgcctcca gcagggccac tggcatccca 180
gacaggttca gtggcagtgg atccgggaca gacttcactc tcaccatcag cagactggag 240
cctgaagatt ttgcagtgtt ttactgtcag cagggtcagg ttattccccc tacgttcggc 300
caggggacca aagtggaaat caaa 324

<210> 59

<211> 117

<212> PRT

<213> Artificial Sequence

<220>

<223> 4D6; VH

<400> 59

Glu Val Gln Leu Leu Glu Ser Gly Gly Gly Leu Val Gln Pro Gly Gly
1 5 10 15

eolf-seql.txt

Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Phe Thr Phe Ser Ser Tyr
20 25 30

Ala Met Ser Trp Val Arg Gln Ala Pro Gly Lys Gly Leu Glu Trp Val
35 40 45

Ser Ala Ile Ser Gly Ser Gly Gly Ser Thr Tyr Tyr Ala Asp Ser Val
50 55 60

Lys Gly Arg Phe Thr Ile Ser Arg Asp Asn Ser Lys Asn Thr Leu Tyr
65 70 75 80

Leu Gln Met Asn Ser Leu Arg Ala Glu Asp Thr Ala Val Tyr Tyr Cys
85 90 95

Ala Lys Gly Trp Leu Gly Asn Phe Asp Tyr Trp Gly Gln Gly Thr Leu
100 105 110

Val Thr Val Ser Ser
115

<210> 60
<211> 351
<212> DNA
<213> Artificial Sequence

<220>
<223> 4D6; VH

<400> 60
gaggtgcaat tggtggagtc tgggggaggc ttggtacagc ctgggggggtc cctgagactc 60
tcctgtgcag cctccggatt caccttttagc agttatgcca tgagctgggt ccgccaggct 120
ccagggaagg ggctggagtg ggtctcagct attagtggta gtggtggtag cacatactac 180
gcagactccg tgaagggccg gttcaccatc tccagagaca attccaagaa cacgctgtat 240
ctgcagatga acagcctgag agccgaggac acggccgtat attactgtgc gaaaggggtg 300
ctgggtaatt ttgactactg gggccaagga accctgggtca ccgtctcgag t 351

<210> 61
<211> 108
<212> PRT
<213> Artificial Sequence

<220>
<223> 2C6; VL

<400> 61

Glu Ile Val Leu Thr Gln Ser Pro Gly Thr Leu Ser Leu Ser Pro Gly
1 5 10 15

Glu Arg Ala Thr Leu Ser Cys Arg Ala Ser Gln Ser Val Ser Ser Ser
20 25 30

eolf-seql.txt

Tyr Leu Ala Trp Tyr Gln Gln Lys Pro Gly Gln Ala Pro Arg Leu Leu
35 40 45

Ile Tyr Gly Ala Ser Ser Arg Ala Thr Gly Ile Pro Asp Arg Phe Ser
50 55 60

Gly Ser Gly Ser Gly Thr Asp Phe Thr Leu Thr Ile Ser Arg Leu Glu
65 70 75 80

Pro Glu Asp Phe Ala Val Tyr Tyr Cys Gln Gln Gly Gln Gln Ile Pro
85 90 95

Pro Thr Phe Gly Gln Gly Thr Lys Val Glu Ile Lys
100 105

<210> 62
<211> 324
<212> DNA
<213> Artificial Sequence

<220>
<223> 2C6; VL

<400> 62
gaaatcgtgt taacgcagtc tccaggcacc ctgtctttgt ctccagggga aagagccacc 60
ctctcttgca gggccagtca gagtgttagc agcagctact tagcctggta ccagcagaaa 120
cctggccagg ctcccaggct cctcatctat ggagcatcca gcagggccac tggcatccca 180
gacaggttca gtggcagtgg atccgggaca gacttcactc tcaccatcag caggctggag 240
cctgaagatt ttgcagtgtta ttactgtcag cagggtcagc agattccccc tacgttcggc 300
caggggacca aagtggaaat caaa 324

<210> 63
<211> 117
<212> PRT
<213> Artificial Sequence

<220>
<223> 2C6; VH

<400> 63

Glu Val Gln Leu Leu Glu Ser Gly Gly Gly Leu Val Gln Pro Gly Gly
1 5 10 15

Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Ser Thr Phe Ser Ser Tyr
20 25 30

Ala Met Ser Trp Val Arg Gln Ala Pro Gly Lys Gly Leu Glu Trp Val
35 40 45

Ser Ala Ile Ser Gly Ser Ala Gly Tyr Thr Tyr Tyr Ala Asp Ser Val
50 55 60

eolf-seql.txt

Lys Gly Arg Phe Thr Ile Ser Arg Asp Asn Ser Lys Asn Thr Leu Tyr
65 70 75 80

Leu Gln Met Asn Ser Leu Arg Ala Glu Asp Thr Ala Val Tyr Tyr Cys
85 90 95

Ala Lys Gly Trp Phe Gly Asn Phe Asp Tyr Trp Gly Gln Gly Thr Leu
100 105 110

Val Thr Val Ser Ser
115

<210> 64
<211> 351
<212> DNA
<213> Artificial Sequence

<220>
<223> 2C6; VH

<400> 64
gaggtgcaat tggtggagtc tgggggaggc ttggtacagc ctgggggggtc cctgagactc 60
tcctgtgcag cctccggatc caccttttagc agttatgccca tgagctgggt ccgccaggct 120
ccagggaagg ggctggagtg ggtctcagct attagtggga gtgctgggta tacatactac 180
gcagactccg tgaagggccg gttcaccatc tccagagaca attccaagaa cacgctgtat 240
ctgcagatga acagcctgag agccgaggac acggccgtat attactgtgc gaaaggttgg 300
tttggaatt ttgactactg gggccaagga accctgggtca ccgtctcgag t 351

<210> 65
<211> 108
<212> PRT
<213> Artificial Sequence

<220>
<223> 5H5; VL

<400> 65

Glu Ile Val Leu Thr Gln Ser Pro Gly Thr Leu Ser Leu Ser Pro Gly
1 5 10 15

Glu Arg Ala Thr Leu Ser Cys Arg Ala Ser Gln Ser Val Ser Ser Ser
20 25 30

Tyr Leu Ala Trp Tyr Gln Gln Lys Pro Gly Gln Ala Pro Arg Leu Leu
35 40 45

Ile Tyr Gly Ala Ser Ser Arg Ala Thr Gly Ile Pro Asp Arg Phe Ser
50 55 60

Gly Ser Gly Ser Gly Thr Asp Phe Thr Leu Thr Ile Ser Arg Leu Glu
65 70 75 80

eolf-seql.txt

Pro Glu Asp Phe Ala Val Tyr Tyr Cys Gln Gln Gly Asn Gln Ile Pro
85 90 95

Pro Thr Phe Gly Gln Gly Thr Lys Val Glu Ile Lys
100 105

<210> 66
<211> 324
<212> DNA
<213> Artificial Sequence

<220>
<223> 5H5; VL

<400> 66
gaaatcgtgt taacgcagtc tccaggcacc ctgtctttgt ctccagggga aagagccacc 60
ctctcttgca gggccagtca gagtgtagc agcagctact tagcctggta ccagcagaaa 120
cctggccagg ctcccaggct cctcatctat ggagcatcca gcagggccac tggcatccca 180
gacaggttca gtggcagtg atccgggaca gacttcactc tcaccatcag cagactggag 240
cctgaagatt ttgcagtgt ttactgtcag cagggtaatc agattccccc tacgttcggt 300
caggggacca aagtggaaat caaa 324

<210> 67
<211> 116
<212> PRT
<213> Artificial Sequence

<220>
<223> 5H5; VH

<400> 67

Glu Val Gln Leu Leu Glu Ser Gly Gly Gly Leu Val Gln Pro Gly Gly
1 5 10 15

Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Phe Thr Phe Ser Ser Tyr
20 25 30

Thr Met Ser Trp Val Arg Arg Ser Pro Gly Lys Gly Leu Glu Trp Val
35 40 45

Ser Ala Ile Ser Gly Gly Gly Arg Thr Tyr Tyr Ala Asp Ser Val Lys
50 55 60

Gly Arg Phe Thr Ile Ser Arg Asp Asn Ser Lys Asn Thr Leu Tyr Leu
65 70 75 80

Gln Met Asn Ser Leu Arg Ala Glu Asp Thr Ala Val Tyr Tyr Cys Ala
85 90 95

Lys Gly Trp Phe Thr Pro Phe Asp Tyr Trp Gly Gln Gly Thr Leu Val
100 105 110

Thr Val Ser Ser
115

<210> 68
<211> 348
<212> DNA
<213> Artificial Sequence

<220>
<223> 5H5; VH

<400> 68
gaggtgcaat tgttggagtc tgggggaggc ttggtacagc ctgggggggtc cctgagactc 60
tcctgtgcag cctccggatt caccttttagc agttataacca tgagctgggt ccgccggtct 120
ccagggaagg ggctggagtg ggtctcagct attagtgggtg gtggtaggac atactacgca 180
gactccgtga agggccggtt caccatctcc agagacaatt ccaagaacac gctgtatctg 240
cagatgaaca gcctgagagc cgaggacacg gccgtatatt actgtgcgaa aggttggttt 300
acgccttttg actactgggg ccaaggaacc ctggtcaccg tctcgagt 348

<210> 69
<211> 108
<212> PRT
<213> Artificial Sequence

<220>
<223> 2C4; VL

<400> 69

Glu Ile Val Leu Thr Gln Ser Pro Gly Thr Leu Ser Leu Ser Pro Gly
1 5 10 15

Glu Arg Ala Thr Leu Ser Cys Arg Ala Ser Gln Ser Val Ser Ser Asn
20 25 30

Tyr Leu Ala Trp Tyr Gln Gln Lys Pro Gly Gln Ala Pro Arg Leu Leu
35 40 45

Ile Tyr Gly Ala Ser Ile Arg Ala Thr Gly Ile Pro Asp Arg Phe Ser
50 55 60

Gly Ser Gly Ser Gly Thr Asp Phe Thr Leu Thr Ile Ser Arg Leu Glu
65 70 75 80

Pro Glu Asp Phe Ala Val Tyr Tyr Cys Gln Gln Gly Asn Gln Ile Pro
85 90 95

Pro Thr Phe Gly Gln Gly Thr Lys Val Glu Ile Lys
100 105

<210> 70
<211> 324

eolf-seql.txt

<212> DNA
<213> Artificial Sequence

<220>
<223> 2C4; VL

<400> 70
gaaatcgtgt taacgcagtc tccaggcacc ctgtctttgt ctccagggga aagagccacc 60
ctctcttgca gggccagtca gagtgtagc agtaactact tagcctggta ccagcagaaa 120
cctggccagg ctcccaggct cctcatctat ggtgcctcca ttagggccac tggcatccca 180
gacaggttca gtggcagtgg atccgggaca gacttcactc tcaccatcag cagactggag 240
cctgaagatt ttgcagtgtta ttactgtcag cagggtaatc agattccccc tacgttcggt 300
caggggacca aagtggaaat caaa 324

<210> 71
<211> 117
<212> PRT
<213> Artificial Sequence

<220>
<223> 2C4; VH

<400> 71

Glu Val Gln Leu Leu Glu Ser Gly Gly Gly Leu Val Gln Pro Gly Gly
1 5 10 15
Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Phe Thr Phe Ser Ser Tyr
20 25 30
Ala Met Ser Trp Val Arg Gln Ala Pro Gly Lys Gly Leu Glu Trp Val
35 40 45
Ser Ala Ile Ser Gly Ser Gly Gly Ser Thr Tyr Tyr Ala Asp Ser Val
50 55 60
Lys Gly Arg Phe Thr Ile Ser Arg Asp Asn Ser Lys Asn Thr Leu Tyr
65 70 75 80
Leu Gln Met Asn Ser Leu Arg Ala Glu Asp Thr Ala Val Tyr Tyr Cys
85 90 95
Ala Lys Gly Trp Phe Thr Pro Phe Asp Tyr Trp Gly Gln Gly Thr Leu
100 105 110
Val Thr Val Ser Ser
115

<210> 72
<211> 351
<212> DNA
<213> Artificial Sequence

<220>

eolf-seql.txt

<223> 2C4; VH

<400> 72
gaggtgcaat tgttggagtc tgggggaggc ttggtacagc ctgggggggc cctgagactc 60
tcctgtgcag cctccggatt cacctttagc agttatgcca tgagctgggt ccgccaggct 120
ccagggaagg ggctggagtg ggtctcagct attagcggta gtggtggtag cacatactac 180
gcagactccg tgaagggccg gttcaccatc tccagagaca attccaagaa cacgctgtat 240
ctgcagatga acagcctgag agccgaggac acggccgtat attactgtgc gaaagggttg 300
tttacgcctt ttgactactg gggccaagga accctggtca ccgtctcgag t 351

<210> 73
<211> 108
<212> PRT
<213> Artificial Sequence

<220>
<223> 2D9; VL

<400> 73
Glu Ile Val Leu Thr Gln Ser Pro Gly Thr Leu Ser Leu Ser Pro Gly
1 5 10 15
Glu Arg Ala Thr Leu Ser Cys Arg Ala Ser Gln Ser Val Ser Ser Ser
20 25 30
Tyr Leu Ala Trp Tyr Gln Gln Lys Pro Gly Gln Ala Pro Arg Leu Leu
35 40 45
Ile Tyr Gly Ala Ser Ser Arg Ala Thr Gly Ile Pro Asp Arg Phe Ser
50 55 60
Gly Ser Gly Ser Gly Thr Asp Phe Thr Leu Thr Ile Ser Arg Leu Glu
65 70 75 80
Pro Glu Asp Phe Ala Val Tyr Tyr Cys Gln Gln Gly Asn Gln Ile Pro
85 90 95
Pro Thr Phe Gly Gln Gly Thr Lys Val Glu Ile Lys
100 105

<210> 74
<211> 324
<212> DNA
<213> Artificial Sequence

<220>
<223> 2D9; VL

<400> 74
gaaatcgtgt taacgcagtc tccaggcacc ctgtctttgt ctccagggga aagagccacc 60
ctctcttgca gggccagtca gagtgttagc agcagctact tagcctggta ccagcagaaa 120
cctggccagg ctcccaggct cctcatctat ggagcatcca gcagggccac tggcatccca 180

eolf-seql.txt

gacaggttca gtggcagtgg atccgggaca gacttcactc tcaccatcag cagactggag 240
 cctgaagatt ttgcagtgtg ttactgtcag cagggtaatc agattccccc tacgttcggt 300
 caggggacca aagtggaaat caaa 324

<210> 75
 <211> 117
 <212> PRT
 <213> Artificial Sequence

<220>
 <223> 2D9; VH

<400> 75

Glu Val Gln Leu Leu Glu Ser Gly Gly Gly Leu Val Gln Pro Gly Gly
 1 5 10 15

Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Phe Thr Phe Ser Ser Tyr
 20 25 30

Ala Met Ser Trp Val Arg Gln Ala Pro Gly Lys Gly Leu Glu Trp Val
 35 40 45

Ser Ala Ile Ser Gly Ser Gly Gly Ser Thr Tyr Tyr Ala Asp Ser Val
 50 55 60

Lys Gly Arg Phe Thr Ile Ser Arg Asp Asn Ser Lys Asn Thr Leu Tyr
 65 70 75 80

Leu Gln Met Asn Ser Leu Arg Ala Glu Asp Thr Ala Val Tyr Tyr Cys
 85 90 95

Ala Lys Gly Trp Phe Thr Pro Phe Asp Tyr Trp Gly Gln Gly Thr Leu
 100 105 110

Val Thr Val Ser Ser
 115

<210> 76
 <211> 351
 <212> DNA
 <213> Artificial Sequence

<220>
 <223> 2D9; VH

<400> 76

gaggtgcaat tggttgagtc tgggggaggc ttggtacagc ctgggggggc cctgagactc 60

tcctgtgcag cctccggatt cacctttagc agttatgccca tgagctgggt ccgccaggct 120

ccaggaagg ggctggagtg ggtctcagct attagcggta gtggtggtag cacatactac 180

gcagactccg tgaagggccg gttcaccatc tccagagaca attccaagaa cacgctgtat 240

ctgcagatga acagcctgag agccgaggac acggccgtat attactgtgc gaaaggttgg 300

eolf-seql.txt

tttacgcctt ttgactactg gggccaagga accctggtca ccgtctcgag t 351

<210> 77
<211> 108
<212> PRT
<213> Artificial Sequence

<220>
<223> 4B8; VL

<400> 77

Glu Ile Val Leu Thr Gln Ser Pro Gly Thr Leu Ser Leu Ser Pro Gly
1 5 10 15

Glu Arg Ala Thr Leu Ser Cys Arg Ala Ser Gln Ser Val Ser Ser Ser
20 25 30

Tyr Leu Ala Trp Tyr Gln Gln Lys Pro Gly Gln Ala Pro Arg Leu Leu
35 40 45

Ile Tyr Gly Ala Ser Ser Arg Ala Thr Gly Ile Pro Asp Arg Phe Ser
50 55 60

Gly Ser Gly Ser Gly Thr Asp Phe Thr Leu Thr Ile Ser Arg Leu Glu
65 70 75 80

Pro Glu Asp Phe Ala Val Tyr Tyr Cys Gln Gln Gly Gln Val Ile Pro
85 90 95

Pro Thr Phe Gly Gln Gly Thr Lys Val Glu Ile Lys
100 105

<210> 78
<211> 324
<212> DNA
<213> Artificial Sequence

<220>
<223> 4B8; VL

<400> 78

gaaatcgtgt taacgcagtc tccaggcacc ctgtctttgt ctccagggga aagagccacc 60
ctctcttgca gggccagtca gagtgttagc agcagctact tagcctggta ccagcagaaa 120
cctggccagg ctcccaggct cctcatctat ggagcatcca gcagggccac tggcatccca 180
gacaggttca gtggcagtg atccgggaca gacttcactc tcaccatcag cagactggag 240
cctgaagatt ttgcagtgt ttactgtcag cagggtcagg ttattcccc tacgttcggc 300
caggggacca aagtggaaat caaa 324

<210> 79
<211> 117
<212> PRT
<213> Artificial Sequence

eolf-seql.txt

<220>

<223> 4B8; VH

<400> 79

Glu Val Gln Leu Leu Glu Ser Gly Gly Gly Leu Val Gln Pro Gly Gly
1 5 10 15

Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Phe Thr Phe Ser Ser Tyr
20 25 30

Ala Met Ser Trp Val Arg Gln Ala Pro Gly Lys Gly Leu Glu Trp Val
35 40 45

Ser Ala Ile Ser Gly Ser Gly Gly Ser Thr Tyr Tyr Ala Asp Ser Val
50 55 60

Lys Gly Arg Phe Thr Ile Ser Arg Asp Asn Ser Lys Asn Thr Leu Tyr
65 70 75 80

Leu Gln Met Asn Ser Leu Arg Ala Glu Asp Thr Ala Val Tyr Tyr Cys
85 90 95

Ala Lys Gly Trp Leu Gly Asn Phe Asp Tyr Trp Gly Gln Gly Thr Leu
100 105 110

Val Thr Val Ser Ser
115

<210> 80

<211> 351

<212> DNA

<213> Artificial Sequence

<220>

<223> 4B8; VH

<400> 80

gaggtgcaat tgttggagtc tgggggaggc ttggtacagc ctgggggggtc cctgagactc 60

tcctgtgcag cctccggatt caccttttagc agttatgccca tgagctgggt ccgccaggct 120

ccaggaagg ggctggagtg ggtctcagct attagtggta gtggtggtag cacatactac 180

gcagactccg tgaagggccg gttcaccatc tccagagaca attccaagaa cacgctgtat 240

ctgcagatga acagcctgag agccgaggac acggccgtat attactgtgc gaaaggggtgg 300

ctgggtaatt ttgactactg gggccaagga accctgggtca ccgtctcgag t 351

<210> 81

<211> 108

<212> PRT

<213> Artificial Sequence

<220>

<223> 7A1; VL

eolf-seql.txt

<400> 81

Glu Ile Val Leu Thr Gln Ser Pro Gly Thr Leu Ser Leu Ser Pro Gly
1 5 10 15

Glu Arg Ala Thr Leu Ser Cys Arg Ala Ser Gln Ser Val Ser Ser Ser
20 25 30

Tyr Leu Ala Trp Tyr Gln Gln Lys Pro Gly Gln Ala Pro Arg Leu Leu
35 40 45

Ile Tyr Gly Ala Ser Ser Arg Ala Thr Gly Ile Pro Asp Arg Phe Ser
50 55 60

Gly Ser Gly Ser Gly Thr Asp Phe Thr Leu Thr Ile Ser Arg Leu Glu
65 70 75 80

Pro Glu Asp Phe Ala Val Tyr Tyr Cys Gln Gln Gly Gln Gln Ile Pro
85 90 95

Pro Thr Phe Gly Gln Gly Thr Lys Val Glu Ile Lys
100 105

<210> 82

<211> 324

<212> DNA

<213> Artificial Sequence

<220>

<223> 7A1; VL

<400> 82

gaaatcgtgt taacgcagtc tccaggcacc ctgtctttgt ctccagggga aagagccacc	60
ctctcttgca gggccagtca gagtgttagc agcagctact tagcctggta ccagcagaaa	120
cctggccagg ctcccaggct cctcatctat ggagcatcca gcagggccac tggcatccca	180
gacaggttca gtggcagtgg atccgggaca gacttcactc tcaccatcag cagactggag	240
cctgaagatt ttgcagtgtg ttactgtcag cagggtcagc agattccccc tacgttcggc	300
caggggacca aagtggaaat caaa	324

<210> 83

<211> 117

<212> PRT

<213> Artificial Sequence

<220>

<223> 7A1; VH

<400> 83

Glu Val Gln Leu Leu Glu Ser Gly Gly Gly Leu Val Gln Pro Gly Gly
1 5 10 15

Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Phe Thr Phe Ser Ser Tyr
20 25 30

eolf-seql.txt

Ala Met Ser Trp Val Arg Gln Ala Pro Gly Lys Gly Leu Glu Trp Val
35 40 45

Ser Ala Ile Ser Gly Ser Gly Gly Ser Thr Tyr Tyr Ala Asp Ser Val
50 55 60

Lys Gly Arg Phe Thr Ile Ser Arg Asp Asn Ser Lys Asn Thr Leu Tyr
65 70 75 80

Leu Gln Met Asn Ser Leu Arg Ala Glu Asp Thr Ala Val Tyr Tyr Cys
85 90 95

Ala Lys Gly Trp Phe Gly Asn Phe Asp Tyr Trp Gly Gln Gly Thr Leu
100 105 110

Val Thr Val Ser Ser
115

<210> 84
<211> 351
<212> DNA
<213> Artificial Sequence

<220>
<223> 7A1; VH

<400> 84
gaggtgcaat tgttggagtc tgggggaggc ttggtacagc ctgggggggtc cctgagactc 60
tcctgtgcag cctccggatt cacctttagc agttatgcca tgagctgggt ccgccaggct 120
ccaggaagg ggctggagtg ggtctcagct attagtggta gtggtggtag cacatactac 180
gcagactccg tgaagggccg gttcaccatc tccagagaca attccaagaa cacgctgtat 240
ctgcagatga acagcctgag agccgaggac acggccgtat attactgtgc gaaagggttg 300
tttggaatt ttgactactg gggccaagga accctgggtca ccgtctcgag t 351

<210> 85
<211> 108
<212> PRT
<213> Artificial Sequence

<220>
<223> 13C2; VL

<400> 85

Glu Ile Val Leu Thr Gln Ser Pro Gly Thr Leu Ser Leu Ser Pro Gly
1 5 10 15

Glu Arg Ala Thr Leu Ser Cys Arg Ala Ser Gln Ser Val Ser Ser Ser
20 25 30

Tyr Leu Ala Trp Tyr Gln Gln Lys Pro Gly Gln Ala Pro Arg Leu Leu
35 40 45

eolf-seql.txt

Ile Tyr Gly Ala Ser Ser Arg Ala Thr Gly Ile Pro Asp Arg Phe Ser
50 55 60

Gly Ser Gly Ser Gly Thr Asp Phe Thr Leu Thr Ile Ser Arg Leu Glu
65 70 75 80

Pro Glu Asp Phe Ala Val Tyr Tyr Cys Gln Gln Gly Gln Leu Ile Pro
85 90 95

Pro Thr Phe Gly Gln Gly Thr Lys Val Glu Ile Lys
100 105

<210> 86
<211> 324
<212> DNA
<213> Artificial Sequence

<220>
<223> 13C2; VL

<400> 86
gaaatcgtgt taacgcagtc tccaggcacc ctgtctttgt ctccagggga aagagccacc 60
ctctcttgca gggccagtca gagtgttagc agcagctact tagcctggta ccagcagaaa 120
cctggccagg ctcccaggct cctcatctat ggagcatcca gcagggccac tggcatccca 180
gacaggttca gtggcagtgg atccgggaca gacttcactc tcaccatcag cagactggag 240
cctgaagatt ttgcagtgtg ttactgtcag cagggtcagc ttattccccc tacgttcggc 300
caggggacca aagtggaaat caaa 324

<210> 87
<211> 117
<212> PRT
<213> Artificial Sequence

<220>
<223> 13C2; VH

<400> 87

Glu Val Gln Leu Leu Glu Ser Gly Gly Gly Leu Val Gln Pro Gly Gly
1 5 10 15

Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Phe Thr Phe Ser Ser Tyr
20 25 30

Ala Met Ser Trp Val Arg Gln Ala Pro Gly Lys Gly Leu Glu Trp Val
35 40 45

Ser Ala Ile Ser Gly Ser Gly Gly Ser Thr Tyr Tyr Ala Asp Ser Val
50 55 60

Lys Gly Arg Phe Thr Ile Ser Arg Asp Asn Ser Lys Asn Thr Leu Tyr
65 70 75 80

eolf-seql.txt

Leu Gln Met Asn Ser Leu Arg Ala Glu Asp Thr Ala Val Tyr Tyr Cys
85 90 95

Ala Lys Gly Trp Leu Gly Pro Phe Asp Tyr Trp Gly Gln Gly Thr Leu
100 105 110

Val Thr Val Ser Ser
115

<210> 88
<211> 351
<212> DNA
<213> Artificial Sequence

<220>
<223> 13C2; VH

<400> 88
gagggtgcaat tggttgagtc tgggggaggc ttggtacagc ctgggggggtc cctgagactc 60
tcctgtgcag cctccgatt cacctttagc agttatgcca tgagctgggt ccgccaggct 120
ccaggaagg ggctggagtg ggtctcagct attagtggta gtggtggttag cacatactac 180
gcagactccg tgaagggccg gttcaccatc tccagagaca attccaagaa cacgctgtat 240
ctgcagatga acagcctgag agccgaggac acggccgtat attactgtgc gaaagggttg 300
ctgggtcctt ttgactactg gggccaagga accctggtca ccgtctcgag t 351

<210> 89
<211> 108
<212> PRT
<213> Artificial Sequence

<220>
<223> 13E8; VL

<400> 89

Glu Ile Val Leu Thr Gln Ser Pro Gly Thr Leu Ser Leu Ser Pro Gly
1 5 10 15

Glu Arg Ala Thr Leu Ser Cys Arg Ala Ser Gln Ser Val Ser Ser Ser
20 25 30

Tyr Leu Ala Trp Tyr Gln Gln Lys Pro Gly Gln Ala Pro Arg Leu Leu
35 40 45

Ile Tyr Gly Ala Ser Ser Arg Ala Thr Gly Ile Pro Asp Arg Phe Ser
50 55 60

Gly Ser Gly Ser Gly Thr Asp Phe Thr Leu Thr Ile Ser Arg Leu Glu
65 70 75 80

Pro Glu Asp Phe Ala Val Tyr Tyr Cys Gln Gln Gly Leu Asn Ile Pro
85 90 95

eolf-seql.txt

Ser Thr Phe Gly Gln Gly Thr Lys Val Glu Ile Lys
100 105

<210> 90
<211> 324
<212> DNA
<213> Artificial Sequence

<220>
<223> 13E8; VL

<400> 90
gaaatcgtgt taacgcagtc tccaggcacc ctgtctttgt ctccagggga aagagccacc 60
ctctcttgca gggccagtca gagggttagc agcagctact tagcctggta ccagcagaaa 120
cctggccagg ctcccaggct cctcatctat ggagcatcca gcagggccac tggcatccca 180
gacaggttca gtggcagtg atccgggaca gacttcactc tcaccatcag cagactggag 240
cctgaagatt ttgcagtgt ttactgtcag caggggtctga atattccctc gacgttcggc 300
caggggacca aagtggaaat caaa 324

<210> 91
<211> 117
<212> PRT
<213> Artificial Sequence

<220>
<223> 13E8; VH

<400> 91

Glu Val Gln Leu Leu Glu Ser Gly Gly Gly Leu Val Gln Pro Gly Gly
1 5 10 15

Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Phe Thr Phe Ser Ser Tyr
20 25 30

Ala Met Ser Trp Val Arg Gln Ala Pro Gly Lys Gly Leu Glu Trp Val
35 40 45

Ser Ala Ile Ser Gly Ser Gly Gly Ser Thr Tyr Tyr Ala Asp Ser Val
50 55 60

Lys Gly Arg Phe Thr Ile Ser Arg Asp Asn Ser Lys Asn Thr Leu Tyr
65 70 75 80

Leu Gln Met Asn Ser Leu Arg Ala Glu Asp Thr Ala Val Tyr Tyr Cys
85 90 95

Ala Lys Gly Trp Leu Gly Pro Phe Asp Tyr Trp Gly Gln Gly Thr Leu
100 105 110

Val Thr Val Ser Ser
115

eolf-seql.txt

<210> 92
 <211> 351
 <212> DNA
 <213> Artificial Sequence

<220>
 <223> 13E8; VH

<400> 92
 gaggtgcaat tggtggagtc tgggggaggc ttggtacagc ctgggggggtc cctgagactc 60
 tcctgtgcag cctccggatt caccttttagc agttatgcca tgagctgggt ccgccaggct 120
 ccaggggaagg ggctggagtg ggtctcagct attagtggta gtgggtggtag cacatactac 180
 gcagactccg tgaagggccg gttcaccatc tccagagaca attccaagaa cacgctgtat 240
 ctgcagatga acagcctgag agccgaggac acggccgtat attactgtgc gaaaggttgg 300
 ttgggtccgt ttgactactg gggccaagga accctgggtca ccgtctcgag t 351

<210> 93
 <211> 108
 <212> PRT
 <213> Artificial Sequence

<220>
 <223> 14C10; VL

<400> 93

Glu Ile Val Leu Thr Gln Ser Pro Gly Thr Leu Ser Leu Ser Pro Gly
 1 5 10 15

Glu Arg Ala Thr Leu Ser Cys Arg Ala Ser Gln Ser Val Ser Ser Ser
 20 25 30

Tyr Leu Ala Trp Tyr Gln Gln Lys Pro Gly Gln Ala Pro Arg Leu Leu
 35 40 45

Ile Tyr Gly Ala Ser Ser Arg Ala Thr Gly Ile Pro Asp Arg Phe Ser
 50 55 60

Gly Ser Gly Ser Gly Thr Asp Phe Thr Leu Thr Ile Ser Arg Leu Glu
 65 70 75 80

Pro Glu Asp Phe Ala Val Tyr Tyr Cys Gln Gln Gly His Ile Ile Pro
 85 90 95

Pro Thr Phe Gly Gln Gly Thr Lys Val Glu Ile Lys
 100 105

<210> 94
 <211> 324
 <212> DNA
 <213> Artificial Sequence

<220>

eolf-seql.txt

<223> 14C10; VL

<400> 94
gaaatcgtgt taacgcagtc tccaggcacc ctgtctttgt ctccagggga aagagccacc 60
ctctcttgca gggccagtca gagtgtagc agcagctact tagcctggta ccagcagaaa 120
cctggccagg ctcccaggct cctcatctat ggagcatcca gcagggccac tggcatccca 180
gacaggttca gtggcagtg atccgggaca gacttcactc tcaccatcag cagactggag 240
cctgaagatt ttgcagtgt ttactgtcag cagggtcata ttattcccc gacgttcggc 300
caggggacca aagtggaaat caaa 324

<210> 95
<211> 117
<212> PRT
<213> Artificial Sequence

<220>
<223> 14C10; VH

<400> 95
Glu Val Gln Leu Leu Glu Ser Gly Gly Gly Leu Val GlnPro Gly Gly
1 5 10 15
Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Phe Thr Phe Ser Ser Tyr
20 25 30
Ala Met Ser Trp Val Arg Gln Ala Pro Gly Lys Gly Leu Glu Trp Val
35 40 45
Ser Ala Ile Ser Gly Ser Gly Gly Ser Thr Tyr Tyr Ala Asp Ser Val
50 55 60
Lys Gly Arg Phe Thr Ile Ser Arg Asp Asn Ser Lys Asn Thr Leu Tyr
65 70 75 80
Leu Gln Met Asn Ser Leu Arg Ala Glu Asp Thr Ala Val Tyr Tyr Cys
85 90 95
Ala Lys Ala Trp Met Gly Pro Phe Asp Tyr Trp Gly Gln Gly Thr Leu
100 105 110
Val Thr Val Ser Ser
115

<210> 96
<211> 351
<212> DNA
<213> Artificial Sequence

<220>
<223> 14C10; VH

<400> 96
gaggtgcaat tgttgagtc tgggggaggc ttggtacagc ctgggggggc cctgagactc 60

eolf-seql.txt

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tcctgtgcag cctccggatt cacctttagc agttatgcca tgagctgggt cgcaggct      120
ccaggaagg ggctggagt ggtctcagct attagtggta gtggtagtag cacatactac      180
gcagactccg tgaagggccg gttcaccatc tccagagaca attccaagaa cagctgtat      240
ctgcagatga acagcctgag agccgaggac acggccgtat attactgtgc gaaagcttgg      300
atggggcctt ttgactactg gggccaagga accctgggtca ccgtctcgag t          351
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<210> 97
 <211> 108
 <212> PRT
 <213> Artificial Sequence

<220>
 <223> 17A11; VL

<400> 97

Glu Ile Val Leu Thr Gln Ser Pro Gly Thr Leu Ser Leu Ser Pro Gly
 1 5 10 15

Glu Arg Ala Thr Leu Ser Cys Arg Ala Ser Gln Ser Val Ser Ser Ser
 20 25 30

Tyr Leu Ala Trp Tyr Gln Gln Lys Pro Gly Gln Ala Pro Arg Leu Leu
 35 40 45

Ile Tyr Gly Ala Ser Ser Arg Ala Thr Gly Ile Pro Asp Arg Phe Ser
 50 55 60

Gly Ser Gly Ser Gly Thr Asp Phe Thr Leu Thr Ile Ser Arg Leu Glu
 65 70 75 80

Pro Glu Asp Phe Ala Val Tyr Tyr Cys Gln Gln Gly Leu Asn Ile Pro
 85 90 95

Ser Thr Phe Gly Gln Gly Thr Lys Val Glu Ile Lys
 100 105

<210> 98
 <211> 324
 <212> DNA
 <213> Artificial Sequence

<220>
 <223> 17A11; VL

<400> 98

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gaaatcgtgt taacgcagtc tccaggcacc ctgtctttgt ctccagggga aagagccacc      60
ctctcttgca gggccagtca gagggttagc agcagctact tagcctggta ccagcagaaa      120
cctggccagg ctcccaggct cctcatctat ggagcatcca gcagggccac tggcatccca      180
gacaggttca gtggcagtg atccgggaca gacttcactc tcaccatcag cagactggag      240
cctgaagatt ttgcagtgt ttactgtcag cagggtctga atattccctc gacgttcggc      300
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caggggacca aagtggaaat caaa

<210> 99
 <211> 117
 <212> PRT
 <213> Artificial Sequence

<220>
 <223> 17A11; VH

<400> 99

Glu Val Gln Leu Leu Glu Ser Gly Gly Gly Leu Val Gln Pro Gly Gly
 1 5 10 15

Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Phe Thr Phe Ser Ser Tyr
 20 25 30

Ala Met Ser Trp Val Arg Gln Ala Pro Gly Lys Gly Leu Glu Trp Val
 35 40 45

Ser Ala Ile Ser Gly Ser Gly Gly Ser Thr Tyr Tyr Ala Asp Ser Val
 50 55 60

Lys Gly Arg Phe Thr Ile Ser Arg Asp Asn Ser Lys Asn Thr Leu Tyr
 65 70 75 80

Leu Gln Met Asn Ser Leu Arg Ala Glu Asp Thr Ala Val Tyr Tyr Cys
 85 90 95

Ala Lys Gly Trp Leu Gly Pro Phe Asp Tyr Trp Gly Gln Gly Thr Leu
 100 105 110

Val Thr Val Ser Ser
 115

<210> 100
 <211> 351
 <212> DNA
 <213> Artificial Sequence

<220>
 <223> 17A11; VH

<400> 100

gaggtgcaat tgttgagtc tgggggaggc ttggtacagc ctgggggggtc cctgagactc 60
 tcctgtgcag cctccgatt cacctttagc agttatgcca tgagctgggt ccgccaggct 120
 ccagggaagg ggctggagt ggtctcagct attagtggta gtggtggtag cacatactac 180
 gcagactccg tgaagggccg gttcaccatc tccagagaca attccaagaa cacgctgtat 240
 ctgcagatga acagcctgag agccgaggac acggccgtat attactgtgc gaaaggttgg 300
 ttgggtccgt ttgactactg gggccaagga accctggtca ccgtctcgag t 351

eolf-seql.txt

<210> 101
 <211> 108
 <212> PRT
 <213> Artificial Sequence

<220>
 <223> 19G1; VL

<400> 101

Glu Ile Val Leu Thr Gln Ser Pro Gly Thr Leu Ser Leu Ser Pro Gly
 1 5 10 15

Glu Arg Ala Thr Leu Ser Cys Arg Ala Ser Gln Ser Val Thr Ser Ser
 20 25 30

Tyr Leu Ala Trp Tyr Gln Gln Lys Pro Gly Gln Ala Pro Arg Leu Leu
 35 40 45

Ile Asn Val Gly Ser Arg Arg Ala Thr Gly Ile Pro Asp Arg Phe Ser
 50 55 60

Gly Ser Gly Ser Gly Thr Asp Phe Thr Leu Thr Ile Ser Arg Leu Glu
 65 70 75 80

Pro Glu Asp Phe Ala Val Tyr Tyr Cys Gln Gln Gly Ile Met Leu Pro
 85 90 95

Pro Thr Phe Gly Gln Gly Thr Lys Val Glu Ile Lys
 100 105

<210> 102
 <211> 324
 <212> DNA
 <213> Artificial Sequence

<220>
 <223> 19G1; VL

<400> 102
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 ctctcttgca gggccagtca gagggttacc agtagctact tagcctggta ccagcagaaa 120
 cctggccagg ctcccaggct cctcatcaat gtgggctccc gtagggccac tggcatccca 180
 gacaggttca gtggcagtgg atccgggaca gacttcactc tcaccatcag cagactggag 240
 cctgaagatt ttgcagtgtg ttactgtcag cagggtatta tgcttcccc gacgttcggc 300
 caggggacca aagtggaaat caaa 324

<210> 103
 <211> 117
 <212> PRT
 <213> Artificial Sequence

<220>
 <223> 19G1; VH

eolf-seql.txt

<400> 103

Glu Val Gln Leu Leu Glu Ser Gly Gly Gly Leu Val Gln Pro Gly Gly
1 5 10 15

Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Phe Thr Phe Ser Ser Tyr
20 25 30

Ala Met Ser Trp Val Arg Gln Ala Pro Gly Lys Gly Leu Glu Trp Val
35 40 45

Ser Ala Ile Ile Ser Ser Gly Gly Leu Thr Tyr Tyr Ala Asp Ser Val
50 55 60

Lys Gly Arg Phe Thr Ile Ser Arg Asp Asn Ser Lys Asn Thr Leu Tyr
65 70 75 80

Leu Gln Met Asn Ser Leu Arg Ala Glu Asp Thr Ala Val Tyr Tyr Cys
85 90 95

Ala Lys Gly Trp Phe Gly Gly Phe Asn Tyr Trp Gly Gln Gly Thr Leu
100 105 110

Val Thr Val Ser Ser
115

<210> 104

<211> 351

<212> DNA

<213> Artificial Sequence

<220>

<223> 19G1; VH

<400> 104

gaggtgcaat tgttggagtc tgggggaggc ttggtacagc ctgggggggtc cctgagactc	60
tcctgtgcag cctccggatt caccttttagc agttatgcga tgagctgggt ccgccaggct	120
ccagggaagg ggctggagtg ggtctcagcg attattagta gtggtggtct cacatactac	180
gcagactccg tgaagggccg gttcaccatc tccagagaca attccaagaa cacgctgtat	240
ctgcagatga acagcctgag agccgaggac acggccgtat attactgtgc gaaaggggtgg	300
tttgggtggtt ttaactactg gggccaagga accctggtca ccgtctcgtc c	351

<210> 105

<211> 108

<212> PRT

<213> Artificial Sequence

<220>

<223> 20G8; VL

<400> 105

Glu Ile Val Leu Thr Gln Ser Pro Gly Thr Leu Ser Leu Ser Pro Gly
1 5 10 15

eolf-seql.txt

Glu Arg Ala Thr Leu Ser Cys Arg Ala Ser Gln Ser Val Thr Ser Ser
20 25 30

Tyr Leu Ala Trp Tyr Gln Gln Lys Pro Gly Gln Ala Pro Arg Leu Leu
35 40 45

Ile Asn Val Gly Ser Arg Arg Ala Thr Gly Ile Pro Asp Arg Phe Ser
50 55 60

Gly Ser Gly Ser Gly Thr Asp Phe Thr Leu Thr Ile Ser Arg Leu Glu
65 70 75 80

Pro Glu Asp Phe Ala Val Tyr Tyr Cys Gln Gln Gly Ile Met Leu Pro
85 90 95

Pro Thr Phe Gly Gln Gly Thr Lys Val Glu Ile Lys
100 105

<210> 106
<211> 324
<212> DNA
<213> Artificial Sequence

<220>
<223> 20G8; VL

<400> 106	
gaaatcgtgt taacgcagtc tccaggcacc ctgtctttgt ctccagggga aagagccacc	60
ctctcttgca gggccagtca gagtgttacc agtagctact tagcctggta ccagcagaaa	120
cctggccagg ctcccaggct cctcatcaat gtgggctccc gtagggccac tggcatccca	180
gacaggttca gtggcagtgg atccgggaca gacttcactc tcaccatcag cagactggag	240
cctgaagatt ttgcagtgtt ttactgtcag cagggtatta tgcttcccc gacgttcggc	300
caggggacca aagtggaaat caaa	324

<210> 107
<211> 117
<212> PRT
<213> Artificial Sequence

<220>
<223> 20G8; VH

<400> 107

Glu Val Gln Leu Leu Glu Ser Gly Gly Gly Leu Val Gln Pro Gly Gly
1 5 10 15

Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Phe Thr Phe Ser Ser Tyr
20 25 30

Ala Met Ser Trp Val Arg Gln Ala Pro Gly Lys Gly Leu Glu Trp Val
35 40 45

eolf-seql.txt

Ser Ala Ile Ile Gly Ser Gly Ser Arg Thr Tyr Tyr Ala Asp Ser Val
50 55 60

Lys Gly Arg Phe Thr Ile Ser Arg Asp Asn Ser Lys Asn Thr Leu Tyr
65 70 75 80

Leu Gln Met Asn Ser Leu Arg Ala Glu Asp Thr Ala Val Tyr Tyr Cys
85 90 95

Ala Lys Gly Trp Phe Gly Gly Phe Asn Tyr Trp Gly Gln Gly Thr Leu
100 105 110

Val Thr Val Ser Ser
115

<210> 108
<211> 351
<212> DNA
<213> Artificial Sequence

<220>
<223> 20G8; VH

<400> 108
gaggtgcaat tggttgagtc tgggggaggc ttggtacagc ctgggggggtc cctgagactc 60
tcctgtgcag cctccggatt caccttttagc agttatgcaa tgagctgggt ccgccaggct 120
ccaggggaagg ggctggagtg ggtctcagct attattggga gtggtagtcg tacatactac 180
gcagactccg tgaagggccg gttcaccatc tccagagaca attccaagaa cacgctgtat 240
ctgcagatga acagcctgag agccgaggac acggccgtat attactgtgc gaaaggggtg 300
tttggtgggtt ttaactactg gggccaagga accctggtca ccgtctcgtc c 351

<210> 109
<211> 108
<212> PRT
<213> Artificial Sequence

<220>
<223> 4B9; VL

<400> 109

Glu Ile Val Leu Thr Gln Ser Pro Gly Thr Leu Ser Leu Ser Pro Gly
1 5 10 15

Glu Arg Ala Thr Leu Ser Cys Arg Ala Ser Gln Ser Val Thr Ser Ser
20 25 30

Tyr Leu Ala Trp Tyr Gln Gln Lys Pro Gly Gln Ala Pro Arg Leu Leu
35 40 45

Ile Asn Val Gly Ser Arg Arg Ala Thr Gly Ile Pro Asp Arg Phe Ser
50 55 60

eolf-seql.txt

Gly Ser Gly Ser Gly Thr Asp Phe Thr Leu Thr Ile Ser Arg Leu Glu
65 70 75 80

Pro Glu Asp Phe Ala Val Tyr Tyr Cys Gln Gln Gly Ile Met Leu Pro
85 90 95

Pro Thr Phe Gly Gln Gly Thr Lys Val Glu Ile Lys
100 105

<210> 110
<211> 324
<212> DNA
<213> Artificial Sequence

<220>
<223> 4B9; VL

<400> 110
gaaatcgtgt taacgcagtc tccaggcacc ctgtctttgt ctccagggga aagagccacc 60
ctctcttgca gggccagtca gagggttacc agtagctact tagcctggta ccagcagaaa 120
cctggccagg ctcccaggct cctcatcaat gtgggctccc gtagggccac tggcatccca 180
gacaggttca gtggcagtggt atccgggaca gacttcactc tcaccatcag cagactggag 240
cctgaagatt ttgcagtgtt ttactgtcag cagggtatta tgcttcccc gacgttcggc 300
caggggacca aagtggaaat caaa 324

<210> 111
<211> 117
<212> PRT
<213> Artificial Sequence

<220>
<223> 4B9; VH

<400> 111

Glu Val Gln Leu Leu Glu Ser Gly Gly Gly Leu Val Gln Pro Gly Gly
1 5 10 15

Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Phe Thr Phe Ser Ser Tyr
20 25 30

Ala Met Ser Trp Val Arg Gln Ala Pro Gly Lys Gly Leu Glu Trp Val
35 40 45

Ser Ala Ile Ile Gly Ser Gly Ala Ser Thr Tyr Tyr Ala Asp Ser Val
50 55 60

Lys Gly Arg Phe Thr Ile Ser Arg Asp Asn Ser Lys Asn Thr Leu Tyr
65 70 75 80

Leu Gln Met Asn Ser Leu Arg Ala Glu Asp Thr Ala Val Tyr Tyr Cys
85 90 95

eolf-seql.txt

Ala Lys Gly Trp Phe Gly Gly Phe Asn Tyr Trp Gly Gln Gly Thr Leu
100 105 110

Val Thr Val Ser Ser
115

<210> 112
<211> 351
<212> DNA
<213> Artificial Sequence

<220>
<223> 4B9; VH

<400> 112
gaggtgcaat tggttgagtc tgggggaggc ttggtacagc ctgggggggc cctgagactc 60
tcctgtgcag cctccggatt caccttttagc agttatgcta tgagctgggt ccgccaggct 120
ccagggaagg ggctggagtg ggtctcagct attattggta gtggtgctag cacatactac 180
gcagactccg tgaagggccg gttcaccatc tccagagaca attccaagaa cacgctgtat 240
ctgcagatga acagcctgag agccgaggac acggccgtat attactgtgc gaaaggggtg 300
tttggtggtt ttaactactg gggccaagga accctggtca ccgtctcgtc c 351

<210> 113
<211> 108
<212> PRT
<213> Artificial Sequence

<220>
<223> 5B8; VL

<400> 113

Glu Ile Val Leu Thr Gln Ser Pro Gly Thr Leu Ser Leu Ser Pro Gly
1 5 10 15

Glu Arg Ala Thr Leu Ser Cys Arg Ala Ser Gln Ser Val Thr Ser Ser
20 25 30

Tyr Leu Ala Trp Tyr Gln Gln Lys Pro Gly Gln Ala Pro Arg Leu Leu
35 40 45

Ile Asn Val Gly Ser Arg Arg Ala Thr Gly Ile Pro Asp Arg Phe Ser
50 55 60

Gly Ser Gly Ser Gly Thr Asp Phe Thr Leu Thr Ile Ser Arg Leu Glu
65 70 75 80

Pro Glu Asp Phe Ala Val Tyr Tyr Cys Gln Gln Gly Ile Met Leu Pro
85 90 95

Pro Thr Phe Gly Gln Gly Thr Lys Val Glu Ile Lys
100 105

eolf-seql.txt

<210> 114
 <211> 324
 <212> DNA
 <213> Artificial Sequence

<220>
 <223> 5B8; VL

<400> 114
 gaaatcgtgt taacgcagtc tccaggcacc ctgtctttgt ctccagggga aagagccacc 60
 ctctcttgca gggccagtca gagtgttacc agtagctact tagcctggta ccagcagaaa 120
 cctggccagg ctcccaggct cctcatcaat gtgggctccc gtagggccac tggcatccca 180
 gacaggttca gtggcagtgg atccgggaca gacttcactc tcaccatcag cagactggag 240
 cctgaagatt ttgcagtgtg ttactgtcag cagggtatta tgcttcccc gacgttcggc 300
 caggggacca aagtggaaat caaa 324

<210> 115
 <211> 117
 <212> PRT
 <213> Artificial Sequence

<220>
 <223> 5B8; VH

<400> 115
 Glu Val Gln Leu Leu Glu Ser Gly Gly Gly Leu Val Gln Pro Gly Gly
 1 5 10 15
 Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Phe Thr Phe Ser Ser Tyr
 20 25 30
 Ala Met Ser Trp Val Arg Gln Ala Pro Gly Lys Gly Leu Glu Trp Val
 35 40 45
 Ser Ala Ile Trp Gly Gly Gly Arg Ser Thr Tyr Tyr Ala Asp Ser Val
 50 55 60
 Lys Gly Arg Phe Thr Ile Ser Arg Asp Asn Ser Lys Asn Thr Leu Tyr
 65 70 75 80
 Leu Gln Met Asn Ser Leu Arg Ala Glu Asp Thr Ala Val Tyr Tyr Cys
 85 90 95
 Ala Lys Gly Trp Phe Gly Gly Phe Asn Tyr Trp Gly Gln Gly Thr Leu
 100 105 110
 Val Thr Val Ser Ser
 115

<210> 116
 <211> 351

eolf-seql.txt

<212> DNA
<213> Artificial Sequence

<220>
<223> 5B8; VH

<400> 116
gaggtgcaat tgttgagtc tgggggaggc ttggtacagc ctgggggggc cctgagactc 60
tcctgtgcag cctccggatt caccttttagc agttatgcta tgagctgggt ccgccaggct 120
ccagggaagg ggctggagtg ggtctcagct atttgggggtg gtggtcgtag cacatactac 180
gcagactccg tgaagggccg gttcaccatc tccagagaca attccaagaa cagcgtgtat 240
ctgcagatga acagcctgag agccgaggac acggccgtat attactgtgc gaaaggggtg 300
tttggtggtt ttaactactg gggccaagga accctggtca ccgtctcgtc c 351

<210> 117
<211> 108
<212> PRT
<213> Artificial Sequence

<220>
<223> 5F1; VL

<400> 117

Glu Ile Val Leu Thr Gln Ser Pro Gly Thr Leu Ser Leu Ser Pro Gly
1 5 10 15

Glu Arg Ala Thr Leu Ser Cys Arg Ala Ser Gln Ser Val Thr Ser Ser
20 25 30

Tyr Leu Ala Trp Tyr Gln Gln Lys Pro Gly Gln Ala Pro Arg Leu Leu
35 40 45

Ile Asn Val Gly Ser Arg Arg Ala Thr Gly Ile Pro Asp Arg Phe Ser
50 55 60

Gly Ser Gly Ser Gly Thr Asp Phe Thr Leu Thr Ile Ser Arg Leu Glu
65 70 75 80

Pro Glu Asp Phe Ala Val Tyr Tyr Cys Gln Gln Gly Ile Met Leu Pro
85 90 95

Pro Thr Phe Gly Gln Gly Thr Lys Val Glu Ile Lys
100 105

<210> 118
<211> 324
<212> DNA
<213> Artificial Sequence

<220>
<223> 5F1; VL

<400> 118
gaaatcgtgt taacgcagtc tccaggcacc ctgtctttgt ctccagggga aagagccacc 60

eolf-seql.txt

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ctctcttgca gggccagtca gagtgttacc agtagctact tagcctggta ccagcagaaa      120
cctggccagg ctcccaggct cctcatcaat gtggggtccc gtagggccac tggcatccca      180
gacagggttca gtggcagtgg atccgggaca gacttcactc tcaccatcag cagactggag      240
cctgaagatt ttgcagtgtg ttactgtcag cagggtatta tgcttcccc gacgttcggc      300
caggggacca aagtggaaat caaa                                             324
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<210> 119
 <211> 117
 <212> PRT
 <213> Artificial Sequence

<220>
 <223> 5F1; VH

<400> 119

Glu Val Gln Leu Leu Glu Ser Gly Gly Gly Leu Val Gln Pro Gly Gly
 1 5 10 15

Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Phe Thr Phe Ser Ser Tyr
 20 25 30

Ala Met Ser Trp Val Arg Gln Ala Pro Gly Lys Gly Leu Glu Trp Val
 35 40 45

Ser Ala Ile Ile Ser Ser Gly Ala Ser Thr Tyr Tyr Ala Asp Ser Val
 50 55 60

Lys Gly Arg Phe Thr Ile Ser Arg Asp Asn Ser Lys Asn Thr Leu Tyr
 65 70 75 80

Leu Gln Met Asn Ser Leu Arg Ala Glu Asp Thr Ala Val Tyr Tyr Cys
 85 90 95

Ala Lys Gly Trp Phe Gly Gly Phe Asn Tyr Trp Gly Gln Gly Thr Leu
 100 105 110

Val Thr Val Ser Ser
 115

<210> 120
 <211> 351
 <212> DNA
 <213> Artificial Sequence

<220>
 <223> 5F1; VH

<400> 120

gaggtgcaat tggtggagtc tgggggaggc ttggtacagc ctgggggggtc cctgagactc 60

tcctgtgcag cctccgatt cacctttagc agttatgcta tgagctgggt ccgccaggct 120

ccaggggaagg ggctggagtg ggtctcagct attattagta gtggggctag cacatactac 180

eolf-seql.txt

gcagactccg tgaagggccg gttcaccatc tccagagaca attccaagaa cacgctgtat 240
 ctgcagatga acagcctgag agccgaggac acggccgtat attactgtgc gaaaggggtgg 300
 tttggtggtt ttaactactg gggccaagga accctgggtca ccgtctcgtc c 351

<210> 121
 <211> 108
 <212> PRT
 <213> Artificial Sequence

<220>
 <223> 14B3; VL

<400> 121

Glu Ile Val Leu Thr Gln Ser Pro Gly Thr Leu Ser Leu Ser Pro Gly
 1 5 10 15

Glu Arg Ala Thr Leu Ser Cys Arg Ala Ser Gln Ser Val Thr Ser Ser
 20 25 30

Tyr Leu Ala Trp Tyr Gln Gln Lys Pro Gly Gln Ala Pro Arg Leu Leu
 35 40 45

Ile Asn Val Gly Ser Arg Arg Ala Thr Gly Ile Pro Asp Arg Phe Ser
 50 55 60

Gly Ser Gly Ser Gly Thr Asp Phe Thr Leu Thr Ile Ser Arg Leu Glu
 65 70 75 80

Pro Glu Asp Phe Ala Val Tyr Tyr Cys Gln Gln Gly Ile Met Leu Pro
 85 90 95

Pro Thr Phe Gly Gln Gly Thr Lys Val Glu Ile Lys
 100 105

<210> 122
 <211> 324
 <212> DNA
 <213> Artificial Sequence

<220>
 <223> 14B3; VL

<400> 122

gaaatcgtgt taacgcagtc tccaggcacc ctgtctttgt ctccagggga aagagccacc 60
 ctctcttgca gggccagtc gagtggtacc agtagctact tagcctggta ccagcagaaa 120
 cctggccagg ctcccaggct cctcatcaat gtgggctccc gtagggccac tggcatccca 180
 gacaggttca gtggcagtggt atccgggaca gacttcactc tcaccatcag cagactggag 240
 cctgaagatt ttgcagtgtt ttactgtcag cagggtatta tgcttcccc gacgttcggc 300
 caggggacca aagtggaaat caaa 324

eolf-seql.txt

<210> 123
 <211> 117
 <212> PRT
 <213> Artificial Sequence

<220>
 <223> 14B3; VH

<400> 123

Glu Val Gln Leu Leu Glu Ser Gly Gly Gly Leu Val Gln Pro Gly Gly
 1 5 10 15

Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Phe Thr Phe Ser Ser Tyr
 20 25 30

