

MASKED IL-12 CYTOKINES AND THEIR CLEAVAGE PRODUCTS**CROSS-REFERENCE TO RELATED APPLICATIONS**

This application claims the priority benefit of U.S. Provisional Application Serial Nos. 63/003,842, filed
5 April 1, 2020; 63/118,579, filed November 25, 2020; and 63/127,893, filed December 18, 2020; each of
which is incorporated herein by reference in its entirety.

SUBMISSION OF SEQUENCE LISTING ON ASCII TEXT FILE

The content of the following submission on ASCII text file is incorporated herein by reference in its
10 entirety: a computer readable form (CRF) of the Sequence Listing (file name:
737762002840SEQLIST.TXT, date recorded: March 26, 2021, size: 1,000 KB).

FIELD

This invention relates to masked IL-12 cytokines and methods related to the use and manufacture of the
15 same. This invention also relates to cleavage products of said masked IL-12 cytokines, and methods related
to the use of the same.

BACKGROUND

20 Cancer is the second leading cause of death in the United States, accounting for more deaths than the next
five leading causes (chronic respiratory disease, stroke, accidents, Alzheimer's disease and diabetes).
While great strides have been made especially with targeted therapies, there remains a great deal of work
to do in this space. Immunotherapy and a branch of this field, immuno-oncology, is creating viable and
exciting therapeutic options for treating malignancies. Specifically, it is now recognized that one hallmark
25 of cancer is immune evasion and significant efforts have identified targets and developed therapies to these
targets to reactivate the immune system to recognize and treat cancer.

Cytokines can be classified in a variety of ways, such as based on their three-dimensional structure. Some
cytokines are classified as being heterodimeric. Examples of heterodimeric cytokines include IL-12 and
30 IL-23. IL-12 cytokine is a heterodimer comprising p35 and p40 sub-units.

Cytokine therapy is an effective strategy for stimulating the immune system to induce anti-tumor cytotoxicity. In particular, aldesleukin, a recombinant form of interleukin-2 (IL-2), has been approved by the FDA for the treatment of metastatic renal cell carcinoma and melanoma. Unfortunately, cytokines that are administered to patients generally have a very short half-life, thereby requiring frequent dosing. For instance, the product label of aldesleukin, marketed under the brand name Proleukin, states that the drug was shown to have a half-life of 85 minutes in patients who received a 5- minute intravenous (IV) infusion. In addition, administration of high doses of cytokine can cause adverse health outcomes, such as vascular leakage, through systemic immune activation. These findings illustrate the need for developing IL-2 cytokine therapeutics that effectively target tumors without the side effects associated with systemic immune activation.

Provided herein are masked IL-12 cytokines, cleavage products of said masked IL-12 cytokines, and compositions thereof and methods of use thereof for addressing this need.

SUMMARY

The disclosed invention relates to IL-12 cytokines or functional fragments thereof that are engineered to be masked by a masking moiety at one or more receptor binding site(s) of the IL-12 cytokine or functional fragment thereof. The IL-12 cytokines are engineered to be activatable by a protease at a target site, such as in a tumor microenvironment, by including a proteolytically cleavable linker. In the masked cytokine construct, the masking moiety reduces or prevents binding of the IL-12 cytokine or functional fragment thereof to its cognate receptor. Upon proteolytic cleavage of the cleavable linker at the target site, the IL-12 cytokine or functional fragment thereof becomes activated, which renders it capable or more capable of binding to its cognate receptor.

Provided herein is a masked IL-12 cytokine comprising a protein heterodimer comprising:

a first polypeptide chain comprising:

N' HL1-L1-MM C'

and a second polypeptide chain comprising:

N' HL2-L2-C C'

where HL1 is a first half life extension domain, L1 is a first linker, MM is a masking moiety, HL2 is a second half life extension domain, L2 is a second linker, and C is an IL-12 cytokine or functional fragment thereof,

wherein the first half-life extension domain is associated with the second half-life extension domain, and

wherein one of the first linker or the second linker comprises a proteolytically cleavable peptide.

In some embodiments, the IL-12 polypeptide or functional fragment thereof comprises an IL-12p40 polypeptide or functional fragment thereof covalently linked to an IL-12p35 polypeptide or functional fragment thereof.

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In some embodiments, the IL-12p40 – IL-12p35 linker is between 5 and 20 amino acids in length.

In some embodiments, the IL-12p40 – IL-12p35 linker is rich in amino acid residues G and S.

10 In some embodiments, the IL-12p40 – IL-12p35 linker comprises SEQ ID NO: 3.

In some embodiments, the IL-12p40 polypeptide comprises SEQ ID NO: 1 or an amino acid sequence having at least one amino acid modification as compared to the amino acid sequence of SEQ ID NO: 1.

15 In some embodiments, the IL-12p40 polypeptide comprises SEQ ID NO: 1.

In some embodiments, the IL-12p40 polypeptide comprises at least one amino acid modification to the GAG-binding domain (KSKREKKDRV) as compared to the amino acid sequence of SEQ ID NO: 1.

In some embodiments, the IL-12p40 polypeptide comprises SEQ ID NO: 57.

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In some embodiments, the IL-12p40 polypeptide comprises SEQ ID NO: 58.

In some embodiments, the IL-12p40 polypeptide comprises an amino acid sequence having one or more cysteine substitution mutations as compared to the amino acid sequence of SEQ ID NO: 1.

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In some embodiments, the IL-12p40 polypeptide comprises SEQ ID NO: 59.

In some embodiments, the IL-12p40 polypeptide comprises SEQ ID NO: 60.

30 In some embodiments, the IL-12p35 polypeptide comprises SEQ ID NO: 2 or an amino acid sequence having at least one amino acid modification as compared to the amino acid sequence of SEQ ID NO: 2.

In some embodiments, the IL-12p35 polypeptide comprises SEQ ID NO: 2.

35 In some embodiments, the IL-12 cytokine or functional fragment thereof comprises SEQ ID NO: 4.

In some embodiments, the IL-12 cytokine or functional fragment thereof comprises SEQ ID NO: 61.

In some embodiments, the IL-12 cytokine or functional fragment thereof comprises SEQ ID NO: 62.

In some embodiments, the IL-12 cytokine or functional fragment thereof comprises SEQ ID NO: 63.

In some embodiments, the IL-12 cytokine or functional fragment thereof comprises SEQ ID NO: 64.

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In some embodiments, the masking moiety comprises an IL-12 cytokine receptor, or a subunit or functional fragment thereof.

10 In some embodiments, the masking moiety comprises the extracellular domain of human IL-12R β 1 or a fragment, portion, or variant thereof that retains or otherwise demonstrates an affinity to IL-12.

In some embodiments, the masking moiety comprises residues 24 to 237 of human IL-12R β 1, namely a sequence having SEQ ID NO: 5.

15 In some embodiments, the masking moiety comprises residues 24 to 545 of human IL-12R β 1, namely a sequence having SEQ ID NO: 6.

In some embodiments, the masking moiety comprises the extracellular domain of human IL-12R β 2 or a fragment, portion, or variant thereof that retains or otherwise demonstrates an affinity to IL-12.

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In some embodiments, the masking moiety comprises residues 24 to 212 of human IL-12R β 2, namely a sequence having SEQ ID NO: 7.

25 In some embodiments, the masking moiety comprises residues 24 to 222 of human IL-12R β 2, namely a sequence having SEQ ID NO: 8, or the masking moiety comprises residues 24 to 227 of human IL-12R β 2, namely a sequence having SEQ ID NO: 11.

In some embodiments, the masking moiety comprises residues 24 to 319 of human IL-12R β 2, namely a sequence having SEQ ID NO: 9.

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In some embodiments, the masking moiety comprises at least one amino acid modification as compared to the sequence of SEQ ID NO: 9, optionally wherein said modifications are cysteine substitution mutations.

In some embodiments, the masking moiety comprises SEQ ID NO: 65.

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In some embodiments, the masking moiety comprises residues 24 to 622 of human IL-12R β 2, namely a sequence having SEQ ID NO: 10.

In some embodiments, the cleavable peptide is from 6 to 10 amino acids in length.

In some embodiments, the cleavable peptide comprises an amino acid sequence of SEQ ID NO: 15.

5 In some embodiments, the cleavable peptide comprises an amino acid sequence of SEQ ID NO: 41.

In some embodiments, the cleavable peptide comprises an amino acid sequence of SEQ ID NO: 42.

In some embodiments, the cleavable peptide comprises an amino acid sequence of SEQ ID NO: 43.

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In some embodiments, the cleavable peptide comprises an amino acid sequence of SEQ ID NO: 44.

In some embodiments, the cleavable peptide comprises an amino acid sequence of SEQ ID NO: 45.

15 In some embodiments, the first polypeptide chain comprises:

N' **HL1-non-cleavable L1-MM** C'

and the second polypeptide chain comprises

N' **HL2-cleavable L2-C** C'

20 In some embodiments, the non-cleavable linker is between 3 and 18 amino acids in length.

In some embodiments, the non-cleavable linker is between 3 and 15 amino acids in length.

In some embodiments, the non-cleavable linker is rich in amino acid residues G and S.

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In some embodiments, the non-cleavable linker includes $[(G)_nS]$, where $n=4$ or 5 .

In some embodiments, the non-cleavable linker comprises an amino acid sequence as shown in SEQ ID NO: 12.

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In some embodiments, the non-cleavable linker comprises an amino acid sequence as shown in SEQ ID NO: 13.

In some embodiments, the non-cleavable linker comprises an amino acid sequence as shown in SEQ ID NO: 14.

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In some embodiments, the non-cleavable linker comprises an amino acid sequence as shown in SEQ ID NO: 54 (GGSGGSGGSGGSGGSSGP).

In some embodiments, the non-cleavable linker comprises an amino acid sequence as shown in SEQ ID NO: 55 (PGGSGP).

- 5 In some embodiments, the non-cleavable linker comprises an amino acid sequence as shown in SEQ ID NO: 56 (GGSPG).

In some embodiments, the cleavable linker comprises a proteolytically cleavable peptide (CP) flanked on both sides by a spacer domain (SD):

10 SD1-CP-SD2

where SD1 and SD2 are different, such that the first polypeptide chain comprises:

N' HL1-non-cleavable L1-MM C'

and the second polypeptide chain comprises:

N' HL2- SD1-CP-SD2 -C C'

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In some embodiments, the first spacer domain (SD1) is between 3 and 10 amino acids in length.

In some embodiments, SD1 comprises SEQ ID NO: 16.

- 20 In some embodiments, SD1 comprises SEQ ID NO: 17.

In some embodiments, the second spacer domain (SD2) is between 3 and 6 amino acids in length.

In some embodiments, SD2 comprises SEQ ID NO: 18.

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In some embodiments, the proteolytically cleavable linker comprises SD1-CP-SD2 where SD1 is a first spacer domain, CP is a cleavable peptide and SD2 is a second spacer domain, and wherein CP has an amino acid sequence as shown in SEQ ID NO: 44 and SD2 has an amino acid sequence as shown in SEQ ID NO: 18.

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In some embodiments, the proteolytically cleavable linker comprises SD1-CP-SD2 where SD1 is a first spacer domain, CP is a cleavable peptide and SD2 is a second spacer domain, and wherein CP has an amino acid sequence as shown in SEQ ID NO: 45 and SD2 has an amino acid sequence as shown in SEQ ID NO: 18.

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In some embodiments, the cleavable linker comprises SEQ ID NO: 19.

In some embodiments, the cleavable linker comprises SEQ ID NO: 20.

In some embodiments, the cleavable linker comprises SEQ ID NO: 46. (GGSGGSMPYDLYHPSPG)

- 5 In some embodiments, the cleavable linker comprises SEQ ID NO: 47.
(GGSGSGGSMPYDLYHPSPG)

In some embodiments, the cleavable linker comprises SEQ ID NO: 48. (GGSGGSDSGGFMLTSGP)

- 10 In some embodiments, the cleavable linker comprises SEQ ID NO: 49.
(GGSGGSDSGGFMLTSGP)

In some embodiments, the cleavable linker comprises SEQ ID NO: 50. (GGSGGSRAAAVKSPSPG)

- 15 In some embodiments, the cleavable linker comprises SEQ ID NO: 51.
(GGSGGSRAAAVKSPSPG)

In some embodiments, the cleavable linker comprises SEQ ID NO: 52. (GGSGGSISSGLLSGRSSGP)

- 20 In some embodiments, the cleavable linker comprises SEQ ID NO: 53.
(GGSGGSISSGLLSGRSSGP)

- In some embodiments, the first half-life extension domain comprises a first IgG1 Fc domain or a
fragment thereof and the second half-life extension domain comprises a second IgG1 Fc domain or a
25 fragment thereof.

In some embodiments, the first and/ or second Fc domains each contain one or more modifications that
promote the non-covalent association of the first and the second half-life extension domains.

- 30 In some embodiments, the first half-life extension domain comprises SEQ ID NO: 25 (Y349C; T366S;
L38A; Y407V; and N297A) and the second half-life extension domain comprises SEQ ID NO 26 (S354C,
T366W and N297A).

- In some embodiments, the first half-life extension domain comprises SEQ ID NO: 27 (Y349C; T366S;
35 L38A; Y407V, N297A and I253A) and the second half-life extension domain comprises SEQ ID NO: 28
(S354C, T366W, N297A and I253A).

In some embodiments, the first polypeptide chain comprises an amino acid sequence of SEQ ID NO: 34 and a second polypeptide chain comprises an amino acid sequence of SEQ ID NO: 40.

5 Provided herein is a cleavage product capable of binding to IL-12R, the cleavage product comprising an IL-12 cytokine or functional fragment thereof, preparable by proteolytic cleavage of the cleavable peptide in a masked IL-12 cytokine as defined in any one of the statements or embodiments described herein.

10 Provided herein is a cleavage product of a masked IL-12 cytokine, where the cleavage product is capable of binding to IL-12R, the cleavage product comprising a polypeptide comprising:
PCP-SD2-C

wherein PCP is a portion of a proteolytically cleavable peptide; SD2 is a spacer domain; and C is an IL-12 cytokine or functional fragment thereof.

15 In some embodiments, PCP is a portion of a proteolytically cleavable peptide as described herein.

In some embodiments, SD2 is a spacer domain as described herein.

In some embodiments, C is an IL-12 cytokine or functional fragment thereof as described herein.

20 In some embodiments, the cleavage product comprises an amino acid sequence having about or at least about 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% sequence identity to an amino acid sequence selected from the group consisting of SEQ ID NO: 29.

25 In some embodiments, the cleavage product comprises an amino acid sequence of SEQ ID NO: 29.

Provided herein is a nucleic acid encoding any one of the masked IL-12 cytokines described herein.

30 Provided herein is a nucleic acid encoding one of the chains of any one of the masked IL-12 cytokines described herein.

Provided herein is a vector comprising a nucleic acid described herein.

Provided herein is a vector comprising a nucleic acid encoding a masked IL-12 cytokine described herein.

35 Provided herein is a vector comprising a nucleic acid encoding one of the chains of a masked IL-12 cytokine described herein.

Provided herein is a host cell comprising a nucleic acid described herein.

In one embodiment, the host cell is a HEK cell. In another embodiment, the host cell is a CHO cell.

Provided herein is a composition comprising any one of the masked IL-12 cytokines described herein.

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Provided herein is a pharmaceutical composition comprising any one of the masked IL-12 cytokines described herein and a pharmaceutically acceptable carrier.

In some embodiments, the pharmaceutical composition is in single unit dosage form.

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In some embodiments, the pharmaceutical composition is formulated for intravenous administration and is in single unit dosage form.

In some embodiments, the pharmaceutical composition is formulated for injection and is in single unit dosage form.

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In some embodiments, the pharmaceutical composition is a liquid and is in single unit dosage form.

Provided herein is a kit comprising a masked IL-12 cytokine as described herein, or a composition described herein, or a pharmaceutical composition described herein.

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Provided herein is a method of producing a masked IL-12 cytokine as described herein comprising culturing a host cell described herein under a condition that produces the masked IL-12 cytokine.

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Provided herein is a nucleic acid encoding a cleavage product described herein.

Provided herein is a composition comprising a cleavage product described herein.

Provided herein is a pharmaceutical composition comprising a cleavage product described herein, and a pharmaceutically acceptable carrier.

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Provided herein is a masked IL-12 cytokine described herein for use in medicine.

Provided herein is a cleavage product described herein for use in medicine.

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Provided herein is a method of treating or preventing cancer in a subject, the method comprising administering to the subject an effective amount of a masked IL-12 cytokine described herein.

Provided herein is a method of treating or preventing cancer in a subject, the method comprising administering to the subject an effective amount of a composition described herein.

5 Provided herein is a method of treating or preventing cancer in a subject, the method comprising administering to the subject an effective amount of a pharmaceutical composition described herein.

10 Provided herein is a method of treating or preventing cancer in a subject, the method comprising administering to the subject an effective amount of a masked IL-12 cytokine described herein, whereby the masked cytokine is proteolytically cleaved *in vivo* to produce a cleavage product described herein.

Provided herein is a method of treating or preventing cancer in a subject, the method comprising a step of producing a cleavage product *in vivo* that is capable of binding to its cognate receptor, wherein the cleavage product is described herein.

15 In some embodiments, the cancer is a solid tumor.

Provided herein is a masked IL-12 cytokine described herein for use in treating or preventing cancer.

20 Provided herein is a masked IL-12 cytokine described herein for use in a method of treating or preventing cancer, the method comprising administering to the subject an effective amount of the masked IL-12 cytokine, whereby the masked cytokine is proteolytically cleaved *in vivo* to produce a cleavage product as described herein.

In some embodiments, the cancer is a solid tumor.

25 Provided herein is a cleavage product described herein for use in treating or preventing cancer.

30 Provided herein is a cleavage product described herein for use in a method of treating or preventing cancer, the method comprising a step of administering a masked cytokine described herein to a patient, thereby producing the cleavage product by proteolytic cleavage of the masked cytokine *in vivo*.

Provided herein is a cleavage product described herein for use in a method of treating or preventing cancer in a subject, the method comprising a step of producing the cleavage product by *in vivo* proteolytic cleavage from a masked cytokine described herein that has been administered to the subject.

35 In some embodiments, the cancer is a solid tumor.

Provided herein is a pharmaceutical composition described herein for use in treating or preventing cancer.

In some embodiments, the cancer is a solid tumor.

5 A BRIEF DESCRIPTION OF THE DRAWINGS

FIG. 1 shows the structure of exemplary embodiments of a masked cytokine that includes a masking moiety, a cytokine or functional fragment thereof ("cytokine"), a half-life extension domain, and a first linker that includes a first cleavable peptide ("1CP"), a first N-terminal spacer domain ("1NSD"), and a first C-terminal spacer domain ("1CSD"). These exemplary embodiments also include a second linker that includes a second cleavable peptide ("2CP"), a second N-terminal spacer domain ("2NSD"), and a second C-terminal spacer domain ("2CSD"). As shown by the arrows, while the exemplary embodiments shows the masking moiety linked to the first linker, and the cytokine or functional fragment thereof is linked to the first linker and the second linker, the masking moiety and the cytokine or functional fragment thereof can be interchanged such that the cytokine or functional fragment thereof is linked to the first linker, and the masking moiety is linked to the first linker and the second linker. **FIG. 1** shows the structure of an exemplary embodiment of a masked cytokine as a monomer.

FIG. 2 shows the structure of an exemplary embodiment of a masked cytokine that includes a masking moiety, a cytokine or functional fragment thereof ("cytokine"), a first half-life extension domain, and a second half-life extension domain. The exemplary embodiment shown in **FIG. 2** also includes a first linker that includes a first cleavable peptide ("1CP"), a first N-terminal spacer domain ("1NSD"), and a first C-terminal spacer domain ("1CSD"), and a second linker that includes a second cleavable peptide ("2CP"), a second N-terminal spacer domain ("2NSD"), and a second C-terminal spacer domain ("2CSD"). The exemplary first and second half-life extension domains include "knobs into holes" modifications that promote the association of the first half-life extension domain with the second half-life extension domain, as shown by the "hole" in the first half-life extension domain and the "knob" in the second half-life extension domain. The first half-life extension domain and the second half-life extension domain are also shown as associating, at least in part, due to the formation of disulfide bonds. It is to be understood that although the "hole" is depicted as part of the first half-life extension domain (linked to the masking moiety) and the "knob" is depicted as part of the second half-life extension domain (linked to the cytokine), the "hole" and the "knob" can alternatively be included in the second half-life extension domain and the first half-life extension domain, respectively, so that the "hole" is a part of the second half-life extension domain (linked to the cytokine) and the "knob" is part of the first half-life extension domain (linked to masking moiety).

FIGs. 3A-3B show exemplary embodiments of masked cytokines prior to (left) and after (right) cleavage

by a protease, such as at the tumor microenvironment. **FIGs. 3A-3B** show exemplary embodiments of a masked IL-2 cytokine. Cleavage by a protease releases a masking moiety (e.g., IL-2R β , as shown in **FIG. 3B**), or releases an IL-2 (**FIG. 3A**).

FIG 4 shows SDS-PAGE analysis on flow-through (FT) samples (i.e., proteins that did not bind to the Protein A column) and the eluted (E) samples (i.e., proteins that bound to the Protein A column and were eluted from it) following production and purification of IL-2 constructs (AK304, AK305, AK307, AK308, AK309, AK310, AK311, AK312, AK313, AK314, and AK315).

FIGs. 5A-5D show results from SPR analysis that tested the binding of an exemplary masked IL-2 polypeptide constructs (AK168), or a rhIL2 control, to CD25-Fc. **FIG. 5A** shows the interaction between AK168 and CD25-Fc, **FIG. 5B** shows the interaction between AK168 activated with MMP and CD25-Fc, and **FIG. 5C** shows the interaction between a recombinant human IL-2 (rhIL-2) control and CD25-Fc. **FIG. 5D** provides a table summarizing the data obtained for the association constant (k_a), dissociation constant (k_d), equilibrium dissociation constant (K_D), as well as the Chi2 value and U-value for each interaction.

FIGs. 6A-6D shows results from SPR analysis that tested the binding of an exemplary masked IL-2 polypeptide constructs (AK111), or a rhIL-2 control, to CD122-Fc. **FIG. 6A** shows the interaction between AK111 and CD122-Fc, **FIG. 6B** shows the interaction between AK111 activated with protease and CD122-Fc, and **FIG. 6C** shows the interaction between a recombinant human IL-2 (rhIL-2) control and CD122-Fc. **FIG. 6D** provides a table summarizing the data obtained for the association constant (k_a), dissociation constant (k_d), equilibrium dissociation constant (K_D), as well as the Chi2 value and U-value for each interaction.

FIG. 7A shows an exemplary embodiment of a masked cytokines prior to (left) and after (right) cleavage by a protease, such as at the tumor microenvironment. **FIG. 7B** shows SDS-PAGE analysis of an exemplary masked IL-2 polypeptide construct that was incubated in the absence (left lane) or presence (right lane) of the MMP10 protease, which demonstrates the release of IL-2 from the Fc portion.

FIGs. 8A-8D show STAT5 activation (%) in PBMCs treated with the construct AK032, AK035, AK041, or rhIL-2 as a control. The levels of STAT5 activation (%) are shown for NK cells, CD8+ T cells, effector T cells (Teff), and regulatory T cells (Treg), as determined following incubation with rhIL-2 (**FIG. 8A**), AK032 (**FIG. 8B**), AK035 (**FIG. 8C**), or AK041 (**FIG. 8D**).

FIGs. 9A-9C show STAT5 activation (%) in PBMCs treated with the construct AK081 or AK032. The AK081 construct with and without prior exposure to MMP10 was tested. An isotype control as well as a

no IL-2 negative control was also tested. The levels of STAT5 activation (%) are shown for NK cells (FIG. 9A), CD8+ T cells (FIG. 9C), and CD4+ T cells (FIG. 9B).

FIGs. 10A-10D show the results from STAT5 activation studies in PBMCs using constructs AK081 and AK111, as well as controls that included an rhIL-2 and anti-RSV antibody. A no-treatment control was also tested. EC50 (pM) is also shown for the rhIL-2, AK081, and AK111 treatments. STAT5 activation (%) is shown for CD4+FoxP3+CD25+ cells (FIG. 10A), CD8+ cells (FIG. 10B), and CD4+FoxP3-CD25- cells (FIG. 10C). FIG. 10D provides EC50 (pM) and fold-change data for the AK081, AK111 constructs, as well as the rhIL-2 control.

FIGs. 11A-11D show the results from STAT5 activation studies in PBMCs using constructs AK167 and AK168, as well as controls that included an rhIL-2 and anti-RSV antibody. A no-treatment control was also tested. EC50 (pM) is also shown for the rhIL-2, AK167, and AK168 treatments. STAT5 activation (%) is shown for CD4+FoxP3+CD25+ cells (FIG. 11A), CD8+ cells (FIG. 11B), and CD4+FoxP3-CD25- cells (FIG. 11C). FIG. 11D provides EC50 (pM) and fold-change data for the AK167 and AK168 constructs, as well as the rhIL-2 control.

FIGs. 12A-12D show STAT5 activation (%) in PBMCs treated with the construct AK165 or AK166, or an isotype control or an IL-2-Fc control, that were (+ MMP10) or were not previously exposed to the MMP10 protease. The key as shown in FIG. 12A also applies to FIG. 12B, and the key as shown in FIG. 12C also applies to FIG. 12D. STAT5 activation (%) is shown for CD4+FoxP3+ T regulatory cells (FIG. 12A), CD4+FoxP3- T helper cells (FIG. 12B), CD8+ cytotoxic T cells (FIG. 12C), and CD56+ NK cells (FIG. 12D).

FIGs. 13A-13C show STAT5 activation (%) in PBMCs treated with the construct AK109 or AK110, or an isotype control or an IL-2-Fc control, that were (+ MMP10) or were not previously exposed to the MMP10 protease. The key as shown in FIG. 12B also applies to FIG. 13A. STAT5 activation (%) is shown for NK cells (FIG. 13A), CD8 cells (FIG. 13B), and CD4 cells (FIG. 13C).

FIGs. 14A-14D show the results from STAT5 activation studies in PBMCs using the constructs AK211, AK235, AK253, AK306, AK310, AK314, and AK316, as well as an rhIL-2 control. STAT5 activation (%) is shown for CD3+CD4+FoxP3+ cells (FIG. 14A), CD3+CD4+FoxP3- cells (FIG. 14B), and CD3+CD8+ cells (FIG. 14C). FIG. 14D provides EC50 data for each of the tested constructs as well as the rhIL-2 control.

FIGs. 15A-15D show the results from STAT5 activation studies in PBMCs using the constructs AK081, AK167, AK216, AK218, AK219, AK220, and AK223 that have been activated by protease, as well as an

rhIL-2 control. STAT5 activation (%) is shown for CD4+FoxP3+CD25+ regulatory T cells (**FIG. 15A**), CD4+FoxP3-CD25- cells (**FIG. 15B**), and CD8+ cells (**FIG. 15C**). **FIG. 15D** provides EC50 data for each of the tested constructs as well as the rhIL-2 control.

FIGs. 16A-16C show STAT5 activation (%) in PBMCs treated with the construct AK081, AK189, AK190, or AK210, or an anti-RSV control. The key as shown in **FIG. 16A** also applies to **FIGs. 16B** and **16C**. STAT5 activation (%) is shown for regulatory T cells (**FIG. 16A**), CD4 helper T cells (**FIG. 16B**), and CD8 cells (**FIG. 16C**).

FIGs. 17A-17C show STAT5 activation (%) in PBMCs treated with the construct AK167, AK191, AK192, or AK193, or an anti-RSV control. The key as shown in **FIG. 17A** also applies to **FIGs. 17B** and **17C**. STAT5 activation (%) is shown for regulatory T cells (**FIG. 17A**), CD4 helper T cells (**FIG. 17B**), and CD8 cells (**FIG. 17C**).

FIGs. 18A-18D show results from pharmacokinetic studies carried out in tumor-bearing mice using the construct AK032, AK081, AK111, AK167, or AK168, or an anti-RSV control. **FIG. 18A** provides a simplistic depiction of the structure of each of the constructs tested. **FIG. 18B** shows Fc levels in plasma (µg/mL) by detecting human IgG, **FIG. 18C** shows Fc-CD122 levels in plasma (µg/mL) by detecting human CD 122, and **FIG. 18D** shows Fc-IL2 levels in plasma (µg/mL) by detecting human IL- 2. Prior to the detection step, an anti-human IG was used as the capture antibody.

FIGs. 19A-19D show results from pharmacokinetic studies carried out in tumor-bearing mice using the construct AK167, AK191 AK197, AK203, AK209, or AK211, or an anti-RSV control. **FIG. 19A** provides a simplistic depiction of the structure of each of the constructs tested. **FIG. 19B** shows Fc levels in plasma (µg/mL) by detecting human IgG, **FIG. 19C** shows Fc-IL2 levels in plasma (µg/mL) by detecting human IL-2, and **FIG. 19D** shows Fc-CD122 levels in plasma (µg/mL) by detecting human CD 122. Prior to the detection step, an anti-human IG was used as the capture antibody.

FIGs. 20A-20L show results from studies testing the in vivo responses of CD4, CD8, NK, and Treg percentages in spleen, blood, and tumor, using the AK032, AK081, AK111, AK167, or AK168 construct, or an anti-RSV IgG control. For spleen tissue, % CD8 cells of CD3 cells (**FIG. 20A**), % CD4 of CD3 cells (**FIG. 20B**), % NK cells of CD3- cells (**FIG. 20C**), % FoxP3 of CD4 cells (**FIG. 20D**) is shown. For blood, % CD8 cells of CD3 cells (**FIG. 20E**), % CD4 of CD3 cells (**FIG. 20F**), % NK cells of CD3- cells (**FIG. 20G**), % FoxP3 of CD4 cells (**FIG. 20H**) is shown. For tumor tissue, % CD8 cells of CD3 cells (**FIG. 20I**), % CD4 of CD3 cells (**FIG. 20J**), % NK cells of CD3- cells (**FIG. 20K**), % FoxP3 of CD4 cells (**FIG. 20L**) is shown.

FIGs. 21A-21L show results from studies testing the in vivo responses of CD4, CD8, NK, and Treg percentages in spleen, blood, and tumor, using the AK167, AK168, AK191, AK197, AK203, AK209, or AK211 construct, or an anti-RSV IgG control. For spleen tissue, % CD8 cells of CD3 cells (**FIG. 21A**), % CD4 of CD3 cells (**FIG. 21B**), % NK cells of CD3- cells (**FIG. 21C**), % FoxP3 of CD4 cells (**FIG. 21D**) is shown. For blood, % CD8 cells of CD3 cells (**FIG. 21E**), % CD4 of CD3 cells (**FIG. 21F**), % NK cells of CD3- cells (**FIG. 21G**), % FoxP3 of CD4 cells (**FIG. 21H**) is shown. For tumor tissue, % CD8 cells of CD3 cells (**FIG. 21I**), % CD4 of CD3 cells (**FIG. 21J**), % NK cells of CD3- cells (**FIG. 21K**), % FoxP3 of CD4 cells (**FIG. 21L**) is shown.

FIGs. 22A-22L show results from studies testing the in vivo responses of CD4, CD8, NK, and Treg percentages in spleen, blood, and tumor, using the AK235, AK191, AK192, AK193, AK210, AK189, AK190, or AK211 construct, or an anti-RSV IgG control. For spleen tissue, % CD8 cells of CD3 cells (**FIG. 22A**), % CD4 of CD3 cells (**FIG. 22B**), % NK cells of CD3- cells (**FIG. 22C**), % FoxP3 of CD4 cells (**FIG. 22D**) is shown. For blood, % CD8 cells of CD3 cells (**FIG. 22E**), % CD4 of CD3 cells (**FIG. 22F**), % NK cells of CD3- cells (**FIG. 22G**), % FoxP3 of CD4 cells (**FIG. 22H**) is shown. For tumor tissue, % CD8 cells of CD3 cells (**FIG. 22I**), % CD4 of CD3 cells (**FIG. 22J**), % NK cells of CD3- cells (**FIG. 22K**), % FoxP3 of CD4 cells (**FIG. 22L**) is shown.

FIGs. 23A-23I show results from in vivo T cell activation in spleen, blood, and tumor, using the AK235, AK191, AK192, AK193, AK210, AK189, AK190, or AK211 construct. T cell activation was measured as the mean fluorescence intensity (MFI) of CD25 in CD8+ T cells (**FIG. 23A**; **FIG. 23B**; **FIG. 23G**), CD4+ T cells (**FIG. 23B**; **FIG. 23E**; **FIG. 23H**), or Foxp3+ cells (**FIG. 23C**; **FIG. 23F**; **FIG. 23D**) in the spleen, blood, and tumor. Statistical analysis was performed using One-way ANOVA as compared to the non-cleavable AK211 construct.

FIGs. 24A-24D show the results from studies testing the in vivo cleavage of the exemplary masked IL-2 polypeptide constructs AK168 (cleavable peptide sequence: MPYDLYHP) and AK209 (cleavable peptide sequence: VPLSLY; SEQ ID NO: 15). **FIG. 24E** shows results from a pharmacokinetic study of total plasma IgG concentration ($\mu\text{g/mL}$) for total levels of the AK167, AK168, and AK209 constructs, and for levels of non-cleaved forms of each construct.

FIGs. 25A-25D show results from an in vivo study that assessed vascular leakage using the exemplary masked IL-2 polypeptide construct AK111 or AK168, or the non-masked IL-2 polypeptide construct AK081 or AK167, or an anti-RSV control. **FIG. 25A** shows the percentage (%) of body weight loss, and **FIGs. 25B, 25C, and 25D** shows the weight in grams of the liver, lung, and spleen, respectively, for each.

FIGs. 26A and 26B show results from an in vivo study that assessed vascular leakage as indicated by

measuring the extent of dye leakage into liver and lung tissue following administration of the AK081, AK111, AK167, or AK168 construct, or an anti-RSV control. The extent of dye leakage into liver (**FIG. 26A**) and lung (**FIG. 26B**) was measured based on absorbance at 650nm.

5 **FIGs. 27A and 27B** show results from an in vivo study that assessed vascular leakage as indicated by measuring the extent of mononuclear cell perivascular invasion into the liver and lung tissue following administration of the AK081, AK111, AK167, or AK168 construct, or an anti-RSV control. The average number of mononuclear cells in the liver (**FIG. 27A**) and the average number of mononuclear cells in the lung (**FIG. 27B**) depicted for each.

10 **FIGs. 28A and 28B** show results from a syngeneic tumor model study that assessed tumor volume and body weight over the course of treatment with the AK032, AK081, AK111, AK167, or AK168 construct, or an anti-RSV control. **FIG. 28A** shows data on tumor volume over the course of treatment, and **FIG. 28B** shows data on the percentage (%) change in body weight over the course of the treatment.

FIGs. 29A and 29B shows AK471 with I253A FcRn mutation induced robust CD8 T cells expansion in the TME while remaining inactive in the periphery.

15 **FIGs. 30A-30C** show AK471 has slightly shorter half-life compared to aglyco-hlgG1

FIGs. 31A-31C shows there is no evidence of cleavage or decapitation with AK471 in the plasma

FIG 32 shows exemplary IL-12 construct formats.

20 **FIGs. 33A and 33B** show exemplary cleavage processes for exemplary molecules AK380, AK381 and AK384 (**FIG34A**), and AK383, AK386, AK434, AK447, AK448, AK446, AK528 and AK529 (**FIG34B**).

25 **FIGs. 34A-34D** depict the masking of IL-12 towards IL-12RB1, using SPR analysis that tested the binding of exemplary masked IL-12 polypeptide constructs (AK384 and AK386) to rhIL-12RB1-Fc. **FIG. 34A** depicts the interaction between AK384 and IL-12RB1-Fc, **FIG. 34B** depicts the interaction between AK386 and IL-12RB1-Fc, and **FIG. 34C** depicts the interaction between a recombinant human IL-12 (rhIL-12) control and IL-12RB1-Fc. **FIG. 34D** provides a table summarizing the data obtained for the association constant (k_a), dissociation constant (k_d), equilibrium dissociation constant (K_D), as well as the χ^2 value and U-value for each interaction. Due to the shape of the curves, accurate kinetics could not be determined and thus the K_D is estimated based on the on-rate. These results demonstrate that these

exemplary masked IL-12 polypeptide constructs (AK384 and AK386) did not demonstrate detectable binding to IL-12RB1-Fc, while the wild-type rhIL-12 control did demonstrate detectable binding.

FIGs. 35A-35D depict the masking of IL-12 towards IL-12RB2, using SPR analysis that tested the binding of exemplary masked IL-12 polypeptide constructs (AK384 and AK386) to rhIL-12RB2-Fc.

5 **FIG. 35A** depicts the interaction between AK384 and IL-12RB2-Fc, **FIG. 35B** depicts the interaction between AK386 and IL-12RB2-Fc, and **FIG. 35C** depicts the interaction between a recombinant human IL-12 (rhIL-12) control and IL-12RB2-Fc. **FIG. 35D** provides a table summarizing the data obtained for the association constant (k_a), dissociation constant (k_d), equilibrium dissociation constant (KD), as well as the χ^2 value and U-value for each interaction. Due to the shape of the curves, accurate kinetics could
10 not be determined and thus the KD is estimated based on the on-rate. These results demonstrate that an exemplary masked IL-12 polypeptide construct (AK386) did demonstrate weak yet detectable binding to IL-12RB1-Fc, while the wild-type rhIL-12 control and an exemplary IL-12 polypeptide construct (AK384) did demonstrate detectable binding.

FIGs 36-40 show the results from Example 6. **FIGs 41-43** show the results from Example 7. **FIGs 44-15 52E** show the results from Example 8.

FIGs. 53A-53D and **FIGs. 54A-54F** show the results of a SDS-PAGE and HEK-Blue IL-2 bioassay using exemplary IL-15 constructs AK904 and AK910 that do not include a peptide substrate, and constructs AK932, AK938, AK930 and AK936 that do include a peptide substrate. **FIGs 53A-53D** shows the SDS-PAGE gel results. **FIGs. 54A-54F** show the HEK-Blue IL-2 bioassay results.

20 **FIGs 55-68** show the results of Example 11. The PK/PD of exemplary murinized molecules AK944, AK945, AK947 and control AK948 were analysed *in vivo* in two tumor models, MB49 and B16F10.

FIGs 69-71B show the results of Example 12. The PK, PD, hematology and serum chemistry of exemplary
25 molecules AK667, AK921, AK923 and control AK671 were analysed in cynomolgus monkeys.

DETAILED DESCRIPTION

By using a masking moiety, the systemic side effects of an administered IL-12 cytokine or functional fragment thereof can be reduced by interfering with the binding capability of the IL-12 cytokine or
30 functional fragment thereof to its cognate receptor.

Interleukin 12 receptor is a type I cytokine receptor, binding interleukin 12. It consists of beta 1 and beta 2 subunits.

By masking the IL-12 cytokine or functional fragment thereof using a linker that includes a proteolytically cleavable peptide, the binding capability that is interfered with by using the masking moiety can be restored by cleavage of the cleavable peptide at the tumor microenvironment. Thus, the masked IL-12 cytokines
5 provided herein are engineered to precisely target pharmacological activity to the tumor microenvironment by exploiting one of the hallmarks of cancer, high local concentrations of active protease. This feature of the tumor microenvironment is used to transform a systemically inert molecule into a locally active IL-12 cytokine or functional fragment thereof in the form of an IL-12 cleavage product. Activation of the IL-12 cytokine or functional fragment thereof at the tumor microenvironment significantly reduces systemic
10 toxicities that can be associated with drugs that are administered to a subject in active form. Thus, the masked IL-2 cytokines of the invention may be viewed as a pro-drug.

Masked IL-12 cytokines described herein have been found to show various advantageous properties. Masked IL-12 cytokines described anywhere herein have been found to be capable of activating immune
15 cells (proliferation and expansion) upon proteolytic cleavage, preferentially in the tumor microenvironment and at lower levels in the periphery. Masked IL-12 cytokines described anywhere herein have been found to be capable of promoting tumor eradication (i.e. show anti-tumor activity) and inhibition of metastasis upon proteolytic cleavage. Masked IL-12 cytokines described anywhere herein have been found to demonstrate advantageous prolonged drug exposure. Masked IL-12 cytokines described herein have been
20 found to demonstrate advantageous stability. Masked IL-12 cytokines described herein have been found to demonstrate advantageous tolerability. Further, masked IL-12 cytokines described herein have been found to demonstrate advantageous potency.

1. 'HETERODIMERIC' MASKED CYTOKINES

25

Provided herein, in some embodiments, is a masked cytokine comprising a masking moiety in a first polypeptide chain and an IL-12 cytokine or functional fragment thereof in a second polypeptide chain. Such masked cytokines may be referred to as 'heterodimeric' masked cytokines.

30 In some embodiments, the masked cytokine comprises a protein heterodimer comprising:

- a) a first polypeptide chain comprising a masking moiety linked to a first half-life extension domain via a first linker; and
- b) a second polypeptide chain comprising an IL-12 cytokine or functional fragment thereof linked to a second half-life extension domain via a second linker,

35 wherein the first half-life extension domain is associated with the second half-life extension domain, and wherein one of the first linker or the second linker comprises a proteolytically cleavable peptide.

The masking moiety, half-life extension domains, IL-12 cytokine or functional fragment thereof, linkers and type of association between the first half-life extension domain and the second half-life extension domain may be any one of those described herein, and any combination of those described herein.

5 In some embodiments, in the first polypeptide chain, the first half life extension domain is linked to the amino terminus of the first linker and the carboxy terminus of the first linker is linked to the amino terminus of the masking moiety and, in the second polypeptide chain, the second half life extension domain is linked to the amino terminus of the second linker and the carboxy terminus of the second linker is linked to the amino terminus of the IL-12 cytokine or functional fragment thereof. This is shown schematically below
10 where:

the first polypeptide chain comprises:

N' HL1-L1-MM C'

and the second polypeptide chain comprises:

N' HL2-L2-C C'

15 where HL1 is the first half life extension domain, L1 is the first linker, MM is the masking moiety, HL2 is the second half life extension domain, L2 is the second linker, and C is the IL-12 cytokine or functional fragment thereof.

1.1 IL-12 Cytokines

20

Provided herein is an IL-12 cytokine or functional fragment thereof for use in a masked cytokine or cleavage product thereof. A cytokine plays a role in cellular signalling, particularly in cells of the immune system. IL-12 is an interleukin, which is a type of cytokine signalling molecule in the immune system that regulates activities of white blood cells.

25

Endogenous IL-12 exists as two distinct molecules IL-12 p40 and IL-12 p35, that dimerize in the cell during biosynthesis.

The full sequences of IL-12 p40 and IL-12 p35 are (pro-peptides cleaved off during biosynthesis are shown)
30 in bold):

IL-12 p40 subunit:

MCHQQLVISWFSLVFLASPLVAIWELKKDVYVVELDWYPDAPGEMVVLTCDTPEEDGITW
TLDQSSEVLGSGKTLTIQVKEFGDAGQYTCHKGGEVLSHSLLLHKKEDGIWSTDILKDQ
 35 **KEPKNKTFLRCEAKNYSGRFTCWWLTTISTDLTFSVKSSRGSSDPQGVTCGAATLSAERV**
RGDNKEYEYSVEQEDSACPAAEESLPIEVMVDAVHKLKYENYTSSFFIRDIIKPDPPKN
LQLKPLKNSRQVEVSWEYPDTWSTPHSYFSLTFCVQVQGKSKREKKDRVFTDKTSATVIC
RKNASISVRAQDRYYSSSWSEWASVPCS

40 IL-12 p35 subunit:

MCPARSLLLVA TLVLLDHL SLARNLPVATPD PGMFPCLHHSQNLLRA VSNMLQKARQTLE
 FYPCTSEEDHEDITKDKTSTVEACLPLELTKNESCLNSRETSFITNGSCLASRKTSFMM
 ALCLSSIIYEDLKMYQVEFKTMNAKLLMDPKRQIFLDQNMLAVIDELMQALNFNSETVPQK
 SSLEEDFYKTKIKLCILLHAFRIRAVTIDRVMSYLNAS

5

The mature forms are as follows:

IL-12 p40 subunit:

IWELKKDVYVVELDWYPDAPGEMVVLTCDTPEEDGITWTL DQSSEVLGSGKTLTIQVKEFGDA
 10 GQYTCHKGGEVLSHSLLLHKKEDGIWSTDILKDKQEPKNKTF LRCEAKNYSGRFTCWWLTTIS
 TDLTFSVKSSRGSSDPQGVTCGAATLSAERVRGDNKEYEYSVEQCEDSACPAAEESLPIEVMVD
 AVHKLKYENYTSSFFIRDIKPDPPKNLQLKPLKNSRQVEVSWEYPTWSTPHSYFSLTFCVQVQ
 GKSKREKKDRVFTDKTSATVICRKNASISVRAQDRYSSSWSEWASVPCS

15 IL-12 p35 subunit:

RNLPVATPD PGMFPCLHHSQNLLRA VSNMLQKARQTLEFYPCTSEEDHEDITKDKTSTVEACL P
 LELTKNESCLNSRETSFITNGSCLASRKTSFMMALCLSSIIYEDLKMYQVEFKTMNAKLLMDPKR
 QIFLDQNMLAVIDELMQALNFNSETVPQKSSLEEDFYKTKIKLCILLHAFRIRAVTIDRVMSYLN
 AS

20

They are expressed as two chains that covalently dimerize during biosynthesis through a disulfide bound between the two subunits: Cysteine C199 of the p40 subunit associates with Cysteine C96 of the p35 subunit.

25

"Functional fragments" of an IL-12 cytokine comprise a portion of a full length cytokine protein which retains or has modified cytokine receptor binding capability (e.g., within at least 50%, 80%, 90%, 95%, 96%, 97%, 98%, 99% or 100% activity compared to the full length cytokine protein). Cytokine receptor binding capability can be shown, for example, by the capability of a cytokine to bind to the cytokine's
 30 cognate receptor or a component thereof.

In some embodiments, the IL-12 cytokine or functional fragment thereof is any naturally occurring interleukin-2 (IL-12) protein or modified variant thereof capable of binding to an interleukin-12 receptor.

35

In some embodiments, the IL-12 polypeptide or functional fragment thereof comprises an IL-12p40 polypeptide or functional fragment thereof covalently linked to an IL-12p35 polypeptide or functional fragment thereof.

40

The IL-12p40 polypeptide or functional fragment thereof may be attached to the first half life extension domain such that the first polypeptide chain comprises:

N' HL1-L1-MM C'

and the second polypeptide chain comprises:

N' HL2-L2-[IL-12p40-linker-IL-12p35] C'

where 'IL-12p40' is the IL-12p40 polypeptide or functional fragment thereof and 'IL-12p35' is the IL-12p35 polypeptide or functional fragment thereof.

In some embodiments, the IL-12p40 polypeptide comprises SEQ ID NO: 1. In some embodiments, the IL-12p40 polypeptide comprises an amino acid sequence having at least one amino acid modification as compared to the amino acid sequence of SEQ ID NO: 1. Each of the at least one amino acid modifications can be any amino acid modification, such as a substitution, insertion, or deletion. In some embodiments, the IL-12 cytokine or functional fragment thereof comprises an amino acid sequence having at least 1, at least 2, at least 3, at least 4, at least 5, at least 6, at least 7, at least 8, at least 9, or at least 10 amino acid substitutions as compared to the amino acid sequence of SEQ ID NO: 1. In some embodiments, the IL-12 cytokine or functional fragment thereof comprises an amino acid sequence having at least 5 amino acid substitutions as compared to the amino acid sequence of SEQ ID NO: 1.

The IL-12p40 polypeptide comprises a glycosaminoglycan (GAG)-binding domain. GAGs, such as heparin and heparan sulphate, have been shown to bind numerous growth factors and cytokines, including IL-12. The physiological significance of this binding is two-fold. First, GAGs can serve as co-receptors on cell surfaces to maintain high, local concentrations of cytokines. Second, GAGs can regulate bioactivities of growth factors and cytokines through multiple mechanisms including dimerization and protection from proteolytic degradation.

The GAG-binding domain in the mature form of the IL-12 p40 subunit is shown below in bold:

IWELKKDVYVVELDWYPDAPGEMVVLTCDTPEEDGITWTLTDSSEVLGSGKTLTIQVKEFGDA
 GQYTCHKGGEVLSHSLLLHKKEDGIWSTDILKDQ
 25 KEPKNKTFLRCEAKNYSGRFTCWLTITISTDLTFSVKSSRGSSDPQGVTCGAATLSAERV
 RGDNKEYEYSVEQEDSACPAAEESLPIEVMVDAVHKLKYENYTSSFFIRDIHKPDPPKN
 LQLKPLKNSRQVEVSWEYPDTWSTPHSYFSLTFCVQVQ**GKSKREKKDRV**FDTKTSATVIC
 RKNASISVRAQDRYYSSSWSEWASVPCS

Modifications to the GAG-binding domain (KSKREKKDRV) has been shown herein to increase the PK profile of constructs comprising an IL-12 cytokine with a mutated GAG-binding domain, without any decrease in cytokine activity. Thus, in some embodiments, the IL-12p40 polypeptide comprises at least one amino acid modification to the GAG-binding domain. In some embodiments, the modification to the GAG-binding domain is a deletion mutation. In some embodiments, the modification to the GAG-binding domain is a deletion mutation and at least one substitution mutation.

In some embodiments, the GAG-binding domain comprises the amino acid sequence KDINTERV. In some embodiments, the IL-12p40 polypeptide comprises the amino acid sequence SEQ ID NO: 57. In some

embodiments, the GAG-binding domain comprises the amino acid sequence KDNTEGRV. In some embodiments, the IL-12p40 polypeptide comprises the amino acid sequence SEQ ID NO: 58.

5 In some embodiments, the GAG-binding domain consists of the amino acid sequence KDNTERV. In some embodiments, the IL-12p40 polypeptide comprises the amino acid sequence SEQ ID NO: 57. In some embodiments, the GAG-binding domain consists of the amino acid sequence KDNTEGRV. In some embodiments, the IL-12p40 polypeptide comprises the amino acid sequence SEQ ID NO: 58.

10 In some embodiments, the IL-12p40 polypeptide comprises an amino acid sequence having one or more cysteine substitutions as compared to the amino acid sequence of SEQ ID NO: 1. In some embodiments, the IL-12p40 polypeptide comprises an amino acid sequence having an amino acid substitution at position C252 as compared to the amino acid sequence of SEQ ID NO: 1. In some embodiments, the amino acid substitution at position C252 is C252S. In some embodiments, the IL-12p40 polypeptide comprises an amino acid sequence of SEQ ID NO: 59. In some embodiments, the IL-12p40 polypeptide comprises an amino acid sequence having about or at least about 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% sequence identity to the amino acid sequence of SEQ ID NO: 59. In some embodiments, the IL-12p40 polypeptide consists of an amino acid sequence of SEQ ID NO: 59.

20 In some embodiments, the IL-12p40 polypeptide comprises an amino acid sequence having one or more cysteine substitutions as compared to the amino acid sequence of SEQ ID NO: 1, and at least one amino acid modification to the GAG-binding domain. In some embodiments, the IL-12p40 polypeptide comprises an amino acid substitution at position C252S as compared to the amino acid sequence of SEQ ID NO: 1, and the GAG-binding domain comprises the amino acid sequence KDNTERV. In some embodiments, the IL-12p40 polypeptide comprises an amino acid substitution at position C252S as compared to the amino acid sequence of SEQ ID NO: 1, and the GAG-binding domain comprises the amino acid sequence KDNTEGRV. In some embodiments, the IL-12p40 polypeptide comprises an amino acid sequence of SEQ ID NO: 60. In some embodiments, the IL-12p40 polypeptide comprises an amino acid sequence having about or at least about 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% sequence identity to the amino acid sequence of SEQ ID NO: 60. In some embodiments, the IL-12p40 polypeptide consists of an amino acid sequence of SEQ ID NO: 60.

35 In some embodiments, the IL-12p35 polypeptide comprises SEQ ID NO: 2. In some embodiments, the IL-12p35 polypeptide comprises an amino acid sequence having at least one amino acid modification as compared to the amino acid sequence of SEQ ID NO: 2. Each of the at least one amino acid modifications can be any amino acid modification, such as a substitution, insertion, or deletion. In some embodiments, the IL-12 cytokine or functional fragment thereof comprises an amino acid sequence having at least 1, at least 2, at least 3, at least 4, at least 5, at least 6, at least 7, at least 8, at least 9, or at least 10 amino acid substitutions as compared to the amino acid sequence of SEQ ID NO: 2. In some embodiments, the IL-12

cytokine or functional fragment thereof comprises an amino acid sequence having at least 5 amino acid substitutions as compared to the amino acid sequence of SEQ ID NO: 2.

In some embodiments, the IL-12p40 – IL-12p35 linker is between 5 and 20 amino acids in length.

5

In some embodiments, the IL-12p40 – IL-12p35 linker is rich in amino acid residues G and S.

In some embodiments, the IL-12p40 – IL-12p35 linker only includes amino acid residue types selected from the group consisting of G and S.

10

In some embodiments, the IL-12p40 – IL-12p35 linker includes $[(G)_nS]$, where $n=4$ or 5 .

In some embodiments, the IL-12p40 – IL-12p35 linker includes a (GGGGS) repeat.

15 In some embodiments, IL-12p40 – IL-12p35 linker comprises SEQ ID NO: 3. (GGGGSGGGSGGGGS)

In some embodiments, the IL-12 cytokine or functional fragment thereof comprises SEQ ID NO: 4. In some embodiments, the IL-12 cytokine or functional fragment thereof comprises an amino acid sequence having at least one amino acid modification as compared to the amino acid sequences of SEQ ID NO: 1 and 2.

20 Each of the at least one amino acid modifications can be any amino acid modification, such as a substitution, insertion, or deletion. In some embodiments, the IL-12 cytokine or functional fragment thereof comprises an amino acid sequence having at least 1, at least 2, at least 3, at least 4, at least 5, at least 6, at least 7, at least 8, at least 9, or at least 10 amino acid substitutions as compared to the amino acid sequences of SEQ ID NO: 1 and 2. In some embodiments, the IL-12 cytokine or functional fragment thereof comprises an amino acid sequence having at least 5 amino acid substitutions as compared to the amino acid sequences of SEQ ID NO: 1 and 2.

25

In some embodiments, the IL-12 cytokine or functional fragment thereof comprises the amino acid sequence of SEQ ID NO: 4. In some embodiments, the IL-12 cytokine or functional fragment thereof comprises an amino acid sequence having about or at least about 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% sequence identity to the amino acid sequence of SEQ ID NO: 4.

30

In some embodiments, the IL-12 cytokine or functional fragment thereof comprises the amino acid sequence of SEQ ID NO: 61. In some embodiments, the IL-12 cytokine or functional fragment thereof comprises an amino acid sequence having about or at least about 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% sequence identity to the amino acid sequence of SEQ ID NO: 61.

35

In some embodiments, the IL-12 cytokine or functional fragment thereof comprises the amino acid sequence of SEQ ID NO: 62. In some embodiments, the IL-12 cytokine or functional fragment thereof comprises an amino acid sequence having about or at least about 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% sequence identity to the amino acid sequence of SEQ ID NO: 62.

In some embodiments, the IL-12 cytokine or functional fragment thereof comprises the amino acid sequence of SEQ ID NO: 63. In some embodiments, the IL-12 cytokine or functional fragment thereof comprises an amino acid sequence having about or at least about 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% sequence identity to the amino acid sequence of SEQ ID NO: 63.

In some embodiments, the IL-12 cytokine or functional fragment thereof comprises the amino acid sequence of SEQ ID NO: 64. In some embodiments, the IL-12 cytokine or functional fragment thereof comprises an amino acid sequence having about or at least about 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% sequence identity to the amino acid sequence of SEQ ID NO: 64.

1.2 Masking Moieties

Provided herein is a masking moiety for use in a masked cytokine. It will be understood that the masking moiety is cleaved from the masked cytokine to form the cleavage product thereof. The masking moiety masks the IL-12 cytokine or functional fragment thereof in the masked cytokine thereby reducing or preventing binding of the IL-cytokine or functional fragment thereof to its cognate receptor.

The IL-12 receptor, beta 1, or IL-12R β 1 is a subunit of the IL-12 receptor complex. IL-12R β 1 is also known as CD212. This protein binds to interleukin-12 (IL-12) with a low affinity. This protein forms a disulfide-linked oligomer, which is required for its IL-12 binding activity. The IL-12 receptor, beta 2, or IL-12R β 2 is a subunit of the IL-12 receptor complex. The coexpression of IL-12R β 1 and IL-12R β 2 protein has been shown to lead to the formation of high-affinity IL-12 binding sites.

Methods for determining the extent of binding of a protein (e.g., cytokine) to a cognate protein (e.g., cytokine receptor) are well known in the art.

In some embodiments, the masking moiety comprises an extracellular domain of an IL-12 cytokine receptor, or a subunit or functional fragment thereof.

Interleukin-12 receptor subunit beta-1, also called CD212 has the sequence:

MEPLVTWVVP~~LLFLFLLSRQGAA~~CRTSECCFQDPPYPDADSGSASGPRDLRCYRISSDRY
ECSWFOYEGPTAGVSHFLRCCLSSGRCCYFAAGSATRLOFSDOAGVSVLYTVTLWVESHAR
 5 NOTEKSPEVTLOLYNSVKYEPPLGDIKVS~~KL~~AGOLRMEWETPDNOVGAEVQFRHRT~~PSSP~~
WKLGDGCPQDDDTESCLCLEMNV~~AOEFOLRRROI~~GSOGSSWSKWSSPV~~CVPPENPPPOQ~~
VRFSVEOLGODGRRRLTLKEOPTOLEPEGCOGLAPGTEVTYRLOLHMLSCPC~~KAKATRT~~
LHLGKMPYLSGAAYNVAVISSNOFGPGLNOTWTHPADTHTEPV~~ALNISVGTNGTMYWPA~~
RAQSMTYCIEWQPVGODGGLATCSLTAPQDPDPAGMATYSWSRESGAMGQEK~~CYITIFA~~
 10 SAHPEKLT~~LWSTVLSTYHFGGNASAGTPHHVSVKNHSLDSVSDWAPSLLSTCPGVLKE~~
YVTRCRDEDSKOVSEHPVOPTETOVTL~~SGLRAGVAYTVOVRADTAWLRGVWSOPORFIE~~
VOVSDWLIFFASLGSFLSILLVGVLYGLGLNRAARHLCPPLTPCASSAIEFP~~GCKETWQ~~
WINPVDFQEEASLQEA~~LVVEMSWDKGERTEPLEKTELPEGAPELALDTELSLEDGDRCKA~~
KM

15

Interleukin-12 receptor subunit beta-2 has the sequence:

MAHTFRGCSLAFMFHITWLLIKAKIDACKRGDVTVKPSHVILLGSTVNITCSLKPROGCF
HYSRRNKLILYKFDRRINFHHHGSLSNOVTGLPLGTTLFVCKLACINSDEIOICGAEIFV
 20 GVAPQEQONLS~~CIQKGEQGVACTW~~ERGRDTHLYTEYTLQ~~LSGPKNLWQKOCKDIYCDY~~
LDFGINLTPESPESNFTAKVTA~~VNSLGSSSSLPSTFTELDIVRPLPPWDIRIKFOKASVS~~
RC~~TL~~YWRDEGLVLLNRLRYRPSNSRLWNM~~VN~~TKAKGRHDLLDLKPFTEYEFQISSKLHL
YKGSWSDWSESLRAOTPEFEPTGMLDVWYMKRHIDYSROOISLFWKNLSVSEARGKILHY
QVTLOELTGKKAMTONITGHTSWTTVIPRTGNWAVAVSAANSKGSSLPTRINIMNLCEAG
 25 LLAPROVSANSEGMDN~~ILVTWOPPRKDP~~SAVOEYVVEWRELHPGGDT~~OVPLNWLRSRPN~~
V~~S~~ALISENIKSYICYEIRVYALSGDOGGCSSILGNSKHKAPLSGPHINAITEEKGSILIS
WNSIPVOEOMGCLLHYRIYWKERDSNSQPOLCEIPYRVSNSHPINSLOPRVTYVLWMTA
LTAA~~GESSHGNEREF~~CLOGKANWMAFVAPSICIAIMV~~GIFSTHYFOOKVFVLLAALRPO~~
WCSREIPDPANSTCAKKYPIAEK~~TQLPLDRLLIDWPTPEDPEPLVISEVLHOVTPVFRH~~
 30 PPCSNWPOREKGIQGHQASEKDMMHSA~~SSPPPPRALOESROLV~~DLYKVLESRGSDPKPE
NPACPWTVLPAGDLPTH~~DGYLPSNIDDLPSHEAPLADSLEELEPOHISLSVFPSSSLHPL~~
TFSCGDKLTLBQLKMRCDSLML

The bold indicates the pro-peptide, the italics with underline indicates the extracellular domain, the italics
 35 indicates the transmembrane domain and the bold with underline indicates the cytoplasmic domain.

In some embodiments, the masking moiety comprises the extracellular domain of human IL-12R β 1 or a
 fragment, portion, or variant thereof that retains or otherwise demonstrates an affinity to IL-12.

40 In some embodiments, the masking moiety comprises an amino acid sequence having an amino acid
 sequence of human IL-12R β 1 with one to four amino acid substitutions. In some embodiments, the masking
 moiety comprises an amino acid sequence having an amino acid sequence of human IL-12R β 1 with one or
 two amino acid substitutions.

In some embodiments, the masking moiety comprises residues 24 to 237 of human IL-12R β 1, namely a sequence having SEQ ID NO: 5 or a fragment, portion, or variant thereof that retains or otherwise demonstrates an affinity to IL-12. In some embodiments, the masking moiety comprises IL-12R β 1 having SEQ ID NO: 5. In some embodiments, the masking moiety comprises an amino acid sequence having about or at least about 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% sequence identity to any one of the amino acid sequence of SEQ ID NO: 5. In some embodiments, the masking moiety comprises an amino acid sequence having the amino acid sequence of SEQ ID NO: 5 with one to four amino acid substitutions. In some embodiments, the masking moiety comprises an amino acid sequence having the amino acid sequence of SEQ ID NO: 5 with one or two amino acid substitutions.

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In some embodiments, the masking moiety comprises residues 24 to 545 of human IL-12R β 1, namely a sequence having SEQ ID NO: 6 or a fragment, portion, or variant thereof that retains or otherwise demonstrates an affinity to IL-12. In some embodiments, the masking moiety comprises IL-12R β 1 having SEQ ID NO: 6. In some embodiments, the masking moiety comprises an amino acid sequence having about or at least about 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% sequence identity to any one of the amino acid sequence of SEQ ID NO: 6. In some embodiments, the masking moiety comprises an amino acid sequence having the amino acid sequence of SEQ ID NO: 6 with one to four amino acid substitutions. In some embodiments, the masking moiety comprises an amino acid sequence having the amino acid sequence of SEQ ID NO: 6 with one or two amino acid substitutions.

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In some embodiments, the masking moiety comprises the extracellular domain of human IL-12R β 2 or a fragment, portion, or variant thereof that retains or otherwise demonstrates an affinity to IL-12. In some embodiments, the masking moiety comprises an amino acid sequence having an amino acid sequence of human IL-12R β 2 with one to four amino acid substitutions. In some embodiments, the masking moiety comprises an amino acid sequence having an amino acid sequence of human IL-12R β 2 with one or two amino acid substitutions.

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In some embodiments, the masking moiety comprises residues 24 to 212 of human IL-12R β 2, namely a sequence having SEQ ID NO: 7. In some embodiments, the masking moiety comprises an amino acid sequence having about or at least about 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% sequence identity to any one of the amino acid sequence of SEQ ID NO: 7. In some embodiments, the masking moiety comprises an amino acid sequence having the amino acid sequence of SEQ ID NO: 7 with one to four amino acid substitutions. In some embodiments, the masking moiety comprises an amino acid sequence having the amino acid sequence of SEQ ID NO: 7 with one or two amino acid substitutions.

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In some embodiments, the masking moiety comprises residues 24 to 222 of human IL-12R β 2, namely a sequence having SEQ ID NO: 8. In some embodiments, the masking moiety comprises an amino acid

sequence having about or at least about 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% sequence identity to any one of the amino acid sequence of SEQ ID NO: 8. In some embodiments, the masking moiety comprises an amino acid sequence having the amino acid sequence of SEQ ID NO: 8 with one to four amino acid substitutions. In some embodiments, the masking moiety
5 comprises an amino acid sequence having the amino acid sequence of SEQ ID NO: 8 with one or two amino acid substitutions.

In some embodiments, the masking moiety comprises residues 24 to 319 of human IL-12R β 2, namely a sequence having SEQ ID NO: 9. In some embodiments, the masking moiety comprises an amino acid
10 sequence having about or at least about 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% sequence identity to any one of the amino acid sequence of SEQ ID NO: 9. In some embodiments, the masking moiety comprises an amino acid sequence having the amino acid sequence of SEQ ID NO: 9 with one to four amino acid substitutions. In some embodiments, the masking moiety
15 comprises an amino acid sequence having the amino acid sequence of SEQ ID NO: 9 with one or two amino acid substitutions.

In some embodiments, the masking moiety comprises residues 24 to 319 of human IL-12R β 2, namely a sequence having SEQ ID NO: 9, with one or more cysteine substitutions. In some embodiments, the masking moiety comprises residues 24 to 319 of human IL-12R β 2, namely a sequence having SEQ ID NO:
20 9, with an amino acid substitution at position C242. In some embodiments, the amino acid substitution is at position C242 is C242S. In some embodiments, the masking moiety comprises an amino acid sequence of SEQ ID NO: 65. In some embodiments, the masking moiety comprises an amino acid sequence having about or at least about 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% sequence identity to the amino acid sequence of SEQ ID NO: 65. In some embodiments, the masking
25 moiety consists of an amino acid sequence of SEQ ID NO: 65. In some embodiments, the masking moiety comprises residues 24 to 622 of human IL-12R β 2, namely a sequence having SEQ ID NO: 10. In some embodiments, the masking moiety comprises an amino acid sequence having about or at least about 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% sequence identity to any one of the amino acid sequence of SEQ ID NO: 10. In some embodiments, the masking moiety
30 comprises an amino acid sequence having the amino acid sequence of SEQ ID NO: 10 with one to four amino acid substitutions. In some embodiments, the masking moiety comprises an amino acid sequence having the amino acid sequence of SEQ ID NO: 10 with one or two amino acid substitutions.

In some embodiments, the masking moiety comprises residues 24 to 227 of human IL-12R β 2, namely a
35 sequence having SEQ ID NO: 11. In some embodiments, the masking moiety comprises an amino acid sequence having about or at least about 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% sequence identity to any one of the amino acid sequence of SEQ ID NO: 11. In some embodiments, the masking moiety comprises an amino acid sequence having the amino acid sequence

of SEQ ID NO: 11 with one to four amino acid substitutions. In some embodiments, the masking moiety comprises an amino acid sequence having the amino acid sequence of SEQ ID NO: 11 with one or two amino acid substitutions.

5 1.3 Linkers

Provided herein are linkers for use in a masked cytokine or cleavage product thereof. A linker as provided herein refers to a peptide of two more amino acids that is used to link two functional components together in the masked cytokines described herein.

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The masked cytokine comprises a first linker and a second linker, where one of the first linker or the second linker comprises a proteolytically cleavable peptide.

In some embodiments, the second linker comprises a proteolytically cleavable peptide (linker herein referred to as a 'proteolytically cleavable linker') and the first linker does not comprise a proteolytically cleavable peptide (linker herein referred to as a 'non-proteolytically cleavable linker') such that the first polypeptide chain comprises:

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N' HL1-non-cleavable L1-MM C'

and the second polypeptide chain comprises

20

N' HL2-cleavable L2-C C'

In some embodiments, the first linker comprises a proteolytically cleavable peptide (linker herein referred to as a 'proteolytically cleavable linker' or 'cleavable linker') and the second linker does not comprise a proteolytically cleavable peptide (linker herein referred to as a 'non-proteolytically cleavable linker' or

25

'non-cleavable linker') such that the first polypeptide chain comprises:

N' HL1- cleavable L1-MM C'

and the second polypeptide chain comprises

N' HL2- non-cleavable L2-C C'

30 The non-cleavable linkers and cleavable linkers of some embodiments are described in more detail below.

1.3.1 Non-Proteolytically Cleavable Linkers

In some embodiments, the non-cleavable linker is between 3 and 18 amino acids in length.

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In some embodiments, the non-cleavable linker is between 3 and 15 amino acids in length.

In some embodiments, the non-cleavable linker is rich in amino acid residues G and S.

In some embodiments, the non-cleavable linker only includes amino acid residue types selected from the group consisting of G and S.

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In some embodiments, the non-cleavable linker includes $[(G)_nS]$, where $n=4$ or 5 .

In some embodiments, the non-cleavable linker comprises an amino acid sequence as shown in SEQ ID NO: 12 (GGGGS).

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In some embodiments, the non-cleavable linker comprises an amino acid sequence as shown in SEQ ID NO: 13 (GGGGSGGGGS).

In some embodiments, the non-cleavable linker comprises an amino acid sequence as shown in SEQ ID NO: 14 (GGSGGSGGGGGS).

15

In some embodiments, the non-cleavable linker comprises an amino acid sequence as shown in SEQ ID NO: 54 (GGSGGSGGSGGSGSSGP).

In some embodiments, the non-cleavable linker comprises an amino acid sequence as shown in SEQ ID NO: 55 (PGGSGP).

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In some embodiments, the non-cleavable linker comprises an amino acid sequence as shown in SEQ ID NO: 56 (GGSPG).

25

In some embodiments, wherein the second linker comprises a proteolytically cleavable peptide such that the second linker is a proteolytically cleavable linker and the first linker does not comprise a proteolytically cleavable peptide such that the first linker is a non- proteolytically cleavable linker, the non-cleavable linker is between 3 and 18 amino acids in length. In some embodiments, the non-cleavable linker comprises an amino acid sequence as shown in SEQ ID NO: 12 (GGGGS). In some embodiments, the non-cleavable linker comprises an amino acid sequence as shown in SEQ ID NO: 13 (GGGGSGGGGS). In some embodiments, the non-cleavable linker comprises an amino acid sequence as shown in SEQ ID NO: 14 (GGSGGSGGGGGS). In some embodiments, the non-cleavable linker comprises an amino acid sequence as shown in SEQ ID NO: 54 (GGSGGSGGSGGSGSSGP). In some embodiments, the non-cleavable linker comprises an amino acid sequence as shown in SEQ ID NO: 55 (PGGSGP). In some embodiments, the non-cleavable linker comprises an amino acid sequence as shown in SEQ ID NO: 56 (GGSPG).

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In some embodiments, it is desirable for the first and second polypeptide chains to be of the same or a similar length to facilitate the first half life extension domain associating with the second half life extension domain and the masking moiety masking the IL-12 cytokine or functional fragment thereof in the assembled construct. As such where the masking moiety is a shorter amino acid sequence than the IL-12 cytokine or functional fragment thereof, the difference in length may be compensated fully or in part by using a longer linker L1.

1.3.2 Proteolytically Cleavable Linkers

In some embodiments, the cleavable linker is from 10 to 25 amino acids in length.

In some embodiments, the cleavable linker comprises a proteolytically cleavable peptide (CP) flanked on both sides by a spacer domain (SD) as shown below:

SD-CP-SD

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Cleavable Peptides

The cleavable linker comprises a cleavable peptide.

A cleavable peptide is a polypeptide that includes a protease cleavage site, such that the cleavable peptide is proteolytically cleavable. Proteases are enzymes that cleave and hydrolyse the peptide bonds between two specific amino acid residues of target substrate proteins. A “cleavage site” as used herein refers to a recognizable site for cleavage of a portion of the cleavable peptide found in any of the linkers that comprise a cleavable peptide described herein. Thus, a cleavage site may be found in the sequence of a cleavable peptide as described herein. In some embodiments, the cleavage site is an amino acid sequence that is recognized and cleaved by a cleaving agent.

In some embodiments, the protease cleavage site is a tumor-associated protease cleavage site. A “tumor-associated protease cleavage site” as provided herein is an amino acid sequence recognized by a protease whose expression is specific or upregulated for a tumor cell or tumor cell environment thereof.

The tumor cell environment is complex and can comprise multiple different proteases. As such, the precise site at which a given cleavable peptide will be cleaved in the tumor cell environment may vary between tumor types, between patients with the same tumor type and even between cleavage products formed in the same tumor dependent on the specific tumor cell environment. Moreover, even after cleavage, further modification of the initial cleavage product, e.g. by removal of one or two terminal amino acids, may occur by the further action of proteases in the tumor cell environment. A distribution of cleavage products can

thus be expected to form in the tumor cell environment of a patient following administration of a single structure of a masked cytokine as described herein.

It will be understood that a cleavage site as referred to herein refers to a site between two specific amino acid residues within the cleavable peptide that are a target for a protease known to be associated with a tumor cell environment. In this sense, there may be more than one cleavage site present in a cleavable peptide as described herein where different proteases cleave the cleavable peptide at different cleavage sites. It is also possible that more than one protease may act on the same cleavage site within a cleavable peptide. Discussion of protease cleavage sites can be found in the art.

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Thus, the cleavable peptides disclosed herein may be cleaved by one or more proteases.

In some embodiments, the cleavable peptide is a substrate for a protease that is co-localized in a region or a tissue expressing the IL-12 cytokine receptor.

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In some embodiments, the cleavable peptide is a 5-mer (i.e. peptide 5 amino acids in length), 6-mer (i.e. peptide 6 amino acids in length), 7-mer (i.e. peptide 7 amino acids in length), 8-mer (i.e. peptide 8 amino acids in length), 9-mer (i.e. peptide 9 amino acids in length), 10-mer (i.e. peptide 10 amino acids in length), 11-mer (i.e. peptide 11 amino acids in length), 12-mer (i.e. peptide 12 amino acids in length), 13-mer (i.e. peptide 13 amino acids in length), 14-mer (i.e. peptide 14 amino acids in length), 15-mer (i.e. peptide 15 amino acids in length), 16-mer (i.e. peptide 16 amino acids in length), 17-mer (i.e. peptide 17 amino acids in length), or 18-mer (i.e. peptide 18 amino acids in length).

20

In some embodiments, the cleavable peptide is from 5 to 18 amino acids in length. In some embodiments, the cleavable peptide is from 6 to 10 amino acids in length.

In some embodiments, the cleavable peptide within the cleavable linker comprises an amino acid sequence selected from the group consisting of:

Sequence (* indicates a cleavage site within the cleavable peptide)
MPYD*LYHP
DSGG*FMLT
HEQ*LTV
RAAA*VKSP
VPLS*LY
ISSGLL*SGRS

DLLA*VVAAS

Purely by way of example, in the above table, * indicates a known or observed protease cleavage site within the cleavable peptide.

5 In some embodiments, the cleavable peptide comprises an amino acid sequence of SEQ ID NO: 15. (VPLS*LY), for example the cleavable peptide may comprise an amino acid sequence of SEQ ID NO: 210 (VPLSLYSG). In some embodiments, the cleavable peptide comprises an amino acid sequence of SEQ ID NO: 41. (MPYD*LYHP). In some embodiments, the cleavable peptide comprises an amino acid sequence of SEQ ID NO: 42. (DSGG*FMLT). In some embodiments, the cleavable peptide comprises an amino acid
10 sequence of SEQ ID NO: 43. (RAAA*VKSP). In some embodiments, the cleavable peptide comprises an amino acid sequence of SEQ ID NO: 44. (ISSGLL*SGRS), for example the cleavable peptide may comprise an amino acid sequence of SEQ ID NO: 211 (ISSGLLSGRSDQP). In some embodiments, the cleavable peptide comprises an amino acid sequence of SEQ ID NO: 45. (DLLA*VVAAS).

15 In some embodiments, the cleavable peptide consists of an amino acid sequence of SEQ ID NO: 15. (VPLS*LY). In some embodiments, the cleavable peptide consists of an amino acid sequence of SEQ ID NO: 210 (VPLSLYSG). In some embodiments, the cleavable peptide consists of an amino acid sequence of SEQ ID NO: 41. (MPYD*LYHP). In some embodiments, the cleavable peptide consists of an amino acid sequence of SEQ ID NO: 42. (DSGG*FMLT). In some embodiments, the cleavable peptide consists
20 of an amino acid sequence of SEQ ID NO: 43. (RAAA*VKSP). In some embodiments, the cleavable peptide consists of an amino acid sequence of SEQ ID NO: 44. (ISSGLL*SGRS). In some embodiments, the cleavable peptide consists of an amino acid sequence of SEQ ID NO: 211 (ISSGLLSGRSDQP). In some embodiments, the cleavable peptide consists of an amino acid sequence of SEQ ID NO: 45. (DLLA*VVAAS).

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Cleavable peptides having an amino acid sequence as shown in SEQ ID NOs: 44 or 45 have been found to demonstrate very specific cleavage in the tumor cell environment compared to non-tumor cell environment. Thus, when these cleavable peptides are incorporated into a masked IL-12 cytokine as disclosed anywhere herein, any systemic side effects of the administered IL-12 cytokine or functional fragment thereof may be
30 further reduced.

Spacer Domains

A spacer domain may consist of one or more amino acids. The function of the spacer domains, where present, is to link the proteolytically cleavable peptide (CP) to the other functional components in the
35 constructs described herein.

It will be understood that spacer domains do not alter the biological interaction of the proteolytically cleavable peptide with proteases in the tumor-cell environment or in non-tumor cell environment. In other words, even in the presence of spacer domains the inventive proteolytically cleavable peptides disclosed herein retain their advantageous tumor specificity.

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In some embodiments, the spacer domains flanking the proteolytically cleavable peptide are different.

In some embodiments, the spacer domains are rich in amino acid residues G, S and P.

10 In some embodiments, the spacer domains only includes amino acid residue types selected from the group consisting of G, S and P.

In some embodiments, the cleavable linker comprises:

N' SD1-CP-SD2 C'

15 where SD1 is a first spacer domain and SD2 is a second spacer domain.

