MASKED IL-2 CYTOKINES AND THEIR CLEAVAGE PRODUCTS

CROSS-REFERENCE TO RELATED APPLICATIONS

This application claims the priority benefit of U.S. Provisional Application Serial Nos. 63/003,824, filed April 1, 2020; and 63/118,571, filed November 25, 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 entirety: a computer readable form (CRF) of the Sequence Listing (file name: 737762002740SEQLIST.TXT, date recorded: March 26, 2021, size: 650 KB).

FIELD

This invention relates to masked IL-2 cytokines and methods related to the use and manufacture of the same. This invention also relates to cleavage products of said masked IL-2 cytokines, and methods related to the use of the same.

BACKGROUND

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 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.

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-2 cytokines, cleavage products of said masked IL-2 cytokines, and compositions thereof and methods of use thereof for addressing this need.

SUMMARY

The disclosed invention relates to IL-2 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-2 cytokine or functional fragment thereof. The IL-2 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-2 cytokine or functional fragment thereof to its cognate receptor. Upon proteolytic cleavage of the cleavable linker at the target site, the IL-2 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-2 cytokine comprising 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-2 cytokine or functional fragment thereof linked to a second half-life extension domain via a second linker,

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 is a proteolytically cleavable linker comprising a proteolytically cleavable peptide.

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

In some embodiments, the first Fc domain comprises a CH3 domain or a fragment thereof and the second Fc domain comprises a CH3 domain or a fragment thereof.

In some embodiments, the first and second half-life extension domains are each an IgG1 Fc domain or 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, numbered according to the Kabat EU numbering system.

In some embodiments, the first and second half-life extension domains are each an IgG1 Fc domain or fragment thereof and each comprise the amino substitution N297A, numbered according to the Kabat EU numbering system.

In some embodiments, the first and second half-life extension domains are each an IgG1 Fc domain or fragment thereof and each comprise the amino substitution I253A, numbered according to the Kabat EU numbering system.

In some embodiments, the first half-life extension domain comprises the amino acid sequence of SEQ ID NO: 9, and the second half-life extension domain thereof comprises the amino acid sequence of SEQ ID NO: 12.

In some embodiments, the first half-life extension domain comprises the amino acid sequence of SEQ ID NO: 10 and the second half-life extension domain thereof comprises the amino acid sequence of SEQ ID NO: 13.

In some embodiments, the IL-2 cytokine or functional fragment thereof is modified compared to the sequence of a mature IL-2 having SEQ ID NO: 2.

In some embodiments, the modified IL-2 cytokine or functional fragment thereof comprises modifications R38A, F42A, Y45A, and E62A relative to the sequence of a mature IL-2 having SEQ ID NO: 2.

In some embodiments, the modified IL-2 cytokine or functional fragment thereof comprises the modification C125A relative to the sequence of a mature IL-2 having SEQ ID NO: 2.

In some embodiments, the modified IL-2 cytokine or functional fragment thereof comprises R38A, F42A, Y45A, E62A and C125A relative to the sequence of a mature IL-2 having SEQ ID NO: 2.

In some embodiments, the IL-2 cytokine or functional fragment thereof comprises an amino acid sequence of SEQ ID NO: 3.

In some embodiments, the masking moiety comprises IL-2Rβ or a fragment, portion or variant thereof.

In some embodiments, the IL-2Rβ or a fragment, portion or variant thereof comprises an amino acid sequence of SEQ ID NO: 4.

In some embodiments, wherein the IL-2Rβ or a fragment, portion or variant thereof comprises an amino acid sequence of SEQ ID NO: 5.

In some embodiments, 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.

In some embodiments, the first linker comprises a proteolytically cleavable peptide such that the first linker is a proteolytically cleavable linker and the second linker does not comprise a proteolytically cleavable peptide such that the second linker is a non- proteolytically cleavable linker.

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

In some embodiments, the cleavable peptide within the proteolytically cleavable linker comprises an amino acid sequence selected from the group consisting of SEQ ID NOs: 24, 25, 26, 27 and 28.

In some embodiments, the cleavable peptide within the proteolytically cleavable linker comprises SEQ ID NO: 118.

In some embodiments, the cleavable peptide within the proteolytically cleavable linker comprises SEQ ID NO: 119.

In some embodiments, the proteolytically cleavable linker comprises a proteolytically cleavable peptide flanked on both sides by a spacer domain.

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.

In some embodiments, the proteolytically cleavable linker comprises an amino acid sequence selected from the group consisting of SEQ ID NOs: 16, 17, 18, 19, 20, 21 and 22.

In some embodiments, the proteolytically cleavable linker comprises an amino acid sequence selected from the group consisting of SEQ ID NO: 19.

In some embodiments, the proteolytically cleavable linker comprises an amino acid sequence selected from the group consisting of SEQ ID NO: 17.

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: 118 and SD2 has an amino acid sequence as shown in SEQ ID NO: 29.

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: 119 and SD2 has an amino acid sequence as shown in SEQ ID NO: 29.

In some embodiments, the SD2 is from 3 to 6 amino acids in length.

In some embodiments, the proteolytically cleavable linker comprises an amino acid sequence selected from the group consisting of SEQ ID NO: 115.

In some embodiments, the proteolytically cleavable linker comprises an amino acid sequence selected from the group consisting of SEQ ID NO: 116.

In some embodiments, the proteolytically cleavable linker comprises an amino acid sequence selected from the group consisting of SEQ ID NO: 117.

In some embodiments, the proteolytically cleavable linker comprises an amino acid sequence selected from the group consisting of SEQ ID NO: 112.

In some embodiments, the proteolytically cleavable linker comprises an amino acid sequence selected from the group consisting of SEQ ID NO: 113.

In some embodiments, the proteolytically cleavable linker comprises an amino acid sequence selected from the group consisting of SEQ ID NO: 114.

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

In some embodiments, the non-proteolytically cleavable linker is between 3 and 8 amino acids in length.

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

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

In some embodiments, the non-proteolytically cleavable linker comprises an amino acid sequence of SEQ ID NO: 23.

In some embodiments, the masked IL-2 cytokine comprises a first polypeptide chain of SEQ ID NO: 38.

In some embodiments, the masked IL-2 cytokine comprises a first polypeptide chain of SEQ ID NO: 39.

In some embodiments, the masked IL-2 cytokine comprises a first polypeptide chain of SEQ ID NO: 125.

In some embodiments, the masked IL-2 cytokine comprises a first polypeptide chain of SEQ ID NO: 126.

In some embodiments, the masked IL-2 cytokine comprises a first polypeptide chain of SEQ ID NO: 127.

In some embodiments, the masked IL-2 cytokine comprises a first polypeptide chain of SEQ ID NO: 39 and a second polypeptide chain of SEQ ID NO: 49.

In some embodiments, the masked IL-2 cytokine comprises a first polypeptide chain of SEQ ID NO: 40 and a second polypeptide chain of SEQ ID NO: 51.

In some embodiments, the masked IL-2 cytokine comprises a first polypeptide chain of SEQ ID NO: 38 and a second polypeptide chain of SEQ ID NO: 128.

In some embodiments, the masked IL-2 cytokine comprises a first polypeptide chain of SEQ ID NO: 38 and a second polypeptide chain of SEQ ID NO: 129.

In some embodiments, the masked IL-2 cytokine comprises a first polypeptide chain of SEQ ID NO: 38 and a second polypeptide chain of SEQ ID NO: 130.

In some embodiments, the masked IL-2 cytokine comprises a first polypeptide chain of SEQ ID NO: 125 and a second polypeptide chain of SEQ ID NO: 51.

In some embodiments, the masked IL-2 cytokine comprises a first polypeptide chain of SEQ ID NO: 126 and a second polypeptide chain of SEQ ID NO: 51.

In some embodiments, the masked IL-2 cytokine comprises a first polypeptide chain of SEQ ID NO: 127 and a second polypeptide chain of SEQ ID NO: 51.

Provided herein is a masked IL-2 cytokine comprising a masking moiety and an IL-2 cytokine or functional fragment thereof, wherein the masking moiety masks the IL-2 cytokine or functional fragment thereof thereby reducing or preventing binding of the IL-cytokine or functional fragment thereof to its cognate receptor, and wherein a proteolytically cleavable peptide is present between the IL-2 fragment or functional fragment thereof and masking moiety.

In some embodiments, the masking moiety and IL-2 cytokine or functional fragment thereof are linked in a single polypeptide chain.

In some embodiments, the masked IL-2 cytokine comprises a polypeptide chain comprising formula 1:

N' **HL-L2-C-L1-MM** C'

(1)

where HL is the half life extension domain, L1 is the first linker, MM is the masking moiety, L2 is the second linker, and C is the IL-2 cytokine or functional fragment thereof, wherein at least the first linker comprises a proteolytically cleavable peptide.

In some embodiments, the masked IL-2 cytokine comprises a polypeptide chain comprising formula 2:

N' **HL-L2-MM-L1-C** C'

(2)

where HL is the half life extension domain, L1 is the first linker, MM is the masking moiety, L2 is the second linker, and C is the IL-2 cytokine or functional fragment thereof, wherein at least the first linker comprises a proteolytically cleavable peptide.

In some embodiments, the masking moiety comprises IL-2Rβ or a fragment, portion or variant thereof.

In some embodiments, the IL-2Rβ or a fragment, portion or variant thereof has mutations at amino acid positions C122 and C168 as compared to IL-2β of SEQ ID NO: 4.

In some embodiments, the IL-2Rβ or a fragment, portion or variant thereof has mutations C122S and C168S as compared to IL-2β of SEQ ID NO: 4.

In some embodiments, the half life extension domain (HL) comprises first and second half-life extension domains which are each an IgG1 Fc domain or 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, numbered according to the Kabat EU numbering system.

In some embodiments, the first and second half-life extension domains are each an IgG1 Fc domain or fragment thereof and each comprise the amino substitution N297A, numbered according to the Kabat EU numbering system.

In some embodiments, the first and second half-life extension domains are each an IgG1 Fc domain or fragment thereof and each comprise the amino substitution I253A, numbered according to the Kabat EU numbering system.

In some embodiments, the first half-life extension domain comprises the amino acid sequence of SEQ ID NO: 9, and the second half-life extension domain thereof comprises the amino acid sequence of SEQ ID NO: 12.

In some embodiments, the first half-life extension domain comprises the amino acid sequence of SEQ ID NO: 10 and the second half-life extension domain thereof comprises the amino acid sequence of SEQ ID NO: 13.

In some embodiments, the cleavable peptide within the proteolytically cleavable linker comprises SEQ ID NO: 118.

In some embodiments, the cleavable peptide within the proteolytically cleavable linker comprises SEQ ID NO: 119.

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: 118 and SD2 has an amino acid sequence as shown in SEQ ID NO: 29.

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: 119 and SD2 has an amino acid sequence as shown in SEQ ID NO: 29.

In some embodiments, the SD2 is from 3 to 6 amino acids in length.

In some embodiments, the proteolytically cleavable linker comprises an amino acid sequence selected from the group consisting of SEQ ID NO: 115.

In some embodiments, the proteolytically cleavable linker comprises an amino acid sequence selected from the group consisting of SEQ ID NO: 116.

In some embodiments, the proteolytically cleavable linker comprises an amino acid sequence selected from the group consisting of SEQ ID NO: 112.

In some embodiments, the proteolytically cleavable linker comprises an amino acid sequence selected from the group consisting of SEQ ID NO: 113.

In some embodiments, the proteolytically cleavable linker comprises an amino acid sequence selected from the group consisting of SEQ ID NO: 114.

Provided herein is a cleavage product capable of binding to its cognate receptor, the cleavage product comprising an IL-2 cytokine or functional fragment thereof, preparable by proteolytic cleavage of the cleavable peptide in the masked IL-2 cytokine as defined in of any one of the statements or embodiments described herein.

Provided herein is a cleavage product of a masked IL-2 cytokine, where the cleavage product is capable of binding to its cognate receptor, the cleavage product comprising a polypeptide comprising formula 3:

PCP-SD-C

(3)

wherein PCP is a portion of a proteolytically cleavable peptide; SD is a spacer domain; and C is an IL-2 cytokine or functional fragment thereof.

In some embodiments, the IL-2 cytokine or functional fragment thereof is modified compared to the sequence of a mature IL-2 polypeptide having SEQ ID NO: 2.

In some embodiments, the modified IL-2 cytokine or functional fragment thereof comprises modifications R38A, F42A, Y45A, and E62A relative to the sequence of a mature IL-2 having SEQ ID NO: 2.

In some embodiments, the modified IL-2 cytokine or functional fragment thereof comprises the modification C125A relative to the sequence of a mature IL-2having SEQ ID NO: 2.

In some embodiments, the modified IL-2 cytokine or functional fragment thereof comprises R38A, F42A, Y45A, E62A and C125A.

In some embodiments, the IL-2 cytokine or functional fragment thereof comprises an amino acid sequence of SEQ ID NO: 3.

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

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

In some embodiments, the spacer domain comprises an amino acid sequence of any one of SEQ ID NO: 29, 30 and 31.

In some embodiments, the portion of the proteolytically cleavable peptide is a portion of an amino acid

sequence of any one of SEQ ID NOs: 24, 25, 26, 27 and 28.

In some embodiments, the portion of the proteolytically cleavable peptide is a portion of an amino acid sequence of SEQ ID NO: 118.

In some embodiments, the portion of the proteolytically cleavable peptide is a portion of an amino acid sequence of SEQ ID NO: 119.

In some embodiments, the cleavage product comprises the amino acid sequence of SEQ ID NO: 56.

In some embodiments, the cleavage product comprises the amino acid sequence of SEQ ID NO: 137.

Provided herein is a cleavage product of a masked IL-2 cytokine, where the cleavage product is capable of binding to its cognate receptor, the cleavage product comprising a protein heterodimer comprising:
a first polypeptide chain comprising a polypeptide comprising formula 4:

HL1-SD-PCP
(4)

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

a second polypeptide chain comprising a polypeptide comprising formula 5:

HL2-L2-C
(5)

wherein HL2 is a second half-life extension domain; L2 is a linker; and C is an IL-2 cytokine or functional fragment thereof; and
wherein the first half-life extension domain is associated with the second half-life extension domain.

In some embodiments, the IL-2 cytokine or functional fragment thereof is modified compared to the sequence of a mature IL-2 having SEQ ID NO: 2.

In some embodiments, the modified IL-2 cytokine or functional fragment thereof comprises modifications R38A, F42A, Y45A, and E62A relative to the sequence of a mature IL-2 having SEQ ID NO: 2.

In some embodiments, the modified IL-2 cytokine or functional fragment thereof comprises the modification C125A relative to the sequence of a mature IL-2 having SEQ ID NO: 2.

In some embodiments, the modified IL-2 cytokine or functional fragment thereof comprises R38A, F42A, Y45A, E62A and C125A.

In some embodiments, the IL-2 cytokine or functional fragment thereof comprises an amino acid sequence of SEQ ID NO: 3.

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

In some embodiments, the first Fc domain comprises a CH3 domain or a fragment thereof and the second Fc domain comprises a CH3 domain or a fragment thereof.

In some embodiments, the first and second half-life extension domains are each an IgG1 Fc domain or 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 and second half-life extension domains are each an IgG1 Fc domain or fragment thereof and each comprise the amino substitution N297A, numbered according to the Kabat EU numbering system.

In some embodiments, the first and second half-life extension domains are each an IgG1 Fc domain or fragment thereof and each comprise the amino substitutions N297A and I253A, numbered according to the Kabat EU numbering system.

In some embodiments, the first half-life extension domain comprises the amino acid sequence of SEQ ID NO: 9, and the second half-life extension domain thereof comprises the amino acid sequence of SEQ ID NO: 12.

In some embodiments, the first half-life extension domain comprises the amino acid sequence of SEQ ID NO: 10 and the second half-life extension domain thereof comprises the amino acid sequence of SEQ ID NO: 13.

In some embodiments, the second linker comprises an amino acid sequence of SEQ ID NO: 23.

In some embodiments, the spacer domain is rich in amino acids residues G, S and P.

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

In some embodiments, the spacer domain comprises an amino acid sequence of SEQ ID NOs: 32, 33, 34, 35, 36 and 37.

In some embodiments, the portion of the proteolytically cleavable peptide is a portion of an amino acid sequence of any one of SEQ ID NOs: 24, 25, 26, 27 and 28.

In some embodiments, the portion of the proteolytically cleavable peptide is a portion of an amino acid sequence of SEQ ID NO: 118.

In some embodiments, the portion of the proteolytically cleavable peptide is a portion of an amino acid sequence of SEQ ID NO: 119.

In some embodiments, the cleavage product comprises a first polypeptide chain having an amino acid sequence of SEQ ID NO: 136 and a second polypeptide chain having an amino acid sequence of SEQ ID NO: 135.

In some embodiments, the cleavage product comprises a first polypeptide chain having an amino acid sequence of SEQ ID NO: 139 and a second polypeptide chain having an amino acid sequence of SEQ ID NO: 138.

In some embodiments, the cleavage product comprises a first polypeptide chain having an amino acid sequence of SEQ ID NO: 141 and a second polypeptide chain having an amino acid sequence of SEQ ID NO: 140.

In some embodiments, the cleavage product comprises a first polypeptide chain having an amino acid sequence of SEQ ID NO: 143 and a second polypeptide chain having an amino acid sequence of SEQ ID NO: 142.

Provided herein is a nucleic acid encoding any one of the masked IL-2 cytokines described herein.

Provided herein is a nucleic acid encoding one of the chains of any one of the masked IL-2 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-2 cytokine described herein.

Provided herein is a vector comprising a nucleic acid encoding one of the chains of a masked IL-2 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-2 cytokines described herein.

Provided herein is a pharmaceutical composition comprising any one of the masked IL-2 cytokines described herein, and a pharmaceutically acceptable carrier.

Provided herein is a kit comprising any one of the masked IL-2 cytokines, or the compositions, or the pharmaceutical compositions described herein.

Provided herein is a method of producing any one of the masked IL-2 cytokines described herein, comprising culturing a host cell described herein under a condition that produces the masked IL-2 cytokine.

Provided herein is a nucleic acid encoding any one of the cleavage products described herein.

Provided herein is a composition comprising any one of the cleavage products described herein.

Provided herein is a pharmaceutical composition comprising any one of the cleavage products described herein, and a pharmaceutically acceptable carrier.

Provided herein is a masked IL-2 cytokine as described herein for use in medicine.

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

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-2 cytokine as 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 as 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 pharmaceutical composition as 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 masked IL-2 cytokine as described herein, whereby the masked cytokine is proteolytically cleaved *in vivo* to produce a cleavage product as 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, where the cleavage product is as described herein.

Provided herein is a masked IL-2 cytokine as described herein for use in treating or preventing cancer.

Provided herein is a masked IL-2 cytokine as 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-2 cytokine, whereby the masked cytokine is proteolytically cleaved *in vivo* to produce a cleavage product as described herein.

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

Provided herein is a cleavage product as described herein for use in treating or preventing cancer, the method comprising a step of administering a masked cytokine as 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 as 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 as described herein that has been administered to the subject.

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-2Rβ, 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 construct (AK168), or a rhIL-2 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 (rhIL2) control and CD25-Fc. **FIG. 5D** provides a table summarizing the data obtained for the association constant (ka), dissociation constant (kd), equilibrium dissociation constant (KD), as well as the Chi2 value and U-value for each interaction.

FIGs. 6A-6D show results from SPR analysis that tested the binding of an exemplary masked IL-2 polypeptide constructs (AK111), or a rhIL2 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 (ka), dissociation constant (kd), equilibrium dissociation constant (KD), 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. 23D; FIG. 23G**), CD4+ T cells (**FIG. 23B; FIG. 23E; FIG. 23H**), or Foxp3+ cells (**FIG. 23C; FIG. 23F; FIG. 23I**) 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; SEQ ID NO: 24) and AK209 (cleavable peptide sequence: VPLSLY; SEQ ID NO: 28). **FIG. 24E** shows 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.

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** show 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.

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.

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 show AK471 with I253A FcRn mutation induced robust CD8 T cells expansion in the TME while remaining inactive in the periphery.

FIGs. 30A-30C show AK471 has slightly shorter half-life compared to aglyco-hIgG1

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

FIGs. 32A and 32B show results of Example 5.

FIGs. 33A-33D show results of Example 5.

FIGs. 34A and 34B shows results of Example 6i. **FIGs. 35A and 35B** show results of Example 6ii. **FIGs. 36A and 36B** show results of Example 6iii. **FIGs. 37A and 37B** show results of Example 6iv. **FIGs. 38A and 38B** show results of Example 6v. **FIGs. 39A and 39B** show results of Example 6vi. **FIGs. 40A-40D** show results of Example 6vii. **FIGs. 41A and 41B** show results of Example 6viii. **FIGs. 42A and 42B** show results of Example6ix. **FIGs. 43A and 43B** show results of Example 6x.

FIGs. 44A-44D and **FIGs. 45A-45F** 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 44A-44D** show the SDS-PAGE gel results. **FIGs. 45A-45F** show the HEK-Blue IL-2 bioassay results.

DETAILED DESCRIPTION

By using a masking moiety, the systemic side effects of an administered IL-2 cytokine or functional fragment thereof can be reduced by interfering with the binding capability of the IL-2 cytokine or functional fragment thereof to its cognate receptor.

The IL-2 cytokine receptor is an IL-2 receptor complex that comprises three separate and non-covalently linked chains: the IL-2Rα chain (also referred to as CD25), the IL-2Rβ chain (also referred to as CD122), and the IL-2Rγ chain (also referred to as CD132). The three receptor chains can assemble in different combinations and orders to generate low, intermediate, and high affinity IL-2 receptors. The α chain alone binds IL-2 with low affinity, the combination of β and γ together form a complex that binds IL-2 with intermediate affinity, and combination of all three receptor chains (α , β and γ) form a complex that binds IL-2 with high affinity.

For instance, high-dose recombinant IL-2 (aldesleukin) has been approved by the FDA for the treatment of metastatic renal cell carcinoma and melanoma, but has been associated with severe cardiovascular, hepatic, pulmonary, gastrointestinal, neurologic, and haematological side effects. Preclinical studies showed, for instance, that IL-2-induced pulmonary edema is caused by the interaction between IL-2 and the IL-2Rα (CD25) subunit of the IL-2 receptor on lung endothelial cells, and that this IL-2-mediated pulmonary edema could be abrogated by interfering with the ability of the IL-2 to bind IL-2Rα. See Krieg et al. (2010) PNAS, 107(26): 11906-11911. Thus, in some embodiments, a masking moiety is employed that reduces or prevents binding of the IL-2 cytokine or functional fragment thereof to IL-2Rα. To further reduce systemic effects, in some embodiments, binding of the IL-2 cytokine or fragment thereof to the IL-2Rβ and/or IL-2Rγ subunits of the IL-2 receptor may also be reduced or prevented by the masking moiety in the masked cytokine.

By masking the IL-2 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-2 cytokines 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-2 cytokine or functional fragment thereof in the form of an IL-2 cleavage product. Activation of the IL-2 cytokine or functional fragment thereof at the tumor microenvironment significantly reduces systemic 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-2 cytokines described herein have been found to show various advantageous properties. Masked IL-2 cytokines described anywhere herein have been found to be capable of activating immune cells (proliferation and expansion) upon proteolytic cleavage, preferentially in the tumor microenvironment and at lower levels in the periphery. Masked IL-2 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-2 cytokines described anywhere herein have been found to demonstrate advantageous prolonged drug exposure. Masked IL-2 cytokines described herein have been found to demonstrate advantageous stability. Masked IL-2 cytokines described herein have been found to demonstrate advantageous tolerability. Further, masked IL-2 cytokines described herein have been found to demonstrate advantageous potency.

1. 'HETERODIMERIC' MASKED CYTOKINES

Provided herein, in some embodiments, is a masked cytokine comprising a masking moiety in a first polypeptide chain and an IL-2 cytokine or functional fragment thereof in a second polypeptide chain. Such masked cytokines may be referred to as 'heterodimeric' masked cytokines.

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-2 cytokine or functional fragment thereof linked to a second half-life extension domain via a second linker,

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

The masking moiety, half-life extension domains, IL-2 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.

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-2 cytokine or functional fragment thereof. This is shown schematically N- to C-terminal in formulae 6 (first polypeptide chain) and 5 (second polypeptide chain) below:

N' **HL1-L1-MM** C'
(6)

N' **HL2-L2-C** C'
(5)

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-2 cytokine or functional fragment thereof.

1.1 IL-2 Cytokines

Provided herein is an IL-2 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-2 is an interleukin, which is a type of cytokine signalling molecule in the immune system that regulates activities of white blood cells.

In eukaryotic cells, naturally occurring IL-2 is synthesized as a precursor polypeptide of 153 amino acids, which has SEQ ID NO: 1. This is then processed into mature IL-2 by the removal of amino acid residues 1-20. This results in a mature form of IL-2 consisting of 133 amino acids (amino acid residues 21-153), which has SEQ ID NO: 2. "Functional fragments" of an IL-2 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 cognate receptor or a component thereof (e.g., one or more chain(s) of a heterotrimeric receptor complex).

In some embodiments, the IL-2 cytokine or functional fragment thereof is any naturally occurring interleukin-2 (IL-2) protein or modified variant thereof capable of binding to an interleukin-2 receptor, particularly the IL-2Rα chain. In the context of IL-2 cytokine binding, the target protein could be IL-2R (comprising the IL-2Rα, IL-2Rβ, and IL-2Rγ chains), the IL-2Rα chain, the IL-2Rβ chain, or the IL-2Rα/β dimeric complex. In some embodiments, the IL-2 cytokine or functional fragment thereof comprises the amino acid sequence of amino acid residues 21-153 of SEQ ID NO: 1. In some embodiments, the IL-2 polypeptide or functional fragment thereof comprises the amino acid sequence of mature IL-2, SEQ ID NO: 2.

In some embodiments, the IL-2 cytokine or functional fragment thereof 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-2 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-2 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-2 cytokine or functional fragment thereof comprises an amino acid sequence having one or more amino acid substitutions as compared to the amino acid sequence of wild-type IL-2 of SEQ ID NO: 2 that reduces the affinity of the IL-2 peptide or functional fragment thereof for IL-2Rα (CD25). In some embodiments, the IL-2 cytokine or functional fragment thereof comprises an amino acid sequence having one or more amino acid substitutions as compared to the amino acid sequence of SEQ ID NO: 2, such that one or more of amino acid residues 38, 42, 45, and 62 is an alanine (A). In some embodiments, the IL-2 cytokine or functional fragment thereof comprises an amino acid sequence having one or more amino acid substitutions as compared to the amino acid sequence of SEQ ID NO: 2, such that amino acid residues 38, 42, 45, and 62 are an alanine (A).

In some embodiments, the IL-2 cytokine or functional fragment thereof comprises amino acid sequence substitution C125A as compared to the amino acid sequence of SEQ ID NO: 2.

In some embodiments, the IL-2 cytokine or functional fragment thereof comprises an amino acid sequence having one or more amino acid substitutions as compared to the amino acid sequence of SEQ ID NO: 2, such that amino acid residues 38, 42, 45, and 62 are an alanine (A) and amino acid residue 125 is a alanine (A). In some embodiments, the IL-2 cytokine or functional fragment thereof comprises an amino acid sequence having amino acid residues R38, F42, Y45, and E62 substituted for alanine in the amino acid sequence of SEQ ID NO: 2. In some embodiments, the IL-2 cytokine or functional fragment thereof comprises an amino acid sequence having amino acid residues R38, F42, Y45, and E62 substituted for alanine (A) and amino acid residue C125 substituted for alanine (A) in the amino acid sequence of SEQ ID NO: 2.

In some embodiments, the IL-2 cytokine or functional fragment thereof comprises the amino acid sequence of SEQ ID NO: 3. In some embodiments, the IL-2 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: 3.

In some embodiments, the IL-2 cytokine or functional fragment thereof has one or more amino acid residues e.g. residues 1-3 s removed as compared to the amino acid sequence of the mature IL-2 of SEQ ID 2, for the purpose of removing an O-glycosylation site. In some embodiments, the IL-2 cytokine or functional fragment thereof has one or more amino acid residues substituted as compared to the amino acid sequence of the mature IL-2 of SEQ ID 2, for the purpose of removing an O-glycosylation site. In some embodiments, the IL-2 cytokine or functional fragment thereof has one or more amino acid residues inserted, e.g. in the region of residues 1-3, as compared to the amino acid sequence of the mature IL-2 of SEQ ID 2, for the purpose of removing an O-glycosylation site. In some embodiments, the IL-2 cytokine or functional fragment thereof does not have an O-glycosylation site within residues 1-3.

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-2 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. In some embodiments, the masking moiety reduces or prevents binding of the IL-2 cytokine or functional fragment thereof to IL-2Rα (CD25). In some embodiments, the masking moiety as provided herein refers to a moiety capable of binding to, or otherwise exhibiting an affinity for the IL-2 cytokine or functional fragment thereof, such as an anti-IL-2 antibody or IL-2 cognate receptor protein. 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 IL-2 cytokine receptor, or a subunit or functional fragment thereof.

In some embodiments, the masking moiety comprises IL-2Rβ (also referred to as CD122) or a fragment, portion, or variant thereof that retains or otherwise demonstrates an affinity to IL-2.

In some embodiments, the masking moiety comprises the amino acid sequence of SEQ ID NO: 4. 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: 4. In some embodiments, the masking moiety comprises an amino acid sequence having the amino acid sequence of SEQ ID NO: 4 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: 4 with one or two amino acid substitutions .

In some embodiments, the IL-2Rβ or a fragment, portion or variant thereof has mutation at amino acid position C122 as compared to IL-2Rβ of SEQ ID NO: 4.

In some embodiments, the IL-2Rβ or a fragment, portion or variant thereof has mutation C122S at amino acid position 122 as compared to IL-2Rβ of SEQ ID NO: 4.

In some embodiments, the masking moiety comprises an amino acid sequence of SEQ ID NO: 4 with a C122 mutation.

In some embodiments, the masking moiety comprises an amino acid sequence of SEQ ID NO: 4 with a C122S mutation.

In some embodiments, the IL-2Rβ or a fragment, portion or variant thereof has mutation at amino acid position C168 as compared to IL-2Rβ of SEQ ID NO: 4.

In some embodiments, the IL-2Rβ or a fragment, portion or variant thereof has mutation C168S at amino acid position 168 as compared to IL-2Rβ of SEQ ID NO: 4.

In some embodiments, the masking moiety comprises an amino acid sequence of SEQ ID NO: 4 with a C168 mutation.

In some embodiments, the masking moiety comprises an amino acid sequence of SEQ ID NO: 4 with a C168S mutation.

The masked cytokine of any one of claims, wherein the IL-2Rβ or a fragment, portion or variant thereof has mutation at amino acid positions C122 and C168 as compared to IL-2Rβ of SEQ ID NO: 4.

The masked cytokine of any one of claims, wherein the IL-2Rβ or a fragment, portion or variant thereof has mutation C122S and C168S as compared to IL-2Rβ of SEQ ID NO: 4.

In some embodiments, the masking moiety comprises an amino acid sequence of SEQ ID NO: 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.

The masked cytokine comprises a first linker and a second linker, where at least 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'). In some embodiments, the first polypeptide chain comprises formula 7 and the second polypeptide chain comprises formula 8 below:

N' **HL1-non-cleavable L1-MM** C'
(7)

N' **HL2-cleavable L2-C** C'
(8)

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 'non-cleavable linker'). In some embodiments, the first polypeptide chain comprises formula 9 and the second polypeptide chain comprises formula 10 below:

N' **HL1- cleavable L1-MM** C'
(9)

N' **HL2- non-cleavable L2-C** C'
(10)

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.

In some embodiments, the non-cleavable linker is between 3 and 8 amino acids in length. In some embodiments, the non-cleavable linker is between 4 and 6 amino acids in length.

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

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

In some embodiments, the non-cleavable linker includes a 'GS' repeat.

In some embodiments, the non-cleavable linker includes an N' terminal 'P' residue.

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

In some embodiments, the non-cleavable linker comprises an amino acid sequence as shown in SEQ ID NO: 23 (GGSSPPGGGSSGGGSGP).

In some embodiments, the non-cleavable linker comprises an amino acid sequence GGS.
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 8 amino acids in length. In some embodiments, the non-cleavable linker is between 4 and 6 amino acids in length. In some embodiments, the non-cleavable linker comprises an amino acid sequence as shown in SEQ ID NO: 14 (PGSGS).

In some embodiments, wherein the first linker comprises a proteolytically cleavable peptide such that the first linker is a proteolytically cleavable linker and the second linker does not comprise a proteolytically cleavable peptide such that the second 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: 23 (GGSSPPGGGSSGGGSGP).

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 8 amino acids in length. In some embodiments, the non-cleavable linker comprises an amino acid sequence GGS.

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-2 cytokine or functional fragment thereof in the assembled construct. As such where the masking moiety is a shorter amino acid sequence than the IL-2 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 in formula 11:

SD-CP-SD
(11)

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.

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-2 cytokine receptor, particularly IL-2Rα.

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).

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 SEQ ID NOs: 24, 25, 26, 27 and 28. In some embodiments, the cleavable peptide within the cleavable linker comprises an amino acid sequence selected from the group consisting of SEQ ID NOs: 24, 25, 26, 27, 28 and 118 and 119.

SEQ ID NO:	Sequence (* indicates a cleavage site within the cleavable peptide)
24	MPYD*LYHP

25	DSGG*FMLT
26	HEQ*LTV
27	RAAA*VKSP
28	VPLS*LY
118	DLLA*VVAAS
119	ISSGLL*SG*RS

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

In some embodiments, the cleavable peptide comprises an amino acid sequence of SEQ ID NO: 24. In some embodiments, the cleavable peptide comprises an amino acid sequence of SEQ ID NO: 25. In some embodiments, the cleavable peptide comprises an amino acid sequence of SEQ ID NO: 26. In some embodiments, the cleavable peptide comprises an amino acid sequence of SEQ ID NO: 27. In some embodiments, the cleavable peptide comprises an amino acid sequence of SEQ ID NO: 28, for example the cleavable peptide may comprise an amino acid sequence of SEQ ID NO: 324 (VPLSLYSG). In some embodiments, the cleavable peptide comprises an amino acid sequence of SEQ ID NO: 118. In some embodiments, the cleavable peptide comprises an amino acid sequence of SEQ ID NO: 119, for example the cleavable peptide may comprise an amino acid sequence of SEQ ID NO: 323 (ISSGLLSGRSDQP).

In some embodiments, the cleavable peptide consists of an amino acid sequence selected from the group consisting of SEQ ID NOs: 24, 25, 26, 27 and 28. In some embodiments, the cleavable peptide consists of an amino acid sequence selected from the group consisting of SEQ ID NOs: 24, 25, 26, 27, 28, 118 and 119. In some embodiments, the cleavable peptide consists of an amino acid sequence of SEQ ID NO: 24. In some embodiments, the cleavable peptide consists of an amino acid sequence of SEQ ID NO: 25. In some embodiments, the cleavable peptide consists of an amino acid sequence of SEQ ID NO: 26. In some embodiments, the cleavable peptide consists of an amino acid sequence of SEQ ID NO: 27. In some embodiments, the cleavable peptide consists of an amino acid sequence of SEQ ID NO: 28. In some embodiments, the cleavable peptide consists of an amino acid sequence of SEQ ID NO: 118. In some embodiments, the cleavable peptide consists of an amino acid sequence of SEQ ID NO: 119. In some embodiments, the cleavable peptide consists of an amino acid sequence of SEQ ID NO: 323 (ISSGLLSGRSDQP). In some embodiments, the cleavable peptide consists of an amino acid sequence of SEQ ID NO: 324 (VPLSLYSG).

Cleavable peptides having an amino acid sequence as shown in SEQ ID NOs: 118 or 119 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-2 cytokine as

disclosed anywhere herein, any systemic side effects of the administered IL-2 cytokine or functional fragment thereof may be 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 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.

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.

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 formula 12:

N' **SD1-CP-SD2** C'

(12)

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

In some embodiments, the cleavable linker comprises formula 12:

N' **SD1-CP-SD2** C'

(12)

In some embodiments, the first polypeptide chain comprises formula 7 and the second polypeptide chain comprises formula 13 below:

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

(7)

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

(13)

In some embodiments, the first polypeptide chain comprises formula 14 and the second polypeptide chain comprises formula 10 below:

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

(14)

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

(10)

In some embodiments, SD1 consists of a glycine (G).

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 4 and 9 amino acids in length. In some embodiments, the first spacer domain (SD1) is between 3 and 6 amino acids in length.

In some embodiments, SD1 comprises SEQ ID NO: 32, 33, 34, 35, 36 or 37. In some embodiments, SD1 comprises SEQ ID NO: 32, 33, 34, 35, 36, 120, 121, 122, 123 or 124. In some embodiments, SD1 comprises SEQ ID NO: 32, 33, 34, 35, 36, 120, 121, 122, 123, 124, 179 (PSGSSPG) or 185 (SGSPS).

In some embodiments, SD1 consists of SEQ ID NO: 32, 33, 34, 35, 36 or 37. In some embodiments, SD1 consists of SEQ ID NO: 32, 33, 34, 35, 36, 120, 121, 122, 123 or 124. In some embodiments, SD1 consists of SEQ ID NO: 32, 33, 34, 35, 36, 120, 121, 122, 123, 124, 179 (PSGSSPG) or 185 (SGSPS).

SEQ ID NO of SD1	Sequence
32	GGSSPP
33	GSGP
34	GSPG
35	GGSG
36	GPPSGSSPG
37	GPPSGSSP
120	GGPS

121	GSGPS
122	GSSGGP
123	GSP
124	GSGSPS
179	PSGSSPG
185	SGSPS

In some embodiments, the SD2 consists of GP.

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

In some embodiments, the sequence of the C-terminus of SD2 is SEQ ID NO: 29.

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: 29, 30 or 31.

In some embodiments, SD2 consists of SEQ ID NO: 29, 30 or 31.

SEQ ID NO of SD2	Sequence
29	SGP
30	SGGG
31	GSGGG

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

Linker structure	SD1 sequence	SD2 sequence
SD1-CP-SD2	GPPSGSSPG	SGGG
SD1-CP-SD2	GPPSGSSPG	GSGGG
SD1-CP-SD2	GPPSGSSP	SGGG
SD1-CP-SD2	GGSSPP	SGP
SD1-CP-SD2	GSPG	SGP
SD1-CP-SD2	GGSG	SGP
SD1-CP-SD2	GSGP	SGP
SD1-CP-SD2	GGPS	SGP
SD1-CP-SD2	GSGPS	SGP
SD1-CP-SD2	GSSGGP	SGP

SD1-CP-SD2	GSP	SGP
SD1-CP-SD2	GSGSPS	SGP
SD1-CP-SD2	G	SGP

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: 118. 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.

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: 119. 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.

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: 323. 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.

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: 118 and SD2 has an amino acid sequence as shown in SEQ ID NO: 29. 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.

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: 119 and SD2 has an amino acid sequence as shown in SEQ ID NO: 29. 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.

Exemplary cleavable linkers are shown below:

SEQ ID NO of Cleavable linker	Cleavable linker sequence (cleavable peptide shown in bold)
15	GPPSGSSPG**DSGGFMLT**SGGG
16	GPPSGSSPG**VPLSLY**GSGGG
17	GPPSGSSP**MPYDLYHP**SGGG
18	GGSSPP**MPYDLYHP**SGP
19	GSPG**VPLSLY**SGP
20	GGSG**RAAAVKSP**SGP
21	GGSG**HEQLTV**SGP
22	GSGP**DSGGFMLT**SGP
115	GGPS**DLLAVVAAS**SGP
116	GSGPS**DLLAVVAAS**SGP
117	GSSGGP**DLLAVVAAS**SGP
112	GSP**DLLAVVAAS**SGP
113	GSPG**DLLAVVAAS**SGP
114	GSGSPS**DLLAVVAAS**SGP
175	GI**SSGLLSGRSDQP**SGP
177	G**ISSGLLSGRS**SGP

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

In some embodiments, the cleavable linker comprises SEQ ID NO: 17.

In some embodiments, the cleavable linker comprises SEQ ID NO: 19 and the non-cleavable linker comprises SEQ ID NO: 14.

In some embodiments, the cleavable linker comprises SEQ ID NO: 115 and the non-cleavable linker comprises SEQ ID NO: 14.

In some embodiments, the cleavable linker comprises SEQ ID NO: 116 and the non-cleavable linker comprises SEQ ID NO: 14.

In some embodiments, the cleavable linker comprises SEQ ID NO: 117 and the non-cleavable linker comprises SEQ ID NO: 14.

In some embodiments, the cleavable linker comprises SEQ ID NO: 17 and the non-cleavable linker comprises SEQ ID NO: 23.

In some embodiments, the cleavable linker comprises SEQ ID NO: 112 and the non-cleavable linker comprises SEQ ID NO: 23.

In some embodiments, the cleavable linker comprises SEQ ID NO: 113 and the non-cleavable linker comprises SEQ ID NO: 23.

In some embodiments, the cleavable linker comprises SEQ ID NO: 114 and the non-cleavable linker comprises SEQ ID NO: 23.

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 comprises SEQ ID NO: 115 and the non-cleavable linker comprises SEQ ID NO: 14. In some embodiments, the cleavable linker comprises SEQ ID NO: 116 and the non-cleavable linker comprises SEQ ID NO: 14. In some embodiments, the cleavable linker comprises SEQ ID NO: 117 and the non-cleavable linker comprises SEQ ID NO: 14.

In some embodiments, wherein the first linker comprises a proteolytically cleavable peptide such that the first linker is a proteolytically cleavable linker and the second linker does not comprise a proteolytically cleavable peptide such that the second linker is a non- proteolytically cleavable linker, the cleavable linker comprises SEQ ID NO: 112 and the non-cleavable linker comprises SEQ ID NO: 23. In some embodiments, the cleavable linker comprises SEQ ID NO: 113 and the non-cleavable linker comprises SEQ ID NO: 23. In some embodiments, the cleavable linker comprises SEQ ID NO: 114 and the non-cleavable linker comprises SEQ ID NO: 23.

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, then the proteolytically cleavable peptide linker does not have the amino acid sequence GGSGISSGLLSGRSSSGP or GISSGLLSGRSSSGP.

In some embodiments, the proteolytically cleavable linker comprises a cleavable peptide consisting of an amino acid sequence of SEQ ID NO: 118. (DLLA*VVAAS).

In some embodiments, the proteolytically cleavable linker comprises a cleavable peptide consisting of an amino acid sequence of SEQ ID NO: 119. (ISSGLL*SGRS).

Linker combinations disclosed in exemplary AK molecules may be used with any IL-2 cytokine or fragment thereof disclosed herein. Linker combinations disclosed in exemplary AK molecules may be used with any masking moiety disclosed herein. Linker combinations disclosed in exemplary AK molecules may be used with any half-life extension domains. In other words, the linker disclosed in exemplary AK molecules may be used in combinations with any IL-2 cytokine or fragment thereof disclosed herein, masking moiety disclosed herein and/or half-life extension domain disclosed herein.

1.4 Half-life Extension Domains

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 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 fragments.

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

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.

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).

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.

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
GLYSLSSVVTVPSSSLGTQTYICNVNHKPSNTKVDKKVEPKSC**DKTHTCPPCPAPELLGG**
PSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYN
STYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSRDE
LTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRW
QQGNVFSCSVMHEALHNHYTQKSLSLSPGK
(SEQ ID NO: 6)

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: 6 (the 'parent sequence'), such that the first and second half-life extension domains each comprise SEQ ID NO: 6 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: 6 shown in bold above, optionally with one or more amino acid modifications, i.e.:

DKTHTCPPCPAPELLGG

PSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYN
STYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSRDE
LTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRW
QQGNVFSCSVMHEALHNHYTQKSLSLSPG
(SEQ ID NO: 7)

In some embodiments, the first and second half-life extension domains comprise SEQ ID NO: 7 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: 7 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 8 and 11 shown below:

First half-life extension domain (Y349C; T366S; L38A; and Y407V) SEQ ID NO 8:

DKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHE
DPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWL
NGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVCTLPPSRDELTKNQ
VSLSCAVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLVSKL
TVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPG

Second half-life extension domain (S354C and T366W) SEQ ID NO 11:

DKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSH
EDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDW
LNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPCRDELTK
NQVSLWCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFL
YSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPG

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 9:

DKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHE
DPEVKFNWYVDGVEVHNAKTKPREEQYASTYRVVSVLTVLHQDWL
NGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVCTLPPSRDELTKNQ

VSLSCAVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLVSKL
TVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPG

Second half-life extension domain (S354C, T366W and N297A) SEQ ID NO 12:

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 10:

DKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMASRTPEVTCVVVDVSHEDP
EVKFNWYVDGVEVHNAKTKPREEQYASTYRVVSVLTVLHQDWLNGKE
YKCKVSNKALPAPIEKTISKAKGQPREPQVCTLPPSRDELTKNQVSLSCA
VKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLVSKLTVDKSRW
QQGNVFSCSVMHEALHNHYTQKSLSLSPG

Second half-life extension domain (S354C, T366W, N297A and I253A) SEQ ID NO 13:

DKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMASRTPEVTCVVVDVSHEDP
EVKFNWYVDGVEVHNAKTKPREEQYASTYRVVSVLTVLHQDWLNGKEY
KCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPCRDELTKNQVSLWCLVK
GFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRW
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: 7, 8, 9 and 10.

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: 7, 11, 12 and 13.

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: 7, 8, 9 and 10. 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: 7, 11, 12 and 13. 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.

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 Q3111; 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).

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-2 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-2 cytokine or functional fragment thereof comprises the amino acid sequence of SEQ ID NO: 3 and the masking moiety comprises the amino acid sequence of SEQ ID NO: 4.

In some embodiments, the IL-2 cytokine or functional fragment thereof comprises the amino acid sequence of SEQ ID NO: 3 and the masking moiety comprises the amino acid sequence of SEQ ID NO: 5.

In some embodiments, the IL-2 cytokine or functional fragment thereof comprises the amino acid sequence of SEQ ID NO: 3 and the first half-life extension domain comprises SEQ ID NO: 9 (Y349C; T366S; L38A; Y407V; and N297A) and the second half-life extension domain comprises SEQ ID NO 12 (S354C, T366W and N297A).

In some embodiments, the IL-2 cytokine or functional fragment thereof comprises the amino acid sequence of SEQ ID NO: 3 and the first half-life extension domain comprises SEQ ID NO: 10 (Y349C; T366S; L38A; Y407V, N297A and I253A) and the second half-life extension domain comprises SEQ ID NO: 13 (S354C, T366W, N297A and I253A).

In some embodiments, the IL-2 cytokine or functional fragment thereof comprises the amino acid sequence of SEQ ID NO: 3 and the non-cleavable linker comprises an amino acid sequence as shown in SEQ ID NO: 14.

In some embodiments, the masking moiety comprises the amino acid sequence of SEQ ID NO: 4 and the first half-life extension domain comprises SEQ ID NO: 9 (Y349C; T366S; L38A; Y407V; and N297A) and the second half-life extension domain comprises SEQ ID NO 12 (S354C, T366W and N297A).

In some embodiments, the masking moiety comprises the amino acid sequence of SEQ ID NO: 5 and the first half-life extension domain comprises SEQ ID NO: 9 (Y349C; T366S; L38A; Y407V; and N297A) and the second half-life extension domain comprises SEQ ID NO 12 (S354C, T366W and N297A).

In some embodiments, the masking moiety comprises the amino acid sequence of SEQ ID NO: 4 and the first half-life extension domain comprises SEQ ID NO: 10 (Y349C; T366S; L38A; Y407V, N297A and I253A) and the second half-life extension domain comprises SEQ ID NO: 13 (S354C, T366W, N297A and I253A).

In some embodiments, the masking moiety comprises the amino acid sequence of SEQ ID NO: 5 and the first half-life extension domain comprises SEQ ID NO: 10 (Y349C; T366S; L38A; Y407V, N297A and I253A) and the second half-life extension domain comprises SEQ ID NO: 13 (S354C, T366W, N297A and I253A).

In some embodiments, the masking moiety comprises the amino acid sequence of SEQ ID NO: 4 and the non-cleavable linker comprises an amino acid sequence as shown in SEQ ID NO: 14.

In some embodiments, the masking moiety comprises the amino acid sequence of SEQ ID NO: 5 and the non-cleavable linker comprises an amino acid sequence as shown in SEQ ID NO: 14.

In some embodiments, the first half-life extension domain comprises SEQ ID NO: 9 (Y349C; T366S; L38A; Y407V; and N297A) and the second half-life extension domain comprises SEQ ID NO 12 (S354C,

T366W and N297A) and the non-cleavable linker comprises an amino acid sequence as shown in SEQ ID NO: 14.

In some embodiments, the first half-life extension domain comprises SEQ ID NO: 9 (Y349C; T366S; L38A; Y407V; and N297A) and the second half-life extension domain comprises SEQ ID NO 12 (S354C, T366W and N297A) and the non-cleavable linker comprises an amino acid sequence as shown in SEQ ID NO: 14.

In some embodiments, the IL-2 cytokine or functional fragment thereof comprises the amino acid sequence of SEQ ID NO: 3 and the masking moiety comprises the amino acid sequence of SEQ ID NO: 4 and the first half-life extension domain comprises SEQ ID NO: 9 (Y349C; T366S; L38A; Y407V; and N297A) and the second half-life extension domain comprises SEQ ID NO 12 (S354C, T366W and N297A).

In some embodiments, the IL-2 cytokine or functional fragment thereof comprises the amino acid sequence of SEQ ID NO: 3 and the masking moiety comprises the amino acid sequence of SEQ ID NO: 5 and the first half-life extension domain comprises SEQ ID NO: 9 (Y349C; T366S; L38A; Y407V; and N297A) and the second half-life extension domain comprises SEQ ID NO 12 (S354C, T366W and N297A).

In some embodiments, the IL-2 cytokine or functional fragment thereof comprises the amino acid sequence of SEQ ID NO: 3 and the masking moiety comprises the amino acid sequence of SEQ ID NO: 4 and the first half-life extension domain comprises SEQ ID NO: 10 (Y349C; T366S; L38A; Y407V, N297A and I253A) and the second half-life extension domain comprises SEQ ID NO: 13 (S354C, T366W, N297A and I253A)

In some embodiments, the IL-2 cytokine or functional fragment thereof comprises the amino acid sequence of SEQ ID NO: 3 and the masking moiety comprises the amino acid sequence of SEQ ID NO: 5 and the first half-life extension domain comprises SEQ ID NO: 10 (Y349C; T366S; L38A; Y407V, N297A and I253A) and the second half-life extension domain comprises SEQ ID NO: 13 (S354C, T366W, N297A and I253A).

In some embodiments, the IL-2 cytokine or functional fragment thereof comprises the amino acid sequence of SEQ ID NO: 3 and the masking moiety comprises the amino acid sequence of SEQ ID NO: 4 and the non-cleavable linker comprises an amino acid sequence as shown in SEQ ID NO: 14.

In some embodiments, the IL-2 cytokine or functional fragment thereof comprises the amino acid sequence of SEQ ID NO: 3 and the masking moiety comprises the amino acid sequence of SEQ ID NO: 5 and the non-cleavable linker comprises an amino acid sequence as shown in SEQ ID NO: 14.

In some embodiments, the masking moiety comprises the amino acid sequence of SEQ ID NO: 4 and the first half-life extension domain comprises SEQ ID NO: 9 (Y349C; T366S; L38A; Y407V; and N297A) and the second half-life extension domain comprises SEQ ID NO 12 (S354C, T366W and N297A) and the non-cleavable linker comprises an amino acid sequence as shown in SEQ ID NO: 14.

In some embodiments, the masking moiety comprises the amino acid sequence of SEQ ID NO: 5 and the first half-life extension domain comprises SEQ ID NO: 9 (Y349C; T366S; L38A; Y407V; and N297A) and the second half-life extension domain comprises SEQ ID NO 12 (S354C, T366W and N297A) and the non-cleavable linker comprises an amino acid sequence as shown in SEQ ID NO: 14.

In some embodiments, the masking moiety comprises the amino acid sequence of SEQ ID NO: 4 and the first half-life extension domain comprises SEQ ID NO: 10 (Y349C; T366S; L38A; Y407V, N297A and I253A) and the second half-life extension domain comprises SEQ ID NO: 13 (S354C, T366W, N297A and I253A) and the non-cleavable linker comprises an amino acid sequence as shown in SEQ ID NO: 14.

In some embodiments, the masking moiety comprises the amino acid sequence of SEQ ID NO: 5 and the first half-life extension domain comprises SEQ ID NO: 10 (Y349C; T366S; L38A; Y407V, N297A and I253A) and the second half-life extension domain comprises SEQ ID NO: 13 (S354C, T366W, N297A and I253A) and the non-cleavable linker comprises an amino acid sequence as shown in SEQ ID NO: 14.

In some embodiments, the IL-2 cytokine or functional fragment thereof comprises the amino acid sequence of SEQ ID NO: 3 and the masking moiety comprises the amino acid sequence of SEQ ID NO: 4 and the first half-life extension domain comprises SEQ ID NO: 9 (Y349C; T366S; L38A; Y407V; and N297A) and the second half-life extension domain comprises SEQ ID NO 12 (S354C, T366W and N297A) and the non-cleavable linker comprises an amino acid sequence as shown in SEQ ID NO: 14.

In some embodiments, the IL-2 cytokine or functional fragment thereof comprises the amino acid sequence of SEQ ID NO: 3 and the masking moiety comprises the amino acid sequence of SEQ ID NO: 5 and the first half-life extension domain comprises SEQ ID NO: 9 (Y349C; T366S; L38A; Y407V; and N297A) and the second half-life extension domain comprises SEQ ID NO 12 (S354C, T366W and N297A) and the non-cleavable linker comprises an amino acid sequence as shown in SEQ ID NO: 14.

In some embodiments, the IL-2 cytokine or functional fragment thereof comprises the amino acid sequence of SEQ ID NO: 3 and the masking moiety comprises the amino acid sequence of SEQ ID NO: 4 and the first half-life extension domain comprises SEQ ID NO: 10 (Y349C; T366S; L38A; Y407V, N297A and

I253A) and the second half-life extension domain comprises SEQ ID NO: 13 (S354C, T366W, N297A and I253A) and the non-cleavable linker comprises an amino acid sequence as shown in SEQ ID NO: 14.

In some embodiments, the IL-2 cytokine or functional fragment thereof comprises the amino acid sequence of SEQ ID NO: 3 and the masking moiety comprises the amino acid sequence of SEQ ID NO: 5 and the first half-life extension domain comprises SEQ ID NO: 10 (Y349C; T366S; L38A; Y407V, N297A and I253A) and the second half-life extension domain comprises SEQ ID NO: 13 (S354C, T366W, N297A and I253A) and the non-cleavable linker comprises an amino acid sequence as shown in SEQ ID NO: 14.

In some embodiments, the IL-2 cytokine or functional fragment thereof comprises the amino acid sequence of SEQ ID NO: 3 and the masking moiety comprises the amino acid sequence of SEQ ID NO: 4 and the first half-life extension domain comprises SEQ ID NO: 9 (Y349C; T366S; L38A; Y407V; and N297A) and the second half-life extension domain comprises SEQ ID NO 12 (S354C, T366W and N297A) and the non-cleavable linker comprises an amino acid sequence as shown in SEQ ID NO: 23.

2. CLEAVAGE PRODUCT

Provided herein is a cleavage product of a 'heterodimeric' masked IL-2 cytokines described herein.

The masked IL-2 cytokines described herein comprise a cleavable linker. Upon proteolytic cleavage of the cleavable linker at the cleavage site, a cleavage product comprising the IL-2 cytokine or functional fragment thereof is formed. The IL-2 cytokine or functional fragment thereof in the cleavage product is activated since it is no longer masked by the masking moiety. The IL-2 cytokine or functional fragment thereof in the cleavage product is therefore capable of binding to the target protein.

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-2 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.

Provided herein is a cleavage product capable of binding to IL-2R, the cleavage product comprising an IL-2 cytokine or functional fragment thereof, preparable by proteolytic cleavage of the cleavable peptide in a masked IL-2 cytokine as described anywhere herein.

Also provided herein is a cleavage product of a masked IL-2 cytokine, where the cleavage product is capable of binding to IL-2R, the cleavage product comprising an IL-2 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-2 cytokine, where each cleavage product within the distribution of cleavage products (i) is capable of binding to IL-2R and (ii) comprises an IL-2 cytokine or functional fragment thereof as defined anywhere herein.

Also provided herein is a cleavage product of a masked IL-2 cytokine, where the cleavage product is capable of binding to IL-2R, the cleavage product comprising a polypeptide comprising formula 3:

PCP-SD-C

(3)

wherein PCP is a portion of a proteolytically cleavable peptide; SD is a spacer domain; and C is an IL-2 cytokine or functional fragment thereof.

In some embodiments, the cleavage product has an amino acid sequence with at least 90% homology to the mature IL-2 of SEQ ID NO: 2.

Further provided herein is a cleavage product of a masked IL-2 cytokine, where the cleavage product is capable of binding to IL-2R, 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

(5)

wherein HL2 is a second half-life extension domain; L2 is a non-cleavable linker; and C is an IL-2 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-2 cytokine, where each cleavage product within the distribution of cleavage products (i) is capable of binding to IL-2R 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

(5)

wherein HL2 is a second half-life extension domain; L2 is a non-cleavable linker; and C is an IL-2 cytokine or functional fragment thereof; and wherein the first half-life extension domain is associated with the second half-life extension domain.