Ala Met Ser Trp Val Arg Gln Ala Pro Gly Lys Gly Leu Glu Trp Val
 35 40 45

Ser Ala Ile Leu Ala Ser Gly Ala Ile Thr Tyr Tyr Ala Asp Ser Val
 50 55 60

Lys Gly Arg Phe Thr Ile Ser Arg Asp Asn Ser Lys Asn Thr Leu Tyr
 65 70 75 80

Leu Gln Met Asn Ser Leu Arg Ala Glu Asp Thr Ala Val Tyr Tyr Cys
 85 90 95

Ala Lys Gly Trp Phe Gly Gly Phe Asn Tyr Trp Gly Gln Gly Thr Leu
 100 105 110

Val Thr Val Ser Ser
 115

<210> 124
 <211> 351
 <212> DNA
 <213> Artificial Sequence

<220>
 <223> 14B3; VH

<400> 124

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 tcctgtgcag cctccggatt cacctttagc agttatgcta tgagctgggt ccgccaggct 120
 ccagggaagg ggctggagtg ggtctcagct attttggtc gttggtgcgat cacatactac 180
 gcagactccg tgaagggccg gttcaccatc tccagagaca attccaagaa cacgctgtat 240
 ctgcagatga acagcctgag agccgaggac acggccgtat attactgtgc gaaaggggtg 300
 tttggtgggt ttaactactg gggccaagga accctggtca ccgtctcgtc c 351

<210> 125
 <211> 108
 <212> PRT
 <213> Artificial Sequence

eolf-seql.txt

<220>

<223> 16F1; VL

<400> 125

Glu Ile Val Leu Thr Gln Ser Pro Gly Thr Leu Ser Leu Ser Pro Gly
1 5 10 15

Glu Arg Ala Thr Leu Ser Cys Arg Ala Ser Gln Ser Val Thr Ser Ser
20 25 30

Tyr Leu Ala Trp Tyr Gln Gln Lys Pro Gly Gln Ala Pro Arg Leu Leu
35 40 45

Ile Asn Val Gly Ser Arg Arg Ala Thr Gly Ile Pro Asp Arg Phe Ser
50 55 60

Gly Ser Gly Ser Gly Thr Asp Phe Thr Leu Thr Ile Ser Arg Leu Glu
65 70 75 80

Pro Glu Asp Phe Ala Val Tyr Tyr Cys Gln Gln Gly Ile Met Leu Pro
85 90 95

Pro Thr Phe Gly Gln Gly Thr Lys Val Glu Ile Lys
100 105

<210> 126

<211> 324

<212> DNA

<213> Artificial Sequence

<220>

<223> 16F1; VL

<400> 126

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cctggccagg ctcccaggct cctcatcaat gtgggctccc gtagggccac tggcatccca 180
gacaggttca gtggcagtgg atccgggaca gacttcactc tcaccatcag cagactggag 240
cctgaagatt ttgcagtgtt ttactgtcag cagggtatta tgcttcccc gacgttcggc 300
caggggacca aagtggaaat caaa 324

<210> 127

<211> 117

<212> PRT

<213> Artificial Sequence

<220>

<223> 16F1; VH

<400> 127

Glu Val Gln Leu Leu Glu Ser Gly Gly Gly Leu Val Gln Pro Gly Gly
1 5 10 15

eolf-seql.txt

Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Phe Thr Phe Ser Ser Tyr
20 25 30

Ala Met Ser Trp Val Arg Gln Ala Pro Gly Lys Gly Leu Glu Trp Val
35 40 45

Ser Gly Ile Ile Gly Ser Gly Gly Ile Thr Tyr Tyr Ala Asp Ser Val
50 55 60

Lys Gly Arg Phe Thr Ile Ser Arg Asp Asn Ser Lys Asn Thr Leu Tyr
65 70 75 80

Leu Gln Met Asn Ser Leu Arg Ala Glu Asp Thr Ala Val Tyr Tyr Cys
85 90 95

Ala Lys Gly Trp Phe Gly Gly Phe Asn Tyr Trp Gly Gln Gly Thr Leu
100 105 110

Val Thr Val Ser Ser
115

<210> 128
<211> 351
<212> DNA
<213> Artificial Sequence

<220>
<223> 16F1; VH

<400> 128
gaggtgcaat tggttgagtc tgggggaggc ttggtacagc ctgggggggtc cctgagactc 60
tcctgtgcag cctccggatt cacctttagc agttatgcta tgagctgggt ccgccaggct 120
ccagggaagg ggctggagtg ggtctcaggt attattggta gtggtggtat cacatactac 180
gcagactccg tgaagggccg gttcaccatc tccagagaca attccaagaa cacgctgtat 240
ctgcagatga acagcctgag agccgaggac acggccgtat attactgtgc gaaaggggtg 300
tttgggtggtt ttaactactg gggccaagga accctgggtca ccgtctcgtc c 351

<210> 129
<211> 108
<212> PRT
<213> Artificial Sequence

<220>
<223> 16F8; VL

<400> 129

Glu Ile Val Leu Thr Gln Ser Pro Gly Thr Leu Ser Leu Ser Pro Gly
1 5 10 15

Glu Arg Ala Thr Leu Ser Cys Arg Ala Ser Gln Ser Val Thr Ser Ser
20 25 30

eolf-seql.txt

Tyr Leu Ala Trp Tyr Gln Gln Lys Pro Gly Gln Ala Pro Arg Leu Leu
35 40 45

Ile Asn Val Gly Ser Arg Arg Ala Thr Gly Ile Pro Asp Arg Phe Ser
50 55 60

Gly Ser Gly Ser Gly Thr Asp Phe Thr Leu Thr Ile Ser Arg Leu Glu
65 70 75 80

Pro Glu Asp Phe Ala Val Tyr Tyr Cys Gln Gln Gly Ile Met Leu Pro
85 90 95

Pro Thr Phe Gly Gln Gly Thr Lys Val Glu Ile Lys
100 105

<210> 130
<211> 324
<212> DNA
<213> Artificial Sequence

<220>
<223> 16F8; VL

<400> 130
gaaatcgtgt taacgcagtc tccaggcacc ctgtctttgt ctccagggga aagagccacc 60
ctctcttgca gggccagtca gagtgttacc agtagctact tagcctggta ccagcagaaa 120
cctggccagg ctcccaggct cctcatcaat gtgggctccc gtagggccac tggcatccca 180
gacaggttca gtggcagtgg atccgggaca gacttcactc tcaccatcag cagactggag 240
cctgaagatt ttgcagtgtg ttactgtcag cagggtatta tgcttcccc gacgttcggc 300
caggggacca aagtggaaat caaa 324

<210> 131
<211> 117
<212> PRT
<213> Artificial Sequence

<220>
<223> 16F8; VH

<400> 131

Glu Val Gln Leu Leu Glu Ser Gly Gly Gly Leu Val Gln Pro Gly Gly
1 5 10 15

Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Phe Thr Phe Ser Ser Tyr
20 25 30

Ala Met Ser Trp Val Arg Gln Ala Pro Gly Lys Gly Leu Glu Trp Val
35 40 45

Ser Ala Ile Leu Gly Ser Gly Gly Ser Thr Tyr Tyr Ala Asp Ser Val
50 55 60

eolf-seql.txt

Lys Gly Arg Phe Thr Ile Ser Arg Asp Asn Ser Lys Asn Thr Leu Tyr
65 70 75 80

Leu Gln Met Asn Ser Leu Arg Ala Glu Asp Thr Ala Val Tyr Tyr Cys
85 90 95

Ala Lys Gly Trp Phe Gly Gly Phe Asn Tyr Trp Gly Gln Gly Thr Leu
100 105 110

Val Thr Val Ser Ser
115

<210> 132
<211> 351
<212> DNA
<213> Artificial Sequence

<220>
<223> 16F8; VH

<400> 132
gaggtgcaat tggtggagtc tgggggaggc ttggtacagc ctgggggggtc cctgagactc 60
tcctgtgcag cctccggatt caccttttagc agttatgccca tgagctgggt ccgccaggct 120
ccagggaagg ggctggagtg ggtctcagct attcttggtg gtggtggttag cacatactac 180
gcagactccg tgaagggccg gttcaccatc tccagagaca attccaagaa cacgctgtat 240
ctgcagatga acagcctgag agccgaggac acggccgtat attactgtgc gaaaggggtgg 300
tttgggtggtt ttaactactg gggccaagga accctgggtca ccgtctcgtc c 351

<210> 133
<211> 108
<212> PRT
<213> Artificial Sequence

<220>
<223> 03C9; VL

<400> 133

Glu Ile Val Leu Thr Gln Ser Pro Gly Thr Leu Ser Leu Ser Pro Gly
1 5 10 15

Glu Arg Ala Thr Leu Ser Cys Arg Ala Ser Gln Ser Val Thr Ser Ser
20 25 30

Tyr Leu Ala Trp Tyr Gln Gln Lys Pro Gly Gln Ala Pro Arg Leu Leu
35 40 45

Ile Asn Val Gly Ser Arg Arg Ala Thr Gly Ile Pro Asp Arg Phe Ser
50 55 60

Gly Ser Gly Ser Gly Thr Asp Phe Thr Leu Thr Ile Ser Arg Leu Glu
65 70 75 80

eolf-seql.txt

Pro Glu Asp Phe Ala Val Tyr Tyr Cys Gln Gln Gly Ile Met Leu Pro
85 90 95

Pro Thr Phe Gly Gln Gly Thr Lys Val Glu Ile Lys
100 105

<210> 134
<211> 324
<212> DNA
<213> Artificial Sequence

<220>
<223> 03C9; VL

<400> 134
gaaatcgtgt taacgcagtc tccaggcacc ctgtctttgt ctccagggga aagagccacc 60
ctctcttgca gggccagtca gagtgttacc agtagctact tagcctggta ccagcagaaa 120
cctggccagg ctcccaggct cctcatcaat gtgggctccc gtagggccac tggcatccca 180
gacaggttca gtggcagtggt atccgggaca gacttcactc tcaccatcag cagactggag 240
cctgaagatt ttgcagtgtta ttactgtcag cagggtatta tgcttcccc gacgttcggc 300
caggggacca aagtggaaat caaa 324

<210> 135
<211> 117
<212> PRT
<213> Artificial Sequence

<220>
<223> 03C9; VH

<400> 135

Glu Val Gln Leu Leu Glu Ser Gly Gly Gly Leu Val Gln Pro Gly Gly
1 5 10 15

Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Phe Thr Phe Ser Ser Phe
20 25 30

Ala Met Ser Trp Val Arg Gln Ser Pro Gly Lys Gly Leu Glu Trp Val
35 40 45

Ser Ala Ile Ile Gly Ser Gly Ser Asn Thr Tyr Tyr Ala Asp Ser Val
50 55 60

Lys Gly Arg Phe Thr Ile Ser Arg Asp Asn Ser Lys Asn Thr Leu Tyr
65 70 75 80

Leu Gln Met Asn Ser Leu Arg Ala Glu Asp Thr Ala Val Tyr Tyr Cys
85 90 95

Ala Lys Gly Trp Phe Gly Gly Phe Asn Tyr Trp Gly Gln Gly Thr Leu
100 105 110

Val Thr Val Ser Ser
115

<210> 136
<211> 351
<212> DNA
<213> Artificial Sequence

<220>
<223> 03C9; VH

<400> 136
gaggtgcaat tgttggagtc tgggggaggc ttggtacagc ctgggggggtc cctgagactc 60
tcctgtgcag cctccggatt caccttttagc agttttgccca tgagctgggt ccgtcagtct 120
ccagggaagg ggctggagtg ggtctcagct attattggta gtggtagtaa cacatactac 180
gcagactccg tgaagggccg gttcaccatc tccagagaca attccaagaa cacgctgtat 240
ctgcagatga acagcctgag agccgaggac acggccgtat attactgtgc gaaaggggtgg 300
tttgggtggtt ttaactactg gggccaagga accctgggtca ccgtctcgtc c 351

<210> 137
<211> 108
<212> PRT
<213> Artificial Sequence

<220>
<223> 02D7; VL

<400> 137

Glu Ile Val Leu Thr Gln Ser Pro Gly Thr Leu Ser Leu Ser Pro Gly
1 5 10 15

Glu Arg Ala Thr Leu Ser Cys Arg Ala Ser Gln Ser Val Thr Ser Ser
20 25 30

Tyr Leu Ala Trp Tyr Gln Gln Lys Pro Gly Gln Ala Pro Arg Leu Leu
35 40 45

Ile Asn Val Gly Ser Arg Arg Ala Thr Gly Thr Pro Asp Arg Phe Ser
50 55 60

Gly Ser Gly Ser Gly Thr Asp Phe Thr Leu Thr Ile Ser Arg Leu Glu
65 70 75 80

Pro Glu Asp Phe Ala Val Tyr Tyr Cys Gln Gln Ala Ile Met Leu Pro
85 90 95

Pro Thr Phe Gly Gln Gly Thr Lys Val Glu Ile Lys
100 105

<210> 138
<211> 324

eolf-seql.txt

<212> DNA
<213> Artificial Sequence

<220>
<223> 02D7; VL

<400> 138
gaaatcgtgt taacgcagtc tccaggcacc ctgtctttgt ctccagggga aagagccacc 60
ctctcttgca gggccagtca gagtggtacc agtagctact tagcctggta ccagcagaaa 120
cctggccagg ctcccaggct cctcatcaat gtgggctccc gtagggccac tggcacccca 180
gacaggttca gtggcagtgg atccgggaca gacttcactc tcaccatcag cagactggag 240
cctgaagatt ttgcagtgtg ttactgtcag caggctatta tgcttcctcc gacgttcggc 300
caggggacca aagtggaaat caaa 324

<210> 139
<211> 117
<212> PRT
<213> Artificial Sequence

<220>
<223> 02D7; VH

<400> 139
Glu Val Gln Leu Leu Glu Ser Gly Gly Gly Leu Val Gln Pro Gly Gly
1 5 10 15
Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Phe Thr Phe Ser Ser Tyr
20 25 30
Ala Met Ser Trp Val Arg Gln Ala Pro Gly Lys Gly Leu Glu Trp Val
35 40 45
Ser Ala Ile Ser Gly Ser Gly Gly Ser Thr Tyr Tyr Ala Asp Ser Val
50 55 60
Lys Gly Arg Phe Thr Ile Ser Arg Asp Asn Ser Lys Asn Thr Leu Tyr
65 70 75 80
Leu Gln Met Asn Ser Leu Arg Ala Glu Asp Thr Ala Val Tyr Tyr Cys
85 90 95
Ala Lys Gly Trp Phe Gly Gly Phe Asn Tyr Trp Gly Gln Gly Thr Leu
100 105 110
Val Thr Val Ser Ser
115

<210> 140
<211> 351
<212> DNA
<213> Artificial Sequence

<220>

<223> 02D7; VH

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<400> 140
gaggtgcaat tgttggagtc tgggggaggc ttggtacagc ctgggggggc cctgagactc      60
tcctgtgcag cctccggatt cacctttagc agttatgcca tgagctgggt ccgccaggct      120
ccagggaagg ggctggagtg ggtctcagct attagtggta gtggtggtag cacatactac      180
gcagactccg tgaagggccg gttcaccatc tccagagaca attccaagaa cacgctgtat      240
ctgcagatga acagcctgag agccgaggac acggccgtat attactgtgc gaaaggggtg      300
tttggtggtt ttaactactg gggccaagga accctggtca ccgtctcgtc c              351

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<210> 141
<211> 108
<212> PRT
<213> Artificial Sequence

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<220>
<223> 28H1; VL

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<400> 141
Glu Ile Val Leu Thr Gln Ser Pro Gly Thr Leu Ser Leu Ser Pro Gly
1           5           10          15

Glu Arg Ala Thr Leu Ser Cys Arg Ala Ser Gln Ser Val Ser Arg Ser
          20          25          30

Tyr Leu Ala Trp Tyr Gln Gln Lys Pro Gly Gln Ala Pro Arg Leu Leu
          35          40          45

Ile Ile Gly Ala Ser Thr Arg Ala Thr Gly Ile Pro Asp Arg Phe Ser
          50          55          60

Gly Ser Gly Ser Gly Thr Asp Phe Thr Leu Thr Ile Ser Arg Leu Glu
65          70          75          80

Pro Glu Asp Phe Ala Val Tyr Tyr Cys Gln Gln Gly Gln Val Ile Pro
          85          90          95

Pro Thr Phe Gly Gln Gly Thr Lys Val Glu Ile Lys
          100         105

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<210> 142
<211> 324
<212> DNA
<213> Artificial Sequence

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<220>
<223> 28H1; VL

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<400> 142
gaaatcgtgt taacgcagtc tccaggcacc ctgtctttgt ctccagggga aagagccacc      60
ctctcttgca gggccagtca gagtgttagc cgcagctact tagcctggta ccagcagaaa      120
cctggccagg ctcccaggct cctcatcatt ggggcctcca ccagggccac tggcatccca      180

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eolf-seql.txt

gacaggttca gtggcagtgg atccgggaca gacttcactc tcaccatcag cagactggag 240
 cctgaagatt ttgcagtgtg ttactgtcag caggggtcagg ttattccccc tacgttcggc 300
 caggggacca aagtggaaat caaa 324

<210> 143
 <211> 116
 <212> PRT
 <213> Artificial Sequence

<220>
 <223> 28H1; VH

<400> 143

Glu Val Gln Leu Leu Glu Ser Gly Gly Gly Leu Val Gln Pro Gly Gly
 1 5 10 15

Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Phe Thr Phe Ser Ser His
 20 25 30

Ala Met Ser Trp Val Arg Gln Ala Pro Gly Lys Gly Leu Glu Trp Val
 35 40 45

Ser Ala Ile Trp Ala Ser Gly Glu Gln Tyr Tyr Ala Asp Ser Val Lys
 50 55 60

Gly Arg Phe Thr Ile Ser Arg Asp Asn Ser Lys Asn Thr Leu Tyr Leu
 65 70 75 80

Gln Met Asn Ser Leu Arg Ala Glu Asp Thr Ala Val Tyr Tyr Cys Ala
 85 90 95

Lys Gly Trp Leu Gly Asn Phe Asp Tyr Trp Gly Gln Gly Thr Leu Val
 100 105 110

Thr Val Ser Ser
 115

<210> 144
 <211> 348
 <212> DNA
 <213> Artificial Sequence

<220>
 <223> 28H1; VH

<400> 144

gaggtgcaat tggttgagtc tgggggaggc ttggtacagc ctgggggggtc cctgagactc 60

tcctgtgcag cctccggatt cacctttagc agtcatgcta tgagctgggt ccgccaggct 120

ccaggaagg ggctggagt ggtctcagc atttgggcta gtggggagca atactacgca 180

gactccgtga agggccggtt caccatctcc agagacaatt ccaagaacac gctgtatctg 240

cagatgaaca gcctgagagc cgaggacacg gccgtatatt actgtgcgaa aggggtggctg 300

eolf-seql.txt

ggtaattttg actactgggg ccaaggaacc ctggtcaccg tctcgagt

348

<210> 145
<211> 108
<212> PRT
<213> Artificial Sequence

<220>
<223> 22A3; VL

<400> 145

Glu Ile Val Leu Thr Gln Ser Pro Gly Thr Leu Ser Leu Ser Pro Gly
1 5 10 15

Glu Arg Ala Thr Leu Ser Cys Arg Ala Ser Gln Ser Val Thr Ser Ser
20 25 30

Tyr Leu Ala Trp Tyr Gln Gln Lys Pro Gly Gln Ala Pro Arg Leu Leu
35 40 45

Ile Asn Val Gly Ser Arg Arg Ala Thr Gly Ile Pro Asp Arg Phe Ser
50 55 60

Gly Ser Gly Ser Gly Thr Asp Phe Thr Leu Thr Ile Ser Arg Leu Glu
65 70 75 80

Pro Glu Asp Phe Ala Val Tyr Tyr Cys Gln Gln Gly Ile Met Leu Pro
85 90 95

Pro Thr Phe Gly Gln Gly Thr Lys Val Glu Ile Lys
100 105

<210> 146
<211> 324
<212> DNA
<213> Artificial Sequence

<220>
<223> 22A3; VL

<400> 146

gaaatcgtgt taacgcagtc tccaggcacc ctgtctttgt ctccagggga aagagccacc 60
ctctcttgca gggccagtca gagggttacc agtagctact tagcctggta ccagcagaaa 120
cctggccagg ctcccaggct cctcatcaat gtgggctccc gtagggccac tggcatccca 180
gacaggttca gtggcagtg atccgggaca gacttcactc tcaccatcag cagactggag 240
cctgaagatt ttgcagtgt ttactgtcag cagggtatta tgcttcccc gacgttcggc 300
caggggacca aagtggaaat caaa 324

<210> 147
<211> 117
<212> PRT
<213> Artificial Sequence

eolf-seql.txt

<220>

<223> 22A3; VH

<400> 147

Glu Val Gln Leu Leu Glu Ser Gly Gly Gly Leu Val Gln Pro Gly Gly
1 5 10 15

Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Phe Thr Phe Ser Ser Tyr
20 25 30

Ala Met Ser Trp Val Arg Gln Ala Pro Gly Lys Gly Leu Glu Trp Val
35 40 45

Ser Ala Ile Ile Gly Ser Gly Ser Ile Thr Tyr Tyr Ala Asp Ser Val
50 55 60

Lys Gly Arg Phe Thr Ile Ser Arg Asp Asn Ser Lys Asn Thr Leu Tyr
65 70 75 80

Leu Gln Met Asn Ser Leu Arg Ala Glu Asp Thr Ala Val Tyr Tyr Cys
85 90 95

Ala Lys Gly Trp Phe Gly Gly Phe Asn Tyr Trp Gly Gln Gly Thr Leu
100 105 110

Val Thr Val Ser Ser
115

<210> 148

<211> 351

<212> DNA

<213> Artificial Sequence

<220>

<223> 22A3; VH

<400> 148

gaggtgcaat tgttggagtc tgggggaggc ttggtacagc ctgggggggtc cctgagactc 60

tcctgtgcag cctccggatt cacctttagc agttatgccca tgagctgggt ccgccaggct 120

ccaggaagg ggctggagtg ggtctcagct attatttgta gtggtagtat cacatactac 180

gcagactccg tgaagggccg gttcaccatc tccagagaca attccaagaa cacgctgtat 240

ctgcagatga acagcctgag agccgaggac acggccgtat attactgtgc gaaaggggtg 300

tttggtggtt ttaactactg gggccaagga accctggtca ccgtctcgag t 351

<210> 149

<211> 108

<212> PRT

<213> Artificial Sequence

<220>

<223> 29B11; VL

eolf-seql.txt

<400> 149

Glu Ile Val Leu Thr Gln Ser Pro Gly Thr Leu Ser Leu Ser Pro Gly
1 5 10 15

Glu Arg Ala Thr Leu Ser Cys Arg Ala Ser Gln Ser Val Thr Ser Ser
20 25 30

Tyr Leu Ala Trp Tyr Gln Gln Lys Pro Gly Gln Ala Pro Arg Leu Leu
35 40 45

Ile Asn Val Gly Ser Arg Arg Ala Thr Gly Ile Pro Asp Arg Phe Ser
50 55 60

Gly Ser Gly Ser Gly Thr Asp Phe Thr Leu Thr Ile Ser Arg Leu Glu
65 70 75 80

Pro Glu Asp Phe Ala Val Tyr Tyr Cys Gln Gln Gly Ile Met Leu Pro
85 90 95

Pro Thr Phe Gly Gln Gly Thr Lys Val Glu Ile Lys
100 105

<210> 150

<211> 324

<212> DNA

<213> Artificial Sequence

<220>

<223> 29B11; VL

<400> 150

gaaatcgtgt taacgcagtc tccaggcacc ctgtctttgt ctccagggga aagagccacc	60
ctctcttgca gggccagtca gagtgttacc agtagctact tagcctggta ccagcagaaa	120
cctggccagg ctcccaggct cctcatcaat gtgggctccc gtagggccac tggcatccca	180
gacaggttca gtggcagtggt atccgggaca gacttcactc tcaccatcag cagactggag	240
cctgaagatt ttgcagtgtta ttactgtcag caggggtatta tgcttcccc gacgttcggc	300
caggggacca aagtggaaat caaa	324

<210> 151

<211> 117

<212> PRT

<213> Artificial Sequence

<220>

<223> 29B11; VH

<400> 151

Glu Val Gln Leu Leu Glu Ser Gly Gly Gly Leu Val Gln Pro Gly Gly
1 5 10 15

Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Phe Thr Phe Ser Ser Tyr
20 25 30

eolf-seql.txt

Ala Met Ser Trp Val Arg Gln Ala Pro Gly Lys Gly Leu Glu Trp Val
35 40 45

Ser Ala Ile Ile Gly Ser Gly Gly Ile Thr Tyr Tyr Ala Asp Ser Val
50 55 60

Lys Gly Arg Phe Thr Ile Ser Arg Asp Asn Ser Lys Asn Thr Leu Tyr
65 70 75 80

Leu Gln Met Asn Ser Leu Arg Ala Glu Asp Thr Ala Val Tyr Tyr Cys
85 90 95

Ala Lys Gly Trp Phe Gly Gly Phe Asn Tyr Trp Gly Gln Gly Thr Leu
100 105 110

Val Thr Val Ser Ser
115

<210> 152
<211> 351
<212> DNA
<213> Artificial Sequence

<220>
<223> 29B11; VH

<400> 152
gaggtgcaat tgttggagtc tgggggaggc ttggtacagc ctgggggggtc cctgagactc 60
tcctgtgcag cctccggatt cacctttagc agttatgcta tgagctgggt ccgccaggct 120
ccaggaagg ggctggagtg ggtctcagct attattggtg gtggtggtat cacatactac 180
gcagactccg tgaagggccg gttcaccatc tccagagaca attccaagaa cacgctgtat 240
ctgcagatga acagcctgag agccgaggac acggccgtat attactgtgc gaaaggggtg 300
tttgggtggtt ttaactactg gggccaagga accctgggtca ccgtctcgag t 351

<210> 153
<211> 108
<212> PRT
<213> Artificial Sequence

<220>
<223> 23C10; VL

<400> 153

Glu Ile Val Leu Thr Gln Ser Pro Gly Thr Leu Ser Leu Ser Pro Gly
1 5 10 15

Glu Arg Ala Thr Leu Ser Cys Arg Ala Ser Gln Ser Val Ser Arg Ser
20 25 30

Tyr Leu Ala Trp Tyr Gln Gln Lys Pro Gly Gln Ala Pro Arg Leu Leu
35 40 45

eolf-seql.txt

Ile Ile Gly Ala Ser Thr Arg Ala Thr Gly Ile Pro Asp Arg Phe Ser
50 55 60

Gly Ser Gly Ser Gly Thr Asp Phe Thr Leu Thr Ile Ser Arg Leu Glu
65 70 75 80

Pro Glu Asp Phe Ala Val Tyr Tyr Cys Gln Gln Gly Gln Val Ile Pro
85 90 95

Pro Thr Phe Gly Gln Gly Thr Lys Val Glu Ile Lys
100 105

<210> 154
<211> 324
<212> DNA
<213> Artificial Sequence

<220>
<223> 23C10; VL

<400> 154
gaaatcgtgt taacgcagtc tccaggcacc ctgtctttgt ctccagggga aagagccacc 60
ctctcttgca gggccagtca gagtgttagc cgcagctact tagcctggta ccagcagaaa 120
cctggccagg ctcccaggct cctcatcatt ggggcctcca ccagggccac tggcatccca 180
gacaggttca gtggcagtgg atccgggaca gacttcactc tcaccatcag cagactggag 240
cctgaagatt ttgcagtgtg ttactgtcag cagggtcagg ttattccccc tacgttcggc 300
caggggacca aagtggaaat caaa 324

<210> 155
<211> 117
<212> PRT
<213> Artificial Sequence

<220>
<223> 23C10; VH

<400> 155

Glu Val Gln Leu Leu Glu Ser Gly Gly Gly Leu Val Gln Pro Gly Gly
1 5 10 15

Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Phe Thr Phe Ser Ser Ser
20 25 30

Ala Met Ser Trp Val Arg Gln Ala Pro Gly Lys Gly Leu Glu Trp Val
35 40 45

Ser Ala Ile Ser Thr Asn Gly Asn Tyr Thr Tyr Tyr Ala Asp Ser Val
50 55 60

Lys Gly Arg Phe Thr Ile Ser Arg Asp Asn Ser Lys Asn Thr Leu Tyr
65 70 75 80

eolf-seql.txt

Leu Gln Met Asn Ser Leu Arg Ala Glu Asp Thr Ala Val Tyr Tyr Cys
85 90 95

Ala Lys Gly Trp Leu Gly Asn Phe Asp Tyr Trp Gly Gln Gly Thr Leu
100 105 110

Val Thr Val Ser Ser
115

<210> 156
<211> 351
<212> DNA
<213> Artificial Sequence

<220>
<223> 23C10; VH

<400> 156
gaggtgcaat tggtggagtc tgggggaggc ttggtacagc ctgggggggct cctgagactc 60
tcctgtgcag cctccggatt cacctttagc agttctgcca tgagctgggt ccgccaggct 120
ccaggaagg ggctggagtg ggtctcagct attagtacta atggttaatta tacatactac 180
gcagactccg tgaagggccg gttcaccatc tccagagaca attccaagaa cacgctgtat 240
ctgcagatga acagcctgag agccgaggac acggccgtat attactgtgc gaaaggggtg 300
ctgggtaatt ttgactactg gggccaagga accctgggtca ccgtctcgag t 351

<210> 157
<211> 107
<212> PRT
<213> Artificial Sequence

<220>
<223> 2B10_C3B6; VL

<400> 157

Asp Ile Gln Met Thr Gln Ser Pro Ser Ser Leu Ser Ala Ser Val Gly
1 5 10 15

Asp Arg Val Thr Ile Thr Cys Arg Ala Ser Gln Gly Ile Arg Asn Asp
20 25 30

Leu Gly Trp Tyr Gln Gln Lys Pro Gly Lys Ala Pro Lys Arg Leu Ile
35 40 45

Tyr Ala Ala Ser Ser Leu Gln Ser Gly Val Pro Ser Arg Phe Ser Gly
50 55 60

Ser Gly Ser Gly Thr Glu Phe Thr Leu Thr Ile Ser Ser Leu Gln Pro
65 70 75 80

Glu Asp Phe Ala Thr Tyr Tyr Cys Leu Gln Asn Gly Leu Gln Pro Ala
85 90 95

eolf-seql.txt

Thr Phe Gly Gln Gly Thr Lys Val Glu Ile Lys
100 105

<210> 158
<211> 321
<212> DNA
<213> Artificial Sequence

<220>
<223> 2B10_C3B6; VL

<400> 158
gatatccaga tgaccagtc tccatcctcc ctgtctgcat ctgtcggaga ccgggtcacc 60
atcacctgcc gggcaagtca gggcattaga aatgatttag gctggtacca gcagaagcca 120
gggaaagccc ctaagcgcct gatctatgct gcatccagtt tgcagagtgg cgtcccatca 180
aggttcagcg gcagtggatc cgggacagag ttcactctca ccatcagcag cttgcagcct 240
gaagattttg ccacctatta ctgcttgag aatgggtctgc agcccgcgac gtttggccag 300
ggcaccaaag tcgagatcaa g 321

<210> 159
<211> 121
<212> PRT
<213> Artificial Sequence

<220>
<223> 2B10_C3B6; VH

<400> 159
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 Gly Thr Phe Ser Ser Tyr
20 25 30
Ala Ile Ser Trp Val Arg Gln Ala Pro Gly Gln Gly Leu Glu Trp Met
35 40 45
Gly Ala Ile Ile Pro Ile Leu Gly Ile Ala Asn Tyr Ala Gln Lys Phe
50 55 60
Gln 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 Leu Tyr Gly Tyr Ala Tyr Tyr Gly Ala Phe Asp Tyr Trp Gly
100 105 110
Gln Gly Thr Thr Val Thr Val Ser Ser
115 120

eolf-seql.txt

<210> 160
 <211> 363
 <212> DNA
 <213> Artificial Sequence

<220>
 <223> 2B10_C3B6; VH

<400> 160
 caggtgcaat tgggtgcagtc tggggctgag gtgaagaagc ctgggtcctc ggtgaaggtc 60
 tcctgcaagg cctccggagg cacattcagc agctacgcta taagctgggt gcgacaggcc 120
 cctggacaag ggctcgagtg gatgggagct atcatccga tccttggtat cgcaaactac 180
 gcacagaagt tccagggcag ggtcaccatt actgcagaca aatccacgag cacagcctac 240
 atggagctga gcagcctgag atctgaggac accgccgtgt attactgtgc gagactgtac 300
 ggttacgctt actacggtgc ttttgactac tggggccaag ggaccaccgt gaccgtctcc 360
 tca 363

<210> 161
 <211> 107
 <212> PRT
 <213> Artificial Sequence

<220>
 <223> 2B10_6A12; VL

<400> 161

Asp Ile Gln Met Thr Gln Ser Pro Ser Ser Leu Ser Ala Ser Val Gly
 1 5 10 15

Asp Arg Val Thr Ile Thr Cys Arg Ala Ser Gln Gly Ile Arg Asn Asp
 20 25 30

Leu Gly Trp Tyr Gln Gln Lys Pro Gly Lys Ala Pro Lys Arg Leu Ile
 35 40 45

Tyr Ala Ala Ser Ser Leu Gln Ser Gly Val Pro Ser Arg Phe Ser Gly
 50 55 60

Ser Gly Ser Gly Thr Glu Phe Thr Leu Thr Ile Ser Ser Leu Gln Pro
 65 70 75 80

Glu Asp Phe Ala Thr Tyr Tyr Cys Leu Gln Asn Gly Leu Gln Pro Ala
 85 90 95

Thr Phe Gly Gln Gly Thr Lys Val Glu Ile Lys
 100 105

<210> 162
 <211> 321
 <212> DNA
 <213> Artificial Sequence

eolf-seql.txt

<220>

<223> 2B10_6A12; VL

<400> 162

gatatccaga tgaccagtc tccatcctcc ctgtctgcat ctgtcggaga ccgggtcacc 60
 atcacctgcc gggcaagtca gggcattaga aatgatttag gctggtacca gcagaagcca 120
 gggaaagccc ctaagcgcct gatctatgct gcatccagtt tgcagagtgg cgtcccatca 180
 aggttcagcg gcagtggatc cgggacagag ttcactctca ccatcagcag cttgcagcct 240
 gaagattttg ccacctatta ctgcttgag aatgggtctgc agcccgcgac gtttggccag 300
 ggcaccaaag tcgagatcaa g 321

<210> 163

<211> 121

<212> PRT

<213> Artificial Sequence

<220>

<223> 2B10_6A12; VH

<400> 163

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 Gly Thr Phe Ser Ser Tyr
 20 25 30
 Ala Ile Ser Trp Val Arg Gln Ala Pro Gly Gln Gly Leu Glu Trp Met
 35 40 45
 Gly Val Ile Ile Pro Ile Leu Gly Thr Ala Asn Tyr Ala Gln Lys Phe
 50 55 60
 Gln 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 Leu Tyr Gly Tyr Ala Tyr Tyr Gly Ala Phe Asp Tyr Trp Gly
 100 105 110
 Gln Gly Thr Thr Val Thr Val Ser Ser
 115 120

<210> 164

<211> 363

<212> DNA

<213> Artificial Sequence

<220>

<223> 2B10_6A12; VH

eolf-seql.txt

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<400> 164
caggtgcaat tgggtgcagtc tggggctgag gtgaagaagc ctgggtcctc ggtgaaggtc      60
tcctgcaagg cctccggagg cacattcagc agctatgcta taagctgggt gcgacaggcc      120
cctggacaag ggctcgagtg gatgggagtg atcatcccta tccttggtac cgcaaactac      180
gcacagaagt tccagggcag ggtcaccatt actgcagaca aatccacgag cacagcctac      240
atggagctga gcagcctgag atctgaggac accgccgtgt attactgtgc gagactgtac      300
ggttacgctt actacggtgc ttttgactac tggggccaag ggaccaccgt gaccgtctcc      360
tca                                                                    363
```

```
<210> 165
<211> 107
<212> PRT
<213> Artificial Sequence
```

```
<220>
<223> 2B10_C3A6; VL
```

```
<400> 165
Asp Ile Gln Met Thr Gln Ser Pro Ser Ser Leu Ser Ala Ser Val Gly
1           5           10           15

Asp Arg Val Thr Ile Thr Cys Arg Ala Ser Gln Gly Ile Arg Asn Val
          20           25           30

Leu Gly Trp Tyr Gln Gln Lys Pro Gly Lys Ala Pro Lys Arg Leu Ile
          35           40           45

Tyr Asp Ser Ser Ser Leu Gln Ser Gly Val Pro Ser Arg Phe Ser Gly
          50           55           60

Gly Gly Ser Gly Thr Glu Phe Thr Leu Thr Ile Ser Ser Leu Gln Pro
65           70           75           80

Glu Asp Phe Ala Thr Tyr Tyr Cys Leu Gln Asn Gly Leu Gln Pro Ala
          85           90           95

Thr Phe Gly Gln Gly Thr Lys Val Glu Ile Lys
          100          105
```

```
<210> 166
<211> 321
<212> DNA
<213> Artificial Sequence
```

```
<220>
<223> 2B10_C3A6; VL
```

```
<400> 166
gacatccaga tgaccagtc tccttcctcc ctgtctgcat ctgtcggaga ccgggtcacc      60
atcacctgcc gggcaagtca gggcattcgt aatgttttag gctggtacca gcagaagcca      120
gggaaagccc ctaagcgcct gatctatgat tcgtccagtt tgcagagtgg cgtcccatca      180
```

eolf-seql.txt

aggttcagcg gcggtggatc cgggacagag ttcactctca ccatcagcag cttgcagcct 240
gaagattttg ccacctatta ctgcttgacag aatgggtctgc agcccgcgac gtttggccag 300
ggcaccaaag tcgagatcaa g 321

<210> 167
<211> 121
<212> PRT
<213> Artificial Sequence

<220>
<223> 2B10_C3A6; VH

<400> 167

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 Gly Thr Phe Ser Ser Tyr
20 25 30

Ala Ile Ser Trp Val Arg Gln Ala Pro Gly Gln Gly Leu Glu Trp Met
35 40 45

Gly Gly Ile Ile Pro Ile Phe Gly Thr Ala Asn Tyr Ala Gln Lys Phe
50 55 60

Gln 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 Leu Tyr Gly Tyr Ala Tyr Tyr Gly Ala Phe Asp Tyr Trp Gly
100 105 110

Gln Gly Thr Thr Val Thr Val Ser Ser
115 120

<210> 168
<211> 363
<212> DNA
<213> Artificial Sequence

<220>
<223> 2B10_C3A6; VH

<400> 168

caggtgcaat tggtgcagtc tggggctgag gtgaagaagc ctgggtcctc ggtgaaggtc 60
tcctgcaagg cctccggagg cacattcagc agctacgcta taagctgggt gcgacaggcc 120
cctggacaag ggctcgagtg gatgggaggg atcatcccta tctttggtac agcaaactac 180
gcacagaagt tccagggcag ggtcaccatt actgcagaca aatccacgag cacagcctac 240
atggagctga gcagcctgag atctgaggac accgccgtgt attactgtgc gagactgtac 300

eolf-seql.txt

ggttacgctt actacggtgc ttttgactac tggggccaag ggaccaccgt gaccgtctcc 360
tca 363

<210> 169
<211> 107
<212> PRT
<213> Artificial Sequence

<220>
<223> 2B10_D1A2_wt; VL

<400> 169

Asp Ile Gln Met Thr Gln Ser Pro Ser Ser Leu Ser Ala Ser Val Gly
1 5 10 15

Asp Arg Val Thr Ile Thr Cys Arg Ala Ser Gln Gly Ile Arg Asn Val
20 25 30

Leu Gly Trp Tyr Gln Gln Lys Pro Gly Lys Ala Pro Lys Arg Leu Ile
35 40 45

Tyr Asp Ala Tyr Ser Leu Gln Ser Gly Val Pro Ser Arg Phe Ser Gly
50 55 60

Gly Gly Ser Gly Thr Glu Phe Thr Leu Thr Ile Ser Ser Leu Gln Pro
65 70 75 80

Glu Asp Phe Ala Thr Tyr Tyr Cys Leu Gln Asn Gly Leu Gln Pro Ala
85 90 95

Thr Phe Gly Gln Gly Thr Lys Val Glu Ile Lys
100 105

<210> 170
<211> 321
<212> DNA
<213> Artificial Sequence

<220>
<223> 2B10_D1A2_wt; VL

<400> 170

gatatccaga tgaccagtc tccatcctcc ctgtctgcat ctgtcggaga ccgggtcacc 60
atcacctgcc gggcaagtca ggggattcgt aatgttttag gctggtacca gcagaagcca 120
gggaaagccc ctaagcgcct gatctatgat gcttacagct tgcagagtgg cgtcccatca 180
aggttcagcg gcggtggatc cgggacagag ttcactctca ccatcagcag cttgcagcct 240
gaagattttg ccacctatta ctgcttgag aatgggtctgc agcccgcgac gtttggccag 300
ggcaccaaag tcgagatcaa g 321

<210> 171
<211> 121

<212> PRT
 <213> Artificial Sequence

<220>
 <223> 2B10_D1A2_wt; VH

<400> 171

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 Gly Thr Phe Ser Ser Tyr
 20 25 30

Ala Ile Ser Trp Val Arg Gln Ala Pro Gly Gln Gly Leu Glu Trp Met
 35 40 45

Gly Gly Ile Ile Pro Ile Phe Gly Thr Ala Asn Tyr Ala Gln Lys Phe
 50 55 60

Gln 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 Leu Tyr Gly Tyr Ala Tyr Tyr Gly Ala Phe Asp Tyr Trp Gly
 100 105 110

Gln Gly Thr Thr Val Thr Val Ser Ser
 115 120

<210> 172
 <211> 363
 <212> DNA
 <213> Artificial Sequence

<220>
 <223> 2B10_D1A2_wt; VH

<400> 172
 caggtgcaat tgggtgcagtc tggggctgag gtgaagaagc ctgggtcctc ggtgaaggtc 60
 tcctgcaagg cctccgagg cacattcagc agctacgcta taagctgggt gcgacaggcc 120
 cctggacaag ggctcgagt gatgggaggg atcatcccta tctttggtac agcaaactac 180
 gcacagaagt tccagggcag ggtcaccatt actgcagaca aatccacgag cacagcctac 240
 atggagctga gcagcctgag atctgaggac accgccgtgt attactgtgc gagactgtac 300
 ggttacgctt actacggtgc ttttgactac tggggccaag ggaccaccgt gaccgtctcc 360
 tca 363

<210> 173
 <211> 107
 <212> PRT
 <213> Artificial Sequence

eolf-seql.txt

<220>

<223> 2B10_D1A2_VD; VL

<400> 173

Asp Ile Gln Met Thr Gln Ser Pro Ser Ser Leu Ser Ala Ser Val Gly
1 5 10 15

Asp Arg Val Thr Ile Thr Cys Arg Ala Ser Gln Gly Ile Arg Asn Asp
20 25 30

Leu Gly Trp Tyr Gln Gln Lys Pro Gly Lys Ala Pro Lys Arg Leu Ile
35 40 45

Tyr Asp Ala Tyr Ser Leu Gln Ser Gly Val Pro Ser Arg Phe Ser Gly
50 55 60

Gly Gly Ser Gly Thr Glu Phe Thr Leu Thr Ile Ser Ser Leu Gln Pro
65 70 75 80

Glu Asp Phe Ala Thr Tyr Tyr Cys Leu Gln Asn Gly Leu Gln Pro Ala
85 90 95

Thr Phe Gly Gln Gly Thr Lys Val Glu Ile Lys
100 105

<210> 174

<211> 321

<212> DNA

<213> Artificial Sequence

<220>

<223> 2B10_D1A2_VD; VL

<400> 174

gacatccaga tgaccagtc tccatcctcc ctgtctgcat ctgtcggaga ccgggtcacc 60
atcacctgcc gggcaagtca ggggattcgt aatgatttag gctggtacca gcagaagcca 120
gggaaagccc ctaagcgcct gatctatgat gcttacagct tgcagagtgg cgtcccatca 180
aggttcagcg gcggtggatc cgggacagag ttcactctca ccatcagcag cttgcagcct 240
gaagattttg ccacctatta ctgcttgag aatgggtctgc agcccgcgac gtttgccag 300
ggcaccaaag tcgagatcaa g 321

<210> 175

<211> 121

<212> PRT

<213> Artificial Sequence

<220>

<223> 2B10_D1A2_VD; VH

<400> 175

Gln Val Gln Leu Val Gln Ser Gly Ala Glu Val Lys Lys Pro Gly Ser
1 5 10 15

eolf-seql.txt

Ser Val Lys Val Ser Cys Lys Ala Ser Gly Gly Thr Phe Ser Ser Tyr
20 25 30

Ala Ile Ser Trp Val Arg Gln Ala Pro Gly Gln Gly Leu Glu Trp Met
35 40 45

Gly Gly Ile Ile Pro Ile Phe Gly Thr Ala Asn Tyr Ala Gln Lys Phe
50 55 60

Gln 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 Leu Tyr Gly Tyr Ala Tyr Tyr Gly Ala Phe Asp Tyr Trp Gly
100 105 110

Gln Gly Thr Thr Val Thr Val Ser Ser
115 120

<210> 176
<211> 363
<212> DNA
<213> Artificial Sequence

<220>
<223> 2B10_D1A2_VD; VH

<400> 176
caggtgcaat tgggtgcagtc tggggctgag gtgaagaagc ctgggtcctc ggtgaaggtc 60
tcctgcaagg cctccggagg cacattcagc agctacgcta taagctgggt gcgacaggcc 120
cctggacaag ggctcgagtg gatgggaggg atcatcccta tctttggtac agcaaactac 180
gcacagaagt tccagggcag ggtcaccatt actgcagaca aatccacgag cacagcctac 240
atggagctga gcagcctgag atctgaggac accgccgtgt attactgtgc gagactgtac 300
ggttacgctt actacggtgc ttttgactac tggggccaag ggaccaccgt gaccgtctcc 360
tca 363

<210> 177
<211> 107
<212> PRT
<213> Artificial Sequence

<220>
<223> 2B10_07D8; VL

<400> 177

Asp Ile Gln Met Thr Gln Ser Pro Ser Ser Leu Ser Ala Ser Val Gly
1 5 10 15

eolf-seql.txt

Asp Arg Val Thr Ile Thr Cys Arg Ala Ser Gln Ser Ile Arg Asn Val
20 25 30

Leu Gly Trp Tyr Gln Gln Lys Pro Gly Lys Ala Pro Lys Arg Leu Ile
35 40 45

Tyr Asp Val Ser Ser Leu Gln Ser Gly Val Pro Ser Arg Phe Ser Gly
50 55 60

Gly Gly Ser Gly Thr Glu Phe Thr Leu Thr Ile Ser Ser Leu Gln Pro
65 70 75 80

Glu Asp Phe Ala Thr Tyr Tyr Cys Leu Gln Asn Gly Leu Gln Pro Ala
85 90 95

Thr Phe Gly Gln Gly Thr Lys Val Glu Ile Lys
100 105

<210> 178
<211> 321
<212> DNA
<213> Artificial Sequence

<220>
<223> 2B10_07D8; VL

<400> 178
gatatccaga tgaccagtc tccatcctcc ctgtctgcat ctgtcggaga ccgggtcacc 60
atcacctgcc gggcaagtca gagcattcgt aatgttttag gctggtacca gcagaagcca 120
gggaaagccc ctaagcgcct gatctatgat gtgtccagtt tgcagagtgg cgtcccatca 180
aggttcagcg gcggtggatc cgggacagag ttcactctca ccatcagcag cttgcagcct 240
gaagattttg ccacctatta ctgcttgcag aatggtctgc agcccgcgac gtttggccag 300
ggcaccaaag tcgagatcaa g 321

<210> 179
<211> 121
<212> PRT
<213> Artificial Sequence

<220>
<223> 2B10_07D8; VH

<400> 179
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 Gly Thr Phe Ser Ser Tyr
20 25 30

Ala Ile Ser Trp Val Arg Gln Ala Pro Gly Gln Gly Leu Glu Trp Met
35 40 45

eolf-seql.txt

Gly Gly Ile Ile Pro Ile Phe Gly Thr Ala Asn Tyr Ala Gln Lys Phe
50 55 60

Gln 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 Leu Tyr Gly Tyr Ala Tyr Tyr Gly Ala Phe Asp Tyr Trp Gly
100 105 110

Gln Gly Thr Thr Val Thr Val Ser Ser
115 120

<210> 180
<211> 363
<212> DNA
<213> Artificial Sequence

<220>
<223> 2B10_07D8; VH

<400> 180
caggtgcaat tgggtgcagtc tggggctgag gtgaagaagc ctgggtcctc ggtgaaggtc 60
tcctgcaagg cctccggagg cacattcagc agctacgcta taagctgggt gcgacaggcc 120
cctggacaag ggctcgagt gatgggaggg atcatcccta tctttggtac agcaaactac 180
gcacagaagt tccagggcag ggtcaccatt actgcagaca aatccacgag cacagcctac 240
atggagctga gcagcctgag atctgaggac accgccgtgt attactgtgc gagactgtac 300
ggttacgctt actacggtgc ttttgactac tggggccaag ggaccaccgt gaccgtctcc 360
tca 363

<210> 181
<211> 107
<212> PRT
<213> Artificial Sequence

<220>
<223> 2B10_01F7; VL

<400> 181

Asp Ile Gln Met Thr Gln Ser Pro Ser Ser Leu Ser Ala Ser Val Gly
1 5 10 15

Asp Arg Val Thr Ile Thr Cys Arg Ala Ser Gln Gly Ile Arg Asn Val
20 25 30

Leu Gly Trp Tyr Gln Gln Lys Pro Gly Lys Ala Pro Lys Arg Leu Ile
35 40 45

Tyr Asp Ala Ser Ser Leu Gln Ser Gly Val Pro Ser Arg Phe Ser Gly
50 55 60

eolf-seql.txt

Gly Gly Ser Gly Thr Glu Phe Thr Leu Thr Ile Ser Ser Leu Gln Pro
65 70 75 80

Glu Asp Phe Ala Thr Tyr Tyr Cys Leu Gln Asn Gly Leu Gln Pro Ala
85 90 95

Thr Phe Gly Gln Gly Thr Lys Val Glu Ile Lys
100 105

<210> 182
<211> 321
<212> DNA
<213> Artificial Sequence

<220>
<223> 2B10_01F7; VL

<400> 182
gatatccaga tgaccagtc tccatcctcc ctgtctgcat ctgtcggaga ccgggtcacc 60
atcacctgcc gggcaagtca gggcattcgt aatgttttag gctggtacca gcagaagcca 120
gggaaagccc ctaagcgcct gatctatgat gcgtccagtt tgcagagtgg cgtcccatca 180
aggttcagcg gcggtggatc cgggacagag ttcactctca ccatcagcag cttgcagcct 240
gaagattttg ccacctatta ctgcctgcag aatgggtctgc agcccgcgac gtttggccag 300
ggcaccaaag tcgagatcaa g 321

<210> 183
<211> 121
<212> PRT
<213> Artificial Sequence

<220>
<223> 2B10_01F7; VH

<400> 183

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 Gly Thr Phe Ser Ser Tyr
20 25 30

Ala Ile Ser Trp Val Arg Gln Ala Pro Gly Gln Gly Leu Glu Trp Met
35 40 45

Gly Gly Ile Ile Pro Ile Phe Gly Thr Ala Asn Tyr Ala Gln Lys Phe
50 55 60

Gln 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

eolf-seql.txt

Ala Arg Leu Tyr Gly Tyr Ala Tyr Tyr Gly Ala Phe Asp Tyr Trp Gly
100 105 110

Gln Gly Thr Thr Val Thr Val Ser Ser
115 120

<210> 184
<211> 363
<212> DNA
<213> Artificial Sequence

<220>
<223> 2B10_01F7; VH

<400> 184
caggtgcaat tgggtgcagtc tggggctgag gtgaagaagc ctgggtcctc ggtgaaggtc 60
tcctgcaagg cctccggagg cacattcagc agctacgcta taagctgggt gcgacaggcc 120
cctggacaag ggctcgagtg gatgggaggg atcatcccta tctttggtac agcaaactac 180
gcacagaagt tccagggcag ggtcaccatt actgcagaca aatccacgag cacagcctac 240
atggagctga gcagcctgag atctgaggac accgccgtgt attactgtgc gagactgtac 300
ggttacgctt actacggtgc ttttgactac tggggccaag ggaccaccgt gaccgtctcc 360
tca 363

<210> 185
<211> 107
<212> PRT
<213> Artificial Sequence

<220>
<223> 2B10_6H10; VL

<400> 185
Asp Ile Gln Met Thr Gln Ser Pro Ser Ser Leu Ser Ala Ser Val Gly
1 5 10 15

Asp Arg Val Thr Ile Thr Cys Arg Ala Ser Gln Gly Ile Arg Asn Val
20 25 30

Leu Gly Trp Tyr Gln Gln Lys Pro Gly Lys Ala Pro Lys Arg Leu Ile
35 40 45

Gln Ala Ala Thr Ser Leu Gln Ser Gly Val Pro Ser Arg Phe Ser Gly
50 55 60

Gly Gly Ser Gly Thr Glu Phe Thr Leu Thr Ile Ser Ser Leu Gln Pro
65 70 75 80

Glu Asp Phe Ala Thr Tyr Tyr Cys Leu Gln Asn Gly Leu Gln Pro Ala
85 90 95

eolf-seql.txt

Thr Phe Gly Gln Gly Thr Lys Val Glu Ile Lys
 100 105

<210> 186
 <211> 321
 <212> DNA
 <213> Artificial Sequence

<220>
 <223> 2B10_6H10; VL

<400> 186
 gacatccaga tgaccagtc tccatcctcc ctgtctgcat ctgtcggaga ccgggtcacc 60
 atcacctgcc gggcaagtca gggcattcgt aatgttttag gctggtacca gcagaagcca 120
 gggaaagccc ctaagcgcct gatccaggct gctaccagtt tgcagagtgg cgtcccatca 180
 aggttcagcg gcggtggatc cgggacagag ttcactctca ccatcagcag cttgcagcct 240
 gaagattttg ccacctatta ctgcttgag aatgggtctgc agcccgcgac gtttggccag 300
 ggcaccaaag tcgagatcaa g 321

<210> 187
 <211> 121
 <212> PRT
 <213> Artificial Sequence

<220>
 <223> 2B10_6H10; VH

<400> 187

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 Gly Thr Phe Ser Ser Tyr
 20 25 30

Ala Ile Ser Trp Val Arg Gln Ala Pro Gly Gln Gly Leu Glu Trp Met
 35 40 45

Gly Gly Ile Ile Pro Ile Phe Gly Thr Ala Asn Tyr Ala Gln Lys Phe
 50 55 60

Gln 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 Leu Tyr Gly Tyr Ala Tyr Tyr Gly Ala Phe Asp Tyr Trp Gly
 100 105 110

Gln Gly Thr Thr Val Thr Val Ser Ser
 115 120

eolf-seql.txt

<210> 188
 <211> 363
 <212> DNA
 <213> Artificial Sequence

<220>
 <223> 2B10_6H10; VH

<400> 188
 caggtgcaat tgggtgcagtc tggggctgag gtgaagaagc ctgggtcctc ggtgaaggtc 60
 tcctgcaagg cctccggagg cacattcagc agctacgcta taagctgggt gcgacaggcc 120
 cctggacaag ggctcgagt gatgggaggg atcatcccta tctttggtac agcaaactac 180
 gcacagaagt tccagggcag ggtcaccatt actgcagaca aatccacgag cacagcctac 240
 atggagctga gcagcctgag atctgaggac accgccgtgt attactgtgc gagactgtac 300
 gggtacgctt actacggtgc ttttgactac tggggccaag ggaccaccgt gaccgtctcc 360
 tca 363

<210> 189
 <211> 108
 <212> PRT
 <213> Artificial Sequence

<220>
 <223> CH1A1A; VL

<400> 189
 Asp Ile Gln Met Thr Gln Ser Pro Ser Ser Leu Ser Ala Ser Val Gly
 1 5 10 15
 Asp Arg Val Thr Ile Thr Cys Lys Ala Ser Ala Ala Val Gly Thr Tyr
 20 25 30
 Val Ala Trp Tyr Gln Gln Lys Pro Gly Lys Ala Pro Lys Leu Leu Ile
 35 40 45
 Tyr Ser Ala Ser Tyr Arg Lys Arg Gly Val Pro Ser Arg Phe Ser Gly
 50 55 60
 Ser Gly Ser Gly Thr Asp Phe Thr Leu Thr Ile Ser Ser Leu Gln Pro
 65 70 75 80
 Glu Asp Phe Ala Thr Tyr Tyr Cys His Gln Tyr Tyr Thr Tyr Pro Leu
 85 90 95
 Phe Thr Phe Gly Gln Gly Thr Lys Leu Glu Ile Lys
 100 105

<210> 190
 <211> 324
 <212> DNA
 <213> Artificial Sequence

<220>

<223> CH1A1A; VL