In some embodiments, the cleavable linker comprises:

N' SD1-CP-SD2 C'

20 In some embodiments, the first polypeptide chain comprises:

N' HL1-non-cleavable L1-MM C'

and the second polypeptide chain comprises:

N' HL2- SD1-CP-SD2 -C C'

25 In some embodiments, the first polypeptide chain comprises:

N' HL1- SD1-CP-SD2 -MM C'

and the second polypeptide chain comprises:

N' HL2- non-cleavable L2-C C'

30 In some embodiments, the N-terminus of SD1 is a glycine (G).

In some embodiments, the first spacer domain (SD1) is between 3 and 10 amino acids in length. In some embodiments, the first spacer domain (SD1) is between 5 and 9 amino acids in length.

35 In some embodiments, SD1 comprises SEQ ID NO: 16. (GGSGGS)

In some embodiments, SD1 comprises SEQ ID NO: 17. (GGSGGSGGS)

In some embodiments, the C-terminus sequence of SD2 is **-GP C'**.

In some embodiments, the second spacer domain (SD2) is between 3 and 6 amino acids in length.

- 5 In some embodiments, SD2 comprises SEQ ID NO: 18. (SGP)

Exemplary combinations of SD1 and SD2 in a cleavable linker are shown below:

Linker structure	SD1 sequence	SD2 sequence
SD1-CP-SD2	GGSGGS	SGP
SD1-CP-SD2	GGSGGSGGS	SGP

- 10 In some embodiments, the proteolytically cleavable linker comprises SD1-CP-SD2 where SD1 is a first spacer domain, CP is a cleavable peptide and SD2 is a second spacer domain, and wherein CP has an amino acid sequence as shown in SEQ ID NO: 44. In some embodiments, the spacer domains are rich in amino acid residues G, S and P. In some embodiments, the spacer domains only include amino acid residue types selected from the group consisting of G, S and P.

- 15 In some embodiments, the proteolytically cleavable linker comprises SD1-CP-SD2 where SD1 is a first spacer domain, CP is a cleavable peptide and SD2 is a second spacer domain, and wherein CP has an amino acid sequence as shown in SEQ ID NO: 45. In some embodiments, the spacer domains are rich in amino acid residues G, S and P. In some embodiments, the spacer domains only include amino acid residue types selected from the group consisting of G, S and P.

- 20 In some embodiments, the proteolytically cleavable linker comprises SD1-CP-SD2 where SD1 is a first spacer domain, CP is a cleavable peptide and SD2 is a second spacer domain, and wherein CP has an amino acid sequence as shown in SEQ ID NO: 44 and SD2 has an amino acid sequence as shown in SEQ ID NO: 18. In some embodiments, the SD1 is from 3 to 6 amino acids in length. In some embodiments, the spacer domains are rich in amino acid residues G, S and P. In some embodiments, the spacer domains only include amino acid residue types selected from the group consisting of G, S and P.
- 25

- In some embodiments, the proteolytically cleavable linker comprises SD1-CP-SD2 where SD1 is a first spacer domain, CP is a cleavable peptide and SD2 is a second spacer domain, and wherein CP has an amino acid sequence as shown in SEQ ID NO: 45 and SD2 has an amino acid sequence as shown in SEQ ID NO: 18. In some embodiments, the SD1 is from 3 to 6 amino acids in length. In some embodiments, wherein the spacer domains are rich in amino acid residues G, S and P. In some embodiments, the spacer domains only include amino acid residue types selected from the group consisting of G, S and P.
- 30

- 35 In some embodiments, the cleavable linker comprises SEQ ID NO: 19. (GGSGGSVP~~LS~~LYSGP)

In some embodiments, the cleavable linker comprises SEQ ID NO: 20. (GGSGGSGGS**VPLSLY**SGP)

In some embodiments, the cleavable linker comprises SEQ ID NO: 46. (GGSGGS**MPYDLYHP**SGP)

5

In some embodiments, the cleavable linker comprises SEQ ID NO: 47.
(GGSGGSGGS**MPYDLYHP**SGP)

In some embodiments, the cleavable linker comprises SEQ ID NO: 48. (GGSGGS**DSGGFMLT**SGP)

10

In some embodiments, the cleavable linker comprises SEQ ID NO: 49.
(GGSGGSGGS**DSGGFMLT**SGP)

In some embodiments, the cleavable linker comprises SEQ ID NO: 50. (GGSGGS**RAAAVKSP**SGP)

15

In some embodiments, the cleavable linker comprises SEQ ID NO: 51.
(GGSGGSGGS**RAAAVKSP**SGP)

In some embodiments, the cleavable linker comprises SEQ ID NO: 52. (GGSGGS**ISSGLLSGRSS**GP)

20

In some embodiments, the cleavable linker comprises SEQ ID NO: 53.
(GGSGGSGGS**ISSGLLSGRSS**GP).

In some embodiments, the non-cleavable linker comprises an amino acid sequence as shown in SEQ ID NO: 12 (GGGGS) and the cleavable linker comprises SEQ ID NO: 19 (GGSGGS**VPLSLY**SGP).

25

In some embodiments, the non-cleavable linker comprises an amino acid sequence as shown in SEQ ID NO: 13 (GGGGSGGGGS) and the cleavable linker comprises SEQ ID NO: 19 (GGSGGS**VPLSLY**SGP).

In some embodiments, the non-cleavable linker comprises an amino acid sequence as shown in SEQ ID NO: 14 (GGSGGGSGGGGS) and the cleavable linker comprises SEQ ID NO: 19 (GGSGGS**VPLSLY**SGP).

30

In some embodiments, the non-cleavable linker comprises an amino acid sequence as shown in SEQ ID NO: 12 (GGGGS) and the cleavable linker comprises SEQ ID NO: 20 (GGSGGSGGS**VPLSLY**SGP).

In some embodiments, the non-cleavable linker comprises an amino acid sequence as shown in SEQ ID NO: 13 (GGGGSGGGGS) and the cleavable linker comprises SEQ ID NO: 20 (GGSGSGSGSVPLSLYSGP).

- 5 In some embodiments, the non-cleavable linker comprises an amino acid sequence as shown in SEQ ID NO: 14 (GGSGSGSGGGGGGS) and the cleavable linker comprises SEQ ID NO: 20 (GGSGSGSGSVPLSLYSGP),

In some embodiments, wherein the second linker comprises a proteolytically cleavable peptide such that the second linker is a proteolytically cleavable linker and the first linker does not comprise a proteolytically cleavable peptide such that the first linker is a non- proteolytically cleavable linker, the cleavable linker
10 comprises SEQ ID NO: 46 and the non-cleavable linker comprises SEQ ID NO: 55. In some embodiments, the cleavable linker comprises SEQ ID NO: 47 and the non-cleavable linker comprises SEQ ID NO: 55. In some embodiments, the cleavable linker comprises SEQ ID NO: 48 and the non-cleavable linker comprises SEQ ID NO: 55. In some embodiments, the cleavable linker comprises SEQ ID NO: 49 and the non-
15 cleavable linker comprises SEQ ID NO: 56. In some embodiments, the cleavable linker comprises SEQ ID NO: 50 and the non-cleavable linker comprises SEQ ID NO: 55. In some embodiments, the cleavable linker comprises SEQ ID NO: 50 and the non-cleavable linker comprises SEQ ID NO: 14. In some embodiments, the cleavable linker comprises SEQ ID NO: 51 and the non-cleavable linker comprises SEQ ID NO: 56. In some embodiments, the cleavable linker comprises SEQ ID NO: 51 and the non-cleavable linker comprises
20 SEQ ID NO: 14. In some embodiments, the cleavable linker comprises SEQ ID NO: 52 and the non-cleavable linker comprises SEQ ID NO: 55. In some embodiments, the cleavable linker comprises SEQ ID NO: 52 and the non-cleavable linker comprises SEQ ID NO: 14. In some embodiments, the cleavable linker comprises SEQ ID NO: 53 and the non-cleavable linker comprises SEQ ID NO: 56. In some embodiments, the cleavable linker comprises SEQ ID NO: 53 and the non-cleavable linker comprises SEQ ID NO: 14.

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In some embodiments, the proteolytically cleavable linker comprises a cleavable peptide consisting of an amino acid sequence of SEQ ID NO: 44. (ISSGLL*SGRS).

- 30 In some embodiments, the proteolytically cleavable linker comprises a cleavable peptide consisting of an amino acid sequence of SEQ ID NO: 45. (DLLA*VVAAS).

Linker combinations disclosed herein and disclosed in exemplary AK molecules may be used with any IL-12 cytokine or fragment thereof disclosed herein. Linker combinations disclosed herein and disclosed in exemplary AK molecules may be used with any masking moiety disclosed herein. Linker combinations
35 disclosed herein and disclosed in exemplary AK molecules may be used with any half-life extension domains. In other words, the linkers disclosed in exemplary AK molecules may be used in combinations

with any IL-12 cytokine or fragment thereof disclosed herein, masking moiety disclosed herein and/or half-life extension domain disclosed herein.

1.4 Half-life Extension Domains

5

Provided herein are half life extension domains for use in a masked cytokine or cleavage product thereof. A long half-life in vivo is important for therapeutic proteins. Unfortunately, cytokines that are administered to a subject generally have a short half-life since they are normally cleared rapidly from the subject by mechanisms including clearance by the kidney and endocytic degradation. Thus, in the masked cytokine
10 provided herein, a half-life extension domain is linked to the masked cytokine for the purpose of extending the half-life of the cytokine in vivo.

The term “half-life extension domain” refers to a domain that extends the half-life of the target component in serum. The term “half-life extension domain” encompasses, for example, antibodies and antibody
15 fragments.

The masked cytokine provided herein comprises a first half-life extension domain that is associated with a second half-life extension domain.

20 In some embodiments, the first half-life extension domain and the second half-life extension domain are non-covalently associated.

In some embodiments, the first half-life extension domain and the second half-life extension domain are covalently bound.
25

In some embodiments, the first half-life extension domain is linked to the second half-life extension domain via one or more disulphide bonds.

In some embodiments, the first half-life extension domain is linked to the second half-life extension domain via a half life extension domain linker (HLDL).
30

In some embodiments, the first half-life extension domain and the second half-life extension domain are non-covalently associated and, further, the first half-life extension domain is linked to the second half-life extension domain via a disulphide bond.
35

In some embodiments, the first half-life extension domain comprises a first antibody or fragment thereof, and second half-life extension domain comprises a second antibody or fragment thereof.

An antibody or fragment thereof that is capable of FcRn-mediated recycling, can be reduce or otherwise delay clearance of the masked cytokine from a subject, thereby prolonging the half-life of the administered masked cytokine. In some embodiments, the antibody or fragment thereof is any antibody or fragment thereof that is capable of FcRn-mediated recycling, such as any heavy chain polypeptide or portion thereof (e.g., Fc domain or fragment thereof) that is capable of FcRn-mediated recycling.

The antibody or fragment thereof can be any antibody or fragment thereof. However, in some embodiments of a masked cytokine comprising a first half-life extension domain and a second half-life extension domain, either the first half-life extension domain or the second half-life extension domain may comprise an antibody or fragment thereof that does not bind to the FcRn receptor, such as a light chain polypeptide. For example, in some embodiments of the masked cytokine, a first half-life extension domain comprises an antibody or fragment thereof that comprises a light chain polypeptide or portion thereof that does not directly interact with the FcRn receptor, but the masked cytokine nonetheless has an extended half-life due to comprising a second half-life extension domain that is capable of interacting with the FcRn receptor, such as by comprising a heavy chain polypeptide. It is recognized in the art that FcRn-mediated recycling requires binding of the FcRn receptor to the Fc region of the antibody or fragment thereof. For instance, studies have shown that residues I253, S254, H435, and Y436 (numbering according to the Kabat EU index numbering system) are important for the interaction between the human Fc region and the human FcRn complex. See, e.g., Firan, M., et al., *Int. Immunol.* 13 (2001) 993-1002; Shields, R.L., et al., *J. Biol. Chem.* 276 (2001) 6591-6604. Various mutants of residues 248-259, 301-317, 376-382, and 424-437 (numbering according to the Kabat EU index numbering system) have also been examined and reported. Yeung, Y.A., et al. (*J. Immunol.* 182 (2009) 7667-7671).

In some embodiments, the antibody or fragment thereof comprises either a heavy chain polypeptide or a light chain polypeptide. In some embodiments, the antibody or fragment thereof comprises a portion of either a heavy chain polypeptide or a light chain polypeptide. In some embodiments, the antibody or fragment thereof comprises an Fc domain or fragment thereof. In some embodiments, the antibody or fragment thereof comprises a CH2 and CH3 domain or a fragment thereof. In some embodiments, the antibody or fragment thereof comprises the constant domain of the heavy chain polypeptide. In some embodiments, the antibody or fragment thereof comprises the constant domain of the light chain polypeptide. In some embodiments, the antibody or fragment thereof comprises a heavy chain polypeptide or fragment thereof (e.g., an Fc domain or fragment thereof). In some embodiments, the antibody or fragment thereof comprises a light chain polypeptide.

In some embodiments, the first half-life extension domain comprises a first Fc domain or a fragment thereof and the second half-life extension domain comprises a second Fc domain or a fragment thereof.

In some embodiments, the first and/or second Fc domains each contain one or more modifications that promote the non-covalent association of the first and the second half-life extension domains. In some embodiments, the first half-life extension domain comprises an IgG1 Fc domain or fragment thereof including the mutations Y349C; T366S; L38A; and Y407V to form a 'hole' in the first half-life extension domain and the second half-life extension domain comprises an IgG1 Fc domain or fragment thereof including the mutations S354C and T366W to form the 'knob' in the second half-life extension domain.

In some embodiments, the first and second half-life extension domains are each an IgG1, IgG2 or IgG4 Fc domain or fragment thereof. In some embodiments, the first and second half-life extension domains are each an IgG1 Fc domain or fragment thereof. Human IgG1 Immunoglobulin heavy constant gamma 1 has the sequence:

ASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSS
GLYSLSSVVPSSSLGTQTYICNVNHKPSNTKVDKKVEPKSCDKTHTCPPCPAPELLGG
PSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYN
STYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSRDE
LTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTTPVLDSDGSFFLYSKLTVDKSRW
QQGNVFSCSVMHEALHNHYTQKSLSLSPGK
(SEQ ID NO: 21)

In some embodiments, the first and second half-life extension domains are derived from the sequence for human IgG1 Immunoglobulin heavy constant gamma 1 having SEQ ID NO: 21 (the 'parent sequence'), such that the first and second half-life extension domains each comprise SEQ ID NO: 21 or fragment thereof, with one or more amino acid modifications.

In some embodiments, the first and second half-life extension domains each comprise the portion of SEQ ID NO: 21 shown in bold above, optionally with one or more amino acid modifications, i.e.:

DKTHTCPPCPAPELLGG
PSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYN
STYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSRDE
LTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTTPVLDSDGSFFLYSKLTVDKSRW
QQGNVFSCSVMHEALHNHYTQKSLSLSPG
(SEQ ID NO: 22)

In some embodiments, the first and second half-life extension domains comprise SEQ ID NO: 22 with amino substitutions to promote association of the first and second half-life extension domains according to the 'knob into holes' approach. In some embodiments, the sequence SEQ ID NO: 22 contains mutations Y349C; T366S; L38A; and Y407V (numbered according to the Kabat EU numbering system) to form the 'hole' in the first half-life extension domain and mutations S354C and T366W (numbered according to the

Kabat EU numbering system) to form the 'knob' in the second half-life extension domain. These modified sequences have SEQ ID NOs 23 and 24 shown below:

First half-life extension domain (Y349C; T366S; L38A; and Y407V) SEQ ID NO 23:

5

DKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHE
DPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWL
NGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVCTLPSSRDELTKNQ
VSLSCAVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLVSKL
TVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPG

10

Second half-life extension domain (S354C and T366W) SEQ ID NO 24:

15

DKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSH
EDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDW
LNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPCRDELTK
NQVSLWCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFL
YSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPG

20

In some embodiments, the first and second half-life extension domains each further comprise amino substitution N297A, numbered according to the Kabat EU numbering system:

First half-life extension domain (Y349C; T366S; L38A; Y407V and N297A) SEQ ID NO 25:

25

DKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHE
DPEVKFNWYVDGVEVHNAKTKPREEQYASTYRVVSVLTVLHQDWL
NGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVCTLPSSRDELTKNQ
VSLSCAVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLVSKL
TVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPG

30

Second half-life extension domain (S354C, T366W and N297A) SEQ ID NO 26:

35

DKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSH
EDPEVKFNWYVDGVEVHNAKTKPREEQYASTYRVVSVLTVLHQDW
LNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPCRDELTK
NQVSLWCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFL
YSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPG

In some embodiments, the first and second half-life extension domains each further comprise the amino substitution I253A, numbered according to the Kabat EU numbering system.

In some embodiments, the first and second half-life extension domains each further comprise both the amino substitutions N297A and I253A, numbered according to the Kabat EU numbering system:

First half-life extension domain (Y349C; T366S; L38A; Y407V, N297A and I253A) SEQ ID NO 27:

DKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMASRTPEVTCVVVDVSHEDP
EVKFNWYVDGVEVHNAKTKPREEQYASTYRVVSVLTVLHQDWLNGKE
YKCKVSNKALPAPIEKTISKAKGQPREPQVCTLPSSRDELTKNQVSLSCA
VKGFYPSDIAVEWESNGQPENNYKTTTPVLDSGDSFFLVSKLTVDKSRW
QQGNVFSCSVMHEALHNHYTQKSLSLSPG

Second half-life extension domain (S354C, T366W, N297A and I253A) SEQ ID NO 28:

DKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMASRTPEVTCVVVDVSHEDP
EVKFNWYVDGVEVHNAKTKPREEQYASTYRVVSVLTVLHQDWLNGKEY
KCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPCRDELTKNQVSLWCLVK
GFYPSDIAVEWESNGQPENNYKTTTPVLDSGDSFFLYSKLTVDKSRW
QQGNVFSCSVMHEALHNHYTQKSLSLSPG

In some embodiments, the first half-life extension domain comprises an amino acid sequence having about or at least about 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% sequence identity to any one of the amino acid sequence of any one of SEQ ID NOs: 22, 23, 25, and 27.

In some embodiments, the second half-life extension domain comprises an amino acid sequence having about or at least about 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% sequence identity to any one of the amino acid sequence of any one of SEQ ID NOs: 22, 24, 26 and 28.

In some embodiments, the first half-life extension domain comprises an amino acid sequence having one or more modifications, such as one or more amino acid substitutions, additions, or deletions, as compared to the amino acid sequence of any one of SEQ ID NOs: 22, 23, 25, and 27. In some embodiments, the second half-life extension domain comprises an amino acid sequence having one or more modifications, such as one or more amino acid substitutions, additions, or deletions, as compared to the amino acid sequence of any one of SEQ ID NOs: 22, 24, 26 and 28. The one or more modifications can be any

modifications or alterations described herein, including, in some embodiments, any modifications or alterations disclosed herein that promote heterodimerization of polypeptide chains and/or suppresses homodimerization of polypeptide chains, alter effector function, or enhance effector function.

- 5 In some embodiments, the Fc domain or fragment thereof comprises one or more amino acid substitutions altering effector function. In some embodiments, the half-life extension domain is an IgG1 Fc domain or fragment thereof and comprises one or more amino acid substitutions selected from the group consisting of N297A, N297G, N297Q, L234A, L235A, C220S, C226S, C229S, P238S, E233P, L234V, L234F, L235E, P331S, S267E, L328F, D265A, and P329G, numbered according to the Kabat EU numbering system. In some embodiments, the half-life extension domain is an IgG2 Fc domain or fragment thereof and comprises the amino substitution(s): V234A and G237A; H268Q, V309L, A330S, and A331S; and/or V234A, G237A, P238S, H268A, V309L, and A330S, numbered according to the Kabat EU numbering system. In some embodiments, the half-life extension domain is an IgG2 Fc domain or fragment thereof and comprises one or more amino acid substitutions selected from the group consisting of V234A, G237A, H268Q, V309L, A330S, A331S, P238S, H268A, and V309L, numbered according to the Kabat EU numbering system. In some embodiments, the half-life extension domain is an IgG4 Fc domain or fragment thereof and comprises the amino substitution(s): L235A, G237A, and E318A; S228P, L234A, and L235A; H268Q, V309L, A330S, and P331S; and/or S228P and L235A, numbered according to the Kabat EU numbering system. In some embodiments, the half-life extension domain is an IgG2 Fc domain or fragment thereof and comprises one or more amino acid substitutions selected from the group consisting of L235A, G237A, E318A, S228P, L234A, H268Q, V309L, A330S, and P331S, numbered according to the Kabat EU numbering system.

- In some embodiments, the half-life extension domain comprises Fc domain or fragment thereof that comprises one or more amino acid substitutions enhancing effector function. In some embodiments, the half-life extension domain is an IgG1 Fc domain or fragment thereof and comprises the amino acid substitution(s): S298A, E333A, and K334A; S239D and I332E; S239D, A330L, and I332E; P247I and A339D or A339Q; D280H and K290S; D280H, K290S, and either S298D or S298V; F243L, R292P, and Y300L; F243L, R292P, Y300L, and P396L; F243L, R292P, Y300L, V305I, and P396L; G236A, S239D, and I332E; K326A and E333A; K326W and E333S; K290E, S298G, and T299A; K290E, S298G, T299A, and K326E; K290N, S298G, and T299A; K290N, S298G, T299A, and K326E; K334V; L235S, S239D, and K334V; K334V and Q331M, S239D, F243V, E294L, or S298T; E233L, Q311M, and K334V; L234I, Q311M, and K334V; K334V and S298T, A330M, or A330F; K334V, Q311M, and either A330M or A330F; K334V, S298T, and either A330M or A330F; K334V, S239D, and either A330M or S298T; L234Y, Y296W, and K290Y, F243V, or E294L; Y296W and either L234Y or K290Y; S239D, A330S, and I332E, V264I; F243L and V264I; L328M; I332E; L328M and I332E; V264I and I332E; S239E and I332E; S239Q and I332E; S239E; A330Y; I332D; L328I and I332E; L328Q and I332E; V264T; V240I; V266I; S239D; S239D and I332D; S239D and I332N; S239D and I332Q; S239E and I332D; S239E and I332N;

- S239E and I332Q; S239N and I332D; S239N and I332E; S239Q and I332D; A330Y and I332E; V264I, A330Y, and I332E; A330L and I332E; V264I, A330L, and I332E; L234E, L234Y, or L234I; L235D, L235S, L235Y, or L235I; S239T; V240M; V264Y; A330I; N325T; I332E and L328D, L328V, L328T, or L328I; V264I, I332E, and either S239E or S239Q; S239E, V264I, A330Y, and I332E; A330Y, I332E, and either S239D or S239N; A330L, I332E, and either S239D or S239N; V264I, S298A, and I332E; S298A, I332E, and either S239D or S239N; S239D, V264I, and I332E; S239D, V264I, S298A, and I332E; S239D, V264I, A330L, and I332E; S239D, I332E, and A330I; P230A; P230A, E233D, and I332E; E272Y; K274T, K274E, K274R, K274L, or K274Y; F275W; N276L; Y278T; V302I; E318R; S324D, S324I or S324V; K326I or K326T; T335D, T335R, or T335Y; V240I and V266I; S239D, A330Y, I332E, and L234I; S239D, A330Y, I332E, and L235D; S239D, A330Y, I332E, and V240I; S239D, A330Y, I332E, and V264T; and/or S239D, A330Y, I332E, and either K326E or K326T, numbered according to the Kabat EU numbering system. In some embodiments, the half-life extension domain is an IgG1 Fc domain or fragment thereof and comprises one or more amino acid substitution(s) selected from the group consisting of: P230A, E233D, L234E, L234Y, L234I, L235D, L235S, L235Y, L235I, S239D, S239E, S239N, S239Q, S239T, V240I, V240M, F243L, V264I, V264T, V264Y, V266I, E272Y, K274T, K274E, K274R, K274L, K274Y, F275W, N276L, Y278T, V302I, E318R, S324D, S324I, S324V, N325T, K326I, K326T, L328M, L328I, L328Q, L328D, L328V, L328T, A330Y, A330L, A330I, I332D, I332E, I332N, I332Q, T335D, T335R, and T335Y.
- In some embodiments, the half-life extension domain comprises one or more amino acid substitution(s) that enhance binding of the half-life extension domain to FcRn. In some embodiments, the one or more amino acid substitution(s) increase binding affinity of an Fc-containing polypeptide (e.g., a heavy chain polypeptide or an Fc domain or fragment thereof) to FcRn at acidic pH. In some embodiments, the half-life extension domain comprises one or more amino acid substitution(s) selected from the group consisting of M428F; T250Q and M428F; M252Y, S254T, and T256E; P257I and N434H; D376V and N434H; P257I and Q311I; N434A; N434W; M428F and N434S; V259I and V308F; M252Y, S254T, and T256E; V259I, V308F and M428F; T307Q and N434A; T307Q and N434S; T307Q, E380A, and N434A; V308P and N434A; N434H; and V308P.
- For manufacturing purposes, a signal peptide may be engineered upstream of the half life domain to improve secretion of the protein. The signal peptide is selected according to the cell line's requirements as is known in the art. It will be understood that the signal peptide is not expressed as part of the protein that will be purified and formulated as drug product.

1.4.1 Heterodimerization Modifications

The half-life extension domains described herein may include one or more modifications that promote heterodimerization of two different half-life extension domains. In some embodiments, it is desirable to

promote heterodimerization of the first and second half-life extension domains such that production of the masked cytokine in its correct heterodimeric form is produced efficiently. As such, one or more amino acid modifications can be made to the first half-life extension domain and one or more amino acid modifications can be made to the second half-life extension domain using any strategy available in the art, including any strategy as described in Klein et al. (2012), MAbs, 4(6): 653-663. Exemplary strategies and modifications are described in detail below.

Knobs-into-Holes Approach

One strategy for promoting heterodimerization of two different half-life extension domains is an approach termed the “knobs-into-holes”.

In some embodiments, the masked cytokine comprises a first half-life extension domain and a second half-life extension domain, each of which comprises a CH3 domain. In some embodiments, the half-life extension domain comprising a CH3 domain is a heavy chain polypeptide or a fragment thereof (e.g., an Fc domain or fragment thereof). The CH3 domains of the two half-life extension domains can be altered by the “knobs-into-holes” technology, which is described in detail with several examples in, e.g., WO 1996/027011; Ridgway, J.B. et al, Protein Eng. (1996) 9(7): 617-621; Merchant, A.M., et al, Nat. Biotechnol. (1998) 16(7): 677-681. See also Klein et al. (2012), MAbs, 4(6): 653-663. Using the knob-into-holes method, the interaction surfaces of the two CH3 domains are altered to increase the heterodimerization of the two half-life extension domains containing the two altered CH3 domains. This occurs by introducing a bulky residue into the CH3 domain of one of the half-life extension domains, which acts as the “knob.” Then, in order to accommodate the bulky residue, a “hole” is formed in the other half-life extension domain that can accommodate the knob. Either of the altered CH3 domains can be the “knob” while the other can be the “hole.” The introduction of a disulfide bridge further stabilizes the heterodimers (Merchant, A.M., et al, Nat. Biotechnol. (1998) 16(7); Atwell, S., et al, J. Mol. Biol. (1997) 270(1): 26-35) as well as increases yield.

It has been reported that heterodimerization yields above 97% can be achieved by introducing the S354C and T366W mutations in a heavy chain to create the “knob” and by introducing the Y349C, T366S, L368A, and Y407V mutations in a heavy chain to create the “hole” (numbering of the residues according to the Kabat EU numbering system). Carter et al. (2001), J. Immunol. Methods, 248: 7-15; Klein et al. (2012), MAbs, 4(6): 653-663.

In some embodiments comprising a first half-life extension domain and a second half-life extension domain, the first half-life extension domain comprises a heavy chain polypeptide or portion thereof (e.g., an Fc domain or fragment thereof) that comprises the amino acid mutations S354C and T366W (numbered according to the Kabat EU numbering system), and the second half-life extension domain comprises a

heavy chain polypeptide or portion thereof (e.g., an Fc domain or fragment thereof) that comprises the amino acid mutations Y349C, T366S, L368A, and Y407V (numbered according to the Kabat EU numbering system). In some embodiments comprising a first half-life extension domain and a second half-life extension domain, the first half-life extension domain comprises a heavy chain polypeptide or portion thereof (e.g., an Fc domain or fragment thereof) that comprises the amino acid mutations Y349C, T366S, L368A, and Y407V (numbered according to the Kabat EU numbering system), and the second half-life extension domain comprises a heavy chain polypeptide or portion thereof (e.g., an Fc domain or fragment thereof) that comprises the amino acid mutations S354C and T366W (numbered according to the Kabat EU numbering system).

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Additional examples of substitutions that can be made to form knobs and holes include those described in US20140302037A1, the contents of which are herein incorporated by reference. For example, in some embodiments, any of the following amino acid substitutions can be made to a first half-life extension domain ("first domain") and a paired second half-life extension domain ("second domain") that each contain an Fc domain: (a) Y407T in the first domain and T366Y in the second domain; (b) Y407A in the first domain and T366W in the second domain; (c) F405A in the first domain and T394W in the second domain; (d) F405W in the first domain and T394S in the second domain; (e) Y407T in the first domain and T366Y in the second domain; (f) T366Y and F405A in the first domain and T394W and Y407T in the second domain; (g) T366W and F405W in the first domain and T394S and Y407A in the second domain; (h) F405W and Y407A in the first domain and T366W and T394S in the second domain; or (i) T366W in the first domain and T366S, L368A, and Y407V in the second domain, numbered according to the Kabat EU numbering system.

In some embodiments, any of the following amino acid substitutions can be made to a first half-life extension domain ("first domain") and a paired second half-life extension domain ("second domain") that each contain an Fc domain: (a) Y407T in the second domain and T366Y in the first domain; (b) Y407A in the second domain and T366W in the first domain; (c) F405A in the second domain and T394W in the first domain; (d) F405W in the second domain and T394S in the first domain; (e) Y407T in the second domain and T366Y in the first domain; (f) T366Y and F405A in the second domain and T394W and Y407T in the first domain; (g) T366W and F405W in the second domain and T394S and Y407A in the first domain; (h) F405W and Y407A in the second domain and T366W and T394S in the first domain; or (i) T366W in the second domain and T366S, L368A, and Y407V in the first domain, numbered according to the Kabat EU numbering system.

In embodiments comprising a first half-life extension domain and a second half-life extension domain that each comprise an Fc domain, any of the heterodimerizing alterations described herein can be used in the Fc domains to promote heterodimerization of any of the masked cytokines described herein.

1.5 Exemplary masked cytokines

Masked cytokines according to the disclosure can combine a IL-12 cytokine or functional fragment thereof as described anywhere herein; a masking moiety as described anywhere herein; first and second half life domains as described anywhere herein; and cleavable and non-cleavable linkers as described anywhere herein.

Furthermore, in an embodiment, any specific sequence disclosed herein may optionally comprise further amino acid substitutions, such as one, two or three substitutions. In another embodiment, sequences having at least 90% homology, preferably 95%, more preferably 99%, to any specific sequence disclosed herein for a domain of the masked cytokines are also encompassed by the invention.

In some embodiments, the IL-12 cytokine or functional fragment thereof comprises the amino acid sequence of SEQ ID NO: 4, the masking moiety comprises human IL-12R β 1 or a fragment, portion, or variant thereof that retains or otherwise demonstrates an affinity to IL-12, and the first half-life extension domain comprises SEQ ID NO: 25 (Y349C; T366S; L38A; Y407V; and N297A) and the second half-life extension domain comprises SEQ ID NO 26 (S354C, T366W and N297A).

In some embodiments, the IL-12 cytokine or functional fragment thereof comprises the amino acid sequence of SEQ ID NO: 4, the masking moiety comprises residues 24 to 237 of human IL-12R β 1, namely a sequence having SEQ ID NO: 5 or a fragment, portion, or variant thereof that retains or otherwise demonstrates an affinity to IL-12, and the first half-life extension domain comprises SEQ ID NO: 25 (Y349C; T366S; L38A; Y407V; and N297A) and the second half-life extension domain comprises SEQ ID NO 26 (S354C, T366W and N297A).

In some embodiments, the IL-12 cytokine or functional fragment thereof comprises the amino acid sequence of SEQ ID NO: 4, the masking moiety comprises residues 24 to 545 of human IL-12R β 1, namely a sequence having SEQ ID NO: 6 or a fragment, portion, or variant thereof that retains or otherwise demonstrates an affinity to IL-12, and the first half-life extension domain comprises SEQ ID NO: 25 (Y349C; T366S; L38A; Y407V; and N297A) and the second half-life extension domain comprises SEQ ID NO 26 (S354C, T366W and N297A).

In some embodiments, the IL-12 cytokine or functional fragment thereof comprises the amino acid sequence of SEQ ID NO: 4, the masking moiety comprises human IL-12R β 2 or a fragment, portion, or variant thereof that retains or otherwise demonstrates an affinity to IL-12, and the first half-life extension domain comprises SEQ ID NO: 25 (Y349C; T366S; L38A; Y407V; and N297A) and the second half-life extension domain comprises SEQ ID NO 26 (S354C, T366W and N297A).

In some embodiments, the IL-12 cytokine or functional fragment thereof comprises the amino acid sequence of SEQ ID NO: 61, the masking moiety comprises human IL-12R β 2 or a fragment, portion, or variant thereof that retains or otherwise demonstrates an affinity to IL-12, and the first half-life extension domain comprises SEQ ID NO: 25 (Y349C; T366S; L38A; Y407V; and N297A) and the second half-life extension domain comprises SEQ ID NO 26 (S354C, T366W and N297A).

In some embodiments, the IL-12 cytokine or functional fragment thereof comprises the amino acid sequence of SEQ ID NO: 62, the masking moiety comprises human IL-12R β 2 or a fragment, portion, or variant thereof that retains or otherwise demonstrates an affinity to IL-12, and the first half-life extension domain comprises SEQ ID NO: 25 (Y349C; T366S; L38A; Y407V; and N297A) and the second half-life extension domain comprises SEQ ID NO 26 (S354C, T366W and N297A).

In some embodiments, the IL-12 cytokine or functional fragment thereof comprises the amino acid sequence of SEQ ID NO: 63, the masking moiety comprises human IL-12R β 2 or a fragment, portion, or variant thereof that retains or otherwise demonstrates an affinity to IL-12, and the first half-life extension domain comprises SEQ ID NO: 25 (Y349C; T366S; L38A; Y407V; and N297A) and the second half-life extension domain comprises SEQ ID NO 26 (S354C, T366W and N297A).

In some embodiments, the IL-12 cytokine or functional fragment thereof comprises the amino acid sequence of SEQ ID NO: 64, and the first half-life extension domain comprises SEQ ID NO: 25 (Y349C; T366S; L38A; Y407V; and N297A) and the second half-life extension domain comprises SEQ ID NO 26 (S354C, T366W and N297A).

In some embodiments, the IL-12 cytokine or functional fragment thereof comprises the amino acid sequence of SEQ ID NO: 64, the masking moiety comprises human IL-12R β 2 or a fragment, portion, or variant thereof that retains or otherwise demonstrates an affinity to IL-12, and the first half-life extension domain comprises SEQ ID NO: 25 (Y349C; T366S; L38A; Y407V; and N297A) and the second half-life extension domain comprises SEQ ID NO 26 (S354C, T366W and N297A).

In some embodiments, the IL-12 cytokine or functional fragment thereof comprises the amino acid sequence of SEQ ID NO: 4, the masking moiety comprises residues 24 to 212 of human IL-12R β 2, namely a sequence having SEQ ID NO: 7, and the first half-life extension domain comprises SEQ ID NO: 25 (Y349C; T366S; L38A; Y407V; and N297A) and the second half-life extension domain comprises SEQ ID NO 26 (S354C, T366W and N297A).

In some embodiments, the IL-12 cytokine or functional fragment thereof comprises the amino acid sequence of SEQ ID NO: 4, the masking moiety comprises residues 24 to 222 of human IL-12R β 2, namely a sequence having SEQ ID NO: 8, and the first half-life extension domain comprises SEQ ID NO: 25

(Y349C; T366S; L38A; Y407V; and N297A) and the second half-life extension domain comprises SEQ ID NO 26 (S354C, T366W and N297A).

5 In some embodiments, the IL-12 cytokine or functional fragment thereof comprises the amino acid sequence of SEQ ID NO: 4, the masking moiety comprises residues 24 to 319 of human IL-12R β 2, namely a sequence having SEQ ID NO: 9, and the first half-life extension domain comprises SEQ ID NO: 25 (Y349C; T366S; L38A; Y407V; and N297A) and the second half-life extension domain comprises SEQ ID NO 26 (S354C, T366W and N297A).

10 In some embodiments, the IL-12 cytokine or functional fragment thereof comprises the amino acid sequence of SEQ ID NO: 4, the masking moiety comprises residues 24 to 319 of human IL-12R β 2, namely a sequence having SEQ ID NO: 65, and the first half-life extension domain comprises SEQ ID NO: 25 (Y349C; T366S; L38A; Y407V; and N297A) and the second half-life extension domain comprises SEQ ID NO 26 (S354C, T366W and N297A).

15 In some embodiments, the IL-12 cytokine or functional fragment thereof comprises the amino acid sequence of SEQ ID NO: 63, the masking moiety comprises residues 24 to 319 of human IL-12R β 2, namely a sequence having SEQ ID NO: 65, and the first half-life extension domain comprises SEQ ID NO: 25 (Y349C; T366S; L38A; Y407V; and N297A) and the second half-life extension domain comprises SEQ ID NO 26 (S354C, T366W and N297A).

20 In some embodiments, the IL-12 cytokine or functional fragment thereof comprises the amino acid sequence of SEQ ID NO: 64, the masking moiety comprises residues 24 to 319 of human IL-12R β 2, namely a sequence having SEQ ID NO: 65, and the first half-life extension domain comprises SEQ ID NO: 25 (Y349C; T366S; L38A; Y407V; and N297A) and the second half-life extension domain comprises SEQ ID NO 26 (S354C, T366W and N297A).

25 In some embodiments, the IL-12 cytokine or functional fragment thereof comprises the amino acid sequence of SEQ ID NO: 64, the masking moiety comprises residues 24 to 319 of human IL-12R β 2, namely a sequence having SEQ ID NO: 65, and the first half-life extension domain comprises SEQ ID NO: 25 (Y349C; T366S; L38A; Y407V; and N297A) and the second half-life extension domain comprises SEQ ID NO 26 (S354C, T366W and N297A).

30 In some embodiments, the IL-12 cytokine or functional fragment thereof comprises the amino acid sequence of SEQ ID NO: 4, the masking moiety comprises residues 24 to 622 of human IL-12R β 2, namely a sequence having SEQ ID NO: 10, and the first half-life extension domain comprises SEQ ID NO: 25 (Y349C; T366S; L38A; Y407V; and N297A) and the second half-life extension domain comprises SEQ ID NO 26 (S354C, T366W and N297A).

35 In some embodiments, the IL-12 cytokine or functional fragment thereof comprises the amino acid sequence of SEQ ID NO: 4, the masking moiety comprises residues 24 to 622 of human IL-12R β 2, namely a sequence having SEQ ID NO: 10, and the first half-life extension domain comprises SEQ ID NO: 25 (Y349C; T366S; L38A; Y407V; and N297A) and the second half-life extension domain comprises SEQ ID NO 26 (S354C, T366W and N297A).

In some embodiments, the IL-12 cytokine or functional fragment thereof comprises the amino acid sequence of SEQ ID NO: 4, the masking moiety comprises residues 24 to 227 of human IL-12R β 2, namely a sequence having SEQ ID NO: 11, and the first half-life extension domain comprises SEQ ID NO: 25 (Y349C; T366S; L38A; Y407V; and N297A) and the second half-life extension domain comprises SEQ ID NO 26 (S354C, T366W and N297A).

In some embodiments, the IL-12 cytokine or functional fragment thereof comprises the amino acid sequence of SEQ ID NO: 4, the masking moiety comprises human IL-12R β 1 or a fragment, portion, or variant thereof that retains or otherwise demonstrates an affinity to IL-12 and the first half-life extension domain comprises SEQ ID NO: 27 (Y349C; T366S; L38A; Y407V, N297A and I253A) and the second half-life extension domain comprises SEQ ID NO: 28 (S354C, T366W, N297A and I253A).

In some embodiments, the IL-12 cytokine or functional fragment thereof comprises the amino acid sequence of SEQ ID NO: 4, the masking moiety comprises residues 24 to 237 of human IL-12R β 1, namely a sequence having SEQ ID NO: 5 or a fragment, portion, or variant thereof that retains or otherwise demonstrates an affinity to IL-12 and the first half-life extension domain comprises SEQ ID NO: 27 (Y349C; T366S; L38A; Y407V, N297A and I253A) and the second half-life extension domain comprises SEQ ID NO: 28 (S354C, T366W, N297A and I253A).

In some embodiments, the IL-12 cytokine or functional fragment thereof comprises the amino acid sequence of SEQ ID NO: 4, the masking moiety comprises residues 24 to 545 of human IL-12R β 1, namely a sequence having SEQ ID NO: 6 or a fragment, portion, or variant thereof that retains or otherwise demonstrates an affinity to IL-12 and the first half-life extension domain comprises SEQ ID NO: 27 (Y349C; T366S; L38A; Y407V, N297A and I253A) and the second half-life extension domain comprises SEQ ID NO: 28 (S354C, T366W, N297A and I253A).

In some embodiments, the IL-12 cytokine or functional fragment thereof comprises the amino acid sequence of SEQ ID NO: 4, the masking moiety comprises human IL-12R β 2 or a fragment, portion, or variant thereof that retains or otherwise demonstrates an affinity to IL-12 and the first half-life extension domain comprises SEQ ID NO: 27 (Y349C; T366S; L38A; Y407V, N297A and I253A) and the second half-life extension domain comprises SEQ ID NO: 28 (S354C, T366W, N297A and I253A).

In some embodiments, the IL-12 cytokine or functional fragment thereof comprises the amino acid sequence of SEQ ID NO: 4, the masking moiety comprises residues 24 to 212 of human IL-12R β 2, namely a sequence having SEQ ID NO: 7 and the first half-life extension domain comprises SEQ ID NO: 27 (Y349C; T366S; L38A; Y407V, N297A and I253A) and the second half-life extension domain comprises SEQ ID NO: 28 (S354C, T366W, N297A and I253A).

In some embodiments, the IL-12 cytokine or functional fragment thereof comprises the amino acid sequence of SEQ ID NO: 4, the masking moiety comprises residues 24 to 222 of human IL-12R β 2, namely a sequence having SEQ ID NO: 8 and the first half-life extension domain comprises SEQ ID NO: 27 (Y349C; T366S; L38A; Y407V, N297A and I253A) and the second half-life extension domain comprises
5 SEQ ID NO: 28 (S354C, T366W, N297A and I253A).

In some embodiments, the IL-12 cytokine or functional fragment thereof comprises the amino acid sequence of SEQ ID NO: 4, the masking moiety comprises residues 24 to 319 of human IL-12R β 2, namely a sequence having SEQ ID NO: 9 and the first half-life extension domain comprises SEQ ID NO: 27
10 (Y349C; T366S; L38A; Y407V, N297A and I253A) and the second half-life extension domain comprises SEQ ID NO: 28 (S354C, T366W, N297A and I253A).

In some embodiments, the IL-12 cytokine or functional fragment thereof comprises the amino acid sequence of SEQ ID NO: 4, the masking moiety comprises residues 24 to 622 of human IL-12R β 2, namely
15 a sequence having SEQ ID NO: 10 and the first half-life extension domain comprises SEQ ID NO: 27 (Y349C; T366S; L38A; Y407V, N297A and I253A) and the second half-life extension domain comprises SEQ ID NO: 28 (S354C, T366W, N297A and I253A).

In some embodiments, the IL-12 cytokine or functional fragment thereof comprises the amino acid
20 sequence of SEQ ID NO: 4, the masking moiety comprises residues 24 to 227 of human IL-12R β 2, namely a sequence having SEQ ID NO: 11 and the first half-life extension domain comprises SEQ ID NO: 27 (Y349C; T366S; L38A; Y407V, N297A and I253A) and the second half-life extension domain comprises SEQ ID NO: 28 (S354C, T366W, N297A and I253A).

25 In some embodiments, the IL-12 cytokine or functional fragment thereof comprises the amino acid sequence of SEQ ID NO: 64 and the masking moiety comprises residues 24 to 319 of human IL-12R β 2, namely a sequence having SEQ ID NO: 65.

In some embodiments, the IL-12 cytokine or functional fragment thereof comprises the amino acid
30 sequence of SEQ ID NO: 64, the masking moiety comprises residues 24 to 319 of human IL-12R β 2, namely a sequence having SEQ ID NO: 65, the non-cleavable linker comprises the amino acid sequence of SEQ ID NO: 14, and the first half-life extension domain comprises SEQ ID NO: 25 (Y349C; T366S; L38A; Y407V; and N297A) and the second half-life extension domain comprises SEQ ID NO 26 (S354C, T366W and N297A).

35 In some embodiments, the IL-12 cytokine or functional fragment thereof comprises the amino acid sequence of SEQ ID NO: 64, the masking moiety comprises residues 24 to 319 of human IL-12R β 2, namely a sequence having SEQ ID NO: 65, the non-cleavable linker comprises the amino acid sequence of SEQ

ID NO: 55, and the first half-life extension domain comprises SEQ ID NO: 25 (Y349C; T366S; L38A; Y407V; and N297A) and the second half-life extension domain comprises SEQ ID NO 26 (S354C, T366W and N297A).

5 In some embodiments, the IL-12 cytokine or functional fragment thereof comprises the amino acid sequence of SEQ ID NO: 64, the masking moiety comprises residues 24 to 319 of human IL-12R β 2, namely a sequence having SEQ ID NO: 65, the non-cleavable linker comprises the amino acid sequence of SEQ ID NO: 56, and the first half-life extension domain comprises SEQ ID NO: 25 (Y349C; T366S; L38A; Y407V; and N297A) and the second half-life extension domain comprises SEQ ID NO 26 (S354C, T366W
10 and N297A).

In some embodiments, the IL-12 cytokine or functional fragment thereof comprises the amino acid sequence of SEQ ID NO: 64, the masking moiety comprises residues 24 to 319 of human IL-12R β 2, namely a sequence having SEQ ID NO: 65, the cleavable peptide comprises the amino acid sequence of SEQ ID
15 NO: 41, and the first half-life extension domain comprises SEQ ID NO: 25 (Y349C; T366S; L38A; Y407V; and N297A) and the second half-life extension domain comprises SEQ ID NO 26 (S354C, T366W and N297A).

In some embodiments, the IL-12 cytokine or functional fragment thereof comprises the amino acid
20 sequence of SEQ ID NO: 64, the masking moiety comprises residues 24 to 319 of human IL-12R β 2, namely a sequence having SEQ ID NO: 65, the cleavable peptide comprises the amino acid sequence of SEQ ID NO: 43, and the first half-life extension domain comprises SEQ ID NO: 25 (Y349C; T366S; L38A; Y407V; and N297A) and the second half-life extension domain comprises SEQ ID NO 26 (S354C, T366W and N297A).

25 In some embodiments, the IL-12 cytokine or functional fragment thereof comprises the amino acid sequence of SEQ ID NO: 64, the masking moiety comprises residues 24 to 319 of human IL-12R β 2, namely a sequence having SEQ ID NO: 65, the cleavable peptide comprises the amino acid sequence of SEQ ID NO: 44, and the first half-life extension domain comprises SEQ ID NO: 25 (Y349C; T366S; L38A; Y407V; and N297A) and the second half-life extension domain comprises SEQ ID NO 26 (S354C, T366W and
30 N297A).

In some embodiments, the IL-12 cytokine or functional fragment thereof comprises the amino acid
35 sequence of SEQ ID NO: 64, the masking moiety comprises residues 24 to 319 of human IL-12R β 2, namely a sequence having SEQ ID NO: 65, the cleavable linker comprises the amino acid sequence of SEQ ID NO: 51, and the first half-life extension domain comprises SEQ ID NO: 25 (Y349C; T366S; L38A; Y407V; and N297A) and the second half-life extension domain comprises SEQ ID NO 26 (S354C, T366W and N297A).

In some embodiments, the IL-12 cytokine or functional fragment thereof comprises the amino acid sequence of SEQ ID NO: 64, the masking moiety comprises residues 24 to 319 of human IL-12R β 2, namely a sequence having SEQ ID NO: 65, the cleavable linker comprises the amino acid sequence of SEQ ID NO: 53, and the first half-life extension domain comprises SEQ ID NO: 25 (Y349C; T366S; L38A; Y407V; and N297A) and the second half-life extension domain comprises SEQ ID NO 26 (S354C, T366W and N297A).

In some embodiments, the IL-12 cytokine or functional fragment thereof comprises the amino acid sequence of SEQ ID NO: 64, the masking moiety comprises residues 24 to 319 of human IL-12R β 2, namely a sequence having SEQ ID NO: 65, the cleavable linker comprises the amino acid sequence of SEQ ID NO: 46, and the first half-life extension domain comprises SEQ ID NO: 25 (Y349C; T366S; L38A; Y407V; and N297A) and the second half-life extension domain comprises SEQ ID NO 26 (S354C, T366W and N297A).

In some embodiments, the IL-12 cytokine or functional fragment thereof comprises the amino acid sequence of SEQ ID NO: 64, the masking moiety comprises residues 24 to 319 of human IL-12R β 2, namely a sequence having SEQ ID NO: 65, the non-cleavable linker comprises the amino acid sequence of SEQ ID NO: 56, the cleavable linker comprises the amino acid sequence of SEQ ID NO: 51, and the first half-life extension domain comprises SEQ ID NO: 25 (Y349C; T366S; L38A; Y407V; and N297A) and the second half-life extension domain comprises SEQ ID NO 26 (S354C, T366W and N297A).

In some embodiments, the IL-12 cytokine or functional fragment thereof comprises the amino acid sequence of SEQ ID NO: 64, the masking moiety comprises residues 24 to 319 of human IL-12R β 2, namely a sequence having SEQ ID NO: 65, the non-cleavable linker comprises the amino acid sequence of SEQ ID NO: 14, the cleavable linker comprises the amino acid sequence of SEQ ID NO: 53, and the first half-life extension domain comprises SEQ ID NO: 25 (Y349C; T366S; L38A; Y407V; and N297A) and the second half-life extension domain comprises SEQ ID NO 26 (S354C, T366W and N297A).

In some embodiments, the IL-12 cytokine or functional fragment thereof comprises the amino acid sequence of SEQ ID NO: 64, the masking moiety comprises residues 24 to 319 of human IL-12R β 2, namely a sequence having SEQ ID NO: 65, the non-cleavable linker comprises the amino acid sequence of SEQ ID NO: 55, the cleavable linker comprises the amino acid sequence of SEQ ID NO: 46, and the first half-life extension domain comprises SEQ ID NO: 25 (Y349C; T366S; L38A; Y407V; and N297A) and the second half-life extension domain comprises SEQ ID NO 26 (S354C, T366W and N297A).

In some embodiments of the masked cytokine, the first polypeptide chain comprises:

N' HL1-L1-MM C'

and the second polypeptide chain comprises:

N' HL2-L2-[IL-12p35-linker-IL-12p40] C'

where 'IL-12p40' is the IL-12p40 polypeptide or functional fragment thereof and 'IL-12p35' is the IL-12p35 polypeptide or functional fragment thereof. The first half life extension domain (HL1), the first linker (L1), the masking moiety (MM), the second half life extension domain (HL2), the second linker (L2) and IL-12 cytokine or fragment thereof ([IL-12p35-linker-IL-12p40]) may be as defined anywhere herein.

5

In some embodiments, the masked cytokine comprises a first polypeptide chain comprising an amino acid sequence of SEQ ID NO:34 and a second polypeptide chain comprising an amino acid sequence of SEQ ID NO: 40.

10 In some embodiments, the masked cytokine comprises a first polypeptide chain comprising an amino acid sequence of SEQ ID NO: 81 and a second polypeptide chain comprising an amino acid sequence of SEQ ID NO: 40.

In some embodiments, the masked cytokine comprises a first polypeptide chain comprising an amino acid sequence of SEQ ID NO: 34 and a second polypeptide chain comprising an amino acid sequence of SEQ ID NO: 88.

15 In some embodiments, the masked cytokine comprises a first polypeptide chain comprising an amino acid sequence of SEQ ID NO: 81 and a second polypeptide chain comprising an amino acid sequence of SEQ ID NO: 88.

In some embodiments, the masked cytokine comprises a first polypeptide chain comprising an amino acid sequence of SEQ ID NO: 82 and a second polypeptide chain comprising an amino acid sequence of SEQ ID NO: 89.

25

In some embodiments, the masked cytokine comprises a first polypeptide chain comprising an amino acid sequence of SEQ ID NO: 83 and a second polypeptide chain comprising an amino acid sequence of SEQ ID NO: 90.

30 In some embodiments, the masked cytokine comprises a first polypeptide chain comprising an amino acid sequence of SEQ ID NO: 83 and a second polypeptide chain comprising an amino acid sequence of SEQ ID NO: 91.

In some embodiments, the masked cytokine comprises a first polypeptide chain comprising an amino acid sequence of SEQ ID NO: 82 and a second polypeptide chain comprising an amino acid sequence of SEQ ID NO: 92.

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In some embodiments, the masked cytokine comprises a first polypeptide chain comprising an amino acid sequence of SEQ ID NO: 83 and a second polypeptide chain comprising an amino acid sequence of SEQ ID NO: 93.

- 5 In some embodiments, the masked cytokine comprises a first polypeptide chain comprising an amino acid sequence of SEQ ID NO: 82 and a second polypeptide chain comprising an amino acid sequence of SEQ ID NO: 94.

- 10 In some embodiments, the masked cytokine comprises a first polypeptide chain comprising an amino acid sequence of SEQ ID NO: 84 and a second polypeptide chain comprising an amino acid sequence of SEQ ID NO: 93.

- 15 In some embodiments, the masked cytokine comprises a first polypeptide chain comprising an amino acid sequence of SEQ ID NO: 84 and a second polypeptide chain comprising an amino acid sequence of SEQ ID NO: 94.

- 20 In some embodiments, the masked cytokine comprises a first polypeptide chain comprising an amino acid sequence of SEQ ID NO: 83 and a second polypeptide chain comprising an amino acid sequence of SEQ ID NO: 95.

- In some embodiments, the masked cytokine comprises a first polypeptide chain comprising an amino acid sequence of SEQ ID NO: 82 and a second polypeptide chain comprising an amino acid sequence of SEQ ID NO: 96.

- 25 In some embodiments, the masked cytokine comprises a first polypeptide chain comprising an amino acid sequence of SEQ ID NO: 83 and a second polypeptide chain comprising an amino acid sequence of SEQ ID NO: 97.

- 30 In some embodiments, the masked cytokine comprises a first polypeptide chain comprising an amino acid sequence of SEQ ID NO: 82 and a second polypeptide chain comprising an amino acid sequence of SEQ ID NO: 98.

- 35 In some embodiments, the masked cytokine comprises a first polypeptide chain comprising an amino acid sequence of SEQ ID NO: 84 and a second polypeptide chain comprising an amino acid sequence of SEQ ID NO: 97.

In some embodiments, the masked cytokine comprises a first polypeptide chain comprising an amino acid sequence of SEQ ID NO: 84 and a second polypeptide chain comprising an amino acid sequence of SEQ ID NO: 98.

5 2. CLEAVAGE PRODUCT

Provided herein is a cleavage product of a 'heterodimeric' masked IL-12 cytokines described herein.

10 The masked IL-12 cytokines described herein comprise a cleavable linker. Upon proteolytic cleavage of the cleavable linker at the cleavage site, a cleavage product comprising the IL-12 cytokine or functional fragment thereof is formed. The IL-12 cytokine or functional fragment thereof in the cleavage product is activated since it is no longer masked by the masking moiety. The IL-12 cytokine or functional fragment thereof in the cleavage product is therefore capable of binding to the target protein.

15 The tumor cell environment is complex and can comprise multiple different proteases. As such, the precise site at which a given cleavable peptide within a masked IL-12 cytokine will be cleaved in the tumor cell environment may vary between tumor types, between patients with the same tumor type and even between cleavage products formed in the same tumor. Moreover, even after cleavage, further modification of the initial cleavage product, e.g. by removal of one or two terminal amino acids, may occur by the further action of proteases in the tumor cell environment. A distribution of cleavage products can thus be expected to form in the tumor cell environment of a patient following administration of a masked cytokine as described herein.

25 Provided herein is a cleavage product capable of binding to IL-12R, the cleavage product comprising an IL-12 cytokine or functional fragment thereof, preparable by proteolytic cleavage of the cleavable peptide in a masked IL-12 cytokine as described anywhere herein.

30 Also provided herein is a cleavage product of a masked IL-12 cytokine, where the cleavage product is capable of binding to IL-12R, the cleavage product comprising an IL-12 cytokine or functional fragment thereof as defined anywhere herein. Also provided herein is a distribution of cleavage products obtained or obtainable from a single structure of a masked IL-12 cytokine, where each cleavage product within the distribution of cleavage products (i) is capable of binding to IL-12R and (ii) comprises an IL-12 cytokine or functional fragment thereof as defined anywhere herein.

35 Also provided herein is a cleavage product of a masked IL-12 cytokine, where the cleavage product is capable of binding to IL-12R, the cleavage product comprising a polypeptide comprising:

PCP-SD-C

wherein PCP is a portion of a proteolytically cleavable peptide; SD is a spacer domain; and C is an IL-12 cytokine or functional fragment thereof.

5 Further provided herein is a cleavage product of a masked IL-12 cytokine, where the cleavage product is capable of binding to IL-12R, the cleavage product comprising a protein heterodimer comprising:

- a) a first polypeptide chain comprising a first half-life extension domain; and
- b) a second polypeptide chain comprising a polypeptide comprising formula 5:

HL2-L2-C

10 (5)

wherein HL2 is a second half-life extension domain; L2 is a non-cleavable linker; and C is an IL-12 cytokine or functional fragment thereof; and wherein the first half-life extension domain is associated with the second half-life extension domain. Also provided herein is a distribution of cleavage products obtained or obtainable from a single structure of a masked IL-12 cytokine, where each cleavage product within the distribution of cleavage products (i) is capable of binding to IL-12R and (ii) comprises a protein heterodimer comprising:

- a) a first polypeptide chain comprising a first half-life extension domain; and
- b) a second polypeptide chain comprising a polypeptide comprising formula 5:

HL2-L2-C

20 (5)

wherein HL2 is a second half-life extension domain; L2 is a non-cleavable linker; and C is an IL-12 cytokine or functional fragment thereof; and wherein the first half-life extension domain is associated with the second half-life extension domain.

25 Further provided herein is a cleavage product of a masked IL-12 cytokine, where the cleavage product is capable of binding to IL-12R, the cleavage product comprising a protein heterodimer comprising:

- a) a first polypeptide chain comprising a polypeptide comprising:

HL1-SD-PCP

wherein HL1 is a first half-life extension domain; SD is a spacer domain; and PCP is a portion of a proteolytically cleavable peptide; and

- b) a second polypeptide chain comprising a polypeptide comprising:

HL2-L2-C

wherein HL2 is a second half-life extension domain; L2 is a non-cleavable linker; and C is an IL-12 cytokine or functional fragment thereof; and

35 wherein the first half-life extension domain is associated with the second half-life extension domain.

Within the cleavage product, the masking moiety, half-life extension domains, IL-12 cytokine or functional fragment thereof, linkers, space domains and type of association between the first half-life extension

domain and the second half-life extension domain may be any one of those described herein, and any combination of those described herein.

The location of the cleavable peptide determines the structure of the resulting cleavage product comprising the IL-12 cytokine.

A “portion of a proteolytically cleavable peptide”, refers to a part of the original proteolytically cleavable peptide sequence after cleavage at the cleavage site has occurred. After cleavage, further modification of the initial cleavage product, e.g. by removal of one or two terminal amino acids, may also occur by the further action of proteases in the tumor cell environment. As such, cleavage products within the distribution of cleavage products that might be formed in the tumor cell environment of a patient following administration of a masked cytokine might not contain any portion of the proteolytically cleavable peptide.

In some embodiments, a “portion” refers to 1 amino acid, 2 amino acids, 3 amino acids, 4 amino acids, 5 amino acids or 6 amino acids of the original proteolytically cleavable peptide sequence. In some embodiments, a “portion” refers to 2 amino acids of the original proteolytically cleavable peptide sequence. In some embodiments, a “portion” refers to 3 amino acids of the original proteolytically cleavable peptide sequence. In some embodiments, a “portion” refers to 4 amino acids of the original proteolytically cleavable peptide sequence.

In some embodiments, the ‘portion’ of the proteolytically cleavable peptide is from 3 to 6 amino acids in length. In some embodiments, the ‘portion’ of the proteolytically cleavable peptide is 3 or 4 amino acids in length.

Cleavage sites for cleavable linkers disclosed herein are disclosed below:

Sequence (* indicates a cleavage site within the cleavable peptide)
MPYD*LYHP
DSGG*FMLT
HEQ*LTV
RAAA*VKSP
VPLS*LY
ISSGLL*SGRS
DLLA*VVAAS

Purely by way of example, in the above table, * indicates a known or observed protease cleavage site within the cleavable peptide.

Accordingly, herein disclosed is the cleavage product of any one of the masked cytokines disclosed herein.

5

In some embodiments, the cleavage product comprises an amino acid sequence having about or at least about 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% sequence identity to an amino acid sequence selected from the group consisting of SEQ ID NO: 29.

10 In some embodiments, the cleavage product comprises an amino acid sequence having an amino acid sequence comprising SEQ ID NO: 29.

In some embodiments, the cleavage product comprises an amino acid sequence having about or at least about 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% sequence
15 identity to an amino acid sequence selected from the group consisting of SEQ ID NO: 66.

In some embodiments, the cleavage product comprises an amino acid sequence having an amino acid sequence comprising SEQ ID NO: 66.

20 In some embodiments, the cleavage product comprises an amino acid sequence having about or at least about 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% sequence identity to an amino acid sequence selected from the group consisting of SEQ ID NO: 67.

In some embodiments, the cleavage product comprises an amino acid sequence having an amino acid
25 sequence comprising SEQ ID NO: 67.

In some embodiments, the cleavage product comprises an amino acid sequence having about or at least about 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% sequence
identity to an amino acid sequence selected from the group consisting of SEQ ID NO: 68.

30

In some embodiments, the cleavage product comprises an amino acid sequence having an amino acid sequence comprising SEQ ID NO: 68.

In some embodiments, the cleavage product comprises an amino acid sequence having about or at least
35 about 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% sequence identity to an amino acid sequence selected from the group consisting of SEQ ID NO: 69.

In some embodiments, the cleavage product comprises an amino acid sequence having an amino acid sequence comprising SEQ ID NO: 69.

5 In some embodiments, the cleavage product comprises an amino acid sequence having about or at least about 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% sequence identity to an amino acid sequence selected from the group consisting of SEQ ID NO: 70.

10 In some embodiments, the cleavage product comprises an amino acid sequence having an amino acid sequence comprising SEQ ID NO: 70.

In some embodiments, the cleavage product comprises an amino acid sequence having about or at least about 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% sequence identity to an amino acid sequence selected from the group consisting of SEQ ID NO: 71.

15 In some embodiments, the cleavage product comprises an amino acid sequence having an amino acid sequence comprising SEQ ID NO: 71.

20 In some embodiments, the cleavage product comprises an amino acid sequence having about or at least about 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% sequence identity to an amino acid sequence selected from the group consisting of SEQ ID NO: 72.

In some embodiments, the cleavage product comprises an amino acid sequence having an amino acid sequence comprising SEQ ID NO: 72.

25 In some embodiments, the cleavage product comprises an amino acid sequence having about or at least about 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% sequence identity to an amino acid sequence selected from the group consisting of SEQ ID NO: 73.

30 In some embodiments, the cleavage product comprises an amino acid sequence having an amino acid sequence comprising SEQ ID NO: 73.

35 In some embodiments, the cleavage product comprises an amino acid sequence having about or at least about 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% sequence identity to an amino acid sequence selected from the group consisting of SEQ ID NO: 74.

In some embodiments, the cleavage product comprises an amino acid sequence having an amino acid sequence comprising SEQ ID NO: 74.

In some embodiments, the cleavage product comprises an amino acid sequence having about or at least about 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% sequence identity to an amino acid sequence selected from the group consisting of SEQ ID NO: 75.

- 5 In some embodiments, the cleavage product comprises an amino acid sequence having an amino acid sequence comprising SEQ ID NO: 75.

- In some embodiments, the cleavage product comprises an amino acid sequence having about or at least about 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% sequence
10 identity to an amino acid sequence selected from the group consisting of SEQ ID NO: 76.

In some embodiments, the cleavage product comprises an amino acid sequence having an amino acid sequence comprising SEQ ID NO: 76.

- 15 In some embodiments, the cleavage product comprises an amino acid sequence having about or at least about 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% sequence identity to an amino acid sequence selected from the group consisting of SEQ ID NO: 77.

- In some embodiments, the cleavage product comprises an amino acid sequence having an amino acid
20 sequence comprising SEQ ID NO: 77.

- In some embodiments, the cleavage product comprises an amino acid sequence having about or at least about 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% sequence
25 identity to an amino acid sequence selected from the group consisting of SEQ ID NO: 78.

In some embodiments, the cleavage product comprises an amino acid sequence having an amino acid sequence comprising SEQ ID NO: 78.

- In some embodiments, the cleavage product comprises an amino acid sequence having about or at least
30 about 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% sequence identity to an amino acid sequence selected from the group consisting of SEQ ID NO: 79.

- In some embodiments, the cleavage product comprises an amino acid sequence having an amino acid
35 sequence comprising SEQ ID NO: 79.

In some embodiments, the cleavage product comprises an amino acid sequence having about or at least about 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% sequence
identity to an amino acid sequence selected from the group consisting of SEQ ID NO: 80.

In some embodiments, the cleavage product comprises an amino acid sequence having an amino acid sequence comprising SEQ ID NO: 80.

5 3. BINDING ASSAYS

The strength, or affinity of immunological binding interactions, such as between a cytokine or functional fragment thereof and a binding partner (e.g., a target protein, such as a cytokine receptor) for which the cytokine or functional fragment thereof is specific, can be expressed in terms of the dissociation constant (Kd) of the interaction, wherein a smaller Kd represents a greater affinity. The binding of the IL-12 cytokine to the IL-12 cytokine receptor can be expressed in terms of the Kd. In some embodiments, the immunological binding interactions are between a masked cytokine (in the presence or absence of a protease) and a target protein, such as a cytokine receptor. Immunological binding properties of proteins can be quantified using methods well known in the art. For example, one method comprises measuring the rates of cytokine receptor (e.g., IL-12R)/cytokine (e.g., IL-12) complex formation and dissociation, wherein those rates depend on the concentrations of the complex partners, the affinity of the interaction, and geometric parameters that equally influence the rate in both directions. Both the "on rate constant" (Kon) and the "off rate constant" (Koff) can be determined by calculation of the concentrations and the actual rates of association and dissociation. The ratio of Koff/Kon enables the cancelation of all parameters not related to affinity, and is equal to the dissociation constant Kd. See Davies et al., Annual Rev Biochem. 59:439-473, (1990).

In some aspects, a masked cytokine described herein binds to a target protein with about the same or higher affinity upon cleavage with a protease as compared to the parental cytokine that comprises a masking moiety but does not comprise a cleavable peptide. The target protein can be any cytokine receptor.

In some embodiments, a masked cytokine provided herein that does not comprise a cleavable peptide in the linker has a dissociation constant (Kd) of $\leq 1\text{M}$, $\leq 150\text{ nM}$, $\leq 100\text{ nM}$, $\leq 50\text{ nM}$, $\leq 10\text{ nM}$, $\leq 1\text{ nM}$, $\leq 0.1\text{ nM}$, $\leq 0.01\text{ nM}$, 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) with the target protein. In some embodiments, a masked cytokine provided herein that comprises a cleavable peptide in the linker has a dissociation constant (Kd) of $\leq 1\text{M}$, $\leq 150\text{ nM}$, $\leq 100\text{ nM}$, $\leq 50\text{ nM}$, $\leq 10\text{ nM}$, $\leq 1\text{ nM}$, $\leq 0.1\text{ nM}$, $\leq 0.01\text{ nM}$, 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) with the target protein prior to cleavable with a protease. In some embodiments, a masked cytokine provided herein that comprises a cleavable peptide in the linker has a dissociation constant (Kd) of $\leq 1\text{M}$, $\leq 150\text{ nM}$, $\leq 100\text{ nM}$, $\leq 50\text{ nM}$, $\leq 10\text{ nM}$, $\leq 1\text{ nM}$, $\leq 0.1\text{ nM}$, $\leq 0.01\text{ nM}$, 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) with the target protein upon cleavage with a protease. In some embodiments, the cytokine or functional fragment thereof of a

masked cytokine provided herein has a dissociation constant (K_d) of $\geq 500M$, $\geq 250M$, $\geq 200M$, $\geq 150M$, $\geq 100M$, $\geq 50M$, $\geq 10M$, $\geq 1M$, $\geq 500\text{ nM}$, $\geq 250\text{ nM}$, $\geq 150\text{ nM}$, $\geq 100\text{ nM}$, $\geq 50\text{ nM}$, $\geq 10\text{ nM}$, $\geq 1\text{ nM}$, $\geq 0.1\text{ nM}$, $\geq 0.01\text{ nM}$, or $\geq 0.001\text{ nM}$ with the masking moiety of the masked cytokine. In some embodiments, the cytokine or functional fragment thereof of a masked cytokine provided herein has a dissociation constant (K_d) that is between about 200M and about 50 nM, such as about or at least about 175M, about or at least about 150M, about or at least about 125M, about or at least about 100M, about or at least about 75M, about or at least about 50M, about or at least about 25M, about or at least about 5M, about or at least about 1M, about or at least about 750 nM, about or at least about 500 nM, about or at least about 250 nM, about or at least about 150 nM, about or at least about 100 nM, about or at least about 75 nM, or about or at least about 50 nM. Assays for assessing binding affinity are well known in the art.

In some aspects, masked cytokines that exhibit a desired occlusion ratio are provided. The term "occlusion ratio" as used herein refers a ratio of (a) a maximum detected level of a parameter under a first set of conditions to (b) a minimum detected value of that parameter under a second set of conditions. In the context of a masked IL-12 polypeptide, the occlusion ratio refers to the ratio of (a) a maximum detected level of target protein (e.g., IL-12R protein) binding to the masked IL-12 polypeptide in the presence of at least one protease capable of cleaving the cleavable peptide of the masked IL-12 polypeptide to (b) a minimum detected level of target protein (e.g., IL-12R protein) binding to the masked IL-12 polypeptide in the absence of the protease. Thus, the occlusion ratio for a masked cytokine can be calculated by dividing the EC_{50} of the masked cytokine pre-cleavage by the EC_{50} of the masked cytokine post-cleavage. The occlusion ratio of a masked cytokine can also be calculated as the ratio of the dissociation constant of the masked cytokine before cleavage with a protease to the dissociation constant of the masked cytokine after cleavage with a protease. In some embodiments, a greater occlusion ratio for the masked cytokine indicates that target protein bound by the masked cytokine occurs to a greater extent (e.g., predominantly occurs) in the presence of a protease capable of cleaving the cleavable peptide of the masked cytokine than in the absence of a protease.

In some embodiments, masked cytokines with an optimal occlusion ratio are provided herein. In some embodiments, an optimal occlusion ratio of a masked cytokine indicates the masked cytokine has desirable properties useful for the methods or compositions contemplated herein. In some embodiments, a masked cytokine provided herein exhibits an optimal occlusion ratio of about 2 to about 10,000, e.g., about 80 to about 100. In a further embodiment of any of the masked cytokine provided herein, the occlusion ratio is about 2 to about 7,500, about 2 to about 5,000, about 2 to about 2,500, about 2 to about 2,000, about 2 to about 1,000, about 2 to about 900, about 2 to about 800, about 2 to about 700, about 2 to about 600, about 2 to about 500, about 2 to about 400, about 2 to about 300, about 2 to about 200, about 2 to about 100, about 2 to about 50, about 2 to about 25, about 2 to about 15, about 2 to about 10, about 5 to about 10, about 5 to about 15, about 5 to about 20, about 10 to about 100, about 20 to about 100, about 30 to about 100, about 40 to about 100, about 50 to about 100, about 60 to about 100, about 70 to about 100, about 80 to

about 100, or about 100 to about 1,000. In some embodiments, a masked cytokine provided herein exhibits an optimal occlusion ratio of about 2 to about 1,000. Binding of a masked IL-12 polypeptide to a target protein before cleavage and/or after cleavage with a protease can be determined using techniques well known in the art such as by ELISA.

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In some embodiments, a masking moiety described herein binds to a cytokine or functional fragment thereof as described herein with lower affinity than the affinity between the cytokine or functional fragment thereof and a target protein (e.g., cytokine receptor). In certain embodiments, a masking moiety provided herein binds to a cytokine or functional fragment thereof as described herein with a dissociation constant (K_d) of
10 $\geq 500\text{M}$, $\geq 250\text{M}$, $\geq 200\text{M}$, $\geq 150\text{M}$, $\geq 100\text{M}$, $\geq 50\text{M}$, $\geq 10\text{M}$, $\geq 1\text{M}$, $\geq 500\text{ nM}$, $\geq 250\text{ nM}$, $\geq 150\text{ nM}$, $\geq 100\text{ nM}$, $\geq 50\text{ nM}$, $\geq 10\text{ nM}$, $\geq 1\text{ nM}$, $\geq 0.1\text{ nM}$, $\geq 0.01\text{ nM}$, or $\geq 0.001\text{ nM}$.

4. MASKED IL-12 CYTOKINE PRODUCTION

15 The masked cytokines described herein are prepared using techniques available in the art, exemplary methods of which are described.

4.1 Antibody Production

20 Some embodiments of the masked IL-12 cytokine comprise an antibody or fragment thereof. The following sections provide further detail on the production of antibodies and antibody fragments, variants, and derivatives thereof, that may be used in some embodiments of the masked IL-12 cytokine provided herein. In some embodiments, the masked cytokine is in the form of a dimer produced by two copies of a masked IL-12 cytokine that are associated through disulfide bonds.

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1. Antibody Fragments

The present invention encompasses, in some embodiments, antibody fragments. The antibody fragments can be any antibody fragments, such as an Fc domain, a portion of the heavy chain, a portion of the light chain, an Fab, an Fv, or an scFv, among other fragments. Antibody fragments may be generated by
30 traditional means, such as enzymatic digestion, or by recombinant techniques. In certain circumstances, there are advantages of linking antibody fragments, rather than whole antibodies, to the masked cytokines described herein. For a review of certain antibody fragments, see Hudson et al. (2003) Nat. Med. 9:129-134.

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Various techniques have been developed for the production of antibody fragments. Traditionally, these fragments were derived via proteolytic digestion of intact antibodies (see, e.g., Morimoto et al., Journal of

Biochemical and Biophysical Methods 24:107-117 (1992); and Brennan et al., Science, 229:81 (1985)). However, these fragments can now be produced directly by recombinant host cells. Fab, Fv and ScFv antibody fragments can all be expressed in and secreted from E. coli and other cell types, such as HEK293 and CHO cells, thus allowing the facile production of large amounts of these fragments. Alternatively, Fab-SH fragments can be directly recovered from culture media and chemically coupled to form F(ab)2 fragments (Carter et al., Bio/Technology 10: 163-167 (1992)). According to another approach, F(ab)2 fragments can be isolated directly from recombinant host cell culture. Fab and F(ab)2 fragments with increased in vivo half-life comprising FcRN / salvage receptor binding epitope residues are described in U.S. Pat. No. 5,869,046. Other techniques for the production of antibody fragments for use in the masked cytokines will be apparent to the skilled practitioner. In certain embodiments, a masked cytokine comprises a single chain Fv fragment (scFv). See WO 93/16185; U.S. Pat. Nos. 5,571,894; and 5,587,458. scFv fusion proteins may be constructed to yield fusion of an effector protein at either the amino or the carboxy terminus of an scFv. See Antibody Engineering, ed. Borrebaeck, supra. Also, in some embodiments, bi-scFv comprising two scFvs linked via a polypeptide linker can be used with the masked cytokines.

The present invention includes, in some embodiments, a linear antibody (e.g., as described in U.S. Pat. No. 5,641,870) or a single chain immunoglobulin comprising heavy and light chain sequences of the antibody linked via an appropriate linker. Such linear antibodies or immunoglobulins may be monospecific or bispecific. Such a single chain immunoglobulin can be dimerized to thereby maintain a structure and activities similar to those of the antibody, which is originally a tetramer. Also, in some embodiments, the antibody or fragment thereof may be an antibody that has a single heavy chain variable region and has no light chain sequence. Such an antibody is called a single domain antibody (sdAb) or a nanobody. These antibodies are also encompassed in the meaning of the functional fragment of the antibody according to the present invention. Antibody fragments can be linked to the masked cytokines described herein according to the guidance provided herein.

2. *Humanized Antibodies*

The invention encompasses, in some embodiments, humanized antibodies or antibody fragments thereof. In some embodiments, the humanized antibodies can be any antibodies, including any antibody fragment. Various methods for humanizing non-human antibodies are known in the art. For example, a humanized antibody can have one or more amino acid residues introduced into it from a source which is non-human. These non-human amino acid residues are often referred to as "import" residues, which are typically taken from an "import" variable domain. Humanization can be essentially performed following the method of Winter (Jones et al. (1986) Nature 321:522-525; Riechmann et al. (1988) Nature 332:323-327; Verhoeyen et al. (1988) Science 239:1534-1536), by substituting hypervariable region sequences for the corresponding sequences of a human antibody. Accordingly, such "humanized" antibodies are chimeric antibodies (U.S. Pat. No. 4,816,567) wherein substantially less than an intact human variable domain has been substituted

by the corresponding sequence from a non-human species. In practice, humanized antibodies are typically human antibodies in which some hypervariable region residues and possibly some FR residues are substituted by residues from analogous sites in rodent antibodies. Humanized antibodies can be linked to the masked cytokines described herein according to the guidance provided herein.