Further provided herein is a cleavage product of a masked IL-2 cytokine, where the cleavage product is capable of binding to IL-2R, the cleavage product comprising a protein heterodimer comprising:

a) a first polypeptide chain comprising a polypeptide comprising formula 4:

HL1-SD-PCP

(4)

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 formula 5:

HL2-L2-C

(5)

wherein HL2 is a second half-life extension domain; L2 is a non-cleavable linker; and C is an IL-2 cytokine or functional fragment thereof; and

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-2 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-2 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:

SEQ ID NO:	Sequence (* indicates a cleavage site within the cleavable peptide)
24	MPYD*LYHP
25	DSGG*FMLT
26	HEQ*LTV
27	RAAA*VKSP
28	VPLS*LY
118	DLLA*VVAAS
119	ISSGLL*SG*RS

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.

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 NOs: 52, 53, 54, 55 and 56. 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 NOs: 52, 53, 54, 55, 56 and 137. 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 of SEQ ID NO: 52. 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 of SEQ

ID NO: 53. 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 of SEQ ID NO: 54. 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 of SEQ ID NO: 55. 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 of SEQ ID NO: 56. 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 of SEQ ID NO: 137.

In some embodiments, the cleavage product has an amino acid sequence selected from the group consisting of SEQ ID NOs: 52, 53, 54, 55 and 56. In some embodiments, the cleavage product has an amino acid sequence selected from the group consisting of SEQ ID NOs: 52, 53, 54, 55, 56 and 137. In some embodiments, the cleavage product has an amino acid sequence of SEQ ID NO: 52. In some embodiments, the cleavage product has an amino acid sequence of SEQ ID NO: 53. In some embodiments, the cleavage product has an amino acid sequence of SEQ ID NO: 54. In some embodiments, the cleavage product has an amino acid sequence of SEQ ID NO: 55. In some embodiments, the cleavage product has an amino acid sequence of SEQ ID NO: 56. In some embodiments, the cleavage product has an amino acid sequence of SEQ ID NO: 137.

% homology of the amino acid sequence of these cleavage products to mature IL-2 of SEQ ID NO: 2 is shown in table 1 below:

Table 1

Description	% Homology to mature IL-2 SEQ ID NO: 2
AK168 Cleavage Product ("AK168CP")	91.4
AK191 Cleavage Product 1("AK191CP")	91.4
AK197 Cleavage Product ("AK197CP")	92.1
AK203 Cleavage Product 3("AK203CP")	91.4
AK209 Cleavage Product ("AK209CP)	92.8
mature IL-2 (SEQ ID NO: 2)	100

In some embodiments, the cleavage product comprises a first polypeptide chain having 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 of SEQ ID NO: 136 and a second polypeptide chain having 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 of SEQ ID NO: 135. In some embodiments, the cleavage product comprises a first polypeptide chain having 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 of SEQ ID NO: 139 and a second polypeptide chain having 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 of SEQ ID NO: 138. In some embodiments, the cleavage product comprises a first polypeptide chain having 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 of SEQ ID NO: 141 and a second polypeptide chain having 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 of SEQ ID NO: 140. In some embodiments, the cleavage product comprises a first polypeptide chain having 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 of SEQ ID NO: 143 and a second polypeptide chain having 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 of SEQ ID NO: 142.

In some embodiments, the cleavage product has a first polypeptide chain having an amino acid sequence of SEQ ID NO: 136 and a second polypeptide chain having an amino acid sequence of SEQ ID NO: 135. In some embodiments, the cleavage product has a first polypeptide chain having an amino acid sequence of SEQ ID NO: 139 and a second polypeptide chain having an amino acid sequence of SEQ ID NO: 138. In some embodiments, the cleavage product has a first polypeptide chain having an amino acid sequence of SEQ ID NO: 141 and a second polypeptide chain having an amino acid sequence of SEQ ID NO: 140. In some embodiments, the cleavage product has a first polypeptide chain having an amino acid sequence of SEQ ID NO: 143 and a second polypeptide chain having an amino acid sequence of SEQ ID NO: 142.

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-2 cytokine to the IL-2 cytokine receptor (e.g., IL-2R or a component thereof, such as IL-2Rα, IL-2Rβ, IL-2Rγ, or combinations thereof), 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. In the context of IL-2 cytokine binding, the target protein could be IL-2R (comprising the IL-2Rα, IL-2Rβ, and IL-2Rγ chains), the IL-2Rα chain, the IL-2Rβ chain, or the IL-2Rα/β dimeric complex. 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-2R)/cytokine (e.g., IL-2) 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, the target protein is IL-2R (comprising the IL-2Rα, IL-2Rβ, and IL-2Rγ chains). In some embodiments, the target protein is IL-2Rα. In some embodiments, the target protein is IL-2Rβ. In some embodiments, the target protein is the IL-2Rα/β dimeric complex.

In some embodiments, a masked cytokine provided herein that does not comprise a cleavable peptide in the linker has a dissociation constant (Kd) of $\leq$ 1M, $\leq$150 nM, $\leq$100 nM, $\leq$50 nM, $\leq$ 10 nM, $\leq$1 nM, $\leq$0.1 nM, $\leq$0.01 nM, or $\leq$ 0.001 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$1M, $\leq$150 nM, $\leq$ 100 nM, $\leq$50 nM, $\leq$10 nM, $\leq$1 nM, $\leq$0.1 nM, $\leq$ 0.01 nM, or 0.001 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$1M, $\leq$150 nM, $\leq$ 100 nM, $\leq$50 nM, $\leq$ 10 nM, $\leq$1 nM, $\leq$0.1 nM, $\leq$0.01nM, or $\leq$0.001 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 (Kd) of $\geq$ 500M, $\geq$ 250M, $\geq$ 200M, $\geq$ 150M, $\geq$ 100M, $\geq$ 50M, $\geq$ 10M, $\geq$ 1M, $\geq$ 500 nM, $\geq$ 250 nM, $\geq$ 150 nM, $\geq$ 100 nM, $\geq$ 50 nM, $\geq$ 10 nM, $\geq$ 1 nM, $\geq$ 0.1 nM, $\geq$ 0.01 nM, or $\geq$ 0.001 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 (Kd) 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-2 polypeptide, the occlusion ratio refers to the ratio of (a) a maximum detected level of target protein (e.g., IL-2R protein) binding to the masked IL-2 polypeptide in the presence of at least one protease capable of cleaving the cleavable peptide of the masked IL-2 polypeptide to (b) a minimum detected level of target protein (e.g., IL-2R protein) binding to the masked IL-2 polypeptide in the absence of the protease. Thus, the occlusion ratio for a masked cytokine can be calculated by dividing the EC50 of the masked cytokine pre-cleavage by the EC50 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-2 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.

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 (Kd) of ≥ 500M, ≥ 250M, ≥ 200M, ≥ 150M, ≥ 100M, ≥ 50M, ≥ 10M, ≥ 1M, ≥ 500 nM, ≥ 250 nM, ≥ 150 nM, ≥ 100 nM, ≥ 50 nM, ≥ 10 nM, ≥ 1 nM, ≥ 0.1 nM, ≥ 0.01 nM, or ≥ 0.001 nM.

4. MASKED CYTOKINES WITH VARIANT MASKING MOIETIES

Provided herein, are masked cytokines with variant masking moieties.

In some embodiments, the masked IL-2 cytokine comprises a masking moiety and an IL-2 cytokine or functional fragment thereof, wherein the masking moiety masks the IL-2 cytokine or functional fragment thereof thereby reducing or preventing binding of the IL-cytokine or functional fragment thereof to its cognate receptor, and wherein a proteolytically cleavable peptide is present between the IL-2 fragment or functional fragment thereof and masking moiety.

An IL-2Rβ polypeptide, or a functional fragment thereof, is provided herein, where the IL-2Rβ polypeptide has an amino acid substitution at position C122.

An IL-2Rβ polypeptide, or a functional fragment thereof, is provided herein, where the IL-2Rβ polypeptide has amino acid substitution C122S.

An IL-2Rβ polypeptide, or a functional fragment thereof, is provided herein, where the IL-2Rβ polypeptide has an amino acid substitution at position C122 as compared to IL-2Rβ of SEQ ID NO: 4.

An IL-2Rβ polypeptide, or a functional fragment thereof, is provided herein, where the IL-2Rβ polypeptide has amino acid substitution C122S as compared to IL-2Rβ of SEQ ID NO: 4.

An IL-2Rβ polypeptide is provided herein, comprising the amino acid sequence of SEQ ID NO: 4 with a C122 mutation.

An IL-2Rβ polypeptide is provided herein, comprising the amino acid sequence of SEQ ID NO: 11 with a C122S mutation.

An IL-2Rβ polypeptide, or a functional fragment thereof, is provided herein, where the IL-2Rβ polypeptide has an amino acid substitution at position C168.

An IL-2Rβ polypeptide, or a functional fragment thereof, is provided herein, where the IL-2Rβ polypeptide has amino acid substitution C168S.

An IL-2Rβ polypeptide, or a functional fragment thereof, is provided herein, where the IL-2Rβ polypeptide has an amino acid substitution at position C168 as compared to IL-2Rβ of SEQ ID NO: 4.

An IL-2Rβ polypeptide, or a functional fragment thereof, is provided herein, where the IL-2Rβ polypeptide has amino acid substitution C168S as compared to IL-2Rβ of SEQ ID NO: 4.

An IL-2Rβ polypeptide is provided herein, comprising the amino acid sequence of SEQ ID NO: 4 with a C168 mutation.

An IL-2Rβ polypeptide is provided herein, comprising the amino acid sequence of SEQ ID NO: 4 with a C68S mutation.

An IL-2Rβ polypeptide, or a functional fragment thereof, is provided herein, where the IL-2Rβ polypeptide has amino acid substitutions at positions C122 and C168.

An IL-2Rβ polypeptide, or a functional fragment thereof, is provided herein, where the IL-2Rβ polypeptide has amino acid substitutions C122S and C168S.

An IL-2Rβ polypeptide, or a functional fragment thereof, is provided herein, where the IL-2Rβ polypeptide has amino acid substitutions at positions C122 and C168 as compared to IL-2Rβ of SEQ ID NO: 4.

An IL-2Rβ polypeptide, or a functional fragment thereof, is provided herein, where the IL-2Rβ polypeptide has amino acid substitutions C122S and C168S as compared to IL-2Rβ of SEQ ID NO: 4.

An IL-2Rβ polypeptide, or a functional fragment thereof, is provided herein, where the IL-2Rβ polypeptide comprises an amino acid of SEQ ID NO: 5.

A masked cytokine comprising a masking moiety and an IL-2 cytokine or functional fragment thereof, is provided herein, wherein the masking moiety masks the IL-2 cytokine or functional fragment thereof thereby reducing or preventing binding of the IL-2 cytokine or functional fragment thereof to its cognate receptor, and where a proteolytically cleavable peptide is present between the IL-2 cytokine or functional fragment thereof and the masking moiety, and the masking moiety is an IL-2Rβ polypeptide, or a functional fragment thereof, as defined anywhere herein.

4.1 'Heterodimeric' Masked Cytokines

In some embodiments, the masked IL-2 cytokine comprises a masking moiety in a first polypeptide chain and an IL-2 cytokine or functional fragment thereof in a second polypeptide chain. In some embodiments, the masked IL-2 cytokine is as described anywhere herein. In some embodiments, the masked IL-2 cytokine comprises formulae 6 (first polypeptide chain) and 5 (second polypeptide chain) below:

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

(6)

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

(5)

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-2 cytokine or functional fragment thereof, wherein at least the first linker or the second linker comprises a proteolytically cleavable peptide. In some embodiments, the first half life extension domain, first linker, masking moiety, second half life extension domain, second linker, and IL-2 cytokine or functional fragment thereof are as described anywhere herein.

Cleavable peptides having an amino acid sequence as shown in SEQ ID NOs: 118 or 119 have been found to demonstrate very specific cleavage in the tumor cell environment compared to non-tumor cell environment. These cleavable peptides may therefore be used advantageously in combination with the variant masking moieties disclosed herein.

4.2 'Linear' Masked Cytokines

In some embodiments, the masked IL-2 cytokine comprises a masking moiety and an IL-2 cytokine or functional fragment thereof linked in a single polypeptide chain. In some embodiments, the masked IL-2 cytokine comprises a polypeptide chain comprising formula 1:

N' **HL-L2-C-L1-MM** C'

(1)

where HL is the half life extension domain, L1 is the first linker, MM is the masking moiety, L2 is the second linker, and C is the IL-2 cytokine or functional fragment thereof, wherein at least the first linker comprises a proteolytically cleavable peptide.

In some embodiments, the masked IL-2 cytokine comprises a polypeptide chain comprising formula 2:

N' **HL-L2-MM-L1-C** C'

(2)

where HL is the half life extension domain, L1 is the first linker, MM is the masking moiety, L2 is the second linker, and C is the IL-2 cytokine or functional fragment thereof, wherein at least the first linker comprises a proteolytically cleavable peptide. In some embodiments, the first linker is a cleavable linker as described anywhere herein. In some embodiments, the second linker is a non-cleavable linker as described anywhere herein. In some embodiments, the IL-2 cytokine or functional fragment thereof is as described anywhere herein. . In some embodiments, the half life extension domain (HL) comprises an Fc region of an antibody (i.e. the C-terminal region of an immunoglobulin heavy chain) or a fragment thereof comprising dimerized Fc domains (HL1-HL2). Although the boundaries of the Fc region of an immunoglobulin heavy chain might vary, the human IgG heavy-chain Fc region is usually defined to stretch from an amino acid residue at position Cys226, or from Pro230, to the carboxyl-terminus thereof. In some embodiments, the dimerized Fc domains of an antibody (HL1-HL2) comprises a first half life extension domain and a second half life extension domain as described anywhere herein, where 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, HL2 is a component of the polypeptide chain and HL1 is dimerized to HL2.

Cleavable peptides having an amino acid sequence as shown in SEQ ID NOs: 118 or 119 have been found to demonstrate very specific cleavage in the tumor cell environment compared to non-tumor cell environment.

In some embodiments, HL2 is a component of the polypeptide chain and HL1 is dimerized thereto such that:

a first polypeptide chain comprises:

N' **HL1** C'

and a second polypeptide chain comprises:

N' **HL2-L2-MM-L1-C** C'

4.3 Variant masking moieties

In some embodiments, the masking moiety is as described anywhere herein. In some embodiments, the masking moiety comprises IL-2Rβ or a fragment, portion or variant thereof. In some embodiments, the masking moiety comprises the amino acid sequence of SEQ ID NO: 4. 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: 4. In some embodiments, the masking moiety comprises an amino acid sequence having the amino acid sequence of SEQ ID NO: 4 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: 4 with one or two amino acid substitutions. In some embodiments, the IL-2Rβ or a fragment, portion or variant thereof has mutation C122S at amino acid position 122 as compared to IL-2Rβ of SEQ ID NO: 4. In some embodiments, the masking moiety comprises an amino acid sequence of SEQ ID NO: 4 with a C122S mutation. In some embodiments, the IL-2Rβ or a fragment, portion or variant thereof has mutation C168S at amino acid position 168 as compared to IL-2Rβ of SEQ ID NO: 4. In some embodiments, the masking moiety comprises an amino acid sequence of SEQ ID NO: 4 with a C168S mutation. In some embodiments, the IL-2Rβ or a fragment, portion or variant thereof has mutations C122S and C168S as compared to IL-2Rβ of SEQ ID NO: 4. In some embodiments, the masking moiety comprises an amino acid sequence of SEQ ID NO: 5.

5. MASKED CYTOKINES WITH VARIANT HALF LIFE EXTENSION DOMAINS

Provided herein, are masked cytokines with variant half life extension domains.

In some embodiments, the masked IL-2 cytokine comprises a masking moiety and an IL-2 cytokine or functional fragment thereof, wherein the masking moiety masks the IL-2 cytokine or functional fragment thereof thereby reducing or preventing binding of the IL-cytokine or functional fragment thereof to its cognate receptor, and wherein a proteolytically cleavable peptide is present between the IL-2 fragment or functional fragment thereof and masking moiety.

An IgG1 Fc domain or fragment thereof is provided herein comprising the amino acid substitution I253A, numbered according to the Kabat EU numbering system.

An IgG1 Fc domain or fragment thereof is provided herein comprising the amino acid substitutions N297A and I253A, numbered according to the Kabat EU numbering system.

A dimer comprising a first polypeptide sequence comprising an IgG1 Fc domain or fragment thereof is provided herein comprising the amino acid substitution I253A and a second polypeptide sequence comprising an IgG1 Fc domain or fragment thereof comprising the amino acid substitution I253A.

A dimer comprising a first polypeptide sequence comprising an IgG1 Fc domain or fragment thereof is provided herein comprising the amino acid substitutions N297A and I253A and a second polypeptide sequence comprising an IgG1 Fc domain or fragment thereof comprising the amino acid substitutions N297A and I253A.

A dimer is provided herein comprising a first polypeptide sequence comprising SEQ ID NO: 10 and a second polypeptide sequence comprising SEQ ID NO: 13.

A masked cytokine comprising a masking moiety, an IL-2 cytokine or functional fragment thereof, and a half life extension domain is provided herein wherein the masking moiety masks the IL-2 cytokine or functional fragment thereof thereby reducing or preventing binding of the IL-2 cytokine or functional fragment thereof to its cognate receptor, and where a proteolytically cleavable peptide is present between the IL-2 cytokine or functional fragment thereof and the masking moiety, and half life extension domain comprises dimerized IgG1 Fc domains, as defined in anywhere herein.

5.1 'Heterodimeric' Masked Cytokines

In some embodiments, the masked IL-2 cytokine comprises a masking moiety in a first polypeptide chain and an IL-2 cytokine or functional fragment thereof in a second polypeptide chain. In some embodiments, the masked IL-2 cytokine is as described anywhere herein. In some embodiments, the masked IL-2 cytokine comprises formulae 6 (first polypeptide chain) and 5 (second polypeptide chain) below:

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

(6)

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

(5)

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-2 cytokine or functional fragment thereof, wherein at least the first linker or the second linker comprises a proteolytically cleavable peptide. In some embodiments, the first half life extension domain, first linker, masking moiety, second

half life extension domain, second linker, and IL-2 cytokine or functional fragment thereof are as described anywhere herein.

Cleavable peptides having an amino acid sequence as shown in SEQ ID NOs: 118 or 119 have been found to demonstrate very specific cleavage in the tumor cell environment compared to non-tumor cell environment.

5.2 'Linear' Masked Cytokines

In some embodiments, the masked IL-2 cytokine comprises a masking moiety and IL-2 cytokine or functional fragment thereof linked in a single polypeptide chain. In some embodiments, the masked IL-2 cytokine comprises a polypeptide chain comprising formula 1:

N' **HL-L2-C-L1-MM** C'

(1)

where HL is the half life extension domain, L1 is the first linker, MM is the masking moiety, L2 is the second linker, and C is the IL-2 cytokine or functional fragment thereof, wherein at least the first linker comprises a proteolytically cleavable peptide. In some embodiments, the masked IL-2 cytokine comprises a polypeptide chain comprising formula 2:

N' **HL-L2-MM-L1-C** C'

(2)

where HL is the half life extension domain, L1 is the first linker, MM is the masking moiety, L2 is the second linker, and C is the IL-2 cytokine or functional fragment thereof, wherein at least the first linker comprises a proteolytically cleavable peptide. In some embodiments, the IL-2 cytokine or functional fragment thereof is as described anywhere herein. In some embodiments, the masking moiety is as described anywhere herein. In some embodiments, the half life extension domain (HL) comprises an Fc region of an antibody (i.e. the C-terminal region of an immunoglobulin heavy chain) or a fragment thereof comprising dimerized Fc domains (HL1-HL2). Although the boundaries of the Fc region of an immunoglobulin heavy chain might vary, the human IgG heavy-chain Fc region is usually defined to stretch from an amino acid residue at position Cys226, or from Pro230, to the carboxyl-terminus thereof. In some embodiments, the dimerized Fc domains of an antibody (HL1-HL2) comprises a first half life extension domain and a second half life extension domain as described anywhere herein, where 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, HL2 is a component of the polypeptide chain and HL1 is dimerized to HL2.

In some embodiments, HL2 is a component of the polypeptide chain and HL1 is dimerized thereto such that:

a first polypeptide chain comprises:

N' **HL1** C'

and a second polypeptide chain comprises:

N' **HL2-L2-MM-L1-C** C'

Cleavable peptides having an amino acid sequence as shown in SEQ ID NOs: 118 or 119 have been found to demonstrate very specific cleavage in the tumor cell environment compared to non-tumor cell environment. These cleavable peptides may therefore be used advantageously in combination with the variant half life extension domains disclosed herein.

5.3 Variant half life extension domains

In some embodiments, the first and second half-life extension domains are each an IgG1 Fc domain or fragment thereof. In some embodiments, the first half-life extension domain comprises an IgG1 Fc domain or fragment thereof including the mutation I253A and the second half-life extension domain comprises an IgG1 Fc domain or fragment thereof including the mutation I253A. 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: 6 (the 'parent sequence'), such that the first and second half-life extension domains each comprise SEQ ID NO: 7 or fragment thereof, with one or more amino acid modifications. In some embodiments, the first and second half-life extension domains comprise SEQ ID NO: 7 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: 7 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. 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. 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. 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: 7, 8, 9 and 10. 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: 7, 11, 12 and 13.

6. MASKED IL-2 CYTOKINE PRODUCTION

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

6.1 Antibody Production

Some embodiments of the masked IL-2 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-2 cytokine provided herein. In some embodiments, the masked cytokine is in the form of a dimer produced by two copies of a masked IL-2 cytokine that are associated through disulfide bonds.

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 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.

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.

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 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 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 transchromosomic 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; 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 the masked cytokines described herein according to the guidance provided herein.

4. Bispecific Antibodies

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 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.

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 demonshating 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.

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.

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 IgGl 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 FcyR 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.

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/Q3111, 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 mutations enhance antibody binding to FcRn at low pH but do not change the antibody affinity at neutral pH.

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 potential glycosylation site. O-linked glycosylation refers to the attachment of one of the sugars N-acetylgalactosamine, galactose, or xylose to a hydroxyamino acid, most commonly serine or threonine, although 5-hydroxyproline or 5-hydroxylysine may also be used.

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).

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, 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.

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: 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; W02005/053742; 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 Al, Presta, L; and WO 2004/056312 Al, Adams et al., especially at Example 11), and knockout cell lines, such as alpha-1,6- fucosyltransferase gene, FUT8, knockout CHO cells (Yamane-Ohnuki et al. Biotech. Bioeng.

87: 614 (2004)), and cells overexpressing (31,4-N-acetylglycosminyltransferase III (GnT-III) and Golgi p-mannosidase II (Manll).

In any of the embodiments herein, the masked cytokine can be engineered to improve antibody-dependent cell-mediated cytotoxicity (ADCC) activity. In some embodiments, the masked cytokine may be produced in a cell line having a alphal,6-fucosyltransferase (Fut8) knockout. In some embodiments, the host cells have been modified to have reduced intrinsic alphal ,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 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 Lecl3 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 some embodiments, small interfering RNA (siRNA) against genes relevant to alphal,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-acetylglycosminyltransferase III (GnT-III). In further embodiments, the cell line additionally overexpresses Golgi p-mannosidase II (Manll). 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 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., IgGl, 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).

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 2 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 2, or as further described below in reference to amino acid classes, may be introduced and the products screened.

Table 2:

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), lie (I), Pro (P), Phe (F), Trp (W), Met (M)

(2) uncharged polar: Gly (G), Ser (S), Thr (T), Cys (C), Tyr (Y), Asn (N), Gin (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:

(1) hydrophobic: Norleucine, Met, Ala, Val, Leu, he;

(2) neutral hydrophilic: Cys, Ser, Thr, Asn, Gin;

(3) acidic: Asp, Glu;

(4) basic: His, Lys, Arg;
(5) residues that influence chain orientahon: 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 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 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 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 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 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.

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.

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.

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 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 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 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 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 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 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. 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 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 by reference. See, also, Idusogie et al. J. Immunol. 164: 4178-4184 (2000).

6.2 Masked IL-2 Cytokine-Drug Conjugates

The invention also provides masked IL-2 cytokine-drug conjugates (MCDCs) comprising a masked IL-2 cytokine provided herein, which can be any IL-2 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.

In some embodiments, the one or more drugs conjugated to the masked IL-2 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 Bl); 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 (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 ak, Cancer Res. 53:3336-3342 (1993); and Lode et ak, Cancer Res. 58:2925-2928 (1998)); an anthracycline such as daunomycin or doxorubicin (see Kratz et ak, Current Med. Chem. 13:477-

523 (2006); Jeffrey et ak, Bioorganic & Med. Chem. Letters 16:358-362 (2006); Torgov et ak, Bioconj. Chem. 16:717-721 (2005); Nagy et ak, Proc. Natl. Acad. Sci. USA 97:829-834 (2000); Dubowchik et ak, Bioorg. & Med. Chem. Letters 12:1529-1532 (2002); King et ak, 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.

In another embodiment, the one or more drugs conjugated to the masked IL-2 cytokine includes, but is not limited to, an inhibitor of tubulin polymerization (e.g., maytansinoids and auristatins), DNA damaging agents (e.g., pyrrolobenzodiazepine (PBD) dimers, calicheamicins, duocarmycins and indolinobenzodiazepine dimers), and DNA synthesis inhibitors (e.g., exatecan derivative Dxd).

In another embodiment, a masked IL-2 cytokine-drug conjugate comprises a masked IL-2 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, Phytolaca americana proteins (PAPI, PAPII, and PAP-S), momordica charantia inhibitor, curcin, crotin, sapaonaria officinalis inhibitor, gelonin, mitogellin, restrictocin, phenomycin, enomycin, and the tricothecenes.

In another embodiment, a masked IL-2 cytokine-drug conjugate comprises a masked IL-2 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, Rel86, Rel88, Sml53, B1212, 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 1123, 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.

In some embodiments, a masked IL-2 cytokine-drug conjugate comprises a masked IL-2 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 (TER) agonist.

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][l,4]thiazine-6-carboxamide, or a derivative or variant thereof. *See, e.g.,* Sali et al. (2015) PloS 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-2 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 HC1), 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 l,5-difluoro-2,4-dinitrobenzene). For example, a ricin immunotoxin can be prepared as described in Vitetta et ah, Science 238:1098 (1987). Carbon-14-labeled l-isothiocyanatobenzyl-3- methyldiethylene triaminepentaacetic 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 ah, 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- vinylsulfonejbenzoate) which are commercially available (e.g., from Pierce Biotechnology, Inc., Rockford, IL., U.S.A).

6.3 Vectors, Host Cells, and Recombinant Methods

For recombinant production of a IL-2 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-2 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-2 masked cytokine. In some embodiments, more than one vector is used to encode the masked IL-2 cytokine.

1. Generating Masked IL-2 Cytokines Using Prokaryotic Host Cells

a. Vector Construction

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 PGR techniques, or obtained from other sources. Once obtained, sequences encoding the components of the masked cytokine 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 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.

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 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 ah, U.S. Pat. No. 5,648,237.

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.

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.

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.
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.

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.

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 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 Archaebacteria and Eubacteria, such as Gram-negative or Gram-positive organisms. Examples of useful bacteria include 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. 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 genotypes are known in the art and described in, for example, Bass et ah, 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 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 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 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, 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 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.

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.

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 ah, J. Immunol. Methods (2002), 263:133-147). A 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, 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 horn 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 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 horn 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 ak, U.S. Pat. No. 6,083,715; Georgiou et ak, 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 ak (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 ak (1998), supra; Georgiou et ak, U.S. Pat. No.

5,264,365; Georgiou et ak, U.S. Pat. No. 5,508,192; Kara et ak, 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 aureas 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 hanscription 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

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.

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.

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.

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).

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 mammalian cells are described in U.S. Pat. No. 5,122,464 and U.S. Pat. No. 5,891,693.

d. Promoter Component

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 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 (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 cytomegalovirus is conveniently obtained as a Hindlll 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 ah, Nature 297:598-601 (1982), describing expression of human [3-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.

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 mammalian genes (globin, elastase, albumin, a-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 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 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

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 ah, J. Gen Virol. 36:59 (1977)); baby hamster kidney cells (BHK, ATCC CCL 10); Chinese hamster ovary cells/-DHFR (CHO, Urlaub et ah, 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 cells (Hep G2, HB 8065); murine mammary tumor (MMT 060562, ATCC CCL51); TRI cells (Mather et ah, 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 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),

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 ah, Meth. Enz. 58:44 (1979), Barnes et ah, 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 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 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 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 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 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 IgGl, IgG2, or IgG4 heavy chains (Lindmark et ak, J. Immunol. Methods 62:1-13 (1983)). Protein G is recommended for all murine isotypes and for human y3 (Guss et ak, EMBO J. 5:15671575 (1986)). The matrix to which the affinity ligand is attached may be agarose, but other matrices are available. Mechanically stable matrices such as controlled pore glass or poly(styrenedivinyl)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

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.

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).

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.

7. COMPOSITIONS

In some aspects, also provided herein are compositions comprising any of the IL-2 masked cytokines described herein. In some embodiments, the composition comprises any of the exemplary embodiments of masked IL-2 cytokine described herein. In some embodiments, the composition comprises a dimer of any of the masked IL-2 cytokines described herein. In some embodiments, the composition is a pharmaceutical composition. In some embodiments, the composition comprises a masked IL-2 cytokine and further 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.

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 & Wiklins, Pub., Gennaro Ed., Philadelphia, Pa. 2000). Acceptable carriers, excipients, or stabilizers are nontoxic to recipients at the 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

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 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-cresol, p-cresol, methyl p-hydroxybenzoate, propyl p-hydroxybenzoate, 2-phenoxyethanol, butyl p-hydroxybenzoate, 2-phenylethanol, ethanol, chlorobutanol, thiomerosal, bronopol, benzoic acid, imidurea, chlorohexidine, sodium dehydroacetate, chlorocresol, ethyl p-hydroxybenzoate, and chlorphenesine (3p-chlorphenoxypropane-l ,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) and 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, myoinisitose, myoinisitol, galactose, galactitol, glycerol, cyclitols (e.g., inositol), polyethylene glycol; sulfur containing reducing agents, such as urea, glutathione, thioctic acid, sodium thioglycolate, thioglycerol, a-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.), lauromacrogol 400, polyoxyl 40 stearate, polyoxyethylene hydrogenated castor oil 10, 50 and 60, glycerol monostearate, sucrose fatty acid ester, methyl celluose 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 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 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-2 cytokines described herein can be used alone or in combination with other therapeutic agents such is in the methods described herein. The term "in combination with" encompasses two or more therapeutic agents (e.g., a masked IL-2 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-2 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-2 cytokine and administration of the one or more additional therapeutic agents). In some embodiments, "in combination with" refers to sequential administration, in which case administration of the masked IL-2 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-2 cytokine and administration of the one or more

additional therapeutic agents). Agents contemplated herein include, but are not limited to, a cytotoxic agent, 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 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.

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.

8. METHODS OF TREATMENT

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-2 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 embodiments, methods are provided for treating or preventing disease in a subject comprising administering to the subject an effective amount of any masked IL-2 cytokine described herein or compositions thereof, wherein the masked IL-2 cytokine is activated upon cleavage by an enzyme. In some embodiments, the masked IL-2 cytokine is activated at a tumor microenvironment. The masked IL-2 cytokine is therapeutically active after it has cleaved. Thus, in some embodiments, the active agent is the cleavage product.

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 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-2 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-2 cytokine described herein is about 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-2 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-2 cytokine described herein is about 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-2 cytokine and the next administration of the masked IL-2 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-2 cytokine, wherein the interval between administrations may vary. For example, in some 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-2 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-2 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.

In some embodiments, a masked IL-2 polypeptide described herein is administered at a flat dose. In some embodiments, a masked IL-2 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-2 polypeptide is administered to a subject at a dosage of about 25mg to about 50mg, about 50mg to about 75mg, about 75mg to about l00mg, about l00mg 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 425mt to about 450mg, about 450mg, to about 475mg, or about 475mg to about 500mg per dose.

In some embodiments, a masked IL-2 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-l0mg/kg) of masked IL-2 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-2 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 embodiments, a masked IL-2 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-2 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-2 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 l00mg/kg. Any of the dosing frequencies described above may be used.

A method of treatment contemplated herein is the treatment of a disorder or disease such as cancer with any of the masked IL-2 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., 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-2 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-2 masked cytokine or 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, 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 molecule, a PSMA targeting molecule, an MSFN targeting molecule, an NY-ES0-1 targeting molecule, a B7H3 targeting molecule, a B7H4 targeting molecule, a BCMA targeting molecule, a CD29 targeting molecule, a CD151targeting 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 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-23R 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- 1(3 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 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 GITR agonist, a 0X40 agonist, a VISTA agonist, a CD137 agonist, a LAG3 inhibitor, a TIM3 inhibitor, a TIGIT inhibitor, and an IL-2R inhibitor.

In some embodiments, provided herein is a method of treatment or prevention of a cancer by administration of any masked IL-2 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 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

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 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, 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 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-κB inhibitor. The NF-κB inhibitor can be any agent that inhibits the activity of the NF-κB pathway. In some embodiments, the NF-κB inhibitor is selected from the group consisting of an IKK complex inhibitor, an IκB degradation inhibitor, an NF-κB nuclear translocation inhibitor, a p65 acetylation inhibitor, an NF-κB DNA binding inhibitor, an NF-κB transactivation inhibitor, and a p53 induction inhibitor.

In some embodiments, the IKK complex inhibitor is selected from the group consisting of TPCA-1, NF-κB Activation Inhibitor VI (BOT-64), BMS-345541, amlexanox, SC-514 (GK-01140), IMD-0354, and IKK-16. In some embodiments, the IκB 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-κB 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-κB 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-κB 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-κB inhibitor is selected from the group consisting of TPCA-1, NF-κB 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-2 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.

9. ARTICLES OF MANUFACTURE OR KITS

In another aspect, an article of manufacture or kit is provided which comprises any masked IL-2 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-2 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-2 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.

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.

10. 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.

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-2 polypeptide" optionally includes a combination of two or more such polypeptides, and the like.

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.

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 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 *a* and y chains and four CH domains for p and s 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 (CHI). 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. Sties, Abba I. Terr and Tristram G. Parsolw (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 a, 8, e, y and p, respectively. The y and *a* 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: IgGl, IgG2, IgG3, IgG4, IgAl and IgA2. IgGl 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.

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. 5,641,870, Example 2; Zapata et ah, 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.

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 (CHI). 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 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 CHI 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 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.

"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 subshtuyions as part of the sequence identity. Alignment for purposes of determining percent amino acid sequence identity can be achieved in various ways that are within the skill in the art, for instance, using publicly available computer software such as BLAST, BLAST-2, ALIGN or Megalign (DNASTAR) software. Those skilled in the art can determine appropriate parameters for aligning sequences, including any algorithms needed to achieve maximal alignment over the full length of the sequences being compared. For 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 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: Clq 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 (Kd). 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 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™.

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.

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.

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.

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.

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. 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 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 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, pigs, hamsters, gerbils, mice, ferrets, rats, cats, etc. In some embodiments, the individual or subject is human.

11. EXAMPLES

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.

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-masked versions of the construct were not also tested.

Example 1: Engineering of Masked IL-2 Polypeptides

Masked IL-2 polypeptide constructs 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 #	Cytokine or functional fragment thereof (C)	Linker (L1)	Masking moiety (MM)	Linker (L2)	Half-life extension domain (H)	Structure (N- to C-terminal direction)	Amino Acid Sequence
AK032	SEQ ID NO: 62	-	-	-	SEQ ID NO: 65	H-C	SEQ ID NO: 67
AK035	SEQ ID NO: 3	-	-	-	SEQ ID NO: 65	H-C	SEQ ID NO: 68

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 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 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 #	Masking moiety (MM1)	Linker (L1)	Cytokine or functional fragment thereof (C)	Linker (L2)	Masking moiety (MM2)	Linker (L3)	Half-life extension domain (H)	Structure (N- to C- terminal direction)	Amino Acid Sequence
AK041	SEQ ID NO: 60	SEQ ID NO: 61	SEQ ID NO: 62	SEQ ID NO: 63	SEQ ID NO: 64	SEQ ID NO: 17	SEQ ID NO: 65	H-L1-MM1-L2-C-L3-MM2	SEQ ID NO: 66

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 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 includes the amino acid sequence of SEQ ID NO: 38, and the IL-2 polypeptide or functional fragment thereof is linked to the second half-life extension domain and includes the amino acid sequence of SEQ ID NO: 48, 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 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 includes the amino acid sequence of SEQ ID NO: 48, and the second half-life extension domain includes the amino acid sequence of SEQ ID NO: 79. 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 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 #	Cytokine or functional fragment thereof (C)	Linker (L1)	Masking moiety (MM)	Linker (L2)	Half-life extension domain (H)	Structure (N- to C-terminal direction)	Amino Acid Sequence
AK081	SEQ ID NO: 62	SEQ ID NO: 18	-	-	SEQ ID NO: 12	H-L1-C	SEQ ID NO: 85
	-	-	-	-	SEQ ID NO: 79	H	SEQ ID NO: 79
AK109	-	SEQ ID NO: 17	SEQ ID NO: 4	-	SEQ ID NO: 80	H-L1-MM	SEQ ID NO: 86
	SEQ ID NO: 62	-	-	-	SEQ ID NO: 81	H-C	SEQ ID NO: 87
AK110	-	SEQ ID NO: 17	SEQ ID NO: 4	-	SEQ ID NO: 82	H-L1-MM	SEQ ID NO: 88
	SEQ ID NO: 62	-	-	-	SEQ ID NO: 83	H-C	SEQ ID NO: 89
AK111	SEQ ID NO: 62	SEQ ID NO: 18	-	-	SEQ ID NO: 12	H-L1-C	SEQ ID NO: 85
	-	SEQ ID NO: 14	SEQ ID NO: 4	-	SEQ ID NO: 9	H-L1-MM	SEQ ID NO: 38
AK165	SEQ ID NO: 62	SEQ ID NO: 18	-	-	SEQ ID NO: 83	H-L1-C	SEQ ID NO: 90
	-	-	-	-	SEQ ID NO: 84	H	SEQ ID NO: 91
AK166	SEQ ID NO: 62	SEQ ID NO: 18	-	-	SEQ ID NO: 83	H-L1-C	SEQ ID NO: 90
	-	SEQ ID NO: 75	SEQ ID NO: 4	-	SEQ ID NO:82	H-L1-MM	SEQ ID NO: 92
AK167	SEQ ID NO: 3	SEQ ID NO: 18	-	-	SEQ ID NO: 12	H-L1-C	SEQ ID NO: 45
	-	-	-	-	SEQ ID NO: 79	H	SEQ ID NO: 79
AK168	SEQ ID NO: 3	SEQ ID NO: 18	-	-	SEQ ID NO: 12	H-L1-C	SEQ ID NO:45
	-	SEQ ID NO: 14	SEQ ID NO: 4	-	SEQ ID NO: 9	H-L1-MM	SEQ ID NO: 38
AK189	SEQ ID NO: 62	SEQ ID NO: 76	-	-	SEQ ID NO: 12	H-L1-C	SEQ ID NO: 93
	-	SEQ ID NO: 14	SEQ ID NO: 4	-	SEQ ID NO: 9	H-L1-MM	SEQ ID NO: 38

Construct #	Cytokine or functional fragment thereof (C)	Linker (L1)	Masking moiety (MM)	Linker (L2)	Half-life extension domain (H)	Structure (N- to C-terminal direction)	Amino Acid Sequence
AK190	SEQ ID NO: 62	SEQ ID NO: 77	-	-	SEQ ID NO: 12	H-L1-C	SEQ ID NO: 94
	-	SEQ ID NO: 14	SEQ ID NO: 4	-	SEQ ID NO: 9	H-L1-MM	SEQ ID NO: 38
AK191	SEQ ID NO: 3	SEQ ID NO: 20	-	-	SEQ ID NO: 12	H-L1-C	SEQ ID NO: 46
	-	SEQ ID NO: 14	SEQ ID NO: 4	-	SEQ ID NO: 9	H-L1-MM	SEQ ID NO: 38
AK192	SEQ ID NO: 3	SEQ ID NO: 76	-	-	SEQ ID NO: 12	H-L1-C	SEQ ID NO:95
	-	SEQ ID NO: 14	SEQ ID NO: 4	-	SEQ ID NO: 9	H-L1-MM	SEQ ID NO: 38
AK193	SEQ ID NO: 3	SEQ ID NO: 77	-	-	SEQ ID NO: 12	H-L1-C	SEQ ID NO: 96
	-	SEQ ID NO: 14	SEQ ID NO: 4	-	SEQ ID NO: 9	H-L1-MM	SEQ ID NO: 38
AK197	SEQ ID NO: 3	SEQ ID NO: 21	-	-	SEQ ID NO: 12	H-L1-C	SEQ ID NO: 47
	-	SEQ ID NO: 14	SEQ ID NO: 4	-	SEQ ID NO: 9	H-L1-MM	SEQ ID NO: 38
AK203	SEQ ID NO: 3	SEQ ID NO: 22	-	-	SEQ ID NO: 12	H-L1-C	SEQ ID NO: 48
	-	SEQ ID NO: 14	SEQ ID NO: 4	-	SEQ ID NO:9	H-L1-MM	SEQ ID NO: 38
AK209	SEQ ID NO: 3	SEQ ID NO: 78	-	-	SEQ ID NO: 12	H-L1-C	SEQ ID NO: 49
	-	SEQ ID NO: 14	SEQ ID NO: 4	-	SEQ ID NO: 9	H-L1-MM	SEQ ID NO: 38
AK210	SEQ ID NO: 62	SEQ ID NO: 20	-	-	SEQ ID NO: 12	H-L1-C	SEQ ID NO: 97
	-	SEQ ID NO: 14	SEQ ID NO: 4	-	SEQ ID NO: 9	H-L1-MM	SEQ ID NO: 38
AK211	SEQ ID NO: 3	SEQ ID NO: 23	-	-	SEQ ID NO: 12	H-L1-C	SEQ ID NO: 98
	-	SEQ ID NO: 14	SEQ ID NO: 4	-	SEQ ID NO: 9	H-L1-MM	SEQ ID NO: 38
AK215	SEQ ID NO: 69	SEQ ID NO: 18	-	-	SEQ ID NO: 12	H-L1-C	SEQ ID NO: 99
	-	SEQ ID NO: 14	SEQ ID NO: 4	-	SEQ ID NO: 9	H-L1-MM	SEQ ID NO:38

Construct #	Cytokine or functional fragment thereof (C)	Linker (L1)	Masking moiety (MM)	Linker (L2)	Half-life extension domain (H)	Structure (N- to C-terminal direction)	Amino Acid Sequence
AK216	SEQ ID NO: 70	SEQ ID NO: 18	-	-	SEQ ID NO: 12	H-L1-C	SEQ ID NO: 100
	-	SEQ ID NO: 14	SEQ ID NO: 4	-	SEQ ID NO: 9	H-L1-MM	SEQ ID NO: 38
AK218	SEQ ID NO: 71	SEQ ID NO: 18	-	-	SEQ ID NO: 12	H-L1-C	SEQ ID NO: 101
	-	SEQ ID NO: 14	SEQ ID NO: 4	-	SEQ ID NO: 9	H-L1-MM	SEQ ID NO:38
AK219	SEQ ID NO: 72	SEQ ID NO: 18	-	-	SEQ ID NO: 12	H-L1-C	SEQ ID NO: 102
	-	SEQ ID NO: 14	SEQ ID NO: 4	-	SEQ ID NO: 9	H-L1-MM	SEQ ID NO: 38
AK220	SEQ ID NO: 873	SEQ ID NO: 18	-	-	SEQ ID NO: 12	H-L1-C	SEQ ID NO: 103
	-	SEQ ID NO: 14	SEQ ID NO: 4	-	SEQ ID NO: 9	H-L1-MM	SEQ ID NO: 38
AK223	SEQ ID NO: 74	SEQ ID NO: 18	-	-	SEQ ID NO: 12	H-L1-C	SEQ ID NO: 104
	-	SEQ ID NO: 14	SEQ ID NO: 4	-	SEQ ID NO: 9	H-L1-MM	SEQ ID NO: 38
AK235	SEQ ID NO: 3	SEQ ID NO: 78	-	-	SEQ ID NO: 12	H-L1-C	SEQ ID NO: 49
	-	-	-	-	SEQ ID NO: 79	H	SEQ ID NO: 79
AK253	SEQ ID NO: 3	SEQ ID NO: 23	-	-	SEQ ID NO: 12	H-L1-C	SEQ ID NO: 98
	-	-	-	-	SEQ ID NO: 79	H	SEQ ID NO: 79
AK304	SEQ ID NO: 69	SEQ ID NO: 78	-	-	SEQ ID NO: 12	H-L1-C	SEQ ID NO: 105
	-	-	-	-	SEQ ID NO: 79	H	SEQ ID NO: 79
AK305	SEQ ID NO: 69	SEQ ID NO: 78	-	-	SEQ ID NO: 12	H-L1-C	SEQ ID NO: 105
	-	SEQ ID NO: 14	SEQ ID NO: 4	-	SEQ ID NO: 9	H-L1-MM	SEQ ID NO: 38
AK306	SEQ ID NO: 70	SEQ ID NO: 78	-	-	SEQ ID NO: 12	H-L1-C	SEQ ID NO: 106
	-	-	-	-	SEQ ID NO: 79	H	SEQ ID NO: 79

Construct #	Cytokine or functional fragment thereof (C)	Linker (L1)	Masking moiety (MM)	Linker (L2)	Half-life extension domain (H)	Structure (N- to C-terminal direction)	Amino Acid Sequence
AK307	SEQ ID NO: 70	SEQ ID NO: 78	-	-	SEQ ID NO: 12	H-L1-C	SEQ ID NO: 106
	-	SEQ ID NO: 14	SEQ ID NO:4	-	SEQ ID NO: 9	H-L1-MM	SEQ ID NO: 38
AK308	SEQ ID NO: 71	SEQ ID NO: 78	-	-	SEQ ID NO: 12	H-L1-C	SEQ ID NO: 107
	-	-	-	-	SEQ ID NO: 79	H	SEQ ID NO: 79
AK309	SEQ ID NO: 71	SEQ ID NO: 78	-	-	SEQ ID NO: 12	H-L1-C	SEQ ID NO: 107
	-	SEQ ID NO: 14	SEQ ID NO: 4	-	SEQ ID NO: 9	H-L1-MM	SEQ ID NO: 38
AK310	SEQ ID NO: 72	SEQ ID NO: 78	-	-	SEQ ID NO: 12	H-L1-C	SEQ ID NO: 108
	-	-	-	-	SEQ ID NO: 79	H	SEQ ID NO: 79
AK311	SEQ ID NO: 72	SEQ ID NO: 78	-	-	SEQ ID NO: 12	H-L1-C	SEQ ID NO: 108
	-	SEQ ID NO: 14	SEQ ID NO:4	-	SEQ ID NO: 9	H-L1-MM	SEQ ID NO: 38
AK312	SEQ ID NO: 73	SEQ ID NO:78	-	-	SEQ ID NO: 12	H-L1-C	SEQ ID NO: 109
	-	-	-	-	SEQ ID NO: 79	H	SEQ ID NO: 79
AK313	SEQ ID NO: 73	SEQ ID NO: 78	-	-	SEQ ID NO: 12	H-L1-C	SEQ ID NO: 109
	-	SEQ ID NO: 14	SEQ ID NO: 4	-	SEQ ID NO: 9	H-L1-MM	SEQ ID NO: 38
AK314	SEQ ID NO: 74	SEQ ID NO:78	-	-	SEQ ID NO: 12	H-L1-C	SEQ ID NO: 110
	-	-	-	-	SEQ ID NO: 79	H	SEQ ID NO: 79
AK315	SEQ ID NO: 74	SEQ ID NO: 78	-	-	SEQ ID NO: 12	H-L1-C	SEQ ID NO: 110
	-	SEQ ID NO: 14	SEQ ID NO: 4	-	SEQ ID NO: 9	H-L1-MM	SEQ ID NO: 38
AK316	SEQ ID NO: 62	SEQ ID NO: 78	-	-	SEQ ID NO: 12	H-L1-C	SEQ ID NO: 112
	-	SEQ ID NO: 14	SEQ ID NO: 4	-	SEQ ID NO: 9	H-L1-MM	SEQ ID NO: 38

Example 2: In vitro characterization of masked IL-2 polypeptides

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

Production

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 and added to the Expi293 cells with a cell density of 1.8 – 2.8 x 10^6 cells/mL or 0.85-1.20 x 10^6 cells/m, 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 x g and the supernatant was filtered through a 0.2 µm membrane. Protein A resin (CaptivA, Repligen CA-PRI-0005) was added to the filtered supernatant and incubated for at least 2 hours at 4 °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 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 plated at 5e4 cells/well in 150 uL 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 uL 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 Quantiblue (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. ELISA plates are coated with a receptor subunit, such as IL-2Rα (also referred to as CD25), IL- 2Rβ (also referred to as CD122), or IL-2Rγ (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

The binding affinity of the masked IL-2 polypeptide constructs generated in Example 1 is assessed. The binding affinity of the masked IL-2 polypeptide constructs is determined in conditions with and without protease cleavage.

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 (ka), dissociation constant (kd), equilibrium dissociation constant (KD), as well as the Chi^2 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 (ka), dissociation constant (kd), equilibrium dissociation constant (KD), as well as the Chi^2 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		
AK219	Weak binding		
AK220	Weak or no binding detected		
AK223	No binding detected		

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/uL in MMP cleavage buffer and activated with 1mM APMA for 2 h at 37 °C. 5 µL of protease (250 ng total) of the activated protease was incubated with 1uM of masked cytokine 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 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; 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. 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 generated in Example 1 is assessed in vivo using mouse models.

Mice are treated intravenously, intraperitoneally 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.

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 $x10^5$ cells per mouse) were injected subcutaneously into the right flank of each mouse. Upon reaching ~100 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 (μg/mL) by detecting human IgG, FIG. 18C shows Fc-CD122 levels in plasma (μg/mL) by detecting human CD122, and FIG. 18D shows Fc-IL2 levels in plasma (μg/mL) by detecting human IL-2.

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 (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 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

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 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 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.

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 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 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.

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.

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.

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.

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 $x10^5$ cells per mouse) were injected subcutaneously into the right flank of each mouse. Upon reaching ~100 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 harvested. Cell suspensions were prepared from spleens by mechanical disruption and 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 (Milenyi) 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.

In Vivo Cleavage

The in vivo cleavage of masked IL-2 cytokine 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.

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 (Poly5176, 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; SEQ ID NO: 24) and AK209 (cleavable peptide sequence: VPLSLY; SEQ ID NO: 28). 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.

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.

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 or the masked IL-15 polypeptide 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 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.

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 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 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 (Length*(Width^2)/2) using dial calipers and body weights were recorded twice weekly. FIGs. 28A and 28B show results from a 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.

Bioactivity in cynomolgus monkeys

The in vivo bioactivity of the masked IL-2 polypeptide constructs 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 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, aldesluekin, 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 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.

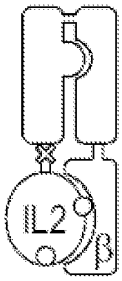
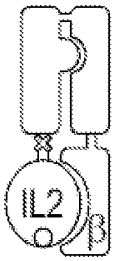
To test the safety profile of exemplary masked IL-2 polypeptide constructs in non-human primates, a dose ranging study is performed in accordance with the following method. Groups of 3 healthy male cynomolgus monkeys (Macaca fascicularis) are randomly assigned to receive a single intravenous bolus dose of 2 mL/kg of activatable (i.e., cleavable) masked IL-2 polypeptide proteins or non-cleavable masked IL-2 polypeptide

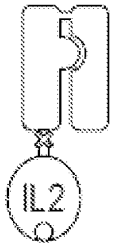
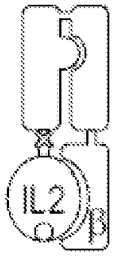
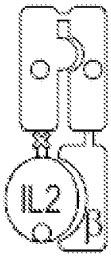
proteins at 10, 30 and 100 nmol/kg in 100 mM sodium citrate buffer (pH 5.5). A third group receives the parental non-masked, cleavable protein at 3, 10 and 30 nmol/kg as a positive control. This third group is dosed at a lower range to account for higher potency of the parental non-masked molecules. 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 ~100 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	
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 and passing through a 40 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 (Milenyi) 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

Summary of Cys to Ser mutations on CD122

The two free cysteines on the CD122 masking domain were mutated to serines to increase protein stability and mitigate developability risks including, without being limited as to theory, aggregation, oxidation, and immunogenicity. The mutant was evaluated in an accelerated stability study, where the control and the Cys to Ser mutant was incubated for a prolonged time (3 weeks), with elevated temperature (40°C), and in multiple pHs. Various analyses were performed to assess the impact of the cysteine mutations. The results demonstrate that the Cys to Ser mutant clearly enhanced the protein stability as evidenced by significantly reduced aggregation under stress. After 3 weeks incubation at pH8.0, the constructs with the cysteines mutated exhibit low levels of aggregation as compared to the control constructs, which do not contain the cysteine mutations, that have greater than fifty (50) percent aggregation as measured by SEC-HPLC. CE-SDS demonstrated that the construct with the mutated cysteines remains unaggregated (>99%) for pH6.0 and pH8.0 incubation, where the control constructs contained levels of aggregation up to fifteen (15) percent l.

In addition, constructs with the mutated cysteines in the CD122 masking protein interact with the IL-2 protein in a similar manner as the control constructs, which contain a wild-type CD122 masking protein (i.e. without mutation of the cysteine residues). In addition, the constructs with the mutated cysteines in the CD122 masking protein are similar in both functional assays and pharmacodynamics studies as the control constructs, which contain a CD122 masking protein without the cysteine mutations.

Experimental Protocols

Stability Study

Samples were incubated in a Galaxy 170 S air incubator set to 40°C. Three buffer systems were tested: 20 mM Citrate pH 5.0, 20 mM histidine pH 6.0, and 20 mM tris pH 8.0. The pH of each was calibrated at room temperature (approximately 27C) and buffers were adjusted to within 0.05 pH units with HCl/NaOH. Buffers were filtered by 0.22 um bottle top filters. Samples were buffer exchanged approximately 3000-fold into starting buffer via spin concentration. Sample aliquots were removed under sterile conditions at day 0, 1, 3, 7, 14, and 21, and stored at -80°C before being evaluated in the below analytical tests.

SEC-HPLC

An HPLC system was used to assess the aggregation level in the incubated samples; the system was calibrated with along with molecular weight standards. Levels of high molecular weight species ("HMWS") were measured in each sample. Increases in HMWS indicated increasing levels of aggregation.

The results of these studies is shown in Figures 32A and 32B. The key represents 'AK' molecule numbers, where AK341 is a Cys to Ser mutant and AK209 is a control.

CE-SDS

CE-SDS was run on a labchip machine. In general, a reducing agent was used for experiments under reducing conditions. Samples were subjected to high heat before samples were loaded into 96-well PCR plate. Recombinant human IL-2 was used as a low molecular weight protein control. Levels of HMWS were measured in each sample. Increases in HMWS indicated increasing levels of aggregation.

The results of these studies is shown in Figures 33A-33D. The key represents 'AK' molecule numbers, where AK341 is a Cys to Ser mutant and AK209 is a control.

Example 6

The constructs used are as follows:

AK #	Protease Substrate	Protease Site on	Half-life extension
AK209	VPLSLY	IL-2	agly-hIgG1
AK341*	VPLSLY	IL-2	agly-hIgG1
AK438	VPLSLY	CD122	agly-hIgG1
AK471	VPLSLY	IL-2	FcRn I253A
AK508	VPLSLY	CD122	FcRn I253A
AK504	VPLSLY	IL-2	hIgG4
AK511	VPLSLY	CD122	hIgG4
AK203	DSGGFMLT	IL-2	agly-hIgG1
AK442	DSGGFMLT	CD122	agly-hIgG1
AK168	MPYDLYHP	IL-2	agly-hIgG1
AK252	MPYDLYHP	CD122	agly-hIgG1
AK509	MPYDLYHP	IL-2	FcRn I253A
AK510	MPYDLYHP	CD122	FcRn I253A
AK191	RAAAVKSP	IL-2	agly-hIgG1
AK503	RAAAVKSP	CD122	agly-hIgG1
AK211 - Non-cleavable			
AK253 - parental (no mask); no cleavage site; always active			

AK341* Contains two cys → ser mutations on CD122

i. Anti-tumor activity - AK438 and AK442

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 x105 cells per mouse) were injected subcutaneously into the right flank of each mouse. Upon reaching ~100 mm3 sized tumors (day 0), the mice were randomized to receive Fc-IL-2 constructs in PBS. Mice were dosed intravenously on days 0, 3 and 6. Tumor volume was calculated (Length*(Width^2)/2) using dial calipers and body weights were recorded twice weekly. Mice were sacrificed upon reaching humane end points of tumor burden (2000 mm3) or body weight loss due to toxicity (20%).