```

<400> 190
gatatccaga tgaccagtc tccatcctcc ctgtctgcat ctgtgggaga cagagtcacc      60
atcacttgca aggccagtc ggctgtgggt acgtatgttg cgtgggtatca gcagaaacca      120
gggaaagcac ctaagctcct gatctattcg gcatactacc gcaaaagggg agtcccatca      180
aggttcagtg gcagtggatc tgggacagat ttcactctca ccatcagcag tctgcaacct      240
gaagatttcg caacttacta ctgtcaccaa tattacacct atcctctatt cacgtttggc      300
cagggcacca agctcgagat caag                                           324

```

<210> 191

<211> 121

<212> PRT

<213> Artificial Sequence

<220>

<223> CH1A1A; VH

<400> 191

Gln Val Gln Leu Val Gln Ser Gly Ala Glu Val Lys Lys Pro Gly Ala
 1 5 10 15

Ser Val Lys Val Ser Cys Lys Ala Ser Gly Tyr Thr Phe Thr Glu Phe
 20 25 30

Gly Met Asn Trp Val Arg Gln Ala Pro Gly Gln Gly Leu Glu Trp Met
 35 40 45

Gly Trp Ile Asn Thr Lys Thr Gly Glu Ala Thr Tyr Val Glu Glu Phe
 50 55 60

Lys Gly Arg Val Thr Phe Thr Thr Asp Thr Ser Thr Ser Thr Ala Tyr
 65 70 75 80

Met Glu Leu Arg Ser Leu Arg Ser Asp Asp Thr Ala Val Tyr Tyr Cys
 85 90 95

Ala Arg Trp Asp Phe Ala Tyr Tyr Val Glu Ala Met Asp Tyr Trp Gly
 100 105 110

Gln Gly Thr Thr Val Thr Val Ser Ser
 115 120

<210> 192

<211> 363

<212> DNA

<213> Artificial Sequence

<220>

<223> CH1A1A; VH

<400> 192

```

caggtgcagc tgggtgcagtc tggcgccgaa gtgaagaaac ctggagctag tgtgaagggtg      60

```


eolf-seql.txt

```

tcctgcaagg ccagcggcta caccttcacc gagttcggca tgaactgggt ccgacaggct      120
ccaggccagg gcctcgaatg gatgggctgg atcaacacca agaccggcga ggccacctac      180
gtggaagagt tcaagggcag agtgaccttc accacggaca ccagcaccag caccgcctac      240
atggaactgc ggagcctgag aagcgacgac accgccgtgt actactgcdc cagatgggac      300
ttcgcctatt acgtggaagc catggactac tggggccagg gcaccaccgt gaccgtgtct      360
agc                                                                           363

```

```

<210> 193
<211> 446
<212> PRT
<213> Artificial Sequence

```

```

<220>
<223> 28H1 Fab HC-Fc hole (LALA P329G)

```

```

<400> 193

```

```

Glu Val Gln Leu Leu Glu Ser Gly Gly Gly Leu Val Gln Pro Gly Gly
1           5           10           15

```

```

Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Phe Thr Phe Ser Ser His
20           25           30

```

```

Ala Met Ser Trp Val Arg Gln Ala Pro Gly Lys Gly Leu Glu Trp Val
35           40           45

```

```

Ser Ala Ile Trp Ala Ser Gly Glu Gln Tyr Tyr Ala Asp Ser Val Lys
50           55           60

```

```

Gly Arg Phe Thr Ile Ser Arg Asp Asn Ser Lys Asn Thr Leu Tyr Leu
65           70           75           80

```

```

Gln Met Asn Ser Leu Arg Ala Glu Asp Thr Ala Val Tyr Tyr Cys Ala
85           90           95

```

```

Lys Gly Trp Leu Gly Asn Phe Asp Tyr Trp Gly Gln Gly Thr Leu Val
100          105          110

```

```

Thr Val Ser Ser Ala Ser Thr Lys Gly Pro Ser Val Phe Pro Leu Ala
115          120          125

```

```

Pro Ser Ser Lys Ser Thr Ser Gly Gly Thr Ala Ala Leu Gly Cys Leu
130          135          140

```

```

Val Lys Asp Tyr Phe Pro Glu Pro Val Thr Val Ser Trp Asn Ser Gly
145          150          155          160

```

```

Ala Leu Thr Ser Gly Val His Thr Phe Pro Ala Val Leu Gln Ser Ser
165          170          175

```

eofl-seql.txt

Gly Leu Tyr Ser₁₈₀ Leu Ser Ser Val Val₁₈₅ Thr Val Pro Ser Ser₁₉₀ Ser Leu

Gly Thr Gln₁₉₅ Thr Tyr Ile Cys Asn₂₀₀ Val Asn His Lys Pro₂₀₅ Ser Asn Thr

Lys Val₂₁₀ Asp Lys Lys Val Glu₂₁₅ Pro Lys Ser Cys Asp₂₂₀ Lys Thr His Thr

Cys₂₂₅ Pro Pro Cys Pro Ala₂₃₀ Pro Glu Ala Ala Gly₂₃₅ Gly Pro Ser Val Phe₂₄₀

Leu Phe Pro Pro Lys₂₄₅ Pro Lys Asp Thr Leu₂₅₀ Met Ile Ser Arg Thr₂₅₅ Pro

Glu Val Thr Cys₂₆₀ Val Val Val Asp Val₂₆₅ Ser His Glu Asp Pro₂₇₀ Glu Val

Lys Phe Asn₂₇₅ Trp Tyr Val Asp Gly₂₈₀ Val Glu Val His Asn₂₈₅ Ala Lys Thr

Lys Pro₂₉₀ Arg Glu Glu Gln Tyr₂₉₅ Asn Ser Thr Tyr Arg₃₀₀ Val Val Ser Val

Leu₃₀₅ Thr Val Leu His Gln₃₁₀ Asp Trp Leu Asn Gly₃₁₅ Lys Glu Tyr Lys Cys₃₂₀

Lys Val Ser Asn Lys₃₂₅ Ala Leu Gly Ala Pro₃₃₀ Ile Glu Lys Thr Ile₃₃₅ Ser

Lys Ala Lys Gly₃₄₀ Gln Pro Arg Glu Pro₃₄₅ Gln Val Cys Thr Leu₃₅₀ Pro Pro

Ser Arg Asp₃₅₅ Glu Leu Thr Lys Asn₃₆₀ Gln Val Ser Leu Ser₃₆₅ Cys Ala Val

Lys Gly₃₇₀ Phe Tyr Pro Ser Asp₃₇₅ Ile Ala Val Glu Trp₃₈₀ Glu Ser Asn Gly

Gln₃₈₅ Pro Glu Asn Asn Tyr₃₉₀ Lys Thr Thr Pro Pro₃₉₅ Val Leu Asp Ser Asp₄₀₀

Gly Ser Phe Phe Leu₄₀₅ Val Ser Lys Leu Thr Val Asp Lys Ser Arg₄₁₅ Trp

Gln Gln Gly Asn₄₂₀ Val Phe Ser Cys Ser₄₂₅ Val Met His Glu Ala₄₃₀ Leu His

Asn His Tyr₄₃₅ Thr Gln Lys Ser Leu₄₄₀ Ser Leu Ser Pro Gly₄₄₅ Lys

eolf-seql.txt

<210> 194
 <211> 1338
 <212> DNA
 <213> Artificial Sequence

<220>
 <223> 28H1 Fab HC-Fc hole (LALA P329G)

<400> 194
 gaagtgcagc tgctggaatc cggcggaggc ctggtgcagc ctggcggatc tctgagactg 60
 tcctgcgccg cctccggctt caccttctcc tcccacgcca tgtcctgggt ccgacaggct 120
 cctggcaaag gcctggaatg ggtgtccgcc atctgggcct ccggcgagca gtactacgcc 180
 gactctgtga agggccggtt caccatctcc cgggacaact ccaagaacac cctgtacctg 240
 cagatgaact ccctgcgggc cgaggacacc gccgtgtact actgtgccaa gggctggctg 300
 ggcaacttcg actactgggg acagggcacc ctggtcaccg tgtccagcgc tagcaccaag 360
 ggccccctccg tgttccccct ggcccccagc agcaagagca ccagcggcgg cacagccgct 420
 ctgggctgcc tgggtcaagga ctacttcccc gagcccgtga ccgtgtcctg gaacagcgga 480
 gccctgacct ccggcgtgca caccttcccc gccgtgctgc agagtctctg cctgtatagc 540
 ctgagcagcg tggtcaccgt gccttctagc agcctgggca ccagacctc catctgcaac 600
 gtgaaccaca agcccagcaa caccaagggtg gacaagaagg tggagcccaa gagctgcgac 660
 aaaactcaca catgcccacc gtgcccagca cctgaagctg cagggggacc gtcagtcttc 720
 ctcttcccc caaaaccaa ggacaccctc atgatctccc ggaccctga ggtcacatgc 780
 gtggtggtgg acgtgagcca cgaagaccct gaggtcaagt tcaactggta cgtggacggc 840
 gtggagggtg ataatgcaa gacaaagccg cgggaggagc agtacaacag cacgtaccgt 900
 gtggtcagcg tcctcaccgt cctgcaccag gactggctga atggcaagga gtacaagtgc 960
 aaggtctcca acaaagccct cggcgcccc atcgagaaaa ccatctccaa agccaaaggg 1020
 cagccccgag aaccacaggt gtgcaccctg ccccatccc gggatgagct gaccaagaac 1080
 caggtcagcc tctcgtgcgc agtcaaaggc ttctatccca gcgacatcgc cgtggagtgg 1140
 gagagcaatg ggagccgga gaacaactac aagaccacgc ctcccgtgct ggactccgac 1200
 ggctccttct tcctcgtgag caagctcacc gtggacaaga gcaggtggca gcaggggaac 1260
 gtcttctcat gtcctgtgat gcatgaggct ctgcacaacc actacacgca gaagagcctc 1320
 tccctgtctc cgggtaaa 1338

<210> 195
 <211> 592
 <212> PRT
 <213> Artificial Sequence

<220>
 <223> 28H1 Fab HC-Fc knob (LALA P329G)-IL-2 qm

<400> 195

Glu Val Gln Leu Leu Glu Ser Gly Gly Gly Leu Val Gln Pro Gly Gly
 1 5 10 15

eolf-seql.txt

Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Phe Thr Phe Ser Ser His
20 25 30

Ala Met Ser Trp Val Arg Gln Ala Pro Gly Lys Gly Leu Glu Trp Val
35 40 45

Ser Ala Ile Trp Ala Ser Gly Glu Gln Tyr Tyr Ala Asp Ser Val Lys
50 55 60

Gly Arg Phe Thr Ile Ser Arg Asp Asn Ser Lys Asn Thr Leu Tyr Leu
65 70 75 80

Gln Met Asn Ser Leu Arg Ala Glu Asp Thr Ala Val Tyr Tyr Cys Ala
85 90 95

Lys Gly Trp Leu Gly Asn Phe Asp Tyr Trp Gly Gln Gly Thr Leu Val
100 105 110

Thr Val Ser Ser Ala Ser Thr Lys Gly Pro Ser Val Phe Pro Leu Ala
115 120 125

Pro Ser Ser Lys Ser Thr Ser Gly Gly Thr Ala Ala Leu Gly Cys Leu
130 135 140

Val Lys Asp Tyr Phe Pro Glu Pro Val Thr Val Ser Trp Asn Ser Gly
145 150 155 160

Ala Leu Thr Ser Gly Val His Thr Phe Pro Ala Val Leu Gln Ser Ser
165 170 175

Gly Leu Tyr Ser Leu Ser Ser Val Val Thr Val Pro Ser Ser Ser Leu
180 185 190

Gly Thr Gln Thr Tyr Ile Cys Asn Val Asn His Lys Pro Ser Asn Thr
195 200 205

Lys Val Asp Lys Lys Val Glu Pro Lys Ser Cys Asp Lys Thr His Thr
210 215 220

Cys Pro Pro Cys Pro Ala Pro Glu Ala Ala 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

eolf-seql.txt

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
 305 310 315 320
 Lys Val Ser Asn Lys Ala Leu Gly 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
 Cys Arg Asp Glu Leu Thr Lys Asn Gln Val Ser Leu Trp 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 Gly Gly Gly
 435 440 445
 Gly Ser Gly Gly Gly Gly Ser Gly Gly Gly Gly Ala Pro Ala Ser Ser
 450 455 460
 Ser Thr Lys Lys Thr Gln Leu Gln Leu Glu His Leu Leu Leu Asp Leu
 465 470 475 480
 Gln Met Ile Leu Asn Gly Ile Asn Asn Tyr Lys Asn Pro Lys Leu Thr
 485 490 495
 Arg Met Leu Thr Ala Lys Phe Ala Met Pro Lys Lys Ala Thr Glu Leu
 500 505 510
 Lys His Leu Gln Cys Leu Glu Glu Glu Leu Lys Pro Leu Glu Glu Val
 515 520 525
 Leu Asn Gly Ala Gln Ser Lys Asn Phe His Leu Arg Pro Arg Asp Leu
 530 535 540
 Ile Ser Asn Ile Asn Val Ile Val Leu Glu Leu Lys Gly Ser Glu Thr
 545 550 555 560

eolf-seql.txt

Thr Phe Met Cys Glu Tyr Ala Asp Glu Thr Ala Thr Ile Val Glu Phe
565 570 575

Leu Asn Arg Trp Ile Thr Phe Ala Gln Ser Ile Ile Ser Thr Leu Thr
580 585 590

<210> 196
<211> 1776
<212> DNA
<213> Artificial Sequence

<220>
<223> 28H1 Fab HC-Fc knob (LALA P329G)-IL-2 qm

<400> 196
gaagtgcagc tgctggaatc cggcggaggc ctggtgcagc ctggcggatc tctgagactg 60
tcctgcgccg cctccggctt caccttctcc tcccacgcca tgtcctgggt ccgacaggct 120
cctggcaaag gcctggaatg ggtgtccgcc atctgggcct cgggcgagca gtactacgcc 180
gactctgtga agggccggtt caccatctcc cgggacaact ccaagaacac cctgtacctg 240
cagatgaact ccctgcgggc cgaggacacc gccgtgtact actgtgccaa gggctggctg 300
ggcaacttcg actactgggg acagggcacc ctggtcaccg tgtccagcgc tagcaccaag 360
ggcccatcgg tcttccccct ggcaccctcc tccaagagca cctctggggg cacagcggcc 420
ctgggctgcc tggtaagga ctacttcccc gaaccggtga cgggtgtcgtg gaactcaggc 480
gccctgacca gcggcgtgca caccttcccc gctgtcctac agtcctcagg actctactcc 540
ctcagcagcg tggtgaccgt gccctccagc agcttgggca ccagaccta catctgcaac 600
gtgaatcaca agcccagcaa caccaagggt gacaagaaag ttgagcccaa atcttgtgac 660
aaaactcaca catgcccacc gtgcccagca cctgaagctg cagggggacc gtcagtcttc 720
ctcttcccc caaaaccaa ggacaccctc atgatctccc ggaccctga ggtcacatgc 780
gtggtggtgg acgtgagcca cgaagaccct gaggtcaagt tcaactggta cgtggacggc 840
gtggaggtgc ataatgcaa gacaaagccg cgggaggagc agtacaacag cacgtaccgt 900
gtggtcagcg tcctcaccgt cctgcaccag gactggctga atggcaagga gtacaagtgc 960
aaggtctcca acaaagccct cggcgcccc atcgagaaaa ccatctcaa agccaaaggg 1020
cagccccgag aaccacaggt gtacaccctg ccccatgcc gggatgagct gaccaagaac 1080
caggtcagcc tgtggtgcct ggtcaaaggc ttctatccca gcgacatcgc cgtggagtgg 1140
gagagcaatg ggcagccgga gaacaactac aagaccacgc ctcccgtgct ggactccgac 1200
ggctccttct tcctctacag caagctcacc gtggacaaga gcagggtggca gcaggggaac 1260
gtcttctcat gtcctgtgat gcatgaggct ctgcacaacc actacacgca gaagagcctc 1320
tcctgtctc cgggtggcgg cggaggctcc ggaggcggag gttctggcgg aggtggcgct 1380
cctgcctcct ccagaccaa gaaaaccag ctccagctgg aacatctcct gctggatctg 1440
cagatgatcc tgaacggcat caacaactac aagaaccca agctgaccg gatgctgacc 1500

eolf-seql.txt

```

gccaagttcg ccatgccc aa gaagccacc gagctgaaac atctgcagtg cctggaagag      1560
gaactgaagc ctctggaaga ggtgctgaac ggcgccagtg ccaagaactt ccacctgagg      1620
cctcgggacc tgatctccaa catcaacgtg atcgtgctgg aactgaaggg ctccgagaca      1680
accttcatgt gcgagtacgc cgacgagaca gctaccatcg tggaatttct gaaccggtgg      1740
atcaccttcg cccagtccat catctccacc ctgacc                                1776

```

```

<210> 197
<211> 592
<212> PRT
<213> Artificial Sequence

```

```

<220>
<223> 28H1 Fab HC-Fc knob (LALA P329G)-IL-2 wt
<400> 197

```

```

Glu Val Gln Leu Leu Glu Ser Gly Gly Gly Leu Val Gln Pro Gly Gly
1          5          10          15

```

```

Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Phe Thr Phe Ser Ser His
          20          25          30

```

```

Ala Met Ser Trp Val Arg Gln Ala Pro Gly Lys Gly Leu Glu Trp Val
          35          40          45

```

```

Ser Ala Ile Trp Ala Ser Gly Glu Gln Tyr Tyr Ala Asp Ser Val Lys
          50          55          60

```

```

Gly Arg Phe Thr Ile Ser Arg Asp Asn Ser Lys Asn Thr Leu Tyr Leu
65          70          75          80

```

```

Gln Met Asn Ser Leu Arg Ala Glu Asp Thr Ala Val Tyr Tyr Cys Ala
          85          90          95

```

```

Lys Gly Trp Leu Gly Asn Phe Asp Tyr Trp Gly Gln Gly Thr Leu Val
          100          105          110

```

```

Thr Val Ser Ser Ala Ser Thr Lys Gly Pro Ser Val Phe Pro Leu Ala
          115          120          125

```

```

Pro Ser Ser Lys Ser Thr Ser Gly Gly Thr Ala Ala Leu Gly Cys Leu
          130          135          140

```

```

Val Lys Asp Tyr Phe Pro Glu Pro Val Thr Val Ser Trp Asn Ser Gly
145          150          155          160

```

```

Ala Leu Thr Ser Gly Val His Thr Phe Pro Ala Val Leu Gln Ser Ser
          165          170          175

```

```

Gly Leu Tyr Ser Leu Ser Ser Val Val Thr Val Pro Ser Ser Ser Leu
          180          185          190

```

eolf-seql.txt

Gly Thr Gln Thr Tyr Ile Cys Asn Val Asn His Lys Pro Ser Asn Thr
195 200 205

Lys Val Asp Lys Lys Val Glu Pro Lys Ser Cys Asp Lys Thr His Thr
210 215 220

Cys Pro Pro Cys Pro Ala Pro Glu Ala Ala 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 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
305 310 315 320

Lys Val Ser Asn Lys Ala Leu Gly 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

Cys Arg Asp Glu Leu Thr Lys Asn Gln Val Ser Leu Trp 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 Gly Gly Gly
435 440 445

Gly Ser Gly Gly Gly Gly Ser Gly Gly Gly Gly Ala Pro Thr Ser Ser
450 455 460

eolf-seql.txt

Ser Thr Lys Lys Thr Gln Leu Gln Leu Glu His Leu Leu Leu Asp Leu
465 470 475 480

Gln Met Ile Leu Asn Gly Ile Asn Asn Tyr Lys Asn Pro Lys Leu Thr
485 490 495

Arg Met Leu Thr Phe Lys Phe Tyr Met Pro Lys Lys Ala Thr Glu Leu
500 505 510

Lys His Leu Gln Cys Leu Glu Glu Glu Leu Lys Pro Leu Glu Glu Val
515 520 525

Leu Asn Leu Ala Gln Ser Lys Asn Phe His Leu Arg Pro Arg Asp Leu
530 535 540

Ile Ser Asn Ile Asn Val Ile Val Leu Glu Leu Lys Gly Ser Glu Thr
545 550 555 560

Thr Phe Met Cys Glu Tyr Ala Asp Glu Thr Ala Thr Ile Val Glu Phe
565 570 575

Leu Asn Arg Trp Ile Thr Phe Ala Gln Ser Ile Ile Ser Thr Leu Thr
580 585 590

<210> 198
<211> 1776
<212> DNA
<213> Artificial Sequence

<220>
<223> 28H1 Fab HC-Fc knob (LALA P329G)-IL-2 wt

<400> 198
gaagtgcagc tgctggaatc cggcggaggc ctggtgcagc ctggcggatc tctgagactg 60
tcctgcgccg cctccggctt caccttctcc tcccacgcc a tgcctgggt cgcacaggct 120
cctggcaaag gcctggaatg ggtgtccgcc atctgggcct cggcgagca gtactacgcc 180
gactctgtga agggccggtt caccatctcc cgggacaact ccaagaacac cctgtacctg 240
cagatgaact ccctgcgggc cgaggacacc gccgtgtact actgtgccaa gggctggctg 300
ggcaacttcg actactgggg acagggcacc ctggtcaccg tgtccagcgc tagcaccaag 360
ggcccatcgg tcttccccct ggcaccctcc tccaagagca cctctggggg cacagcggcc 420
ctgggctgcc tggtaagga ctacttccc gaaccggtga cgggtgtcgtg gaactcaggc 480
gccctgacca gcggcgtgca caccttccc gctgtcctac agtcctcagg actctactcc 540
ctcagcagcg tggtgaccgt gccctccagc agcttgggca cccagacct a catctgcaac 600
gtgaatcaca agcccagcaa caccaagggtg gacaagaaag ttgagcccaa atcttgtgac 660
aaaactcaca catgcccacc gtgcccagca cctgaagctg cagggggacc gtcagtcttc 720
ctcttcccc caaaaccaa ggacaccctc atgatctccc ggaccctga ggtcacatgc 780

eolf-seql.txt

```

gtggtggtgg acgtgagcca cgaagaccct gaggtcaagt tcaactggta cgtggacggc      840
gtggaggtgc ataatgcca gacaaagccg cgggaggagc agtacaacag cacgtaccgt      900
gtggtcagcg tcctcacctg cctgcaccag gactggctga atggcaagga gtacaagtgc      960
aaggtctcca acaaagccct cggcgccccc atcgagaaaa ccatctccaa agccaaaggg    1020
cagccccgag aaccacaggt gtacaccctg ccccatgcc gggatgagct gaccaagaac    1080
caggtcagcc tgtggtgcct ggtcaaaggc ttctatccca gcgacatcgc cgtggagtgg    1140
gagagcaatg ggcagccgga gaacaactac aagaccacgc ctcccgctgt ggactccgac    1200
ggctccttct tcctctacag caagctcacc gtggacaaga gcaggtggca gcaggggaac    1260
gtcttctcat gctccgtgat gcatgaggct ctgcacaacc actacacgca gaagagcctc    1320
tccctgtctc cgggtggcgg cggaggctcc ggaggcggag gttctggcgg aggtggcgct    1380
cctacatcct ccagcaccaa gaaaaccag ctccagctgg aacatctcct gctggatctg    1440
cagatgatcc tgaacggcat caacaactac aagaacccca agctgaccgg gatgctgacc    1500
ttcaagttct acatgcccaa gaaggccacc gagctgaaac atctgcagtg cctggaagag    1560
gaactgaagc ctctggaaga ggtgctgaac ctggcccagt ccaagaactt ccacctgagg    1620
cctcgggacc tgatctccaa catcaacgtg atcgtgctgg aactgaaggg ctccgagaca    1680
accttcatgt gcgagtacgc cgacgagaca gctaccatcg tggaatttct gaaccggtgg    1740
atcaccttcg cccagtccat catctccacc ctgacc                                1776

```

<210> 199
 <211> 574
 <212> PRT
 <213> Artificial Sequence

<220>
 <223> 28H1 Fab HC-Fc knob (LALA P329G)-IL-15 (E53A N79A)
 <400> 199

Glu Val Gln Leu Leu Glu Ser Gly Gly Gly Leu Val Gln Pro Gly Gly
 1 5 10 15

Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Phe Thr Phe Ser Ser His
 20 25 30

Ala Met Ser Trp Val Arg Gln Ala Pro Gly Lys Gly Leu Glu Trp Val
 35 40 45

Ser Ala Ile Trp Ala Ser Gly Glu Gln Tyr Tyr Ala Asp Ser Val Lys
 50 55 60

Gly Arg Phe Thr Ile Ser Arg Asp Asn Ser Lys Asn Thr Leu Tyr Leu
 65 70 75 80

Gln Met Asn Ser Leu Arg Ala Glu Asp Thr Ala Val Tyr Tyr Cys Ala
 85 90 95

eolf-seql.txt

Lys Gly Trp Leu Gly Asn Phe Asp Tyr Trp Gly Gln Gly Thr Leu Val
 100 105 110
 Thr Val Ser Ser Ala Ser Thr Lys Gly Pro Ser Val Phe Pro Leu Ala
 115 120 125
 Pro Ser Ser Lys Ser Thr Ser Gly Gly Thr Ala Ala Leu Gly Cys Leu
 130 135 140
 Val Lys Asp Tyr Phe Pro Glu Pro Val Thr Val Ser Trp Asn Ser Gly
 145 150 155 160
 Ala Leu Thr Ser Gly Val His Thr Phe Pro Ala Val Leu Gln Ser Ser
 165 170 175
 Gly Leu Tyr Ser Leu Ser Ser Val Val Thr Val Pro Ser Ser Ser Leu
 180 185 190
 Gly Thr Gln Thr Tyr Ile Cys Asn Val Asn His Lys Pro Ser Asn Thr
 195 200 205
 Lys Val Asp Lys Lys Val Glu Pro Lys Ser Cys Asp Lys Thr His Thr
 210 215 220
 Cys Pro Pro Cys Pro Ala Pro Glu Ala Ala 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 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
 305 310 315 320
 Lys Val Ser Asn Lys Ala Leu Gly 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
 Cys Arg Asp Glu Leu Thr Lys Asn Gln Val Ser Leu Trp Cys Leu Val
 355 360 365

eolf-seql.txt

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 Ser Gly Gly
435 440 445

Gly Gly Ser Gly Gly Gly Gly Ser Gly Gly Gly Gly Asn Trp Val Asn
450 455 460

Val Ile Ser Asp Leu Lys Lys Ile Glu Asp Leu Ile Gln Ser Met His
465 470 475 480

Ile Asp Ala Thr Leu Tyr Thr Glu Ser Asp Val His Pro Ser Cys Lys
485 490 495

Val Thr Ala Met Lys Cys Phe Leu Leu Glu Leu Gln Val Ile Ser Leu
500 505 510

Ala Ser Gly Asp Ala Ser Ile His Asp Thr Val Glu Asn Leu Ile Ile
515 520 525

Leu Ala Asn Asn Ser Leu Ser Ser Asn Gly Ala Val Thr Glu Ser Gly
530 535 540

Cys Lys Glu Cys Glu Glu Leu Glu Glu Lys Asn Ile Lys Glu Phe Leu
545 550 555 560

Gln Ser Phe Val His Ile Val Gln Met Phe Ile Asn Thr Ser
565 570

<210> 200
<211> 1722
<212> DNA
<213> Artificial Sequence

<220>
<223> 28H1 Fab HC-Fc knob (LALA P329G)-IL-15 (E53A N79A)

<400> 200
gaagtgcagc tgctggaatc cggcggaggc ctggtgcagc ctggcggatc tctgagactg 60

tcctgcgccg cctccggctt caccttctcc tcccacgcca tgtcctgggt cgcacaggct 120

cctggcaaag gcctggaatg ggtgtccgcc atctgggcct cggcgagca gtactacgcc 180

eolf-seql.txt

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gactctgtga agggccggtt caccatctcc cgggacaact ccaagaacac cctgtacctg      240
cagatgaact ccctgcgggc cgaggacacc gccgtgtact actgtgccaa gggctggctg      300
ggcaacttcg actactgggg acagggcacc ctggtcaccg tgtccagcgc tagcaccaag      360
ggcccatcgg tcttccccct ggcaccctcc tccaagagca cctctggggg cacagcggcc      420
ctgggctgcc tgggtcaagga ctacttcccc gaaccggtga cgggtgtcgtg gaactcaggg      480
gccctgacca gcggcgtgca caccttcccc gctgtcctac agtcctcagg actctactcc      540
ctcagcagcg tggtgaccgt gccctccagc agcttgggca cccagacctc catctgcaac      600
gtgaatcaca agcccagcaa caccaaggtg gacaagaaag ttgagcccaa atcttgtgac      660
aaaactcaca catgcccacc gtgcccagca cctgaagctg caggggggacc gtcagtcttc      720
ctcttcccc caaaacccaa ggacaccctc atgatctccc ggaccctga ggtcacatgc      780
gtgggtgggtg acgtgagcca cgaagaccct gaggtcaagt tcaactggta cgtggacggc      840
gtggaggtgc ataatgcaa gacaaagccg cgggaggagc agtacaacag cacgtaccgt      900
gtggtcagcg tcctcaccgt cctgcaccag gactggctga atggcaagga gtacaagtgc      960
aaggctctcca acaaagccct cggcgcccc atcgagaaaa ccatctccaa agccaaaggg     1020
cagccccgag aaccacaggt gtacaccctg ccccatgcc gggatgagct gaccaagaac     1080
caggtcagcc tgtggtgcct ggtcaaaggc ttctatccca gcgacatcgc cgtggagtgg     1140
gagagcaatg ggcagccgga gaacaactac aagaccacgc ctcccgtgct ggactccgac     1200
ggctccttct tcctctacag caagctcacc gtggacaaga gcagggtggca gcaggggaac     1260
gtcttctcat gtcctgtgat gcatgaggct ctgcacaacc actacacgca gaagagcctc     1320
tcctgtctc cgggttccgg cggcggaggc tccggaggcg gaggttctgg cggaggtggc     1380
aactgggtga atgtaataag tgatttgaag aaaattgaag atcttattca atctatgcat     1440
attgatgcta ctttatatac ggaaagtgat gttcacccca gttgcaaagt aacagcaatg     1500
aagtgtcttc tcttgagatt acaagttatt tcaattgcgt cgggagatgc aagtattcat     1560
gatacagtag aaaatctgat catcctagca aacaacagtt tgtcttctaa tggggctgta     1620
acagaatctg gatgcaaaga atgtgaggaa ctggaggaaa aaaatattaa agaatttttg     1680
cagagttttg tacatatgtg ccaaagtgtc atcaacactt ct                          1722

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<210> 201
 <211> 447
 <212> PRT
 <213> Artificial Sequence

<220>
 <223> 4G8 Fab HC-Fc hole (LALA P329G)

<400> 201

Glu Val Gln Leu Leu Glu Ser Gly Gly Gly Leu Val Gln Pro Gly Gly
 1 5 10 15

eolf-seql.txt

Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Phe Thr Phe Ser Ser Tyr
20 25 30

Ala Met Ser Trp Val Arg Gln Ala Pro Gly Lys Gly Leu Glu Trp Val
35 40 45

Ser Ala Ile Ser Gly Ser Gly Gly Ser Thr Tyr Tyr Ala Asp Ser Val
50 55 60

Lys Gly Arg Phe Thr Ile Ser Arg Asp Asn Ser Lys Asn Thr Leu Tyr
65 70 75 80

Leu Gln Met Asn Ser Leu Arg Ala Glu Asp Thr Ala Val Tyr Tyr Cys
85 90 95

Ala Lys Gly Trp Leu Gly Asn Phe Asp Tyr Trp Gly Gln Gly Thr Leu
100 105 110

Val Thr Val Ser Ser Ala Ser Thr Lys Gly Pro Ser Val Phe Pro Leu
115 120 125

Ala Pro Ser Ser Lys Ser Thr Ser Gly Gly Thr Ala Ala Leu Gly Cys
130 135 140

Leu Val Lys Asp Tyr Phe Pro Glu Pro Val Thr Val Ser Trp Asn Ser
145 150 155 160

Gly Ala Leu Thr Ser Gly Val His Thr Phe Pro Ala Val Leu Gln Ser
165 170 175

Ser Gly Leu Tyr Ser Leu Ser Ser Val Val Thr Val Pro Ser Ser Ser
180 185 190

Leu Gly Thr Gln Thr Tyr Ile Cys Asn Val Asn His Lys Pro Ser Asn
195 200 205

Thr Lys Val Asp Lys Lys Val Glu Pro Lys Ser Cys Asp Lys Thr His
210 215 220

Thr Cys Pro Pro Cys Pro Ala Pro Glu Ala Ala Gly Gly Pro Ser Val
225 230 235 240

Phe Leu Phe Pro Pro Lys Pro Lys Asp Thr Leu Met Ile Ser Arg Thr
245 250 255

Pro Glu Val Thr Cys Val Val Val Asp Val Ser His Glu Asp Pro Glu
260 265 270

Val Lys Phe Asn Trp Tyr Val Asp Gly Val Glu Val His Asn Ala Lys
275 280 285

eolf-seql.txt

Thr Lys Pro Arg Glu Glu Gln Tyr Asn Ser Thr Tyr Arg Val Val Ser
 290 295 300

Val Leu Thr Val Leu His Gln Asp Trp Leu Asn Gly Lys Glu Tyr Lys
 305 310 315 320

Cys Lys Val Ser Asn Lys Ala Leu Gly Ala Pro Ile Glu Lys Thr Ile
 325 330 335

Ser Lys Ala Lys Gly Gln Pro Arg Glu Pro Gln Val Cys Thr Leu Pro
 340 345 350

Pro Ser Arg Asp Glu Leu Thr Lys Asn Gln Val Ser Leu Ser Cys Ala
 355 360 365

Val Lys Gly Phe Tyr Pro Ser Asp Ile Ala Val Glu Trp Glu Ser Asn
 370 375 380

Gly Gln Pro Glu Asn Asn Tyr Lys Thr Thr Pro Pro Val Leu Asp Ser
 385 390 395 400

Asp Gly Ser Phe Phe Leu Val Ser Lys Leu Thr Val Asp Lys Ser Arg
 405 410 415

Trp Gln Gln Gly Asn Val Phe Ser Cys Ser Val Met His Glu Ala Leu
 420 425 430

His Asn His Tyr Thr Gln Lys Ser Leu Ser Leu Ser Pro Gly Lys
 435 440 445

<210> 202
 <211> 1341
 <212> DNA
 <213> Artificial Sequence

<220>
 <223> 4G8 Fab HC-Fc hole (LALA P329G)

<400> 202
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 tcctgtgcag cctccggatt caccttttagc agttatgcca tgagctgggt ccgccaggct 120
 ccagggaagg ggctggagtg ggtctcagct attagtggta gtggtggtag cacatactac 180
 gcagactccg tgaagggccg gttcaccatc tccagagaca attccaagaa cacgctgtat 240
 ctgcagatga acagcctgag agccgaggac acggccgtat attactgtgc gaaaggggtg 300
 ctgggtaatt ttgactactg gggccaagga accctgggtca ccgtctcgag tgctagcacc 360
 aagggcccct ccgtgttccc cctggccccc agcagcaaga gcaccagcgg cggcacagcc 420
 gctctgggct gcctgggtcaa ggactacttc cccgagcccg tgaccgtgtc ctggaacagc 480
 ggagccctga cctccggcgt gcacaccttc cccgccgtgc tgcagagttc tggcctgtat 540
 agcctgagca gcgtgggtcac cgtgccttct agcagcctgg gcaccagac ctacatctgc 600

eolf-seql.txt

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aacgtgaacc acaagcccag caacaccaag gtggacaaga aggtggagcc caagagctgc      660
gacaaaactc acacatgccc accgtgcccga gcacctgaag ctgcaggggg accgtcagtc      720
ttcctcttcc ccccaaaacc caaggacacc ctcatgatct cccggacccc tgaggtcaca      780
tgcgtggtgg tggacgtgag ccacgaagac cctgaggtca agttcaactg gtacgtggac      840
ggcgtggagg tgcataatgc caagacaaag ccgcggggagg agcagtacaa cagcacgtac      900
cgtgtggtca gcgtcctcac cgtcctgcac caggactggc tgaatggcaa ggagtacaag      960
tgcaaggtct ccaacaaagc cctcggcgcc cccatcgaga aaaccatctc caaagccaaa     1020
gggcagcccc gagaaccaca ggtgtgcacc ctgcccccat cccgggatga gctgaccaag     1080
aaccaggtca gcctctcgtg cgcagtcaaa ggcttctatc ccagcgacat cgccgtggag     1140
tgggagagca atgggcagcc ggagaacaac tacaagacca cgcctcccgt gctggactcc     1200
gacggctcct tcttctcgt gagcaagctc accgtggaca agagcaggtg gcagcagggg     1260
aacgtcttct catgctccgt gatgcatgag gctctgcaca accactacac gcagaagagc     1320
ctctccctgt ctccgggtaa a                                             1341

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<210> 203
 <211> 593
 <212> PRT
 <213> Artificial Sequence

<220>
 <223> 4G8 Fab HC-Fc knob (LALA P329G)-IL-2 qm
 <400> 203

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Glu Val Gln Leu Leu Glu Ser Gly Gly Gly Leu Val Gln Pro Gly Gly
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Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Phe Thr Phe Ser Ser Tyr
          20          25          30

Ala Met Ser Trp Val Arg Gln Ala Pro Gly Lys Gly Leu Glu Trp Val
          35          40          45

Ser Ala Ile Ser Gly Ser Gly Gly Ser Thr Tyr Tyr Ala Asp Ser Val
          50          55          60

Lys Gly Arg Phe Thr Ile Ser Arg Asp Asn Ser Lys Asn Thr Leu Tyr
65          70          75          80

Leu Gln Met Asn Ser Leu Arg Ala Glu Asp Thr Ala Val Tyr Tyr Cys
          85          90          95

Ala Lys Gly Trp Leu Gly Asn Phe Asp Tyr Trp Gly Gln Gly Thr Leu
          100          105          110

Val Thr Val Ser Ser Ala Ser Thr Lys Gly Pro Ser Val Phe Pro Leu
          115          120          125

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eolf-seql.txt

Ala Pro Ser Ser Lys Ser Thr Ser Gly Gly Thr Ala Ala Leu Gly Cys
130 135 140

Leu Val Lys Asp Tyr Phe Pro Glu Pro Val Thr Val Ser Trp Asn Ser
145 150 155 160

Gly Ala Leu Thr Ser Gly Val His Thr Phe Pro Ala Val Leu Gln Ser
165 170 175

Ser Gly Leu Tyr Ser Leu Ser Ser Val Val Thr Val Pro Ser Ser Ser
180 185 190

Leu Gly Thr Gln Thr Tyr Ile Cys Asn Val Asn His Lys Pro Ser Asn
195 200 205

Thr Lys Val Asp Lys Lys Val Glu Pro Lys Ser Cys Asp Lys Thr His
210 215 220

Thr Cys Pro Pro Cys Pro Ala Pro Glu Ala Ala Gly Gly Pro Ser Val
225 230 235 240

Phe Leu Phe Pro Pro Lys Pro Lys Asp Thr Leu Met Ile Ser Arg Thr
245 250 255

Pro Glu Val Thr Cys Val Val Val Asp Val Ser His Glu Asp Pro Glu
260 265 270

Val Lys Phe Asn Trp Tyr Val Asp Gly Val Glu Val His Asn Ala Lys
275 280 285

Thr Lys Pro Arg Glu Glu Gln Tyr Asn Ser Thr Tyr Arg Val Val Ser
290 295 300

Val Leu Thr Val Leu His Gln Asp Trp Leu Asn Gly Lys Glu Tyr Lys
305 310 315 320

Cys Lys Val Ser Asn Lys Ala Leu Gly Ala Pro Ile Glu Lys Thr Ile
325 330 335

Ser Lys Ala Lys Gly Gln Pro Arg Glu Pro Gln Val Tyr Thr Leu Pro
340 345 350

Pro Cys Arg Asp Glu Leu Thr Lys Asn Gln Val Ser Leu Trp Cys Leu
355 360 365

Val Lys Gly Phe Tyr Pro Ser Asp Ile Ala Val Glu Trp Glu Ser Asn
370 375 380

Gly Gln Pro Glu Asn Asn Tyr Lys Thr Thr Pro Pro Val Leu Asp Ser
385 390 395 400

eolf-seql.txt

Asp Gly Ser Phe Phe Leu Tyr Ser Lys Leu Thr Val Asp Lys Ser Arg
405 410 415

Trp Gln Gln Gly Asn Val Phe Ser Cys Ser Val Met His Glu Ala Leu
420 425 430

His Asn His Tyr Thr Gln Lys Ser Leu Ser Leu Ser Pro Gly Gly Gly
435 440 445

Gly Gly Ser Gly Gly Gly Gly Ser Gly Gly Gly Gly Ala Pro Ala Ser
450 455 460

Ser Ser Thr Lys Lys Thr Gln Leu Gln Leu Glu His Leu Leu Leu Asp
465 470 475 480

Leu Gln Met Ile Leu Asn Gly Ile Asn Asn Tyr Lys Asn Pro Lys Leu
485 490 495

Thr Arg Met Leu Thr Ala Lys Phe Ala Met Pro Lys Lys Ala Thr Glu
500 505 510

Leu Lys His Leu Gln Cys Leu Glu Glu Glu Leu Lys Pro Leu Glu Glu
515 520 525

Val Leu Asn Gly Ala Gln Ser Lys Asn Phe His Leu Arg Pro Arg Asp
530 535 540

Leu Ile Ser Asn Ile Asn Val Ile Val Leu Glu Leu Lys Gly Ser Glu
545 550 555 560

Thr Thr Phe Met Cys Glu Tyr Ala Asp Glu Thr Ala Thr Ile Val Glu
565 570 575

Phe Leu Asn Arg Trp Ile Thr Phe Ala Gln Ser Ile Ile Ser Thr Leu
580 585 590

Thr

<210> 204
<211> 1779
<212> DNA
<213> Artificial Sequence

<220>
<223> 4G8 Fab HC-Fc knob (LALA P329G)-IL-2 qm

<400> 204
gaggtgcaat tggtggagtc tgggggaggc ttggtacagc ctgggggggtc cctgagactc 60

tcctgtgcag cctccgatt cacctttagc agttatgcca tgagctgggt ccgccaggct 120

ccaggggaagg ggctggagtg ggtctcagct attagtggta gtggtggtag cacatactac 180

eolf-seql.txt

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gcagactccg tgaagggccg gttcaccatc tccagagaca attccaagaa cacgctgtat 240
ctgcagatga acagcctgag agccgaggac acggccgtat attactgtgc gaaaggggtgg 300
ctgggtaatt ttgactactg gggccaagga accctgggtca ccgtctcgag tgctagcacc 360
aagggcccat cggctctccc cctggcaccc tcctccaaga gcacctctgg gggcacagcg 420
gccctgggct gcctgggtcaa ggactacttc cccgaaccgg tgacgggtgtc gtggaactca 480
ggcgccctga ccagcggcgt gcacaccttc ccggctgtcc tacagtcctc aggactctac 540
tcctcagca gcgtgggtgac cgtgccctcc agcagcttgg gcaccagac ctacatctgc 600
aacgtgaatc acaagcccag caacaccaag gtggacaaga aagttgagcc caaatcttgt 660
gacaaaactc acacatgccc accgtgcccga gcacctgaag ctgcaggggg accgtcagtc 720
ttcctcttcc ccccaaaacc caaggacacc ctcatgatct cccggacccc tgaggtcaca 780
tgcgtgggtg tggacgtgag ccacgaagac cctgaggtca agttcaactg gtacgtggac 840
ggcgtggagg tgcataatgc caagacaaag ccgcgggagg agcagtacaa cagcacgtac 900
cgtgtgggtca gcgtcctcac cgtcctgcac caggactggc tgaatggcaa ggagtacaag 960
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gggcagcccc gagaaccaca ggtgtacacc ctgcccccat gccgggatga gctgaccaag 1080
aaccagggtca gcctgtgggtg cctgggtcaaa ggcttctatc ccagcgacat cgccgtggag 1140
tgggagagca atgggcagcc ggagaacaac tacaagacca cgcctcccgt gctggactcc 1200
gacggctcct tcttctctta cagcaagctc accgtggaca agagcagggtg gcagcagggg 1260
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gctcctgcct cctccagcac caagaaaacc cagctccagc tggaacatct cctgctggat 1440
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accgccaagt tcgccatgcc caagaaggcc accgagctga aacatctgca gtgcctggaa 1560
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aggcctcggg acctgatctc caacatcaac gtgatcgtgc tggaactgaa gggctccgag 1680
acaaccttca tgtgcgagta cgccgacgag acagctacca tcgtggaatt tctgaaccgg 1740
tggatcacct tcgcccagtc catcatctcc accctgacc 1779
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<210> 205
 <211> 215
 <212> PRT
 <213> Artificial Sequence

<220>
 <223> 4G8 Fab LC

<400> 205

Glu Ile Val Leu Thr Gln Ser Pro Gly Thr Leu Ser Leu Ser Pro Gly
 1 5 10 15

eolf-seql.txt

Glu Arg Ala Thr Leu Ser Cys Arg Ala Ser Gln Ser Val Ser Arg Ser
20 25 30

Tyr Leu Ala Trp Tyr Gln Gln Lys Pro Gly Gln Ala Pro Arg Leu Leu
35 40 45

Ile Ile Gly Ala Ser Thr Arg Ala Thr Gly Ile Pro Asp Arg Phe Ser
50 55 60

Gly Ser Gly Ser Gly Thr Asp Phe Thr Leu Thr Ile Ser Arg Leu Glu
65 70 75 80

Pro Glu Asp Phe Ala Val Tyr Tyr Cys Gln Gln Gly Gln Val Ile Pro
85 90 95

Pro Thr Phe Gly Gln Gly Thr Lys Val Glu Ile Lys Arg Thr Val Ala
100 105 110

Ala Pro Ser Val Phe Ile Phe Pro Pro Ser Asp Glu Gln Leu Lys Ser
115 120 125

Gly Thr Ala Ser Val Val Cys Leu Leu Asn Asn Phe Tyr Pro Arg Glu
130 135 140

Ala Lys Val Gln Trp Lys Val Asp Asn Ala Leu Gln Ser Gly Asn Ser
145 150 155 160

Gln Glu Ser Val Thr Glu Gln Asp Ser Lys Asp Ser Thr Tyr Ser Leu
165 170 175

Ser Ser Thr Leu Thr Leu Ser Lys Ala Asp Tyr Glu Lys His Lys Val
180 185 190

Tyr Ala Cys Glu Val Thr His Gln Gly Leu Ser Ser Pro Val Thr Lys
195 200 205

Ser Phe Asn Arg Gly Glu Cys
210 215

<210> 206
<211> 645
<212> DNA
<213> Artificial Sequence

<220>
<223> 4G8 Fab LC

<400> 206
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ctgtcctgca gaggctccca gtccgtgtcc cggctcctacc tcgcctggta tcagcagaag 120

cccggccagg cccctcggct gctgatcatc ggcgctctta ccagagccac cggcatcct 180

eolf-seql.txt

gaccggttct ccggctctgg ctccggcacc gacttcaccc tgaccatctc ccggctggaa 240
 cccgaggact tcgccgtgta ctactgccag cagggccagg tcatccctcc cacctttggc 300
 cagggcacca aggtggaaat caagcgtacg gtggctgcac catctgtctt catcttcccg 360
 ccatctgatg agcagttgaa atctggaact gcctctgttg tgtgcctgct gaataacttc 420
 tatcccagag aggccaaagt acagtggaag gtggataacg cctccaatc gggtaactcc 480
 caggagagtg tcacagagca ggacagcaag gacagcacct acagcctcag cagcaccctg 540
 acgctgagca aagcagacta cgagaaacac aaagtctacg cctgcgaagt caccatcag 600
 ggctgagct cgcccgctac aaagagcttc aacaggggag agtgt 645

<210> 207
 <211> 447
 <212> PRT
 <213> Artificial Sequence

<220>
 <223> 4B9 Fab HC-Fc hole (LALA P329G)

<400> 207

Glu Val Gln Leu Leu Glu Ser Gly Gly Gly Leu Val Gln Pro Gly Gly
 1 5 10 15

Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Phe Thr Phe Ser Ser Tyr
 20 25 30

Ala Met Ser Trp Val Arg Gln Ala Pro Gly Lys Gly Leu Glu Trp Val
 35 40 45

Ser Ala Ile Ile Gly Ser Gly Ala Ser Thr Tyr Tyr Ala Asp Ser Val
 50 55 60

Lys Gly Arg Phe Thr Ile Ser Arg Asp Asn Ser Lys Asn Thr Leu Tyr
 65 70 75 80

Leu Gln Met Asn Ser Leu Arg Ala Glu Asp Thr Ala Val Tyr Tyr Cys
 85 90 95

Ala Lys Gly Trp Phe Gly Gly Phe Asn Tyr Trp Gly Gln Gly Thr Leu
 100 105 110

Val Thr Val Ser Ser Ala Ser Thr Lys Gly Pro Ser Val Phe Pro Leu
 115 120 125

Ala Pro Ser Ser Lys Ser Thr Ser Gly Gly Thr Ala Ala Leu Gly Cys
 130 135 140

Leu Val Lys Asp Tyr Phe Pro Glu Pro Val Thr Val Ser Trp Asn Ser
 145 150 155 160

eolf-seql.txt

Gly Ala Leu Thr Ser₁₆₅ Gly Val His Thr Phe₁₇₀ Pro Ala Val Leu Gln Ser₁₇₅

Ser Gly Leu Tyr₁₈₀ Ser Leu Ser Ser Val₁₈₅ Val Thr Val Pro Ser₁₉₀ Ser Ser

Leu Gly Thr₁₉₅ Gln Thr Tyr Ile Cys₂₀₀ Asn Val Asn His Lys₂₀₅ Pro Ser Asn

Thr Lys₂₁₀ Val Asp Lys Lys Val₂₁₅ Glu Pro Lys Ser Cys₂₂₀ Asp Lys Thr His

Thr Cys₂₂₅ Pro Pro Cys₂₃₀ Pro Ala Pro Glu Ala₂₃₅ Ala Gly Gly Pro Ser Val₂₄₀

Phe Leu Phe Pro₂₄₅ Lys Pro Lys Asp Thr₂₅₀ Leu Met Ile Ser Arg₂₅₅ Thr

Pro Glu Val Thr₂₆₀ Cys Val Val Val Asp₂₆₅ Val Ser His Glu Asp₂₇₀ Pro Glu

Val Lys Phe₂₇₅ Asn Trp Tyr Val Asp₂₈₀ Gly Val Glu Val His₂₈₅ Asn Ala Lys

Thr Lys₂₉₀ Pro Arg Glu Glu Gln₂₉₅ Tyr Asn Ser Thr Tyr₃₀₀ Arg Val Val Ser

Val₃₀₅ Leu Thr Val Leu His₃₁₀ Gln Asp Trp Leu Asn₃₁₅ Gly Lys Glu Tyr Lys₃₂₀

Cys Lys Val Ser₃₂₅ Lys Ala Leu Gly Ala₃₃₀ Pro Ile Glu Lys Thr₃₃₅ Ile

Ser Lys Ala Lys₃₄₀ Gly Gln Pro Arg Glu₃₄₅ Pro Gln Val Cys Thr₃₅₀ Leu Pro

Pro Ser Arg₃₅₅ Asp Glu Leu Thr Lys₃₆₀ Asn Gln Val Ser Leu₃₆₅ Ser Cys Ala

Val Lys₃₇₀ Gly Phe Tyr Pro Ser₃₇₅ Asp Ile Ala Val Glu₃₈₀ Trp Glu Ser Asn

Gly₃₈₅ Gln Pro Glu Asn Asn₃₉₀ Tyr Lys Thr Thr Pro₃₉₅ Pro Val Leu Asp Ser₄₀₀

Asp Gly Ser Phe₄₀₅ Phe Leu Val Ser Lys₄₁₀ Leu Thr Val Asp Lys Ser₄₁₅ Arg

Trp Gln Gln Gly₄₂₀ Asn Val Phe Ser Cys₄₂₅ Ser Val Met His₄₃₀ Glu Ala Leu

eolf-seql.txt

His Asn His Tyr Thr Gln Lys Ser Leu Ser Leu Ser Pro Gly Lys
 435 440 445

<210> 208
 <211> 1341
 <212> DNA
 <213> Artificial Sequence

<220>
 <223> 4B9 Fab HC-Fc hole (LALA P329G)

<400> 208
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 tcttgccg ccagcggctt caccttcagc agctacgcca tgagctgggt ccgccaggcc 120
 cctggcaagg gactggaatg ggtgtccgcc atcatcggt ctggcgccag cacctactac 180
 gccgacagcg tgaagggccg gttcaccatc agccgggaca acagcaagaa caccctgtac 240
 ctgcagatga acagcctgcg ggccgaggac accgccgtgt actactgcmc caagggatgg 300
 ttcggcggct tcaactactg gggacagggc accctggtca cagtgtccag cgctagcacc 360
 aagggcccct ccgtgttccc cctggcccc agcagcaaga gcaccagcgg cggcacagcc 420
 gctctgggct gcctgggtcaa ggactacttc cccgagccc tgaccgtgtc ctggaacagc 480
 ggagccctga cctccggcgt gcacaccttc cccgccgtgc tgcagagtgc tggcctgtat 540
 agcctgagca gcgtgggtcac cgtgccttct agcagcctgg gcaccagac ctacatctgc 600
 aacgtgaacc acaagcccag caacaccaag gtggacaaga aggtggagcc caagagctgc 660
 gacaaaactc acacatgccc accgtgccc gcacctgaag ctgcaggggg accgtcagtc 720
 ttcctcttcc cccaaaacc caaggacacc ctcatgatct cccggacccc tgaggtcaca 780
 tgcgtgggtg tggacgtgag ccacgaagac cctgaggtca agttcaactg gtacgtggac 840
 ggcgtggagg tgcataatgc caagacaaag ccgcgggagg agcagtacaa cagcacgtac 900
 cgtgtgggtca gcgtcctcac cgtcctgcac caggactggc tgaatggcaa ggagtacaag 960
 tgcaaggtct ccaacaaagc cctcggcgcc cccatcgaga aaaccatctc caaagccaaa 1020
 gggcagcccc gagaaccaca ggtgtgcacc ctgcccccat cccgggatga gctgaccaag 1080
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 tgggagagca atgggcagcc ggagaacaac tacaagacca cgcctcccgt gctggactcc 1200
 gacggctcct tcttctcgt gagcaagctc accgtggaca agagcaggtg gcagcagggg 1260
 aacgtcttct catgctccgt gatgcatgag gctctgcaca accactacac gcagaagagc 1320
 ctctccctgt ctccgggtaa a 1341

<210> 209
 <211> 593
 <212> PRT
 <213> Artificial Sequence

<220>
 <223> 4B9 Fab HC-Fc knob (LALA P329G)-IL-2 qm

eolf-seql.txt

<400> 209

Glu Val Gln Leu Leu Glu Ser Gly Gly Gly Leu Val Gln Pro Gly Gly
 1 5 10 15
 Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Phe Thr Phe Ser Ser Tyr
 20 25 30
 Ala Met Ser Trp Val Arg Gln Ala Pro Gly Lys Gly Leu Glu Trp Val
 35 40 45
 Ser Ala Ile Ile Gly Ser Gly Ala Ser Thr Tyr Tyr Ala Asp Ser Val
 50 55 60
 Lys Gly Arg Phe Thr Ile Ser Arg Asp Asn Ser Lys Asn Thr Leu Tyr
 65 70 75 80
 Leu Gln Met Asn Ser Leu Arg Ala Glu Asp Thr Ala Val Tyr Tyr Cys
 85 90 95
 Ala Lys Gly Trp Phe Gly Gly Phe Asn Tyr Trp Gly Gln Gly Thr Leu
 100 105 110
 Val Thr Val Ser Ser Ala Ser Thr Lys Gly Pro Ser Val Phe Pro Leu
 115 120 125
 Ala Pro Ser Ser Lys Ser Thr Ser Gly Gly Thr Ala Ala Leu Gly Cys
 130 135 140
 Leu Val Lys Asp Tyr Phe Pro Glu Pro Val Thr Val Ser Trp Asn Ser
 145 150 155 160
 Gly Ala Leu Thr Ser Gly Val His Thr Phe Pro Ala Val Leu Gln Ser
 165 170 175
 Ser Gly Leu Tyr Ser Leu Ser Ser Val Val Thr Val Pro Ser Ser Ser
 180 185 190
 Leu Gly Thr Gln Thr Tyr Ile Cys Asn Val Asn His Lys Pro Ser Asn
 195 200 205
 Thr Lys Val Asp Lys Lys Val Glu Pro Lys Ser Cys Asp Lys Thr His
 210 215 220
 Thr Cys Pro Pro Cys Pro Ala Pro Glu Ala Ala Gly Gly Pro Ser Val
 225 230 235 240
 Phe Leu Phe Pro Pro Lys Pro Lys Asp Thr Leu Met Ile Ser Arg Thr
 245 250 255
 Pro Glu Val Thr Cys Val Val Val Asp Val Ser His Glu Asp Pro Glu
 260 265 270

eolf-seql.txt

Val Lys Phe Asn Trp Tyr Val Asp Gly Val Glu Val His Asn Ala Lys
275 280 285

Thr Lys Pro Arg Glu Glu Gln Tyr Asn Ser Thr Tyr Arg Val Val Ser
290 295 300

Val Leu Thr Val Leu His Gln Asp Trp Leu Asn Gly Lys Glu Tyr Lys
305 310 315 320

Cys Lys Val Ser Asn Lys Ala Leu Gly Ala Pro Ile Glu Lys Thr Ile
325 330 335

Ser Lys Ala Lys Gly Gln Pro Arg Glu Pro Gln Val Tyr Thr Leu Pro
340 345 350

Pro Cys Arg Asp Glu Leu Thr Lys Asn Gln Val Ser Leu Trp Cys Leu
355 360 365

Val Lys Gly Phe Tyr Pro Ser Asp Ile Ala Val Glu Trp Glu Ser Asn
370 375 380

Gly Gln Pro Glu Asn Asn Tyr Lys Thr Thr Pro Pro Val Leu Asp Ser
385 390 395 400

Asp Gly Ser Phe Phe Leu Tyr Ser Lys Leu Thr Val Asp Lys Ser Arg
405 410 415

Trp Gln Gln Gly Asn Val Phe Ser Cys Ser Val Met His Glu Ala Leu
420 425 430

His Asn His Tyr Thr Gln Lys Ser Leu Ser Leu Ser Pro Gly Gly Gly
435 440 445

Gly Gly Ser Gly Gly Gly Gly Ser Gly Gly Gly Gly Ala Pro Ala Ser
450 455 460

Ser Ser Thr Lys Lys Thr Gln Leu Gln Leu Glu His Leu Leu Leu Asp
465 470 475 480

Leu Gln Met Ile Leu Asn Gly Ile Asn Asn Tyr Lys Asn Pro Lys Leu
485 490 495

Thr Arg Met Leu Thr Ala Lys Phe Ala Met Pro Lys Lys Ala Thr Glu
500 505 510

Leu Lys His Leu Gln Cys Leu Glu Glu Glu Leu Lys Pro Leu Glu Glu
515 520 525

Val Leu Asn Gly Ala Gln Ser Lys Asn Phe His Leu Arg Pro Arg Asp
530 535 540

eolf-seql.txt

Leu Ile Ser Asn Ile Asn Val Ile Val Leu Glu Leu Lys Gly Ser Glu
545 550 555 560

Thr Thr Phe Met Cys Glu Tyr Ala Asp Glu Thr Ala Thr Ile Val Glu
565 570 575

Phe Leu Asn Arg Trp Ile Thr Phe Ala Gln Ser Ile Ile Ser Thr Leu
580 585 590

Thr

<210> 210
<211> 1779
<212> DNA
<213> Artificial Sequence

<220>
<223> 4B9 Fab HC-Fc knob (LALA P329G)-IL-2 qm

<400> 210
gaggtgcagc tgctcgaaag cggcggagga ctggtgcagc ctggcggcag cctgagactg 60
tcttgcgccg ccagcggcctt caccttcagc agctacgcc a tgagctgggt ccgccaggcc 120
cctggcaagg gactggaatg ggtgtccgcc atcatcggct ctggcgccag cacctactac 180
gccgacagcg tgaagggccg gttcaccatc agccgggaca acagcaagaa caccctgtac 240
ctgcagatga acagcctgcg ggccgaggac accgccgtgt actactgcgc caagggatgg 300
ttcggcggct tcaactactg gggacagggc accctgggtca cagtgtccag cgctagcacc 360
aagggcccat cggtcttccc cctggcaccc tcttccaaga gcacctctgg gggcacagcg 420
gccctgggct gcctgggtcaa ggactacttc cccgaaccgg tgacggtgtc gtggaactca 480
ggcgccctga ccagcggcgt gcacaccttc ccggctgtcc tacagtcctc aggactctac 540
tccctcagca gcgtggtgac cgtgccctcc agcagcttgg gcacccagac ctacatctgc 600
aacgtgaatc acaagcccag caacaccaag gtggacaaga aagttgagcc caaatcttgt 660
gacaaaactc acacatgccc accgtgccc a gcacctgaag ctgcaggggg accgtcagtc 720
ttcctcttcc ccccaaaacc caaggacacc ctcatgatct cccggacccc tgaggtcaca 780
tgctgtgtgg tggacgtgag ccacgaagac cctgaggtca agttcaactg gtacgtggac 840
ggcgtggagg tgcataatgc caagacaaag ccgcgggagg agcagtacaa cagcacgtac 900
cgtgtgggtca gcgtcctcac cgtcctgcac caggactggc tgaatggcaa ggagtacaag 960
tgcaaggtct ccaacaaagc cctcggcgcc cccatcgaga aaaccatctc caaagccaaa 1020
gggcagcccc gagaaccaca ggtgtacacc ctgcccccat gccgggatga gctgaccaag 1080
aaccaggtca gcctgtggtg cctgggtcaaa ggcttctatc ccagcgacat cgccgtggag 1140
tgggagagca atgggcagcc ggagaacaac tacaagacca cgctcccgt gctggactcc 1200
gacggctcct tcttctctca cagcaagctc accgtggaca agagcaggtg gcagcagggg 1260

eolf-seql.txt