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3. *Human Antibodies*

Human antibodies of some embodiments of the invention can be constructed by combining Fv clone variable domain sequence(s) selected from human-derived phage display libraries with known human
10 constant domain sequences(s). Alternatively, human monoclonal antibodies of some embodiments of the invention can be made by the hybridoma method, e.g., by using mouse, rat, bovine (e.g., cow), or rabbit cells, for example, to produce the human monoclonal antibodies. In some embodiments, the human antibodies and human monoclonal antibodies can be antibodies that bind to any antigen. In some
15 embodiments, human monoclonal antibodies of the invention can be made by immunizing a non-human animal that comprises human immunoglobulin loci with the target antigen, and isolating the antibody from the immunized animal or from cells derived from the immunized animal. Examples of suitable non-human animals include a transgenic or transchromosomal animal, such as HuMAb Mouse® (Medarex, Inc.), KM Mouse®, "TC mice," and Xenomouse™. See, e.g., Lonberg, et al. (1994) Nature 368: 856-859; Fishwild, D. et al. (1996) Nature Biotechnology 14: 845-851; WO2002/43478; U.S. Pat. Nos. 5,939,598; 6,075,181;
20 6,114,598; 6,150,584; 6,162,963; and Tomizuka et al. (2000) Proc. Natl. Acad. Sci. USA 97:722-727.

[0420] Human myeloma and murine-human heteromyeloma cell lines for the production of human monoclonal antibodies have been described, for example, by Kozbor J. Immunol., 133: 3001 (1984); Brodeur et al., Monoclonal Antibody Production Techniques and Applications, pp. 51-63 (Marcel Dekker, Inc., New York, 1987); and Boerner et al., J. Immunol., 147: 86 (1991). Human antibodies can be linked to
25 the masked cytokines described herein according to the guidance provided herein.

4. *Bispecific Antibodies*

30 Bispecific antibodies are monoclonal antibodies that have binding specificities for at least two different antigens. In certain embodiments, bispecific antibodies are human or humanized antibodies. In some embodiments, one of the binding specificities is for a first antigen and the other binding specificity is for a second antigen, which may be either two different epitopes on the same target protein, or two different epitopes on two different target proteins. Bispecific antibodies may also be used to localize cytotoxic agents
35 to cells which express the first antigen and/or the second antigen. Bispecific antibodies may also be used to recruit cells, such as T cells or natural killer cells, to kill certain cells, e.g., cancer cells. Bispecific antibodies can be prepared as full-length antibodies or antibody fragments (e.g. F(ab')₂ bispecific

antibodies). Bispecific antibodies can be linked to the masked cytokines described herein according to the guidance provided herein.

Methods for making bispecific antibodies are known in the art. See Milstein and Cuello, *Nature*, 305: 537 (1983), WO 93/08829 published May 13, 1993, Traunecker et al., *EMBO J.*, 10: 3655 (1991); Kontermann and Brinkmann, *Drug Discovery Today*, 20(7):838-847. For further details of generating bispecific antibodies see, for example, Suresh et al., *Methods in Enzymology*, 121:210 (1986). Bispecific antibodies include cross-linked or "heteroconjugate" antibodies. For example, one of the antibodies in the heteroconjugate can be coupled to avidin, the other to biotin. Heteroconjugate antibodies may be made using any convenient cross-linking method. Suitable cross-linking agents are well known in the art, and are disclosed in U.S. Pat. No. 4,676,980, along with a number of cross-linking techniques.

5. *Single-Domain Antibodies*

In some embodiments, a single-domain antibody is linked to the masked cytokine in accordance with the guidance provided herein. The single-domain antibody can be any antibody. A single-domain antibody is a single polypeptide chain 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, Mass.; see, e.g., U.S. Pat. No. 6,248,516 B1). In some embodiments, a single-domain antibody consists of all or a portion of the heavy chain variable domain of an antibody. In some embodiment, the single domain antibody is a camelid-derived antibody obtained by immunization of a camelid with the target antigen. In some embodiments, the single domain antibody is a shark-derived antibody obtained by immunization of a shark with the target antigen. In some embodiments, the single domain antibody is a Nanobody (see, e.g., WO 2004041865A2 and US20070269422A1).

6. *Antibody Variants*

In some embodiments, amino acid sequence modification(s) of the antibodies or fragments thereof described herein are contemplated. For example, it may be desirable to improve the FcRn-binding affinity and/or pH-dependent FcRn-binding affinity of the antibody. It may also be desirable to promote heterodimerization of antibody heavy chains by introducing certain amino acid modifications. Methods for promoting heterodimerization of antibody chains, including certain modifications that can be made to facilitate heterodimerization, is described by Klein et al. (2012), *MAbs*, 4(6): 653-663.

Amino acid sequence variants of the antibody may be prepared by introducing appropriate changes into the nucleotide sequence encoding the antibody, or by peptide synthesis. Such modifications include, for example, deletions from, and/or insertions into and/or substitutions of, residues within the amino acid

sequences of the antibody. Any combination of deletion, insertion, and substitution can be made to arrive at the final construct, provided that the final construct possesses the desired characteristics. The amino acid alterations may be introduced in the subject antibody amino acid sequence at the time that sequence is made.

5

A useful method for identification of certain residues or regions of the antibody that are preferred locations for mutagenesis is called "alanine scanning mutagenesis" as described by Cunningham and Wells (1989) Science, 244:1081-1085. Here, a residue or group of target residues are identified (e.g., charged residues such as arg, asp, his, lys, and glu) and replaced by a neutral or negatively charged amino acid (e.g., alanine or polyalanine) to affect the interaction of the amino acids with antigen. Those amino acid locations demonstrating functional sensitivity to the substitutions then are refined by introducing further or other variants at, or for, the sites of substitution. Thus, while the site for introducing an amino acid sequence variation is predetermined, the nature of the mutation per se need not be predetermined. For example, to analyze the performance of a mutation at a given site, ala scanning or random mutagenesis is conducted at the target codon or region and the expressed immunoglobulins are screened for the desired activity.

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Amino acid sequence insertions include amino- and/or carboxyl-terminal fusions ranging in length from one residue to polypeptides containing a hundred or more residues, as well as intrasequence insertions of single or multiple amino acid residues. Examples of terminal insertions include an antibody with an N-terminal methionyl residue. Other insertional variants of the antibody molecule include the fusion to the N- or C-terminus of the antibody to an enzyme or a polypeptide which increases the serum half-life of the antibody.

20

In some embodiments, the masked cytokine is modified to eliminate, reduce, or otherwise hinder protease cleavage near the hinge region. The "hinge region" of an IgG is generally defined as including E216 and terminating at P230 of human IgG1 according to the EU index as in Kabat, but, functionally, the flexible portion of the chain may be considered to include additional residues termed the upper and lower hinge regions, such as from E216 to G237 (Roux et al., 1998 J Immunol 161:4083) and the lower hinge has been referred to as residues 233 to 239 of the Fc region where FcγR binding was generally attributed. Modifications to any of the masked cytokines described herein, can be performed, for example, according to the methods described in US 20150139984A1, which is incorporated herein by reference, as well as by incorporating any of the modifications described therein.

25
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In some embodiments, FcRn mutations that improve pharmacokinetics include, but are not limited to, M428L, T250Q/M428L, M252Y/S254T/T256E, P257I/N434H, D376V/N434H, P257I/Q311I, N434A, N434W, M428L/N434S, V259I/V308F, M252Y/S254T/T256E, V259I/V308F/M428L, T307Q/N434A, T307Q/N434S, T307Q/E380A/N434A, V308P/N434A, N434H, V308P. In some embodiments, such

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mutations enhance antibody binding to FcRn at low pH but do not change the antibody affinity at neutral pH.

5 In certain embodiments, an antibody or fragment thereof is altered to increase or decrease the extent to which the antibody is glycosylated. Glycosylation of polypeptides is typically either N-linked or O-linked. N-linked refers to the attachment of a carbohydrate moiety to the side chain of an asparagine residue. The tripeptide sequences asparagine-X-serine and asparagine-X-threonine, where X is any amino acid except proline, are the recognition sequences for enzymatic attachment of the carbohydrate moiety to the asparagine side chain. Thus, the presence of either of these tripeptide sequences in a polypeptide creates a
10 potential glycosylation site. O-linked glycosylation refers to the attachment of one of the sugars N-acetylglucosamine, galactose, or xylose to a hydroxyamino acid, most commonly serine or threonine, although 5-hydroxyproline or 5-hydroxylysine may also be used.

15 Addition or deletion of glycosylation sites to the masked cytokine is conveniently accomplished by altering the amino acid sequence such that one or more of the above-described tripeptide sequences (for N-linked glycosylation sites) is created or removed. The alteration may also be made by the addition, deletion, or substitution of one or more serine or threonine residues to the sequence of the original antibody (for O-linked glycosylation sites).

20 Where the antibody or fragment thereof comprises an Fc region, the carbohydrate attached thereto may be altered. For example, antibodies with a mature carbohydrate structure that lacks fucose attached to an Fc region of the antibody are described in US Pat Appl No US 2003/0157108 (Presta, L.). See also US 2004/0093621 (Kyowa Hakko Kogyo Co., Ltd). Antibodies with a bisecting N- acetylglucosamine (GlcNAc) in the carbohydrate attached to an Fc region of the antibody are referenced in WO 2003/011878,
25 Jean-Mairet et al. and U.S. Pat. No. 6,602,684, Umana et al. Antibodies with at least one galactose residue in the oligosaccharide attached to an Fc region of the antibody are reported in WO 1997/30087, Patel et al. See, also, WO 1998/58964 (Raju, S.) and WO 1999/22764 (Raju, S.) concerning antibodies with altered carbohydrate attached to the Fc region thereof. See also US 2005/0123546 (Umana et al.) on antigen-binding molecules with modified glycosylation.

30 In certain embodiments, a glycosylation variant comprises an Fc region, wherein a carbohydrate structure attached to the Fc region lacks fucose or has reduced fucose. Such variants have improved ADCC function. Optionally, the Fc region further comprises one or more amino acid substitutions therein which further improve ADCC, for example, substitutions at positions 298, 333, and/or 334 of the Fc region (Eu numbering of residues). Examples of publications related to "defucosylated" or "fucose-deficient" antibodies include:
35 US 2003/0157108; WO 2000/61739; WO 2001/29246; US 2003/0115614; US 2002/0164328; US 2004/0093621; US 2004/0132140; US 2004/0110704; US 2004/0110282; US 2004/0109865; WO 2003/085119; WO 2003/084570; WO 2005/035586; WO 2005/035778; WO2005/053742; Okazaki et al. J.

Mol. Biol. 336:1239-1249 (2004); Yamane-Ohnuki et al. Biotech. Bioeng. 87: 614 (2004). Examples of cell lines producing defucosylated antibodies include Lee 13 CHO cells deficient in protein fucosylation (Ripka et al. Arch. Biochem. Biophys. 249:533-545 (1986); US Pat Appl No US 2003/0157108 A1, Presta, L.; and WO 2004/056312 A1, Adams et al., especially at Example 11), and knockout cell lines, such as
 5 alpha-1,6- fucosyltransferase gene, FUT8, knockout CHO cells (Yamane-Ohnuki et al. Biotech. Bioeng. 87: 614 (2004)), and cells overexpressing (31,4-N-acetylglycosaminyltransferase III (GnT-III) and Golgi p-mannosidase II (ManII).

In any of the embodiments herein, the masked cytokine can be engineered to improve antibody-dependent
 10 cell-mediated cytotoxicity (ADCC) activity. In some embodiments, the masked cytokine may be produced in a cell line having a alpha1,6-fucosyltransferase (Fut8) knockout. In some embodiments, the host cells have been modified to have reduced intrinsic alpha1,6-fucosylation activity. Examples of methods for modifying the fucosylation pathways in mammalian host cells can be found in, e.g., Yamane-Ohnuki and Satoh, MAbs, 1(3): 230-236 (2009), the contents of which are incorporated herein by reference. Examples of methods and compositions for partially or completely inactivating the expression of the FUT8 gene can
 15 be found in, e.g., US Pub. No. 20160194665A 1; WO2006133148A2, the contents of which are incorporated herein by reference. In some embodiments, the masked cytokine is produced in the Lec13 variant of CHO cells (see, e.g., Shields et al., J. Biol. Chem., 277(30):26733-40 (2002)) or the YB2/0 cell line having reduced FUT8 activity (see, e.g., Shinkawa et al., J. Biol. Chem., 278(5): 3466-73 (2003)). In
 20 some embodiments, small interfering RNA (siRNA) against genes relevant to alpha1,6-fucosylation can be introduced (see, e.g., Mori et al., Biotechnol. Bioeng. 88(7): 901-908 (2004); Imai-Nishiya et al., BMC Biotechnol. 7: 84 (2007); Omasa et al., J. Biosci. Bioeng., 106(2): 168-173 (2008)). In some further embodiments, the masked cytokine may be produced in a cell line overexpressing (31,4-N-acetylglycosaminyltransferase III (GnT-III). In further embodiments, the cell line additionally overexpresses
 25 Golgi p-mannosidase II (ManII). In some of the embodiments herein, the masked cytokine may comprise at least one amino acid substitution in the Fc region that improves ADCC activity.

In some embodiments, the masked cytokine is altered to improve its serum half-life. To increase the serum half-life of the cytokine, one may incorporate a FcRN /salvage receptor binding epitope into a linked
 30 antibody (especially an antibody fragment) as described in U.S. Pat. No. 5,739,277, for example. As used herein, the term "salvage receptor binding epitope" refers to an epitope of the Fc region of an IgG molecule (e.g., IgG1, IgG2, IgG3, or IgG4) that is responsible for increasing the in vivo serum half-life of the IgG molecule (US 2003/0190311, U.S. Pat. No. 6,821,505; U.S. Pat. No. 6,165,745; U.S. Pat. No. 5,624,821; U.S. Pat. No. 5,648,260; U.S. Pat. No. 6,165,745; U.S. Pat. No. 5,834,597).

35 Another type of variant is an amino acid substitution variant. These variants have at least one amino acid residue in the antibody molecule replaced by a different residue. Sites of interest for substitutional mutagenesis include the hypervariable regions, but FR alterations are also contemplated. Conservative

substitutions are shown in Table 3 under the heading of “preferred substitutions.” If such substitutions result in a desirable change in biological activity, then more substantial changes, denominated “exemplary substitutions” in Table 3, or as further described below in reference to amino acid classes, may be introduced and the products screened.

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Original Residue	Exemplary Substitutions	Preferred Substitutions
Ala (A)	Val; Leu; Ile	Val
Arg (R)	Lys; Gln; Asn	Lys
Asn (N)	Gln; His; Asp; Lys; Arg	Gln
Asp (D)	Glu; Asn	Glu
Cys (C)	Ser; Ala	Ser
Gln (Q)	Asn; Glu	Asn
Glu (E)	Asp; Gln	Asp
Gly (G)	Ala	Ala
His (H)	Asn; Gln; Lys; Arg	Arg
Ile (I)	Leu; Val; Met; Ala; Phe; Norleucine	Leu
Leu (L)	Norleucine; Ile; Val; Met; Ala; Phe	Ile
Lys (K)	Arg; Gln; Asn	Arg
Met (M)	Leu; Phe; Ile	Leu
Phe (F)	Trp; Leu; Val; Ile; Ala; Tyr	Tyr
Pro (P)	Ala	Ala
Ser (S)	Thr	Thr
Thr (T)	Val; Ser	Ser
Trp (W)	Tyr; Phe	Tyr
Tyr (Y)	Trp; Phe; Thr; Ser	Phe
Val (V)	Ile; Leu; Met; Phe; Ala; Norleucine	Leu

Substantial modifications in the biological properties of the antibody are accomplished by selecting substitutions that differ significantly in their effect on maintaining (a) the structure of the polypeptide backbone in the area of the substitution, for example, as a sheet or helical conformation, (b) the charge or hydrophobicity of the molecule at the target site, or c) the bulk of the side chain. Amino acids may be grouped according to similarities in the properties of their side chains (in A. L. Lehninger, in *Biochemistry*, second ed., pp. 73-75, Worth Publishers, New York (1975)):

- (1) non-polar: Ala (A), Val (V), Leu (L), Ile (I), Pro (P), Phe (F), Trp (W), Met (M)
- (2) uncharged polar: Gly (G), Ser (S), Thr (T), Cys (C), Tyr (Y), Asn (N), Gln (Q)
- (3) acidic: Asp (D), Glu (E)
- (4) basic: Lys (K), Arg (R), His (H)

Alternatively, naturally occurring residues may be divided into groups based on common side-chain properties:

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- (1) hydrophobic: Norleucine, Met, Ala, Val, Leu, Ile;
- (2) neutral hydrophilic: Cys, Ser, Thr, Asn, Gln;
- (3) acidic: Asp, Glu;
- (4) basic: His, Lys, Arg;
- 5 (5) residues that influence chain orientation: Gly, Pro;
- (6) aromatic: Trp, Tyr, Phe.

Non-conservative substitutions will entail exchanging a member of one of these classes for another class. Such substituted residues also may be introduced into the conservative substitution sites or, into the
10 remaining (non-conserved) sites.

Another type of substitutional variant involves the substitution of a naturally occurring amino acid residue for a non-naturally occurring amino acid residue. Non-naturally occurring amino acid residues can be incorporated, e.g., through tRNA recoding, or through any of the methods as described, e.g., in WO
15 2016154675A1, which is incorporated herein by reference.

One type of substitutional variant involves substituting one or more hypervariable region residues of a parent antibody (e.g., a humanized or human antibody). Generally, the resulting variant(s) selected for further development will have modified (e.g., improved) biological properties relative to the parent
20 antibody from which they are generated. A convenient way for generating such substitutional variants involves affinity maturation using phage display, yeast display, or mammalian display. Briefly, several hypervariable region sites (e.g., 6-7 sites) are mutated to generate all possible amino acid substitutions at each site. The antibodies thus generated are displayed from filamentous phage particles as fusions to at least part of a phage coat protein (e.g., the gene III product of M13) packaged within each particle. The
25 phage-displayed variants are then screened for their biological activity (e.g., binding affinity). In order to identify candidate hypervariable region sites for modification, scanning mutagenesis (e.g., alanine scanning) can be performed to identify hypervariable region residues contributing significantly to antigen binding. Alternatively, or additionally, it may be beneficial to analyze a crystal structure of the antigen-antibody complex to identify contact points between the antibody and antigen. Such contact residues and
30 neighbouring residues are candidates for substitution according to techniques known in the art, including those elaborated herein. Once such variants are generated, the panel of variants is subjected to screening using techniques known in the art, including those described herein, and antibodies with superior properties in one or more relevant assays may be selected for further development.

35 Nucleic acid molecules encoding amino acid sequence variants of the masked cytokines are prepared by a variety of methods known in the art. These methods include, but are not limited to, isolation from a natural source (in the case of naturally occurring amino acid sequence variants) or preparation by oligonucleotide-

mediated (or site-directed) mutagenesis, PCR mutagenesis, and cassette mutagenesis of an earlier prepared variant or a non-variant version of the antibody, for example.

5 It may be desirable to introduce one or more amino acid modifications in an Fc region of antibodies of the invention, thereby generating an Fc region variant. The Fc region variant may comprise a human Fc region sequence (e.g., a human IgG1, IgG2, IgG3 or IgG4 Fc region) comprising an amino acid modification (e.g. a substitution) at one or more amino acid positions including that of a hinge cysteine.

10 In some embodiments, a masked cytokine provided herein includes an antibody or fragment thereof having an IgG1, IgG2, IgG3, or IgG4 isotype with enhanced effector function. In some embodiments, a masked cytokine provided herein includes an antibody or fragment thereof having an IgG1 isotype with enhanced effector function. In some embodiments, a masked cytokine provided herein has an IgG1 isotype with enhanced effector function. In some embodiments, the masked cytokine is afucosylated. In some
15 embodiments, the masked cytokine has increased levels of mannose moieties. In some embodiments, the masked cytokine has increased levels of bisecting glycan moieties. In some embodiments, the IgG1 comprises amino acid mutations.

In some embodiments, a masked cytokine provided herein includes an antibody having an IgG1 isotype (e.g., a human IgG1 isotype). In some embodiments, the IgG1 comprises one or more amino acid
20 substitutions that enhance effector function. In one embodiment, the IgG1 comprises the amino acid substitutions S298A, E333A, and K334A wherein the amino acid residues are numbered according to the EU index as in Kabat. In one embodiment, the IgG1 comprises the amino acid substitutions S239D and I332E wherein the amino acid residues are numbered according to the EU index as in Kabat. In one embodiment, the IgG1 comprises the amino acid substitutions S239D, A330L, and I332E wherein
25 the amino acid residues are numbered according to the EU index as in Kabat. In one embodiment, the IgG1 comprises the amino acid substitutions P247I and A339D or A339Q wherein the amino acid residues are numbered according to the EU index as in Kabat. In one embodiment, the IgG1 comprises the amino acid substitutions D280H, K290S with or without S298D or S298V wherein the amino acid residues are numbered according to the EU index as in Kabat. In one embodiment, the IgG1 comprises the amino acid
30 substitutions F243L, R292P, and Y300L wherein the amino acid residues are numbered according to the EU index as in Kabat. In one embodiment, the IgG1 comprises the amino acid substitutions F243L, R292P, Y300L, and P396L wherein the amino acid residues are numbered according to the EU index as in Kabat. In one embodiment, the IgG1 comprises the amino acid substitutions F243L, R292P, Y300L, V305I, and P396L wherein the amino acid residues are numbered according to the EU index as in Kabat. In one
35 embodiment, the IgG1 comprises the amino acid substitutions G236A, S239D, and I332E wherein the amino acid residues are numbered according to the EU index as in Kabat. In one embodiment, the IgG1 comprises the amino acid substitutions K326A and E333A wherein the amino acid residues are numbered according to the EU index as in Kabat. In one embodiment, the IgG1 comprises the amino acid substitutions

K326W and E333S wherein the amino acid residues are numbered according to the EU index as in Kabat. In one embodiment, the IgG1 comprises the amino acid substitutions K290E, S298G, T299A, with or without K326E wherein the amino acid residues are numbered according to the EU index as in Kabat. In one embodiment, the IgG1 comprises the amino acid substitutions K290N, S298G, T299A, with or without K326E wherein the amino acid residues are numbered according to the EU index as in Kabat. In one embodiment, the IgG1 comprises the amino acid substitution K334V wherein the amino acid residues are numbered according to the EU index as in Kabat. In one embodiment, the IgG1 comprises the amino acid substitutions L235S, S239D, and K334V wherein the amino acid residues are numbered according to the EU index as in Kabat. In one embodiment, the IgG1 comprises the amino acid substitutions K334V and Q331M, S239D, F243V, E294L, or S298T wherein the amino acid residues are numbered according to the EU index as in Kabat. In one embodiment, the IgG1 comprises the amino acid substitutions E233L, Q311M, and K334V wherein the amino acid residues are numbered according to the EU index as in Kabat. In one embodiment, the IgG1 comprises the amino acid substitutions L234I, Q311M, and K334V wherein the amino acid residues are numbered according to the EU index as in Kabat. In one embodiment, the IgG1 comprises the amino acid substitutions K334V and S298T, A330M, or A330F wherein the amino acid residues are numbered according to the EU index as in Kabat. In one embodiment, the IgG1 comprises the amino acid substitutions K334V, Q311M, and either A330M or A330F wherein the amino acid residues are numbered according to the EU index as in Kabat. In one embodiment, the IgG1 comprises the amino acid substitutions K334V, S298T, and either A330M or A330F wherein the amino acid residues are numbered according to the EU index as in Kabat. In one embodiment, the IgG1 comprises the amino acid substitutions K334V, S239D, and either A330M or S298T wherein the amino acid residues are numbered according to the EU index as in Kabat. In one embodiment, the IgG1 comprises the amino acid substitutions L234Y, Y296W, and K290Y, F243V, or E294L wherein the amino acid residues are numbered according to the EU index as in Kabat. In one embodiment, the IgG1 comprises the amino acid substitutions Y296W and either L234Y or K290Y wherein the amino acid residues are numbered according to the EU index as in Kabat. In one embodiment, the IgG1 comprises the amino acid substitutions S239D, A330S, and I332E wherein the amino acid residues are numbered according to the EU index as in Kabat.

In some embodiments, the IgG1 comprises one or more amino acid substitutions that decrease or inhibit effector function. In one embodiment, the IgG1 comprises the amino acid substitution N297A, N297G, or N297Q wherein the amino acid residues are numbered according to the EU index as in Kabat. In one embodiment, the IgG1 comprises the amino acid substitution L234A or L235A wherein the amino acid residues are numbered according to the EU index as in Kabat. In one embodiment, the IgG1 comprises the amino acid substitutions C220S, C226S, C229S, and P238S wherein the amino acid residues are numbered according to the EU index as in Kabat. In one embodiment, the IgG1 comprises the amino acid substitutions C226S, C229S, E233P, L234V, and L235A wherein the amino acid residues are numbered according to the EU index as in Kabat. In one embodiment, the IgG1 comprises the amino acid substitutions L234F, L235E, and P331S wherein the amino acid residues are numbered according to the EU index as in Kabat.

In one embodiment, the IgG1 comprises the amino acid substitutions S267E and L328F wherein the amino acid residues are numbered according to the EU index as in Kabat.

In accordance with this description and the teachings of the art, it is contemplated that in some
 5 embodiments, an antibody or fragment thereof of the masked cytokine may comprise one or more
 alterations as compared to the wild type counterpart antibody, e.g. in the Fc region. For example, it is
 thought that certain alterations can be made in the Fc region that would result in altered (i.e., either improved
 or diminished) C1q binding and/or Complement Dependent Cytotoxicity (CDC), e.g., as described in
 WO99/51642. See also Duncan & Winter *Nature* 322:738-40 (1988); U.S. Pat. No. 5,648,260; U.S. Pat.
 10 No. 5,624,821; and WO94/29351 concerning other examples of Fc region variants. WO00/42072 (Presta)
 and WO 2004/056312 (Lowman) describe antibody variants with improved or diminished binding to FcRs.
 The content of these patent publications are specifically incorporated herein by reference. See also Shields
 et al. *J. Biol. Chem.* 9(2): 6591-6604 (2001). Antibodies with increased half-lives and improved binding
 to the neonatal Fc receptor (FcRn), which is responsible for the transfer of maternal IgGs to the fetus (Guyer
 15 et al., *J. Immunol.* 117:587 (1976) and Kim et al., *J. Immunol.* 24:249 (1994)), are described in
 US2005/0014934A1 (Hinton et al.). These antibodies comprise an Fc region with one or more substitutions
 therein which improve binding of the Fc region to FcRn. Polypeptide variants with altered Fc region amino
 acid sequences and increased or decreased C1q binding capability are described in U.S. Pat. No.
 6,194,551B1, WO99/51642. The contents of those patent publications are specifically incorporated herein
 20 by reference. See, also, Idusogie et al. *J. Immunol.* 164: 4178-4184 (2000).

4.2 Masked IL-12 Cytokine-Drug Conjugates

The invention also provides masked IL-12 cytokine-drug conjugates (MCDCs) comprising a masked IL-
 25 12 cytokine provided herein, which can be any IL-12 masked cytokine disclosed herein, conjugated to one
 or more agents. In some embodiments, the one or more agents is a cytotoxic agent, such as a
 chemotherapeutic agent or drug, growth inhibitory agent, toxin (e.g., protein toxin, enzymatically active
 toxin of bacterial, fungal, plant, or animal origin, or fragments thereof), or radioactive isotopes. In some
 embodiments, the one or more agents is an immune stimulant.

30 In some embodiments, the one or more drugs conjugated to the masked IL-12 cytokine includes, but is not
 limited to, a maytansinoid (see U.S. Patent Nos. 5,208,020, 5,416,064 and European Patent EP 0 425 235
 B1); an auristatin such as monomethylauristatin drug moieties DE and DF (MMAE and MMAF) (see U.S.
 Patent Nos. 5,635,483 and 5,780,588, and 7,498,298); a dolastatin; a calicheamicin or derivative thereof
 35 (see U.S. Patent Nos. 5,712,374, 5,714,586, 5,739,116, 5,767,285, 5,770,701, 5,770,710, 5,773,001, and
 5,877,296; Hinman et al., *Cancer Res.* 53:3336-3342 (1993); and Lode et al., *Cancer Res.* 58:2925-2928
 (1998)); an anthracycline such as daunomycin or doxorubicin (see Kratz et al., *Current Med. Chem.* 13:477-
 523 (2006); Jeffrey et al., *Bioorganic & Med. Chem. Letters* 16:358-362 (2006); Torgov et al., *Bioconj.*

Chem. 16:717-721 (2005); Nagy et al., Proc. Natl. Acad. Sci. USA 97:829-834 (2000); Dubowchik et al., Bioorg. & Med. Chem. Letters 12:1529-1532 (2002); King et al., J. Med. Chem. 45:4336-4343 (2002); and U.S. Patent No. 6,630,579; methotrexate; vindesine; a taxane such as docetaxel, paclitaxel, larotaxel, tesetaxel, and ortataxel; a trichothecene; and CC1065.

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In another embodiment, the one or more drugs conjugated to the masked IL-12 cytokine includes, but is not limited to, an inhibitor of tubulin polymerization (e.g., maytansinoids and auristatins), DNA damaging agents (e.g., pyrrolbenzodiazepine (PBD) dimers, calicheamicins, duocarmycins and indolizobenzodiazepine dimers), and DNA synthesis inhibitors (e.g., exatecan derivative Dxd).

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In another embodiment, a masked IL-12 cytokine-drug conjugate comprises a masked IL-12 cytokine as described herein conjugated to an enzymatically active toxin or fragment thereof, including, but not limited to, diphtheria A chain, nonbinding active fragments of diphtheria toxin, exotoxin A chain (from *Pseudomonas aeruginosa*), ricin A chain, abrin A chain, modeccin A chain, alpha-sarcin, Aleurites fordii proteins, dianthin proteins, *Phytolacca americana* proteins (PAPI, PAPII, and PAP-S), momordica charantia inhibitor, curcumin, croton, saponaria officinalis inhibitor, gelonin, mitogellin, restrictocin, phenomycin, enomycin, and the trichothecenes.

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In another embodiment, a masked IL-12 cytokine-drug conjugate comprises a masked IL-12 cytokine as described herein conjugated to a radioactive atom to form a radioconjugate. A variety of radioactive isotopes are available for the production of radioconjugates. Examples include At211,1131,1125, Y90, Re186, Re188, Sm153, Bi212, P32, Pb212 and radioactive isotopes of Lu. When the radioconjugate is used for detection, it may comprise a radioactive atom for scintigraphic studies, for example Tc99m or I123, or a spin label for nuclear magnetic resonance (NMR) imaging (also known as magnetic resonance imaging, MRI), such as iodine-123 again, iodine-131, indium-111, fluorine-19, carbon-13, nitrogen-15, oxygen-17, gadolinium, manganese or iron.

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In some embodiments, a masked IL-12 cytokine-drug conjugate comprises a masked IL-12 cytokine as described herein conjugated to one or more immune stimulants. In some embodiments, the immune stimulant is a stimulator of interferon genes (STING) agonist or a toll-like receptor (TLR) agonist.

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The STING agonist can be any agonist of STING. In some embodiments, the STING agonist is a cyclic dinucleotide (CDN). The CDN can be any CDN or derivative or variant thereof. In some embodiments, the STING agonist is a CDN selected from the group consisting of cGAMP, c-di-AMP, c-di-GMP, cAIMP, and c-di-IMP. In some embodiments, the STING agonist is a derivative or variant of a CDN selected from the group consisting of cGAMP, c-di-AMP, c-di-GMP, cAIMP, and c-di-IMP. In some embodiments, the STING agonist is 4-(2-chloro-6-fluorobenzyl)-N-(furan-2-ylmethyl)-3-oxo-3,4-dihydro-2H-benzo[b][1,4]thiazine-6-carboxamide, or a derivative or variant thereof. See, e.g., Sali et al. (2015) PLoS

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Pathog., 11(12): e!005324.

The TLR agonist can be an agonist of any TLR, such as TLR1, TLR2, TLR3, TLR4, TLR5, TLR6, TLR7, TLR8, TLR9, or TLR10. In some embodiments, the TLR agonist is an agonist of a TLR expressed on the cell surface, such as TLR1, TLR2, TLR4, or TLR5. In some embodiments, the TLR agonist is an agonist of a TLR expressed intracellularly, such as TLR3, TLR7, TLR8, TLR9, or TLR10.

Conjugates of a masked IL-12 cytokine and a cytotoxic agent may be made using a variety of bifunctional protein coupling agents such as N-succinimidyl-3-(2-pyridyldithio) propionate (SPDP), succinimidyl-4-(N-maleimidomethyl) cyclohexane-1-carboxylate (SMCC), iminothiolane (IT), bifunctional derivatives of imidoesters (such as dimethyl adipimidate HCl), active esters (such as disuccinimidyl suberate), aldehydes (such as glutaraldehyde), bis-azido compounds (such as bis (p- azidobenzoyl) hexanediamine), bis-diazonium derivatives (such as bis-(p-diazoniumbenzoyl)- ethylenediamine), diisocyanates (such as toluene 2,6-diisocyanate), and bis-active fluorine compounds (such as 1,5-difluoro-2,4-dinitrobenzene). For example, a ricin immunotoxin can be prepared as described in Vitetta et al, Science 238:1098 (1987). Carbon-14-labeled 1-isothiocyanatobenzyl-3- methyl diethylene triaminopentaacetic acid (MX-DTPA) is an exemplary chelating agent for conjugation of radionucleotide to an antibody. See W094/11026. The linker may be a "cleavable linker" facilitating release of a cytotoxic drug in the cell. For example, an acid-labile linker, peptidase-sensitive linker, photolabile linker, dimethyl linker or disulfide-containing linker (Chari et al, Cancer Res. 52:127-131 (1992); U.S. Patent No. 5,208,020) may be used.

The MCDCs herein expressly contemplate, but are not limited to such conjugates prepared with cross-linker reagents including, but not limited to, BMPS, EMCS, GMBS, HBVS, LC-SMCC, MBS, MPBH, SBAP, SIA, SIAB, SMCC, SMPB, SMPH, sulfo-EMCS, sulfo-GMBS, sulfo-KMUS, sulfo-MBS, sulfo-SIAB, sulfo-SMCC, and sulfo-SMPB, and SVSB (succinimidyl-(4- vinylsulfone)benzoate) which are commercially available (e.g., from Pierce Biotechnology, Inc., Rockford, IL., U.S.A).

4.3 Vectors, Host Cells, and Recombinant Methods

For recombinant production of a IL-12 masked cytokine of the invention, the one or more nucleic acids encoding it is isolated and inserted into a replicable vector for further cloning (amplification of the DNA) or for expression. DNA encoding the masked IL-12 cytokine, including components thereof, is readily isolated and sequenced using conventional procedures. Many vectors are available. The choice of vector depends in part on the host cell to be used. Generally, host cells are of either prokaryotic or eukaryotic (generally mammalian) origin. It will be appreciated that constant regions of any isotype of antibody or fragment thereof, when applicable, can be used for this purpose, including IgG, IgM, IgA, IgD, and IgE constant regions, and that such constant regions can be obtained from any human or animal species. In some embodiments, one vector is used to encode the IL-12 masked cytokine. In some embodiments, more

than one vector is used to encode the masked IL-12 cytokine.

1. Generating Masked IL-12 Cytokines Using Prokaryotic Host Cells

a. Vector Construction

- 5 Polynucleotide sequences encoding polypeptide components of the masked cytokines of the invention can be obtained using standard recombinant techniques. Desired polynucleotide sequences of an antibody or antibody fragment thereof may be isolated and sequenced from antibody producing cells such as hybridoma cells. Alternatively, polynucleotides can be synthesized using nucleotide synthesizer or PCR techniques, or obtained from other sources. Once obtained, sequences encoding the components of the masked cytokine
- 10 are inserted into a recombinant vector capable of replicating and expressing heterologous polynucleotides in prokaryotic hosts. Many vectors that are available and known in the art can be used for the purpose of the present invention. Selection of an appropriate vector will depend mainly on the size of the nucleic acids to be inserted into the vector and the particular host cell to be transformed with the vector. Each vector contains various components, depending on its function (amplification or expression of heterologous
- 15 polynucleotide, or both) and its compatibility with the particular host cell in which it resides. The vector components generally include, but are not limited to: an origin of replication, a selection marker gene, a promoter, a ribosome binding site (RBS), a signal sequence, the heterologous nucleic acid insert and a transcription terminator sequence.
- 20 In general, plasmid vectors containing replicon and control sequences which are derived from species compatible with the host cell are used in connection with these hosts. The vector ordinarily carries a replication site, as well as marking sequences which are capable of providing phenotypic selection in transformed cells. For example, *E. coli* is typically transformed using pBR322, a plasmid derived from an *E. coli* species. pBR322 contains genes-encoding ampicillin (Amp) and tetracycline (Tet) resistance and
- 25 thus provides easy means for identifying transformed cells. pBR322, its derivatives, or other microbial plasmids or bacteriophage may also contain, or be modified to contain, promoters which can be used by the microbial organism for expression of endogenous proteins. Examples of pBR322 derivatives used for expression of particular antibodies are described in detail in Carter et al, U.S. Pat. No. 5,648,237.
- 30 In addition, phage vectors containing replicon and control sequences that are compatible with the host microorganism can be used as transforming vectors in connection with these hosts. For example, bacteriophage such as 7GEM.TM.-11 may be utilized in making a recombinant vector which can be used to transform susceptible host cells such as *E. coli* LE392.
- 35 The expression vector of the invention may comprise two or more promoter-cistron pairs, encoding each of the polypeptide components. A promoter is an untranslated regulatory sequence located upstream (5') to a cistron that modulates its expression. Prokaryotic promoters typically fall into two classes, inducible and

constitutive. Inducible promoter is a promoter that initiates increased levels of transcription of the cistron under its control in response to changes in the culture condition, e.g. the presence or absence of a nutrient or a change in temperature.

- 5 A large number of promoters recognized by a variety of potential host cells are well known. The selected promoter can be operably linked to cistron DNA encoding either chain of the masked cytokine by removing the promoter from the source DNA via restriction enzyme digestion and inserting the isolated promoter sequence into the vector of the invention. Both the native promoter sequence and many heterologous promoters may be used to direct amplification and/or expression of the target genes.
- 10 In some embodiments, heterologous promoters are utilized, as they generally permit greater transcription and higher yields of expressed target gene as compared to the native target polypeptide promoter.

Promoters suitable for use with prokaryotic hosts include the PhoA promoter, the [3- galactamase and lactose promoter systems, a tryptophan (trp) promoter system and hybrid promoters such as the tac or the trc promoter. However, other promoters that are functional in bacteria (such as other known bacterial or phage promoters) are suitable as well. Their nucleotide sequences have been published, thereby enabling a skilled worker operably to ligate them to cistrons encoding, for example, the target light and heavy chains for masked cytokines comprising a light and heavy chain (Siebenlist et al. (1980) Cell 20: 269) using linkers or adaptors to supply any required restriction sites.

- 20 In one aspect of the invention, each cistron within the recombinant vector comprises a secretion signal sequence component that directs translocation of the expressed polypeptides across a membrane. In general, the signal sequence may be a component of the vector, or it may be a part of the target polypeptide DNA that is inserted into the vector. The signal sequence selected for the purpose of this invention should be one that is recognized and processed (i.e. cleaved by a signal peptidase) by the host cell. For prokaryotic host cells that do not recognize and process the signal sequences native to the heterologous polypeptides, the signal sequence is substituted by a prokaryotic signal sequence selected, for example, from the group consisting of the alkaline phosphatase, penicillinase, Ipp, or heat-stable enterotoxin II (STII) leaders, LamB, PhoE, PelB, OmpA and MBP. In one embodiment of the invention, the signal sequences used in both cistrons of the expression system are STII signal sequences or variants thereof.

- 35 In another aspect, the production of the polypeptide components according to the invention can occur in the cytoplasm of the host cell, and therefore does not require the presence of secretion signal sequences within each cistron. In that regard, for embodiments comprising immunoglobulin light and heavy chains, for example, the light and heavy chains are expressed with or without the sequences for the masking moiety, linker sequence, etc., folded and assembled to form functional immunoglobulins within the cytoplasm. Certain host strains (e.g., the E. coli trxB-strains) provide cytoplasm conditions that are favorable for disulfide bond formation, thereby permitting proper folding and assembly of expressed protein subunits.

Proba and Pluckthun Gene, 159:203 (1995).

Masked cytokines of the invention can also be produced by using an expression system in which the quantitative ratio of expressed polypeptide components can be modulated in order to maximize the yield of
5 secreted and properly assembled antibodies of the invention. Such modulation is accomplished at least in part by simultaneously modulating translational strengths for the polypeptide components.

Prokaryotic host cells suitable for expressing masked cytokines of the invention include Archaeobacteria and Eubacteria, such as Gram-negative or Gram-positive organisms. Examples of useful bacteria include
10 Escherichia (e.g., E. coli), Bacilli (e.g., B. subtilis), Enterobacteria, Pseudomonas species (e.g., P. aeruginosa), Salmonella typhimurium, Serratia marcescans, Klebsiella, Proteus, Shigella, Rhizobia, Vitreoscilla, or Paracoccus. In one embodiment, gram-negative cells are used. In one embodiment, E. coli cells are used as hosts for the invention. Examples of E. coli strains include strain W3110 (Bachmann, Cellular and Molecular Biology, vol. 2 (Washington, D.C.: American Society for Microbiology, 1987), pp.
15 1190-1219; ATCC Deposit No. 27,325) and derivatives thereof, including strain 33D3 having genotype W3110 AfhuA (AtonA) ptr3 lac Iq lacL8 AompTA(nmpc-fepE) degP41 kanR (U.S. Pat. No. 5,639,635). Other strains and derivatives thereof, such as E. coli 294 (ATCC 31,446), E. coli B, E. colik 1776 (ATCC 31,537) and E. coli RV308(ATCC 31,608) are also suitable. These examples are illustrative rather than limiting. Methods for constructing derivatives of any of the above-mentioned bacteria having defined
20 genotypes are known in the art and described in, for example, Bass et al, Proteins, 8:309-314 (1990). It is generally necessary to select the appropriate bacteria taking into consideration replicability of the replicon in the cells of a bacterium. For example, E. coli, Serratia, or Salmonella species can be suitably used as the host when well-known plasmids such as pBR322, pBR325, pACYC177, or pKN410 are used to supply the replicon. Typically, the host cell should secrete minimal amounts of proteolytic enzymes, and additional
25 protease inhibitors may desirably be incorporated in the cell culture.

b. Masked Cytokine Production

Host cells are transformed with the above-described expression vectors and cultured in conventional nutrient media modified as appropriate for inducing promoters, selecting transformants, or amplifying the
30 genes encoding the desired sequences.

Transformation means introducing DNA into the prokaryotic host so that the DNA is replicable, either as an extrachromosomal element or by chromosomal integrant. Depending on the host cell used, transformation is done using standard techniques appropriate to such cells. The calcium treatment
35 employing calcium chloride is generally used for bacterial cells that contain substantial cell-wall barriers. Another method for transformation employs polyethylene glycol/DMSO. Yet another technique used is electroporation.

Prokaryotic cells used to produce the masked cytokines of the invention are grown in media known in the art and suitable for culture of the selected host cells. Examples of suitable media include luria broth (LB) plus necessary nutrient supplements. In some embodiments, the media also contains a selection agent,
5 chosen based on the construction of the expression vector, to selectively permit growth of prokaryotic cells containing the expression vector. For example, ampicillin is added to media for growth of cells expressing ampicillin resistant gene.

Any necessary supplements besides carbon, nitrogen, and inorganic phosphate sources may also be
10 included at appropriate concentrations introduced alone or as a mixture with another supplement or medium such as a complex nitrogen source. Optionally, the culture medium may contain one or more reducing agents selected from the group consisting of glutathione, cysteine, cystamine, thioglycollate, dithioerythritol and dithiothreitol.

15 The prokaryotic host cells are cultured at suitable temperatures. In certain embodiments, for *E. coli* growth, growth temperatures range from about 20° C. to about 39° C; from about 25° C. to about 37° C.; or about 30° C. The pH of the medium may be any pH ranging from about 5 to about 9, depending mainly on the host organism. In certain embodiments, for *E. coli*, the pH is from about 6.8 to about 7.4, or about 7.0.

20 If an inducible promoter is used in the expression vector of the invention, protein expression is induced under conditions suitable for the activation of the promoter. In one aspect of the invention, *PhoA* promoters are used for controlling transcription of the polypeptides. Accordingly, the transformed host cells are cultured in a phosphate-limiting medium for induction. In certain embodiments, the phosphate-limiting medium is the C.R.A.P. medium (see, e.g., Simmons et al, *J. Immunol. Methods* (2002), 263:133-147). A
25 variety of other inducers may be used, according to the vector construct employed, as is known in the art.

In one embodiment, the expressed masked cytokines of the present invention are secreted into and recovered from the periplasm of the host cells. Protein recovery typically involves disrupting the microorganism, generally by such means as osmotic shock, sonication or lysis. Once cells are disrupted,
30 cell debris or whole cells may be removed by centrifugation or filtration. The proteins may be further purified, for example, by affinity resin chromatography. Alternatively, proteins can be transported into the culture media and isolated therein. Cells may be removed from the culture and the culture supernatant being filtered and concentrated for further purification of the proteins produced. The expressed polypeptides can be further isolated and identified using commonly known methods such as polyacrylamide gel
35 electrophoresis (PAGE) and Western blot assay.

In one aspect of the invention, masked cytokine production is conducted in large quantity by a fermentation process. Various large-scale fed-batch fermentation procedures are available for production of recombinant

proteins. Large-scale fermentations have at least 1000 liters of capacity, and in certain embodiments, about 1,000 to 100,000 liters of capacity. These fermenters use agitator impellers to distribute oxygen and nutrients, especially glucose. Small scale fermentation refers generally to fermentation in a fermentor that is no more than approximately 100 liters in volumetric capacity, and can range from about 1 liter to about 100 liters.

In a fermentation process, induction of protein expression is typically initiated after the cells have been grown under suitable conditions to a desired density, e.g., an OD₅₅₀ of about 180-220, at which stage the cells are in the early stationary phase. A variety of inducers may be used, according to the vector construct employed, as is known in the art and described above. Cells may be grown for shorter periods prior to induction. Cells are usually induced for about 12-50 hours, although longer or shorter induction time may be used.

To improve the production yield and quality of the polypeptides of the invention, various fermentation conditions can be modified. For example, to improve the proper assembly and folding of, for example, secreted antibody polypeptides, additional vectors overexpressing chaperone proteins, such as Dsb proteins (DsbA, DsbB, DsbC, DsbD and or DsbG) or FkpA (a peptidylprolyl cis,trans-isomerase with chaperone activity) can be used to co-transform the host prokaryotic cells. The chaperone proteins have been demonstrated to facilitate the proper folding and solubility of heterologous proteins produced in bacterial host cells. Chen et al. (1999) J. Biol. Chem. 274:19601-19605; Georgiou et al, U.S. Pat. No. 6,083,715; Georgiou et al, U.S. Pat. No. 6,027,888; Bothmann and Pluckthun (2000) J. Biol. Chem. 275:17100-17105; Ramm and Pluckthun (2000) J. Biol. Chem. 275:17106-17113; Arie et al (2001) Mol. Microbiol. 39:199-210.

To minimize proteolysis of expressed heterologous proteins (especially those that are proteolytically sensitive), certain host strains deficient for proteolytic enzymes can be used for the present invention. For example, host cell strains may be modified to effect genetic mutation(s) in the genes encoding known bacterial proteases such as Protease III, OmpT, DegP, Tsp, Protease I, Protease Mi, Protease V, Protease VI and combinations thereof. Some E. coli protease-deficient strains are available and described in, for example, Joly et al (1998), supra; Georgiou et al, U.S. Pat. No. 5,264,365; Georgiou et al, U.S. Pat. No. 5,508,192; Kara et al, Microbial Drug Resistance, 2:63-72 (1996).

In some embodiments, E. coli strains deficient for proteolytic enzymes and transformed with plasmids overexpressing one or more chaperone proteins are used as host cells in the expression system of the invention.

c. *Masked Cytokine Purification*

In some embodiments, the masked cytokine produced herein is further purified to obtain preparations that are substantially homogeneous for further assays and uses. Standard protein purification methods known in the art can be employed. The following procedures are exemplary of suitable purification procedures: fractionation on immunoaffinity or ion-exchange columns, ethanol precipitation, reverse phase HPLC, chromatography on silica or on a cation-exchange resin such as DEAE, chromatofocusing, SDS-PAGE, ammonium sulfate precipitation, and gel filtration using, for example, Sephadex G-75.

In some embodiments, Protein A immobilized on a solid phase is used for immunoaffinity purification of the masked cytokines of the invention. Protein A is a 41 kD cell wall protein from *Staphylococcus aureus* which binds with a high affinity to the Fc region of antibodies. Lindmark et al (1983) *J. Immunol. Meth.* 62:1-13. The solid phase to which Protein A is immobilized can be a column comprising a glass or silica surface, or a controlled pore glass column or a silicic acid column. In some applications, the column is coated with a reagent, such as glycerol, to possibly prevent nonspecific adherence of contaminants.

As the first step of purification, a preparation derived from the cell culture as described above can be applied onto a Protein A immobilized solid phase to allow specific binding of the masked cytokine of interest to Protein A. The solid phase would then be washed to remove contaminants non-specifically bound to the solid phase. Finally, the masked cytokine of interest is recovered from the solid phase by elution.

Other methods of purification that provide for high affinity binding to a component of the masked cytokine can be employed in accordance with standard protein purification methods known in the art.

2. *Generating Masked Cytokines Using Eukaryotic Host Cells*

A vector for use in a eukaryotic host cell generally includes one or more of the following non-limiting components: a signal sequence, an origin of replication, one or more marker genes, an enhancer element, a promoter, and a transcription termination sequence.

a. *Signal Sequence Component*

A vector for use in a eukaryotic host cell may also contain a signal sequence or other polypeptide having a specific cleavage site at the N-terminus of the mature protein or polypeptide of interest. The heterologous signal sequence selected may be one that is recognized and processed (i.e., cleaved by a signal peptidase) by the host cell. In mammalian cell expression, mammalian signal sequences as well as viral secretory leaders, for example, the herpes simplex gD signal, are available.

The DNA for such a precursor region is ligated in reading frame to DNA encoding the masked cytokine.

b. *Origin of Replication*

Generally, an origin of replication component is not needed for mammalian expression vectors. For example, the SV40 origin may typically be used only because it contains the early promoter.

c. Selection Gene Component

5 Expression and cloning vectors may contain a selection gene, also termed a selectable marker. Typical selection genes encode proteins that (a) confer resistance to antibiotics or other toxins, e.g., ampicillin, neomycin, methotrexate, or tetracycline, (b) complement auxotrophic deficiencies, where relevant, or (c) supply critical nutrients not available from complex media.

10 One example of a selection scheme utilizes a drug to arrest growth of a host cell. Those cells that are successfully transformed with a heterologous gene produce a protein conferring drug resistance and thus survive the selection regimen. Examples of such dominant selection use the drugs neomycin, mycophenolic acid and hygromycin.

15 Another example of suitable selectable markers for mammalian cells are those that enable the identification of cells competent to take up the masked cytokine encoding nucleic acid, such as DHFR, thymidine kinase, metallothionein-I and -II, primate metallothionein genes, adenosine deaminase, ornithine decarboxylase, etc.

20 For example, in some embodiments, cells transformed with the DHFR selection gene are first identified by culturing all of the transformants in a culture medium that contains methotrexate (Mtx), a competitive antagonist of DHFR. In some embodiments, an appropriate host cell when wild-type DHFR is employed is the Chinese hamster ovary (CHO) cell line deficient in DHFR activity (e.g., ATCC CRL- 9096).

25 Alternatively, host cells (particularly wild-type hosts that contain endogenous DHFR) transformed or co-transformed with DNA sequences encoding a masked cytokine, wild-type DHFR protein, and another selectable marker such as aminoglycoside 3'-phosphotransferase (APH) can be selected by cell growth in medium containing a selection agent for the selectable marker such as an aminoglycosidic antibiotic, e.g., kanamycin, neomycin, or G418. See U.S. Pat. No. 4,965,199. Host cells may include NS0, including cell lines deficient in glutamine synthetase (GS). Methods for the use of GS as a selectable marker for
30 mammalian cells are described in U.S. Pat. No. 5,122,464 and U.S. Pat. No. 5,891,693.

d. Promoter Component

35 Expression and cloning vectors usually contain a promoter that is recognized by the host organism and is operably linked to nucleic acid encoding a masked cytokine of interest, which can be any masked cytokine described herein. Promoter sequences are known for eukaryotes. For example, virtually all eukaryotic genes have an AT-rich region located approximately 25 to 30 bases upstream from the site where transcription is

initiated. Another sequence found 70 to 80 bases upstream from the start of transcription of many genes is a CNCAAT region where N may be any nucleotide. At the 3' end of most eukaryotic genes is an AATAAA sequence that may be the signal for addition of the poly A tail to the 3' end of the coding sequence. In certain embodiments, any or all of these sequences may be suitably inserted into eukaryotic expression
5 vectors.

Transcription from vectors in mammalian host cells is controlled, for example, by promoters obtained from the genomes of viruses such as polyoma virus, fowlpox virus, adenovirus (such as Adenovirus 2), bovine papilloma virus, avian sarcoma virus, cytomegalovirus, a retrovirus, hepatitis-B virus and Simian Virus 40
10 (SV40), from heterologous mammalian promoters, e.g., the actin promoter or an immunoglobulin promoter, from heat-shock promoters, provided such promoters are compatible with the host cell systems.

The early and late promoters of the SV40 virus are conveniently obtained as an SV40 restriction fragment that also contains the SV40 viral origin of replication. The immediate early promoter of the human
15 cytomegalovirus is conveniently obtained as a HindIII E restriction fragment. A system for expressing DNA in mammalian hosts using the bovine papilloma virus as a vector is disclosed in U.S. Pat. No. 4,419,446. A modification of this system is described in U.S. Pat. No. 4,601,978. See also Reyes et al., Nature 297:598-601 (1982), describing expression of human β -interferon cDNA in murine cells under the control of a thymidine kinase promoter from herpes simplex virus. Alternatively, the Rous Sarcoma Virus long terminal repeat can be used as the promoter.
20

e. Enhancer Element Component

Transcription of DNA encoding a masked cytokine of this invention by higher eukaryotes is often increased by inserting an enhancer sequence into the vector. Many enhancer sequences are now known from
25 mammalian genes (globin, elastase, albumin, α -fetoprotein, and insulin). Typically, however, one will use an enhancer from a eukaryotic cell virus. Examples include the SV40 enhancer on the late side of the replication origin (bp 100-270), the human cytomegalovirus early promoter enhancer, the murine cytomegalovirus early promoter enhancer, the polyoma enhancer on the late side of the replication origin, and adenovirus enhancers. See also Yaniv, Nature 297:17-18 (1982) (describing enhancer elements for
30 activation of eukaryotic promoters). The enhancer may be spliced into the vector at a position 5' or 3' to the masked cytokine-encoding sequence, but is generally located at a site 5' from the promoter.

f. Transcription Termination Component

Expression vectors used in eukaryotic host cells may also contain sequences necessary for the termination
35 of transcription and for stabilizing the mRNA. Such sequences are commonly available from the 5' and, occasionally 3', untranslated regions of eukaryotic or viral DNAs or cDNAs. These regions contain

nucleotide segments transcribed as polyadenylated fragments in the untranslated portion of the mRNA encoding a masked cytokine. One useful transcription termination component is the bovine growth hormone polyadenylation region. *See* W094/11026 and the expression vector disclosed therein.

g. Selection and Transformation of Host Cells

5 Suitable host cells for cloning or expressing the DNA in the vectors herein include higher eukaryote cells described herein, including vertebrate host cells. Propagation of vertebrate cells in culture (tissue culture) has become a routine procedure. Examples of useful mammalian host cell lines are monkey kidney CV1 line transformed by SV40 (COS-7, ATCC CRL 1651); human embryonic kidney line (293 or 293 cells subcloned for growth in suspension culture, Graham et al, J. Gen Virol. 36:59 (1977)); baby hamster kidney
10 cells (BHK, ATCC CCL 10); Chinese hamster ovary cells/-DHFR (CHO, Urlaub et al, Proc. Natl. Acad. Sci. USA 77:4216 (1980)); murine sertoli cells (TM4, Mather, Biol. Reprod. 23:243-251 (1980)); monkey kidney cells (CV1 ATCC CCL 70); African green monkey kidney cells (VERO-76, ATCC CRL-1587); human cervical carcinoma cells (HELA, ATCC CCL 2); canine kidney cells (MDCK, ATCC CCL 34); buffalo rat liver cells (BEL 3A, ATCC CRL 1442); human lung cells (W138, ATCC CCL 75); human liver
15 cells (Hep G2, HB 8065); murine mammary tumor (MMT 060562, ATCC CCL51); TRI cells (Mather et al, Annals N.Y. Acad. Sci. 383:44-68 (1982)); MRC 5 cells; FS4 cells; and a human hepatoma line (Hep G2).

Host cells are transformed with the above-described-expression or cloning vectors for masked cytokine
20 production and cultured in conventional nutrient media modified as appropriate for inducing promoters, selecting transformants, or amplifying the genes encoding the desired sequences.

h. Culturing Host Cells

The host cells used to produce masked cytokines of this invention may be cultured in a variety of media. Commercially available media such as Ham's F10 (Sigma), Minimal Essential Medium ((MEM), Sigma),
25 RPMI-1640 (Sigma), and Dulbecco's Modified Eagle's Medium ((DMEM), Sigma) are suitable for culturing the host cells. In addition, any of the media described in Ham et al, Meth. Enz. 58:44 (1979), Barnes et al, Anal. Biochem. 102:255 (1980), U.S. Pat. No. 4,767,704; 4,657,866; A,921,162; 4,560,655; or 5,122,469; WO 90/03430; WO 87/00195; or U.S. Pat. Re. 30,985 may be used as
30 culture media for the host cells. Any of these media may be supplemented as necessary with hormones and/or other growth factors (such as insulin, transferrin, or epidermal growth factor), salts (such as sodium chloride, calcium, magnesium, and phosphate), buffers (such as HEPES), nucleotides (such as adenosine and thymidine), antibiotics (such as GENTAMYCIN™ drug), trace elements (defined as inorganic compounds usually present at final concentrations in the micromolar range), and glucose or an equivalent energy source. Any other supplements may also be included at appropriate concentrations that would be
35 known to those skilled in the art. The culture conditions, such as temperature, pH, and the like, are those

previously used with the host cell selected for expression, and will be apparent to the ordinarily skilled artisan.

i. Purification of Masked Cytokines

When using recombinant techniques, the masked cytokines can be produced intracellularly, or directly
5 secreted into the medium. If the masked cytokine is produced intracellularly, as a first step, the particulate debris, either host cells or lysed fragments, may be removed, for example, by centrifugation or ultrafiltration. Where the masked cytokine is secreted into the medium, supernatants from such expression systems may be first concentrated using a commercially available protein concentration filter, for example,
an Amicon or Millipore Pellicon ultrafiltration unit. A protease inhibitor such as PMSF may be included in
10 any of the foregoing steps to inhibit proteolysis, and antibiotics may be included to prevent the growth of adventitious contaminants.

The masked cytokine composition prepared from the cells can be purified using, for example,
hydroxylapatite chromatography, gel electrophoresis, dialysis, and affinity chromatography, with affinity
15 chromatography being a convenient technique. The suitability of protein A as an affinity ligand depends on the species and isotype of any immunoglobulin Fc domain, if any, that is present in the masked cytokine. Protein A can be used to purify antibodies that are based on human IgG1, IgG2, or IgG4 heavy chains (Lindmark et al, J. Immunol. Methods 62:1-13 (1983)). Protein G is recommended for all murine isotypes and for human $\gamma 3$ (Guss et al, EMBO J. 5:1567-1575 (1986)). The matrix to which the affinity ligand is
20 attached may be agarose, but other matrices are available. Mechanically stable matrices such as controlled pore glass or poly(styrene-divinyl)benzene allow for faster flow rates and shorter processing times than can be achieved with agarose. Where the masked cytokine comprises a CH3 domain, the Bakerbond ABX™ resin (J. T. Baker, Phillipsburg, N.J.) is useful for purification.

Other techniques for protein purification such as fractionation on an ion-exchange column, ethanol
25 precipitation, Reverse Phase HPLC, chromatography on silica, chromatography on heparin SEPHAROSE™ chromatography on an anion or cation exchange resin (such as a polyaspartic acid column), chromatofocusing, SDS-PAGE, and ammonium sulfate precipitation are also available depending on the masked cytokine to be recovered.

30 Following any preliminary purification step(s), the mixture comprising the masked cytokine of interest and contaminants may be subjected to further purification, for example, by low pH hydrophobic interaction chromatography using an elution buffer at a pH between about 2.5-4.5, performed at low salt concentrations (e.g., from about 0-0.25M salt).

35 In general, various methodologies for preparing masked cytokines for use in research, testing, and clinical use are well-established in the art, consistent with the above-described methodologies and/or as deemed

appropriate by one skilled in the art for a particular masked cytokine of interest.

5. COMPOSITIONS

5 In some aspects, also provided herein are compositions comprising any of the IL-12 masked cytokines described herein. In some embodiments, the composition comprises any of the exemplary embodiments of masked IL-12 cytokine described herein. In some embodiments, the composition comprises a dimer of any of the masked IL-12 cytokines described herein. In some embodiments, the composition is a pharmaceutical composition. In some embodiments, the composition comprises a masked IL-12 cytokine and further
10 comprises one or more of the components as described in detail below. For example, in some embodiments, the composition comprises one or more pharmaceutically acceptable carriers, excipients, stabilizers, buffers, preservatives, tonicity agents, non-ionic surfactants or detergents, or other therapeutic agents or active compounds, or combinations thereof. The various embodiments of the composition are sometimes referred to herein as formulations.

15

Therapeutic formulations are prepared for storage by mixing the active ingredient having the desired degree of purity with optional pharmaceutically acceptable carriers, excipients or stabilizers (Remington: The Science and Practice of Pharmacy, 20th Ed., Lippincott Williams & Wilkins, Pub., Gennaro Ed., Philadelphia, Pa. 2000). Acceptable carriers, excipients, or stabilizers are nontoxic to recipients at the
20 dosages and concentrations employed, and include buffers, antioxidants including ascorbic acid, methionine, Vitamin E, sodium metabisulfite; preservatives, isotonicifiers, stabilizers, metal complexes (e.g. Zn-protein complexes); chelating agents such as EDTA and/or non-ionic surfactants.

Buffers can be used to control the pH in a range which optimizes the therapeutic effectiveness, especially
25 if stability is pH dependent. Buffers can be present at concentrations ranging from about 50 mM to about 250 mM. Suitable buffering agents for use with the present invention include both organic and inorganic acids and salts thereof. For example, citrate, phosphate, succinate, tartrate, fumarate, gluconate, oxalate, lactate, acetate. Additionally, buffers may be comprised of histidine and trimethylamine salts such as Tris.

Preservatives can be added to prevent microbial growth, and are typically present in a range from about
30 0.2%-1.0% (w/v). Examples of suitable preservatives commonly used with therapeutics include octadecyldimethylbenzyl ammonium chloride; hexamethonium chloride; benzalkonium halides (e.g., chloride, bromide, iodide), benzethonium chloride; thimerosal, phenol, butyl or benzyl alcohol; alkyl parabens such as methyl or propyl paraben; catechol; resorcinol; cyclohexanol, 3-pentanol, m- cresol, o-
35 cresol, p-cresol, methyl p-hydroxybenzoate, propyl p-hydroxybenzoate, 2-phenoxyethanol, butyl p-hydroxybenzoate, 2-phenylethanol, ethanol, chlorobutanol, thiomersal, bronopol, benzoic acid, inidurea, chlorohexidine, sodium dehydroacetate, chlorocresol, ethyl p-hydroxybenzoate, and chlorphenesine (3p-

chlorophenoxypropane-1,2- diol).

Tonicity agents, sometimes known as “stabilizers” can be present to adjust or maintain the tonicity of liquid in a composition. When used with large, charged biomolecules such as proteins and antibodies, they are often termed “stabilizers” because they can interact with the charged groups of the amino acid side chains, thereby lessening the potential for inter and intra-molecular interactions.

Tonicity agents can be present in any amount between about 0.1% to about 25% by weight or between about 1 to about 5% by weight, taking into account the relative amounts of the other ingredients. In some embodiments, tonicity agents include polyhydric sugar alcohols, trihydric or higher sugar alcohols, such as glycerin, erythritol, arabitol, xylitol, sorbitol and mannitol.

Additional excipients include agents which can serve as one or more of the following: (1) bulking agents, (2) solubility enhancers, (3) stabilizers and (4) agents preventing denaturation or adherence to the container wall. Such excipients include: polyhydric sugar alcohols (enumerated above); amino acids such as alanine, glycine, glutamine, asparagine, histidine, arginine, lysine, ornithine, leucine, 2-phenylalanine, glutamic acid, threonine, etc.; organic sugars or sugar alcohols such as sucrose, lactose, lactitol, trehalose, stachyose, mannose, sorbose, xylose, ribose, ribitol, myoinositol, galactose, galactitol, glycerol, cyclitols (e.g., inositol), polyethylene glycol; sulfur containing reducing agents, such as urea, glutathione, thioctic acid, sodium thioglycolate, thioglycerol, α -monothioglycerol and sodium thio sulfate; low molecular weight proteins such as human serum albumin, bovine serum albumin, gelatin or other immunoglobulins; hydrophilic polymers such as polyvinylpyrrolidone; monosaccharides (e.g., xylose, mannose, fructose, glucose; disaccharides (e.g., lactose, maltose, sucrose); trisaccharides such as raffinose; and polysaccharides such as dextrin or dextran.

Non-ionic surfactants or detergents (also known as “wetting agents”) can be present to help solubilize the therapeutic agent as well as to protect the therapeutic protein against agitation-induced aggregation, which also permits the formulation to be exposed to shear surface stress without causing denaturation of the active therapeutic protein or antibody. Non-ionic surfactants are present in a range of about 0.05 mg/ml to about 1.0 mg/ml or about 0.07 mg/ml to about 0.2 mg/ml. In some embodiments, non-ionic surfactants are present in a range of about 0.001% to about 0.1% w/v or about 0.01% to about 0.1% w/v or about 0.01% to about 0.025% w/v.

Suitable non-ionic surfactants include polysorbates (20, 40, 60, 65, 80, etc.), polyoxamers (184, 188, etc.), PLURONIC® polyols, TRITON®, polyoxyethylene sorbitan monoethers (TWEEN®-20, TWEEN®-80, etc.), lauramcrogol 400, polyoxyl 40 stearate, polyoxyethylene hydrogenated castor oil 10, 50 and 60, glycerol monostearate, sucrose fatty acid ester, methyl cellulose and carboxymethyl cellulose. Anionic detergents that can be used include sodium lauryl sulfate, dioctyle sodium sulfosuccinate and dioctyl

sodium sulfonate. Cationic detergents include benzalkonium chloride or benzethonium chloride.

In order for the formulations to be used for in vivo administration, they must be sterile. The formulation may be rendered sterile by filtration through sterile filtration membranes. The therapeutic compositions
5 herein generally are placed into a container having a sterile access port, for example, an intravenous solution bag or vial having a stopper pierceable by a hypodermic injection needle.

The route of administration is in accordance with known and accepted methods, such as by single or multiple bolus or infusion over a long period of time in a suitable manner, e.g., injection or infusion by
10 subcutaneous, intravenous, intraperitoneal, intramuscular, intraarterial, intralesional or intraarticular routes, topical administration, inhalation or by sustained release or extended-release means.

Any of the masked IL-12 cytokines described herein can be used alone or in combination with other therapeutic agents such as in the methods described herein. The term "in combination with" encompasses
15 two or more therapeutic agents (e.g., a masked IL-12 cytokine and a therapeutic agent) that are included in the same or separate formulations. In some embodiments, "in combination with" refers to "simultaneous" administration, in which case administration of the masked IL-12 cytokine of the invention occurs simultaneously to the administration of the one or more additional therapeutic agents (e.g., at the same time or within one hour between administration (s) of the masked IL-12 cytokine and administration of the one
20 or more additional therapeutic agents). In some embodiments, "in combination with" refers to sequential administration, in which case administration of the masked IL-12 cytokine of the invention occurs prior to and/or following, administration of the one or more additional therapeutic agents (e.g., greater than one hour between administration (s) of the masked IL-12 cytokine and administration of the one or more additional therapeutic agents). Agents contemplated herein include, but are not limited to, a cytotoxic agent,
25 a cytokine, an agent targeting an immune checkpoint molecule, an agent targeting an immune stimulatory molecule, a growth inhibitory agent, an immune stimulatory agent, an anti-inflammatory agent, or an anti-cancer agent.

The formulation herein may also contain more than one active compound as necessary for the particular indication being treated, preferably those with complementary activities that do not adversely affect each other. Alternatively, or in addition, the composition may comprise a cytotoxic agent, cytokine, agent
30 targeting an immune checkpoint molecule or stimulatory molecule, growth inhibitory agent, an immune stimulatory agent, an anti-inflammatory agent, or an anti-cancer agent. Such molecules are suitably present in combination in amounts that are effective for the purpose intended.

35 The formulation may be presented in any suitable state, such as a liquid formulation, a solid state (lyophilized) formulation, or a frozen formulation. Approaches for preparing each of these types of formulations for therapeutic use are well known in the art.

6. METHODS OF TREATMENT

5 Provided herein are methods for treating or preventing a disease in a subject comprising administering to the subject an effective amount of any masked IL-12 cytokine described herein or compositions thereof. In some embodiments, methods are provided for treating or preventing a disease in a subject comprising administering to the subject any composition described herein. In some embodiments, the subject (e.g., a human patient) has been diagnosed with cancer or is at risk of developing such a disorder. In some
10 embodiments, methods are provided for treating or preventing disease in a subject comprising administering to the subject an effective amount of any masked IL-12 cytokine described herein or compositions thereof, wherein the masked IL-12 cytokine is activated upon cleavage by an enzyme. In some embodiments, the masked IL-12 cytokine is activated at a tumor microenvironment. The masked IL-12 cytokine is therapeutically active after it has cleaved. Thus, in some embodiments, the active agent is the cleavage product.

15 For the prevention or treatment of disease, the appropriate dosage of an active agent will depend on the type of disease to be treated, as defined herein, the severity and course of the disease, whether the agent is administered for preventive or therapeutic purposes, previous therapy, the subject's clinical history and response to the agent, and the discretion of the attending physician. The agent is suitably administered to
20 the subject at one time or over a series of treatments.

In some embodiments of the methods described herein, an interval between administrations of a masked IL-12 cytokine described herein is about one week or longer. In some embodiments of the methods described herein, an interval between administrations of a masked IL-12 cytokine described herein is about
25 two days or longer, about three days or longer, about four days or longer, about five days or longer, or about six days or longer. In some embodiments of the methods described herein, an interval between administrations of a masked IL-12 cytokine described herein is about one week or longer, about two weeks or longer, about three weeks or longer, or about four weeks or longer. In some embodiments of the methods described herein, an interval between administrations of a masked IL-12 cytokine described herein is about
30 one month or longer, about two months or longer, or about three months or longer. As used herein, an interval between administrations refers to the time period between one administration of the masked IL-12 cytokine and the next administration of the masked IL-12 cytokine. As used herein, an interval of about one month includes four weeks. In some embodiments, the treatment includes multiple administrations of the masked IL-12 cytokine, wherein the interval between administrations may vary. For example, in some
35 embodiments, the interval between the first administration and the second administration is about one week, and the intervals between the subsequent administrations are about two weeks. In some embodiments, the interval between the first administration and the second administration is about two days, three days, four

days, or five days, or six days, and the intervals between the subsequent administrations are about one week.

In some embodiments, the masked IL-12 cytokine is administered on multiple occasions over a period of time. The dosage that is administered to the subject on multiple occasions can, in some embodiments, be the same dosage for each administration, or, in some embodiments, the masked cytokine can be administered to the subject at two or more different dosages. For example, in some embodiments, a masked IL-12 cytokine is initially administered at one dosage on one or more occasions and is later administered at a second dosage on one or more occasions beginning at a later time point.

10 In some embodiments, a masked IL-12 polypeptide described herein is administered at a flat dose. In some embodiments, a masked IL-12 polypeptide described herein is administered to a subject at a dosage from about 25 mg to about 500 mg per dose. In some embodiments, the masked IL-12 polypeptide is administered to a subject at a dosage of about 25mg to about 50mg, about 50mg to about 75mg, about 75mg to about 100mg, about 100mg to about 125mg, about 125mg to about 150mg, about 150mg to about 175mg, about 175mg to about 200mg, about 200mg to about 225mg, about 225mg to about 250mg, about 250mg to about 275mg, about 275mg to about 300mg, about 300mg to about 325mg, about 325mg to about 350mg, about 350mg to about 375mg, about 375mg to about 400mg, about 400mg to about 425mg, about 425mg to about 450mg, about 450mg, to about 475mg, or about 475mg to about 500mg per dose.

20 In some embodiments, a masked IL-12 polypeptide described herein is administered to a subject at a dosage based on the subject's weight or body surface area (BSA). Depending on the type and severity of the disease, about 1 µg/kg to 15 mg/kg (e.g. 0.1 mg/kg-10mg/kg) of masked IL-12 polypeptide 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 µg/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 masked IL-12 polypeptide would be in the range from about 0.05 mg/kg to about 10 mg/kg. Thus, one or more doses of about 0.5 mg/kg, 2.0 mg/kg, 4.0 mg/kg or 10 mg/kg (or any combination thereof) may be administered to the patient. In some 30 embodiments, a masked IL-12 polypeptide described herein is administered to a subject at a dosage from about 0.1 mg/kg to about 10 mg/kg or about 1.0 mg/kg to about 10 mg/kg. In some embodiments, a masked IL-12 polypeptide described herein is administered to a subject at a dosage of about any of 0.1 mg/kg, 0.5 mg/kg, 1.0 mg/kg, 1.5 mg/kg, 2.0 mg/kg, 2.5 mg/kg, 3.0 mg/kg, 3.5 mg/kg, 4.0 mg/kg, 4.5 mg/kg, 5.0 mg/kg, 5.5 mg/kg, 6.0 mg/kg, 6.5 mg/kg, 7.0 mg/kg, 7.5 mg/kg, 8.0 mg/kg, 8.5 mg/kg, 9.0 mg/kg, 9.5 mg/kg, or 10.0 mg/kg. In some embodiments, a masked IL-12 polypeptide described herein is administered to a subject at a dosage of about or at least about 0.1 mg/kg, about or at least about 0.5 mg/kg, about or at least about 1.0 mg/kg, about or at least about 1.5 mg/kg, about or at least about 2.0 mg/kg, about or at least about 2.5 mg/kg, about or at least about 3.0 mg/kg, about or at least about 3.5 mg/kg, about or at least about

4.0 mg/kg, about or at least about 4.5 mg/kg, about or at least about 5.0 mg/kg, about or at least about 5.5 mg/kg, about or at least about 6.0 mg/kg, about or at least about 6.5 mg/kg, about or at least about 7.0 mg/kg, about or at least about 7.5 mg/kg, about or at least about 8.0 mg/kg, about or at least about 8.5 mg/kg, about or at least about 9.0 mg/kg, about or at least about 9.5 mg/kg, about or at least about 10.0 mg/kg, about or at least about 15.0 mg/kg, about or at least about 20mg/kg, about or at least about 30mg/kg, about or at least about 40mg/kg, about or at least about 50mg/kg, about or at least about 60mg/kg, about or at least about 70mg/kg, about or at least about 80mg/kg, about or at least about 90mg/kg, or about or at least about 100mg/kg. Any of the dosing frequencies described above may be used.

10 A method of treatment contemplated herein is the treatment of a disorder or disease such as cancer with any of the masked IL-12 cytokines or compositions described herein. Disorders or diseases that are treatable with the formulations of this present invention include leukemia, lymphoma, head and neck cancer, colorectal cancer, prostate cancer, pancreatic cancer, melanoma, breast cancer, neuroblastoma, lung cancer, ovarian cancer, osteosarcoma, bladder cancer, cervical cancer, liver cancer, kidney cancer, skin cancer (e.g.,
15 Merkel cell carcinoma) or testicular cancer.