Results are shown in Figures 34A and B.

ii. Peripheral (spleen) vs tumor CD8 T cell expansion - AK438 and AK442

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 x10^5 cells per mouse) were injected subcutaneously into the right flank of each mouse. Upon reaching ~100 mm^3 sized tumors (day 0), the mice were randomized to receive AK253 at very low dose level and all other Fc-IL-2 constructs at high dose level in PBS. Mice were dosed intravenously on days 0, 3 and 6.

Immunophenotyping on day 7 was performed using a FACS-based method from peripheral blood. Red blood cells 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) and Ki-67 (clone SOLA15, eBioscience). Data

acquisition was carried out on the MACSQuant Analyzer flow cytometer (Milenyi) and data were analyzed using the FlowJo. A one-way ANOVA with Bonferonni's post-test was performed to determine the statistical significance of treatment vs. control AK211) (*P<0.05; **P<0.01; ***P<0.001; ****P<0.0001).

Results are shown in Figures 35A and B.

iii. Anti-tumor activity – AK252, AK438, AK209 and AK471

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 x105 cells per mouse) were injected subcutaneously into the right flank of each mouse. Upon reaching ~100 mm3 sized tumors (day 0), the mice were randomized to receive AK253 at very low dose level and all other Fc-IL-2 constructs at high dose level in PBS. Mice were dosed intravenously on days 0, 3 and 6. Tumor volume was calculated (Length*(Width^2)/2) using dial calipers and body weights were recorded twice weekly. Mice were sacrificed upon reaching humane end points of tumor burden (2000 mm3) or body weight loss due to toxicity (20%).

Results are shown in Figures 36A and 36B.

iv. Peripheral (spleen) vs tumor CD8 T cell expansion – AK252, AK438, AK209, AK471

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 x105 cells per mouse) were injected subcutaneously into the right flank of each mouse. Upon reaching ~100 mm3 sized tumors (day 0), the mice were randomized to receive AK253 at very low dose level and all other Fc-IL-2 constructs at high dose level in PBS. Mice were dosed intravenously on days 0, 3 and 6.

Immunophenotyping on day 7 was performed using a FACS-based method from peripheral blood. Red blood cells 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) and Ki-67 (clone SOLA15, eBioscience). Data acquisition was carried out on the MACSQuant Analyzer flow cytometer (Milenyi) and data were analyzed using the FlowJo. A one-way ANOVA with Bonferonni's post-test was performed to determine the statistical significance of treatment vs. control AK211) (*P<0.05; **P<0.01; ***P<0.001; ****P<0.0001).

Results are shown in Figures 37A and 37B.

v. Anti-tumor activity - AK252, AK442, AK203, AK508 and AK510

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 x105 cells per mouse) were injected subcutaneously into the right flank of each mouse. Upon reaching ~100 mm3 sized tumors (day 0), the mice were randomized to receive AK253 at very low dose level and all other Fc-IL-2 constructs at high dose level in PBS. Mice were dosed intravenously on days 0, 3 and 6. Tumor volume was calculated (Length*(Width^2)/2) using dial calipers and body weights were recorded twice weekly. Mice were sacrificed upon reaching humane end points of tumor burden (2000 mm3) or body weight loss due to toxicity (20%).

Results are shown in Figures 38A and 38B.

vi. Peripheral (spleen) vs tumor CD8 T cell expansion - AK252, AK442, AK203, AK508 and AK510

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 x105 cells per mouse) were injected subcutaneously into the right flank of each mouse. Upon reaching ~100 mm3 sized tumors (day 0), the mice were randomized to receive AK253 at very low dose level and all other Fc-IL-2 constructs at high dose level in PBS. Mice were dosed intravenously on days 0, 3 and 6.

Immunophenotyping on day 7 was performed using a FACS-based method from peripheral blood. Red blood cells 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) and Ki-67 (clone SOLA15, eBioscience). Data acquisition was carried out on the MACSQuant Analyzer flow cytometer (Milenyi) and data were analyzed using the FlowJo. A one-way ANOVA with Bonferonni's post-test was performed to determine the statistical significance of treatment vs. control AK211) (*P<0.05; **P<0.01; ***P<0.001; ****P<0.0001).

Results are shown in Figures 39A and 39B.

vii. Anti-tumor activity - AK252, AK508, AK509, AK510, AK511

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 x105 cells per mouse) were injected subcutaneously into the right flank of each mouse. Upon reaching ~100 mm3 sized tumors (day 0), the mice were randomized to receive AK253 at very low dose level and all other Fc-IL-2 constructs at high dose level in PBS. Mice were dosed intravenously on days 0, 3 and 6. Tumor volume was calculated (Length*(Width^2)/2) using dial calipers and body weights were recorded twice weekly. Mice were sacrificed upon reaching humane end points of tumor burden (2000 mm3) or body weight loss due to toxicity (20%).

Results are shown in Figures 40A-40D.

viii. Peripheral (spleen) vs tumor CD8 T cell expansion - AK252, AK508, AK509, AK510, AK511

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 x105 cells per mouse) were injected subcutaneously into the right flank of each mouse. Upon reaching ~100 mm3 sized tumors (day 0), the mice were randomized to receive AK253 at very low dose level and all other Fc-IL-2 constructs at high dose level in PBS. Mice were dosed intravenously on days 0, 3 and 6.

Immunophenotyping on day 7 was performed using a FACS-based method from peripheral blood. Red blood cells 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) and Ki-67 (clone SOLA15, eBioscience). Data acquisition was carried out on the MACSQuant Analyzer flow cytometer (Milenyi) and data were analyzed using the FlowJo. A one-way ANOVA with Bonferonni's post-test was performed to determine the statistical significance of treatment vs. control AK211) (*P<0.05; **P<0.01; ***P<0.001; ****P<0.0001). AK252++ produced in-house lot# AK252-06B, AK252 produced by ATUM lot# AK252-A-01A.

Results shown in Figures 41A and 41B.

ix. Anti-tumor activity – AK252, AK438, AK442, AK209, AK341

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 x105 cells per mouse) were injected subcutaneously into the right flank of each mouse. Upon reaching ~100 mm3 sized tumors (day 0), the mice were randomized to receive AK253 at very low dose level and all other Fc-IL-2 constructs at high dose level in PBS. Mice were dosed intravenously on days 0, 3 and 6. Tumor volume was calculated (Length*(Width^2)/2) using dial calipers and body weights were recorded twice weekly. Mice were sacrificed upon reaching humane end points of tumor burden (2000 mm3) or body weight loss due to toxicity (20%).

Results are shown in Figures 42A and 42B.

x. Splenomegaly and lung edema - AK252, AK438, AK442, AK209, AK341

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 x105 cells per mouse) were injected subcutaneously into the right flank of each mouse. Upon reaching ~100 mm3 sized tumors (day 0), the mice were randomized to receive

AK253 at very low dose level and all other Fc-IL-2 constructs at high dose level in PBS. Mice were dosed intravenously on days 0, 3 and 6. Tissues were harvested and weighed on day 6.

Results are shown in Figures 43A and 43B.

Example 7

i. Cleavage of peptides by NAT vs. RCC culture supernatant

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.

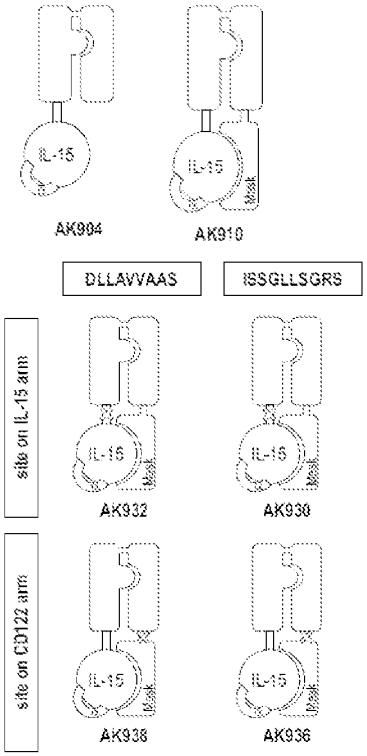
Results are as follows:

Substrate	AMSP-MS 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	RSG**VPLS*LYSG**SGGGK	0	3/5		15min
AK-18	RSG**MP*YDLY*HP**SGK	5/5	5/5	15min	15min
AK-21	RGP**DSGGF*ML*T**SGK	3/5	5/5	15min	15min
AK-28	RGSG**HEQLTV**SGGSK	0	0		
AK-49	RSG**R*AAAVKSP**SGK	0	3/5		15-60-240min
AK-02	RGSG**ISSGLLSGRS*D*N*H**SGK	5/5	5/5	15-60min	15-60min
AK-50	RG**DLLAVVA*AS**GGK	0	5/5		15-60min
AK-88	RGG**ISSGLL*SG*RS**GK	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.

Example 8

The following constructs were used in this example:



Details on the domain features and sequences of each AK molecule is as follows:

AK904	1st polypeptide chain: Fc(Hole)	DKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMIS RTPEVTCVVVDVSHEDPEVKFNWYVDGVEVH NAKTKPREEQYASTYRVVSVLTVLHQDWLNG KEYKCKVSNKALPAPIEKTISKAKGQPREPQVC TLPPSRDELTKNQVSLSCAVKGFYPSDIAVEWE SNGQPENNYKTTPPVLDSDGSFFLVSKLTVDK SRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGK	DNA158
	2nd polypeptide chain: Fc(knob)-IL15 V1 Non-cleavable (N71Q, N79Q)	DKTHTCPPCPAPELLGGPSVFLFPPKPKDTL MISRTPEVTCVVVDVSHEDPEVKFNWYVD GVEVHNAKTKPREEQYASTYRVVSVLTVL HQDWLNGKEYKCKVSNKALPAPIEKTISKA KGQPREPQVYTLPPCRDELTKNQVSLWCLV KGFYPSDIAVEWESNGQPENNYKTTPPVLD SDGSFFLYSKLTVDKSRWQQGNVFSCSVMHE ALHNHYTQKSLSLSPGGGSSPPGGGSSGGGSG PSGSPGNWVNVISDLKKIEDLIQSMHIDATLY	AK904

		TESDVHPSCKVTAMKCFLLELQVISLESGDASI HDTVENLIILAQNSLSSNGQVTESGCKECEEL EEKNIKEFLQSFVHIVQMFINTS	
AK910	1st polypeptide chain: Fc(Hole) CD122(C122S, C168S)	DKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPE VTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPRE EQYASTYRVVSVLTVLHQDWLNGKEYKCKVSNKAL PAPIEKTISKAKGQPREPQVCTLPPSRDELTKNQVSLS CAVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDG SFFLVSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQ KSLSLSPGPGSGSAVNGTSQFTCFYNSRANISCVWSQ DGALQDTSCQVHAWPDRRRWNQTCELLPVSQASW ACNLILGAPDSQKLTTVDIVTLRVLCREGVRWRVMA IQDFKPFENLRLMAPISLQVVHVETHRSNISWEISQAS HYFERHLEFEARTLSPGHTWEEAPLLTLKQKQEWIS LETLTPDTQYEFQVRVKPLQGEFTTWSPWSQPLAF RTKPAALGKD	DNA440
	2nd polypeptide chain: Fc(knob)-IL15 V1 Non-cleavable (N71Q, N79Q)	DKTHTCPPCPAPELLGGPSVFLFPPKPKDTL MISRTPEVTCVVVDVSHEDPEVKFNWYVD GVEVHNAKTKPREEQYASTYRVVSVLTVL HQDWLNGKEYKCKVSNKALPAPIEKTISKA KGQPREPQVYTLPPCRDELTKNQVSLWCLV KGFYPSDIAVEWESNGQPENNYKTTPPVLD SDGSFFLYSKLTVDKSRWQQGNVFSCSVMHE ALHNHYTQKSLSLSPGGGSSPPGGGSSGGGSG PSGSPGNWVNVISDLKKIEDLIQSMHIDATLY TESDVHPSCKVTAMKCFLLELQVISLESGDASI HDTVENLIILAQNSLSSNGQVTESGCKECEEL EEKNIKEFLQSFVHIVQMFINTS	DNA904
AK932	1st polypeptide chain: Fc(Hole) CD122(C122S, C168S)	DKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPE VTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPRE EQYASTYRVVSVLTVLHQDWLNGKEYKCKVSNKAL PAPIEKTISKAKGQPREPQVCTLPPSRDELTKNQVSLS CAVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDG SFFLVSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQ KSLSLSPGPGSGSAVNGTSQFTCFYNSRANISCVWSQ DGALQDTSCQVHAWPDRRRWNQTCELLPVSQASW ACNLILGAPDSQKLTTVDIVTLRVLCREGVRWRVMA IQDFKPFENLRLMAPISLQVVHVETHRSNISWEISQAS	DNA440

		HYFERHLEFEARTLSPGHTWEEAPLLTLKQKQEWIS LETLTPDTQYEFQVRVKPLQGEFTTWSPWSQPLAF RTKPAALGKD	
	2nd polypeptide chain: Fc(knob)-[DLLAVVAAS]-IL15 (N71Q,N79Q) *cleavable peptide bolded	DKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEV TCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREE QYASTYRVVSVLTVLHQDWLNGKEYKCKVSNKALP APIEKTISKAKGQPREPQVYTLPPCRDELTKNQVSLW CLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGS FFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKS LSLSPGSGS**DLLAVVAAS**SGPGSGNWVNVISDLKKIE DLIQSMHIDATLYTESDVHPSCKVTAMKCFLLELQVISL ESGDASIHDTVENLIILAQNSLSSNGQVTESGCKECEE LEEKNIKEFLQSFVHIVQMFINTS	DNA924
AK938	1st polypeptide chain: Fc(hole)-[DLLAVVAAS]-CD122 *cleavable peptide bolded	DKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRT PEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAK TKPREEQYASTYRVVSVLTVLHQDWLNGKEYK CKVSNKALPAPIEKTISKAKGQPREPQVCTLPPS RDELTKNQVSLSCAVKGFYPSDIAVEWESNGQ PENNYKTTPPVLDSDGSFFLVSKLTVDKSRWQQ GNVFSCSVMHEALHNHYTQKSLSLSPGSGSPSG**D LLAVVAAS**SGPGSGSPAVNGTSQFTCFYNSRANISC VWSQDGALQDTSCQVHAWPDRRRWNQTCELLPV SQASWACNLILGAPDSQKLTTVDIVTLRVLCREGV RWRVMAIQDFKPFENLRLMAPISLQVVHVETHRSN ISWEISQASHYFERHLEFEARTLSPGHTWEEAPLLTL KQKQEWISLETLTPDTQYEFQVRVKPLQGEFTT WSPWSQPLAFRTKPAALGKD	DNA822
	2nd polypeptide chain: Fc(knob)-IL15 V1 Non-cleavable (N71Q, N79Q)	DKTHTCPPCPAPELLGGPSVFLFPPKPKDTL MISRTPEVTCVVVDVSHEDPEVKFNWYVD GVEVHNAKTKPREEQYASTYRVVSVLTVL HQDWLNGKEYKCKVSNKALPAPIEKTISKA KGQPREPQVYTLPPCRDELTKNQVSLWCLV KGFYPSDIAVEWESNGQPENNYKTTPPVLD SDGSFFLYSKLTVDKSRWQQGNVFSCSVMHE ALHNHYTQKSLSLSPGGGSSPPGGGSSGGGSG PSGSPGNWVNVISDLKKIEDLIQSMHIDATLY TESDVHPSCKVTAMKCFLLELQVISLESGDASI HDTVENLIILAQNSLSSNGQVTESGCKECEEL	DNA904

		EEKNIKEFLQSFVHIVQMFINTS	
AK930	1st polypeptide chain: Fc(Hole) CD122(C122S, C168S)	DKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYASTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVCTLPPSRDELTKNQVSLSCAVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLVSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGPGSGSAVNGTSQFTCFYNSRANISCVWSQDGALQDTSCQVHAWPDRRRWNQTCELLPVSQASWACNLILGAPDSQKLTTVDIVTLRVLCREGVRWRVMAIQDFKPFENLRLMAPISLQVVHVETHRSNISWEISQASHYFERHLEFEARTLSPGHTWEEAPLLTLKQKQEWISLETLTPDTQYEFQVRVKPLQGEFTTWSPWSQPLAFRTKPAALGKD	DNA440
	2nd polypeptide chain: Fc(knob)-[ISSGLLSGRS]-IL15 (N71Q,N79Q) *cleavable peptide bolded	DKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYASTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPCRDELTKNQVSLWCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGGGSSGGSP**ISSGLLSGRS**SGPGSGSNWVNVISDLKKIEDLIQSMHIDATLYTESDVHPSCKVTAMKCFLLELQVISLESGDASIHDTVENLIILAQNSLSSNGQVTESGCKECEELEEKNIKEFLQSFVHIVQMFINTS	DNA922
AK936	1st polypeptide chain: Fc(hole)-[ISSGLLSGRS]-CD122	DKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYASTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVCTLPPSRDELTKNQVSLSCAVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLVSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGGPPSGSSP**ISSGLLSGRS**SGGGAVNGTSQFTCFYNSRANISCVWSQDGALQDTSCQVHAWPDRRRWNQTCELLPVSQASWACNLILGAPDSQKLTTVDIVTLRVLCREGVRWRVMAIQDFKPFENLRLMAPISLQVVHVETHRSNISWEISQASHYFERHLEFEARTLSPGHTWEEAPLLTLKQKQEWISLETLTPDTQYEFQVRVKPLQGEFTTWSPWSQPLAFRTKPAALGKD	DNA823

	2nd polypeptide chain: Fc(knob)-IL15 V1 Non-cleavable (N71Q, N79Q)	DKTHTCPPCPAPELLGGPSVFLFPPKPKDTL MISRTPEVTCVVVDVSHEDPEVKFNWYVD GVEVHNAKTKPREEQYASTYRVVSVLTVL HQDWLNGKEYKCKVSNKALPAPIEKTISKA KGQPREPQVYTLPPCRDELTKNQVSLWCLV KGFYPSDIAVEWESNGQPENNYKTTPPVLD SDGSFFLYSKLTVDKSRWQQGNVFSCSVMHE ALHNHYTQKSLSLSPGGGSSPPGGGSSGGGSG PSGSPGNWVNVISDLKKIEDLIQSMHIDATLY TESDVHPSCKVTAMKCFLLELQVISLESGDASI HDTVENLIILAQNSLSSNGQVTESGCKECEEL EEKNIKEFLQSFVHIVQMFINTS	DNA904

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.

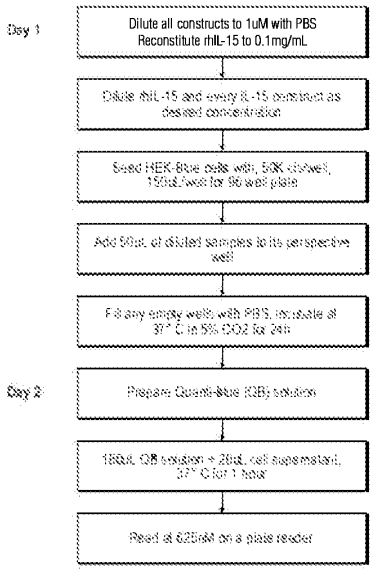
Cleavage was achieved for masked constructs including a peptide substrate.

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 cls/well. Time point for HEK Blue detection were tested: 1h. Construct number: Total 14 constructs that were tested.

Assay Flow chart:



The results are shown in the table below, where a 'X' indicates not fully cleaved and a ' ' indicates cleavage:

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 EC_{50} 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 44A-D. The HEK-Blue IL-2 bioassay results are shown in Figures 45A-F.

The present invention is not intended to be limited in scope to the particular disclosed embodiments, which are provided, for example, to illustrate various aspects of the invention. Various modifications to the compositions and methods described will become apparent from the description and teachings herein. Such variations may be practiced without departing from the true scope and spirit of the disclosure and are intended to fall within the scope of the present disclosure.

12. SEQUENCES

DESCRIPTION	NEW SEQ ID NO.	Exemplary AK number	AMINO ACID SEQUENCE
IL-2 precursor	1		MYRMQLLSCIALSLALVTNSAPTSSSTKKTQLQLEHLLLDLQMILNGIN NYKNPKLTRMLTFKFYMPKKATELKHLQCLEEELKPLEEVLNLAQSKNFHL RPRDLISNINVIVLELKGSETTFMCEYADETATIVEFLNRWITFCQSIISTLT
IL-2 mature	2		APTSSSTKKTQLQLEHLLLDLQMILNGINNYKNPKLTRMLTFKFYMPKK ATELKHLQCLEEELKPLEEVLNLAQSKNFHLRPRDLISNINVIVLELKGSE TTFMCEYADETATIVEFLNRWITFCQSIISTLT
IL-2 (R38A, F42A, Y45A, E62A, C125A)	3	AK168 AK209 AK191 AK197 AK203 AK471 AK442 AK438 AK341 AK530 AK539 AK540	APTSSSTKKTQLQLEHLLLDLQMILNGINNYKNPKLTAMLTAKFAMPKK ATELKHLQCLEEALKPLEEVLNLAQSKNFHLRPRDLISNINVIVLELKGSE TTFMCEYADETATIVEFLNRWITFAQSIISTLT

		AK541 AK523 AK524 AK525	
MM	4	AK168 AK209 AK191 AK197 AK203 AK471 AK442 AK438 AK539 AK540 AK541 AK523 AK524 AK525	AVNGTSQFTCFYNSRANISCVWSQDGALQDTSCQVHAWPDRRRWNQT CELLPVSQASWACNLILGAPDSQKLTTVDIVTLRVLCREGVRWRVMAIQ DFKPFENLRLMAPISLQVVHVETHRCNISWEISQASHYFERHLEFEARTLS PGHTWEEAPLLTLKQKQEWICLETLTPDTQYEFQVRVKPLQGEFTTWSP WSQPLAFRTKPAALGKD
MM (C122S, C168S)	5	AK341 AK530	AVNGTSQFTCFYNSRANISCVWSQDGALQDTSCQVHAWPDRRRWNQT CELLPVSQASWACNLILGAPDSQKLTTVDIVTLRVLCREGVRWRVMAIQ DFKPFENLRLMAPISLQVVHVETHRSNISWEISQASHYFERHLEFEARTLS PGHTWEEAPLLTLKQKQEWISLETLTPDTQYEFQVRVKPLQGEFTTWSP WSQPLAFRTKPAALGKD

Parent IgG1_human heavy chain constant gamma 1	6		ASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSS GLYSLSSVVTVPSSSLGTQTYICNVNHKPSNTKVDKKVEPKSCDKTHTCPPCPAPELLGG PSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYN STYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSRDE LTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRW QQGNVFSCSVMHEALHNHYTQKSLSLSPGK
Parent IgG1_human heavy chain constant gamma 1 – Fc domain	7		DKTHTCPPCPAPELLGG PSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYN STYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSRDE LTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRW QQGNVFSCSVMHEALHNHYTQKSLSLSPG
HL1 (Y349C, T366S, L38A, Y407V)	8		DKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHE DPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWL NGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVCTLPPSRDELTKNQ VSLSCAVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLVSKL TVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPG
HL1 (Y349C, T366S, L38A, Y407V, N297A)	9	AK168 AK209 AK191 AK197 AK203 AK442 AK438	DKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHE DPEVKFNWYVDGVEVHNAKTKPREEQYASTYRVVSVLTVLHQDWL NGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVCTLPPSRDELTKNQ VSLSCAVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLVSKL TVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPG

		AK341 AK530 AK539 AK540 AK541 AK523 AK524 AK525	DKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMASRTPEVTCVVVDVSHEDP EVKFNWYVDGVEVHNAKTKPREEQYASTYRVVSVLTVLHQDWLNGKE YKCKVSNKALPAPIEKTISKAKGQPREPQVCTLPPSRDELTKNQVSLSCA VKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLVSKLTVDKSRW QQGNVFSCSVMHEALHNHYTQKSLSLSPG
HL1 (Y349C, T366S, L38A, Y407V, N297A, I253A)	10	AK471	
HL2 (S354C, T366W)	11		DKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSH EDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDW LNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPCRDELTK NQVSLWCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFL YSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGK
HL2 (S354C, T366W, N297A)	12	AK168 AK209 AK191 AK197 AK203 AK442	DKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSH EDPEVKFNWYVDGVEVHNAKTKPREEQYASTYRVVSVLTVLHQDW LNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPCRDELTK NQVSLWCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFL YSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPG

		AK438 AK341 AK530 AK539 AK540 AK541 AK523 AK524 AK525	DKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMASRTPEVTCVVVDVSHEDP EVKFNWYVDGVEVHNAKTKPREEQYASTYRVVSVLTVLHQDWLNGKEY KCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPCRDELTKNQVSLWCLVK GFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRW QQGNVFSCSVMHEALHNHYTQKSLSLSPG
HL2 (S354C, T366W, N297A, I253A)	13	AK471	
1st linker L1 (non-cleavable)	14	AK168 AK209 AK191 AK197 AK203 AK471 AK341 AK539 AK540	PGSGS

		AK541	
1st linker L1 (cleavable)	15	AK442	GPPSGSSPGDSGGFMLTSGGG
1st linker L1 (cleavable)	16	AK438	GPPSGSSPGVPLSLYGSGGG
1st linker L1 (cleavable)	17	AK530	GPPSGSSPMPYDLYHPSGGG
1st linker L1 (cleavable)	112	AK523	GSPDLLAVVAASSGP
1st linker L1 (cleavable)	113	AK524	GSPGDLLAVVAASSGP
1st linker L1 (cleavable)	114	AK525	GSGSPSDLLAVVAASSGP
2nd linker L2 (cleavable)	18	AK168	GGSSPPMPYDLYHPSGP
2nd linker L2 (cleavable)	19	AK209 AK471 AK341	GSPGVPLSLYSGP
2nd linker L2 (cleavable)	20	AK191	GGSGRAAAVKSPSGP
2nd linker L2 (cleavable)	21	AK197	GGSGHEQLTVSGP
2nd linker L2	22	AK203	GSGPDSGGFMLTSGP

(cleavable)			
2nd linker L2 (non-cleavable)	23	AK442 AK438 AK530 AK523 AK524 AK525	GGSSPPGGGSSGGGSGP
2nd linker L2 (cleavable)	115	AK539	GGPSDLLAVVAASSGP
2nd linker L2 (cleavable)	116	AK540	GSGPSDLLAVVAASSGP
2nd linker L2 (cleavable)	117	AK541	GSSGGPDLLAVVAASSGP
Cleavable peptide	24	AK168 AK530	MPYD*LYHP *indicates cleavage site
Cleavable peptide	25	AK203 AK442	DSGG*FMLT *indicates cleavage site
Cleavable peptide	26	AK197	HEQ*LTV *indicates cleavage site
Cleavable peptide	27	AK191	RAAA*VKSP

			*indicates cleavage site
Cleavable peptide	28	AK209 AK471 AK341 AK438	VPLS*LY *indicates cleavage site
Cleavable peptide	118	AK50 AK539 AK540 AK541 AK523 AK524 AK525	DLLA*VVAAS *indicates cleavage site
Cleavable peptide	119	AK88	ISSGLL*SG*RS *indicates cleavage site
C terminal spacer domain	29	AK168 AK209 AK191 AK197 AK203 AK471 AK348	SGP

		AK539 AK540 AK541 AK523 AK524 AK525	
C terminal spacer domain	30	AK442 AK530	SGGG
C terminal spacer domain	31	AK438	GSGGG
N terminal spacer domain	32	AK168	GGSSPP
N terminal spacer domain	33	AK203	GSGP
N terminal spacer domain	34	AK209 AK341 AK471 AK524	GSPG
N terminal spacer domain	35	AK191 AK197	GGSG
N terminal spacer domain	36	AK442 AK348	GPPSGSSPG
N terminal spacer	37	AK530	GPPSGSSP

domain			
N terminal spacer domain	120	AK539	GGPS
N terminal spacer domain	121	AK540	GSGPS
N terminal spacer domain	122	AK541	GSSGGP
N terminal spacer domain	123	AK523	GSP
N terminal spacer domain	124	AK525	GSGSPS
1st polypeptide chain - A (HL1-L1-MM)	38	AK168	DKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHE
		AK191	DPEVKFNWYVDGVEVHNAKTKPREEQYASTYRVVSVLTVLHQDWL
		AK197	NGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVCTLPPSRDELTKN
		AK203	QVSLSCAVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLV
		AK209	SKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGPGSGSA
		AK539	VNGTSQFTCFYNSRANISCVWSQDGALQDTSCQVHAWPDRRRWN
		AK540	QTCELLPVSQASWACNLILGAPDSQKLTTVDIVTLRVLCREGVRWR
		AK541	VMAIQDFKPFENLRLMAPISLQVVHVETHRCNISWEISQASHYFERHL
			EFEARTLSPGHTWEEAPLLTLKQKQEWICLETLTPDTQYEFQVRVKP
			LQGEFTTWSPWSQPLAFRTKPAALGKD
1st polypeptide chain - B	39	AK341	DKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDP
			EVKFNWYVDGVEVHNAKTKPREEQYASTYRVVSVLTVLHQDWLNGKE

(HL1-L1-MM)			YKCKVSNKALPAPIEKTISKAKGQPREPQVCTLPPSRDELTKNQVSLSCA VKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLVSKLTVDKSRWQ QGNVFSCSVMHEALHNHYTQKSLSLSPGPGSGSAVNGTSQFTCFYNSRAN ISCVWSQDGALQDTSCQVHAWPDRRRWNQTCELLPVSQASWACNLILGAP DSQKLTTVDIVTLRVLCREGVRWRVMAIQDFKPFENLRLMAPISLQVVHVE THRSNISWEISQASHYFERHLEFEARTLSPGHTWEEAPLLTLKQKQEWISLETL TPDTQYEFQVRVKPLQGEFTTWSPWSQPLAFRTKPAALGKD
1st polypeptide chain - C (HL1-L1-MM)	40	AK530	DKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSH EDPEVKFNWYVDGVEVHNAKTKPREEQYASTYRVVSVLTVLHQDW LNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVCTLPPSRDELTKNQ VSLSCAVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLVSKLT VDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGGPPSGSSPMPYD LYHPSGGGAVNGTSQFTCFYNSRANISCVWSQDGALQDTSCQVHAWP DRRRWNQTCELLPVSQASWACNLILGAPDSQKLTTVDIVTLRVLCREG VRWRVMAIQDFKPFENLRLMAPISLQVVHVETHRSNISWEISQASHYFER HLEFEARTLSPGHTWEEAPLLTLKQKQEWISLETLTPDTQYEFQVRVKPL QGEFTTWSPWSQPLAFRTKPAALGKD
1st polypeptide chain - D (HL1-L1-MM)	41	AK442	DKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFN WYVDGVEVHNAKTKPREEQYASTYRVVSVLTVLHQDWLNGKEYKCKVSNKAL PAPIEKTISKAKGQPREPQVCTLPPSRDELTKNQVSLSCAVKGFYPSDIAVEWESN GQPENNYKTTPPVLDSDGSFFLVSKLTVDKSRWQQGNVFSCSVMHEALHNHYT QKSLSLSPGGPPSGSSPGDSGGFMLTSGGGAVNGTSQFTCFYNSRANISCVWSQD GALQDTSCQVHAWPDRRRWNQTCELLPVSQASWACNLILGAPDSQKLTTVDIV TLRVLCREGVRWRVMAIQDFKPFENLRLMAPISLQVVHVETHRCNISWEISQAS

			HYFERHLEFEARTLSPGHTWEEAPLLTLKQKQEWICLETLTPDTQYEFQVRVKP LQGEFTTWSPWSQPLAFRTKPAALGKD
1st polypeptide chain - E (HL1-L1-MM)	42	AK438	DKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNW YVDGVEVHNAKTKPREEQYASTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAP IEKTISKAKGQPREPQVCTLPPSRDELTKNQVSLSCAVKGFYPSDIAVEWESNGQPE NNYKTTPPVLDSDGSFFLVSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSL SPGGPPSGSSPGVPLSLYGSGGGAVNGTSQFTCFYNSRANISCVWSQDGALQDTSC QVHAWPDRRRWNQTCELLPVSQASWACNLILGAPDSQKLTTVDIVTLRVLCREGV RWRVMAIQDFKPFENLRLMAPISLQVVHVETHRCNISWEISQASHYFERHLEFEART LSPGHTWEEAPLLTLKQKQEWICLETLTPDTQYEFQVRVKPLQGEFTTWSPWSQPL AFRTKPAALGKD
1st polypeptide chain - G (HL-L2-C)	43	AK471	DKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMASRTPEVTCVVVDVSHEDP EVKFNWYVDGVEVHNAKTKPREEQYASTYRVVSVLTVLHQDWLNGKE YKCKVSNKALPAPIEKTISKAKGQPREPQVCTLPPSRDELTKNQVSLSCA VKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLVSKLTVDKSRW QQGNVFSCSVMHEALHNHYTQKSLSLSPGPGSGSAVNGTSQFTCFYNSRA NISCVWSQDGALQDTSCQVHAWPDRRRWNQTCELLPVSQASWACNLILG APDSQKLTTVDIVTLRVLCREGVRWRVMAIQDFKPFENLRLMAPISLQVVH VETHRCNISWEISQASHYFERHLEFEARTLSPGHTWEEAPLLTLKQKQEWIC LETLTPDTQYEFQVRVKPLQGEFTTWSPWSQPLAFRTKPAALGKD
1st polypeptide chain – H (HL-L2-C)	44	AK252	DKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPE VKFNWYVDGVEVHNAKTKPREEQYASTYRVVSVLTVLHQDWLNGKEYK CKVSNKALPAPIEKTISKAKGQPREPQVCTLPPSRDELTKNQVSLSCAVKGF YPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLVSKLTVDKSRWQQGNVF

			SCSVMHEALHNHYTQKSLSLSPGGPPSGSSPMPYDLYHPSGGGAVNGTSQF TCFYNSRANISCVWSQDGALQDTSCQVHAWPDRRRWNQTCELLPVSQASW ACNLILGAPDSQKLTTVDIVTLRVLCREGVRWRVMAIQDFKPFENLRLM APISLQVVHVETHRCNISWEISQASHYFERHLEFEARTLSPGHTWEEA PLLTLKQKQEWICLETLTPDTQYEFQVRVKPLQGEFTTWSPWSQPLAFRTKPAALGKD
1st polypeptide chain – I (HL-L1-MM)	125	AK523	DKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNW YVDGVEVHNAKTKPREEQYASTYRVVSVLTVLHQDWLNGKEYKCKVSNKALP APIEKTISKAKGQPREPQVCTLPPSRDELTKNQVSLSCAVKGFYPSDIAVEWESNG QPENNYKTTPPVLDSDGSFFLVSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQK SLSLSPGGSPDLLAVVAASSGPAVNGTSQFTCFYNSRANISCVWSQDGALQDTSC QVHAWPDRRRWNQTCELLPVSQASWACNLILGAPDSQKLTTVDIVTLRVLCREG VRWRVMAIQDFKPFENLRLMAPISLQVVHVETHRCNISWEISQASHYFERHLEFE ARTLSPGHTWEEAPLLTLKQKQEWICLETLTPDTQYEFQVRVKPLQGEFTTWSPW SQPLAFRTKPAALGKD
1st polypeptide chain – J (HL-L1-MM)	126	AK524	DKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNW YVDGVEVHNAKTKPREEQYASTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPI EKTISKAKGQPREPQVCTLPPSRDELTKNQVSLSCAVKGFYPSDIAVEWESNGQPENNY KTTPPVLDSDGSFFLVSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGGS PGDLLAVVAASSGPAVNGTSQFTCFYNSRANISCVWSQDGALQDTSCQVHAWPDRR RWNQTCELLPVSQASWACNLILGAPDSQKLTTVDIVTLRVLCREGVRWRVMAIQDFKP FENLRLMAPISLQVVHVETHRCNISWEISQASHYFERHLEFEARTLSPGHTWEEAPLLT LKQKQEWICLETLTPDTQYEFQVRVKPLQGEFTTWSPWSQPLAFRTKPAALGKD
1st polypeptide chain – K	127	AK525	DKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVD GVEVHNAKTKPREEQYASTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISK

(HL-L1-MM)			AKGQPREPQVCTLPPSRDELTKNQVSLSCAVKGFYPSDIAVEWESNGQPENNYKTTPPV LDSDGSFFLVSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGSGSPSDLLAV VAASSGPAVNGTSQFTCFYNSRANISCVWSQDGALQDTSCQVHAWPDRRRWNQTCELL PVSQASWACNLILGAPDSQKLTTVDIVTLRVLCREGVRWRVMAIQDFKPFENLRLMAPISL QVVHVETHRCNISWEISQASHYFERHLEFEARTLSPGHTWEEAPLLTLKQKQEWICLE TLTPDTQYEFQVRVKPLQGEFTTWSPWSQPLAFRTKPAALGKD
2nd polypeptide chain - A (HL-L2-C)	45	AK168	DKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVS HEDPEVKFNWYVDGVEVHNAKTKPREEQYASTYRVVSVLTVLHQ DWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPCRD ELTKNQVSLWCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSD GSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPG GGSSPPMPYDLYHPSGPAPTSSSTKKTQLQLEHLLLDLQMILNGINN YKNPKLTAMLTAKFAMPKKATELKHLQCLEEALKPLEEVLNLAQ SKNFHLRPRDLISNINVIVLELKGSETTFMCEYADETATIVEFLNRW ITFAQSIISTLT
2nd polypeptide chain - B (HL-L2-C)	46	AK191	DKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVS HEDPEVKFNWYVDGVEVHNAKTKPREEQYASTYRVVSVLTVLHQ DWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPCRDE LTKNQVSLWCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGS FFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGGG SGRAAAVKSPSGPAPTSSSTKKTQLQLEHLLLDLQMILNGINNYKN PKLTAMLTAKFAMPKKATELKHLQCLEEALKPLEEVLNLAQSKNF HLRPRDLISNINVIVLELKGSETTFMCEYADETATIVEFLNRWITFAQ

			SIISTLT
2nd polypeptide chain - C (HL-L2-C)	47	AK197	DKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVS HEDPEVKFNWYVDGVEVHNAKTKPREEQYASTYRVVSVLTVLHQ DWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPCRDE LTKNQVSLWCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDG SFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGG GSGHEQLTVSGPAPTSSSTKKTQLQLEHLLLDLQMILNGINNYKNP KLTAMLTAKFAMPKKATELKHLQCLEEALKPLEEVLNLAQSKNFH LRPRDLISNINVIVLELKGSETTFMCEYADETATIVEFLNRWITFAQS IISTLT
2nd polypeptide chain - D (HL-L2-C)	48	AK203	DKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVD VSHEDPEVKFNWYVDGVEVHNAKTKPREEQYASTYRVVSVLT VLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYT LPPCRDELTKNQVSLWCLVKGFYPSDIAVEWESNGQPENNYKT TPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHY TQKSLSLSPGGSGPDSGGFMLTSGPAPTSSSTKKTQLQLEHLLLD LQMILNGINNYKNPKLTAMLTAKFAMPKKATELKHLQCLEEAL KPLEEVLNLAQSKNFHLRPRDLISNINVIVLELKGSETTFMCEYAD ETATIVEFLNRWITFAQSIISTLT
2nd polypeptide chain - E (HL-L2-C)	49	AK209 AK341	DKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDV SHEDPEVKFNWYVDGVEVHNAKTKPREEQYASTYRVVSVLTVL HQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPC RDELTKNQVSLWCLVKGFYPSDIAVEWESNGQPENNYKTTPPVL DSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLS

			LSPGGSPGVPLSLYSGPAPTSSSTKKTQLQLEHLLLDLQMILNGINN YKNPKLTAMLTAKFAMPKKATELKHLQCLEEALKPLEEVLNLA QSKNFHLRPRDLISNINVIVLELKGSETTFMCEYADETATIVEFLN RWITFAQSIISTLT
2nd polypeptide chain - F (HL-L2-C)	50	AK471	DKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMASRTPEVTCVVVDVSHEDP EVKFNWYVDGVEVHNAKTKPREEQYASTYRVVSVLTVLHQDWLNGKEY KCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPCRDELTKNQVSLWCLVK GFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRW QQGNVFSCSVMHEALHNHYTQKSLSLSPGGSPGVPLSLYSGP APTSSSTKKTQLQLEHLLLDLQMILNGINNYKNPKLTAMLTAKFAMPKK ATELKHLQCLEEALKPLEEVLNLAQSKNFHLRPRDLISNINVIVLELKGSE TTFMCEYADETATIVEFLNRWITFAQSIISTLT
2nd polypeptide chain - G (HL-L2-C)	51	AK442	DKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNW
		AK438	YVDGVEVHNAKTKPREEQYASTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAP
		AK530	IEKTISKAKGQPREPQVYTLPPCRDELTKNQVSLWCLVKGFYPSDIAVEWESNGQPE
		AK252	NNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLS
		AK523	PGGGSSPPGGGSSGGGSGPAPTSSSTKKTQLQLEHLLLDLQMILNGINNYKNPKLTAM
		AK524	LTAKFAMPKKATELKHLQCLEEALKPLEEVLNLAQSKNFHLRPRDLISNINVIVLELKG
		AK525	SETTFMCEYADETATIVEFLNRWITFAQSIISTLT
2nd polypeptide chain - H (HL-L2-C)	128	AK539	DKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNW YVDGVEVHNAKTKPREEQYASTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPI EKTISKAKGQPREPQVYTLPPCRDELTKNQVSLWCLVKGFYPSDIAVEWESNGQP ENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSL SPGGGPSDLLAVVAASSGPAPTSSSTKKTQLQLEHLLLDLQMILNGINNYKNPKLTA

			MLTAKFAMPKKATELKHLQCLEEALKPLEEVLNLAQSKNFHLRPRDLISNINVIVL ELKGSETTFMCEYADETATIVEFLNRWITFAQSIISTLT
2nd polypeptide chain - H (HL-L2-C)	129	AK540	DKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYV DGVEVHNAKTKPREEQYASTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTI SKAKGQPREPQVYTLPPCRDELTKNQVSLWCLVKGFYPSDIAVEWESNGQPENNYK TTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGGSG PSDLLAVVAASSGPAPTSSSTKKTQLQLEHLLLDLQMILNGINNYKNPKLTAMLTAK FAMPKKATELKHLQCLEEALKPLEEVLNLAQSKNFHLRPRDLISNINVIVLELK GSETTFMCEYADETATIVEFLNRWITFAQSIISTLT
2nd polypeptide chain - H (HL-L2-C)	130	AK541	DKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNW YVDGVEVHNAKTKPREEQYASTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAP IEKTISKAKGQPREPQVYTLPPCRDELTKNQVSLWCLVKGFYPSDIAVEWESNGQPEN NYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPG GSSGGPDLLAVVAASSGPAPTSSSTKKTQLQLEHLLLDLQMILNGINNYKNPKLTAMLTA KFAMPKKATELKHLQCLEEALKPLEEVLNLAQSKNFHLRPRDLISNINVIVLELKGSETT FMCEYADETATIVEFLNRWITFAQSIISTLT
Cleavage product CP	52	AK168	LYHPSGPAPTSSSTKKTQLQLEHLLLDLQMILNGINNYKNPKLTA MLTAKFAMPKKATELKHLQCLEEALKPLEEVLNLAQSKNFHLR PRDLISNINVIVLELKGSETTFMCEYADETATIVEFLNRWITFAQ SIISTLT
Cleavage product CP	53	AK191	VKSPSGPAPTSSSTKKTQLQLEHLLLDLQMILNGINNYKNPKLT AMLTAKFAMPKKATELKHLQCLEEALKPLEEVLNLAQSKNFH LRPRDLISNINVIVLELKGSETTFMCEYADETATIVEFLNRWITFA

			QSIISTLT
Cleavage product CP	54	AK197	LTVSGPAPTSSSTKKTQLQLEHLLLDLQMILNGINNYKNPKLTA MLTAKFAMPKKATELKHLQCLEEALKPLEEVLNLAQSKNFHLR PRDLISNINVIVLELKGSETTFMCEYADETATIVEFLNRWITFAQ SIISTLT
Cleavage product CP	55	AK203	FMLTSGPAPTSSSTKKTQLQLEHLLLDLQMILNGINNYKNPKLT AMLTAKFAMPKKATELKHLQCLEEALKPLEEVLNLAQSKNFH LRPRDLISNINVIVLELKGSETTFMCEYADETATIVEFLNRWITF AQSIISTLT
Cleavage product CP	56	AK209	LYSGPAPTSSSTKKTQLQLEHLLLDLQMILNGINNYKNPKLTAM
		AK341	LTAKFAMPKKATELKHLQCLEEALKPLEEVLNLAQSKNFHLR
		AK471	PRDLISNINVIVLELKGSETTFMCEYADETATIVEFLNRWITFA QSIISTLT
Cleavage product CP	131	AK442	DKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVD GVEVHNAKTKPREEQYASTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISK AKGQPREPQVYTLPPCRDELTKNQVSLWCLVKGFYPSDIAVEWESNGQPENNYKTTPPV LDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGGGSSPPGGGSS GGGSGPAPTSSSTKKTQLQLEHLLLDLQMILNGINNYKNPKLTAMLTAKFAMPKKATELK HLQCLEEALKPLEEVLNLAQSKNFHLRPRDLISNINVIVLELKGSETTFMCEYADETATIV EFLNRWITFAQSIISTLT; (2nd polypeptide chain – SEQ ID NO: 131) DKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVV VDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYASTYRVVSVLTVLHQDWLNGKEYK CKVSNKALPAPIEKTISKAKGQPREPQVCTLPPSRDELTKNQVSLSCAVKGFYPSDIAVE

			WESNGQPENNYKTTPPVLDSDGSFFLVSKLTVDKSRWQQGNVFSCSVMHEALHNHY TQKSLSLSPGGPPSGSSPGDSGG (1st polypeptide chain – SEQ ID NO: 132)
Cleavage product CP	133 134	AK438	DKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDG VEVHNAKTKPREEQYASTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKG QPREPQVYTLPPCRDELTKNQVSLWCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDG SFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGGGSSPPGGGSSGGGSGPAPT SSSTKKTQLQLEHLLLDLQMILNGINNYKNPKLTAMLTAKFAMPKKATELKHLQCLEEALKPLEE VLNLAQSKNFHLRPRDLISNINVIVLELKGSETTFMCEYADETATIVEFLNRWITFAQSIISTLT; (2nd polypeptide chain – SEQ ID NO: 133) DKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHN AKTKPREEQYASTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVCT LPPSRDELTKNQVSLSCAVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLVSKLTVD KSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGGPPSGSSPGVPLS (1st polypeptide chain – SEQ ID NO: 134)
Cleavage product CP	135 136	AK530	DKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPE VKFNWYVDGVEVHNAKTKPREEQYASTYRVVSVLTVLHQDWLNGKEYK CKVSNKALPAPIEKTISKAKGQPREPQVYTLPPCRDELTKNQVSLWCLVKG FYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGN VFSCSVMHEALHNHYTQKSLSLSPGGGSSPPGGGSSGGGSGPAPTSSSTKK TQLQLEHLLLDLQMILNGINNYKNPKLTAMLTAKFAMPKKATELKHLQC LEEALKPLEEVLNLAQSKNFHLRPRDLISNINVIVLELKGSETTFMCEYADET ATIVEFLNRWITFAQSIISTLT; (2nd polypeptide chain – SEQ ID NO: 135)

			DKTHTCPPCPAPELLGGPSVFLFPPKPKDTL MISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYASTYR VVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVCTLP PSRDELTKNQVSLSCAVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGS FFLVSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGGPPSGSSPMPYD (1st polypeptide chain - SEQ ID NO: 136)
Cleavage product	137	AK539	VVAASSGPAPTSSSTKKTQLQLEHLLLLDLQMILNGINNYKNPKLTAMLTAKF
CP		AK540	AMPKKATELKHLQCLEEALKPLEEVLNLAQSKNFHLRPRDLISNINVIVLELK
		AK541	GSETTFMCEYADETATIVEFLNRWITFAQSIISTLT
Cleavage product	138	AK523	DKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNW
CP	139		YVDGVEVHNAKTKPREEQYASTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAP IEKTISKAKGQPREPQVYTLPPCRDELTKNQVSLWCLVKGFYPSDIAVEWESNGQPE NNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLS PGGGSSPPGGGSSGGGSGPAPTSSSTKKTQLQLEHLLLLDLQMILNGINNYKNPKLTAM LTAKFAMPKKATELKHLQCLEEALKPLEEVLNLAQSKNFHLRPRDLISNINVIVLELKG SETTFMCEYADETATIVEFLNRWITFAQSIISTLT (2nd polypeptide chain – SEQ ID NO: 138)
			DKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWY VDGVEVHNAKTKPREEQYASTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEK TISKAKGQPREPQVCTLPPSRDELTKNQVSLSCAVKGFYPSDIAVEWESNGQPENNY KTTPPVLDSDGSFFLVSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGGSPDLLA (1st polypeptide chain- – SEQ ID NO: 139)
Cleavage product	140	AK524	DKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNW
CP	141		YVDGVEVHNAKTKPREEQYASTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAP

			IEKTISKAKGQPREPQVYTLPPCRDELTKNQVSLWCLVKGFYPSDIAVEWESNGQPE
			NNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLS
			PGGGSSPPGGGSSGGGSGPAPTSSSTKKTQLQLEHLLLDLQMILNGINNYKNPKLTAM
			LTAKFAMPKKATELKHLQCLEEALKPLEEVLNLAQSKNFHLRPRDLISNINVIVLELKG
			SETTFMCEYADETATIVEFLNRWITFAQSIISTLT (2nd polypeptide chain - SEQ ID NO: 140)
			DKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWY
			VDGVEVHNAKTKPREEQYASTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEK
			TISKAKGQPREPQVCTLPPSRDELTKNQVSLSCAVKGFYPSDIAVEWESNGQPENNY
			KTTPPVLDSDGSFFLVSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGG
			SPGDLLA (1st polypeptide chain – SEQ ID NO: 141)
Cleavage product CP	142	AK525	DKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNW
	143		YVDGVEVHNAKTKPREEQYASTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAP
			IEKTISKAKGQPREPQVYTLPPCRDELTKNQVSLWCLVKGFYPSDIAVEWESNGQPE
			NNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLS
			PGGGSSPPGGGSSGGGSGPAPTSSSTKKTQLQLEHLLLDLQMILNGINNYKNPKLTAM
			LTAKFAMPKKATELKHLQCLEEALKPLEEVLNLAQSKNFHLRPRDLISNINVIVLELKG
			SETTFMCEYADETATIVEFLNRWITFAQSIISTLT (2nd polypeptide chain – SEQ ID NO: 142)
			DKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWY
			VDGVEVHNAKTKPREEQYASTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEK
			TISKAKGQPREPQVCTLPPSRDELTKNQVSLSCAVKGFYPSDIAVEWESNGQPENNY
			KTTPPVLDSDGSFFLVSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGGSG
			SPSDLLA (1st polypeptide chain - SEQ ID NO: 143)

12.1 Other Sequences:

DESCRIPTION	SEQ ID NO:	SEQUENCE
MM1	60	ELCDDDPPEIPHATFKAMAYKEGTMLNCECKRGFRRIKSGSLYMLCTGNSSHSSWDNQC QCTSSATRNTTKQVTPQPEEQKERKTTEMQSPMQPVDQASLPGHCREPPPWENEATERIY HFVVGQMVYYQCVQGYRALHRGPAESVCKMTHGKTRWTQPQLICT
Linker L1	61	PA
IL-2 domain	62	APTSSSTKKTQLQLEHLLLDLQMILNGINNYKNPKLTRMLTFKFYMPKKATELKHLQCLE EELKPLEEVLNLAQSKNFHLRPRDLISNINVIVLELKGSETTFMCEYADETATIVEFLNRWI TFAQSIISTLT
Linker L2	63	GGGGGGGGGGGGGGGGGGGGGGGGGGGGGGG
MM2	64	AVNGTSQFTCFYNSRANISCVWSQDGALQDTSCQVHAWPDRRRWNQTCELLPVSQASW ACNLILGAPDSQKLTTVDIVTLRVLCREGVRWRVMAIQDFKPFENLRLMAPISLQVVHVE THRCNISWEISQASHYFERHLEFEARTLSPGHTWEEAPLLTLKQKQEWICLETLTPDTQYE FQVRVKPLQ
HL	65	DKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVD GVEVHNAKTKPREEQYASTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKA KGQPREPQVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLD SDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPG
Polypeptide chain	66	DKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVD GVEVHNAKTKPREEQYASTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKA KGQPREPQVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLD SDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGPAELCDDDPPEIP HATFKAMAYKEGTMLNCECKRGFRRIKSGSLYMLCTGNSSHSSWDNQCQCTSSATRNTT

		KQVTPQPEEQKERKTTEMQSPMQPVDQASLPGHCREPPPWENEATERIYHFVVGQMVYY QCVQGYRALHRGPAESVCKMTHGKTRWTQPQLICTGGGGGGGGGGGGGGGGG GGGGGGGGGGGGGAPTSSSTKKTQLQLEHLLLDLQMILNGINNYKNPKLTRMLTFKFY MPKKATELKHLQCLEEELKPLEEVLNLAQSKNFHLRPRDLISNINVIVLELKGSETTFMCE YADETATIVEFLNRWITFAQSIISTLTGPPSGSSPMPYDLYHPSGGGAVNGTSQFTCFYNSR ANISCVWSQDGALQDTSCQVHAWPDRRRWNQTCELLPVSQASWACNLILGAPDSQKLT TVDIVTLRVLCREGVRWRVMAIQDFKPFENLRLMAPISLQWHVETHRCNISWEISQASH YFERHLEFEARTLSPGHTWEEAPLLTLKQKQEWICLETLTPDTQYEFQVRVKPLQ
Polypeptide chain	67	DKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVD GVEVHNAKTKPREEQYASTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKA KGQPREPQVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLD SDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGAPTSSSTKKTQLQ LEHLLLDLQMILNGINNYKNPKLTRMLTFKFYMPKKATELKHLQCLEEELKPLEEVLNLA QSKNFHLRPRDLISNINVIVLELKGSETTFMCEYADETATIVEFLNRWITFAQSIISTLT
Polypeptide chain	68	DKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVD GVEVHNAKTKPREEQYASTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKA KGQPREPQVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLD SDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGAPTSSSTKKTQLQ LEHLLLDLQMILNGINNYKNPKLTAMLTAKFAMPKKATELKHLQCLEEALKPLEEVLNL AQSKNFHLRPRDLISNINVIVLELKGSETTFMCEYADETATIVEFLNRWITFAQSIISTLT
IL-2 domain	69	APTSSSTKKTQLQLEHLLLDLQMILNGINNYKNPKLTRMLTFKFYMPKKATELKHLQCLE ERLKPLEEVLNLAQSKNFHLRPRDLISNINVIVLELKGSETTFMCEYADETATIVEFLNRWI TFAQSIISTLT
IL-2 domain	70	APTSSSTKKTQLQLEHLLLDLQMILNGINNYKNPKLTRMLTEKFYMPKKATELKHLQCLE

		EELKPLEEVLNLAQSKNFHLRPRDLISNINVIVLELKGSETTFMCEYADETATIVEFLNRWI TFAQSIISTLT
IL-2 domain	71	APTSSSTKKTQLQLEHLLLLDLQMILNGINNYKNPKLTRMLTFKFYMPKKATELKHLQCLE FSLKPLFFVLNLAQSKNFHLRPRDLISNINVIVLFLKGSETTFMCFYADFTATIVFFLNRWI TFAQSIISTLT
IL-2 domain	72	APTSSSTKKTQLQLEHLLLLDLQMILNGINNYKNPKLTRMLTAKFYMPKKATELKHLQCLE EELKPLEEVLNLAQSKNFHLRPRDLISNINVIVLELKGSETTFMCEYADETATIVEFLNRWI TFAQSIISTLT
IL-2 domain	73	APTSSSTKKTQLQLEHLLLLDLQMILNGINNYKNPKLTRMLTFKFRMPKKATELKHLQCLE EELKPLEEVLNLAQSKNFHLRPRDLISNINVIVLELKGSETTFMCEYADETATIVEFLNRWI TFAQSIISTLT
IL-2 domain	74	APTSSSTKKTQLQLEHLLLLDLQMILNGINNYKNPKLTRMLTSKFYMPKKATELKHLQCLE ESLKPLEEVLNLAQSKNFHLRPRDLISNINVIVLELKGSETTFMCEYADETATIVEFLNRWI TFAQSIISTLT
Linker L1	75	PGSG
Linker L1	76	GGSSPPRAAAVKSPSGP
Linker L1	77	GGPGGPRAAAAVKSPSGP
Linker L1	78	GSPGVPLSLYSGP
HL	79	DKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVD GVEVHNAKTKPREEQYASTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKA KGQPREPQVCTLPPSRDELTKNQVSLSCAVKGFYPSDIAVEWESNGQPENNYKTTPPVLD SDGSFFLVSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGK
HL	80	DKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVD GVEVHNAKTKPREEQYASTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKA

		KGQPREPQVYTLPPCRKELTKNQVSLWCLVKGFYPSDIAVEWESNGQPENNYKTTPPVL
		KSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPG
HL	81	DKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVD
		GVEVHNAKTKPREEQYASTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKA
		KGQPREPQVCTLPPSRDELTKNQVSLSCAVKGFYPSDIAVEWESNGQPENNYDTTPPVLD
		SDGSFFLVSDLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPG
HL	82	DKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVD
		GVEVHNAKTKPREEQYASTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKA
		KGQPREPQVYTLPPCRKKLTKNQVSLWCLVKGFYPSDIAVEWESNGQPENNYKTTPPVL
		DSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPG
HL	83	DKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVD
		GVEVHNAKTKPREEQYASTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKA
		KGQPREPQVCTLPPSRDELTKNQVSLSCAVEGFYPSDIAVEWESNGQPENNYKTTPPVLD
		SDGSFFLVSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQESLSLSPG
HL	84	DKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVD
		GVEVHNAKTKPREEQYASTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKA
		KGQPREPQVYTLPPCRKKLTKNQVSLWCLVKGFYPSDIAVEWESNGQPENNYKTTPPVL
		DSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGK
Polypeptide chain	85	DKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVD
		GVEVHNAKTKPREEQYASTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKA
		KGQPREPQVYTLPPCRDELTKNQVSLWCLVKGFYPSDIAVEWESNGQPENNYKTTPPVL
		DSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGGGSSPPMPYDL
		YHPSGPAPTSSSTKKTQLQLEHLLLDLQMILNGINNYKNPKLTRMLTFKFYMPKKATELK
		HLQCLEEELKPLEEVLNLAQSKNFHLRPRDLISNINVIVLELKGSETTFMCEYADETATIVE

		FLNRWITFAQSIISTLT
Polypeptide chain	86	DKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVD GVEVHNAKTKPREEQYASTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKA KGQPREPQVYTLPPCRKELTKNQVSLWCLVKGFYPSDIAVEWESNGQPENNYKTTPPVL KSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGGPPSGSSPMPY DLYHPSGGGAVNGTSQFTCFYNSRANISCVWSQDGALQDTSCQVHAWPDRRRWNQTCE LLPVSQASWACNLILGAPDSQKLTTVDIVTLRVLCREGVRWRVMAIQDFKPFENLRLMAP ISLQVVHVETHRCNISWEISQASHYFERHLEFEARTLSPGHTWEEAPLLTLKQKQEWICLE TLTPDTQYEFQVRVKPLQGEFTTWSPWSQPLAFRTKPAALGKD
Polypeptide chain	87	DKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVD GVEVHNAKTKPREEQYASTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKA KGQPREPQVCTLPPSRDELTKNQVSLSCAVKGFYPSDIAVEWESNGQPENNYDTTPPVLD SDGSFFLVSDLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGAPTSSSTKKTQLQ LEHLLLDLQMILNGINNYKNPKLTRMLTFKFYMPKKATELKHLQCLEEELKPLEEVLNLA QSKNFHLRPRDLISNINVIVLELKGSETTFMCEYADETATIVEFLNRWITFAQSIISTLT
Polypeptide chain	88	DKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVD GVEVHNAKTKPREEQYASTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKA KGQPREPQVYTLPPCRKKLTKNQVSLWCLVKGFYPSDIAVEWESNGQPENNYKTTPPVL DSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGGPPSGSSPMPY DLYHPSGGGAVNGTSQFTCFYNSRANISCVWSQDGALQDTSCQVHAWPDRRRWNQTCE LLPVSQASWACNLILGAPDSQKLTTVDIVTLRVLCREGVRWRVMAIQDFKPFENLRLMAP ISLQVVHVETHRCNISWEISQASHYFERHLEFEARTLSPGHTWEEAPLLTLKQKQEWICLE TLTPDTQYEFQVRVKPLQGEFTTWSPWSQPLAFRTKPAALGKD
Polypeptide chain	89	DKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVD

		GVEVHNAKTKPREEQYASTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKA KGQPREPQVCTLPPSRDELTKNQVSLSCAVEGFYPSDIAVEWESNGQPENNYKTTPPVLD SDGSFFLVSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQESLSLSPGAPTSSSTKKTQLQ LEHLLLDLQMILNGINNYKNPKLTRMLTFKFYMPKKATELKHLQCLEEELKPLEEVLNLA QSKNFHLRPRDLISNINVIVLELKGSETTFMCEYADETATIVEFLNRWITFAQSIISTLT
Polypeptide chain	90	DKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVD GVEVHNAKTKPREEQYASTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKA KGQPREPQVCTLPPSRDELTKNQVSLSCAVEGFYPSDIAVEWESNGQPENNYKTTPPVLD SDGSFFLVSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQESLSLSPGGGSSPPMPYDLY HPSGPAPTSSSTKKTQLQLEHLLLDLQMILNGINNYKNPKLTRMLTFKFYMPKKATELKH LQCLEEELKPLEEVLNLAQSKNFHLRPRDLISNINVIVLELKGSETTFMCEYADETATIVEF LNRWITFAQSIISTLT
Polypeptide chain	91	DKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVD GVEVHNAKTKPREEQYASTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKA KGQPREPQVYTLPPCRKKLTKNQVSLWCLVKGFYPSDIAVEWESNGQPENNYKTTPPVL DSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGK
Polypeptide chain	92	DKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVD GVEVHNAKTKPREEQYASTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKA KGQPREPQVYTLPPCRKKLTKNQVSLWCLVKGFYPSDIAVEWESNGQPENNYKTTPPVL DSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGPGSGAVNGTSQ FTCFYNSRANISCVWSQDGALQDTSCQVHAWPDRRRWNQTCELLPVSQASWACNLILG APDSQKLTTVDIVTLRVLCREGVRWRVMAIQDFKPFENLRLMAPISLQWHVETHRCNIS WEISQASHYFERHLEFEARTLSPGHTWEEAPLLTLKQKQEWICLETLTPDTQYEFQVRVK PLQGEFTTWSPWSQPLAFRTKPAALGKD

Polypeptide chain	93	DKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVD GVEVHNAKTKPREEQYASTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKA KGQPREPQVYTLPPCRDELTKNQVSLWCLVKGFYPSDIAVEWESNGQPENNYKTTPPVL DSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGGGSSPPMPYDL YHPSGPAPTSSSTKKTQLQLEHLLLDLQMILNGINNYKNPKLTAMLTAKFAMPKKATELK HLQCLEEALKPLEEVLNLAQSKNFHLRPRDLISNINVIVLELKGSETTFMCEYADETATIVE FLNRWITFAQSIISTLT
Polypeptide chain	94	DKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVD GVEVHNAKTKPREEQYASTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKA KGQPREPQVYTLPPCRDELTKNQVSLWCLVKGFYPSDIAVEWESNGQPENNYKTTPPVL DSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGGGPGRAAAV KSPSGPAPTSSSTKKTQLQLEHLLLDLQMILNGINNYKNPKLTRMLTFKFYMPKKATELK HLQCLEEELKPLEEVLNLAQSKNFHLRPRDLISNINVIVLELKGSETTFMCEYADETATIVE FLNRWITFAQSIISTLT
Polypeptide chain	95	DKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVD GVEVHNAKTKPREEQYASTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKA KGQPREPQVYTLPPCRDELTKNQVSLWCLVKGFYPSDIAVEWESNGQPENNYKTTPPVL DSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGGGSSPPRAAAV KSPSGPAPTSSSTKKTQLQLEHLLLDLQMILNGINNYKNPKLTAMLTAKFAMPKKATELK HLQCLEEALKPLEEVLNLAQSKNFHLRPRDLISNINVIVLELKGSETTFMCEYADETATIVE FLNRWITFAQSIISTLT
Polypeptide chain	96	DKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVD GVEVHNAKTKPREEQYASTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKA KGQPREPQVYTLPPCRDELTKNQVSLWCLVKGFYPSDIAVEWESNGQPENNYKTTPPVL

		DSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGGGPGGPRAAAV KSPSGPAPTSSSTKKTQLQLEHLLLDLQMILNGINNYKNPKLTAMLTAKFAMPKKATELK HLQCLEEALKPLEEVLNLAQSKNFHLRPRDLISNINVIVLELKGSETTFMCEYADETATIVE FLNRWITFAQSIISTLT
Polypeptide chain	97	DKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVD GVEVHNAKTKPREEQYASTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKA KGQPREPQVYTLPPCRDELTKNQVSLWCLVKGFYPSDIAVEWESNGQPENNYKTTPPVL DSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGGGSGRAAAVKS PSGPAPTSSSTKKTQLQLEHLLLDLQMILNGINNYKNPKLTRMLTFKFYMPKKATELKHL QCLEEELKPLEEVLNLAQSKNFHLRPRDLISNINVIVLELKGSETTFMCEYADETATIVEFL NRWITFAQSIISTLT
Polypeptide chain	98	DKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVD GVEVHNAKTKPREEQYASTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKA KGQPREPQVYTLPPCRDELTKNQVSLWCLVKGFYPSDIAVEWESNGQPENNYKTTPPVL DSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGGGSSPPGGGSS GGGSGPAPTSSSTKKTQLQLEHLLLDLQMILNGINNYKNPKLTAMLTAKFAMPKKATEL KHLQCLEEALKPLEEVLNLAQSKNFHLRPRDLISNINVIVLELKGSETTFMCEYADETATI VEFLNRWITFAQSIISTLT
Polypeptide chain	99	DKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVD GVEVHNAKTKPREEQYASTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKA KGQPREPQVYTLPPCRDELTKNQVSLWCLVKGFYPSDIAVEWESNGQPENNYKTTPPVL DSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGGGSSPPMPYDL YHPSGPAPTSSSTKKTQLQLEHLLLDLQMILNGINNYKNPKLTRMLTFKFYMPKKATELK HLQCLEERLKPLEEVLNLAQSKNFHLRPRDLISNINVIVLELKGSETTFMCEYADETATIVE

		FLNRWITFAQSIISTLT
Polypeptide chain	100	DKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVD GVEVHNAKTKPREEQYASTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKA KGQPREPQVYTLPPCRDELTKNQVSLWCLVKGFYPSDIAVEWESNGQPENNYKTTPPVL DSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGGGSSPPMPYDL YHPSGPAPTSSSTKKTQLQLEHLLLDLQMILNGINNYKNPKLTRMLTEKFYMPKKATELK HLQCLEEELKPLEEVLNLAQSKNFHLRPRDLISNINVIVLELKGSETTFMCEYADETATIVE FLNRWITFAQSIISTLT
Polypeptide chain	101	DKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVD GVEVHNAKTKPREEQYASTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKA KGQPREPQVYTLPPCRDELTKNQVSLWCLVKGFYPSDIAVEWESNGQPENNYKTTPPVL DSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGGGSSPPMPYDL YHPSGPAPTSSSTKKTQLQLEHLLLDLQMILNGINNYKNPKLTRMLTFKFYMPKKATELK HLQCLEESLKPLEEVLNLAQSKNFHLRPRDLISNINVIVLELKGSETTFMCEYADETATIVE FLNRWITFAQSIISTLT
Polypeptide chain	102	DKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVD GVEVHNAKTKPREEQYASTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKA KGQPREPQVYTLPPCRDELTKNQVSLWCLVKGFYPSDIAVEWESNGQPENNYKTTPPVL DSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGGGSSPPMPYDL YHPSGPAPTSSSTKKTQLQLEHLLLDLQMILNGINNYKNPKLTRMLTAKFYMPKKATELK HLQCLEEELKPLEEVLNLAQSKNFHLRPRDLISNINVIVLELKGSETTFMCEYADETATIVE FLNRWITFAQSIISTLT
Polypeptide chain	103	DKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVD GVEVHNAKTKPREEQYASTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKA

		KGQPREPQVYTLPPCRDELTKNQVSLWCLVKGFYPSDIAVEWESNGQPENNYKTTPPVL
		DSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGGGSSPPMPYDL
		YHPSGPAPTSSSTKKTQLQLEHLLLLDLQMILNGINNYKNPKLTRMLTFKFRMPKKATELK
		HLQCLEEELKPLEEVLNLAQSKNFHLRPRDLISNINVIVLELKGSETTFMCEYADETATIVE
		FLNRWITFAQSIISTLT
Polypeptide chain	104	DKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVD
		GVEVHNAKTKPREEQYASTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKA
		KGQPREPQVYTLPPCRDELTKNQVSLWCLVKGFYPSDIAVEWESNGQPENNYKTTPPVL
		DSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGGGSSPPMPYDL
		YHPSGPAPTSSSTKKTQLQLEHLLLLDLQMILNGINNYKNPKLTRMLTSKFYMPKKATELK
		HLQCLFFSLKPLFEVLNLAQSKNFHLRPRDLISNINVIVLELKGSFTTFMCEYADETATIVE
		FLNRWITFAQSIISTLT
Polypeptide chain	105	DKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVD
		GVEVHNAKTKPREEQYASTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKA
		KGQPREPQVYTLPPCRDELTKNQVSLWCLVKGFYPSDIAVEWESNGQPENNYKTTPPVL
		DSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGGSPGVPLSLYSG
		PAPTSSSTKKTQLQLEHLLLLDLQMILNGINNYKNPKLTRMLTFKFYMPKKATELKHLQCL
		EERLKPLEEVLNLAQSKNFHLRPRDLISNINVIVLELKGSETTFMCEYADETATIVEFLNR
		WITFAQSIISTLT
Polypeptide chain	106	DKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVD
		GVEVHNAKTKPREEQYASTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKA
		KGQPREPQVYTLPPCRDELTKNQVSLWCLVKGFYPSDIAVEWESNGQPENNYKTTPPVL
		DSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGGSPGVPLSLYSG
		PAPTSSSTKKTQLQLEHLLLLDLQMILNGINNYKNPKLTRMLTEKFYMPKKATELKHLQCL

		EEELKPLEEVLNLAQSKNFHLRPRDLISNINVIVLELKGSETTFMCEYADETATIVEFLNRW ITFAQSIISTLT
Polypeptide chain	107	DKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVD GVEVHNAKTKPREEQYASTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKA KGQPREPQVYTLPPCRDELTKNQVSLWCLVKGFYPSDIAVEWESNGQPENNYKTTPPVL DSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGGSPGVPLSLYSG PAPTSSSTKKTQLQLEHLLLDLQMILNGINNYKNPKLTRMLTFKFYMPKKATELKHLQCL EESLKPLEEVLNLAQSKNFHLRPRDLISNINVIVLELKGSETTFMCEYADETATIVEFLNRW ITFAQSIISTLT
Polypeptide chain	108	DKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVD GVEVHNAKTKPREEQYASTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKA KGQPREPQVYTLPPCRDELTKNQVSLWCLVKGFYPSDIAVEWESNGQPENNYKTTPPVL DSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGGSPGVPLSLYSG PAPTSSSTKKTQLQLEHLLLDLQMILNGINNYKNPKLTRMLTAKFYMPKKATELKHLQCL EEELKPLEEVLNLAQSKNFHLRPRDLISNINVIVLELKGSETTFMCEYADETATIVEFLNRW ITFAQSIISTLT
Polypeptide chain	109	DKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVD GVEVHNAKTKPREEQYASTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKA KGQPREPQVYTLPPCRDELTKNQVSLWCLVKGFYPSDIAVEWESNGQPENNYKTTPPVL DSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGGSPGVPLSLYSG PAPTSSSTKKTQLQLEHLLLDLQMILNGINNYKNPKLTRMLTFKFRMPKKATELKHLQCL EEELKPLEEVLNLAQSKNFHLRPRDLISNINVIVLELKGSETTFMCEYADETATIVEFLNRW ITFAQSIISTLT
Polypeptide chain	110	DKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVD

		GVEVHNAKTKPREEQYASTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKA KGQPREPQVYTLPPCRDELTKNQVSLWCLVKGFYPSDIAVEWESNGQPENNYKTTPPVL DSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGGSPGVPLSLYSG PAPTSSSTKKTQLQLEHLLLDLQMILNGINNYKNPKLTRMLTSKFYMPKKKATELKHLQCL EESLKPLEEVLNLAQSKNFHLRPRDLISNINVIVLELKGSETTFMCEYADETATFVEFLNRW ITFAQSIISTLT
Polypeptide chain	111	DKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVD GVEVHNAKTKPREEQYASTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKA KGQPREPQVYTLPPCRDELTKNQVSLWCLVKGFYPSDIAVEWESNGQPENNYKTTPPVL DSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGGSPGVPLSLYSG PAPTSSSTKKTQLQLEHLLLDLQMILNGINNYKNPKLTRMLTFKFYMPKKKATELKHLQCL EEELKPLEEVLNLAQSKNFHLRPRDLISNINVIVLELKGSETTFMCEYADETATIVEFLNRW ITFAQSIISTLT

12.2 **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.

olecule	name	newnames	Component1Sequence	Component2Sequence	Component3Sequence
			DKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDP EVKFNWYVDGVEVHNAKTKPREEQYASTYRVVSVLTVLHQDWLNGKEYK		AVNGTSQFTCFYNSRANISCVWSQDGALQDTSCQVHAWPDRRR WNQTCELLPVSQASWACNLILGAPDSQKLTTVDIVTLRVLCREGVR
368	DNA187	Hole: hFc(N297A)-hCD122	CKVSNKALPAPIEKTISKAKGQPREPQVCTLPPSRDELTKNQVSLSCAVKGF YPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLVSKLTVDKSRWQQGN VFSCSVMHEALHNHYTQKSLSLSPG	PGSGS	WRVMAIQDFKPFENLRLMAPISLQVVHVETHRCNISWEISQASHYF ERHLEFEARTLSPGHTWEEAPLLTLKQKQEWICLETLTPDTQYEFQV RVKPLQGEFTTWSPWSQPLAFRTKPAALGKD
			DKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDP		
		Knob: hFc(N297A)-	EVKFNWYVDGVEVHNAKTKPREEQYASTYRVVSVLTVLHQDWLNGKEYK		
368	DNA476	[NPMGSDPVNFKLLRVVNG]- hIL2(F42S, E62S, C125A)	CKVSNKALPAPIEKTISKAKGQPREPQVYTLPPCRDELTKNQVSLWCLVKG FYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGN VFSCSVMHEALHNHYTQKSLSLSPG	G	NPMGSDPVNFKLLRVVNG
			TIKPCPPCKCPAPNAAGGPSVFIFPPKIKDVLMISLSPIVTCVVVDVSEDDPD		
		Knob: mFclgG2a(LALAPG)-	VQISWFVNNVEVHTAQTQTHREDYNSTLRVVSALPIQHQDWMSGKEFK		APTSSSTKKTQLQLEHLLLDLQMILNGINNYKNPKLTAMLTAKFAMF
375	DNA477	hIL2(R38A, F42A, Y45A, E62A, C125A)	CKVNNKDLGAPIERTISKPKGSVRAPQVYVLPPCEEEMTKKQVTLWCMVT DFMPEDIYVEWTNNGKTELNYKNTEPVLDSDGSYFMYSKLRVEKKNWVE RNSYSCSVVHEGLHNHHTTKSFSRTPG	GGSSPPGGGSSGGGSGP	KKATELKHLQCLEEALKPLEEVLNLAQSKNFHLRPRDLISNINVIVLEL KGSETTFMCEYADETATIVEFLNRWITFAQSIISTLT
			TIKPCPPCKCPAPNAAGGPSVFIFPPKIKDVLMISLSPIVTCVVVDVSEDDPD		
			VQISWFVNNVEVHTAQTQTHREDYNSTLRVVSALPIQHQDWMSGKEFK		
375	DNA479	Hole: mFclgG2a(LALAPG)	CKVNNKDLGAPIERTISKPKGSVRAPQVCVLPPPEEEMTKKQVTLSCAVTD FMPEDIYVEWTNNGKTELNYKNTEPVLDSDGSYFMVSKLRVEKKNWVER NSYSCSVVHEGLHNHHTTKSFSRTPG		
			TIKPCPPCKCPAPNAAGGPSVFIFPPKIKDVLMISLSPIVTCVVVDVSEDDPD		
		Knob: mFclgG2a(LALAPG)-	VQISWFVNNVEVHTAQTQTHREDYNSTLRVVSALPIQHQDWMSGKEFK		
376	DNA478	[VPLSLY]-hIL2(R38A, F42A, Y45A, E62A, C125A)	CKVNNKDLGAPIERTISKPKGSVRAPQVYVLPPCEEEMTKKQVTLWCMVT DFMPEDIYVEWTNNGKTELNYKNTEPVLDSDGSYFMYSKLRVEKKNWVE RNSYSCSVVHEGLHNHHTTKSFSRTPG	GSPG	VPLSLY
			TIKPCPPCKCPAPNAAGGPSVFIFPPKIKDVLMISLSPIVTCVVVDVSEDDPD		
			VQISWFVNNVEVHTAQTQTHREDYNSTLRVVSALPIQHQDWMSGKEFK		
376	DNA479	Hole: mFclgG2a(LALAPG)	CKVNNKDLGAPIERTISKPKGSVRAPQVCVLPPPEEEMTKKQVTLSCAVTD FMPEDIYVEWTNNGKTELNYKNTEPVLDSDGSYFMVSKLRVEKKNWVER NSYSCSVVHEGLHNHHTTKSFSRTPG		

			TIKPCPPCKCPAPNAAGGPSVFIFPPKIKDVLMISLSPIVTCVVVDVSEDDPD	
	Knob: mFclgG2a(LALAPG)-		VQISWFVNNVEVHTAQTQTHREDYNSTLRVVSALPIQHQDWMSGKEFK	APTSSSTKKTQLQLEHLLLDLQMILNGINNYKNPKLTAMLTAKFAMF
377	DNA477	hIL2(R38A, F42A, Y45A, E62A, C125A)	CKVNNKDLGAPIERTISKPKGSVRAPQVYVLPPCEEEMTKKQVTLWCMVT GGSSPPGGGSSGGGSGP	KKATELKHLQCLEEALKPLEEVLNLAQSKNFHLRPRDLISNINVIVLEL
			DFMPEDIYVEWTNNGKTELNYKNTEPVLDSDGSYFMYSKLRVEKKNWVE	KGSETTFMCEYADETATIVEFLNRWITFAQSIISTLT
			RNSYSCSVVHEGLHNHHTTKSFSRTPG	
			TIKPCPPCKCPAPNAAGGPSVFIFPPKIKDVLMISLSPIVTCVVVDVSEDDPD	AVNGTSQFTCFYNSRANISCVWSQDGALQDTSCQVHAWPDRRR
			VQISWFVNNVEVHTAQTQTHREDYNSTLRVVSALPIQHQDWMSGKEFK	WNQTCELLPVSQASWACNLILGAPDSQKLTTVDIVTLRVLCREGVR
377	DNA480	Hole: mFclgG2a(LALAPG)- hCD122	CKVNNKDLGAPIERTISKPKGSVRAPQVCVLPPPEEEMTKKQVTLSCAVTD PGSGS	WRVMAIQDFKPFENLRLMAPISLQVVHVETHRCNISWEISQASHYF
			FMPEDIYVEWTNNGKTELNYKNTEPVLDSDGSYFMVSKLRVEKKNWVER	ERHLEFEARTLSPGHTWEEAPLLTLKQKQEWICLETLTPDTQYEFQV
			NSYSCSVVHEGLHNHHTTKSFSRTPG	RVKPLQGEFTTWSPWSQPLAFRTKPAALGKD
			TIKPCPPCKCPAPNAAGGPSVFIFPPKIKDVLMISLSPIVTCVVVDVSEDDPD	
	Knob: mFclgG2a(LALAPG)-		VQISWFVNNVEVHTAQTQTHREDYNSTLRVVSALPIQHQDWMSGKEFK	
378	DNA478	[VPLSLY]-hIL2(R38A, F42A, Y45A, E62A, C125A)	CKVNNKDLGAPIERTISKPKGSVRAPQVYVLPPCEEEMTKKQVTLWCMVT GSPG	VPLSLY
			DFMPEDIYVEWTNNGKTELNYKNTEPVLDSDGSYFMYSKLRVEKKNWVE	
			RNSYSCSVVHEGLHNHHTTKSFSRTPG	
			TIKPCPPCKCPAPNAAGGPSVFIFPPKIKDVLMISLSPIVTCVVVDVSEDDPD	AVNGTSQFTCFYNSRANISCVWSQDGALQDTSCQVHAWPDRRR
			VQISWFVNNVEVHTAQTQTHREDYNSTLRVVSALPIQHQDWMSGKEFK	WNQTCELLPVSQASWACNLILGAPDSQKLTTVDIVTLRVLCREGVR
378	DNA480	Hole: mFclgG2a(LALAPG)- hCD122	CKVNNKDLGAPIERTISKPKGSVRAPQVCVLPPPEEEMTKKQVTLSCAVTD PGSGS	WRVMAIQDFKPFENLRLMAPISLQVVHVETHRCNISWEISQASHYF
			FMPEDIYVEWTNNGKTELNYKNTEPVLDSDGSYFMVSKLRVEKKNWVER	ERHLEFEARTLSPGHTWEEAPLLTLKQKQEWICLETLTPDTQYEFQV
			NSYSCSVVHEGLHNHHTTKSFSRTPG	RVKPLQGEFTTWSPWSQPLAFRTKPAALGKD
			DKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDP	
			EVKFNWYVDGVEVHNAKTKPREEQYASTYRVVSVLTVLHQDWLNGKEYK	
397	DNA158	Hole: hFc(N297A)	CKVSNKALPAPIEKTISKAKGQPREPQVCTLPPSRDELTKNQVSLSCAVKGF	
			YPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLVSKLTVDKSRWQQGN	
			VFSCSVMHEALHNHYTQKSLSLSPG	
			DKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDP	
			EVKFNWYVDGVEVHNAKTKPREEQYASTYRVVSVLTVLHQDWLNGKEYK	
397	DNA278	Knob: hFc(N297A)-[DSGGFMLT]-hIL2(C125A)	CKVSNKALPAPIEKTISKAKGQPREPQVYTLPPCRDELTKNQVSLWCLVKG GSGP	DSGGFMLT
			FYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGN	
			VFSCSVMHEALHNHYTQKSLSLSPG	

			TIKPCPPCKCPAPNAAGGPSVFIFPPKIKDVLMISLSPIVTCVVVDVSEDDPD		
429	DNA477	Knob: mFclgG2a(LALAPG)-hIL2(R38A, F42A, Y45A, E62A, C125A)	VQISWFVNNVEVHTAQTQTHREDYNSTLRVVSALPIQHQDWMSGKEFK CKVNNKDLGAPIERTISKPKGSVRAPQVYVLPPCEEEMTKKQVTLWCMVT GGSSPPGGGSSGGGSGGP DFMPEDIYVEWTNNGKTELNYKNTEPVLDSDGSYFMYSKLRVEKKNWVE RNSYSCSVVHEGLHNHHTTKSFSRTPG	APTSSSTKKTQLQLEHLLLDLQMILNGINNYKNPKLTAMLTAKFAMF KKATELKHLQCLEEALKPLEEVLNLAQSKNFHLRPRDLISNINVIVLEL KGSETTFMCEYADETATIVEFLNRWITFAQSIISTLT	
			TIKPCPPCKCPAPNAAGGPSVFIFPPKIKDVLMISLSPIVTCVVVDVSEDDPD		
429	DNA520	Hole: mFclgG2a(LALAPG)-NoAnnotationFound	VQISWFVNNVEVHTAQTQTHREDYNSTLRVVSALPIQHQDWMSGKEFK CKVNNKDLGAPIERTISKPKGSVRAPQVCVLPPPEEEMTKKQVTLSCAVTD HHHHHHHH FMPEDIYVEWTNNGKTELNYKNTEPVLDSDGSYFMVSKLRVEKKNWVER NSYSCSVVHEGLHNHHTTKSFSRTPG		
			TIKPCPPCKCPAPNAAGGPSVFIFPPKIKDVLMISLSPIVTCVVVDVSEDDPD		
430	DNA477	Knob: mFclgG2a(LALAPG)-hIL2(R38A, F42A, Y45A, E62A, C125A)	VQISWFVNNVEVHTAQTQTHREDYNSTLRVVSALPIQHQDWMSGKEFK CKVNNKDLGAPIERTISKPKGSVRAPQVYVLPPCEEEMTKKQVTLWCMVT GGSSPPGGGSSGGGSGGP DFMPEDIYVEWTNNGKTELNYKNTEPVLDSDGSYFMYSKLRVEKKNWVE RNSYSCSVVHEGLHNHHTTKSFSRTPG	APTSSSTKKTQLQLEHLLLDLQMILNGINNYKNPKLTAMLTAKFAMF KKATELKHLQCLEEALKPLEEVLNLAQSKNFHLRPRDLISNINVIVLEL KGSETTFMCEYADETATIVEFLNRWITFAQSIISTLT	
			TIKPCPPCKCPAPNAAGGPSVFIFPPKIKDVLMISLSPIVTCVVVDVSEDDPD		AVNGTSQFTCFYNSRANISCVWSQDGALQDTSCQVHAWPDRRR
430	DNA521	Hole: mFclgG2a(LALAPG)-hCD122-NoAnnotationFound	VQISWFVNNVEVHTAQTQTHREDYNSTLRVVSALPIQHQDWMSGKEFK CKVNNKDLGAPIERTISKPKGSVRAPQVCVLPPPEEEMTKKQVTLSCAVTD PGSGS FMPEDIYVEWTNNGKTELNYKNTEPVLDSDGSYFMVSKLRVEKKNWVER NSYSCSVVHEGLHNHHTTKSFSRTPG		WNQTCELLPVSQASWACNLILGAPDSQKLTTVDIVTLRVLCREGVR WRVMAIQDFKPFENLRLMAPISLQVVHVETHRCNISWEISQASHYF ERHLEFEARTLSPGHTWEEAPLLTLKQKQEWICLETLTPDTQYEFQV RVKPLQGEFTTWSPWSQPLAFRTKPAALGKD
			TIKPCPPCKCPAPNAAGGPSVFIFPPKIKDVLMISLSPIVTCVVVDVSEDDPD		
431	DNA477	Knob: mFclgG2a(LALAPG)-hIL2(R38A, F42A, Y45A, E62A, C125A)	VQISWFVNNVEVHTAQTQTHREDYNSTLRVVSALPIQHQDWMSGKEFK CKVNNKDLGAPIERTISKPKGSVRAPQVYVLPPCEEEMTKKQVTLWCMVT GGSSPPGGGSSGGGSGGP DFMPEDIYVEWTNNGKTELNYKNTEPVLDSDGSYFMYSKLRVEKKNWVE RNSYSCSVVHEGLHNHHTTKSFSRTPG	APTSSSTKKTQLQLEHLLLDLQMILNGINNYKNPKLTAMLTAKFAMF KKATELKHLQCLEEALKPLEEVLNLAQSKNFHLRPRDLISNINVIVLEL KGSETTFMCEYADETATIVEFLNRWITFAQSIISTLT	
			TIKPCPPCKCPAPNAAGGPSVFIFPPKIKDVLMISLSPIVTCVVVDVSEDDPD		AVKNCSHLECFYNSRANVSCMWSHEEALNVTTCHVHAKSNLRHW
431	DNA522	Hole: mFclgG2a(LALAPG)-mCD122-NoAnnotationFound	VQISWFVNNVEVHTAQTQTHREDYNSTLRVVSALPIQHQDWMSGKEFK CKVNNKDLGAPIERTISKPKGSVRAPQVCVLPPPEEEMTKKQVTLSCAVTD PGSGS FMPEDIYVEWTNNGKTELNYKNTEPVLDSDGSYFMVSKLRVEKKNWVER NSYSCSVVHEGLHNHHTTKSFSRTPG		NKTCELTLVRQASWACNLILGSFPESQSLTSVDLLDINVVCWEEKG WRRVKTCDFHPFDNLRLVAPHSLQVLHIDTQRCNISWKVSQVSHYI EPYLEFEAARRLLGHSWEDASVLSLKQRQQWLFLEMLIPSTSYEVQV RVKAQRNNTGTWSPWSQPLTFRTRPADPMKE

			TIKPCPPCKCPAPNAAGGPSVFIFPPKIKDVLMISLSPIVTCVVVDVSEDDPD		
	Knob: mFclgG2a(LALAPG)-		VQISWFVNNVEVHTAQTQTHREDYNSTLRVVSALPIQHQDWMSGKEFK		
432	DNA478	[VPLSLY]-hIL2(R38A, F42A, Y45A, E62A, C125A)	CKVNNKDLGAPIERTISKPKGSVRAPQVYVLPPCEEEMTKKQVTLWCMVT GSPG DFMPEDIYVEWTNNGKTELNYKNTEPVLDSDGSYFMYSKLRVEKKNWVE RNSYSCSVVHEGLHNHHTTKSFSRTPG	VPLSLY	
			TIKPCPPCKCPAPNAAGGPSVFIFPPKIKDVLMISLSPIVTCVVVDVSEDDPD		AVNGTSQFTCFYNSRANISCVWSQDGALQDTSCQVHAWPDRRR
			VQISWFVNNVEVHTAQTQTHREDYNSTLRVVSALPIQHQDWMSGKEFK		WNQTCELLPVSQASWACNLILGAPDSQKLTTVDIVTLRVLCREGVR
432	DNA521	Hole: mFclgG2a(LALAPG)- hCD122-NoAnnotationFound	CKVNNKDLGAPIERTISKPKGSVRAPQVCVLPPPEEEMTKKQVTLSCAVTD PGSGS FMPEDIYVEWTNNGKTELNYKNTEPVLDSDGSYFMVSKLRVEKKNWVER NSYSCSVVHEGLHNHHTTKSFSRTPG		WRVMAIQDFKPFENLRLMAPISLQVVHVETHRCNISWEISQASHYF ERHLEFEARTLSPGHTWEEAPLLTLKQKQEWICLETLTPDTQYEFQV RVKPLQGEFTTWSPWSQPLAFRTKPAALGKD
			TIKPCPPCKCPAPNAAGGPSVFIFPPKIKDVLMISLSPIVTCVVVDVSEDDPD		
	Knob: mFclgG2a(LALAPG)-		VQISWFVNNVEVHTAQTQTHREDYNSTLRVVSALPIQHQDWMSGKEFK		
433	DNA478	[VPLSLY]-hIL2(R38A, F42A, Y45A, E62A, C125A)	CKVNNKDLGAPIERTISKPKGSVRAPQVYVLPPCEEEMTKKQVTLWCMVT GSPG DFMPEDIYVEWTNNGKTELNYKNTEPVLDSDGSYFMYSKLRVEKKNWVE RNSYSCSVVHEGLHNHHTTKSFSRTPG	VPLSLY	
			TIKPCPPCKCPAPNAAGGPSVFIFPPKIKDVLMISLSPIVTCVVVDVSEDDPD		AVKNCSHLECFYNSRANVSCMWSHEEALNVTTCHVHAKSNLRHW
			VQISWFVNNVEVHTAQTQTHREDYNSTLRVVSALPIQHQDWMSGKEFK		NKTCELTLVRQASWACNLILGSFPESQSLTSVDLLDINVVCWEEKG
433	DNA522	Hole: mFclgG2a(LALAPG)- mCD122-NoAnnotationFound	CKVNNKDLGAPIERTISKPKGSVRAPQVCVLPPPEEEMTKKQVTLSCAVTD PGSGS FMPEDIYVEWTNNGKTELNYKNTEPVLDSDGSYFMVSKLRVEKKNWVER NSYSCSVVHEGLHNHHTTKSFSRTPG	VPLSLY	WRRVKTCDFHPFDNLRLVAPHSLQVLHIDTQRCNISWKVSQVSHYI EPYLEFEARRRLLGHSWEDASVLSLKQRQQWLFLEMLIPSTSYEVQV RVKAQRNNTGTWSPWSQPLTFRTRPADPMKE
			DKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDP		
	Knob: hFc(N297A)-[VPLSLY]-		EVKFNWYVDGVEVHNAKTKPREEQYASTYRVVSVLTVLHQDWLNGKEYK		
435	DNA263	hIL2(R38A, F42A, Y45A, E62A, C125A)	CKVSNKALPAPIEKTISKAKGQPREPQVYTLPPCRDELTKNQVSLWCLVKG GSPG FYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGN VFSCSVMHEALHNHYTQKSLSLSPG	VPLSLY	
			EVQLLESGGGLVQPGGSLRLSCAASGFTFSLFTMSVVRQAPGKGLEVVV		DKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSH
			SAISGSGGSTYYADSVKGRFTISRDNSKNTLYLQMNSLRAEDTAVYYCAKS		EDPEVKFNWYVDGVEVHNAKTKPREEQYASTYRVVSVLTVLHQDW
435	DNA516	F8ScFvVersion1-Hole: hFc(N297A)-hCD122	THLYLFDYWGQGTLVTVSSGGGGSGGGGSGGGGSEIVLTQSPGTLSLSPG GGS ERATLSCRASQSVSMPFLAWYQQKPGQAPRLLIYGASSRATGIPDRFSGS GSGTDFTLTISRLEPEDFAVYYCQQMRGRPPTFGQGTKVEIK		LNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVCTLPPSRDELTKN QVSLSCAVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLVS KLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPG

			DKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDP	AVNGTSQFTCFYNSRANISCVWSQDGALQDTSCQVHAWPDRRR
			EVKFNWYVDGVEVHNAKTKPREEQYASTYRVVSVLTVLHQDWLNGKEYK	WNQTCELLPVSQASWACNLILGAPDSQKLTTVDIVTLRVLCREGVR
436	DNA187	Hole: hFc(N297A)-hCD122	CKVSNKALPAPIEKTISKAKGQPREPQVCTLPPSRDELTKNQVSLSCAVKGF PGSGS	WRVMAIQDFKPFENLRLMAPISLQVVHVETHRCNISWEISQASHYF
			YPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLVSKLTVDKSRWQQGN	ERHLEFEARTLSPGHTWEEAPLLTLKQKQEWICLETLTPDTQYEFQV
			VFSCSVMHEALHNHYTQKSLSLSPG	RVKPLQGEFTTWSPWSQPLAFRTKPAALGKD
			DKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDP	APTSSSTKKTQLQLEHLLLDLQMILNGINNYKNPKLTAMLTAKFAMIF
436	DNA542	Knob: hFc(N297A)-hIL2(R38A, F42A, Y45A, E62A, C125A)	EVKFNWYVDGVEVHNAKTKPREEQYASTYRVVSVLTVLHQDWLNGKEYK	KKATELKHLQCLEEALKPLEEVLNLAQSKNFHLRPRDLISNINVIVLEL
			CKVSNKALPAPIEKTISKAKGQPREPQVYTLPPCRDELTKNQVSLWCLVKG GISSGLLSGRSDQPSGP	KGSETTFMCEYADETATIVEFLNRWITFAQSIISTLT
			FYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGN	
			VFSCSVMHEALHNHYTQKSLSLSPG	
			DKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDP	AVNGTSQFTCFYNSRANISCVWSQDGALQDTSCQVHAWPDRRR
			EVKFNWYVDGVEVHNAKTKPREEQYASTYRVVSVLTVLHQDWLNGKEYK	WNQTCELLPVSQASWACNLILGAPDSQKLTTVDIVTLRVLCREGVR
437	DNA187	Hole: hFc(N297A)-hCD122	CKVSNKALPAPIEKTISKAKGQPREPQVCTLPPSRDELTKNQVSLSCAVKGF PGSGS	WRVMAIQDFKPFENLRLMAPISLQVVHVETHRCNISWEISQASHYF
			YPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLVSKLTVDKSRWQQGN	ERHLEFEARTLSPGHTWEEAPLLTLKQKQEWICLETLTPDTQYEFQV
			VFSCSVMHEALHNHYTQKSLSLSPG	RVKPLQGEFTTWSPWSQPLAFRTKPAALGKD
			DKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDP	APTSSSTKKTQLQLEHLLLDLQMILNGINNYKNPKLTAMLTAKFAMIF
437	DNA545	Knob: hFc(N297A)-hIL2(R38A, F42A, Y45A, E62A, C125A)	EVKFNWYVDGVEVHNAKTKPREEQYASTYRVVSVLTVLHQDWLNGKEYK	KKATELKHLQCLEEALKPLEEVLNLAQSKNFHLRPRDLISNINVIVLEL
			CKVSNKALPAPIEKTISKAKGQPREPQVYTLPPCRDELTKNQVSLWCLVKG GISSGLLSGRSSGP	KGSETTFMCEYADETATIVEFLNRWITFAQSIISTLT
			FYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGN	
			VFSCSVMHEALHNHYTQKSLSLSPG	
			DKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDP	APTSSSTKKTQLQLEHLLLDLQMILNGINNYKNPKLTAMLTAKFAMIF
			EVKFNWYVDGVEVHNAKTKPREEQYASTYRVVSVLTVLHQDWLNGKEYK	
438	DNA255	Knob: hFc(N297A)-hIL2(R38A, F42A, Y45A, E62A, C125A)	CKVSNKALPAPIEKTISKAKGQPREPQVYTLPPCRDELTKNQVSLWCLVKG GGSSPPGGGSSGGGSGP	KKATELKHLQCLEEALKPLEEVLNLAQSKNFHLRPRDLISNINVIVLEL
			FYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGN	KGSETTFMCEYADETATIVEFLNRWITFAQSIISTLT
			VFSCSVMHEALHNHYTQKSLSLSPG	
			DKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDP	
			EVKFNWYVDGVEVHNAKTKPREEQYASTYRVVSVLTVLHQDWLNGKEYK	
438	DNA543	Hole: hFc(N297A)-[VPLSLY]-hCD122	CKVSNKALPAPIEKTISKAKGQPREPQVCTLPPSRDELTKNQVSLSCAVKGF GPPSGSSPG	VPLSLY
			YPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLVSKLTVDKSRWQQGN	
			VFSCSVMHEALHNHYTQKSLSLSPG	

			DKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDP	
			EVKFNWYVDGVEVHNAKTKPREEQYASTYRVVSVLTVLHQDWLNGKEYK	
439	DNA158	Hole: hFc(N297A)	CKVSNKALPAPIEKTISKAKGQPREPQVCTLPPSRDELTKNQVSLSCAVKGF	
			YPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLVSKLTVDKSRWQQGN	
			VFSCSVMHEALHNHYTQKSLSLSPG	
			DKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDP	
		Knob: hFc(N297A)-[VPLSLY]-hIL2(R38A, F42A, Y45A, E62A, L80F, R81D, L85V, I86V, I92F, C125A)	EVKFNWYVDGVEVHNAKTKPREEQYASTYRVVSVLTVLHQDWLNGKEYK	
439	DNA544		CKVSNKALPAPIEKTISKAKGQPREPQVYTLPPCRDELTKNQVSLWCLVKG GSPG	VPLSLY
			FYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGN	
			VFSCSVMHEALHNHYTQKSLSLSPG	
			DKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDP	AVNGTSQFTCFYNSRANISCVWSQDGALQDTSCQVHAWPDRRR
			EVKFNWYVDGVEVHNAKTKPREEQYASTYRVVSVLTVLHQDWLNGKEYK	WNQTCELLPVSQASWACNLILGAPDSQKLTTVDIVTLRVLCREGVR
440	DNA187	Hole: hFc(N297A)-hCD122	CKVSNKALPAPIEKTISKAKGQPREPQVCTLPPSRDELTKNQVSLSCAVKGF PGSGS	WRVMAIQDFKPFENLRLMAPISLQVVHVETHRCNISWEISQASHYF
			YPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLVSKLTVDKSRWQQGN	ERHLEFEARTLSPGHTWEEAPLLTLKQKQEWICLETLTPDTQYEFQV
			VFSCSVMHEALHNHYTQKSLSLSPG	RVKPLQGEFTTWSPWSQPLAFRTKPAALGKD
			DKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDP	
		Knob: hFc(N297A)-[VPLSLY]-hIL2(R38A, F42A, Y45A, E62A, L80F, R81D, L85V, I86V, I92F, C125A)	EVKFNWYVDGVEVHNAKTKPREEQYASTYRVVSVLTVLHQDWLNGKEYK	
440	DNA544		CKVSNKALPAPIEKTISKAKGQPREPQVYTLPPCRDELTKNQVSLWCLVKG GSPG	VPLSLY
			FYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGN	
			VFSCSVMHEALHNHYTQKSLSLSPG	
			DKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDP	
		Hole: hFc(N297A)-[VPLSLY]-hCD122	EVKFNWYVDGVEVHNAKTKPREEQYASTYRVVSVLTVLHQDWLNGKEYK	
441	DNA543		CKVSNKALPAPIEKTISKAKGQPREPQVCTLPPSRDELTKNQVSLSCAVKGF GPPSGSSPG	VPLSLY
			YPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLVSKLTVDKSRWQQGN	
			VFSCSVMHEALHNHYTQKSLSLSPG	
			DKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDP	APTSSSTKKTQLQLEHLLLDLQMILNGINNYKNPKLTAMLTAKFAMF
		Knob: hFc(N297A)-hIL2(R38A, F42A, Y45A, E62A, L80F, R81D, L85V, I86V, I92F, C125A)	EVKFNWYVDGVEVHNAKTKPREEQYASTYRVVSVLTVLHQDWLNGKEYK	KKATELKHLQCLEEALKPLEEVLNLAQSKNFHFDPRDVVSNINVFVLE
441	DNA546		CKVSNKALPAPIEKTISKAKGQPREPQVYTLPPCRDELTKNQVSLWCLVKG GGSSPPGGGSSGGGSGGP	LKGSETTFMCEYADETATIVEFLNRWITFAQSIISTLT
			FYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGN	
			VFSCSVMHEALHNHYTQKSLSLSPG	

			DKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDP	
442	DNA255	Knob: hFc(N297A)-hIL2(R38A, F42A, Y45A, E62A, C125A)	EVKFNWYVDGVEVHNAKTKPREEQYASTYRVVSVLTVLHQDWLNGKEYK CKVSNKALPAPIEKTISKAKGQPREPQVYTLPPCRDELTKNQVSLWCLVKG GGSSPPGGGSSGGGSGP FYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGN VFSCSVMHEALHNHYTQKSLSLSPG	APTSSSTKKTQLQLEHLLLDLQMILNGINNYKNPKLTAMLTAKFAMF KKATELKHLQCLEEALKPLEEVLNLAQSKNFHLRPRDLISNINVIVLEL KGSETTFMCEYADETATIVEFLNRWITFAQSIISTLT
			DKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDP	
442	DNA553	Hole: hFc(N297A)-[DSGGFMLT]-hCD122	EVKFNWYVDGVEVHNAKTKPREEQYASTYRVVSVLTVLHQDWLNGKEYK CKVSNKALPAPIEKTISKAKGQPREPQVCTLPPSRDELTKNQVSLSCAVKGF GPPSGSSPG YPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLVSKLTVDKSRWQQGN VFSCSVMHEALHNHYTQKSLSLSPG	DSGGFMLT
			DKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDP	
443	DNA187	Hole: hFc(N297A)-hCD122	EVKFNWYVDGVEVHNAKTKPREEQYASTYRVVSVLTVLHQDWLNGKEYK CKVSNKALPAPIEKTISKAKGQPREPQVCTLPPSRDELTKNQVSLSCAVKGF PGSGS YPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLVSKLTVDKSRWQQGN VFSCSVMHEALHNHYTQKSLSLSPG	AVNGTSQFTCFYNSRANISCVWSQDGALQDTSCQVHAWPDRRR WNQTCELLPVSQASWACNLILGAPDSQKLTTVDIVTLRVLCREGVR WRVMAIQDFKPFENLRLMAPISLQVVHVETHRCNISWEISQASHYF ERHLEFEARTLSPGHTWEEAPLLTLKQKQEWICLETLTPDTQYEFQV RVKPLQGEFTTWSPWSQPLAFRTKPAALGKD
			DKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDP	
443	DNA554	Knob: hFc(N297A)-[VPLSLY]-hIL2(E15R, L18C, D20R, R38A, F42A, Y45A, E62A)	EVKFNWYVDGVEVHNAKTKPREEQYASTYRVVSVLTVLHQDWLNGKEYK CKVSNKALPAPIEKTISKAKGQPREPQVYTLPPCRDELTKNQVSLWCLVKG GSPG FYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGN VFSCSVMHEALHNHYTQKSLSLSPG	VPLSLY
			DKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDP	
444	DNA281	Knob: hFc(N297A)-[DSGGFMLT]-hIL2(R38A, F42A, Y45A, E62A, C125A)	EVKFNWYVDGVEVHNAKTKPREEQYASTYRVVSVLTVLHQDWLNGKEYK CKVSNKALPAPIEKTISKAKGQPREPQVYTLPPCRDELTKNQVSLWCLVKG GSGP FYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGN VFSCSVMHEALHNHYTQKSLSLSPG	DSGGFMLT
			DKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDP	
444	DNA440	Hole: hFc(N297A)-hCD122(C122S, C168S)	EVKFNWYVDGVEVHNAKTKPREEQYASTYRVVSVLTVLHQDWLNGKEYK CKVSNKALPAPIEKTISKAKGQPREPQVCTLPPSRDELTKNQVSLSCAVKGF PGSGS YPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLVSKLTVDKSRWQQGN VFSCSVMHEALHNHYTQKSLSLSPG	AVNGTSQFTCFYNSRANISCVWSQDGALQDTSCQVHAWPDRRR WNQTCELLPVSQASWACNLILGAPDSQKLTTVDIVTLRVLCREGVR WRVMAIQDFKPFENLRLMAPISLQVVHVETHRSNISWEISQASHYF ERHLEFEARTLSPGHTWEEAPLLTLKQKQEWISLETLTPDTQYEFQV RVKPLQGEFTTWSPWSQPLAFRTKPAALGKD

			EPKSSDKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVS		AVNGTSQFTCFYNSRANISCVWSQDGALQDTSCQVHAWPDRRR
		Hole: hFcIgG1(N297A + EPKSS)-	HEDPEVKFNWYVDGVEVHNAKTKPREEQYASTYRVVSVLTVLHQDWLN		WNQTCELLPVSQASWACNLILGAPDSQKLTTVDIVTLRVLCREGVR
449	DNA547	Hole: hFc(N297A)-hCD122	GKEYKCKVSNKALPAPIEKTISKAKGQPREPQVCTLPPSRDELTKNQVSLSC	PGSGS	WRVMAIQDFKPFENLRLMAPISLQVVHVETHRCNISWEISQASHYF
			AVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLVSKLTVDKSRW		ERHLEFEARTLSPGHTWEEAPLLTLKQKQEWICLETLTPDTQYEFQV
			QQGNVFSCSVMHEALHNHYTQKSLSLSPG		RVKPLQGEFTTWSPWSQPLAFRTKPAALGKD
			EPKSSDKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVS		
		Knob: hFcIgG1(N297A + EPKSS)-	HEDPEVKFNWYVDGVEVHNAKTKPREEQYASTYRVVSVLTVLHQDWLN		
449	DNA550	hIL2(R38A, F42A, Y45A, E62A,	GKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPCRDELTKNQVSLW	GSPG	VPLSLY
		C125A)	CLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSR		
			WQQGNVFSCSVMHEALHNHYTQKSLSLSPG		
			AKTDKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHE		AVNGTSQFTCFYNSRANISCVWSQDGALQDTSCQVHAWPDRRR
			DPEVKFNWYVDGVEVHNAKTKPREEQYASTYRVVSVLTVLHQDWLNGK		WNQTCELLPVSQASWACNLILGAPDSQKLTTVDIVTLRVLCREGVR
450	DNA548	Hole: hFcIgG1(N297A + AKT)-	EYKCKVSNKALPAPIEKTISKAKGQPREPQVCTLPPSRDELTKNQVSLSCAV	PGSGS	WRVMAIQDFKPFENLRLMAPISLQVVHVETHRCNISWEISQASHYF
		Hole: hFc(N297A)-hCD122	KGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLVSKLTVDKSRWQQ		ERHLEFEARTLSPGHTWEEAPLLTLKQKQEWICLETLTPDTQYEFQV
			GNVFSCSVMHEALHNHYTQKSLSLSPG		RVKPLQGEFTTWSPWSQPLAFRTKPAALGKD
			AKTDKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHE		
		Knob: hFcIgG1(N297A + AKT)-	DPEVKFNWYVDGVEVHNAKTKPREEQYASTYRVVSVLTVLHQDWLNGK		
450	DNA551	[VPLSLY]-hIL2(R38A, F42A, Y45A,	EYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPCRDELTKNQVSLWCLV	GSPG	VPLSLY
		E62A, C125A)	KGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQ		
			GNVFSCSVMHEALHNHYTQKSLSLSPG		
			AKTEPKSSDKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVV		AVNGTSQFTCFYNSRANISCVWSQDGALQDTSCQVHAWPDRRR
			DVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYASTYRVVSVLTVLHQD		WNQTCELLPVSQASWACNLILGAPDSQKLTTVDIVTLRVLCREGVR
451	DNA549	Hole: hFcIgG1(N297A	WLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVCTLPPSRDELTKNQV	PGSGS	WRVMAIQDFKPFENLRLMAPISLQVVHVETHRCNISWEISQASHYF
		+AKTEPKSS)--hCD122	SLSCAVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLVSKLTVDK		ERHLEFEARTLSPGHTWEEAPLLTLKQKQEWICLETLTPDTQYEFQV
			SRWQQGNVFSCSVMHEALHNHYTQKSLSLSPG		RVKPLQGEFTTWSPWSQPLAFRTKPAALGKD
			AKTEPKSSDKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVV		
		Knob : hFcIgG1(N297A +	DVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYASTYRVVSVLTVLHQD		
451	DNA552	AKTEPKSS)-[VPLSLY]-hIL2(R38A,	WLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPCRDELTKNQV	GSPG	VPLSLY
		F42A, Y45A, E62A, C125A)	SLWCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDK		
			SRWQQGNVFSCSVMHEALHNHYTQKSLSLSPG		

452	DNA187	Hole: hFc(N297A)-hCD122	DKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDP EVKFNWYVDGVEVHNAKTKPREEQYASTYRVVSVLTVLHQDWLNGKEYK CKVSNKALPAPIEKTISKAKGQPREPQVCTLPPSRDELTKNQVSLSCAVKGF PGSGS YPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLVSKLTVDKSRWQQGN VFSCSVMHEALHNHYTQKSLSLSPG	AVNGTSQFTCFYNSRANISCVWSQDGALQDTSCQVHAWPDRRR WNQTCELLPVSQASWACNLILGAPDSQKLTTVDIVTLRVLCREGVR WRVMAIQDFKPFENLRLMAPISLQVVHVETHRCNISWEISQASHYF ERHLEFEARTLSPGHTWEEAPLLTLKQKQEWICLETLTPDTQYEFQV RVKPLQGEFTTWSPWSQPLAFRTKPAALGKD
452	DNA563	Knob: hFc(N297A)-[VPLSLY]-hIL2(E15R, L18C, D20R, R38A, F42A, Y45A, E62A, N88L)	DKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDP EVKFNWYVDGVEVHNAKTKPREEQYASTYRVVSVLTVLHQDWLNGKEYK CKVSNKALPAPIEKTISKAKGQPREPQVYTLPPCRDELTKNQVSLWCLVKG GSPG FYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGN VFSCSVMHEALHNHYTQKSLSLSPG	VPLSLY
453	DNA187	Hole: hFc(N297A)-hCD122	DKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDP EVKFNWYVDGVEVHNAKTKPREEQYASTYRVVSVLTVLHQDWLNGKEYK CKVSNKALPAPIEKTISKAKGQPREPQVCTLPPSRDELTKNQVSLSCAVKGF PGSGS YPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLVSKLTVDKSRWQQGN VFSCSVMHEALHNHYTQKSLSLSPG	AVNGTSQFTCFYNSRANISCVWSQDGALQDTSCQVHAWPDRRR WNQTCELLPVSQASWACNLILGAPDSQKLTTVDIVTLRVLCREGVR WRVMAIQDFKPFENLRLMAPISLQVVHVETHRCNISWEISQASHYF ERHLEFEARTLSPGHTWEEAPLLTLKQKQEWICLETLTPDTQYEFQV RVKPLQGEFTTWSPWSQPLAFRTKPAALGKD
453	DNA565	Knob: hFc(N297A)-[VPLSLY]-hIL2(E15L, L18C, D20R, R38A, F42A, Y45A, E62A, N88L)	DKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDP EVKFNWYVDGVEVHNAKTKPREEQYASTYRVVSVLTVLHQDWLNGKEYK CKVSNKALPAPIEKTISKAKGQPREPQVYTLPPCRDELTKNQVSLWCLVKG GSPG FYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGN VFSCSVMHEALHNHYTQKSLSLSPG	VPLSLY
454	DNA187	Hole: hFc(N297A)-hCD122	DKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDP EVKFNWYVDGVEVHNAKTKPREEQYASTYRVVSVLTVLHQDWLNGKEYK CKVSNKALPAPIEKTISKAKGQPREPQVCTLPPSRDELTKNQVSLSCAVKGF PGSGS YPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLVSKLTVDKSRWQQGN VFSCSVMHEALHNHYTQKSLSLSPG	AVNGTSQFTCFYNSRANISCVWSQDGALQDTSCQVHAWPDRRR WNQTCELLPVSQASWACNLILGAPDSQKLTTVDIVTLRVLCREGVR WRVMAIQDFKPFENLRLMAPISLQVVHVETHRCNISWEISQASHYF ERHLEFEARTLSPGHTWEEAPLLTLKQKQEWICLETLTPDTQYEFQV RVKPLQGEFTTWSPWSQPLAFRTKPAALGKD
454	DNA566	Knob: hFc(N297A)-[VPLSLY]-hIL2(E15R, L18C, R38A, F42A, Y45A, E62A, N88L)	DKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDP EVKFNWYVDGVEVHNAKTKPREEQYASTYRVVSVLTVLHQDWLNGKEYK CKVSNKALPAPIEKTISKAKGQPREPQVYTLPPCRDELTKNQVSLWCLVKG GSPG FYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGN VFSCSVMHEALHNHYTQKSLSLSPG	VPLSLY

			DKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDP	AVNGTSQFTCFYNSRANISCVWSQDGALQDTSCQVHAWPDRRR
			EVKFNWYVDGVEVHNAKTKPREEQYASTYRVVSVLTVLHQDWLNGKEYK	WNQTCELLPVSQASWACNLILGAPDSQKLTTVDIVTLRVLCREGVR
455	DNA187	Hole: hFc(N297A)-hCD122	CKVSNKALPAPIEKTISKAKGQPREPQVCTLPPSRDELTKNQVSLSCAVKGF PGSGS	WRVMAIQDFKPFENLRLMAPISLQVVHVETHRCNISWEISQASHYF
			YPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLVSKLTVDKSRWQQGN	ERHLEFEARTLSPGHTWEEAPLLTLKQKQEWICLETLTPDTQYEFQV
			VFSCSVMHEALHNHYTQKSLSLSPG	RVKPLQGEFTTWSPWSQPLAFRTKPAALGKD
			DKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDP	
		Knob: hFc(N297A)-[VPLSLY]-hIL2(L18C, D20R, R38A, F42A, Y45A, E62A, N88L)	EVKFNWYVDGVEVHNAKTKPREEQYASTYRVVSVLTVLHQDWLNGKEYK	
455	DNA567		CKVSNKALPAPIEKTISKAKGQPREPQVYTLPPCRDELTKNQVSLWCLVKG GSPG	VPLSLY
			FYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGN	
			VFSCSVMHEALHNHYTQKSLSLSPG	
			DKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDP	AVNGTSQFTCFYNSRANISCVWSQDGALQDTSCQVHAWPDRRR
			EVKFNWYVDGVEVHNAKTKPREEQYASTYRVVSVLTVLHQDWLNGKEYK	WNQTCELLPVSQASWACNLILGAPDSQKLTTVDIVTLRVLCREGVR
456	DNA187	Hole: hFc(N297A)-hCD122	CKVSNKALPAPIEKTISKAKGQPREPQVCTLPPSRDELTKNQVSLSCAVKGF PGSGS	WRVMAIQDFKPFENLRLMAPISLQVVHVETHRCNISWEISQASHYF
			YPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLVSKLTVDKSRWQQGN	ERHLEFEARTLSPGHTWEEAPLLTLKQKQEWICLETLTPDTQYEFQV
			VFSCSVMHEALHNHYTQKSLSLSPG	RVKPLQGEFTTWSPWSQPLAFRTKPAALGKD
			DKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDP	
		Knob: hFc(N297A)-[VPLSLY]-hIL2(E15F, L18C, D20R, R38A, F42A, Y45A, E62A, N88L)	EVKFNWYVDGVEVHNAKTKPREEQYASTYRVVSVLTVLHQDWLNGKEYK	
456	DNA568		CKVSNKALPAPIEKTISKAKGQPREPQVYTLPPCRDELTKNQVSLWCLVKG GSPG	VPLSLY
			FYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGN	
			VFSCSVMHEALHNHYTQKSLSLSPG	
			VRSGCKPCICTVPEVSSVFIFPPKPKDVLTITLTPKVTCVVVAISKDDPEVQFS	
		Knob: mFcIgG1(DAPG)-hIL2(R38A, F42A, Y45A, E62A, C125A)	WFVDDVEVHTAQTQPREEQFNSTFRSVSELPIMHQDWLNGKEFKCRVN	APTSSSTKKTQLQLEHLLLDLQMILNGINNYKNPKLTAMLTAKFAMF
462	DNA530		SAAFGAPIEKTISKTKGRPKAPQVYTIPPPKEQMAKDKVSLTCMITDFFPED	KKATELKHLQCLEEALKPLEEVLNLAQSKNFHLRPRDLISNINVIVLEL
			ITVEWQWNGQPAENYDNTQPIMDTDGSYFVYSDLNVQKSNWEAGNTF	KGSETTFMCEYADETATIVEFLNRWITFAQSIISTLT
			TCSVLHEGLHNHHTEKSLSHSPG	
			VRSGCKPCICTVPEVSSVFIFPPKPKDVLTITLTPKVTCVVVAISKDDPEVQFS	
			WFVDDVEVHTAQTQPREEQFNSTFRSVSELPIMHQDWLNGKEFKCRVN	
462	DNA532	Hole: mFcIgG1(DAPG)	SAAFGAPIEKTISKTKGRPKAPQVYTIPPPKKQMAKDKVSLTCMITDFFPED	
			ITVEWQWNGQPAENYKNTQPIMKTDGSYFVYSKLNVQKSNWEAGNTFT	
			CSVLHEGLHNHHTEKSLSHSPG	

			VRSGCKPCICTVPEVSSVFIFPPKPKDVLTITLTPKVTCVVVAISKDDPEVQFS		
463	DNA530	Knob: mFclgG1(DAPG)-hIL2(R38A, F42A, Y45A, E62A, C125A)	WFVDDVEVHTAQTQPREEQFNSTFRSVSELPIMHQDWLNGKEFKCRVN SAAFGAPIEKTISKTKGRPKAPQVYTIPPPKEQMAKDKVSLTCMITDFFPED GGSSPPGGGSSGGGSGP ITVEWQWNGQPAENYDNTQPIMDTDGSYFVYSDLNVQKSNWEAGNTF TCSVLHEGLHNHHTEKSLSHSPG		APTSSSTKKTQLQLEHLLLDLQMILNGINNYKNPKLTAMLTAKFAMF KKATELKHLQCLEEALKPLEEVLNLAQSKNFHLRPRDLISNINVIVLEL KGSETTFMCEYADETATIVEFLNRWITFAQSIISTLT
			VRSGCKPCICTVPEVSSVFIFPPKPKDVLTITLTPKVTCVVVAISKDDPEVQFS		
463	DNA533	Hole: mFclgG1(DAPG)-hCD122	WFVDDVEVHTAQTQPREEQFNSTFRSVSELPIMHQDWLNGKEFKCRVN SAAFGAPIEKTISKTKGRPKAPQVYTIPPPKKQMAKDKVSLTCMITDFFPED PGSGS ITVEWQWNGQPAENYKNTQPIMKTDGSYFVYSKLNVQKSNWEAGNTFT CSVLHEGLHNHHTEKSLSHSPG		AVNGTSQFTCFYNSRANISCVWSQDGALQDTSCQVHAWPDRRR WNQTCELLPVSQASWACNLILGAPDSQKLTTVDIVTLRVLCREGVR WRVMAIQDFKPFENLRLMAPISLQVVHVETHRCNISWEISQASHYF ERHLEFEARTLSPGHTWEEAPLLTLKQKQEWICLETLTPDTQYEFQV RVKPLQGEFTTWSPWSQPLAFRTKPAALGKD
			VRSGCKPCICTVPEVSSVFIFPPKPKDVLTITLTPKVTCVVVAISKDDPEVQFS		
464	DNA530	Knob: mFclgG1(DAPG)-hIL2(R38A, F42A, Y45A, E62A, C125A)	WFVDDVEVHTAQTQPREEQFNSTFRSVSELPIMHQDWLNGKEFKCRVN SAAFGAPIEKTISKTKGRPKAPQVYTIPPPKEQMAKDKVSLTCMITDFFPED GGSSPPGGGSSGGGSGP ITVEWQWNGQPAENYDNTQPIMDTDGSYFVYSDLNVQKSNWEAGNTF TCSVLHEGLHNHHTEKSLSHSPG		APTSSSTKKTQLQLEHLLLDLQMILNGINNYKNPKLTAMLTAKFAMF KKATELKHLQCLEEALKPLEEVLNLAQSKNFHLRPRDLISNINVIVLEL KGSETTFMCEYADETATIVEFLNRWITFAQSIISTLT
			VRSGCKPCICTVPEVSSVFIFPPKPKDVLTITLTPKVTCVVVAISKDDPEVQFS		
464	DNA534	Hole: mFclgG1(DAPG)-mCD122	WFVDDVEVHTAQTQPREEQFNSTFRSVSELPIMHQDWLNGKEFKCRVN SAAFGAPIEKTISKTKGRPKAPQVYTIPPPKKQMAKDKVSLTCMITDFFPED PGSGS ITVEWQWNGQPAENYKNTQPIMKTDGSYFVYSKLNVQKSNWEAGNTFT CSVLHEGLHNHHTEKSLSHSPG		AVKNCSHLECFYNSRANVSCMWSHEEALNVTTCHVHAKSNLRHW NKTCELTLVRQASWACNLILGSFPESQSLTSVDLLDINVVCWEEKG WRRVKTCDFHPFDNLRLVAPHSLQVLHIDTQRCNISWKVSQVSHYI EPYLEFEARRRLLGHSWEDASVLSLKQRQQWLFLEMLIPSTSYEVQ\ RVKAQRNNTGTWSPWSQPLTFRTRPADPMKE
			VRSGCKPCICTVPEVSSVFIFPPKPKDVLTITLTPKVTCVVVAISKDDPEVQFS		
465	DNA531	Knob: mFclgG1(DAPG)-[VPLSLY]-hIL2(R38A, F42A, Y45A, E62A, C125A)	WFVDDVEVHTAQTQPREEQFNSTFRSVSELPIMHQDWLNGKEFKCRVN SAAFGAPIEKTISKTKGRPKAPQVYTIPPPKEQMAKDKVSLTCMITDFFPED GSPG ITVEWQWNGQPAENYDNTQPIMDTDGSYFVYSDLNVQKSNWEAGNTF TCSVLHEGLHNHHTEKSLSHSPG		VPLSLY
			VRSGCKPCICTVPEVSSVFIFPPKPKDVLTITLTPKVTCVVVAISKDDPEVQFS		
465	DNA532	Hole: mFclgG1(DAPG)	WFVDDVEVHTAQTQPREEQFNSTFRSVSELPIMHQDWLNGKEFKCRVN SAAFGAPIEKTISKTKGRPKAPQVYTIPPPKKQMAKDKVSLTCMITDFFPED ITVEWQWNGQPAENYKNTQPIMKTDGSYFVYSKLNVQKSNWEAGNTFT CSVLHEGLHNHHTEKSLSHSPG		

466	DNA531	Knob: mFcIgG1(DAPG)-[VPLSLY]-hIL2(R38A, F42A, Y45A, E62A, C125A)	VRSGCKPCICTVPEVSSVFIFPPKPKDVLTITLTPKVTCVVVAISKDDPEVQFS WFVDDVEVHTAQTQPREEQFNSTFRSVSELPIMHQDWLNGKEFKCRVN SAAFGAPIEKTISKTKGRPKAPQVYTIPPPKEQMAKDKVSLTCMITDFFPED ITVEWQWNGQPAENYDNTQPIMDTDGSYFVYSDLNVQKSNWEAGNTF TCSVLHEGLHNHHTEKSLSHSPG GSPG	VPLSLY
466	DNA533	Hole: mFcIgG1(DAPG)-hCD122	VRSGCKPCICTVPEVSSVFIFPPKPKDVLTITLTPKVTCVVVAISKDDPEVQFS WFVDDVEVHTAQTQPREEQFNSTFRSVSELPIMHQDWLNGKEFKCRVN SAAFGAPIEKTISKTKGRPKAPQVYTIPPPKKQMAKDKVSLTCMITDFFPED ITVEWQWNGQPAENYKNTQPIMKTDGSYFVYSKLNVQKSNWEAGNTFT CSVLHEGLHNHHTEKSLSHSPG PGSGS	AVNGTSQFTCFYNSRANISCVWSQDGALQDTSCQVHAWPDRRR WNQTCELLPVSQASWACNLILGAPDSQKLTTVDIVTLRVLCREGVR WRVMAIQDFKPFENLRLMAPISLQVVHVETHRCNISWEISQASHYF ERHLEFEARTLSPGHTWEEAPLLTLKQKQEWICLETLTPDTQYEFQV RVKPLQGEFTTWSPWSQPLAFRTKPAALGKD
467	DNA531	Knob: mFcIgG1(DAPG)-[VPLSLY]-hIL2(R38A, F42A, Y45A, E62A, C125A)	VRSGCKPCICTVPEVSSVFIFPPKPKDVLTITLTPKVTCVVVAISKDDPEVQFS WFVDDVEVHTAQTQPREEQFNSTFRSVSELPIMHQDWLNGKEFKCRVN SAAFGAPIEKTISKTKGRPKAPQVYTIPPPKEQMAKDKVSLTCMITDFFPED ITVEWQWNGQPAENYDNTQPIMDTDGSYFVYSDLNVQKSNWEAGNTF TCSVLHEGLHNHHTEKSLSHSPG GSPG	VPLSLY
467	DNA534	Hole: mFcIgG1(DAPG)-mCD122	VRSGCKPCICTVPEVSSVFIFPPKPKDVLTITLTPKVTCVVVAISKDDPEVQFS WFVDDVEVHTAQTQPREEQFNSTFRSVSELPIMHQDWLNGKEFKCRVN SAAFGAPIEKTISKTKGRPKAPQVYTIPPPKKQMAKDKVSLTCMITDFFPED ITVEWQWNGQPAENYKNTQPIMKTDGSYFVYSKLNVQKSNWEAGNTFT CSVLHEGLHNHHTEKSLSHSPG PGSGS	AVKNCSHLECFYNSRANVSCMWSHEEALNVTTCHVHAKSNLRHW NKTCELTLVRQASWACNLILGSFPESQSLTSVDLLDINVVCWEEKG WRRVKTCDFHPFDNLRLVAPHSLQVLHIDTQRCNISWKVSQVSHYI EPYLEFEARRRLLGHSWEDASVLSLKQRQQWLFLEMLIPSTSYEVQV RVKAQRNNTGTWSPWSQPLTFRTRPADPMKE
468	DNA576	Hole: hFc(N297A, M252Y, S254T, T256E)-hCD122	DKTHTCPPCPAPELLGGPSVFLFPPKPKDTLYITREPEVTCVVVDVSHEDPE VKFNWYVDGVEVHNAKTKPREEQYASTYRVVSVLTVLHQDWLNGKEYK CKVSNKALPAPIEKTISKAKGQPREPQVCTLPPSRDELTKNQVSLSCAVKGF YPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLVSKLTVDKSRWQQGN VFSCSVMHEALHNHYTQKSLSLSPG PGSGS	AVNGTSQFTCFYNSRANISCVWSQDGALQDTSCQVHAWPDRRR WNQTCELLPVSQASWACNLILGAPDSQKLTTVDIVTLRVLCREGVR WRVMAIQDFKPFENLRLMAPISLQVVHVETHRCNISWEISQASHYF ERHLEFEARTLSPGHTWEEAPLLTLKQKQEWICLETLTPDTQYEFQV RVKPLQGEFTTWSPWSQPLAFRTKPAALGKD
468	DNA580	Knob: hFc(N297A, M252Y, S254T, T256E)-[VPLSLY]-hIL2(R38A, F42A, Y45A, E62A, C125A)	DKTHTCPPCPAPELLGGPSVFLFPPKPKDTLYITREPEVTCVVVDVSHEDPE VKFNWYVDGVEVHNAKTKPREEQYASTYRVVSVLTVLHQDWLNGKEYK CKVSNKALPAPIEKTISKAKGQPREPQVYTLPPCRDELTKNQVSLWCLVKG FYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGN VFSCSVMHEALHNHYTQKSLSLSPG GSPG	VPLSLY

			DKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMASRTPEVTCVVVDVSHEDP	AVNGTSQFTCFYNSRANISCVWSQDGALQDTSCQVHAWPDRRR
			EVKFNWYVDGVEVHNAKTKPREEQYASTYRVVSVLTVLHQDWLNGKEYK	WNQTCELLPVSQASWACNLILGAPDSQKLTTVDIVTLRVLCREGVR
469	DNA575	Hole: hFc(N297A, I253A)-hCD122	CKVSNKALPAPIEKTISKAKGQPREPQVCTLPPSRDELTKNQVSLSCAVKGF PGSGS	WRVMAIQDFKPFENLRLMAPISLQVVHVETHRCNISWEISQASHYF
			YPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLVSKLTVDKSRWQQGN	ERHLEFEARTLSPGHTWEEAPLLTLKQKQEWICLETLTPDTQYEFQV
			VFSCSVMHEALHNHYTQKSLSLSPG	RVKPLQGEFTTWSPWSQPLAFRTKPAALGKD
			DKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMASRTPEVTCVVVDVSHEDP	APTSSSTKKTQLQLEHLLLDLQMILNGINNYKNPKLTAMLTAKFAMIF
		Knob: hFc(N297, I253A)-hIL2(R38A, F42A, Y45A, E62A, C125A)	EVKFNWYVDGVEVHNAKTKPREEQYASTYRVVSVLTVLHQDWLNGKEYK	KKATELKHLQCLEEALKPLEEVLNLAQSKNFHLRPRDLISNINVIVLEL
469	DNA577		CKVSNKALPAPIEKTISKAKGQPREPQVYTLPPCRDELTKNQVSLWCLVKG GGSSPPGGGSSGGGSGP	KGSETTFMCEYADETATIVEFLNRWITFAQSIISTLT
			FYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGN	
			VFSCSVMHEALHNHYTQKSLSLSPG	
			DKTHTCPPCPAPELLGGPSVFLFPPKPKDTLYITREPEVTCVVVDVSHEDPE	AVNGTSQFTCFYNSRANISCVWSQDGALQDTSCQVHAWPDRRR
			VKFNWYVDGVEVHNAKTKPREEQYASTYRVVSVLTVLHQDWLNGKEYK	WNQTCELLPVSQASWACNLILGAPDSQKLTTVDIVTLRVLCREGVR
470	DNA576	Hole: hFc(N297A, M252Y, S254T, T256E)-hCD122	CKVSNKALPAPIEKTISKAKGQPREPQVCTLPPSRDELTKNQVSLSCAVKGF PGSGS	WRVMAIQDFKPFENLRLMAPISLQVVHVETHRCNISWEISQASHYF
			YPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLVSKLTVDKSRWQQGN	ERHLEFEARTLSPGHTWEEAPLLTLKQKQEWICLETLTPDTQYEFQV
			VFSCSVMHEALHNHYTQKSLSLSPG	RVKPLQGEFTTWSPWSQPLAFRTKPAALGKD
			DKTHTCPPCPAPELLGGPSVFLFPPKPKDTLYITREPEVTCVVVDVSHEDPE	APTSSSTKKTQLQLEHLLLDLQMILNGINNYKNPKLTAMLTAKFAMIF
		Knob: hFc(N297A, M252Y, S254T, T256E)-hIL2(R38A, F42A, Y45A, E62A, C125A)	VKFNWYVDGVEVHNAKTKPREEQYASTYRVVSVLTVLHQDWLNGKEYK	KKATELKHLQCLEEALKPLEEVLNLAQSKNFHLRPRDLISNINVIVLEL
470	DNA578		CKVSNKALPAPIEKTISKAKGQPREPQVYTLPPCRDELTKNQVSLWCLVKG GGSSPPGGGSSGGGSGP	KGSETTFMCEYADETATIVEFLNRWITFAQSIISTLT
			FYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGN	
			VFSCSVMHEALHNHYTQKSLSLSPG	
			DKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMASRTPEVTCVVVDVSHEDP	AVNGTSQFTCFYNSRANISCVWSQDGALQDTSCQVHAWPDRRR
			EVKFNWYVDGVEVHNAKTKPREEQYASTYRVVSVLTVLHQDWLNGKEYK	WNQTCELLPVSQASWACNLILGAPDSQKLTTVDIVTLRVLCREGVR
471	DNA575	Hole: hFc(N297A, I253A)-hCD122	CKVSNKALPAPIEKTISKAKGQPREPQVCTLPPSRDELTKNQVSLSCAVKGF PGSGS	WRVMAIQDFKPFENLRLMAPISLQVVHVETHRCNISWEISQASHYF
			YPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLVSKLTVDKSRWQQGN	ERHLEFEARTLSPGHTWEEAPLLTLKQKQEWICLETLTPDTQYEFQV
			VFSCSVMHEALHNHYTQKSLSLSPG	RVKPLQGEFTTWSPWSQPLAFRTKPAALGKD
			DKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMASRTPEVTCVVVDVSHEDP	
		Knob: hFc(N297, I253A)-[VPLSLY]: hIL2(R38A, F42A, Y45A, E62A, C125A)	EVKFNWYVDGVEVHNAKTKPREEQYASTYRVVSVLTVLHQDWLNGKEYK	VPLSLY
471	DNA579		CKVSNKALPAPIEKTISKAKGQPREPQVYTLPPCRDELTKNQVSLWCLVKG GSPG	
			FYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGN	
			VFSCSVMHEALHNHYTQKSLSLSPG	

			DKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDP			
475	DNA255	Knob: hFc(N297A)-hIL2(R38A, F42A, Y45A, E62A, C125A)	EVKFNWYVDGVEVHNAKTKPREEQYASTYRVVSVLTVLHQDWLNGKEYK CKVSNKALPAPIEKTISKAKGQPREPQVYTLPPCRDELTKNQVSLWCLVKG FYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGN VFSCSVMHEALHNHYTQKSLSLSPG	GGSSPPGGGSSGGGSGGP	APTSSSTKKTQLQLEHLLLDLQMILNGINNYKNPKLTAMLTAKFAMF KKATELKHLQCLEEALKPLEEVLNLAQSKNFHLRPRDLISNINVIVLEL KGSETTFMCEYADETATIVEFLNRWITFAQSIISTLT	
			DKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDP		AVNGTSQFTCFYNSRANISCVWSQDGALQDTSCQVHAWPDRRR	
475	DNA528	Hole: hFc(N297A)-hCD122(C168S)	EVKFNWYVDGVEVHNAKTKPREEQYASTYRVVSVLTVLHQDWLNGKEYK CKVSNKALPAPIEKTISKAKGQPREPQVCTLPPSRDELTKNQVSLSCAVKGF YPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLVSKLTVDKSRWQQGN VFSCSVMHEALHNHYTQKSLSLSPG	PGSGS	WNQTCELLPVSQASWACNLILGAPDSQKLTTVDIVTLRVLCREGVR WRVMAIQDFKPFENLRLMAPISLQVVHVETHRCNISWEISQASHYF ERHLEFEARTLSPGHTWEEAPLLTLKQKQEWISLETLTPDTQYEFQV RVKPLQGEFTTWSPWSQPLAFRTKPAALGKD	
			DKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDP			
476	DNA263	Knob: hFc(N297A)-[VPLSLY]-hIL2(R38A, F42A, Y45A, E62A, C125A)	EVKFNWYVDGVEVHNAKTKPREEQYASTYRVVSVLTVLHQDWLNGKEYK CKVSNKALPAPIEKTISKAKGQPREPQVYTLPPCRDELTKNQVSLWCLVKG FYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGN VFSCSVMHEALHNHYTQKSLSLSPG	GSPG	VPLSLY	
			DKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDP		AVNGTSQFTCFYNSRANISCVWSQDGALQDTSCQVHAWPDRRR	
476	DNA528	Hole: hFc(N297A)-hCD122(C168S)	EVKFNWYVDGVEVHNAKTKPREEQYASTYRVVSVLTVLHQDWLNGKEYK CKVSNKALPAPIEKTISKAKGQPREPQVCTLPPSRDELTKNQVSLSCAVKGF YPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLVSKLTVDKSRWQQGN VFSCSVMHEALHNHYTQKSLSLSPG	PGSGS	WNQTCELLPVSQASWACNLILGAPDSQKLTTVDIVTLRVLCREGVR WRVMAIQDFKPFENLRLMAPISLQVVHVETHRCNISWEISQASHYF ERHLEFEARTLSPGHTWEEAPLLTLKQKQEWISLETLTPDTQYEFQV RVKPLQGEFTTWSPWSQPLAFRTKPAALGKD	
			DKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDP			
477	DNA158	Hole: hFc(N297A)	EVKFNWYVDGVEVHNAKTKPREEQYASTYRVVSVLTVLHQDWLNGKEYK CKVSNKALPAPIEKTISKAKGQPREPQVCTLPPSRDELTKNQVSLSCAVKGF YPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLVSKLTVDKSRWQQGN VFSCSVMHEALHNHYTQKSLSLSPG			
			DKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDP			
477	DNA554	Knob: hFc(N297A)-[VPLSLY]-hIL2(E15R, L18C, D20R, R38A, F42A, Y45A, E62A)	EVKFNWYVDGVEVHNAKTKPREEQYASTYRVVSVLTVLHQDWLNGKEYK CKVSNKALPAPIEKTISKAKGQPREPQVYTLPPCRDELTKNQVSLWCLVKG FYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGN VFSCSVMHEALHNHYTQKSLSLSPG	GSPG	VPLSLY	

			DKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDP EVKFNWYVDGVEVHNAKTKPREEQYASTYRVVSVLTVLHQDWLNGKEYK CKVSNKALPAPIEKTISKAKGQPREPQVCTLPPSRDELTKNQVSLSCAVKGF YPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLVSKLTVDKSRWQQGN VFSCSVMHEALHNHYTQKSLSLSPG		
484	DNA158	Hole: hFc(N297A)			
			DKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDP EVKFNWYVDGVEVHNAKTKPREEQYASTYRVVSVLTVLHQDWLNGKEYK CKVSNKALPAPIEKTISKAKGQPREPQVYTLPPCRDELTKNQVSLWCLVKG GSPG FYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGN VFSCSVMHEALHNHYTQKSLSLSPG		VPLSLY
484	DNA581	Knob: hFc(N297A)-[VPLSLY]-hIL2(L18C, R38A, F42A, Y45A, E62A)			
			DKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDP EVKFNWYVDGVEVHNAKTKPREEQYASTYRVVSVLTVLHQDWLNGKEYK CKVSNKALPAPIEKTISKAKGQPREPQVCTLPPSRDELTKNQVSLSCAVKGF YPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLVSKLTVDKSRWQQGN VFSCSVMHEALHNHYTQKSLSLSPG		
485	DNA158	Hole: hFc(N297A)			
			DKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDP EVKFNWYVDGVEVHNAKTKPREEQYASTYRVVSVLTVLHQDWLNGKEYK CKVSNKALPAPIEKTISKAKGQPREPQVYTLPPCRDELTKNQVSLWCLVKG GSPG FYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGN VFSCSVMHEALHNHYTQKSLSLSPG		VPLSLY
485	DNA582	Knob: hFc(N297A)-[VPLSLY]-hIL2(H16Y, R38A, F42A, Y45A, E62A, C125A)			
			DKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDP EVKFNWYVDGVEVHNAKTKPREEQYASTYRVVSVLTVLHQDWLNGKEYK CKVSNKALPAPIEKTISKAKGQPREPQVCTLPPSRDELTKNQVSLSCAVKGF YPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLVSKLTVDKSRWQQGN VFSCSVMHEALHNHYTQKSLSLSPG		
486	DNA158	Hole: hFc(N297A)			
			DKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDP EVKFNWYVDGVEVHNAKTKPREEQYASTYRVVSVLTVLHQDWLNGKEYK CKVSNKALPAPIEKTISKAKGQPREPQVYTLPPCRDELTKNQVSLWCLVKG GSPG FYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGN VFSCSVMHEALHNHYTQKSLSLSPG		VPLSLY
486	DNA583	Knob: hFc(N297A)-[VPLSLY]-hIL2(H16E, R38A, F42A, Y45A, E62A, C125A)			

			DKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDP EVKFNWYVDGVEVHNAKTKPREEQYASTYRVVSVLTVLHQDWLNGKEYK CKVSNKALPAPIEKTISKAKGQPREPQVCTLPPSRDELTKNQVSLSCAVKGF YPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLVSKLTVDKSRWQQGN VFSCSVMHEALHNHYTQKSLSLSPG		
487	DNA158	Hole: hFc(N297A)			
487	DNA584	Knob: hFc(N297A)-[VPLSLY]-hIL2(D20L, R38A, F42A, Y45A, E62A, C125A)	DKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDP EVKFNWYVDGVEVHNAKTKPREEQYASTYRVVSVLTVLHQDWLNGKEYK CKVSNKALPAPIEKTISKAKGQPREPQVYTLPPCRDELTKNQVSLWCLVKG GSPG FYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGN VFSCSVMHEALHNHYTQKSLSLSPG	VPLSLY	
488	DNA158	Hole: hFc(N297A)	DKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDP EVKFNWYVDGVEVHNAKTKPREEQYASTYRVVSVLTVLHQDWLNGKEYK CKVSNKALPAPIEKTISKAKGQPREPQVCTLPPSRDELTKNQVSLSCAVKGF YPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLVSKLTVDKSRWQQGN VFSCSVMHEALHNHYTQKSLSLSPG		
488	DNA585	Knob: hFc(N297A)-[VPLSLY]-hIL2(H16Y, L18C, R38A, F42A, Y45A, E62A)	DKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDP EVKFNWYVDGVEVHNAKTKPREEQYASTYRVVSVLTVLHQDWLNGKEYK CKVSNKALPAPIEKTISKAKGQPREPQVYTLPPCRDELTKNQVSLWCLVKG GSPG FYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGN VFSCSVMHEALHNHYTQKSLSLSPG	VPLSLY	
489	DNA158	Hole: hFc(N297A)	DKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDP EVKFNWYVDGVEVHNAKTKPREEQYASTYRVVSVLTVLHQDWLNGKEYK CKVSNKALPAPIEKTISKAKGQPREPQVCTLPPSRDELTKNQVSLSCAVKGF YPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLVSKLTVDKSRWQQGN VFSCSVMHEALHNHYTQKSLSLSPG		
489	DNA586	Knob: hFc(N297A)-[VPLSLY]-hIL2(H16E, L18C, R38A, F42A, Y45A, E62A)	DKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDP EVKFNWYVDGVEVHNAKTKPREEQYASTYRVVSVLTVLHQDWLNGKEYK CKVSNKALPAPIEKTISKAKGQPREPQVYTLPPCRDELTKNQVSLWCLVKG GSPG FYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGN VFSCSVMHEALHNHYTQKSLSLSPG	VPLSLY	

			DKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDP EVKFNWYVDGVEVHNAKTKPREEQYASTYRVVSVLTVLHQDWLNGKEYK CKVSNKALPAPIEKTISKAKGQPREPQVCTLPPSRDELTKNQVSLSCAVKGF YPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLVSKLTVDKSRWQQGN VFSCSVMHEALHNHYTQKSLSLSPG		
490	DNA158	Hole: hFc(N297A)			
			DKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDP EVKFNWYVDGVEVHNAKTKPREEQYASTYRVVSVLTVLHQDWLNGKEYK CKVSNKALPAPIEKTISKAKGQPREPQVYTLPPCRDELTKNQVSLWCLVKG GSPG FYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGN VFSCSVMHEALHNHYTQKSLSLSPG		
490	DNA587	Knob: hFc(N297A)-[VPLSLY]-hIL2(L18C, D20L, R38A, F42A, Y45A, E62A)			VPLSLY
			DKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDP EVKFNWYVDGVEVHNAKTKPREEQYASTYRVVSVLTVLHQDWLNGKEYK CKVSNKALPAPIEKTISKAKGQPREPQVCTLPPSRDELTKNQVSLSCAVKGF YPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLVSKLTVDKSRWQQGN VFSCSVMHEALHNHYTQKSLSLSPG		
491	DNA158	Hole: hFc(N297A)			
			DKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDP EVKFNWYVDGVEVHNAKTKPREEQYASTYRVVSVLTVLHQDWLNGKEYK CKVSNKALPAPIEKTISKAKGQPREPQVYTLPPCRDELTKNQVSLWCLVKG GSPG FYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGN VFSCSVMHEALHNHYTQKSLSLSPG		
491	DNA588	Knob: hFc(N297A)-[VPLSLY]-hIL2(H16Y, L18C, D20L, R38A, F42A, Y45A, E62A)			VPLSLY
			DKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDP EVKFNWYVDGVEVHNAKTKPREEQYASTYRVVSVLTVLHQDWLNGKEYK CKVSNKALPAPIEKTISKAKGQPREPQVCTLPPSRDELTKNQVSLSCAVKGF YPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLVSKLTVDKSRWQQGN VFSCSVMHEALHNHYTQKSLSLSPG		
492	DNA158	Hole: hFc(N297A)			
			DKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDP EVKFNWYVDGVEVHNAKTKPREEQYASTYRVVSVLTVLHQDWLNGKEYK CKVSNKALPAPIEKTISKAKGQPREPQVYTLPPCRDELTKNQVSLWCLVKG GSPG FYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGN VFSCSVMHEALHNHYTQKSLSLSPG		
492	DNA589	Knob: hFc(N297A)-[VPLSLY]-hIL2(H16E, L18C, D20L, R38A, F42A, Y45A, E62A)			VPLSLY

			DKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDP	AVNGTSQFTCFYNSRANISCVWSQDGALQDTSCQVHAWPDRRR
			EVKFNWYVDGVEVHNAKTKPREEQYASTYRVVSVLTVLHQDWLNGKEYK	WNQTCELLPVSQASWACNLILGAPDSQKLTTVDIVTLRVLCREGVR
493	DNA187	Hole: hFc(N297A)-hCD122	CKVSNKALPAPIEKTISKAKGQPREPQVCTLPPSRDELTKNQVSLSCAVKGF PGSGS	WRVMAIQDFKPFENLRLMAPISLQVVHVETHRCNISWEISQASHYF
			YPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLVSKLTVDKSRWQQGN	ERHLEFEARTLSPGHTWEEAPLLTLKQKQEWICLETLTPDTQYEFQV
			VFSCSVMHEALHNHYTQKSLSLSPG	RVKPLQGEFTTWSPWSQPLAFRTKPAALGKD
			DKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDP	
		Knob: hFc(N297A)-[VPLSLY]-hIL2(L18C, R38A, F42A, Y45A, E62A)	EVKFNWYVDGVEVHNAKTKPREEQYASTYRVVSVLTVLHQDWLNGKEYK	
493	DNA581		CKVSNKALPAPIEKTISKAKGQPREPQVYTLPPCRDELTKNQVSLWCLVKG GSPG	VPLSLY
			FYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGN	
			VFSCSVMHEALHNHYTQKSLSLSPG	
			DKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDP	AVNGTSQFTCFYNSRANISCVWSQDGALQDTSCQVHAWPDRRR
			EVKFNWYVDGVEVHNAKTKPREEQYASTYRVVSVLTVLHQDWLNGKEYK	WNQTCELLPVSQASWACNLILGAPDSQKLTTVDIVTLRVLCREGVR
494	DNA187	Hole: hFc(N297A)-hCD122	CKVSNKALPAPIEKTISKAKGQPREPQVCTLPPSRDELTKNQVSLSCAVKGF PGSGS	WRVMAIQDFKPFENLRLMAPISLQVVHVETHRCNISWEISQASHYF
			YPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLVSKLTVDKSRWQQGN	ERHLEFEARTLSPGHTWEEAPLLTLKQKQEWICLETLTPDTQYEFQV
			VFSCSVMHEALHNHYTQKSLSLSPG	RVKPLQGEFTTWSPWSQPLAFRTKPAALGKD
			DKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDP	
		Knob: hFc(N297A)-[VPLSLY]-hIL2(H16Y, R38A, F42A, Y45A, E62A, C125A)	EVKFNWYVDGVEVHNAKTKPREEQYASTYRVVSVLTVLHQDWLNGKEYK	
494	DNA582		CKVSNKALPAPIEKTISKAKGQPREPQVYTLPPCRDELTKNQVSLWCLVKG GSPG	VPLSLY
			FYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGN	
			VFSCSVMHEALHNHYTQKSLSLSPG	
			DKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDP	AVNGTSQFTCFYNSRANISCVWSQDGALQDTSCQVHAWPDRRR
			EVKFNWYVDGVEVHNAKTKPREEQYASTYRVVSVLTVLHQDWLNGKEYK	WNQTCELLPVSQASWACNLILGAPDSQKLTTVDIVTLRVLCREGVR
495	DNA187	Hole: hFc(N297A)-hCD122	CKVSNKALPAPIEKTISKAKGQPREPQVCTLPPSRDELTKNQVSLSCAVKGF PGSGS	WRVMAIQDFKPFENLRLMAPISLQVVHVETHRCNISWEISQASHYF
			YPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLVSKLTVDKSRWQQGN	ERHLEFEARTLSPGHTWEEAPLLTLKQKQEWICLETLTPDTQYEFQV
			VFSCSVMHEALHNHYTQKSLSLSPG	RVKPLQGEFTTWSPWSQPLAFRTKPAALGKD
			DKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDP	
		Knob: hFc(N297A)-[VPLSLY]-hIL2(H16E, R38A, F42A, Y45A, E62A, C125A)	EVKFNWYVDGVEVHNAKTKPREEQYASTYRVVSVLTVLHQDWLNGKEYK	
495	DNA583		CKVSNKALPAPIEKTISKAKGQPREPQVYTLPPCRDELTKNQVSLWCLVKG GSPG	VPLSLY
			FYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGN	
			VFSCSVMHEALHNHYTQKSLSLSPG	

496	DNA187	Hole: hFc(N297A)-hCD122	DKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDP EVKFNWYVDGVEVHNAKTKPREEQYASTYRVVSVLTVLHQDWLNGKEYK CKVSNKALPAPIEKTISKAKGQPREPQVCTLPPSRDELTKNQVSLSCAVKGF PGSGS YPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLVSKLTVDKSRWQQGN VFSCSVMHEALHNHYTQKSLSLSPG	AVNGTSQFTCFYNSRANISCVWSQDGALQDTSCQVHAWPDRRR WNQTCELLPVSQASWACNLILGAPDSQKLTTVDIVTLRVLCREGVR WRVMAIQDFKPFENLRLMAPISLQVVHVETHRCNISWEISQASHYF ERHLEFEARTLSPGHTWEEAPLLTLKQKQEWICLETLTPDTQYEFQV RVKPLQGEFTTWSPWSQPLAFRTKPAALGKD	VPLSLY
496	DNA584	Knob: hFc(N297A)-[VPLSLY]-hIL2(D20L, R38A, F42A, Y45A, E62A, C125A)	DKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDP EVKFNWYVDGVEVHNAKTKPREEQYASTYRVVSVLTVLHQDWLNGKEYK CKVSNKALPAPIEKTISKAKGQPREPQVYTLPPCRDELTKNQVSLWCLVKG GSPG FYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGN VFSCSVMHEALHNHYTQKSLSLSPG	VPLSLY
497	DNA187	Hole: hFc(N297A)-hCD122	DKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDP EVKFNWYVDGVEVHNAKTKPREEQYASTYRVVSVLTVLHQDWLNGKEYK CKVSNKALPAPIEKTISKAKGQPREPQVCTLPPSRDELTKNQVSLSCAVKGF PGSGS YPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLVSKLTVDKSRWQQGN VFSCSVMHEALHNHYTQKSLSLSPG	AVNGTSQFTCFYNSRANISCVWSQDGALQDTSCQVHAWPDRRR WNQTCELLPVSQASWACNLILGAPDSQKLTTVDIVTLRVLCREGVR WRVMAIQDFKPFENLRLMAPISLQVVHVETHRCNISWEISQASHYF ERHLEFEARTLSPGHTWEEAPLLTLKQKQEWICLETLTPDTQYEFQV RVKPLQGEFTTWSPWSQPLAFRTKPAALGKD
497	DNA585	Knob: hFc(N297A)-[VPLSLY]-hIL2(H16Y, L18C, R38A, F42A, Y45A, E62A)	DKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDP EVKFNWYVDGVEVHNAKTKPREEQYASTYRVVSVLTVLHQDWLNGKEYK CKVSNKALPAPIEKTISKAKGQPREPQVYTLPPCRDELTKNQVSLWCLVKG GSPG FYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGN VFSCSVMHEALHNHYTQKSLSLSPG	VPLSLY
498	DNA187	Hole: hFc(N297A)-hCD122	DKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDP EVKFNWYVDGVEVHNAKTKPREEQYASTYRVVSVLTVLHQDWLNGKEYK CKVSNKALPAPIEKTISKAKGQPREPQVCTLPPSRDELTKNQVSLSCAVKGF PGSGS YPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLVSKLTVDKSRWQQGN VFSCSVMHEALHNHYTQKSLSLSPG	AVNGTSQFTCFYNSRANISCVWSQDGALQDTSCQVHAWPDRRR WNQTCELLPVSQASWACNLILGAPDSQKLTTVDIVTLRVLCREGVR WRVMAIQDFKPFENLRLMAPISLQVVHVETHRCNISWEISQASHYF ERHLEFEARTLSPGHTWEEAPLLTLKQKQEWICLETLTPDTQYEFQV RVKPLQGEFTTWSPWSQPLAFRTKPAALGKD
498	DNA586	Knob: hFc(N297A)-[VPLSLY]-hIL2(H16E, L18C, R38A, F42A, Y45A, E62A)	DKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDP EVKFNWYVDGVEVHNAKTKPREEQYASTYRVVSVLTVLHQDWLNGKEYK CKVSNKALPAPIEKTISKAKGQPREPQVYTLPPCRDELTKNQVSLWCLVKG GSPG FYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGN VFSCSVMHEALHNHYTQKSLSLSPG	VPLSLY

			DKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDP	AVNGTSQFTCFYNSRANISCVWSQDGALQDTSCQVHAWPDRRR
			EVKFNWYVDGVEVHNAKTKPREEQYASTYRVVSVLTVLHQDWLNGKEYK	WNQTCELLPVSQASWACNLILGAPDSQKLTTVDIVTLRVLCREGVR
499	DNA187	Hole: hFc(N297A)-hCD122	CKVSNKALPAPIEKTISKAKGQPREPQVCTLPPSRDELTKNQVSLSCAVKGF PGSGS	WRVMAIQDFKPFENLRLMAPISLQVVHVETHRCNISWEISQASHYF
			YPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLVSKLTVDKSRWQQGN	ERHLEFEARTLSPGHTWEEAPLLTLKQKQEWICLETLTPDTQYEFQV
			VFSCSVMHEALHNHYTQKSLSLSPG	RVKPLQGEFTTWSPWSQPLAFRTKPAALGKD
			DKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDP	
		Knob: hFc(N297A)-[VPLSLY]-hIL2(L18C, D20L, R38A, F42A, Y45A, E62A)	EVKFNWYVDGVEVHNAKTKPREEQYASTYRVVSVLTVLHQDWLNGKEYK	
499	DNA587		CKVSNKALPAPIEKTISKAKGQPREPQVYTLPPCRDELTKNQVSLWCLVKG GSPG	VPLSLY
			FYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGN	
			VFSCSVMHEALHNHYTQKSLSLSPG	
			DKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDP	AVNGTSQFTCFYNSRANISCVWSQDGALQDTSCQVHAWPDRRR
			EVKFNWYVDGVEVHNAKTKPREEQYASTYRVVSVLTVLHQDWLNGKEYK	WNQTCELLPVSQASWACNLILGAPDSQKLTTVDIVTLRVLCREGVR
500	DNA187	Hole: hFc(N297A)-hCD122	CKVSNKALPAPIEKTISKAKGQPREPQVCTLPPSRDELTKNQVSLSCAVKGF PGSGS	WRVMAIQDFKPFENLRLMAPISLQVVHVETHRCNISWEISQASHYF
			YPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLVSKLTVDKSRWQQGN	ERHLEFEARTLSPGHTWEEAPLLTLKQKQEWICLETLTPDTQYEFQV
			VFSCSVMHEALHNHYTQKSLSLSPG	RVKPLQGEFTTWSPWSQPLAFRTKPAALGKD
			DKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDP	
		Knob: hFc(N297A)-[VPLSLY]-hIL2(H16Y, L18C, D20L, R38A, F42A, Y45A, E62A)	EVKFNWYVDGVEVHNAKTKPREEQYASTYRVVSVLTVLHQDWLNGKEYK	
500	DNA588		CKVSNKALPAPIEKTISKAKGQPREPQVYTLPPCRDELTKNQVSLWCLVKG GSPG	VPLSLY
			FYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGN	
			VFSCSVMHEALHNHYTQKSLSLSPG	
			DKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDP	AVNGTSQFTCFYNSRANISCVWSQDGALQDTSCQVHAWPDRRR
			EVKFNWYVDGVEVHNAKTKPREEQYASTYRVVSVLTVLHQDWLNGKEYK	WNQTCELLPVSQASWACNLILGAPDSQKLTTVDIVTLRVLCREGVR
501	DNA187	Hole: hFc(N297A)-hCD122	CKVSNKALPAPIEKTISKAKGQPREPQVCTLPPSRDELTKNQVSLSCAVKGF PGSGS	WRVMAIQDFKPFENLRLMAPISLQVVHVETHRCNISWEISQASHYF
			YPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLVSKLTVDKSRWQQGN	ERHLEFEARTLSPGHTWEEAPLLTLKQKQEWICLETLTPDTQYEFQV
			VFSCSVMHEALHNHYTQKSLSLSPG	RVKPLQGEFTTWSPWSQPLAFRTKPAALGKD
			DKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDP	
		Knob: hFc(N297A)-[VPLSLY]-hIL2(H16E, L18C, D20L, R38A, F42A, Y45A, E62A)	EVKFNWYVDGVEVHNAKTKPREEQYASTYRVVSVLTVLHQDWLNGKEYK	
501	DNA589		CKVSNKALPAPIEKTISKAKGQPREPQVYTLPPCRDELTKNQVSLWCLVKG GSPG	VPLSLY
			FYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGN	
			VFSCSVMHEALHNHYTQKSLSLSPG	

			DKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDP		
		Hole: hFc(N297A)-[VPLSLY]-hCD122	EVKFNWYVDGVEVHNAKTKPREEQYASTYRVVSVLTVLHQDWLNGKEYK CKVSNKALPAPIEKTISKAKGQPREPQVCTLPPSRDELTKNQVSLSCAVKGF GPPSGSSPG YPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLVSKLTVDKSRWQQGN VFSCSVMHEALHNHYTQKSLSLSPG	VPLSLY	
502	DNA543				
			DKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMASRTPEVTCVVVDVSHEDP		
		Knob: hFc(N297, I253A)-hIL2(R38A, F42A, Y45A, E62A, C125A)	EVKFNWYVDGVEVHNAKTKPREEQYASTYRVVSVLTVLHQDWLNGKEYK CKVSNKALPAPIEKTISKAKGQPREPQVYTLPPCRDELTKNQVSLWCLVKG GGSSPPGGGSSGGGSGP FYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGN VFSCSVMHEALHNHYTQKSLSLSPG	APTSSSTKKTQLQLEHLLLDLQMILNGINNYKNPKLTAMLTAKFAMIF KKATELKHLQCLEEALKPLEEVLNLAQSKNFHLRPRDLISNINVIVLEL KGSETTFMCEYADETATIVEFLNRWITFAQSIISTLT	
502	DNA577				
			DKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDP		
		Knob: hFc(N297A)-hIL2(R38A, F42A, Y45A, E62A, C125A)	EVKFNWYVDGVEVHNAKTKPREEQYASTYRVVSVLTVLHQDWLNGKEYK CKVSNKALPAPIEKTISKAKGQPREPQVYTLPPCRDELTKNQVSLWCLVKG GGSSPPGGGSSGGGSGP FYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGN VFSCSVMHEALHNHYTQKSLSLSPG	APTSSSTKKTQLQLEHLLLDLQMILNGINNYKNPKLTAMLTAKFAMIF KKATELKHLQCLEEALKPLEEVLNLAQSKNFHLRPRDLISNINVIVLEL KGSETTFMCEYADETATIVEFLNRWITFAQSIISTLT	
503	DNA255				
			DKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDP		
		Hole: hFc(N297A)-[RAAAVKSP]-hCD122	EVKFNWYVDGVEVHNAKTKPREEQYASTYRVVSVLTVLHQDWLNGKEYK CKVSNKALPAPIEKTISKAKGQPREPQVCTLPPSRDELTKNQVSLSCAVKGF GPPSGSSP YPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLVSKLTVDKSRWQQGN VFSCSVMHEALHNHYTQKSLSLSPG	RAAAVKSP	
503	DNA606				
			ESKYGPPCPPCPAPEFLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSQED		
		Hole: hFcIgG4-hCD122	PEVQFNWYVDGVEVHNAKTKPREEQFNSTYRVVSVLTVLHQDWLNGKE YKCKVSNKGLPSSIEKTISKAKGQPREPQVCTLPPSQEEMTKNQVSLSCAVK PGSGS GFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSRLTVDKSRWQEG NVFSCSVMHEALHNHYTQKSLSLSLG	AVNGTSQFTCFYNSRANISCVWSQDGALQDTSCQVHAWPDRRR WNQTCELLPVSQASWACNLILGAPDSQKLTTVDIVTLRVLCREGVR WRVMAIQDFKPFENLRLMAPISLQVVHVETHRCNISWEISQASHYF ERHLEFEARTLSPGHTWEEAPLLTLKQKQEWICLETLTPDTQYEFEQV RVKPLQGEFTTWSPWSQPLAFRTKPAALGKD	
504	DNA603				
			ESKYGPPCPPCPAPEFLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSQED		
		Knob: hFcIgG4-hIL2-[VPLSLY]-hIL2(R38A, F42A, Y45A, E62A, C125A)	PEVQFNWYVDGVEVHNAKTKPREEQFNSTYRVVSVLTVLHQDWLNGKE YKCKVSNKGLPSSIEKTISKAKGQPREPQVYTLPPCQEEMTKNQVSLWCLV GSPG KGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSRLTVDKSRWQE GNVFSCSVMHEALHNHYTQKSLSLSLG	VPLSLY	
504	DNA605				

			ESKYGPPCPPCPAPEFLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSQED		AVNGTSQFTCFYNSRANISCVWSQDGALQDTSCQVHAWPDRRR
			PEVQFNWYVDGVEVHNAKTKPREEQFNSTYRVVSVLTVLHQDWLNGKE		WNQTCELLPVSQASWACNLILGAPDSQKLTTVDIVTLRVLCREGVR
505	DNA603	Hole: hFcIgG4-hCD122	YKCKVSNKGLPSSIEKTISKAKGQPREPQVCTLPPSQEEMTKNQVSLSCAVK	PGSGS	WRVMAIQDFKPFENLRLMAPISLQVVHVETHRCNISWEISQASHYF
			GFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSRLTVDKSRWQEG		ERHLEFEARTLSPGHTWEEAPLLTLKQKQEWICLETLTPDTQYEFQV
			NVFSCSVMHEALHNHYTQKSLSLSLG		RVKPLQGEFTTWSPWSQPLAFRTKPAALGKD
			ESKYGPPCPPCPAPEFLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSQED		
			PEVQFNWYVDGVEVHNAKTKPREEQFNSTYRVVSVLTVLHQDWLNGKE		APTSSSTKKTQLQLEHLLLDLQMILNGINNYKNPKLTAMLTAKFAMIF
505	DNA604	Knob: IgG4 hFc-hIL2(R38A, F42A, Y45A, E62A, C125A)	YKCKVSNKGLPSSIEKTISKAKGQPREPQVYTLPPCQEEMTKNQVSLWCLV	GGSSPPGGGSSGGGSGP	KKATELKHLQCLEEALKPLEEVLNLAQSKNFHLRPRDLISNINVIVLEL
			KGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSRLTVDKSRWQE		KGSETTFMCEYADETATIVEFLNRWITFAQSIISTLT
			GNVFSCSVMHEALHNHYTQKSLSLSLG		
			DKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMASRTPEVTCVVVDVSHEDP		
		Knob: hFc(N297, I253A)-hIL2(R38A, F42A, Y45A, E62A, C125A)	EVKFNWYVDGVEVHNAKTKPREEQYASTYRVVSVLTVLHQDWLNGKEYK		APTSSSTKKTQLQLEHLLLDLQMILNGINNYKNPKLTAMLTAKFAMIF
508	DNA577		CKVSNKALPAPIEKTISKAKGQPREPQVYTLPPCRDELTKNQVSLWCLVKG	GGSSPPGGGSSGGGSGP	KKATELKHLQCLEEALKPLEEVLNLAQSKNFHLRPRDLISNINVIVLEL
			FYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGN		KGSETTFMCEYADETATIVEFLNRWITFAQSIISTLT
			VFSCSVMHEALHNHYTQKSLSLSPG		
			DKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMASRTPEVTCVVVDVSHEDP		
		Hole: hFc(N297A, I253A)-[VPLSLY]-hCD122	EVKFNWYVDGVEVHNAKTKPREEQYASTYRVVSVLTVLHQDWLNGKEYK		
508	DNA609		CKVSNKALPAPIEKTISKAKGQPREPQVCTLPPSRDELTKNQVSLSCAVKGF	GPPSGSSPG	VPLSLY
			YPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLVSKLTVDKSRWQQGN		
			VFSCSVMHEALHNHYTQKSLSLSPG		
			DKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMASRTPEVTCVVVDVSHEDP		AVNGTSQFTCFYNSRANISCVWSQDGALQDTSCQVHAWPDRRR
			EVKFNWYVDGVEVHNAKTKPREEQYASTYRVVSVLTVLHQDWLNGKEYK		WNQTCELLPVSQASWACNLILGAPDSQKLTTVDIVTLRVLCREGVR
509	DNA575	Hole: hFc(N297A, I253A)-hCD122	CKVSNKALPAPIEKTISKAKGQPREPQVCTLPPSRDELTKNQVSLSCAVKGF	PGSGS	WRVMAIQDFKPFENLRLMAPISLQVVHVETHRCNISWEISQASHYF
			YPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLVSKLTVDKSRWQQGN		ERHLEFEARTLSPGHTWEEAPLLTLKQKQEWICLETLTPDTQYEFQV
			VFSCSVMHEALHNHYTQKSLSLSPG		RVKPLQGEFTTWSPWSQPLAFRTKPAALGKD
			DKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMASRTPEVTCVVVDVSHEDP		
		Knob: hFc(N297, I253A)-[MPYDLYHP]-hIL2(R38A, F42A, Y45A, E62A, C125A)	EVKFNWYVDGVEVHNAKTKPREEQYASTYRVVSVLTVLHQDWLNGKEYK		
509	DNA623		CKVSNKALPAPIEKTISKAKGQPREPQVYTLPPCRDELTKNQVSLWCLVKG	GGSSPP	MPYDLYHP
			FYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGN		
			VFSCSVMHEALHNHYTQKSLSLSPG		

			DKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMASRTPEVTCVVVDVSHEDP		
		Knob: hFc(N297, I253A)-	EVKFNWYVDGVEVHNAKTKPREEQYASTYRVVSVLTVLHQDWLNGKEYK		APTSSSTKKTQLQLEHLLLDLQMILNGINNYKNPKLTAMLTAKFAMIF
510	DNA577	hIL2(R38A, F42A, Y45A, E62A, C125A)	CKVSNKALPAPIEKTISKAKGQPREPQVYTLPPCRDELTKNQVSLWCLVKG	GGSSPPGGGSSGGGSGP	KKATELKHLQCLEEALKPLEEVLNLAQSKNFHLRPRDLISNINVIVLEL
			FYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGN		KGSETTFMCEYADETATIVEFLNRWITFAQSIISTLT
			VFSCSVMHEALHNHYTQKSLSLSPG		
			DKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMASRTPEVTCVVVDVSHEDP		
			EVKFNWYVDGVEVHNAKTKPREEQYASTYRVVSVLTVLHQDWLNGKEYK		
510	DNA608	Hole: hFc(N297A, I253A)-[MPYDLYHP]-hCD122	CKVSNKALPAPIEKTISKAKGQPREPQVCTLPPSRDELTKNQVSLSCAVKGF	GPPSGSSP	MPYDLYHP
			YPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLVSKLTVDKSRWQQGN		
			VFSCSVMHEALHNHYTQKSLSLSPG		
			ESKYGPPCPPCPAPEFLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSQED		
			PEVQFNWYVDGVEVHNAKTKPREEQFNSTYRVVSVLTVLHQDWLNGKE		APTSSSTKKTQLQLEHLLLDLQMILNGINNYKNPKLTAMLTAKFAMIF
511	DNA604	Knob: IgG4 hFc-hIL2(R38A, F42A, Y45A, E62A, C125A)	YKCKVSNKGLPSSIEKTISKAKGQPREPQVYTLPPCQEEMTKNQVSLWCLV	GGSSPPGGGSSGGGSGP	KKATELKHLQCLEEALKPLEEVLNLAQSKNFHLRPRDLISNINVIVLEL
			KGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSRLTVDKSRWQE		KGSETTFMCEYADETATIVEFLNRWITFAQSIISTLT
			GNVFSCSVMHEALHNHYTQKSLSLSLG		
			ESKYGPPCPPCPAPEFLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSQED		
			PEVQFNWYVDGVEVHNAKTKPREEQFNSTYRVVSVLTVLHQDWLNGKE		
511	DNA621	Hole: hFcIgG4-[VPLSLY]-hCD122	YKCKVSNKGLPSSIEKTISKAKGQPREPQVCTLPPSQEEMTKNQVSLSCAVK	PSGSSPG	VPLSLY
			GFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSRLTVDKSRWQEG		
			NVFSCSVMHEALHNHYTQKSLSLSLGGP		
			DKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMASRTPEVTCVVVDVSHEDP		
		Knob: hFc(N297, I253A)-	EVKFNWYVDGVEVHNAKTKPREEQYASTYRVVSVLTVLHQDWLNGKEYK		APTSSSTKKTQLQLEHLLLDLQMILNGINNYKNPKLTAMLTAKFAMIF
512	DNA577	hIL2(R38A, F42A, Y45A, E62A, C125A)	CKVSNKALPAPIEKTISKAKGQPREPQVYTLPPCRDELTKNQVSLWCLVKG	GGSSPPGGGSSGGGSGP	KKATELKHLQCLEEALKPLEEVLNLAQSKNFHLRPRDLISNINVIVLEL
			FYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGN		KGSETTFMCEYADETATIVEFLNRWITFAQSIISTLT
			VFSCSVMHEALHNHYTQKSLSLSPG		
			DKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMASRTPEVTCVVVDVSHEDP		
			EVKFNWYVDGVEVHNAKTKPREEQYASTYRVVSVLTVLHQDWLNGKEYK		
512	DNA625	Hole: hFc(N297A, I253A)	CKVSNKALPAPIEKTISKAKGQPREPQVCTLPPSRDELTKNQVSLSCAVKGF		
			YPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLVSKLTVDKSRWQQGN		
			VFSCSVMHEALHNHYTQKSLSLSPG		

			ESKYGPPCPPCPAPEFLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSQED			
513	DNA604	Knob: IgG4 hFc-hIL2(R38A, F42A, Y45A, E62A, C125A)	PEVQFNWYVDGVEVHNAKTKPREEQFNSTYRVVSVLTVLHQDWLNGKE YKCKVSNKGLPSSIEKTISKAKGQPREPQVYTLPPCQEEMTKNQVSLWCLV KGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSRLTVDKSRWQE GNVFSCSVMHEALHNHYTQKSLSLSLG	GGSSPPGGGSSGGGSGGP	APTSSSTKKTQLQLEHLLLDLQMILNGINNYKNPKLTAMLTAKFAMF KKATELKHLQCLEEALKPLEEVLNLAQSKNFHLRPRDLISNINVIVLEL KGSETTFMCEYADETATIVEFLNRWITFAQSIISTLT	
			ESKYGPPCPPCPAPEFLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSQED			
513	DNA626	Hole: hFcIgG4	PEVQFNWYVDGVEVHNAKTKPREEQFNSTYRVVSVLTVLHQDWLNGKE YKCKVSNKGLPSSIEKTISKAKGQPREPQVCTLPPSQEEMTKNQVSLSCAVK GFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSRLTVDKSRWQEG NVFSCSVMHEALHNHYTQKSLSLSLGPG			
			DKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDP			
526	DNA670	Knob: hFc-hIL2(R38A, F42A, Y45A, E62A, C125A)	EVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEY KCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPCRDELTKNQVSLWCLVK GFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQG NVFSCSVMHEALHNHYTQKSLSLSPG	GGSSPPGGGSSGGGSGGP	APTSSSTKKTQLQLEHLLLDLQMILNGINNYKNPKLTAMLTAKFAMF KKATELKHLQCLEEALKPLEEVLNLAQSKNFHLRPRDLISNINVIVLEL KGSETTFMCEYADETATIVEFLNRWITFAQSIISTLT	
			DKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDP			
526	DNA672	Hole: hFc-[VPLSLY]-hCD122	EVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEY KCKVSNKALPAPIEKTISKAKGQPREPQVCTLPPSRDELTKNQVSLSCAVKG FYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLVSKLTVDKSRWQQG NVFSCSVMHEALHNHYTQKSLSLSPG	GPPSGSSPG	VPLSLY	
			DKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDP			
530	DNA255	Knob: hFc(N297A)-hIL2(R38A, F42A, Y45A, E62A, C125A)	EVKFNWYVDGVEVHNAKTKPREEQYASTYRVVSVLTVLHQDWLNGKEYK CKVSNKALPAPIEKTISKAKGQPREPQVYTLPPCRDELTKNQVSLWCLVKG FYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGN VFSCSVMHEALHNHYTQKSLSLSPG	GGSSPPGGGSSGGGSGGP	APTSSSTKKTQLQLEHLLLDLQMILNGINNYKNPKLTAMLTAKFAMF KKATELKHLQCLEEALKPLEEVLNLAQSKNFHLRPRDLISNINVIVLEL KGSETTFMCEYADETATIVEFLNRWITFAQSIISTLT	
			DKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDP			
530	DNA612	Hole: hFc(N297A)-[MPYDLYHP]-hCD122(C122S, C168S)	EVKFNWYVDGVEVHNAKTKPREEQYASTYRVVSVLTVLHQDWLNGKEYK CKVSNKALPAPIEKTISKAKGQPREPQVCTLPPSRDELTKNQVSLSCAVKGF YPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLVSKLTVDKSRWQQGN VFSCSVMHEALHNHYTQKSLSLSPG	GPPSGSSP	MPYDLYHP	

531	DNA255	Knob: hFc(N297A)-hIL2(R38A, F42A, Y45A, E62A, C125A)	DKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDP EVKFNWYVDGVEVHNAKTKPREEQYASTYRVVSVLTVLHQDWLNGKEYK CKVSNKALPAPIEKTISKAKGQPREPQVYTLPPCRDELTKNQVSLWCLVKG GGSSPPGGGSSGGGSGP FYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGN VFSCSVMHEALHNHYTQKSLSLSPG	APTSSSTKKTQLQLEHLLLDLQMILNGINNYKNPKLTAMLTAKFAMF KKATELKHLQCLEEALKPLEEVLNLAQSKNFHLRPRDLISNINVIVLEL KGSETTFMCEYADETATIVEFLNRWITFAQSIISTLT
531	DNA614	Hole: hFc(N297A)-[DSGGFMLT]-hCD122(C122S, C168S)	DKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDP EVKFNWYVDGVEVHNAKTKPREEQYASTYRVVSVLTVLHQDWLNGKEYK CKVSNKALPAPIEKTISKAKGQPREPQVCTLPPSRDELTKNQVSLSCAVKGF GPPSGSSPG YPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLVSKLTVDKSRWQQGN VFSCSVMHEALHNHYTQKSLSLSPG	DSGGFMLT
532	DNA669	Hole: hFc-hCD122	DKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDP EVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEY KCKVSNKALPAPIEKTISKAKGQPREPQVCTLPPSRDELTKNQVSLSCAVKG PGSGS FYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLVSKLTVDKSRWQQG NVFSCSVMHEALHNHYTQKSLSLSPG	AVNGTSQFTCFYNSRANISCVWSQDGALQDTSCQVHAWPDRRR WNQTCELLPVSQASWACNLILGAPDSQKLTTVDIVTLRVLCREGVR WRVMAIQDFKPFENLRLMAPISLQVVHVETHRCNISWEISQASHYF ERHLEFEARTLSPGHTWEEAPLLTLKQKQEWICLETLTPDTQYEFQV RVKPLQGEFTTWSPWSQPLAFRTKPAALGKD
532	DNA671	Knob: hFc-[VPLSLY]-hIL2(R38A, F42A, Y45A, E62A, C125A)	DKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDP EVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEY KCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPCRDELTKNQVSLWCLVK GSPG GFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQG NVFSCSVMHEALHNHYTQKSLSLSPG	VPLSLY

olecule	name	newnames	Component4 Sequence	Component5Sequence	FullSequence
					DKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFN
					WYVDGVEVHNAKTKPREEQYASTYRVVSVLTVLHQDWLNGKEYKCKVSNKALP
					APIEKTISKAKGQPREPQVCTLPPSRDELTKNQVSLSCAVKGFYPSDIAVEWESNG
		Hole:			QPENNYKTTPPVLDSDGSFFLVSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQ
:368	DNA187	hFc(N297A)-hCD122			KSLSLSPGPGSGSAVNGTSQFTCFYNSRANISCVWSQDGALQDTSCQVHAWPDR
					RRWNQTCELLPVSQASWACNLILGAPDSQKLTTVDIVTLRVLCREGVRWRVMAI
					QDFKPFENLRLMAPISLQVVHVETHRCNISWEISQASHYFERHLEFEARTLSPGHT
					WEEAPLLTLKQKQEWICLETLTPDTQYEFQVRVKPLQGEFTTWSPWSQPLAFRT
					KPAALGKD
				APTSSSTKKTQL	
				QLEHLLLDLQMI	
				LNGINNYKNPKL	DKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFN
		Knob:		TRMLTSKFYMP	WYVDGVEVHNAKTKPREEQYASTYRVVSVLTVLHQDWLNGKEYKCKVSNKALP
		hFc(N297A)-[NPMGSDPV	GP	KKATELKHLQCL	APIEKTISKAKGQPREPQVYTLPPCRDELTKNQVSLWCLVKGFYPSDIAVEWESN
:368	DNA476	NFKLLRVVNG]-hIL2(F42S, E62S, C125A)		EESLKPLEEVLNL	GQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYT
				AQSKNFHLRPR	QKSLSLSPGGNPMGSDPVNFKLLRVVNGGPAPTSSSTKKTQLQLEHLLLDLQMIL
				DLISNINVIVLEL	NGINNYKNPKLTRMLTSKFYMPKKATELKHLQCLEESLKPLEEVLNLAQSKNFHLR
				KGSETTFMCEY	PRDLISNINVIVLELKGSETTFMCEYADETATIVEFLNRWITFAQSIISTLT
				ADETATIVEFLN	
				RWITFAQSIISTL	
				T	
					TIKPCPPCKCPAPNAAGGPSVFIFPPKIKDVLMISLSPIVTCVVVDVSEDDPDVQIS
		Knob:			WFVNNVEVHTAQTQTHREDYNSTLRVVSALPIQHQDWMSGKEFKCKVNNKDL
		mFcIgG2a(LALAPG)-			GAPIERTISKPKGSVRAPQQYVLPPCEEEMTKKQVTLWCMVTDFMPEDIYVEWT
:375	DNA477	hIL2(R38A, F42A, Y45A, E62A, C125A)			NNGKTELNYKNTEPVLDSDGSYFMYSKLRVEKKNWVERNSYSCSVVHEGLHNH
					HTTKSFSRTPGGGSSPPGGGSSGGGSGGAPTSSSTKKTQLQLEHLLLDLQMILNGI
					NNYKNPKLTAMLTAKFAMPKKATELKHLQCLEEALKPLEEVLNLAQSKNFHLRPR
					DLISNINVIVLELKGSETTFMCEYADETATIVEFLNRWITFAQSIISTLT
					TIKPCPPCKCPAPNAAGGPSVFIFPPKIKDVLMISLSPIVTCVVVDVSEDDPDVQIS
		Hole:			WFVNNVEVHTAQTQTHREDYNSTLRVVSALPIQHQDWMSGKEFKCKVNNKDL
:375	DNA479	mFcIgG2a(LALAPG)			GAPIERTISKPKGSVRAPQQVCVLPPPEEEMTKKQVTLSCAVTDFMPEDIYVEWTN
					NGKTELNYKNTEPVLDSDGSYFMVSKLRVEKKNWVERNSYSCSVVHEGLHNHHT
					TKSFSRTPG

APTSSSTKKTQL
QLEHLLLDLQMI
LNGINNYKNPKL
TAMLTAKFAMP
KKATELKHLQCL
EEALKPLEEVLN
LAQSKNFHLRPR
DLISNINVIVLEL
KGSETTFMCEY
ADETATIVEFLN
RWITFAQSIISTL
T

		Knob: mFcIgG2a(LALAPG)-[VPLSLY]-SGP hIL2(R38A, F42A, Y45A, E62A, C125A)	TIKPCPPCKCPAPNAAGGPSVFIFPPKIKDVLMISLSPIVTCVVVDVSEDDPDVQIS WFVNNVEVHTAQTQTHREDYNSTLRVVSALPIQHQDWMSGKEFKCKVNNKDL GAPIERTISKPKGSVRAPQVYVLPPCEEEMTKKQVTLWCMVTDFMPEDIYVEWT NNGKTELNYKNTEPVLDSDGSYFMYSKLRVEKKNWVERNSYSCSVVHEGLHNH HTTKSFSRTPGGSPGVPLSLYSGPAPTSSSTKKTQLQLEHLLLDLQMILNGINNYKN PKLTAMLTAKFAMPKKATELKHLQCLEEALKPLEEVLNLAQSKNFHLRPRDLISNI NVIVLELKGSETTFMCEYADETATIVEFLNRWITFAQSIISTLT	
:376	DNA478			
		Hole: mFcIgG2a(LALAPG)	TIKPCPPCKCPAPNAAGGPSVFIFPPKIKDVLMISLSPIVTCVVVDVSEDDPDVQIS WFVNNVEVHTAQTQTHREDYNSTLRVVSALPIQHQDWMSGKEFKCKVNNKDL GAPIERTISKPKGSVRAPQVCVLPPPEEEMTKKQVTLSCAVTDFMPEDIYVEWTN NGKTELNYKNTEPVLDSDGSYFMVSKLRVEKKNWVERNSYSCSVVHEGLHNHHT TKSFSRTPG	
:376	DNA479			
		Knob: mFcIgG2a(LALAPG)-hIL2(R38A, F42A, Y45A, E62A, C125A)	TIKPCPPCKCPAPNAAGGPSVFIFPPKIKDVLMISLSPIVTCVVVDVSEDDPDVQIS WFVNNVEVHTAQTQTHREDYNSTLRVVSALPIQHQDWMSGKEFKCKVNNKDL GAPIERTISKPKGSVRAPQVYVLPPCEEEMTKKQVTLWCMVTDFMPEDIYVEWT NNGKTELNYKNTEPVLDSDGSYFMYSKLRVEKKNWVERNSYSCSVVHEGLHNH HTTKSFSRTPGGGSSPPGGGSSGGGSGGPAPTSSSTKKTQLQLEHLLLDLQMILNGI NNYKNPKLTAMLTAKFAMPKKATELKHLQCLEEALKPLEEVLNLAQSKNFHLRPR DLISNINVIVLELKGSETTFMCEYADETATIVEFLNRWITFAQSIISTLT	
:377	DNA477			
		Hole: mFcIgG2a(LALAPG)-hCD122	TIKPCPPCKCPAPNAAGGPSVFIFPPKIKDVLMISLSPIVTCVVVDVSEDDPDVQIS WFVNNVEVHTAQTQTHREDYNSTLRVVSALPIQHQDWMSGKEFKCKVNNKDL GAPIERTISKPKGSVRAPQVCVLPPPEEEMTKKQVTLSCAVTDFMPEDIYVEWTN NGKTELNYKNTEPVLDSDGSYFMVSKLRVEKKNWVERNSYSCSVVHEGLHNHHT TKSFSRTPGPGSGSAVNGTSQFTCFYNSRANISCVWSQDGALQDTSCQVHAWP DRRRWNQTCELLPVSQASWACNLILGAPDSQKLTTVDIVTLRVLCREGVRWRV MAIQDFKPFENLRLMAPISLQVVHVETHRCNISWEISQASHYFERHLEFEARTLSP GHTWEEAPLLTLKQKQEWICLETLTPDTQYEFQVRVKPLQGEFTTWSPWSQPLA FRTKPAALGKD	
:377	DNA480			

				APTSSSTKKTQL
				QLEHLLLDLQMI
		Knob:		LNGINNYKNPKL
		mFcIgG2a(LA		TAMLTAKFAMP
		LAPG)-		KKATELKHLQCL
:378	DNA478	[VPLSLY]-	SGP	EEALKPLEEVLN
		hIL2(R38A,		LAQSKNFHLRPR
		F42A, Y45A,		DLISNINVIVLEL
		E62A, C125A)		KGSETTFMCEY
				ADETATIVEFLN
				RWITFAQSIISTL
				T
				TIKPCPPCKCPAPNAAGGPSVFIFPPKIKDVLMISLSPIVTCVVVDVSEDDPDVQIS
				WFVNNVEVHTAQTQTHREDYNSTLRVVSALPIQHQDWMSGKEFKCKVNNKDL
				GAPIERTISKPKGSVRAPQVCVLPPPEEEMTKKQVTLSCAVTDFMPEDIYVEWTN
				NGKTELNYKNTEPVLDSDGSYFMVSKLRVEKKNWVERNSYSCSVVHEGLHNHHT
		Hole:		TKSFSRTPGPGSGSAVNGTSQFTCFYNSRANISCVWSQDGALQDTSCQVHAWP
:378	DNA480	mFcIgG2a(LA		DRRRWNQTCELLPVSQASWACNLILGAPDSQKLTTVDIVTLRVLCREGVRWRV
		LAPG)-		MAIQDFKPFENLRLMAPISLQVVHVETHRCNISWEISQASHYFERHLEFEARTLSP
		hCD122		GHTWEEAPLLTLKQKQEWICLETLTPDTQYEFQVRVKPLQGEFTTWSPWSQPLA
				FRTKPAALGKD
				DKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFN
				WYVDGVEVHNAKTKPREEQYASTYRVVSVLTVLHQDWLNGKEYKCKVSNKALP
:397	DNA158	Hole:		APIEKTISKAKGQPREPQVCTLPPSRDELTKNQVSLSCAVKGFYPSDIAVEWESNG
		hFc(N297A)		QPENNYKTTPPVLDSDGSFFLVSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQ
				KSLSLSPG
				APTSSSTKKTQL
				QLEHLLLDLQMI
				LNGINNYKNPKL
				DKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFN
				TRMLTFKFYMP
		Knob:		WYVDGVEVHNAKTKPREEQYASTYRVVSVLTVLHQDWLNGKEYKCKVSNKALP
		hFc(N297A)-		KKATELKHLQCL
:397	DNA278	[DSGGFMLT]-	SGP	APIEKTISKAKGQPREPQVTLPPCRDELTKNQVSLWCLVKGFYPSDIAVEWESN
		hIL2(C125A)		EEELKPLEEVLN
				GQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYT
				LAQSKNFHLRPR
				QKSLSLSPGGSGPDSGGFMLTSGPAPTSSSTKKTQLQLEHLLLDLQMILNGINNYK
				DLISNINVIVLEL
				NPKLTRMLTFKFYMPKKATELKHLQCLEEELKPLEEVLNLAQSKNFHLRPRDLISNI
				KGSETTFMCEY
				NVIVLELKGSETTFMCEYADETATIVEFLNRWITFAQSIISTLT
				ADETATIVEFLN
				RWITFAQSIISTL
				T

		Knob: mFcIgG2a(LALAPG)-hIL2(R38A, F42A, Y45A, E62A, C125A)	TIKPCPPCKCPAPNAAGGPSVFIFPPKIKDVLMISLSPIVTCVVVDVSEDDPDVQIS WFVNNVEVHTAQTQTHREDYNSTLRVVSALPIQHQDWMSGKEFKCKVNNKDL GAPIERTISKPKGSVRAPQVYVLPPCEEEMTKKQVTLWCMVTDFMPEDIYVEWT NNGKTELNYKNTEPVLDSDGSYFMYSKLRVEKKNWVERNSYSCSVVHEGLHNH HTTKSFSRTPGGGSSPPGGGSSGGGSGPAPTSSSTKKTQLQLEHLLLDLQMILNGI NNYKNPKLTAMLTAKFAMPKKATELKHLQCLEEALKPLEEVLNLAQSKNFHLRPR DLISNINVIVLELKGSETTFMCEYADETATIVEFLNRWITFAQSIISTLT
429	DNA477		
		Hole: mFcIgG2a(LALAPG)-NoAnnotationFound	TIKPCPPCKCPAPNAAGGPSVFIFPPKIKDVLMISLSPIVTCVVVDVSEDDPDVQIS WFVNNVEVHTAQTQTHREDYNSTLRVVSALPIQHQDWMSGKEFKCKVNNKDL GAPIERTISKPKGSVRAPQVCVLPPPEEEMTKKQVTLSCAVTDFMPEDIYVEWTN NGKTELNYKNTEPVLDSDGSYFMVSKLRVEKKNWVERNSYSCSVVHEGLHNHHT TKSFSRTPGHHHHHHHHH
429	DNA520		
		Knob: mFcIgG2a(LALAPG)-hIL2(R38A, F42A, Y45A, E62A, C125A)	TIKPCPPCKCPAPNAAGGPSVFIFPPKIKDVLMISLSPIVTCVVVDVSEDDPDVQIS WFVNNVEVHTAQTQTHREDYNSTLRVVSALPIQHQDWMSGKEFKCKVNNKDL GAPIERTISKPKGSVRAPQVYVLPPCEEEMTKKQVTLWCMVTDFMPEDIYVEWT NNGKTELNYKNTEPVLDSDGSYFMYSKLRVEKKNWVERNSYSCSVVHEGLHNH HTTKSFSRTPGGGSSPPGGGSSGGGSGPAPTSSSTKKTQLQLEHLLLDLQMILNGI NNYKNPKLTAMLTAKFAMPKKATELKHLQCLEEALKPLEEVLNLAQSKNFHLRPR DLISNINVIVLELKGSETTFMCEYADETATIVEFLNRWITFAQSIISTLT
430	DNA477		
		Hole: mFcIgG2a(LALAPG)-hCD122-NoAnnotationFound	TIKPCPPCKCPAPNAAGGPSVFIFPPKIKDVLMISLSPIVTCVVVDVSEDDPDVQIS WFVNNVEVHTAQTQTHREDYNSTLRVVSALPIQHQDWMSGKEFKCKVNNKDL GAPIERTISKPKGSVRAPQVCVLPPPEEEMTKKQVTLSCAVTDFMPEDIYVEWTN NGKTELNYKNTEPVLDSDGSYFMVSKLRVEKKNWVERNSYSCSVVHEGLHNHHT TKSFSRTPGPGSGSAVNGTSQFTCFYNSRANISCVWSQDGALQDTSCQVHAWP DRRRWNQTCELLPVSQASWACNLILGAPDSQKLTTVDIVTLRVLCREGVRWRV MAIQDFKPFENLRLMAPISLQVVHVETHRCNISWEISQASHYFERHLEFEARTLSP GHTWEEAPLLTLKQKQEWICLETLTPDTQYEFQVRVKPLQGEFTTWSPWSQPLA FRTKPAALGKDGHHHHHHHHH
430	DNA521		

		Knob: mFcIgG2a(LALAPG)-hIL2(R38A, F42A, Y45A, E62A, C125A)		TIKPCPPCKCPAPNAAGGPSVFIFPPKIKDVLMISLSPIVTCVVVDVSEDDPDVQIS WFVNNVEVHTAQTQTHREDYNSTLRVVSALPIQHQDWMSGKEFKCKVNNKDL GAPIERTISKPKGSVRAPQVYVLPPCEEEMTKKQVTLWCMVTDFMPEDIYVEWT NNGKTELNYKNTEPVLDSDGSYFMYSKLRVEKKNWVERNSYSCSVVHEGLHNH HTTKSFSRTPGGGSSPPGGGSSGGGSGPAPTSSSTKKTQLQLEHLLLDLQMILNGI NNYKNPKLTAMLTAKFAMPKKATELKHLQCLEEALKPLEEVLNLAQSKNFHLRPR DLISNINVIVLELKGSETTFMCEYADETATIVEFLNRWITFAQSIISTLT
:431	DNA477			
		Hole: mFcIgG2a(LALAPG)-mCD122-NoAnnotationFound	GHHHHHHHH	TIKPCPPCKCPAPNAAGGPSVFIFPPKIKDVLMISLSPIVTCVVVDVSEDDPDVQIS WFVNNVEVHTAQTQTHREDYNSTLRVVSALPIQHQDWMSGKEFKCKVNNKDL GAPIERTISKPKGSVRAPQVCVLPPPEEEMTKKQVTLSCAVTDFMPEDIYVEWTN NGKTELNYKNTEPVLDSDGSYFMVSKLRVEKKNWVERNSYSCSVVHEGLHNHHT TKSFSRTPGPGSGSAVKNCSHLECFYNSRANVSCMWSHEEALNVTTCHVHAKSN LRHWNKTCELTLVRQASWACNLILGSFPESQSLTSVDLLDINVVCWEEKGWRRV KTCDFHPFDNLRLVAPHSLQVLHIDTQRCNISWKVSQVSHYIEPYLEFEARRRLLG HSWEDASVLSLKQRQQWLFLEMLIPSTSYEVQVRVKAQRNNTGTWSPWSQPLT FRTRPADPMKEGHHHHHHHH
:431	DNA522			
		Knob: mFcIgG2a(LALAPG)-[VPLSLY]-hIL2(R38A, F42A, Y45A, E62A, C125A)	SGP	APTSSSTKKTQL QLEHLLLDLQMI LNGINNYKNPKL TAMLTAKFAMP KKATELKHLQCL EEALKPLEEVLN LAQSKNFHLRPR DLISNINVIVLEL KGSETTFMCEY ADETATIVEFLN RWITFAQSIISTL T
:432	DNA478			TIKPCPPCKCPAPNAAGGPSVFIFPPKIKDVLMISLSPIVTCVVVDVSEDDPDVQIS WFVNNVEVHTAQTQTHREDYNSTLRVVSALPIQHQDWMSGKEFKCKVNNKDL GAPIERTISKPKGSVRAPQVYVLPPCEEEMTKKQVTLWCMVTDFMPEDIYVEWT NNGKTELNYKNTEPVLDSDGSYFMYSKLRVEKKNWVERNSYSCSVVHEGLHNH HTTKSFSRTPGGSPGVPLSLYSGPAPTSSSTKKTQLQLEHLLLDLQMILNGINNYKN PKLTAMLTAKFAMPKKATELKHLQCLEEALKPLEEVLNLAQSKNFHLRPRDLISNI NVIVLELKGSETTFMCEYADETATIVEFLNRWITFAQSIISTLT

					TIKPCPPCKCPAPNAAGGPSVFIFPPKIKDVLMISLSPIVTCVVVDVSEDDPDVQIS
					WFVNNVEVHTAQTQTHREDYNSTLRVVSALPIQHQDWMSGKEFKCKVNNKDL
			Hole:		GAPIERTISKPKGSVRAPQVCVLPPPEEEMTKKQVTLSCAVTDFMPEDIYVEWTN
			mFcIgG2a(LA		NGKTELNYKNTEPVLDSDGSYFMVSKLRVEKKNWVERNSYSCSVVHEGLHNHHT
:432	DNA521		LAPG)-	GHHHHHHHHH	TKSFSRTPGPGSGSAVNGTSQFTCFYNSRANISCVWSQDGALQDTSCQVHAWP
			hCD122-		DRRRWNQTCELLPVSQASWACNLILGAPDSQKLTTVDIVTLRVLCREGVRWRV
			NoAnnotatio		MAIQDFKPFENLRLMAPISLQVVHVETHRCNISWEISQASHYFERHLEFEARTLSP
			nFound		GHTWEEAPLLTLKQKQEWICLETLTPDTQYEFQVRVKPLQGEFTTWSPWSQPLA
					FRTKPAALGKDGHHHHHHHHH
					APTSSSTKKTQL
					QLEHLLLDLQMI
					LNGINNYKNPKL
			Knob:		TIKPCPPCKCPAPNAAGGPSVFIFPPKIKDVLMISLSPIVTCVVVDVSEDDPDVQIS
			mFcIgG2a(LA		TAMLTAKFAMP
			LAPG)-		WFVNNVEVHTAQTQTHREDYNSTLRVVSALPIQHQDWMSGKEFKCKVNNKDL
			[VPLSLY]-		KKATELKHLQCL
:433	DNA478	SGP	hIL2(R38A,		GAPIERTISKPKGSVRAPQVYVLPPCEEEMTKKQVTLWCMVTDFMPEDIYVEWT
			F42A, Y45A,		EEALKPLEEVLN
			E62A, C125A)		NNGKTELNYKNTEPVLDSDGSYFMYSKLRVEKKNWVERNSYSCSVVHEGLHNH
					LAQSKNFHLRPR
					HTTKSFSRTPGGSPGVPLSLYSGPAPTSSSTKKTQLQLEHLLLDLQMILNGINNYKN
					DLISNINVIVLEL
					PKLTAMLTAKFAMPKKATELKHLQCLEEALKPLEEVLNLAQSKNFHLRPRDLISNI
					KGSETTFMCEY
					NVIVLELKGSETTFMCEYADETATIVEFLNRWITFAQSIISTLT
					ADETATIVEFLN
					RWITFAQSIISTL
					T
					TIKPCPPCKCPAPNAAGGPSVFIFPPKIKDVLMISLSPIVTCVVVDVSEDDPDVQIS
					WFVNNVEVHTAQTQTHREDYNSTLRVVSALPIQHQDWMSGKEFKCKVNNKDL
			Hole:		GAPIERTISKPKGSVRAPQVCVLPPPEEEMTKKQVTLSCAVTDFMPEDIYVEWTN
			mFcIgG2a(LA		NGKTELNYKNTEPVLDSDGSYFMVSKLRVEKKNWVERNSYSCSVVHEGLHNHHT
			LAPG)-		TKSFSRTPGPGSGSAVKNCSHLECFYNSRANVSCMWSHEEALNVTTCHVHAKSN
:433	DNA522	GHHHHHHHHH	mCD122-		LRHWNKTCELTLVRQASWACNLILGSFPESQSLTSVDLLDINVVCWEEKGWRRV
			NoAnnotatio		KTCDFHPFDNLRLVAPHSLQVLHIDTQRCNISWKVSQVSHYIEPYLEFEARRRLLG
			nFound		HSWEDASVLSLKQRQQWLFLEMLIPSTSYEVQVRVKAQRNNTGTWSPWSQPLT
					FRTRPADPMKEGHHHHHHHH

				APTSSSTKKTQL
				QLEHLLLDLQMI
				LNGINNYKNPKL
		Knob:		TAMLTAKFAMP
		hFc(N297A)-		KKATELKHLQCL
:435	DNA263	[VPLSLY]-	SGP	EEALKPLEEVLN
		hIL2(R38A,		LAQSKNFHLRPR
		F42A, Y45A,		DLISNINVIVLEL
		E62A, C125A)		KGSETTFMCEY
				ADETATIVEFLN
				RWITFAQSIISTL
				T
				AVNGTSQFTCF
				YNSRANISCVW
				SQDGALQDTSC
				QVHAWPDRRR
				WNQTCELLPVS
				QASWACNLILG
				APDSQKLTTVDI
				VTLRVLCREGVR
				WRVMAIQDFK
		F8ScFvVersio		PFENLRLMAPIS
:435	DNA516	n1-Hole:	PGSGS	LQVVHVETHRC
		hFc(N297A)-		NISWEISQASHY
		hCD122		FERHLEFEARTL
				SPGHTWEEAPL
				LTLKQKQEWICL
				ETLTPDTQYEFQ
				VRVKPLQGEFTT
				WSPWSQPLAFR
				TKPAALGKD

				DKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFN
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				APIEKTISKAKGQPREPQVYTLPPCRDELTKNQVSLWCLVKGFYPSDIAVEWESN
				GQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYT
				QKSLSLSPGGSPGVPLSLYSGPAPTSSSTKKTQLQLEHLLLDLQMILNGINNYKNPK
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				GGSTYYADSVKGRFTISRDNSKNTLYLQMNSLRAEDTAVYYCAKSTHLYLFDYWG
				QGTLVTVSSGGGGSGGGGSGGGGSEIVLTQSPGTLSLSPGERATLSCRASQSVSM
				PFLAWYQQKPGQAPRLLIYGASSRATGIPDRFSGSGSGTDFTLTISRLEPEDFAVYY
				CQQMRGRPPTFGQGTKVEIKGGSDKTHTCPPCPAPELLGGPSVFLFPPKPKDTL
				MISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYASTYRVVSV
				LTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVCTLPPSRDELTKN
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				WQQGNVFSCSVMHEALHNHYTQKSLSLSPGPGSGSAVNGTSQFTCFYNSRANIS
				CVWSQDGALQDTSCQVHAWPDRRRWNQTCELLPVSQASWACNLILGAPDSQ
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			DKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFN
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			APIEKTISKAKGQPREPQVCTLPPSRDELTKNQVSLSCAVKGFYPSDIAVEWESNG
		Hole:	QPENNYKTTPPVLDSDGSFFLVSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQ
:436	DNA187	hFc(N297A)-	KSLSLSPGPGSGSAVNGTSQFTCFYNSRANISCVWSQDGALQDTSCQVHAWPDR
		hCD122	RRWNQTCELLPVSQASWACNLILGAPDSQKLTTVDIVTLRVLCREGVRWRVMAI
			QDFKPFENLRLMAPISLQVVHVETHRCNISWEISQASHYFERHLEFEARTLSPGHT
			WEEAPLLTLKQKQEWICLETLTPDTQYEFQVRVKPLQGEFTTWSPWSQPLAFRT
			KPAALGKD
			DKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFN
		Knob:	WYVDGVEVHNAKTKPREEQYASTYRVVSVLTVLHQDWLNGKEYKCKVSNKALP
		hFc(N297A)-	APIEKTISKAKGQPREPQVTLPPCRDELTKNQVSLWCLVKGFYPSDIAVEWESN
:436	DNA542	hIL2(R38A,	GQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYT
		F42A, Y45A,	QKSLSLSPGGISSGLLSGRSDQPSGPAPTSSSTKKTQLQLEHLLLDLQMILNGINNY
		E62A, C125A)	KNPKLTAMLTAKFAMPKKATELKHLQCLEEALKPLEEVLNLAQSKNFHLRPRDLIS
			NINVIVLELKGSETTFMCEYADETATIVEFLNRWITFAQSIISTLT
			DKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFN
			WYVDGVEVHNAKTKPREEQYASTYRVVSVLTVLHQDWLNGKEYKCKVSNKALP
			APIEKTISKAKGQPREPQVCTLPPSRDELTKNQVSLSCAVKGFYPSDIAVEWESNG
		Hole:	QPENNYKTTPPVLDSDGSFFLVSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQ
:437	DNA187	hFc(N297A)-	KSLSLSPGPGSGSAVNGTSQFTCFYNSRANISCVWSQDGALQDTSCQVHAWPDR
		hCD122	RRWNQTCELLPVSQASWACNLILGAPDSQKLTTVDIVTLRVLCREGVRWRVMAI
			QDFKPFENLRLMAPISLQVVHVETHRCNISWEISQASHYFERHLEFEARTLSPGHT
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			DKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFN
		Knob:	WYVDGVEVHNAKTKPREEQYASTYRVVSVLTVLHQDWLNGKEYKCKVSNKALP
		hFc(N297A)-	APIEKTISKAKGQPREPQVTLPPCRDELTKNQVSLWCLVKGFYPSDIAVEWESN
:437	DNA545	hIL2(R38A,	GQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYT
		F42A, Y45A,	QKSLSLSPGGISSGLLSGRSSGPAPTSSSTKKTQLQLEHLLLDLQMILNGINNYKNP
		E62A, C125A)	KLTAMLTAKFAMPKKATELKHLQCLEEALKPLEEVLNLAQSKNFHLRPRDLISNIN
			VIVLELKGSETTFMCEYADETATIVEFLNRWITFAQSIISTLT

				DKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFN
				WYVDGVEVHNAKTKPREEQYASTYRVVSVLTVLHQDWLNGKEYKCKVSNKALP
		Knob:		APIEKTISKAKGQPREPQVYTLPPCRDELTKNQVSLWCLVKGFYPSDIAVEWESN
:438	DNA255	hFc(N297A)-hIL2(R38A, F42A, Y45A, E62A, C125A)		GQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYT
				QKSLSLSPGGGSSPPGGGSSGGGSGPAPTSSSTKKTQLQLEHLLLDLQMILNGINN
				YKNPKLTAMLTAKFAMPKKATELKHLQCLEEALKPLEEVLNLAQSKNFHLRPRDLI
				SNINVIVLELKGSETTFMCEYADETATIVEFLNRWITFAQSIISTLT
				AVNGTSQFTCF
				YNSRANISCVW
				SQDGALQDTSC
				QVHAWPDRRR
				WNQTCELLPVS
				QASWACNLILG
				APDSQKLTTVDI
				VTLRVLCREGVR
		Hole:		WRVMAIQDFK
:438	DNA543	hFc(N297A)-[VPLSLY]-hCD122	GSGGG	PFENLRLMAPIS
				LQVVHVETHRC
				NISWEISQASHY
				FERHLEFEARTL
				SPGHTWEEAPL
				LTLKQKQEWICL
				ETLTPDTQYEFQ
				VRVKPLQGEFTT
				WSPWSQPLAFR
				TKPAALGKD
				DKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFN
				WYVDGVEVHNAKTKPREEQYASTYRVVSVLTVLHQDWLNGKEYKCKVSNKALP
:439	DNA158	Hole: hFc(N297A)		APIEKTISKAKGQPREPQVCTLPPSRDELTKNQVSLSCAVKGFYPSDIAVEWESNG
				QPENNYKTTPPVLDSDGSFFLVSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQ
				KSLSLSPG

			APTSSSTKKTQL	
			QLEHLLLDLQMI	
		Knob:	LNGINNYKNPKL	DKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFN
		hFc(N297A)-[VPLSLY]-hIL2(R38A,	TAMLTAKFAMP	WYVDGVEVHNAKTKPREEQYASTYRVVSVLTVLHQDWLNGKEYKCKVSNKALP
			KKATELKHLQCL	APIEKTISKAKGQPREPQVYTLPPCRDELTKNQVSLWCLVKGFYPSDIAVEWESN
:439	DNA544	F42A, Y45A, E62A, L80F, R81D, L85V, I86V, I92F, C125A)	EEALKPLEEVLN	GQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYT
			LAQSKNFHFDP	QKSLSLSPGGSPGVPLSLYSGPAPTSSSTKKTQLQLEHLLLDLQMILNGINNYKNPK
			RDVVSNINVFVL	LTAMLTAKFAMPKKATELKHLQCLEEALKPLEEVLNLAQSKNFHFDPRDVVSNIN
			ELKGSETTFMCE	VFVLELKGSETTFMCEYADETATIVEFLNRWITFAQSIISTLT
			YADETATIVEFL	
			NRWITFAQSIIS	
			TLT	
				DKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFN
				WYVDGVEVHNAKTKPREEQYASTYRVVSVLTVLHQDWLNGKEYKCKVSNKALP
				APIEKTISKAKGQPREPQVCTLPPSRDELTKNQVSLSCAVKGFYPSDIAVEWESNG
		Hole:		QPENNYKTTPPVLDSDGSFFLVSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQ
:440	DNA187	hFc(N297A)-hCD122		KSLSLSPGPGSGSAVNGTSQFTCFYNSRANISCVWSQDGALQDTSCQVHAWPDR
				RRWNQTCELLPVSQASWACNLILGAPDSQKLTTVDIVTLRVLCREGVRWRVMAI
				QDFKPFENLRLMAPISLQVVHVETHRCNISWEISQASHYFERHLEFEARTLSPGHT
				WEEAPLLTLKQKQEWICLETLTPDTQYEFQVRVKPLQGEFTTWSPWSQPLAFRT
				KPAALGKD
			APTSSSTKKTQL	
		Knob:	QLEHLLLDLQMI	
		hFc(N297A)-[VPLSLY]-hIL2(R38A,	LNGINNYKNPKL	DKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFN
			TAMLTAKFAMP	WYVDGVEVHNAKTKPREEQYASTYRVVSVLTVLHQDWLNGKEYKCKVSNKALP
			KKATELKHLQCL	APIEKTISKAKGQPREPQVYTLPPCRDELTKNQVSLWCLVKGFYPSDIAVEWESN
:440	DNA544	F42A, Y45A, E62A, L80F, R81D, L85V, I86V, I92F, C125A)	EEALKPLEEVLN	GQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYT
			LAQSKNFHFDP	QKSLSLSPGGSPGVPLSLYSGPAPTSSSTKKTQLQLEHLLLDLQMILNGINNYKNPK
			RDVVSNINVFVL	LTAMLTAKFAMPKKATELKHLQCLEEALKPLEEVLNLAQSKNFHFDPRDVVSNIN
			ELKGSETTFMCE	VFVLELKGSETTFMCEYADETATIVEFLNRWITFAQSIISTLT
			YADETATIVEFL	
			NRWITFAQSIIS	
			TLT	

AVNGTSQFTCF
YNSRANISCVW
SQDGALQDTSC
QVHAWPDRRR
WNQTCELLPVS

				QASWACNLILG
				APDSQKLTTVDI
				VTLRVLCREGVR
				WRVMAIQDFK
:441	DNA543	Hole: hFc(N297A)-[VPLSLY]-hCD122	GSGGG	PFENLRLMAPIS
				LQVVHVETHRC
				NISWEISQASHY
				FERHLEFEARTL
				SPGHTWEEAPL
				LTLKQKQEWICL
				ETLTPDTQYEFQ
				VRVKPLQGEFTT
				WSPWSQPLAFR
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				WYVDGVEVHNAKTKPREEQYASTYRVVSVLTVLHQDWLNGKEYKCKVSNKALP
		Knob: hFc(N297A)-hIL2(R38A, F42A, Y45A, E62A, L80F, R81D, L85V, I86V, I92F, C125A)		APIEKTISKAKGQPREPQVYTLPPCRDELTKNQVSLWCLVKGFYPSDIAVEWESN
:441	DNA546			GQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYT
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				YKNPKLTAMLTAKFAMPKKATELKHLQCLEEALKPLEEVLNLAQSKNFHFDPRDV
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				DKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFN
				WYVDGVEVHNAKTKPREEQYASTYRVVSVLTVLHQDWLNGKEYKCKVSNKALP
		Knob:		APIEKTISKAKGQPREPQVYTLPPCRDELTKNQVSLWCLVKGFYPSDIAVEWESN
:442	DNA255	hFc(N297A)-hIL2(R38A, F42A, Y45A, E62A, C125A)		GQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYT
				QKSLSLSPGGGSSPPGGGSSGGGSGPAPTSSSTKKTQLQLEHLLLDLQMILNGINN
				YKNPKLTAMLTAKFAMPKKATELKHLQCLEEALKPLEEVLNLAQSKNFHLRPRDLI
				SNINVIVLELKGSETTFMCEYADETATIVEFLNRWITFAQSIISTLT
				AVNGTSQFTCF
				YNSRANISCVW
				SQDGALQDTSC
				QVHAWPDRRR
				WNQTCELLPVS
				QASWACNLILG
				APDSQKLTTVDI
				VTLRVLCREGVR
		Hole:		WRVMAIQDFK
:442	DNA553	hFc(N297A)-[DSGGFMLT]-hCD122	SGGG	PFENLRLMAPIS
				LQVVHVETHRC
				NISWEISQASHY
				FERHLEFEARTL
				SPGHTWEEAPL
				LTLKQKQEWICL
				ETLTPDTQYEFQ
				VRVKPLQGEFTT
				WSPWSQPLAFR
				TKPAALGKD
				DKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFN
				WYVDGVEVHNAKTKPREEQYASTYRVVSVLTVLHQDWLNGKEYKCKVSNKALP
				APIEKTISKAKGQPREPQVCTLPPSRDELTKNQVSLSCAVKGFYPSDIAVEWESNG
				QPENNYKTTPPVLDSDGSFFLVSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQ
:443	DNA187	Hole: hFc(N297A)-hCD122		KSLSLSPGPGSGSAVNGTSQFTCFYNSRANISCVWSQDGALQDTSCQVHAWPDR
				RRWNQTCELLPVSQASWACNLILGAPDSQKLTTVDIVTLRVLCREGVRWRVMAI
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				WEEAPLLTLKQKQEWICLETLTPDTQYEFQVRVKPLQGEFTTWSPWSQPLAFRT
				KPAALGKD

APTSSSTKKTQL
QLRHLCLRLQMI
LNGINNYKNPKL
Knob: hFc(N297A)-[VPLSLY]-hIL2(E15R, L18C, D20R, R38A, F42A, Y45A, E62A)-SGP :443 DNA554 TAMLTAKFAMP DKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFN
KKATELKHLQCL WYVDGVEVHNAKTKPREEQYASTYRVVSVLTVLHQDWLNGKEYKCKVSNKALP
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RWITFCQSIISTL
T

APTSSSTKKTQL
QLEHLLLDLQMI
LNGINNYKNPKL
Knob: hFc(N297A)-[DSGGFMLT]-hIL2(R38A, F42A, Y45A, E62A, C125A)-SGP :444 DNA281 TAMLTAKFAMP DKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFN
KKATELKHLQCL WYVDGVEVHNAKTKPREEQYASTYRVVSVLTVLHQDWLNGKEYKCKVSNKALP
EEALKPLEEVLN APIEKTISKAKGQPREPQVYTLPPCRDELTKNQVSLWCLVKGFYPSDIAVEWESN
LAQSKNFHLRPR GQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYT
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KGSETTFMCEY NPKLTAMLTAKFAMPKKATELKHLQCLEEALKPLEEVLNLAQSKNFHLRPRDLISN
ADETATIVEFLN INVIVLELKGSETTFMCEYADETATIVEFLNRWITFAQSIISTLT
RWITFAQSIISTL
T

Hole: hFc(N297A)-hCD122(C122S, C168S) :444 DNA440 DKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFN
WYVDGVEVHNAKTKPREEQYASTYRVVSVLTVLHQDWLNGKEYKCKVSNKALP
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			EPKSSDKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDP EVKFNWYVDGVEVHNAKTKPREEQYASTYRVVSVLTVLHQDWLNGKEYKCKVS NKALPAPIEKTISKAKGQPREPQVCTLPPSRDELTKNQVSLSCAVKGFYPSDIAVE WESNGQPENNYKTTPPVLDSDGSFFLVSKLTVDKSRWQQGNVFSCSVMHEALH NHYTQKSLSLSPGPGSGSAVNGTSQFTCFYNSRANISCVWSQDGALQDTSCQVH AWPDRRRWNQTCELLPVSQASWACNLILGAPDSQKLTTVDIVTLRVLCREGVR WRVMAIQDFKPFENLRLMAPISLQVVHVETHRCNISWEISQASHYFERHLEFEAR TLSPGHTWEEAPLLTLKQKQEWICLETLTPDTQYEFQVRVKPLQGEFTTWSPWS QPLAFRTKPAALGKD
			APTSSSTKKTQL QLEHLLLDLQMI LNGINNYKNPKL
:449	DNA547	Hole: hFcIgG1(N297A)- EPKSS)- Hole: hFc(N297A)- hCD122	EPKSSDKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDP EVKFNWYVDGVEVHNAKTKPREEQYASTYRVVSVLTVLHQDWLNGKEYKCKVS NKALPAPIEKTISKAKGQPREPQVYTLPPCRDELTKNQVSLWCLVKGFYPSDIAVE WESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALH NHYTQKSLSLSPGGSPGVPLSLYSGPAPTSSSTKKTQLQLEHLLLDLQMILNGINNY KNPKLTAMLTAKFAMPKKATELKHLQCLEEALKPLEEVLNLAQSKNFHLRPRDLIS NINVIVLELKGSETTFMCEYADETATIVEFLNRWITFAQSIISTLT
:449	DNA550	Knob: hFcIgG1(N297A)- EPKSS)- hIL2(R38A, F42A, Y45A, E62A, C125A)	SGP
:450	DNA548	Hole: hFcIgG1(N297A)- AKT)- Hole: hFc(N297A)- hCD122	AKTDKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEV KFNWYVDGVEVHNAKTKPREEQYASTYRVVSVLTVLHQDWLNGKEYKCKVSNK ALPAPIEKTISKAKGQPREPQVCTLPPSRDELTKNQVSLSCAVKGFYPSDIAVEWES NGQPENNYKTTPPVLDSDGSFFLVSKLTVDKSRWQQGNVFSCSVMHEALHNHY TQKSLSLSPGPGSGSAVNGTSQFTCFYNSRANISCVWSQDGALQDTSCQVHAWP DRRRWNQTCELLPVSQASWACNLILGAPDSQKLTTVDIVTLRVLCREGVRWRV MAIQDFKPFENLRLMAPISLQVVHVETHRCNISWEISQASHYFERHLEFEARTLSP GHTWEEAPLLTLKQKQEWICLETLTPDTQYEFQVRVKPLQGEFTTWSPWSQPLA FRTKPAALGKD

				APTSSSTKKTQL
				QLEHLLLDLQMI
		Knob:		LNGINNYKNPKL
		hFcIgG1(N29		TAMLTAKFAMP
		7A + AKT)-		KKATELKHLQCL
:450	DNA551	[VPLSLY]-	SGP	EEALKPLEEVLN
		hIL2(R38A,		LAQSKNFHLRPR
		F42A, Y45A,		DLISNINVIVLEL
		E62A, C125A)		KGSETTFMCEY
				ADETATIVEFLN
				RWITFAQSIISTL
				T

				AKTDKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEV
				KFNWYVDGVEVHNAKTKPREEQYASTYRVVSVLTVLHQDWLNGKEYKCKVSNK
				ALPAPIEKTISKAKGQPREPQVYTLPPCRDELTKNQVSLWCLVKGFYPSDIAVEWE
				SNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNH
				YTQKSLSLSPGGSPGVPLSLYSGPAPTSSSTKKTQLQLEHLLLDLQMILNGINNYKN
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				NVIVLELKGSETTFMCEYADETATIVEFLNRWITFAQSIISTLT

				AKTEPKSSDKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSH
				EDPEVKFNWYVDGVEVHNAKTKPREEQYASTYRVVSVLTVLHQDWLNGKEYKC
				KVSNKALPAPIEKTISKAKGQPREPQVCTLPPSRDELTKNQVSLSCAVKGFYPSDIA
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		Hole:		LHNHYTQKSLSLSPGPGSGSAVNGTSQFTCFYNSRANISCVWSQDGALQDTSCQ
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:451	DNA549	7A		RWRVMAIQDFKPFENLRLMAPISLQVVHVETHRCNISWEISQASHYFERHLEFEA
		+AKTEPKSS)--		RTLSPGHTWEEAPLLTLKQKQEWICLETLTPDTQYEFQVRVKPLQGEFTTWSPW
		hCD122		SQPLAFRTKPAALGKD

				APTSSSTKKTQL
				QLEHLLLDLQMI
		Knob :		LNGINNYKNPKL
		hFcIgG1(N29		TAMLTAKFAMP
		7A +		KKATELKHLQCL
		AKTEPKSS)-		EEALKPLEEVLN
:451	DNA552	[VPLSLY]-	SGP	LAQSKNFHLRPR
		hIL2(R38A,		DLISNINVIVLEL
		F42A, Y45A,		KGSETTFMCEY
		E62A, C125A)		ADETATIVEFLN
				RWITFAQSIISTL
				T

				AKTEPKSSDKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSH
				EDPEVKFNWYVDGVEVHNAKTKPREEQYASTYRVVSVLTVLHQDWLNGKEYKC
				KVSNKALPAPIEKTISKAKGQPREPQVYTLPPCRDELTKNQVSLWCLVKGFYPSDI
				AVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHE
				ALHNHYTQKSLSLSPGGSPGVPLSLYSGPAPTSSSTKKTQLQLEHLLLDLQMILNGI
				NNYKNPKLTAMLTAKFAMPKKATELKHLQCLEEALKPLEEVLNLAQSKNFHLRPR
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				DKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFN
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				APIEKTISKAKGQPREPQVCTLPPSRDELTKNQVSLSCAVKGFYPSDIAVEWESNG
				QPENNYKTTPPVLDSDGSFFLVSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQ
:452	DNA187	Hole: hFc(N297A)-hCD122		KSLSLSPGPGSGSAVNGTSQFTCFYNSRANISCVWSQDGALQDTSCQVHAWPDR
				RRWNQTCELLPVSQASWACNLILGAPDSQKLTTVDIVTLRVLCREGVRWRVMAI
				QDFKPFENLRLMAPISLQVVHVETHRCNISWEISQASHYFERHLEFEARTLSPGHT
				WEEAPLLTLKQKQEWICLETLTPDTQYEFQVRVKPLQGEFTTWSPWSQPLAFRT
				KPAALGKD
				APTSSSTKKTQL
				QLRHLCLRLQMI
		Knob: hFc(N297A)-[VPLSLY]-hIL2(E15R, L18C, D20R, R38A, F42A, Y45A, E62A, N88L) SGP		LNGINNYKNPKL DKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFN
				TAMLTAKFAMP WYVDGVEVHNAKTKPREEQYASTYRVVSVLTVLHQDWLNGKEYKCKVSNKALP
				KKATELKHLQCL APIEKTISKAKGQPREPQVYTLPPCRDELTKNQVSLWCLVKGFYPSDIAVEWESN
:452	DNA563			EEALKPLEEVLN GQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYT
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				DLISLINVIVLELK LTAMLTAKFAMPKKATELKHLQCLEEALKPLEEVLNLAQSKNFHLRPRDLISLINVI
				GSETTFMCEYA VLELKGSETTFMCEYADETATIVEFLNRWITFCQSIISTLT
				DETATIVEFLNR
				WITFCQSIISTLT
				DKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFN
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:453	DNA187	Hole: hFc(N297A)-hCD122		KSLSLSPGPGSGSAVNGTSQFTCFYNSRANISCVWSQDGALQDTSCQVHAWPDR
				RRWNQTCELLPVSQASWACNLILGAPDSQKLTTVDIVTLRVLCREGVRWRVMAI
				QDFKPFENLRLMAPISLQVVHVETHRCNISWEISQASHYFERHLEFEARTLSPGHT
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				KPAALGKD

			APTSSSTKKTQL	
			QLLHLCLRLQMI	
		Knob:	LNGINNYKNPKL	DKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFN
		hFc(N297A)-	TAMLTAKFAMP	WYVDGVEVHNAKTKPREEQYASTYRVVSVLTVLHQDWLNGKEYKCKVSNKALP
		[VPLSLY]-	KKATELKHLQCL	APIEKTISKAKGQPREPQVYTLPPCRDELTKNQVSLWCLVKGFYPSDIAVEWESN
:453	DNA565	hIL2(E15L,	EEALKPLEEVLN	GQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYT
		L18C, D20R,	LAQSKNFHLRPR	QKSLSLSPGGSPGVPLSLYSGPAPTSSSTKKTQLQLLHLCLRLQMILNGINNYKNPK
		R38A, F42A,	DLISLINVIVLELK	LTAMLTAKFAMPKKATELKHLQCLEEALKPLEEVLNLAQSKNFHLRPRDLISLINVI
		Y45A, E62A,	GSETTFMCEYA	VLELKGSETTFMCEYADETATIVEFLNRWITFCQSIISTLT
		N88L)	DETATIVEFLNR	
			WITFCQSIISTLT	
				DKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFN
				WYVDGVEVHNAKTKPREEQYASTYRVVSVLTVLHQDWLNGKEYKCKVSNKALP
				APIEKTISKAKGQPREPQVCTLPPSRDELTKNQVSLSCAVKGFYPSDIAVEWESNG
		Hole:		QPENNYKTTPPVLDSDGSFFLVSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQ
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		hCD122		RRWNQTCELLPVSQASWACNLILGAPDSQKLTTVDIVTLRVLCREGVRWRVMAI
				QDFKPFENLRLMAPISLQVVHVETHRCNISWEISQASHYFERHLEFEARTLSPGHT
				WEEAPLLTLKQKQEWICLETLTPDTQYEFQVRVKPLQGEFTTWSPWSQPLAFRT
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			APTSSSTKKTQL	
			QLRHLCLDLQM	
			ILNGINNYKNPK	
		Knob:	LTAMLTAKFAM	DKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFN
		hFc(N297A)-	PKKATELKHLQC	WYVDGVEVHNAKTKPREEQYASTYRVVSVLTVLHQDWLNGKEYKCKVSNKALP
		[VPLSLY]-	LEEALKPLEEVL	APIEKTISKAKGQPREPQVYTLPPCRDELTKNQVSLWCLVKGFYPSDIAVEWESN
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		L18C, R38A,	PRDLISLINVIVL	QKSLSLSPGGSPGVPLSLYSGPAPTSSSTKKTQLQLRHLCLDLQMILNGINNYKNP
		F42A, Y45A,	ELKGSETTFMCE	KLTAMLTAKFAMPKKATELKHLQCLEEALKPLEEVLNLAQSKNFHLRPRDLISLINV
		E62A, N88L)	YADETATIVEFL	IVLELKGSETTFMCEYADETATIVEFLNRWITFCQSIISTLT
			NRWITFCQSIIST	
			LT	

			DKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFN
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:455	DNA187	Hole: hFc(N297A)-hCD122	KSLSLSPGPGSGSAVNGTSQFTCFYNSRANISCVWSQDGALQDTSCQVHAWPDR
			RRWNQTCELLPVSQASWACNLILGAPDSQKLTTVDIVTLRVLCREGVRWRVMAI
			QDFKPFENLRLMAPISLQVVHVETHRCNISWEISQASHYFERHLEFEARTLSPGHT
			WEEAPLLTLKQKQEWICLETLTPDTQYEFQVRVKPLQGEFTTWSPWSQPLAFRT
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			APTSSSTKKTQL
			QLEHLCLRLQMI
		Knob: hFc(N297A)-[VPLSLY]-hIL2(L18C, D20R, R38A, F42A, Y45A, E62A, N88L) SGP	LNGINNYKNPKL DKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFN
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			KKATELKHLQCL APIEKTISKAKGQPREPQVVTLPPCRDELTKNQVSLWCLVKGFYPSDIAVEWESN
:455	DNA567		EEALKPLEEVLN GQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYT
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			GSETTFMCEYA VLELKGSETTFMCEYADETATIVEFLNRWITFCQSIISTLT
			DETATIVEFLNR
			WITFCQSIISTLT
			DKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFN
			WYVDGVEVHNAKTKPREEQYASTYRVVSVLTVLHQDWLNGKEYKCKVSNKALP
			APIEKTISKAKGQPREPQVCTLPPSRDELTKNQVSLSCAVKGFYPSDIAVEWESNG
			QPENNYKTTPPVLDSDGSFFLVSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQ
:456	DNA187	Hole: hFc(N297A)-hCD122	KSLSLSPGPGSGSAVNGTSQFTCFYNSRANISCVWSQDGALQDTSCQVHAWPDR
			RRWNQTCELLPVSQASWACNLILGAPDSQKLTTVDIVTLRVLCREGVRWRVMAI
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			KPAALGKD