```

aacgtcttct catgctccgt gatgcatgag gctctgcaca accactacac gcagaagagc      1320
ctctccctgt ctccgggtgg cggcggaggc tccggaggcg gaggttcttg cggaggtggc      1380
gctcctgcct cctccagcac caagaaaacc cagctccagc tggaacatct cctgctggat      1440
ctgcagatga tcctgaacgg catcaacaac tacaagaacc ccaagctgac ccggatgctg      1500
accgccaagt tcgccatgcc caagaaggcc accgagctga aacatctgca gtgcctggaa      1560
gaggaactga agcctctgga agaggtgctg aacggcgccc agtccaagaa cttccacctg      1620
aggcctcggg acctgatctc caacatcaac gtgatcgtgc tggaactgaa gggctccgag      1680
acaaccttca tgtgcgagta cgccgacgag acagctacca tcgtggaatt tctgaaccgg      1740
tggatcacct tcgcccagtc catcatctcc accctgacc                               1779

```

```

<210> 211
<211> 215
<212> PRT
<213> Artificial Sequence

```

```

<220>
<223> 3F2 Fab LC

```

```

<400> 211

```

```

Glu Ile Val Leu Thr Gln Ser Pro Gly Thr Leu Ser Leu Ser Pro Gly
1           5           10           15

```

```

Glu Arg Ala Thr Leu Ser Cys Arg Ala Ser Gln Ser Val Thr Ser Ser
20           25           30

```

```

Tyr Leu Ala Trp Tyr Gln Gln Lys Pro Gly Gln Ala Pro Arg Leu Leu
35           40           45

```

```

Ile Asn Val Gly Ser Arg Arg Ala Thr Gly Ile Pro Asp Arg Phe Ser
50           55           60

```

```

Gly Ser Gly Ser Gly Thr Asp Phe Thr Leu Thr Ile Ser Arg Leu Glu
65           70           75           80

```

```

Pro Glu Asp Phe Ala Val Tyr Tyr Cys Gln Gln Gly Ile Met Leu Pro
85           90           95

```

```

Pro Thr Phe Gly Gln Gly Thr Lys Val Glu Ile Lys Arg Thr Val Ala
100          105          110

```

```

Ala Pro Ser Val Phe Ile Phe Pro Pro Ser Asp Glu Gln Leu Lys Ser
115          120          125

```

```

Gly Thr Ala Ser Val Val Cys Leu Leu Asn Asn Phe Tyr Pro Arg Glu
130          135          140

```

```

Ala Lys Val Gln Trp Lys Val Asp Asn Ala Leu Gln Ser Gly Asn Ser
145          150          155          160

```

eolf-seql.txt

Gln Glu Ser Val Thr Glu Gln Asp Ser Lys Asp Ser Thr Tyr Ser Leu
165 170 175

Ser Ser Thr Leu Thr Leu Ser Lys Ala Asp Tyr Glu Lys His Lys Val
180 185 190

Tyr Ala Cys Glu Val Thr His Gln Gly Leu Ser Ser Pro Val Thr Lys
195 200 205

Ser Phe Asn Arg Gly Glu Cys
210 215

<210> 212
<211> 645
<212> DNA
<213> Artificial Sequence

<220>
<223> 3F2 Fab LC

<400> 212
gagatcgtgc tgaccagtc ccccggcacc ctgtctctga gccctggcga gagagccacc 60
ctgtcctgca gagcctccca gtccgtgacc tcctcctacc tcgcctggta tcagcagaag 120
cccgccagg cccctcggct gctgatcaac gtgggcagtc ggagagccac cggcatccct 180
gaccggttct ccggctctgg ctccggcacc gacttcaccc tgaccatctc ccggctggaa 240
cccgaggact tcgccgtgta ctactgccag cagggcatca tgctgcccc cacctttggc 300
cagggcacca aggtggaaat caagcgtacg gtggccgctc cctccgtggt catcttccca 360
ccctccgacg agcagctgaa gtccggcacc gcctccgtcg tgtgcctgct gaacaacttc 420
tacccccgcg aggccaaggt gcagtggaag gtggacaacg cctgcagtc cggcaactcc 480
caggaatccg tcaccgagca ggactccaag gacagcacct actccctgtc ctccaccctg 540
accctgtcca aggccgacta cgagaagcac aaggtgtacg cctgcgaagt gaccaccag 600
ggcctgtcca gcccgtgac caagtccttc aaccggggcg agtgc 645

<210> 213
<211> 446
<212> PRT
<213> Artificial Sequence

<220>
<223> L19 Fab HC-Fc hole (LALA P329G)

<400> 213

Glu Val Gln Leu Leu Glu Ser Gly Gly Gly Leu Val Gln Pro Gly Gly
1 5 10 15

Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Phe Thr Phe Ser Ser Phe
20 25 30

ecolf-seql.txt

Ser Met Ser Trp Val Arg Gln Ala Pro Gly Lys Gly Leu Glu Trp Val
35 40 45

Ser Ser Ile Ser Gly Ser Ser Gly Thr Thr Tyr Tyr Ala Asp Ser Val
50 55 60

Lys Gly Arg Phe Thr Ile Ser Arg Asp Asn Ser Lys Asn Thr Leu Tyr
65 70 75 80

Leu Gln Met Asn Ser Leu Arg Ala Glu Asp Thr Ala Val Tyr Tyr Cys
85 90 95

Ala Lys Pro Phe Pro Tyr Phe Asp Tyr Trp Gly Gln Gly Thr Leu Val
100 105 110

Thr Val Ser Ser Ala Ser Thr Lys Gly Pro Ser Val Phe Pro Leu Ala
115 120 125

Pro Ser Ser Lys Ser Thr Ser Gly Gly Thr Ala Ala Leu Gly Cys Leu
130 135 140

Val Lys Asp Tyr Phe Pro Glu Pro Val Thr Val Ser Trp Asn Ser Gly
145 150 155 160

Ala Leu Thr Ser Gly Val His Thr Phe Pro Ala Val Leu Gln Ser Ser
165 170 175

Gly Leu Tyr Ser Leu Ser Ser Val Val Thr Val Pro Ser Ser Ser Leu
180 185 190

Gly Thr Gln Thr Tyr Ile Cys Asn Val Asn His Lys Pro Ser Asn Thr
195 200 205

Lys Val Asp Lys Lys Val Glu Pro Lys Ser Cys Asp Lys Thr His Thr
210 215 220

Cys Pro Pro Cys Pro Ala Pro Glu Ala Ala 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 Asn Ser Thr Tyr Arg Val Val Ser Val
290 295 300

eolf-seql.txt

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 Gly Ala Pro Ile Glu Lys Thr Ile Ser
325 330 335

Lys Ala Lys Gly Gln Pro Arg Glu Pro Gln Val Cys Thr Leu Pro Pro
340 345 350

Ser Arg Asp Glu Leu Thr Lys Asn Gln Val Ser Leu Ser Cys Ala 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 Val 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> 214
<211> 1338
<212> DNA
<213> Artificial Sequence

<220>
<223> L19 Fab HC-Fc hole (LALA P329G)

<400> 214
gaggtgcagc tggttgagtc tgggggaggc ttggtacagc ctgggggggtc cctgagactc 60
tcctgtgcag cctctggatt caccttttagc agtttttcga tgagctgggt ccgccaggct 120
ccagggaagg ggctggagtg ggtctcatct atttccggta gttcgggtac cacatactac 180
gcagactccg tgaagggccg gttcaccatc tccagagaca attccaagaa cacgctgtat 240
ctgcaaatac acagcctgag agccgaggac acggccgtat attactgtgc gaaaccgttt 300
ccgtattttg actactgggg ccagggaacc ctgggtcaccg tctcgagtgc tagcaccaag 360
ggcccctccg tggtccccct ggccccagc agcaagagca ccagcggcgg cacagccgct 420
ctgggctgcc tgggtcaagga ctacttcccc gagcccgtga ccgtgtcctg gaacagcgga 480
gccctgacct ccggcgtgca caccttcccc gccgtgctgc agagttcttg cctgtatagc 540
ctgagcagcg tggtcaccgt gccttctagc agcctgggca ccagacctc catctgcaac 600
gtgaaccaca agcccagcaa caccaaggtg gacaagaagg tggagcccaa gagctgcgac 660
aaaactcaca catgcccacc gtgcccagca cctgaagctg caggggggacc gtcagtcttc 720

eolf-seql.txt

```

ctcttcccc caaaaccaa ggacaccctc atgatctccc ggaccctga ggtcacatgc      780
gtggtggtgg acgtgagcca cgaagaccct gaggtcaagt tcaactggta cgtggacggc      840
gtggaggtgc ataatgcaa gacaaagccg cgggaggagc agtacaacag cacgtaccgt      900
gtggtcagcg tcctcaccgt cctgcaccag gactggctga atggcaagga gtacaagtgc      960
aaggtctcca acaaagccct cggcgccccc atcgagaaaa ccatctccaa agccaaaggg    1020
cagccccgag aaccacaggt gtgcaccctg ccccatccc gggatgagct gaccaagaac    1080
caggtcagcc tctcgtgcgc agtcaaaggc ttctatccca gcgacatcgc cgtggagtgg    1140
gagagcaatg ggcagccgga gaacaactac aagaccacgc ctcccgtgct ggactccgac    1200
ggctccttct tcctcgtgag caagctcacc gtggacaaga gcaggtggca gcaggggaac    1260
gtcttctcat gtcctgtgat gcatgaggct ctgcacaacc actacacgca gaagagcctc    1320
tccctgtctc cgggtaaa                                1338

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<210> 215
 <211> 592
 <212> PRT
 <213> Artificial Sequence

<220>
 <223> L19 Fab HC-Fc knob (LALA P329G)-IL-2 qm
 <400> 215

Glu Val Gln Leu Leu Glu Ser Gly Gly Gly Leu Val Gln Pro Gly Gly
 1 5 10 15

Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Phe Thr Phe Ser Ser Phe
 20 25 30

Ser Met Ser Trp Val Arg Gln Ala Pro Gly Lys Gly Leu Glu Trp Val
 35 40 45

Ser Ser Ile Ser Gly Ser Ser Gly Thr Thr Tyr Tyr Ala Asp Ser Val
 50 55 60

Lys Gly Arg Phe Thr Ile Ser Arg Asp Asn Ser Lys Asn Thr Leu Tyr
 65 70 75 80

Leu Gln Met Asn Ser Leu Arg Ala Glu Asp Thr Ala Val Tyr Tyr Cys
 85 90 95

Ala Lys Pro Phe Pro Tyr Phe Asp Tyr Trp Gly Gln Gly Thr Leu Val
 100 105 110

Thr Val Ser Ser Ala Ser Thr Lys Gly Pro Ser Val Phe Pro Leu Ala
 115 120 125

Pro Ser Ser Lys Ser Thr Ser Gly Gly Thr Ala Ala Leu Gly Cys Leu
 130 135 140

eolf-seql.txt

Val Lys Asp Tyr Phe Pro Glu Pro Val Thr Val Ser Trp Asn Ser Gly
145 150 155 160

Ala Leu Thr Ser Gly Val His Thr Phe Pro Ala Val Leu Gln Ser Ser
165 170 175

Gly Leu Tyr Ser Leu Ser Ser Val Val Thr Val Pro Ser Ser Ser Leu
180 185 190

Gly Thr Gln Thr Tyr Ile Cys Asn Val Asn His Lys Pro Ser Asn Thr
195 200 205

Lys Val Asp Lys Lys Val Glu Pro Lys Ser Cys Asp Lys Thr His Thr
210 215 220

Cys Pro Pro Cys Pro Ala Pro Glu Ala Ala 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 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
305 310 315 320

Lys Val Ser Asn Lys Ala Leu Gly 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

Cys Arg Asp Glu Leu Thr Lys Asn Gln Val Ser Leu Trp 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

eolf-seql.txt

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 Gly Gly Gly
435 440 445

Gly Ser Gly Gly Gly Gly Ser Gly Gly Gly Gly Ala Pro Ala Ser Ser
450 455 460

Ser Thr Lys Lys Thr Gln Leu Gln Leu Glu His Leu Leu Leu Asp Leu
465 470 475 480

Gln Met Ile Leu Asn Gly Ile Asn Asn Tyr Lys Asn Pro Lys Leu Thr
485 490 495

Arg Met Leu Thr Ala Lys Phe Ala Met Pro Lys Lys Ala Thr Glu Leu
500 505 510

Lys His Leu Gln Cys Leu Glu Glu Glu Leu Lys Pro Leu Glu Glu Val
515 520 525

Leu Asn Gly Ala Gln Ser Lys Asn Phe His Leu Arg Pro Arg Asp Leu
530 535 540

Ile Ser Asn Ile Asn Val Ile Val Leu Glu Leu Lys Gly Ser Glu Thr
545 550 555 560

Thr Phe Met Cys Glu Tyr Ala Asp Glu Thr Ala Thr Ile Val Glu Phe
565 570 575

Leu Asn Arg Trp Ile Thr Phe Ala Gln Ser Ile Ile Ser Thr Leu Thr
580 585 590

<210> 216
<211> 1776
<212> DNA
<213> Artificial Sequence

<220>
<223> L19 Fab HC-Fc knob (LALA P329G)-IL-2 qm

<400> 216
gaggtgcagc tgttggagtc tgggggaggc ttggtacagc ctgggggggtc cctgagactc 60
tcctgtgcag cctctggatt cacctttagc agtttttcga tgagctgggt ccgccaggct 120
ccagggaagg ggctggagtg ggtctcatct atttccggta gttcgggtac cacatactac 180
gcagactccg tgaagggccg gttcaccatc tccagagaca attccaagaa cacgctgtat 240
ctgcaaatac acagcctgag agccgaggac acggccgtat attactgtgc gaaaccgttt 300
ccgtattttg actactgggg ccagggaacc ctggtcaccg tctcgagtgc tagcaccaag 360
ggcccatcgg tcttccccct ggcaccctcc tccaagagca cctctggggg cacagcggcc 420

eolf-seql.txt

```

ctgggctgcc tggtaagga ctacttcccc gaaccggtga cgggtgctgtg gaactcaggc      480
gccctgacca gcggcgtgca caccttcccc gctgtcctac agtcctcagg actctactcc      540
ctcagcagcg tggtgaccgt gccctccagc agcttgggca cccagaccta catctgcaac      600
gtgaatcaca agcccagcaa caccaaggtg gacaagaaag ttgagcccaa atcttgtgac      660
aaaactcaca catgcccacc gtgcccagca cctgaagctg caggggggacc gtcagtcttc      720
ctcttcccc caaaacccaa ggacaccctc atgatctccc ggaccctga ggtcacatgc      780
gtggtggtgg acgtgagcca cgaagaccct gaggtcaagt tcaactggta cgtggacggc      840
gtggaggtgc ataatgcaa gacaaagccg cgggaggagc agtacaacag cacgtaccgt      900
gtggtcagcg tcctcaccgt cctgcaccag gactggctga atggcaagga gtacaagtgc      960
aaggctctca acaaagccct cggcgcccc atcgagaaaa ccatctccaa agccaaaggg     1020
cagccccgag aaccacaggt gtacaccctg ccccatgcc gggatgagct gaccaagaac     1080
caggtcagcc tgtggtgcct ggtcaaaggc ttctatccca gcgacatcg cgtggagtgg     1140
gagagcaatg ggagccgga gaacaactac aagaccacgc ctcccgtgct ggactccgac     1200
ggctccttct tcctctacag caagctcacc gtggacaaga gcaggtggca gcaggggaac     1260
gtcttctcat gctccgtgat gcatgaggct ctgcacaacc actacacgca gaagagcctc     1320
tccctgtctc cgggtggcgg cggaggctcc ggaggcggag gttctggcgg aggtggcgct     1380
cctgcctcct ccagcaccaa gaaaaccag ctccagctgg aacatctcct gctggatctg     1440
cagatgatcc tgaacggcat caacaactac aagaacccca agctgaccg gatgctgacc     1500
gccaagtctg ccatgccc aaaggccacc gagctgaaac atctgcagtg cctggaagag     1560
gaactgaagc ctctggaaga ggtgctgaac ggcgccagc ccaagaactt ccacctgagg     1620
cctcgggacc tgatctccaa catcaacgtg atcgtgctgg aactgaaggg ctccgagaca     1680
accttcatgt gcgagtacgc cgacgagaca gctaccatcg tggaatttct gaaccggtgg     1740
atcaccttcg cccagtccat catctccacc ctgacc                                1776

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<210> 217
 <211> 215
 <212> PRT
 <213> Artificial Sequence

<220>
 <223> L19 Fab LC

<400> 217

Glu Ile Val Leu Thr Gln Ser Pro Gly Thr Leu Ser Leu Ser Pro Gly
 1 5 10 15

Glu Arg Ala Thr Leu Ser Cys Arg Ala Ser Gln Ser Val Ser Ser Ser
 20 25 30

Tyr Leu Ala Trp Tyr Gln Gln Lys Pro Gly Gln Ala Pro Arg Leu Leu
 35 40 45

eolf-seql.txt

Ile Tyr Tyr Ala Ser Ser Arg Ala Thr Gly Ile Pro Asp Arg Phe Ser
50 55 60

Gly Ser Gly Ser Gly Thr Asp Phe Thr Leu Thr Ile Ser Arg Leu Glu
65 70 75 80

Pro Glu Asp Phe Ala Val Tyr Tyr Cys Gln Gln Thr Gly Arg Ile Pro
85 90 95

Pro Thr Phe Gly Gln Gly Thr Lys Val Glu Ile Lys Arg Thr Val Ala
100 105 110

Ala Pro Ser Val Phe Ile Phe Pro Pro Ser Asp Glu Gln Leu Lys Ser
115 120 125

Gly Thr Ala Ser Val Val Cys Leu Leu Asn Asn Phe Tyr Pro Arg Glu
130 135 140

Ala Lys Val Gln Trp Lys Val Asp Asn Ala Leu Gln Ser Gly Asn Ser
145 150 155 160

Gln Glu Ser Val Thr Glu Gln Asp Ser Lys Asp Ser Thr Tyr Ser Leu
165 170 175

Ser Ser Thr Leu Thr Leu Ser Lys Ala Asp Tyr Glu Lys His Lys Val
180 185 190

Tyr Ala Cys Glu Val Thr His Gln Gly Leu Ser Ser Pro Val Thr Lys
195 200 205

Ser Phe Asn Arg Gly Glu Cys
210 215

<210> 218
<211> 645
<212> DNA
<213> Artificial Sequence

<220>
<223> L19 Fab LC

<400> 218
gaaattgtgt tgacgcagtc tccaggcacc ctgtctttgt ctccagggga aagagccacc 60
ctctcctgca gggccagtca gagtgtagc agcagctact tagcctggta ccagcagaaa 120
cctggccagg ctcccaggct cctcatctat tatgcatcca gcagggccac tggcatccca 180
gacaggttca gtggcagtggt gtctgggaca gacttcactc tcaccatcag cagactggag 240
cctgaagatt ttgcagtgtt ttactgtcag cagacgggtc gtattcctcc gacgttcggc 300
caagggacca aggttgaaat caaacgtacg gtggctgcac catctgtctt catcttcccg 360
ccatctgatg agcagttgaa atctggaact gcctctgttg tgtgcctgct gaataacttc 420

eolf-seql.txt

tatcccagag aggccaaagt acagtggaag gtggataacg ccctccaatc gggtaactcc	480
caggagagtg tcacagagca ggacagcaag gacagcacct acagcctcag cagcaccctg	540
acgctgagca aagcagacta cgagaaacac aaagtctacg cctgcgaagt cacccatcag	600
ggcctgagct cgcccgtcac aaagagcttc aacaggggag agtgt	645

<210> 219
 <211> 445
 <212> PRT
 <213> Artificial Sequence

<220>
 <223> DP47 GS Fab HC-Fc hole (LALA P329G)

<400> 219

Glu Val Gln Leu	Leu Glu Ser Gly Gly Gly	Leu Val Gln Pro Gly Gly
1	5	10 15

Ser Leu Arg	Leu Ser Cys Ala Ala	Ser Gly Phe Thr Phe Ser Ser Tyr
	20	25 30

Ala Met Ser Trp Val Arg Gln	Ala Pro Gly Lys Gly	Leu Glu Trp Val
	35 40	45

Ser Ala Ile Ser Gly Ser	Gly Gly Ser Thr Tyr Tyr	Ala Asp Ser Val
	50 55	60

Lys Gly Arg Phe Thr	Ile Ser Arg Asp Asn Ser	Lys Asn Thr Leu Tyr
65	70	75 80

Leu Gln Met Asn	Ser Leu Arg Ala Glu Asp Thr	Ala Val Tyr Tyr Cys
	85	90 95

Ala Lys Gly Ser Gly Phe Asp Tyr	Trp Gly Gln Gly Thr	Leu Val Thr
	100 105	110

Val Ser Ser Ala Ser Thr Lys	Gly Pro Ser Val Phe Pro	Leu Ala Pro
	115 120	125

Ser Ser Lys Ser Thr Ser	Gly Gly Thr Ala Ala	Leu Gly Cys Leu Val
	130 135	140

Lys Asp Tyr Phe Pro	Glu Pro Val Thr Val Ser	Trp Asn Ser Gly Ala
145	150	155 160

Leu Thr Ser Gly Val His Thr Phe Pro	Ala Val Leu Gln Ser Ser Gly	
	165 170	175

Leu Tyr Ser	Leu Ser Ser Val Val Thr	Val Pro Ser Ser Ser Leu Gly
	180	185 190

eofl-seql.txt

Thr Gln Thr Tyr Ile Cys Asn Val Asn His Lys Pro Ser Asn Thr Lys
195 200 205

Val Asp Lys Lys Val Glu Pro Lys Ser Cys Asp Lys Thr His Thr Cys
210 215 220

Pro Pro Cys Pro Ala Pro Glu Ala Ala 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 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

Val Ser Asn Lys Ala Leu Gly Ala Pro Ile Glu Lys Thr Ile Ser Lys
325 330 335

Ala Lys Gly Gln Pro Arg Glu Pro Gln Val Cys Thr Leu Pro Pro Ser
340 345 350

Arg Asp Glu Leu Thr Lys Asn Gln Val Ser Leu Ser Cys Ala 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 Val 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> 220
<211> 1335
<212> DNA
<213> Artificial Sequence

eolf-seql.txt

<220>

<223> DP47 GS Fab HC-Fc hole (LALA P329G)

<400> 220

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gaggtgcaat tgttgagtc tgggggaggc ttggtacagc ctgggggggtc cctgagactc      60
tcctgtgcag cctccggatt cacctttagc agttatgcca tgagctgggt ccgccaggct      120
ccaggggaagg ggctggagtg ggtctcagct attagtggta gtggtggtag cacatactac      180
gcagactccg tgaagggccg gttcaccatc tccagagaca attccaagaa cacgctgtat      240
ctgcagatga acagcctgag agccgaggac acggccgtat attactgtgc gaaaggcagc      300
ggatttgact actggggcca aggaaccctg gtcaccgtct cgagtgctag caccaagggc      360
ccctccgtgt tccccctggc ccccagcagc aagagcacca gcggcggcac agccgctctg      420
ggctgcctgg tcaaggacta cttccccgag cccgtgaccg tgtcctggaa cagcggagcc      480
ctgacctccg gcgtgcacac cttccccgcc gtgctgcaga gttctggcct gtatagcctg      540
agcagcgtgg tcaccgtgcc ttctagcagc ctgggcaccc agacctacat ctgcaacgtg      600
aaccacaagc ccagcaacac caaggtggac aagaaggtgg agcccaagag ctgcgacaaa      660
actcacacat gcccaccgtg cccagcacct gaagctgcag ggggaccgtc agtcttcctc      720
ttccccccaa aaccaagga caccctcatg atctcccga cccctgaggt cacatgcgtg      780
gtggtggacg tgagccacga agaccctgag gtcaagttca actggtacgt ggacggcgtg      840
gaggtgcata atgccaagac aaagccgcgg gaggagcagt acaacagcac gtaccgtgtg      900
gtcagcgtcc tcaccgtcct gcaccaggac tggctgaatg gcaaggagta caagtgaag      960
gtctccaaca aagccctcgg cgccccatc gagaaaacca tctccaaagc caaagggcag     1020
ccccgagaac cacaggtgtg caccctgccc ccatcccggg atgagctgac caagaaccag     1080
gtcagcctct cgtgcgcagt caaaggcttc tatcccagcg acatcgccgt ggagtgggag     1140
agcaatgggc agccggagaa caactacaag accacgcctc ccgtgctgga ctccgacggc     1200
tccttcttcc tcgtgagcaa gtcaccgtg gacaagagca ggtggcagca ggggaacgtc     1260
ttctcatgct ccgtgatgca tgaggctctg cacaaccact acacgcagaa gagcctctcc     1320
ctgtctccgg gtaaa                                           1335

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<210> 221

<211> 591

<212> PRT

<213> Artificial Sequence

<220>

<223> DP47 GS Fab HC-Fc knob (LALA P329G)-IL-2 qm

<400> 221

Glu Val Gln Leu Leu Glu Ser Gly Gly Gly Leu Val Gln Pro Gly Gly
1 5 10 15

Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Phe Thr Phe Ser Ser Tyr
20 25 30

eolf-seql.txt

Ala Met Ser Trp Val Arg Gln Ala Pro Gly Lys Gly Leu Glu Trp Val
35 40 45

Ser Ala Ile Ser Gly Ser Gly Gly Ser Thr Tyr Tyr Ala Asp Ser Val
50 55 60

Lys Gly Arg Phe Thr Ile Ser Arg Asp Asn Ser Lys Asn Thr Leu Tyr
65 70 75 80

Leu Gln Met Asn Ser Leu Arg Ala Glu Asp Thr Ala Val Tyr Tyr Cys
85 90 95

Ala Lys Gly Ser Gly Phe Asp Tyr Trp Gly Gln Gly Thr Leu Val Thr
100 105 110

Val Ser Ser Ala Ser Thr Lys Gly Pro Ser Val Phe Pro Leu Ala Pro
115 120 125

Ser Ser Lys Ser Thr Ser Gly Gly Thr Ala Ala Leu Gly Cys Leu Val
130 135 140

Lys Asp Tyr Phe Pro Glu Pro Val Thr Val Ser Trp Asn Ser Gly Ala
145 150 155 160

Leu Thr Ser Gly Val His Thr Phe Pro Ala Val Leu Gln Ser Ser Gly
165 170 175

Leu Tyr Ser Leu Ser Ser Val Val Thr Val Pro Ser Ser Ser Leu Gly
180 185 190

Thr Gln Thr Tyr Ile Cys Asn Val Asn His Lys Pro Ser Asn Thr Lys
195 200 205

Val Asp Lys Lys Val Glu Pro Lys Ser Cys Asp Lys Thr His Thr Cys
210 215 220

Pro Pro Cys Pro Ala Pro Glu Ala Ala 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

eolf-seql.txt

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 Gly 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 Cys
340 345 350

Arg Asp Glu Leu Thr Lys Asn Gln Val Ser Leu Trp 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 Gly Gly Gly Gly
435 440 445

Ser Gly Gly Gly Gly Ser Gly Gly Gly Gly Ala Pro Ala Ser Ser Ser
450 455 460

Thr Lys Lys Thr Gln Leu Gln Leu Glu His Leu Leu Leu Asp Leu Gln
465 470 475 480

Met Ile Leu Asn Gly Ile Asn Asn Tyr Lys Asn Pro Lys Leu Thr Arg
485 490 495

Met Leu Thr Ala Lys Phe Ala Met Pro Lys Lys Ala Thr Glu Leu Lys
500 505 510

His Leu Gln Cys Leu Glu Glu Glu Leu Lys Pro Leu Glu Glu Val Leu
515 520 525

Asn Gly Ala Gln Ser Lys Asn Phe His Leu Arg Pro Arg Asp Leu Ile
530 535 540

Ser Asn Ile Asn Val Ile Val Leu Glu Leu Lys Gly Ser Glu Thr Thr
545 550 555 560

Phe Met Cys Glu Tyr Ala Asp Glu Thr Ala Thr Ile Val Glu Phe Leu
565 570 575

eolf-seql.txt

Asn Arg Trp Ile Thr Phe Ala Gln Ser Ile Ile Ser Thr Leu Thr
580 585 590

<210> 222
<211> 1773
<212> DNA
<213> Artificial Sequence

<220>
<223> DP47 GS Fab HC-Fc knob (LALA P329G)-IL-2 qm

<400> 222
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tcctgtgcag cctccggatt cacctttagc agttatgcc a tgagctgggt ccgccaggct 120
ccagggaagg ggctggagtg ggtctcagct attagtggta gtggtggtag cacatactac 180
gcagactccg tgaagggccg gttcaccatc tccagagaca attccaagaa cacgctgtat 240
ctgcagatga acagcctgag agccgaggac acggccgtat attactgtgc gaaaggcagc 300
ggatttgact actggggcca aggaaccctg gtcaccgtct cgagtgctag caccaagggc 360
ccatcggtct tccccctggc accctcctcc aagagcacct ctggggggcac agcgggccctg 420
ggctgcctgg tcaaggacta cttccccgaa ccggtgacgg tgtcgtggaa ctcaggcgcc 480
ctgaccagcg gcgtgcacac cttcccggtc gtcctacagt cctcaggact ctactccctc 540
agcagcgtgg tgaccgtgcc ctccagcagc ttgggcaccc agacctacat ctgcaacgtg 600
aatcacaagc ccagcaacac caaggtggac aagaaagttg agcccaaatac ttgtgacaaa 660
actcacacat gccaccgtg cccagcacct gaagctgcag ggggaccgtc agtcttcctc 720
ttccccccaa aaccaagga caccctcatg atctcccga cccctgaggt cacatgcgtg 780
gtggtggacg tgagccacga agaccctgag gtcaagttca actggtacgt ggacggcgtg 840
gaggtgcata atgccaagac aaagccgcgg gaggagcagt acaacagcac gtaccgtgtg 900
gtcagcgtcc tcaccgtcct gcaccaggac tggctgaatg gcaaggagta caagtgaag 960
gtctccaaca aagccctcgg cgccccatc gagaaaacca tctccaaagc caaagggcag 1020
ccccgagaac cacaggtgta caccctgccc ccatgccggg atgagctgac caagaaccag 1080
gtcagcctgt ggtgcctggt caaaggcttc tatccagcg acatcgccgt ggagtgggag 1140
agcaatgggc agccggagaa caactacaag accacgcctc ccgtgctgga ctccgacggc 1200
tccttcttcc tctacagcaa gctcaccgtg gacaagagca ggtggcagca ggggaacgtc 1260
ttctcatgct ccgtgatgca tgaggctctg cacaaccact acacgcagaa gagcctctcc 1320
ctgtctccgg gtggcggcgg aggtccgga ggcggagggt ctggcggagg tggcgctcct 1380
gcctcctcca gcaccaagaa aaccagctc cagctggaac atctcctgct ggatctgcag 1440
atgatcctga acggcatcaa caactacaag aaccccaagc tgaccgggat gctgaccgcc 1500
aagttcgcca tgccaagaa ggccaccgag ctgaaacatc tgcagtgcct ggaagaggaa 1560
ctgaagcctc tggaagaggt gctgaacggc gccagtgcca agaacttcca cctgaggcct 1620

eolf-seql.txt

cgggacctga tctccaacat caacgtgatc gtgctggaac tgaagggctc cgagacaacc 1680
 ttcatgtgcg agtacgccga cgagacagct accatcgtgg aatttctgaa ccggtggatc 1740
 accttcgccc agtccatcat ctccaccctg acc 1773

<210> 223
 <211> 591
 <212> PRT
 <213> Artificial Sequence

<220>
 <223> DP47GL Fab HC-Fc knob (LALA P329G)- IL-2 wt
 <400> 223

Glu Val Gln Leu Leu Glu Ser Gly Gly Gly Leu Val Gln Pro Gly Gly
 1 5 10 15

Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Phe Thr Phe Ser Ser Tyr
 20 25 30

Ala Met Ser Trp Val Arg Gln Ala Pro Gly Lys Gly Leu Glu Trp Val
 35 40 45

Ser Ala Ile Ser Gly Ser Gly Gly Ser Thr Tyr Tyr Ala Asp Ser Val
 50 55 60

Lys Gly Arg Phe Thr Ile Ser Arg Asp Asn Ser Lys Asn Thr Leu Tyr
 65 70 75 80

Leu Gln Met Asn Ser Leu Arg Ala Glu Asp Thr Ala Val Tyr Tyr Cys
 85 90 95

Ala Lys Gly Ser Gly Phe Asp Tyr Trp Gly Gln Gly Thr Leu Val Thr
 100 105 110

Val Ser Ser Ala Ser Thr Lys Gly Pro Ser Val Phe Pro Leu Ala Pro
 115 120 125

Ser Ser Lys Ser Thr Ser Gly Gly Thr Ala Ala Leu Gly Cys Leu Val
 130 135 140

Lys Asp Tyr Phe Pro Glu Pro Val Thr Val Ser Trp Asn Ser Gly Ala
 145 150 155 160

Leu Thr Ser Gly Val His Thr Phe Pro Ala Val Leu Gln Ser Ser Gly
 165 170 175

Leu Tyr Ser Leu Ser Ser Val Val Thr Val Pro Ser Ser Ser Leu Gly
 180 185 190

Thr Gln Thr Tyr Ile Cys Asn Val Asn His Lys Pro Ser Asn Thr Lys
 195 200 205

eolf-seql.txt

Val Asp Lys Lys Val Glu Pro Lys Ser Cys Asp Lys Thr His Thr Cys
210 215 220

Pro Pro Cys Pro Ala Pro Glu Ala Ala 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 Gly 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 Cys
340 345 350

Arg Asp Glu Leu Thr Lys Asn Gln Val Ser Leu Trp 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 Gly Gly Gly Gly
435 440 445

Ser Gly Gly Gly Gly Ser Gly Gly Gly Gly Ala Pro Thr Ser Ser Ser
450 455 460

Thr Lys Lys Thr Gln Leu Gln Leu Glu His Leu Leu Leu Asp Leu Gln
465 470 475 480

eolf-seql.txt

Met Ile Leu Asn Gly Ile Asn Asn Tyr Lys Asn Pro Lys Leu Thr Arg
485 490 495

Met Leu Thr Phe Lys Phe Tyr Met Pro Lys Lys Ala Thr Glu Leu Lys
500 505 510

His Leu Gln Cys Leu Glu Glu Glu Leu Lys Pro Leu Glu Glu Val Leu
515 520 525

Asn Leu Ala Gln Ser Lys Asn Phe His Leu Arg Pro Arg Asp Leu Ile
530 535 540

Ser Asn Ile Asn Val Ile Val Leu Glu Leu Lys Gly Ser Glu Thr Thr
545 550 555 560

Phe Met Cys Glu Tyr Ala Asp Glu Thr Ala Thr Ile Val Glu Phe Leu
565 570 575

Asn Arg Trp Ile Thr Phe Ala Gln Ser Ile Ile Ser Thr Leu Thr
580 585 590

<210> 224
<211> 1773
<212> DNA
<213> Artificial Sequence

<220>
<223> DP47GS Fab HC-Fc knob (LALA P329G)- IL-2 wt

<400> 224
gaggtgcaat tgttggagtc tgggggaggc ttggtacagc ctgggggggtc cctgagactc 60
tcctgtgcag cctccggatt cacctttagc agttatgcca tgagctgggt ccgccaggct 120
ccagggaagg ggctggagtg ggtctcagct attagtggta gtggtggtag cacatactac 180
gcagactccg tgaagggccg gttcaccatc tccagagaca attccaagaa cacgctgtat 240
ctgcagatga acagcctgag agccgaggac acggccgtat attactgtgc gaaaggcagc 300
ggatttgact actggggcca aggaaccctg gtcaccgtct cgagtgctag caccaagggc 360
ccatcggtct tccccctggc accctcctcc aagagcacct ctggggggcac agcggccctg 420
ggctgcctgg tcaaggacta cttccccgaa ccggtgacgg tgtcgtggaa ctcaggcgcc 480
ctgaccagcg gcgtgcacac cttcccggct gtcctacagt cctcaggact ctactccctc 540
agcagcgtgg tgaccgtgcc ctccagcagc ttgggcaccc agacctacat ctgcaacgtg 600
aatcacaagc ccagcaacac caaggtggac aagaaagttg agcccaaadc ttgtgacaaa 660
actcacacat gccaccgtg cccagcacct gaagctgcag ggggaccgtc agtcttcctc 720
ttccccccaa aaccaagga caccctcatg atctcccga cccctgaggt cacatgcgtg 780
gtggtggacg tgagccacga agaccctgag gtcaagttca actggtacgt ggacggcgtg 840
gaggtgcata atgccaagac aaagccgcgg gaggagcagt acaacagcac gtaccgtgtg 900

eolf-seql.txt

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gtcagcgtcc tcaccgtcct gcaccaggac tggctgaatg gcaaggagta caagtgcaag      960
gtctccaaca aagccctcgg cgcccccatc gagaaaacca tctccaaagc caaagggcag      1020
ccccgagaac cacaggtgta caccctgccc ccatgccggg atgagctgac caagaaccag      1080
gtcagcctgt ggtgcctggg caaaggcttc tatcccagcg acatcgccgt ggagtgggag      1140
agcaatgggc agccggagaa caactacaag accacgcctc ccgtgctgga ctccgacggc      1200
tccttcttcc tctacagcaa gctcaccgtg gacaagagca ggtggcagca ggggaacgtc      1260
ttctcatgct ccgtgatgca tgaggctctg cacaaccact acacgcagaa gagcctctcc      1320
ctgtctccgg gtggcggcgg aggctccgga ggcggagggt ctggcggagg tggcgctcct      1380
acatcctcca gcaccaagaa aaccagctc cagctggaac atctcctgct ggatctgcag      1440
atgatcctga acggcatcaa caactacaag aacccaagc tgaccgggat gctgaccttc      1500
aagttctaca tgcccaagaa ggccaccgag ctgaaacatc tgcatgcctt ggaagaggaa      1560
ctgaagcctc tggaagaggt gctgaacctg gccagtgcca agaacttcca cctgaggcct      1620
cgggacctga tctccaacat caacgtgatc gtgctggaac tgaagggtc cgagacaacc      1680
ttcatgtgcg agtacgccga cgagacagct accatcgtgg aatttctgaa ccggtggatc      1740
accttcgccc agtccatcat ctccaccctg acc                                     1773

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<210> 225
 <211> 215
 <212> PRT
 <213> Artificial Sequence

<220>
 <223> DP47GS Fab LC

<400> 225

Glu Ile Val Leu Thr Gln Ser Pro Gly Thr Leu Ser Leu Ser Pro Gly
 1 5 10 15

Glu Arg Ala Thr Leu Ser Cys Arg Ala Ser Gln Ser Val Ser Ser Ser
 20 25 30

Tyr Leu Ala Trp Tyr Gln Gln Lys Pro Gly Gln Ala Pro Arg Leu Leu
 35 40 45

Ile Tyr Gly Ala Ser Ser Arg Ala Thr Gly Ile Pro Asp Arg Phe Ser
 50 55 60

Gly Ser Gly Ser Gly Thr Asp Phe Thr Leu Thr Ile Ser Arg Leu Glu
 65 70 75 80

Pro Glu Asp Phe Ala Val Tyr Tyr Cys Gln Gln Tyr Gly Ser Ser Pro
 85 90 95

Leu Thr Phe Gly Gln Gly Thr Lys Val Glu Ile Lys Arg Thr Val Ala
 100 105 110

eolf-seql.txt

Ala Pro Ser Val Phe Ile Phe Pro Pro Ser Asp Glu Gln Leu Lys Ser
115 120 125

Gly Thr Ala Ser Val Val Cys Leu Leu Asn Asn Phe Tyr Pro Arg Glu
130 135 140

Ala Lys Val Gln Trp Lys Val Asp Asn Ala Leu Gln Ser Gly Asn Ser
145 150 155 160

Gln Glu Ser Val Thr Glu Gln Asp Ser Lys Asp Ser Thr Tyr Ser Leu
165 170 175

Ser Ser Thr Leu Thr Leu Ser Lys Ala Asp Tyr Glu Lys His Lys Val
180 185 190

Tyr Ala Cys Glu Val Thr His Gln Gly Leu Ser Ser Pro Val Thr Lys
195 200 205

Ser Phe Asn Arg Gly Glu Cys
210 215

<210> 226
<211> 645
<212> DNA
<213> Artificial Sequence

<220>
<223> DP47GS Fab LC

<400> 226
gaaatcgtgt taacgcagtc tccaggcacc ctgtctttgt ctccagggga aagagccacc 60
ctctcttgca gggccagtca gagtgttagc agcagctact tagcctggta ccagcagaaa 120
cctggccagg ctcccaggct cctcatctat ggagcatcca gcagggccac tggcatccca 180
gacaggttca gtggcagtgg atccgggaca gacttcactc tcaccatcag cagactggag 240
cctgaagatt ttgcagtgtg ttactgtcag cagtatggta gctcaccgct gacgttcggc 300
caggggacca aagtggaaat caaacgtacg gtggctgcac catctgtctt catcttcccg 360
ccatctgatg agcagttgaa atctggaact gcctctgttg tgtgcctgct gaataacttc 420
tatcccagag aggccaaagt acagtggaag gtggataacg ccctccaatc gggtaactcc 480
caggagagtg tcacagagca ggacagcaag gacagcacct acagcctcag cagcaccctg 540
acgctgagca aagcagacta cgagaaacac aaagtctacg cctgcgaagt cacccatcag 600
ggcctgagct cgcccgtcac aaagagcttc aacaggggag agtgt 645

<210> 227
<211> 451
<212> PRT
<213> Artificial Sequence

<220>

<223> CH1A1A Fab HC-Fc hole (wt)

<400> 227

Gln Val Gln Leu Val Gln Ser Gly Ala Glu Val Lys Lys Pro Gly Ala
1 5 10 15

Ser Val Lys Val Ser Cys Lys Ala Ser Gly Tyr Thr Phe Thr Glu Phe
20 25 30

Gly Met Asn Trp Val Arg Gln Ala Pro Gly Gln Gly Leu Glu Trp Met
35 40 45

Gly Trp Ile Asn Thr Lys Thr Gly Glu Ala Thr Tyr Val Glu Glu Phe
50 55 60

Lys Gly Arg Val Thr Phe Thr Thr Asp Thr Ser Thr Ser Thr Ala Tyr
65 70 75 80

Met Glu Leu Arg Ser Leu Arg Ser Asp Asp Thr Ala Val Tyr Tyr Cys
85 90 95

Ala Arg Trp Asp Phe Ala Tyr Tyr Val Glu Ala Met Asp Tyr Trp Gly
100 105 110

Gln Gly Thr Thr Val Thr Val Ser Ser Ala Ser Thr Lys Gly Pro Ser
115 120 125

Val Phe Pro Leu Ala Pro Ser Ser Lys Ser Thr Ser Gly Gly Thr Ala
130 135 140

Ala Leu Gly Cys Leu Val Lys Asp Tyr Phe Pro Glu Pro Val Thr Val
145 150 155 160

Ser Trp Asn Ser Gly Ala Leu Thr Ser Gly Val His Thr Phe Pro Ala
165 170 175

Val Leu Gln Ser Ser Gly Leu Tyr Ser Leu Ser Ser Val Val Thr Val
180 185 190

Pro Ser Ser Ser Leu Gly Thr Gln Thr Tyr Ile Cys Asn Val Asn His
195 200 205

Lys Pro Ser Asn Thr Lys Val Asp Lys Lys Val Glu Pro Lys Ser Cys
210 215 220

Asp Lys Thr His Thr Cys Pro Pro Cys Pro Ala Pro Glu Leu Leu Gly
225 230 235 240

Gly Pro Ser Val Phe Leu Phe Pro Pro Lys Pro Lys Asp Thr Leu Met
245 250 255

eolf-seql.txt

Ile Ser Arg Thr Pro Glu Val Thr Cys Val Val Val Asp Val Ser His
260 265 270

Glu Asp Pro Glu Val Lys Phe Asn Trp Tyr Val Asp Gly Val Glu Val
275 280 285

His Asn Ala Lys Thr Lys Pro Arg Glu Glu Gln Tyr Asn Ser Thr Tyr
290 295 300

Arg Val Val Ser Val Leu Thr Val Leu His Gln Asp Trp Leu Asn Gly
305 310 315 320

Lys Glu Tyr Lys Cys Lys Val Ser Asn Lys Ala Leu Pro Ala Pro Ile
325 330 335

Glu Lys Thr Ile Ser Lys Ala Lys Gly Gln Pro Arg Glu Pro Gln Val
340 345 350

Cys Thr Leu Pro Pro Ser Arg Asp Glu Leu Thr Lys Asn Gln Val Ser
355 360 365

Leu Ser Cys Ala Val Lys Gly Phe Tyr Pro Ser Asp Ile Ala Val Glu
370 375 380

Trp Glu Ser Asn Gly Gln Pro Glu Asn Asn Tyr Lys Thr Thr Pro Pro
385 390 395 400

Val Leu Asp Ser Asp Gly Ser Phe Phe Leu Val Ser Lys Leu Thr Val
405 410 415

Asp Lys Ser Arg Trp Gln Gln Gly Asn Val Phe Ser Cys Ser Val Met
420 425 430

His Glu Ala Leu His Asn His Tyr Thr Gln Lys Ser Leu Ser Leu Ser
435 440 445

Pro Gly Lys
450

<210> 228
<211> 1353
<212> DNA
<213> Artificial Sequence

<220>
<223> CH1A1A Fab HC-Fc hole (wt)

<400> 228
caggtgcagc tgggtgcagtc tggcgccgaa gtgaagaaac ctggagctag tgtgaagggtg 60
tcctgcaagg ccagcggcta caccttcacc gagttcggca tgaactgggt ccgacaggct 120
ccaggccagg gcctcgaatg gatgggctgg atcaacacca agaccggcga ggccacctac 180
gtggaagagt tcaagggcag agtgaccttc accacggaca ccagcaccag caccgcctac 240

eolf-seql.txt

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atggaactgc ggagcctgag aagcgacgac accgccgtgt actactgcgc cagatgggac      300
ttcgcctatt acgtggaagc catggactac tggggccagg gcaccaccgt gaccgtgtct      360
agcgctagca ccaagggccc aagcgtgttc cctctggccc ccagcagcaa gagcacaagc      420
ggcggaacag ccgccctggg ctgcctggtc aaggactact tccccgagcc cgtgacagtg      480
tcctggaaca gcggagccct gaccagcggc gtgcacacct ttccagccgt gctgcagagc      540
agcggcctgt acagcctgag cagcgtggtc acagtgccta gcagcagcct gggcacccag      600
acctacatct gcaacgtgaa ccacaagccc agcaacacca aggtggacaa gaaggtggag      660
cccaagagct gcgacaagac ccacacctgt ccccttgctc ctgcccctga gctgctgggc      720
ggacccagcg tgttcctgtt cccccaaag cccaaggaca ccctgatgat cagccggacc      780
cccgaagtga cctgcgtggt ggtggacgtg tcccacgagg accctgaagt gaagttcaat      840
tggtacgtgg acggcgtgga ggtgcacaat gccaaagacca agccccggga ggaacagtac      900
aacagcacct accgggtggt gtccgtgctg accgtgctgc accaggactg gctgaacggc      960
aaagagtaca agtgcaaggt ctccaacaag gccctgcctg ccccatcga gaaaaccatc     1020
agcaaggcca agggccagcc cagagaaccc caggtgtgca ccctgcccc cagcagagat     1080
gagctgacca agaaccaggt gtccctgagc tgtgccgtca agggcttcta cccagcgat     1140
atcgccgtgg agtgggagag caacggccag cctgagaaca actacaagac cccccccct     1200
gtgctggaca gcgacggcag cttcttctctg gtgtccaaac tgaccgtgga caagagccgg     1260
tggcagcagg gcaacgtgtt cagctgcagc gtgatgcacg aggccctgca caaccactac     1320
accagaagt ccctgagcct gagccccggc aag                                     1353