In some embodiments, provided herein is a method of treatment or prevention of a cancer by administration of any masked IL-12 cytokines or compositions described herein. In some embodiments, provided herein is a method of treatment or prevention of a cancer by administration of any IL-12 masked cytokine or
20 composition described herein in combination with an anticancer agent. The anti-cancer agent can be any agent capable of reducing cancer growth, interfering with cancer cell replication, directly or indirectly killing cancer cells, reducing metastasis, reducing tumor blood supply, or reducing cell survival. In some embodiments, the anti-cancer agent is selected from the group consisting of a PD-1 inhibitor, an EGFR inhibitor, a HER2 inhibitor, a VEGFR inhibitor, a CTLA-4 inhibitor, a BTLA inhibitor, a B7H4 inhibitor,
25 a B7H3 inhibitor, a CSFIR inhibitor, an HVEM inhibitor, a CD27 inhibitor, a KIR inhibitor, an NKG2A inhibitor, an NKG2D agonist, a TWEAK inhibitor, an ALK inhibitor, a CD52 targeting antibody, a CCR4 targeting antibody, a PD-L1 inhibitor, a KIT inhibitor, a PDGFR inhibitor, a BAFF inhibitor, an HD AC inhibitor, a VEGF ligand inhibitor, a CD19 targeting molecule, a FOFR1 targeting molecule, a DFF3 targeting molecule, a DKK1 targeting molecule, a MUC1 targeting molecule, a MUG 16 targeting
30 molecule, a PSMA targeting molecule, an MSFN targeting molecule, an NY-ESO-1 targeting molecule, a B7H3 targeting molecule, a B7H4 targeting molecule, a BCMA targeting molecule, a CD29 targeting molecule, a CD151 targeting molecule, a CD 123 targeting molecule, a CD33 targeting molecule, a CD37 targeting molecule, a CDH19 targeting molecule, a CEA targeting molecule, a Claudin 18.2 targeting molecule, a CFEC12A targeting molecule, an EGFRVIII targeting molecule, an EPCAM targeting
35 molecule, an EPHA2 targeting molecule, an FCRH5 targeting molecule, an FLT3 targeting molecule, a GD2 targeting molecule, a glypican 3 targeting molecule, a gpA33 targeting molecule, a GPRC5D targeting molecule, an IL-123R targeting molecule, an IL-1RAP targeting molecule, a MCSP targeting molecule, a RON targeting molecule, a ROR1 targeting molecule, a STEAP2 targeting molecule, a Tfr targeting

molecule, a CD166 targeting molecule, a TPBG targeting molecule, a TROP2 targeting molecule, a proteasome inhibitor, an ABE inhibitor, a CD30 inhibitor, a FLT3 inhibitor, a MET inhibitor, a RET inhibitor, an IL-13 inhibitor, a MEK inhibitor, a ROS1 inhibitor, a BRAE inhibitor, a CD38 inhibitor, a RANKE inhibitor, a B4GALNT1 inhibitor, a SLAMF7 inhibitor, an IDH2 inhibitor, an mTOR inhibitor, a
 5 CD20 targeting antibody, a BTK inhibitor, a PI3K inhibitor, a FLT3 inhibitor, a PARP inhibitor, a CDK4 inhibitor, a CDK6 inhibitor, an EGFR inhibitor, a RAF inhibitor, a JAK1 inhibitor, a JAK2 inhibitor, a JAK3 inhibitor, an IL-6 inhibitor, a IL-17 inhibitor, a Smoothened inhibitor, an IL-6R inhibitor, a BCL2 inhibitor, a PTCH inhibitor, a PIGF inhibitor, a TGFB inhibitor, a CD28 agonist, a CD3 agonist, CD40 agonist, a GTR agonist, a OX40 agonist, a VISTA agonist, a CD137 agonist, a LAG3 inhibitor, a TIM3
 10 inhibitor, a TIGIT inhibitor, and an IL-12R inhibitor.

In some embodiments, provided herein is a method of treatment or prevention of a cancer by administration of any masked IL-12 cytokine described herein in combination with an anti-inflammatory agent. The anti-inflammatory agent can be any agent capable of preventing, counteracting, inhibiting, or otherwise reducing
 15 inflammation.

In some embodiments, the anti-inflammatory agent is a cyclooxygenase (COX) inhibitor. The COX inhibitor can be any agent that inhibits the activity of COX-1 and/or COX-2. In some embodiments, the COX inhibitor selectively inhibits COX-1 (i.e., the COX inhibitor inhibits the activity of COX-1 more than
 20 it inhibits the activity of COX-2). In some embodiments, the COX inhibitor selectively inhibits COX-2 (i.e., the COX inhibitor inhibits the activity of COX-2 more than it inhibits the activity of COX-1). In some embodiments, the COX inhibitor inhibits both COX-1 and COX-2.

In some embodiments, the COX inhibitor is a selective COX-1 inhibitor and is selected from the group consisting of SC-560, FR122047, P6, mofezolac, TFAP, flurbiprofen, and ketoprofen. In some
 25 embodiments, the COX inhibitor is a selective COX-2 inhibitor and is selected from the group consisting of celecoxib, rofecoxib, meloxicam, piroxicam, deracoxib, parecoxib, valdecoxib, etoricoxib, a chromene derivative, a chroman derivative, N-(2-cyclohexyloxynitrophenyl) methane sulfonamide, parecoxib, lumiracoxib, RS 57067, T-614, BMS-347070, JTE-522, S-2474, SVT- 2016, CT-3, ABT-963, SC-58125,
 30 nimesulide, flosulide, NS-398, L- 745337, RWJ-63556, L-784512, darbufelone, CS-502, LAS-34475, LAS- 34555, S-33516, diclofenac, mefenamic acid, and SD-8381. In some embodiments, the COX inhibitor is selected from the group consisting of ibuprofen, naproxen, ketorolac, indomethacin, aspirin, naproxen, tolmetin, piroxicam, and meclofenamate. In some embodiments, the COX inhibitor is selected from the
 35 group consisting of SC-560, FR122047, P6, mofezolac, TFAP, flurbiprofen, ketoprofen, celecoxib, rofecoxib, meloxicam, piroxicam, deracoxib, parecoxib, valdecoxib, etoricoxib, a chromene derivative, a chroman derivative, N-(2-cyclohexyloxynitrophenyl) methane sulfonamide, parecoxib, lumiracoxib, RS 57067, T-614, BMS-347070, JTE-522, S-2474, SVT- 2016, CT-3, ABT-963, SC-58125, nimesulide, flosulide, NS-398, L- 745337, RWJ-63556, L-784512, darbufelone, CS-502, LAS-34475, LAS- 34555, S-

33516, diclofenac, mefenamic acid, SD-8381, ibuprofen, naproxen, ketorolac, indomethacin, aspirin, naproxen, tolmetin, piroxicam, and meclofenamate.

- In some embodiments, the anti-inflammatory agent is an NF- κ B inhibitor. The NF- κ B inhibitor can be any agent that inhibits the activity of the NF- κ B pathway. In some embodiments, the NF- κ B inhibitor is selected from the group consisting of an IKK complex inhibitor, an I κ B degradation inhibitor, an NF- κ B nuclear translocation inhibitor, a p65 acetylation inhibitor, an NF- κ B DNA binding inhibitor, an NF- κ B transactivation inhibitor, and a p53 induction inhibitor.
- In some embodiments, the IKK complex inhibitor is selected from the group consisting of TPCA-1, NF- κ B Activation Inhibitor VI (BOT-64), BMS-345541, amlexanox, SC-514 (GK-01140), IMD-0354, and IKK-16. In some embodiments, the I κ B degradation inhibitor is selected from the group consisting of BAY-11-7082, MG-115, MG-132, lactacystin, epoxomicin, parthenolide, carfilzomib, and MLN-4924 (pevonedistat). In some embodiments, the NF- κ B nuclear translocation inhibitor is selected from the group consisting of JSH-23 and rolipram. In some embodiments, the p65 acetylation inhibitor is selected from the group consisting of gallic acid and anacardic acid. In some embodiments, the NF- κ B DNA binding inhibitor is selected from the group consisting of GYY-4137, p-XSC, CV-3988, and prostaglandin E2 (PGE2). In some embodiments, the NF- κ B transactivation inhibitor is selected from the group consisting of LY-294002, wortmannin, and mesalamine. In some embodiments, the p53 induction inhibitor is selected from the group consisting of quinacrine and flavopiridol. In some embodiments, the NF- κ B inhibitor is selected from the group consisting of TPCA-1, NF- κ B Activation Inhibitor VI (BOT-64), BMS-345541, amlexanox, SC-514 (GK-01140), IMD-0354, IKK-16, BAY-11-7082, MG-115, MG-132, lactacystin, epoxomicin, parthenolide, carfilzomib, MLN-4924 (pevonedistat), JSH-23 rolipram, gallic acid, anacardic acid, GYY-4137, p-XSC, CV-3988, prostaglandin E2 (PGE2), LY-294002, wortmannin, mesalamine, quinacrine, and flavopiridol.

- In some embodiments, provided herein is a method of treatment or prevention of a cancer by administration of any masked IL-12 cytokine or composition described herein in combination with an anticancer therapeutic protein. The anti-cancer therapeutic protein can be any therapeutic protein capable of reducing cancer growth, interfering with cancer cell replication, directly or indirectly killing cancer cells, reducing metastasis, reducing tumor blood supply, or reducing cell survival. Exemplary anti-cancer therapeutic proteins may come in the form of an antibody or fragment thereof, an antibody derivative, a bispecific antibody, a chimeric antigen receptor (CAR) T cell, a fusion protein, or a bispecific T-cell engager (BiTE). In some embodiments, provided herein is a method of treatment or prevention of a cancer by administration of any masked IL-2 cytokine or composition described herein in combination with CAR-NK (Natural Killer) cells.

7. ARTICLES OF MANUFACTURE OR KITS

In another aspect, an article of manufacture or kit is provided which comprises any masked IL-12 cytokine described herein. The article of manufacture or kit may further comprise instructions for use of the cytokines in the methods of the invention. Thus, in certain embodiments, the article of manufacture or kit comprises instructions for the use of a masked cytokine in methods for treating or preventing a disorder (e.g., a cancer) in an individual comprising administering to the individual an effective amount of a masked cytokine. For example, in certain embodiments, the article of manufacture or kit comprises instructions for the use of a masked IL-12 polypeptide in methods for treating or preventing a disorder (e.g., a cancer) in an individual comprising administering to the individual an effective amount of a masked IL-12 polypeptide. In certain embodiments, the individual is a human. In some embodiments, the individual has a disease selected from the group consisting of include leukemia, lymphoma, head and neck cancer, colorectal cancer, prostate cancer, pancreatic cancer, melanoma, breast cancer, neuroblastoma, lung cancer, ovarian cancer, osteosarcoma, bladder cancer, cervical cancer, liver cancer, kidney cancer, skin cancer or testicular cancer.

The article of manufacture or kit may further comprise a container. Suitable containers include, for example, bottles, vials (e.g., dual chamber vials), syringes (such as single or dual chamber syringes), test tubes, and intravenous (IV) bags. The container may be formed from a variety of materials such as glass or plastic. The container holds the formulation. In some embodiments, the formulation is a lyophilized formulation. In some embodiments, the formulation is a frozen formulation. In some embodiments, the formulation is a liquid formulation.

The article of manufacture or kit may further comprise a label or a package insert, which is on or associated with the container, may indicate directions for reconstitution and/or use of the formulation. The label or package insert may further indicate that the formulation is useful or intended for subcutaneous, intravenous, or other modes of administration for treating or preventing a disorder (e.g., a cancer) in an individual. The container holding the formulation may be a single-use vial or a multi-use vial, which allows for repeat administrations of the reconstituted formulation. The article of manufacture or kit may further comprise a second container comprising a suitable diluent. The article of manufacture or kit may further include other materials desirable from a commercial, therapeutic, and user standpoint, including other buffers, diluents, filters, needles, syringes, and package inserts with instructions for use.

In a specific embodiment, the present invention provides kits for a single dose-administration unit. Such kits comprise a container of an aqueous formulation of therapeutic cytokine, including both single or multi-chambered pre-filled syringes. Exemplary pre-filled syringes are available from Vetter GmbH, Ravensburg, Germany.

The article of manufacture or kit herein optionally further comprises a container comprising a second medicament, wherein the masked cytokine is a first medicament, and which article or kit further comprises instructions on the label or package insert for treating the subject with the second medicament, in an effective amount.

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In another embodiment, provided herein is an article of manufacture or kit comprising the formulations described herein for administration in an auto-injector device. An auto-injector can be described as an injection device that upon activation, will deliver its contents without additional necessary action from the patient or administrator. They are particularly suited for self-medication of therapeutic formulations when the delivery rate must be constant and the time of delivery is greater than a few moments.

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8. DEFINITIONS

Unless defined otherwise, all terms of art, notations and other technical and scientific terms or terminology used herein are intended to have the same meaning as is commonly understood by one of ordinary skill in the art to which the claimed subject matter pertains. In some cases, terms with commonly understood meanings are defined herein for clarity and/or for ready reference, and the inclusion of such definitions herein should not necessarily be construed to represent a substantial difference over what is generally understood in the art.

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It is to be understood that this invention is not limited to particular compositions or biological systems, which can, of course, vary. It is also to be understood that the terminology used herein is for the purpose of describing particular embodiments only, and is not intended to be limiting. As used in this specification and the appended claims, the singular forms "a," "an," and "the" include plural referents unless the content clearly dictates otherwise. Thus, for example, reference to "an IL-12 polypeptide" optionally includes a combination of two or more such polypeptides, and the like.

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The term "about" as used herein refers to the usual error range for the respective value readily known to the skilled person in this technical field. Reference to "about" a value or parameter herein includes (and describes) embodiments that are directed to that value or parameter per se.

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It is understood that aspects and embodiments of the invention described herein include "comprising," "consisting," and "consisting essentially of" aspects and embodiments.

As used herein, the term "and/or" refers to any one of the items, any combination of the items, or all of the items with which the term is associated. For instance, the phrase "A, B, and/or C" is intended to encompass each of the following embodiments: A, B, and C; A, B, or C; A or B; A or C; B or C; A and B; A and C; B

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and C; A and B or C; B and A or C; C and A or B; A (alone); B (alone); and C (alone).

The term “antibody” includes polyclonal antibodies, monoclonal antibodies (including full length antibodies which have an immunoglobulin Fc region), antibody compositions with polyepitopic specificity, multispecific antibodies (e.g., bispecific antibodies, diabodies, and single-chain molecules, as well as antibody fragments (e.g., Fab, F(ab')₂, and Fv). The term “immunoglobulin” (Ig) is used interchangeably with “antibody” herein.

The term “diabodies” refers to small antibody fragments with two antigen-binding sites, which comprise a heavy chain variable (VH) domain connected to a light chain variable (VL) domain in the same polypeptide chain (VH-VL).

The basic 4-chain antibody unit is a heterotetrameric glycoprotein composed of two identical light (L) chains and two identical heavy (H) chains. An IgM antibody consists of 5 of the basic heterotetramer units along with an additional polypeptide called a J chain, and contains 10 antigen binding sites, while IgA antibodies comprise from 2-5 of the basic 4-chain units which can polymerize to form polyvalent assemblages in combination with the J chain. In the case of IgGs, the 4-chain unit is generally about 150,000 daltons. Each L chain is linked to an H chain by one covalent disulfide bond, while the two H chains are linked to each other by one or more disulfide bonds depending on the H chain isotype. Each H and L chain also has regularly spaced intrachain disulfide bridges. Each H chain has at the N-terminus, a variable domain (VH) followed by three constant domains (CH) for each of the α and γ chains and four CH domains for μ and δ isotypes. Each L chain has at the N-terminus, a variable domain (VL) followed by a constant domain at its other end. The VL is aligned with the VH and the CL is aligned with the first constant domain of the heavy chain (CH1). Particular amino acid residues are believed to form an interface between the light chain and heavy chain variable domains. The pairing of a VH and VL together forms a single antigen-binding site. For the structure and properties of the different classes of antibodies, see e.g., Basic and Clinical Immunology, 8th Edition, Daniel P. Stites, Abba I. Terr and Tristram G. Parslow (eds), Appleton & Lange, Norwalk, CT, 1994, page 71 and Chapter 6.

The L chain from any vertebrate species can be assigned to one of two clearly distinct types, called kappa and lambda, based on the amino acid sequences of their constant domains. Depending on the amino acid sequence of the constant domain of their heavy chains (CH), immunoglobulins can be assigned to different classes or isotypes. There are five classes of immunoglobulins: IgA, IgD, IgE, IgG and IgM, having heavy chains designated α , δ , ϵ , γ and μ , respectively. The γ and α classes are further divided into subclasses on the basis of relatively minor differences in the CH sequence and function, e.g., humans express the following subclasses: IgG1, IgG2, IgG3, IgG4, IgA1 and IgA2. IgG1 antibodies can exist in multiple polymorphic variants termed allotypes (reviewed in Jefferis and Lefranc 2009. mAbs Vol 1 Issue 4 1-7) any of which are suitable for use in the invention. Common allotypic variants in human populations are

those designated by the letters a,f,n,z.

An "isolated" antibody is one that has been identified, separated and/or recovered from a component of its production environment (e.g., naturally or recombinantly). In some embodiments, the isolated polypeptide is free of association with all other components from its production environment. Contaminant components of its production environment, such as that resulting from recombinant transfected cells, are materials that would typically interfere with research, diagnostic or therapeutic uses for the antibody, and may include enzymes, hormones, and other proteinaceous or non-proteinaceous solutes. In some embodiments, the polypeptide is purified: (1) to greater than 95% by weight of antibody as determined by, for example, the Lowry method, and in some embodiments, to greater than 99% by weight; (1) to a degree sufficient to obtain at least 15 residues of N-terminal or internal amino acid sequence by use of a spinning cup sequenator, or (3) to homogeneity by SDS-PAGE under non-reducing or reducing conditions using Coomassie blue or silver stain. Isolated antibody includes the antibody in situ within recombinant cells since at least one component of the antibody's natural environment will not be present. Ordinarily, however, an isolated polypeptide or antibody is prepared by at least one purification step.

The term "monoclonal antibody" as used herein refers to an antibody obtained from a population of substantially homogeneous antibodies, i.e., the individual antibodies comprising the population are identical except for possible naturally occurring mutations and/or post-translation modifications (e.g., isomerizations, amidations) that may be present in minor amounts. In some embodiments, monoclonal antibodies have a C-terminal cleavage at the heavy chain and/or light chain. For example, 1, 2, 3, 4, or 5 amino acid residues are cleaved at the C-terminus of heavy chain and/or light chain. In some embodiments, the C-terminal cleavage removes a C-terminal lysine from the heavy chain. In some embodiments, monoclonal antibodies have an N-terminal cleavage at the heavy chain and/or light chain. For example, 1, 2, 3, 4, or 5 amino acid residues are cleaved at the N-terminus of heavy chain and/or light chain. In some embodiments truncated forms of monoclonal antibodies can be made by recombinant techniques. In some embodiments, monoclonal antibodies are highly specific, being directed against a single antigenic site. In some embodiments, monoclonal antibodies are highly specific, being directed against multiple antigenic sites (such as a bispecific antibody or a multispecific antibody). The modifier "monoclonal" indicates the character of the antibody as being obtained from a substantially homogeneous population of antibodies, and is not to be construed as requiring production of the antibody by any particular method. For example, the monoclonal antibodies to be used in accordance with the present invention may be made by a variety of techniques, including, for example, the hybridoma method, recombinant DNA methods, phage-display technologies, and technologies for producing human or human-like antibodies in animals that have parts or all of the human immunoglobulin loci or genes encoding human immunoglobulin sequences.

The terms "full-length antibody," "intact antibody" or "whole antibody" are used interchangeably to refer to an antibody in its substantially intact form, as opposed to an antibody fragment. Specifically, whole

antibodies include those with heavy and light chains including an Fc region. The constant domains may be native sequence constant domains (e.g., human native sequence constant domains) or amino acid sequence variants thereof. In some cases, the intact antibody may have one or more effector functions.

5 An "antibody fragment" comprises a portion of an intact antibody, such as the antigen binding region and/or the variable region of the intact antibody, and/or the constant region of the intact antibody. Examples of an antibody fragment include the Fc region of the antibody, a portion of the Fc region, or a portion of the antibody comprising the Fc region. Examples of antigen-binding antibody fragments include domain antibodies (dAbs), Fab, Fab', F(ab')₂ and Fv fragments; diabodies; linear antibodies (see U.S. Pat. No. 10 5,641,870, Example 2; Zapata et al, Protein Eng. 8(10): 1057-1062 [1995]); single-chain antibody molecules, and multispecific antibodies formed from antibody fragments. Single heavy chain antibodies or single light chain antibodies can be engineered, or in the case of the heavy chain, can be isolated from camelids, shark, libraries or mice engineered to produce single heavy chain molecules.

15 Papain digestion of antibodies produced two identical antigen-binding fragments, called "Fab" fragments, and a residual "Fc" fragment, a designation reflecting the ability to crystallize readily. The Fab fragment consists of an entire L chain along with the variable region domain of the H chain (VH), and the first constant domain of one heavy chain (CH1). Each Fab fragment is monovalent with respect to antigen binding, i.e., it has a single antigen-binding site. Pepsin treatment of an antibody yields a single large 20 F(ab')₂ fragment which roughly corresponds to two disulfide linked Fab fragments having different antigen-binding activity and is still capable of cross-linking antigen. Fab' fragments differ from Fab fragments by having a few additional residues at the carboxy terminus of the CH1 domain including one or more cysteines from the antibody hinge region. Fab'-SH is the designation herein for Fab' in which the cysteine residue(s) of the constant domains bear a free thiol group. F(ab')₂ antibody fragments originally 25 were produced as pairs of Fab' fragments which have hinge cysteines between them. Other chemical couplings of antibody fragments are also known. The Fc fragment comprises the carboxy-terminal portions of both H chains held together by disulfides. The effector functions of antibodies are determined by sequences and glycan in the Fc region, the region which is also recognized by Fc receptors (FcR) found on certain types of cells.

30 "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 35 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 example, 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 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.

Antibody "effector functions" refer to those biological activities attributable to the Fc region (a native sequence Fc region or amino acid sequence variant Fc region) of an antibody, and vary with the antibody isotype. Examples of antibody effector functions include: C1q binding and complement dependent cytotoxicity; Fc receptor binding; antibody-dependent cell-mediated cytotoxicity (ADCC); phagocytosis; down regulation of cell surface receptors (e.g., B cell receptors); and B cell activation.

"Binding affinity" as used herein refers to the strength of the non-covalent interactions between a single binding site of a molecule (e.g., a cytokine) and its binding partner (e.g., a cytokine receptor). In some embodiments, the affinity of a binding protein (e.g., a cytokine) can generally be represented by a dissociation constant (K_d). Affinity can be measured by common methods known in the art, including those described herein.

An "isolated" nucleic acid molecule encoding the cytokine polypeptides described herein is a nucleic acid molecule that is identified and separated from at least one contaminant nucleic acid molecule with which it is ordinarily associated in the environment in which it was produced. In some embodiments, the isolated nucleic acid is free of association with all components associated with the production environment. The isolated nucleic acid molecules encoding the polypeptides and cytokine polypeptides herein is in a form other than in the form or setting in which it is found in nature. Isolated nucleic acid molecules therefore are distinguished from nucleic acid encoding the polypeptides and cytokine polypeptides herein existing naturally in cells.

The term "pharmaceutical formulation" refers to a preparation that is in such form as to permit the biological activity of the active ingredient to be effective, and that contains no additional components that are unacceptably toxic to a subject to which the formulation would be administered. Such formulations are sterile.

"Carriers" as used herein include pharmaceutically acceptable carriers, excipients, or stabilizers that are

5 nontoxic to the cell or mammal being exposed thereto at the dosages and concentrations employed. Often the physiologically acceptable carrier is an aqueous pH buffered solution. Examples of physiologically acceptable carriers include buffers such as phosphate, citrate, and other organic acids; antioxidants including ascorbic acid; low molecular weight (less than about 10 residues) polypeptide; proteins, such as serum albumin, gelatin, or immunoglobulins; hydrophilic polymers such as polyvinylpyrrolidone; amino acids such as glycine, glutamine, asparagine, arginine or lysine; monosaccharides, disaccharides, and other carbohydrates including glucose, mannose, or dextrins; chelating agents such as EDTA; sugar alcohols such as mannitol or sorbitol; salt-forming counterions such as sodium; and/or nonionic surfactants such as TWEEN™, polyethylene glycol (PEG), and PLURONICS™.

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As used herein, the term “treatment” refers to clinical intervention designed to alter the natural course of the individual or cell being treated during the course of clinical pathology. Desirable effects of treatment include decreasing the rate of disease progression, ameliorating or palliating the disease state, and remission or improved prognosis. An individual is successfully “treated”, for example, if one or more symptoms associated with a disorder (e.g., a neoplastic disease) are mitigated or eliminated. For example, an individual is successfully “treated” if treatment results in increasing the quality of life of those suffering from a disease, decreasing the dose of other medications required for treating the disease, reducing the frequency of recurrence of the disease, lessening severity of the disease, delaying the development or progression of the disease, and/or prolonging survival of individuals.

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As used herein, “in conjunction with” or “in combination with” refers to administration of one treatment modality in addition to another treatment modality. As such, “in conjunction with” or “in combination with” refers to administration of one treatment modality before, during or after administration of the other treatment modality to the individual.

25

As used herein, the term “prevention” includes providing prophylaxis with respect to occurrence or recurrence of a disease in an individual. An individual may be predisposed to, susceptible to a disorder, or at risk of developing a disorder, but has not yet been diagnosed with the disorder. In some embodiments, masked cytokines described herein are used to delay development of a disorder.

30

As used herein, an individual “at risk” of developing a disorder may or may not have detectable disease or symptoms of disease, and may or may not have displayed detectable disease or symptoms of disease prior to the treatment methods described herein. “At risk” denotes that an individual has one or more risk factors, which are measurable parameters that correlate with development of the disease, as known in the art. An individual having one or more of these risk factors has a higher probability of developing the disorder than an individual without one or more of these risk factors.

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An “effective amount” refers to at least an amount effective, at dosages and for periods of time necessary,

to achieve the desired or indicated effect, including a therapeutic or prophylactic result.

An effective amount can be provided in one or more administrations. A “therapeutically effective amount” is at least the minimum concentration required to effect a measurable improvement of a particular disorder.

5 A therapeutically effective amount herein may vary according to factors such as the disease state, age, sex, and weight of the patient, and the ability of the antibody to elicit a desired response in the individual. A therapeutically effective amount may also be one in which any toxic or detrimental effects of the masked cytokine are outweighed by the therapeutically beneficial effects. A “prophylactically effective amount” refers to an amount effective, at the dosages and for periods of time necessary, to achieve the desired
10 prophylactic result. Typically, but not necessarily, since a prophylactic dose is used in subjects prior to or at the earlier stage of disease, the prophylactically effective amount can be less than the therapeutically effective amount.

“Chronic” administration refers to administration of the medicament(s) in a continuous as opposed to acute
15 mode, so as to main the initial therapeutic effect (activity) for an extended period of time. “Intermittent” administration is treatment that is not consecutively done without interruption, but rather is cyclic in nature.

As used herein, an “individual” or a “subject” is a mammal. A “mammal” for purposes of treatment includes humans, domestic and farm animals, and zoo, sports, or pet animals, such as dogs, horses, rabbits, cattle,
20 pigs, hamsters, gerbils, mice, ferrets, rats, cats, etc. In some embodiments, the individual or subject is human.

9. EXAMPLES

25 The invention will be more fully understood by reference to the following examples. They should not, however, be construed as limiting the scope of the invention. It is understood that the examples and embodiments described herein are for illustrative purposes only and that various modifications or changes in light thereof will be suggested to persons skilled in the art and are to be included within the spirit and purview of this application and scope of the appended claims.

30 Although some examples describe the engineering, production, and/or testing of “masked” versions of an IL-2 polypeptide construct, some examples also employ parental “non-masked” versions of the IL-2 polypeptide construct, such as for comparison, or other constructs that include one or more of the components described herein that are tested as controls for comparison. Accordingly, the description of, for instance, testing done on masked IL-2 polypeptide constructs does not necessarily mean that non-
35 masked versions of the construct were not also tested.

Example 1: Engineering of Masked IL-2 Polypeptides

Masked IL-2 polypeptide are generated in accordance with the teachings herein. In the subsequent examples, some experiments involve use of the masked IL-2 polypeptide constructs in monomer form, and some experiments involve use of the masked IL-2 constructs in dimer form, such as a dimer formed through disulfide bonds linking two copies of the same masked polypeptide construct (homodimer), or a heterodimer formed by two different polypeptides (*see, e.g.*, Table 5).

Masked IL-2 polypeptide constructs are generated that include an IL-2 polypeptide or functional fragment thereof, a masking moiety, and a half-life extension domain, such as an antibody or fragment thereof (e.g., an Fc region, heavy chain, and/or light chain). Some IL-2 polypeptide constructs are also generated that include an IL-2 polypeptide or functional fragment thereof linked to a half-life extension domain without also including a masking moiety. Some of the constructs also include a linker that comprises a cleavable peptide and links the masking moiety to the IL-2 polypeptide or functional fragment thereof, thereby resulting in an activatable masked IL-2 polypeptide construct. Some of the constructs also include a linker that links the IL-2 polypeptide or functional fragment thereof to the half- life extension domain. Some of the constructs also include a linker that links the IL-2 polypeptide or functional fragment thereof to the masking moiety. The masked IL-2 polypeptide constructs that do not include a cleavable peptide in the linker that links the IL-2 polypeptide or functional fragment thereof to the masking moiety are also referred to as non-activatable masked IL-2 polypeptide constructs or non- activatable IL-2 polypeptide constructs because they do not include a cleavable peptide. The structure and composition of exemplary IL-2 polypeptide constructs are provided in Table 3.

Table 3:

Construct #	Structure (N- to C-terminal direction)
AK032	H-C
AK035	H-C

Also generated are masked IL-2 polypeptide constructs that include an IL-2 polypeptide or functional fragment thereof, a first masking moiety, a second masking moiety, and a half-life extension domain, such as albumin, an antibody or fragment thereof (e.g., an Fc region, heavy chain, and/or light chain), an albumin-binding peptide, an IgG-binding peptide, or a polyamino acid sequence. Some of the constructs also include a linker that links the first masking moiety to the IL-2 polypeptide or functional fragment

thereof. Some of the constructs also include a linker that links the second masking moiety to the IL-2 polypeptide or functional fragment thereof. Some of the constructs include a cleavable peptide in the linker linking the first masking moiety to the IL-2 polypeptide or functional fragment thereof and/or the linker linking the second masking moiety to the IL-2 polypeptide or functional fragment thereof, thereby
5 resulting in an activatable masked IL-2 polypeptide construct. Some of the constructs also include a linker linking the second masking moiety to the half-life extension domain. The masked IL-2 polypeptide constructs that do not include a cleavable peptide in either of the linkers that link the IL-2 polypeptide or functional fragment thereof to the first masking moiety or the second masking moiety are also referred to as non-activatable masked IL-2 polypeptide constructs or non-activatable IL-2 polypeptide constructs
10 because they do not include a cleavable peptide. The structure and composition of exemplary IL-2 polypeptide constructs are provided in Table 4.

Table 4:

Construct #	Structure (N- to C- terminal direction)
AK041	H-L1-MM1-L2-C-L3-MM2

15

Also generated are masked IL-2 polypeptide constructs that include an IL-2 polypeptide or functional fragment thereof, a masking moiety, a first half-life extension domain, and a second half-life extension domain, an antibody or fragment thereof (e.g., an Fc region, heavy chain, and/or light chain). The masking moiety is linked to the first half-life extension domain, the IL-2 polypeptide or functional
20 fragment thereof is linked to the second half-life extension domain, and the first half-life extension domain and the second half-life extension domain contain modifications promoting the association of the first and the second half-life extension domain. In one exemplary embodiment, the masking moiety is linked to the first half-life extension domain, and the IL-2 polypeptide or functional fragment thereof is linked to the second half-life extension domain, and the first half-life extension domain and the second
25 half-life extension domain contain modifications promoting the association of the first and the second half-life extension domain. In one exemplary embodiment of a non-masked IL-2 polypeptide construct, the embodiment comprises an IL-2 polypeptide or functional fragment thereof linked to a first half-life extension domain, and comprises a second half-life extension domain, where the IL-2 polypeptide or functional fragment thereof is linked to the first half-life extension domain, and the second half-life
30 extension domain. Some of the constructs also include a linker that links the masking moiety to the first half-life extension domain, and/or a linker that links the IL-2 polypeptide or functional fragment thereof to the second half-life extension domain. The first and second half-life extension domain of some of the constructs are also linked. In some constructs, the first and second half-life extension domain of some of

the constructs are linked by a linker. Some of the constructs include a cleavable peptide in the linker linking the masking moiety to the first half-life extension domain and/or the linker linking the IL-2 polypeptide or functional fragment thereof to the second half-life extension domain, thereby resulting in an activatable masked IL-2 polypeptide construct. The masked IL-2 polypeptide constructs that do not
5 include a cleavable peptide in either the linker that links the IL-2 polypeptide or functional fragment thereof to the second half-life extension domain or the linker that links the masking moiety to the first half-life extension domain are also referred to as non-activatable masked IL-2 polypeptide constructs or non-activatable IL-2 polypeptide constructs because they do not include a cleavable peptide. The structure and composition of exemplary IL-2 polypeptide constructs are provided in Table 5.

Table 5

Construct #	Structure (N- to C-terminal direction)
AK081	H-L1-C
	H
AK109	H-L1-MM
	H-C
AK110	H-L1-MM
	H-C
AK111	H-L1-C
	H-L1-MM
AK165	H-L1-C
	H
AK166	H-L1-C
	H-L1-MM
AK167	H-L1-C
	H
AK168	H-L1-C
	H-L1-MM
AK189	H-L1-C
	H-L1-MM
AK190	H-L1-C
	H-L1-MM

Construct #	Structure (N- to C-terminal direction)
AK191	H-L1-C
	H-L1-MM
AK192	H-L1-C
	H-L1-MM
AK193	H-L1-C
	H-L1-MM
AK197	H-L1-C
	H-L1-MM
AK203	H-L1-C
	H-L1-MM
AK209	H-L1-C
	H-L1-MM
AK210	H-L1-C
	H-L1-MM
AK211	H-L1-C
	H-L1-MM
AK215	H-L1-C
	H-L1-MM
AK216	H-L1-C
	H-L1-MM
AK218	H-L1-C
	H-L1-MM

Construct #	Structure (N- to C-terminal direction)
AK219	H-L1-C
	H-L1-MM
AK220	H-L1-C
	H-L1-MM
AK223	H-L1-C
	H-L1-MM
AK235	H-L1-C
	H
AK253	H-L1-C
	H
AK304	H-L1-C
	H
AK305	H-L1-C
	H-L1-MM
AK306	H-L1-C
	H
AK307	H-L1-C
	H-L1-MM
AK308	H-L1-C
	H
AK309	H-L1-C
	H-L1-MM

Construct #	Structure (N- to C-terminal direction)
AK310	H-L1-C
	H
AK311	H-L1-C
	H-L1-MM
AK312	H-L1-C
	H
AK313	H-L1-C
	H-L1-MM
AK314	H-L1-C
	H
AK315	H-L1-C
	H-L1-MM
AK316	H-L1-C
	H-L1-MM

Example 2: In vitro characterization of masked IL-2

The masked IL-2 polypeptide constructs generated in Example 1 are characterized using several cellular and functional assays in vitro.

Production

- 5 Plasmids encoding the constructs (e.g., masked IL-2 polypeptide constructs) were transfected into either Expi293 cells (Life Technologies A14527) or HEK293-6E cells (National Research Council; NRC). Transfections were performed using 1 mg of total DNA using PEIpro (Polyplus Transfection, 115-100) in a 1:1 ratio with the total DNA. The DNA and PEI were each added to 50 mL of OptiMem (Life Technologies 31985088) medium and sterile filtered. The DNA and PEI were combined for 10 minutes
- 10 and added to the Expi293 cells with a cell density of $1.8 - 2.8 \times 10^6$ cells/mL or $0.85 - 1.20 \times 10^6$ cells/mL, for expi293 cells or HEK293 cells, respectively, and a viability of at least 95%. The HEK293-6E transfection was performed with a cell density of and a viability of at least 95%, following the same protocol used for the Expi293 transfections. After 5-7 days, the cells were pelleted by centrifugation at $3000 \times g$ and the supernatant was filtered through a $0.2 \mu m$ membrane. Protein A resin (CaptivA,
- 15 Repligen CA-PRI-0005) was added to the filtered supernatant and incubated for at least 2 hours at $4^\circ C$ with shaking. The resin was packed into a column, washed with 15 column volumes of 20 mM citrate, pH 6.5, and then washed with 15 column volumes of 20 mM citrate, 500 mM sodium chloride, pH 6.5. The bound protein was eluted from the column with 20 mM citrate, 100 mM NaCl, pH 2.9.

- The titer (mg/L) of exemplary constructs produced, including parental (e.g., non-masked) and masked
- 20 constructs, is provided in Table 6, below.

Table 6

Construct ID	Titer (mg/L)	Construct ID	Titer (mg/L)
AK032	5.8	AK312	154
AK035	16.7	AK313	81.2
AK081	23.5		
AK111	12.7		
AK165	13.5		
AK166	17.1		
AK167	56.4		
AK168	36.1		
AK203	83.2		
AK209	27.3		
AK211	43.8		
AK235	35.9		

Construct ID	Titer (mg/L)	Construct ID	Titer (mg/L)
AK253	41.4		
AK304	19.9		
AK305	53.2		
AK306	29.3		
AK307	62.9		
AK314	60		
AK315	59.8		
AK316	69.2		
AK308	74.5		
AK309	90.8		
AK310	44		
AK311	64.9		

SDS-PAGE Analysis

For SDS-PAGE analysis, protein samples were made with 4x Laemmli sample buffer (BioRad Catalog Number 1610747). For the reduced samples, 0.1 M Bond Breaker TCEP Solution (Thermo Scientific 77720) was added and the samples were heated for 5 minutes at 65 °C. The proteins were loaded into a 12-well NuPage 4-12 % Bis-Tris Protein Gel (Invitrogen NP0322BOX), with 4 µg of protein loaded per well. The gel was stained using SimplyBlue SafeStain (Invitrogen LC6065).

As depicted in FIG. 4, SDS-PAGE analysis was performed on the flow-through (FT) samples (i.e., proteins that did not bind to the Protein A column) and the eluted (E) samples (i.e., proteins that bound to the Protein A column and were eluted from it) following production and purification of exemplary constructs (AK304, AK305, AK307, AK308, AK309, AK310, AK311, AK312, AK313, AK314, and AK315). This exemplary data demonstrates that constructs as described herein can be successfully produced and purified.

Reporter Bioassays

Reporter bioassays are performed on masked IL-2 polypeptide constructs, along with non-masked parental constructs or other controls, to monitor activation of a downstream pathway, such as the JAK-STAT pathway.

In some studies, HEK-Blue IL-2 reporter cells (Invivogen) were used to test activation of the JAK-STAT pathway in accordance with the following method. HEK-Blue IL-2 cells passage 6 (p6) (97% live) were washed 2x with assay medium (DMEM + 10% heat-inactivated FBS), plated in 3 plates at 5e4 cells/well in 150 µL of assay medium, and rested in assay medium for about 2 hours to allow adherence to plate. Each construct tested was diluted to 300 pM in assay medium, then diluted 1:2 down the plate. 50 µL of

each dilution was added, for a final starting concentration of 75 pM. HEK-Blue IL-2 cell supernatant was harvested after 24 hours, an incubated with Quantiblow (180 uL + 20 uL supernatant), plus 3 wells/plate of assay medium, at 37 deg C for 1 hour. The absorbance was read using a Biotek Neo2 at 625 nm.

In some studies, CTLL2 cells were used to test activation of the JAK-STAT pathway in accordance with the following method. CTLL2 cells were plated at 40,000 cell per well in RPMI with 10% FBS. Dilutions of the constructs of interest were added and incubated at 37 degrees. After 6 hours, the Bio-Glo reagent was added and luminescence measured with a BioTek Synergy Neo2 plate reader.

Receptor Binding

The binding of the masked IL-2 polypeptide constructs generated in Example 1 is assessed. For the masked IL-2 polypeptide constructs, in some experiments, ELISA plates are coated with a receptor subunit, such as IL-2R α (also referred to as CD25), IL-2R β (also referred to as CD122), or IL-2R γ (also referred to as CD132), or combinations thereof. Dilutions of masked IL-2 polypeptide constructs are allowed to bind to the receptor subunit(s) and are detected using an anti-huFc-HRP detection antibody. The binding of the masked IL-2 polypeptide constructs is determined in conditions with and without protease cleavage.

On-Cell Receptor Binding

The on-cell receptor binding of the masked IL-2 polypeptide constructs generated in Example 1 is assessed. Dilutions of masked IL-2 polypeptide constructs are allowed to bind to peripheral blood lymphocytes or tissue culture cells, such as CTLL2 cells and are detected by fluorescence activated cell sorting (FACS) using an anti-huFc-FITC or anti-albumin-FITC detection antibody. The binding of the masked IL-2 polypeptide constructs is determined in conditions with and without protease cleavage.

Receptor Binding Affinity

For SPR studies testing binding of masked and non-masked IL-2 polypeptide constructs, Reichert Carboxymethyl Dextran Hydrogel Surface Sensor Chips were coated and immobilized with the construct of interest (e.g., a masked IL-2 polypeptide construct or non-masked IL-2 polypeptide construct) at 30ug/ml in 10mM Sodium Acetate, pH 5.0 via amine coupling with EDC and NHS. Dilutions of CD25-Fc or Fc-CD122 in PBST (CD25: 16 nM, 8 nM, 4 nM, 2 nM, 1 nM and CD122: 500 nM, 250 nM, 125 nM, 62.5 nM, 31.25 nM) were prepared. Using a Reichert 4Channel SPR, dilutions of CD25 or CD122 were flowed over the clips with the immobilized construct to determine the on rate at 25 degrees C. At equilibrium (approximately 3 minutes), the flow buffer was changed to PBST, to determine the off rates over 6 minutes. Between each run the chip was regenerated with 10 mM glycine, pH 2.0.

FIGs. 5A-5D depicts the efficacy of mutations on IL-2 which prevent binding to its alpha-receptor, using SPR analysis that tested the binding of an exemplary masked IL-2 polypeptide construct (AK168) to

CD25-Fc. FIG. 5A depicts the interaction between AK168 and CD25-Fc, FIG. 5B depicts the interaction between AK168 activated with MMP and CD25-Fc, and FIG. 5C depicts the interaction between a recombinant human IL-2 (rhIL-2) control and CD25-Fc. FIG. 5D provides a table summarizing the data obtained for the association constant (k_a), dissociation constant (k_d), equilibrium dissociation constant (KD), as well as the Chi2 value and U-value for each interaction. These results demonstrate that this exemplary masked IL-2 polypeptide construct (AK168) did not demonstrate detectable binding to CD25-Fc, while the wild-type rhIL-2 control did demonstrate detectable binding.

FIGs. 6A-6D depicts the masking of IL-2 towards its beta-receptor as well as restoration of binding post activation with protease, using SPR analysis that tested the binding of an exemplary masked IL-2 polypeptide construct (AK111) to CD122-Fc. FIG. 6A depicts the interaction between AK111 and CD122-Fc, FIG. 6B depicts the interaction between AK111 activated with MMP and CD122-Fc, and FIG. 6C depicts the interaction between a recombinant human IL-2 (rhIL-2) control and CD122-Fc. FIG. 6D provides a table summarizing the data obtained for the association constant (k_a), dissociation constant (k_d), equilibrium dissociation constant (KD), as well as the Chi2 value and U-value for each interaction. These results demonstrate that this exemplary masked IL-2 polypeptide construct (AK111) did not demonstrate detectable binding to CD122-Fc unless it has been activated with protease, while the rhIL-2 control did demonstrate detectable binding. Additional exemplary SPR data is provided below in Table 7 for various constructs tested, including masked and non-masked constructs. For some structures, when applicable, the KD was determined for the construct with or without having been previously cleaved by a protease.

Table 7

Construct	KD for CD25 (without protease cleavage)	KD for CD122 (without protease cleavage)	KD for CD122 (after protease cleavage)
rhIL2	0.878 nM	124 nM	N/A
AK032	1.76 nM	260 nM	N/A
AK035	No binding detected	110 nM	N/A
AK081	0.875 nM	347 nM	N/A
AK109	1.67 nM	No binding detected	118 nM
AK110	0.911 nM	No binding detected	195 nM
AK111	0.4 nM	No binding detected	235 nM
AK168	No binding detected	Not determined	175 nM
AK215	No binding detected		
AK216	No binding detected		
AK218	Weak binding		
rhIL2	0.878 nM	124 nM	N/A
AK032	1.76 nM	260 nM	N/A

AK035	No binding detected	110 nM	N/A
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Cleavage

The cleavage rate of the masked IL-2 polypeptide constructs is assessed by conducting receptor-binding assays, as described above, after incubation of the masked IL-2 peptide constructs in the presence or absence of a protease, and with the protease, if any, inactivated at various time points, such as by the addition of EDTA. The cleavage rate is also assessed using reducing and non-reducing polyacrylamide gel electrophoresis (PAGE) and by mass spectrometry whole mass and peptide map analyses. The cleavage rate is also assessed using an ex vivo assay in which the masked IL-2 polypeptide constructs are exposed to human, mouse, or cynomolgus monkey peripheral blood lymphocytes, or normal human tissue or human tumor tissue.

For some protease activation studies, MMP10 was diluted to 50 ng/ μ L in MMP cleavage buffer and activated with 1mM APMA for 2 h at 37 °C. 5 μ L of protease (250 ng total) of the activated protease was incubated with 1 μ M of masked cytokine constructs (e.g., masked IL-2 polypeptide constructs) and incubated at 37 degrees for 2 hours. Cleavage was assessed by SDS-PAGE using AnykD™ Criterion™ TGX Stain-Free™ Protein Gels. A similar approach is taken to test cleavage by other proteases.

FIG. 7A depicts an exemplary structure of a masked IL-2 polypeptide prior to (left) and after (right) cleavage by a protease, such as a protease associated with the tumor environment. FIG. 7B depicts SDS-PAGE analysis of an exemplary masked IL-2 polypeptide construct that was incubated in the absence (left lane) or presence (right lane) of the MMP10 protease.

Proliferation

Proliferation of IL-2 responsive tissue culture cell lines, such as CTLL2, YT, TF1B, LGL, HH, and CT6, following treatment with the masked IL-2 polypeptide constructs generated in Example 1 is assessed. For experiments involving the masked IL-2 polypeptide constructs, cells are plated in 96 well tissue culture plates in media lacking IL-2 for 2-4 hours and then treated with the masked IL-2 polypeptide constructs at various concentrations. After incubation at 37 degrees for 24-48 hours, the cell number is determined by the addition of MTS, alamar blue, luciferase, or a similar metabolic detection reagent, and the colorimetric, fluorescent or luciferase readout detected by a plate spectrophotometer reader.

The proliferation of immune cells following treatment with the masked IL-2 polypeptide constructs generated in Example 1 is also assessed. Human, mouse, or cynomolgus peripheral blood mononuclear cells (PBMCs) are treated with the constructs at various concentrations, and the proliferation of cell types, such as Natural Killer (NK) cells, CD8+ T cells, CD4+ T cells, and/or Treg cells, is determined by staining for the particular cell type and analysis via fluorescence activated cell sorting (FACS). In some experiments, some PBMCs are treated with controls for comparison. In some experiments, some PBMCs

are treated with aldesleukin as a control for the masked IL-2 polypeptide treatment. In some experiments, the masked IL-2 polypeptide constructs are tested in conditions with and without protease cleavage (e.g., activation). In some experiments, the NK cells are stained as CD45+ CD3- CD56+, the CD8+ T cells are stained as CD45+ CD3+ CD8+, the CD4+ T cells are stained as CD45+ CD3+ CD4+ CD25-, and the Treg cells are stained as CD45+ CD3+ CD4+ CD25+ FOXP3+. In some experiments, the PBMCs are treated for a period of five days. In some experiments, the PBMCs are also stained with Ki67, a marker of cell proliferation. In some experiments, the PBMCs are labeled with CFSE (Sigma-Aldrich) prior to treatment and proliferation is measured by determining the extent of CFSE dilution. In some experiments, each construct, as well as aldesleukin and/or other controls, is administered at one or more concentrations, such as one or more concentrations ranging from 0.0001 nM to 500 nM.

STAT5 Activation

The activation of Signal Transducer and Activator of Transcription 5 (STAT5) following treatment with the masked IL-2 polypeptide constructs generated in Example 1 is also assessed. PBMCs are treated with the constructs for a specified period of time and are then immediately fixed to preserve the phosphorylation status of proteins, such as STAT5. In some experiments, some PBMCs are treated with controls for comparison. In some experiments, some PBMCs are treated with aldesleukin as a control for the masked IL-2 polypeptide treatment. In some experiments, the masked IL-2 polypeptide constructs are tested in conditions with and without protease cleavage (e.g., activation). In some experiments, the PBMCs are treated for 10 minutes, 15 minutes, 20 minutes, or 25 minutes. In some experiments, each construct, as well as aldesleukin and/or other controls, is administered at one or more concentrations, such as one or more concentrations ranging from 0.0001 nM to 500 nM. In some experiments, the fixed and permeabilized PBMCs are then stained with an antibody specific for phosphorylated STAT5 (phospho-STAT5) and are analyzed by flow cytometry. In some experiments, total and phosphorylated levels of STAT5 are measured. The phospho-STAT5 status of certain cell types, such as NK cells, CD8+ T cells, CD4+ T cells, and/or Treg cells, is determined by staining for the particular cell type. In some experiments, the NK cells are stained as CD45+ CD3- CD56+, the CD8+ T cells are stained as CD45+ CD3+ CD8+, the CD4+ T cells are stained as CD45+ CD3+ CD4+ CD25-, and the Treg cells are stained as CD45+ CD3+ CD4+ CD25+ FOXP3+.

The activation of STAT5 in the mouse cell lines, such as CTLL-2 cells, following treatment with the masked IL-2 polypeptide constructs generated in Example 1 is also assessed. In some experiments, some CTLL-2 cells are treated with controls for comparison. In some experiments, some CTLL-2 cells are treated with aldesleukin as a control for the masked IL-2 polypeptide treatment. In some experiments, the masked IL-2 polypeptide constructs are tested in conditions with and without protease cleavage (e.g., activation). In some experiments, the CTLL-2 cells are treated for 10 minutes, 15 minutes, 20 minutes, or 25 minutes, and are then fixed to preserve the phosphorylation status of proteins, such as STAT5. In some experiments, each construct, as well as aldesleukin and/or other controls, is administered at one or

more concentrations. In some experiments, total and phosphorylated levels of STAT5 are measured. In some studies, the levels of intracellular STAT5 activation (pSTAT5 signal) induced by IL-2 was determined by the following method. Frozen human PBMCs were thawed in water bath and added to 39 mL pre-warmed media (RPMI1640 medium plus 10% FBS, 1%P/S, 1% NEA), spun and reconstitute in media at 10E6 cells/mL. Cells were plated at 5E5 per well cells in a 96 well plate. IL-2 (e.g., rhIL-2 or an exemplary IL-2-containing polypeptide construct) diluted in medium was added to each well, and incubated at 37 °C for 20 min. Cells were then fix with 200ul/well Fixation buffer (eBiosciences) at 4 °C, overnight. After centrifugation, the fixed cells were resuspended in 200ul cold BD Phosflow buffer and incubated at 4°C for 30 min. After washing the cells twice, they were treated with Biolegend Human TruStain FcX (2.5 uL in 50 uL total per sample in Staining buffer) for 5 min on ice. Staining antibodies were added; 5ul pSTAT5- APC (pY694, BD), 10ul CD56-BV421 (5.1H11, Biolegend), 10ul CD4-PerCP/Cy5.5 (A161A1, Biolegend), and 10ul CD3-FITC (UCHT1, Biolegend) and incubated for 30 min, on ice, protected from light. Cells were washed 2 times and resuspended, and analyzed by flow cytometry.

FIGs. 8A-8D depict the results from STAT5 activation studies, as described above, using the exemplary constructs AK032, AK035, AK041, or rhIL-2 as a control. The levels of STAT5 activation (%) are shown for NK cells, CD8+ T cells, effector T cells (Teff), and regulatory T cells (Treg). The AK032 and AK035 constructs include an IL-2 polypeptide linked to an Fc domain, and the AK041 construct includes an IL-2 polypeptide linked to a CD25 domain and a CD122 domain. As shown, engineered IL-2 polypeptide constructs can, in some embodiments, reduce activation of Treg cells while retaining or enhancing activation of CD8+ T cells and NK cells.

FIGs. 9A-9C depict the results from STAT5 activation studies, as described above, using the exemplary constructs AK081 and AK032. The AK081 construct with and without prior exposure to MMP10 was tested. An isotype control as well as a no IL-2 negative control was also tested. The levels of STAT5 activation (%) are shown for NK cells, CD8+ T cells, and CD4+ T cells. The AK032 and AK081 constructs include an IL-2 polypeptide linked to an Fc domain, and the AK081 construct includes a cleavable peptide in the linker connecting the IL-2 polypeptide to the Fc domain. As shown, the non-masked monomeric AK081 IL-2 polypeptide construct stimulates STAT5 activation of PBMCs with or without protease activation similarly to the non-masked dimeric AK032 IL-2 polypeptide construct.

FIGs. 10A-10D depict the results from STAT5 activation studies, as described above, using the exemplary constructs AK081 and AK111, as well as controls that included an rhIL-2 and anti-RSV antibody. A no-treatment control was also tested. The AK111 construct is an exemplary masked IL-2 polypeptide construct that includes a wildtype form of an IL-2 polypeptide (except for a C125A mutation). As shown in FIGs. 10A-10D, the masked IL-2 polypeptide construct AK111 demonstrated reduced STAT5 activation as compared to the non-masked IL-2 polypeptide construct AK081. FIG. 10D provides EC50 (pM) and fold- change data for the AK081, AK111 constructs, as well as the rhIL-2

control.

FIGs. 11A-11D depict the results from STAT5 activation studies, as described above, using the exemplary constructs AK167 and AK168, as well as controls that included an rhIL-2 and anti-RSV antibody. A no-treatment control was also tested. The AK168 construct is an exemplary masked IL-2 polypeptide construct that includes a mutant form of an IL-2 polypeptide that eliminates or reduces CD25 binding. The AK167 construct is a parental, non-masked form of the AK168 construct that includes the same mutant IL-2 polypeptide. As shown in FIGs. 11A-11C, the non-masked AK167 construct demonstrated reduced STAT5 activation as compared to the rhIL-2 control, and the masked IL-2 polypeptide construct AK168 did not induce detectable STAT5 activation. FIG. 11D provides EC₅₀ (pM) and fold-change data for the AK167, AK168 constructs, as well as the rhIL-2 control. The EC₅₀ of the AK168 construct was non-detectable (n.d.).

FIGs. 12A-12D depict the results from STAT5 activation studies, as described above, using the exemplary constructs AK165 and AK166, as well as an isotype control and an IL-2-Fc control, that were (+ MMP10) or were not previously exposed to the MMP10 protease. The AK166 construct is an exemplary masked IL-2 polypeptide construct that includes a wildtype form of an IL-2 polypeptide (except for a C125A mutation). The AK165 construct is a parental, non-masked form of the AK166 construct that includes the same IL-2 polypeptide. The key as shown in FIG. 12A also applies to FIG. 12B, and the key as shown in FIG. 12C also applies to FIG. 12D. As shown in FIGs. 12A-12D STAT5 activation was greatly diminished for the masked AK166 construct (without protease cleavage), but was restored to levels resembling the IL2- Fc control following exposure to the activating protease MMP10. FIGs. 13A-13C depict the results from STAT5 activation studies, as described above, using the exemplary constructs AK109 and AK110, as well as an isotype control and an IL-2-Fc control, that were (+ MMP10) or were not previously exposed to the MMP10 protease. The AK109 and AK110 construct are exemplary masked IL-2 polypeptide constructs that include half-life extension domains having different heterodimerization mutations. The key as shown in FIG. 13B also applies to FIG. 13A. As shown in FIGs. 13A-13C, STAT5 activation was greatly diminished for the masked AK109 and AK110 construct (without protease cleavage), but was greatly increased to levels approaching the IL2-Fc control following exposure to the activating protease MMP10.

FIGs. 14A-14D depict the results from STAT5 activation studies, as described above, using the constructs AK211, AK235, AK253, AK306, AK310, AK314, and AK316, as well as an an rhIL-2 control. This includes constructs that are parental, non-masked constructs (AK235, AK253, AK306, AK310, AK314) that include various mutations that modulate CD25 binding. FIG. 14D provides EC₅₀ data for each of the tested constructs as well as the rhIL-2 control.

FIGs. 15A-15D depict the results from STAT5 activation studies, as described above, using the constructs

AK081, AK167, AK216, AK218, AK219, AK220, and AK223 that have been activated by protease, as well as an an rhIL-2 control. A no-treatment control was also tested. This includes masked IL-2 polypeptide constructs (AK216, AK218, AK219, AK220, and AK223) that include various mutations that modulate CD25 binding. The constructs were previously exposed to an activating protease prior to testing their ability to activate STAT5. FIG. 15D provides EC50 data for each of the tested constructs as well as the rhIL-2 control.

FIGs. 16A-16C depict the results from STAT5 activation studies, as described above, using the constructs AK081, AK189, AK190, and AK210, as well as an an anti-RSV control. This includes masked IL-2 polypeptide constructs (AK189, AK190, AK210) that include an IL-2 polypeptide having a C125A mutation and include the same cleavable peptide sequence (RAAAVKSP) but having different linker sequences due to differences in the amino acid residues on the N-terminus of the protease cleavage sequence. The key as shown in FIG. 16A also applies to FIGs. 16B and 16C.

FIGs. 17A-17C depict the results from STAT5 activation studies, as described above, using the constructs AK167, AK191, AK192, and AK193, as well as an an anti-RSV control. This includes masked IL-2 polypeptide constructs (AK189, AK190, AK210) that include an IL-2 polypeptide having R38A, F42A, Y45A, E62A, and C125A mutations and include the same cleavable peptide sequence (RAAAVKSP; SEQ ID NO: 27) but having different linker sequences due to differences in the amino acid residues on the N-terminus of the protease cleavage sequence. The key as shown in FIG. 17A also applies to FIGs. 17B and 17C.

Example 3: In vivo characterization of masked IL-2

Pharmacokinetics

The pharmacokinetics of the masked IL-2 polypeptide constructs and generated in Example 1 is assessed in vivo using mouse models.

Mice are treated intravenously or subcutaneously with the constructs and the concentration of the construct in the plasma is measured over time. In some experiments, some mice are treated with controls for comparison. In some experiments, some mice are treated with aldesleukin as a control for masked IL-2 polypeptide treatment. In some experiments, the mice that are treated have tumors. In some experiments, the mice that are treated are tumor-free. In some experiments, mice are treated with the constructs and blood is drawn at various times over the course of treatment, which may include drawing blood prior to the initiation of treatment and processing it to obtain plasma. In some experiments, blood is drawn at various time points over the course of two weeks, three weeks, or four weeks or more of treatment. In some experiments, the mean plasma concentration of the administered constructs, as well as aldesleukin and/or other controls, is measured. Masked IL-2 polypeptide constructs are detected in the

plasma samples after dilution into PBS Tween with IL-2- and human Fc-specific ELISAs and are quantified using a standard curve generated for each construct. The percentage of full length and cleaved constructs is determined by western blot with anti-huFc-HRP and anti-huIL-2-HRP and by whole mass and peptide mass spectrometry.

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The pharmacokinetics of the masked IL-2 polypeptide constructs in tumors is also assessed in vivo using mouse models. Mice having tumors are treated intravenously or subcutaneously with the constructs and the concentration of the construct in tumors of the mice is assessed. In some experiments, some mice are treated with controls for comparison. In some experiments, some mice are treated with aldesleukin as a control for masked IL-2 polypeptide treatment. Tumors are analyzed for the presence of the constructs as well as the presence of particular proteases. In some experiments, the tumors are analyzed for the presence and percentage of full length and cleaved constructs.

Some pharmacokinetic studies were carried out according to the following method. C57BL/6 female mice were purchased from Charles River Laboratories and were 8-10 weeks old at the start of study. MC38 tumor cells (5×10^5 cells per mouse) were injected subcutaneously into the right flank of each mouse. Upon reaching $\sim 100 \text{ mm}^3$ sized tumors (day 0), the mice received a single 2 mg/kg intravenous dose of the construct of interest (e.g., a non-masked parental IL-2 polypeptide construct, a masked IL-2 polypeptide construct, or a non-cleavable masked IL-2 polypeptide construct) in PBS. Constructs tested include, for instance, AK032, AK081, AK111, AK167, AK168, AK191, AK197, AK203, AK209, and AK211. Plasma were collected at 5 min, days 1, 2 and 5 after dosing. Drug levels were determined using ELISAs utilizing anti-human IgG (clone M1310G05, Biolegend) as the capture antibody and various detection antibodies. HRP or biotin conjugated detection antibodies against human IgG (ab97225, Abcam) or CD122 (clone 9A2, Ancell) and IL-2 (Poly5176, Biolegend) were utilized to detect total and non-cleaved drug levels, respectively.

FIGs. 18A-18D describe results from pharmacokinetic studies carried out, as described above, in tumor-bearing mice using the constructs AK032, AK081, AK111, AK167, and AK168, as well as an anti-RSV control. FIG. 18A provides a simplistic depiction of the structure of each of the constructs tested. As indicated, AK111 and AK168 are exemplary masked IL-2 polypeptide constructs. The AK167 and AK168 constructs include mutations (R38A, F42A, Y45A, and E62A) that eliminate or reduce binding to CD25. FIG. 18A shows Fc levels in plasma ($\mu\text{g/mL}$) by detecting human IgG, FIG. 18C shows Fc-CD122 levels in plasma ($\mu\text{g/mL}$) by detecting human CD122, and FIG. 18D shows Fc-IL2 levels in plasma ($\mu\text{g/mL}$) by detecting human IL-2.

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FIGs. 19A-19D describe results from pharmacokinetic studies carried out, as described above, in tumor-bearing mice using the constructs AK167, AK191, AK197, AK203, AK209, and AK211, as well as an anti-RSV control. FIG. 19A provides a simplistic depiction of the structure of each of the constructs

tested. As indicated, AK168, AK191, AK197, AK203, and AK209 are exemplary masked IL-2 polypeptide constructs that each include a different cleavable peptide sequence in the linker connecting the IL-2 polypeptide to the half-life extension domain. FIG. 19B shows Fc levels in plasma ($\mu\text{g/mL}$) by detecting human IgG, FIG. 19C shows Fc-IL2 levels in plasma ($\mu\text{g/mL}$) by detecting human IL-2, and
5 FIG. 19D shows Fc-CD122 levels in plasma ($\mu\text{g/mL}$) by detecting human CD122. As shown in FIGs. 19B, 19C and 19D, the Fc levels, Fc-IL2 levels, and Fc-CD122 levels in the plasma are similar among the masked IL-2 polypeptide constructs tested.

Bioactivity in mice

10 The in vivo bioactivity of the masked IL-2 polypeptide constructs generated in Example 1 is assessed in vivo using mouse models, such as C57BL/6 mice. Mice are treated with the constructs and in vivo bioactivity is assessed. In some experiments, some mice are treated with controls for comparison. In some experiments, some mice are treated with aldesleukin as a control for masked IL-2 polypeptide treatment. In some experiments, the mice that are treated have tumors. In some experiments, the mice that
15 are treated are tumor-free. In some experiments, the dose- dependent expansion of immune cells is assessed in the mice. In some experiments, the mice are treated with various doses of a construct, aldesleukin, or other control. In some experiments, the mice are treated over the course of two weeks. Blood is collected from the mice at various time points and is then stained using antibodies to immune cell markers of interest. In some experiments, the longitudinal kinetics of the proliferation and expansion
20 of certain circulating cell types, such as CD8+ T cells, NK cells, and Treg cells, is also determined, as well as the ratio of CD8+ T cells and NK cells to CD4+ CD25+ FoxP3+ Treg cells. In some experiments, the mice are assessed for vascular leakage, such as by assessing for edema and lymphocyte infiltration in certain organs like the lung and liver as determined by organ wet weight and histology.

25 In some studies, vascular leakage was assessed in order to assess potential toxicity-related effects mediated by IL-2 based therapies by performing the following method. Repeated dose toxicity studies were conducted using C57BL/6 female mice that were purchased from Charles River Laboratories and were 8-10 weeks old weighing 18-22 grams at the start of study. Groups of 5 mice received daily intraperitoneal injections of masked and non-masked IL-2 constructs in PBS daily for 4 or 5 days. The
30 constructs tested included AK081, AK111, AK167, and AK168. A control antibody was also administered as a control. Two hours after the last dose, all mice received an intravenous injection of 0.1 ml of 1% Evans blue (Sigma, cat# E2129) in PBS. Two hours after Evans blue administration, mice were anesthetized and perfused with 10 U/ml heparin in PBS. Spleen, lung and liver were harvested and fixed in 3 ml of 4% PFA 2 days at 4°C prior to measuring the absorbance of the supernatant at 650 nm with
35 NanoDrop OneC (Thermo Fisher Scientific, Waltham, MA) as an indicator of vascular leak of Evans blue. Fixed organs were embedded in paraffin, sectioned, and stained with hematoxylin and eosin. Histopathological studies and quantification were carried out by NovoVita Histopath Laboratory, LLC. (Allston, MA) according to standard procedures. FIGs. 25A-50D depict results from an in vivo study as

described above for assessing vascular leakage using the exemplary masked IL-2 polypeptide constructs AK111 and AK168, as well as the non-masked IL-2 polypeptide constructs AK081 and AK167, and an anti-RSV control. FIG. 25A shows the percentage (%) of body weight loss, and FIGs. 25B, 25C and 25D shows the weight in grams of the liver, lung, and spleen, respectively, for each.

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Vascular leakage as indicated by measuring the extent of dye leakage into tissues was also assessed for the AK081, AK111, AK167, and AK168 constructs, along with an anti-RSV control, with results shown in FIGs. 26A and 26B for the liver and lung, respectively. The extent of dye leakage was measured based on absorbance at 650nm.

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Vascular leakage as indicated by measuring the extent of mononuclear cell perivascular invasion into the liver and lung was also assessed for the AK081, AK111, AK167, and AK168 constructs, along with an anti-RSV control, with results shown in FIGs. 27A and 27B for the liver and lung, respectively. The average number of mononuclear cells in the liver (FIG. 27A) and the average number of mononuclear cells in the lung (FIG. 27B) depicted for each. As shown in FIG. 27B, for instance, the masked IL-2 polypeptide constructs AK111 and AK168 did not result in a detectable number of mononuclear cells in the lung, unlike the non-masked constructs AK081 and AK167.

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Infiltrating Immune Cell Phenotype

The phenotype of immune cells infiltrating tumors in vivo in mouse models treated with the masked IL-2 polypeptide constructs generated in Example 1 is assessed. Mice are treated with the constructs and the phenotype of tumor-infiltrating immune cells is assessed. In some experiments, some mice are treated with controls for comparison. In some experiments, some mice are treated with aldesleukin as a control for masked IL-2 polypeptide treatment. Mice bearing tumors are treated with a construct, aldesleukin, or another control, and tumors, tissues such as liver, lung, and spleen, and blood, are collected at various time points following the initial dose, such as five days, seven days, or ten days after the initial dose. In some experiments, immune cells are isolated from the tumors, tissues, and blood, and are subject to phenotypic assessment using flow cytometry. In some experiments, the isolated immune cells are assessed using markers of interest, such as those for CD8⁺ T cells, Memory CD8⁺ T cells, activated NK cells, CD4⁺ T cells, and CD4⁺ Treg cells.

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In some studies, the phenotype of immune cells infiltrating tumors in vivo was assessed using the following method. C57BL/6 female mice were purchased from Charles River Laboratories and were 8-10 weeks old at the start of study. MC38 tumor cells (5×10^5 cells per mouse) were injected subcutaneously into the right flank of each mouse. Upon reaching $\sim 100 \text{ mm}^3$ sized tumors (day 0), the mice received a single 2 mg/kg intravenous dose of the construct of interest (e.g., a non-masked parental IL-2 polypeptide construct, a masked IL-2 polypeptide construct, or a non-cleavable masked IL-2 polypeptide construct) in PBS. On day 5, mice were euthanized by CO₂ asphyxiation and tumors, livers, spleens and blood were

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harvested. Cell suspensions were prepared from spleens by mechanical disruption and passing through a 40 µm cell strainer. The tumor tissues were enzymatically digested using Miltenyi Tumor Dissociation Kit reagents (Miltenyi cat# 130-096-730) and the gentleMACS Dissociator (Miltenyi) was used for the mechanical dissociation steps. Red blood cells in the spleen and tumor cell suspensions and blood were lysed using ACK buffer (Gibco cat# A10492). The cell suspensions were stained with the following antibodies: CD45 (clone 30-F11, eBioscience), CD3 (clone 2C11, Biolegend), CD8 (clone 53-6.7, BD Biosciences), CD4 (clone RM-45, BD Biosciences), FOXP3 (MF-14, Biolegend), CD25 (3C7, Biolegend), CD44 (clone IM7, eBioscience), and Nkp46 (29A1.4, eBioscience). Data acquisition was carried out on the MACSQuant Analyzer flow cytometer (Miltenyi) and data were analyzed using the FlowJo.

Results from studies testing the in vivo responses of CD4, CD8, NK, and Treg percentages in spleen, blood, and tumor, as carried out as described above, using the AK032, AK081, AK111, AK167, and AK168 constructs, as well as an anti-RSV IgG control, are shown in FIGs. 20A-20L. AK111 and AK168 are exemplary masked IL-2 polypeptide constructs.

Results from studies testing the in vivo responses of CD4, CD8, NK, and Treg percentages in spleen, blood, and tumor, as carried out as described above, using the AK167, AK168, AK191, AK197, AK203, AK209, and AK211 constructs, as well as an anti-RSV IgG control, are shown in FIGs. 21A-21L. AK168, AK191, AK197, AK203, and AK209 are exemplary masked IL-2 polypeptide constructs that each include a different cleavable peptide sequence in the linker connecting the IL-2 polypeptide to the half-life extension domain. Statistical analysis was performed using One-way ANOVA as compared to the non-cleavable AK211 construct.

Results from studies testing the in vivo responses of CD4, CD8, NK, and Treg percentages in spleen, blood, and tumor, as carried out as described above, using the AK235, AK191, AK192, AK193, AK210, AK189, AK190, and AK211 constructs are shown in FIGs. 22A-22L. AK191, AK192, AK193, AK210, AK189, and AK190 are exemplary masked IL-2 polypeptide constructs that each include a cleavable peptide sequence in the linker connecting the IL-2 polypeptide to the half-life extension domain. The linker sequence also differs among these constructs, depending on the linker sequence utilized. AK189, AK190, and AK210 include an IL-2 polypeptide having a C125A mutation, and AK191, AK192, and AK193 include an IL-2 polypeptide having C125A, R38A, F42A, Y45A, and E62A mutations. The AK235 construct is a non-masked construct and the AK211 construct includes a non-cleavable linker sequence. Statistical analysis was performed using One-way ANOVA as compared to the non-cleavable AK211 construct.

Results from studies testing the in vivo T cell activation in spleen, blood, and tumor, as carried out as described above, using the AK235, AK191, AK192, AK193, AK210, AK189, AK190, and AK211

constructs, as described above, are shown in FIGs. 23A-23I. T cell activation was measured as the mean fluorescence intensity (MFI) of CD25 in CD8+ T cells, CD4+ T cells, or Foxp3+ cells in the spleen, blood, and tumor. Statistical analysis was performed using One-way ANOVA as compared to the non-cleavable AK211 construct.

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In Vivo Cleavage

The in vivo cleavage of masked IL-2 cytokine constructs (e.g., masked IL-2 polypeptide constructs) is assessed. In some studies, a control antibody is administered for comparison. In some studies, in vivo cleavage is assessed by administering the construct of interest in a mouse and, after a certain period of time, capturing human IgG and then measuring the levels of, e.g., human IgG, CD122, and IL-2.

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In some studies testing the in vivo cleavage of masked IL-2 polypeptide constructs, drug levels (i.e., levels of the administered construct, including cleavage byproducts) were determined using ELISAs utilizing anti-human IgG (clone M1310G05, Biolegend) as the capture antibody and various detection antibodies. HRP or biotin conjugated detection antibodies against human IgG (ab97225, Abcam) or CD122 (clone 9A2, Ancell) and IL-2 (Poly 5176, Biolegend) were utilized to detect total and non-cleaved drug levels, respectively. The concentrations of cleaved and released IL-2 is calculated by subtracting non-cleaved (i.e., intact) from total drug concentrations. FIGs. 24A-24D depict the results from studies testing the in vivo cleavage of the exemplary masked IL-2 polypeptide constructs AK168 (cleavable peptide sequence: MPYDLYHP) and AK209 (cleavable peptide sequence: VPLSLY; SEQ ID NO: 15). The AK167 construct is a cleavable non-masked IL-2 polypeptide construct that includes the same IL-2 polypeptide as the masked AK168 construct. As shown in FIGs. 24A-24D, both the masked (AK168 and AK209) and non-masked (AK167) constructs were effectively cleaved, and both cleavable peptide sequences were cleaved. FIG. 24E depicts results from a pharmacokinetic study of total plasma IgG concentration (µg/mL) for total levels of the AK167, AK168, and AK209 constructs, and for levels of non-cleaved forms of each construct.

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Tumor Eradication and Inhibition of Metastasis

The ability of the masked IL-2 polypeptide constructs generated in Example 1 to promote tumor eradication and to inhibit metastasis is assessed in vivo using mouse models, such as syngeneic MC38, CT26, and B16F10 tumor models.

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Mice are implanted with tumor cells subcutaneously, and tumors are allowed to grow to a palpable size. Tumor-bearing mice are treated with the masked IL-2 constructs and tumor volume is measured over the course of treatment. In some experiments, some mice are treated with controls for comparison. In some experiments, some mice are treated with aldesleukin as a control for masked IL-2 polypeptide treatment. Tumor volume is measured periodically over the course of treatment. In some experiments, body weight is also measured periodically over the course of treatment. In some experiments, plasma samples are

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produced over the course of the treatment and analyzed for pharmacokinetics, pharmacodynamics, cleavage, and blood markers, such as those for CD8+ T cells, Memory CD8+ T cells, activated NK cells, CD4+ T cells, and CD4+ Treg cells.

- 5 The capability of the masked IL-2 polypeptide constructs to inhibit metastasis is also assessed in vivo using mouse models suitable for metastasis studies, such as syngeneic CT26 tumor models for assessing lung metastasis. Mice are implanted with tumor cells subcutaneously. In some experiments, tumors are allowed to grow to a palpable size prior to treatment. In some experiments, treatment begins before tumors grow to palpable size. Tumor-bearing mice are treated with the masked IL-2 constructs are
10 assessed for tumor cell metastasis into tissues such as lungs, liver, and lymph nodes.

In some studies, a syngeneic tumor model was used to assess the ability of masked IL-2 polypeptide constructs to reduce tumor volume in accordance with the following method. C57BL/6 female mice were purchased from Charles River Laboratories and were 8-10 weeks old at the start of study. MC38 tumor
15 cells (5 x10⁵ cells per mouse) were injected subcutaneously into the right flank of each mouse. Upon reaching ~125 mm³ sized tumors (day 0), the mice were randomized to receive 2 mg/kg doses of AK081, AK111, AK167, or AK168, or an anti-RSV antibody as a control, in PBS. Mice were dosed intraperitoneally, three times a week for 6 doses. Tumor volume was calculated ($\text{Length} \times (\text{Width}^2)/2$) using dial calipers and body weights were recorded twice weekly. FIGs. 28A and 28B show results from a
20 syngeneic tumor model study that assessed tumor volume and body weight over the course of treatment. As shown in FIG.28A, treatment using exemplary IL-2 polypeptide constructs, including the masked constructs AK111 and AK168, resulted in tumor growth inhibition over time as compared to the anti-RSV control. As shown in FIG. 28B, there was a general lack of body weight reduction observed when the mice were treated with the masked constructs AK111 and AK168.

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Bioactivity in cynomolgus monkeys

The in vivo bioactivity of the masked IL-2 polypeptide constructs a generated in Example 1 is assessed in vivo in cynomolgus monkeys. Cynomolgus monkeys are treated with the constructs and in vivo bioactivity, pharmacokinetics, and cleavage is assessed. In some experiments, some monkeys are treated
30 with controls for comparison. In some experiments, some monkeys are treated with aldesleukin as a control for masked IL-2 polypeptide treatment. In some experiments, the monkeys are treated with various doses of the construct, aldesleukin, or other control. Blood is collected from the monkeys at various time points and is then evaluated for certain cell types, such as CD8+ T cells, Memory CD8+ T cells, activated NK cells, CD4+ T cells, and CD4+ Treg cells, and/or markers of interest, such as for the
35 dose-response of total lymphocytes, Ki67+, and of soluble CD25. In some experiments, the longitudinal kinetics of the proliferation and expansion of certain circulating T and NK cell types is assessed. In some experiments, pharmacokinetics and cleavage of the masked IL-2 polypeptide constructs are determined by ELISA, PAGE, and mass spectrometry.

To test the safety profile of exemplary masked IL-2 polypeptide constructs in non-human primates, a dose ranging study is performed in accordance with the following method. Groups of 3 healthy male cynomolgus monkeys (*Macaca fascicularis*) are randomly assigned to receive a single intravenous bolus dose of 2 mL/kg of activatable (i.e., cleavable) masked IL-2 polypeptide proteins or non-cleavable masked IL-2 polypeptide proteins at 10, 30 and 100 nmol/kg in 100 mM sodium citrate buffer (pH 5.5). A third group receives the parental non-masked, cleavable protein at 3, 10 and 30 nmol/kg as a positive control. This third group is dosed at a lower range to account for higher potency of the parental non-masked molecules. Doses are calculated in moles to account for differences in molecular weight. Blood samples are collected before dosing and 1, 24, 48, 72, 96, 168, 264 and 336 hours post-dosing. An automated hematology analyzer is used to monitor changes in lymphocyte subsets and serum chemistry. Total and intact (i.e., non-cleaved) drug levels are measured from plasma using custom ELISA as described above. Soluble CD25 levels are measured with an ELISA (R&D systems, cat# DR2A00) to monitor immune stimulation. Plasma levels of inflammatory cytokines are quantified using custom multiplexed electrochemiluminescence assay (Meso Scale Discovery). Blood pressure is monitored as an indicator of vascular leak syndrome. PK is analyzed using an ELISA that captures IL-2 and detects human Fc and by an ELISA that captures human Fc and detects human Fc.

Example 4:

C57BL/6 female mice were purchased from Charles River Laboratories and were 8-10 weeks old at the start of study. MC38 tumor cells (5×10^5 cells per mouse) were injected subcutaneously into the right flank of each mouse. Upon reaching $\sim 100 \text{ mm}^3$ sized tumors (day 0), the mice received a single high dose intraperitoneal dose of various Fc-IL-2 constructs in PBS. Plasma were collected at 5 min, days 3, 5 and 7 after dosing.