			APTSSSTKKTQL
			QLFHLCLRLQMI
		Knob:	LNGINNYKNPKL DKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFN
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		[VPLSLY]-	KKATELKHLQCL APIEKTISKAKGQPREPQVYTLPPCRDELTKNQVSLWCLVKGFYPSDIAVEWESN
456	DNA568	hIL2(E15F, L18C, D20R, R38A, F42A, Y45A, E62A, N88L) SGP	EEALKPLEEVLN GQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYT LAQSKNFHLRPR QKSLSLSPGGSPGVPLSLYSGPAPTSSSTKKTQLQLFHLCLRLQMILNGINNYKNPK DLISLINVIVLELK LTAMLTAKFAMPKKATELKHLQCLEEALKPLEEVLNLAQSKNFHLRPRDLISLINVI GSETTFMCEYA VLELKGSETTFMCEYADETATIVEFLNRWITFCQSIISTLT DETATIVEFLNR WITFCQSIISTLT
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		Knob:	DDVEVHTAQTQPREEQFNSTFRSVSELPIMHQDWLNGKEFKCRVNSAAFGAPIE
		mFcIgG1(DAP	KTISKTKGRPKAPQVYTIPPPKEQMAKDKVSLTCMITDFFPEDITVEWQWNGQP
462	DNA530	G)- hIL2(R38A, F42A, Y45A, E62A, C125A)	AENYDNTQPIMDTDGSYFVYSDLNVQKSNWEAGNTFTCSVLHEGLHNHHTEKS LSHSPGGGSSPPGGGSSGGGSGGSGPAPTSSSTKKTQLQLEHLLLDLQMILNGINNYK NPKLTAMLTAKFAMPKKATELKHLQCLEEALKPLEEVLNLAQSKNFHLRPRDLISN INVIVLELKGSETTFMCEYADETATIVEFLNRWITFAQSIISTLT
			VRSGCKPCICTVPEVSSVFIFPPKPKDVLTITLTPKVTCVVVAISKDDPEVQFSWFV
		Hole:	DDVEVHTAQTQPREEQFNSTFRSVSELPIMHQDWLNGKEFKCRVNSAAFGAPIE
		mFcIgG1(DAP	KTISKTKGRPKAPQVVTIPPPKKQMAKDKVSLTCMITDFFPEDITVEWQWNGQP
462	DNA532	G)	AENYKNTQPIMKTDGSYFVYSKLNVQKSNWEAGNTFTCSVLHEGLHNHHTEKSL SHSPG
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		Knob:	DDVEVHTAQTQPREEQFNSTFRSVSELPIMHQDWLNGKEFKCRVNSAAFGAPIE
		mFcIgG1(DAP	KTISKTKGRPKAPQVYTIPPPKEQMAKDKVSLTCMITDFFPEDITVEWQWNGQP
463	DNA530	G)- hIL2(R38A, F42A, Y45A, E62A, C125A)	AENYDNTQPIMDTDGSYFVYSDLNVQKSNWEAGNTFTCSVLHEGLHNHHTEKS LSHSPGGGSSPPGGGSSGGGSGGSGPAPTSSSTKKTQLQLEHLLLDLQMILNGINNYK NPKLTAMLTAKFAMPKKATELKHLQCLEEALKPLEEVLNLAQSKNFHLRPRDLISN INVIVLELKGSETTFMCEYADETATIVEFLNRWITFAQSIISTLT

			VRSGCKPCICTVPEVSSVFIFPPKPKDVLTITLTPKVTCVVVAISKDDPEVQFSWFV
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			KTISKTKGRPKAPQVYTIPPPKKQMAKDKVSLTCMITDFFPEDITVEWQWNGQP
			AENYKNTQPIMKTDGSYFVYSKLNVQKSNWEAGNTFTCSVLHEGLHNHHTEKSL
:463	DNA533	Hole: mFcIgG1(DAP G)-hCD122	SHSPGPGSGSAVNGTSQFTCFYNSRANISCVWSQDGALQDTSCQVHAWPDRRR
			WNQTCELLPVSQASWACNLILGAPDSQKLTTVDIVTLRVLCREGVRWRVMAIQ
			DFKPFENLRLMAPISLQVVHVETHRCNISWEISQASHYFERHLEFEARTLSPGHT
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			KPAALGKD
			VRSGCKPCICTVPEVSSVFIFPPKPKDVLTITLTPKVTCVVVAISKDDPEVQFSWFV
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		Knob: mFcIgG1(DAP G)-	KTISKTKGRPKAPQVYTIPPPKEQMAKDKVSLTCMITDFFPEDITVEWQWNGQP
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			DDVEVHTAQTQPREEQFNSTFRSVSELPIMHQDWLNGKEFKCRVNSAAFGAPIE
			KTISKTKGRPKAPQVYTIPPPKKQMAKDKVSLTCMITDFFPEDITVEWQWNGQP
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			WNKTCELTLVRQASWACNLILGSFPESQSLTSVDLLDINVVCWEEKGWRRVKTC
			DFHPFDNLRLVAPHSLQVLHIDTQRCNISWKVSQVSHYIEPYLEFEARRRLLGHS
			WEDASVLSLKQRQQWLFLEMLIPSTSYEVQVRVKAQRNNTGTWSPWSQPLTFR
			TRPADPMKE
			APTSSSTKKTQL
			QLEHLLLDLQMI
			LNGINNYKNPKL
		Knob: mFcIgG1(DAP G)-[VPLSLY]- hIL2(R38A, F42A, Y45A, E62A, C125A)	VRSGCKPCICTVPEVSSVFIFPPKPKDVLTITLTPKVTCVVVAISKDDPEVQFSWFV
			TAMLTAKFAMP
			DDVEVHTAQTQPREEQFNSTFRSVSELPIMHQDWLNGKEFKCRVNSAAFGAPIE
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			MLTAKFAMPKKATELKHLQCLEEALKPLEEVLNLAQSKNFHLRPRDLISNINVIVLE
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			ADETATIVEFLN
			RWITFAQSIISTL
			T

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:465	DNA532	Hole: mFcIgG1(DAP G)	APTSSSTKKTQL QLEHLLLDLQMI LNGINNYKNPKL TAMLTAKFAMP KKATELKHLQCL EEALKPLEEVLN LAQSKNFHLRPR DLISNINVIVLEL KGSETTFMCEY ADETATIVEFLN RWITFAQSIISTL T
:466	DNA531	Knob: mFcIgG1(DAP G)-[VPLSLY]- hIL2(R38A, F42A, Y45A, E62A, C125A) SGP	VRSGCKPCICTVPEVSSVFIFPPKPKDVLTITLTPKVTCVVVAISKDDPEVQFSWFV DDVEVHTAQTQPREEQFNSTFRSVSELPIMHQDWLNGKEFKCRVNSAAFGAPIE KTISKTKGRPKAPQVVTIPPPKEQMAKDKVSLTCMITDFFPEDITVEWQWNGQP AENYDNTQPIMDTDGSYFVYSDLNVQKSNWEAGNTFTCSVLHEGLHNHHTEKS LSHSPGGSPGVPLSLYSGPAPTSSSTKKTQLQLEHLLLDLQMILNGINNYKNPKLTA MLTAKFAMPKKATELKHLQCLEEALKPLEEVLNLAQSKNFHLRPRDLISNINVIVLE LKGSETTFMCEYADETATIVEFLNRWITFAQSIISTLT
:466	DNA533	Hole: mFcIgG1(DAP G)-hCD122	VRSGCKPCICTVPEVSSVFIFPPKPKDVLTITLTPKVTCVVVAISKDDPEVQFSWFV DDVEVHTAQTQPREEQFNSTFRSVSELPIMHQDWLNGKEFKCRVNSAAFGAPIE KTISKTKGRPKAPQVVTIPPPKKQMAKDKVSLTCMITDFFPEDITVEWQWNGQP AENYKNTQPIMKTDGSYFVYSKLNVQKSNWEAGNTFTCSVLHEGLHNHHTEKSL SHSPGPGSGSAVNGTSQFTCFYNSRANISCVWSQDGALQDTSCQVHAWPDRRR WNQTCELLPVSQASWACNLILGAPDSQKLTTVDIVTLRVLCREGVRWRVMAIQ DFKPFENLRLMAPISLQVVHVETHRCNISWEISQASHYFERHLEFEARTLSPGHT WEEAPLLTLKQKQEWICLETLTPDTQYEFQVRVKPLQGEFTTWSPWSQPLAFRT KPAALGKD
:467	DNA531	Knob: mFcIgG1(DAP G)-[VPLSLY]- hIL2(R38A, F42A, Y45A, E62A, C125A) SGP	APTSSSTKKTQL QLEHLLLDLQMI LNGINNYKNPKL TAMLTAKFAMP KKATELKHLQCL EEALKPLEEVLN LAQSKNFHLRPR DLISNINVIVLEL KGSETTFMCEY ADETATIVEFLN RWITFAQSIISTL T
			VRSGCKPCICTVPEVSSVFIFPPKPKDVLTITLTPKVTCVVVAISKDDPEVQFSWFV DDVEVHTAQTQPREEQFNSTFRSVSELPIMHQDWLNGKEFKCRVNSAAFGAPIE KTISKTKGRPKAPQVVTIPPPKEQMAKDKVSLTCMITDFFPEDITVEWQWNGQP AENYDNTQPIMDTDGSYFVYSDLNVQKSNWEAGNTFTCSVLHEGLHNHHTEKS LSHSPGGSPGVPLSLYSGPAPTSSSTKKTQLQLEHLLLDLQMILNGINNYKNPKLTA MLTAKFAMPKKATELKHLQCLEEALKPLEEVLNLAQSKNFHLRPRDLISNINVIVLE LKGSETTFMCEYADETATIVEFLNRWITFAQSIISTLT

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			KTISKTKGRPKAPQVYTIPPPKKQMAKDKVSLTCMITDFFPEDITVEWQWNGQP
			AENYKNTQPIMKTDGSYFVYSKLNVQKSNWEAGNTFTCSVLHEGLHNHHTEKSL
:467	DNA534	Hole: mFcIgG1(DAPG)-mCD122	SHSPGPGSGSAVKNCSHLECFYNSRANVSCMWSHEEALNVTTCHVHAKSNLRH
			WNKTCELTLVRQASWACNLILGSFPESQSLTSVDLLDINVVCWEEKGWRRVKTC
			DFHPFDNLRLVAPHSLQVLHIDTQRCNISWKVSQVSHYIEPYLEFEARRRLLGHS
			WEDASVLSLKQRQQWLFLEMLIPSTSYEVQVRVKAQRNNTGTWSPWSQPLTFR
			TRPADPMKE
			DKTHTCPPCPAPELLGGPSVFLFPPKPKDTLYITREPEVTCVVVDVSHEDPEVKFN
			WYVDGVEVHNAKTKPREEQYASTYRVVSVLTVLHQDWLNGKEYKCKVSNKALP
		Hole: hFc(N297A, M252Y, S254T, T256E)-hCD122	APIEKTISKAKGQPREPQVCTLPPSRDELTKNQVSLSCAVKGFYPSDIAVEWESNG
			QPENNYKTTPPVLDSDGSFFLVSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQ
:468	DNA576		KSLSLSPGPGSGSAVNGTSQFTCFYNSRANISCVWSQDGALQDTSCQVHAWPDR
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			QDFKPFENLRLMAPISLQVVHVETHRCNISWEISQASHYFERHLEFEARTLSPGHT
			WEEAPLLTLKQKQEWICLETLTPDTQYEFQVRVKPLQGEFTTWSPWSQPLAFRT
			KPAALGKD
			APTSSSTKKTQL
			QLEHLLLDLQMI
		Knob: hFc(N297A, M252Y, S254T, T256E)-[VPLSLY]-hIL2(R38A, F42A, Y45A, E62A, C125A)	LNGINNYKNPKL DKTHTCPPCPAPELLGGPSVFLFPPKPKDTLYITREPEVTCVVVDVSHEDPEVKFN
			TAMLTAKFAMP WYVDGVEVHNAKTKPREEQYASTYRVVSVLTVLHQDWLNGKEYKCKVSNKALP
			KKATELKHLQCL APIEKTISKAKGQPREPQVYTLPPCRDELTKNQVSLWCLVKGFYPSDIAVEWESN
			EEALKPLEEVLN GQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYT
:468	DNA580		LAQSKNFHLRPR QKSLSLSPGGSPGVPLSLYSGPAPTSSSTKKTQLQLEHLLLDLQMILNGINNYKNPK
			DLISNINVIVLEL LTAMLTAKFAMPKKATELKHLQCLEEALKPLEEVLNLAQSKNFHLRPRDLISNINVI
			KGSETTFMCEY VLELKGSETTFMCEYADETATIVEFLNRWITFAQSIISTLT
			ADETATIVEFLN
			RWITFAQSIISTL
			T

			DKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMASRTPEVTCVVVDVSHEDPEVKF NWYVDGVEVHNAKTKPREEQYASTYRVVSVLTVLHQDWLNGKEYKCKVSNKAL PAPIEKTISKAKGQPREPQVCTLPPSRDELTKNQVSLSCAVKGFYPSDIAVEWESN GQPENNYKTTPPVLDSDGSFFLVSKLTVDKSRWQQGNVFSCSVMHEALHNHYT QKSLSLSPGPGSGSAVNGTSQFTCFYNSRANISCVWSQDGALQDTSCQVHAWP DRRRWNQTCELLPVSQASWACNLILGAPDSQKLTTVDIVTLRVLCREGVRWRV MAIQDFKPFENLRLMAPISLQVVHVETHRCNISWEISQASHYFERHLEFEARTLSP GHTWEEAPLLTLKQKQEWICLETLTPDTQYEFQVRVKPLQGEFTTWSPWSQPLA FRTKPAALGKD
:469	DNA575	Hole: hFc(N297A, I253A)- hCD122	DKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMASRTPEVTCVVVDVSHEDPEVKF NWYVDGVEVHNAKTKPREEQYASTYRVVSVLTVLHQDWLNGKEYKCKVSNKAL PAPIEKTISKAKGQPREPQVYTLPPCRDELTKNQVSLWCLVKGFYPSDIAVEWESN GQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYT QKSLSLSPGGGSSPPGGGSSGGGSGAPAPTSSSTKKTQLQLEHLLLDLQMILNGINN YKNPKLTAMLTAKFAMPKKATELKHLQCLEEALKPLEEVLNLAQSKNFHLRPRDLI SNINVIVLELKGSETTFMCEYADETATIVEFLNRWITFAQSIISTLT
:469	DNA577	Knob: hFc(N297, I253A)- hIL2(R38A, F42A, Y45A, E62A, C125A)	DKTHTCPPCPAPELLGGPSVFLFPPKPKDTLYITREPEVTCVVVDVSHEDPEVKFN WYVDGVEVHNAKTKPREEQYASTYRVVSVLTVLHQDWLNGKEYKCKVSNKALP APIEKTISKAKGQPREPQVCTLPPSRDELTKNQVSLSCAVKGFYPSDIAVEWESNG QPENNYKTTPPVLDSDGSFFLVSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQ KSLSLSPGPGSGSAVNGTSQFTCFYNSRANISCVWSQDGALQDTSCQVHAWPDR RRWNQTCELLPVSQASWACNLILGAPDSQKLTTVDIVTLRVLCREGVRWRVMAI QDFKPFENLRLMAPISLQVVHVETHRCNISWEISQASHYFERHLEFEARTLSPGHT WEEAPLLTLKQKQEWICLETLTPDTQYEFQVRVKPLQGEFTTWSPWSQPLAFRT KPAALGKD
:470	DNA576	Hole: hFc(N297A, M252Y, S254T, T256E)- hCD122	
:470	DNA578	Knob: hFc(N297A, M252Y, S254T, T256E)- hIL2(R38A, F42A, Y45A, E62A, C125A)	DKTHTCPPCPAPELLGGPSVFLFPPKPKDTLYITREPEVTCVVVDVSHEDPEVKFN WYVDGVEVHNAKTKPREEQYASTYRVVSVLTVLHQDWLNGKEYKCKVSNKALP APIEKTISKAKGQPREPQVYTLPPCRDELTKNQVSLWCLVKGFYPSDIAVEWESN GQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYT QKSLSLSPGGGSSPPGGGSSGGGSGAPAPTSSSTKKTQLQLEHLLLDLQMILNGINN YKNPKLTAMLTAKFAMPKKATELKHLQCLEEALKPLEEVLNLAQSKNFHLRPRDLI SNINVIVLELKGSETTFMCEYADETATIVEFLNRWITFAQSIISTLT

				DKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMASRTPEVTCVVVDVSHEDPEVKF NWYVDGVEVHNAKTKPREEQYASTYRVVSVLTVLHQDWLNGKEYKCKVSNKAL PAPIEKTISKAKGQPREPQVCTLPPSRDELTKNQVSLSCAVKGFYPSDIAVEWESN GQPENNYKTTPPVLDSDGSFFLVSKLTVDKSRWQQGNVFSCSVMHEALHNHYT QKSLSLSPGPGSGSAVNGTSQFTCFYNSRANISCVWSQDGALQDTSCQVHAWP DRRRWNQTCELLPVSQASWACNLILGAPDSQKLTTVDIVTLRVLCREGVRWRV MAIQDFKPFENLRLMAPISLQVVHVETHRCNISWEISQASHYFERHLEFEARTLSP GHTWEEAPLLTLKQKQEWICLETLTPDTQYEFQVRVKPLQGEFTTWSPWSQPLA FRTKPAALGKD
:471	DNA575	Hole: hFc(N297A, I253A)- hCD122		APTSSSTKKTQL QLEHLLLDLQMI LNGINNYKNPKL
:471	DNA579	Knob: hFc(N297, I253A)- [VPLSLY]- hIL2(R38A, F42A, Y45A, E62A, C125A)	SGP	DKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMASRTPEVTCVVVDVSHEDPEVKF NWYVDGVEVHNAKTKPREEQYASTYRVVSVLTVLHQDWLNGKEYKCKVSNKAL PAPIEKTISKAKGQPREPQVYTLPPCRDELTKNQVSLWCLVKGFYPSDIAVEWESN GQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYT QKSLSLSPGGGSPGVPLSLYSGPAPTSSSTKKTQLQLEHLLLDLQMILNGINNYKNPK LTAMLTAKFAMPKKATELKHLQCLEEALKPLEEVLNLAQSKNFHLRPRDLISNINVI VLELKGSETTFMCEYADETATIVEFLNRWITFAQSIISTLT
:475	DNA255	Knob: hFc(N297A)- hIL2(R38A, F42A, Y45A, E62A, C125A)		DKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFN WYVDGVEVHNAKTKPREEQYASTYRVVSVLTVLHQDWLNGKEYKCKVSNKALP APIEKTISKAKGQPREPQVYTLPPCRDELTKNQVSLWCLVKGFYPSDIAVEWESN GQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYT QKSLSLSPGGGSSPPGGGSSGGGSGSPAPTSSSTKKTQLQLEHLLLDLQMILNGINN YKNPKLTAMLTAKFAMPKKATELKHLQCLEEALKPLEEVLNLAQSKNFHLRPRDLI SNINVIVLELKGSETTFMCEYADETATIVEFLNRWITFAQSIISTLT
:475	DNA528	Hole: hFc(N297A)- hCD122(C168 S)		DKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFN WYVDGVEVHNAKTKPREEQYASTYRVVSVLTVLHQDWLNGKEYKCKVSNKALP APIEKTISKAKGQPREPQVCTLPPSRDELTKNQVSLSCAVKGFYPSDIAVEWESNG QPENNYKTTPPVLDSDGSFFLVSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQ KSLSLSPGPGSGSAVNGTSQFTCFYNSRANISCVWSQDGALQDTSCQVHAWPDR RRWNQTCELLPVSQASWACNLILGAPDSQKLTTVDIVTLRVLCREGVRWRVMAI QDFKPFENLRLMAPISLQVVHVETHRCNISWEISQASHYFERHLEFEARTLSPGHT WEEAPLLTLKQKQEWISLETLTPDTQYEFQVRVKPLQGEFTTWSPWSQPLAFRT KPAALGKD

				APTSSSTKKTQL
				QLEHLLLDLQMI
				LNGINNYKNPKL
		Knob: hFc(N297A)-[VPLSLY]-hIL2(R38A, F42A, Y45A, E62A, C125A)	SGP	DKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFN
				WYVDGVEVHNAKTKPREEQYASTYRVVSVLTVLHQDWLNGKEYKCKVSNKALP
:476	DNA263			KKATELKHLQCL
				APIEKTISKAKGQPREPQVYTLPPCRDELTKNQVSLWCLVKGFYPSDIAVEWESN
				EEALKPLEEVLN
				GQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYT
				LAQSKNFHLRPR
				QKSLSLSPGGSPGVPLSLYSGPAPTSSSTKKTQLQLEHLLLDLQMILNGINNYKNPK
				DLISNINVIVLEL
				LTAMLTAKFAMPKKATELKHLQCLEEALKPLEEVLNLAQSKNFHLRPRDLISNINVI
				KGSETTFMCEY
				VLELKGSETTFMCEYADETATIVEFLN
				ADETATIVEFLN
				RWITFAQSIISTL
				RWITFCQSIISTL
				T
				DKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFN
				WYVDGVEVHNAKTKPREEQYASTYRVVSVLTVLHQDWLNGKEYKCKVSNKALP
				APIEKTISKAKGQPREPQVCTLPPSRDELTKNQVSLSCAVKGFYPSDIAVEWESNG
		Hole: hFc(N297A)-hCD122(C168S)		QPENNYKTTPPVLDSDGSFFLVSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQ
:476	DNA528			KSLSLSPGPGSGSAVNGTSQFTCFYNSRANISCVWSQDGALQDTSCQVHAWPDR
				RRWNQTCELLPVSQASWACNLILGAPDSQKLTTVDIVTLRVLCREGVRWRVMAI
				QDFKPFENLRLMAPISLQVVHVETHRCNISWEISQASHYFERHLEFEARTLSPGHT
				WEEAPLLTLKQKQEWISLETLTPDTQYEFQVRVKPLQGEFTTWSPWSQPLAFRT
				KPAALGKD
				DKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFN
				WYVDGVEVHNAKTKPREEQYASTYRVVSVLTVLHQDWLNGKEYKCKVSNKALP
		Hole: hFc(N297A)		APIEKTISKAKGQPREPQVCTLPPSRDELTKNQVSLSCAVKGFYPSDIAVEWESNG
:477	DNA158			QPENNYKTTPPVLDSDGSFFLVSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQ
				KSLSLSPG
				APTSSSTKKTQL
				QLRHLCLRLQMI
				LNGINNYKNPKL
		Knob: hFc(N297A)-[VPLSLY]-hIL2(E15R, L18C, D20R, R38A, F42A, Y45A, E62A)	SGP	DKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFN
				WYVDGVEVHNAKTKPREEQYASTYRVVSVLTVLHQDWLNGKEYKCKVSNKALP
:477	DNA554			KKATELKHLQCL
				APIEKTISKAKGQPREPQVYTLPPCRDELTKNQVSLWCLVKGFYPSDIAVEWESN
				EEALKPLEEVLN
				GQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYT
				LAQSKNFHLRPR
				QKSLSLSPGGSPGVPLSLYSGPAPTSSSTKKTQLQLRHLCLRLQMILNGINNYKNPK
				DLISNINVIVLEL
				LTAMLTAKFAMPKKATELKHLQCLEEALKPLEEVLNLAQSKNFHLRPRDLISNINVI
				KGSETTFMCEY
				VLELKGSETTFMCEYADETATIVEFLNRWITFCQSIISTLT
				ADETATIVEFLN
				RWITFCQSIISTL
				T

					DKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFN WYVDGVEVHNAKTKPREEQYASTYRVVSVLTVLHQDWLNGKEYKCKVSNKALP APIEKTISKAKGQPREPQVCTLPPSRDELTKNQVSLSCAVKGFYPSDIAVEWESNG QPENNYKTTPPVLDSDGSFFLVSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQ KSLSLSPG
:484	DNA158	Hole: hFc(N297A)			
					APTSSSTKKTQL QLEHLCLDLQM ILNGINNYKNPK
		Knob: hFc(N297A)- [VPLSLY]- hIL2(L18C, R38A, F42A, Y45A, E62A)			DKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFN WYVDGVEVHNAKTKPREEQYASTYRVVSVLTVLHQDWLNGKEYKCKVSNKALP APIEKTISKAKGQPREPQVYTLPPCRDELTKNQVSLWCLVKGFYPSDIAVEWESN GQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYT QKSLSLSPGGSPGVPLSLYSGPAPTSSSTKKTQLQLEHLCLDLQMILNGINNYKNPK LTAMLTAKFAMPKKATELKHLQCLEEALKPLEEVLNLAQSKNFHLRPRDLISNINVI VLELKGSETTFMCEYADETATIVEFLNRWITFCQSIISTLT
:484	DNA581		SGP		
					DKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFN WYVDGVEVHNAKTKPREEQYASTYRVVSVLTVLHQDWLNGKEYKCKVSNKALP APIEKTISKAKGQPREPQVCTLPPSRDELTKNQVSLSCAVKGFYPSDIAVEWESNG QPENNYKTTPPVLDSDGSFFLVSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQ KSLSLSPG
:485	DNA158	Hole: hFc(N297A)			
					APTSSSTKKTQL QLEYLLLDLQMI LNGINNYKNPKL
		Knob: hFc(N297A)- [VPLSLY]- hIL2(H16Y, R38A, F42A, Y45A, E62A, C125A)			DKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFN WYVDGVEVHNAKTKPREEQYASTYRVVSVLTVLHQDWLNGKEYKCKVSNKALP APIEKTISKAKGQPREPQVYTLPPCRDELTKNQVSLWCLVKGFYPSDIAVEWESN GQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYT QKSLSLSPGGSPGVPLSLYSGPAPTSSSTKKTQLQLEYLLLDLQMILNGINNYKNPK LTAMLTAKFAMPKKATELKHLQCLEEALKPLEEVLNLAQSKNFHLRPRDLISNINVI VLELKGSETTFMCEYADETATIVEFLNRWITFAQSIISTLT
:485	DNA582		SGP		
					DKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFN WYVDGVEVHNAKTKPREEQYASTYRVVSVLTVLHQDWLNGKEYKCKVSNKALP APIEKTISKAKGQPREPQVCTLPPSRDELTKNQVSLSCAVKGFYPSDIAVEWESNG QPENNYKTTPPVLDSDGSFFLVSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQ KSLSLSPG
:486	DNA158	Hole: hFc(N297A)			

				APTSSSTKKTQL
				QLEELLLDLQMI
				LNGINNYKNPKL
		Knob: hFc(N297A)-[VPLSLY]-hIL2(H16E, R38A, F42A, Y45A, E62A, C125A)	SGP	DKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFN WYVDGVEVHNAKTKPREEQYASTYRVVSVLTVLHQDWLNGKEYKCKVSNKALP KKATELKHLQCL APIEKTISKAKGQPREPQVYTLPPCRDELTKNQVSLWCLVKGFYPSDIAVEWESN EEALKPLEEVLN GQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYT LAQSKNFHLRPR QKSLSLSPGGSPGVPLSLYSGPAPTSSSTKKTQLQLEELLLDLQMILNGINNYKNPK DLISNINVIVLEL LTAMLTAKFAMPKKATELKHLQCLEEALKPLEEVLNLAQSKNFHLRPRDLISNINVI KGSETTFMCEY VLELKGSETTFMCEYADETATIVEFLN ADETATIVEFLN RWITFAQSIISTL RWITFAQSIISTL T T
:486	DNA583			
		Hole: hFc(N297A)		DKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFN WYVDGVEVHNAKTKPREEQYASTYRVVSVLTVLHQDWLNGKEYKCKVSNKALP APIEKTISKAKGQPREPQVCTLPPSRDELTKNQVSLSCAVKGFYPSDIAVEWESNG QPENNYKTTPPVLDSDGSFFLVSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQ KSLSLSPG
:487	DNA158			
				APTSSSTKKTQL
				QLEHLLLLLQMI
				LNGINNYKNPKL
		Knob: hFc(N297A)-[VPLSLY]-hIL2(D20L, R38A, F42A, Y45A, E62A, C125A)	SGP	DKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFN WYVDGVEVHNAKTKPREEQYASTYRVVSVLTVLHQDWLNGKEYKCKVSNKALP APIEKTISKAKGQPREPQVVTLPPCRDELTKNQVSLWCLVKGFYPSDIAVEWESN GQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYT QKSLSLSPGGSPGVPLSLYSGPAPTSSSTKKTQLQLEHLLLLLQMILNGINNYKNPK LTAMLTAKFAMPKKATELKHLQCLEEALKPLEEVLNLAQSKNFHLRPRDLISNINVI VLELKGSETTFMCEYADETATIVEFLN RWITFAQSIISTL T
:487	DNA584			
		Hole: hFc(N297A)		DKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFN WYVDGVEVHNAKTKPREEQYASTYRVVSVLTVLHQDWLNGKEYKCKVSNKALP APIEKTISKAKGQPREPQVCTLPPSRDELTKNQVSLSCAVKGFYPSDIAVEWESNG QPENNYKTTPPVLDSDGSFFLVSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQ KSLSLSPG
:488	DNA158			

				APTSSSTKKTQL
				QLEYLCLDLQMI
				LNGINNYKNPKL
		Knob:		DKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFN
		hFc(N297A)-		TAMLTAKFAMP
		[VPLSLY]-		KKATELKHLQCL
:488	DNA585	hIL2(H16Y,	SGP	EEALKPLEEVLN
		L18C, R38A,		LAQSKNFHLRPR
		F42A, Y45A,		DLISNINVIVLEL
		E62A)		KGSETTFMCEY
				ADETATIVEFLN
				RWITFCQSIISTL
				T
				DKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFN
				WYVDGVEVHNAKTKPREEQYASTYRVVSVLTVLHQDWLNGKEYKCKVSNKALP
				APIEKTISKAKGQPREPQVCTLPPSRDELTKNQVSLSCAVKGFYPSDIAVEWESNG
				QPENNYKTTPPVLDSDGSFFLVSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQ
				KSLSLSPG
:489	DNA158	Hole:		
		hFc(N297A)		
				APTSSSTKKTQL
				QLEELCLDLQMI
				LNGINNYKNPKL
		Knob:		DKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFN
		hFc(N297A)-		TAMLTAKFAMP
		[VPLSLY]-		KKATELKHLQCL
:489	DNA586	hIL2(H16E,	SGP	EEALKPLEEVLN
		L18C, R38A,		LAQSKNFHLRPR
		F42A, Y45A,		DLISNINVIVLEL
		E62A)		KGSETTFMCEY
				ADETATIVEFLN
				RWITFCQSIISTL
				T
				DKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFN
				WYVDGVEVHNAKTKPREEQYASTYRVVSVLTVLHQDWLNGKEYKCKVSNKALP
				APIEKTISKAKGQPREPQVCTLPPSRDELTKNQVSLSCAVKGFYPSDIAVEWESNG
				QPENNYKTTPPVLDSDGSFFLVSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQ
				KSLSLSPG
:490	DNA158	Hole:		
		hFc(N297A)		

				APTSSSTKKTQL
				QLEHLCLLLQMI
				LNGINNYKNPKL
		Knob:		DKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFN
		hFc(N297A)-		TAMLTAKFAMP
		[VPLSLY]-		WYVDGVEVHNAKTKPREEQYASTYRVVSVLTVLHQDWLNGKEYKCKVSNKALP
		hIL2(L18C,		KKATELKHLQCL
:490	DNA587	D20L, R38A,	SGP	APIEKTISKAKGQPREPQVYTLPPCRDELTKNQVSLWCLVKGFYPSDIAVEWESN
		F42A, Y45A,		EEALKPLEEVLN
		E62A)		GQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYT
				LAQSKNFHLRPR
				QKSLSLSPGGSPGVPLSLYSGPAPTSSSTKKTQLQLEHLCLLLQMILNGINNYKNPK
				DLISNINVIVLEL
				LTAMLTAKFAMPKKATELKHLQCLEEALKPLEEVLNLAQSKNFHLRPRDLISNINVI
				KGSETTFMCEY
				VLELKGSETTFMCEYADETATIVEFLN
				ADETATIVEFLN
				RWITFCQSIISTL
				T
				DKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFN
				WYVDGVEVHNAKTKPREEQYASTYRVVSVLTVLHQDWLNGKEYKCKVSNKALP
				APIEKTISKAKGQPREPQVCTLPPSRDELTKNQVSLSCAVKGFYPSDIAVEWESNG
				QPENNYKTTPPVLDSDGSFFLVSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQ
				KSLSLSPG
				APTSSSTKKTQL
				QLEYLCLLLQMI
				LNGINNYKNPKL
		Knob:		DKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFN
		hFc(N297A)-		TAMLTAKFAMP
		[VPLSLY]-		WYVDGVEVHNAKTKPREEQYASTYRVVSVLTVLHQDWLNGKEYKCKVSNKALP
		hIL2(H16Y,		KKATELKHLQCL
:491	DNA588	L18C, D20L,	SGP	APIEKTISKAKGQPREPQVYTLPPCRDELTKNQVSLWCLVKGFYPSDIAVEWESN
		R38A, F42A,		EEALKPLEEVLN
		Y45A, E62A)		GQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYT
				LAQSKNFHLRPR
				QKSLSLSPGGSPGVPLSLYSGPAPTSSSTKKTQLQLEYLCLLLQMILNGINNYKNPK
				DLISNINVIVLEL
				LTAMLTAKFAMPKKATELKHLQCLEEALKPLEEVLNLAQSKNFHLRPRDLISNINVI
				KGSETTFMCEY
				VLELKGSETTFMCEYADETATIVEFLN
				ADETATIVEFLN
				RWITFCQSIISTL
				T
				DKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFN
				WYVDGVEVHNAKTKPREEQYASTYRVVSVLTVLHQDWLNGKEYKCKVSNKALP
				APIEKTISKAKGQPREPQVCTLPPSRDELTKNQVSLSCAVKGFYPSDIAVEWESNG
				QPENNYKTTPPVLDSDGSFFLVSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQ
				KSLSLSPG
:492	DNA158	Hole: hFc(N297A)		

			APTSSSTKKTQL
			QLEELCLLLQMI
		Knob:	LNGINNYKNPKL
		hFc(N297A)-	DKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFN
		[VPLSLY]-	TAMLTAKFAMP WYVDGVEVHNAKTKPREEQYASTYRVVSVLTVLHQDWLNGKEYKCKVSNKALP
		hIL2(H16E,	KKATELKHLQCL APIEKTISKAKGQPREPQVYTLPPCRDELTKNQVSLWCLVKGFYPSDIAVEWESN
:492	DNA589	L18C, D20L,	EEALKPLEEVLN GQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYT
		R38A, F42A,	LAQSKNFHLRPR QKSLSLSPGGSPGVPLSLYSGPAPTSSSTKKTQLQLEELCLLLQMILNGINNYKNPK
		Y45A, E62A)	DLISNINVIVLEL LTAMLTAKFAMPKKATELKHLQCLEEALKPLEEVLNLAQSKNFHLRPRDLISNINVI
			KGSETTFMCEY VLELKGSETTFMCEYADETATIVEFLNRWITFCQSIISTLT
			ADETATIVEFLN
			RWITFCQSIISTL
			T
			DKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFN
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			APIEKTISKAKGQPREPQVCTLPPSRDELTKNQVSLSCAVKGFYPSDIAVEWESNG
			QPENNYKTTPPVLDSDGSFFLVSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQ
		Hole:	KSLSLSPGPGSGSAVNGTSQFTCFYNSRANISCVWSQDGALQDTSCQVHAWPDR
:493	DNA187	hFc(N297A)-	RRWNQTCELLPVSQASWACNLILGAPDSQKLTTVDIVTLRVLCREGVRWRVMAI
		hCD122	QDFKPFENLRLMAPISLQVVHVETHRCNISWEISQASHYFERHLEFEARTLSPGHT
			WEEAPLLTLKQKQEWICLETLTPDTQYEFQVRVKPLQGEFTTWSPWSQPLAFRT
			KPAALGKD
			APTSSSTKKTQL
			QLEHLCLDLQM
			ILNGINNYKNPK
		Knob:	DKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFN
		hFc(N297A)-	LTAMLTAKFAM WYVDGVEVHNAKTKPREEQYASTYRVVSVLTVLHQDWLNGKEYKCKVSNKALP
		[VPLSLY]-	PKKATELKHLQC APIEKTISKAKGQPREPQVYTLPPCRDELTKNQVSLWCLVKGFYPSDIAVEWESN
:493	DNA581	hIL2(L18C,	LEEALKPLEEVL GQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYT
		R38A, F42A,	NLAQSKNFHLR QKSLSLSPGGSPGVPLSLYSGPAPTSSSTKKTQLQLEHLCLDLQMILNGINNYKNPK
		Y45A, E62A)	PRDLISNINVIVL LTAMLTAKFAMPKKATELKHLQCLEEALKPLEEVLNLAQSKNFHLRPRDLISNINVI
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			YADETATIVEFL
			NRWITFCQSIIST
			LT

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				APIEKTISKAKGQPREPQVCTLPPSRDELTKNQVSLSCAVKGFYPSDIAVEWESNG
				QPENNYKTTPPVLDSDGSFFLVSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQ
:494	DNA187	Hole: hFc(N297A)-hCD122		KSLSLSPGPGSGSAVNGTSQFTCFYNSRANISCVWSQDGALQDTSCQVHAWPDR
				RRWNQTCELLPVSQASWACNLILGAPDSQKLTTVDIVTLRVLCREGVRWRVMAI
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				QLEYLLLDLQMI
				LNGINNYKNPKL
		Knob: hFc(N297A)-[VPLSLY]-hIL2(H16Y, R38A, F42A, Y45A, E62A, C125A)	SGP	DKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFN
				TAMLTAKFAMP
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				APIEKTISKAKGQPREPQVYTLPPCRDELTKNQVSLWCLVKGFYPSDIAVEWESN
				EEALKPLEEVLN
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				KGSETTFMCEY
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				ADETATIVEFLN
				RWITFAQSIISTL
				T
				DKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFN
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:495	DNA187	Hole: hFc(N297A)-hCD122		KSLSLSPGPGSGSAVNGTSQFTCFYNSRANISCVWSQDGALQDTSCQVHAWPDR
				RRWNQTCELLPVSQASWACNLILGAPDSQKLTTVDIVTLRVLCREGVRWRVMAI
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				KPAALGKD

				APTSSSTKKTQL
				QLEELLLDLQMI
		Knob:		LNGINNYKNPKL
		hFc(N297A)-		DKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFN
		[VPLSLY]-		TAMLTAKFAMP WYVDGVEVHNAKTKPREEQYASTYRVVSVLTVLHQDWLNGKEYKCKVSNKALP
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		Y45A, E62A,		LAQSKNFHLRPR QKSLSLSPGGSPGVPLSLYSGPAPTSSSTKKTQLQLEELLLDLQMILNGINNYKNPK
		C125A)		DLISNINVIVLEL LTAMLTAKFAMPKKATELKHLQCLEEALKPLEEVLNLAQSKNFHLRPRDLISNINVI
				KGSETTFMCEY VLELKGSETTFMCEYADETATIVEFLNRWITFAQSIISTLT
				ADETATIVEFLN
				RWITFAQSIISTL
				T
				DKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFN
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				QPENNYKTTPPVLDSDGSFFLVSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQ
		Hole:		KSLSLSPGPGSGSAVNGTSQFTCFYNSRANISCVWSQDGALQDTSCQVHAWPDR
:496	DNA187	hFc(N297A)-		RRWNQTCELLPVSQASWACNLILGAPDSQKLTTVDIVTLRVLCREGVRWRVMAI
		hCD122		QDFKPFENLRLMAPISLQVVHVETHRCNISWEISQASHYFERHLEFEARTLSPGHT
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				KPAALGKD
				APTSSSTKKTQL
				QLEHLLLLLQMI
		Knob:		LNGINNYKNPKL
		hFc(N297A)-		DKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFN
		[VPLSLY]-		TAMLTAKFAMP WYVDGVEVHNAKTKPREEQYASTYRVVSVLTVLHQDWLNGKEYKCKVSNKALP
		hIL2(D20L,		KKATELKHLQCL APIEKTISKAKGQPREPQVYTLPPCRDELTKNQVSLWCLVKGFYPSDIAVEWESN
:496	DNA584	R38A, F42A,	SGP	EEALKPLEEVLN GQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYT
		Y45A, E62A,		LAQSKNFHLRPR QKSLSLSPGGSPGVPLSLYSGPAPTSSSTKKTQLQLEHLLLLLQMILNGINNYKNPK
		C125A)		DLISNINVIVLEL LTAMLTAKFAMPKKATELKHLQCLEEALKPLEEVLNLAQSKNFHLRPRDLISNINVI
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				ADETATIVEFLN
				RWITFAQSIISTL
				T

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				APIEKTISKAKGQPREPQVCTLPPSRDELTKNQVSLSCAVKGFYPSDIAVEWESNG
				QPENNYKTTPPVLDSDGSFFLVSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQ
:497	DNA187	Hole: hFc(N297A)-hCD122		KSLSLSPGPGSGSAVNGTSQFTCFYNSRANISCVWSQDGALQDTSCQVHAWPDR
				RRWNQTCELLPVSQASWACNLILGAPDSQKLTTVDIVTLRVLCREGVRWRVMAI
				QDFKPFENLRLMAPISLQVVHVETHRCNISWEISQASHYFERHLEFEARTLSPGHT
				WEEAPLLTLKQKQEWICLETLTPDTQYEFQVRVKPLQGEFTTWSPWSQPLAFRT
				KPAALGKD
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				QLEYLCLDLQMI
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		Knob: hFc(N297A)-[VPLSLY]-hIL2(H16Y, L18C, R38A, F42A, Y45A, E62A)	SGP	DKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFN
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				ADETATIVEFLN
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:498	DNA187	Hole: hFc(N297A)-hCD122		KSLSLSPGPGSGSAVNGTSQFTCFYNSRANISCVWSQDGALQDTSCQVHAWPDR
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		Knob: hFc(N297A)-[VPLSLY]-hIL2(H16E, L18C, R38A, F42A, Y45A, E62A)	DKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFN WYVDGVEVHNAKTKPREEQYASTYRVVSVLTVLHQDWLNGKEYKCKVSNKALP APIEKTISKAKGQPREPQVYTLPPCRDELTKNQVSLWCLVKGFYPSDIAVEWESN GQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYT QKSLSLSPGGSPGVPLSLYSGPAPTSSSTKKTQLQLEELCLDLQMILNGINNYKNPK LTAMLTAKFAMPKKATELKHLQCLEEALKPLEEVLNLAQSKNFHLRPRDLISNINVI VLELKGSETTFMCEYADETATIVEFLNRWITFCQSIISTLT	498
DNA586	SGP			
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DNA187				

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RWITFCQSIISTL
T

		Knob: hFc(N297A)-[VPLSLY]-hIL2(L18C, D20L, R38A, F42A, Y45A, E62A)	DKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFN WYVDGVEVHNAKTKPREEQYASTYRVVSVLTVLHQDWLNGKEYKCKVSNKALP APIEKTISKAKGQPREPQVYTLPPCRDELTKNQVSLWCLVKGFYPSDIAVEWESN GQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYT QKSLSLSPGGSPGVPLSLYSGPAPTSSSTKKTQLQLEHLCLLLQMILNGINNYKNPK LTAMLTAKFAMPKKATELKHLQCLEEALKPLEEVLNLAQSKNFHLRPRDLISNINVI VLELKGSETTFMCEYADETATIVEFLNRWITFCQSIISTLT	499
DNA587	SGP			

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:500	DNA187	Hole: hFc(N297A)-hCD122		KSLSLSPGPGSGSAVNGTSQFTCFYNSRANISCVWSQDGALQDTSCQVHAWPDR
				RRWNQTCELLPVSQASWACNLILGAPDSQKLTTVDIVTLRVLCREGVRWRVMAI
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				KPAALGKD
				APTSSSTKKTQL
				QLEYLCLLLQMI
				LNGINNYKNPKL
		Knob: hFc(N297A)-[VPLSLY]-hIL2(H16Y, L18C, D20L, R38A, F42A, Y45A, E62A)	SGP	DKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFN
				WYVDGVEVHNAKTKPREEQYASTYRVVSVLTVLHQDWLNGKEYKCKVSNKALP
				KKATELKHLQCL
				APIEKTISKAKGQPREPQVVTLPPCRDELTKNQVSLWCLVKGFYPSDIAVEWESN
				EEALKPLEEVLN
:500	DNA588			GQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYT
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				DLISNINVIVLEL
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				ADETATIVEFLN
				RWITFCQSIISTL
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				APIEKTISKAKGQPREPQVCTLPPSRDELTKNQVSLSCAVKGFYPSDIAVEWESNG
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:501	DNA187	Hole: hFc(N297A)-hCD122		KSLSLSPGPGSGSAVNGTSQFTCFYNSRANISCVWSQDGALQDTSCQVHAWPDR
				RRWNQTCELLPVSQASWACNLILGAPDSQKLTTVDIVTLRVLCREGVRWRVMAI
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				KPAALGKD

				APTSSSTKKTQL
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		Knob:		LNGINNYKNPKL
		hFc(N297A)-		TAMLTAKFAMP
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:501	DNA589	hIL2(H16E,	SGP	EEALKPLEEVLN
		L18C, D20L,		LAQSKNFHLRPR
		R38A, F42A,		DLISNINVIVLEL
		Y45A, E62A)		KGSETTFMCEY
				ADETATIVEFLN
				RWITFCQSIISTL
				T

DKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFN
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		Hole:		YNSRANISCVW
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		[VPLSLY]-		QVHAWPDRRR
:502	DNA543	hCD122	GSGGG	WNQTCELLPVS

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:502	DNA577	Knob: hFc(N297, I253A)-hIL2(R38A, F42A, Y45A, E62A, C125A)	QKSLSLSPGGGSSPPGGGSSGGGSGPAPTSSSTKKTQLQLEHLLLDLQMILNGINN
			YKNPKLTAMLTAKFAMPKKATELKHLQCLEEALKPLEEVLNLAQSKNFHLRPRDLI
			SNINVIVLELKGSETTFMCEYADETATIVEFLNRWITFAQSIISTLT
			DKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFN
			WYVDGVEVHNAKTKPREEQYASTYRVVSVLTVLHQDWLNGKEYKCKVSNKALP
			APIEKTISKAKGQPREPQVYTLPPCRDELTKNQVSLWCLVKGFYPSDIAVEWESN
			GQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYT
:503	DNA255	Knob: hFc(N297A)-hIL2(R38A, F42A, Y45A, E62A, C125A)	QKSLSLSPGGGSSPPGGGSSGGGSGPAPTSSSTKKTQLQLEHLLLDLQMILNGINN
			YKNPKLTAMLTAKFAMPKKATELKHLQCLEEALKPLEEVLNLAQSKNFHLRPRDLI
			SNINVIVLELKGSETTFMCEYADETATIVEFLNRWITFAQSIISTLT
			AVNGTSQFTCF
			YNSRANISCVW
			SQDGALQDTSC
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			SPGHTWEEAPL
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			ETLTPDTQYEFQ
			VRVKPLQGEFTT
			WSPWSQPLAFR
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			APIEKTISKAKGQPREPQVYTLPPCRDELTKNQVSLWCLVKGFYPSDIAVEWESN
			GQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYT
:503	DNA255	Knob: hFc(N297A)-hIL2(R38A, F42A, Y45A, E62A, C125A)	QKSLSLSPGGGSSPPGGGSSGGGSGPAPTSSSTKKTQLQLEHLLLDLQMILNGINN
			YKNPKLTAMLTAKFAMPKKATELKHLQCLEEALKPLEEVLNLAQSKNFHLRPRDLI
			SNINVIVLELKGSETTFMCEYADETATIVEFLNRWITFAQSIISTLT
			AVNGTSQFTCF
			YNSRANISCVW
			SQDGALQDTSC
			QVHAWPDRRR
			WNQTCELLPVS
			QASWACNLILG
			APDSQKLTTVDI
			VTLRVLCREGVR
			WRVMAIQDFK
:503	DNA606	Hole: hFc(N297A)-[RAAAVKSP]-hCD122 SGGG	PFENLRLMAPIS
			LQVVHVETHRC
			NISWEISQASHY
			FERHLEFEARTL
			SPGHTWEEAPL
			LTLKQKQEWICL
			ETLTPDTQYEFQ
			VRVKPLQGEFTT
			WSPWSQPLAFR
			TKPAALGKD

			ESKYGPPCPPCPAPEFLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSQEDPEVQ
			FNWYVDGVEVHNAKTKPREEQFNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKG
			LPSSIEKTISKAKGQPREPQVCTLPPSQEEMTKNQVSLSCAVKGFYPSDIAVEWES
			NGQPENNYKTTPPVLDSDGSFFLYSRLTVDKSRWQEGNVFSCSVMHEALHNHYT
:504	DNA603	Hole: hFcIgG4-hCD122	QKSLSLSLGPGSGSAVNGTSQFTCFYNSRANISCVWSQDGALQDTSCQVHAWP
			DRRRWNQTCELLPVSQASWACNLILGAPDSQKLTTVDIVTLRVLCREGVRWRV
			MAIQDFKPFENLRLMAPISLQVVHVETHRCNISWEISQASHYFERHLEFEARTLSP
			GHTWEEAPLLTLKQKQEWICLETLTPDTQYEFQVRVKPLQGEFTTWSPWSQPLA
			FRTKPAALGKD
			APTSSSTKKTQL
			QLEHLLLDLQMI
			LNGINNYKNPKL
		Knob: hFcIgG4-hIL2-[VPLSLY]-SGP hIL2(R38A, F42A, Y45A, E62A, C125A)	ESKYGPPCPPCPAPEFLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSQEDPEVQ
			FNWYVDGVEVHNAKTKPREEQFNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKG
			KKATELKHLQCL
			EEALKPLEEVLN
:504	DNA605		LAQSKNFHLRPR
			DLISNINVIVLEL
			KGSETTFMCEY
			ADETATIVEFLN
			RWITFAQSIISTL
			T
			LPSSIEKTISKAKGQPREPQVYTLPPCQEEMTKNQVSLWCLVKGFYPSDIAVEWES
			NGQPENNYKTTPPVLDSDGSFFLYSRLTVDKSRWQEGNVFSCSVMHEALHNHYT
			QKSLSLSLGGSPGVPLSLYSGPAPTSSSTKKTQLQLEHLLLDLQMILNGINNYKNPK
			LTAMLTAKFAMPKKATELKHLQCLEEALKPLEEVLNLAQSKNFHLRPRDLISNINVI
			VLELKGSETTFMCEYADETATIVEFLNRWITFAQSIISTLT
			ESKYGPPCPPCPAPEFLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSQEDPEVQ
			FNWYVDGVEVHNAKTKPREEQFNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKG
			LPSSIEKTISKAKGQPREPQVCTLPPSQEEMTKNQVSLSCAVKGFYPSDIAVEWES
			NGQPENNYKTTPPVLDSDGSFFLYSRLTVDKSRWQEGNVFSCSVMHEALHNHYT
:505	DNA603	Hole: hFcIgG4-hCD122	QKSLSLSLGPGSGSAVNGTSQFTCFYNSRANISCVWSQDGALQDTSCQVHAWP
			DRRRWNQTCELLPVSQASWACNLILGAPDSQKLTTVDIVTLRVLCREGVRWRV
			MAIQDFKPFENLRLMAPISLQVVHVETHRCNISWEISQASHYFERHLEFEARTLSP
			GHTWEEAPLLTLKQKQEWICLETLTPDTQYEFQVRVKPLQGEFTTWSPWSQPLA
			FRTKPAALGKD
			ESKYGPPCPPCPAPEFLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSQEDPEVQ
		Knob: IgG4 hFc-hIL2(R38A, F42A, Y45A, E62A, C125A)	FNWYVDGVEVHNAKTKPREEQFNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKG
			LPSSIEKTISKAKGQPREPQVYTLPPCQEEMTKNQVSLWCLVKGFYPSDIAVEWES
:505	DNA604		NGQPENNYKTTPPVLDSDGSFFLYSRLTVDKSRWQEGNVFSCSVMHEALHNHYT
			QKSLSLSLGGGSSPPGGGSSGGGSGGSGPAPTSSSTKKTQLQLEHLLLDLQMILNGINN
			YKNPKLTAMLTAKFAMPKKATELKHLQCLEEALKPLEEVLNLAQSKNFHLRPRDLI
			SNINVIVLELKGSETTFMCEYADETATIVEFLNRWITFAQSIISTLT

				DKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMASRTPEVTCVVVDVSHEDPEVKF
				NWYVDGVEVHNAKTKPREEQYASTYRVVSVLTVLHQDWLNGKEYKCKVSNKAL
				PAPIEKTISKAKGQPREPQVYTLPPCRDELTKNQVSLWCLVKGFYPSDIAVEWESN
:508	DNA577	Knob: hFc(N297, I253A)-hIL2(R38A, F42A, Y45A, E62A, C125A)		GQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYT
				QKSLSLSPGGGSSPPGGGSSGGGSGPAPTSSSTKKTQLQLEHLLLDLQMILNGINN
				YKNPKLTAMLTAKFAMPKKATELKHLQCLEEALKPLEEVLNLAQSKNFHLRPRDLI
				SNINVIVLELKGSETTFMCEYADETATIVEFLNRWITFAQSIISTLT
				AVNGTSQFTCF
				YNSRANISCVW
				SQDGALQDTSC
				QVHAWPDRRR
				WNQTCELLPVS
				QASWACNLILG
				APDSQKLTTVDI
				VTLRVLCREGVR
				WRVMAIQDFK
:508	DNA609	Hole: hFc(N297A, I253A)-[VPLSLY]-hCD122	GSGGG	PFENLRLMAPIS
				LQVVHVETHRC
				NISWEISQASHY
				FERHLEFEARTL
				SPGHTWEEAPL
				LTLKQKQEWICL
				ETLTPDTQYEFQ
				VRVKPLQGEFTT
				WSPWSQPLAFR
				TKPAALGKD
				DKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMASRTPEVTCVVVDVSHEDPEVKF
				NWYVDGVEVHNAKTKPREEQYASTYRVVSVLTVLHQDWLNGKEYKCKVSNKAL
				PAPIEKTISKAKGQPREPQVCTLPPSRDELTKNQVSLSCAVKGFYPSDIAVEWESN
:509	DNA575	Hole: hFc(N297A, I253A)-hCD122		GQPENNYKTTPPVLDSDGSFFLVSKLTVDKSRWQQGNVFSCSVMHEALHNHYT
				QKSLSLSPGPGSGSAVNGTSQFTCFYNSRANISCVWSQDGALQDTSCQVHAWP
				DRRRWNQTCELLPVSQASWACNLILGAPDSQKLTTVDIVTLRVLCREGVRWRV
				MAIQDFKPFENLRLMAPISLQVVHVETHRCNISWEISQASHYFERHLEFEARTLSP
				GHTWEEAPLLTLKQKQEWICLETLTPDTQYEFQVRVKPLQGEFTTWSPWSQPLA
				FRTKPAALGKD

APTSSSTKKTQL
QLEHLLLDLQMI
LNGINNYKNPKL
TAMLTAKFAMP
KKATELKHLQCL
EEALKPLEEVLN
LAQSKNFHLRPR
DLISNINVIVLEL
KGSETTFMCEY
ADETATIVEFLN
RWITFAQSIISTL
T

:509	DNA623	Knob: hFc(N297, I253A)-[MPYDLYHP]- hIL2(R38A, F42A, Y45A, E62A, C125A)	DKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMASRTPEVTCVVVDVSHEDPEVKF NWYVDGVEVHNAKTKPREEQYASTYRVVSVLTVLHQDWLNGKEYKCKVSNKAL PAPIEKTISKAKGQPREPQVYTLPPCRDELTKNQVSLWCLVKGFYPSDIAVEWESN GQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYT QKSLSLSPGGGSSPPMPYDLYHPSGPAPTSSSTKKTQLQLEHLLLDLQMILNGINN YKNPKLTAMLTAKFAMPKKATELKHLQCLEEALKPLEEVLNLAQSKNFHLRPRDLI SNINVIVLELKGSETTFMCEYADETATIVEFLNRWITFAQSIISTLT
:510	DNA577	Knob: hFc(N297, I253A)-hIL2(R38A, F42A, Y45A, E62A, C125A)	DKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMASRTPEVTCVVVDVSHEDPEVKF NWYVDGVEVHNAKTKPREEQYASTYRVVSVLTVLHQDWLNGKEYKCKVSNKAL PAPIEKTISKAKGQPREPQVYTLPPCRDELTKNQVSLWCLVKGFYPSDIAVEWESN GQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYT QKSLSLSPGGGSSPPGGGSSGGGSGGSGPAPTSSSTKKTQLQLEHLLLDLQMILNGINN YKNPKLTAMLTAKFAMPKKATELKHLQCLEEALKPLEEVLNLAQSKNFHLRPRDLI SNINVIVLELKGSETTFMCEYADETATIVEFLNRWITFAQSIISTLT