```

<210> 229
 <211> 599
 <212> PRT
 <213> Artificial Sequence

<220>
 <223> CH1A1A Fab HC-Fc knob (wt)-IL-2 qm
 <400> 229

Gln Val Gln Leu Val Gln Ser Gly Ala Glu Val Lys Lys Pro Gly Ala
 1 5 10 15

Ser Val Lys Val Ser Cys Lys Ala Ser Gly Tyr Thr Phe Thr Glu Phe
 20 25 30

Gly Met Asn Trp Val Arg Gln Ala Pro Gly Gln Gly Leu Glu Trp Met
 35 40 45

Gly Trp Ile Asn Thr Lys Thr Gly Glu Ala Thr Tyr Val Glu Glu Phe
 50 55 60

Lys Gly Arg Val Thr Phe Thr Thr Asp Thr Ser Thr Ser Thr Ala Tyr
 65 70 75 80

eolf-seql.txt

Met Glu Leu Arg Ser₈₅ Leu Arg Ser Asp₉₀ Thr Ala Val Tyr Tyr₉₅ Cys

Ala Arg Trp Asp₁₀₀ Phe Ala Tyr Tyr Val₁₀₅ Glu Ala Met Asp Tyr₁₁₀ Trp Gly

Gln Gly Thr₁₁₅ Thr Val Thr Val Ser₁₂₀ Ser Ala Ser Thr Lys₁₂₅ Gly Pro Ser

Val Phe₁₃₀ Pro Leu Ala Pro Ser₁₃₅ Ser Lys Ser Thr Ser₁₄₀ Gly Gly Thr Ala

Ala₁₄₅ Leu Gly Cys Leu Val₁₅₀ Lys Asp Tyr Phe Pro₁₅₅ Glu Pro Val Thr Val₁₆₀

Ser Trp Asn Ser Gly₁₆₅ Ala Leu Thr Ser Gly₁₇₀ Val His Thr Phe Pro₁₇₅ Ala

Val Leu Gln Ser₁₈₀ Ser Gly Leu Tyr Ser₁₈₅ Leu Ser Ser Val Val₁₉₀ Thr Val

Pro Ser Ser₁₉₅ Ser Leu Gly Thr Gln₂₀₀ Thr Tyr Ile Cys Asn₂₀₅ Val Asn His

Lys Pro₂₁₀ Ser Asn Thr Lys Val₂₁₅ Asp Lys Lys Val Glu₂₂₀ Pro Lys Ser Cys

Asp₂₂₅ Lys Thr His Thr Cys₂₃₀ Pro Pro Cys Pro Ala₂₃₅ Pro Glu Leu Leu Gly₂₄₀

Gly Pro Ser Val Phe₂₄₅ Leu Phe Pro Pro Lys₂₅₀ Pro Lys Asp Thr Leu₂₅₅ Met

Ile Ser Arg Thr₂₆₀ Pro Glu Val Thr Cys₂₆₅ Val Val Val Asp Val₂₇₀ Ser His

Glu Asp Pro₂₇₅ Glu Val Lys Phe Asn₂₈₀ Trp Tyr Val Asp Gly₂₈₅ Val Glu Val

His Asn Ala Lys Thr Lys Pro₂₉₅ Arg Glu Glu Gln Tyr₃₀₀ Asn Ser Thr Tyr

Arg Val Val Ser Val Leu₃₁₀ Thr Val Leu His Gln₃₁₅ Asp Trp Leu Asn Gly₃₂₀

Lys Glu Tyr Lys Cys₃₂₅ Lys Val Ser Asn Lys₃₃₀ Ala Leu Pro Ala Pro₃₃₅ Ile

Glu Lys Thr Ile₃₄₀ Ser Lys Ala Lys Gly₃₄₅ Gln Pro Arg Glu Pro₃₅₀ Gln Val

eolf-seql.txt

Tyr Thr Leu Pro Pro Cys Arg Asp Glu Leu Thr Lys Asn Gln Val Ser
 355 360 365
 Leu Trp Cys Leu Val Lys Gly Phe Tyr Pro Ser Asp Ile Ala Val Glu
 370 375 380
 Trp Glu Ser Asn Gly Gln Pro Glu Asn Asn Tyr Lys Thr Thr Pro Pro
 385 390 395 400
 Val Leu Asp Ser Asp Gly Ser Phe Phe Leu Tyr Ser Lys Leu Thr Val
 405 410 415
 Asp Lys Ser Arg Trp Gln Gln Gly Asn Val Phe Ser Cys Ser Val Met
 420 425 430
 His Glu Ala Leu His Asn His Tyr Thr Gln Lys Ser Leu Ser Leu Ser
 435 440 445
 Pro Gly Lys Ser Gly Gly Gly Gly Ser Gly Gly Gly Ser Gly Gly
 450 455 460
 Gly Gly Ala Pro Ala Ser Ser Ser Thr Lys Lys Thr Gln Leu Gln Leu
 465 470 475 480
 Glu His Leu Leu Leu Asp Leu Gln Met Ile Leu Asn Gly Ile Asn Asn
 485 490 495
 Tyr Lys Asn Pro Lys Leu Thr Arg Met Leu Thr Ala Lys Phe Ala Met
 500 505 510
 Pro Lys Lys Ala Thr Glu Leu Lys His Leu Gln Cys Leu Glu Glu Glu
 515 520 525
 Leu Lys Pro Leu Glu Glu Val Leu Asn Gly Ala Gln Ser Lys Asn Phe
 530 535 540
 His Leu Arg Pro Arg Asp Leu Ile Ser Asn Ile Asn Val Ile Val Leu
 545 550 555 560
 Glu Leu Lys Gly Ser Glu Thr Thr Phe Met Cys Glu Tyr Ala Asp Glu
 565 570 575
 Thr Ala Thr Ile Val Glu Phe Leu Asn Arg Trp Ile Thr Phe Ala Gln
 580 585 590
 Ser Ile Ile Ser Thr Leu Thr
 595

<210> 230
 <211> 1797

eolf-seql.txt

<212> DNA
<213> Artificial Sequence

<220>
<223> CH1A1A Fab HC-Fc knob (wt)-IL-2 qm

<400> 230
caggtgcagc tgggtgcagtc tggcgccgaa gtgaagaaac ctggagctag tgtgaaggtg 60
tcctgcaagg ccagcgggcta caccttcacc gagttcggca tgaactgggt ccgacaggct 120
ccaggccagg gcctcgaatg gatgggctgg atcaacacca agaccggcga ggccacctac 180
gtggaagagt tcaagggcag agtgacctc accacggaca ccagcaccag caccgcctac 240
atggaactgc ggagcctgag aagcgacgac accgccgtgt actactgcmc cagatgggac 300
ttcgcctatt acgtggaagc catggactac tggggccagg gcaccaccgt gaccgtgtct 360
agcgctagca ccaagggccc aagcgtgttc cctctggccc ccagcagcaa gagcacaagc 420
ggcggaacag ccgccctggg ctgcctggtc aaggactact tccccgagcc cgtgacagtg 480
tcctggaaca gcggagccct gaccagcggc gtgcacacct ttccagccgt gctgcagagc 540
agcggcctgt acagcctgag cagcgtggtc acagtgccta gcagcagcct gggcaccag 600
acctacatct gcaacgtgaa ccacaagccc agcaacacca aggtggacaa gaaggtggag 660
cccaagagct gcgacaagac ccacacctgt ccccttgtc ctgcccctga gctgctgggc 720
ggaccagcg tgttcctgtt cccccaaag cccaaggaca ccctgatgat cagccggacc 780
cccgaagtga cctgcgtggt ggtggacgtg tcccacgagg accctgaagt gaagttcaat 840
tggtacgtgg acggcgtgga ggtgcacaat gccaaagacca agccccggga ggaacagtac 900
aacagcacct accgggtggt gtccgtgctg accgtgctgc accaggactg gctgaacggc 960
aaagagtaca agtgcaaggt ctccaacaag gccctgcctg ccccatcga gaaaaccatc 1020
agcaaggcca agggccagcc cagagaacct caggtgtaca ccctgcccc ctgcagagat 1080
gagctgacca agaaccagggt gtccctgtgg tgtctggtca agggcttcta cccagcgat 1140
atcgccgtgg agtgggagag caacggccag cctgagaaca actacaagac cccccccct 1200
gtgctggaca gcgacggcag cttcttctg tactccaaac tgaccgtgga caagagccgg 1260
tggcagcagg gcaacgtgtt cagctgcagc gtgatgcacg aggccctgca caaccactac 1320
accagaagt ccctgagcct gagccccggc aagtccggag gcggaggctc cggcggcgga 1380
ggttctggcg gaggtggcgc tcctgcctcc tccagcacca agaaaacca gctccagctg 1440
gaacatctcc tgctggatct gcagatgatc ctgaacggca tcaacaacta caagaacccc 1500
aagctgacct ggatgctgac cgccaagttc gccatgccc agaaggccac cgagctgaaa 1560
catctgcagt gcctggaaga ggaactgaag cctctggaag aggtgctgaa cggcgcccag 1620
tccaagaact tccacctgag gcctcgggac ctgatctcca acatcaacgt gatcgtgctg 1680
gaactgaagg gctccgagac aaccttcatt tgcgagtacg ccgacgagac agtaccatc 1740
gtggaatttc tgaaccggtg gatcacctc gccagtgcca tcattctccac cctgacc 1797

eolf-seql.txt

<210> 231
 <211> 215
 <212> PRT
 <213> Artificial Sequence

<220>
 <223> 2F1 Fab LC

<400> 231

Asp Ile Gln Met Thr Gln Ser Pro Ser Ser Leu Ser Ala Ser Val Gly
 1 5 10 15

Asp Arg Val Thr Ile Thr Cys Lys Ala Ser Ala Ala Val Gly Thr Tyr
 20 25 30

Val Ala Trp Tyr Gln Gln Lys Pro Gly Lys Ala Pro Lys Leu Leu Ile
 35 40 45

Tyr Ser Ala Ser Tyr Arg Lys Arg Gly Val Pro Ser Arg Phe Ser Gly
 50 55 60

Ser Gly Ser Gly Thr Asp Phe Thr Leu Thr Ile Ser Ser Leu Gln Pro
 65 70 75 80

Glu Asp Phe Ala Thr Tyr Tyr Cys His Gln Tyr Tyr Thr Tyr Pro Leu
 85 90 95

Phe Thr Phe Gly Gln Gly Thr Lys Leu Glu Ile Lys Arg Thr Val Ala
 100 105 110

Ala Pro Ser Val Phe Ile Phe Pro Pro Ser Asp Glu Gln Leu Lys Ser
 115 120 125

Gly Thr Ala Ser Val Val Cys Leu Leu Asn Asn Phe Tyr Pro Arg Glu
 130 135 140

Ala Lys Val Gln Trp Lys Val Asp Asn Ala Leu Gln Ser Gly Asn Ser
 145 150 155 160

Gln Glu Ser Val Thr Glu Gln Asp Ser Lys Asp Ser Thr Tyr Ser Leu
 165 170 175

Ser Ser Thr Leu Thr Leu Ser Lys Ala Asp Tyr Glu Lys His Lys Val
 180 185 190

Tyr Ala Cys Glu Val Thr His Gln Gly Leu Ser Ser Pro Val Thr Lys
 195 200 205

Ser Phe Asn Arg Gly Glu Cys
 210 215

<210> 232
 <211> 645

eolf-seql.txt

<212> DNA
<213> Artificial Sequence

<220>
<223> 2F1 Fab LC

<400> 232
gatatccaga tgaccagtc tccatcctcc ctgtctgcat ctgtgggaga cagagtcacc 60
atcacttgca aggccagtc ggctgtgggt acgtatgttg cgtgggtatca gcagaaacca 120
gggaaagcac ctaagctcct gatctattcg gcacccctacc gcaaaagggg agtcccatca 180
aggttcagtg gcagtggatc tgggacagat ttcactctca ccatcagcag tctgcaacct 240
gaagatttcg caacttacta ctgtcaccaa tattacacct atcctctatt cacgtttggc 300
cagggcacca agctcgagat caagcgtacg gtggctgcac catctgtctt catcttcccg 360
ccatctgatg agcagttgaa atctggaact gcctctgttg tgtgcctgct gaataacttc 420
tatcccagag aggccaaagt acagtggaag gtggataacg cctccaatc gggtaactcc 480
caggagagtg tcacagagca ggacagcaag gacagcacct acagcctcag cagcaccctg 540
acgctgagca aagcagacta cgagaaacac aaagtctacg cctgcgaagt caccatcag 600
ggcctgagct cgcccgtcac aaagagcttc aacaggggag agtgt 645

<210> 233
<211> 451
<212> PRT
<213> Artificial Sequence

<220>
<223> 2B10 Fab HC-Fc knob (LALA P329G)

<400> 233
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 Gly Thr Phe Ser Ser Tyr
20 25 30
Ala Ile Ser Trp Val Arg Gln Ala Pro Gly Gln Gly Leu Glu Trp Met
35 40 45
Gly Gly Ile Ile Pro Ile Phe Gly Thr Ala Asn Tyr Ala Gln Lys Phe
50 55 60
Gln 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 Leu Tyr Gly Tyr Ala Tyr Tyr Gly Ala Phe Asp Tyr Trp Gly
100 105 110

co1f-seql.txt

Gln Gly Thr Thr Val Thr Val Ser Ser Ala Ser Thr Lys Gly Pro Ser
115 120 125

Val Phe Pro Leu Ala Pro Ser Ser Lys Ser Thr Ser Gly Gly Thr Ala
130 135 140

Ala Leu Gly Cys Leu Val Lys Asp Tyr Phe Pro Glu Pro Val Thr Val
145 150 155 160

Ser Trp Asn Ser Gly Ala Leu Thr Ser Gly Val His Thr Phe Pro Ala
165 170 175

Val Leu Gln Ser Ser Gly Leu Tyr Ser Leu Ser Ser Val Val Thr Val
180 185 190

Pro Ser Ser Ser Leu Gly Thr Gln Thr Tyr Ile Cys Asn Val Asn His
195 200 205

Lys Pro Ser Asn Thr Lys Val Asp Lys Lys Val Glu Pro Lys Ser Cys
210 215 220

Asp Lys Thr His Thr Cys Pro Pro Cys Pro Ala Pro Glu Ala Ala Gly
225 230 235 240

Gly Pro Ser Val Phe Leu Phe Pro Pro Lys Pro Lys Asp Thr Leu Met
245 250 255

Ile Ser Arg Thr Pro Glu Val Thr Cys Val Val Val Asp Val Ser His
260 265 270

Glu Asp Pro Glu Val Lys Phe Asn Trp Tyr Val Asp Gly Val Glu Val
275 280 285

His Asn Ala Lys Thr Lys Pro Arg Glu Glu Gln Tyr Asn Ser Thr Tyr
290 295 300

Arg Val Val Ser Val Leu Thr Val Leu His Gln Asp Trp Leu Asn Gly
305 310 315 320

Lys Glu Tyr Lys Cys Lys Val Ser Asn Lys Ala Leu Gly Ala Pro Ile
325 330 335

Glu Lys Thr Ile Ser Lys Ala Lys Gly Gln Pro Arg Glu Pro Gln Val
340 345 350

Tyr Thr Leu Pro Pro Cys Arg Asp Glu Leu Thr Lys Asn Gln Val Ser
355 360 365

Leu Trp Cys Leu Val Lys Gly Phe Tyr Pro Ser Asp Ile Ala Val Glu
370 375 380

eolf-seql.txt

Trp Glu Ser Asn Gly Gln Pro Glu Asn Asn Tyr Lys Thr Thr Pro Pro
385 390 395 400

Val Leu Asp Ser Asp Gly Ser Phe Phe Leu Tyr Ser Lys Leu Thr Val
405 410 415

Asp Lys Ser Arg Trp Gln Gln Gly Asn Val Phe Ser Cys Ser Val Met
420 425 430

His Glu Ala Leu His Asn His Tyr Thr Gln Lys Ser Leu Ser Leu Ser
435 440 445

Pro Gly Lys
450

<210> 234
<211> 1353
<212> DNA
<213> Artificial Sequence

<220>
<223> 2B10 Fab HC-Fc knob (LALA P329G)

<400> 234
caggtgcaat tgggtgcagtc tggggctgag gtgaagaagc ctgggtcctc ggtgaaggtc 60
tcctgcaagg cctccggagg cacattcagc agctacgcta taagctgggt gcgacaggcc 120
cctggacaag ggctcgagtg gatgggaggg atcatcccta tcttttggtac agcaaactac 180
gcacagaagt tccagggcag ggtcaccatt actgcagaca aatccacgag cacagcctac 240
atggagctga gcagcctgag atctgaggac accgccgtgt attactgtgc gagactgtac 300
ggttacgctt actacggtgc ttttgactac tggggccaag ggaccaccgt gaccgtctcc 360
tcagctagca ccaagggccc atcggctctc cccctggcac cctcctccaa gagcacctct 420
gggggcacag cggccctggg ctgcctggtc aaggactact tccccgaacc ggtgacgggtg 480
tcgtggaact caggcgccct gaccagcggc gtgcacacct tcccggctgt cctacagtcc 540
tcaggactct actccctcag cagcgtggtg accgtgccct ccagcagctt gggcaccag 600
acctacatct gcaacgtgaa tcacaagccc agcaacacca aggtggacaa gaaagttgag 660
cccaaattct gtgacaaaac tcacacatgc ccaccgtgcc cagcacctga agctgcaggg 720
ggaccgtcag tcttcctctt cccccaaaa cccaaggaca cctcatgat ctcccggacc 780
cctgaggtca catgcgtggt ggtggacgtg agccacgaag accctgaggt caagttcaac 840
tggtacgtgg acggcgtgga ggtgcataat gccaaagaaa agccgcggga ggagcagtag 900
aacagcacgt accgtgtggt cagcgtcctc accgtcctgc accaggactg gctgaatggc 960
aaggagtaca agtgcaaggt ctccaacaaa gccctcggcg ccccatcga gaaaaccatc 1020
tccaaagcca aagggcagcc ccgagaacca caggtgtaca ccctgcccc atgccgggat 1080
gagctgacca agaaccaggt cagcctgtgg tgcttggtca aaggcttcta tcccagcgac 1140
atcgccgtgg agtgggagag caatgggcag ccggagaaca actacaagac cagcctccc 1200

eolf-seql.txt

gtgctggact ccgacggctc cttcttcctc tacagcaagc tcaccgtgga caagagcagg 1260
 tggcagcagg ggaacgtctt ctcattgctcc gtgatgcatg aggctctgca caaccactac 1320
 acgcagaaga gcctctccct gtctccgggt aaa 1353

<210> 235
 <211> 805
 <212> PRT
 <213> Artificial Sequence

<220>
 <223> 2B10 Fab HC-Fc hole (LALA P329G)-scIL-10
 <400> 235

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 Gly Thr Phe Ser Ser Tyr
 20 25 30

Ala Ile Ser Trp Val Arg Gln Ala Pro Gly Gln Gly Leu Glu Trp Met
 35 40 45

Gly Gly Ile Ile Pro Ile Phe Gly Thr Ala Asn Tyr Ala Gln Lys Phe
 50 55 60

Gln 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 Leu Tyr Gly Tyr Ala Tyr Tyr Gly Ala Phe Asp Tyr Trp Gly
 100 105 110

Gln Gly Thr Thr Val Thr Val Ser Ser Ala Ser Thr Lys Gly Pro Ser
 115 120 125

Val Phe Pro Leu Ala Pro Ser Ser Lys Ser Thr Ser Gly Gly Thr Ala
 130 135 140

Ala Leu Gly Cys Leu Val Lys Asp Tyr Phe Pro Glu Pro Val Thr Val
 145 150 155 160

Ser Trp Asn Ser Gly Ala Leu Thr Ser Gly Val His Thr Phe Pro Ala
 165 170 175

Val Leu Gln Ser Ser Gly Leu Tyr Ser Leu Ser Ser Val Val Thr Val
 180 185 190

Pro Ser Ser Ser Leu Gly Thr Gln Thr Tyr Ile Cys Asn Val Asn His
 195 200 205

eolf-seql.txt

Lys Pro Ser Asn Thr Lys Val Asp Lys Lys Val Glu Pro Lys Ser Cys
 210 215 220
 Asp Lys Thr His Thr Cys Pro Pro Cys Pro Ala Pro Glu Ala Ala Gly
 225 230 235 240
 Gly Pro Ser Val Phe Leu Phe Pro Pro Lys Pro Lys Asp Thr Leu Met
 245 250 255
 Ile Ser Arg Thr Pro Glu Val Thr Cys Val Val Val Asp Val Ser His
 260 265 270
 Glu Asp Pro Glu Val Lys Phe Asn Trp Tyr Val Asp Gly Val Glu Val
 275 280 285
 His Asn Ala Lys Thr Lys Pro Arg Glu Glu Gln Tyr Asn Ser Thr Tyr
 290 295 300
 Arg Val Val Ser Val Leu Thr Val Leu His Gln Asp Trp Leu Asn Gly
 305 310 315 320
 Lys Glu Tyr Lys Cys Lys Val Ser Asn Lys Ala Leu Gly Ala Pro Ile
 325 330 335
 Glu Lys Thr Ile Ser Lys Ala Lys Gly Gln Pro Arg Glu Pro Gln Val
 340 345 350
 Cys Thr Leu Pro Pro Ser Arg Asp Glu Leu Thr Lys Asn Gln Val Ser
 355 360 365
 Leu Ser Cys Ala Val Lys Gly Phe Tyr Pro Ser Asp Ile Ala Val Glu
 370 375 380
 Trp Glu Ser Asn Gly Gln Pro Glu Asn Asn Tyr Lys Thr Thr Pro Pro
 385 390 395 400
 Val Leu Asp Ser Asp Gly Ser Phe Phe Leu Val Ser Lys Leu Thr Val
 405 410 415
 Asp Lys Ser Arg Trp Gln Gln Gly Asn Val Phe Ser Cys Ser Val Met
 420 425 430
 His Glu Ala Leu His Asn His Tyr Thr Gln Lys Ser Leu Ser Leu Ser
 435 440 445
 Pro Gly Gly Gly Gly Gly Ser Gly Gly Gly Gly Ser Gly Gly Gly Gly
 450 455 460
 Ser Ser Pro Gly Gln Gly Thr Gln Ser Glu Asn Ser Cys Thr His Phe
 465 470 475 480

eolf-seql.txt

Pro Gly Asn Leu Pro Asn Met Leu Arg Asp Leu Arg Asp Ala Phe Ser
485 490 495

Arg Val Lys Thr Phe Phe Gln Met Lys Asp Gln Leu Asp Asn Leu Leu
500 505 510

Leu Lys Glu Ser Leu Leu Glu Asp Phe Lys Gly Tyr Leu Gly Cys Gln
515 520 525

Ala Leu Ser Glu Met Ile Gln Phe Tyr Leu Glu Glu Val Met Pro Gln
530 535 540

Ala Glu Asn Gln Asp Pro Asp Ile Lys Ala His Val Asn Ser Leu Gly
545 550 555 560

Glu Asn Leu Lys Thr Leu Arg Leu Arg Leu Arg Arg Cys His Arg Phe
565 570 575

Leu Pro Cys Glu Asn Lys Ser Lys Ala Val Glu Gln Val Lys Asn Ala
580 585 590

Phe Asn Lys Leu Gln Glu Lys Gly Ile Tyr Lys Ala Met Ser Glu Phe
595 600 605

Asp Ile Phe Ile Asn Tyr Ile Glu Ala Tyr Met Thr Met Lys Ile Arg
610 615 620

Asn Gly Gly Gly Gly Ser Gly Gly Gly Gly Ser Gly Gly Gly Gly Ser
625 630 635 640

Gly Gly Gly Gly Ser Ser Pro Gly Gln Gly Thr Gln Ser Glu Asn Ser
645 650 655

Cys Thr His Phe Pro Gly Asn Leu Pro Asn Met Leu Arg Asp Leu Arg
660 665 670

Asp Ala Phe Ser Arg Val Lys Thr Phe Phe Gln Met Lys Asp Gln Leu
675 680 685

Asp Asn Leu Leu Leu Lys Glu Ser Leu Leu Glu Asp Phe Lys Gly Tyr
690 695 700

Leu Gly Cys Gln Ala Leu Ser Glu Met Ile Gln Phe Tyr Leu Glu Glu
705 710 715 720

Val Met Pro Gln Ala Glu Asn Gln Asp Pro Asp Ile Lys Ala His Val
725 730 735

Asn Ser Leu Gly Glu Asn Leu Lys Thr Leu Arg Leu Arg Leu Arg Arg
740 745 750

eolf-seql.txt

Cys His Arg Phe Leu Pro Cys Glu Asn Lys Ser Lys Ala Val Glu Gln
755 760 765

Val Lys Asn Ala Phe Asn Lys Leu Gln Glu Lys Gly Ile Tyr Lys Ala
770 775 780

Met Ser Glu Phe Asp Ile Phe Ile Asn Tyr Ile Glu Ala Tyr Met Thr
785 790 795 800

Met Lys Ile Arg Asn
805

<210> 236
<211> 2415
<212> DNA
<213> Artificial Sequence

<220>
<223> 2B10 Fab HC-Fc hole (LALA P329G)-scIL-10

<400> 236
caggtgcaat tggtgcagtc tggggctgag gtgaagaagc ctgggtcctc ggtgaaggtc 60
tcctgcaagg cctccggagg cacattcagc agctacgcta taagctgggt gcgacaggcc 120
cctggacaag ggctcgagtg gatgggaggg atcatcccta tctttggtac agcaaactac 180
gcacagaagt tccagggcag ggtcaccatt actgcagaca aatccacgag cacagcctac 240
atggagctga gcagcctgag atctgaggac accgccgtgt attactgtgc gagactgtac 300
ggttacgctt actacggtgc ttttgactac tggggccaag ggaccaccgt gaccgtctcc 360
tcagctagca ccaagggccc atcggtcttc cccctggcac cctcctccaa gagcacctct 420
gggggcacag cggccctggg ctgcctggtc aaggactact tccccgaacc ggtgacgggtg 480
tcgtggaact caggcgccct gaccagcggc gtgcacacct tcccggtgt cctacagtcc 540
tcaggactct actccctcag cagcgtggtg accgtgccct ccagcagctt gggcacccag 600
acctacatct gcaacgtgaa tcacaagccc agcaacacca aggtggacaa gaaagttgag 660
cccaaattctt gtgacaaaac tcacacatgc ccaccgtgcc cagcacctga agctgcaggg 720
ggaccgtcag tcttctctct cccccaaaa cccaaggaca cctcatgat ctcccggacc 780
cctgaggtca catgcgtggt ggtggacgtg agccacgaag accctgaggt caagttcaac 840
tggtacgtgg acggcgtgga ggtgcataat gccaagacaa agccgcggga ggagcagtag 900
aacagcacgt accgtgtggt cagcgtcctc accgtcctgc accaggactg gctgaatggc 960
aaggagtaca agtgcaaggt ctccaacaaa gccctcggcg ccccatcga gaaaaccatc 1020
tccaaagcca aagggcagcc ccgagaacca caggtgtgca cctgcccc atcccggat 1080
gagctgacca agaaccaggt cagcctctcg tgcgcagtca aaggcttcta tcccagcgac 1140
atcgccgtgg agtgggagag caatgggcag ccggagaaca actacaagac cagcctccc 1200
gtgctggact ccgacggctc cttcttctc gtgagcaagc tcaccgtgga caagagcagg 1260

eolf-seql.txt

tggcagcagg ggaacgtctt ctcattgctcc gtgatgcatg aggctctgca caaccactac	1320
acgcagaaga gcctctccct gtctccgggt ggccggcgag gctccggagg cggaggatct	1380
gggggaggcg gaagtagccc gggccagggc acccagagcg agaacagctg caccacttc	1440
cccggcaacc tgccaacat gctgcgggac ctgagggacg ccttcagcag agtgaaaacc	1500
ttcttccaga tgaaggacca gctggacaac ctgctgctga aagagagcct gctggaagat	1560
ttcaagggct acctgggctg tcaggccctg agcgagatga tccagttcta cctggaagaa	1620
gtgatgcccc aggccgagaa ccaggacccc gacatcaagg cccacgtgaa cagcctgggc	1680
gagaacctga aaacctgcg gctgagactg cggcggtgcc acagatttct gccctgagag	1740
aacaagagca aggccgtgga acaggtgaag aacgccttca acaagctgca ggaaaagggc	1800
atctacaagg ccatgtccga gttcgacatc ttcattcaact acatcgaagc ttacatgacc	1860
atgaagatca gaaacggcgg aggcggatct ggccggcggtg gaagtggagg cggaggatct	1920
gggggaggcg gaagtagccc gggccagggc acccagagcg agaacagctg caccacttc	1980
cccggcaacc tgccaacat gctgcgggac ctgagggacg ccttcagcag agtgaaaacc	2040
ttcttccaga tgaaggacca gctggacaac ctgctgctga aagagagcct gctggaagat	2100
ttcaagggct acctgggctg tcaggccctg agcgagatga tccagttcta cctggaagaa	2160
gtgatgcccc aggccgagaa ccaggacccc gacatcaagg cccacgtgaa cagcctgggc	2220
gagaacctga aaacctgcg gctgagactg cggcggtgcc acagatttct gccctgagag	2280
aacaagagca aggccgtgga acaggtgaag aacgccttca acaagctgca ggaaaagggc	2340
atctacaagg ccatgtccga gttcgacatc ttcattcaact acatcgaggc ctacatgaca	2400
atgaaaatcc gcaat	2415

<210> 237
 <211> 631
 <212> PRT
 <213> Artificial Sequence

<220>
 <223> 2B10 Fab HC-Fc hole (LALA P329G)-IL-10M1

<400> 237

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 Gly Thr Phe Ser Ser Tyr	
20 25 30	

Ala Ile Ser Trp Val Arg Gln Ala Pro Gly Gln Gly Leu Glu Trp Met	
35 40 45	

Gly Gly Ile Ile Pro Ile Phe Gly Thr Ala Asn Tyr Ala Gln Lys Phe	
50 55 60	

eofl-seql.txt

Gln 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 Leu Tyr Gly Tyr Ala Tyr Tyr Gly Ala Phe Asp Tyr Trp Gly
100 105 110

Gln Gly Thr Thr Val Thr Val Ser Ser Ala Ser Thr Lys Gly Pro Ser
115 120 125

Val Phe Pro Leu Ala Pro Ser Ser Lys Ser Thr Ser Gly Gly Thr Ala
130 135 140

Ala Leu Gly Cys Leu Val Lys Asp Tyr Phe Pro Glu Pro Val Thr Val
145 150 155 160

Ser Trp Asn Ser Gly Ala Leu Thr Ser Gly Val His Thr Phe Pro Ala
165 170 175

Val Leu Gln Ser Ser Gly Leu Tyr Ser Leu Ser Ser Val Val Thr Val
180 185 190

Pro Ser Ser Ser Leu Gly Thr Gln Thr Tyr Ile Cys Asn Val Asn His
195 200 205

Lys Pro Ser Asn Thr Lys Val Asp Lys Lys Val Glu Pro Lys Ser Cys
210 215 220

Asp Lys Thr His Thr Cys Pro Pro Cys Pro Ala Pro Glu Ala Ala Gly
225 230 235 240

Gly Pro Ser Val Phe Leu Phe Pro Pro Lys Pro Lys Asp Thr Leu Met
245 250 255

Ile Ser Arg Thr Pro Glu Val Thr Cys Val Val Val Asp Val Ser His
260 265 270

Glu Asp Pro Glu Val Lys Phe Asn Trp Tyr Val Asp Gly Val Glu Val
275 280 285

His Asn Ala Lys Thr Lys Pro Arg Glu Glu Gln Tyr Asn Ser Thr Tyr
290 295 300

Arg Val Val Ser Val Leu Thr Val Leu His Gln Asp Trp Leu Asn Gly
305 310 315 320

Lys Glu Tyr Lys Cys Lys Val Ser Asn Lys Ala Leu Gly Ala Pro Ile
325 330 335

eof-seql.txt

Glu Lys Thr Ile Ser Lys Ala Lys Gly Gln Pro Arg Glu Pro Gln Val
340 345 350

Cys Thr Leu Pro Pro Ser Arg Asp Glu Leu Thr Lys Asn Gln Val Ser
355 360 365

Leu Ser Cys Ala Val Lys Gly Phe Tyr Pro Ser Asp Ile Ala Val Glu
370 375 380

Trp Glu Ser Asn Gly Gln Pro Glu Asn Asn Tyr Lys Thr Thr Pro Pro
385 390 395 400

Val Leu Asp Ser Asp Gly Ser Phe Phe Leu Val Ser Lys Leu Thr Val
405 410 415

Asp Lys Ser Arg Trp Gln Gln Gly Asn Val Phe Ser Cys Ser Val Met
420 425 430

His Glu Ala Leu His Asn His Tyr Thr Gln Lys Ser Leu Ser Leu Ser
435 440 445

Pro Gly Gly Gly Gly Gly Ser Gly Gly Gly Gly Ser Gly Gly Gly Gly
450 455 460

Ser Ser Pro Gly Gln Gly Thr Gln Ser Glu Asn Ser Cys Thr His Phe
465 470 475 480

Pro Gly Asn Leu Pro Asn Met Leu Arg Asp Leu Arg Asp Ala Phe Ser
485 490 495

Arg Val Lys Thr Phe Phe Gln Met Lys Asp Gln Leu Asp Asn Leu Leu
500 505 510

Leu Lys Glu Ser Leu Leu Glu Asp Phe Lys Gly Tyr Leu Gly Cys Gln
515 520 525

Ala Leu Ser Glu Met Ile Gln Phe Tyr Leu Glu Glu Val Met Pro Gln
530 535 540

Ala Glu Asn Gln Asp Pro Asp Ile Lys Ala His Val Asn Ser Leu Gly
545 550 555 560

Glu Asn Leu Lys Thr Leu Arg Leu Arg Leu Arg Arg Cys His Arg Phe
565 570 575

Leu Pro Cys Glu Asn Gly Gly Gly Ser Gly Gly Lys Ser Lys Ala Val
580 585 590

Glu Gln Val Lys Asn Ala Phe Asn Lys Leu Gln Glu Lys Gly Ile Tyr
595 600 605

eolf-seql.txt

Lys Ala Met Ser Glu Phe Asp Ile Phe Ile Asn Tyr Ile Glu Ala Tyr
610 615 620

Met Thr Met Lys Ile Arg Asn
625 630

<210> 238
<211> 1893
<212> DNA
<213> Artificial Sequence

<220>
<223> 2B10 Fab HC-Fc hole (LALA P329G)-IL-10M1

<400> 238
caggtgcaat tgggtgcagtc tggggctgag gtgaagaagc ctgggtcctc ggtgaaggtc 60
tcctgcaagg cctccgagg cacattcagc agctacgcta taagctgggt gcgacaggcc 120
cctggacaag ggctcgagt gatgggaggg atcatcccta tctttggtac agcaaactac 180
gcacagaagt tccagggcag ggtcaccatt actgcagaca aatccacgag cacagcctac 240
atggagctga gcagcctgag atctgaggac accgccgtgt attactgtgc gagactgtac 300
ggttacgctt actacggtgc ttttgactac tggggccaag ggaccaccgt gaccgtctcc 360
tcagctagca ccaagggccc atcggctctt cccctggcac cctcctccaa gagcacctct 420
gggggcacag cggccctggg ctgcctggtc aaggactact tccccgaacc ggtgacggtg 480
tcgtggaact caggcgccct gaccagcggc gtgcacacct tcccggctgt cctacagtcc 540
tcaggactct actccctcag cagcgtggtg accgtgccct ccagcagctt gggcacccag 600
acctacatct gcaacgtgaa tcacaagccc agcaacacca aggtggacaa gaaagttgag 660
cccaaattct gtgacaaaac tcacacatgc ccaccgtgcc cagcacctga agctgcaggg 720
ggaccgtcag tcttctctt cccccaaaa cccaaggaca cctcatgat ctcccggacc 780
cctgaggtca catgctggtt ggtggacgtg agccacgaag accctgaggt caagttcaac 840
tggtacgtgg acggcgtgga ggtgcataat gccaaagaaa agccgcggga ggagcagtac 900
aacagcacgt accgtgtggt cagcgtcctc accgtcctgc accaggactg gctgaatggc 960
aaggagtaca agtgcaaggt ctccaacaaa gccctcggcg ccccatcga gaaaaccatc 1020
tcaaagcca aagggcagcc ccgagaacca caggtgtgca cctgcccc atcccgggat 1080
gagctgacca agaaccaggt cagcctctcg tgcgcagtca aaggcttcta tcccagcgac 1140
atcgccgtgg agtgggagag caatgggcag ccggagaaca actacaagac cagcctccc 1200
gtgctggact ccgacggctc cttcttctc gtgagcaagc tcaccgtgga caagagcagg 1260
tggcagcagg ggaacgtctt ctcattgtcc gtgatgcatg aggtcttgca caaccactac 1320
acgcagaaga gcctctccct gtctccgggt ggcggcggag gctccggagg cggaggaagt 1380
ggcggcgggt gcagctctcc aggccagggc acccagagcg agaacagctg caccacttc 1440
cccggcaacc tgccaacat gctgcgggac ctgagggacg ctttcagcag agtgaaaacc 1500
ttcttccaga tgaaggacca gctggacaac ctgctgctga aagagagcct gctggaagat 1560

eolf-seql.txt

ttcaagggct acctgggctg tcaggccctg agcgagatga tccagttcta cctggaagaa 1620
 gtgatgcccc aggccgagaa ccaggacccc gacatcaagg cccacgtgaa cagcctgggc 1680
 gagaacctga aaacctgctg gctgagactg cggcggtgcc acagatttct gccctgagag 1740
 aacggcggag gctctggcgg aaagtccaag gccgtggaac aggtgaagaa cgccttcaac 1800
 aagctgcagg aaaagggcat ctacaaggcc atgagcgagt tcgacatctt catcaactac 1860
 atcgaagctt acatgacaat gaagatacga aac 1893

<210> 239
 <211> 214
 <212> PRT
 <213> Artificial Sequence

<220>
 <223> 2B10 Fab LC

<400> 239

Asp Ile Gln Met Thr Gln Ser Pro Ser Ser Leu Ser Ala Ser Val Gly
 1 5 10 15

Asp Arg Val Thr Ile Thr Cys Arg Ala Ser Gln Gly Ile Arg Asn Asp
 20 25 30

Leu Gly Trp Tyr Gln Gln Lys Pro Gly Lys Ala Pro Lys Arg Leu Ile
 35 40 45

Tyr Ala Ala Ser Ser Leu Gln Ser Gly Val Pro Ser Arg Phe Ser Gly
 50 55 60

Ser Gly Ser Gly Thr Glu Phe Thr Leu Thr Ile Ser Ser Leu Gln Pro
 65 70 75 80

Glu Asp Phe Ala Thr Tyr Tyr Cys Leu Gln Asn Gly Leu Gln Pro Ala
 85 90 95

Thr Phe Gly Gln Gly Thr Lys Val Glu Ile 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

eof-seql.txt

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> 240
<211> 642
<212> DNA
<213> Artificial Sequence

<220>
<223> 2B10 Fab LC

<400> 240
gatatccaga tgaccagtc tccatcctcc ctgtctgcat ctgtcggaga ccgggtcacc 60
atcacctgcc gggcaagtca gggcattaga aatgatttag gctggtacca gcagaagcca 120
gggaaagccc ctaagcgcct gatctatgct gcatccagtt tgcagagtgg cgtcccatca 180
aggttcagcg gcggtggatc cgggacagag ttcactctca ccatcagcag cttgcagcct 240
gaagattttg ccacctatta ctgcttgagc aatgggtctgc agcccgcgac gtttggccag 300
ggcaccaaag tcgagatcaa gcgtacggtg gctgcacat ctgtcttcat cttcccgcca 360
tctgatgagc agttgaaatc tggaactgcc tctgttgtgt gcctgctgaa taacttctat 420
cccagagagg ccaaagtaca gtggaaggtg gataacgccc tccaatcggg taactcccag 480
gagagtgtca cagagcagga cagcaaggac agcacctaca gcctcagcag caccctgacg 540
ctgagcaaag cagactacga gaaacacaaa gtctacgcct gcgaagtcac ccatcagggc 600
ctgagctcgc ccgtcacaaa gagcttcaac aggggagagt gt 642

<210> 241
<211> 447
<212> PRT
<213> Artificial Sequence

<220>
<223> 4G8 Fab HC-Fc knob (LALA P329G)

<400> 241

Glu Val Gln Leu Leu Glu Ser Gly Gly Gly Leu Val Gln Pro Gly Gly
1 5 10 15

Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Phe Thr Phe Ser Ser Tyr
20 25 30

Ala Met Ser Trp Val Arg Gln Ala Pro Gly Lys Gly Leu Glu Trp Val
35 40 45

Ser Ala Ile Ser Gly Ser Gly Gly Ser Thr Tyr Tyr Ala Asp Ser Val
50 55 60

eolf-seql.txt

Lys Gly Arg Phe Thr Ile Ser Arg Asp Asn Ser Lys Asn Thr Leu Tyr
 65 70 75 80
 Leu Gln Met Asn Ser Leu Arg Ala Glu Asp Thr Ala Val Tyr Tyr Cys
 85 90 95
 Ala Lys Gly Trp Leu Gly Asn Phe Asp Tyr Trp Gly Gln Gly Thr Leu
 100 105 110
 Val Thr Val Ser Ser Ala Ser Thr Lys Gly Pro Ser Val Phe Pro Leu
 115 120 125
 Ala Pro Ser Ser Lys Ser Thr Ser Gly Gly Thr Ala Ala Leu Gly Cys
 130 135 140
 Leu Val Lys Asp Tyr Phe Pro Glu Pro Val Thr Val Ser Trp Asn Ser
 145 150 155 160
 Gly Ala Leu Thr Ser Gly Val His Thr Phe Pro Ala Val Leu Gln Ser
 165 170 175
 Ser Gly Leu Tyr Ser Leu Ser Ser Val Val Thr Val Pro Ser Ser Ser
 180 185 190
 Leu Gly Thr Gln Thr Tyr Ile Cys Asn Val Asn His Lys Pro Ser Asn
 195 200 205
 Thr Lys Val Asp Lys Lys Val Glu Pro Lys Ser Cys Asp Lys Thr His
 210 215 220
 Thr Cys Pro Pro Cys Pro Ala Pro Glu Ala Ala Gly Gly Pro Ser Val
 225 230 235 240
 Phe Leu Phe Pro Pro Lys Pro Lys Asp Thr Leu Met Ile Ser Arg Thr
 245 250 255
 Pro Glu Val Thr Cys Val Val Val Asp Val Ser His Glu Asp Pro Glu
 260 265 270
 Val Lys Phe Asn Trp Tyr Val Asp Gly Val Glu Val His Asn Ala Lys
 275 280 285
 Thr Lys Pro Arg Glu Glu Gln Tyr Asn Ser Thr Tyr Arg Val Val Ser
 290 295 300
 Val Leu Thr Val Leu His Gln Asp Trp Leu Asn Gly Lys Glu Tyr Lys
 305 310 315 320
 Cys Lys Val Ser Asn Lys Ala Leu Gly Ala Pro Ile Glu Lys Thr Ile
 325 330 335

eolf-seql.txt

Ser Lys Ala Lys Gly Gln Pro Arg Glu Pro Gln Val Tyr Thr Leu Pro
340 345 350

Pro Cys Arg Asp Glu Leu Thr Lys Asn Gln Val Ser Leu Trp Cys Leu
355 360 365

Val Lys Gly Phe Tyr Pro Ser Asp Ile Ala Val Glu Trp Glu Ser Asn
370 375 380

Gly Gln Pro Glu Asn Asn Tyr Lys Thr Thr Pro Pro Val Leu Asp Ser
385 390 395 400

Asp Gly Ser Phe Phe Leu Tyr Ser Lys Leu Thr Val Asp Lys Ser Arg
405 410 415

Trp Gln Gln Gly Asn Val Phe Ser Cys Ser Val Met His Glu Ala Leu
420 425 430

His Asn His Tyr Thr Gln Lys Ser Leu Ser Leu Ser Pro Gly Lys
435 440 445

<210> 242
<211> 1341
<212> DNA
<213> Artificial Sequence

<220>
<223> 4G8 Fab HC-Fc knob (LALA P329G)

<400> 242
gaggtgcaat tgttggagtc tgggggaggc ttggtacagc ctgggggggtc cctgagactc 60
tcctgtgcag cctccggatt cacctttagc agttatgcca tgagctgggt ccgccaggct 120
ccagggaagg ggctggagtg ggtctcagct attagtggta gtggtggtag cacatactac 180
gcagactccg tgaagggccg gttcaccatc tccagagaca attccaagaa cacgctgtat 240
ctgcagatga acagcctgag agccgaggac acggccgtat attactgtgc gaaaggggtg 300
ctgggtaatt ttgactactg gggccaagga accctgggtca ccgtctcgag tgctagcacc 360
aagggcccat cggtcttccc cctggcacc cctccaaga gcacctctgg gggcacagcg 420
gccctgggct gcctgggtcaa ggactacttc cccgaaccgg tgacggtgtc gtggaactca 480
ggcgcctga ccagcggcgt gcacaccttc ccggctgtcc tacagtcctc aggactctac 540
tccctcagca gcgtgggtgac cgtgccctcc agcagcttgg gcaccagac ctacatctgc 600
aacgtgaatc acaagcccag caacaccaag gtggacaaga aagttgagcc caaatcttgt 660
gacaaaactc acacatgccc accgtgccc gcacctgaag ctgcaggggg accgtcagtc 720
ttcctcttcc ccccaaacc caaggacacc ctcatgatct cccggacccc tgaggtcaca 780
tgctgtgtgg tggacgtgag ccacgaagac cctgaggtca agttcaactg gtacgtggac 840
ggcgtggagg tgcataatgc caagacaaag ccgcgggagg agcagtacaa cagcacgtac 900

eolf-seql.txt

```

cgtgtggtca gcgtcctcac cgtcctgcac caggactggc tgaatggcaa ggagtacaag      960
tgcaaggtct ccaacaaagc cctcggcgcc cccatcgaga aaaccatctc caaagccaaa      1020
gggcagcccc gagaaccaca ggtgtacacc ctgcccccat gccgggatga gctgaccaag      1080
aaccagggtca gcctgtggtg cctggtcaaa ggcttctatc ccagcgacat cgccgtggag      1140
tgggagagca atgggcagcc ggagaacaac tacaagacca cgcctcccgt gctggactcc      1200
gacggctcct tcttcctcta cagcaagctc accgtggaca agagcagggtg gcagcagggg      1260
aacgtcttct catgctccgt gatgcatgag gctctgcaca accactacac gcagaagagc      1320
ctctccctgt ctccgggtaa a                                              1341

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<210> 243
 <211> 801
 <212> PRT
 <213> Artificial Sequence

<220>
 <223> 4G8 Fab HC-Fc hole (LALA P329G)-scIL-10

<400> 243

Glu Val Gln Leu Leu Glu Ser Gly Gly Gly Leu Val Gln Pro Gly Gly
 1 5 10 15

Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Phe Thr Phe Ser Ser Tyr
 20 25 30

Ala Met Ser Trp Val Arg Gln Ala Pro Gly Lys Gly Leu Glu Trp Val
 35 40 45

Ser Ala Ile Ser Gly Ser Gly Gly Ser Thr Tyr Tyr Ala Asp Ser Val
 50 55 60

Lys Gly Arg Phe Thr Ile Ser Arg Asp Asn Ser Lys Asn Thr Leu Tyr
 65 70 75 80

Leu Gln Met Asn Ser Leu Arg Ala Glu Asp Thr Ala Val Tyr Tyr Cys
 85 90 95

Ala Lys Gly Trp Leu Gly Asn Phe Asp Tyr Trp Gly Gln Gly Thr Leu
 100 105 110

Val Thr Val Ser Ser Ala Ser Thr Lys Gly Pro Ser Val Phe Pro Leu
 115 120 125

Ala Pro Ser Ser Lys Ser Thr Ser Gly Gly Thr Ala Ala Leu Gly Cys
 130 135 140

Leu Val Lys Asp Tyr Phe Pro Glu Pro Val Thr Val Ser Trp Asn Ser
 145 150 155 160

eolf-seql.txt

Gly Ala Leu Thr Ser₁₆₅ Gly Val His Thr Phe₁₇₀ Pro Ala Val Leu Gln Ser₁₇₅

Ser Gly Leu Tyr₁₈₀ Ser Leu Ser Ser Val₁₈₅ Val Thr Val Pro Ser₁₉₀ Ser Ser

Leu Gly Thr₁₉₅ Gln Thr Tyr Ile Cys₂₀₀ Asn Val Asn His Lys₂₀₅ Pro Ser Asn

Thr Lys₂₁₀ Val Asp Lys Lys Val₂₁₅ Glu Pro Lys Ser Cys₂₂₀ Asp Lys Thr His

Thr Cys₂₂₅ Pro Pro Cys₂₃₀ Pro Ala Pro Glu Ala₂₃₅ Ala Gly Gly Pro Ser Val₂₄₀

Phe Leu Phe Pro₂₄₅ Lys Pro Lys Asp Thr₂₅₀ Leu Met Ile Ser Arg₂₅₅ Thr

Pro Glu Val Thr₂₆₀ Cys Val Val Val Asp₂₆₅ Val Ser His Glu Asp₂₇₀ Pro Glu

Val Lys Phe₂₇₅ Asn Trp Tyr Val Asp₂₈₀ Gly Val Glu Val His₂₈₅ Asn Ala Lys

Thr Lys₂₉₀ Pro Arg Glu Glu Gln₂₉₅ Tyr Asn Ser Thr Tyr₃₀₀ Arg Val Val Ser

Val₃₀₅ Leu Thr Val Leu His₃₁₀ Gln Asp Trp Leu Asn₃₁₅ Gly Lys Glu Tyr Lys₃₂₀

Cys Lys Val Ser₃₂₅ Lys Ala Leu Gly Ala₃₃₀ Pro Ile Glu Lys Thr₃₃₅ Ile

Ser Lys Ala Lys₃₄₀ Gly Gln Pro Arg Glu₃₄₅ Pro Gln Val Cys Thr₃₅₀ Leu Pro

Pro Ser Arg₃₅₅ Asp Glu Leu Thr Lys₃₆₀ Asn Gln Val Ser Leu₃₆₅ Ser Cys Ala

Val Lys₃₇₀ Gly Phe Tyr Pro Ser₃₇₅ Asp Ile Ala Val Glu₃₈₀ Trp Glu Ser Asn

Gly₃₈₅ Gln Pro Glu Asn Asn₃₉₀ Tyr Lys Thr Thr Pro₃₉₅ Pro Val Leu Asp Ser₄₀₀

Asp Gly Ser Phe₄₀₅ Phe Leu Val Ser Lys₄₁₀ Leu Thr Val Asp Lys Ser₄₁₅ Arg

Trp Gln Gln Gly₄₂₀ Asn Val Phe Ser Cys₄₂₅ Ser Val Met His₄₃₀ Glu Ala Leu

eolf-seql.txt

His Asn His Tyr Thr Gln Lys Ser Leu Ser Leu Ser Pro Gly Gly Gly
435 440 445

Gly Gly Ser Gly Gly Gly Gly Ser Gly Gly Gly Gly Ser Ser Pro Gly
450 455 460

Gln Gly Thr Gln Ser Glu Asn Ser Cys Thr His Phe Pro Gly Asn Leu
465 470 475 480

Pro Asn Met Leu Arg Asp Leu Arg Asp Ala Phe Ser Arg Val Lys Thr
485 490 495

Phe Phe Gln Met Lys Asp Gln Leu Asp Asn Leu Leu Leu Lys Glu Ser
500 505 510

Leu Leu Glu Asp Phe Lys Gly Tyr Leu Gly Cys Gln Ala Leu Ser Glu
515 520 525

Met Ile Gln Phe Tyr Leu Glu Glu Val Met Pro Gln Ala Glu Asn Gln
530 535 540

Asp Pro Asp Ile Lys Ala His Val Asn Ser Leu Gly Glu Asn Leu Lys
545 550 555 560

Thr Leu Arg Leu Arg Leu Arg Arg Cys His Arg Phe Leu Pro Cys Glu
565 570 575

Asn Lys Ser Lys Ala Val Glu Gln Val Lys Asn Ala Phe Asn Lys Leu
580 585 590

Gln Glu Lys Gly Ile Tyr Lys Ala Met Ser Glu Phe Asp Ile Phe Ile
595 600 605

Asn Tyr Ile Glu Ala Tyr Met Thr Met Lys Ile Arg Asn Gly Gly Gly
610 615 620

Gly Ser Gly Gly Gly Gly Ser Gly Gly Gly Gly Ser Gly Gly Gly Gly
625 630 635 640

Ser Ser Pro Gly Gln Gly Thr Gln Ser Glu Asn Ser Cys Thr His Phe
645 650 655

Pro Gly Asn Leu Pro Asn Met Leu Arg Asp Leu Arg Asp Ala Phe Ser
660 665 670

Arg Val Lys Thr Phe Phe Gln Met Lys Asp Gln Leu Asp Asn Leu Leu
675 680 685

Leu Lys Glu Ser Leu Leu Glu Asp Phe Lys Gly Tyr Leu Gly Cys Gln
690 695 700

eofl-seql.txt

Ala Leu Ser Glu Met Ile Gln Phe Tyr Leu Glu Glu Val Met Pro Gln
705 710 715 720

Ala Glu Asn Gln Asp Pro Asp Ile Lys Ala His Val Asn Ser Leu Gly
725 730 735

Glu Asn Leu Lys Thr Leu Arg Leu Arg Arg Arg Cys His Arg Phe
740 745 750

Leu Pro Cys Glu Asn Lys Ser Lys Ala Val Glu Gln Val Lys Asn Ala
755 760 765

Phe Asn Lys Leu Gln Glu Lys Gly Ile Tyr Lys Ala Met Ser Glu Phe
770 775 780

Asp Ile Phe Ile Asn Tyr Ile Glu Ala Tyr Met Thr Met Lys Ile Arg
785 790 795 800

Asn

<210> 244
<211> 2403
<212> DNA
<213> Artificial Sequence

<220>
<223> 4G8 Fab HC-Fc hole (LALA P329G)-scIL-10

<400> 244
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tcctgtgcag cctccggatt caccttttagc agttatgcca tgagctgggt ccgccaggct 120
ccagggaagg ggctggagtg ggtctcagct attagtggta gtggtggttag cacatactac 180
gcagactccg tgaagggccg gttcaccatc tccagagaca attccaagaa cacgctgtat 240
ctgcagatga acagcctgag agccgaggac acggccgtat attactgtgc gaaaggggtg 300
ctgggtaatt ttgactactg gggccaagga accctgggtca ccgtctcgag tgctagcacc 360
aagggcccat cgggtcttccc cctggcaccc tcctccaaga gcacctctgg gggcacagcg 420
gccctgggct gcctgggtcaa ggactacttc cccgaaccgg tgacggtgtc gtggaactca 480
ggcgccctga ccagcggcgt gcacaccttc ccggctgtcc tacagtcctc aggactctac 540
tccctcagca gcgtgggtgac cgtgccctcc agcagcttgg gcacccagac ctacatctgc 600
aacgtgaatc acaagcccag caacaccaag gtggacaaga aagttgagcc caaatcttgt 660
gacaaaactc acacatgccc accgtgcccga gcacctgaag ctgcaggggg accgtcagtc 720
ttcctcttcc ccccaaaacc caaggacacc ctcatgatct cccggacccc tgaggtcaca 780
tgcgtgggtg tggacgtgag ccacgaagac cctgaggtca agttcaactg gtacgtggac 840
ggcgtggagg tgcataatgc caagacaaag ccgcgggagg agcagtacaa cagcacgtac 900
cgtgtgggtca gcgtcctcac cgtcctgcac caggactggc tgaatggcaa ggagtacaag 960

eolf-seql.txt

tgcaaggtct	ccaacaaagc	cctcggcgcc	cccatcgaga	aaaccatctc	caaagccaaa	1020
gggcagcccc	gagaaccaca	ggtgtgcacc	ctgcccccat	cccgggatga	gctgaccaag	1080
aaccaggtca	gcctctcgtg	cgcagtcaaa	ggcttctatc	ccagcgacat	cgccgtggag	1140
tgggagagca	atgggcagcc	ggagaacaac	tacaagacca	cgcctcccgt	gctggactcc	1200
gacggctcct	tcttcctcgt	gagcaagctc	accgtggaca	agagcaggtg	gcagcagggg	1260
aacgtcttct	catgctccgt	gatgcatgag	gctctgcaca	accactacac	gcagaagagc	1320
ctctccctgt	ctccgggtgg	cggcggaggc	tccggaggcg	gaggatctgg	gggaggcgga	1380
agtagcccgg	gccagggcac	ccagagcgag	aacagctgca	cccacttccc	cggcaacctg	1440
cccaacatgc	tgcgggacct	gagggacgcc	ttcagcagag	tgaaaacctt	cttccagatg	1500
aaggaccagc	tggacaacct	gctgctgaaa	gagagcctgc	tggaagattt	caagggctac	1560
ctgggctgtc	aggccctgag	cgagatgata	cagttctacc	tggaagaagt	gatgccccag	1620
gccgagaacc	aggaccccg	catcaaggcc	cacgtgaaca	gcctgggcca	gaacctgaaa	1680
accctgcggc	tgagactgag	gcggtgccac	agatttctgc	cctgcgagaa	caagagcaag	1740
gccgtggaac	aggtgaagaa	cgccttcaac	aagctgcagg	aaaagggcat	ctacaaggcc	1800
atgtccgagt	tcgacatctt	catcaactac	atcgaagctt	acatgaccat	gaagatcaga	1860
aacggcggag	gcggatctgg	cggcgggtga	agtggaggcg	gaggatctgg	gggaggcgga	1920
agtagcccgg	gccagggcac	ccagagcgag	aacagctgca	cccacttccc	cggcaacctg	1980
cccaacatgc	tgcgggacct	gagggacgcc	ttcagcagag	tgaaaacctt	cttccagatg	2040
aaggaccagc	tggacaacct	gctgctgaaa	gagagcctgc	tggaagattt	caagggctac	2100
ctgggctgtc	aggccctgag	cgagatgata	cagttctacc	tggaagaagt	gatgccccag	2160
gccgagaacc	aggaccccg	catcaaggcc	cacgtgaaca	gcctgggcca	gaacctgaaa	2220
accctgcggc	tgagactgag	gcggtgccac	agatttctgc	cctgcgagaa	caagagcaag	2280
gccgtggaac	aggtgaagaa	cgccttcaac	aagctgcagg	aaaagggcat	ctacaaggcc	2340
atgtccgagt	tcgacatctt	catcaactac	atcaggccct	acatgacaat	gaaaatccgc	2400
aat						2403

<210> 245
 <211> 627
 <212> PRT
 <213> Artificial Sequence

<220>
 <223> 4G8 Fab HC-Fc hole (LALA P329G)-IL-10M1

<400> 245

Glu	Val	Gln	Leu	Leu	Glu	Ser	Gly	Gly	Gly	Leu	Val	Gln	Pro	Gly	Gly
1				5					10					15	

Ser	Leu	Arg	Leu	Ser	Cys	Ala	Ala	Ser	Gly	Phe	Thr	Phe	Ser	Ser	Tyr
			20					25					30		

eolf-seql.txt

Ala Met Ser Trp Val Arg Gln Ala Pro Gly Lys Gly Leu Glu Trp Val
35 40 45

Ser Ala Ile Ser Gly Ser Gly Gly Ser Thr Tyr Tyr Ala Asp Ser Val
50 55 60

Lys Gly Arg Phe Thr Ile Ser Arg Asp Asn Ser Lys Asn Thr Leu Tyr
65 70 75 80

Leu Gln Met Asn Ser Leu Arg Ala Glu Asp Thr Ala Val Tyr Tyr Cys
85 90 95

Ala Lys Gly Trp Leu Gly Asn Phe Asp Tyr Trp Gly Gln Gly Thr Leu
100 105 110

Val Thr Val Ser Ser Ala Ser Thr Lys Gly Pro Ser Val Phe Pro Leu
115 120 125

Ala Pro Ser Ser Lys Ser Thr Ser Gly Gly Thr Ala Ala Leu Gly Cys
130 135 140

Leu Val Lys Asp Tyr Phe Pro Glu Pro Val Thr Val Ser Trp Asn Ser
145 150 155 160

Gly Ala Leu Thr Ser Gly Val His Thr Phe Pro Ala Val Leu Gln Ser
165 170 175

Ser Gly Leu Tyr Ser Leu Ser Ser Val Val Thr Val Pro Ser Ser Ser
180 185 190

Leu Gly Thr Gln Thr Tyr Ile Cys Asn Val Asn His Lys Pro Ser Asn
195 200 205

Thr Lys Val Asp Lys Lys Val Glu Pro Lys Ser Cys Asp Lys Thr His
210 215 220

Thr Cys Pro Pro Cys Pro Ala Pro Glu Ala Ala Gly Gly Pro Ser Val
225 230 235 240

Phe Leu Phe Pro Pro Lys Pro Lys Asp Thr Leu Met Ile Ser Arg Thr
245 250 255

Pro Glu Val Thr Cys Val Val Val Asp Val Ser His Glu Asp Pro Glu
260 265 270

Val Lys Phe Asn Trp Tyr Val Asp Gly Val Glu Val His Asn Ala Lys
275 280 285

Thr Lys Pro Arg Glu Glu Gln Tyr Asn Ser Thr Tyr Arg Val Val Ser
290 295 300

eolf-seql.txt

Val Leu Thr Val Leu His Gln Asp Trp Leu Asn Gly Lys Glu Tyr Lys
305 310 315 320

Cys Lys Val Ser Asn Lys Ala Leu Gly Ala Pro Ile Glu Lys Thr Ile
325 330 335

Ser Lys Ala Lys Gly Gln Pro Arg Glu Pro Gln Val Cys Thr Leu Pro
340 345 350

Pro Ser Arg Asp Glu Leu Thr Lys Asn Gln Val Ser Leu Ser Cys Ala
355 360 365

Val Lys Gly Phe Tyr Pro Ser Asp Ile Ala Val Glu Trp Glu Ser Asn
370 375 380

Gly Gln Pro Glu Asn Asn Tyr Lys Thr Thr Pro Pro Val Leu Asp Ser
385 390 395 400

Asp Gly Ser Phe Phe Leu Val Ser Lys Leu Thr Val Asp Lys Ser Arg
405 410 415

Trp Gln Gln Gly Asn Val Phe Ser Cys Ser Val Met His Glu Ala Leu
420 425 430

His Asn His Tyr Thr Gln Lys Ser Leu Ser Leu Ser Pro Gly Gly Gly
435 440 445

Gly Gly Ser Gly Gly Gly Gly Ser Gly Gly Gly Gly Ser Ser Pro Gly
450 455 460

Gln Gly Thr Gln Ser Glu Asn Ser Cys Thr His Phe Pro Gly Asn Leu
465 470 475 480

Pro Asn Met Leu Arg Asp Leu Arg Asp Ala Phe Ser Arg Val Lys Thr
485 490 495

Phe Phe Gln Met Lys Asp Gln Leu Asp Asn Leu Leu Leu Lys Glu Ser
500 505 510

Leu Leu Glu Asp Phe Lys Gly Tyr Leu Gly Cys Gln Ala Leu Ser Glu
515 520 525

Met Ile Gln Phe Tyr Leu Glu Glu Val Met Pro Gln Ala Glu Asn Gln
530 535 540

Asp Pro Asp Ile Lys Ala His Val Asn Ser Leu Gly Glu Asn Leu Lys
545 550 555 560

Thr Leu Arg Leu Arg Leu Arg Arg Cys His Arg Phe Leu Pro Cys Glu
565 570 575

eolf-seql.txt

Asn Gly Gly Gly Ser Gly Gly Lys Ser Lys Ala Val Glu Gln Val Lys
580 585 590

Asn Ala Phe Asn Lys Leu Gln Glu Lys Gly Ile Tyr Lys Ala Met Ser
595 600 605

Glu Phe Asp Ile Phe Ile Asn Tyr Ile Glu Ala Tyr Met Thr Met Lys
610 615 620

Ile Arg Asn
625

<210> 246
<211> 1881
<212> DNA
<213> Artificial Sequence

<220>
<223> 4G8 Fab HC-Fc hole (LALA P329G)-IL-10M1

<400> 246
gaggtgcaat tggtggagtc tgggggaggc ttggtacagc ctgggggggtc cctgagactc 60
tcctgtgcag cctccggatt cacctttagc agttatgcc a tgagctgggt ccgccaggct 120
ccagggaagg ggctggagtg ggtctcagct attagtggta gtgggtggtag cacatactac 180
gcagactccg tgaagggccg gttcaccatc tccagagaca attccaagaa cacgctgtat 240
ctgcagatga acagcctgag agccgaggac acggccgtat attactgtgc gaaaggggtgg 300
ctgggtaatt ttgactactg gggccaagga accctgggtca ccgtctcgag tgctagcacc 360
aagggcccat cggtcttccc cctggcaccc tccccaaga gcacctctgg gggcacagcg 420
gccctgggct gcctgggtcaa ggactacttc cccgaaccgg tgacgggtgtc gtggaactca 480
ggcgccctga ccagcggcgt gcacaccttc ccggctgtcc tacagtcctc aggactctac 540
tccctcagca gcgtgggtgac cgtgccctcc agcagcttgg gcacccagac ctacatctgc 600
aacgtgaatc acaagcccag caacaccaag gtggacaaga aagttgagcc caaatcttgt 660
gacaaaactc acacatgccc accgtgccc a gcacctgaag ctgcaggggg accgtcagtc 720
ttcctcttcc ccccaaaacc caaggacacc ctcatgatct cccggacccc tgaggtcaca 780
tgctgtgggtg tggacgtgag ccacgaagac cctgaggtca agttcaactg gtacgtggac 840
ggcgtggagg tgcataatgc caagacaaag ccgcgggagg agcagtacaa cagcacgtac 900
cgtgtgggtca gcgtcctcac cgtcctgcac caggactggc tgaatggcaa ggagtacaag 960
tgcaaggctc ccaacaaagc cctcggcgcc cccatcgaga aaaccatctc caaagccaaa 1020
gggcagcccc gagaaccaca ggtgtgcacc ctgcccccat cccgggatga gctgaccaag 1080
aaccaggtca gcctctcgtg cgcagtcaaa ggcttctatc ccagcgacat cgccgtggag 1140
tgggagagca atgggcagcc ggagaacaac tacaagacca cgcctcccgt gctggactcc 1200
gacggctcct tcttctcgt gagcaagctc accgtggaca agagcaggtg gcagcagggg 1260

eolf-seql.txt