The constructs used are:

AK443 (VPLSLY) IL-2 dead	
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AK211	
AK235 (VPLSLY)	
AK209 (VPLSLY)	
AK471 (VPLSLY) FcRn mutant	

Immunophenotyping was performed using a FACS-based method. On day 5, mice were euthanized by CO₂ asphyxiation and tumors, livers, spleens and blood were harvested. Cell suspensions were prepared

from spleens by mechanical disruption and passing through a 40 μ m cell strainer. The tumor tissues were enzymatically digested using Miltenyi Tumor Dissociation Kit reagents (Miltenyi cat# 130-096-730) and the gentleMACS Dissociator (Miltenyi) was used for the mechanical dissociation steps. Red blood cells in the spleen and tumor cell suspensions and blood were lysed using ACK buffer (Gibco cat# A10492).

The cell suspensions were stained with the following antibodies: CD45 (clone 30-F11, eBioscience), CD3 (clone 2C11, Biolegend), CD8 (clone 53-6.7, BD Biosciences), CD4 (clone RM-45, BD Biosciences). Data acquisition was carried out on the MACSQuant Analyzer flow cytometer (Miltenyi) and data were analyzed using the FlowJo.

Drug levels were determined using ELISAs utilizing anti-human IgG (clone M1310G05, Biolegend) as the capture antibody and various detection antibodies. HRP or biotin conjugated detection antibodies against human IgG (ab97225, Abcam) or CD122 (clone 9A2, Ancell) and IL-2 (Poly5176, Biolegend) were utilized to detect total and non-cleaved drug levels, respectively.

AK471 with I253A FcRn mutation induced robust CD8 T cells expansion in the TME while remaining inactive in the periphery as shown in Figures 29A and 29B.

AK471 has slightly shorter half-life compared to aglyco-hIgG1 as shown in Figures 30 A, B and C.

There is no evidence of cleavage or decapitation with AK471 in the plasma (Figures 31 A, B and C).

Example 5: IL-12 Heteroalkusion

Cleavage and binding to recombinant IL-12Receptors, IL-12Rb1 and IL-12Rb2 by SPR:

Sensor Chips were coated and immobilized with IL-12 receptors. Dilutions of IL-12 constructs were flowed over the chips with the immobilized IL-12 receptors to determine the on rate at 25 degrees C. At equilibrium (approximately 3-4 minutes), the flow buffer was changed to PBST, to determine the off rates over 6 minutes. Between each run the chip was regenerated. The tables represent the SPR data (Figures 34 and 35). ND is for "not determined" as the masking of the IL-12 prevented binding to the receptors; therefore, binding numbers were not able to be determined.

Testing IL-12 molecules with HEK-Blue IL-12 cells:

HEK-Blue IL-12 reporter cells developed by Invivogen have been specifically designed to monitor the activation of the JAK-STAT pathway. These cells were generated by stable transfection of HEK293 cells with the human , IL-12Rb1, and IL-12Rb2 genes, along with the human JAK2 and STAT4 genes to obtain a fully functional IL-12 signaling pathway. In addition, a STAT4-inducible SEAP reporter gene

was also introduced. Upon stimulation, HEK-Blue™ IL-12 cells trigger the activation of STAT4 and the subsequent secretion of SEAP. The levels of STAT4-induced SEAP can be readily monitored using QUANTI-Blue™. HEK-Blue™ IL-12 cells can be used to validate the functionality, toxicity, and variable dosage effects of human or murine IL-12. HEK Blue IL-12 cells were grown in passage media until ~80% confluent. Washed single-cell suspension in assay media was plated and serial dilutions of IL-12 molecules in assay media were added to cells. Plate was incubated at 37 °C for 24 hours. After 24 h, Quanti-Blue solution (Invivogen) was prepared and cell supernatant was added to the Quanti-Blue solution and incubated for 1-2 h at 37 °C. Absorbance at 625 nm measured. Data analysis was performed in Graphpad Prism, version 8.3. Background was subtracted from raw data and the data were fit nonlinearly: [Agonist] vs. response – Variable slope (four parameters). EC50 value of each IL-12 construct was reported.

Example 6

This example investigates whether mutation of GAG-binding domain on IL-12 constructs alter PK, two GAG-binding mutant variants (AK600 and AK601) was compared to WT IL-12 construct (AK598) in C57BL/6 non-tumor bearing mice. AK600 has a KDNTERV and AK601 has a KDNTEGRV GAG-binding domain mutants, respectively.

AK598, AK600 and AK601 all have the following construct structure:



The animals were single dosed at either 1 or 10 mg/kg through i.v injection. Plasma drug levels were measured using human Fc capture (Southern Biotech IgG cat#2049-01 Goat Anti-Human IgG, Monkey ads-UNLB) human Fc detect(ab97225) and/or human Fc capture/ anti-human IL-12(ab83448) detect ELISA.

	Test Molecule	GAG mutant	M.W. (kDa)	Apprx. Dosing (mg/kg)	Actual Dosing (nMoles/kg)	Actual Dosing (mg/kg)	Batch information
1	AK600	KDNTERV	143.8	10	69.5	10	02A
2	AK600	KDNTERV	143.8	1	6.95	1	02A
3	AK601	KDNTGRV	143.9	10	69.5	10	01A
4	AK601	KDNTGRV	143.9	1	6.95	1	01A
5	AK598	WT	144.3	10	69.5	10	02A
6	AK598	WT	144.3	1	6.95	1	02A

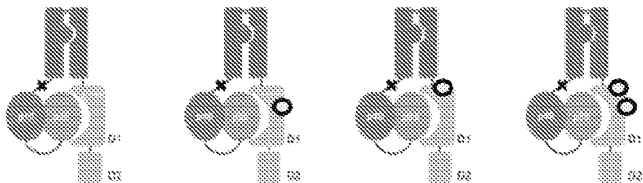
It was found from ELISA that GAG-binding mutation from both AK600 and AK601 improves drug exposure in Cmax and AUC compared to AK598. No difference in PK profiles was observed between
5 AK600 and AK601. Both AK600 and AK601 have similar half-life as AK598, which is about 2 days.

Results are shown in Figure 36-40.

Example 7

10 Free cysteine residues can cause intermolecular cross-linking and aggregation. This example tests whether amino acid mutations of Cysteine to Serine has an effect on aggregation and stability.

15 The following constructs were used in this example:



Molecule	AK386	AK604	AK605	AK606
Cytokine	IL-12	IL-12	IL-12	IL-12
Scaffold	Clamp	Clamp	Clamp	Clamp
Mutation	WT	C242S	C252S	C242S/ C252S

Sequences for AK386, AK604, AK605 and AK606 are in the sequence table in Section 10.

20 Proteins were incubated with the indicated buffer at 40C for 3 days or 12 days. Then the molecules were analyzed by HPLC size exclusion chromatography and by SDS-PAGE and Coomassie staining.

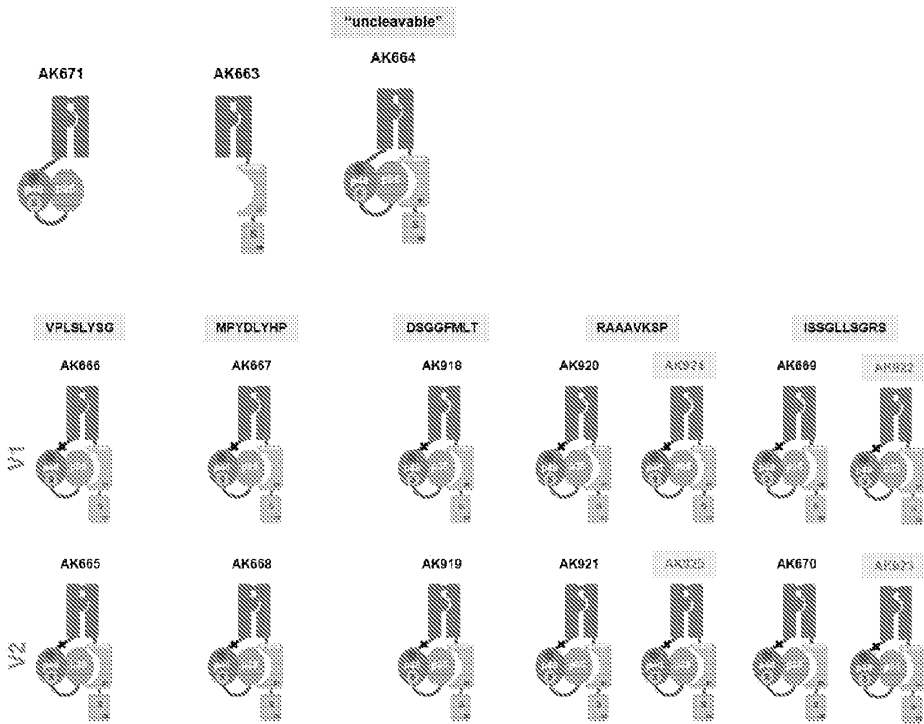
At day 3, only enough aggregation was present to rank stability in 2 buffer conditions, where AK606 ranked the best. Trend towards being more stable with Cys → Ser mutations. At day 12, AK606 ranked the best in 6 additional buffer conditions. Cys → Ser mutations appear to confer stability. SDS-PAGE shows Cys242 causes more covalent aggregation than Cys252. Day 0/12 shown, AK386 and AK605 show much more covalent aggregation than AK604 and AK606.

Results are shown in Figures 41-43.

Example 8

This example demonstrates the masking and cleavage of exemplary IL-12 constructs.

The following constructs were used in this example:



AK671 is an unmasked molecule, AK663 does not comprise a cytokine, and AK664 is non-cleavable. These three molecules serve as controls.

The cleavage peptide for each construct is show at the top of each column.

AK666, AK667, AK918, AK920 and AK669 are ‘version 1’ constructs. AK665, AK668, AK919, AK921, AK670 are ‘version 2’ constructs. AK924, AK922, AK925 and AK923 are ‘version 3’ constructs.

The cleavable linker (protease site linker), i.e. between the HL2 and the IL-12 domain, and the non-cleavable linker (b2 receptor linker) between HL1 and the masking moiety for each version is shown below:

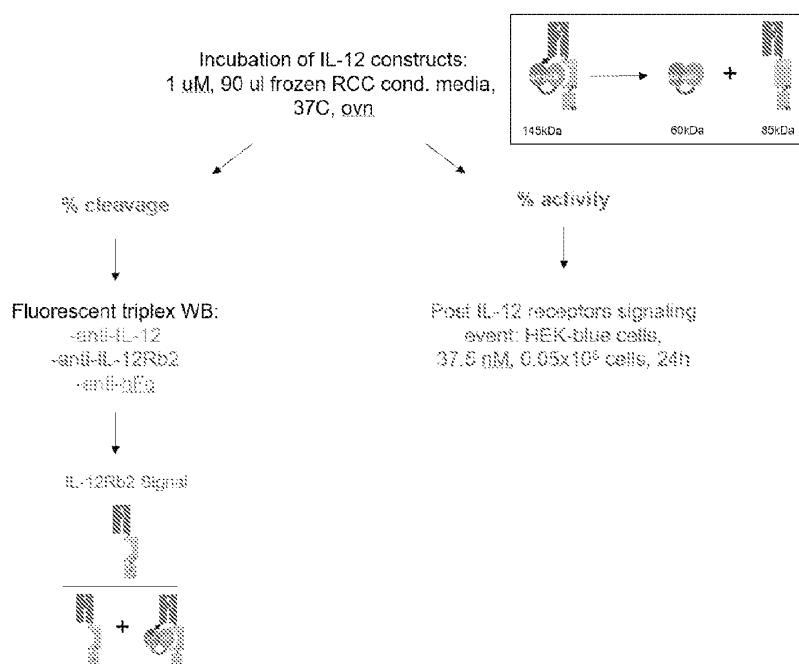
Version	Protease site linker	Version	b2 receptor linker
V1	GGSGGSXXXXXXXXSGP	V1	PGGSGP
V2	GGSGGSGGGSXXXXXXXXSGP	V2	GGSPG
		V3	GGSGGGSG

Where applicable, all of these constructs comprise a KDNTEGRV mutation to the GAG binding domain of the IL-12p40 subunit, a C252S mutation of the IL-12p40 subunit, and a C242S mutation of the IL-12RB2 domain. Exact sequences for each construct are shown in the sequence tables in Section 10.

i) Ex vivo cleavage assay (WB/IL-12 signalling)

1uM of IL-12 construct were incubated with 90ul of conditioned media overnight or 90ul of plasma, for the following times (d1-d2-d4-d7-d9-d11) at 37C. The cleavage rate is calculated as a ratio of: cleaved construct/ (cleaved construct + intact construct), using a western blot anti-human IL-12 and anti-human IL-12Rb. The activation of these constructs by human tissue conditioned media is assessed using a post-IL-12 receptor signalling assay where 0.05x106 HEK-Blue cells are incubated with 37.5nM of constructs, for 24h.

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Results are shown in Figure 44 and in the tables below.

- 5 Molecules with the following cleavage sites exhibited readily detectable cleavage in the tumor supernatants:
- RAAAVKSP
 - ISSGLLSGRS
 - MPYDLYHP

- 10 The cleavage sites sensitivity was observed in the following order:

RAAAVKSP>ISSGLLSGRS>MPYDLYHP

- 15 Therefore, the IL-12 constructs that harbor these cleavage sites represent good candidates for tumor selective activation in RCC and other types of cancers.

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Cutoff 1%, n=30

% Cleavage (WB)

% Activity (signaling assay)

ISSGLLSGRS

	AK669	AK670	AK922	AK923
# of samples with >1% Cleavage, activity	2	5	2	2
Frequency of cleavage, activation (%)	6.7	16.7	6.7	6.7
% Activation (Median)	5.5	1.8	5.8	8.2

ISSGLLSGRS

	AK669	AK670	AK922	AK923
# of samples with >1% Cleavage, activity	3	9	1	8
Frequency of cleavage, activation (%)	10.0	30.0	3.3	26.7
% Activation (Median)	1.7	2.2	5.8	1.2

RAAAVKSP

	AK920	AK921	AK924	AK925
# of samples with >1% Cleavage, activity	7	4	3	3
Frequency of cleavage, activation (%)	23.3	13.3	10.0	10.0
% Activation (Median)	1.8	2.0	13.6	2.6

RAAAVKSP

	AK920	AK921	AK924	AK925
# of samples with >1% Cleavage, activity	7	5	6	7
Frequency of cleavage, activation (%)	23.3	16.7	20.0	23.3
% Activation (Median)	1.9	2.5	1.8	1.7

MPYDLYHP

	AK667	AK668
# of samples with >1% Cleavage, activity	2	2
Frequency of cleavage, activation (%)	6.7	6.7
% Activation (Median)	2.3	2.7

MPYDLYHP

	AK667	AK668
# of samples with >1% Cleavage, activity	5	8
Frequency of cleavage, activation (%)	16.7	26.7
% Activation (Median)	1.5	1.7

VPLSLYSG

	AK665	AK666
# of samples with >1% Cleavage, activity	7	1
Frequency of cleavage, activation (%)	23	3
% Activation (Median)	2.5	1

VPLSLYSG

	AK665	AK666
# of samples with >1% Cleavage, activity	7	4
Frequency of cleavage, activation (%)	23	13
% Activation (Median)	2.1	1.6

DSGGFMLT

	AK918	AK919
# of samples with >1% Cleavage, activity	5	4
Frequency of cleavage, activation (%)	17	13
% Activation (Median)	1.8	2.1

DSGGFMLT

	AK918	AK919
# of samples with >1% Cleavage, activity	3	5
Frequency of cleavage, activation (%)	10	17
% Activation (Median)	1.7	1.3

ii) In vitro cleavage analysis: HEK Blue IL-12 and SDS-PAGE analysis

20

Testing IL-12 molecules with HEK-Blue IL-12 cells:

25

HEK-Blue IL-12 reporter cells developed by Invivogen have been specifically designed to monitor the activation of the JAK-STAT pathway. These cells were generated by stable transfection of HEK293 cells with the human IL-12R β 1 and IL-12R β 2 genes, along with the human TyK2, JAK2, and STAT4 genes to obtain a fully functional IL-12 signaling pathway. In addition, a STAT4-inducible SEAP reporter gene was also introduced. Upon stimulation, HEK-Blue™ IL-12 cells trigger the activation of STAT4 and the

subsequent secretion of SEAP. The levels of STAT4-induced SEAP can be readily monitored using QUANTI-Blue™. HEK-Blue IL-12 cells can be used to validate the functionality, toxicity, and variable dosage effects of human or murine IL-12. HEK Blue IL-12 cells were grown in passage media until ~80% confluent. Washed single-cell suspension in assay media was plated and serial dilutions of IL-12 molecules in assay media were added to cells. Plate was incubated at 37 °C for 24 h. After 24 h, Quanti-Blue solution (Invivogen) was prepared and cell supernatant was added to the Quanti-Blue solution and incubated for 1-2 h at 37 °C. Absorbance at 625 nm measured. Data analysis was performed in Graphpad Prism, version 8.3. Background was subtracted from raw data and the data were fit nonlinearly: [Agonist] vs. response – Variable slope (four parameters). EC50 value of each IL-12 construct was reported.

Masking:

Results are shown in the tables below and in Figures 45, 46A and B.

Construct	Run 1, EC ₅₀	Aklusion	Run 2, EC ₅₀	Aklusion	Average Aklusion
AK671	24.7	N/A	15.2	N/A	N/A
rhIL-12	9.6	N/A	8.2	N/A	N/A
AK386 null	477.9	19.4	367.5	14.9	17.1
AK664 null	1654.0	75.2	1677.0	68.0	71.6
AK665 null	1303.0	52.9	1491.0	60.5	56.7
AK666-01A null	1775.0	72.0	2009.0	81.5	76.8
AK666-02A null	1725.0	70.0	1537.0	62.4	66.2
AK667 null	3104.0	125.9	2035.0	82.6	104.2
AK668 null	1383.0	56.1	1370.0	55.6	55.8
AK669 null	895.6	36.3	1193.0	48.4	42.4
AK670 null	740.9	30.1	862.1	35.0	32.5
AK922 null	1183.0	48.0	1101.0	44.7	46.3
AK923 null	1562.0	63.4	1188.0	48.2	55.8
AK918 null	2886.0	117.1	3116.0	126.4	121.7
AK919 null	1230.0	49.9	1475.0	59.8	54.9
AK920 null	1116.0	45.3	1116.0	45.3	45.3
AK921 null	1698.0	68.9	1352.0	54.8	61.9
AK924 null	1030.0	41.8	766.4	31.1	36.4
AK925 null	995.1	40.4	914.8	37.1	38.7

Parental AK671 is less potent than rhIL-12 (but not significantly, i.e. 3-fold). All masked constructs are more akluded than AK386. AK667 and AK918 are both >100-fold akluded.

As compared to AK386, the new molecules that have the GAG-binding domain mutation, the cysteines to serines mutations, new optimized linkers, as well as different cleavage sites, all exhibit improved masking.

Cleavage:

Cleavage of the constructs was testing using exemplary proteases MMP7, 9 and 10.

Batch 1

AK ID	Protein Lot #	300 ng MMP	Total construct cleaved, ug
AK663	AK663-01A	7	8.8
AK664	AK664-01A	7	14.8
AK665	AK665-01A	7	14.8
AK666	AK666-01A	7	14.8
AK667	AK667-01A	10	14.9
AK668	AK668-01A	10	14.9
AK669	AK669-01A	2	14.9
AK670	AK670-01A	2	14.9
AK671	AK671-01A	7	11.3
AK686	AK686-03A	7	14.9
AK674	AK674-01A	7	15.1

Results are shown in Figures 47A-B and 48A-K

5

Batch 2

AK ID	Protein Lot #	300 ng MMP	Total construct cleaved, ug
AK667	AK667-01A	7, 9, 10	14.86
AK671	AK671-01A	7, 9, 10	11.31
AK918	AK918-01A	7	14.84
AK919	AK919-01A	7	14.85
AK920	AK920-01A	9	14.83
AK921	AK921-01A	9	14.84
AK926	AK926-03A	7	14.90

Results are shown in Figures 49A-B and 50A-G

10

Batch 3

AK ID	Protein Lot #	300 ng MMP	Total construct cleaved, ug
AK986	AK986-04A	7, 10	14.9
AK922	AK922-01A	7	14.9
AK923	AK923-01A	7	14.9
AK924	AK924-01A	10	14.8
AK925	AK925-01A	10	14.9
AK671	AK671-02A	N/A	N/A

Results are shown in Figures 51 and 52A-E

Overall, the new molecules with different cleavage sites are all susceptible to MMP cleavage in vitro. For all the molecules, there is a restoration of activity post cleavage. These compounds represent good candidates for tumor selective activable IL-12 molecules.

5

Example 9

i. Cleavage of peptides by NAT vs. RCC culture supernatant

- 10 Sequences comprising cleavage peptides (shown in bold below) were incubated in either 'NAT' (Normal Adjacent Tissue) or 'RCC' (Renal Cell Carcinoma) culture supernatants, to test the specificity of each peptide's cleavage.

To this end, peptide sequencing by mass spectrometry was used to identify cleaved fragments produced for the synthetic peptides shown in the table below, using a published technique called multiplexed substrate profiling by mass spectrometry (MSP-MS) (O'Donoghue A.J. et al. Nat Methods. 2012 Nov;9(11):1095-100.) Cleavages were monitored in these reactions over time, and the peptides found to be cleaved in the earliest time points were deemed to be most sensitive to proteolytic activity in the conditioned media samples.

20

Results are as follows:

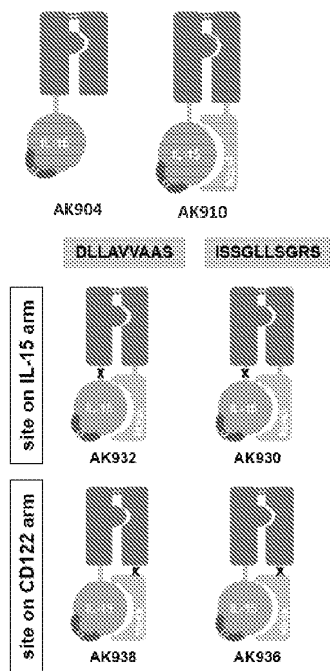
Substrate	Synthetic Peptide Sequence (bolded sequences show the cleavable peptide; * indicates cleavage site)	NAT	RCC	Earliest cleaved time point - NAT	Earliest cleaved time point - RCC
AK-15	RSGVPLS*LYSGSGGGK	0	3/5		15min
AK-18	RSGMP*YDLY*HPSGK	5/5	5/5	15min	15min
AK-21	RGPDSGGF*ML*TSBK	3/5	5/5	15min	15min
AK-28	RSGGHEQLTVSGGSK	0	0		
AK-49	RSGR*AAAVKSPSGK	0	3/5		15-60- 240min
AK-02	RSGISSGLLSGRS*D*N*HSGK	5/5	5/5	15-60min	15-60min
AK-50	RGDLLAVVA*ASGGK	0	5/5		15-60min
AK-88	RGGISSGLL*SG*RSBK	0	5/5		15-60min

Cleavage peptides DLLAVVA*AS and ISSGLL*SG*RS were found to be the most specific. Sequences comprising these peptides did not cleave in the NAT culture, but cleaved in every run in the RCC culture.

25

Example 10

The following constructs were used in this example:



AK91 0	1 st polypeptide chain: Fc(Hole) CD122(C122S, C168S)	DKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPE VTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPRE EQYASTYRVVSVLTVLHQDWLNGKEYKCKVSNKAL PAPIEKTISKAKGQPREPQVCTLPSSRDELTKNQVSL CAVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDG SFFLVSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQ KSLSLSPGPGSGSAVNGTSQFTCFYNSRANISCVWSQ DQALQDTSCQVHAWPDRRRWNQTCELLPVSQASW ACNLILGAPDSQKLTTVDIVTLRVLCREGVRWRVMA IQDFKPFENLRMAPISLQVVHVETHRSNISWEISQAS HYFERHLEFEARTLSPGHTWEEAPLLTLKQKQEWIS LETLPDTPQYEFQVRVKPLQGEFTTWSPWSQPLAF RTKPAALGKD	DNA44 0
	2 nd polypeptide chain: Fc(knob)-IL15 V1 Non- cleavable (N71Q, N79Q)	DKTHTCPPCPAPELLGGPSVFLFPPKPKDTL MISRTPEVTCVVVDVSHEDPEVKFNWYVD GVEVHNAKTKPREEQYASTYRVVSVLTVL HQDWLNGKEYKCKVSNKALPAPIEKTISKA KGQPREPQVYTLPPCRDELTKNQVSLWCLV KGFYPSDIAVEWESNGQPENNYKTTPPVLD SDGSFFLYSKLTVDKSRWQQGNVFSCSVMHE ALHNHYTQKSLSLSPGGGSSPPGGGSSGGGSG PSGSPGNWVNVISDLKKIEDLIQSMHIDATLY TESDVHPSCKVTAMKCFLELQVISLESQDASI HDTVENLILAQNSLSSNGQVTEGCKECEL EEKNIKEFLQSFVHIVQMFINIS	DNA90 4
AK93 2	1 st polypeptide chain: Fc(Hole) CD122(C122S, C168S)	DKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPE VTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPRE EQYASTYRVVSVLTVLHQDWLNGKEYKCKVSNKAL PAPIEKTISKAKGQPREPQVCTLPSSRDELTKNQVSL CAVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDG SFFLVSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQ KSLSLSPGPGSGSAVNGTSQFTCFYNSRANISCVWSQ DQALQDTSCQVHAWPDRRRWNQTCELLPVSQASW ACNLILGAPDSQKLTTVDIVTLRVLCREGVRWRVMA IQDFKPFENLRMAPISLQVVHVETHRSNISWEISQAS HYFERHLEFEARTLSPGHTWEEAPLLTLKQKQEWIS LETLPDTPQYEFQVRVKPLQGEFTTWSPWSQPLAF RTKPAALGKD	DNA44 0

	2 nd polypeptide chain: Fc(knob)- [DLLAVVAAS]-IL15 (N71Q,N79Q) *cleavable peptide bolded	DKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEV TCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREE QYASTYRVVSVLTVLHQDWLNGKEYKCKVSNKALP APIEKTISKAKGQPREPQVYTLPPCRDELTKNQVSLW CLVKGFYPSDIAVEWESNGQPENNYKTTPVLDSDGS FFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKS LSLSPGSGS DLAVVAASS GP GSGNWNVISDLKKIE DLIQSMHIDATLYTESDVHPSCVTAMKCFLELQVISL ESGDASIHTVENLILAQNSLSSNGQVTESGCKECEE LEEKNIKEFLQSFVHIVQMFINIS	DNA92 4
AK93 8	1 st polypeptide chain: Fc(hole)- [DLLAVVAAS]-CD122 *cleavable peptide bolded	DKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRT PEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAK TKPREEQYASTYRVVSVLTVLHQDWLNGKEYK CKVSNKALPAPIEKTISKAKGQPREPQVCTLPPS RDELTKNQVSLSCAVKGFYPSDIAVEWESNGQ PENNYKTTPVLDSDGSFFLVSKLTVDKSRWQQ GNVFSCSVMHEALHNHYTQKSLSLSPGSGSPSG D LLAVVAASS GP GSGSPA VNGTSQFTCFYNSRANISC VWSQDQALQDTSCQVHAWPDRRRWNQTCELLPV SQASWACNLILGAPDSQKLTTVDIVTLRVLCREGV RWRVMAIQDFKPFENLRMAPISLQVVHVETHRSN ISWEISQASHYFERHLEFEARTLSPGHTWEEAPLLTL KQKQEWISLETLPDTQYEFQVRVKPLQGEFTT WSPWSQPLAFRTKPAALGKD	DNA82 2
	2 nd polypeptide chain: Fc(knob)-IL15 V1 Non- cleavable (N71Q, N79Q)	DKTHTCPPCPAPELLGGPSVFLFPPKPKDTL MISRTPEVTCVVVDVSHEDPEVKFNWYVD GVEVHNAKTKPREEQYASTYRVVSVLTVL HQDWLNGKEYKCKVSNKALPAPIEKTISKA KGQPREPQVYTLPPCRDELTKNQVSLWCLV KGFYPSDIAVEWESNGQPENNYKTTPVLD SDGSFFLYSKLTVDKSRWQQGNVFSCSVMHE ALHNHYTQKSLSLSPGGGSSPPGGGSSGGGSG PSGSPGNWNVISDLKKIEDLIQSMHIDATLY TESDVHPSCVTAMKCFLELQVISLES GDASI HDTVENLILAQNSLSSNGQVTESGCKECEEEL EEKNIKEFLQSFVHIVQMFINIS	DNA90 4
AK93 0	1 st polypeptide chain:	DKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPE VTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPRE	DNA44

	Fc(Hole) CD122(C122S, C168S)	EQYASTYRVVSVLTVLHQDWLNGKEYKCKVSNKAL PAPIEKTISKAKGQPREPQVCTLPSSRDELTKNQVSL CAVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDG SFFLVSKLTVDKSRWQQGNVFSCSVMEALHNHYTQ KSLSLSPGGSGSAVNGTSQFTCFYNSRANISCVWSQ DQALQDTSCQVHAWPDRRRWNQTCELLPVSQASW ACNLILGAPDSQKLTTVDIVTLRVLCREGVRWRVMA IQDFKPFENLRMAPISLQVVHVETHRSNISWEISQAS HYFERHLEFEARTLSPGHTWEEAPLLTLKQKQEWIS LETLPDTQYEFQVRVKPLQGEFTTWSPWSQPLAF RTKPAALGKD	0
	2 nd polypeptide chain: Fc(knob)- [ISSGLLSGRS] -IL15 (N71Q,N79Q) *cleavable peptide bolded	DKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISR TPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNA KTKPREEQYASTYRVVSVLTVLHQDWLNGKEY KCKVSNKALPAPIEKTISKAKGQPREPQVYTLPP CRDELTKNQVSLWCLVKGFYPSDIAVEWESNGQ PENNYKTTPPVLDSDGSFFLVSKLTVDKSRWQQ GNVFSCSVMEALHNHYTQKSLSLSPGGGSSGGS PISSGLLSGRSSGPGSGSNWVNVISDLKKIEDLIQSMH IDATLYTESDVHPSCKVTAMKCFLELQVISLESGDA SIHDTVENLILAQNSLSSNGQVTESGCKECEELE EKNIKEFLQSFVHIVQMFINTS	DNA92 2
AK93 6	1 st polypeptide chain: Fc(hole)- [ISSGLLSGRS] -CD122	DKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRT EVTCTVVVDVSHEDPEVKFNWYVDGVEVHNAKTK PREEQYASTYRVVSVLTVLHQDWLNGKEYKCKVS NKALPAPIEKTISKAKGQPREPQVCTLPSSRDELTKN QVSLSCAVKGFYPSDIAVEWESNGQPENNYKTTPPV LDSDGSFFLVSKLTVDKSRWQQGNVFSCSVMEAL HNHYTQKSLSLSPGGPPSGSS PISSGLLSGRSSGGGAV NGTSQFTCFYNSRANISCVWSQDQALQDTSCQVHAW PDRRRWNQTCELLPVSQASWACNLILGAPDSQKL TTVDIVTLRVLCREGVRWRVMAIQDFKPFENLRMA PISLQVVHVETHRSNISWEISQASHYFERHLEFEAR TLSPGHTWEEAPLLTLKQKQEWISLETLPDTQYE FQVRVKPLQGEFTTWSPWSQPLAFRTKPAALGKD	DNA82 3
	2 nd polypeptide chain:	DKTHTCPPCPAPELLGGPSVFLFPPKPKDTL MISRTPEVTCVVVDVSHEDPEVKFNWYVD GVEVHNAKTKPREEQYASTYRVVSVLTVL	DNA90

	Fc(knob)-IL15 VI Non- cleavable (N71Q, N79Q)	HQDWLNGKEYKCKVSNKALPAPIEKTISKA KGQPREPQVYTLPPCRDELTKNQVSLWCLV KGFYPSDIAVEWESNGQPENNYKTTPPVLD SDGSFFLYSKLTVDKSRWQQGNVFSVSMHE ALHNHYTQKSLSLSPGGGSSPPGGGSSGGGSG PSGSPGNWVNVISDLKKIEDLIQSMHIDATLY TESDVHPSCCKVTAMKCFLLLELQVISLESGDASI HDTVENLILAQNSLSSNGQVTESGCKECEEL EEKNIKEFLQSFVHIVQMFINTS	4
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Importantly, AK932 and AK930, and their 'flipped' counterparts AK938 and AK936 include a peptide substrate (the sequence of which is depicted in the box above each molecule and bolded in the sequence table table). AK904 is a non-cleavable unmasked construct, and AK910 is a non-cleavable masked construct, both acting as negative controls.

The above AK molecules include an IL-15 domain, however it will be appreciated that however the results and conclusions of this data are equally relevant for IL-2 constructs.

Cleavage was achieved for masked constructs including a peptide substrate.

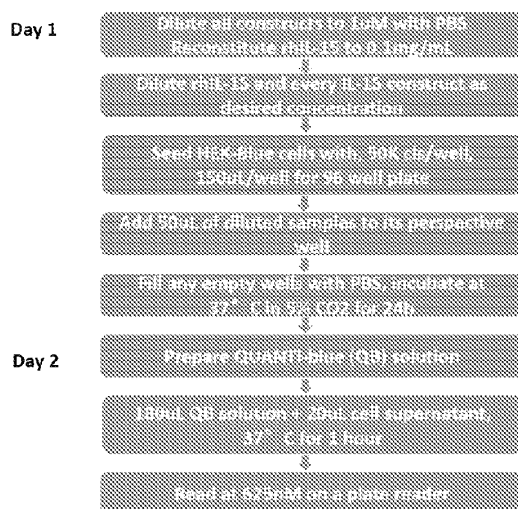
Constructs were incubated with MMP7, 9 and 10. Cleavage for each construct was analysed by SDS-PAGE and confirmed by HEK-Blue IL-2 bioassay.

The HEK-Blue assay was carried out as follows:

Conditions: Cell plate: 96 well plate. Cell density: 50K cels/well. Time point for HEK Blue detection were tested: 1h. Construct number: Total 14 constructs that were tested.

Assay Flow chart:

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The results are shown in the table below, where a 'X' indicates not fully cleaved and a '√' indicates cleavage:

5

ID	MMP	Cleavage
AK904	7	X
	9	X
	10	X
AK910	7	X
	9	X
	10	X
AK932	7	√
	9	-
	10	-
AK938	7	√
	9	-
	10	-
AK930	7 (36hr)	√
	9	-
	10	-
AK936	7	√
	9	-
	10	-

The specific EC₅₀ readout results from the HEK-Blue IL-2 bioassay are shown in the table below.

ID	MMP	EC50 (pM)	Max
AK904 (1:1:2)	-	14.78	1.44
	7	17.08	1.37
	9	16.00	1.43
	10	22.93	1.45

AK910 (1:1:2)	-	1219.34	1.31
	7	284.17	1.42
	9	519.09	1.40
	10	490.52	1.40
AK932 (1:1:2)	-	2403.11	1.22
	7	9.30	1.43
	9	-	-
	10	-	-
AK938 (1:1:2)	-	885.13	1.31
	7	18.03	1.38
	9	-	-
	10	-	-
AK930 (1:1:2)	-	1858.76	1.22
	7	8.00	1.41
	9	-	-
	10	-	-
AK936 (1:1:2)	-	785.85	1.37
	7	16.11	1.40
	9	-	-
	10	-	-

The SDS-PAGE gel results are shown in Figures 53A-D. The HEK-Blue IL-2 bioassay results are shown in Figures 54A-F.

5 **Example 11**

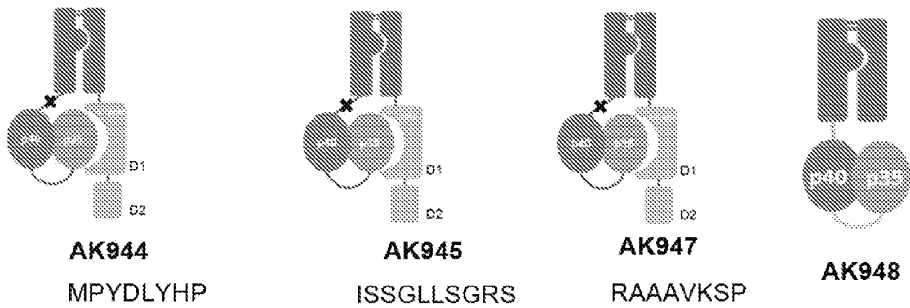
To understand the pharmacokinetic and pharmacodynamic profile of exemplary tumor-targeted hybrid murinized molecules in vivo, three molecules constructed with human Fc and mouse IL-12 with different cleavage sites were tested.

10

AK944(MPYDLYHP), AK945(ISSGLLSGRS), AK947(RAAAVKSP) were used in 2 tumor models, MB49 and B16F10, and parental AK948 without masking was used as control.

Details of the constructs used in this example are as follows:

15



Sequences of the mature mouse IL-12 p40 and IL-12 p35 subunits, and mouse IL-12R masking moiety used in the molecules are as follows:

Mouse IL-12 p40 subunit:

5 MWELEKDVYVVEVDWTPDAPGETVNLTCDTPEEDDITWTSQQRHGVIGSGKLTITVKEFLDA
GQYTCHKGGETLSHSHLLLHKKENGIWSTEILKNFNKNTFLKCEAPNYSGRFTCSWLVRNMDL
KFNKSSSSSPDSRAVTCGMASLSAEKVTLQDRDYEKYSVSCQEDVTCPTAEETLPIELALEARQQ
NKYENYSTSFFIRDIKPDPPKNLQMKPLKNSQVEVSWEYPDSWSTPHSYFSLKFFVRIQRKKEK
MKETEEGCNQKGAFLVEKTSTEVQCKGGNVCVQAQDRYYNSSCSKWACVPCRVRS

10

Mouse IL-12 p35 subunit:

RVIPVSGPARCLSQSRNLLKTTDDMVKTAREKLKHYSCTAEDIDHEDITRDQTSTLKTCLPLELH
KNESCLATRETSSSTRGSLPPQKTSMMTLCLGSIYEDLKMYQTEFQAINAALQNHNHQIILD
KGMLVAIDELMQSLNHNGETLRQKPPVGEADPYRVKMKLCILLHAFSTRVVTINRVMGYLSSA

15

Mouse IL-12R masking moiety:

LYHPSGPMWELEKDVYVVEVDWTPDAPGETVNLTCDTPEEDDITWTSQQRHGVIGSGKLTITV
KEFLDAGQYTCHKGGETLSHSHLLLHKKENGIWSTEILKNFNKNTFLKCEAPNYSGRFTCSWLVRNMDL
KFNKSSSSSPDSRAVTCGMASLSAEKVTLQDRDYEKYSVSCQEDVTCPTAEETLPIEL
20 ALEARQQNKYENYSTSFFIRDIKPDPPKNLQMKPLKNSQVEVSWEYPDSWSTPHSYFSLKFFVRI
QRKKEKMKETEEGCNQKGAFLVEKTSTEVQCKGGNVCVQAQDRYYNSSCSKWACVPCRVRS
GGGSGGGSGGGSGSRVIPVSGPARCLSQSRNLLKTTDDMVKTAREKLKHYSCTAEDIDHEDITR
DQTSTLKTCLPLELHKNESCLATRETSSSTRGSLPPQKTSMMTLCLGSIYEDLKMYQTEFQAIN
AALQNHNHQIILDKGMLVAIDELMQSLNHNGETLRQKPPVGEADPYRVKMKLCILLHAFSTRV
25 VTINRVMGYLSSA

Full sequence information for each molecule is shown in the table below (cleavable linkers shown in bold, non-cleavable linkers shown in underline):

AK944	1 st polypeptide chain Hole: hFc (N297A)-mouseIL12RB2	DKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISR TPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNA KTKPREEQYASTYRVVSVLTVLHQDWLNGKEY KCKVSNKALPAPIEKTISKAKGQPREPQVCTLPPS RDELTKNQVSLSCAVKGFYPSDIAVEWESNGQP ENNYKTTTPVLDSDGSFFLVSKLTVDKSRWQQG NVFSCSVMEALHNHYTQKSLSLSPGGGSGGG SGNIDVCKLGTVTVPAPVIPLGSAANISCSLNP KQCCHYPSSNELILLKFVNDVLVENLHGKKVH DHTGHSSTFQVTNLSLGMTLTVCKLNCNSQKK PPVPVCGVEISVGAPEPPQNISCVQEGENGTV ACSWNSGKVITYLKTNYTLQLSGPNNLTCQKQC FSDNRQNCNRLDLGINLSPDLAESRFIVRVTAI NDLGNSSSLPHTFTFLDIVIPLPPWDIRINFLNA SGSRGTLQWEDEGQVVLNQLRYQPLNSTSWNM VNATNAKGKYDLRDLRPFTEYEFQISSKLHLSG	DNA909
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		GSWSNWSESLRTRTPEE	
	2 nd polypeptide chain Knob: hFc (N297A)- [MPYDLYHP]- mouseIL12	DKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYASTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKISKAKGQPREPQVYTLPPCRDELTKNQVSLWCLVKGFYPSDI AVEWESNGQPENNYKTTPVLDSGGSFFLYSKLTVDKSRWQOGNVFSCSVMEALHNHYTQKSLSLSPGGGSGGSGGSMPLYDLYHPSGPMWELEKDVYVVEVDWTPDAPGETVNLTCDTPEEDDITWTSQQRHGVIGSGKLTITVKEFLDAGQYTCHKGGETLSHSHLLLHKKENGIWSTEILKNFKNKTKFLKCEAPNYSGRFTCSWLVRQNM DLKFNKSSSSSPDSRAVTCGMASLSAEKVTLDQRDYEKYSVSCQEDVTCPTAEETLPIELALEARQQNKYENYSTSFFIRDIKPDPPKNLQMKPLKNSQVEVSWEYPDSWSTPHSYFSLKFFVRIQRKKEKMKETEEGCNQKGAFLVEKTSDEVQCKGGNVCVQAQDRYYNSSCSKWACVPCRVRSGGGGSGGGGSGGGGSRVIPVSGPARCLSQRNLLKTTDDMVKTAREKLKHYSCTAEDIDHEDITRDQSTLKTCLPLELHKNESCLATRETSSTRGSCLPQKTSMMTLCLGSIYEDLKMYQTEFQAINAALQNHNHQQIILDKGMLVAIDELMQSLNHNGETLRQKPPVGEADPYRVKMKLCILLHAFSTRVVTINRVMGYLSSA	DNA911
AK945	1 st polypeptide chain Hole: hFc (N297A)- mouseIL12RB2	DKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPVTCVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYASTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKISKAKGQPREPQVCTLPSSRDELTKNQVSLSCAVKGFYPSDIAVEWESNGQPENNYKTTPVLDSGGSFFLVSKLTVDKSRWQOGNVFSCSVMEALHNHYTQKSLSLSPGGGSGGGSGNIDVCKLGTVTVPAPVPLGSAANISCSLNP KQGC SHYPSSNELILLKFVNDVLVENLHGKKVHDHTGHSSTFQVTNLSLGMTLFVCKLNCNSQKKPPVPVCGVEISVGVAPEPPQNISCVOEGENGTVACSWNSGKVTYLKTNITLQLSGPNNLTCQKQCFSDNRQNCNRLDLGINLSPDLAESRFIVRVTAINDLGNSSSLPHTFTFLDIVIPLPPWDIRINFLNASSRGTLQWEDEGQVVLNQLRYQPLNSTSWNMVNATNAKGKYDLRDLRPFTEYEFQISSKLHLSGGSWSNWSESLRTRTPEE	DNA909
	2 nd polypeptide chain Knob: hFc (N297A)-	DKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPVTCVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYASTYRVVSVLTVLHQDWLNGKEYCKVSNKALPAPIEKISKAKGQPREPQVYTLPPCRDELTKNQVSLWCLVKGFYPSDIAVEWESNGQ	DNA912

	[RAAAVKSP]- mouseIL12	ENNYKTTTPVLDSDGSFFLYSKLTVDKSRWQQG NVFSCSVMEALHNHYTQKSLSLSPGGGSGGSG GSISSGLLSGRSSGPMWELEKD VYVVEVDWTPD APGETVNLTCDTPEEDDITWTSQDRHGVIGSGKT LTITVKEFLDAGQYTCHKGGETLSHSHLLLHKKE NGIWSTEILKNFKNKTFKCEAPNYSGRFTCSWL VQRNMDLKFNKSSSSSPDSRAVTCGMASLSAEK VTLDQRDYEKYSVSCQEDVTCPTAEETLPIELALE ARQONKYENYSTSFIRDIIKPDPPKNLQMKPLKN SQVEVSWEYPDSWSTPHSYFSLKFFVRIQRKKEK MKETEEGCNQKGAFLVEKTSTEVQCKGGNVCVQ AQDRYNNSSCSKWACVPCRVRSGGGSGGGGS GGGGSRVIPVSGPARCLSQSRNLLKTDDMVKT AREKLKHYSCTAEDIDHEDITRDQSTLKTCLPL ELHKNESCLATRETSTTRGSLPPQKTSMMTL CLGSIYEDLKMYQTEFQAINAALQNHNHQQIILD KGMLVAIDELMQSLNHNGETLRQKPPVGEADPY RVKMKLCILHAFSTRVVTINRVMGYLSSA	
AK947	1 st polypeptide chain Hole: hFc (N297A)- mouseIL12RB2	DKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISR TPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNA KTKPREEQYASTYRVVSVLTVLHQDWLNGKEY KCKVSNKALPAPIEKTISKAKGQPREPQVCTLPPS RDELTKNQVSLSCAVKGFYPSDIAVEWESNGQP ENNYKTTTPVLDSDGSFFLYSKLTVDKSRWQQG NVFSCSVMEALHNHYTQKSLSLSPGGGSGGGG SGNIDVCKLGTVTVPAPVIPLGSAANISCSLNP KQGCSHYPPSSNELILLKFVNDVLVENLHGKKVH DHTGHSSTFQVTNLSLGMTLFCVCKLNCNSQKK PPVPVCGVEISVGVAPPEPPQNISCVEGEGNTV ACSWNSGKVTYLKNTYTLQLSGPNNLTCQKQC FSDNRQNCNRLDLGINLSPDLAESRFIVRVTAI NDLGNSSSLPHTFTFLDIVIPLPPWDIRINFLNA SGSRGTLQWEDEGQVVLNQLRYQPLNSTSWNM VNATNAKGKYDLRLRPFTEYEFQISSKLHLSG GSWSNWSESLRTRTPEE	DNA909
	2 nd polypeptide chain Knob: hFc (N297A)- [RAAAVKSP]- mouseIL12	DKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRT PEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKT KPREEQYASTYRVVSVLTVLHQDWLNGKEYKCK VSNKALPAPIEKTISKAKGQPREPQVYTLPPCRDEL TKNQVSLWCLVKGFYPSDIAVEWESNGQPENNYK TTPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSV MEALHNHYTQKSLSLSPGGGSGGGSGSRAAAVK SPSGPMWELEKD VYVVEV DWTPDAPGETVNLTCDTPEEDDITWTSQDRHGVIG SGKTLTITVKEFLDAGQYTCHKGGETLSHSHLLLH KKENGIWSTEILKNFKNKTFKCEAPNYSGRFTCS WLVRNMDLKFNKSSSSSPDSRAVTCGMASLSA EKVTLDQRDYEKYSVSCQEDVTCPTAEETLPIELA	DNA914

		LEARQQNKYENYSTSFFIRDIIKPDPPKNLQMKPL KNSQVEVSWEPDSWSTPHSYFSLKFFVRIQRKK EKMKETEEGCNQKGAFLVEKTSTEVQCKGGNVC VQAQDRYYNSSCSKWACVPCRVRSGGGGSGGG GSGGGGSRVIPVSGPARCLSQSRNLLKTDDMVK TAREKLKHYSCTAEDIDHEDITRDQTSTLKTCLPLE LHKNESCLATRETSSSTRGSCLPQKTSLMMTLCL GSIYEDLKMYQTEFQAINAALQNHNHQQIILDKGM LVAIDELMQSLNHNGETLRQKPPVGEADPYRVKM KLCILLHAFSTRVVTINRVMGYLSSA	
AK948	1 st polypeptide chain Hole: hFc(N297A)	DKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISR TPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNA KTKPREEQYASTYRVVSVLTVLHQDWLNGKEYK CKVSNKALPAPIEKISKAKGQPREPQVCTLPISR DELTKNQVSLSCAVKGFYPSDIAVEWESNGQPEN NYKTTTPVLDSDGSFFLVSKLTVDKSRWQQGNVF SCSVMEALHNHYTQKSLSLSPGK	DNA158
	2 nd polypeptide chain Knob: hFc(N297A)- [DSGGFMLT]- mouseIL12	DKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRT PEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKT KPREEQYASTYRVVSVLTVLHQDWLNGKEYKCKV SNKALPAPIEKISKAKGQPREPQVYTLPPCRDEL TKNQVSLWCLVKGFYPSDIAVEWESNGQPENNYKT TPPVLDSDGSFFLVSKLTVDKSRWQQGNVFSCSV MEALHNHYTQKSLSLSPG GGSGGSGGSDSGGFMLT SGPMWELEKDVYVVEVDWTPDAPGETVNLTCDT PEEDDITWTSQDRHGVIGSGKTLTITVKEFLDAGQ YTCHKGGETLSHSHLLLHKKENGIWSTEILKNFKN KTFLKCEAPNYSGRFTCSWLQVRNMDLKFNIKSS SSSPDSRAVTCGMASLSAEKVTLDQRDYKEYSVS CQEDVTCPTAEETLPIELALEARQQNKYENYSTSFF IRDIIKPDPPKNLQMKPLKNSQVEVSWEPDSWSTP HSYFSLKFFVRIQRKKEKMKETEEGCNQKGAFLVE KTSTEVQCKGGNVCVQAQDRYYNSSCSKWACVPC RVRSGGGGSGGGGSGGGGSRVIPVSGPARCLSQS RNLLKTDDMVKTAREKLKHYSCTAEDIDHEDITR DQTSTLKTCLPLELHKNESCLATRETSSSTRGSCLP PQKTSLMMTLCLGSIYEDLKMYQTEFQAINAALQ NHNHQQIILDKGMLVAIDELMQSLNHNGETLRQK PPVGEADPYRVKMKLCILLHAFSTRVVTINRVMG YLSSA	DNA913

- 5 In this study, C57BL/6 mice were inoculated subcutaneously with 1×10^6 MB49 or 0.5×10^6 B16F10 tumor cells. Treatments started when tumor sizes reached 300mm^3 . The administration of molecules was through single i.v. injections at 0.5 or 3 mg/kg. The animals were euthanized five days post treatment started. Body weight and organ weight were recorded:

	Test Molecule	Cleavage site	M.W. (kDa)	Apprx. Dosing (mg/kg)	Actual Dosing (nMoles/kg)	Actual Dosing (mg/kg)	Batch information
1	Mouse recombinant IL-12		75	0.5	3.6	0.27	
2	AK948 (parental)		110.6	0.1	0.9	0.1	01A
3	AK948 (parental)		110.6	0.5	3.6	0.4	01A
4	AK944	MPYDLYHP	145.7	1	3.6	0.5	01A
5	AK945	ISSGLLSGRS	145.7	1	3.6	0.5	02A
6	AK947	RAAAVKSP	145.5	1	3.6	0.5	02A
7	AK944	MPYDLYHP	145.7	3	20.6	3	01A
8	AK945	ISSGLLSGRS	145.7	3	20.6	3	02A
9	AK947	RAAAVKSP	145.5	3	20.6	3	02A
10	Vehicle						

PD details

- Take down at D5 (total 50 mice)
- Immunophenotyping: CD8, CD4, Treg, CD25, PD-1, KI-67, NK, ICOS*
- FACS
 - Blood, spleen and tumor liver*
 - Snap freeze spleen, kidney, tumor

*B16 only

PK readouts

- Therapy start at 300mm³, (n=5)
- Single dose (i.v)
- Plasma collection
 - 25 µL: 5 min, 2hr, 6hr, 24 hr, D3, D5
 - PK: Fc capture Fc detect in MSD format
 - mIFN-γ ELISA D3

Models:

- MB49
- B16F10*

Material details

- C57BL/6 (n=5 per group, 50 in total)
- Material needed: 2mg/molecule/model

The following readouts were analysed:

5

- *Tumor growth inhibition*: Tumor volume significantly decreased in AK945 and AK947 in B16 model.
- *Tumor weight*: Single dose AK945 significantly decrease tumor weights at low dose in MB49.
- *% of body weight change*: All masked molecules show protection against body weight loss.
- *Spleen weight*: Spleen weights are increased in MB49 model but not B16. The difference in spleen weight from vehicle group between models could be tumor specific driven.
- *Lung weight* (MB49 model only): Lung weights are not affected by IL-12 variants in MB49 model.

10

15

The results are shown in Figures 55-59.

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FACS analysis was also used to investigate immune responses in tumor microenvironments in compared with multiple peripheral organs, including blood and spleen. Activation and/or expansion of CD8 T-cell, CD8 T-cell/T-reg ratio in the tumor microenvironment is of particular interest.

The following PD readouts were analysed:

25

- *CD45 in TME*

- *CD8 T cell*: CD8 T cell increased in tumor microenvironment with AK947 at high dose. AK947 also expand CD8 T cell peripheral.
- *CD8/Treg ratio*: CD8/Treg ratio is increased in tumor microenvironment.
- *CD8 T cell proliferation*: No difference in CD8 T cell proliferation in TME. CD8 T cell are more proliferative in TME than in peripheral.
- *CD8 activation marker*: CD8 T cells are more activated in tumor microenvironment.

The results are shown in Figures 60-65

Finally, serum mouse ALT measurement was measured at day 5; mouse IFN- γ and TNF- α ELISA was performed using day 3 plasma to investigate downstream signalling activated by the tumor-targeted molecules.

The following readouts were analysed:

- *Toxicity - ALT assay*: IL-12 variants do not induce liver toxicity at low dose
- *D3 serum cytokines*: Serum IFN γ expression is significantly lowered in masked IL-12 treated mice. There is dose dependent increase in IFN- γ for all IL-12 variants, however, it is significantly lower than unmasked parental molecules. High dose IL-12 variants induce serum TNF α expression comparable to parental 3 days post treatment started.
- *PK: Fc capture, Fc detect*: Fc detect PK ELISA indicate no difference in drug accumulation in plasma in B16 model. PK analysis was performed using Fc capture, Fc detect ELISA.

The results are shown in Figures 66-68.

Example 12

The purpose of this study is to determine the safety, and compare pharmacokinetics and pharmacodynamic of exemplarily tumor-targeted molecules in cynomolgus monkeys.

Three GAG-binding mutation containing molecules were constructed with human Fc and human IL-12 with different cleavage sites, AK921, AK923, AK667 that has cleavage sites RAAAVKSP, ISSGLLSGRS and MPYDLYHP, respectively, were tested in this study. AK671, parental un-masked molecule with GAG-binding mutation is used as positive control. The structures of these molecules are shown in Example 8, and exact sequences in the sequence tables in Section 10.

Animals will be dosed by intravenous injection at 4 mL/kg on Days 0, 7, 14, and 21 at ~ 1.0 mL/min for a total of 4 doses. Plasma will be collected at various time point (until Day 56) for a full PK analysis.

PK analysis will be performed using Fc capture, Fc detect ELISA. Hematology, serum chemistry will also be performed. FACS analysis will be performed at Day 0 (pre-dose), Day 5 and Day 12 for the following markers: CD3, CD4, CD8, CD16, CD25, CD45, CD127, CD278, CD159a, FoxP3, and Ki67.

The results are shown in shows Figures 69-71.

- *PK*: Measurements of test articles in plasma were made using Meso Scale Discovery (MSD®) technology that employed anti-human IgG as the capture reagent and anti-human IgG as the detection reagent. Figure 69 showed drug level in plasmas from the first 7 days.
5
- *PD via flow cytometry*: Flow cytometry analysis was performed on peripheral blood pre-dose and at various timepoints post dose for T and NK cell proliferation status. MFI of proliferation marker Ki-67 was assessed in NK cells and CD8 T cells and peak changes were observed on day 14 post first dose administration. Ki-67 MFI of unmask AK671 was significantly increased in both NK cells and CD8 T cells in blood, whereas masked molecules did not show significant peripheral NK
10 or T cell activation. A one-way ANOVA Bonferonni's multiple comparison post-test was performed to determine the statistical significance of treatment vs Vehicle (*P<0.05; **P<0.01; ***P<0.001; ****P<0.0001). See results in Figure 70
- *Hematology and Serum chemistry*: Hematology and serum chemistry were performed on day 1 and day 5. (a) Transient decrease in absolute lymphocyte counts (day 1), RBC (day 8) and percentage of Hematocrit (HCT)(day 8) in blood was observed in all groups. All the observations were within normal range for both masked and unmasked molecules. (b) Unmasked AK671 increased serum ALT, AST and TBILI (biomarkers of liver function test), on Day 5 post first dose (not significant).
15
20 Masked molecules of AK667, AK921 and AK923 show relatively lower levels of ALT, AST and TBILI in serum. See results in Figures 71A and 71B (arrows indicate time of dosing).

10. SEQUENCES

Cleavable Linkers:

Component	SEQ ID NO	Sequence	DC
N terminal spacer domain	16	GGSGGS	AK380
			AK434
			AK666
			AK667
			AK918
			AK920
			AK924
			AK669
			AK922
			AK381
			AK382
			AK383
			AK384
			AK386
			AK446
			AK447
	17	GGSGSGGS	AK448
			AK528
			AK529
			AK604
			AK605
			AK606
			AK665
			AK668

Peptide substrate	[VPLSLY]	15	VPLS*LY (* indicates cleavage site)	AK919 AK921 AK925 AK670 AK923 AK380 AK381 AK382 AK383 AK384 AK386 AK434 AK446 AK447 AK448 AK528 AK529 AK604 AK605 AK606 AK666 AK665
				AK667 AK668
				AK918 AK919
	[MPYDLYHP]	41	MPYD*LYHP (* indicates cleavage site)	AK920 AK924 AK921 AK925
	[DSGGFMLT]	42	DSGG*FMLT (* indicates cleavage site)	
	[RAAAVKSP]	43	RAAA*VKSP (* indicates cleavage site)	

C terminal spacer domain	[ISSGLLSGRS]	44	ISSGLL*SGRS (* indicates cleavage site)	AK669 AK922 AK670 AK923
	[DLLAVVAAS]	45	DLLA*VVAAS (* indicates cleavage site)	
		18	SGP	AK380 AK381 AK382 AK383 AK384 AK386 AK434 AK446 AK447 AK448 AK528 AK529 AK604 AK605 AK606 AK666 AK667 AK918 AK920 AK924 AK669 AK922 AK665 AK668 AK919 AK921 AK925 AK670

Cleavable linker (L2)	19	GGSGGSVPLSLYSGP	AK923 AK380 AK434 AK666
	20	GGSGSGGSVPLSLYSGP	AK381 AK382 AK383 AK384 AK386 AK446 AK447 AK448 AK604 AK605 AK606 AK665 AK667 AK668
	46	GGSGGSMPYDLYHPSGP	AK918 AK919
	47	GGSGSGGSMPYDLYHPSGP	AK920 AK924 AK921 AK925
	48	GGSGSDSGGFMLTSGP	AK669 AK922 AK670 AK923
	49	GGSGSGGSDSGGFMLTSGP	
	50	GGSGSRAAAVKSPSGP	
	51	GGSGSGGSRAAAVKSPSGP	
	52	GGSGGSISGLLSGRSSGP	
	53	GGSGSGGSISGLLSGRSSGP	

Non-cleavable linkers:

Component Name	SEQ ID NO	Sequence	DC
Non-cleavable linker (L1)	12	GGGS	AK448
	13	GGGSGGGGS	AK380
			AK381
			AK382
			AK383
			AK384
			AK386
			AK434
			AK446
			AK528
			AK529
			AK604
			AK605
			AK606
			AK663
	14	GGSGGSG	AK447
			AK598
			AK600
			AK601
			AK924
			AK922
			AK925
			AK923
	54	GGSGSGSGSGSGSGP	AK598
			AK600
			AK601
			AK664
	55	PGSGSP	AK671
			AK666
			AK667

	56	GGSPG
		AK918 AK920 AK669 AK665 AK668 AK919 AK921 AK670 AK664

Cytokines:

Component	SEQ ID NO	Sequence	Molecule
hIL12B p40 subunit	1	IWELKKDVYVVELDWYPDAPGEMVVLTCDTPEEDGITWTLDSSEVLGSGKTLTIQVKEFGD AGQYTCHKGGEVLSHSLLLHKKEDGIWSTDILKDQKEPKNKTFLRCEAKNYSGRFTCWVLT TISTDLTFSVKSSRGSSDPQGVTCGAATLSAERVGRDNKEYEYSVECQEDSACPAAEESLPIEV MVDAVHKLKYENYTSFFIRDIKPPPKNLQLKPLKNSRQVEYSWEYPTWSTPHSYFSLTFC VQVQGKSKREKKDRVFTDKTSAIVICRKNASISVRAQDRYYSSSWSEWASVPCS	AK380 AK381 AK382 AK383 AK384 AK386 AK434 AK446 AK447 AK448 AK528 AK529 AK598 AK604 AK601
	57	IWELKKDVYVVELDWYPDAPGEMVVLTCDTPEEDGITW TLDSSEVLGSGKTLTIQVKEFGDAGQYTCCHKGGEVLSHSLLLHKKEDGIWSTDILKDQ	

IL-12 p40 subunit [KDNT ERV] IL-12		KEPKNKTFLRCEAKNYSGRFTCWVLTITSTDLTFSVKSSRGSSDPQGVTCGAATLSAERV RGDNKEYEYSVECEQEDSACPAAEESLPIEVMDAVHKLKYENYTSFFIRDIHKPDPKKN LQLKPLKNSRQVEVSWEPDWTSTPHSYFSLTFCVQVQVGKDNTERTVFTDKTSA TVIC RKNASISVRAQDRYYSSSWSEWASVPCS	AK600
	58	IWELKKDVVVELDWYDPAPGEMVVLTCDTPEEDGITW TLDQSSEVLGSGKTLTIQVKEFGAGQYTCHKGGEVLSHSLLLHHKKEDGIWSTDIKLDQ KEPKNKTFLRCEAKNYSGRFTCWVLTITSTDLTFSVKSSRGSSDPQGVTCGAATLSAERV RGDNKEYEYSVECEQEDSACPAAEESLPIEVMDAVHKLKYENYTSFFIRDIHKPDPKKN LQLKPLKNSRQVEVSWEPDWTSTPHSYFSLTFCVQVQVGKDNTERTVFTDKTSA TVIC RKNASISVRAQDRYYSSSWSEWASVPCS	
IL-12 p40 subunit [C252S]	59	IWELKKDVVVELDWYDPAPGEMVVLTCDTPEEDGITWLTLDQSSEVLGSGKTLTIQVKEFGD AGQYTCHKGGEVLSHSLLLHHKKEDGIWSTDIKLDQKEPKNKTFLRCEAKNYSGRFTCWVLT TISTDLTFSVKSSRGSSDPQGVTCGAATLSAERVGRDNKEYEYSVECEQEDSACPAAEESLPIEV MVDVHKLKYENYTSFFIRDIHKPDPKKNLQLKPLKNSRQVEVSWEPDWTSTPHSYFSLTFS VQVQGKSKREKKDRVFTDKTSA TVICRKNASISVRAQDRYYSSSWSEWASVPCS	AK605 AK606
	60	IWELKKDVVVELDWYDPAPGEMVVLTCDTPEEDGITWLTLDQSSEVLGSGKTLTIQVKEFGD AGQYTCHKGGEVLSHSLLLHHKKEDGIWSTDIKLDQKEPKNKTFLRCEAKNYSGRFTCWVLT TISTDLTFSVKSSRGSSDPQGVTCGAATLSAERVGRDNKEYEYSVECEQEDSACPAAEESLPIEV MVDVHKLKYENYTSFFIRDIHKPDPKKNLQLKPLKNSRQVEVSWEPDWTSTPHSYFSLTFS VQVQGKDNTERTVFTDKTSA TVICRKNASISVRAQDRYYSSSWSEWASVPCS	AK666 AK667 AK918 AK920 AK924 AK669 AK922 AK665 AK668 AK919 AK921 AK925 AK670 AK923 AK671 AK664
p40 subunit [KDNT EGRV] +			

Linker	3	GGGGSGGGSGGGGS	AK380 AK381 AK382 AK383 AK384 AK386 AK434 AK446 AK447 AK448 AK528 AK529 AK598 AK600 AK601 AK604 AK605 AK606 AK666 AK667 AK918 AK920 AK924 AK669 AK922 AK665 AK668 AK919 AK921 AK925 AK670 AK923 AK671 AK664
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hIL12A	IL-12 p35 subunit	2	RNLPVATPDGMPCLHHSQNLLRAVSNMLQKARQILEFYPCCTSEEDHEDIKDKTSTIVEAC LPLELIKNESCLNSRETSTFITNGSCLASRKTSFMMALCLSSIEDLKMYQVEFKTMNAKLLMD PKRQIFLDQNNMLAVIDELMQALNFNSETVPQKSSLEEDFYKTKIKLCILHAFRIRAVTIDRVM SYLNAS	AK944
				AK945
				AK947
				AK948
				AK380
				AK381
				AK382
				AK383
				AK384
				AK386
				AK434
				AK446
				AK447
				AK448
				AK528
				AK529
				AK598
				AK600
				AK601
				AK604
				AK605
				AK606
				AK666
				AK667
				AK918
				AK920
				AK924
				AK669
				AK922
				AK665
				AK668
				AK919
				AK921

Cytokine domain	hiL12B- hiL12A	4	IWELKKDYYVVVELDWYPDAPGEMVVLTCDTPEEDGITWTLDSQSEVLGSGKTLTIQVKEFGD AGQYTCHKGGEVLSHSLLLHHKKEDGIWSTDILKDQKEPKNKTFLRCEAKNYSGRFTCWWLT TISTDLTFSVKSSRGSSDPQGVTCGAATLSAERVGRDNKEYEYVECEQEDSACPAAEESLPIEV MVDAVHKLKYENYTSFFIRDIKDPKLNQLKPLKNSRQVEVSWEYPTWTSTPHSYFSLTF CVQVQGGKSKREKKDRVFTDKTSATVICRKNASISVRAQDRYYSSSWSEWASVPCSGGGGS GGGSGGGGSRNLPVATPDGMPFCLHHSQNLRAVSNMLQKARQITLFEYFCTSEIDHE DITKDKTSTVEACLPLELTKNESCLNSRETSTFTNGSCLASRKTSMFMALCLSSIYEDLKMYQV E FKTMNAKLLMDPKRQIFLDQNNMLAVIDELMQALNFNSETVPQKSSLEEDPFYKTIKILCLLH A FRIRAVTIDRVMSYLNAS	AK925 AK670 AK923 AK671 AK664 AK380 AK381 AK382 AK383 AK384 AK386 AK434 AK446 AK447 AK448 AK528 AK529 AK598 AK604
	hiL12B- hiL12A [KDNT ERV]	61	IWELKKDYYVVVELDWYPDAPGEMVVLTCDTPEEDGITWTLDSQSEVLGSGKTLTIQVKEFGD AGQYTCHKGGEVLSHSLLLHHKKEDGIWSTDILKDQKEPKNKTFLRCEAKNYSGRFTCWWLT TISTDLTFSVKSSRGSSDPQGVTCGAATLSAERVGRDNKEYEYVECEQEDSACPAAEESLPIEV MVDAVHKLKYENYTSFFIRDIKDPKLNQLKPLKNSRQVEVSWEYPTWTSTPHSYFSLTF CVQVQGGKDNTERVFTDKTSATVICRKNASISVRAQDRYYSSSWSEWASVPCSGGGGS GGGSGGGGSRNLPVATPDGMPFCLHHSQNLRAVSNMLQKARQITLFEYFCTSEIDHE DITKDKTSTVEACLPLELTKNESCLNSRETSTFTNGSCLASRKTSMFMALCLSSIYEDLKMYQV E	AK601

hL12B- hL12A [KDNT EGRV]		FKTMNAKLLMDPKRQIFLDQNNMLAVIDELMQALNFNSETVPQKSSLEEPDFYKTKIKLCILLH A FRIRAVTIDRVMSYLNAS	
	62	IWELKKDYYVVVVELDWYPDAPGEMVVLTCDTPEEDGITWTLDSQSEVLGSGKTLTIQVKEFGD AGQYTCHKGGEVLSHSLLLHKKEDGIWSTDILKDQKEPKNKTFLRCEAKNYSGRFTCWWT TISTDLTFSVKSSRGSDPQGVTCGAATLSAERVGRDNKEYEYSVEQEDSACPAAEESLPIEV MVDAVHKLKYENYTSFFIRDIKPPKLNQLKPLKNSRQVEVSWEYPTWSTPHSYFSLTF CVQVQKDNTEGRVFTDKTSAVICRKNASISVRAQDRYYSSSWSEWASVPCSGGGGG GGGGGGGSRNLPVATPDPGMFPCLLHHSQNLLRAVSNMLQKARQTLFYPCTSEEDHE DITKDKTSTVEACLPLELTKNESCLNSRETSTFTNGSCLASRKTSMMALCLSSIYEDLKMYQV E FKTMNAKLLMDPKRQIFLDQNNMLAVIDELMQALNFNSETVPQKSSLEEPDFYKTKIKLCILLH A FRIRAVTIDRVMSYLNAS	AK600
	63	IWELKKDYYVVVVELDWYPDAPGEMVVLTCDTPEEDGITWTLDSQSEVLGSGKTLTIQVKEFGD AGQYTCHKGGEVLSHSLLLHKKEDGIWSTDILKDQKEPKNKTFLRCEAKNYSGRFTCWWT TISTDLTFSVKSSRGSDPQGVTCGAATLSAERVGRDNKEYEYSVEQEDSACPAAEESLPIEV MVDAVHKLKYENYTSFFIRDIKPPKLNQLKPLKNSRQVEVSWEYPTWSTPHSYFSLTF VQVQKSKREKDRVFTDKTSAVICRKNASISVRAQDRYYSSSWSEWASVPCSGGGGGGG GGGGGGGSRNLPVATPDPGMFPCLLHHSQNLLRAVSNMLQKARQTLFYPCTSEEDHEDITDK TSTVEACLPLELTKNESCLNSRETSTFTNGSCLASRKTSMMALCLSSIYEDLKMYQVEFKTMN AKLLMDPKRQIFLDQNNMLAVIDELMQALNFNSETVPQKSSLEEPDFYKTKIKLCILLHAFRIRA VTIDRVMSYLNAS	AK605 AK606

			PLGDIKVSKL AGQLRMEWETPDNQVGAEVQFRHRTSPSPWKL GDCGPQDDDDTESCLCP LEMNV AQEFQLRRRLQSGQSSWSK WSSPVCVPPENPPQPVRESVEQLQDQGRRL TLKEQPTQLELEPGCQGLAPGTEVYRLQLHMLSCPCKAKATRTLHLGKMPYLSGAAY NVA VISSNQFGPLNQTHIPADTHTEPVALNISVGTTMYWPARAQSMTCYIEW QPVGDGGLATCSLTAQDPDPAGMATYSW/SRESGAMGQEK CYITIFASAHPEKLT WSTVLSYHFGGNASAAAGTPHHVSVKNHSLDSVSDWAPSLSTCPGVLEKYVVRDE DSKQVSEHPVQPTETQVTL SGLRAGVATVQVRADTAWLRGVWSQPQRFSIEVQVSD	AK382
IL12RB2(24 -212)	7		KIDACKRGDVTVKPSHVILLGSTVNITCSLKPRQGCFFHSRRNKLILYKFDRRINFHHGHS NSQVTGLPLGTTLFVCKLACINSDEIQICGAEHFVGAPEQPQNLSCIQKGEQGTVACTWE RGRDTHLYTEYTLQLSGPKNL TWQKQCKDIYCDYLDGFINLTPESPESNFTAKVTAVNSL GSSSL	AK528
IL12RB2(24 -222)	8		KIDACKRGDVTVKPSHVILLGSTVNITCSLKPRQGCFFHSRRNKLILYKFDRRINFHHGHS SQVTGLPLGTTLFVCKLACINSDEIQICGAEHFVGAPEQPQNLSCIQKGEQGTVACTWER GRDTHLYTEYTLQLSGPKNL TWQKQCKDIYCDYLDGFINLTPESPESNFTAKVTAVNSLGS SSSLPSTFTFLDIV	AK446
IL12RB2(24 -319)	9		KIDACKRGDVTVKPSHVILLGSTVNITCSLKPRQGCFFHSRRNKLILYKFDRRINFHHGHS NSQVTGLPLGTTLFVCKLACINSDEIQICGAEHFVGAPEQPQNLSCIQKGEQGTVACTW ERGRDTHLYTEYTLQLSGPKNL TWQKQCKDIYCDYLDGFINLTPESPESNFTAKVTAVNSL GSSSLPSTFTFLDIVRPLPPWDIRIKFQKASVSRCTLYWRDEGLVLLNRLRYRPSNSRLWNM VNVTKAKGRHDLDDLKPFTEYEFQISSKLHL YKGSWSDWSESIRAQTPEE	AK386 AK434 AK447 AK448 AK598 AK600 AK601 AK605
IL12RB2(24 -319) [C242S]	65		KIDACKRGDVTVKPSHVILLGSTVNITCSLKPRQGCFFHSRRNKLILYKFDRRINFHHGHS NSQVTGLPLGTTLFVCKLACINSDEIQICGAEHFVGAPEQPQNLSCIQKGEQGTVACTWER GRDTHLYTEYTLQLSGPKNL TWQKQCKDIYCDYLDGFINLTPESPESNFTAKVTAVNSL SSSLPSTFTFLDIVRPLPPWDIRIKFQKASVSRCTLYWRDEGLVLLNRLRYRPSNSRLWNM VNVTKAKGRHDLDDLKPFTEYEFQISSKLHL YKGSWSDWSESIRAQTPEE	AK604 AK606 AK666 AK667 AK918 AK920 AK924 AK669 AK922 AK665 AK668 AK919

				AK921 AK925 AK670 AK923 AK663 AK664
IL12RB2(24-622)	10		KIDACKRGDVTVKPSHVILLGSTVNITCSLKPRQGCIFYSRNKLILYKFDRRINFHHGHSLSNQVTGLPLGTTLVFVCKLACINSDEIQICGAEIFVGVAPEQPONLSQIQKGEQGTACTWGERGRDTHLYTEYTLQLSGPKNLTWQKQCKDIYCDYLDYFNGINLTPEPESNFTAKVTAVNSLGSSSL PSTFTFLDIVRPLPPWDIRIKFQKASVSRCTLYWRDEGLVLLNRLRYRPSNSRLWNMNVNVTAKGRHDLIDLKPFTEYEFQISSKHLHYKGSWSDWSESLRAQTPEEPTGMLDVWYMKRHIDYSRQISLFWKNLSVSEARGKILHYQVTQLQELTGKAMTQNTGHTSWTTVIPRTGNWAVA VSAANSKGSSLPTRINIMNLCEAGLLAPROVSANSEGMDNILVTWQPPKDPQSAVQEYVVE WRELHPGGDTQVPLNWLRSRPNVNSALISENKSICYEIRVYALSGDQGGSSILGNSKHKAP LSGPHINAITEEKGSLISWNSIPVQEQMGCLLHYRIYWKERDSNSQPLCEIPYRVSQNSHPIN SLQPRVITYVLWMTALTAAGESSHGNEREFCLOQKAN	AK383
IL12RB2(24-227)	11		KIDACKRGDVTVKPSHVILLGSTVNITCSLKPRQGCIFYSRNKLILYKFDRRINFHHGHSLSNQVTGVTGLTTLFVCKLACINSDEIQICGAEIFVGVAPEQPONLSQIQKGEQGTACTWGERGRDTHLYTEYTLQLSGPKNLTWQKQCKDIYCDYLDYFNGINLTPEPESNFTAKVTAVNSLGSSSLPP	AK529

Half-life extension domains:

Component	SEQ ID NO	Sequence	BC
1st Half life extension Hole: hFc(N297A)	25	DKTHTCPPCPAPPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYASTYRVVSVLTVLHQDWLNGKEYKCK VSNKALPAPIEKTIISKAKGQPREPQVCTLPPSRDELTKNQVSLSCAVKGFYPS DIAVEWESNGQPENNYKTTTPVLDSGGSFLLVSKLTVDKSRWQQGNVVFSCS	AK380 AK381 AK382 AK383

domain (HL1)		VMHEALHNHYTQKSLSPG	AK384 AK386 AK434 AK446 AK447 AK448 AK528 AK529 AK598 AK600 AK601 AK604 AK605 AK606 AK666 AK667 AK918 AK920 AK924 AK669 AK922 AK665 AK668 AK919 AK921 AK925 AK670 AK923 AK671 AK663 AK664
2nd Half life extension	Knob: hFc(N297A) 26	DKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPE VKFNWYVDGVEVHNAKTKPREEQYASTYRVVSVLTVLTQHQLNGKEYCK VSNKALPAPIEKTIISKAKGQPREPQVYTLPPCRDELTKNQVSLWCLVKGFYPS	AK380 AK381 AK382

domain (HL2)	DIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFS CS VMHEALHNHYTQKSLSLSPG	AK383 AK384 AK386 AK434 AK446 AK447 AK448 AK528 AK529 AK598 AK600 AK601 AK604 AK605 AK606 AK666 AK667 AK918 AK920 AK924 AK669 AK922 AK665 AK668 AK919 AK921 AK925 AK670 AK923 AK671 AK663 AK664
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Human IgG1 heavy chain constant gamma 1	21	ASTKGPSVFPLAPSSKSTSGGTAALGLCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSS GLYSSSVVTVFPSSSLGTQTYICNVNHKPSNTIKVDDKKVEPKSCDKTHITCPCPAPELLGG PSVFLPPKPKDITLMISRTPEVTCVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYN STYRVVSVLTVLHQDWLNGKEYCKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSRDE LTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTTPVLDSDGSFFLYSKLTVDKSRW QQGNVFSCSVMHEALHNHYTQKSLSLSPGK	
Human IgG1 heavy chain constant gamma 1 Fc domain	22	DKTHTCPPCPAPELLGG PSVFLPPKPKDITLMISRTPEVTCVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYN STYRVVSVLTVLHQDWLNGKEYCKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSRDE LTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTTPVLDSDGSFFLYSKLTVDKSRW QQGNVFSCSVMHEALHNHYTQKSLSLSPG	
Human IgG1 heavy chain constant gamma 1 Fc domain (Y349C; T366S; L38A; and Y407V)	23	DKTHTCPPCPAPELLGGPSVFLFPPKPKDITLMISRTPEVTCVVDVVSHE DPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWL NGKEYCKCKVSNKALPAPIEKTISKAKGQPREPQVCTLPISRDELTKNQ VSLSCAVKGFYPSDIAVEWESNGQPENNYKTTTPVLDSDGSFFLYSKL TVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPG	
Human IgG1 heavy chain constant gamma 1 Fc domain (S354C and T366W)	24	DKTHTCPPCPAPELLGGPSVFLFPPKPKDITLMISRTPEVTCVVDVVS EDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDW LNGKEYCKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPCRDELTK NQVSLWCLVKGFYPSDIAVEWESNGQPENNYKTTTPVLDSDGSFFL YSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPG	

Human IgG1 heavy chain constant gamma 1 Fc domain (Y349C, T366S, L38A, Y407V, N297A and I253A)	27	DKTHTCPPCPAPELLGGPSVFLFPPKPKDITLMASRTPEVTCVVDVSHEDP EVKFNWYVDGVEVHNAKTKPREEQYASTYRVVSVLTVLHQDWLNGKE YKCKVSNKALPAPIEKTISKAKGQPREPQVCTLPISRDELTKNQVSLSCA VKGFYPSDIAVEWESNGQPENNYKTTTPVLDSDGSFFLVSKLTVDKSRW QQGNVFSCSVMHEALHNHYTQKSLSLSPG	
Human IgG1 heavy chain constant gamma 1 Fc domain (S354C, T366W, N297A and I253A)	28	DKTHTCPPCPAPELLGGPSVFLFPPKPKDITLMASRTPEVTCVVDVSHEDP EVKFNWYVDGVEVHNAKTKPREEQYASTYRVVSVLTVLHQDWLNGKEY KCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPCRDELTKNQVSLWCLVK GFYPSDIAVEWESNGQPENNYKTTTPVLDSDGSFFLVSKLTVDKSRW QQGNVFSCSVMHEALHNHYTQKSLSLSPG	

Cleavage products:

Product released by cleavage	SEQ ID NO	DC
LYSGPIWELKKDYYVVELDWYYPDAPGEMVVLTCDTPEEDGITWTLTQSSSEVLGSG KTLTIQVKEFGDAGQYTCHKGGEVLSHSLLLHHKKEDGIWSTDILKDQKEPKNKITFL RCEAKNYSGRFTCWWLTTISTDLTFSVKSSRGSSDPQGVTCGAATLSAERVGRDN KEYEYSVEQEDSACPAAEESLPIEVMDAVHKLKYENYTSSFFIRDIKPDPPKNLQ LKPLNSRQVEVSWEYPTWSTPHSYFSLTFCVQVQGKSKREKKDRVFTDKTSAT VICRKNASISVRAQDRYYSSSWSEWASVPCSGGGGGGGGSRNLPVATPD PGMFPCLLHHSQNLRAVSNMLQKARQTLFYPCTSEEDHEDITKDKTSTVEACL LELTKNESCLNSRETSTFNGSCLASRKTSMFMALCLSSIYEDLKMYQVEFKTMNAK LLMDPKRQIFLDQNMLAVIDELMQALNFNSETVPOKSSLEEDFYKTKIKLCILLHA FRIRAVTIDRVMSYLNAS	29	AK380 AK381 AK383 AK434 AK447 AK448 AK528 AK529 AK382 AK384 AK386 AK446 AK604
LYSGPIWELKKDYYVVELDWYYPDAPGEMVVLTCDTPEEDGITWTLTQSSSEVLGSG KTLTIQVKEFGDAGQYTCHKGGEVLSHSLLLHHKKEDGIWSTDILKDQKEPKNKITFL RCEAKNYSGRFTCWWLTTISTDLTFSVKSSRGSSDPQGVTCGAATLSAERVGRDN EYEYSVEQEDSACPAAEESLPIEVMDAVHKLKYENYTSSFFIRDIKPDPPKNLQ KPLNSRQVEVSWEYPTWSTPHSYFSLTFCVQVQGKSKREKKDRVFTDKTSATVI CRKNASISVRAQDRYYSSSWSEWASVPCSGGGGGGGGSRNLPVATPDPMF PCLHHSQNLRAVSNMLQKARQTLFYPCTSEEDHEDITKDKTSTVEACLPLETKN ESCLNSRETSTFNGSCLASRKTSMFMALCLSSIYEDLKMYQVEFKTMNAKLLMDPK RQIFLDQNMLAVIDELMQALNFNSETVPOKSSLEEDFYKTKIKLCILLHAFRIRAVTI DRVMSYLNAS	66	AK605 AK606
LYSGPIWELKKDYYVVELDWYYPDAPGEMVVLTCDTPEEDGITWTLTQSSSEVLGSG KTLTIQVKEFGDAGQYTCHKGGEVLSHSLLLHHKKEDGIWSTDILKDQKEPKNKITFL RCEAKNYSGRFTCWWLTTISTDLTFSVKSSRGSSDPQGVTCGAATLSAERVGRDN KEYEYSVEQEDSACPAAEESLPIEVMDAVHKLKYENYTSSFFIRDIKPDPPKNLQ LKPLNSRQVEVSWEYPTWSTPHSYFSLTFCVQVQGKSKREKKDRVFTDKTSATVI CRKNASISVRAQDRYYSSSWSEWASVPCSGGGGGGGGSRNLPVATPDPMF MFPCLHHSQNLRAVSNMLQKARQTLFYPCTSEEDHEDITKDKTSTVEACLPLE	67	AK665

LTKNESCLNSRETSTFNGSCLASRKTSMFMALCLSSIYEDLKMYQVEFKTMNAKLL MDPKRQIFLDQNMLAVIDELMQALNFNSETVPQKSSLEEDFYKTKIKLCILLHAF RIRAVTIDRVMSYLNAS			
LYSGPIWELKKDVVVELDWYPDAPGEMVVLTCDTPEEDGITWTLQSSSEVLGSG KTLTIQVKEFGDAGQYCHKGGEVLSHSLILLHKKEDGIWSTDILKDQKEPKNKTF LRCEAKNYSGRFTCWWTITSTDLTFSVKSSRGSDPQGVTCGAATLSAERVGRDN LRCENYSEVCEQEDSACPAAEESLPIEMVMDAVHKLKYENYTSFFIRDIKPDPPKNLQ KEYEVSVEQEDSACPAAEESLPIEMVMDAVHKLKYENYTSFFIRDIKPDPPKNLQ LKLKNSRQVEVSWEPDTWSTPHSYFSLTFSVQVQKDNTEGRVFTDKTSA TVIC RKNASISVRAQDRYYSSSWSEWASVPCSGGGGGGGGGGSRNLPVATPDPGM FPLHHSQNLRAVSNMLQKARQTLFYPCTSEEDHEDITDKTSTVEACLPLELT KNESCLNSRETSTFNGSCLASRKTSMFMALCLSSIYEDLKMYQVEFKTMNAKLLM DPKROIFLDQNMLAVIDELMQALNFNSETVPQKSSLEEDFYKTKIKLCILLHAFRIR AVTIDRVMSYLNAS	68		AK666
LYHPSGPIWELKKDVVVELDWYPDAPGEMVVLTCDTPEEDGITWTLQSSSEVLG SGKTLTIQVKEFGDAGQYCHKGGEVLSHSLILLHKKEDGIWSTDILKDQKEPKNK TFLRCEAKNYSGRFTCWWTITSTDLTFSVKSSRGSDPQGVTCGAATLSAERVGRG DNKEYEVSVEQEDSACPAAEESLPIEMVMDAVHKLKYENYTSFFIRDIKPDPPKN LQKPLKNSRQVEVSWEPDTWSTPHSYFSLTFSVQVQKDNTEGRVFTDKTSA T VICRKNASISVRAQDRYYSSSWSEWASVPCSGGGGGGGGGGSRNLPVATPDP GMFPCPLHHSQNLRAVSNMLQKARQTLFYPCTSEEDHEDITDKTSTVEACLPLE LTKNESCLNSRETSTFNGSCLASRKTSMFMALCLSSIYEDLKMYQVEFKTMNAKLL MDPKRQIFLDQNMLAVIDELMQALNFNSETVPQKSSLEEDFYKTKIKLCILLHAFRI RAVTIDRVMSYLNAS	69		AK667
LYHPSGPIWELKKDVVVELDWYPDAPGEMVVLTCDTPEEDGITWTLQSSSEVLG SGKTLTIQVKEFGDAGQYCHKGGEVLSHSLILLHKKEDGIWSTDILKDQKEPKNK TFLRCEAKNYSGRFTCWWTITSTDLTFSVKSSRGSDPQGVTCGAATLSAERVGRG DNKEYEVSVEQEDSACPAAEESLPIEMVMDAVHKLKYENYTSFFIRDIKPDPPKN LQKPLKNSRQVEVSWEPDTWSTPHSYFSLTFSVQVQKDNTEGRVFTDKTSA TVIC RKNASISVRAQDRYYSSSWSEWASVPCSGGGGGGGGGGSRNLPVATPDPGMF PCLHHSQNLRAVSNMLQKARQTLFYPCTSEEDHEDITDKTSTVEACLPLELT SCLNSRETSTFNGSCLASRKTSMFMALCLSSIYEDLKMYQVEFKTMNAKLLMDPKR QIFLDQNMLAVIDELMQALNFNSETVPQKSSLEEDFYKTKIKLCILLHAFRIRAVTI DRVMSYLNAS	70		AK668
SGRSSGPIWELKKDVVVELDWYPDAPGEMVVLTCDTPEEDGITWTLQSSSEVLG SGKTLTIQVKEFGDAGQYCHKGGEVLSHSLILLHKKEDGIWSTDILKDQKEPKNK	71		AK669

FLRCEAKNYSGRFTCWWLTTISTDLTFSVKSSRGSDPQGVTCGAATLSAERVGRD NKEYEYSEVCEQEDSACPAAEESLPIEVMDAVHKLKYENYTSFFIRDIKDPKPKNL QLKPLKNSRQVEVSWEPDTWSTPHSYFSLTFSVQVQKDNTEGRVFTDKTSA TV ICRKNASISVRAQDRYYSSSWSEWASVPCSGGGGGGGGSRNLPVATPDPG MFPCLLHHSQNLRAVSNMLQKARQTLFYPCTSEEDHEDITKDKTSTVEACLPLEL TKNESCLNSRETSTNGSCLASRKTSMFMALCLSSIIYEDLKMYQVEFKTMNAKLL MDPKRQIFLDQNMMLAVIDELMQALNFNSETVTPQKSSLEEDFYKTKIKLCILLHAFR IRAVTIDRVMSYLNAS		
SGRSSGPIWELKKDVVVELDWYPDAPGEMVVLTCDTPEEDGITWTLDOSSSEVLGS GKTLTIQVKEFGDAGQYTCCHKGGEVLSHSLLLHKKEDGIWSTDILKDQKEPKNKT FLRCEAKNYSGRFTCWWLTTISTDLTFSVKSSRGSDPQGVTCGAATLSAERVGRDN KEYEYSEVCEQEDSACPAAEESLPIEVMDAVHKLKYENYTSFFIRDIKDPKPKNLQ LKPLKNSRQVEVSWEPDTWSTPHSYFSLTFSVQVQKDNTEGRVFTDKTSA TVIC RKNASISVRAQDRYYSSSWSEWASVPCSGGGGGGGGSRNLPVATPDPGM FPCLLHHSQNLRAVSNMLQKARQTLFYPCTSEEDHEDITKDKTSTVEACLPLELT KNESCLNSRETSTNGSCLASRKTSMFMALCLSSIIYEDLKMYQVEFKTMNAKLLMD PKRQIFLDQNMMLAVIDELMQALNFNSETVTPQKSSLEEDFYKTKIKLCILLHAFRIR VITIDRVMSYLNAS	72	AK 670
SGRSSGPIWELKKDVVVELDWYPDAPGEMVVLTCDTPEEDGITWTLDOSSSEVLGS GKTLTIQVKEFGDAGQYTCCHKGGEVLSHSLLLHKKEDGIWSTDILKDQKEPKNKT FLRCEAKNYSGRFTCWWLTTISTDLTFSVKSSRGSDPQGVTCGAATLSAERVGRD NKEYEYSEVCEQEDSACPAAEESLPIEVMDAVHKLKYENYTSFFIRDIKDPKPKNL QLKPLKNSRQVEVSWEPDTWSTPHSYFSLTFSVQVQKDNTEGRVFTDKTSA TVI CRKNASISVRAQDRYYSSSWSEWASVPCSGGGGGGGGSRNLPVATPDPG MFPCLLHHSQNLRAVSNMLQKARQTLFYPCTSEEDHEDITKDKTSTVEACLPLEL TKNESCLNSRETSTNGSCLASRKTSMFMALCLSSIIYEDLKMYQVEFKTMNAKLL MDPKRQIFLDQNMMLAVIDELMQALNFNSETVTPQKSSLEEDFYKTKIKLCILLHAF RIRAVTIDRVMSYLNAS	73	AK 922
SGRSSGPIWELKKDVVVELDWYPDAPGEMVVLTCDTPEEDGITWTLDOSSSEVLGS GKTLTIQVKEFGDAGQYTCCHKGGEVLSHSLLLHKKEDGIWSTDILKDQKEPKNKT LRCEAKNYSGRFTCWWLTTISTDLTFSVKSSRGSDPQGVTCGAATLSAERVGRDNK EYSEYSEVCEQEDSACPAAEESLPIEVMDAVHKLKYENYTSFFIRDIKDPKPKNLQ KPLKNSRQVEVSWEPDTWSTPHSYFSLTFSVQVQKDNTEGRVFTDKTSA TVICRK NASISVRAQDRYYSSSWSEWASVPCSGGGGGGGGSRNLPVATPDPGMFPCLL HHSQNLRAVSNMLQKARQTLFYPCTSEEDHEDITKDKTSTVEACLPLELTKNESC LNSRETSTNGSCLASRKTSMFMALCLSSIIYEDLKMYQVEFKTMNAKLLMDPKRQIF	74	AK 923

LDQNMMLAVIDELMQALNFNSETVPQKSSLEEDPFYKTKIKLCILLHAFRIRAVTIDRV MSYLNAS		
FMLTSGPIWELKDDVYVVELDWYPDAPGEMVVLTCDTPEEDGITWTLQDSSEVLG SGKTLTIQVKEFGDAGQYTCCHKGGEVLSHSLLLHKKEDGIWSTDILKDQKEPKNKTF TFLRCEAKNYSGRFTCWWTITISTDLTFSVKSSRGSDPQGVTCGAATLSAERVGRDNK DNKEYEYSEVCEQEDSACPAAEESLPIEVMDVAVHKLKYENYTSFFIRDIKPDPPK NLQKPLKNSRQVEVSWEPDTWSTPHSYFSLTFSVQVQCKDNTEGRVFTDKTSA TVICRKNASIVRAQDRYYSSSWSEWASVPCSGGGGGGGGGGSRNLPVATPD PGMFCLHHSQNLRLRAVSNMLQKARQTLFYPCTSEEDHEDITKDKTSTVEACL LELTKNESCLNSRETSTFNGSCLASRKTSMFMALCLSSYEDLKMYYQVEFKTMNA KLLMDPKRQIFLDQNMMLAVIDELMQALNFNSETVPQKSSLEEDPFYKTKIKLCILLH AFRIRAVTIDRVMSYLNAS	75	AK918
FMLTSGPIWELKDDVYVVELDWYPDAPGEMVVLTCDTPEEDGITWTLQDSSEVLG GKTLTIQVKEFGDAGQYTCCHKGGEVLSHSLLLHKKEDGIWSTDILKDQKEPKNKTF LRCEAKNYSGRFTCWWTITISTDLTFSVKSSRGSDPQGVTCGAATLSAERVGRDNK EYEVSEVCEQEDSACPAAEESLPIEVMDVAVHKLKYENYTSFFIRDIKPDPPKNLQ KPLKNSRQVEVSWEPDTWSTPHSYFSLTFSVQVQCKDNTEGRVFTDKTSA TVICR KNASIVRAQDRYYSSSWSEWASVPCSGGGGGGGGGGSRNLPVATPDPMFP CLHHSQNLRLRAVSNMLQKARQTLFYPCTSEEDHEDITKDKTSTVEACLPLELTKNE SCLNSRETSTFNGSCLASRKTSMFMALCLSSYEDLKMYYQVEFKTMNAKLLMDPKR QIFLDQNMMLAVIDELMQALNFNSETVPQKSSLEEDPFYKTKIKLCILLHAFRIRAVTI DRVMSYLNAS	76	AK919
VKSPSGPIWELKDDVYVVELDWYPDAPGEMVVLTCDTPEEDGITWTLQDSSEVLG KTLTIQVKEFGDAGQYTCCHKGGEVLSHSLLLHKKEDGIWSTDILKDQKEPKNKTF CEAKNYSGRFTCWWTITISTDLTFSVKSSRGSDPQGVTCGAATLSAERVGRDNKEY EYEVSEVCEQEDSACPAAEESLPIEVMDVAVHKLKYENYTSFFIRDIKPDPPKNLQKPL KNSRQVEVSWEPDTWSTPHSYFSLTFSVQVQCKDNTEGRVFTDKTSA TVICRKNAS ISVRAQDRYYSSSWSEWASVPCSGGGGGGGGGGSRNLPVATPDPMFPCLHH SQNLRLRAVSNMLQKARQTLFYPCTSEEDHEDITKDKTSTVEACLPLELTKNESCLNS RETSTFNGSCLASRKTSMFMALCLSSYEDLKMYYQVEFKTMNAKLLMDPKRQIFLDQ NMLAVIDELMQALNFNSETVPQKSSLEEDPFYKTKIKLCILLHAFRIRAVTIDRVMSYLNAS	77	AK920
VKSPSGPIWELKDDVYVVELDWYPDAPGEMVVLTCDTPEEDGITWTLQDSSEVLG KTLTIQVKEFGDAGQYTCCHKGGEVLSHSLLLHKKEDGIWSTDILKDQKEPKNKTF CEAKNYSGRFTCWWTITISTDLTFSVKSSRGSDPQGVTCGAATLSAERVGRDNKEYE YSEVCEQEDSACPAAEESLPIEVMDVAVHKLKYENYTSFFIRDIKPDPPKNLQKPLKN SRQVEVSWEPDTWSTPHSYFSLTFSVQVQCKDNTEGRVFTDKTSA TVICRKNASISV	78	AK921

RAQDRYYSSSWSEWASVPCSGGGGGGGGGSRNLPAVATPDPGMFPCLLHHSQNLL RAVSNMLQKARQTLFYPCTSEEDHEDITKDKTSTVEACLPLELTKNESCLNSRETSTF ITNGSCLASRKTSEMMALCLSSYEDLKMYQVEFKTMNAKLLMDPKRQIFLDQNMLA VIDELMQALNFNSETVPQKSSLEPDPFYKTKIKLCILHAFRIRAVTIDRVMSYLNAS		
VKSPSGPIWELKKDVVYVVELDWYPDAPGEMVVLTCDTPEEDGITWTLDSQSEVLGS GKTLTIQVKEFGDAGQYTKHKGGEVLSHSLLLHKKEDGIWSTDLKDKQKEPKNKT FLRCEAKNYSGRFTCWWTITSTDLTFSVKSSRGSSDPQGVTCGAATLSAERVGRDN KEYEYSVEQEDSACPAAEESLPIEVMDAVHKLKYENYTSFFIRDIKPDPPKNLQ LKPLKNSRQVEVSWEPDWTSTPHSYFSLTFSVQVQKDNTEGRVFTDKTSA TVICR KNASISVRAQDRYYSSSWSEWASVPCSGGGGGGGGGSRNLPAVATPDPGMFP CLHHSQNLLRAVSNMLQKARQTLFYPCTSEEDHEDITKDKTSTVEACLPLELTKNE SCLNSRETSTFNGSCLASRKTSEMMALCLSSYEDLKMYQVEFKTMNAKLLMDPKR QIFLDQNMLAVIDELMQALNFNSETVPQKSSLEPDPFYKTKIKLCILHAFRIRAVTI DRVMSYLNAS	79	AK924
VKSPSGPIWELKKDVVYVVELDWYPDAPGEMVVLTCDTPEEDGITWTLDSQSEVLGS GKTLTIQVKEFGDAGQYTKHKGGEVLSHSLLLHKKEDGIWSTDLKDKQKEPKNKT FLRCEAKNYSGRFTCWWTITSTDLTFSVKSSRGSSDPQGVTCGAATLSAERVGRDN KEYEYSVEQEDSACPAAEESLPIEVMDAVHKLKYENYTSFFIRDIKPDPPKNLQ LKPLKNSRQVEVSWEPDWTSTPHSYFSLTFSVQVQKDNTEGRVFTDKTSA TVICR KNASISVRAQDRYYSSSWSEWASVPCSGGGGGGGGGSRNLPAVATPDPGMFP CLHHSQNLLRAVSNMLQKARQTLFYPCTSEEDHEDITKDKTSTVEACLPLELTKNE LNSRETSTFNGSCLASRKTSEMMALCLSSYEDLKMYQVEFKTMNAKLLMDPKRQIF LDQNMLAVIDELMQALNFNSETVPQKSSLEPDPFYKTKIKLCILHAFRIRAVTIDRV MSYLNAS	80	AK925

Full sequences:

Component	SEQ ID	Full Sequence		DC
	NO			

HL1-L1-MM	Hole: hFc(N297A)- hCD212(24-237)	30	DKTHTCPPCAPPELLGGPSVFLFPKPKDMLMISRTPEVTCVVVDVSHEDPEVKFNW YVDGVEVHNATKPREEQYASTYRVSVSLTVLHQDWLNGKEYCKVSNKALPAPIE KTISKAKGQPREPQVCTLPSPRDELTKNQVLSLCAVKGFYPSDIAVEWESNGQPENN YKTTTPVLDSDGSFFLVSKLTVDKSRWQOGNVFSCVMHEALHNHYTQKSLSLSPG GGGSGGGGSCRTSECCFQDPPYPDADSGSASGPRDLRCYRISDRYECVSWQYEGP TAGVSHFLRCCLSSGRCCYFAAGSATRLQFSDQAGVSVLYTVTLWVESWARNQTEK SPEVTLQLYNSVKYEPPLGDIKVSCLAGQLRMEWETPDNQVGAEVQFRHRTSPSP WKLGDGCPQDDDTESCLCPLEMNVAQEFQLRRRQLGSGQSSWSKWSSPVCVPPENP	AK384	DNA410
	Hole: hFc(N297A)- hCD212(24-545)	31	DKTHTCPPCAPPELLGGPSVFLFPKPKDMLMISRTPEVTCVVVDVSHEDPEVKFN WYVDGVEVHNATKPREEQYASTYRVSVSLTVLHQDWLNGKEYCKVSNKALP APIEKTISKAKGQPREPQVCTLPSPRDELTKNQVLSLCAVKGFYPSDIAVEWESNG QPENNYKTTTPVLDSDGSFFLVSKLTVDKSRWQOGNVFSCVMHEALHNHYT QKSLSLSPGGGGGSCRTSECCFQDPPYPDADSGSASGPRDLRCYRISDRYECVSWQYEGP TAGVSHFLRCCLSSGRCCYFAAGSATRLQFSDQAGVSVLYTVTLWVESWARNQTEK SPEVTLQLYNSVKYEPPLGDIKVSCLAGQLRMEWETPDNQVGAEVQFRHRTSPSP WKLGDGCPQDDDTESCLCPLEMNVAQEFQLRRRQLGSGQSSWSKWSSPVCVPPENP	AK380 AK381 AK382	DNA409
	Hole: hFc(N297A)- IL12RB2(24-212)	32	DKTHTCPPCAPPELLGGPSVFLFPKPKDMLMISRTPEVTCVVVDVSHEDPEVKFN WYVDGVEVHNATKPREEQYASTYRVSVSLTVLHQDWLNGKEYCKVSNKALPAP IEKTISKAKGQPREPQVCTLPSPRDELTKNQVLSLCAVKGFYPSDIAVEWESNGQPE NNYKTTTPVLDSDGSFFLVSKLTVDKSRWQOGNVFSCVMHEALHNHYTQKSLSLSP GGGSGGGGSKIDACKRGDVTVPKPSHVILLGSTVNITCSLKPRQGCCHYSRRNKLI LYKFRDRINFHHHSHLSNSQVTLPLGLTFLVCKLACINSDEIQICGAEIFVGVAPQEP QNLSCIQKGEQGTVACTWGRDTHLYTEYTLQLSGPKNLTWQKQCKDIYCDYLD GINLTPESPESNFTAKVTA VNSLGSSSSL	AK528	DNA629

Hole: hF _Q (N297A)- IL12RB2(24-222)	33	DKTHTCPPCAPPELLGGPSVFLFPPKPKD TLMISRTPEVTCVVVDVSHEDPEVKFNWY VDGVEVHNAAKTPREEQYASTYRVVSVLTVLHQDWLNGKEYCKVSNKALPAPIEKT SKAKGQPREPQVCTLPSPRDELTKNQVSLSCAVKGFYPSDIAVEWESNGQPENNYKT PPVLDSDGSFELLVSKLTVDKSRWQQGNVFCSCVMHEALHNHYTQKSLSLSPGGGG SGGGGSKIDACKRGDVTVPKSHVILLGSTVNTCSLKPRQGCCHYSRRNKLILYKFDRI NFHHGHSLSNSQVTGLPLGTTFLVCKLACINSDEIQICGAEIFVGVAPQPNLS GTVACTWERGRDTHLYTEYTLQLSGPKNLTWQKQCKDIYCDYLDGFINLTPESNFT AKVTAVNSLGSSSSLPSTFELDIV	AK446	DNA539
Hole: hF _Q (N297A)- IL12RB2(24-319)	34	DKTHTCPPCAPPELLGGPSVFLFPPKPKD TLMISRTPEVTCVVVDVSHEDPEVKFNWY VDGVEVHNAAKTPREEQYASTYRVVSVLTVLHQDWLNGKEYCKVSNKALPAPIEKT ISKAKGQPREPQVCTLPSPRDELTKNQVSLSCAVKGFYPSDIAVEWESNGQPENNYK TTPPVLDSDGSFELLVSKLTVDKSRWQQGNVFCSCVMHEALHNHYTQKSLSLSPGGG GGGGGSKIDACKRGDVTVPKSHVILLGSTVNTCSLKPRQGCCHYSRRNKLILYKF DRRINFHHGHSLSNSQVTGLPLGTTFLVCKLACINSDEIQICGAEIFVGVAPQPNLS CIQKGEQGTVACTWERGRDTHLYTEYTLQLSGPKNLTWQKQCKDIYCDYLDGFINL TPESNFTAKVTAVNSLGSSSSLPSTFELDIVRPLPPWDIRIKFQKASVSRCTLYWR DEGLVLLNRLRYRPSNRLWNMNVNVTAKGRHDLIDLKPFTEYEFQISSKHLHYKGSW SDWSESRAQTPEE	AK386 AK434 AK605	DNA426
	35	DKTHTCPPCAPPELLGGPSVFLFPPKPKD TLMISRTPEVTCVVVDVSHEDPEVKF NWYVDGVEVHNAAKTPREEQYASTYRVVSVLTVLHQDWLNGKEYCKVSNKA LPAPIEKTISKAKGQPREPQVCTLPSPRDELTKNQVSLSCAVKGFYPSDIAVEWES NGQPENNYKTTPPVLDSDGSFELLVSKLTVDKSRWQQGNVFCSCVMHEALHNH YTQKSLSLSPGGGSKIDACKRGDVTVPKSHVILLGSTVNTCSLKPRQGCCHY SRRNKLILYKFDRIINFHHGHSLSNSQVTGLPLGTTFLVCKLACINSDEIQICGAEIFV GVAPQPNLS CKDIYCDYLDGFINLTPESNFTAKVTAVNSLGSSSSLPSTFELDIVRPLPPW DIRIKFQKASVSRCTLYWRDEGLVLLNRLRYRPSNRLWNMNVNVTAKGRHDL IDLKPFTEYEFQISSKHLHYKGSWSDWSESRAQTPEE	AK448	DNA541

36		DKTHTCPPCAPPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVK FNWYVDGVEVHNAKTKPREEQYASTYRVVSVLTVLHQDWLNGKEYKCKVSN KALPAPIEKTISKAKGQPREPQVCTLPSPRDELTKNQVSLSCAVKGFYPSDIAVE WESNGQPENNYKTTTPVLDSDGSFFLVSKLTVDKSRWQQGNVFCSSVMHEA LHNHYTQKSLSLSPGSGGGGSKIDACKRGDVTVPKPSHVLGSLVNITCSLKPR QGCFHYSRRNKLLIKFDRRINFHHGHSLSNSQVTLGLPLGTTLLFVCKLACINSDEIQI CGAEIFVGVAPEQPQNLSICQKGEQGTACTWGERGRDTHLYTEYTLQLSGPKN LTWQKQCKDIYCDYLDGFINLTPESPESNFTAKVTAVNSLGSSSSLPSTFTFLDIV RPLPPWDIRIKFQKASVSRCTLYWRDEGLVLLNRLRYRPSNSRLWNMNVNVT AKGRHDILLDKPFTYEYFQISSKLLHYKGSWSDWSESLRAQTPEE	AK447 AK598 AK600 AK601	DNA540
37	Hole: hf _q (N297A)- IL12RB2(24-622)	DKTHTCPPCAPPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPE VKFNWYVDGVEVHNAKTKPREEQYASTYRVVSVLTVLHQDWLNGKEYKCK VSNKALPAPIEKTISKAKGQPREPQVCTLPSPRDELTKNQVSLSCAVKGFYP SDIAVEWESNGQPENNYKTTTPVLDSDGSFFLVSKLTVDKSRWQQGNVFS CSVMHEALHNHYTQKSLSLSPGSGGGGSKIDACKRGDVTVPKPSHVL LGSTVNITCSLKPRQCFHYSRRNKLLIKFDRRINFHHGHSLSNSQVTLGLPLG TTLLFVCKLACINSDEIQICGAEIFVGVAPEQPQNLSICQKGEQGTACTWGER GRDTHLYTEYTLQLSGPKNLTWQKQCKDIYCDYLDGFINLTPESPESNFTAK VTAVNSLGSSSSLPSTFTFLDIVRPLPPWDIRIKFQKASVSRCTLYWRDEGLV LLNRLRYRPSNSRLWNMNVNVTAKGRHDILLDKPFTYEYFQISSKLLHYKGS WSDWSESLRAQTPEEPTGMLDVWYMKRHDYSRQQISLFWKNLSVSEAR GKILHYQVTLQELTGCKAMTQNTGHTSWTTPRTGNWAVAVSAANSKG SSLPTRINIMNLCEAGLLAPQVSAANSEGMNILLVTPQPRKDPASAVQEVV EWRELHPGGDTQVPLNWLRSRPNVVSALISENIKSYICEYRYYALSQDQGGC SSILGNSKHKAPLSGPHINATEEKGSILISWNSIPVQEQMGCLLHYRIYWKER DSNSQPQLCEIPYRVVSQNSHPNSLQPRVTYVLMWMTALTAAGESSHGNERE FCLQKKAN	AK383	DNA425
38	Hole: hf _q (N297A)- IL12RB2(24-227)	DKTHTCPPCAPPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVK FNWYVDGVEVHNAKTKPREEQYASTYRVVSVLTVLHQDWLNGKEYKCKVSN KALPAPIEKTISKAKGQPREPQVCTLPSPRDELTKNQVSLSCAVKGFYPSDIAVE WESNGQPENNYKTTTPVLDSDGSFFLVSKLTVDKSRWQQGNVFCSSVMHEA LHNHYTQKSLSLSPGSGGGGSKIDACKRGDVTVPKPSHVLGSLVNITCS LKPRQCFHYSRRNKLLIKFDRRINFHHGHSLSNSQVTLGLPLGTTLLFVCKLACIN SDEIQICGAEIFVGVAPEQPQNLSICQKGEQGTACTWGERGRDTHLYTEYTLQL SGPKNLTWQKQCKDIYCDYLDGFINLTPESPESNFTAKVTAVNSLGSSSSLPSTF TFLDIVRPLPP	AK529	DNA644

Hole: hf _c (N297A)- IL12RB2(24-319) [C242S]	81	DKTHTCPPCAPPELLGGPSVFLFPPKPKDITLMISRTPEVTCVVVDVSHEDPEVK FNWYVDGVEVHNAKTKPREEQASTYRVSVLTVLHQDWLNGKEYKCKVVS NKAAPAEIKTISKAKGQPREPQVCTLPSPRDELTKNQVSLSCAVKGFYPSDIA VEWESNGQPENNYKTTPVLDSDGSFELVSKLTVDKSRWQQGNVFCSSVMH EALHNHYTQKSLSLSPGSGGSGGSKIDACKRGDVTVKPSHVILLGSTVNI TCSLKPRQGCFFHYSRRNKLILYKFDRRINFHHGHSLSNSQVTLGLTGLTFVCK LACINSEIQICGAEIFVGAPEQPQNLSIQKGEQGTVACTWGRDTHLYTE YTLQLSGPKNLTWQKQCKDIYCDYLDGFINLTPESPESNFTAKVTAVNSLSSSS SLPSTFTFLDIVRPLPPWDIRIKFQKASVSRSTLYWRDEGLVLLNRLRYRPSNSR LWNMNVNVTAKGRHDLDDLKPFTEYEFQISSKLHLKGSWSDWSESLRAQTPEE	AK604 AK606	DNA729
Hole: hf _c (N297A)- IL12RB2(24-319) [C242S]	82	DKTHTCPPCAPPELLGGPSVFLFPPKPKDITLMISRTPEVTCVVVDVSHEDPEVK FNWYVDGVEVHNAKTKPREEQASTYRVSVLTVLHQDWLNGKEYKCKVVS NKAAPAEIKTISKAKGQPREPQVCTLPSPRDELTKNQVSLSCAVKGFYPSDIAV EWESNGQPENNYKTTPVLDSDGSFELVSKLTVDKSRWQQGNVFCSSVMHEA LHNHYTQKSLSLSPGSGGSGPKIDACKRGDVTVKPSHVILLGSTVNITCSLKPRQ GCFHYSRRNKLILYKFDRRINFHHGHSLSNSQVTLGLTGLTFVCKLACINSEI QICGAEIFVGAPEQPQNLSIQKGEQGTVACTWGRDTHLYTEYTLQLSGP KNLTWQKQCKDIYCDYLDGFINLTPESPESNFTAKVTAVNSLSSSSLPSTFTFL DIVRPLPPWDIRIKFQKASVSRSTLYWRDEGLVLLNRLRYRPSNSRLWNMNVNVT KAKGRHDLDDLKPFTEYEFQISSKLHLKGSWSDWSESLRAQTPEE	AK665 AK668 AK670 AK919 AK921	DNA835
Hole: hf _c (N297A)- IL12RB2(24-319) [C242S]	83	DKTHTCPPCAPPELLGGPSVFLFPPKPKDITLMISRTPEVTCVVVDVSHEDPEVK FNWYVDGVEVHNAKTKPREEQASTYRVSVLTVLHQDWLNGKEYKCKVVS NKAAPAEIKTISKAKGQPREPQVCTLPSPRDELTKNQVSLSCAVKGFYPSDIA VEWESNGQPENNYKTTPVLDSDGSFELVSKLTVDKSRWQQGNVFCSSVMH EALHNHYTQKSLSLSPGSGGSGPKIDACKRGDVTVKPSHVILLGSTVNITCSLK PRQGCFFHYSRRNKLILYKFDRRINFHHGHSLSNSQVTLGLTGLTFVCKLACINS DEIQICGAEIFVGAPEQPQNLSIQKGEQGTVACTWGRDTHLYTEYTLQLS GPKNLTWQKQCKDIYCDYLDGFINLTPESPESNFTAKVTAVNSLSSSSLPSTF TFLDIVRPLPPWDIRIKFQKASVSRSTLYWRDEGLVLLNRLRYRPSNSRLWNM VNVTKAKGRHDLDDLKPFTEYEFQISSKLHLKGSWSDWSESLRAQTPEE	AK666 AK667 AK669 AK918 AK920	DNA838

		VSNMLQKARQTLFYPCTSEEDHEDITKDKTSTVEACPLELTKNESCLNSRETS FITNGSCLASRKTSFMMALCLSSIEDLKMVQVEFKTMNAKLLMDPKRQIFLDQ NMLAVIDELMQALNFNSETVPOKSSLEEDFYKTKIKLCILLHAFIRAVTIDRVM SYLNAS	AK382	DNA408
Knob: hFc(N297A)- [non-cleavable]- IL-12	85	DKTHTCPPCAPPELLGGPSVFLFPPKPKDTLMISRTPEVTC VVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYASTY RVVSVLTVLHQDWLNGKEYCKVSNKALPAPIEKTIISKAK GQPREPQVYTLPPCRDELTKNQVSLWCLVKGFYPSDIAVE WESNGQPENNYKTPPVLDSDGSFFLYSKLTVDKSRWQQ GNVFCSCVMHEALHNHYTQKSLSLSPGGGGSGGGSGSG GSSGPIWELKKDYVVELDWPDPAPGEMVVLTCDTPEED GITWLDQSSEVLGSGKTLTQVKEFGDAGQYTCBKGGEVL SHSLLLHKKEDGIWSTDILKDKKEPKNKTLRCEAKNYS RFTCWWTITSTDLTFSVKSSRSGDPQGVTCGAATLSAERV RGDNKEYEYSVEQEDSACPAAEESLPIEVMDAVHKLKYE NYTSSFFIRDIKPPKNIQLKPKNSRQVSEVSEYPTWS TPHSYFSLTFCVQVQKSKREKKDRVFTDKTSATVICRKNAS ISVRAQDRYYSSSWSEWASVPCSGGGGGSGGGSGGSRNL PVATPDGPMFPCLHSONLLRAVSNMLQKARQTLFYPCTSEE IDHEDITKDKTSTVEACPLELTKNESCLNSRETSFINGSLASRKT SFMMALCLSSIEDLKMVQVEFKTMNAKLLMDPKRQIFLDQNMIL AVIDELMQALNFNSETVPOKSSLEEDFYKTKIKLCILLHAFIRA VTIDRVMSYLNAS	AK598	DNA707
Knob: hFc(N297A)- [non-cleavable]- IL-12 GAG mutant KDNTERV	86	DKTHTCPPCAPPELLGGPSVFLFPPKPKDTLMISRTPEVTCVV DVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYASTYRVVSVL TVLHQDWLNGKEYCKVSNKALPAPIEKTIISKAKGQPREPQV YTLPPCRDELTKNQVSLWCLVKGFYPSDIAVEWESNGQPEN YKTTTPVLDSDGSFFLYSKLTVDKSRWQQGNVFCSCVMHEAL HNHYTQKSLSLSPGGGGSGGGSGGGSGGPIWELKKDYVVV ELDWYDPAPGEMVVLTCDTPEEDGITWLDQSSEVLGSGKTLT IQVKEFGDAGQYTCBKGGEVLSSHLLHKKEDGIWSTDILKDKQ KEPKNKTLRCEAKNYSGRFTCWWTITSTDLTFSVKSSRSGSD PQGVTCGAATLSAERVGRDNKEYEYSVEQEDSACPAAEESLP IEVMYDAVHKLKYEYNTSSFFIRDIKPPKNIQLKPKNSRQV	AK600	DNA708

		EVSWYPTDITWSTPHSYSLTFCVQVQKNDTERVFDKTSATV ICRKNASISVRAQDRYYSWSEWASVPCSGGGGGGGGGGG GGSRNLPAVTPDGMFPCLHHSQNLRLRAVSNMLQKARQITLIFY PCTSEEDHEDITKDKTSTVEACLPLELTKNESCLNSRSTSFITNGS CLASRKTSMMALCLSSIYEDLKMVQVEFKTMNAKLLMDPKRQI FLDQNMALVIDELMQALNFNSETVPQKSSLEEDFYKTKIKLCIL LHAERIRAVTIDRVMSYLNAS	AK601	DNA709
Knob: hF _Q (N297A)- [non-cleavable]- IL-12 GAG mutant KDNTGRV	87	DKTHTPCPAPELLGGPSVFLFPPKPKDITLMISRTPEVTCVVVDVS HEDPEVKFNWYVDGVEVHNAKTKPREEQYASTYRVVSVLTVLHQ DWLNGKEYKCKVSNKALPAPIEKTKSKAKGPQREPQVYTLPPCRDE LTKNQVSLWCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDG SFFLYSKLTVDKSRWQQGNVFCSSVMHEALHNHYTQKSLSLSPGG GGSGGGGGGGSGGPIWELKKDYVVVELDWYDPAGEMVVLIT CDTPEEDGHTWLDQSSEVLGSGKTLTIQVKEFGDAGQYCHKGGE VLSHSLLLHKKEDGIWSTDILKDKQEPKNTTLRCEAKNYSGRFT CWWLTTISTDLTFSVSSRSGSDPQGVTCGAATLSAERVGRDNKEY EYSVECEDDSACPAAEESLPIEVMDVAVHKLKYENYTSFFIRDIKP DPKKNLQKPLKNSRQVEVSEWYEDTWTSTPHSYSLTFCVQVQGGK DNTEGRVFDKTSATVICRKNASISVRAQDRYYSWSEWASVPCSG GGSGGGGGGGGGSRNLPAVTPDGMFPCLHHSQNLRLRAVSNMLQ KARQITLIFYPCTSEEDHEDITKDKTSTVEACLPLELTKNESCLNSRET SFTNGSCLASRKTSMMALCLSSIYEDLKMVQVEFKTMNAKLLMDP KRQIFLDQNMALVIDELMQALNFNSETVPQKSSLEEDFYKTKIKLCIL HAFRIRAVTIDRVMSYLNAS	AK605 AK606	DNA719
Knob: hF _Q (N297A)- [VPLSLY]- hIL12B-hIL12A [C252S]	88	DKTHTPCPAPELLGGPSVFLFPPKPKDITLMISRTPEVTCVVVDVSHED PEVKFNWYVDGVEVHNAKTKPREEQYASTYRVVSVLTVLHQDWLNG KEYCKVSNKALPAPIEKTKSKAKGPQREPQVYTLPPCRDELTKNQVSL WCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVD KSRWQQGNVFCSSVMHEALHNHYTQKSLSLSPGGGGGGGGSVPLSLY SGPIWELKKDYVVVELDWYDPAGEMVVLITCDTPEEDGHTWLDQSSE VLGSGKTLTIQVKEFGDAGQYCHKGGEVLSHSLLLHKKEDGIWSTD ILKDKQEPKNTTLRCEAKNYSGRFTCWWLTTISTDLTFSVSSRSGSD PQGVTCGAATLSAERVGRDNKEYEYSVECEDDSACPAAEESLPIEVMDV DAVHKLKYENYTSFFIRDIKPDPKKNLQKPLKNSRQVEVSEWYEDT WSTPHSYFSLTFSVQVQGGKSKREKKDRVFDKTSATVICRKNASISVRA QDRYYSWSEWASVPCSGGGGGGGGGGGGGSRNLPAVTPDGMFPCC		

		LHHSQNLLRAVSNMLQKARQTFLEYPCTSEEDHEDITKDKTSTVEACL PLELTKNESCLNSRETSTFTNGSCLASRKTSFMMALCLSSIEDLKMYQV EFKTMNAKLLMDPKRQIFLDQNMLAVIDELMQALNFNSETVPQKSSLEE PDFYKTKIKLCILLHAFRIRAVTIDRVMSYLNAS	AK665	DNA830
Knob: hFc(N297A)- [VPLSLYSG]-IL- 12 (p40C252S) GAG mutant KDNTGRV	89	DKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHED PEVKFNWYVDGVEVHNAKTKPREEQYASTYRVVSVLTVLTQHDWLNKG EYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPCRDELTKNQVSLWC LVKGFPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSR WQOQGNVFCSCVMHEALHNYHQKLSLSPGGGGGGSGGVPLSLYSGP IWELKDDVYVVELDWPDPAGEMVVLTCDTPEEDGITWLDQSSEVLG SGKTLTIQKEFGDAGQYCHKGGEVLSHLLLLHKKEDGIWSTDILKDKQ KEPKNKTFELRCEAKNYSGRFTCWWTITISDITFSVKSSRGSDPQGVTC GAATLSAERVGRDNKEYEYSEVCEQEDSACPAAEESLPIEVMVDAVHKLK YENYTSFFIRDIKPPKLNQKPLKNSRQVEVSWEPDTWSTPHSYFS LTFVQVQKDNTEGRVFTDKTSATVICRKNASVRAQDRYYSWSE WASVPCSGGGGGGGGGGRNLPVATPDGMPFCLHHSQNLLRAVS NMLQKARQTFLEYPCTSEEDHEDITKDKTSTVEACLPLELTKNESCLNSR ETSTFTNGSCLASRKTSFMMALCLSSIEDLKMYQVEFKTMNAKLLMDP KRQIFLDQNMLAVIDELMQALNFNSETVPQKSSLEEDFYKTKIKLCILLH AFRIRAVTIDRVMSYLNAS	AK666	DNA831
Knob: hFc(N297A)- [VPLSLYSG]-IL- 12 (p40C252S) GAG mutant KDNTGRV	90	DKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDP EVKFNWYVDGVEVHNAKTKPREEQYASTYRVVSVLTVLTQHDWLNKGEY KCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPCRDELTKNQVSLWCLV KGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQ QGNVFCSCVMHEALHNYHQKLSLSPGGGGGGSGGVPLSLYSGPIWELKKD VYVVELDWPDPAGEMVVLTCDTPEEDGITWLDQSSEVLGSGKTLTIQV KEFGDAGQYCHKGGEVLSHLLLLHKKEDGIWSTDILKDKQKEPKNKTFELR CEAKNYSGRFTCWWTITISDITFSVKSSRGSDPQGVTCGAATLSAERV GDNKEYEYSEVCEQEDSACPAAEESLPIEVMVDAVHKLKYENYTSFFIRDI KPDPPKLNQKPLKNSRQVEVSWEPDTWSTPHSYFSLTFVQVQKDKNT EGRVFTDKTSATVICRKNASVRAQDRYYSWSEWASVPCSGGGGGGGG GSGGGGSRNLPVATPDGMPFCLHHSQNLLRAVSNMLQKARQTFLEYPCT SEEDHEDITKDKTSTVEACLPLELTKNESCLNSRETSTFTNGSCLASRKTSFM MALCLSSIEDLKMYQVEFKTMNAKLLMDPKRQIFLDQNMLAVIDELMQA		

		LNENSETVPQKSSLEEDFYKTKIKLCILLHAFRIRAVTIDRVMSYLNAS		
Knob: hFc(N297A)- [MPYDLYHP]- IL-12 (p40C252S) GAG mutant KDNTGRV	91	DKTHTCPPCAPPELLGGPSVFLFPPKPKDITLMISRTPEVTCVVVDVSHEDPE VKFNWYVDGVEVHNAKTKPREEQASTYRVVSVLTVLHQDWLNGKEYKC KVSNAKALPAIEKTSKAKGQPREPQVYTLPPCRDELTKNQVSLWCLVKGFY PSDIAVEWESNGQPENNYKTTPVLDSDGSFFLYSKLTVDKSRWQQGNVFS CSVMHEALHNHYTQKSLSLSPGGSGGSMYPYDLYHPSGPIWELKKDYYV ELDWYPDAPGEMVVLTCDTPEEDGITWLDQSSEVLGSGKTLTIQVKEFGD AGQYTCCHKGGVLSHLLHKKEDGIWSTDIKDKQKEPKNKTFLRCEAKN YSGRFTCWWLTTISDITFSVKSRSRGSDDPGVTCGAATLSAERVGRDNKEY EYSVEQEDSACPAAEESLPIEVMVDAVHKLKYNITSSFFIRDIHKDPKKNL QLKPLKNSRQVEVSWEPDTWSTPHSYFSLTFSVQVQGGKDNTEGRVFTDKTS ATVICRKNASISVRAQDRYYSSSWSEWASVPCSGGGGGGGSGGSRNLP VATPDPGMFPCPLHHSQNLRAVSNMLQKARQTLFYPCTSEEDHEDITKDK TSTVEACLPLELTKNESCLNSRETSHITNGSCLASRKTSMMALCLSSIYEDLK MYQVEFKTMNAKLLMDPKRQIFLDQNNMLAVIDELMQALNFNSETVPQKSS LEEDFYKTKIKLCILLHAFRIRAVTIDRVMSYLNAS	AK667	DNA842
Knob: hFc(N297A)- [MPYDLYHP]- IL-12 (p40C252S) GAG mutant KDNTGRV	92	DKTHTCPPCAPPELLGGPSVFLFPPKPKDITLMISRTPEVTCVVVDVSHEDPEVK FNWYVDGVEVHNAKTKPREEQASTYRVVSVLTVLHQDWLNGKEYCKVS NKALPAIEKTSKAKGQPREPQVYTLPPCRDELTKNQVSLWCLVKGFYPSDI AVEWESNGQPENNYKTTPVLDSDGSFFLYSKLTVDKSRWQQGNVFSVSM HEALHNHYTQKSLSLSPGGSGGSMYPYDLYHPSGPIWELKKDYYVVE LDWYPDAPGEMVVLTCDTPEEDGITWLDQSSEVLGSGKTLTIQVKEFGDA GQYTCCHKGGVLSHLLHKKEDGIWSTDIKDKQKEPKNKTFLRCEAKNY SGRFTCWWLTTISDITFSVKSRSRGSDDPGVTCGAATLSAERVGRDNKEYE YSVEQEDSACPAAEESLPIEVMVDAVHKLKYNITSSFFIRDIHKDPKKNLQ LKPLKNSRQVEVSWEPDTWSTPHSYFSLTFSVQVQGGKDNTEGRVFTDKTSA TVICRKNASISVRAQDRYYSSSWSEWASVPCSGGGGGGGSGGSRNLP VATPDPGMFPCPLHHSQNLRAVSNMLQKARQTLFYPCTSEEDHEDITKDK TSTVEACLPLELTKNESCLNSRETSHITNGSCLASRKTSMMALCLSSIYEDLK MYQVEFKTMNAKLLMDPKRQIFLDQNNMLAVIDELMQALNFNSETVPQKSS LEEDFYKTKIKLCILLHAFRIRAVTIDRVMSYLNAS	AK668	DNA843

Knob: hFc(N297A)- [ISSGLLSGRS]- IL-12 (p40C252S) GAG mutant KDNTTEGRV	93	DKTHTCPPCAPPELLGGPSVFLFPPKPKDITLMISRTPEVTCVVVDVSHEDPEV KFNWYVDGVEVHNAKTKPREEQASTYRVVSVLTVTLHQDWLNGKEYCK VSNKALPAPIEKTISKAKGQPREPQVYTLPPCRDELTKNQVSLWCLVKGFYP SDIAVEWESNGQPENNYKTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFC SVMHEALHNHYTQKSLSLSPGSGSGSSISGLLSGRSSGPIWELKKDYVVE LDWYPDAPGEMVVLTCDTPEEDGITWLDQSSEVLGSGKTLTIQVKEFGDA GQYTCHKGGEVLSHLLHKKEDGIWSTDILKDKQKEPKNKTLRCEAKNY SGRFTCWWLTTISTDLTFSVKSRRGSSDPQGVTCGAATLSAERVRGDNKEYE YSVEQEDSACPAEESLPIEVMDAVHKLKYNENYTSFFIRDIKPDPPKNL QLKPLKNSRQVEVSWYPTDTPHSHYFSLTFSVQVQGGKDNTEGRVFTDKT SATVICRKNASISVRAQDRYYSSSWSEWASVPCSGGGSGGGSGGGSRNL PVATPDGPMFPCLHHSQNLRAVSNMLOKARQITLFEYPCITSEEDHEDITKD KTSTVEACLPLELTKNESCLNSRETSTFTNGSCLASRKTSTFMMALCLSYEDLK MYQVEFKTMNAKLLMDPKRQIFLDQNNMLAVIDELMQALNFNSETVPQKSS LEEPDFYKTKIKLCILLHAFRIRAVTIDRVMSYLNAS	AK669 AK922 DNA850
Knob: hFc(N297A)- [ISSGLLSGRS]- IL-12 (p40C252S) GAG mutant KDNTTEGRV	94	DKTHTCPPCAPPELLGGPSVFLFPPKPKDITLMISRTPEVTCVVVDVSHEDPEV KFNWYVDGVEVHNAKTKPREEQASTYRVVSVLTVTLHQDWLNGKEYCK VSNKALPAPIEKTISKAKGQPREPQVYTLPPCRDELTKNQVSLWCLVKGFYPS DIAVEWESNGQPENNYKTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFCSS VMHEALHNHYTQKSLSLSPGSGSGSGSSISGLLSGRSSGPIWELKKDYVY VELDWYPDAPGEMVVLTCDTPEEDGITWLDQSSEVLGSGKTLTIQVKEFG DAGQYTCHKGGEVLSHLLHKKEDGIWSTDILKDKQKEPKNKTLRCEAK NYSGRFTCWWLTTISTDLTFSVKSRRGSSDPQGVTCGAATLSAERVRGDNKE YEYSEVQEDSACPAEESLPIEVMDAVHKLKYNENYTSFFIRDIKPDPPK NLQLKPLKNSRQVEVSWYPTDTPHSHYFSLTFSVQVQGGKDNTEGRVFTD KTSATVICRKNASISVRAQDRYYSSSWSEWASVPCSGGGSGGGSGGGSS RNLPAVTPDPMFPCLHHSQNLRAVSNMLOKARQITLFEYPCITSEEDHED ITKDKTSTVEACLPLELTKNESCLNSRETSTFTNGSCLASRKTSTFMMALCLSSI YEDLKMYQVEFKTMNAKLLMDPKRQIFLDQNNMLAVIDELMQALNFNSE TVPOKSSLEEDFYKTKIKLCILLHAFRIRAVTIDRVMSYLNAS	AK670 AK923 DNA851
Knob: hFc(N297A)- [DSGGFMLTJ]- IL-12 (p40C252S) GAG mutant KDNTTEGRV	95	DKTHTCPPCAPPELLGGPSVFLFPPKPKDITLMISRTPEVTCVVVDVSHEDPEV KFNWYVDGVEVHNAKTKPREEQASTYRVVSVLTVTLHQDWLNGKEYCK KVSNAKALPAPIEKTISKAKGQPREPQVYTLPPCRDELTKNQVSLWCLVKGF YPSDIAVEWESNGQPENNYKTPPVLDSDGSFFLYSKLTVDKSRWQQGNVF SCSVMHEALHNHYTQKSLSLSPGSGSGSGSSISGLLSGRSSGPIWELKKDYVY VELDWYPDAPGEMVVLTCDTPEEDGITWLDQSSEVLGSGKTLTIQVKEFGD	AK918 DNA844

Knob: hF(N297A)- [DSGGFMT]- IL-12 (p40C252S) GAG mutant KDNTGRV	96	AGQYTCCHKGGVLSHSLLLHHKKEDGIWSTDILKDQKEPKNKTFRLCEAKNY SGRFTCWLTITISDLTFSVKSRRGSSDPQVTCGAATLSAERVGRDNKEYE YSVEQEDSACPAEESLPIEVMDVAVHLKLYENYTSFFIRDIHKDPKPNLQ LKPLKNSRQVEVSEWYPTWSTPHSYFSLTFSVQVQKDNTEGRVFTDKTSA TVICRKNASISVRAQDRYYSSSWSEWASVPCSGGGGGSGGSGSRNLPV ATPDGMFPCLHHSQNLLRAVSNMLQKARQTLFYPCTSEEDHEDITKDK TSTVEACLPLELTKNESCLNSRETSHINGSCLSARKTSEMMALCLSSIEDLKM YQVEFTMNAKLLMDPKRQIFLDQNMMLAVIDELMQALNFNSETVPQKSS LEEDFYKTKIKLCILLHAFIRAVTIDRVMSYLNAS	AK919	DNA845
Knob: hF(N297A)- [RAAAVKSP]- IL-12 (p40C252S) GAG mutant KDNTGRV	97	DKHTHTCPAPPELLGGPSVFLFPPKPKDITLMISRTPEVTCVVVDVSHEDP EVKFNWYVDGVEVHNAKTKPREEQASTYRVVSVLTVLHQDWLNGKE YKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPCRDELTKNQVSLWCL VKGFYPSDIAVEWESNGQPENNYKTTPVLDSDGSFFLYSKLTVDKSRWQ QGNVFCSCVMHEALHNYTQKSLSLSPGGGGSGGSDSGGFMLTSGPI WELKKDYYVVELDWYDPAGEMVVLTCDTPEEDGITWLDQSSEVLGS GKTLTIQVKEFGDAGQYTHKGGEVLSHLLHHKKEDGIWSTDILKDQK EPKNKTLRLCEAKNYSGRFTCWWTITISDLTFSVKSRRGSSDPQVTCGA ATLSAERVGRDNKEYESVEQEDSACPAEESLPIEVMDVAVHLKLYE NYTSFFIRDIHKDPKPNLQKPLKNSRQVEVSEWYPTWSTPHSYFSLTFS VQVQKDNTEGRVFTDKTATVICRKNASISVRAQDRYYSSSWSEWASVP CSGGGGSGGGGGSGSRNLPVATPDGMFPCLHHSQNLLRAVSNMLQK ARQTLFYPCTSEEDHEDITKDKTSTVEACLPLELTKNESCLNSRETSTFN GSCLASRKTSEMMALCLSSIEDLKMVQVEFKTMNAKLLMDPKRQIFLD QNMLAVIDELMQALNFNSETVPQKSSLEEDFYKTKIKLCILLHAFIRAV TIDRVMSYLNAS	AK920 AK924	DNA846

		PCSGGGGGGGGGSRNLPVATPDGMPFCLHHSQNLLRAVSNMLQ KARQTLFYPCTSEEDHEDITKDKTSTVEACLPLELTKNESCLNSRSTFI TNGSCLASRKTSFMALCLSSIYEDLKMVQVEFKTMNAKLLMDPKRQIF LDQNMMLAVIDELMQALNFNSETVPQKSSLEEPDFYKTKIKLCILHAFRIR AVTIDRVMSYLNAS		
Knob: hFc(N297A)- [RAAAVK SPI- IL-12 (p40C252S) GAG mutant KDNTGRV	98	DKTHTCPPCAPPELLGGPSVFLFPPKPKDITLMISRTPEVTCVVVDVSHEDPEV KFNWYVDGVEVHNAKTKPREEQYASTYRVVSVLTVLHQDWLNGKEYKC KVSNAKALPAIEKTISKAKGQPREPQVYTLPPCRDELTKNQVSLWCLVKGF YPSDIAVEWESNGQPENNYKTTPPVLDSDGSFYLTKLTVDKSRWQQGNV FSCVMHEALHNHYTQKSLSLSPGGGGGGGSRAAAASVSPGPIWELKKD VYVVVELDWYPDAPGEMVVLTCDTPEEDGHTWLDQSSEVLGSGKTLTIQV KEFGDAGQVYCHKGGEVLSHSLILLHKKEDGIWSTDILKDKQEPKNTFL RCEAKNYSGRFTCWWLTTISTDLTFSVKSRRGSSDPQGVTCGAATLSAERV RGDNKEYEYSVEQCQEDSACPAAEESLPIEVVMVDAVHKLKYENYTSFFIRD IHKPPPKNIQLKPLKNSRQVEVSWEYPTWSTPHSYFSLSFVSQVQGGKN TEGRVFTDKTSATVICRKNASISVRAQDRYYSSSWSEWASVPCSGGGGGGG GGSGGGGGSRNLPVATPDGMPFCLHHSQNLLRAVSNMLQKARQTLFYP TSEEDHEDITKDKTSTVEACLPLELTKNESCLNSRSTFTNGSCLASRKTSF MMALCLSSIYEDLKMVQVEFKTMNAKLLMDPKRQIFLDQNMMLAVIDELM QALNFNSETVPQKSSLEEPDFYKTKIKLCILHAFRIRAVTIDRVMSYLNAS	AK921 AK925	DNA847

11. LIST OF CONSTRUCTS

The table below shows the full sequences for molecules labelled by 'AK' reference number. The component parts of the sequence are also shown as well as the order in which they are assembled in the chains of the molecules. Individual chains are labelled by a 'DNA' reference number.

cule name	newnames	Fc sequence	Linker	Component3Sequence
3	DNA406	DKTHTCPCPAPELLGGPSVFLFPPKPKDTLMISRT PEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTK PREEQYASTYRVVSVLTVLHQDWLNGKEYKCKVSN Knob: hFc(N297A)- [VPLSLY]-hil12B- hil12A	GGSGGS	VPLSLY
		KALPAPIEKTISKAKGQPREPQVYTLPPCRDELTKN QVSLWCLVKGFYPSDIAVEWESNGQPENNYKTTTP PVLDSGGSFFLYSKLTVDKSRWQQGNVFCFSVMH EALHNHYTQKSLSLSPG		
3	DNA409	DKTHTCPCPAPELLGGPSVFLFPPKPKDTLMISRT PEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTK PREEQYASTYRVVSVLTVLHQDWLNGKEYKCKVSN Hole: hFc(N297A)- hCD212(24-545)	GGSGSGGGGS	CRTSECCFQDPPYPDADSGSASGPRDLRCYRISSDRECS EGPTAGVSHFLRCCLSSGRCCYFAAGSATRLQFSDQAGV TVTLWVESWARNQTEKSPVTLQLVNSVKYEPPLGDIKV GQLRMEWETPDNQVGAEVQFRHRTSPSPWKLGDGCP- DTESCLCPLEMNVAQEFQLRRRQLGSGSGSSWSKWSSPA PENPPQPQVRFVSEQLGQDGRRLTLKEQPTQLELPEGC APGTEVYRLQLHMLSCPCAKATRTLHLGKMPYLSGAF AVISSNQFGPLNQTWHIPADTHTEPVALNISVGTNGTT WPARAQSMTYCIEWQPVGQDGGGLATCSLTAPQDPPD/ ATYSWSRESGAMGQEKCYITIFASAHPEKLTLSVTLST GGNAAAGTPHHVSVKNHSLDSVVDWAPSLSTCPGV VVRCRDEDSKQVSEHPVQPTETQVTLGLRAGVAYTVQ' TAWLRGVWSQPQRFSEIVQVSD
		KALPAPIEKTISKAKGQPREPQVCTLLPPSRDELTKN QVSLCAVKGFPYSDIAVEWESNGQPENNYKTTTP VLDSDGSFFLVSKLTVDKSRWQQGNVFCFSVMHE ALHNHYTQKSLSLSPG		

1	DNA407	Knob: hFc(N297A)- [VPLSLY]-hil.12B- hil.12A	DKTHTCPPCPAPELLGGPSVLFPPKPKDTLMISRT PEVTCVVDVSHEDPEVKFNWYVDGVEVHNAKTK PREEQYASTYRVVSVLTVLHQDWLNGKEYKCKVSN KALPAIEKTIKAKGQPREPQVYTLPPCRDELTKN QVSLWCCLKGFYPSDIAVEWESNGQPENNYKTTTP PVLDSDGSFFLYSKLTVDKSRWQQGNVFCFSVMH EALHNHYTQKSLSLSPG	GGSGSGSGS	VPLSLY
1	DNA409	Hole: hFc(N297A)- hCD212(24-545)	DKTHTCPPCPAPELLGGPSVLFPPKPKDTLMISRT PEVTCVVDVSHEDPEVKFNWYVDGVEVHNAKTK PREEQYASTYRVVSVLTVLHQDWLNGKEYKCKVSN KALPAIEKTIKAKGQPREPQVCTLPSPRDELTKN QVSLSCAVKGFYPSDIAVEWESNGQPENNYKTTTP VLDSGGSFFLVSKLTVDKSRWQQGNVFCFSVMHE ALHNHYTQKSLSLSPG	GGSGSGSGGS	

CRTSECCFQPPYPDADSGSASGPRDLRCYRISSDRYEC
EGPTAGVSHFLRCLSSGRCCYFAAGSATRLQFSDQAGV
TVTLWVESWARNQTEKSPVTLQLYNSVKYEPPLGDIKV
GQLRMEWETPDNQVGAEVQFRHRTPTSSPWKLGDCGP
DTESCLCLEMNVAQEFQLRRRQLGSGSGSSWSKWSSPA
PENPPQPQVRFVSVEQLGQDGRRLTLKEOPTQLELPEGC
APGTEVTYRLQLHMLSCPCAKATRTLHLGKMPYLSGA
AVISSNQFGPLNQTLWHIPADTHTEPVALNISVGTNGTT
WPARAQSMITYCEWQPVGGDGGGLATCSLTAPQDPDP
ATYSWSRESGAMGQEKCYITIFASAHPEKLTWSTVLST
GGNASAACTPHHVSVKNHSLDSVSDWAPSLSTCPGV
VWRCDEDSKQVSEHPVQPTETQVTLTSLGLRAGVAYTVQ
TAWLRGVWSQQRFSIEVQVSD

3	DNA407	Knob: hFc(N297A)- [VPLSLY]-hIL12B- hIL12A	DKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRT PEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTK PREEQYASTYRVVSVLTVLHQDWLNGKEYKCKVSN KALPAPIEKTISKAKGQPREPQVYTLPPCRDELTKN QVSLWCCLVKGFPYSDIAVEWESNGQPENNYKTTTP PVLDSDGSGFFLYSKLTVDKSRWQQGQNVFSCSVMH EALHNHYTQKSLSLSPG	GGGGSGSGGS	VPLSLY
3	DNA425	Hole: hFc(N297A)- IL12RB2(24-622)	DKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRT PEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTK PREEQYASTYRVVSVLTVLHQDWLNGKEYKCKVSN KALPAPIEKTISKAKGQPREPQVCTLPISRDELTKN QVSLSCAVKGFYPSDIAVEWESNGQPENNYKTTTP VLDSDGSGFFLVSKLTVDKSRWQQGQNVFSCSVMIH ALHNHYTQKSLSLSPG	GGGGSGGGGS	

KIDACKRGDVTVKPSHVILLGSTVNITCSLKPRQGCIFYSF
LILYKFDRRINFHHGHSLSNSQVTGLPLGTTTLFVCKLACINS
CGAEIFVGVAPEQPQNLSQKQGEQGTVACTWGERGDT
EYTLQSGPKNLTWQKQCKDIYCDYLDYDFGINLTPESPESN
VTAVNSLSSSSSLPSTFTFLDIVRPLPPWDIRIKFQKASVSI
YWRDEGLVLLNRLRVRPSNRLWNVMVNTKAKGRHDL
PFTYEYFQISSKHLHYKGSWSDWSESLRAQTPEEPTGMI
WYMKRHDYSRQQISLFWKNLSVSEARGKILHYQVTLQE
GKAMTQNTIGHTSWTTVIPRTGNWAVAVSAANSKGSS
NIMNLCEAGLLAPRQVSANSEGMNDNILVTWQPPRKDP
EYVVEWRELHPGGDTQVPLNWLRSRPYNVSALISENIKS
EIRVYALSGDQGGCSSILGNSKHKAPLSGPHINAITEEKG
WNSIPVQEQMGCLLHYRIYWKERDSNSQPLCEIPYRV
HPINSLQPRVTYVVLWMTALTAAAGESHGNEREFCCLQGGK

4	DNA407	Knob: hFc(N297A)- [VPLSLY]-hil12B- hil12A	DKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRT PEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTK PREEQYASTYRVVSVLTVLHQDWLNGKEYKCKVSN KALPAPIEKTISKAKGQPREPQVYTLPPCRDELTKN QVSLWCLVKGFYPSDIAVEWESNGQPENNYKTPP PVLDSGGSFFLYSKLTVDKSRWQQGNVFCFSVMH EALHNHYTQKSLSLSPG	GGSGSGGS	VPLSLY
			CRTSECCFQDPPYPDADSGSASGPRDLRCYRISSDRYEC EGPTAGVSHFLRCCLSSGRCCYFAAGSATRLQFSDOAGV TVTLWVESWARNQTEKSPVTLQLYNSVKYEPPLGDIKV GQLRMWETPDNQVGAEVQFRHRTSPSPWKLGDGCP DTESCLCPLEMNVAQEFQLRRRLQSGSGSSWSKWSSPA PENP		
4	DNA410	Hole: hFc(N297A)- hCD212(24-237)	DKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRT PEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTK PREEQYASTYRVVSVLTVLHQDWLNGKEYKCKVSN KALPAPIEKTISKAKGQPREPQVCTLPSPSRDELTKN QVSLSCAVKGFYPSDIAVEWESNGQPENNYKTPP VLDSDGSFFLVSKLTVDKSRWQQGNVFCFSVMHE ALHNHYTQKSLSLSPG	GGSGSGGS	
5	DNA407	Knob: hFc(N297A)- [VPLSLY]-hil12B- hil12A	DKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRT PEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTK PREEQYASTYRVVSVLTVLHQDWLNGKEYKCKVSN KALPAPIEKTISKAKGQPREPQVYTLPPCRDELTKN QVSLWCLVKGFYPSDIAVEWESNGQPENNYKTPP PVLDSGGSFFLYSKLTVDKSRWQQGNVFCFSVMH EALHNHYTQKSLSLSPG	GGSGSGGS	VPLSLY

		DKHTHTCPPCPAPELLGGPSVFLFPPKPKDITLMISRT	KIDACKRGDVTVPKPSHVILLGSTVNITCSLKPRQGCFFHYSF
		PEVTCVVDVSHEDPEVKFNWYVDGVEVHNAKTK	LILYKFDRRINFHHGHSLSNSQVTGLPLGTTILFVCKLACINS
		PREEQYASTYRVVSVLTVLHQDWLNGKEYKCKVSN	CGAEIFVGVAPEQPQNLSICIQKGEQGTVACTWGERGRT
		KALPAPIEKTISKAKGQPREPQVCTILPPSRDELTKN	EYTLQLSGPKNLTWQKQCKDIYCDYLDYDFGINLTPESPEN
		QVSLSCAVKGFYPSDIAVEWESNGQPENNYKTTTP	VTAVNSLGSSSSSLPSTFTFLDIVRPLPPWDIRIKFQKASVSI
		VLDSDGSFFLVSKLTVDKSRWQQGNVFSCSVMHE	YWRDEGLVLLNRLRYRPSNSRLWNMVMNVTKAKGRHDL
		ALHNHYTQKSLSLSPG	PFTYEYEFQISSKLLHLYKGSWSDWSESIRAQTPEE
5	DNA426		
		Hole: hFc(N297A)-	
		IL12RB2(24-319)	

4	DNA406	Knob: hFc(N297A)-
		[VPLSLY]-hIL12B-
		hIL12A

VPLSLY

GGSGGS

DKHTHTCPPCPAPELLGGPSVFLFPPKPKDITLMISRT
PEVTCVVDVSHEDPEVKFNWYVDGVEVHNAKTK
PREEQYASTYRVVSVLTVLHQDWLNGKEYKCKVSN
KALPAPIEKTISKAKGQPREPQVCTILPPSRDELTKN
QVSLWCCLKGFYPSDIAVEWESNGQPENNYKTTTP
PVLDSDGSFFLVSKLTVDKSRWQQGNVFSCSVMH
EALHNHYTQKSLSLSPG

		DKHTHTCPPCPAPELLGGPSVFLFPPKPKDITLMISRT	KIDACKRGDVTVPKPSHVILLGSTVNITCSLKPRQGCFFHYSF
		PEVTCVVDVSHEDPEVKFNWYVDGVEVHNAKTK	LILYKFDRRINFHHGHSLSNSQVTGLPLGTTILFVCKLACINS
		PREEQYASTYRVVSVLTVLHQDWLNGKEYKCKVSN	CGAEIFVGVAPEQPQNLSICIQKGEQGTVACTWGERGRT
		KALPAPIEKTISKAKGQPREPQVCTILPPSRDELTKN	EYTLQLSGPKNLTWQKQCKDIYCDYLDYDFGINLTPESPEN
		QVSLSCAVKGFYPSDIAVEWESNGQPENNYKTTTP	VTAVNSLGSSSSSLPSTFTFLDIVRPLPPWDIRIKFQKASVSI
		VLDSDGSFFLVSKLTVDKSRWQQGNVFSCSVMHE	YWRDEGLVLLNRLRYRPSNSRLWNMVMNVTKAKGRHDL
		ALHNHYTQKSLSLSPG	PFTYEYEFQISSKLLHLYKGSWSDWSESIRAQTPEE
4	DNA426		
		Hole: hFc(N297A)-	
		IL12RB2(24-319)	

GGSGGS

DKHTHTCPPCPAPELLGGPSVFLFPPKPKDITLMISRT
PEVTCVVDVSHEDPEVKFNWYVDGVEVHNAKTK
PREEQYASTYRVVSVLTVLHQDWLNGKEYKCKVSN
KALPAPIEKTISKAKGQPREPQVCTILPPSRDELTKN
QVSLSCAVKGFYPSDIAVEWESNGQPENNYKTTTP
VLDSDGSFFLVSKLTVDKSRWQQGNVFSCSVMHE
ALHNHYTQKSLSLSPG

5	DNA407	Knob: hFc(N297A)- [VPLSLY]-hIL12B- hIL12A	DKTHTCPCPAPELLGGPSVFLFPPKPKDITLMISRT PEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTK PREEQYASTYRVVSVLTVLHQDWLNGKEYKCKVSN KALPAPIEKTISKAKGQPREPQVYTLPPCRDELTKN QVSLWCLVKGFYPSDIAVEWESNGQPENNYKTPP PVLDSGGSFFLYSKLTVDKSRWQQGNVFCFSVMH EALHNHYTQKSLSLSPG	GGSGSGSGS	VPLSLY
5	DNA539	Hole: hFc(N297A)- IL12RB2(24-222)	DKTHTCPCPAPELLGGPSVFLFPPKPKDITLMISRT PEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTK PREEQYASTYRVVSVLTVLHQDWLNGKEYKCKVSN KALPAPIEKTISKAKGQPREPQVCTLPSPRDELTKN QVSLSCAVKGFYPSDIAVEWESNGQPENNYKTPP VLDSDGSFFLYSKLTVDKSRWQQGNVFCFSVMHE ALHNHYTQKSLSLSPG	GGSGSGSGGS	KIDACKRGDVTVPKPSHVILLGSTVNITCSLKPRQGCFHYSF LILYKFDRRINFHHGHSLSNSQVTGLPLGTTILFVCKLACINS CGAEIFVGVAPEQPQNLSCIQKGEQGTVACTWGERGRDT EYTLQLSGPKNLTWQKQCKDIYCDYLDGFINLTPESPESN VTAVNSLGSSSSLPSTFTFLDIV
7	DNA407	Knob: hFc(N297A)- [VPLSLY]-hIL12B- hIL12A	DKTHTCPCPAPELLGGPSVFLFPPKPKDITLMISRT PEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTK PREEQYASTYRVVSVLTVLHQDWLNGKEYKCKVSN KALPAPIEKTISKAKGQPREPQVYTLPPCRDELTKN QVSLWCLVKGFYPSDIAVEWESNGQPENNYKTPP PVLDSGGSFFLYSKLTVDKSRWQQGNVFCFSVMH EALHNHYTQKSLSLSPG	GGSGSGSGGS	VPLSLY

		DKTHTCPPCPAPELLGGPSVFLFPPPKPKDTLMISRT PEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTK PREEQYASTYRVVSVLTVLHQDWLNGKEYKCKVSN KALPAPIEKTISKAKGQPREPQVCTLPISRDELTKN QVSLSCAVKGFYPSDIAVEWESNGQPENNYKTTTP VLDSGSGFFLVSKLTVDKSRWQQGNVFCFSVMHE ALHNHYTQKSLSLSPG	KIDACKRGDVTVKPSHVILLGSTVNITCSLKPRQGCFHYSF LILYKFDRRINFHHGHSLSNSQVTGLPLGTTILFVCKLACINS CGAEIFVGVAPEQPPQNLSICIQKEQGTVACTWGERGDT EYTLQLSGPKNLTWQKQCKDIYCDYLDGFINLTPESPESN VTAVNSLGSSSSLPSTFTFLDIVRPLPPWDIRIKFQKASVSI YWRDEGLVLLNRLRYRPSNSRLWNMVMNVTKAKGRHDL PFTTEYEFQJSSKHLHYKGSWSDWSESIRAQTPEE
7	DNA540	Hole: hFc(N297A)- IL12RB2(24-319)	

		DKTHTCPPCPAPELLGGPSVFLFPPPKPKDTLMISRT PEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTK PREEQYASTYRVVSVLTVLHQDWLNGKEYKCKVSN KALPAPIEKTISKAKGQPREPQVCTLPISRDELTKN QVSLWCLVKGFYPSDIAVEWESNGQPENNYKTTTP PVLDSGSGFFLYSKLTVDKSRWQQGNVFCFSVMH EALHNHYTQKSLSLSPG	VPLSLY
3	DNA407	Knob: hFc(N297A)- [VPLSLY]-hIL12B- hIL12A	

		DKTHTCPPCPAPELLGGPSVFLFPPPKPKDTLMISRT PEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTK PREEQYASTYRVVSVLTVLHQDWLNGKEYKCKVSN KALPAPIEKTISKAKGQPREPQVCTLPISRDELTKN QVSLSCAVKGFYPSDIAVEWESNGQPENNYKTTTP VLDSGSGFFLVSKLTVDKSRWQQGNVFCFSVMHE ALHNHYTQKSLSLSPG	KIDACKRGDVTVKPSHVILLGSTVNITCSLKPRQGCFHYSF LILYKFDRRINFHHGHSLSNSQVTGLPLGTTILFVCKLACINS CGAEIFVGVAPEQPPQNLSICIQKEQGTVACTWGERGDT EYTLQLSGPKNLTWQKQCKDIYCDYLDGFINLTPESPESN VTAVNSLGSSSSLPSTFTFLDIVRPLPPWDIRIKFQKASVSI YWRDEGLVLLNRLRYRPSNSRLWNMVMNVTKAKGRHDL PFTTEYEFQJSSKHLHYKGSWSDWSESIRAQTPEE
3	DNA541	Hole: hFc(N297A)- IL12RB2(24-319)	