```

aacgtcttct catgctccgt gatgcatgag gctctgcaca accactacac gcagaagagc      1320
ctctccctgt ctccgggtgg cggcggaggc tccggaggcg gaggaagtgg cggcgggtgg      1380
agctctccag gccagggcac ccagagcgag aacagctgca cccacttccc cggcaacctg      1440
cccaacatgc tgcgggacct gagggacgcc ttcagcagag tgaaaacctt cttccagatg      1500
aaggaccagc tggacaacct gctgctgaaa gagagcctgc tggaagattt caagggctac      1560
ctgggctgtc aggccctgag cgagatgatc cagttctacc tggaagaagt gatgccccag      1620
gccgagaacc aggaccccgga catcaaggcc cacgtgaaca gcctgggcga gaacctgaaa      1680
accctgcggc tgagactgcg gcggtgccac agatttctgc cctgcgagaa cggcggaggc      1740
tctggcggaa agtccaaggc cgtggaacag gtgaagaacg cttcaacaa gctgcaggaa      1800
aaggcatct acaaggccat gagcgagttc gacatcttca tcaactacat cgaagcttac      1860
atgacaatga agatacgaaa c                                     1881

```

<210> 247
 <211> 366
 <212> PRT
 <213> Artificial Sequence

<220>
 <223> IL-2 qm-Fc knob (LALA P329G) (IL-2 N-terminal)
 <400> 247

Ala Pro Ala Ser Ser Ser Thr Lys Lys Thr Gln Leu Gln Leu Glu His
1 5 10 15

Leu Leu Leu Asp Leu Gln Met Ile Leu Asn Gly Ile Asn Asn Tyr Lys
20 25 30

Asn Pro Lys Leu Thr Arg Met Leu Thr Ala Lys Phe Ala Met Pro Lys
35 40 45

Lys Ala Thr Glu Leu Lys His Leu Gln Cys Leu Glu Glu Glu Leu Lys
50 55 60

Pro Leu Glu Glu Val Leu Asn Gly Ala Gln Ser Lys Asn Phe His Leu
65 70 75 80

Arg Pro Arg Asp Leu Ile Ser Asn Ile Asn Val Ile Val Leu Glu Leu
85 90 95

Lys Gly Ser Glu Thr Thr Phe Met Cys Glu Tyr Ala Asp Glu Thr Ala
100 105 110

Thr Ile Val Glu Phe Leu Asn Arg Trp Ile Thr Phe Ala Gln Ser Ile
115 120 125

Ile Ser Thr Leu Thr Ser Gly Gly Gly Gly Ser Asp Lys Thr His Thr
130 135 140

eolf-seql.txt

Cys Pro Pro Cys Pro Ala Pro Glu Ala Ala Gly Gly Pro Ser Val Phe
145 150 155 160

Leu Phe Pro Pro Lys Pro Lys Asp Thr Leu Met Ile Ser Arg Thr Pro
165 170 175

Glu Val Thr Cys Val Val Val Asp Val Ser His Glu Asp Pro Glu Val
180 185 190

Lys Phe Asn Trp Tyr Val Asp Gly Val Glu Val His Asn Ala Lys Thr
195 200 205

Lys Pro Arg Glu Glu Gln Tyr Asn Ser Thr Tyr Arg Val Val Ser Val
210 215 220

Leu Thr Val Leu His Gln Asp Trp Leu Asn Gly Lys Glu Tyr Lys Cys
225 230 235 240

Lys Val Ser Asn Lys Ala Leu Gly Ala Pro Ile Glu Lys Thr Ile Ser
245 250 255

Lys Ala Lys Gly Gln Pro Arg Glu Pro Gln Val Tyr Thr Leu Pro Pro
260 265 270

Cys Arg Asp Glu Leu Thr Lys Asn Gln Val Ser Leu Trp Cys Leu Val
275 280 285

Lys Gly Phe Tyr Pro Ser Asp Ile Ala Val Glu Trp Glu Ser Asn Gly
290 295 300

Gln Pro Glu Asn Asn Tyr Lys Thr Thr Pro Pro Val Leu Asp Ser Asp
305 310 315 320

Gly Ser Phe Phe Leu Tyr Ser Lys Leu Thr Val Asp Lys Ser Arg Trp
325 330 335

Gln Gln Gly Asn Val Phe Ser Cys Ser Val Met His Glu Ala Leu His
340 345 350

Asn His Tyr Thr Gln Lys Ser Leu Ser Leu Ser Pro Gly Lys
355 360 365

<210> 248
<211> 1098
<212> DNA
<213> Artificial Sequence

<220>
<223> IL-2 qm-Fc knob (LALA P329G) (IL-2 N-terminal)

<400> 248
gctcctgcct cctccagcac caagaaaacc cagctccagc tggaacatct cctgctggat

eolf-seql.txt

```

ctgcagatga tcctgaacgg catcaacaac tacaagaacc ccaagctgac ccggatgctg      120
accgccaagt tcgccatgcc caagaaggcc accgagctga aacatctgca gtgcctggaa      180
gaggaactga agcctctgga agaggtgctg aacggcgccc agtccaagaa cttccacctg      240
aggcctcggg acctgatctc caacatcaac gtgatcgtgc tggaactgaa gggctccgag      300
acaaccttca tgtgcgagta cgccgacgag acagctacca tcgtggaatt tctgaaccgg      360
tggatcacct tcgcccagtc catcatctcc accctgacct ccggtggtgg cggatccgac      420
aaaactcaca catgcccacc gtgcccagca cctgaagctg caggggggacc gtcagtcttc      480
ctcttcccc caaaaccaa ggacaccctc atgatctccc ggaccctga ggtcacatgc      540
gtggtggtgg acgtgagcca cgaagaccct gaggtcaagt tcaactggta cgtggacggc      600
gtggaggtgc ataatgcaa gacaaagccg cgggaggagc agtacaacag cacgtaccgt      660
gtggtcagcg tcctcaccgt cctgcaccag gactggctga atggcaagga gtacaagtgc      720
aaggtctcca acaaagccct cggcgcccc atcgagaaaa ccatctccaa agccaaaggg      780
cagccccgag aaccacaggt gtacaccctg ccccatgcc gggatgagct gaccaagaac      840
caggtcagcc tgtggtgcct ggtcaaaggc ttctatccca gcgacatcgc cgtggagtgg      900
gagagcaatg ggcagccgga gaacaactac aagaccacgc ctcccgtgct ggactccgac      960
ggctccttct tcctctacag caagctcacc gtggacaaga gcaggtggca gcaggggaac     1020
gtcttctcat gtcctgtgat gcatgaggct ctgcacaacc actacacgca gaagagcctc     1080
tccctgtctc cgggtaaa                                     1098

```

<210> 249
 <211> 384
 <212> PRT
 <213> Artificial Sequence

<220>
 <223> IL-7-Fc knob (LALA P329G) (IL-7 N-terminal)
 <400> 249

Asp Cys Asp Ile Glu Gly Lys Asp Gly Lys Gln Tyr Glu Ser Val Leu
 1 5 10 15

Met Val Ser Ile Asp Gln Leu Leu Asp Ser Met Lys Glu Ile Gly Ser
 20 25 30

Asn Cys Leu Asn Asn Glu Phe Asn Phe Phe Lys Arg His Ile Cys Asp
 35 40 45

Ala Asn Lys Glu Gly Met Phe Leu Phe Arg Ala Ala Arg Lys Leu Arg
 50 55 60

Gln Phe Leu Lys Met Asn Ser Thr Gly Asp Phe Asp Leu His Leu Leu
 65 70 75 80

eof-seql.txt

Lys Val Ser Glu Gly Thr Thr Ile Leu Leu Asn Cys Thr Gly Gln Val
85 90 95

Lys Gly Arg Lys Pro Ala Ala Leu Gly Glu Ala Gln Pro Thr Lys Ser
100 105 110

Leu Glu Glu Asn Lys Ser Leu Lys Glu Gln Lys Lys Leu Asn Asp Leu
115 120 125

Cys Phe Leu Lys Arg Leu Leu Gln Glu Ile Lys Thr Cys Trp Asn Lys
130 135 140

Ile Leu Met Gly Thr Lys Glu His Gly Gly Gly Gly Ser Asp Lys Thr
145 150 155 160

His Thr Cys Pro Pro Cys Pro Ala Pro Glu Ala Ala Gly Gly Pro Ser
165 170 175

Val Phe Leu Phe Pro Pro Lys Pro Lys Asp Thr Leu Met Ile Ser Arg
180 185 190

Thr Pro Glu Val Thr Cys Val Val Val Asp Val Ser His Glu Asp Pro
195 200 205

Glu Val Lys Phe Asn Trp Tyr Val Asp Gly Val Glu Val His Asn Ala
210 215 220

Lys Thr Lys Pro Arg Glu Glu Gln Tyr Asn Ser Thr Tyr Arg Val Val
225 230 235 240

Ser Val Leu Thr Val Leu His Gln Asp Trp Leu Asn Gly Lys Glu Tyr
245 250 255

Lys Cys Lys Val Ser Asn Lys Ala Leu Gly Ala Pro Ile Glu Lys Thr
260 265 270

Ile Ser Lys Ala Lys Gly Gln Pro Arg Glu Pro Gln Val Tyr Thr Leu
275 280 285

Pro Pro Cys Arg Asp Glu Leu Thr Lys Asn Gln Val Ser Leu Trp Cys
290 295 300

Leu Val Lys Gly Phe Tyr Pro Ser Asp Ile Ala Val Glu Trp Glu Ser
305 310 315 320

Asn Gly Gln Pro Glu Asn Asn Tyr Lys Thr Thr Pro Pro Val Leu Asp
325 330 335

Ser Asp Gly Ser Phe Phe Leu Tyr Ser Lys Leu Thr Val Asp Lys Ser
340 345 350

eolf-seql.txt

Arg Trp Gln Gln Gly Asn Val Phe Ser Cys Ser Val Met His Glu Ala
355 360 365

Leu His Asn His Tyr Thr Gln Lys Ser Leu Ser Leu Ser Pro Gly Lys
370 375 380

<210> 250
<211> 1152
<212> DNA
<213> Artificial Sequence

<220>
<223> IL-7-Fc knob (LALA P329G) (IL-7 N-terminal)

<400> 250
gattgtgata ttgaaggtaa agatggcaaa caatatgaga gtgttctaataa gggtcagcatc 60
gatcaattat tggacagcat gaaagaaatt ggtagcaatt gcctgaataa tgaatttaac 120
tttttttaaaa gacatatctg tgatgctaataa aaggaaggta tgttttttatt ccgtgctgct 180
cgcaagttga ggcaatttct taaaatgaat agcactgggtg attttgatct ccacttatta 240
aaagtttcag aaggcacaac aatactgttg aactgcactg gccagggttaa aggaagaaaa 300
ccagctgccc tgggtgaagc ccaaccaaca aagagtttgg aagaaaataa atcttttaag 360
gaacagaaaa aactgaatga cttgtgtttc ctaaagagac tattacaaga gataaaaaact 420
tgtttggaata aaattttgat gggcactaaa gaacacgggtg gtggcggtatc cgacaaaact 480
cacacatgcc caccgtgccc agcacctgaa gctgcagggg gaccgtcagt cttcctcttc 540
cccccaaaac ccaaggacac cctcatgata tcccggaccc ctgagggtcac atgcgtgggtg 600
gtggacgtga gccacgaaga ccctgaggtc aagttcaact ggtacgtgga cggcgtggag 660
gtgcataatg ccaagacaaa gccgcgggag gagcagtaca acagcacgta ccgtgtgggtc 720
agcgtcctca ccgtcctgca ccaggactgg ctgaatggca aggagtacaa gtgcaagggtc 780
tccaacaaaag ccctcggcgc ccccatcgag aaaaccatct ccaaagccaa agggcagccc 840
cgagaaccac aggtgtacac cctgccccca tgccgggatg agctgaccaa gaaccagggtc 900
agcctgtggt gcctgggtcaa aggtttctat cccagcgaca tcgccgtgga gtgggagagc 960
aatgggcagc cggagaacaa ctacaagacc acgcctcccg tgctggactc cgacgggtcc 1020
ttcttctctc acagcaagct caccgtggac aagagcaggt ggcagcaggg gaacgtcttc 1080
tcatgtccg tgatgcatga ggctctgcac aaccactaca cgcagaagag cctctccctg 1140
tctccgggta aa 1152

<210> 251
<211> 398
<212> PRT
<213> Artificial Sequence

<220>
<223> IFN-a-Fc knob (LALA P329G) (IFN-a N-terminal)

<400> 251

co1f-seq1.txt

Ser Cys Asp Leu Pro Gln Thr His Ser Leu Gly Ser Arg Arg Thr Leu
1 5 10 15

Met Leu Leu Ala Gln Met Arg Lys Ile Ser Leu Phe Ser Cys Leu Lys
20 25 30

Asp Arg His Asp Phe Gly Phe Pro Gln Glu Glu Phe Gly Asn Gln Phe
35 40 45

Gln Lys Ala Glu Thr Ile Pro Val Leu His Glu Met Ile Gln Gln Ile
50 55 60

Phe Asn Leu Phe Ser Thr Lys Asp Ser Ser Ala Ala Trp Asp Glu Thr
65 70 75 80

Leu Leu Asp Lys Phe Tyr Thr Glu Leu Tyr Gln Gln Leu Asn Asp Leu
85 90 95

Glu Ala Cys Val Ile Gln Gly Val Gly Val Thr Glu Thr Pro Leu Met
100 105 110

Lys Glu Asp Ser Ile Leu Ala Val Arg Lys Tyr Phe Gln Arg Ile Thr
115 120 125

Leu Tyr Leu Lys Glu Lys Lys Tyr Ser Pro Cys Ala Trp Glu Val Val
130 135 140

Arg Ala Glu Ile Met Arg Ser Phe Ser Leu Ser Thr Asn Leu Gln Glu
145 150 155 160

Ser Leu Arg Ser Lys Glu Gly Gly Gly Gly Ser Asp Lys Thr His Thr
165 170 175

Cys Pro Pro Cys Pro Ala Pro Glu Ala Ala Gly Gly Pro Ser Val Phe
180 185 190

Leu Phe Pro Pro Lys Pro Lys Asp Thr Leu Met Ile Ser Arg Thr Pro
195 200 205

Glu Val Thr Cys Val Val Val Asp Val Ser His Glu Asp Pro Glu Val
210 215 220

Lys Phe Asn Trp Tyr Val Asp Gly Val Glu Val His Asn Ala Lys Thr
225 230 235 240

Lys Pro Arg Glu Glu Gln Tyr Asn Ser Thr Tyr Arg Val Val Ser Val
245 250 255

Leu Thr Val Leu His Gln Asp Trp Leu Asn Gly Lys Glu Tyr Lys Cys
260 265 270

eolf-seql.txt

Lys Val Ser Asn Lys Ala Leu Gly Ala Pro Ile Glu Lys Thr Ile Ser
275 280 285

Lys Ala Lys Gly Gln Pro Arg Glu Pro Gln Val Tyr Thr Leu Pro Pro
290 295 300

Cys Arg Asp Glu Leu Thr Lys Asn Gln Val Ser Leu Trp Cys Leu Val
305 310 315 320

Lys Gly Phe Tyr Pro Ser Asp Ile Ala Val Glu Trp Glu Ser Asn Gly
325 330 335

Gln Pro Glu Asn Asn Tyr Lys Thr Thr Pro Pro Val Leu Asp Ser Asp
340 345 350

Gly Ser Phe Phe Leu Tyr Ser Lys Leu Thr Val Asp Lys Ser Arg Trp
355 360 365

Gln Gln Gly Asn Val Phe Ser Cys Ser Val Met His Glu Ala Leu His
370 375 380

Asn His Tyr Thr Gln Lys Ser Leu Ser Leu Ser Pro Gly Lys
385 390 395

<210> 252
<211> 1194
<212> DNA
<213> Artificial Sequence

<220>
<223> IFN-a-Fc knob (LALA P329G) (IFN-a N-terminal)

<400> 252
agctgtgacc tgcctcagac acacagcctg ggcagccggc ggaccctgat gctgctggcc 60
cagatgcgga agatcagcct gttcagctgc ctgaaggacc ggcacgactt cggcttcctt 120
caggaagagt tcggcaacca gttccagaag gccgagacaa tccccgtgct gcacgagatg 180
atccagcaga ttttcaacct gttcagcacc aaggacagca gcgccgcctg ggacgagaca 240
ctgctggaca agttctacac cgagctgtac cagcagctga acgacctgga agcctgctgtg 300
atccagggcg tgggctgtac cgagacaccc ctgatgaagg aagatagcat cctggccgtg 360
cggaagtatt tccagcggat caccctgtac ctgaaagaga agaagtacag cccctgcgcc 420
tgggaggtcg tgcgggccga gatcatgcgg agcttcagcc tgagcaccaa cctgcaggaa 480
agcctgcgga gcaaagaggg tgggtggcga tccgacaaaa ctcacacatg cccaccgtgc 540
ccagcacctg aagctgcagg gggaccgtca gtcttctct tcccccaaa acccaaggac 600
accctcatga tctcccgga cctgaggtc acatgcgttg tgggtggacgt gagccacgaa 660
gaccctgagg tcaagttcaa ctggtacgtg gacggcgttg aggtgcataa tgccaagaca 720
aagccgcggg aggagcagta caacagcacg taccgtgtgg tcagcgtcct caccgtcctg 780
caccaggact ggctgaatgg caaggagtac aagtgcgaagg tctccaacaa agccctcggc 840

eolf-seql.txt

```

gcccccatcg agaaaacccat ctccaaagcc aaagggcagc cccgagaacc acaggtgtac      900
accctgcccc catgccggga tgagctgacc aagaaccagg tcagcctgtg gtgcctggtc      960
aaaggcttct atcccagcga catcgccgtg gagggggaga gcaatgggca gccggagaac     1020
aactacaaga ccacgcctcc cgtgctggac tccgacggct ctttcttct ctacagcaag     1080
ctcaccgtgg acaagagcag gtggcagcag gggaacgtct tctcatgctc cgtgatgcat     1140
gaggctctgc acaaccacta cacgcagaag agcctctccc tgtctccggg taaa          1194

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<210> 253
 <211> 593
 <212> PRT
 <213> Artificial Sequence

<220>
 <223> 28H1 Fab HC-Fc (LALA P329G)-IL-2 qm

<400> 253

Glu Val Gln Leu Leu Glu Ser Gly Gly Gly Leu Val Gln Pro Gly Gly
 1 5 10 15

Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Phe Thr Phe Ser Ser His
 20 25 30

Ala Met Ser Trp Val Arg Gln Ala Pro Gly Lys Gly Leu Glu Trp Val
 35 40 45

Ser Ala Ile Trp Ala Ser Gly Glu Gln Tyr Tyr Ala Asp Ser Val Lys
 50 55 60

Gly Arg Phe Thr Ile Ser Arg Asp Asn Ser Lys Asn Thr Leu Tyr Leu
 65 70 75 80

Gln Met Asn Ser Leu Arg Ala Glu Asp Thr Ala Val Tyr Tyr Cys Ala
 85 90 95

Lys Gly Trp Leu Gly Asn Phe Asp Tyr Trp Gly Gln Gly Thr Leu Val
 100 105 110

Thr Val Ser Ser Ala Ser Thr Lys Gly Pro Ser Val Phe Pro Leu Ala
 115 120 125

Pro Ser Ser Lys Ser Thr Ser Gly Gly Thr Ala Ala Leu Gly Cys Leu
 130 135 140

Val Lys Asp Tyr Phe Pro Glu Pro Val Thr Val Ser Trp Asn Ser Gly
 145 150 155 160

Ala Leu Thr Ser Gly Val His Thr Phe Pro Ala Val Leu Gln Ser Ser
 165 170 175

eofl-seql.txt

Gly Leu Tyr Ser₁₈₀ Leu Ser Ser Val Val₁₈₅ Thr Val Pro Ser Ser₁₉₀ Ser Leu

Gly Thr Gln₁₉₅ Thr Tyr Ile Cys Asn₂₀₀ Val Asn His Lys Pro₂₀₅ Ser Asn Thr

Lys Val₂₁₀ Asp Lys Lys Val Glu₂₁₅ Pro Lys Ser Cys Asp₂₂₀ Lys Thr His Thr

Cys₂₂₅ Pro Pro Cys Pro Ala₂₃₀ Pro Glu Ala Ala Gly₂₃₅ Gly Pro Ser Val Phe₂₄₀

Leu Phe Pro Pro Lys₂₄₅ Pro Lys Asp Thr Leu₂₅₀ Met Ile Ser Arg Thr₂₅₅ Pro

Glu Val Thr Cys₂₆₀ Val Val Val Asp Val₂₆₅ Ser His Glu Asp Pro₂₇₀ Glu Val

Lys Phe Asn₂₇₅ Trp Tyr Val Asp Gly₂₈₀ Val Glu Val His Asn₂₈₅ Ala Lys Thr

Lys Pro₂₉₀ Arg Glu Glu Gln Tyr₂₉₅ Asn Ser Thr Tyr Arg₃₀₀ Val Val Ser Val

Leu Thr Val Leu His Gln₃₁₀ Asp Trp Leu Asn Gly₃₁₅ Lys Glu Tyr Lys Cys₃₂₀

Lys Val Ser Asn Lys₃₂₅ Ala Leu Gly Ala Pro₃₃₀ Ile Glu Lys Thr Ile₃₃₅ Ser

Lys Ala Lys Gly₃₄₀ Gln Pro Arg Glu Pro₃₄₅ Gln Val Tyr Thr Leu₃₅₀ Pro Pro

Ser Arg Asp₃₅₅ Glu Leu Thr Lys Asn₃₆₀ Gln Val Ser Leu Thr₃₆₅ Cys Leu Val

Lys Gly₃₇₀ Phe Tyr Pro Ser Asp₃₇₅ Ile Ala Val Glu Trp₃₈₀ Glu Ser Asn Gly

Gln Pro Glu Asn Asn Tyr₃₉₀ Lys Thr Thr Pro Pro₃₉₅ Val Leu Asp Ser Asp₄₀₀

Gly Ser Phe Phe Leu₄₀₅ Tyr Ser Lys Leu Thr Val Asp Lys Ser Arg₄₁₅ Trp

Gln Gln Gly Asn₄₂₀ Val Phe Ser Cys Ser₄₂₅ Val Met His Glu Ala₄₃₀ Leu His

Asn His Tyr₄₃₅ Thr Gln Lys Ser Leu₄₄₀ Ser Leu Ser Pro Gly₄₄₅ Gly Gly Gly

eolf-seql.txt

Gly Ser Gly Gly Gly Gly Ser Gly Gly Gly Gly Ser Ala Pro Ala Ser
450 455 460

Ser Ser Thr Lys Lys Thr Gln Leu Gln Leu Glu His Leu Leu Leu Asp
465 470 475 480

Leu Gln Met Ile Leu Asn Gly Ile Asn Asn Tyr Lys Asn Pro Lys Leu
485 490 495

Thr Arg Met Leu Thr Ala Lys Phe Ala Met Pro Lys Lys Ala Thr Glu
500 505 510

Leu Lys His Leu Gln Cys Leu Glu Glu Glu Leu Lys Pro Leu Glu Glu
515 520 525

Val Leu Asn Gly Ala Gln Ser Lys Asn Phe His Leu Arg Pro Arg Asp
530 535 540

Leu Ile Ser Asn Ile Asn Val Ile Val Leu Glu Leu Lys Gly Ser Glu
545 550 555 560

Thr Thr Phe Met Cys Glu Tyr Ala Asp Glu Thr Ala Thr Ile Val Glu
565 570 575

Phe Leu Asn Arg Trp Ile Thr Phe Ala Gln Ser Ile Ile Ser Thr Leu
580 585 590

Thr

<210> 254
<211> 1779
<212> DNA
<213> Artificial Sequence

<220>
<223> 28H1 Fab HC-Fc (LALA P329G)-IL-2 qm

<400> 254
gaagtgcagc tgctggaatc cggcggaggc ctggtgcagc ctggcggatc tctgagactg 60
tcctgcgccg cctccggctt caccttctcc tcccacgcca tgtcctgggt ccgacaggct 120
cctggcaaag gcctggaatg ggtgtccgcc atctgggcct ccggcgagca gtactacgcc 180
gactctgtga agggccggtt caccatctcc cgggacaact ccaagaacac cctgtacctg 240
cagatgaact ccctgcgggc cgaggacacc gccgtgtact actgtgcca gggctggctg 300
ggcaacttcg actactgggg acagggcacc ctggtcaccg tgtccagcgc tagcaccaag 360
ggcccatcgg tcttccccct ggcaccctcc tccaagagca cctctggggg cacagcggcc 420
ctgggctgcc tggtaagga ctacttcccc gaaccgggtga cgggtgctgtg gaactcaggc 480
gccctgacca gcggcgtgca caccttcccc gctgtcctac agtcctcagg actctactcc 540
ctcagcagcg tgggtgaccgt gccctccagc agcttgggca cccagacctc catctgcaac 600

eolf-seql.txt

```

gtgaatcaca agcccagcaa caccaaggtg gacaagaaag ttgagcccaa atcttgtgac      660
aaaactcaca catgcccacc gtgcccagca cctgaagctg caggggggacc gtcagtcttc      720
ctcttcccc caaaacccaa ggacaccctc atgatctccc ggaccctga ggtcacatgc      780
gtggtggtgg acgtgagcca cgaagaccct gaggtcaagt tcaactggta cgtggacggc      840
gtggaggtgc ataatgcaa gacaaagccg cgggaggagc agtacaacag cacgtaccgt      900
gtggtcagcg tcctcacctg cctgcaccag gactggctga atggcaagga gtacaagtgc      960
aaggtctcca acaaagccct cggcgcccc atcgagaaaa ccatctcaa agccaaaggg     1020
cagccccgag aaccacaggt gtacaccctg ccccatccc gggatgagct gaccaagaac     1080
caggtcagcc tgacctgcct ggtcaaaggc ttctatccca gcgacatcgc cgtggagtgg     1140
gagagcaatg ggagccgga gaacaactac aagaccacgc ctcccgtgct ggactccgac     1200
ggctccttct tcctctacag caagctcacc gtggacaaga gcaggtggca gcaggggaac     1260
gtcttctcat gctccgtgat gcatgaggct ctgcacaacc actacacgca gaagagcctc     1320
tccctgtctc cgggtggcgg cggaggctcc ggaggcggag gttctggcgg aggtggctcc     1380
gcacctgcct caagttctac aaagaaaaca cagctacaac tggagcattt actgctggat     1440
ttacagatga ttttgaatgg aattaataat tacaagaatc ccaaactcac caggatgctc     1500
acagccaagt ttgccatgcc caagaaggcc acagaactga aacatcttca gtgtctagaa     1560
gaagaactca aacctctgga ggaagtgcta aatggcgctc aaagcaaaaa ctttacttta     1620
agaccaggg acttaatcag caatatcaac gtaatagttc tggaactaaa gggatctgaa     1680
acaacattca tgtgtgaata tgctgatgag acagcaacca ttgtagaatt tctgaacaga     1740
tggattacct ttgcccaaag catcatctca acactgact                               1779

```

<210> 255
 <211> 473
 <212> PRT
 <213> Artificial Sequence

<220>
 <223> Murine IL-2R-beta-Fc(hole) fusion protein

<400> 255

Met Asp Met Arg Val Pro Ala Gln Leu Leu Gly Leu Leu Leu Trp
 1 5 10 15

Phe Pro Leu Leu Leu Trp Phe Pro Gly Ala Arg Cys Ala Val Lys
 20 25 30

Asn Cys Ser His Leu Glu Cys Phe Tyr Asn Ser Arg Ala Asn Val Ser
 35 40 45

Cys Met Trp Ser His Glu Glu Ala Leu Asn Val Thr Thr Cys His Val
 50 55 60

eolf-seql.txt

His Ala Lys Ser Asn Leu Arg His Trp Asn Lys Thr Cys Glu Leu Thr
65 70 75 80

Leu Val Arg Gln Ala Ser Trp Ala Cys Asn Leu Ile Leu Gly Ser Phe
85 90 95

Pro Glu Ser Gln Ser Leu Thr Ser Val Asp Leu Leu Asp Ile Asn Val
100 105 110

Val Cys Trp Glu Glu Lys Gly Trp Arg Arg Val Lys Thr Cys Asp Phe
115 120 125

His Pro Phe Asp Asn Leu Arg Leu Val Ala Pro His Ser Leu Gln Val
130 135 140

Leu His Ile Asp Thr Gln Arg Cys Asn Ile Ser Trp Lys Val Ser Gln
145 150 155 160

Val Ser His Tyr Ile Glu Pro Tyr Leu Glu Phe Glu Ala Arg Arg Arg
165 170 175

Leu Leu Gly His Ser Trp Glu Asp Ala Ser Val Leu Ser Leu Lys Gln
180 185 190

Arg Gln Gln Trp Leu Phe Leu Glu Met Leu Ile Pro Ser Thr Ser Tyr
195 200 205

Glu Val Gln Val Arg Val Lys Ala Gln Arg Asn Asn Thr Gly Thr Trp
210 215 220

Ser Pro Trp Ser Gln Pro Leu Thr Phe Arg Thr Arg Pro Ala Asp Pro
225 230 235 240

Met Lys Glu Gly Ala Gln Asp Lys Thr His Thr Cys Pro Pro Cys Pro
245 250 255

Ala Pro Glu Leu Leu Gly Gly Pro Ser Val Phe Leu Phe Pro Pro Lys
260 265 270

Pro Lys Asp Thr Leu Met Ile Ser Arg Thr Pro Glu Val Thr Cys Val
275 280 285

Val Val Asp Val Ser His Glu Asp Pro Glu Val Lys Phe Asn Trp Tyr
290 295 300

Val Asp Gly Val Glu Val His Asn Ala Lys Thr Lys Pro Arg Glu Glu
305 310 315 320

Gln Tyr Asn Ser Thr Tyr Arg Val Val Ser Val Leu Thr Val Leu His
325 330 335

eolf-seql.txt

Gln Asp Trp Leu Asn Gly Lys Glu Tyr Lys Cys Lys Val Ser Asn Lys
340 345 350

Ala Leu Pro Ala Pro Ile Glu Lys Thr Ile Ser Lys Ala Lys Gly Gln
355 360 365

Pro Arg Glu Pro Gln Val Cys Thr Leu Pro Pro Ser Arg Asp Glu Leu
370 375 380

Thr Lys Asn Gln Val Ser Leu Ser Cys Ala Val Lys Gly Phe Tyr Pro
385 390 395 400

Ser Asp Ile Ala Val Glu Trp Glu Ser Asn Gly Gln Pro Glu Asn Asn
405 410 415

Tyr Lys Thr Thr Pro Pro Val Leu Asp Ser Asp Gly Ser Phe Phe Leu
420 425 430

Val Ser Lys Leu Thr Val Asp Lys Ser Arg Trp Gln Gln Gly Asn Val
435 440 445

Phe Ser Cys Ser Val Met His Glu Ala Leu His Asn His Tyr Thr Gln
450 455 460

Lys Ser Leu Ser Leu Ser Pro Gly Lys
465 470

<210> 256
<211> 1422
<212> DNA
<213> Artificial Sequence

<220>
<223> Murine IL-2R-beta-Fc(hole) fusion protein

<400> 256
atggacatga ggggtccccgc tcagctcctg ggcctcctgc tgctctgggt cccctcctg 60
ctgctctggt tcccagggtgc cagggtgtgca gtgaaaaact gttcccatct tgaatgcttc 120
tacaactcaa gagccaatgt ctcttgcattg tggagccatg aagaggctct gaatgtcaca 180
acctgccacg tccatgccaa gtcgaacctg cgacactgga acaaaacctg tgagctaact 240
cttgtgaggc aggcattcctg ggcctgcaac ctgacccctg ggtcggttccc agagtcccag 300
tcactgacct ccgtggacct ccttgacata aatgtggtgt gctgggaaga gaagggttgg 360
cgtagggtaa agacctgcga cttccatccc tttgacaacc ttcgcctggt ggcccctcat 420
tccctccaag ttctgcacat tgatacccag agatgtaaca taagctggaa ggtctcccag 480
gtctctcact acattgaacc atacttggaa tttgaggccc gtagacgtct tctgggccac 540
agctgggagg atgcatccgt attaagcctc aagcagagac agcagtggct cttcttggag 600
atgctgatcc ctagtacctc atatgaggtc cagggtgagg tcaaagctca acgaaacaat 660
accgggacct ggagtccttg gagccagccc ctgacctttc ggacaaggcc agcagatccc 720

eolf-seql.txt

```

atgaaggagg gagctcagga caaaactcac acatgcccac cgtgcccagc acctgaactc      780
ctgggggggac cgtcagtcctt cctcttcccc ccaaaaccca aggacaccct catgatctcc      840
cggacccctg aggtcacatg cgtgggtggtg gacgtgagcc acgaagaccc tgaggtcaag      900
ttcaactggt acgtggacgg cgtggaggtg cataatgcca agacaaagcc gcgggaggag      960
cagtacaaca gcacgtaccg tgtgggtcagc gtcctcaccg tcctgcacca ggactggctg     1020
aatggcaagg agtacaagtg caaggtctcc aacaaagccc tcccagcccc catcgagaaa     1080
accatctcca aagccaaagg gcagccccga gaaccacagg tgtgcaccct gcccccatcc     1140
cgggatgagc tgaccaagaa ccaggtcagc ctctcgtgcg cagtcaaagg cttctatccc     1200
agcgacatcg ccgtggagtg ggagagcaat gggcagccgg agaacaacta caagaccacg     1260
cctcccgtagc tggactccga cggctccttc ttcctcgtga gcaagctcac cgtggacaag     1320
agcaggtggc agcaggggaa cgtcttctca tgctccgtga tgcattgaggc tctgcacaac     1380
cactacacgc agaagagcct ctccctgtct ccgggtaaat ga                        1422

```

<210> 257
 <211> 500
 <212> PRT
 <213> Artificial Sequence

<220>
 <223> Murine IL-2R-gamma-Fc(knob) fusion protein
 <400> 257

Met Asp Met Arg Val Pro Ala Gln Leu Leu Gly Leu Leu Leu Leu Trp
 1 5 10 15

Phe Pro Leu Leu Leu Trp Phe Pro Gly Ala Arg Cys Trp Ser Ser
 20 25 30

Lys Val Leu Met Ser Ser Ala Asn Glu Asp Ile Lys Ala Asp Leu Ile
 35 40 45

Leu Thr Ser Thr Ala Pro Glu His Leu Ser Ala Pro Thr Leu Pro Leu
 50 55 60

Pro Glu Val Gln Cys Phe Val Phe Asn Ile Glu Tyr Met Asn Cys Thr
 65 70 75 80

Trp Asn Ser Ser Ser Glu Pro Gln Ala Thr Asn Leu Thr Leu His Tyr
 85 90 95

Arg Tyr Lys Val Ser Asp Asn Asn Thr Phe Gln Glu Cys Ser His Tyr
 100 105 110

Leu Phe Ser Lys Glu Ile Thr Ser Gly Cys Gln Ile Gln Lys Glu Asp
 115 120 125

eolf-seql.txt

Ile Gln Leu Tyr Gln Thr Phe Val Val Gln Leu Gln Asp Pro Gln Lys
 130 135 140
 Pro Gln Arg Arg Ala Val Gln Lys Leu Asn Leu Gln Asn Leu Val Ile
 145 150 155 160
 Pro Arg Ala Pro Glu Asn Leu Thr Leu Ser Asn Leu Ser Glu Ser Gln
 165 170 175
 Leu Glu Leu Arg Trp Lys Ser Arg His Ile Lys Glu Arg Cys Leu Gln
 180 185 190
 Tyr Leu Val Gln Tyr Arg Ser Asn Arg Asp Arg Ser Trp Thr Glu Leu
 195 200 205
 Ile Val Asn His Glu Pro Arg Phe Ser Leu Pro Ser Val Asp Glu Leu
 210 215 220
 Lys Arg Tyr Thr Phe Arg Val Arg Ser Arg Tyr Asn Pro Ile Cys Gly
 225 230 235 240
 Ser Ser Gln Gln Trp Ser Lys Trp Ser Gln Pro Val His Trp Gly Ser
 245 250 255
 His Thr Val Glu Glu Asn Pro Ser Leu Phe Ala Leu Glu Ala Gly Ala
 260 265 270
 Gln Asp Lys Thr His Thr Cys Pro Pro Cys Pro Ala Pro Glu Leu Leu
 275 280 285
 Gly Gly Pro Ser Val Phe Leu Phe Pro Pro Lys Pro Lys Asp Thr Leu
 290 295 300
 Met Ile Ser Arg Thr Pro Glu Val Thr Cys Val Val Val Asp Val Ser
 305 310 315 320
 His Glu Asp Pro Glu Val Lys Phe Asn Trp Tyr Val Asp Gly Val Glu
 325 330 335
 Val His Asn Ala Lys Thr Lys Pro Arg Glu Glu Gln Tyr Asn Ser Thr
 340 345 350
 Tyr Arg Val Val Ser Val Leu Thr Val Leu His Gln Asp Trp Leu Asn
 355 360 365
 Gly Lys Glu Tyr Lys Cys Lys Val Ser Asn Lys Ala Leu Pro Ala Pro
 370 375 380
 Ile Glu Lys Thr Ile Ser Lys Ala Lys Gly Gln Pro Arg Glu Pro Gln
 385 390 395 400

eolf-seql.txt

Val Tyr Thr Leu Pro Pro Cys Arg Asp Glu Leu Thr Lys Asn Gln Val
405 410 415

Ser Leu Trp Cys Leu Val Lys Gly Phe Tyr Pro Ser Asp Ile Ala Val
420 425 430

Glu Trp Glu Ser Asn Gly Gln Pro Glu Asn Asn Tyr Lys Thr Thr Pro
435 440 445

Pro Val Leu Asp Ser Asp Gly Ser Phe Phe Leu Tyr Ser Lys Leu Thr
450 455 460

Val Asp Lys Ser Arg Trp Gln Gln Gly Asn Val Phe Ser Cys Ser Val
465 470 475 480

Met His Glu Ala Leu His Asn His Tyr Thr Gln Lys Ser Leu Ser Leu
485 490 495

Ser Pro Gly Lys
500

<210> 258
<211> 1503
<212> DNA
<213> Artificial Sequence

<220>
<223> Murine IL-2R-gamma-Fc(knob) fusion protein

<400> 258
atggacatga ggggtccccgc tcagctcctg ggcctcctgc tgctctgggt cccctcctg 60
ctgctctggt tcccagggtgc cagggtgttg agttccaagg tcctcatgtc cagtgcgaat 120
gaagacatca aagctgattt gatcctgact tctacagccc ctgaacacct cagtgtcctt 180
actctgcccc ttccagaggt tcagtgcttt gtgttcaaca tagagtacat gaattgcact 240
tggaatagca gttctgagcc tcaggcaacc aacctcagc tgcactatag gtacaaggta 300
tctgataata atacattcca ggagtgcagt cactatttgt tctccaaaga gattacttct 360
ggctgtcaga tacaaaaaga agatatccag ctctaccaga catttggtgt ccagctccag 420
gacccccaga aacccagag gcgagctgta cagaagctaa acctacagaa tcttgatgc 480
ccacggggtc cagaaaatct aacctcagc aatctgagtg aatcccagct agagctgaga 540
tggaagagca gacatattaa agaacgctgt ttacaatact tgggtgcagta ccggagcaac 600
agagatcgaa gctggacgga actaatagtg aatcatgaac ctagattctc cctgcctagt 660
gtggatgagc tgaaacggta cacatttcgg gttcggagcc gctataacct aatctgtgga 720
agttctcaac agtggagtaa atggagccag cctgtccact gggggagtc tactgtagag 780
gagaatcctt ccttgtttgc actggaagct ggagctcagg acaaaactca cacatgccca 840
ccgtgccag cacctgaact cctgggggga ccgtcagtct tcctcttccc cccaaaacct 900
aaggacaccc tcatgatctc ccggaccct gaggtcacat gcgtggtggt ggacgtgagc 960

eolf-seql.txt

```

cacgaagacc ctgaggtcaa gttcaactgg tacgtggacg gcgtggaggt gcataatgcc      1020
aagacaaagc cgcgggagga gcagtacaac agcacgtacc gtgtggtcag cgtcctcacc      1080
gtcctgcacc aggactggct gaatggcaag gagtacaagt gcaaggtctc caacaaagcc      1140
ctcccagccc ccatcgagaa aaccatctcc aaagccaaag ggcagccccg agaaccacag      1200
gtgtacaccc tgcccccatt cggggatgag ctgaccaaga accaggtcag cctgtggtgc      1260
ctgggtcaaag gcttctatcc cagcgacatc gccgtggagt gggagagcaa tgggcagccg      1320
gagaacaact acaagaccac gcctcccgtg ctggactccg acggctcctt cttcctctac      1380
agcaagctca ccgtggacaa gagcaggtgg cagcagggga acgtcttctc atgctccgtg      1440
atgcatgagg ctctgcacaa ccactacacg cagaagagcc tctccctgtc tccgggtaaa      1500
tga                                                                    1503

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```

<210> 259
<211> 213
<212> PRT
<213> Artificial Sequence

```

```

<220>
<223> Murine IL-2R alpha subunit + Avi-tag + His-tag
<400> 259

```

```

Met Gly Trp Ser Cys Ile Ile Leu Phe Leu Val Ala Thr Ala Thr Gly
1          5          10          15

Val His Ser Glu Leu Cys Leu Tyr Asp Pro Pro Glu Val Pro Asn Ala
          20          25          30

Thr Phe Lys Ala Leu Ser Tyr Lys Asn Gly Thr Ile Leu Asn Cys Glu
          35          40          45

Cys Lys Arg Gly Phe Arg Arg Leu Lys Glu Leu Val Tyr Met Arg Cys
          50          55          60

Leu Gly Asn Ser Trp Ser Ser Asn Cys Gln Cys Thr Ser Asn Ser His
65          70          75          80

Asp Lys Ser Arg Lys Gln Val Thr Ala Gln Leu Glu His Gln Lys Glu
          85          90          95

Gln Gln Thr Thr Thr Asp Met Gln Lys Pro Thr Gln Ser Met His Gln
          100          105          110

Glu Asn Leu Thr Gly His Cys Arg Glu Pro Pro Pro Trp Lys His Glu
          115          120          125

Asp Ser Lys Arg Ile Tyr His Phe Val Glu Gly Gln Ser Val His Tyr
          130          135          140

```

eolf-seql.txt

Glu Cys Ile Pro Gly Tyr Lys Ala Leu Gln Arg Gly Pro Ala Ile Ser
145 150 155 160

Ile Cys Lys Met Lys Cys Gly Lys Thr Gly Trp Thr Gln Pro Gln Leu
165 170 175

Thr Cys Val Asp Glu Gln Leu Tyr Phe Gln Gly Gly Ser Gly Leu Asn
180 185 190

Asp Ile Phe Glu Ala Gln Lys Ile Glu Trp His Glu Ala Arg Ala His
195 200 205

His His His His His
210

<210> 260
<211> 642
<212> DNA
<213> Artificial Sequence

<220>
<223> Murine IL-2R alpha subunit + Avi-tag + His-tag

<400> 260
atgggatgga gctgtatcat cctcttcttg gtagcaacag ctaccggtgt gcattccgaa 60
ctgtgtctgt atgaccacc cgaggtcccc aatgccacat tcaaagccct ctcctacaag 120
aacggcacca tcctaaactg tgaatgcaag agaggtttcc gaagactaaa ggaattggtc 180
tatatgcgtt gcttaggaaa ctcttgagc agcaactgcc agtgcaccag caactcccat 240
gacaaatcga gaaagcaagt tacagctcaa cttgaacacc agaaagagca acaaaccaca 300
acagacatgc agaagccaac acagtctatg caccaagaga accttacagg tctactgcagg 360
gagccacctc cttggaaaca tgaagattcc aagagaatct atcatttcgt ggaaggacag 420
agtgttcact acgagtgtat tccgggatac aaggctctac agagaggtcc tgctattagc 480
atctgcaaga tgaagtgtgg gaaaacgggg tggactcagc cccagctcac atgtgtcgac 540
gaacagttat attttcaggg cggctcaggc ctgaacgaca tcttcgaggc ccagaagatc 600
gagtggcacg aggctcgagc tcaccacat caccatcact ga 642

<210> 261
<211> 480
<212> PRT
<213> Artificial Sequence

<220>
<223> Cynomolgous IL-2R-beta-Fc(knob) fusion protein + Avi-tag

<400> 261

Met Gly Trp Ser Cys Ile Ile Leu Phe Leu Val Ala Thr Ala Thr Gly
1 5 10 15

Val His Ser Ala Val Asn Gly Thr Ser Arg Phe Thr Cys Phe Tyr Asn
20 25 30

eolf-seql.txt

Ser Arg Ala Asn Ile Ser Cys Val Trp Ser Gln Asp Gly Ala Leu Gln
35 40 45

Asp Thr Ser Cys Gln Val His Ala Trp Pro Asp Arg Arg Arg Trp Asn
50 55 60

Gln Thr Cys Glu Leu Leu Pro Val Ser Gln Ala Ser Trp Ala Cys Asn
65 70 75 80

Leu Ile Leu Gly Thr Pro Asp Ser Gln Lys Leu Thr Ala Val Asp Ile
85 90 95

Val Thr Leu Arg Val Met Cys Arg Glu Gly Val Arg Trp Arg Met Met
100 105 110

Ala Ile Gln Asp Phe Lys Pro Phe Glu Asn Leu Arg Leu Met Ala Pro
115 120 125

Ile Ser Leu Gln Val Val His Val Glu Thr His Arg Cys Asn Ile Ser
130 135 140

Trp Lys Ile Ser Gln Ala Ser His Tyr Phe Glu Arg His Leu Glu Phe
145 150 155 160

Glu Ala Arg Thr Leu Ser Pro Gly His Thr Trp Glu Glu Ala Pro Leu
165 170 175

Met Thr Leu Lys Gln Lys Gln Glu Trp Ile Cys Leu Glu Thr Leu Thr
180 185 190

Pro Asp Thr Gln Tyr Glu Phe Gln Val Arg Val Lys Pro Leu Gln Gly
195 200 205

Glu Phe Thr Thr Trp Ser Pro Trp Ser Gln Pro Leu Ala Phe Arg Thr
210 215 220

Lys Pro Ala Ala Leu Gly Lys Asp Thr Gly Ala Gln Asp Lys Thr His
225 230 235 240

Thr Cys Pro Pro Cys Pro Ala Pro Glu Leu Leu Gly Gly Pro Ser Val
245 250 255

Phe Leu Phe Pro Pro Lys Pro Lys Asp Thr Leu Met Ile Ser Arg Thr
260 265 270

Pro Glu Val Thr Cys Val Val Val Asp Val Ser His Glu Asp Pro Glu
275 280 285

Val Lys Phe Asn Trp Tyr Val Asp Gly Val Glu Val His Asn Ala Lys
290 295 300

eolf-seql.txt

Thr Lys Pro Arg Glu Glu Gln Tyr Asn Ser Thr Tyr Arg Val Val Ser
305 310 315 320

Val Leu Thr Val Leu His Gln Asp Trp Leu Asn Gly Lys Glu Tyr Lys
325 330 335

Cys Lys Val Ser Asn Lys Ala Leu Pro Ala Pro Ile Glu Lys Thr Ile
340 345 350

Ser Lys Ala Lys Gly Gln Pro Arg Glu Pro Gln Val Tyr Thr Leu Pro
355 360 365

Pro Cys Arg Asp Glu Leu Thr Lys Asn Gln Val Ser Leu Trp Cys Leu
370 375 380

Val Lys Gly Phe Tyr Pro Ser Asp Ile Ala Val Glu Trp Glu Ser Asn
385 390 395 400

Gly Gln Pro Glu Asn Asn Tyr Lys Thr Thr Pro Pro Val Leu Asp Ser
405 410 415

Asp Gly Ser Phe Phe Leu Tyr Ser Lys Leu Thr Val Asp Lys Ser Arg
420 425 430

Trp Gln Gln Gly Asn Val Phe Ser Cys Ser Val Met His Glu Ala Leu
435 440 445

His Asn His Tyr Thr Gln Lys Ser Leu Ser Leu Ser Pro Gly Lys Ser
450 455 460

Gly Gly Leu Asn Asp Ile Phe Glu Ala Gln Lys Ile Glu Trp His Glu
465 470 475 480

<210> 262
<211> 1443
<212> DNA
<213> Artificial Sequence

<220>
<223> Cynomolgous IL-2R-beta-Fc(knob) fusion protein + Avi-tag

<400> 262
atgggatgga gctgtatcat cctcttcttg gtagcaacag ctaccggtgt gcattccgcg 60
gtcaacggca cttcccggtt cacatgcttc tacaactcga gagccaacat ctcctgtgtc 120
tgagaccaag atggggctct gcaggacact tcctgccaaag tccacgcctg gccggacaga 180
cggcgggtgga accaaacctg tgagctgctc cctgtgagtc aagcatcctg ggcttgcaac 240
ctgatcctcg gaaccccaga ttctcagaaa ctgaccgcag tggatatcgt caccctgagg 300
gtgatgtgcc gtgaaggggt gcgatggagg atgatggcca tccaggactt caaaccttt 360
gagaaccttc gcctgatggc ccccatctcc ctccaagtcg tccacgtgga gaccacaga 420

eolf-seql.txt

```

tgcaacataa gctggaaaat ctcccaagcc tcccactact ttgaaagaca cctggagttt      480
gaggccccga cgctgtcccc aggccacacc tgggaggagg cccccctgat gaccctcaag      540
cagaagcagg aatggatctg cctggagacg ctcacccag acaccagta tgagtttcag      600
gtgcgggtca agcctctgca aggcgagttc acgacctgga gcccctggag ccagcccctg      660
gccttcagga caaagcctgc agcccttggg aaggacaccg gagctcagga caaaactcac      720
acatgcccac cgtgcccagc acctgaactc ctggggggac cgtcagtctt cctcttcccc      780
ccaaaacca aggacaccct catgatctcc cggaccctg aggtcacatg cgtggtggtg      840
gacgtgagcc acgaagaccc tgaggtcaag ttcaactggt acgtggacgg cgtggaggtg      900
cataatgcca agacaaagcc gcgggaggag cagtacaaca gcacgtaccg tgtggtcagc      960
gtcctcaccg tcctgcacca ggactggctg aatggcaagg agtacaagtg caaggtctcc     1020
aacaagccc tcccagcccc catcgagaaa accatctcca aagccaaagg gcagccccga     1080
gaaccacagg tgtacaccct gcccctatgc cgggatgagc tgaccaagaa ccaggtcagc     1140
ctgtggtgcc tggtaaagg cttctatccc agcgacatcg ccgtggagtg ggagagcaat     1200
gggcagccgg agaacaacta caagaccag cctcccgtgc tggactccga cggctccttc     1260
ttcctctaca gcaagctcac cgtggacaag agcagggtggc agcaggggaa cgtcttctca     1320
tgctccgtga tgcattgagg tctgcacaac cactacacgc agaagagcct ctccctgtct     1380
ccgggtaaata ccggaggcct gaacgacatc ttcgaggccc agaagattga atggcacgag     1440
tga                                                                    1443