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		DKTHTCPPCPAPELLGGPSVLFPPKPKDTLMISRT	KIDACKRGDVTVKPSHVILLGSTVNITCSLKPRQGCFFHYSF
		PEVTCVVDVSHEDPEVKFNWYVDGVEVHNAKTK	LILYKFDRRINFHHGHSLSNSQVTGLPLGTTILFVCKLACINS
		PREEQYASTYRVVSVLTVLHQDWLNGKEYKCKVSN	CGAEIFVGVAPEQPQNLSCIQKGEQGTVACTWGERGRT
		KALPAPIEKTISKAKGQPREPQVCTLPPSRDELTKN	EYTLQLSGPKNLTWQKQCKDIYCDYLDYLDGINLTPESPESN
		QVSLSCAVKGFYPSDIAVEWESNGQPENNYKTTTPP	VTAVNSLGSSSSLPSTFTFLDIVRPLPP
		VLDSGGSFFLVSKLTVDKSRWQQGNVFSCSVMHE	
		ALHNHYTQKSLSLSPG	
3	DNA644	Hole: hFc(N297A)- IL12RB2(24-227)	

cule name	newnames	Component4Sequ ence	IL-12 p40 subunit	Linker	IL-12 p35 subunit
3	DNA406	Knob: hFc(N297A)- [VPLSLYJ-hIL12B- hIL12A	SGP		
			IWELKKDVYVVELDWYPDAPGEMVVLTCDTPEE DGITWTLTLDQSSEVLGSGKTLTIQVKEFGDAGQYT CHKGGEVLSHSLLHKKEDGIWSTDILKDQKEPK NKTFLRCEAKNYSGRFTCWVWLTITISTDLTFVSKSS RGSSDPQGVTCGAATLSAERVVRGDNKEYEYSVE CQEDSACPAAEESLPIEVMVDAVHKLKVENYTSS FFIRDIKPDPPKNLQLKPLKNSRQVEVSWEYPTD WSTPHSYFSLTFCVQVQGGKSKREKKDORVFTDKTS ATVICRKNASISVRAQDRYYSSSWSEWASVPCS	GGGSGGGGS GGGS	RNLPVATPDPGMFPCLHHSQNLRA VSNMLQKARQTLEFYPCTSEEDHEDI TKDKTSTVEACLPLELTKNESCLNSRE TSFITNGSCLASRKTSFMMALCLSSIYE DLKMYQVEFKTMNAKLLMDPKRQIF LDQNNMLAVIDELMQALNFNSETVPQ KSSLEEPDFYKTKIKLCILLHAFRIRAVTI DRVMSYLNAS

3 DNA409 Hole: hFc(N297A)-
hCD212(24-545)

IWELKDVYVVELDWYPDAPGEMVVLTCDTPEE
DGITWTLDQSSEVLGSGKTLTIQVKEFGDAGQYT
CHKGGEVLSHSLLLHKKEDGIWSTDILKDQKEPK
NKTLRCEAKNYSGRFTCWVLTITISTDLTFSVKSS
RGSSDPQGVTCGAATLSAERVGRDNKEYEYSVE
CQEDSACPAAEESLPIEVMVDAVHKLKYENYTSS
FFIRDIKPDPPKNLQLKPLKNSRQVEVSWEYPT
WSTPHSYFSLTFCVQVQVGKSKREKKDRVFTDKTS
ATVICRKNASISVRAQDRYYSSSWSEWASVPCS

RNLPVATPDPMFPCLHHSQNLRLRA
VSNMLQKARQTLEFYPTCTSEEDHEDI
TKDKTSTVEACLPLELTKNESCNSRE
TSFITNGSCLASRKTSFMMALCLSSIYE
DLKMYQVEFKTMNAKLLMDPKRQIF
LDQNMILAVIDELMQALNFNSETVTPQ
KSSLEEDFYKTKIKLCILLHAFRIRAVTI
DRVMSYLNAS

1 DNA407 Knob: hFc(N297A)-
[VPLSLY]-hIL12B- SGP
hIL12A

1 DNA409 Hole: hFc(N297A)-
hCD212(24-545)

IWELKDVYVVELDWYPDAPGEMVVLTCDTPEE
DGITWTLDQSSEVLGSGKTLTIQVKEFGDAGQYT
CHKGGEVLSHSLLLHKKEDGIWSTDILKDQKEPK
NKTLRCEAKNYSGRFTCWVLTITISTDLTFSVKSS
RGSSDPQGVTCGAATLSAERVGRDNKEYEYSVE
CQEDSACPAAEESLPIEVMVDAVHKLKYENYTSS
FFIRDIKPDPPKNLQLKPLKNSRQVEVSWEYPT
WSTPHSYFSLTFCVQVQVGKSKREKKDRVFTDKTS
ATVICRKNASISVRAQDRYYSSSWSEWASVPCS

RNLPVATPDPMFPCLHHSQNLRLRA
VSNMLQKARQTLEFYPTCTSEEDHEDI
TKDKTSTVEACLPLELTKNESCNSRE
TSFITNGSCLASRKTSFMMALCLSSIYE
DLKMYQVEFKTMNAKLLMDPKRQIF
LDQNMILAVIDELMQALNFNSETVTPQ
KSSLEEDFYKTKIKLCILLHAFRIRAVTI
DRVMSYLNAS

3 DNA407 Knob: hFc(N297A)-
[VPLSLY]-hIL12B- SGP
hIL12A

3 DNA425 Hole: hFc(N297A)-
IL12RB2(24-622)

4 DNA407 Knob: hFc(N297A)-
[VPLSLY]-hIL12B- SGP
hIL12A

IWELKDVVVELDWYPDAPGEMVVLTCDTPEE
DGITWTLDQSSEVLGSGKTLTIQVKEFGDAGQYT
CHKGGEVLSHSLLLHHKKEDGIWSTDILKDQKEPK
NKTFLRCEAKNYSGRFTCWWLTTISTDLTFSVKSS
RGSSDPQGVTCGAATLSAERVVRGDNKEYEYSVE
CQEDSACPAAEESLPIEVMVVDVAVHKLKVENYTSS
FFIRDIKPDPPKNLQLKPLKNSRQVEVSWEYPDT
WSTPHSYFSLTFCVQVQGKSKREKKDORVFTDKTS
ATVICRKNASISVRAQDRYYSSSWSEWASVPCS

GGGGGGGGGS
GGGS

RNLVPATPDPMFPCLHHSQNLRLRA
VSNMLQKARQTLFYPCTSEEDHEDI
TKDKTSTVEACLPLELTKNESCLNSRE
TSFITNGSCLASRKTSFMMALCLSSIYE
DLKNMYQVEFKTMNAKLLMDPKRQJF
LDQNMILAVIDELMQALNFNSETVPQ
KSSLEEPDFYKTKIKLCILLHAFRIRAVTI
DRVMSYLNAS

4 DNA410 Hole: hFc(N297A)-
hCD212(24-237)

IWELKDVVVVELDWYPDAPGEMVVLTCDTPEE
DGITWTLDSSEVLGSGKTLTIQVKEFGDAGQYT
CHKGGEVLSHLLHKKEDGIWSTDILKDQKEPK
NKTFLRCEAKNYSGRFTCWWLTTISTDLTFSVKSS
RGSSDPQGVTCGAATLSAERVVRGDNKEYEYSVE
CQEDSACPAAEESLPIEVMDAVHKLKYENYTSS
FFIRDIKDPKPNLQLKPLKNSRQVEVSWEYPT
WSTPHSYFSLTFCVQVQGGKREKKDRVFTDKTS
ATVICRKNASISVRAQDRYYSSSWSEWASVPCS

RNLPVATPDPGMFPCLHHSQNLLRA
VSNMLQKARQTLEFYPTCTSEEDHEDI
TKDKTSTVEACLPLELTKNESCLNSRE
TSFITNGSCLASRKTSFMMALCLSSIYE
DLKMYQVEFTMNAKLLMDPKRQJIF
LDQNMILAVIDELMQALNFNSETVPQ
KSSLPEPDFYKTKIKLCILLHAFRIRAVTI
DRVMSYLNAS

Knob: hFc(N297A)-
[VPLSLY]-hIL12B- SGP
hIL12A

5 DNA407

Hole: hFc(N297A)-
IL12RB2(24-319)

5 DNA426

IWELKDVVVVELDWYPDAPGEMVVLTCDTPEE
DGITWTLDSSEVLGSGKTLTIQVKEFGDAGQYT
CHKGGEVLSHLLHKKEDGIWSTDILKDQKEPK
NKTFLRCEAKNYSGRFTCWWLTTISTDLTFSVKSS
RGSSDPQGVTCGAATLSAERVVRGDNKEYEYSVE
CQEDSACPAAEESLPIEVMDAVHKLKYENYTSS
FFIRDIKDPKPNLQLKPLKNSRQVEVSWEYPT
WSTPHSYFSLTFCVQVQGGKREKKDRVFTDKTS
ATVICRKNASISVRAQDRYYSSSWSEWASVPCS

RNLPVATPDPGMFPCLHHSQNLLRA
VSNMLQKARQTLEFYPTCTSEEDHEDI
TKDKTSTVEACLPLELTKNESCLNSRE
TSFITNGSCLASRKTSFMMALCLSSIYE
DLKMYQVEFTMNAKLLMDPKRQJIF
LDQNMILAVIDELMQALNFNSETVPQ
KSSLPEPDFYKTKIKLCILLHAFRIRAVTI
DRVMSYLNAS

Knob: hFc(N297A)-
[VPLSLY]-hIL12B- SGP
hIL12A

4 DNA406

IHWELKKDVVVELDWYPDAPGEMVVLCTDTPEE
 DGTITWLDQSSSEVLGSGKTLTIQVKEFGDAGQYT
 CHKGGEVLSHLLHHKKEDGIWSTDILKDQKEPK
 NINKTLRCEAKNYSGRFTCWMLTITDILTFSVKSS
 RGGSSDPQGVTCGAATLSAERVGRGDNKEYEYSVE
 CQEDSACPAAEESLPIEVVMVDVHKLKYENYTS
 FFFIRDIHKDPDKNLQLKPLKNSRQVEVSWEYDPT
 WWSSTPHSYFSLTFCVQVQVGSKREKKDRVFTDKTS
 ATVICRKNASISVRAQDRYSSSWSEWASVPCS

	Knob: hFc(N297A)-
5	DNA407 [VPLSLY]-hIL12B-SGP
	hIL12A

5 DNA539
Hole: hFc(N297A)-
IL12RB2(24-222)

IWELKDVVVELDWYPDAPGEMVVLTCDTPEE
DGITWTLQSSSEVLGSGKTLTIQKEFGDAGQYT
CHKGGEVLSHSLLLHKKEDGIWSTDILKDQKEPK
NKTLRCEAKNYSGRFTCWWLTTISTDLTFSVKSS
RGSSDPQGVTCGAATLSAERVGRDNKEYEYSVE
CQEDSACPAAEESLPIEVMMVDVHKLKYENYTSS
FFIRDIKPPKPNLQLKPLKNSRQVEVSWEYVPT
WSTPHSYFSLTFCVQVQGGKREKKDRVFTDKTS
ATVICRKNASISVRAQDRYYSSSWSEWASVPCS

GGGGSGGGGS
GGGS

RNLPVATPDPGMFPCLHHSQNLRA
VSNMLQKARQTLEFYPTCTSEEDHEDI
TKDKTSTVEACLPLELTKNESCLNSRE
TSFITNGSCLASRKTSFMMALCLSSIYE
DLKMYQVEFKTMNAKLLMDPKRQIF
LDQNMILAVIDELMQALNFNSETVPQ
KSSLEEPDFYKTKIKLCILLHAFRIRAVTI
DRVM'SYLNAS

Knob: hFc(N297A)-
[VPLSLV]-hIL12B- SGP
hIL12A

7 DNA407

Hole: hFc(N297A)-
IL12RB2(24-319)

7 DNA540

IWELKDVVVELDWYPDAPGEMVVLTCDTPEE
DGITWTLQSSSEVLGSGKTLTIQKEFGDAGQYT
CHKGGEVLSHSLLLHKKEDGIWSTDILKDQKEPK
NKTLRCEAKNYSGRFTCWWLTTISTDLTFSVKSS
RGSSDPQGVTCGAATLSAERVGRDNKEYEYSVE
CQEDSACPAAEESLPIEVMMVDVHKLKYENYTSS
FFIRDIKPPKPNLQLKPLKNSRQVEVSWEYVPT
WSTPHSYFSLTFCVQVQGGKREKKDRVFTDKTS
ATVICRKNASISVRAQDRYYSSSWSEWASVPCS

GGGGSGGGGS
GGGS

RNLPVATPDPGMFPCLHHSQNLRA
VSNMLQKARQTLEFYPTCTSEEDHEDI
TKDKTSTVEACLPLELTKNESCLNSRE
TSFITNGSCLASRKTSFMMALCLSSIYE
DLKMYQVEFKTMNAKLLMDPKRQIF
LDQNMILAVIDELMQALNFNSETVPQ
KSSLEEPDFYKTKIKLCILLHAFRIRAVTI
DRVM'SYLNAS

Knob: hFc(N297A)-
[VPLSLV]-hIL12B- SGP
hIL12A

3 DNA407

3 DNA541 Hole: hFc(N297A)-
IL12RB2(24-319)

IWELKDVVVELDWYPDAPGEMVVLTCDTPEE
DGITWTLDSSEVLGSGKTLTIQKFGDAGQYT
CHKGGEVLSHSLLLHHKKEGWIWSTDILKDQKEPK
NKTLRCEAKNYSGRFTCWWLTTISTDLTFSVKSS
GGGGSGGGGS
GGGS
RGSSDPQGVTCGAATLSAERVVRGDNKEYEYSVE
CQEDSACPAAEESLPIEVMVDAVHKLKVENYTSS
FFIRDIKPDPPKNLQLKPLKNSRQVEVSWEYPDT
WSTPHSYFSLTFCVQVQGGKREKKORVFTDKTS
ATVICRKNASISVRAQDRYYSSSWSEWASVPCS

RNLPVATPDPMFPCLHHSQNLLRA
VSNMLQKARQTLEFYPCTSEEDHEDI
TKDKTSTVEACLPLELTKNESCLNSRE
TSFITNGSCLASRKTSFMMALCLSSIYE
DLKMYQVEFKTMNAKLLMDPKRQIF
LDQNMILAVIDELMQALNFNSETVPQ
KSSLLEEPDFYKTKIKLCILLHAFRIRAVTI
DRVMSYLNAS

Knob: hFc(N297A)-
[VPLSLY]-hil12B- SGP
hil12A

3 DNA407

3 DNA629 Hole: hFc(N297A)-
IL12RB2(24-212)

IWELKKDVVVELDWYPDAPGEMVVLTCDTPEE
DGITWTLDSSEVLGSGKTLTIQVKEFGDAQYT
CHKGGEVLSHSLLLHKKEGDIWSTDILKDQKEPK
NKTFLRCEAKNYSGRFTCWWLTTISTDLTFSVKSS
RGSSDPQGVTCGAATLSAERVGRGNKEYEYSVE
CQEDSACPAAEESLPIEVMVDAVHKLKYENYTSS
FFIRDIKPDPPKNLQLKPLKNSRQVEVSWEYPT
WSTPHSYFSLTFCVQVQVGKSKREKKDRVFTDKTS
ATVICRKNASISVRAQDRYSSSWSEWASVPCS

GGGGSGGGGS
GGGS

RNLPVATPDPMFPCLHHSQNLRA
VSNMLQKARQTLEFYPCTSEEDHEDI
TKDKTSTVEACLPLELTKNESCLNSRE
TSFITNGSCLASRKTSFMMALCLSSIYE
DLKMYQVEFTMNAKLLMDPKRQIF
LDQNMILAVIDELMQALNFNSETVPQ
KSSLEEPDFYKTKIKLCILLHAFRIRAVTI
DRVMSYLNAS

Knob: hFc(N297A)-
[VPLSLY]-hIL12B- SGP
hIL12A

3 DNA407

Hole: hFc(N297A)-
IL12RB2(24-227)

3 DNA644

cule name	newnames	Released by cleavage
3	DNA406	Knob: hFc(N297A)- [VPLSLY]-hil12B- hil12A
		LYSGPIWELKDVVVELDWYDPDAPGEMVWLTCDTPEEDGITWTLTQSSSEVLGSGKTLTIQVKEFGDAGQYTCH KGGEVLSHSLILLHKKEDGIWSTDIKDDQKEPKNTFLRCEAKNYSGRFTCWVLTITSTDLTFSVKSSRGSSDPQG VTCGAATLSAERVGRGNKEYEYSVECCQEDSACPAAEESLPIEVMVDVHKLKYENYTSFFIRDIKPPKPNLQLK PLKNSRQVEVSWVEYPTWTSTPHSYFSLTFCVQVQGKSKREKKDRVFTDKTSATVICRKNASISVRAQDRYYSSSW SEWASVPCSGGGGGGGGGGSRNLPAVTPDPMFPCLHHSQNLRAVSNMLQKARQTLFYPCTSEED HEDITKDKTSTVEACLPLELTKNESCINRSRSTFITNGSCLASRKTSMALCLSIYEDLKMYQVEFKTMNAKLIM DPKRQJFLDQNMMLAVIDELMQALNFNSETVYPQKSSLEEPDFYKTKIKLCILLHAFRIRAVTIDRVMSYLNAS
3	DNA409	Hole: hFc(N297A)- hCD212(24-545)
		LYSGPIWELKDVVVELDWYDPDAPGEMVWLTCDTPEEDGITWTLTQSSSEVLGSGKTLTIQVKEFGDAGQYTCH KGGEVLSHSLILLHKKEDGIWSTDIKDDQKEPKNTFLRCEAKNYSGRFTCWVLTITSTDLTFSVKSSRGSSDPQG VTCGAATLSAERVGRGNKEYEYSVECCQEDSACPAAEESLPIEVMVDVHKLKYENYTSFFIRDIKPPKPNLQLK PLKNSRQVEVSWVEYPTWTSTPHSYFSLTFCVQVQGKSKREKKDRVFTDKTSATVICRKNASISVRAQDRYYSSSW SEWASVPCSGGGGGGGGGGSRNLPAVTPDPMFPCLHHSQNLRAVSNMLQKARQTLFYPCTSEED HEDITKDKTSTVEACLPLELTKNESCINRSRSTFITNGSCLASRKTSMALCLSIYEDLKMYQVEFKTMNAKLIM DPKRQJFLDQNMMLAVIDELMQALNFNSETVYPQKSSLEEPDFYKTKIKLCILLHAFRIRAVTIDRVMSYLNAS
1	DNA407	Knob: hFc(N297A)- [VPLSLY]-hil12B- hil12A
		LYSGPIWELKDVVVELDWYDPDAPGEMVWLTCDTPEEDGITWTLTQSSSEVLGSGKTLTIQVKEFGDAGQYTCH KGGEVLSHSLILLHKKEDGIWSTDIKDDQKEPKNTFLRCEAKNYSGRFTCWVLTITSTDLTFSVKSSRGSSDPQG VTCGAATLSAERVGRGNKEYEYSVECCQEDSACPAAEESLPIEVMVDVHKLKYENYTSFFIRDIKPPKPNLQLK PLKNSRQVEVSWVEYPTWTSTPHSYFSLTFCVQVQGKSKREKKDRVFTDKTSATVICRKNASISVRAQDRYYSSSW SEWASVPCSGGGGGGGGGGSRNLPAVTPDPMFPCLHHSQNLRAVSNMLQKARQTLFYPCTSEED HEDITKDKTSTVEACLPLELTKNESCINRSRSTFITNGSCLASRKTSMALCLSIYEDLKMYQVEFKTMNAKLIM DPKRQJFLDQNMMLAVIDELMQALNFNSETVYPQKSSLEEPDFYKTKIKLCILLHAFRIRAVTIDRVMSYLNAS

1 DNA409 Hole: hFc(N297A)-
hCD212(24-545)

LYSGPIWELKKDVVVVWELDWYPDAPGEMVVLTCDTPEEDGITWTLDOSSVLSGSKTLTIQVKEFGDAGQYTCH
KGGEVLSHSLILLHKKEDGIWSTDILKDQKEPKNKTFLRCEAKNYSGRFTCWWLTTISTDLTFSVKSSRGSSDPQG
VTCGAATLSAERVVRGDNKEYEYSEVCEQEDSACPAAEESLPIEVMDAVHKLKYENYTSFFFIRDIKPDPPKNLQLK
PLKNSRQVEVSWEYPTWTSTPHSYFSLTCVQVQGGKSKREKDRVFTDKTSATVICRKNASISVRAQDRYYSSSW
SEWASVPCSGGGGGGGGGGGSRNLPVATPDPGMFPCLLHHSQNLRAVSNMLQKARQTLFYPCTSEED
HEDITKDKTSTVEACLPLELTKNESCLNSRETSTFTNGSCLASRKTSMALCLSIYEDLKM/YQVEFFKTMNAKLLM
DPKRQIFLDQNMILAVIDELMQALNFNSETVTPQKSSLEEDFYTKIKIKLCILLHAFRIRAVTTIDRVMMSYLNAS

3 DNA407 Knob: hFc(N297A)-
[VPLSLY]-hIL12B-
hIL12A

3 DNA425 Hole: hFc(N297A)-
IL12RB2(24-622)

LYSGPIWELKDVVWVWELDWYPDAPGEMVVLTCDTPEEDGITWTLQDSSEVLGSGKTLTIQVKEFGDAGQYTCH
KGGEVLSHSLILLHKKEDGIWSTDLKDQKEPKNKTFLRCEAKNYSGRFTCWWLTTISTDLTFSVKSSRGSSDPQG
Knob: hFc(N297A)- VTCGAATLSAERVRGDNKEYEYSECEQEDSACPAAEESLPIEVMMVDVAVHKLKYENYTSFFIRDIKPPKLNQLK
[VPLSLY]-hIL12B- PLKNSRQVEVSWEYPTWSTPHSYFSLTFCVQVQGSKREKKDRVFTDKTSATVICRKNASISVRAQDRYYSSSW
hIL12A SEWASVPCSGGGGGGGGGGGSRNLPVATPDGMPFCLHHSQNLRAVSNMLQKARQTLFYPCTSEED
HEDITKDKTSTVEACLPLELTKNESCLNSRSTSFITNGSCLASRKTSMALCLSIYEDLKMVQVEFKTMNAKLLM
DPKRQIFLDQNMILAVIDELMQALNFNSETVPOKSSLEEDFYKTKIKLCILLHAFRIRAVTIDRVMMSYLNAS

4 DNA407 Knob: hFc(N297A)-
[VPLSLY]-hIL12B-
hIL12A

4 DNA410 Hole: hFc(N297A)-
hCD212(24-237)

LYSGPIWELKDVVWVWELDWYPDAPGEMVVLTCDTPEEDGITWTLQDSSEVLGSGKTLTIQVKEFGDAGQYTCH
KGGEVLSHSLILLHKKEDGIWSTDLKDQKEPKNKTFLRCEAKNYSGRFTCWWLTTISTDLTFSVKSSRGSSDPQG
Knob: hFc(N297A)- VTCGAATLSAERVRGDNKEYEYSECEQEDSACPAAEESLPIEVMMVDVAVHKLKYENYTSFFIRDIKPPKLNQLK
[VPLSLY]-hIL12B- PLKNSRQVEVSWEYPTWSTPHSYFSLTFCVQVQGSKREKKDRVFTDKTSATVICRKNASISVRAQDRYYSSSW
hIL12A SEWASVPCSGGGGGGGGGGGSRNLPVATPDGMPFCLHHSQNLRAVSNMLQKARQTLFYPCTSEED
HEDITKDKTSTVEACLPLELTKNESCLNSRSTSFITNGSCLASRKTSMALCLSIYEDLKMVQVEFKTMNAKLLM
DPKRQIFLDQNMILAVIDELMQALNFNSETVPOKSSLEEDFYKTKIKLCILLHAFRIRAVTIDRVMMSYLNAS

5 DNA407 Knob: hFc(N297A)-
[VPLSLY]-hIL12B-
hIL12A

5 DNA426 Hole: hFc(N297A)-
IL12RB2(24-319)

4 DNA406 Knob: hFc(N297A)-
[VPLSLY]-hIL12B-
hIL12A
LYSGPIWELKDVVVVELDWYPDAPGEMVVLTCDTPEEDGITWTLDSSEVLGSGKTLTIQVKEFGDAGQYTCH
KGGEVLSHSLILLHHKKEDGIWSTDILKDQKEPKNKTFLRCEAKNYSGRFTCWWTITISTDLTFSVKSSRGSSDPQG
VTCGAATLSAERVVRGDNKEYEYSVECEQEDSACPAAEESLPIEVMDAVHKLKYENYTSFFFIRDIKPDPPKNLQLK
PLKNSRQVEVSWEYPTWTSTPHSYFSLTFCVQVQGGKSKREKKDRVFTDKTSATVICRKNASISVRAQDRYYSSSW
SEWASVPCSGGGGGGGGGGSRNLPVATPDPMFPCLHHSQNLRAVSNMLQKARQTLFYPCTSEED
HEDITKDKTSTVEACLPLELTKNESCLNSRETSTFITNGSCLASRKTSMALCLSIYEDLKM/YQVEFKTMNAKLIM
DPKRQIFLDQNMILAVIDELMQALNFNSETVPQKSSLEEDFYKTIKIKLILLHAFRIRAVTIDRVMSYLNAS

4 DNA426 Hole: hFc(N297A)-
IL12RB2(24-319)

5 DNA407 Knob: hFc(N297A)-
[VPLSLY]-hIL12B-
hIL12A
LYSGPIWELKDVVVVELDWYPDAPGEMVVLTCDTPEEDGITWTLDSSEVLGSGKTLTIQVKEFGDAGQYTCH
KGGEVLSHSLILLHHKKEDGIWSTDILKDQKEPKNKTFLRCEAKNYSGRFTCWWTITISTDLTFSVKSSRGSSDPQG
VTCGAATLSAERVVRGDNKEYEYSVECEQEDSACPAAEESLPIEVMDAVHKLKYENYTSFFFIRDIKPDPPKNLQLK
PLKNSRQVEVSWEYPTWTSTPHSYFSLTFCVQVQGGKSKREKKDRVFTDKTSATVICRKNASISVRAQDRYYSSSW
SEWASVPCSGGGGGGGGGGSRNLPVATPDPMFPCLHHSQNLRAVSNMLQKARQTLFYPCTSEED
HEDITKDKTSTVEACLPLELTKNESCLNSRETSTFITNGSCLASRKTSMALCLSIYEDLKM/YQVEFKTMNAKLIM
DPKRQIFLDQNMILAVIDELMQALNFNSETVPQKSSLEEDFYKTIKIKLILLHAFRIRAVTIDRVMSYLNAS

5 DNA539 Hole: hFc(N297A)-
IL12RB2(24-222)

7 DNA407 Knob: hFc(N297A)-
[VPLSLY]-hil12B-
hil12A

LYSGPIWELKDVVWVWELDWYPDAPGEMVLTCDTPEEDGITWTLDOSSSEVLGSGKTLTIQVKEFGDAGQYTCH
KGGEVLSHSLLLLHKKEDGIWSTDLKDQKEPKNKTFLRCEAKNYSGRFTCWWTITISTDLTFSVKSSRGSSDPQG
VTCGAAATLSAERVVRGDNKEYEYSVEQEDSACPAAEESLPIEVMVDAVHKLKYENVTSSFFIRDIKPPPKNLQLK
PLKNSRQVEVSWWEYPDWTSTPHSYFSLTFCVQVQGGKREKDRVFTDKTSATVICRKNASISVRAQDRYYSSSW
SEWASVPCSGGGGGGGGGGSRNLPVATPDGPMFPCLLHHSQNLRAVSNMLQKARQTLFYPCTSEED
HEDITKDKTSTVEACLPLELTKNESCLNSRETSTFITNGSCLASRKTSFMMALCLSIYEDLKM/YQVEFKTMNAKLLM
DPKROIFLDQNMMLAVIDELMQALNFNSETVYPQKSSLEEDFYKTKIKLCILLHAFRIRAVTIDRVMSYLNAS

7 DNA540 Hole: hFc(N297A)-
IL12RB2(24-319)

3 DNA407 Knob: hFc(N297A)-
[VPLSLY]-hil12B-
hil12A

LYSGPIWELKDVVWVWELDWYPDAPGEMVLTCDTPEEDGITWTLDOSSSEVLGSGKTLTIQVKEFGDAGQYTCH
KGGEVLSHSLLLLHKKEDGIWSTDLKDQKEPKNKTFLRCEAKNYSGRFTCWWTITISTDLTFSVKSSRGSSDPQG
VTCGAAATLSAERVVRGDNKEYEYSVEQEDSACPAAEESLPIEVMVDAVHKLKYENVTSSFFIRDIKPPPKNLQLK
PLKNSRQVEVSWWEYPDWTSTPHSYFSLTFCVQVQGGKREKDRVFTDKTSATVICRKNASISVRAQDRYYSSSW
SEWASVPCSGGGGGGGGGGSRNLPVATPDGPMFPCLLHHSQNLRAVSNMLQKARQTLFYPCTSEED
HEDITKDKTSTVEACLPLELTKNESCLNSRETSTFITNGSCLASRKTSFMMALCLSIYEDLKM/YQVEFKTMNAKLLM
DPKROIFLDQNMMLAVIDELMQALNFNSETVYPQKSSLEEDFYKTKIKLCILLHAFRIRAVTIDRVMSYLNAS

3	DNA541	Hole: hFc(N297A)- IL12RB2(24-319)	LYSGPIWELKDVVVELDWYPDAPGEMVVLTCDTPEEDGITWTLDSSEVLGSGKTLTIQVKEFGDAGQYTCH KGGEVLSHSLILLHKKEDGIWSTDILKDQKEPKNKTFLRCEAKNYSGRFTCWWTITISTDLTFSVKSSRGSSDPQG VTCGAATLSAERVGRGDNKEYEYSVECEQDSACPAAEESLPIEVMVDAVHKLKYENYTSFFFIRDIKPPKNLQLK PLKNSRQVEVSWEYPTWTSTPHSYFSLTFCVQVQGSKREKKDRVFTDKTSATVICRKNASISVRAQDRYVSSSW SEWASVPCSGGGGGGGGGGSRNLPVATPDGMPFCLHHSQNLRAVSNMLQKARQTLFYPCTSEED HEDITKDKTSTVEACLPLELTKNESCLNSRETSTFITNGSCLASRKTSMALCLSIYEDLKMVQVEFKTMNAKLIM DPKRQIFLDQNMILAVIDELMQALNFNSETVPQKSSLEEDFYKTKIKLCILLHAFRIRAVTIDRVMSYLNAS
3	DNA407	Knob: hFc(N297A)- [VPLSLY]-hIL12B- hIL12A	
3	DNA629	Hole: hFc(N297A)- IL12RB2(24-212)	LYSGPIWELKDVVVELDWYPDAPGEMVVLTCDTPEEDGITWTLDSSEVLGSGKTLTIQVKEFGDAGQYTCH KGGEVLSHSLILLHKKEDGIWSTDILKDQKEPKNKTFLRCEAKNYSGRFTCWWTITISTDLTFSVKSSRGSSDPQG VTCGAATLSAERVGRGDNKEYEYSVECEQDSACPAAEESLPIEVMVDAVHKLKYENYTSFFFIRDIKPPKNLQLK PLKNSRQVEVSWEYPTWTSTPHSYFSLTFCVQVQGSKREKKDRVFTDKTSATVICRKNASISVRAQDRYVSSSW SEWASVPCSGGGGGGGGGGSRNLPVATPDGMPFCLHHSQNLRAVSNMLQKARQTLFYPCTSEED HEDITKDKTSTVEACLPLELTKNESCLNSRETSTFITNGSCLASRKTSMALCLSIYEDLKMVQVEFKTMNAKLIM DPKRQIFLDQNMILAVIDELMQALNFNSETVPQKSSLEEDFYKTKIKLCILLHAFRIRAVTIDRVMSYLNAS
3	DNA407	Knob: hFc(N297A)- [VPLSLY]-hIL12B- hIL12A	

3 DNA644 Hole: hFc(N297A)-
IL12RB2(24-227)

cule name	newnames	FullSequence
3	DNA406	DKTHTCPCPAPELLGGPSVFLFPPKPKDITLMISRTPEVTCVVVDVSHEDPEVKFNWVYDVGVEVHNAKTKPREEQYAS
		TYRVVSVLTVLTQHQLWLNQKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPCRDELTKNQVSLWCLVKGFYPSDI
		AVEWESNGQPENNYKTTTPVLDSDGSEFLYSKLTVDKSRWQQGNVFSCSVMEALHNHYTQKSLSLSPGGGGGGSV
	Knob: hFc(N297A)- [VPLSLY]-hIL12B- hIL12A	PLSLYSGPIWELKDVVVELDWYDPDAPGEMVVLTCDTPEEDGITWLDQSSEVLGSGKTLTIQVKEFGDAGQYTCHK
		GGEVLSHSLLLHKKEDGIWSTDILKDQKEPKNKTFLRCEAKNYSGRFTCWWLTTISTDLTFSVKSSRGSSDPQGVTCG
		AATLSAERVGRGNKEYEYSVECCEDSACPAAEESLPIEVMDAVHKLKYENYTSFFIRDIHKDPKPNLQLKPLKNSRQ
		VEVSWEYPTWSTPHSYFSLTFCVQVQGKSKREKKDRVFTDKTSATVICRKNASISVRAQDRYYSSSWSEWASVPCSG
		GGSGGGGGGGGSRNLPVATPDPMFPCLHHSQNLRAVSNMLQKARQTLFYPCTSEEDHEDTKDKTSTVEAC
		LPLELTKNESCINSRETSFITNGSCLSRKTSFMMALCLSIYEDLKNYQVEFTMNAKLLMDPKRQIFLDQNMILAVID
		ELMQALNFNSETVPQKSSLEEDFYTKIKICILLHAFIRAVTIDRVMISYLNAS

DKHTHTCPAPPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYAS
TYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSRDELTKNQVSLSCAVKGFPYPSDI
AVEWESNGQPENNYKTTTPVLDSDGSEFLVSKLTVDKSRWQQGNVFSCSVMEALHNHYTKSLSPGGGGSGG
GGGSCRTSECCFQDPYPADSDGASGPRDLRCYRISSDRYECWSQYEGPTAGVSHFLRCLSSGRCCYFAAGSATRL
QFSDQAGVSVLYTVTLWVESWARNQTEKSPVTLQLYNSVKYEPPLGDIKVKSLAGQLRMEWETPDNQVGAEVQFR
HRTSSPWKLGDGCPQDDTESCLCPLEMNVAQEFQLRRRLGSGQSSWSKWSPPVCVPPENPPQPQVRFSEVQL
GQDGRRLTLKEQPTQLELPEGCQGLAPGTEVTVRLQLHMLSCPCAKATRTLHLGKMPYLSGAAYNVAVISSNQFG
PGLNQTHIPADTHTEPVALNISVGTNGTTMYWPARAQSMITYCIEWQPVGQDGLATCSLTAPQDPPAGMATY
SWSRESGAMGQEKCYITIFASAHPEKLTLSVTLSTYHFGGNASAAAGTPHHVSVKNHSLDSVSDWAPSLSTCPG
VLKEYVVRCDREDSKQVSEHPVQPTETQVTLISGLRAGVATTVQVRADTAWLRGVWSQPQRFSIEVQVSD

Hole: hFc(N297A)-
hCD212(24-545)

DNA409

3

DKHTHTCPAPPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYAS
TYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPCRDELTKNQVSLWCLVKGFYPSDI
AVEWESNGQPENNYKTTTPVLDSDGSEFLVSKLTVDKSRWQQGNVFSCSVMEALHNHYTKSLSPGGGGSGG
GSVPLSLYSGPIWELKDVVVELDWYDPDAPGEMVVLTCDTPEEDGITWTLDSQSEVLGSGGKTLTIQVKEFGDAQYT
CHKGGEVLSHLSLLHKKEDGIWSTDILKDQKEPKNTFLRCEAKNYSGRFTCWWTITSTDLTFSVKSSRGSSDPQGV
TCGAATLSAERVGRDNKEYEYSEVCEQDSACPAEESLPIEVMVDVAVHKLKYENYTSFFIRDIKPPKLNQLKPLKN
SRQVEVSWEPDWTSTPHSYFSLTCVQVQGKSKREKKDRVFTDKTSATVICRKNASISVRAQDRYSSSSSEWASV
PCSGGGGGGGGGGSRNLVATPDGPMFPCLHHSQNLRAVSNMLQKARQTLFYPCTSEEDHEDITDKTST
VEACPLIELTKNESCINSRETSFITNGSCLASRKTSMALCLSIYEDLKMYQVEFKTMNAKLLMDPKRQIFLDQNMIL
AVIDELMQALNFNSETVPQKSSLEEDPFYKTIKILCLLHAFIRAVTIDRVMMSYLNAS

Knob: hFc(N297A)-
[VPLSLY]-hil12B-
hil12A

DNA407

1

DKTHTCPCPAPELLGGPSVLEFPKPKDITLMISRTPEVTCVVVDVSHEDPEVKFNWVVDGVEVHNAKTKPREEQYAS
TYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVCTLPSPSRDELTKNQVSLSCAVKGFYPSDI
AVEWESNGQPENNYKTTTPVLDSDGSFFLVSKLTVDKSRWQQGNVFCSCVMHEALHNHYTQKSLSLSPGGGGSG
GGGSCRTSECCFQDPYPDPADSGSASGPRDLRCYRISDRYECSWQYEGPTAGVSHFLRCCLSGRCCCYFAAGSATRL
QFSDQAGVSVLYTVTLWVESWARNQTEKSPEVTQLYNSVKYEPPLGDIKVKLAGQLRMETPDNQVGAEVQFR
HRTSPSPWKLGDCGPQDDDTESCLCPLEMINVAQEFQLRRRLGSGQSSWSKWSSPVCVPPENPPQPQVRFSEQL
GQDGRRLTLKEQPTQLEPEGCGGLAPGTEVTYRLQLHMLSCPCAKATRTLHLGKMPYLSGAAYNAVVISNQFG
PGLNQTHIPADTHTEPVALNISVGTNGTTMYWPARAQSMITYCIEWQPVGGQGLATCSLTAPQDPPAGMATY
SWSRESGAM/GQEKCVYITIFASAHPEKLTWSTVLSITYHFGGNASAAAGTPHHVSVKNHSLDSVSDWAPSLSTCPG
VLKEYVVRCRDEDSKQVSEHPVQPTETQVTLGLRAGVAYTVQVRADTAWLRGVWSQPQRFSIEVQVSD

1 DNA409 Hole: hFc(N297A)-
hCD212(24-545)

DKTHTCPPCPAPPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQVAS
TYRVVSVLTVHLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPCRDELTKNQVSLWCLVKGFYPSDI
AVEWESNGQPENNYKTTTPVLDSDGSFFLYSKLTVDKSRWQQGNVFCSCVMHEALHNYHTQKSLSLSPGGSGGSG
GSVPLSLYSGPIWELKKDVVVVELDWYPDAPGEMVVLTCDTPEEDGITWILDQSSEVLGSGKTLTIQVKEFGDAGQYT
CHKGGEVLSHSLLLHKKEDGIWSTDILKDQKEPKNKTFLRCEAKNYSGRFTCWWLTTISTDLTFSVKSSRGSSDPQGV
TCGAATLSAERVGRDNKEYEYSVECEQEDSACPAAEESLPIEVMDAVHKLKYENYTSFFIRDIHKPDPKKNLQKLKN
SRQVEVSWEYPTDWTSTPHSYFSLTFCVQYQGGKREKKDRVFTDKTSATVICRKNASISVRAQDRYSSSWSEWASV
PCSGGGGGGGGGGGSRNLPVATPDGMPFCLHHSQNLRAVSNMLQKARQTFEPYCTSEEDHEDITKDKTST
VEACPIELTKNESCLNSRETSTFITNGSCLASRKTSMALCLSIYEDLKMYYQVEFTMINAKLLMDPKRQIFLDQNM
AVIDELMQALNFNSETVPQKSSLEEPDFYKTKIKLCILLHAFRIRAVTIDRVMSYLNAS

Knob: hFc(N297A)-
[VPLSLY]-hil12B-
hil12A

3 DNA407

DKTHTCPPCPAPPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVDVSHEDPEVKFNWVVDGVEVHNAKTKPREEQYAS
TYRVVSVLTVQLHQLDNLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVCTLPSPSRDELTKNQVSLSCAVKGFYPSDI
AVEWESNGQPENNYKTTTPPVLDSDGSFFFLVSKLTVDKSRWQQGNVFCSCVMHEALHNNHYTQKSLSLSPGGGGSG
GGGSKIDACKRGGDVTVKPSHVIILGSTVNIITCSLKPRQGCFFHYSRRNKLILYKFDRRINFHHGHSLSNSQVTLPLGTTLF
VCKLACINSDEIQICGAEIFVGVAPEQPQNLSICIQKGEQGTACTWVERGRDTHLYTEYTLQLSGPKNLTWQKQCKDIY
CDYLDGFINLTPESPESNFTAKVTAVNSLGSSSSLPSTFTFLDIVRPLPPWDIRIKFQKASVSRCTLYWRDEGLVLLNRLRY
RPSNSRLWNMNVNVTAKGRHDLLDLKPFTEYEFQISSKLHLYKGSWSDWSESIRAQTPREEPTGMLDVWYMKRHHID
YSRQQISLFWKNLSVSEARGKILHYQVTILQELTGGKAMTQNTGHTSWTTPRTGNWAVAVSAANSKGSSLPTRINI
MNLCEAGILAPRQV/SANSEGMNDILVTWQPPRKDP/SAVQEVVWRELHPGGDTQVPLNWLRSRPNVNSALISENI
KSYICYEIRVYALSGDQGGCSSILGNSKKAPLSGPHINAITEEKGSIILISWNSIPVQEQMGCLLHYRIYWKERDSNSQP
QLCEIPYRVSQNSHPINSLQPRVTYVLWMTALTAAGESSHGNEREFCLQGGKAN

Hole: hFc(N297A)-
IL12RB2(24-622)

3 DNA42.5

DKHTCPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYAS
TYRVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPCRDELTKNQVSLWCLVKGFYPSDI
AVEWESNGQPENNYKTTTPVLDSDGSEFFLYSKLTVDKSRWQQGNVFCSCVMHEALHNYHTQKSLSLSPGGGGSGG
GSVPLSLYSGPIWELKKDYYVWELDWYDPDAPGEMVWLTCDTPEEDGITTLDQSSSEVLGSGGKTLTIQVKEFGDAGQYT
CHKGGEVLHSHLSLLLHKKEDGIWSTDILKDQKEPKNKTFLRCEAKNYSGRFTCWWTITITDITFVSXSSRSGSDPQGV
TCGAATLSAERVGRGDNKEYEYSEVCEQDSACPAEESLPIEVMDVAVHKLKYENYTSFFIRDIHKPDPKNIQLKPLKN
SRQVEVSWEYPDTWSTPHSYFSLTFCVQVQGGKSKREKKDRVFTDKTSATVICRKNASISVRAQDRYSSSWSEWASV
PCSGGGSGGGGGGGGSRNLPVATPDGMPFCLHHSQNLRAVSNMLQKARQTLFEPYCTSEEDHEDITKDKTST
VEACPLELTKNESCLNSRETSFITNGSCLASRKTSMALCLSSIEDLKMYQVEKTMNAKLLMDPKRQIFLDQNMIL
AVIDELMQALNFNSETVPQKSSLEEDFYKTKIKLCILLHAFRIRAVTIDRVMSVNLNAS

Knob: hFc(N297A)-
[VPLSLY]-hil.12B-
hil.12A

4 DNA407

DKHTCPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYAS
TYRVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVCTLPSSRDELTKNQVSLSCAVKGFYPSDI
AVEWESNGQPENNYKTTTPVLDSDGSEFFLYSKLTVDKSRWQQGNVFCSCVMHEALHNYHTQKSLSLSPGGGGSGG
GGGSCRTSECCFQDPYPDADSGSASGPRDLRCYRISSDRYECWSWQYEGPTAGVSHFLRCCLSSGRCCYFAAGSATRL
QFSDQAGVSVLYTVTLWVESWARNQTEKSPEVTLQLYNSVKYEPPLGDIKIVSKLAGQLRMEWETPDNQVGAEVQFR
HRTSPSPWKLGDGCPQDDDTESCLCPLMNVAQEFQLRRRQLGSGGSSWSKWSSSPVCVPPENP

Hole: hFc(N297A)-
hCD212(24-237)

4 DNA410

DKTHTCPCPAPELLGGPSVLFPPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYAS
TYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPCRDELTKNQVSLWCLVKGFYPSDI
AVEWESNGQPENNYKTTTPVLDSDGSFFLYSLKTVDKSRWQQGNVFCSCVMHEALHNYHTQKSLSLSPGGGGSGG
GSVPLSLYSGPIWELKKDVVVELDWYDPDAPGEMVVLTCDTPEEDGITTWLDQSSSEVLGSGGKTLTIQVKEFGDAGQYT
CHKGGEVLSHSLLLHKKEDGIWSTDILKDKQKEPKNKTFLRCEAKNYSGRFTCWVWLTITSTDLTFSVKSSRGSDDPQGV
TCGAATLSAERVGRDNKEYEYSEVCEQDSACPAAEESLPIEVMMVDVAVHKLKYENVTSSFFIRDIHKPDPKNIQLKPLKN
SRQVEVSWEYPTDWTSTPHSYFSLTFCVQYQGGKREKKDRVFTDKTSATVICRKNASISVRAQDRYSSSSWSEWASV
PCSGGGGGGGGGGGGSRNLPVATPDGMPFCLHHSQNLRAVSNMLQKARQTLFYPCTSEEDHEDITKDKTST
VEACPLIELTKNESCINSRETSFITNGSCLASRKTSMALCLSSYEDLKMYQVEKTMNAKLLMDPKRQIFLDQNMIL
AVIDELMQALNFNSETVPQKSSLEEDFYKTKIKLCILLHAFIRAVTIDRVMISYLNAS

Knob: hFc(N297A)-
[VPLSLY]-hil12B-
hil12A

DNA407

5

DKTHTCPCPAPELLGGPSVLFPPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYAS
TYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVCTLPSPRDELTKNQVSLSCAVKGFYPSDI
AVEWESNGQPENNYKTTTPVLDSDGSFFLYSLKTVDKSRWQQGNVFCSCVMHEALHNYHTQKSLSLSPGGGGSGG
GGGSKIDACKRGDVTVKPSHVILLGSTVNITCSLKPRQGGCFHYSRRNKILYKFDRRINFHHGHSLSNSQVTLGLPLGTTLLF
VCKLACINSDEIQICGAEIFVGVAPEQPQNLSIQKGEQGTVACTWGERGRDTHLYTEYTLQLSGPKNLTWQKQCKDIY
CDYLDGFINLTPESPESNFTAKVTAVNSLGSSSSLPSTFTFLDIVRPLPPWDIRIKFQKASVSRCTLYWVRDEGLVLLNRLRY
RPSNSRLWNM/VNVTAKGRHDLLDLKPTEYEFQISSKLHLKYGSWSDWSESIRAQTPPEE

Hole: hFc(N297A)-
IL12RB2(24-319)

DNA426

5

DKTHTCPCPAPELLGGPSVLEFPKPKDITLMISRTPEVTCVVVDVSHEDPEVKFNWVVDGVEVHNAKTKPREEQYAS
TYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPCDELTKNQVSLWCLVKGFYPSDI
AVEWESNGQPENNYKTTTPVLDSDGSEFFLYSKLTVDKSRWQQGNVFCSCVMHEALHNYHTQKSLSLSPGGGGSGSV
PLSLYSGPIWELKDVVVELDWYDPDAPGEMVVLCDTPEEDGKITWLDQSEVILGSGKTLTIQVKEFGDAGQYTCHK
GGEVLSHSLLLHKKEDGIWSTDILKDKQKEPKNKTFLRCEAKNYSGRFTCWWTITSTDLTFSVKSSRGSSDPQGVTCG
AATLSAERVGRDNKEYEYSECEQDSACPAEESLPIEVMVDVAVHKLKYENYTSFFIRDIKPDPPKNLQKLKNSRQ
VEVSWEPDTWSTPHSYFSLTFCVQVQGKSKREKKDRVFTDKTSAIVCRKNASISVRAQDRYSSSSWSEWASVPCSG
GGSGGGGGGGGSRNLPAVATPDPGMFPCLHHSQNLRAVSNMLQKARQTLFFYPCTSEEDHEDITKDKTSTVEAC
LPLELTKNESCINSRETSFTINGSCLASRKTSPMMALCLSIYEDLKMVQVEFKTMNAKLLMDPKRQIFLDQNMILAVID
ELMQALNFNSETVPQKSSLEEDFYTKIKICILLHAFRIRAVTIDRVMSYLNAS

Knob: hFc(N297A)-
[VPLSLY]-hil12B-
hil12A

DNA406

4

DKTHTCPCPAPELLGGPSVLEFPKPKDITLMISRTPEVTCVVVDVSHEDPEVKFNWVVDGVEVHNAKTKPREEQYAS
TYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVCTLPSPRDELTKNQVSLSCAVKGFYPSDI
AVEWESNGQPENNYKTTTPVLDSDGSEFFLYSKLTVDKSRWQQGNVFCSCVMHEALHNYHTQKSLSLSPGGGGSGG
GGGSKIDACKRGDVTVKPSHVILLGSTVNITCSLKPRQGCFFHYSRRNKILLYKFDRRINFHHGHSLSNSQVTLPLGTTLLF
VCKLACINSDEIQIGCAEIVGVAPEQPQNLSICIQKEGQGTVACTWVERGRDTHLYTEYTLQLSGPKNLTWQKQCKDIY
CDYLDGFINLTPESPESNFTAKVTAVNSLGSSSSLPSTFTFLDIVRPLPPWDIRIKFQKASVSRCTLYWRDEGLVLLNRLRY
RPSNSRLWNMNVNVTAKGRHDLLDLKPFTEYEFQISSKLHLYKGSWSDWSESIRAQTPEE

Hole: hFc(N297A)-
IL12RB2(24-319)

DNA426

4

DKTHTCPPCAPPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVDVSHEDPEVKFNWVVDGVEVHNAKTKPREEQYAS
TYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPCRDELTKNQVSLWCLVKGFYPSDI
AVEWESNGQPENNYKTTTPVLDSDGSEFFLYSKLTVDKSRWQQGNVFCSCVMHEALHNYHTQKSLSLSPGGGGSGG
GSVPLSLYSGPIWELKKDVVVELDWYDPDAGEEMVVLTCDTPEEDGITTLDQSSEVLGSGGKTLTIQVKEFGDAGQYT
CHKGGEVLSHSLLLHKKEDGIWSTDIKDQKEPKNKTFLRCEAKNYSGRFTCWVWLTITSTDLTFSVKSSRSGSSDPQGV
TCGAATLSAERVGRDNKEYEYSEVCEQDSACPAAEESLPIEVMMVDVHKLKYENVTSSFFIRDIHKPDPKNIQLKPLKN
SRQVEVSWEPDWTSTPHSYFSLTFCVQVQKSKREKKDRVFTDKTSATVICRKNASISVRAQDRYSSSWSEWASV
PCSGGGGGGGGGGGSRNLPVATPDGMPFCLHHSQNLRAVSNMLQKARQTFEPYCTSEEDHEDITKDKTST
VEACPIELTKNESCLNSRETSTFITNGSCLASRKTSMALCLSSIEDLKMYQVEFKTMNAKLLMDPKRQIFLDQNMIL
AVIDELMQALNFNSETVPQKSSLEEDFYKTKIKLCILLHAFRIRAVTIDRVMISYLNAS

Knob: hFc(N297A)-
[VPLSLY]-hIL12B-
hIL12A

5 DNA407

DKTHTCPPCAPPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVDVSHEDPEVKFNWVVDGVEVHNAKTKPREEQYAS
TYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSRDELTKNQVSLSCAVKGFYPSDI
AVEWESNGQPENNYKTTTPVLDSDGSEFFLYSKLTVDKSRWQQGNVFCSCVMHEALHNYHTQKSLSLSPGGGGSGG
GGGSKIDACKRGDVTVKPSHVILLGSTVNITCSLKPRQGCFFHYSRRNKLILYKFDRIINFFHGHSLNSQVTLPLGTTLLF
VCKLACINSDEIQCGAEIFVGVAPEQPQNLSICIQKGEQGTACTWERGRDTHLYTEVTLQLSGPKNLTWQKQCKDIY
CDYLDGGINLTPESPESNFTAKVTAVNSLGSSSSLPSTFTFLDIV

Hole: hFc(N297A)-
IL12RB2(24-222)

5 DNA539

DKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWVVDGVEVHNAKTKPREEQYAS
TYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPCDELTKNQVSLWCLVKGFYPSDI
AVEWESNGQPENNYKTTTPVLDSGSEFLVSKLTVDKSRWQQGNVFCSCVMHEALHNYHTQKSLSLSPGGGGSGG
GSVPLSLYSGPIWELKKDVVVELDWYDPADGEMVVLTCDTPEEDGTTWTLDSQSEVLGSGKTLTIQVKEFGDAGQYT
CHKGGEVLSHSLLLHKKEDGIWSTDILKDQKEPKNKTFLRCEAKNYSGRFTCWWTITSTDLTFSVKSSRSGSSDPQGV
TCGAATLSAERVGRDNKEYEYSECEQDSACPAEESLPIEVMVDVAVHLKLYENVTSSFFIRDIHKPPKNIQLKPLKN
SRQVEVSWEPDTWSTPHSYSLTFCVQVQGGKREKKDRVFTDKTSATVICRKNASISVRAQDRYSSSWSEWASV
PCSGGGGGGGGGGGGSRNLPVATPDGMFPCLHHSQNLRAVSNMLQKARQTLFYPCTSEEDHEDITKDKTST
VEACLIELTKNESCLNSRETSTFITNGSCLASRKTSMALCLSSIVEDLKMYQVEFKTMINAKLLMDPKRQIFLDQNMIL
AVIDELMQALNFNSETVPQKSSLEEDFYKTKIKLCILLHAFRIRAVTTIDRVMSYLNAS

Knob: hFc(N297A)-
[VPLSLY]-hil12B-
hil12A

7 DNA407

DKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWVVDGVEVHNAKTKPREEQYAS
TYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVCTLPSPDELTKNQVSLSCAVKGFYPSDI
AVEWESNGQPENNYKTTTPVLDSGSEFLVSKLTVDKSRWQQGNVFCSCVMHEALHNYHTQKSLSLSPGGGGSGG
SGKIDACKRGDVTVPKSHVILLGSTVNITCSLKPRQGCFFHYSRRNKLILYKFDRRINHHGHSLSNQVTGLPLGTTLFVCK
LACINSDEIQICGAEIFVGAPEQPQNLSCIQKGEQGTACTWERGRDTHLYTEYTLQLSGPKNLTWQKQCKDNYCDY
LDFGINLTPESPESNFTAKVTAVNSLSSSLPSTFTFELDIVRPLPPWDIRIKFQKASVSRCTLYWRDEGLVLLNRLRYRPS
NSRLWNMVMNVTKAKGRHDLDDLKPFTEYEFQISSKLLHLYKGSWSDWSSESRAQTPEE

Hole: hFc(N297A)-
IL12RB2(24-319)

7 DNA540

DKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWVYDVGVEVHNAKTKPREEQYAS
TYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPCDELTKNQVSLWCLVKGFYPSDI
AVEWESNGQPENNYKTTTPVLDSGSEFFLYSKLTVDKSRWQQGNVFCSCVMHEALHNYHTQKSLSLSPGGGGSGGSG
GSVPLSLYSGPIWELKKDVVVVELDWYPDAPGEMVVLTCDTPEEDGITWTLDSQSEVLGSGKTLTIQVKEFGDAGQYT
CHKGGEVLSHSLLLHHKKEGWIWSTDILKDQKEPKNKTFLRCEAKNYSGRFTCWWWLTTITDITLTFSVKSSRSGSDPQGV
TCGAATLSAERVGRDNKEYEYSEVCEQEDSACPAAEESLPIEVMMVDVAVHKLKYENYTSFFIRDIHKPPKNIQLKPLKN
SRQVEVSWEPDTWSTPHSYFSLTFCVQVQGGKSKREKKDRVFTDKTSATVICRKNASISVRAQDRYSSSWSEWASV
PCSGGGGGGGGGGGSRNLPVATPDGMFPCLHHSQNLRAVSNMLQKARQTLFYPCTSEEDHEDITKDKTST
VEACPLIELTKNESCLNSRETSTFITNGSCLASRKTSMALCLSSIVEDLKMYYQVEFKTMINAKLLMDPKRQIFLDQNMIL
AVIDELMQALNFNSETVPQKSSLEEDFYKTKIKLCILLHAFRIRAVTIDRVMSYLNAS

Knob: hFc(N297A)-
[VPLSLY]-hil12B-
hil12A
3 DNA407

DKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWVYDVGVEVHNAKTKPREEQYAS
TYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSRDELTKNQVSLSCAVKGFYPSDI
AVEWESNGQPENNYKTTTPVLDSGSEFFLYSKLTVDKSRWQQGNVFCSCVMHEALHNYHTQKSLSLSPGGGGSGSKI
DACKRGDVTVPKPSHVILLGSTVNITCSLKPRQGCIFYSRNKLILYKFDRIINFHHGHSLSNSQVTGLPLGTTILFVCKLACI
NSDEIQICGAEIFVGVAPEQPQNLSIQKGEQGTACTWERGRDTHLYTEYTLQLSGPKNLTWQKQCKDIYCDYLDG
INLTPESPESNFTAKVTAVNSLGSSSSLPSTFTFLDIVRPLPPWDIRIKFQKASVSRCTLYWRDEGLVLLNRLRYRPSNSRL
WNNMVNVTAKGRHDLDLKPFTEYEFQISSKLHLYKGSWSDWSESRAQTPEE

Hole: hFc(N297A)-
IL12RB2(24-319)
3 DNA541

DKTHITCPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWVYDGVVEVHNAKTKPREEQYAS
TYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPCRDELTKNQVSLWCLVKGFYPSDI
AVEWESNGQPENNYKTTTPVLDSDGSEFFLYSKLTVDKSRWQQGNVFCSCVMHEALHNNHYTKSLSPGGGGSGSG
GSVPLSLYSGPIWELKKDQVYVVELDWYDPDAPGEMVWLTCDTPEEDGITTLDQSSEVLGSGGKTLTIQVKEFGDAGQYT
CHKGGEVLHSHLLLLHKKEDGIWSTDILKQKEPKNKTFLRCEAKNYSGRFTCWWLTTISTDLTFSVKSSRSGSDPPQGV
TCGAAATLSAERVGRDNKEYEYSVECEQDSACPAAEESLPIEVMDAVHKLKYENYTSFFIRDIHKPDPKNIQLKPLKN
SRQVEVSWEYPDTWSTPHSYFSLTFCVQVQGGKSKREKKORVFTDKTSATVICRKNASISVRAQDRYSSSWSEWASV
PCSGGGSGGGGGGGSRNLPVATPDGMFPCLHHSQNLRAVSNMLQKARQTLFYPCTSEEDHEDITKDKTST
VEACLPLELTKNESCLNSRETSTFITNGSCLASRKTSTMMALCLSIYEDLKMYYQVEFTMNAKLLMDPKRQIFLDQNMIL
AVIDELMQALNFNSETVPQKSSLEEPDFYKTKIKLCILLHAFRIRAVTIDRVMSYLNAS

Knob: hFc(N297A)-
[VPLSLY]-hil12B-
hil12A

3 DNA407

DKTHITCPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWVYDGVVEVHNAKTKPREEQYAS
TYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVCTLPSPRDELTKNQVSLSCAVKGFYPSDI
AVEWESNGQPENNYKTTTPVLDSDGSEFFLYSKLTVDKSRWQQGNVFCSCVMHEALHNNHYTKSLSPGGGGSGSG
GGGSKIDACKRGDVTVKPSHVILLGSTVNITCSLKPRQGCFFHYSRRNKLILYKFDRRINFHHGHSLSNSQVTLGLPLGTTLF
VCKLACINSDEIQCGAEIFVGVAPEQPQNLSIQKGEQGTACTWGERGRDTHLYTEYTLQLSGPKNLTWQKQCKDIY
CDYLDGGINLTPESPESNFTAKVTAVNSLGSSSSL

Hole: hFc(N297A)-
IL12RB2(24-212)

3 DNA629

DKTHTCPCPAPPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYAS
TYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPCDELTKNQVSLWCLVKGFYPSDI
AVEWESNGQPENNYKTTTPVLDSDGSEFFLVSKLTVDKSRWQQGNVFSCSVMEALHNHYTQKSLSLSPGGGGSGG
GSVPLSLYSGPIWELKDDVYVVELDWYYPDAPGEMVVLTCDTPEEDGTTWLTLDQSSEVLGSGKTLTIQVKEFGDAGQYT
CHKGGEVLSHSLLLHKKEDGIWSTDILKDKQKEPKNTFLRCEAKNYSGRFTCWWTITITDLSFVSKSRGSSDPQGV
TCGAATLSAERVGRDNKEYEYSECEQDSACPAEESLPIEVMVDVAVHKLKYENYTSFFFIIRDIIKPPKNLQLKPLKN
SRQVEVSWEYPTDWTSTPHSYFSLTFCVQVQGGKSKREKKDRVFTDKTSATVICRKNASISVRAQDRYSSSWSEWASV
PCSGGGGGGGGGGGGGSRNLPVATPDGMFPCLHHSQNLRAVSNMLQKARQTLFPCTSEEDHEDITDKTST
VEACPLIELTKNESCLNSRETSTFITNGSCLASRKTSTFMMALCLSSIVEDLKMYYQVEKTMVNAKLLMDPKRQIFLDQNMIL
AVIDELMQALNFNSETVPQKSSLEEDFYKTKIKLCILLHAFIRAVTIDRVMSYLNAS

Knob: hFc(N297A)-
[VPLSLY]-hil12B-
hil12A

3 DNA407

DKTHTCPCPAPPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYAS
TYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVCTLPSPRDELTKNQVSLSCAVKGFYPSDI
AVEWESNGQPENNYKTTTPVLDSDGSEFFLVSKLTVDKSRWQQGNVFSCSVMEALHNHYTQKSLSLSPGGGGSGG
GGGSKIDACKRGDVTVKPSHVILLGSTVNIITSLKPRQGCFFHYSRRNKLILYKFDRIHFHGHSLNSQVTLPLGTTLF
VCKLACINSDEIQCGAEIFVGVAPEQPQNLSCIQKGEQGTACTWERGRDTHLYTEVTLQLSGPKNLTWQKQCKDIY
CDYLDGGINLTPESPESNFTAKVTAVNSLGSSSSLPSTFTFLDIVRPLPP

Hole: hFc(N297A)-
IL12RB2(24-227)

3 DNA644

ne	newnames	Component1Sequence	Component2Sequence
.58	Hole: hFc(N297A)	DKTHTCPCPAPELLGGPSVFLFPPKPKDTLMISRT PEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKT KPREEQYASTYRVSVLTVLHQDWLNGKEYKCKV SNKALPAPIEKTISKAKGQPREPQVCTLPISRDEL KNQVLSLSCAVKGFYPSDIAVEWESNGQPENNYKT TPPVLDSDGSFFLVSKLTVDKSRWQQGNVFCSV MHEALHNHYTQKSLSLSPG	
.06	Knob: hFc(N297A)- [VPLSLY]-hIL12B- hIL12A	DKTHTCPCPAPELLGGPSVFLFPPKPKDTLMISRT PEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKT KPREEQYASTYRVSVLTVLHQDWLNGKEYKCKV SNKALPAPIEKTISKAKGQPREPQVYTLPCRDEL KNQVSLWCLVKGFPYSDIAVEWESNGQPENNYKT TPPVLDSDGSFFLVSKLTVDKSRWQQGNVFCSV MHEALHNHYTQKSLSLSPG	GGSGGS

50.

Hole: hFc(N297A)-
hCD212(24-545)

DKTHTCPCPAPELLGGPSVFLFPPKPKDTLMISRT
PEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKT
KPREEQYASTYRVSVLTVLHQDWLNGKEYKCKV
SNKALPAPIEKTISKAKGQPREPQVCTLPISRDELTTGGGGSGGGGS
KNQVLSLCAVKGFPYSDIAVEWESNGQPENNYKT
TPPVLDSDGSFFLVSKLTVDKSRWQQGNVFCFCSV
MIEALHNHYTQKSLSLSPG

Hole: hFc(N297A)-
hCD212(24-237)

.10

DKTHTCPCPAPELLGGPSVFLFPPKPKDTLMISRT
PEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKT
KPREEQYASTYRVSVLTVLHQDWLNGKEYKCKV
SNKALPAPIEKTISKAKGQPREPQVCTLPISRDELTTGGGGSGGGGS
KNQVLSLCAVKGFPYSDIAVEWESNGQPENNYKT
TPPVLDSDGSFFLVSKLTVDKSRWQQGNVFCFCSV
MIEALHNHYTQKSLSLSPG

Hole: hFc(N297A)-
IL12RB2(24-622)

.25

Hole: hFc(N297A)-
IL12RB2(24-319)

39

Hole: hFc(N297A)-
IL12RB2(24-222)

40

Hole: hFc(N297A)-
IL12RB2(24-319)

41	Hole: hFc(N297A)- IL12RB2(24-319)	DKTHTCPCPAPELLGGPSVFLFPPKPKDTLMISRT PEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKT KPREEQYASTYRVSVLT/LHQDWLNGKEYCKV SNKALPAPIEKTISKAKGQPREPQVCTLPISRDEL KNQVLSLCAV/KGFYPSDIAVEWESNGQPENNYKT TPPVLDSDGSFFLVSKLTVDKSRWQQGNVFC MHEALHNHYTQKSLSLSPG	GGGGG
42	Hole: hFc(N297A)- IL12RB2(24-212)	DKTHTCPCPAPELLGGPSVFLFPPKPKDTLMISRT PEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKT KPREEQYASTYRVSVLT/LHQDWLNGKEYCKV SNKALPAPIEKTISKAKGQPREPQVCTLPISRDEL KNQVLSLCAV/KGFYPSDIAVEWESNGQPENNYKT TPPVLDSDGSFFLVSKLTVDKSRWQQGNVFC MHEALHNHYTQKSLSLSPG	GGGGGGGGG
44	Hole: hFc(N297A)- IL12RB2(24-222)	DKTHTCPCPAPELLGGPSVFLFPPKPKDTLMISRT PEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKT KPREEQYASTYRVSVLT/LHQDWLNGKEYCKV SNKALPAPIEKTISKAKGQPREPQVCTLPISRDEL KNQVLSLCAV/KGFYPSDIAVEWESNGQPENNYKT TPPVLDSDGSFFLVSKLTVDKSRWQQGNVFC MHEALHNHYTQKSLSLSPG	GGGGGGGGG

27	Hole: hFc(N297A)- IL12RB2(24-210)	DKTHTCPPCPAPPELLGGPSVFLFPPKPKDTLMISRT PEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKT KPREEQYASTYRVSVLTVHQDWLNGKEYKCKV SNKALPAPIEKTISKAKGQPREPQVCTLPISRDEL KNQVLSLCAVKGFPYSDIAVEWESNGQPENNYKT TPPVLDSDGSFFLVSKLTVDKSRWQQGNVFC MHEALHNHYTQKSLSLSPG	GGGGSGGGGS
28	Hole: hFc(N297A)- IL12RB2(24-211)	DKTHTCPPCPAPPELLGGPSVFLFPPKPKDTLMISRT PEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKT KPREEQYASTYRVSVLTVHQDWLNGKEYKCKV SNKALPAPIEKTISKAKGQPREPQVCTLPISRDEL KNQVLSLCAVKGFPYSDIAVEWESNGQPENNYKT TPPVLDSDGSFFLVSKLTVDKSRWQQGNVFC MHEALHNHYTQKSLSLSPG	GGGGSGGGGS
29	Hole: hFc(N297A)- IL12RB2(24-212)	DKTHTCPPCPAPPELLGGPSVFLFPPKPKDTLMISRT PEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKT KPREEQYASTYRVSVLTVHQDWLNGKEYKCKV SNKALPAPIEKTISKAKGQPREPQVCTLPISRDEL KNQVLSLCAVKGFPYSDIAVEWESNGQPENNYKT TPPVLDSDGSFFLVSKLTVDKSRWQQGNVFC MHEALHNHYTQKSLSLSPG	GGGGSGGGGS

Hole: hfc(N297A)-
IL12RB2(24-213)

31

Hole: hFc(N297A)-
IL12RB2(24-214)

32

Hole: hFc(N297A)-
IL12RB2(24-215)

33 Hole: hFc(N297A)-
IL12RB2(24-216)
DKTHTCPAPPELLGGPSVFLFPPKPKDTLMISRT
PEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKT
KPREEQYASTYRVSVLT/LHQDWLNGKEYKCKV
SNKALPAPIEKTISKAKGQPREPQVCTLPISRDEL
KNQVLSLCAV/KGFYPSDIAVEWESNGQPENNYKT
TPPVLDSDGSFFLVSKLTVDKSRWQQGNVFC
MHEALHNHYTQKSLSLSPG
GGGGSGGGGS

34 Hole: hFc(N297A)-
IL12RB2(24-217)
DKTHTCPAPPELLGGPSVFLFPPKPKDTLMISRT
PEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKT
KPREEQYASTYRVSVLT/LHQDWLNGKEYKCKV
SNKALPAPIEKTISKAKGQPREPQVCTLPISRDEL
KNQVLSLCAV/KGFYPSDIAVEWESNGQPENNYKT
TPPVLDSDGSFFLVSKLTVDKSRWQQGNVFC
MHEALHNHYTQKSLSLSPG
GGGGSGGGGS

35 Hole: hFc(N297A)-
IL12RB2(24-218)
DKTHTCPAPPELLGGPSVFLFPPKPKDTLMISRT
PEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKT
KPREEQYASTYRVSVLT/LHQDWLNGKEYKCKV
SNKALPAPIEKTISKAKGQPREPQVCTLPISRDEL
KNQVLSLCAV/KGFYPSDIAVEWESNGQPENNYKT
TPPVLDSDGSFFLVSKLTVDKSRWQQGNVFC
MHEALHNHYTQKSLSLSPG
GGGGSGGGGS

36 Hole: hFc(N297A)-
IL12RB2(24-219) DKTHTPCPCAPELLGGPSVFLFPKPKDTLMIISRT
PEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKT
KPREEQYASTYRVVSVLTVLHQDWLNGKEYKCKV
SNKALPAIEKTSISKAKGQPREPQVCTLLPSRDEL
TKNQVSLTSCAVKGFPYSDIAVEWESNGQQPENNYKT
TPPVLDSDGSFFLVSKLTVDKSRWQQGNVVFCSV
MIHEALHNHYTQKSLSLSPG

37

Hole: hFc(N297A)-
IL12Rβ2(24-220)

DKTHTPCPCAPELLGGPSVFLFPKPKDTLMISRT
PEVTCVVDSHEDPEVKFNWYVDGVEVHNAKT
KPREEQYASTYRVVSVLTVLHQDWLNGKEYCKKV
SNKALPAIEKTSKAKGQPREPQVCTLLPSRDEL
KNQVLSCAVKGFYPSDIAVEWESNGQPENNYKT
TPPVLDSDGSFFLVSKLTVDKSRWQQGNVVFCSV
MHEALHNHYTQKSLSLSPG

GGGGSGGGGS

DKTHTPCPCAPPELLGGPSVFLFPPKPKDTLMISRT
PEVTCWVDVSHEDPEVKFNWYVDGVEVHNAKT
KPREEQYASTYRVVSVLTVLHQDWLNGKEYKCKV
SNKALPAPIEKTSKAKGQPREPQVCTLLPSRDEL
KNQVLSCAVKGFPYSDIAVEWESNGQPENNYKT
TPPVLDSGSGFFLVSKLTVDKSRWQQGNVVFCSV
MIHEALHNHYTQKSLSLSPG

39 Hole: hFc(N297A)-
IL12RB2(24-222)
DKTHTCPCPAPELLGGPSVFLFPPKPKDTLMISRT
PEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKT
KPREEQYASTYRVSVLT/LHQDWLNGKEYCKV
SNKALPAPIEKTISKAKGQPREPQVCTLPISRDEL
KNQVLSLCAV/KGFYPSDIAVEWESNGQPENNYKT
TPPVLDSDGSFFLVSKLTVDKSRWQQGNVFC
MHEALHNHYTQKSLSLSPG
GGGGSGGGGS

40 Hole: hFc(N297A)-
IL12RB2(24-223)
DKTHTCPCPAPELLGGPSVFLFPPKPKDTLMISRT
PEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKT
KPREEQYASTYRVSVLT/LHQDWLNGKEYCKV
SNKALPAPIEKTISKAKGQPREPQVCTLPISRDEL
KNQVLSLCAV/KGFYPSDIAVEWESNGQPENNYKT
TPPVLDSDGSFFLVSKLTVDKSRWQQGNVFC
MHEALHNHYTQKSLSLSPG
GGGGSGGGGS

41 Hole: hFc(N297A)-
IL12RB2(24-224)
DKTHTCPCPAPELLGGPSVFLFPPKPKDTLMISRT
PEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKT
KPREEQYASTYRVSVLT/LHQDWLNGKEYCKV
SNKALPAPIEKTISKAKGQPREPQVCTLPISRDEL
KNQVLSLCAV/KGFYPSDIAVEWESNGQPENNYKT
TPPVLDSDGSFFLVSKLTVDKSRWQQGNVFC
MHEALHNHYTQKSLSLSPG
GGGGSGGGGS

42
Hole: hFc(N297A)-
IL12RB2(24-225)

43
Hole: hFc(N297A)-
IL12RB2(24-226)

44
Hole: hFc(N297A)-
IL12RB2(24-227)

Hole: hFc(N297A)-
IL12RB2(24-228)

DKTHTPCPAPELLGGPSVFLFPKPKDTLMIISRT
PEVTCVVDSHEDPEVKFNWYVDGVEVHNAKT
KPREEQYASTYRVVSVLTVLHQDWLNGKEYCKKV
SNKALPAIEKTSKAKGQPREPQVCTLLPSRDEL
KNQVLSCAVKGFPYSDIAVEWESNGQDENNYKT
TPPVLDSGSGFFLVSKLTVDKSRWQQGNVVFCSV
MHEALHNHYTQKSLSLSPG

Hole: hFc(N297A)-
IL12RB2(24-230)
47
DKHTTCCPAPPELLGGPSVFLFPPKPKDTLMISRT
PEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKT
KPREEQYASTYRVVSVLTVLHQDWLNGKEYCKKV
SNKALPAIEKTSISKAGQPREPQVCTLLPSRDEL
KNQVLSCAVKGFGYPSDIAVEWESNGQPENNYKT
TPPVLDSDGSFFLVSKLTVDKSRWQQGNVVFCSV
MHEALHNHYTQKSLSLSPG
GGGGGGGGGG

48
Hole: hFc(N297A)-
IL12RB2(24-231)
DKTHTPCPCAPELLGGPSVFLFPPKPKDTLMIISRT
PEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKT
KPREEQYASTYRVVSVLTVLHQDWLNGKEYCKV
SNKALPAIEKTSISKAKGQPREPQVCTLLPSRDEL
KNQVLSCAVKGFYPSDIAVEWESNGQPENNYKT
TPPVLDSDGSFFLVSKLTVDKSRWQQGNVVFCSV
MIHEALHNHYTQKSLSLSPG

49

50 Hole: hFc(N297A)-
IL12RB2(24-233) GGGGGGGGGG

DKTHTCPCPAPELLGGPSVFLFPKPKDTLMIISRT
PEVTCWVDVSHEDPEVKFNWYVDGVEVHNAKT
KPREEQYASTYRVVSVLTVLHQDWLNGKEYCKKV
SNKALPAPIEKTSKAKGQPREPQVCTLLPSRDEL
KNQVLSCAVKGFYPSDIAVEWESNGQPENNYKT
TPPVLDSDGSFFLVSKLTVDKSRWQQGNVVFCSV
MIHEALHNHYTQKSLSLSPG

ne	newnames	Component3Sequence	Component4Se quence	IL-12 p40 subunit
.58	Hole: hFc(N297A)			
.05	Knob: hFc(N297A)- [VPLSLY]-hIL12B- hIL12A		SGP	IWELKDVVVELDWYPDAPGEMVVLTCDTPEEDGIT WTLDSSEVLGSGKTLTIQVKEFGDAGQYCHKGGEVL SHSLLLHKKEDGIWSTDILKDQKEPKNKTFLRCEAKNYS GRFTCWWLTTISTDLTFSVKSSRGSSDPQGVTCGAATL SAERVGRDNKEYEYSVEQCEDSACPAAEESLPIEVMVD AVHKLKYNYSSTFFIRDIKPDPPKLNQLKPLKNSRQVE VSWEYPTWTSTPHSYFSLTFCVQVQGKSKREKKDRVFT DKTSATVICRKNASISVRAQDRYYSSSWSEWASVPCS
.07	Knob: hFc(N297A)- [VPLSLY]-hIL12B- hIL12A		SGP	IWELKDVVVELDWYPDAPGEMVVLTCDTPEEDGIT WTLDSSEVLGSGKTLTIQVKEFGDAGQYCHKGGEVL SHSLLLHKKEDGIWSTDILKDQKEPKNKTFLRCEAKNYS GRFTCWWLTTISTDLTFSVKSSRGSSDPQGVTCGAATL SAERVGRDNKEYEYSVEQCEDSACPAAEESLPIEVMVD AVHKLKYNYSSTFFIRDIKPDPPKLNQLKPLKNSRQVE VSWEYPTWTSTPHSYFSLTFCVQVQGKSKREKKDRVFT DKTSATVICRKNASISVRAQDRYYSSSWSEWASVPCS