```

<210> 263
 <211> 489
 <212> PRT
 <213> Artificial Sequence

<220>
 <223> Cynomolgous IL-2R-gamma-Fc(hole) fusion protein
 <400> 263

Met Gly Trp Ser Cys Ile Ile Leu Phe Leu Val Ala Thr Ala Thr Gly
 1 5 10 15

Val His Ser Leu Asn Thr Thr Ile Leu Thr Pro Asn Gly Asn Glu Asp
 20 25 30

Ala Thr Thr Asp Phe Phe Leu Thr Ser Met Pro Thr Asp Ser Leu Ser
 35 40 45

Val Ser Thr Leu Pro Leu Pro Glu Val Gln Cys Phe Val Phe Asn Val
 50 55 60

Glu Tyr Met Asn Cys Thr Trp Asn Ser Ser Ser Glu Pro Gln Pro Thr
 65 70 75 80

eolf-seql.txt

Asn Leu Thr Leu His Tyr Trp Tyr Lys Asn Ser Asp Asn Asp Lys Val
85 90 95

Gln Lys Cys Ser His Tyr Leu Phe Ser Glu Glu Ile Thr Ser Gly Cys
100 105 110

Gln Leu Gln Lys Lys Glu Ile His Leu Tyr Gln Thr Phe Val Val Gln
115 120 125

Leu Gln Asp Pro Arg Glu Pro Arg Arg Gln Ala Thr Gln Met Leu Lys
130 135 140

Leu Gln Asn Leu Val Ile Pro Trp Ala Pro Glu Asn Leu Thr Leu Arg
145 150 155 160

Lys Leu Ser Glu Ser Gln Leu Glu Leu Asn Trp Asn Asn Arg Phe Leu
165 170 175

Asn His Cys Leu Glu His Leu Val Gln Tyr Arg Thr Asp Trp Asp His
180 185 190

Ser Trp Thr Glu Gln Ser Val Asp Tyr Arg His Lys Phe Ser Leu Pro
195 200 205

Ser Val Asp Gly Gln Lys Arg Tyr Thr Phe Arg Val Arg Ser Arg Phe
210 215 220

Asn Pro Leu Cys Gly Ser Ala Gln His Trp Ser Glu Trp Ser His Pro
225 230 235 240

Ile His Trp Gly Ser Asn Ser Ser Lys Glu Asn Pro Phe Leu Phe Ala
245 250 255

Leu Glu Ala Gly Ala Gln Asp Lys Thr His Thr Cys Pro Pro Cys Pro
260 265 270

Ala Pro Glu Leu Leu Gly Gly Pro Ser Val Phe Leu Phe Pro Pro Lys
275 280 285

Pro Lys Asp Thr Leu Met Ile Ser Arg Thr Pro Glu Val Thr Cys Val
290 295 300

Val Val Asp Val Ser His Glu Asp Pro Glu Val Lys Phe Asn Trp Tyr
305 310 315 320

Val Asp Gly Val Glu Val His Asn Ala Lys Thr Lys Pro Arg Glu Glu
325 330 335

Gln Tyr Asn Ser Thr Tyr Arg Val Val Ser Val Leu Thr Val Leu His
340 345 350

eolf-seql.txt

Gln Asp Trp Leu Asn Gly Lys Glu Tyr Lys Cys Lys Val Ser Asn Lys
355 360 365

Ala Leu Gly Ala Pro Ile Glu Lys Thr Ile Ser Lys Ala Lys Gly Gln
370 375 380

Pro Arg Glu Pro Gln Val Cys Thr Leu Pro Pro Ser Arg Asp Glu Leu
385 390 395 400

Thr Lys Asn Gln Val Ser Leu Ser Cys Ala Val Lys Gly Phe Tyr Pro
405 410 415

Ser Asp Ile Ala Val Glu Trp Glu Ser Asn Gly Gln Pro Glu Asn Asn
420 425 430

Tyr Lys Thr Thr Pro Pro Val Leu Asp Ser Asp Gly Ser Phe Phe Leu
435 440 445

Val Ser Lys Leu Thr Val Asp Lys Ser Arg Trp Gln Gln Gly Asn Val
450 455 460

Phe Ser Cys Ser Val Met His Glu Ala Leu His Asn His Tyr Thr Gln
465 470 475 480

Lys Ser Leu Ser Leu Ser Pro Gly Lys
485

<210> 264
<211> 1470
<212> DNA
<213> Artificial Sequence

<220>
<223> Cynomolgous IL-2R-gamma-Fc(hole) fusion protein

<400> 264
atgggatgga gctgtatcat cctcttcttg gtagcaacag ctaccggtgt gcattccctg 60
aacacgacaa ttctgacgcc caatgggaat gaagacgcca caactgattt cttcctgacc 120
tctatgccca ctgactccct cagtgtttcc actctgcccc tcccagaggt tcagtgtttt 180
gtgttcaatg tcgagtacat gaattgcact tggaacagca gctctgagcc ccagcctacc 240
aacctcactc tgcattattg gtacaagaat tcggataatg ataaagtcca gaagtgcagc 300
cactatctat tctctgaaga aatcacttct ggctgtcagt tgcaaaaaaa ggagatccac 360
ctctacaaa cgtttgttgt tcagctccag gaccacggg aaccaggag acaggccaca 420
cagatgctaa aactgcagaa tctggtgatc ccctgggctc cggagaacct aacacttcgc 480
aaactgagtg aatcccagct agaactgaac tggaacaaca gattcttgaa cactgtttg 540
gagcacttgg tgcagtaccg gactgactgg gaccacagct ggactgaaca atcagtggat 600
tatagacata agttctcctt gcctagtgtg gatgggcaga aacgctacac gtttcgtgtc 660
cggagccgct ttaaccact ctgtggaagt gctcagcatt ggagtgaatg gagccacca 720

eolf-seql.txt

```

atccactggg ggagcaatag ttcaaaagag aatcctttcc tgtttgcatt ggaagccgga      780
gctcaggaca aaactcacac atgccaccg  tgcccagcac ctgaactcct ggggggaccg      840
tcagtcttcc tcttcccccc aaaacccaag gacaccctca tgatctcccg gacccttgag      900
gtcacatgcg tggtggtgga cgtgagccac gaagaccctg aggtcaagtt caactggtac      960
gtggacggcg tggaggtgca taatgccaag acaaagccgc gggaggagca gtacaacagc     1020
acgtaccctg tggtcagcgt cctcaccgtc ctgcaccagg actggctgaa tggcaaggag     1080
tacaagtgca aggtctccaa caaagccctc ggcgccccca tcgagaaaac catctccaaa     1140
gccaaagggc agccccgaga accacaggtg tgcaccctgc ccccatcccg ggatgagctg     1200
accaagaacc aggtcagcct ctcgtgcgca gtcaaaggct tctatcccag cgacatcgcc     1260
gtggagtggg agagcaatgg gcagccggag aacaactaca agaccacgcc tcccgtgctg     1320
gactccgacg gtccttctt cctcgtgagc aagctcaccg tggacaagag caggtggcag     1380
caggggaacg tcttctcatg ctccgtgatg catgaggctc tgcacaacca ctacacgcag     1440
aagagcctct ccctgtctcc gggtaaatga      1470

```

<210> 265
 <211> 217
 <212> PRT
 <213> Artificial Sequence

<220>
 <223> Cynomolgous IL-2R alpha subunit + Avi-tag + His-tag
 <400> 265

Met Gly Trp Ser Cys Ile Ile Leu Phe Leu Val Ala Thr Ala Thr Gly
 1 5 10 15

Glu Leu Cys Asp Asp Asp Pro Pro Lys Ile Thr His Ala Thr Phe Lys
 20 25 30

Ala Met Ala Tyr Lys Glu Gly Thr Met Leu Asn Cys Glu Cys Lys Arg
 35 40 45

Gly Phe Arg Arg Ile Lys Ser Gly Ser Pro Tyr Met Leu Cys Thr Gly
 50 55 60

Asn Ser Ser His Ser Ser Trp Asp Asn Gln Cys Gln Cys Thr Ser Ser
 65 70 75 80

Ala Ala Arg Asn Thr Thr Lys Gln Val Thr Pro Gln Pro Glu Glu Gln
 85 90 95

Lys Glu Arg Lys Thr Thr Glu Met Gln Ser Gln Met Gln Leu Ala Asp
 100 105 110

Gln Val Ser Leu Pro Gly His Cys Arg Glu Pro Pro Pro Trp Glu Asn
 115 120 125

eolf-seql.txt

Glu Ala Thr Glu Arg Ile Tyr His Phe Val Val Gly Gln Thr Val Tyr
130 135 140

Tyr Gln Cys Val Gln Gly Tyr Arg Ala Leu His Arg Gly Pro Ala Glu
145 150 155 160

Ser Val Cys Lys Met Thr His Gly Lys Thr Arg Trp Thr Gln Pro Gln
165 170 175

Leu Ile Cys Thr Gly Glu Val Asp Glu Gln Leu Tyr Phe Gln Gly Gly
180 185 190

Ser Gly Leu Asn Asp Ile Phe Glu Ala Gln Lys Ile Glu Trp His Glu
195 200 205

Ala Arg Ala His His His His His His
210 215

<210> 266
<211> 654
<212> DNA
<213> Artificial Sequence

<220>
<223> Cynomolgous IL-2R alpha subunit + Avi-tag + His-tag

<400> 266
atgggatgga gctgtatcat cctcttcttg gtagcaacag ctaccggtga gctctgtgac 60
gatgacccgc caaaaatcac acatgccaca ttcaaagcca tggcctacaa ggaaggaacc 120
atgttgaact gtgaatgcaa gagaggtttc cgcagaataa aaagcgggtc accctatatg 180
ctctgtacag gaaactctag ccactcgtcc tgggacaacc aatgtcaatg cacaagctct 240
gctgctcgga acacaacaaa acaagtgaca cctcaacctg aagaacagaa agaaagaaaa 300
accacagaaa tgcaaagtca aatgcagctg gcggaccaag tgagccttcc aggtcactgc 360
agggaacctc caccgtggga aaatgaagcc acagaaagaa tttatcattt cgtggtgggg 420
cagacggttt actaccagtg cgtccaggga tacagggtc tacacagagg tcctgctgag 480
agcgtctgca aaatgaccca cggaagaca agatggacct agccccagct catatgcaca 540
ggtgaagtcg acgaacagtt atattttcag ggcggctcag gcctgaacga catcttcgag 600
gccagaaga tcgagtggca cgaggctcga gctcaccacc atcaccatca ctga 654

<210> 267
<211> 474
<212> PRT
<213> Artificial Sequence

<220>
<223> Human IL-10R1-Fc fusion + Avi-tag

<400> 267

eo1f-seq1.txt

His Gly Thr Glu Leu Pro Ser Pro Pro Ser Val Trp Phe Glu Ala Glu
1 5 10 15

Phe Phe His His Ile Leu His Trp Thr Pro Ile Pro Asn Gln Ser Glu
20 25 30

Ser Thr Cys Tyr Glu Val Ala Leu Leu Arg Tyr Gly Ile Glu Ser Trp
35 40 45

Asn Ser Ile Ser Asn Cys Ser Gln Thr Leu Ser Tyr Asp Leu Thr Ala
50 55 60

Val Thr Leu Asp Leu Tyr His Ser Asn Gly Tyr Arg Ala Arg Val Arg
65 70 75 80

Ala Val Asp Gly Ser Arg His Ser Asn Trp Thr Val Thr Asn Thr Arg
85 90 95

Phe Ser Val Asp Glu Val Thr Leu Thr Val Gly Ser Val Asn Leu Glu
100 105 110

Ile His Asn Gly Phe Ile Leu Gly Lys Ile Gln Leu Pro Arg Pro Lys
115 120 125

Met Ala Pro Ala Asn Asp Thr Tyr Glu Ser Ile Phe Ser His Phe Arg
130 135 140

Glu Tyr Glu Ile Ala Ile Arg Lys Val Pro Gly Asn Phe Thr Phe Thr
145 150 155 160

His Lys Lys Val Lys His Glu Asn Phe Ser Leu Leu Thr Ser Gly Glu
165 170 175

Val Gly Glu Phe Cys Val Gln Val Lys Pro Ser Val Ala Ser Arg Ser
180 185 190

Asn Lys Gly Met Trp Ser Lys Glu Glu Cys Ile Ser Leu Thr Arg Gln
195 200 205

Tyr Phe Thr Val Thr Asn Val Asp Glu Gln Leu Tyr Phe Gln Gly Gly
210 215 220

Ser Pro Lys Ser Ala Asp Lys Thr His Thr Cys Pro Pro Cys Pro Ala
225 230 235 240

Pro Glu Leu Leu Gly Gly Pro Ser Val Phe Leu Phe Pro Pro Lys Pro
245 250 255

Lys Asp Thr Leu Met Ile Ser Arg Thr Pro Glu Val Thr Cys Val Val
260 265 270

eolf-seql.txt

Val Asp Val Ser His Glu Asp Pro Glu Val Lys Phe Asn Trp Tyr Val
275 280 285

Asp Gly Val Glu Val His Asn Ala Lys Thr Lys Pro Arg Glu Glu Gln
290 295 300

Tyr Asn Ser Thr Tyr Arg Val Val Ser Val Leu Thr Val Leu His Gln
305 310 315 320

Asp Trp Leu Asn Gly Lys Glu Tyr Lys Cys Lys Val Ser Asn Lys Ala
325 330 335

Leu Pro Ala Pro Ile Glu Lys Thr Ile Ser Lys Ala Lys Gly Gln Pro
340 345 350

Arg Glu Pro Gln Val Tyr Thr Leu Pro Pro Ser Arg Asp Glu Leu Thr
355 360 365

Lys Asn Gln Val Ser Leu Thr Cys Leu Val Lys Gly Phe Tyr Pro Ser
370 375 380

Asp Ile Ala Val Glu Trp Glu Ser Asn Gly Gln Pro Glu Asn Asn Tyr
385 390 395 400

Lys Thr Thr Pro Pro Val Leu Asp Ser Asp Gly Ser Phe Phe Leu Tyr
405 410 415

Ser Lys Leu Thr Val Asp Lys Ser Arg Trp Gln Gln Gly Asn Val Phe
420 425 430

Ser Cys Ser Val Met His Glu Ala Leu His Asn His Tyr Thr Gln Lys
435 440 445

Ser Leu Ser Leu Ser Pro Gly Gly Gly Ser Gly Gly Leu Asn Asp Ile
450 455 460

Phe Glu Ala Gln Lys Ile Glu Trp His Glu
465 470

<210> 268
<211> 1422
<212> DNA
<213> Artificial Sequence

<220>
<223> Human IL-10R1-Fc fusion + Avi-tag

<400> 268
catgggacag agctgcccag ccctccgtct gtgtggtttg aagcagaatt tttccaccac 60
atcctccact ggacacccat cccaaatcag tctgaaagta cctgctatga agtggcactc 120
ctgaggtatg gaatagagtc ctggaactcc atctccaact gtagccagac cctgtcctat 180
gaccttaccg cagtgcctt ggacctgtac cacagcaatg gctaccgggc cagagtgcgg 240

eolf-seql.txt

```

gctgtggacg gcagccggca ctccaactgg accgtcacca acacccgctt ctctgtggat      300
gaagtgactc tgacagttgg cagtgtgaac ctagagatcc acaatggctt catcctcggg      360
aagattcagc taccagggcc caagatggcc cccgcaaatg acacatatga aagcatcttc      420
agtcacttcc gagagtatga gattgccatt cgcaagggtgc cgggaaactt cacgttcaca      480
cacaagaaag taaaacatga aaacttcagc ctctaacct ctggagaagt gggagagttc      540
tgtgtccagg tgaaaccatc tgtcgcttcc cgaagtaaca aggggatgtg gtctaaagag      600
gagtgcattc ccctcaccag gcagtatttc accgtgacca acgtcgacga acagttatat      660
tttcagggcg gtcacccaa atctgcagac aaaactcaca catgcccacc gtgcccagca      720
cctgaactcc tgggggggacc gtcagtcttc ctcttcccc caaaacccaa ggacaccctc      780
atgatctccc ggaccctga ggtcacatgc gtggtggtgg acgtgagcca cgaagaccct      840
gaggtcaagt tcaactggta cgtggacggc gtggaggtgc ataatgccaa gacaaagccg      900
cgggaggagc agtacaacag cacgtaccgt gtggtcagcg tcctcaccgt cctgcaccag      960
gactggctga atggcaagga gtacaagtgc aaggtctcca acaaagccct cccagccccc     1020
atcgagaaaa ccatctccaa agccaaaggg cagccccgag aaccacaggt gtacaccctg     1080
cccccatccc gggatgagct gaccaagaac caggtcagcc tgacctgcct ggtcaaaggc     1140
ttctatccca gcgacatcgc cgtggagtgg gagagcaatg ggcagccgga gaacaactac     1200
aagaccacgc ctcccgctgt ggactccgac ggctccttct tcctctacag caagctcacc     1260
gtggacaaga gcaggtggca gcaggggaac gtcttctcat gtcctgtgat gcatgaggct     1320
ctgcacaacc actacacgca gaagagcctc tccctgtctc cgggtggcgg gtccggaggc     1380
ctgaacgaca tcttcgaggc ccagaagatt gaatggcacg ag                          1422

```

<210> 269
 <211> 593
 <212> PRT
 <213> Artificial Sequence

<220>
 <223> 28H1 Fab HC-Fc knob (LALA P329G)-IL-2 qm (2)
 <400> 269

```

Glu Val Gln Leu Leu Glu Ser Gly Gly Gly Leu Val Gln Pro Gly Gly
1           5           10          15

Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Phe Thr Phe Ser Ser His
          20          25          30

Ala Met Ser Trp Val Arg Gln Ala Pro Gly Lys Gly Leu Glu Trp Val
          35          40          45

Ser Ala Ile Trp Ala Ser Gly Glu Gln Tyr Tyr Ala Asp Ser Val Lys
          50          55          60

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eo1f-seq1.txt

Gly Arg Phe Thr Ile Ser Arg Asp Asn Ser Lys Asn Thr Leu Tyr Leu
 65 70 75 80
 Gln Met Asn Ser Leu Arg Ala Glu Asp Thr Ala Val Tyr Tyr Cys Ala
 85 90 95
 Lys Gly Trp Leu Gly Asn Phe Asp Tyr Trp Gly Gln Gly Thr Leu Val
 100 105 110
 Thr Val Ser Ser Ala Ser Thr Lys Gly Pro Ser Val Phe Pro Leu Ala
 115 120 125
 Pro Ser Ser Lys Ser Thr Ser Gly Gly Thr Ala Ala Leu Gly Cys Leu
 130 135 140
 Val Lys Asp Tyr Phe Pro Glu Pro Val Thr Val Ser Trp Asn Ser Gly
 145 150 155 160
 Ala Leu Thr Ser Gly Val His Thr Phe Pro Ala Val Leu Gln Ser Ser
 165 170 175
 Gly Leu Tyr Ser Leu Ser Ser Val Val Thr Val Pro Ser Ser Ser Leu
 180 185 190
 Gly Thr Gln Thr Tyr Ile Cys Asn Val Asn His Lys Pro Ser Asn Thr
 195 200 205
 Lys Val Asp Lys Lys Val Glu Pro Lys Ser Cys Asp Lys Thr His Thr
 210 215 220
 Cys Pro Pro Cys Pro Ala Pro Glu Ala Ala 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 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
 305 310 315 320
 Lys Val Ser Asn Lys Ala Leu Gly Ala Pro Ile Glu Lys Thr Ile Ser
 325 330 335

eolf-seql.txt

Lys Ala Lys Gly Gln Pro Arg Glu Pro Gln Val Tyr Thr Leu Pro Pro
340 345 350

Cys Arg Asp Glu Leu Thr Lys Asn Gln Val Ser Leu Trp 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 Gly Gly Gly
435 440 445

Gly Ser Gly Gly Gly Gly Ser Gly Gly Gly Gly Ser Ala Pro Ala Ser
450 455 460

Ser Ser Thr Lys Lys Thr Gln Leu Gln Leu Glu His Leu Leu Leu Asp
465 470 475 480

Leu Gln Met Ile Leu Asn Gly Ile Asn Asn Tyr Lys Asn Pro Lys Leu
485 490 495

Thr Arg Met Leu Thr Ala Lys Phe Ala Met Pro Lys Lys Ala Thr Glu
500 505 510

Leu Lys His Leu Gln Cys Leu Glu Glu Glu Leu Lys Pro Leu Glu Glu
515 520 525

Val Leu Asn Gly Ala Gln Ser Lys Asn Phe His Leu Arg Pro Arg Asp
530 535 540

Leu Ile Ser Asn Ile Asn Val Ile Val Leu Glu Leu Lys Gly Ser Glu
545 550 555 560

Thr Thr Phe Met Cys Glu Tyr Ala Asp Glu Thr Ala Thr Ile Val Glu
565 570 575

Phe Leu Asn Arg Trp Ile Thr Phe Ala Gln Ser Ile Ile Ser Thr Leu
580 585 590

Thr

eolf-seql.txt

<210> 270
 <211> 1779
 <212> DNA
 <213> Artificial Sequence

<220>
 <223> 28H1 Fab HC-Fc knob (LALA P329G)-IL-2 qm (2)

```

<400> 270
gaagtgcagc tgctggaatc cggcggaggc ctggtgcagc ctggcggatc tctgagactg      60
tcctgcgccg cctccggctt caccttctcc tcccacgcc a tgcctgggt cgcacaggct      120
cctggcaaag gcctggaatg ggtgtccgcc atctgggcct cggcgagca gtactacgcc      180
gactctgtga agggccggtt caccatctcc cgggacaact ccaagaacac cctgtacctg      240
cagatgaact ccctgcgggc cgaggacacc gccgtgtact actgtgcca gggctggctg      300
ggcaacttcg actactgggg acagggcacc ctggtcaccg tgtccagcgc tagcaccaag      360
ggcccatcgg tcttccccct ggcaccctcc tccaagagca cctctggggg cacagcggcc      420
ctgggctgcc tgggtcaagga ctacttcccc gaaccggtga cgggtgctgt gaactcaggc      480
gccctgacca gcggcgtgca caccttcccg gctgtcctac agtcctcagg actctactcc      540
ctcagcagcg tggtgaccgt gccctccagc agcttgggca ccagaccta catctgcaac      600
gtgaatcaca agcccagcaa caccaagggt gacaagaaag ttgagcccaa atcttgtgac      660
aaaactcaca catgccacc gtgccagca cctgaagctg cagggggacc gtcagtcttc      720
ctcttcccc caaaaccaa ggacaccctc atgatctccc ggaccctga ggtcacatgc      780
gtggtggtgg acgtgagcca cgaagaccct gaggtcaagt tcaactggta cgtggacggc      840
gtggagggtg ataatgcaa gacaaagccg cgggaggagc agtacaacag cacgtaccgt      900
gtggtcagcg tcctcaccgt cctgcaccag gactggctga atggcaagga gtacaagtgc      960
aaggtctcca acaaagccct cggcgcccc atcgagaaaa ccatctcaa agccaaaggg     1020
cagccccgag aaccacaggt gtacaccctg ccccatgcc gggatgagct gaccaagaac     1080
caggtcagcc tgtggtgcct ggtcaaaggc ttctatccca gcgacatcgc cgtggagtgg     1140
gagagcaatg ggcagccgga gaacaactac aagaccacgc ctcccgctgt ggactccgac     1200
ggctccttct tcctctacag caagctcacc gtggacaaga gcagggtggca gcaggggaac     1260
gtcttctcat gtcctgtgat gcatgaggct ctgcacaacc actacacgca gaagagcctc     1320
tccctgtctc cgggtggcgg cggaggctcc ggaggcggag gttctggcgg aggtggctcc     1380
gcacctgcct caagttctac aaagaaaaca cagctacaac tggagcattt actgctggat     1440
ttacagatga ttttgaatgg aattaataat tacaagaatc ccaaactcac caggatgctc     1500
acagccaagt ttgccatgcc caagaaggcc acagaactga aacatcttca gtgtctagaa     1560
gaagaactca aacctctgga ggaagtgtca aatggcgctc aaagcaaaaa ctttacttta     1620
agaccagggt acttaatcag caatatcaac gtaatagttc tggaactaaa gggatctgaa     1680
acaacattca tgtgtgaata tgctgatgag acagcaacca ttgtagaatt tctgaacaga     1740
tggtattacct ttgccc aaag catcatctca acactgact                                1779
    
```

eolf-seql.txt

<210> 271
 <211> 594
 <212> PRT
 <213> Artificial Sequence

<220>
 <223> 4B9 Fab HC-Fc knob (LALA P329G)-IL-2 qm (2)
 <400> 271

Glu Val Gln Leu Leu Glu Ser Gly Gly Gly Leu Val Gln Pro Gly Gly
 1 5 10 15

Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Phe Thr Phe Ser Ser Tyr
 20 25 30

Ala Met Ser Trp Val Arg Gln Ala Pro Gly Lys Gly Leu Glu Trp Val
 35 40 45

Ser Ala Ile Ile Gly Ser Gly Ala Ser Thr Tyr Tyr Ala Asp Ser Val
 50 55 60

Lys Gly Arg Phe Thr Ile Ser Arg Asp Asn Ser Lys Asn Thr Leu Tyr
 65 70 75 80

Leu Gln Met Asn Ser Leu Arg Ala Glu Asp Thr Ala Val Tyr Tyr Cys
 85 90 95

Ala Lys Gly Trp Phe Gly Gly Phe Asn Tyr Trp Gly Gln Gly Thr Leu
 100 105 110

Val Thr Val Ser Ser Ala Ser Thr Lys Gly Pro Ser Val Phe Pro Leu
 115 120 125

Ala Pro Ser Ser Lys Ser Thr Ser Gly Gly Thr Ala Ala Leu Gly Cys
 130 135 140

Leu Val Lys Asp Tyr Phe Pro Glu Pro Val Thr Val Ser Trp Asn Ser
 145 150 155 160

Gly Ala Leu Thr Ser Gly Val His Thr Phe Pro Ala Val Leu Gln Ser
 165 170 175

Ser Gly Leu Tyr Ser Leu Ser Ser Val Val Thr Val Pro Ser Ser Ser
 180 185 190

Leu Gly Thr Gln Thr Tyr Ile Cys Asn Val Asn His Lys Pro Ser Asn
 195 200 205

Thr Lys Val Asp Lys Lys Val Glu Pro Lys Ser Cys Asp Lys Thr His
 210 215 220

eofl-seql.txt

Thr Cys Pro Pro Cys Pro Ala Pro Glu Ala Ala Gly Gly Pro Ser Val
225 230 235 240

Phe Leu Phe Pro Pro Lys Pro Lys Asp Thr Leu Met Ile Ser Arg Thr
245 250 255

Pro Glu Val Thr Cys Val Val Val Asp Val Ser His Glu Asp Pro Glu
260 265 270

Val Lys Phe Asn Trp Tyr Val Asp Gly Val Glu Val His Asn Ala Lys
275 280 285

Thr Lys Pro Arg Glu Glu Gln Tyr Asn Ser Thr Tyr Arg Val Val Ser
290 295 300

Val Leu Thr Val Leu His Gln Asp Trp Leu Asn Gly Lys Glu Tyr Lys
305 310 315 320

Cys Lys Val Ser Asn Lys Ala Leu Gly Ala Pro Ile Glu Lys Thr Ile
325 330 335

Ser Lys Ala Lys Gly Gln Pro Arg Glu Pro Gln Val Tyr Thr Leu Pro
340 345 350

Pro Cys Arg Asp Glu Leu Thr Lys Asn Gln Val Ser Leu Trp Cys Leu
355 360 365

Val Lys Gly Phe Tyr Pro Ser Asp Ile Ala Val Glu Trp Glu Ser Asn
370 375 380

Gly Gln Pro Glu Asn Asn Tyr Lys Thr Thr Pro Pro Val Leu Asp Ser
385 390 395 400

Asp Gly Ser Phe Phe Leu Tyr Ser Lys Leu Thr Val Asp Lys Ser Arg
405 410 415

Trp Gln Gln Gly Asn Val Phe Ser Cys Ser Val Met His Glu Ala Leu
420 425 430

His Asn His Tyr Thr Gln Lys Ser Leu Ser Leu Ser Pro Gly Gly Gly
435 440 445

Gly Gly Ser Gly Gly Gly Gly Ser Gly Gly Gly Gly Ser Ala Pro Ala
450 455 460

Ser Ser Ser Thr Lys Lys Thr Gln Leu Gln Leu Glu His Leu Leu Leu
465 470 475 480

Asp Leu Gln Met Ile Leu Asn Gly Ile Asn Asn Tyr Lys Asn Pro Lys
485 490 495

eolf-seql.txt

Leu Thr Arg Met Leu Thr Ala Lys Phe Ala Met Pro Lys Lys Ala Thr
500 505 510

Glu Leu Lys His Leu Gln Cys Leu Glu Glu Glu Leu Lys Pro Leu Glu
515 520 525

Glu Val Leu Asn Gly Ala Gln Ser Lys Asn Phe His Leu Arg Pro Arg
530 535 540

Asp Leu Ile Ser Asn Ile Asn Val Ile Val Leu Glu Leu Lys Gly Ser
545 550 555 560

Glu Thr Thr Phe Met Cys Glu Tyr Ala Asp Glu Thr Ala Thr Ile Val
565 570 575

Glu Phe Leu Asn Arg Trp Ile Thr Phe Ala Gln Ser Ile Ile Ser Thr
580 585 590

Leu Thr

<210> 272
<211> 1782
<212> DNA
<213> Artificial Sequence

<220>
<223> 4B9 Fab HC-Fc knob (LALA P329G)-IL-2 qm (2)

<400> 272
gaggtgcagc tgctcgaaag cggcggagga ctggtgcagc ctggcggcag cctgagactg 60
tcttgccg ccagcggctt caccttcagc agctacgcca tgagctgggt ccgccaggcc 120
cctggcaagg gactggaatg ggtgtccgcc atcatcggct ctggcgccag cacctactac 180
gccgacagcg tgaagggccg gttcaccatc agccgggaca acagcaagaa caccctgtac 240
ctgcagatga acagcctgcg ggccgaggac accgccgtgt actactgcgc caagggatgg 300
ttcggcggct tcaactactg gggacagggc accctggtca cagtgtccag cgctagcacc 360
aagggcccat cgggtcttccc cctggcaccc tcctccaaga gcacctctgg gggcacagcg 420
gccctgggct gcctgggtcaa ggactacttc cccgaaccgg tgacggtgtc gtggaactca 480
ggcgccctga ccagcggcgt gcacaccttc ccggctgtcc tacagtcctc aggactctac 540
tccctcagca gcgtggtgac cgtgccctcc agcagcttgg gcaccagac ctacatctgc 600
aacgtgaatc acaagcccag caacaccaag gtggacaaga aagttgagcc caaatcttgt 660
gacaaaactc acacatgccc accgtgcccga gcacctgaag ctgcaggggg accgtcagtc 720
ttcctcttcc ccccaaaacc caaggacacc ctcatgatct cccggacccc tgaggtcaca 780
tgcgtggtgg tggacgtgag ccacgaagac cctgaggtca agttcaactg gtacgtggac 840
ggcgtggagg tgcataatgc caagacaaag ccgcgggagg agcagtacaa cagcacgtac 900
cgtgtggtca gcgtcctcac cgtcctgcac caggactggc tgaatggcaa ggagtacaag 960

eolf-seql.txt

```

tgcaaggtct ccaacaaagc cctcggcgcc cccatcgaga aaaccatctc caaagccaaa 1020
gggcagcccc gagaaccaca ggtgtacacc ctgcccccat gccgggatga gctgaccaag 1080
aaccaggtca gcctgtggtg cctgggtcaaa ggcttctatc ccagcgacat cgccgtggag 1140
tgggagagca atgggcagcc ggagaacaac tacaagacca cgcctcccgt gctggactcc 1200
gacggctcct tcttctctta cagcaagctc accgtggaca agagcaggtg gcagcagggg 1260
aacgtcttct catgctccgt gatgcatgag gctctgcaca accactacac gcagaagagc 1320
ctctccctgt ctccgggtgg cggcggaggc tccggaggcg gaggttcttg cggaggtggc 1380
tccgcacctg cctcaagttc tacaagaaa acacagctac aactggagca tttactgctg 1440
gatttacaga tgattttgaa tggaattaat aattacaaga atcccaaact caccaggatg 1500
ctcacagcca agtttgccat gcccaagaag gccacagaac tgaaacatct tcagtgtcta 1560
gaagaagaac tcaaacctct ggaggaagtg ctaaattggcg ctcaaagcaa aaactttcac 1620
ttaagacca gggacttaat cagcaatatc aacgtaatag ttctggaact aaagggatct 1680
gaaacaacat tcatgtgtga atatgctgat gagacagcaa ccattgtaga atttctgaac 1740
agatggatta cctttgccca aagcatcatc tcaacactga ct 1782

```

<210> 273
 <211> 594
 <212> PRT
 <213> Artificial Sequence

<220>
 <223> 4B9 Fab HC-Fc knob (LALA P329G)-IL-2 wt
 <400> 273

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Glu Val Gln Leu 5 Leu Glu Ser Gly Gly 10 Gly Leu Val Gln Pro 15 Gly Gly
1
Ser Leu Arg 20 Leu Ser Cys Ala Ala 25 Ser Gly Phe Thr Phe 30 Ser Ser Tyr
Ala Met 35 Ser Trp Val Arg Gln Ala Pro Gly Lys Gly 45 Leu Glu Trp Val
Ser Ala 50 Ile Ile Gly Ser 55 Gly Ala Ser Thr Tyr 60 Tyr Ala Asp Ser Val
Lys Gly 65 Arg Phe Thr 70 Ile Ser Arg Asp Asn 75 Ser Lys Asn Thr Leu Tyr 80
Leu Gln Met 85 Asn Ser Leu Arg Ala Glu 90 Asp Thr Ala Val Tyr 95 Tyr Cys
Ala Lys Gly 100 Trp Phe Gly Gly Phe 105 Asn Tyr Trp Gly Gln 110 Gly Thr Leu

```

co1f-seql.txt

Val Thr Val Ser Ser Ala Ser Thr Lys Gly Pro Ser Val Phe Pro Leu
115 120 125

Ala Pro Ser Ser Lys Ser Thr Ser Gly Gly Thr Ala Ala Leu Gly Cys
130 135 140

Leu Val Lys Asp Tyr Phe Pro Glu Pro Val Thr Val Ser Trp Asn Ser
145 150 155 160

Gly Ala Leu Thr Ser Gly Val His Thr Phe Pro Ala Val Leu Gln Ser
165 170 175

Ser Gly Leu Tyr Ser Leu Ser Ser Val Val Thr Val Pro Ser Ser Ser
180 185 190

Leu Gly Thr Gln Thr Tyr Ile Cys Asn Val Asn His Lys Pro Ser Asn
195 200 205

Thr Lys Val Asp Lys Lys Val Glu Pro Lys Ser Cys Asp Lys Thr His
210 215 220

Thr Cys Pro Pro Cys Pro Ala Pro Glu Ala Ala Gly Gly Pro Ser Val
225 230 235 240

Phe Leu Phe Pro Pro Lys Pro Lys Asp Thr Leu Met Ile Ser Arg Thr
245 250 255

Pro Glu Val Thr Cys Val Val Val Asp Val Ser His Glu Asp Pro Glu
260 265 270

Val Lys Phe Asn Trp Tyr Val Asp Gly Val Glu Val His Asn Ala Lys
275 280 285

Thr Lys Pro Arg Glu Glu Gln Tyr Asn Ser Thr Tyr Arg Val Val Ser
290 295 300

Val Leu Thr Val Leu His Gln Asp Trp Leu Asn Gly Lys Glu Tyr Lys
305 310 315 320

Cys Lys Val Ser Asn Lys Ala Leu Gly Ala Pro Ile Glu Lys Thr Ile
325 330 335

Ser Lys Ala Lys Gly Gln Pro Arg Glu Pro Gln Val Tyr Thr Leu Pro
340 345 350

Pro Cys Arg Asp Glu Leu Thr Lys Asn Gln Val Ser Leu Trp Cys Leu
355 360 365

Val Lys Gly Phe Tyr Pro Ser Asp Ile Ala Val Glu Trp Glu Ser Asn
370 375 380

eofl-seql.txt

Gly Gln Pro Glu Asn Asn Tyr Lys Thr Thr Pro Pro Val Leu Asp Ser
385 390 395 400

Asp Gly Ser Phe Phe Leu Tyr Ser Lys Leu Thr Val Asp Lys Ser Arg
405 410 415

Trp Gln Gln Gly Asn Val Phe Ser Cys Ser Val Met His Glu Ala Leu
420 425 430

His Asn His Tyr Thr Gln Lys Ser Leu Ser Leu Ser Pro Gly Gly Gly
435 440 445

Gly Gly Ser Gly Gly Gly Gly Ser Gly Gly Gly Gly Ser Ala Pro Thr
450 455 460

Ser Ser Ser Thr Lys Lys Thr Gln Leu Gln Leu Glu His Leu Leu Leu
465 470 475 480

Asp Leu Gln Met Ile Leu Asn Gly Ile Asn Asn Tyr Lys Asn Pro Lys
485 490 495

Leu Thr Arg Met Leu Thr Phe Lys Phe Tyr Met Pro Lys Lys Ala Thr
500 505 510

Glu Leu Lys His Leu Gln Cys Leu Glu Glu Glu Leu Lys Pro Leu Glu
515 520 525

Glu Val Leu Asn Leu Ala Gln Ser Lys Asn Phe His Leu Arg Pro Arg
530 535 540

Asp Leu Ile Ser Asn Ile Asn Val Ile Val Leu Glu Leu Lys Gly Ser
545 550 555 560

Glu Thr Thr Phe Met Cys Glu Tyr Ala Asp Glu Thr Ala Thr Ile Val
565 570 575

Glu Phe Leu Asn Arg Trp Ile Thr Phe Ala Gln Ser Ile Ile Ser Thr
580 585 590

Leu Thr

<210> 274
<211> 1782
<212> DNA
<213> Artificial Sequence

<220>
<223> 4B9 Fab HC-Fc knob (LALA P329G)-IL-2 wt

<400> 274
gaggtgcagc tgctcgaaag cggcggagga ctggtgcagc ctggcggcag cctgagactg 60

tcttgcgccg ccagcggcctt caccttcagc agctacgcca tgagctgggt ccgccaggcc 120
Page 207

eolf-seql.txt

```

cctggcaagg gactggaatg ggtgtccgcc atcatcggt ctggcgccag cacctactac      180
gccgacagcg tgaagggccg gttcaccatc agccgggaca acagcaagaa caccctgtac      240
ctgcagatga acagcctgcg ggccgaggac accgccgtgt actactgcg caagggatgg      300
ttcggcggct tcaactactg gggacagggc accctggtca cagtgtccag cgctagcacc      360
aagggcccat cggctcttccc cctggcacc cctccaaga gcacctctgg gggcacagcg      420
gccctgggct gcctgggtcaa ggactacttc cccgaaccgg tgacgggtgtc gtggaactca      480
ggcgccctga ccagcggcgt gcacaccttc ccggctgtcc tacagtcttc aggactctac      540
tccctcagca gcgtggtgac cgtgccctcc agcagcttgg gcacccagac ctacatctgc      600
aacgtgaatc acaagcccag caacaccaag gtggacaaga aagttgagcc caaatcttgt      660
gacaaaactc acacatgccc accgtgccc gcacctgaag ctgcaggggg accgtcagtc      720
ttcctcttcc ccccaaaacc caaggacacc ctcatgatct cccggacccc tgaggtcaca      780
tgcgtggtgg tggacgtgag ccacgaagac cctgaggtca agttcaactg gtacgtggac      840
ggcgtggagg tgcataatgc caagacaaag ccgcgggagg agcagtacaa cagcacgtac      900
cgtgtggtca gcgtcctcac cgtcctgcac caggactggc tgaatggcaa ggagtacaag      960
tgcaaggtct ccaacaaagc cctcggcgcc cccatcgaga aaaccatctc caaagccaaa     1020
gggcagcccc gagaaccaca ggtgtacacc ctgcccccat gccgggatga gctgaccaag     1080
aaccaggtca gcctgtggtg cctgggtcaaa ggcttctatc ccagcgacat cgccgtggag     1140
tgggagagca atgggcagcc ggagaacaac tacaagacca cgctcccgt gctggactcc     1200
gacggctcct tcttctcta cagcaagctc accgtggaca agagcaggtg gcagcagggg     1260
aacgtcttct catgctccgt gatgcatgag gctctgcaca accactacac gcagaagagc     1320
ctctccctgt ctccgggtgg cggcggaggc tccggaggcg gaggttcttg aggcggaggc     1380
tccgcaccta cttcaagttc taaaagaaa acacagctac aactggagca tttactgctg     1440
gatttacaga tgattttgaa tggaattaat aattacaaga atcccaaact caccaggatg     1500
ctcacattta agttttacat gcccaagaag gccacagaac tgaaacatct tcagtgtcta     1560
gaagaagaac tcaaacctct ggaggaagtg ctaaatttag ctcaaagcaa aaactttcac     1620
ttaagacca gggacttaat cagcaatatc aacgtaatag ttctggaact aaagggatct     1680
gaaacaacat tcatgtgtga atatgctgat gagacagcaa ccattgtaga atttctgaac     1740
agatggatta ctttgccca aagcatcatc tcaacactga ct                          1782

```

<210> 275
 <211> 599
 <212> PRT
 <213> Artificial Sequence

<220>
 <223> CH1A1A 98/99 2F1 Fab HC-Fc knob (LALA P329G)-IL-2 qm
 <400> 275

eof-seql.txt

Gln Val Gln Leu Val Gln Ser Gly Ala Glu Val Lys Lys Pro Gly Ala
1 5 10 15

Ser Val Lys Val Ser Cys Lys Ala Ser Gly Tyr Thr Phe Thr Glu Phe
20 25 30

Gly Met Asn Trp Val Arg Gln Ala Pro Gly Gln Gly Leu Glu Trp Met
35 40 45

Gly Trp Ile Asn Thr Lys Thr Gly Glu Ala Thr Tyr Val Glu Glu Phe
50 55 60

Lys Gly Arg Val Thr Phe Thr Thr Asp Thr Ser Thr Ser Thr Ala Tyr
65 70 75 80

Met Glu Leu Arg Ser Leu Arg Ser Asp Asp Thr Ala Val Tyr Tyr Cys
85 90 95

Ala Arg Trp Asp Phe Ala Tyr Tyr Val Glu Ala Met Asp Tyr Trp Gly
100 105 110

Gln Gly Thr Thr Val Thr Val Ser Ser Ala Ser Thr Lys Gly Pro Ser
115 120 125

Val Phe Pro Leu Ala Pro Ser Ser Lys Ser Thr Ser Gly Gly Thr Ala
130 135 140

Ala Leu Gly Cys Leu Val Lys Asp Tyr Phe Pro Glu Pro Val Thr Val
145 150 155 160

Ser Trp Asn Ser Gly Ala Leu Thr Ser Gly Val His Thr Phe Pro Ala
165 170 175

Val Leu Gln Ser Ser Gly Leu Tyr Ser Leu Ser Ser Val Val Thr Val
180 185 190

Pro Ser Ser Ser Leu Gly Thr Gln Thr Tyr Ile Cys Asn Val Asn His
195 200 205

Lys Pro Ser Asn Thr Lys Val Asp Lys Lys Val Glu Pro Lys Ser Cys
210 215 220

Asp Lys Thr His Thr Cys Pro Pro Cys Pro Ala Pro Glu Leu Leu Gly
225 230 235 240

Gly Pro Ser Val Phe Leu Phe Pro Pro Lys Pro Lys Asp Thr Leu Met
245 250 255

Ile Ser Arg Thr Pro Glu Val Thr Cys Val Val Val Asp Val Ser His
260 265 270

eolf-seql.txt

Glu Asp Pro Glu Val Lys Phe Asn Trp Tyr Val Asp Gly Val Glu Val
275 280 285

His Asn Ala Lys Thr Lys Pro Arg Glu Glu Gln Tyr Asn Ser Thr Tyr
290 295 300

Arg Val Val Ser Val Leu Thr Val Leu His Gln Asp Trp Leu Asn Gly
305 310 315 320

Lys Glu Tyr Lys Cys Lys Val Ser Asn Lys Ala Leu Pro Ala Pro Ile
325 330 335

Glu Lys Thr Ile Ser Lys Ala Lys Gly Gln Pro Arg Glu Pro Gln Val
340 345 350

Tyr Thr Leu Pro Pro Cys Arg Asp Glu Leu Thr Lys Asn Gln Val Ser
355 360 365

Leu Trp Cys Leu Val Lys Gly Phe Tyr Pro Ser Asp Ile Ala Val Glu
370 375 380

Trp Glu Ser Asn Gly Gln Pro Glu Asn Asn Tyr Lys Thr Thr Pro Pro
385 390 395 400

Val Leu Asp Ser Asp Gly Ser Phe Phe Leu Tyr Ser Lys Leu Thr Val
405 410 415

Asp Lys Ser Arg Trp Gln Gln Gly Asn Val Phe Ser Cys Ser Val Met
420 425 430

His Glu Ala Leu His Asn His Tyr Thr Gln Lys Ser Leu Ser Leu Ser
435 440 445

Pro Gly Lys Ser Gly Gly Gly Gly Ser Gly Gly Gly Gly Ser Gly Gly
450 455 460

Gly Gly Ala Pro Ala Ser Ser Ser Thr Lys Lys Thr Gln Leu Gln Leu
465 470 475 480

Glu His Leu Leu Leu Asp Leu Gln Met Ile Leu Asn Gly Ile Asn Asn
485 490 495

Tyr Lys Asn Pro Lys Leu Thr Arg Met Leu Thr Ala Lys Phe Ala Met
500 505 510

Pro Lys Lys Ala Thr Glu Leu Lys His Leu Gln Cys Leu Glu Glu Glu
515 520 525

Leu Lys Pro Leu Glu Glu Val Leu Asn Gly Ala Gln Ser Lys Asn Phe
530 535 540

eolf-seql.txt

His Leu Arg Pro Arg Asp Leu Ile Ser Asn Ile Asn Val Ile Val Leu
545 550 555 560

Glu Leu Lys Gly Ser Glu Thr Thr Phe Met Cys Glu Tyr Ala Asp Glu
565 570 575

Thr Ala Thr Ile Val Glu Phe Leu Asn Arg Trp Ile Thr Phe Ala Gln
580 585 590

Ser Ile Ile Ser Thr Leu Thr
595

<210> 276
<211> 1797
<212> DNA
<213> Artificial Sequence

<220>
<223> CH1A1A 98/99 2F1 Fab HC-Fc knob (LALA P329G)-IL-2 qm

<400> 276
caggtgcagc tgggtgcagtc tggcgccgaa gtgaagaaac ctggagctag tgtgaaggtg 60
tcctgcaagg ccagcggcta caccttcacc gagttcggca tgaactgggt ccgacaggct 120
ccaggccagg gcctcgaatg gatgggctgg atcaacacca agaccggcga ggccacctac 180
gtggaagagt tcaagggcag agtgacctc accacggaca ccagcaccag caccgcctac 240
atggaactgc ggagcctgag aagcgacgac accgccgtgt actactgcg cagatgggac 300
ttcgcctatt acgtggaagc catggactac tggggccagg gcaccaccgt gaccgtgtct 360
agcgctagca ccaagggccc aagcgtgttc cctctggccc ccagcagcaa gagcacaagc 420
ggcggaacag ccgccctggg ctgcctggtc aaggactact tccccgagcc cgtgacagtg 480
tcctggaaca gcggagccct gaccagcggc gtgcacacct ttccagccgt gctgcagagc 540
agcggcctgt acagcctgag cagcgtggtc acagtgccta gcagcagcct gggcacccag 600
acctacatct gcaacgtgaa ccacaagccc agcaacacca aggtggacaa gaaggtggag 660
cccaagagct gcgacaagac ccacacctgt ccccttgctc ctgcccctga gctgctgggc 720
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cccgaagtga cctgcgtggt ggtggacgtg tcccacgagg accctgaagt gaagttcaat 840
tggtacgtgg acggcgtgga ggtgcacaat gccaagacca agccccggga ggaacagtac 900
aacagcacct accgggtggt gtccgtgctg accgtgctgc accaggactg gctgaacggc 960
aaagagtaca agtgcaaggt ctccaacaag gccctgcctg ccccatcga gaaaaccatc 1020
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gagctgacca agaaccaggt gtccctgtgg tgtctggtca agggcttcta cccagcgat 1140
atcgccgtgg agtgggagag caacggccag cctgagaaca actacaagac cccccccct 1200
gtgctggaca gcgacggcag cttcttctg tactccaaac tgaccgtgga caagagccgg 1260
tggcagcagg gcaacgtgtt cagctgcagc gtgatgcagc aggccctgca caaccactac 1320

eolf-seql.txt

```

accagaagt ccctgagcct gagccccggc aagtccggag gcggaggctc cggcggcgga      1380
ggttctggcg gaggtggcgc tcctgcctcc tccagcacca agaaaaccca gctccagctg      1440
gaacatctcc tgctggatct gcagatgatc ctgaacggca tcaacaacta caagaacccc      1500
aagctgaccc ggatgctgac cgccaagttc gccatgccca agaaggccac cgagctgaaa      1560
catctgcagt gcctggaaga ggaactgaag cctctggaag aggtgctgaa cggcgcccag      1620
tccaagaact tccacctgag gcctcgggac ctgatctcca acatcaacgt gatcgtgctg      1680
gaactgaagg gctccgagac aaccttcatg tgcgagtacg ccgacgagac agctaccatc      1740
gtggaatttc tgaaccggtg gatcaccttc gcccagtcca tcatctccac cctgacc      1797

```

```

<210> 277
<211> 598
<212> PRT
<213> Artificial Sequence

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<220>
<223> CH1A1A 98/99 2F1 Fab HC-Fc knob (LALA P329G)-IL-2 qm (2)
<400> 277

```

```

Gln Val Gln Leu Val Gln Ser Gly Ala Glu Val Lys Lys Pro Gly Ala
1          5          10          15

```

```

Ser Val Lys Val Ser Cys Lys Ala Ser Gly Tyr Thr Phe Thr Glu Phe
          20          25          30

```

```

Gly Met Asn Trp Val Arg Gln Ala Pro Gly Gln Gly Leu Glu Trp Met
          35          40          45

```

```

Gly Trp Ile Asn Thr Lys Thr Gly Glu Ala Thr Tyr Val Glu Glu Phe
50          55          60

```

```

Lys Gly Arg Val Thr Phe Thr Thr Asp Thr Ser Thr Ser Thr Ala Tyr
65          70          75          80

```

```

Met Glu Leu Arg Ser Leu Arg Ser Asp Asp Thr Ala Val Tyr Tyr Cys
          85          90          95

```

```

Ala Arg Trp Asp Phe Ala Tyr Tyr Val Glu Ala Met Asp Tyr Trp Gly
          100          105          110

```

```

Gln Gly Thr Thr Val Thr Val Ser Ser Ala Ser Thr Lys Gly Pro Ser
          115          120          125

```

```

Val Phe Pro Leu Ala Pro Ser Ser Lys Ser Thr Ser Gly Gly Thr Ala
          130          135          140

```

```

Ala Leu Gly Cys Leu Val Lys Asp Tyr Phe Pro Glu Pro Val Thr Val
          145          150          155          160

```

eo1f-seq1.txt

Ser Trp Asn Ser Gly Ala Leu Thr Ser Gly Val His Thr Phe Pro Ala
165 170 175

Val Leu Gln Ser Ser Gly Leu Tyr Ser Leu Ser Ser Val Val Thr Val
180 185 190

Pro Ser Ser Ser Leu Gly Thr Gln Thr Tyr Ile Cys Asn Val Asn His
195 200 205

Lys Pro Ser Asn Thr Lys Val Asp Lys Lys Val Glu Pro Lys Ser Cys
210 215 220

Asp Lys Thr His Thr Cys Pro Pro Cys Pro Ala Pro Glu Ala Ala Gly
225 230 235 240

Gly Pro Ser Val Phe Leu Phe Pro Pro Lys Pro Lys Asp Thr Leu Met
245 250 255

Ile Ser Arg Thr Pro Glu Val Thr Cys Val Val Val Asp Val Ser His
260 265 270

Glu Asp Pro Glu Val Lys Phe Asn Trp Tyr Val Asp Gly Val Glu Val
275 280 285

His Asn Ala Lys Thr Lys Pro Arg Glu Glu Gln Tyr Asn Ser Thr Tyr
290 295 300

Arg Val Val Ser Val Leu Thr Val Leu His Gln Asp Trp Leu Asn Gly
305 310 315 320

Lys Glu Tyr Lys Cys Lys Val Ser Asn Lys Ala Leu Gly Ala Pro Ile
325 330 335

Glu Lys Thr Ile Ser Lys Ala Lys Gly Gln Pro Arg Glu Pro Gln Val
340 345 350

Tyr Thr Leu Pro Pro Cys Arg Asp Glu Leu Thr Lys Asn Gln Val Ser
355 360 365

Leu Trp Cys Leu Val Lys Gly Phe Tyr Pro Ser Asp Ile Ala Val Glu
370 375 380

Trp Glu Ser Asn Gly Gln Pro Glu Asn Asn Tyr Lys Thr Thr Pro Pro
385 390 395 400

Val Leu Asp Ser Asp Gly Ser Phe Phe Leu Tyr Ser Lys Leu Thr Val
405 410 415

Asp Lys Ser Arg Trp Gln Gln Gly Asn Val Phe Ser Cys Ser Val Met
420 425 430

eolf-seql.txt

His Glu Ala Leu His Asn His Tyr Thr Gln Lys Ser Leu Ser Leu Ser
435 440 445

Pro Gly Gly Gly Gly Gly Ser Gly Gly Gly Gly Ser Gly Gly Gly Gly
450 455 460

Ser Ala Pro Ala Ser Ser Ser Thr Lys Lys Thr Gln Leu Gln Leu Glu
465 470 475 480

His Leu Leu Leu Asp Leu Gln Met Ile Leu Asn Gly Ile Asn Asn Tyr
485 490 495

Lys Asn Pro Lys Leu Thr Arg Met Leu Thr Ala Lys Phe Ala Met Pro
500 505 510

Lys Lys Ala Thr Glu Leu Lys His Leu Gln Cys Leu Glu Glu Glu Leu
515 520 525

Lys Pro Leu Glu Glu Val Leu Asn Gly Ala Gln Ser Lys Asn Phe His
530 535 540

Leu Arg Pro Arg Asp Leu Ile Ser Asn Ile Asn Val Ile Val Leu Glu
545 550 555 560

Leu Lys Gly Ser Glu Thr Thr Phe Met Cys Glu Tyr Ala Asp Glu Thr
565 570 575

Ala Thr Ile Val Glu Phe Leu Asn Arg Trp Ile Thr Phe Ala Gln Ser
580 585 590

Ile Ile Ser Thr Leu Thr
595

<210> 278
<211> 1794
<212> DNA
<213> Artificial Sequence

<220>
<223> CH1A1A 98/99 2F1 Fab HC-Fc knob (LALA P329G)-IL-2 qm (2)