CRTSECCFQDPPYPADSGSASGPRDLRCYRISSDRYECSWQ
YEGPTAGVSHFLRCCLSSGRCCYFAAGSATRLQFSDQAGVSV
LYTVTLWVESWARNQTEKSPVTLQLYNSVKYEPPLGDIKVS
KLAGQLRMEWETPDNQVGAEVQFRHRTSPSPWKLGDGCP
QDDDTESCLCPLMNVAQEFQLRRRLQSGSGSSWSKWSSP
VCVPPENPPQPVRFVSQLGQDGRRLTLKEQPTQLELPE
GCQGLAPGTEVYRLQLHMLSCPKAKATRTLHLGKMPYLS
GAAYNAVISSNQFGPLNQTHIPADTHTEPVALNISVGT
NGTTMYWPARAQSMITYCIEWQPVGQDGLATCSLTAPQD
PDPAGMATYSWSRESGAMGOEKCYITIFASAHPEKLTLS
TVLSTYHFGGNASAAAGTPHHVSVKNHSLDSVVDWAPSLLS
TCPGVLKEYVVRCDREDSKQVSEHPVQPTETQVTLGRLAGV
AYTVQVRADTAWLRGVWSQPQRFSEIVQVSD

Hole: hFc(N297A)-
hCD212(24-545)

.09

CRTSECCFQDPPYPADSGSASGPRDLRCYRISSDRYECSWQ
YEGPTAGVSHFLRCCLSSGRCCYFAAGSATRLQFSDQAGVSV
LYTVTLWVESWARNQTEKSPVTLQLYNSVKYEPPLGDIKVS
KLAGQLRMEWETPDNQVGAEVQFRHRTSPSPWKLGDGCP
QDDDTESCLCPLMNVAQEFQLRRRLQSGSGSSWSKWSSP
VCVPPENP

Hole: hFc(N297A)-
hCD212(24-237)

.10

KIDACKRGDVTVPKPSHVILLGSTVNITCSLKPRQGCIFYSRRN
KILLYKFDRRINFHHGHSLSNSQVTGLPLGTTLFVCKLACINSDE
IQICGAEIFVGVAPEQPQNLSQIQKGEQGTACTWGERGDT
HLYTEYTLQJSGPKNLTWQCKDIYCDYLDGFINLTPESPES
NFTAKVTAVNSLGSSSSLPTFTFLDIVRPLPPWDIRIKFQKAS
VSRCTLYWRDEGLVLLNRLRYRPSNRLWNMNVTKAKGR
HDLLDKPFTFEYEFQISSKLHLYKGSWSDWSESRAQTPEEP
TGMLDVWVMKRHDYSRQJSLFWKNLSVSEARGKILHYQV
TLQELTGGKAMTONITGHTSWTTVIPRTGNWAVAVSAANS
KGSSLPTRINIMNLCAGLLAPRQVSANSEGMNDNILVTWQP
PRKDPQSAVQEVVWRELHPGGDTQVPLNWLRSRPNVSA
LISENIKSYICYEIRVYALSGDQGGSSILGNSKHKAPLSGPHIN
AITEEKGSILISWNSIPVQEQMGCLLHYRIYWKERDSNSQPQL
CEIPYRVSONSHPINSLQPRVTYVLMWMTALTAAGESSHGNE
REFCLQGGKAN

Hole: hFc(N297A)-
IL12RB2(24-622)

.25

KIDACKRGDVTVPKPSHVILLGSTVNITCSLKPRQGCIFYSRRN
KILLYKFDRRINFHHGHSLSNSQVTGLPLGTTLFVCKLACINSDE
IQICGAEIFVGVAPEQPQNLSQIQKGEQGTACTWGERGDT
HLYTEYTLQJSGPKNLTWQCKDIYCDYLDGFINLTPESPES
NFTAKVTAVNSLGSSSSLPTFTFLDIVRPLPPWDIRIKFQKAS
VSRCTLYWRDEGLVLLNRLRYRPSNRLWNMNVTKAKGR
HDLLDKPFTFEYEFQISSKLHLYKGSWSDWSESRAQTPEE

Hole: hFc(N297A)-
IL12RB2(24-319)

.26

39 Hole: hfc(N297A)-
IL12RB2(24-222)

KIDACKRGDVTVPKSHVLLGSTVYNITCSLKPRQGGCFHYSRRN
KILILYKFDRRNFHHGHSLSQVTLGLPTGTLFVCKLACINSDE
QIOICGAIEFVGVAPEQPQNLSQIQKGEQGTVACTWGERDGT
HLHYEYTLQLSGPKNLTWQKQCKDIYCDYLDGFINLTSPES
NFTAKVTAVNSLGSSSLPSTFTFLDIVRPLPPWDIRIKFQKAS
VVSRCITVYWRDEGLVLNRLRYRPSNSRLWNMVNVTKAKGR
HDLDLKPFTEVEFQISSKLHLYGKGSWSDWSFSLRAQTPEE

40 Hole: hFc(N297A)-
IL12RB2(24-319)

KIDACKRGDVTVPKSHVLLGSTVNITCSLKPRQGGCFHYSRRN
KILILYKFDRRINFHHGSLNSQVTLGLPTTLFVCKLACINSDE
QIQICGAIEFVGVAPEQPQNLSQIKQGEQGTVACTWFERGDT
HLHYEYTLQLSGPKNLTWQKQCKDIYCDYLFNGILTPESPES
NFTAKVTAVNSLGSSSLPSTFTFLDIVRPLPPWDIRIKFQKAS
VSRCTLYVRDEGLVILNRLRYRPSNSRLWNMVNVTKAKGR
HDLDLDKPTEYEFQJSSKLHLYKGWSWDWSESRAQTPEE

41
Hole: hFc(N297A)-
L12RB2(24-319)

129 KIDACKRGDVTVPKPSHVILLGSTVNITCSLKPRQGCCHYSRRN
 KLILYKFDRRINFHHGHSLSNSQVTGLPLGTTLFVCKLACINSDE
 IQICGAEIFVGVAPEQPQNLSCIQKGEQGTACTWGERGDT
 HLYTEYTLQLSGPKNLTWQKQCKDIYCDYLDGGINLTPEPES
 NFTAKVTAVNSLGSSSL

Hole: hFc(N297A)-
IL12RB2(24-212)

144 KIDACKRGDVTVPKPSHVILLGSTVNITCSLKPRQGCCHYSRRN
 KLILYKFDRRINFHHGHSLSNSQVTGLPLGTTLFVCKLACINSDE
 IQICGAEIFVGVAPEQPQNLSCIQKGEQGTACTWGERGDT
 HLYTEYTLQLSGPKNLTWQKQCKDIYCDYLDGGINLTPEPES
 NFTAKVTAVNSLGSSSLPSTFTFLDIV

Hole: hFc(N297A)-
IL12RB2(24-222)

127 KIDACKRGDVTVPKPSHVILLGSTVNITCSLKPRQGCCHYSRRN
 KLILYKFDRRINFHHGHSLSNSQVTGLPLGTTLFVCKLACINSDE
 IQICGAEIFVGVAPEQPQNLSCIQKGEQGTACTWGERGDT
 HLYTEYTLQLSGPKNLTWQKQCKDIYCDYLDGGINLTPEPES
 NFTAKVTAVNSLGSSS

Hole: hFc(N297A)-
IL12RB2(24-210)

KIDACKRGDVTVPKPSHVILLGSTVNITCSLKPRQGCIFYSRRN
KILLYKFDRRINFHHGHSLSNSQVTGLPLGTTLFVCKLACINSDE
IQICGAEIFVGVAPEQPONLSQKGEQGTACTWGERGDT
HLYTEYTLQLSGPKNLTWQKQCKDIYCDYLDGGINLTPEPES
NFTAKVTAVNSLGSS

Hole: hFc(N297A)-
IL12RB2(24-211)

28

KIDACKRGDVTVPKPSHVILLGSTVNITCSLKPRQGCIFYSRRN
KILLYKFDRRINFHHGHSLSNSQVTGLPLGTTLFVCKLACINSDE
IQICGAEIFVGVAPEQPONLSQKGEQGTACTWGERGDT
HLYTEYTLQLSGPKNLTWQKQCKDIYCDYLDGGINLTPEPES
NFTAKVTAVNSLGSSSL

Hole: hFc(N297A)-
IL12RB2(24-212)

29

KIDACKRGDVTVPKPSHVILLGSTVNITCSLKPRQGCIFYSRRN
KILLYKFDRRINFHHGHSLSNSQVTGLPLGTTLFVCKLACINSDE
IQICGAEIFVGVAPEQPONLSQKGEQGTACTWGERGDT
HLYTEYTLQLSGPKNLTWQKQCKDIYCDYLDGGINLTPEPES
NFTAKVTAVNSLGSSSLP

Hole: hFc(N297A)-
IL12RB2(24-213)

30

KIDACKRGDVTVPKPSHVILLGSTVNITCSLKPRQGCIFYSRRN
KLILYKFDRRINFHHGHSLSNSQVTGLPLGTTLFVCKLACINSDE
IQICGAEIFVGVAPQPNLSQKGEQGTACTWGERGDT
HLYTEYTLQLSGPKNLTWQKQCKDIYCDYLDYDFGINLTPESPES
NFTAKVTAVNSLGSSSSLPST

Hole: hFc(N297A)-
IL12RB2(24-214)

31

KIDACKRGDVTVPKPSHVILLGSTVNITCSLKPRQGCIFYSRRN
KLILYKFDRRINFHHGHSLSNSQVTGLPLGTTLFVCKLACINSDE
IQICGAEIFVGVAPQPNLSQKGEQGTACTWGERGDT
HLYTEYTLQLSGPKNLTWQKQCKDIYCDYLDYDFGINLTPESPES
NFTAKVTAVNSLGSSSSLPST

Hole: hFc(N297A)-
IL12RB2(24-215)

32

KIDACKRGDVTVPKPSHVILLGSTVNITCSLKPRQGCIFYSRRN
KLILYKFDRRINFHHGHSLSNSQVTGLPLGTTLFVCKLACINSDE
IQICGAEIFVGVAPQPNLSQKGEQGTACTWGERGDT
HLYTEYTLQLSGPKNLTWQKQCKDIYCDYLDYDFGINLTPESPES
NFTAKVTAVNSLGSSSSLPSTF

Hole: hFc(N297A)-
IL12RB2(24-216)

33

KIDACKRGDVTVPKPSHVILLGSTVNITCSLKPRQGCIFYSRRN
KLILYKFDRRINFHHGHSLSNSQVTGLPLGTTTLFVCKLACINSDE
IQICGAEIFVGVAPQPNLSCIQKGEQGTACTWGERGDT
HLYTEYTLQLSGPKNLTWQKQCKDIYCDYLDGFINLTPESPES
NFTAKVTAVNSLGSSSSLPSTFT

Hole: hFc(N297A)-
IL12RB2(24-217)

34

KIDACKRGDVTVPKPSHVILLGSTVNITCSLKPRQGCIFYSRRN
KLILYKFDRRINFHHGHSLSNSQVTGLPLGTTTLFVCKLACINSDE
IQICGAEIFVGVAPQPNLSCIQKGEQGTACTWGERGDT
HLYTEYTLQLSGPKNLTWQKQCKDIYCDYLDGFINLTPESPES
NFTAKVTAVNSLGSSSSLPSTFT

Hole: hFc(N297A)-
IL12RB2(24-218)

35

KIDACKRGDVTVPKPSHVILLGSTVNITCSLKPRQGCIFYSRRN
KLILYKFDRRINFHHGHSLSNSQVTGLPLGTTTLFVCKLACINSDE
IQICGAEIFVGVAPQPNLSCIQKGEQGTACTWGERGDT
HLYTEYTLQLSGPKNLTWQKQCKDIYCDYLDGFINLTPESPES
NFTAKVTAVNSLGSSSSLPSTFT

Hole: hFc(N297A)-
IL12RB2(24-219)

36

KIDACKRGDVTVPKPSHVILLGSTVNITCSLKPRQGCIFYSRRN
KLILYKFDRRINFHHGHSLSNSQVTGLPLGTTTLFVCKLACINSDE
IQICGAEIFVGVAPQPNLSCIQKGEQGTACTWGERGDT
HLYTEYTLQLSGPKNLTWQKQCKDIYCDYLDGFINLTPESPES
NFTAKVTAVNSLGSSSSLPSTFT

Hole: hFc(N297A)-
IL12RB2(24-220)

37

KIDACKRGDVTVPKPSHVILLGSTVNITCSLKPRQGCIFYSRRN
KLILYKFDRRINFHHGHSLSNSQVTGLPLGTTLFVCKLACINSDE
IQICGAEIFVGVAPEQPQNLSQKGEQGTACTWGERGDT
HLYTEYTLQLSGPKNLTWQKQCKDIYCDYLDGFINLTPESPES
NFTAKVTAVNSLGSSSSLPSTFTFLDI

Hole: hFc(N297A)-
IL12RB2(24-221)

38

KIDACKRGDVTVPKPSHVILLGSTVNITCSLKPRQGCIFYSRRN
KLILYKFDRRINFHHGHSLSNSQVTGLPLGTTLFVCKLACINSDE
IQICGAEIFVGVAPEQPQNLSQKGEQGTACTWGERGDT
HLYTEYTLQLSGPKNLTWQKQCKDIYCDYLDGFINLTPESPES
NFTAKVTAVNSLGSSSSLPSTFTFLDIV

Hole: hFc(N297A)-
IL12RB2(24-222)

39

KIDACKRGDVTVPKPSHVILLGSTVNITCSLKPRQGCIFYSRRN
KLILYKFDRRINFHHGHSLSNSQVTGLPLGTTLFVCKLACINSDE
IQICGAEIFVGVAPEQPQNLSQKGEQGTACTWGERGDT
HLYTEYTLQLSGPKNLTWQKQCKDIYCDYLDGFINLTPESPES
NFTAKVTAVNSLGSSSSLPSTFTFLDIVR

Hole: hFc(N297A)-
IL12RB2(24-223)

40

KIDACKRGDVTVPKPSHVILLGSTVNITCSLKPRQGCIFYSRRN
KLILYKFDRRINFHHGHSLSNSQVTGLPLGTTLVCKLACINSDE
IQICGAEIFVGVAPEQPQNLSQKGEQGTACTWGERGDT
HLYTEYTLQLSGPKNLTWQKCKDIYCDYLDYDFGINLTPESPES
NFTAKVTAVNSLGSSSSLPSTFTFLDIVRPL

Hole: hFc(N297A)-
IL12RB2(24-224)

41

KIDACKRGDVTVPKPSHVILLGSTVNITCSLKPRQGCIFYSRRN
KLILYKFDRRINFHHGHSLSNSQVTGLPLGTTLVCKLACINSDE
IQICGAEIFVGVAPEQPQNLSQKGEQGTACTWGERGDT
HLYTEYTLQLSGPKNLTWQKCKDIYCDYLDYDFGINLTPESPES
NFTAKVTAVNSLGSSSSLPSTFTFLDIVRPL

Hole: hFc(N297A)-
IL12RB2(24-225)

42

KIDACKRGDVTVPKPSHVILLGSTVNITCSLKPRQGCIFYSRRN
KLILYKFDRRINFHHGHSLSNSQVTGLPLGTTLVCKLACINSDE
IQICGAEIFVGVAPEQPQNLSQKGEQGTACTWGERGDT
HLYTEYTLQLSGPKNLTWQKCKDIYCDYLDYDFGINLTPESPES
NFTAKVTAVNSLGSSSSLPSTFTFLDIVRPL

Hole: hFc(N297A)-
IL12RB2(24-226)

43

KIDACKRGDVTVPKPSHVILLGSTVNITCSLKPRQGCIFYHRRN
KULYKFDRRINFHHGHSLSQVTGLPLGTTLFVCKLACINSDE
IQICGAEIFVGVAPEQPQNLSICQKGEQGTACTWGERGDT
HLYTEYTLQLSGPKNLTWQKQCKDIYCDYLDGFINLTPESPES
NFTAKVTAVNSLGSSSSLPSTFTFLDIVRPLPP

Hole: hFc(N297A)-
IL12RB2(24-227)

44

KIDACKRGDVTVPKPSHVILLGSTVNITCSLKPRQGCIFYHRRN
KULYKFDRRINFHHGHSLSQVTGLPLGTTLFVCKLACINSDE
IQICGAEIFVGVAPEQPQNLSICQKGEQGTACTWGERGDT
HLYTEYTLQLSGPKNLTWQKQCKDIYCDYLDGFINLTPESPES
NFTAKVTAVNSLGSSSSLPSTFTFLDIVRPLPPW

Hole: hFc(N297A)-
IL12RB2(24-228)

45

KIDACKRGDVTVPKPSHVILLGSTVNITCSLKPRQGCIFYHRRN
KULYKFDRRINFHHGHSLSQVTGLPLGTTLFVCKLACINSDE
IQICGAEIFVGVAPEQPQNLSICQKGEQGTACTWGERGDT
HLYTEYTLQLSGPKNLTWQKQCKDIYCDYLDGFINLTPESPES
NFTAKVTAVNSLGSSSSLPSTFTFLDIVRPLPPWD

Hole: hFc(N297A)-
IL12RB2(24-229)

46

47 KIDACKRGDVTVPKPSHVILLGSTVNITCSLKPRQGCIFYSRRN
 KLILYKFDRRINFHHGHSLSNSQVTGLPLGTTLFVCKLACINSDE
 IQICGAEIFVGVAPEQPQNLSICIQKGEQGTACTWGERGDT
 HLYTEYTLQLSGPKNLTWQKQCKDIYCDYLDGFINLTPESPES
 NFTAKVTAVNSLGSSSSLPSTFTFLDIVRPLPPWDI
Hole: hFc(N297A)-
IL12RB2(24-230)

48 KIDACKRGDVTVPKPSHVILLGSTVNITCSLKPRQGCIFYSRRN
 KLILYKFDRRINFHHGHSLSNSQVTGLPLGTTLFVCKLACINSDE
 IQICGAEIFVGVAPEQPQNLSICIQKGEQGTACTWGERGDT
 HLYTEYTLQLSGPKNLTWQKQCKDIYCDYLDGFINLTPESPES
 NFTAKVTAVNSLGSSSSLPSTFTFLDIVRPLPPWDIR
Hole: hFc(N297A)-
IL12RB2(24-231)

49 KIDACKRGDVTVPKPSHVILLGSTVNITCSLKPRQGCIFYSRRN
 KLILYKFDRRINFHHGHSLSNSQVTGLPLGTTLFVCKLACINSDE
 IQICGAEIFVGVAPEQPQNLSICIQKGEQGTACTWGERGDT
 HLYTEYTLQLSGPKNLTWQKQCKDIYCDYLDGFINLTPESPES
 NFTAKVTAVNSLGSSSSLPSTFTFLDIVRPLPPWDIRI
Hole: hFc(N297A)-
IL12RB2(24-232)

50 KIDACKRGDVTVPKPSHVILLGSTVNITCSLKPRQGCIFYSRRN
 KLILYKFDRRINFHHGHSLSNSQVTGLPLGTTLFVCKLACINSDE
 IQICGAEIFVGVAPEQPQNLSICIQKGEQGTACTWGERGDT
 HLYTEYTLQLSGPKNLTWQKQCKDIYCDYLDGFINLTPESPES
 NFTAKVTAVNSLGSSSSLPSTFTFLDIVRPLPPWDIRIK
Hole: hFc(N297A)-
IL12RB2(24-233)

ne	newnames	Linker	IL-12 p35 subunit	Released by cleavage
.06	Knob: hFc(N297A)- [VPLSLY]-hIL12B- hIL12A	GGGGGGG GSGGGGS	RNLPVATPDPGMFPCLHHSQNLLRAVS NMLQKARQTLEFYPTCTSEEDHEDITKDK TSTVEACLPLELTKNESCLNSRETSTFITNGS CLASRKTSMMLCLSSIEDLKMYQVEF KTMNAKLLMDPKRQIFLDQNMMLAVIDEL MQALNFNSETVPQKSSLEEPDFYKTKIKL CILLHAFRIRAVTIDRVMSYLNAS	LYSGPIWELKKDVYVVELDWYPDAPGEMVVLTCDTPEEDGITWTLDQSSEVLGSGK TLTIQVKEFGDAGQYTCHKGGEVLSHLLHLHKEDGIWSTDILKDQKEPKNKTFLRC EAKNYSGRFTCWWLTTISTDLTFSVKSSRGSDPQGVTCGAATLSAERVGRDNKEY EYSVECEQEDSACPAAEESLPIEVMVDVHKLKYENYTSFFIRDIKDPDPKNLQKPL KNSRQVEYSWEYPTWTSTPHSYFSLTFCVQVQKSKREKDRVFTDKTSATVICRK NASISVRAQDRYSSSWSEWASVPCSGGGGGGGGGGGSRNLPVATPDPGMF PCLHHSQNLLRAVSNMLQKARQTLEFYPTCTSEEDHEDITKDKTSTVEACLPLELTKN ESCLNSRETSTFITNGSCLASRKTSMMLCLSSIEDLKMYQVEFKTMNAKLLMDPK RQIFLDQNMMLAVIDELMQALNFNSETVPQKSSLEEPDFYKTKIKLCILLHAFRIRAVTI DRVMSYLNAS

Knob: hFc(N297A)-
[VPLSLY]-hIL12B-
hIL12A

.07

GGGGGGG
GGGGGS

LYSGPIWELKKDVVVVELDWYYPDAPGEMVVLTCDTPEEDGITWTLDSSEVLGSGK
TLTIQVKEFGDAGQYTCHKGGEVLSHSLLLHHKKEDGIWSTDILKDQKEPKNKTFLRC
EAKNYSGRFTCWWLTTTISTDLTFSVKSSRGSDPQGVTCGAATLSAERVRGDNKEY
EYSVECCQEDSACPAAEESLPIEVMMVDVHKLKYENYSSFFIRDIHKDPKPNLQKPL
KNSRQVEVSWEYPTWTSTPHSYFSLTFCVQVQKSKREKKDRVFTDKTSATVICRK
NASISVRAQDRYSSSWSEWASVPCSGGGGGGGGGGGGGSRNLPVATPDPGMF
PCLHHSQNLRLRAVSNMLQKARQTLFYPCTSEEDHEDITKDKTSTVEACLPLELTKN
ESCLNSRETSFITNGSCLASRKTSMMLALCLSIYEDLKMYQVEFKTMNAKLLMDPK
RQIFLDQNNMLAVIDELMQALNFNSETVPQKSSLEEPDFYKTKIKLCLLHAFRIRAVTI
DRVMSYLNAS

ne	newnames	Linker	FullSequence
.58	Hole: hFc(N297A)		DKTHTCPCPAPPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQVASTYR VVSIVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTIKAKGQPREPQVCTLPSPSRDELTKNQVSLSCAVKGFYPSDIAVEWES NGQPENNYKTTPPVLDSDGSFFFLVSKLTVDKSRWQQGNVFCFSVMHEALHNHYTQKSLSLSPG
.05	Knob: hFc(N297A)- [VPLSLY]-hIL12B- hIL12A	GGGGGGG GSGGGGS	DKTHTCPCPAPPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQVASTYR VVSIVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTIKAKGQPREPQVCTLPSPSRDELTKNQVSLWCLVKGFYPSDIAVEWE SNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFCFSVMHEALHNHYTQKSLSLSPGGGGGGSVPLSLYSGPI WELKKDVVVELDWYDPAGEMVVLCTDTPDEEDGITWLDQSEVLGSGKTLTIQVKEFGDAGQYTCCHKGGEVLSHSLLL HKKEDGIWSTDILKDQKEPKNKTFLRCEAKNYSGRFTCWWLTTISTDLTFSVKSSRGSDPQGVTCGAATLSAERVGRDNK EYESVECCEDSACPAAEESLPIEVMDAVHKLKYENYTSFFIRDIKPPDKNLQLKPLKNSRQVEVSWEYPTWTSTPHSY FSLTFCVQVQGSKREKKDRVFTDKTSATVICRKNASISVRAQDRYYSSSWSEWASVPCSGGGGGGGGGGGSRNLPV ATPDPMFPCLHHSQNLRAVSNMLOKARQTLFYPCTSEEDHEDITKDKTSTVEACLPLELTKNESCLNSRETSTFTNGSC LASRKTSMMALCLSSIEDLKMYYQVEFKTMNAKILLMDPKRQIFLDQNMMLAVIDELMQALNFNSETVPQKSSLEEDPFYKT KIKLCILLHAFRIRAVTIDRVMSYLNAS

DKTHTCPPAPPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVDVSHEDPEVKFNWVVDGVEVHNAKTKPREEQVASTYR
VVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPCRDELTKNQVSLWCLVKGFYPSDIAVEWE
SNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFCSCVMHEALHNYHTQKSLSLSPGGGSGSGGSPVLSLYS
GPIWELKDVVYVVELDWYPDAPGEMVWLTCDTPEEDGITWTLDSSEVLGSGKTLTIQVKEFGDAGQYTC HKGGEVLSHS
LLLLHKEDGIWSTDILKDQKEPKNTFLRCEAKNYSGRFTCWWTITITDITFSVKSSRGSSDPQGVTCGAATLSAERVRG
DNKEYEYSECEQEDSACPAAEESLPIEVMVDAVHKLKYENVTSSFFIRDIKPDPPKNLQLKPLKNSRQVEVSWEPDTWSTP
HSYFSLTFCVQVQVGKSKREKKDRVFTDKTSATVICRKNASIVRAQDRYSSSWSEWASVPCSGGGGSGGGGSGGGSRN
LPVATPDGPMFPCLLHHSQNLLRAVSNMLQKARQTLFYPCTSEIDHEDITKDKTSTVEACLPIELTKNESCLNSRETSTFITN
GSCLASRKTSFMMA LCSSYIEDLKMVQVEFKTMNAKLLMDPKRQIFLDQNM/LAVIDELMQALNFNSETVPQKSSLEEPDF
YKTKIKLILLHA FRIRAVTIDRVMSYLNAS

Knob: hFc(N297A)- GGGGSGGG
[VPLSLY]-hIL12B- GSGGGGS
hIL12A

.07

DKTHTCPPAPPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVDVSHEDPEVKFNWVVDGVEVHNAKTKPREEQVASTYR
VVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVCTLPSPRDELTKNQVSLCAVKGFYPSDIAVEWES
NGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFCSCVMHEALHNYHTQKSLSLSPGGGSGGGGSGGSCRTSEC
CFQDPPYPDADSGSASGPRDLRCYRISSDRYECSWQYEGPTAGVSHFLRCCLSSGRCCYFAAGSATRLQFSDQAAGSVLYT
VTLWVESWARNQTEKSPVTLQLYNSVKYEPPLGDIKVKLAGQLRMEWETPDNQVGAEVQFRHRTPPSSPWKLGDCGP
QDDDTESCLCPLMNVAQEFQLRRRLQSGSGSSWSKWSSPVCVPPENPPQPVQVRFVSEQLGQDGRRLTLKEQPTQLEL
PEGCQGLAPGTEVTRYRLQLHMLSCPCAKATRTLHLGKMPYLSGAAYNVAVISSNQFGPGLNQTHIPADTHTEPVALNI
SVGTNGTTMYWPARAQSMTYCIEWQPVGQDGGGLATCSLTAPQDPDPAGMATYWSRESGAMGQEKCYITIFASAHIP
EKLTLWSTVLSTYHFGGNASAAAGTPHHVSVKNHSLDSVSDWAPSLSTCPGVLKEVYVRCRDEDSKQVSEHPVQPTETQ
VTLGLRAGVAVTVQVRADTAWLRGVWSQPQRFSEVQVSD

Hole: hFc(N297A)-
hCD212(24-545)

.09

DKTHTCPPCPAPPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYASTYR
VVSIVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVCTLPISRDELTKNQVSLSCAVKGFYPSDIAVEWES
NGQPENNYKTTPPVLDSDGSFLLVSKLTVDKSRWQQGNVFCSCVMHEALHNHYTQKSLSLSPGSGGGGGGGGSCRTSEC
CFQDPPYPDADSGSASGPRDLRCYRISSDRYECSWQYEGPTAGVSHFLRCLSSGRCCYFAAGSATRLQFSDOAGVSVLYT
VTLWVESWARNQTEKSPVTLQLYNSVKYEPPLGDIKIVSLAGQLRMEWETPDNQVGAEQVFRHRTSPSPWKLGDCCGP
QDDDTESCLCLEMNVAAQEFQLRRRQLSGSQSSWSKWSPPVCVPPENP

10 Hole: hFc(N297A)-
hCD212(24-237)

DKTHTCPPCPAPPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYASTYR
VVSIVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVCTLPISRDELTKNQVSLSCAVKGFYPSDIAVEWES
NGQPENNYKTTPPVLDSDGSFLLVSKLTVDKSRWQQGNVFCSCVMHEALHNHYTQKSLSLSPGSGGGGGGGGSKIDACK
RGDVTVKPSHVILLGSTVNITCSLKPRQGCIFYSRNKLILYKFDRRINFHHGHSLSQVTLGLPLGTTTLFVCKLACINSDEIQIC
GAEIFVGVAPEQPQNLSICIQGEQGTVACTWGERGRDTHLYTEYTLQLSGPKNLTWQKQCKDIYCDYLDGFINLTPESPESN
FTAKVTAVNSLGSSSSLPSTFTFLDIVRPLPPWDIRIKFQKASVSRCTLYWRDEGLVLLNRLRYRPSNSRLWNMNVTKAKG
RHDLLDLKPTFEYEFQISSKLHLYKGSWSDWSESLRAQTPEEPTGMILDVWYMKRHIDYSRQQISLFWKNLSVSEARGKILH
YQVTLQELTGGKAMTQNTGHTSWTTVIPRTGNWAVAVSAANSKGSSLIPTTRINIMNLCEAGLLAPRQVYSANSEGMNDNIL
VTWQPPRKDPSPAVQEVVVEWRELHPGGDTQVPLNWLRSRPNVNSALISENIKSYICYEIRVYVALSGDQGGCSSLGNSKHK
APLSGPHINAITEEKGSLISWNSIPVQEQMGCLLHYRIYWKERDSNSQPLCEIPYRVSQNSHPINSIQPRVTYVWLWMTAL
TAAGESSHGNEREFCLQGGKAN

25 Hole: hFc(N297A)-
IL12RB2(24-622)

26 Hole: hFc(N297A)-
IL12RB2(24-319)

DKTHTCPPAPPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQVASTYR
VVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVCTLPSPRDELTKNQVSLSCAVKGFFYPSDIAVEWES
NGQPENNYKTTPPVLDSDGSFFFLVSKLTVDKSRWQQGNVFCSCVMHEALHNNHYTKSLSPGGGGGGGSKIDACK
RGDVTVKPSHVIILGSTVNITCSLKPRQGCFFHYSRRNKLILYKFDRIINHHGHSLSNSQVTGLPLGTTTLFVCKLACINSDEIQIC
GAEIFVGVAPEQPQNLSCIQKGEQGTACTWGERGRDTHLYTEYTLQSGPKNLTWQKQCKDIYCDYLDGGINLTPESPESN
FTAKVTAVNSLGSSSSLPSTFTFLDIVRPLPPWDIRIKFQKASVSRCTLYWRDEGLVLLNRLRYRPSNSRLWNMVMNVTAKAG
RHDLLDKPFTEYEFQISSKLHLYKGSWSDWSESLRAQTPEE

39 Hole: hFc(N297A)-
IL12RB2(24-222)

DKTHTCPPAPPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQVASTYR
VVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVCTLPSPRDELTKNQVSLSCAVKGFFYPSDIAVEWES
NGQPENNYKTTPPVLDSDGSFFFLVSKLTVDKSRWQQGNVFCSCVMHEALHNNHYTKSLSPGGGGGGGSKIDACK
RGDVTVKPSHVIILGSTVNITCSLKPRQGCFFHYSRRNKLILYKFDRIINHHGHSLSNSQVTGLPLGTTTLFVCKLACINSDEIQIC
GAEIFVGVAPEQPQNLSCIQKGEQGTACTWGERGRDTHLYTEYTLQSGPKNLTWQKQCKDIYCDYLDGGINLTPESPESN
FTAKVTAVNSLGSSSSLPSTFTFLDIV

40 Hole: hFc(N297A)-
IL12RB2(24-319)

DKTHTCPPAPPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQVASTYR
VVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVCTLPSPRDELTKNQVSLSCAVKGFFYPSDIAVEWES
NGQPENNYKTTPPVLDSDGSFFFLVSKLTVDKSRWQQGNVFCSCVMHEALHNNHYTKSLSPGGGGGGGSKIDACKRG
DVTVKPSHVIILGSTVNITCSLKPRQGCFFHYSRRNKLILYKFDRIINHHGHSLSNSQVTGLPLGTTTLFVCKLACINSDEIQICGA
EIFVGVAPEQPQNLSCIQKGEQGTACTWGERGRDTHLYTEYTLQSGPKNLTWQKQCKDIYCDYLDGGINLTPESPESNFTA
KVTAVNSLGSSSSLPSTFTFLDIVRPLPPWDIRIKFQKASVSRCTLYWRDEGLVLLNRLRYRPSNSRLWNMVMNVTAKGRHD
LLDLKPFTEYEFQISSKLHLYKGSWSDWSESLRAQTPEE

41 Hole: hFc(N297A)-
IL12RB2(24-319)

DKTHTCPPCAPPELLGGPSVFLFPPKPKD TLMISRTPEVTCVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQVASTYR
VVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVCTLPSPRDELTKNQVSLSCAVKGFFPSDIAVEWES
NGQPENNYKTTPPVLDSDGSFFFLVSKLTVDKSRWQQGNVFCSCVMHEALHNNHYTKSLSPGSGGSGKIDACKRGGDVT
VKPSHVIILGSTVNITCSLKPRQGCFFHYSRRNKLILYKFDRRINFHHGHSLSNSQVTGLPLGTTLFVCKLACINSDEIQICGAEIFV
GVAPEQPNLSLSCIQKGEQGTACTWGERGRDTHLYTEYTLQLSGPKNLTWQKQCKDIYCDYLDGFINLTPESPESNFTAKVT
AVNSLGSSSSLPSTFTFLDIVRPLPPWDIRIKFQKASVSRCTLWVRDEGLVLLNRLRYRPSNSRLWNMVMNVTAKAGRHDLLD
LKPFTYEFEQISSKLHLKGSWSDWSESLRAQTPEE

29 Hole: hFc(N297A)-
IL12RB2(24-212)

DKTHTCPPCAPPELLGGPSVFLFPPKPKD TLMISRTPEVTCVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQVASTYR
VVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVCTLPSPRDELTKNQVSLSCAVKGFFPSDIAVEWES
NGQPENNYKTTPPVLDSDGSFFFLVSKLTVDKSRWQQGNVFCSCVMHEALHNNHYTKSLSPGSGGSGGSGKIDACK
RGDVTVKPSHVIILGSTVNITCSLKPRQGCFFHYSRRNKLILYKFDRRINFHHGHSLSNSQVTGLPLGTTLFVCKLACINSDEIQIC
GAEIFVGVAPEQPNLSLSCIQKGEQGTACTWGERGRDTHLYTEYTLQLSGPKNLTWQKQCKDIYCDYLDGFINLTPESPESN
FTAKVTAVNSLGSSSSL

44 Hole: hFc(N297A)-
IL12RB2(24-222)

DKTHTCPPCAPPELLGGPSVFLFPPKPKD TLMISRTPEVTCVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQVASTYR
VVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVCTLPSPRDELTKNQVSLSCAVKGFFPSDIAVEWES
NGQPENNYKTTPPVLDSDGSFFFLVSKLTVDKSRWQQGNVFCSCVMHEALHNNHYTKSLSPGSGGSGGSGKIDACK
RGDVTVKPSHVIILGSTVNITCSLKPRQGCFFHYSRRNKLILYKFDRRINFHHGHSLSNSQVTGLPLGTTLFVCKLACINSDEIQIC
GAEIFVGVAPEQPNLSLSCIQKGEQGTACTWGERGRDTHLYTEYTLQLSGPKNLTWQKQCKDIYCDYLDGFINLTPESPESN
FTAKVTAVNSLGSSSSLPSTFTFLDIVRPLPP

DKTHTCPPCPAPPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQVASTYR
VVSIVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVCTLPSPSRDELTKNQVSLSCAVKGFGYPSDIAVEWES
NGQPENNYKTTPPVLDSDGSFFFLVSKLTVDKSRWQQGNVFCSCVMHEALHNNHYTKSLSPGGGGGGGGGSKIDACK
RGDVTVKPSHVIILLGSTVNITCSLKPRQGCFFHYSRRNKLILYKFDRLRINFHHGHSLSNSQVTGLPLGTTTLFVCKLACINSDEIQIC
GAEIFVGVAPEQPQNLSICIQGEQGTACTWGERGRDTHLYTEVTLQSLGPKNLTWQKQCKDIYCDYLDGGINLTPESPESN
FTAKVTAVNSLGSSS

27
Hole: hFc(N297A)-
IL12RB2(24-210)

DKTHTCPPCPAPPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQVASTYR
VVSIVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVCTLPSPSRDELTKNQVSLSCAVKGFGYPSDIAVEWES
NGQPENNYKTTPPVLDSDGSFFFLVSKLTVDKSRWQQGNVFCSCVMHEALHNNHYTKSLSPGGGGGGGGGSKIDACK
RGDVTVKPSHVIILLGSTVNITCSLKPRQGCFFHYSRRNKLILYKFDRLRINFHHGHSLSNSQVTGLPLGTTTLFVCKLACINSDEIQIC
GAEIFVGVAPEQPQNLSICIQGEQGTACTWGERGRDTHLYTEVTLQSLGPKNLTWQKQCKDIYCDYLDGGINLTPESPESN
FTAKVTAVNSLGSSS

28
Hole: hFc(N297A)-
IL12RB2(24-211)

DKTHTCPPCPAPPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQVASTYR
VVSIVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVCTLPSPSRDELTKNQVSLSCAVKGFGYPSDIAVEWES
NGQPENNYKTTPPVLDSDGSFFFLVSKLTVDKSRWQQGNVFCSCVMHEALHNNHYTKSLSPGGGGGGGGGSKIDACK
RGDVTVKPSHVIILLGSTVNITCSLKPRQGCFFHYSRRNKLILYKFDRLRINFHHGHSLSNSQVTGLPLGTTTLFVCKLACINSDEIQIC
GAEIFVGVAPEQPQNLSICIQGEQGTACTWGERGRDTHLYTEVTLQSLGPKNLTWQKQCKDIYCDYLDGGINLTPESPESN
FTAKVTAVNSLGSSSL

29
Hole: hFc(N297A)-
IL12RB2(24-212)

DKTHTCPPCPAPPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQVASTYR
VVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVCTLPSPRDELTKNQVSLSCAVKGIFYPSDIAVEWES
NGQPENNYKTTPPVLDSDGSFFFLVSKLTVDKSRWQQGNVFCSCVMHEALHNNHYTEKSLSPGSGGGSGGGGSKIDACK
RGDVTVKPSHVIILLGSTVNITCSLKPRQGCIFYHRRNKLILYKFDRIINHHGHSLSNSQVTGLPLGTTTLFVCKLACINSDEIQIC
GAEIFVGVAPEQPQNLSICIQGEQGTACTWGERGRDTHLYTEVTLQSLGPKNLTWQKQCKDIYCDYLDYDFGINLTPESPESN
FTAKVTAVNSLGSSSSLP

Hole: hFc(N297A)-
IL12RB2(24-213)

30

DKTHTCPPCPAPPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQVASTYR
VVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVCTLPSPRDELTKNQVSLSCAVKGIFYPSDIAVEWES
NGQPENNYKTTPPVLDSDGSFFFLVSKLTVDKSRWQQGNVFCSCVMHEALHNNHYTEKSLSPGSGGGSGGGGSKIDACK
RGDVTVKPSHVIILLGSTVNITCSLKPRQGCIFYHRRNKLILYKFDRIINHHGHSLSNSQVTGLPLGTTTLFVCKLACINSDEIQIC
GAEIFVGVAPEQPQNLSICIQGEQGTACTWGERGRDTHLYTEVTLQSLGPKNLTWQKQCKDIYCDYLDYDFGINLTPESPESN
FTAKVTAVNSLGSSSSLPS

Hole: hFc(N297A)-
IL12RB2(24-214)

31

DKTHTCPPCPAPPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQVASTYR
VVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVCTLPSPRDELTKNQVSLSCAVKGIFYPSDIAVEWES
NGQPENNYKTTPPVLDSDGSFFFLVSKLTVDKSRWQQGNVFCSCVMHEALHNNHYTEKSLSPGSGGGSGGGGSKIDACK
RGDVTVKPSHVIILLGSTVNITCSLKPRQGCIFYHRRNKLILYKFDRIINHHGHSLSNSQVTGLPLGTTTLFVCKLACINSDEIQIC
GAEIFVGVAPEQPQNLSICIQGEQGTACTWGERGRDTHLYTEVTLQSLGPKNLTWQKQCKDIYCDYLDYDFGINLTPESPESN
FTAKVTAVNSLGSSSSLPST

Hole: hFc(N297A)-
IL12RB2(24-215)

32

DKTHTCPPCPAPPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQVASTYR
VVSFLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVCTLPSPSRDELTKNQVSLSCAVKGIFYPSDIAVEWES
NGQPENNYKTTPPVLDSDGSFFFLVSKLTVDKSRWQQGNVFCSCVMHEALHNNHYTKSLSPGGGGGGGGGSKIDACK
RGDVTVKPSHVIILLGSTVNITCSLKPRQGCIFYHRRNKLILYKFDRIINHHGHSLSNSQVTGLPLGTTTLFVCKLACINSDEIQIC
GAEIFVGVAPEQPQNLSICIQGEQGTACTWGERGRDTHLYTEVTLQSLGPKNLTWQKQCKDIYCDYLDGGINLTPESPESN
FTAKVTAVNSLGSSSSLPSTF

33 Hole: hFc(N297A)-
IL12RB2(24-216)

DKTHTCPPCPAPPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQVASTYR
VVSFLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVCTLPSPSRDELTKNQVSLSCAVKGIFYPSDIAVEWES
NGQPENNYKTTPPVLDSDGSFFFLVSKLTVDKSRWQQGNVFCSCVMHEALHNNHYTKSLSPGGGGGGGGGSKIDACK
RGDVTVKPSHVIILLGSTVNITCSLKPRQGCIFYHRRNKLILYKFDRIINHHGHSLSNSQVTGLPLGTTTLFVCKLACINSDEIQIC
GAEIFVGVAPEQPQNLSICIQGEQGTACTWGERGRDTHLYTEVTLQSLGPKNLTWQKQCKDIYCDYLDGGINLTPESPESN
FTAKVTAVNSLGSSSSLPSTF

34 Hole: hFc(N297A)-
IL12RB2(24-217)

DKTHTCPPCPAPPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQVASTYR
VVSFLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVCTLPSPSRDELTKNQVSLSCAVKGIFYPSDIAVEWES
NGQPENNYKTTPPVLDSDGSFFFLVSKLTVDKSRWQQGNVFCSCVMHEALHNNHYTKSLSPGGGGGGGGGSKIDACK
RGDVTVKPSHVIILLGSTVNITCSLKPRQGCIFYHRRNKLILYKFDRIINHHGHSLSNSQVTGLPLGTTTLFVCKLACINSDEIQIC
GAEIFVGVAPEQPQNLSICIQGEQGTACTWGERGRDTHLYTEVTLQSLGPKNLTWQKQCKDIYCDYLDGGINLTPESPESN
FTAKVTAVNSLGSSSSLPSTF

35 Hole: hFc(N297A)-
IL12RB2(24-218)

DKTHTCPPCPAPPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQVASTYR
VVSFLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVCTLPSPRDELTKNQVSLSCAVKGIFYPSDIAVEWES
NGQPENNYKTTPPVLDSDGSFFFLVSKLTVDKSRWQQGNVFCSCVMHEALHNNHYTKSLSPGSGGGSGGGGSKIDACK
RGDVTVKPSHVIILLGSTVNITCSLKPRQGCIFYHRRNKLILYKFDRIINHHHSHLSNSQVTGLPLGTTTLFVCKLACINSDEIQIC
GAEIFVGVAPEQPQNLSICIQGEQGTACTWGERGRDTHLYTEVTLQSLGPKNLTWQKQCKDIYCDYLDGGINLTPESPESN
FTAKVTAVNSLGSSSSLPSTFTFL

36 Hole: hFc(N297A)-
IL12RB2(24-219)

DKTHTCPPCPAPPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQVASTYR
VVSFLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVCTLPSPRDELTKNQVSLSCAVKGIFYPSDIAVEWES
NGQPENNYKTTPPVLDSDGSFFFLVSKLTVDKSRWQQGNVFCSCVMHEALHNNHYTKSLSPGSGGGSGGGGSKIDACK
RGDVTVKPSHVIILLGSTVNITCSLKPRQGCIFYHRRNKLILYKFDRIINHHHSHLSNSQVTGLPLGTTTLFVCKLACINSDEIQIC
GAEIFVGVAPEQPQNLSICIQGEQGTACTWGERGRDTHLYTEVTLQSLGPKNLTWQKQCKDIYCDYLDGGINLTPESPESN
FTAKVTAVNSLGSSSSLPSTFTFLD

37 Hole: hFc(N297A)-
IL12RB2(24-220)

DKTHTCPPCPAPPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQVASTYR
VVSFLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVCTLPSPRDELTKNQVSLSCAVKGIFYPSDIAVEWES
NGQPENNYKTTPPVLDSDGSFFFLVSKLTVDKSRWQQGNVFCSCVMHEALHNNHYTKSLSPGSGGGSGGGGSKIDACK
RGDVTVKPSHVIILLGSTVNITCSLKPRQGCIFYHRRNKLILYKFDRIINHHHSHLSNSQVTGLPLGTTTLFVCKLACINSDEIQIC
GAEIFVGVAPEQPQNLSICIQGEQGTACTWGERGRDTHLYTEVTLQSLGPKNLTWQKQCKDIYCDYLDGGINLTPESPESN
FTAKVTAVNSLGSSSSLPSTFTFLDI

38 Hole: hFc(N297A)-
IL12RB2(24-221)

DKTHTCPPCPAPPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQVASTYR
VVSFLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVCTLPSPRDELTKNQVSLSCAVKGFGYPSDIAVEWES
NGQPENNYKTTPPVLDSDGSFFFLVSKLTVDKSRWQQGNVFCSCVMHEALHNNHYTKSLSPGGGGGGGGGSKIDACK
RGDVTVKPSHVIILLGSTVNITCSLKPRQGCFFHYSRRNKLILYKFDRIINFHGHSLNSQVTGLPLGTTTLFVCKLACINSDEIQIC
GAEIFVGVAPEQPQNLSICIQGEQGTVACTWGERGRDTHLYTEVTLQSLGPKNLTWQKQCKDIYCDYLDGGINLTPESPESN
FTAKVTAVNSLGSSSSLPSTFTFLDIV

39 Hole: hFc(N297A)-
IL12RB2(24-222)

DKTHTCPPCPAPPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQVASTYR
VVSFLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVCTLPSPRDELTKNQVSLSCAVKGFGYPSDIAVEWES
NGQPENNYKTTPPVLDSDGSFFFLVSKLTVDKSRWQQGNVFCSCVMHEALHNNHYTKSLSPGGGGGGGGGSKIDACK
RGDVTVKPSHVIILLGSTVNITCSLKPRQGCFFHYSRRNKLILYKFDRIINFHGHSLNSQVTGLPLGTTTLFVCKLACINSDEIQIC
GAEIFVGVAPEQPQNLSICIQGEQGTVACTWGERGRDTHLYTEVTLQSLGPKNLTWQKQCKDIYCDYLDGGINLTPESPESN
FTAKVTAVNSLGSSSSLPSTFTFLDIVR

40 Hole: hFc(N297A)-
IL12RB2(24-223)

DKTHTCPPCPAPPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQVASTYR
VVSFLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVCTLPSPRDELTKNQVSLSCAVKGFGYPSDIAVEWES
NGQPENNYKTTPPVLDSDGSFFFLVSKLTVDKSRWQQGNVFCSCVMHEALHNNHYTKSLSPGGGGGGGGGSKIDACK
RGDVTVKPSHVIILLGSTVNITCSLKPRQGCFFHYSRRNKLILYKFDRIINFHGHSLNSQVTGLPLGTTTLFVCKLACINSDEIQIC
GAEIFVGVAPEQPQNLSICIQGEQGTVACTWGERGRDTHLYTEVTLQSLGPKNLTWQKQCKDIYCDYLDGGINLTPESPESN
FTAKVTAVNSLGSSSSLPSTFTFLDIVRP

41 Hole: hFc(N297A)-
IL12RB2(24-224)

DKTHTCPPCPAPPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQVASTYR
VVSIVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVCTLPSPSRDELTKNQVSLSCAVKGFGYPSDIAVEWES
NGQPENNYKTTPPVLDSDGSFFFLVSKLTVDKSRWQQGNVFCSCVMHEALHNNHYTKLSLSLSPGGGGGGGGSKIDACK
RGDVTVKPSHVIILLGSTVNITCSLKPRQGCFFHYSRRNKLILYKFDRIINHHGHSLSNSQVTGLPLGTTTLFVCKLACINSDEIQIC
GAEIFVGVAPEQPQNLSICIQGEQGTACTWGERGRDTHLYTEVTLQSLGPKNLTWQKQCKDIYCDYLDGGINLTPESPESN
FTAKVTAVNSLGSSSSLPSTFTFLDIVRPL

42 Hole: hFc(N297A)-
IL12RB2(24-225)

DKTHTCPPCPAPPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQVASTYR
VVSIVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVCTLPSPSRDELTKNQVSLSCAVKGFGYPSDIAVEWES
NGQPENNYKTTPPVLDSDGSFFFLVSKLTVDKSRWQQGNVFCSCVMHEALHNNHYTKLSLSLSPGGGGGGGGSKIDACK
RGDVTVKPSHVIILLGSTVNITCSLKPRQGCFFHYSRRNKLILYKFDRIINHHGHSLSNSQVTGLPLGTTTLFVCKLACINSDEIQIC
GAEIFVGVAPEQPQNLSICIQGEQGTACTWGERGRDTHLYTEVTLQSLGPKNLTWQKQCKDIYCDYLDGGINLTPESPESN
FTAKVTAVNSLGSSSSLPSTFTFLDIVRPLP

43 Hole: hFc(N297A)-
IL12RB2(24-226)

DKTHTCPPCPAPPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQVASTYR
VVSIVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVCTLPSPSRDELTKNQVSLSCAVKGFGYPSDIAVEWES
NGQPENNYKTTPPVLDSDGSFFFLVSKLTVDKSRWQQGNVFCSCVMHEALHNNHYTKLSLSLSPGGGGGGGGSKIDACK
RGDVTVKPSHVIILLGSTVNITCSLKPRQGCFFHYSRRNKLILYKFDRIINHHGHSLSNSQVTGLPLGTTTLFVCKLACINSDEIQIC
GAEIFVGVAPEQPQNLSICIQGEQGTACTWGERGRDTHLYTEVTLQSLGPKNLTWQKQCKDIYCDYLDGGINLTPESPESN
FTAKVTAVNSLGSSSSLPSTFTFLDIVRPLP

44 Hole: hFc(N297A)-
IL12RB2(24-227)

DKTHTCPPCPAPPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQVASTYR
VVSIVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVCTLPSPSRDELTKNQVSLSCAVKGFGYPSDIAVEWES
NGQPENNYKTTPPVLDSDGSFFFLVSKLTVDKSRWQQGNVFCSCVMHEALHNNHYTKSLSPGGGGGGGGGSKIDACK
RGDVTVKPSHVIILLGSTVNITCSLKPRQGCFFHYSRRNKLILYKFDRIINHHHSHLSNSQVTGLPLGTTTLFVCKLACINSDEIQIC
GAEIFVGVAPEQPQNLSICIQGEQGTACTWGERGRDTHLYTEVTLQSLGPKNLTWQKQCKDIYCDYLDGGINLTPESPESN
FTAKVTAVNSLGSSSSLPSTFTFLDIVRPLPPW

45 Hole: hFc(N297A)-
IL12RB2(24-228)

DKTHTCPPCPAPPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQVASTYR
VVSIVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVCTLPSPSRDELTKNQVSLSCAVKGFGYPSDIAVEWES
NGQPENNYKTTPPVLDSDGSFFFLVSKLTVDKSRWQQGNVFCSCVMHEALHNNHYTKSLSPGGGGGGGGGSKIDACK
RGDVTVKPSHVIILLGSTVNITCSLKPRQGCFFHYSRRNKLILYKFDRIINHHHSHLSNSQVTGLPLGTTTLFVCKLACINSDEIQIC
GAEIFVGVAPEQPQNLSICIQGEQGTACTWGERGRDTHLYTEVTLQSLGPKNLTWQKQCKDIYCDYLDGGINLTPESPESN
FTAKVTAVNSLGSSSSLPSTFTFLDIVRPLPPWD

46 Hole: hFc(N297A)-
IL12RB2(24-229)

DKTHTCPPCPAPPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQVASTYR
VVSIVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVCTLPSPSRDELTKNQVSLSCAVKGFGYPSDIAVEWES
NGQPENNYKTTPPVLDSDGSFFFLVSKLTVDKSRWQQGNVFCSCVMHEALHNNHYTKSLSPGGGGGGGGGSKIDACK
RGDVTVKPSHVIILLGSTVNITCSLKPRQGCFFHYSRRNKLILYKFDRIINHHHSHLSNSQVTGLPLGTTTLFVCKLACINSDEIQIC
GAEIFVGVAPEQPQNLSICIQGEQGTACTWGERGRDTHLYTEVTLQSLGPKNLTWQKQCKDIYCDYLDGGINLTPESPESN
FTAKVTAVNSLGSSSSLPSTFTFLDIVRPLPPWDI

47 Hole: hFc(N297A)-
IL12RB2(24-230)

DKTHTCPPCPAPPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQVASTYR
VVSIVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVCTLPSPSRDELTKNQVSLSCAVKGIFYPSDIAVEWES
NGQPENNYKTTPPVLDSDGSFFFLVSKLTVDKSRWQQGNVFCSCVMHEALHNNHYTKSLSPGGGGGGGGGSKIDACK
RGDVTVKPSHVIILLGSTVNITCSLKPRQGCIFYHYSRRNKLILYKFDRIINHHGHSLNSQVTGLPLGTTTLFVCKLACINSDEIQIC
GAEIFVGVAPEQPQNLSICIQGEQGTACTWGERGRDTHLYTEVTLQSLGPKNLTWQKQCKDIYCDYLDGGINLTPESPESN
FTAKVTAVNSLGSSSSLPSTFTFLDIVRPLPPWDIR

48 Hole: hFc(N297A)-
IL12RB2(24-231)

DKTHTCPPCPAPPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQVASTYR
VVSIVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVCTLPSPSRDELTKNQVSLSCAVKGIFYPSDIAVEWES
NGQPENNYKTTPPVLDSDGSFFFLVSKLTVDKSRWQQGNVFCSCVMHEALHNNHYTKSLSPGGGGGGGGGSKIDACK
RGDVTVKPSHVIILLGSTVNITCSLKPRQGCIFYHYSRRNKLILYKFDRIINHHGHSLNSQVTGLPLGTTTLFVCKLACINSDEIQIC
GAEIFVGVAPEQPQNLSICIQGEQGTACTWGERGRDTHLYTEVTLQSLGPKNLTWQKQCKDIYCDYLDGGINLTPESPESN
FTAKVTAVNSLGSSSSLPSTFTFLDIVRPLPPWDIR

49 Hole: hFc(N297A)-
IL12RB2(24-232)

DKTHTCPPCPAPPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQVASTYR
VVSIVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVCTLPSPSRDELTKNQVSLSCAVKGIFYPSDIAVEWES
NGQPENNYKTTPPVLDSDGSFFFLVSKLTVDKSRWQQGNVFCSCVMHEALHNNHYTKSLSPGGGGGGGGGSKIDACK
RGDVTVKPSHVIILLGSTVNITCSLKPRQGCIFYHYSRRNKLILYKFDRIINHHGHSLNSQVTGLPLGTTTLFVCKLACINSDEIQIC
GAEIFVGVAPEQPQNLSICIQGEQGTACTWGERGRDTHLYTEVTLQSLGPKNLTWQKQCKDIYCDYLDGGINLTPESPESN
FTAKVTAVNSLGSSSSLPSTFTFLDIVRPLPPWDIR

50 Hole: hFc(N297A)-
IL12RB2(24-233)

Module	Unique sequences		Co-expression partner	
	DNA	Name	DNA	Name
	DNA627	Hole: hFc(N297A)-IL12RB2(24-210)	DNA407	Knob: hFc(N297A)-[VPLSLY]-hIL12B-hIL12A
	DNA628	Hole: hFc(N297A)-IL12RB2(24-211)	DNA407	Knob: hFc(N297A)-[VPLSLY]-hIL12B-hIL12A
	DNA630	Hole: hFc(N297A)-IL12RB2(24-213)	DNA407	Knob: hFc(N297A)-[VPLSLY]-hIL12B-hIL12A
	DNA631	Hole: hFc(N297A)-IL12RB2(24-214)	DNA407	Knob: hFc(N297A)-[VPLSLY]-hIL12B-hIL12A
	DNA632	Hole: hFc(N297A)-IL12RB2(24-215)	DNA407	Knob: hFc(N297A)-[VPLSLY]-hIL12B-hIL12A
	DNA633	Hole: hFc(N297A)-IL12RB2(24-216)	DNA407	Knob: hFc(N297A)-[VPLSLY]-hIL12B-hIL12A
	DNA634	Hole: hFc(N297A)-IL12RB2(24-217)	DNA407	Knob: hFc(N297A)-[VPLSLY]-hIL12B-hIL12A
	DNA635	Hole: hFc(N297A)-IL12RB2(24-218)	DNA407	Knob: hFc(N297A)-[VPLSLY]-hIL12B-hIL12A
	DNA636	Hole: hFc(N297A)-IL12RB2(24-219)	DNA407	Knob: hFc(N297A)-[VPLSLY]-hIL12B-hIL12A
	DNA637	Hole: hFc(N297A)-IL12RB2(24-220)	DNA407	Knob: hFc(N297A)-[VPLSLY]-hIL12B-hIL12A
	DNA638	Hole: hFc(N297A)-IL12RB2(24-221)	DNA407	Knob: hFc(N297A)-[VPLSLY]-hIL12B-hIL12A
	DNA639	Hole: hFc(N297A)-IL12RB2(24-222)	DNA407	Knob: hFc(N297A)-[VPLSLY]-hIL12B-hIL12A
	DNA640	Hole: hFc(N297A)-IL12RB2(24-223)	DNA407	Knob: hFc(N297A)-[VPLSLY]-hIL12B-hIL12A
	DNA641	Hole: hFc(N297A)-IL12RB2(24-224)	DNA407	Knob: hFc(N297A)-[VPLSLY]-hIL12B-hIL12A
	DNA642	Hole: hFc(N297A)-IL12RB2(24-225)	DNA407	Knob: hFc(N297A)-[VPLSLY]-hIL12B-hIL12A

DNA643	Hole: hFc(N297A)-IL12RB2(24-226)	DNA407	Knob: hFc(N297A)-[VPLSLY]-hIL12B-hIL12A
DNA645	Hole: hFc(N297A)-IL12RB2(24-228)	DNA407	Knob: hFc(N297A)-[VPLSLY]-hIL12B-hIL12A
DNA646	Hole: hFc(N297A)-IL12RB2(24-229)	DNA407	Knob: hFc(N297A)-[VPLSLY]-hIL12B-hIL12A
DNA647	Hole: hFc(N297A)-IL12RB2(24-230)	DNA407	Knob: hFc(N297A)-[VPLSLY]-hIL12B-hIL12A
DNA648	Hole: hFc(N297A)-IL12RB2(24-231)	DNA407	Knob: hFc(N297A)-[VPLSLY]-hIL12B-hIL12A
DNA649	Hole: hFc(N297A)-IL12RB2(24-232)	DNA407	Knob: hFc(N297A)-[VPLSLY]-hIL12B-hIL12A
DNA650	Hole: hFc(N297A)-IL12RB2(24-233)	DNA407	Knob: hFc(N297A)-[VPLSLY]-hIL12B-hIL12A

CLAIMS

What is claimed is:

1. A masked IL-12 cytokine comprises a protein heterodimer comprising:
a first polypeptide chain comprising:
$$\text{N}^{\prime} \text{HL1-L1-MM C}^{\prime}$$

and a second polypeptide chain comprising:
$$\text{N}^{\prime} \text{HL2-L2-C C}^{\prime}$$

where HL1 is a first half life extension domain, L1 is a first linker, MM is a masking moiety, HL2 is a second half life extension domain, L2 is a second linker, and C is an IL-12 cytokine or functional fragment thereof,
wherein the first half-life extension domain is associated with the second half-life extension domain, and
wherein one of the first linker or the second linker comprises a proteolytically cleavable peptide.
2. The masked cytokine of claim 1, wherein the IL-12 polypeptide or functional fragment thereof comprises an IL-12p40 polypeptide or functional fragment thereof covalently linked to an IL-12p35 polypeptide or functional fragment thereof.
3. The masked cytokine of claim 2, wherein the IL-12p40 – IL-12p35 linker is between 5 and 20 amino acids in length.
4. The masked cytokine of claim 2 or 3, wherein the IL-12p40 – IL-12p35 linker is rich in amino acid residues G and S.
5. The masked cytokine of any one of claims 2-4, wherein the IL-12p40 – IL-12p35 linker comprises SEQ ID NO: 3. (GGGGSGGGGSGGGGS)
6. The masked cytokine of any one of claims 2-5, wherein the IL-12p40 polypeptide comprises SEQ ID NO: 1 or an amino acid sequence having at least one amino acid modification as compared to the amino acid sequence of SEQ ID NO: 1.
7. The masked cytokine of claim 6, wherein IL-12p40 polypeptide comprises SEQ ID NO: 1.

8. The masked cytokine of claim 6, wherein IL-12p40 polypeptide comprises at least one amino acid modification to the GAG-binding domain (KSKREKKDRV) as compared to the amino acid sequence of SEQ ID NO: 1.
9. The masked cytokine of claim 8, wherein IL-12p40 polypeptide comprises SEQ ID NO: 57.
10. The masked cytokine of claim 8, wherein IL-12p40 polypeptide comprises SEQ ID NO: 58.
11. The masked cytokine of any one of claims 6-10, wherein IL-12p40 polypeptide comprises an amino acid sequence having one or more cysteine substitution mutations as compared to the amino acid sequence of SEQ ID NO: 1.
12. The masked cytokine of claim 11, wherein the IL-12p40 polypeptide comprises SEQ ID NO: 59.
13. The masked cytokine of claim 11, wherein the IL-12p40 polypeptide comprises SEQ ID NO: 60.
14. The masked cytokine of any one of claims 2-13, wherein the IL-12p35 polypeptide comprises SEQ ID NO: 2 or an amino acid sequence having at least one amino acid modification as compared to the amino acid sequence of SEQ ID NO: 2.
15. The masked cytokine of claim 14, wherein the IL-12p35 polypeptide comprises SEQ ID NO: 2.
16. The masked cytokine of any one of claims 1 to 7, wherein the IL-12 cytokine or functional fragment thereof comprises SEQ ID NO: 4.
17. The masked cytokine of any one of claims 1 to 10, wherein the IL-12 cytokine or functional fragment thereof comprises SEQ ID NO: 61.
18. The masked cytokine of any one of claims 1 to 10, wherein the IL-12 cytokine or functional fragment thereof comprises SEQ ID NO: 62.
19. The masked cytokine of any one of claims 1 to 12, wherein the IL-12 cytokine or functional fragment thereof comprises SEQ ID NO: 63.
20. The masked cytokine of any one of claims 1 to 13, wherein the IL-12 cytokine or functional fragment thereof comprises SEQ ID NO: 64.

21. The masked cytokine of any one of the preceding claims, wherein the masking moiety comprises an IL-12 cytokine receptor, or a subunit or functional fragment thereof.
22. The masked cytokine of any one of the preceding claims, wherein the masking moiety comprises the extracellular domain of human IL-12R β 1 or a fragment, portion, or variant thereof that retains or otherwise demonstrates an affinity to IL-12.
23. The masked cytokine of claim 22, wherein the masking moiety comprises residues 24 to 237 of human IL-12R β 1, namely a sequence having SEQ ID NO: 5.
24. The masked cytokine of claim 22, wherein the masking moiety comprises residues 24 to 545 of human IL-12R β 1, namely a sequence having SEQ ID NO: 6.
25. The masked cytokine of any one of claims 1-21, wherein the masking moiety comprises the extracellular domain of human IL-12R β 2 or a fragment, portion, or variant thereof that retains or otherwise demonstrates an affinity to IL-12.
26. The masked cytokine of claim 25, wherein the masking moiety comprises residues 24 to 212 of human IL-12R β 2, namely a sequence having SEQ ID NO: 7.
27. The masked cytokine of claim 25, wherein the masking moiety comprises residues 24 to 222 of human IL-12R β 2, namely a sequence having SEQ ID NO: 8 or wherein the masking moiety comprises residues 24 to 227 of human IL-12R β 2, namely a sequence having SEQ ID NO: 11.
28. The masked cytokine of claim 25, wherein the masking moiety comprises residues 24 to 319 of human IL-12R β 2, namely a sequence having SEQ ID NO: 9.
29. The masked cytokine of claim 28, wherein the masking moiety comprises at least one amino acid modification as compared to the sequence of SEQ ID NO: 9, optionally wherein said modifications are cysteine substitution mutations.
30. The masked cytokine of claim 29, wherein the masking moiety comprises SEQ ID NO: 65
31. The masked cytokine of claim 25, wherein the masking moiety comprises residues 24 to 622 of human IL-12R β 2, namely a sequence having SEQ ID NO: 10.
32. The masked cytokine of any one of the preceding claims, wherein the cleavable peptide is from 6 to 10 amino acids in length.

33. The masked cytokine of any one of the preceding claims, wherein the cleavable peptide comprises an amino acid sequence of SEQ ID NO: 15.
34. The masked cytokine of any one of the preceding claims, wherein the cleavable peptide comprises an amino acid sequence of SEQ ID NO: 41.
35. The masked cytokine of any one of the preceding claims, wherein the cleavable peptide comprises an amino acid sequence of SEQ ID NO: 42.
36. The masked cytokine of any one of the preceding claims, wherein the cleavable peptide comprises an amino acid sequence of SEQ ID NO: 43.
37. The masked cytokine of any one of the preceding claims, wherein the cleavable peptide comprises an amino acid sequence of SEQ ID NO: 44.
38. The masked cytokine of any one of the preceding claims, wherein the cleavable peptide comprises an amino acid sequence of SEQ ID NO: 45.
39. The masked cytokine of any one of the preceding claims, wherein the first polypeptide chain comprises:
- N' **HL1-non-cleavable L1-MM** C'
- and the second polypeptide chain comprises
- N' **HL2-cleavable L2-C** C'
40. The masked cytokine of claim 39, wherein the non-cleavable linker is between 3 and 18 amino acids in length.
41. The masked cytokine of claim 39, wherein the non-cleavable linker is between 3 and 15 amino acids in length.
42. The masked cytokine of any one of claims 30 to 41, wherein the non-cleavable linker is rich in amino acid residues G and S.
43. The masked cytokine of any one of claims 39 to 42, wherein the non-cleavable linker includes [(G)_nS], where n=4 or 5.

44. The masked cytokine of any one of claims 39 to 43, wherein the non-cleavable linker comprises an amino acid sequence as shown in SEQ ID NO: 12 (GGGGS).
45. The masked cytokine of any one of claims 39 to 43, wherein the non-cleavable linker comprises an amino acid sequence as shown in SEQ ID NO: 13 (GGGGSGGGGS).
46. The masked cytokine of any one of claims 39 to 43, wherein the non-cleavable linker comprises an amino acid sequence as shown in SEQ ID NO: 14 (GGSGGSGGGGS).
47. The masked cytokine of any one of claims 39 to 42, wherein the non-cleavable linker comprises an amino acid sequence as shown in SEQ ID NO: 54 (GGSGGSGGSGGSGSSGP).
48. The masked cytokine of any one of claims 39 to 42, wherein the non-cleavable linker comprises an amino acid sequence as shown in SEQ ID NO: 55 (PGSGP).
49. The masked cytokine of any one of claims 39 to 42, wherein the non-cleavable linker comprises an amino acid sequence as shown in SEQ ID NO: 56 (GGSPG).
50. The masked cytokine of any one of claims 39 to 49, wherein the cleavable linker comprises a proteolytically cleavable peptide (CP) flanked on both sides by a spacer domain (SD):
SD1-CP-SD2
where SD1 and SD2 are different, such that the first polypeptide chain comprises:
N' **HL1-non-cleavable L1-MM** C'
and the second polypeptide chain comprises:
N' **HL2- SD1-CP-SD2 -C** C'
51. The masked cytokine of claim 50, wherein the first spacer domain (SD1) is between 3 and 10 amino acids in length.
52. The masked cytokine of claims 50 or 51, wherein SD1 comprises SEQ ID NO: 16. (GGSGGS)
53. The masked cytokine of claims 50 or 51, wherein SD1 comprises SEQ ID NO: 17. (GGSGGSGGS)
54. The masked cytokine of any one of claims 50 to 53, wherein the second spacer domain (SD2) is between 3 and 6 amino acids in length.
55. The masked cytokine of any one of claims 50 to 54, wherein SD2 comprises SEQ ID NO: 18. (SGP)

56. The masked IL-2 cytokine of claim 50, wherein the proteolytically cleavable linker comprises SD1-CP-SD2 where SD1 is a first spacer domain, CP is a cleavable peptide and SD2 is a second spacer domain, and wherein CP has an amino acid sequence as shown in SEQ ID NO: 44 and SD2 has an amino acid sequence as shown in SEQ ID NO: 18.
57. The masked IL-2 cytokine of claim 50, wherein the proteolytically cleavable linker comprises SD1-CP-SD2 where SD1 is a first spacer domain, CP is a cleavable peptide and SD2 is a second spacer domain, and wherein CP has an amino acid sequence as shown in SEQ ID NO: 45 and SD2 has an amino acid sequence as shown in SEQ ID NO: 18.
58. The masked cytokine of any one of claims 50 to 54, wherein the cleavable linker comprises SEQ ID NO: 19. (GGSGGS**VPLSLY**SGP)
59. The masked cytokine of any one of claims 50 to 54, wherein the cleavable linker comprises SEQ ID NO: 20. (GGSGGSGGS**VPLSLY**SGP)
60. The masked cytokine of claim 50, wherein the cleavable linker comprises SEQ ID NO: 46. (GGSGGSM**PYDLYHP**SGP)
61. The masked cytokine of claim 50, wherein the cleavable linker comprises SEQ ID NO: 47. (GGSGGSGGS**MPYDLYHP**SGP)
62. The masked cytokine of claim 50, wherein the cleavable linker comprises SEQ ID NO: 48. (GGSGGSDSG**GFM**LTSGP)
63. The masked cytokine of claim 50, wherein the cleavable linker comprises SEQ ID NO: 49. (GGSGGSGGSDSG**GFM**LTSGP)
64. The masked cytokine of claim 50, wherein the cleavable linker comprises SEQ ID NO: 50. (GGSGGSRAA**AVKSP**SGP)
65. The masked cytokine of claim 50, wherein the cleavable linker comprises SEQ ID NO: 51. (GGSGGSGGSRAA**AVKSP**SGP)
66. The masked cytokine of claim 50, wherein the cleavable linker comprises SEQ ID NO: 52. (GGSGGSIS**SGLLS**GRSSGP)

67. The masked cytokine of claim 50, wherein the cleavable linker comprises SEQ ID NO: 53. (GGSGGSGGSISSGLLSGRSSGP)
68. The masked cytokine of any one of the preceding claims, wherein the first half-life extension domain comprises a first IgG1 Fc domain or a fragment thereof and the second half-life extension domain comprises a second IgG1 Fc domain or a fragment thereof.
69. The masked cytokine of any one of the preceding claims, wherein the first and/ or second Fc domains each contain one or more modifications that promote the non-covalent association of the first and the second half-life extension domains.
70. The masked cytokine of any one of the preceding claims, wherein the first half-life extension domain comprises SEQ ID NO: 25 (Y349C; T366S; L38A; Y407V; and N297A) and the second half-life extension domain comprises SEQ ID NO 26 (S354C, T366W and N297A).
71. The masked cytokine of any one of the preceding claims, wherein the first half-life extension domain comprises SEQ ID NO: 27 (Y349C; T366S; L38A; Y407V, N297A and I253A) and the second half-life extension domain comprises SEQ ID NO: 28 (S354C, T366W, N297A and I253A).
72. The masked cytokine of claim 1, wherein the first polypeptide chain comprises an amino acid sequence of SEQ ID NO:34 and a second polypeptide chain comprises an amino acid sequence of SEQ ID NO: 40.
73. A cleavage product capable of binding to IL-12R, the cleavage product comprising an IL-12 cytokine or functional fragment thereof, preparable by proteolytic cleavage of the cleavable peptide in a masked IL-12 cytokine as defined in any of claims 1-42.
74. A cleavage product of a masked IL-12 cytokine, where the cleavage product is capable of binding to IL-12R, the cleavage product comprising a polypeptide comprising:
PCP-SD2-C
wherein PCP is a portion of a proteolytically cleavable peptide; SD2 is a spacer domain; and C is an IL-12 cytokine or functional fragment thereof.
75. The cleavage product of claim 74, wherein PCP is a portion of a proteolytically cleavable peptide as defined in any of claims 32 to 38.

76. The cleavage product of claims 74 or 75, wherein SD2 is a spacer domain as defined in any of claims 54 to 55.
77. The cleavage product of any one of claims 74 to 76, wherein C is an IL-12 cytokine or functional fragment thereof as defined in any of claims 2-20.
78. The cleavage product of any one of claims 74 to 77, wherein the cleavage product comprises an amino acid sequence having about or at least about 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% sequence identity to an amino acid sequence selected from the group consisting of SEQ ID NO: 29.
79. The cleavage product of claim 78, wherein the cleavage product comprises an amino acid sequence of SEQ ID NO: 29.
80. A nucleic acid encoding the masked IL-12 cytokine of any one of claims 1-72 or encoding one of the polypeptide chains of a masked IL-12 cytokine of any one of claims 1 to 72.
81. A vector comprising the nucleic acid of claim 80.
82. A host cell comprising nucleic acid encoding the masked IL-12 cytokine of any one of claims 1 to 72.
83. A composition comprising the masked IL-12 cytokine of any one of claims 1-72.
84. A pharmaceutical composition comprising the masked IL-12 cytokine of any one of claims 1-72 and a pharmaceutically acceptable carrier.
85. The pharmaceutical composition of claim 84, wherein the pharmaceutical composition is in single unit dosage form.
86. The pharmaceutical composition of claim 84, wherein the pharmaceutical composition is formulated for intravenous administration and is in single unit dosage form.
87. The pharmaceutical composition of claim 84, wherein the pharmaceutical composition is formulated for injection and is in single unit dosage form.
88. The pharmaceutical composition of claim 84, wherein the pharmaceutical composition is a liquid and is in single unit dosage form.

89. A kit comprising the masked IL-12 cytokine of any one of claims 1-41, or the composition of claim 83, or the pharmaceutical composition of claims 84 to 88.
90. A method of producing a masked IL-12 cytokine as defined in any one of claims 1 to 72 comprising culturing the host cell of claim 82 under a condition that produces the masked IL-12 cytokine.
91. A nucleic acid encoding the cleavage product of any one of claims 73 to 79.
92. A composition comprising the cleavage product of any one of claims 73 to 79.
93. A pharmaceutical composition comprising the cleavage product of any one of claims 73 to 79, and a pharmaceutically acceptable carrier.
94. A masked IL-12 cytokine as defined in any one of claims 1 to 72 for use in medicine.
95. A cleavage product as defined in any one of claims 73 to 79 for use in medicine.
96. A method of treating or preventing cancer in a subject, the method comprising administering to the subject an effective amount of a masked IL-12 cytokine according to any one of claims 1 to 72.
97. A method of treating or preventing cancer in a subject, the method comprising administering to the subject an effective amount of a composition according to claims 83 or 92.
98. A method of treating or preventing cancer in a subject, the method comprising administering to the subject an effective amount of a pharmaceutical composition according to any one of claims 84 to 88 and 93.
99. A method of treating or preventing cancer in a subject, the method comprising administering to the subject an effective amount of a masked IL-12 cytokine as defined in any one of claims 1 to 72, whereby the masked cytokine is proteolytically cleaved *in vivo* to produce a cleavage product as defined in any one of claims 73 to 79.
100. A method of treating or preventing cancer in a subject, the method comprising a step of producing a cleavage product *in vivo* that is capable of binding to its cognate receptor, where the cleavage product is as defined in any one of claims 73 to 79.

101. A method according to any one of claims 96-100, wherein the cancer is a solid tumor.
102. A masked IL-12 cytokine as defined in any one of claims 1 to 72 for use in treating or preventing cancer.
103. A masked IL-12 cytokine as defined in any one of claims 1 to 72 for use in a method of treating or preventing cancer, the method comprising administering to the subject an effective amount of the masked IL-12 cytokine, whereby the masked cytokine is proteolytically cleaved *in vivo* to produce a cleavage product as defined in any one of claims 73 to 79.
104. A masked IL-12 cytokine for use according to claim 103, wherein the cancer is a solid tumor.
105. A cleavage product as defined in any one of claims 73 to 79 for use in treating or preventing cancer.
106. A cleavage product as defined in any one of claims 73 to 79 for use in a method of treating or preventing cancer, the method comprising a step of administering a masked cytokine as defined in any one of claims 1 to 72 to a patient, thereby producing the cleavage product by proteolytic cleavage of the masked cytokine *in vivo*.
107. A cleavage product as defined in any one of claims 73 to 79 for use in a method of treating or preventing cancer in a subject, the method comprising a step of producing the cleavage product by *in vivo* proteolytic cleavage from a masked cytokine as defined in any one of claims 1 to 72 that has been administered to the subject.
108. A cleavage product for use according to any one of claims 105 to 107, wherein the cancer is a solid tumor.
109. A pharmaceutical composition according to any one of claims 84 to 88 and 93, for use in treating or preventing cancer.
110. A pharmaceutical composition for use according to claim 109, wherein the cancer is a solid tumor.