<400> 278
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tcctgcaagg ccagcggcta caccttcacc gagttcggca tgaactgggt ccgacaggct 120
ccaggccagg gcctcgaatg gatgggctgg atcaacacca agaccggcga ggccacctac 180
gtggaagagt tcaagggcag agtgacctc accacggaca ccagcaccag caccgcctac 240
atggaactgc ggagcctgag aagcgacgac accgccgtgt actactgcgc cagatgggac 300
ttgcctatt acgtggaagc catggactac tggggccagg gcaccaccgt gaccgtgtct 360
agcgctagca ccaagggccc atcggctctc cccctggcac cctcctccaa gagcacctct 420
gggggacacag cggccctggg ctgcctggtc aaggactact tccccgaacc ggtgacgggtg 480

eolf-seql.txt

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tcgtggaact caggcgccct gaccagcggc gtgcacacct tcccggtgt cctacagtcc      540
tcaggactct actccctcag cagcgtgggtg accgtgccct ccagcagctt gggcaccacag      600
acctacatct gcaacgtgaa tcacaagccc agcaacacca aggtggacaa gaaagttgag      660
cccaaactct gtgacaaaac tcacacatgc ccaccgtgcc cagcacctga agctgcaggg      720
ggaccgtcag tcttctctct ccccccaaaa cccaaggaca cctcatgat ctcccgacc      780
cctgaggtca catgcgtggt ggtggacgtg agccacgaag accctgaggt caagttcaac      840
tggtacgtgg acggcgtgga ggtgcataat gccaaagaaa agccgcggga ggagcagtac      900
aacagcacgt accgtgtggt cagcgtcctc accgtcctgc accaggactg gctgaatggc      960
aaggagtaca agtgcaaggt ctccaacaaa gccctcggcg ccccatcga gaaaaccatc     1020
tcaaagcca aagggcagcc ccgagaacca caggtgtaca cctgcccc atgccgggat     1080
gagctgacca agaaccaggt cagcctgtgg tgcttggtca aaggcttcta tcccagcgac     1140
atcgccgtgg agtgggagag caatgggcag ccggagaaca actacaagac cagcctccc     1200
gtgctggact ccgacggctc cttcttcctc tacagcaagc tcaccgtgga caagagcagg     1260
tggcagcagg ggaacgtctt ctcatgtctc gtgatgcatg aggctctgca caaccactac     1320
acgcagaaga gcctctccct gtctccgggt ggcggcggag gctccggagg cggaggttct     1380
ggcggagggtg gctccgcacc tgctcaagt tctacaaaga aaacacagct acaactggag     1440
catttactgc tggatttaca gatgattttg aatggaatta ataattacaa gaatcccaaa     1500
ctcaccagga tgctcacagc caagtttgcc atgccaaga aggccacaga actgaaacat     1560
cttcagtgtc tagaagaaga actcaaacct ctggaggaag tgctaaatgg cgctcaaagc     1620
aaaaactttc acttaagacc cagggactta atcagcaata tcaacgtaat agttctggaa     1680
ctaaagggat ctgaaacaac attcatgtgt gaatatgctg atgagacagc aaccattgta     1740
gaatttctga acagatggat tacctttgcc caaagcatca tctcaacact gact          1794

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<210> 279
 <211> 598
 <212> PRT
 <213> Artificial Sequence

<220>
 <223> CH1A1A 98/99 2F1 Fab HC-Fc knob (LALA P329G)-IL-2 wt
 <400> 279

Gln Val Gln Leu Val Gln Ser Gly Ala Glu Val Lys Lys Pro Gly Ala
 1 5 10 15

Ser Val Lys Val Ser Cys Lys Ala Ser Gly Tyr Thr Phe Thr Glu Phe
 20 25 30

Gly Met Asn Trp Val Arg Gln Ala Pro Gly Gln Gly Leu Glu Trp Met
 35 40 45

eofl-seql.txt

Gly Trp Ile Asn Thr Lys Thr Gly Glu Ala Thr Tyr Val Glu Glu Phe
50 55 60

Lys Gly Arg Val Thr Phe Thr Thr Asp Thr Ser Thr Ser Thr Ala Tyr
65 70 75 80

Met Glu Leu Arg Ser Leu Arg Ser Asp Asp Thr Ala Val Tyr Tyr Cys
85 90 95

Ala Arg Trp Asp Phe Ala Tyr Tyr Val Glu Ala Met Asp Tyr Trp Gly
100 105 110

Gln Gly Thr Thr Val Thr Val Ser Ser Ala Ser Thr Lys Gly Pro Ser
115 120 125

Val Phe Pro Leu Ala Pro Ser Ser Lys Ser Thr Ser Gly Gly Thr Ala
130 135 140

Ala Leu Gly Cys Leu Val Lys Asp Tyr Phe Pro Glu Pro Val Thr Val
145 150 155 160

Ser Trp Asn Ser Gly Ala Leu Thr Ser Gly Val His Thr Phe Pro Ala
165 170 175

Val Leu Gln Ser Ser Gly Leu Tyr Ser Leu Ser Ser Val Val Thr Val
180 185 190

Pro Ser Ser Ser Leu Gly Thr Gln Thr Tyr Ile Cys Asn Val Asn His
195 200 205

Lys Pro Ser Asn Thr Lys Val Asp Lys Lys Val Glu Pro Lys Ser Cys
210 215 220

Asp Lys Thr His Thr Cys Pro Pro Cys Pro Ala Pro Glu Ala Ala Gly
225 230 235 240

Gly Pro Ser Val Phe Leu Phe Pro Pro Lys Pro Lys Asp Thr Leu Met
245 250 255

Ile Ser Arg Thr Pro Glu Val Thr Cys Val Val Val Asp Val Ser His
260 265 270

Glu Asp Pro Glu Val Lys Phe Asn Trp Tyr Val Asp Gly Val Glu Val
275 280 285

His Asn Ala Lys Thr Lys Pro Arg Glu Glu Gln Tyr Asn Ser Thr Tyr
290 295 300

Arg Val Val Ser Val Leu Thr Val Leu His Gln Asp Trp Leu Asn Gly
305 310 315 320

ecolf-seql.txt

Lys Glu Tyr Lys Cys Lys Val Ser Asn Lys Ala Leu Gly Ala Pro Ile
325 330 335

Glu Lys Thr Ile Ser Lys Ala Lys Gly Gln Pro Arg Glu Pro Gln Val
340 345 350

Tyr Thr Leu Pro Pro Cys Arg Asp Glu Leu Thr Lys Asn Gln Val Ser
355 360 365

Leu Trp Cys Leu Val Lys Gly Phe Tyr Pro Ser Asp Ile Ala Val Glu
370 375 380

Trp Glu Ser Asn Gly Gln Pro Glu Asn Asn Tyr Lys Thr Thr Pro Pro
385 390 395 400

Val Leu Asp Ser Asp Gly Ser Phe Phe Leu Tyr Ser Lys Leu Thr Val
405 410 415

Asp Lys Ser Arg Trp Gln Gln Gly Asn Val Phe Ser Cys Ser Val Met
420 425 430

His Glu Ala Leu His Asn His Tyr Thr Gln Lys Ser Leu Ser Leu Ser
435 440 445

Pro Gly Gly Gly Gly Gly Ser Gly Gly Gly Gly Ser Gly Gly Gly Gly
450 455 460

Ser Ala Pro Thr Ser Ser Ser Thr Lys Lys Thr Gln Leu Gln Leu Glu
465 470 475 480

His Leu Leu Leu Asp Leu Gln Met Ile Leu Asn Gly Ile Asn Asn Tyr
485 490 495

Lys Asn Pro Lys Leu Thr Arg Met Leu Thr Phe Lys Phe Tyr Met Pro
500 505 510

Lys Lys Ala Thr Glu Leu Lys His Leu Gln Cys Leu Glu Glu Glu Leu
515 520 525

Lys Pro Leu Glu Glu Val Leu Asn Leu Ala Gln Ser Lys Asn Phe His
530 535 540

Leu Arg Pro Arg Asp Leu Ile Ser Asn Ile Asn Val Ile Val Leu Glu
545 550 555 560

Leu Lys Gly Ser Glu Thr Thr Phe Met Cys Glu Tyr Ala Asp Glu Thr
565 570 575

Ala Thr Ile Val Glu Phe Leu Asn Arg Trp Ile Thr Phe Ala Gln Ser
580 585 590

Ile Ile Ser Thr Leu Thr
595

<210> 280
<211> 1794
<212> DNA
<213> Artificial Sequence

<220>
<223> CH1A1A 98/99 2F1 Fab HC-Fc knob (LALA P329G)-IL-2 wt

```

<400> 280
caggtgcagc tgggtgcagtc tggcgccgaa gtgaagaaac ctggagctag tgtgaagggtg      60
tcctgcaagg ccagcggcta caccttcacc gagttcggca tgaactgggt ccgacaggct      120
ccaggccagg gcctcgaatg gatgggctgg atcaacacca agaccggcga ggccacctac      180
gtggaagagt tcaagggcag agtgacctc accacggaca ccagcaccag caccgcctac      240
atggaactgc ggagcctgag aagcgacgac accgccgtgt actactgcmc cagatgggac      300
ttcgcctatt acgtggaagc catggactac tggggccagg gcaccaccgt gaccgtgtct      360
agcgctagca ccaagggccc atcggctctc cccctggcac cctcctcaa gagcacctct      420
gggggacacag cggccctggg ctgcctggtc aaggactact tccccgaacc ggtgacgggtg      480
tcgtggaact caggcgccct gaccagcggc gtgcacacct tcccggctgt cctacagtcc      540
tcaggactct actccctcag cagcgtggtg accgtgccct ccagcagctt gggcacccag      600
acctacatct gcaacgtgaa tcacaagccc agcaacacca aggtggacaa gaaagttgag      660
cccaaattct gtgacaaaac tcacacatgc ccaccgtgcc cagcacctga agctgcaggg      720
ggaccgtcag tcttcctctt cccccaaaa cccaaggaca ccctcatgat ctcccggacc      780
cctgagggtca catgcgtggt ggtggacgtg agccacgaag accctgaggt caagttcaac      840
tggtacgtgg acggcgtgga ggtgcataat gccaagacaa agccgcggga ggagcagtac      900
aacagcacgt accgtgtggt cagcgtcctc accgtcctgc accaggactg gctgaatggc      960
aaggagtaca agtgcaaggt ctccaacaaa gccctcggcg ccccatcga gaaaaccatc     1020
tccaaagcca aagggcagcc ccgagaacca caggtgtaca cctgcccc atgccgggat     1080
gagctgacca agaaccaggt cagcctgtgg tgcctggtca aaggcttcta tcccagcgac     1140
atgccgtgag agtgggagag caatgggcag ccggagaaca actacaagac cagcctccc     1200
gtgctggact ccgacggctc cttcttcctc tacagcaagc tcaccgtgga caagagcagg     1260
tggcagcagg ggaacgtctt ctcatgtctc gtgatgcatg aggctctgca caaccactac     1320
acgcagaaga gcctctccct gtctccgggt ggcggcggag gctccggagg cggaggttct     1380
ggaggcggag gctccgcacc tacttcaagt tctacaaaga aaacacagct acaactggag     1440
catttactgc tggatttaca gatgattttg aatggaatta ataattacaa gaatcccaaa     1500
ctcaccagga tgctcacatt taagttttac atgcccaaga aggccacaga actgaaacat     1560
cttcagtgtc tagaagaaga actcaaacct ctggaggaag tgctaaattt agctcaaagc     1620
aaaaactttc acttaagacc cagggactta atcagcaata tcaacgtaat agttctggaa     1680

```

eolf-seql.txt

ctaaagggat ctgaaacaac attcatgtgt gaatatgctg atgagacagc aaccattgta 1740
gaatttctga acagatggat tacctttgcc caaagcatca tctcaacact gact 1794

<210> 281
<211> 451
<212> PRT
<213> Artificial Sequence

<220>
<223> CH1A1A 98/99 2F1 Fab HC-Fc hole (LALA P329G)
<400> 281

Gln Val Gln Leu Val Gln Ser Gly Ala Glu Val Lys Lys Pro Gly Ala
1 5 10 15

Ser Val Lys Val Ser Cys Lys Ala Ser Gly Tyr Thr Phe Thr Glu Phe
20 25 30

Gly Met Asn Trp Val Arg Gln Ala Pro Gly Gln Gly Leu Glu Trp Met
35 40 45

Gly Trp Ile Asn Thr Lys Thr Gly Glu Ala Thr Tyr Val Glu Glu Phe
50 55 60

Lys Gly Arg Val Thr Phe Thr Thr Asp Thr Ser Thr Ser Thr Ala Tyr
65 70 75 80

Met Glu Leu Arg Ser Leu Arg Ser Asp Asp Thr Ala Val Tyr Tyr Cys
85 90 95

Ala Arg Trp Asp Phe Ala Tyr Tyr Val Glu Ala Met Asp Tyr Trp Gly
100 105 110

Gln Gly Thr Thr Val Thr Val Ser Ser Ala Ser Thr Lys Gly Pro Ser
115 120 125

Val Phe Pro Leu Ala Pro Ser Ser Lys Ser Thr Ser Gly Gly Thr Ala
130 135 140

Ala Leu Gly Cys Leu Val Lys Asp Tyr Phe Pro Glu Pro Val Thr Val
145 150 155 160

Ser Trp Asn Ser Gly Ala Leu Thr Ser Gly Val His Thr Phe Pro Ala
165 170 175

Val Leu Gln Ser Ser Gly Leu Tyr Ser Leu Ser Ser Val Val Thr Val
180 185 190

Pro Ser Ser Ser Leu Gly Thr Gln Thr Tyr Ile Cys Asn Val Asn His
195 200 205

eolf-seql.txt

Lys Pro Ser Asn Thr Lys Val Asp Lys Lys Val Glu Pro Lys Ser Cys
210 215 220

Asp Lys Thr His Thr Cys Pro Pro Cys Pro Ala Pro Glu Ala Ala Gly
225 230 235 240

Gly Pro Ser Val Phe Leu Phe Pro Pro Lys Pro Lys Asp Thr Leu Met
245 250 255

Ile Ser Arg Thr Pro Glu Val Thr Cys Val Val Val Asp Val Ser His
260 265 270

Glu Asp Pro Glu Val Lys Phe Asn Trp Tyr Val Asp Gly Val Glu Val
275 280 285

His Asn Ala Lys Thr Lys Pro Arg Glu Glu Gln Tyr Asn Ser Thr Tyr
290 295 300

Arg Val Val Ser Val Leu Thr Val Leu His Gln Asp Trp Leu Asn Gly
305 310 315 320

Lys Glu Tyr Lys Cys Lys Val Ser Asn Lys Ala Leu Gly Ala Pro Ile
325 330 335

Glu Lys Thr Ile Ser Lys Ala Lys Gly Gln Pro Arg Glu Pro Gln Val
340 345 350

Cys Thr Leu Pro Pro Ser Arg Asp Glu Leu Thr Lys Asn Gln Val Ser
355 360 365

Leu Ser Cys Ala Val Lys Gly Phe Tyr Pro Ser Asp Ile Ala Val Glu
370 375 380

Trp Glu Ser Asn Gly Gln Pro Glu Asn Asn Tyr Lys Thr Thr Pro Pro
385 390 395 400

Val Leu Asp Ser Asp Gly Ser Phe Phe Leu Val Ser Lys Leu Thr Val
405 410 415

Asp Lys Ser Arg Trp Gln Gln Gly Asn Val Phe Ser Cys Ser Val Met
420 425 430

His Glu Ala Leu His Asn His Tyr Thr Gln Lys Ser Leu Ser Leu Ser
435 440 445

Pro Gly Lys
450

<210> 282
<211> 1353
<212> DNA
<213> Artificial Sequence

eolf-seql.txt

<220>

<223> CH1A1A 98/99 2F1 Fab HC-Fc hole (LALA P329G)

<400> 282

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caggtgcagc tgggtgcagtc tggcgccgaa gtgaagaaac ctggagctag tgtgaagggtg      60
tcctgcaagg ccagcggcta caccttcacc gagttcggca tgaactgggt cgcacaggct      120
ccaggccagg gcctcgaatg gatgggctgg atcaacacca agaccggcga ggccacctac      180
gtggaagagt tcaagggcag agtgaccttc accacggaca ccagcaccag caccgcctac      240
atggaactgc ggagcctgag aagcgacgac accgccgtgt actactgcmc cagatgggac      300
ttcgcctatt acgtggaagc catggactac tggggccagg gcaccaccgt gaccgtgtct      360
agcgctagca ccaagggccc ctccgtgttc cccctggccc ccagcagcaa gagcaccagc      420
ggcggcacag ccgctctggg ctgcctggtc aaggactact tccccgagcc cgtgaccgtg      480
tcctggaaca gcggagccct gacctccggc gtgcacacct tccccgccgt gctgcagagt      540
tctggcctgt atagcctgag cagcgtggtc accgtgcctt ctagcagcct gggcacccag      600
acctacatct gcaacgtgaa ccacaagccc agcaacacca aggtggacaa gaaggtggag      660
cccaagagct gcgacaaaac tcacacatgc ccaccgtgcc cagcacctga agctgcaggg      720
ggaccgtcag tcttctcttt cccccaaaa cccaaggaca ccctcatgat ctcccgacc      780
cctgagggtca catgcgtggg ggtggacgtg agccacgaag accctgaggt caagttcaac      840
tggtacgtgg acggcgtgga ggtgcataat gccaaagaaa agccgcggga ggagcagtag      900
aacagcacgt accgtgtggg cagcgtcctc accgtcctgc accaggactg gctgaatggc      960
aaggagtaca agtgcaaggc ctccaacaaa gccctcggcg ccccatcga gaaaaccatc     1020
tccaaagcca aagggcagcc ccgagaacca caggtgtgca ccctgcccc atcccgggat     1080
gagctgacca agaaccaggc cagcctctcg tgcgcagtca aaggcttcta tcccagcgac     1140
atcgccgtgg agtgggagag caatgggcag ccggagaaca actacaagac cagcctccc     1200
gtgctggact ccgacggctc cttcttctc gtgagcaagc tcaccgtgga caagagcagg     1260
tggcagcagg ggaacgtctt ctcatgtcc gtgatgcatg aggtcttgca caaccactac     1320
acgcagaaga gcctctccct gtctccgggt aaa                                1353
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<210> 283

<211> 215

<212> PRT

<213> Artificial Sequence

<220>

<223> CH1A1A 98/99 2F1 Fab LC

<400> 283

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Asp Ile Gln Met Thr Gln Ser Pro Ser Ser Leu Ser Ala Ser Val Gly
1           5           10           15
```

```
Asp Arg Val Thr Ile Thr Cys Lys Ala Ser Ala Ala Val Gly Thr Tyr
          20          25          30
```

eolf-seql.txt

Val Ala Trp Tyr Gln Gln Lys Pro Gly Lys Ala Pro Lys Leu Leu Ile
35 40 45

Tyr Ser Ala Ser Tyr Arg Lys Arg Gly Val Pro Ser Arg Phe Ser Gly
50 55 60

Ser Gly Ser Gly Thr Asp Phe Thr Leu Thr Ile Ser Ser Leu Gln Pro
65 70 75 80

Glu Asp Phe Ala Thr Tyr Tyr Cys His Gln Tyr Tyr Thr Tyr Pro Leu
85 90 95

Phe Thr Phe Gly Gln Gly Thr Lys Leu Glu Ile Lys Arg Thr Val Ala
100 105 110

Ala Pro Ser Val Phe Ile Phe Pro Pro Ser Asp Glu Gln Leu Lys Ser
115 120 125

Gly Thr Ala Ser Val Val Cys Leu Leu Asn Asn Phe Tyr Pro Arg Glu
130 135 140

Ala Lys Val Gln Trp Lys Val Asp Asn Ala Leu Gln Ser Gly Asn Ser
145 150 155 160

Gln Glu Ser Val Thr Glu Gln Asp Ser Lys Asp Ser Thr Tyr Ser Leu
165 170 175

Ser Ser Thr Leu Thr Leu Ser Lys Ala Asp Tyr Glu Lys His Lys Val
180 185 190

Tyr Ala Cys Glu Val Thr His Gln Gly Leu Ser Ser Pro Val Thr Lys
195 200 205

Ser Phe Asn Arg Gly Glu Cys
210 215

<210> 284
<211> 645
<212> DNA
<213> Artificial Sequence

<220>
<223> CH1A1A 98/99 2F1 Fab LC

<400> 284
gatatccaga tgaccagtc tccatcctcc ctgtctgcat ctgtgggaga cagagtcacc 60
atcacttgca aggccagtgc ggctgtgggt acgtatgttg cgtggtatca gcagaaacca 120
gggaaagcac ctaagctcct gatctattcg gcacccctacc gcaaaagggg agtcccatca 180
aggttcagtg gcagtggatc tgggacagat ttcactctca ccatcagcag tctgcaacct 240
gaagatttcg caacttacta ctgtcaccaa tattacacct atcctctatt cacgtttggc 300

eolf-seql.txt

cagggcacca agctcgagat caagcgtacg gtggctgcac catctgtctt catcttcccg 360
ccatctgatg agcagttgaa atctggaact gcctctgttg tgtgcctgct gaataacttc 420
tatcccagag aggccaaagt acagtggaag gtggataacg ccctccaatc gggtaactcc 480
caggagagtg tcacagagca ggacagcaag gacagcacct acagcctcag cagcaccctg 540
acgctgagca aagcagacta cgagaaacac aaagtctacg cctgcgaagt caccatcag 600
ggcctgagct cgcccgtcac aaagagcttc aacaggggag agtgt 645

<210> 285
<211> 598
<212> PRT
<213> Artificial Sequence

<220>
<223> 2B10 Fab HC-Fc knob (LALA P329G)-IL-2 qm
<400> 285

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 Gly Thr Phe Ser Ser Tyr
20 25 30

Ala Ile Ser Trp Val Arg Gln Ala Pro Gly Gln Gly Leu Glu Trp Met
35 40 45

Gly Gly Ile Ile Pro Ile Phe Gly Thr Ala Asn Tyr Ala Gln Lys Phe
50 55 60

Gln 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 Leu Tyr Gly Tyr Ala Tyr Tyr Gly Ala Phe Asp Tyr Trp Gly
100 105 110

Gln Gly Thr Thr Val Thr Val Ser Ser Ala Ser Thr Lys Gly Pro Ser
115 120 125

Val Phe Pro Leu Ala Pro Ser Ser Lys Ser Thr Ser Gly Gly Thr Ala
130 135 140

Ala Leu Gly Cys Leu Val Lys Asp Tyr Phe Pro Glu Pro Val Thr Val
145 150 155 160

Ser Trp Asn Ser Gly Ala Leu Thr Ser Gly Val His Thr Phe Pro Ala
165 170 175

eolf-seql.txt

Val Leu Gln Ser Ser Gly Leu Tyr Ser Leu Ser Ser Val Val Thr Val
180 185 190

Pro Ser Ser Ser Leu Gly Thr Gln Thr Tyr Ile Cys Asn Val Asn His
195 200 205

Lys Pro Ser Asn Thr Lys Val Asp Lys Lys Val Glu Pro Lys Ser Cys
210 215 220

Asp Lys Thr His Thr Cys Pro Pro Cys Pro Ala Pro Glu Ala Ala Gly
225 230 235 240

Gly Pro Ser Val Phe Leu Phe Pro Pro Lys Pro Lys Asp Thr Leu Met
245 250 255

Ile Ser Arg Thr Pro Glu Val Thr Cys Val Val Val Asp Val Ser His
260 265 270

Glu Asp Pro Glu Val Lys Phe Asn Trp Tyr Val Asp Gly Val Glu Val
275 280 285

His Asn Ala Lys Thr Lys Pro Arg Glu Glu Gln Tyr Asn Ser Thr Tyr
290 295 300

Arg Val Val Ser Val Leu Thr Val Leu His Gln Asp Trp Leu Asn Gly
305 310 315 320

Lys Glu Tyr Lys Cys Lys Val Ser Asn Lys Ala Leu Gly Ala Pro Ile
325 330 335

Glu Lys Thr Ile Ser Lys Ala Lys Gly Gln Pro Arg Glu Pro Gln Val
340 345 350

Tyr Thr Leu Pro Pro Cys Arg Asp Glu Leu Thr Lys Asn Gln Val Ser
355 360 365

Leu Trp Cys Leu Val Lys Gly Phe Tyr Pro Ser Asp Ile Ala Val Glu
370 375 380

Trp Glu Ser Asn Gly Gln Pro Glu Asn Asn Tyr Lys Thr Thr Pro Pro
385 390 395 400

Val Leu Asp Ser Asp Gly Ser Phe Phe Leu Tyr Ser Lys Leu Thr Val
405 410 415

Asp Lys Ser Arg Trp Gln Gln Gly Asn Val Phe Ser Cys Ser Val Met
420 425 430

His Glu Ala Leu His Asn His Tyr Thr Gln Lys Ser Leu Ser Leu Ser
435 440 445

eolf-seql.txt

Pro Gly Gly Gly Gly Gly Ser Gly Gly Gly Gly Ser Gly Gly Gly Gly
450 455 460

Ser Ala Pro Ala Ser Ser Ser Thr Lys Lys Thr Gln Leu Gln Leu Glu
465 470 475 480

His Leu Leu Leu Asp Leu Gln Met Ile Leu Asn Gly Ile Asn Asn Tyr
485 490 495

Lys Asn Pro Lys Leu Thr Arg Met Leu Thr Ala Lys Phe Ala Met Pro
500 505 510

Lys Lys Ala Thr Glu Leu Lys His Leu Gln Cys Leu Glu Glu Glu Leu
515 520 525

Lys Pro Leu Glu Glu Val Leu Asn Gly Ala Gln Ser Lys Asn Phe His
530 535 540

Leu Arg Pro Arg Asp Leu Ile Ser Asn Ile Asn Val Ile Val Leu Glu
545 550 555 560

Leu Lys Gly Ser Glu Thr Thr Phe Met Cys Glu Tyr Ala Asp Glu Thr
565 570 575

Ala Thr Ile Val Glu Phe Leu Asn Arg Trp Ile Thr Phe Ala Gln Ser
580 585 590

Ile Ile Ser Thr Leu Thr
595

<210> 286
<211> 1794
<212> DNA
<213> Artificial Sequence

<220>
<223> 2B10 Fab HC-Fc knob (LALA P329G)-IL-2 qm

<400> 286
caggtgcaat tgggtgcagtc tggggctgag gtgaagaagc ctgggtcctc ggtgaaggtc 60
tcctgcaagg cctccgagg cacattcagc agctacgcta taagctgggt gcgacaggcc 120
cctggacaag ggctcgagt gatgggaggg atcatcccta tctttggtac agcaaactac 180
gcacagaagt tccagggcag ggtcaccatt actgcagaca aatccacgag cacagcctac 240
atggagctga gcagcctgag atctgaggac accgccgtgt attactgtgc gagactgtac 300
ggttacgctt actacggtgc ttttgactac tggggccaag ggaccaccgt gaccgtctcc 360
tcagctagca ccaagggccc atcgggtcttc cccctggcac cctcctccaa gagcacctct 420
gggggcacag cggccctggg ctgcctggtc aaggactact tccccgaacc ggtgacgggtg 480
tcgtggaact caggcgcct gaccagcggc gtgcacacct tcccggctgt cctacagtcc 540
tcaggactct actccctcag cagcgtggtg accgtgccct ccagcagctt gggcacccag 600

eolf-seql.txt

```

acctacatct gcaacgtgaa tcacaagccc agcaacacca aggtggacaa gaaagttgag      660
cccaaattctt gtgacaaaac tcacacatgc ccaccgtgcc cagcacctga agctgcaggg      720
ggaccgtcag tcttcctctt ccccccaaaa cccaaggaca ccctcatgat ctcccggacc      780
cctgaggtca catgcgtggt ggtggacgtg agccacgaag accctgaggt caagttcaac      840
tggtacgtgg acggcgtgga ggtgcataat gccaaagaaa agccgcggga ggagcagtag      900
aacagcacgt accgtgtggt cagcgtcctc accgtcctgc accaggactg gctgaatggc      960
aaggagtaca agtgcaaggt ctccaacaaa gccctcggcg ccccatcga gaaaaccatc     1020
tccaaagcca aagggcagcc ccgagaacca caggtgtaca ccctgcccc atgccgggat     1080
gagctgacca agaaccaggt cagcctgtgg tgcctggtca aaggcttcta tcccagcgac     1140
atcgccgtgg agtgggagag caatgggcag ccggagaaca actacaagac cagcctccc     1200
gtgctggact ccgacggctc cttcttcctc tacagcaagc tcaccgtgga caagagcagg     1260
tggcagcagg ggaacgtctt ctcattgtcc gtgatgcatg aggtcttga caaccactac     1320
acgcagaaga gcctctccct gtctccgggt ggcggcggag gctccggagg cggaggttct     1380
ggcggaggtg gctccgcacc tgcctcaagt tctacaaaga aaacacagct acaactggag     1440
catttactgc tggatttaca gatgattttg aatggaatta ataattaca gaatcccaaa     1500
ctcaccagga tgctcacagc caagtttgcc atgcccaaga agccacaga actgaaacat     1560
cttcagtgtc tagaagaaga actcaaact ctggaggaag tgctaaatgg cgctcaaagc     1620
aaaaactttc acttaagacc cagggactta atcagcaata tcaacgtaat agttctggaa     1680
ctaaagggat ctgaaacaac attcatgtgt gaatatgctg atgagacagc aaccattgta     1740
gaatttctga acagatggat tacctttgcc caaagcatca tctcaacact gact          1794

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<210> 287
 <211> 451
 <212> PRT
 <213> Artificial Sequence

<220>
 <223> 2B10 Fab HC-Fc hole (LALA P329G)

<400> 287

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 Gly Thr Phe Ser Ser Tyr
 20 25 30

Ala Ile Ser Trp Val Arg Gln Ala Pro Gly Gln Gly Leu Glu Trp Met
 35 40 45

Gly Gly Ile Ile Pro Ile Phe Gly Thr Ala Asn Tyr Ala Gln Lys Phe
 50 55 60

eofl-seql.txt

Gln 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 Leu Tyr Gly Tyr Ala Tyr Tyr Gly Ala Phe Asp Tyr Trp Gly
100 105 110

Gln Gly Thr Thr Val Thr Val Ser Ser Ala Ser Thr Lys Gly Pro Ser
115 120 125

Val Phe Pro Leu Ala Pro Ser Ser Lys Ser Thr Ser Gly Gly Thr Ala
130 135 140

Ala Leu Gly Cys Leu Val Lys Asp Tyr Phe Pro Glu Pro Val Thr Val
145 150 155 160

Ser Trp Asn Ser Gly Ala Leu Thr Ser Gly Val His Thr Phe Pro Ala
165 170 175

Val Leu Gln Ser Ser Gly Leu Tyr Ser Leu Ser Ser Val Val Thr Val
180 185 190

Pro Ser Ser Ser Leu Gly Thr Gln Thr Tyr Ile Cys Asn Val Asn His
195 200 205

Lys Pro Ser Asn Thr Lys Val Asp Lys Lys Val Glu Pro Lys Ser Cys
210 215 220

Asp Lys Thr His Thr Cys Pro Pro Cys Pro Ala Pro Glu Ala Ala Gly
225 230 235 240

Gly Pro Ser Val Phe Leu Phe Pro Pro Lys Pro Lys Asp Thr Leu Met
245 250 255

Ile Ser Arg Thr Pro Glu Val Thr Cys Val Val Val Asp Val Ser His
260 265 270

Glu Asp Pro Glu Val Lys Phe Asn Trp Tyr Val Asp Gly Val Glu Val
275 280 285

His Asn Ala Lys Thr Lys Pro Arg Glu Glu Gln Tyr Asn Ser Thr Tyr
290 295 300

Arg Val Val Ser Val Leu Thr Val Leu His Gln Asp Trp Leu Asn Gly
305 310 315 320

Lys Glu Tyr Lys Cys Lys Val Ser Asn Lys Ala Leu Gly Ala Pro Ile
325 330 335

eolf-seql.txt

Glu Lys Thr Ile Ser Lys Ala Lys Gly Gln Pro Arg Glu Pro Gln Val
340 345 350

Cys Thr Leu Pro Pro Ser Arg Asp Glu Leu Thr Lys Asn Gln Val Ser
355 360 365

Leu Ser Cys Ala Val Lys Gly Phe Tyr Pro Ser Asp Ile Ala Val Glu
370 375 380

Trp Glu Ser Asn Gly Gln Pro Glu Asn Asn Tyr Lys Thr Thr Pro Pro
385 390 395 400

Val Leu Asp Ser Asp Gly Ser Phe Phe Leu Val Ser Lys Leu Thr Val
405 410 415

Asp Lys Ser Arg Trp Gln Gln Gly Asn Val Phe Ser Cys Ser Val Met
420 425 430

His Glu Ala Leu His Asn His Tyr Thr Gln Lys Ser Leu Ser Leu Ser
435 440 445

Pro Gly Lys
450

<210> 288
<211> 1353
<212> DNA
<213> Artificial Sequence

<220>
<223> 2B10 Fab HC-Fc hole (LALA P329G)

<400> 288
caggtgcaat tgggtgcagtc tggggctgag gtgaagaagc ctgggtcctc ggtgaaggtc 60
tcctgcaagg cctccggagg cacattcagc agctacgcta taagctgggt gcgacaggcc 120
cctggacaag ggctcgagtg gatgggaggg atcatcccta tctttggtac agcaaactac 180
gcacagaagt tccagggcag ggtcaccatt actgcagaca aatccacgag cacagcctac 240
atggagctga gcagcctgag atctgaggac accgccgtgt attactgtgc gagactgtac 300
ggttacgctt actacggtgc ttttgactac tggggccaag ggaccaccgt gaccgtctcc 360
tcagctagca ccaagggccc ctccgtgttc cccctggccc ccagcagcaa gagcaccagc 420
ggcggcacag ccgctctggg ctgcctggtc aaggactact tccccgagcc cgtgaccgtg 480
tcctggaaca gcggagccct gacctccggc gtgcacacct tccccgccgt gctgcagagt 540
tctggcctgt atagcctgag cagcgtggtc accgtgcctt ctagcagcct gggcaccag 600
acctacatct gcaacgtgaa ccacaagccc agcaacacca aggtggacaa gaaggaggag 660
cccaagagct gcgacaaaac tcacacatgc ccaccgtgcc cagcacctga agctgcaggg 720
ggaccgtcag tcttcctctt cccccaaaa cccaaggaca ccctcatgat ctcccggacc 780
cctgaggtca catgcgtggg ggtggacgtg agccacgaag accctgaggt caagttcaac 840

eolf-seql.txt

```

tggtacgtgg acggcgtgga ggtgcataat gccaaagaaa agccgcggga ggagcagtag      900
aacagcacgt accgtgtggt cagcgtcctc accgtcctgc accaggactg gctgaatggc      960
aaggagtaca agtgcaaggt ctccaacaaa gccctcggcg ccccatcga gaaaaccatc     1020
tccaaagcca aagggcagcc ccgagaacca caggtgtgca ccctgcccc atcccgggat     1080
gagctgacca agaaccaggt cagcctctcg tgcgcagtca aaggcttcta tcccagcgac     1140
atcgccgtgg agtgggagag caatgggcag ccggagaaca actacaagac cagcctccc      1200
gtgctggact ccgacggctc cttcttcctc gtgagcaagc tcaccgtgga caagagcagg     1260
tggcagcagg ggaacgtctt ctcagtctcc gtgatgatg aggctctgca caaccactac     1320
acgcagaaga gcctctccct gtctccgggt aaa                                1353

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<210> 289
 <211> 592
 <212> PRT
 <213> Artificial Sequence

<220>
 <223> DP47GS Fab HC-Fc knob (LALA P329G)-IL-2 qm

<400> 289

Glu Val Gln Leu Leu Glu Ser Gly Gly Gly Leu Val Gln Pro Gly Gly
 1 5 10 15

Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Phe Thr Phe Ser Ser Tyr
 20 25 30

Ala Met Ser Trp Val Arg Gln Ala Pro Gly Lys Gly Leu Glu Trp Val
 35 40 45

Ser Ala Ile Ser Gly Ser Gly Gly Ser Thr Tyr Tyr Ala Asp Ser Val
 50 55 60

Lys Gly Arg Phe Thr Ile Ser Arg Asp Asn Ser Lys Asn Thr Leu Tyr
 65 70 75 80

Leu Gln Met Asn Ser Leu Arg Ala Glu Asp Thr Ala Val Tyr Tyr Cys
 85 90 95

Ala Lys Gly Ser Gly Phe Asp Tyr Trp Gly Gln Gly Thr Leu Val Thr
 100 105 110

Val Ser Ser Ala Ser Thr Lys Gly Pro Ser Val Phe Pro Leu Ala Pro
 115 120 125

Ser Ser Lys Ser Thr Ser Gly Gly Thr Ala Ala Leu Gly Cys Leu Val
 130 135 140

Lys Asp Tyr Phe Pro Glu Pro Val Thr Val Ser Trp Asn Ser Gly Ala
 145 150 155 160

eolf-seql.txt

Leu Thr Ser Gly Val His Thr Phe Pro Ala Val Leu Gln Ser Ser Gly
165 170 175

Leu Tyr Ser Leu Ser Ser Val Val Thr Val Pro Ser Ser Ser Leu Gly
180 185 190

Thr Gln Thr Tyr Ile Cys Asn Val Asn His Lys Pro Ser Asn Thr Lys
195 200 205

Val Asp Lys Lys Val Glu Pro Lys Ser Cys Asp Lys Thr His Thr Cys
210 215 220

Pro Pro Cys Pro Ala Pro Glu Ala Ala 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 Gly 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 Cys
340 345 350

Arg Asp Glu Leu Thr Lys Asn Gln Val Ser Leu Trp 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

eolf-seql.txt

His Tyr Thr Gln Lys Ser Leu Ser Leu Ser Pro Gly Gly Gly Gly Gly
435 440 445

Ser Gly Gly Gly Gly Ser Gly Gly Gly Gly Ser Ala Pro Ala Ser Ser
450 455 460

Ser Thr Lys Lys Thr Gln Leu Gln Leu Glu His Leu Leu Leu Asp Leu
465 470 475 480

Gln Met Ile Leu Asn Gly Ile Asn Asn Tyr Lys Asn Pro Lys Leu Thr
485 490 495

Arg Met Leu Thr Ala Lys Phe Ala Met Pro Lys Lys Ala Thr Glu Leu
500 505 510

Lys His Leu Gln Cys Leu Glu Glu Glu Leu Lys Pro Leu Glu Glu Val
515 520 525

Leu Asn Gly Ala Gln Ser Lys Asn Phe His Leu Arg Pro Arg Asp Leu
530 535 540

Ile Ser Asn Ile Asn Val Ile Val Leu Glu Leu Lys Gly Ser Glu Thr
545 550 555 560

Thr Phe Met Cys Glu Tyr Ala Asp Glu Thr Ala Thr Ile Val Glu Phe
565 570 575

Leu Asn Arg Trp Ile Thr Phe Ala Gln Ser Ile Ile Ser Thr Leu Thr
580 585 590

<210> 290
<211> 1776
<212> DNA
<213> Artificial Sequence

<220>
<223> DP47GS Fab HC-Fc knob (LALA P329G)-IL-2 qm

<400> 290
gaggtgcaat tggtggagtc tgggggaggc ttggtacagc ctgggggggtc cctgagactc 60
tcctgtgcag cctccggatt cacctttagc agttatgcca tgagctgggt ccgccaggct 120
ccagggaagg ggctggagtg ggtctcagct attagtggta gtggtggttag cacatactac 180
gcagactccg tgaagggccg gttcaccatc tccagagaca attccaagaa cacgctgtat 240
ctgcagatga acagcctgag agccgaggac acggccgtat attactgtgc gaaaggcagc 300
ggatttgact actggggcca aggaaccctg gtcaccgtct cgagtgctag caccaagggc 360
ccatcggtct tccccctggc accctcctcc aagagcacct ctgggggcac agcggccctg 420
ggctgcctgg tcaaggacta cttccccgaa ccggtgacgg tgtcgtggaa ctcaggcgcc 480
ctgaccagcg gcgtgcacac cttcccggtc gtctacagt cctcaggact ctactccctc 540

eolf-seql.txt

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agcagcgtgg tgaccgtgcc ctccagcagc ttgggcaccc agacctacat ctgcaacgtg      600
aatcacaagc ccagcaacac caaggtggac aagaaagttg agcccaaadc ttgtgacaaa      660
actcacacat gcccaccgtg cccagcacct gaagctgcag ggggaccgtc agtcttcctc      720
ttcccccaa aaccaagga caccctcatg atctcccga cccctgaggt cacatgcgtg      780
gtggtggacg tgagccacga agaccctgag gtcaagttca actggtacgt ggacggcgtg      840
gaggtgcata atgccaagac aaagccgcgg gaggagcagt acaacagcac gtaccgtgtg      900
gtcagcgtcc tcaccgtcct gcaccaggac tggctgaatg gcaaggagta caagtgaag      960
gtctccaaca aagccctcgg cgtcccatc gagaaaacca tctccaaagc caaagggcag     1020
ccccgagaac cacaggtgta caccctgccc ccatgccggg atgagctgac caagaaccag     1080
gtcagcctgt ggtgcctggt caaaggcttc tatcccagcg acatcgccgt ggagtgggag     1140
agcaatgggc agccggagaa caactacaag accacgcctc ccgtgctgga ctccgacggc     1200
tccttcttcc tctacagcaa gctcaccgtg gacaagagca ggtggcagca ggggaacgtc     1260
ttctcatgct ccgtgatgca tgaggctctg cacaaccact acacgcagaa gagcctctcc     1320
ctgtctccgg gtggcggcgg aggtccgga ggcggaggtt ctggcggagg tggctccgca     1380
cctgcctcaa gttctacaaa gaaaacacag ctacaactgg agcatttact gctggattta     1440
cagatgattt tgaatggaat taataattac aagaatccca aactcaccag gatgctcaca     1500
gccaagtttg ccatgccccaa gaaggccaca gaactgaaac atcttcagtg tctagaagaa     1560
gaactcaaac ctctggagga agtgctaaat ggcgctcaaa gcaaaaactt tacttaaga     1620
cccagggact taatcagcaa tatcaacgta atagttctgg aactaaaggg atctgaaaca     1680
acattcatgt gtgaatatgc tgatgagaca gcaaccattg tagaatttct gaacagatgg     1740
attacctttg cccaaagcat catctcaaca ctgact                                1776

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<210> 291
 <211> 592
 <212> PRT
 <213> Artificial Sequence

<220>
 <223> DP47GS Fab HC-Fc knob (LALA P329G)-IL-2 wt (2)
 <400> 291

Glu Val Gln Leu Leu Glu Ser Gly Gly Gly Leu Val Gln Pro Gly Gly
 1 5 10 15

Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Phe Thr Phe Ser Ser Tyr
 20 25 30

Ala Met Ser Trp Val Arg Gln Ala Pro Gly Lys Gly Leu Glu Trp Val
 35 40 45

Ser Ala Ile Ser Gly Ser Gly Gly Ser Thr Tyr Tyr Ala Asp Ser Val
 50 55 60

eolf-seql.txt

Lys Gly Arg Phe Thr Ile Ser Arg Asp Asn Ser Lys Asn Thr Leu Tyr
 65 70 75 80
 Leu Gln Met Asn Ser Leu Arg Ala Glu Asp Thr Ala Val Tyr Tyr Cys
 85 90 95
 Ala Lys Gly Ser Gly Phe Asp Tyr Trp Gly Gln Gly Thr Leu Val Thr
 100 105 110
 Val Ser Ser Ala Ser Thr Lys Gly Pro Ser Val Phe Pro Leu Ala Pro
 115 120 125
 Ser Ser Lys Ser Thr Ser Gly Gly Thr Ala Ala Leu Gly Cys Leu Val
 130 135 140
 Lys Asp Tyr Phe Pro Glu Pro Val Thr Val Ser Trp Asn Ser Gly Ala
 145 150 155 160
 Leu Thr Ser Gly Val His Thr Phe Pro Ala Val Leu Gln Ser Ser Gly
 165 170 175
 Leu Tyr Ser Leu Ser Ser Val Val Thr Val Pro Ser Ser Ser Leu Gly
 180 185 190
 Thr Gln Thr Tyr Ile Cys Asn Val Asn His Lys Pro Ser Asn Thr Lys
 195 200 205
 Val Asp Lys Lys Val Glu Pro Lys Ser Cys Asp Lys Thr His Thr Cys
 210 215 220
 Pro Pro Cys Pro Ala Pro Glu Ala Ala 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 Gly Ala Pro Ile Glu Lys Thr Ile Ser Lys
 325 330 335

eolf-seql.txt

Ala Lys Gly Gln Pro Arg Glu Pro Gln Val Tyr Thr Leu Pro Pro Cys
340 345 350

Arg Asp Glu Leu Thr Lys Asn Gln Val Ser Leu Trp 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 Gly Gly Gly Gly
435 440 445

Ser Gly Gly Gly Gly Ser Gly Gly Gly Gly Ser Ala Pro Thr Ser Ser
450 455 460

Ser Thr Lys Lys Thr Gln Leu Gln Leu Glu His Leu Leu Leu Asp Leu
465 470 475 480

Gln Met Ile Leu Asn Gly Ile Asn Asn Tyr Lys Asn Pro Lys Leu Thr
485 490 495

Arg Met Leu Thr Phe Lys Phe Tyr Met Pro Lys Lys Ala Thr Glu Leu
500 505 510

Lys His Leu Gln Cys Leu Glu Glu Glu Leu Lys Pro Leu Glu Glu Val
515 520 525

Leu Asn Leu Ala Gln Ser Lys Asn Phe His Leu Arg Pro Arg Asp Leu
530 535 540

Ile Ser Asn Ile Asn Val Ile Val Leu Glu Leu Lys Gly Ser Glu Thr
545 550 555 560

Thr Phe Met Cys Glu Tyr Ala Asp Glu Thr Ala Thr Ile Val Glu Phe
565 570 575

Leu Asn Arg Trp Ile Thr Phe Ala Gln Ser Ile Ile Ser Thr Leu Thr
580 585 590

<210> 292
<211> 1776

eolf-seql.txt

<212> DNA
 <213> Artificial Sequence

<220>

<223> DP47GS Fab HC-Fc knob (LALA P329G)-IL-2 wt (2)

<400> 292

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gaggtgcaat tgttggagtc tgggggaggc ttggtacagc ctgggggggtc cctgagactc      60
tcctgtgcag cctccggatt cacctttagc agttatgcc a tgagctgggt ccgccaggct      120
ccagggaagg ggctggagtg ggtctcagct attagtggta gtggtggtag cacatactac      180
gcagactccg tgaagggccg gttcaccatc tccagagaca attccaagaa cacgctgtat      240
ctgcagatga acagcctgag agccgaggac acggccgtat attactgtgc gaaaggcagc      300
ggatttgact actggggcca aggaaccctg gtcaccgtct cgagtgctag caccaagggc      360
ccatcgggtct tccccctggc accctcctcc aagagcacct ctggggggcac agcgggccctg      420
ggctgcctgg tcaaggacta cttccccgaa ccggtgacgg tgtcgtggaa ctcaggcgcc      480
ctgaccagcg gcgtgcacac cttcccggct gtcctacagt cctcaggact ctactccctc      540
agcagcgtgg tgaccgtgcc ctccagcagc ttgggcaccc agacctacat ctgcaacgtg      600
aatcacaagc ccagcaacac caaggtggac aagaaagttg agcccaaadc ttgtgacaaa      660
actcacacat gccaccgtg cccagcacct gaagctgcag ggggaccgtc agtcttcctc      720
ttcccccaa aaccaagga caccctcatg atctcccga cccctgaggt cacatgcgtg      780
gtggtggacg tgagccacga agaccctgag gtcaagttca actggtacgt ggacggcgtg      840
gaggtgcata atgccaagac aaagccgagg gaggagcagt acaacagcac gtaccgtgtg      900
gtcagcgtcc tcaccgtcct gcaccaggac tggctgaatg gcaaggagta caagtgaag      960
gtctccaaca aagccctcgg cgccccatc gagaaaacca tctccaaagc caaagggcag     1020
ccccgagaac cacaggtgta caccctgccc ccatgccggg atgagctgac caagaaccag     1080
gtcagcctgt ggtgcctggt caaaggcttc tatccagcg acatcgccgt ggagtgggag     1140
agcaatgggc agccggagaa caactacaag accacgcctc ccgtgctgga ctccgacggc     1200
tccttcttcc tctacagcaa gctcaccgtg gacaagagca ggtggcagca ggggaacgtc     1260
ttctcatgct ccgtgatgca tgaggctctg cacaaccact acacgcagaa gagcctctcc     1320
ctgtctccgg gtggcggcgg aggctccgga ggcggaggtt ctggaggcgg aggctccgca     1380
cctacttcaa gttctacaaa gaaaacacag ctacaactgg agcatttact gctggattta     1440
cagatgattt tgaatggaat taataattac aagaatccca aactcaccag gatgctcaca     1500
tttaagtttt acatgccc aaaggccaca gaactgaaac atcttcagtg tctagaagaa     1560
gaactcaaac ctctggagga agtgctaaat ttagctcaaa gcaaaaactt tcaactaaga     1620
cccaggggact taatcagcaa tatcaacgta atagtcttg aactaaagg atctgaaaca     1680
acattcatgt gtgaatatgc tgatgagaca gcaaccattg tagaatttct gaacagatgg     1740
attacctttg cccaaagcat catctcaaca ctgact                                  1776

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eolf-seql.txt

<210> 293
 <211> 108
 <212> PRT
 <213> Artificial Sequence

<220>
 <223> CH1A1A 98/99 2F1; VL

<400> 293

Asp Ile Gln Met Thr Gln Ser Pro Ser Ser Leu Ser Ala Ser Val Gly
 1 5 10 15

Asp Arg Val Thr Ile Thr Cys Lys Ala Ser Ala Ala Val Gly Thr Tyr
 20 25 30

Val Ala Trp Tyr Gln Gln Lys Pro Gly Lys Ala Pro Lys Leu Leu Ile
 35 40 45

Tyr Ser Ala Ser Tyr Arg Lys Arg Gly Val Pro Ser Arg Phe Ser Gly
 50 55 60

Ser Gly Ser Gly Thr Asp Phe Thr Leu Thr Ile Ser Ser Leu Gln Pro
 65 70 75 80

Glu Asp Phe Ala Thr Tyr Tyr Cys His Gln Tyr Tyr Thr Tyr Pro Leu
 85 90 95

Phe Thr Phe Gly Gln Gly Thr Lys Leu Glu Ile Lys
 100 105

<210> 294
 <211> 324
 <212> DNA
 <213> Artificial Sequence

<220>
 <223> CH1A1A 98/99 2F1; VL

<400> 294
 gatatccaga tgaccagtc tccatcctcc ctgtctgcat ctgtgggaga cagagtcacc 60
 atcacttgca aggccagtc ggctgtgggt acgtatgttg cgtgggtatca gcagaaacca 120
 gggaaagcac ctaagtcct gatctattcg gcatcctacc gcaaaagggg agtcccatca 180
 aggttcagtg gcagtggatc tgggacagat ttcactctca ccatcagcag tctgcaacct 240
 gaagatttcg caacttacta ctgtcaccaa tattacacct atcctctatt cacgtttggc 300
 cagggcacca agctcgagat caag 324

<210> 295
 <211> 121
 <212> PRT
 <213> Artificial Sequence

<220>
 <223> CH1A1A 98/99 2F1; VH

eolf-seql.txt

<400> 295

Gln Val Gln Leu Val Gln Ser Gly Ala Glu Val Lys Lys Pro Gly Ala
1 5 10 15

Ser Val Lys Val Ser Cys Lys Ala Ser Gly Tyr Thr Phe Thr Glu Phe
20 25 30

Gly Met Asn Trp Val Arg Gln Ala Pro Gly Gln Gly Leu Glu Trp Met
35 40 45

Gly Trp Ile Asn Thr Lys Thr Gly Glu Ala Thr Tyr Val Glu Glu Phe
50 55 60

Lys Gly Arg Val Thr Phe Thr Thr Asp Thr Ser Thr Ser Thr Ala Tyr
65 70 75 80

Met Glu Leu Arg Ser Leu Arg Ser Asp Asp Thr Ala Val Tyr Tyr Cys
85 90 95

Ala Arg Trp Asp Phe Ala Tyr Tyr Val Glu Ala Met Asp Tyr Trp Gly
100 105 110

Gln Gly Thr Thr Val Thr Val Ser Ser
115 120

<210> 296

<211> 363

<212> DNA

<213> Artificial Sequence

<220>

<223> CH1A1A 98/99 2F1; VH

<400> 296

caggtgcagc tgggtgcagtc tggcgccgaa gtgaagaaac ctggagctag tgtgaagggtg	60
tcctgcaagg ccagcggcta caccttcacc gagttcggca tgaactgggt ccgacaggct	120
ccaggccagg gcctcgaatg gatgggctgg atcaacacca agaccggcga ggccacctac	180
gtggaagagt tcaagggcag agtgaccttc accacggaca ccagcaccag caccgcctac	240
atggaactgc ggagcctgag aagcgacgac accgccgtgt actactgcgc cagatgggac	300
ttcgcctatt acgtggaagc catggactac tggggccagg gcaccaccgt gaccgtgtct	360
agc	363

<210> 297

<211> 108

<212> PRT

<213> Artificial Sequence

<220>

<223> DP47GS VL

<400> 297

eolf-seql.txt

Glu Ile Val Leu Thr Gln Ser Pro Gly Thr Leu Ser Leu Ser Pro Gly
1 5 10 15

Glu Arg Ala Thr Leu Ser Cys Arg Ala Ser Gln Ser Val Ser Ser Ser
20 25 30

Tyr Leu Ala Trp Tyr Gln Gln Lys Pro Gly Gln Ala Pro Arg Leu Leu
35 40 45

Ile Tyr Gly Ala Ser Ser Arg Ala Thr Gly Ile Pro Asp Arg Phe Ser
50 55 60

Gly Ser Gly Ser Gly Thr Asp Phe Thr Leu Thr Ile Ser Arg Leu Glu
65 70 75 80

Pro Glu Asp Phe Ala Val Tyr Tyr Cys Gln Gln Tyr Gly Ser Ser Pro
85 90 95

Leu Thr Phe Gly Gln Gly Thr Lys Val Glu Ile Lys
100 105

<210> 298
<211> 324
<212> DNA
<213> Artificial Sequence

<220>
<223> DP47GS VL

<400> 298
gaaatcgtgt taacgcagtc tccaggcacc ctgtctttgt ctccagggga aagagccacc 60
ctctcttgca gggccagtca gagtgttagc agcagctact tagcctggta ccagcagaaa 120
cctggccagg ctcccaggct cctcatctat ggagcatcca gcagggccac tggcatccca 180
gacaggttca gtggcagtgg atccgggaca gacttcactc tcaccatcag cagactggag 240
cctgaagatt ttgcagtgtt ttactgtcag cagtatggta gctcaccgct gacgttcggc 300
caggggacca aagtggaaat caaa 324

<210> 299
<211> 115
<212> PRT
<213> Artificial Sequence

<220>
<223> DP47GS VH

<400> 299

Glu Val Gln Leu Leu Glu Ser Gly Gly Gly Leu Val Gln Pro Gly Gly
1 5 10 15

Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Phe Thr Phe Ser Ser Tyr
20 25 30

eolf-seql.txt

Ala Met Ser Trp Val Arg Gln Ala Pro Gly Lys Gly Leu Glu Trp Val
35 40 45

Ser Ala Ile Ser Gly Ser Gly Gly Ser Thr Tyr Tyr Ala Asp Ser Val
50 55 60

Lys Gly Arg Phe Thr Ile Ser Arg Asp Asn Ser Lys Asn Thr Leu Tyr
65 70 75 80

Leu Gln Met Asn Ser Leu Arg Ala Glu Asp Thr Ala Val Tyr Tyr Cys
85 90 95

Ala Lys Gly Ser Gly Phe Asp Tyr Trp Gly Gln Gly Thr Leu Val Thr
100 105 110

Val Ser Ser
115

<210> 300
<211> 345
<212> DNA
<213> Artificial Sequence

<220>
<223> DP47GS VH

<400> 300
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tcctgtgcag cctccggatt cacctttagc agttatgcc a tgagctgggt ccgccaggct 120
ccagggaagg ggctggagtg ggtctcagct attagtggta gtggtggtag cacatactac 180
gcagactccg tgaagggccg gttcaccatc tccagagaca attccaagaa cacgctgtat 240
ctgcagatga acagcctgag agccgaggac acggccgtat attactgtgc gaaaggcagc 300
ggatttgact actggggcca aggaaccctg gtcaccgtct cgagt 345