AVNGTSQFTCF
YNSRANISCVW
SQDGALQDTSC
QVHAWPDRRR
WNQTCELLPVS
QASWACNLILG DKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMASRTPEVTCVVVDVSHEDPEVKF
APDSQKLTTVDI NWYVDGVEVHNAKTKPREEQYASTYRVVSVLTVLHQDWLNGKEYKCKVSNKAL
VTLRVLCREGVR PAPIEKTISKAKGQPREPQVCTLPPSRDELTKNQVSLSCAVKGFYPSDIAVEWESN
WRVMAIQDFK GQPENNYKTTPPVLDSDGSFFLVSKLTVDKSRWQQGNVFSCSVMHEALHNHYT
:510 DNA608 Hole: hFc(N297A, I253A)-[MPYDLYHP]-hCD122 PFENLRLMAPIS QKSLSLSPGGPPSGSSPMPYDLYHPSGGGAVNGTSQFTCFYNSRANISCVWSQD
LQVVHVETHRC GALQDTSCQVHAWPDRRRWNQTCELLPVSQASWACNLILGAPDSQKLTTVDIV
NISWEISQASHY TLRVLCREGVRWRVMAIQDFKPFENLRLMAPISLQVVHVETHRCNISWEISQAS
FERHLEFEARTL HYFERHLEFEARTLSPGHTWEEAPLLTLKQKQEWICLETLTPDTQYEFQVRVKPL
SPGHTWEEAPL QGEFTTWSPWSQPLAFRTKPAALGKD
LTLKQKQEWICL
ETLTPDTQYEFQ
VRVKPLQGEFTT
WSPWSQPLAFR
TKPAALGKD

ESKYGPPCPPCPAPEFLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSQEDPEVQ
FNWYVDGVEVHNAKTKPREEQFNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKG
LPSSIEKTISKAKGQPREPQVYTLPPCQEEMTKNQVSLWCLVKGFYPSDIAVEWES
:511 DNA604 Knob: IgG4 hFc-hIL2(R38A, F42A, Y45A, E62A, C125A) NGQPENNYKTTPPVLDSDGSFFLYSRLTVDKSRWQEGNVFSCSVMHEALHNHYT
QKSLSLSLGGGSSPPGGGSSGGGSGPAPTSSSTKKTQLQLEHLLLDLQMILNGINN
YKNPKLTAMLTAKFAMPKKATELKHLQCLEEALKPLEEVLNLAQSKNFHLRPRDLI
SNINVIVLELKGSETTFMCEYADETATIVEFLNRWITFAQSIISTLT

AVNGTSQFTCF
YNSRANISCVW
SQDGALQDTSC
QVHAWPDRRR
WNQTCELLPVS
QASWACNLILG ESKYGPPCPPCPAPEFLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSQEDPEVQ
APDSQKLTTVDI FNWYVDGVEVHNAKTKPREEQFNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKG
VTLRVLCREGVR LPSSIEKTISKAKGQPREPQVCTLPPSQEEMTKNQVSLSCAVKGFYPSDIAVEWES
WRVMAIQDFK NGQPENNYKTTPPVLDSDGSFFLYSRLTVDKSRWQEGNVFSCSVMHEALHNHYT
PFENLRLMAPIS QKSLSLSLGGPPSGSSPGVPLSLYGSGGGAVNGTSQFTCFYNSRANISCVWSQDG
LQVVHVETHRC ALQDTSCQVHAWPDRRRWNQTCELLPVSQASWACNLILGAPDSQKLTTVDIVT
NISWEISQASHY LRVLCREGVRWRVMAIQDFKPFENLRLMAPISLQVVHVETHRCNISWEISQASH
FERHLEFEARTL YFERHLEFEARTLSPGHTWEEAPLLTLKQKQEWICLETLTPDTQYEFQVRVKPLQ
SPGHTWEEAPL GEFTTWSPWSQPLAFRTKPAALGKD
LTLKQKQEWICL
ETLTPDTQYEFQ
VRVKPLQGEFTT
WSPWSQPLAFR
TKPAALGKD

:511 DNA621 Hole: hFcIgG4-[VPLSLY]-hCD122 GSGGG

DKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMASRTPEVTCVVVDVSHEDPEVKF
NWYVDGVEVHNAKTKPREEQYASTYRVVSVLTVLHQDWLNGKEYKCKVSNKAL
PAPIEKTISKAKGQPREPQVYTLPPCRDELTKNQVSLWCLVKGFYPSDIAVEWESN
GQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYT
QKSLSLSPGGGSSPPGGGSSGGGSGPAPTSSSTKKTQLQLEHLLLDLQMILNGINN
YKNPKLTAMLTAKFAMPKKATELKHLQCLEEALKPLEEVLNLAQSKNFHLRPRDLI
SNINVIVLELKGSETTFMCEYADETATIVEFLNRWITFAQSIISTLT

:512 DNA577 Knob: hFc(N297, I253A)-hIL2(R38A, F42A, Y45A, E62A, C125A)

DKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMASRTPEVTCVVVDVSHEDPEVKF
NWYVDGVEVHNAKTKPREEQYASTYRVVSVLTVLHQDWLNGKEYKCKVSNKAL
PAPIEKTISKAKGQPREPQVCTLPPSRDELTKNQVSLSCAVKGFYPSDIAVEWESN
GQPENNYKTTPPVLDSDGSFFLVSKLTVDKSRWQQGNVFSCSVMHEALHNHYT
QKSLSLSPG

:512 DNA625 Hole: hFc(N297A, I253A)

			ESKYGPPCPPCPAPEFLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSQEDPEVQ
			FNWYVDGVEVHNAKTKPREEQFNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKG
			LPSSIEKTISKAKGQPREPQVYTLPPCQEEMTKNQVSLWCLVKGFYPSDIAVEWES
:513	DNA604	Knob: IgG4 hFc-hIL2(R38A, F42A, Y45A, E62A, C125A)	NGQPENNYKTTPPVLDSDGSFFLYSRLTVDKSRWQEGNVFSCSVMHEALHNHYT
			QKSLSLSLGGGSSPPGGGSSGGGSGPAPTSSSTKKTQLQLEHLLLDLQMILNGINN
			YKNPKLTAMLTAKFAMPKKATELKHLQCLEEALKPLEEVLNLAQSKNFHLRPRDLI
			SNINVIVLELKGSETTFMCEYADETATIVEFLNRWITFAQSIISTLT
			ESKYGPPCPPCPAPEFLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSQEDPEVQ
			FNWYVDGVEVHNAKTKPREEQFNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKG
:513	DNA626	Hole: hFcIgG4	LPSSIEKTISKAKGQPREPQVCTLPPSQEEMTKNQVSLSCAVKGFYPSDIAVEWES
			NGQPENNYKTTPPVLDSDGSFFLYSRLTVDKSRWQEGNVFSCSVMHEALHNHYT
			QKSLSLSLGPG
			DKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFN
			WYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALP
			APIEKTISKAKGQPREPQVYTLPPCRDELTKNQVSLWCLVKGFYPSDIAVEWESN
:526	DNA670	Knob: hFc-hIL2(R38A, F42A, Y45A, E62A, C125A)	GQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYT
			QKSLSLSPGGGSSPPGGGSSGGGSGPAPTSSSTKKTQLQLEHLLLDLQMILNGINN
			YKNPKLTAMLTAKFAMPKKATELKHLQCLEEALKPLEEVLNLAQSKNFHLRPRDLI
			SNINVIVLELKGSETTFMCEYADETATIVEFLNRWITFAQSIISTLT

				AVNGTSQFTCF
				YNSRANISCVW
				SQDGALQDTSC
				QVHAWPDRRR
				WNQTCELLPVS
				QASWACNLILG DKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFN
				APDSQKLTTVDI WYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALP
				VTLRVLCREGVR APIEKTISKAKGQPREPQVCTLPPSRDELTKNQVSLSCAVKGFYPSDIAVEWESNG
				WRVMAIQDFK QPENNYKTTPPVLDSDGSFFLVSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQ
:526	DNA672	Hole: hFc-[VPLSLY]-hCD122	GSGGG	PFENLRLMAPIS KSLSLSPGGPPSGSSPGVPLSLYGSGGGAVNGTSQFTCFYNSRANISCVWSQDGA
				LQVVHVETHRC LQDTSCQVHAWPDRRRWNQTCELLPVSQASWACNLILGAPDSQKLTTVDIVTL
				NISWEISQASHY RVLCREGVRWRVMAIQDFKPFENLRLMAPISLQVVHVETHRCNISWEISQASHY
				FERHLEFEARTL FERHLEFEARTLSPGHTWEEAPLLTLKQKQEWICLETLTPDTQYEFQVRVKPLQG
				SPGHTWEEAPL EFTTWSPWSQPLAFRTKPAALGKD
				LTLKQKQEWICL
				ETLTPDTQYEFQ
				VRVKPLQGEFTT
				WSPWSQPLAFR
				TKPAALGKD
				DKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFN
				WYVDGVEVHNAKTKPREEQYASTYRVVSVLTVLHQDWLNGKEYKCKVSNKALP
		Knob: hFc(N297A)-hIL2(R38A, F42A, Y45A, E62A, C125A)		APIEKTISKAKGQPREPQVYTLPPCRDELTKNQVSLWCLVKGFYPSDIAVEWESN
:530	DNA255			GQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYT
				QKSLSLSPGGGSSPPGGGSSGGGSGPAPTSSSTKKTQLQLEHLLLDLQMILNGINN
				YKNPKLTAMLTAKFAMPKKATELKHLQCLEEALKPLEEVLNLAQSKNFHLRPRDLI
				SNINVIVLELKGSETTFMCEYADETATIVEFLNRWITFAQSIISTLT

			AVNGTSQFTCF
			YNSRANISCVW
			SQDGALQDTSC
			QVHAWPDRRR
			WNQTCELLPVS
			QASWACNLILG DKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFN
			APDSQKLTTVDI WYVDGVEVHNAKTKPREEQYASTYRVVSVLTVLHQDWLNGKEYKCKVSNKALP
		Hole:	VTLRVLCREGVR APIEKTISKAKGQPREPQVCTLPPSRDELTKNQVSLSCAVKGFYPSDIAVEWESNG
		hFc(N297A)-	WRVMAIQDFK QPENNYKTTPPVLDSDGSFFLVSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQ
:530	DNA612	[MPYDLYHP]-	PFENLRLMAPIS KSLSLSPGGPPSGSSPMPYDLYHPSGGGAVNGTSQFTCFYNSRANISCVWSQDG
		hCD122(C122	LQVVHVETHRS ALQDTSCQVHAWPDRRRWNQTCELLPVSQASWACNLILGAPDSQKLTTVDIVT
		S, C168S)	NISWEISQASHY LRVLCREGVRWRVMAIQDFKPFENLRLMAPISLQVVHVETHRSNISWEISQASHY
			FERHLEFEARTL FERHLEFEARTLSPGHTWEEAPLLTLKQKQEWISLETLTPDTQYEFQVRVKPLQGE
			SPGHTWEEAPL FTTWSPWSQPLAFRTKPAALGKD
			LTLKQKQEWISL
			ETLTPDTQYEFQ
			VRVKPLQGEFTT
			WSPWSQPLAFR
			TKPAALGKD
			DKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFN
			WYVDGVEVHNAKTKPREEQYASTYRVVSVLTVLHQDWLNGKEYKCKVSNKALP
		Knob:	APIEKTISKAKGQPREPQVYTLPPCRDELTKNQVSLWCLVKGFYPSDIAVEWESN
		hFc(N297A)-	GQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYT
:531	DNA255	hIL2(R38A,	QKSLSLSPGGGSSPPGGGSSGGGSGPAPTSSSTKKTQLQLEHLLLDLQMILNGINN
		F42A, Y45A,	YKNPKLTAMLTAKFAMPKKATELKHLQCLEEALKPLEEVLNLAQSKNFHLRPRDLI
		E62A, C125A)	SNINVIVLELKGSETTFMCEYADETATIVEFLNRWITFAQSIISTLT

AVNGTSQFTCF
YNSRANISCVW
SQDGALQDTSC
QVHAWPDRRR
WNQTCELLPVS

			QASWACNLILG DKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFN
			APDSQKLTTVDI WYVDGVEVHNAKTKPREEQYASTYRVVSVLTVLHQDWLNGKEYKCKVSNKALP
		Hole:	VTLRVLCREGVR APIEKTISKAKGQPREPQVCTLPPSRDELTKNQVSLSCAVKGFYPSDIAVEWESNG
		hFc(N297A)-	WRVMAIQDFK QPENNYKTTPPVLDSDGSFFLVSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQ
:531	DNA614	[DSGGFMLTI-	SGGG PFENLRLMAPIS KSLSLSPGGPPSGSSPGDSGGFMLTSGGGAVNGTSQFTCFYNSRANISCVWSQD
		hCD122(C122	LQVVHVETHRS GALQDTSCQVHAWPDRRRWNQTCELLPVSQASWACNLILGAPDSQKLTTVDIV
		S, C168S)	NISWEISQASHY TLRVLCREGVRWRVMAIQDFKPFENLRLMAPISLQVVHVETHRSNISWEISQASH
			FERHLEFEARTL YFERHLEFEARTLSPGHTWEEAPLLTLKQKQEWISLETLTPDTQYEFQVRVKPLQG
			SPGHTWEEAPL EFTTWSPWSQPLAFRTKPAALGKD
			LTLKQKQEWISL
			ETLTPDTQYEFQ
			VRVKPLQGEFTT
			WSPWSQPLAFR
			TKPAALGKD

			DKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFN
			WYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALP
			APIEKTISKAKGQPREPQVCTLPPSRDELTKNQVSLSCAVKGFYPSDIAVEWESNG
			QPENNYKTTPPVLDSDGSFFLVSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQ
:532	DNA669	Hole: hFc-	KSLSLSPGPGSGSAVNGTSQFTCFYNSRANISCVWSQDGALQDTSCQVHAWPDR
		hCD122	RRWNQTCELLPVSQASWACNLILGAPDSQKLTTVDIVTLRVLCREGVRWRVMAI
			QDFKPFENLRLMAPISLQVVHVETHRCNISWEISQASHYFERHLEFEARTLSPGHT
			WEEAPLLTLKQKQEWICLETLTPDTQYEFQVRVKPLQGEFTTWSPWSQPLAFRT
			KPAALGKD

			APTSSSTKKTQL
			QLEHLLLDLQMI
			LNGINNYKNPKL
			TAMLTAKFAMP
			KKATELKHLQCL
			EEALKPLEEVLN
			LAQSKNFHLRPR
			DLISNINVIVLEL
			KGSETTFMCEY
			ADETATIVEFLN
			RWITFAQSIISTL
			T
:532	DNA671	Knob: hFc-[VPLSLY]-hIL2(R38A, F42A, Y45A, E62A, C125A)	SGP

DKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFN
WYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALP
APIEKTISKAKGQPREPQVYTLPPCRDELTKNQVSLWCLVKGFYPSDIAVEWESN
GQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYT
QKSLSLSPGGSPGVPLSLYSGPAPTSSSTKKTQLQLEHLLLDLQMILNGINNYKNPK
LTAMLTAKFAMPKKATELKHLQCLEEALKPLEEVLNLAQSKNFHLRPRDLISNINVI
VLELKGSETTFMCEYADETATIVEFLNRWITFAQSIISTLT

me	newnames	Component1Sequence	Component2Sequence	Component3Sequence
VA158	Hole: hFc(N297A)	DKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHED PEVKFNWYVDGVEVHNAKTKPREEQYASTYRVVSVLTVLHQDWLNGK EYKCKVSNKALPAPIEKTISKAKGQPREPQVCTLPPSRDELTKNQVSLSCA VKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLVSKLTVDKSRW QQGNVFSCSVMHEALHNHYTQKSLSLSPG		
VA187	Hole: hFc(N297A)-hCD122	DKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHED PEVKFNWYVDGVEVHNAKTKPREEQYASTYRVVSVLTVLHQDWLNGK EYKCKVSNKALPAPIEKTISKAKGQPREPQVCTLPPSRDELTKNQVSLSCA VKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLVSKLTVDKSRW QQGNVFSCSVMHEALHNHYTQKSLSLSPG	PGSGS	AVNGTSQFTCFYNSRANISCVWSQDGALQDTSCQVHAWPDRRRWI TCELLPVSQASWACNLILGAPDSQKLTTVDIVTLRVLCREGVRWRVM QDFKPFENLRLMAPISLQVVHVETHRCNISWEISQASHYFERHLEFEA LSPGHTWEEAPLLTLKQKQEWICLETLTPDTQYEFQVRVKPLQGEFTT SPWSQPLAFRTKPAALGKD

VA255	Knob: hFc(N297A)-hIL2(R38A, F42A, Y45A, E62A, C125A)	DKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHED PEVKFNWYVDGVEVHNAKTKPREEQYASTYRVVSVLTVLHQDWLNGK EYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPCRDELTKNQVSLWC LVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSR WQQGNVFSCSVMHEALHNHYTQKSLSLSPG	GGSSPPGGGSSG GGSGP	APTSSSTKKTQLQLEHLLLDLQMILNGINNYKNPKLTAMLTAKFAMP ATELKHLQCLEEALKPLEEVLNLAQSKNFHLRPRDLISNINVIVLELKGS TFMCEYADETATIVEFLNRWITFAQSIISTLT	
VA263	Knob: hFc(N297A)-[VPLSLY]-hIL2(R38A, F42A, Y45A, E62A, C125A)	DKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHED PEVKFNWYVDGVEVHNAKTKPREEQYASTYRVVSVLTVLHQDWLNGK EYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPCRDELTKNQVSLWC LVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSR WQQGNVFSCSVMHEALHNHYTQKSLSLSPG	GSPG	VPLSLY	
VA278	Knob: hFc(N297A)-[DSGGFMLT]-hIL2(C125A)	DKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHED PEVKFNWYVDGVEVHNAKTKPREEQYASTYRVVSVLTVLHQDWLNGK EYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPCRDELTKNQVSLWC LVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSR WQQGNVFSCSVMHEALHNHYTQKSLSLSPG	GSGP	DSGGFMLT	

vA281	Knob: hFc(N297A)-[DSGGFMLT]-hIL2(R38A, F42A, Y45A, E62A, C125A)	DKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHED PEVKFNWYVDGVEVHNAKTKPREEQYASTYRVVSVLTVLHQDWLNGK EYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPCRDELTKNQVSLWC LVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSR WQQGNVFSCSVMHEALHNHYTQKSLSLSPG	DSGGFMLT	GSGP
vA440	Hole: hFc(N297A)-hCD122(C122S, C168S)	DKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHED PEVKFNWYVDGVEVHNAKTKPREEQYASTYRVVSVLTVLHQDWLNGK EYKCKVSNKALPAPIEKTISKAKGQPREPQVCTLPPSRDELTKNQVSLSCA VKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLVSKLTVDKSRW QQGNVFSCSVMHEALHNHYTQKSLSLSPG	AVNGTSQFTCFYNSRANISCVWSQDGALQDTSCQVHAWPDRRRWI TCELLPVSQASWACNLILGAPDSQKLTTVDIVTLRVLCREGVRWRVM QDFKPFENLRLMAPISLQVVHVETHRSNISWEISQASHYFERHLEFEA LSPGHTWEEAPLLTLKQKQEWISLETLTPDTQYEFQVRVKPLQGEFTT SPWSQPLAFRTKPAALGKD	PGSGS
vA476	Knob: hFc(N297A)-[NPMGSDPVNFK LLRVVNG]-hIL2(F42S, E62S, C125A)	DKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHED PEVKFNWYVDGVEVHNAKTKPREEQYASTYRVVSVLTVLHQDWLNGK EYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPCRDELTKNQVSLWC LVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSR WQQGNVFSCSVMHEALHNHYTQKSLSLSPG	NPMGSDPVNFKLLRVVNG	G

vA477	Knob: mFcIgG2a(LALAPG)-hIL2(R38A, F42A, Y45A, E62A, C125A)	TIKPCPPCKCPAPNAAGGPSVFIFPPKIKDVLMISLSPIVTCVVVDVSEDDP DVQISWFVNNVEVHTAQTQTHREDYNSTLRVVSALPIQHQDWMSGKE FKCKVNNKDLGAPIERTISKPKGSVRAPQVYVLPPCEEEMTKKQVTLWC MVTDFMPEDIYVEWTNNGKTELNYKNTEPVLDSDGSYFMYSKLRVEKK NWVERNSYSCSVVHEGLHNHHTTKSFSRTPG	GGSSPPGGGSSG GGSGP	APTSSSTKKTQLQLEHLLLDLQMILNGINNYKNPKLTAMLTAKFAMPI ATELKHLQCLEEALKPLEEVLNLAQSKNFHLRPRDLISNINVIVLELKGS TFMCEYADETATIVEFLNRWITFAQSIISTLT	
vA478	Knob: mFcIgG2a(LALAPG)-[VPLSLY]-hIL2(R38A, F42A, Y45A, E62A, C125A)	TIKPCPPCKCPAPNAAGGPSVFIFPPKIKDVLMISLSPIVTCVVVDVSEDDP DVQISWFVNNVEVHTAQTQTHREDYNSTLRVVSALPIQHQDWMSGKE FKCKVNNKDLGAPIERTISKPKGSVRAPQVYVLPPCEEEMTKKQVTLWC MVTDFMPEDIYVEWTNNGKTELNYKNTEPVLDSDGSYFMYSKLRVEKK NWVERNSYSCSVVHEGLHNHHTTKSFSRTPG	GSPG	VPLSLY	
vA479	Hole: mFcIgG2a(LALAPG)	TIKPCPPCKCPAPNAAGGPSVFIFPPKIKDVLMISLSPIVTCVVVDVSEDDP DVQISWFVNNVEVHTAQTQTHREDYNSTLRVVSALPIQHQDWMSGKE FKCKVNNKDLGAPIERTISKPKGSVRAPQVCVLPPPEEEMTKKQVTLSCA VTDFMPEDIYVEWTNNGKTELNYKNTEPVLDSDGSYFMVSKLRVEKKN WVERNSYSCSVVHEGLHNHHTTKSFSRTPG			

		TIKPCPPCKCPAPNAAGGPSVFIFPPKIKDVLMISLSPIVTCVVVDVSEDDP	AVNGTSQFTCFYNSRANISCVWSQDGALQDTSCQVHAWPDRRRWI
	Hole:	DVQISWFVNNVEVHTAQTQTHREDYNSTLRVVSALPIQHQDWMSGKE	TCELLPVSQASWACNLILGAPDSQKLTTVDIVTLRVLCREGVRWRVM
vA480	mFcIgG2a(LALAP	FKCKVNNKDLGAPIERTISKPKGSVRAPQQVCVLPPPEEEMTKKQVTLSCA PGSGS	QDFKPFENLRLMAPISLQVVHVETHRCNISWEISQASHYFERHLEFEA
	G)-hCD122	VTDFMPEDIYVEWTNNGKTELNYKNTEPVLDSDGSYFMVSKLRVEKKN	LSPGHTWEEAPLLTLKQKQEWICLETLTPDTQYEFQVRVKPLQGEFTT
		WVERNSYSCSVVHEGLHNHHTTKSFSRTPG	SPWSQPLAFRTKPAALGKD

	F8ScFvVersion1-	EVQLLESGGGLVQPGGSLRLSCAASGFTFSLFTMSVVVRQAPGKGLEVV	DKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHI
	Hole:	VSAISGSGGSTYYADSVKGRFTISRDNSKNTLYLQMNSLRAEDTAVYYCA	PEVKFNWYVDGVEVHNAKTKPREEQYASTYRVVSVLTVLHQDWLNG
vA516	hFc(N297A)-	KSTHLYLFDYWGQGTLVTVSSGGGGSGGGGSGGGGSEIVLTQSPGTLSL GGS	EYKCKVSNKALPAPIEKTISKAKGQPREPQVCTLPPSRDELTKNQVSLS
	hCD122	SPGERATLSCRASQSVSMPFLAWYQQKPGQAPRLLIYGASSRATGIPDR	VKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLVSKLTVDKSF
		FSGSGSGTDFTLTISRLEPEDFAVYYCQQMRGRPPTFGQGTKVEIK	QQGNVFSCSVMHEALHNHYTQKSLSLSPG

	Hole:	TIKPCPPCKCPAPNAAGGPSVFIFPPKIKDVLMISLSPIVTCVVVDVSEDDP	
	mFcIgG2a(LALAP	DVQISWFVNNVEVHTAQTQTHREDYNSTLRVVSALPIQHQDWMSGKE	
vA520	G)-	FKCKVNNKDLGAPIERTISKPKGSVRAPQQVCVLPPPEEEMTKKQVTLSCA HHHHHHHH	
	NoAnnotationFo	VTDFMPEDIYVEWTNNGKTELNYKNTEPVLDSDGSYFMVSKLRVEKKN	
	und	WVERNSYSCSVVHEGLHNHHTTKSFSRTPG	

	Hole:	TIKPCPPCKCPAPNAAGGPSVFIFPPKIKDVLMISLSPIVTCVVVDVSEDDP	
	mFcIgG2a(LALAP	DVQISWFVNNVEVHTAQTQTHREDYNSTLRVVSALPIQHQDWMSGKE	
VA521	G)-hCD122-	FKCKVNNKDLGAPIERTISKPKGSVRAPQVCVLPPPEEEMTKKQVTLSCA	PGSGS
	NoAnnotationFo	VTDFMPEDIYVEWTNNGKTELNYKNTEPVLDSDGSYFMVSKLRVEKKN	
	und	WVERNSYSCSVVHEGLHNHHTTKSFSRTPG	
		AVNGTSQFTCFYNSRANISCVWSQDGALQDTSCQVHAWPDRRRWI	
		TCELLPVSQASWACNLILGAPDSQKLTTVDIVTLRVLCREGVRWRVM	
		QDFKPFENLRLMAPISLQVVHVETHRCNISWEISQASHYFERHLEFEA	
		LSPGHTWEEAPLLTLKQKQEWICLETLTPDTQYEFQVRVKPLQGEFTT	
		SPWSQPLAFRTKPAALGKD	

	Hole:	TIKPCPPCKCPAPNAAGGPSVFIFPPKIKDVLMISLSPIVTCVVVDVSEDDP	
	mFcIgG2a(LALAP	DVQISWFVNNVEVHTAQTQTHREDYNSTLRVVSALPIQHQDWMSGKE	
VA522	G)-mCD122-	FKCKVNNKDLGAPIERTISKPKGSVRAPQVCVLPPPEEEMTKKQVTLSCA	PGSGS
	NoAnnotationFo	VTDFMPEDIYVEWTNNGKTELNYKNTEPVLDSDGSYFMVSKLRVEKKN	
	und	WVERNSYSCSVVHEGLHNHHTTKSFSRTPG	
		AVKNCSHLECFYNSRANVSCMWSHEEALNVTTCHVHAKSNLRHWN	
		CELTLVRQASWACNLILGSFPESQSLTSVDLLDINVVCWEEKGWRRV	
		CDFHPFDNLRLVAPHSLQVLHIDTQRCNISWKVSQVSHYIEPYLEFEA	
		RLLGHSWEDASVLSLKQRQQWLFLEMLIPSTSYEVQVRVKAQRNNTI	
		WSPWSQPLTFRTRPADPMKE	

		DKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHED	
	Hole:	PEVKFNWYVDGVEVHNAKTKPREEQYASTYRVVSVLTVLHQDWLNGK	
VA528	hFc(N297A)-	EYKCKVSNKALPAPIEKTISKAKGQPREPQVCTLPPSRDELTKNQVSLSCA	PGSGS
	hCD122(C168S)	VKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLVSKLTVDKSRW	
		QQGNVFSCSVMHEALHNHYTQKSLSLSPG	
		AVNGTSQFTCFYNSRANISCVWSQDGALQDTSCQVHAWPDRRRWI	
		TCELLPVSQASWACNLILGAPDSQKLTTVDIVTLRVLCREGVRWRVM	
		QDFKPFENLRLMAPISLQVVHVETHRCNISWEISQASHYFERHLEFEA	
		LSPGHTWEEAPLLTLKQKQEWISLETLTPDTQYEFQVRVKPLQGEFTT	
		SPWSQPLAFRTKPAALGKD	

VA530	Knob: mFcIgG1(DAPG)-hIL2(R38A, F42A, Y45A, E62A, C125A)	VRSGCKPCICTVPEVSSVFIFPPKPKDVLTITLTPKVTCVVVAISKDDPEVQ FSWFVDDVEVHTAQTQPREEQFNSTFRSVSELPIMHQDWLNGKEFKCR VNSAAFGAPIEKTISKTKGRPKAPQVYTIPPPKEQMAKDKVSLTCMITDF FPEDITVEWQWNGQPAENYDNTQPIMDTDGSYFVYSDLNVQKSNWE AGNTFTCSVLHEGLHNHHTEKSLSHSPG	GGSSPPGGGSSG GGSGP	APTSSSTKKTQLQLEHLLLDLQMILNGINNYKNPKLTAMLTAKFAMPK ATELKHLQCLEEALKPLEEVLNLAQSKNFHLRPRDLISNINVIVLELKGS TFMCEYADETATIVEFLNRWITFAQSIISTLT	
VA531	Knob: mFcIgG1(DAPG)-[VPLSLY]-hIL2(R38A, F42A, Y45A, E62A, C125A)	VRSGCKPCICTVPEVSSVFIFPPKPKDVLTITLTPKVTCVVVAISKDDPEVQ FSWFVDDVEVHTAQTQPREEQFNSTFRSVSELPIMHQDWLNGKEFKCR VNSAAFGAPIEKTISKTKGRPKAPQVYTIPPPKEQMAKDKVSLTCMITDF FPEDITVEWQWNGQPAENYDNTQPIMDTDGSYFVYSDLNVQKSNWE AGNTFTCSVLHEGLHNHHTEKSLSHSPG	GSPG	VPLSLY	
VA532	Hole: mFcIgG1(DAPG)	VRSGCKPCICTVPEVSSVFIFPPKPKDVLTITLTPKVTCVVVAISKDDPEVQ FSWFVDDVEVHTAQTQPREEQFNSTFRSVSELPIMHQDWLNGKEFKCR VNSAAFGAPIEKTISKTKGRPKAPQVYTIPPPKKQMAKDKVSLTCMITDF FPEDITVEWQWNGQPAENYKNTQPIMKTDGSYFVYSKLNVQKSNWEA GNTFTCSVLHEGLHNHHTEKSLSHSPG			

		VRSGCKPCICTVPEVSSVFIFPPKPKDVLTITLTPKVTCVVVAISKDDPEVQ	
	Hole:	FSWFVDDVEVHTAQTQPREEQFNSTFRSVSELPIMHQDWLNGKEFKCR	
VA533	mFcIgG1(DAPG)-	VNSAAFGAPIEKTISKTKGRPKAPQVYTIPPPKKQMAKDKVSLTCMITDF	PGSGS
	hCD122	FPEDITVEWQWNGQPAENYKNTQPIMKTDGSYFVYSKLNVQKSNWEA	
		GNTFTCSVLHEGLHNHHTEKSLSHSPG	
		AVNGTSQFTCFYNSRANISCVWSQDGALQDTSCQVHAWPDRRRWI	
		TCELLPVSQASWACNLILGAPDSQKLTTVDIVTLRVLCREGVRWRVM	
		QDFKPFENLRLMAPISLQVVHVETHRCNISWEISQASHYFERHLEFEA	
		LSPGHTWEEAPLLTLKQKQEWICLETLTPDTQYEFQVRVKPLQGEFTT	
		SPWSQPLAFRTKPAALGKD	
		VRSGCKPCICTVPEVSSVFIFPPKPKDVLTITLTPKVTCVVVAISKDDPEVQ	
	Hole:	FSWFVDDVEVHTAQTQPREEQFNSTFRSVSELPIMHQDWLNGKEFKCR	
VA534	mFcIgG1(DAPG)-	VNSAAFGAPIEKTISKTKGRPKAPQVYTIPPPKKQMAKDKVSLTCMITDF	PGSGS
	mCD122	FPEDITVEWQWNGQPAENYKNTQPIMKTDGSYFVYSKLNVQKSNWEA	
		GNTFTCSVLHEGLHNHHTEKSLSHSPG	
		AVKNCSHLECFYNSRANVSCMWSHEEALNVTTCHVHAKSNLRHWN	
		CELTLVRQASWACNLILGSFPESQSLTSVDLLDINVVCWEEKGWRRV	
		CDFHPFDNLRLVAPHSLQVLHIDTQRCNISWKVSQVSHYIEPYLEFEA	
		RLLGHSWEDASVLSLKQRQQWLFLEMLIPSTSYEVQVRVKAQRNNT	
		WSPWSQPLTFRTRPADPMKE	
	Knob:	DKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHED	
	hFc(N297A)-	PEVKFNWYVDGVEVHNAKTKPREEQYASTYRVVSVLTVLHQDWLNGK	
VA542	hIL2(R38A, F42A,	EYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPCRDELTKNQVSLWC	GISSGLLSGRSDQP
	Y45A, E62A,	LVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSR	SGP
	C125A)	WQQGNVFSCSVMHEALHNHYTQKSLSLSPG	
		APTSSSTKKTQLQLEHLLLDLQMILNGINNYKNPKLTAMLTAKFAMPI	
		ATELKHLQCLEEALKPLEEVLNLAQSKNFHLRPRDLISNINVIVLELKGS	
		TFMCEYADETATIVEFLNRWITFAQSIISTLT	

		DKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHED			
	Hole:	PEVKFNWYVDGVEVHNAKTKPREEQYASTYRVVSVLTVLHQDWLNGK			
VA543	hFc(N297A)-	EYKCKVSNKALPAPIEKTISKAKGQPREPQVCTLPPSRDELTKNQVSLSCA	GPPSGSSPG	VPLSLY	
	[VPLSLY]-hCD122	VKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLVSKLTVDKSRW			
		QQGNVFSCSVMHEALHNHYTQKSLSLSPG			
	Knob:				
	hFc(N297A)-	DKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHED			
	[VPLSLY]-	PEVKFNWYVDGVEVHNAKTKPREEQYASTYRVVSVLTVLHQDWLNGK			
	hIL2(R38A, F42A,				
VA544	Y45A, E62A,	EYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPCRDELTKNQVSLWC	GSPG	VPLSLY	
	L80F, R81D,	LVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSR			
	L85V, I86V, I92F,	WQQGNVFSCSVMHEALHNHYTQKSLSLSPG			
	C125A)				
	Knob:	DKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHED			
	hFc(N297A)-	PEVKFNWYVDGVEVHNAKTKPREEQYASTYRVVSVLTVLHQDWLNGK			APTSSSTKKTQLQLEHLLLDLQMILNGINNYKNPKLTAMLTAKFAMPI
VA545	hIL2(R38A, F42A,	EYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPCRDELTKNQVSLWC	GISSGLLSGRSSGP	ATELKHLQCLEEALKPLEEVLNLAQSKNFHLRPRDLISNINVIVLELKGS	
	Y45A, E62A,	LVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSR		TFMCEYADETATIVEFLNRWITFAQSIISTLT	
	C125A)	WQQGNVFSCSVMHEALHNHYTQKSLSLSPG			

	Knob:			
	hFc(N297A)-	DKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHED		
	hIL2(R38A, F42A,	PEVKFNWYVDGVEVHNAKTKPREEQYASTYRVVSVLTVLHQDWLNGK	GGSSPPGGGSSG	APTSSSTKKTQLQLEHLLLDLQMILNGINNYKNPKLTAMLTAKFAMPK
VA546	Y45A, E62A,	EYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPCRDELTKNQVSLWC	GGSGP	ATELKHLQCLEEALKPLEEVLNLAQSKNFHFDPRDVVSNINVFVLELKG
	L80F, R81D,	LVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSR		TTFMCEYADETATIVEFLNRWITFAQSIISTLT
	L85V, I86V, I92F,	WQQGNVFSCSVMHEALHNHYTQKSLSLSPG		
	C125A)			
				DKTHTCPPCPAPE
	Hole:	EPKSSDKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDV	KFNWYVDGVEVH	LLGGPSVFLFPPKP
	hFcIgG1(N297A	SHEDPEVKFNWYVDGVEVHNAKTKPREEQYASTYRVVSVLTVLHQDWL	NAKTKPREEQYAS	KDTLMISRTPEVT
VA547	+ EPKSS)-Hole:	NGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVCTLPPSRDELTKNQVSL	TYRVVSVLTVLHQ	PGSGS CVVVDVSHEDPEV
	hFc(N297A)-	SCAVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLVSKLTVDKS	DWLNGKEYKCKV	
	hCD122	RWQQGNVFSCSVMHEALHNHYTQKSLSLSPG	SNKALPAPIEKTIS	
			KAKGQPREPQVC	
			TLPPSRDELTKNQ	
			VSLSCAVKGFYPS	
			DKTHTCPPCPAPE	
			LLGGPSVFLFPPKP	
			KDTLMISRTPEVT	
			CVVVDVSHEDPEV	
	Hole:	AKTDKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVS	KFNWYVDGVEVH	
	hFcIgG1(N297A	HEDPEVKFNWYVDGVEVHNAKTKPREEQYASTYRVVSVLTVLHQDWL	NAKTKPREEQYAS	
VA548	+ AKT)-Hole:	NGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVCTLPPSRDELTKNQVSL	TYRVVSVLTVLHQ	PGSGS
	hFc(N297A)-	SCAVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLVSKLTVDKS	DWLNGKEYKCKV	
	hCD122	RWQQGNVFSCSVMHEALHNHYTQKSLSLSPG	SNKALPAPIEKTIS	
			KAKGQPREPQVC	
			TLPPSRDELTKNQ	
			VSLSCAVKGFYPS	
			DIAVEWESNGQP	

		EPKSSDKTHTCPP	
		CPAPELLGGPSVFL	
		FPPKPKDTLMISRT	
		PEVTCVVVDVSHE	
	Hole:		
	hFcIgG1(N297A	AKTEPKSSDKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVV	DKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSH
	+AKTEPKSS)-	VDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYASTYRVVSVLTVLHQ	PEVKFNWYVDGVEVHNAKTKPREEQYASTYRVVSVLTVLHQDWLN
	Hole:	DWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVCTLPPSRDELTKN	EYKCKVSNKALPAPIEKTISKAKGQPREPQVCTLPPSRDELTKNQVSLS
VA549	hFcIgG1(N297A	QVSLSCAVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLVSKLT	VKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLVSKLTVDKSF
	+ EPKSS)-Hole:	VDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPG	QQGNVFSCSVMHEALHNHYTQKSLSLSPG
	hFc(N297A)-		KCKVSNKALPAPIE
	hCD122		KTISKAKGQPREP
			QVCTLPPSRDELT
			KNQVSLSCAVKGF
			VDCDIAVEWESNG
	Knob:		
	hFcIgG1(N297A	EPKSSDKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDV	
	+ EPKSS)-	SHEDPEVKFNWYVDGVEVHNAKTKPREEQYASTYRVVSVLTVLHQDWL	
VA550	[VPLSLY]-	NGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPCRDELTKNQVSL	GSPG VPLSLY
	hIL2(R38A, F42A,	WCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDK	
	Y45A, E62A,	SRWQQGNVFSCSVMHEALHNHYTQKSLSLSPG	
	C125A)		
	Knob:		
	hFcIgG1(N297A	AKTDKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVS	
	+ AKT)-[VPLSLY]-	HEDPEVKFNWYVDGVEVHNAKTKPREEQYASTYRVVSVLTVLHQDWL	
VA551	hIL2(R38A, F42A,	NGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPCRDELTKNQVSL	GSPG VPLSLY
	Y45A, E62A,	WCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDK	
	C125A)	SRWQQGNVFSCSVMHEALHNHYTQKSLSLSPG	

VA552	Knob : hFclgG1(N297A + AKTEPKSS)- Knob: -[VPLSLY]- hIL2(R38A, F42A, Y45A, E62A, C125A)	AKTEPKSSDKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVV VDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYASTYRVVSVLTVLHQ DWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPCRDELTKN QVSLWCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKL TVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPG	GSPG	VPLSLY
VA553	Hole: hFc(N297A)- [DSGGFMLT]- hCD122	DKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHED PEVKFNWYVDGVEVHNAKTKPREEQYASTYRVVSVLTVLHQDWLNGK EYKCKVSNKALPAPIEKTISKAKGQPREPQVCTLPPSRDELTKNQVSLSCA VKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLVSKLTVDKSRW QQGNVFSCSVMHEALHNHYTQKSLSLSPG	GPPSGSSPG	DSGGFMLT
VA554	Knob: hFc(N297A)- [VPLSLY]- hIL2(E15R, L18C, D20R, R38A, F42A, Y45A, E62A)	DKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHED PEVKFNWYVDGVEVHNAKTKPREEQYASTYRVVSVLTVLHQDWLNGK EYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPCRDELTKNQVSLWC LVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSR WQQGNVFSCSVMHEALHNHYTQKSLSLSPG	GSPG	VPLSLY

	Knob:			
	hFc(N297A)-[VPLSLY]-	DKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHED PEVKFNWYVDGVEVHNAKTKPREEQYASTYRVVSVLTVLHQDWLNGK		VPLSLY
vA563	hIL2(E15R, L18C, D20R, R38A, F42A, Y45A, E62A, N88L)	EYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPCRDELTKNQVSLWC LVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSR WQQGNVFSCSVMHEALHNHYTQKSLSLSPG	GSPG	
	Knob:			
	hFc(N297A)-[VPLSLY]-	DKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHED PEVKFNWYVDGVEVHNAKTKPREEQYASTYRVVSVLTVLHQDWLNGK		VPLSLY
vA565	hIL2(E15L, L18C, D20R, R38A, F42A, Y45A, E62A, N88L)	EYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPCRDELTKNQVSLWC LVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSR WQQGNVFSCSVMHEALHNHYTQKSLSLSPG	GSPG	
	Knob:			
	hFc(N297A)-[VPLSLY]-	DKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHED PEVKFNWYVDGVEVHNAKTKPREEQYASTYRVVSVLTVLHQDWLNGK		VPLSLY
vA566	hIL2(E15R, L18C, R38A, F42A, Y45A, E62A, N88L)	EYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPCRDELTKNQVSLWC LVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSR WQQGNVFSCSVMHEALHNHYTQKSLSLSPG	GSPG	

	Knob:			
	hFc(N297A)-[VPLSLY]-	DKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHED PEVKFNWYVDGVEVHNAKTKPREEQYASTYRVVSVLTVLHQDWLNGK		VPLSLY
VA567	hIL2(L18C, D20R, R38A, F42A, Y45A, E62A, N88L)	EYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPCRDELTKNQVSLWC LVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSR WQQGNVFSCSVMHEALHNHYTQKSLSLSPG	GSPG	
	Knob:			
	hFc(N297A)-[VPLSLY]-	DKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHED PEVKFNWYVDGVEVHNAKTKPREEQYASTYRVVSVLTVLHQDWLNGK		VPLSLY
VA568	hIL2(E15F, L18C, D20R, R38A, F42A, Y45A, E62A, N88L)	EYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPCRDELTKNQVSLWC LVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSR WQQGNVFSCSVMHEALHNHYTQKSLSLSPG	GSPG	
	Hole:			
VA575	hFc(N297A, I253A)-hCD122	DKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMASRTPEVTCVVVDVSHED PEVKFNWYVDGVEVHNAKTKPREEQYASTYRVVSVLTVLHQDWLNGK EYKCKVSNKALPAPIEKTISKAKGQPREPQVCTLPPSRDELTKNQVSLSCA VKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLVSKLTVDKSRW QQGNVFSCSVMHEALHNHYTQKSLSLSPG	PGSGS	AVNGTSQFTCFYNSRANISCVWSQDGALQDTSCQVHAWPDRRRWI TCELLPVSQASWACNLILGAPDSQKLTTVDIVTLRVLCREGVRWRVM QDFKPFENLRLMAPISLQVVHVETHRCNISWEISQASHYFERHLEFEA LSPGHTWEEAPLLTLKQKQEWICLETLTPDTQYEFQVRVKPLQGEFTT SPWSQPLAFRTKPAALGKD

VA576	Hole: hFc(N297A, M252Y, S254T, T256E)-hCD122	DKTHTCPPCPAPELLGGPSVFLFPPKPKDTLYITREPEVTCVVVDVSHEDP EVKFNWYVDGVEVHNAKTKPREEQYASTYRVVSVLTVLHQDWLNGKE YKCKVSNKALPAPIEKTISKAKGQPREPQVCTLPPSRDELTKNQVSLSCAV KGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLVSKLTVDKSRWQ QGNVFSCSVMHEALHNHYTQKSLSLSPG	PGSGS	AVNGTSQFTCFYNSRANISCVWSQDGALQDTSCQVHAWPDRRRWI TCELLPVSQASWACNLILGAPDSQKLTTVDIVTLRVLCREGVRWRVM QDFKPFENLRLMAPISLQVVHVETHRCNISWEISQASHYFERHLEFEA LSPGHTWEEAPLLTLKQKQEWICLETLTPDTQYEFQVRVKPLQGEFTT SPWSQPLAFRTKPAALGKD
VA577	Knob: hFc(N297, I253A)-hIL2(R38A, F42A, Y45A, E62A, C125A)	DKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMASRTPEVTCVVVDVSHED PEVKFNWYVDGVEVHNAKTKPREEQYASTYRVVSVLTVLHQDWLNGK EYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPCRDELTKNQVSLWC LVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSR WQQGNVFSCSVMHEALHNHYTQKSLSLSPG	GGSSPPGGGSSG GGSGP	APTSSSTKKTQLQLEHLLLDLQMILNGINNYKNPKLTAMLTAKFAMPK ATELKHLQCLEEALKPLEEVLNLAQSKNFHLRPRDLISNINVIVLELKGS TFMCEYADETATIVEFLNRWITFAQSIISTLT
VA578	Knob: hFc(N297A, M252Y, S254T, T256E)-hIL2(R38A, F42A, Y45A, E62A, C125A)	DKTHTCPPCPAPELLGGPSVFLFPPKPKDTLYITREPEVTCVVVDVSHEDP EVKFNWYVDGVEVHNAKTKPREEQYASTYRVVSVLTVLHQDWLNGKE YKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPCRDELTKNQVSLWCL VKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRW QQGNVFSCSVMHEALHNHYTQKSLSLSPG	GGSSPPGGGSSG GGSGP	APTSSSTKKTQLQLEHLLLDLQMILNGINNYKNPKLTAMLTAKFAMPK ATELKHLQCLEEALKPLEEVLNLAQSKNFHLRPRDLISNINVIVLELKGS TFMCEYADETATIVEFLNRWITFAQSIISTLT

VA579	Knob: hFc(N297, I253A)-[VPLSLY]-hIL2(R38A, F42A, Y45A, E62A, C125A)	DKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMASRTPEVTCVVVDVSHED PEVKFNWYVDGVEVHNAKTKPREEQYASTYRVVSVLTVLHQDWLNGK EYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPCRDELTKNQVSLWC LVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSR WQQGNVFSCSVMHEALHNHYTQKSLSLSPG	GSPG VPLSLY
VA580	Knob: hFc(N297A, M252Y, S254T, T256E)-[VPLSLY]-hIL2(R38A, F42A, Y45A, E62A, C125A)	DKTHTCPPCPAPELLGGPSVFLFPPKPKDTLYITREPEVTCVVVDVSHEDP EVKFNWYVDGVEVHNAKTKPREEQYASTYRVVSVLTVLHQDWLNGKE YKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPCRDELTKNQVSLWCL VKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRW QQGNVFSCSVMHEALHNHYTQKSLSLSPG	GSPG VPLSLY
VA581	Knob: hFc(N297A)-[VPLSLY]-hIL2(L18C, R38A, F42A, Y45A, E62A)	DKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHED PEVKFNWYVDGVEVHNAKTKPREEQYASTYRVVSVLTVLHQDWLNGK EYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPCRDELTKNQVSLWC LVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSR WQQGNVFSCSVMHEALHNHYTQKSLSLSPG	GSPG VPLSLY

VA582	Knob: hFc(N297A)-[VPLSLY]-hIL2(H16Y, R38A, F42A, Y45A, E62A, C125A)	DKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHED PEVKFNWYVDGVEVHNAKTKPREEQYASTYRVVSVLTVLHQDWLNGK EYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPCRDELTKNQVSLWC LVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSR WQQGNVFSCSVMHEALHNHYTQKSLSLSPG	GSPG	VPLSLY
VA583	Knob: hFc(N297A)-[VPLSLY]-hIL2(H16E, R38A, F42A, Y45A, E62A, C125A)	DKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHED PEVKFNWYVDGVEVHNAKTKPREEQYASTYRVVSVLTVLHQDWLNGK EYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPCRDELTKNQVSLWC LVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSR WQQGNVFSCSVMHEALHNHYTQKSLSLSPG	GSPG	VPLSLY
VA584	Knob: hFc(N297A)-[VPLSLY]-hIL2(D20L, R38A, F42A, Y45A, E62A, C125A)	DKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHED PEVKFNWYVDGVEVHNAKTKPREEQYASTYRVVSVLTVLHQDWLNGK EYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPCRDELTKNQVSLWC LVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSR WQQGNVFSCSVMHEALHNHYTQKSLSLSPG	GSPG	VPLSLY

VA585	Knob: hFc(N297A)-[VPLSLY]-hIL2(H16Y, L18C, R38A, F42A, Y45A, E62A)	DKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHED PEVKFNWYVDGVEVHNAKTKPREEQYASTYRVVSVLTVLHQDWLNGK EYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPCRDELTKNQVSLWC LVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSR WQQGNVFSCSVMHEALHNHYTQKSLSLSPG	GSPG	VPLSLY
VA586	Knob: hFc(N297A)-[VPLSLY]-hIL2(H16E, L18C, R38A, F42A, Y45A, E62A)	DKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHED PEVKFNWYVDGVEVHNAKTKPREEQYASTYRVVSVLTVLHQDWLNGK EYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPCRDELTKNQVSLWC LVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSR WQQGNVFSCSVMHEALHNHYTQKSLSLSPG	GSPG	VPLSLY
VA587	Knob: hFc(N297A)-[VPLSLY]-hIL2(L18C, D20L, R38A, F42A, Y45A, E62A)	DKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHED PEVKFNWYVDGVEVHNAKTKPREEQYASTYRVVSVLTVLHQDWLNGK EYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPCRDELTKNQVSLWC LVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSR WQQGNVFSCSVMHEALHNHYTQKSLSLSPG	GSPG	VPLSLY

VA588	Knob: hFc(N297A)-[VPLSLY]-hIL2(H16Y, L18C, D20L, R38A, F42A, Y45A, E62A)	DKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHED PEVKFNWYVDGVEVHNAKTKPREEQYASTYRVVSVLTVLHQDWLNGK EYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPCRDELTKNQVSLWC LVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSR WQQGNVFSCSVMHEALHNHYTQKSLSLSPG	GSPG	VPLSLY
VA589	Knob: hFc(N297A)-[VPLSLY]-hIL2(H16E, L18C, D20L, R38A, F42A, Y45A, E62A)	DKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHED PEVKFNWYVDGVEVHNAKTKPREEQYASTYRVVSVLTVLHQDWLNGK EYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPCRDELTKNQVSLWC LVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSR WQQGNVFSCSVMHEALHNHYTQKSLSLSPG	GSPG	VPLSLY
VA603	Hole: hFcIgG4-hCD122	ESKYGPPCPPCPAPEFLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSQE DPEVQFNWYVDGVEVHNAKTKPREEQFNSTYRVVSVLTVLHQDWLNG KEYKCKVSNKGLPSSIEKTISKAKGQPREPQVCTLPPSQEEMTKNQVSLSC AVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSRLTVDKSR WQEGNVFSCSVMHEALHNHYTQKSLSLSLG	PGSGS	AVNGTSQFTCFYNSRANISCVWSQDGALQDTSCQVHAWPDRRRWI TCELLPVSQASWACNLILGAPDSQKLTTVDIVTLRVLCREGVRWRVM QDFKPFENLRLMAPISLQVVHVETHRCNISWEISQASHYFERHLEFEA LSPGHTWEEAPLLTLKQKQEWICLETLTPDTQYEFQVRVKPLQGEFTT SPWSQPLAFRTKPAALGKD

VA604	Knob: IgG4 hFc-hIL2(R38A, F42A, Y45A, E62A, C125A)	ESKYGPPCPPCPAPEFLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSQE DPEVQFNWYVDGVEVHNAKTKPREEQFNSTYRVVSVLTVLHQDWLNG KEYKCKVSNKGLPSSIEKTISKAKGQPREPQVYTLPPCQEEMTKNQVSLW CLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSRLTVDKSR WQEGNVFSCSVMHEALHNHYTQKSLSLSLG	GGSSPPGGGSSG GGSGP	APTSSSTKKTQLQLEHLLLDLQMILNGINNYKNPKLTAMLTAKFAMPK ATELKHLQCLEEALKPLEEVLNLAQSKNFHLRPRDLISNINVIVLELKGS TFMCEYADETATIVEFLNRWITFAQSIISTLT
VA605	Knob: hFcIgG4-hIL2-[VPLSLY]-hIL2(R38A, F42A, Y45A, E62A, C125A)	ESKYGPPCPPCPAPEFLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSQE DPEVQFNWYVDGVEVHNAKTKPREEQFNSTYRVVSVLTVLHQDWLNG KEYKCKVSNKGLPSSIEKTISKAKGQPREPQVYTLPPCQEEMTKNQVSLW CLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSRLTVDKSR WQEGNVFSCSVMHEALHNHYTQKSLSLSLG	GSPG	VPLSLY
VA606	Hole: hFc(N297A)-[RAAAVKSP]-hCD122	DKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHED PEVKFNWYVDGVEVHNAKTKPREEQYASTYRVVSVLTVLHQDWLNGK EYKCKVSNKALPAPIEKTISKAKGQPREPQVCTLPPSRDELTKNQVSLSCA VKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLVSKLTVDKSRW QQGNVFSCSVMHEALHNHYTQKSLSLSPG	GPPSGSSP	RAAAVKSP

VA608	Hole: hFc(N297A, I253A)-[MPYDLYHP]-hCD122	DKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMASRTPEVTCVVVDVSHED PEVKFNWYVDGVEVHNAKTKPREEQYASTYRVVSVLTVLHQDWLNGK EYKCKVSNKALPAPIEKTISKAKGQPREPQVCTLPPSRDELTKNQVSLSCA VKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLVSKLTVDKSRW QQGNVFSCSVMHEALHNHYTQKSLSLSPG	GPPSGSSP	MPYDLYHP
VA609	Hole: hFc(N297A, I253A)-[VPLSLY]-hCD122	DKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMASRTPEVTCVVVDVSHED PEVKFNWYVDGVEVHNAKTKPREEQYASTYRVVSVLTVLHQDWLNGK EYKCKVSNKALPAPIEKTISKAKGQPREPQVCTLPPSRDELTKNQVSLSCA VKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLVSKLTVDKSRW QQGNVFSCSVMHEALHNHYTQKSLSLSPG	GPPSGSSPG	VPLSLY
VA612	Hole: hFc(N297A)-[MPYDLYHP]-hCD122(C122S, C168S)	DKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHED PEVKFNWYVDGVEVHNAKTKPREEQYASTYRVVSVLTVLHQDWLNGK EYKCKVSNKALPAPIEKTISKAKGQPREPQVCTLPPSRDELTKNQVSLSCA VKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLVSKLTVDKSRW QQGNVFSCSVMHEALHNHYTQKSLSLSPG	GPPSGSSP	MPYDLYHP

VA614	Hole: hFc(N297A)-[DSGGFMLT]-hCD122(C122S, C168S)	DKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHED PEVKFNWYVDGVEVHNAKTKPREEQYASTYRVVSVLTVLHQDWLNGK EYKCKVSNKALPAPIEKTISKAKGQPREPQVCTLPPSRDELTKNQVSLSCA VKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLVSKLTVDKSRW QQGNVFSCSVMHEALHNHYTQKSLSLSPG	GPPSGSSPG DSGGFMLT
VA621	Hole: hFcIgG4-[VPLSLY]-hCD122	ESKYGPPCPPCPAPEFLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSQE DPEVQFNWYVDGVEVHNAKTKPREEQFNSTYRVVSVLTVLHQDWLNG KEYKCKVSNKGLPSSIEKTISKAKGQPREPQVCTLPPSQEEMTKNQVSLSC AVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSRLTVDKSR WQEGNVFSCSVMHEALHNHYTQKSLSLSLGGP	PSGSSPG VPLSLY
VA623	Knob: hFc(N297, I253A)-[MPYDLYHP]-hIL2(R38A, F42A, Y45A, E62A, C125A)	DKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMASRTPEVTCVVVDVSHED PEVKFNWYVDGVEVHNAKTKPREEQYASTYRVVSVLTVLHQDWLNGK EYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPCRDELTKNQVSLWC LVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSR WQQGNVFSCSVMHEALHNHYTQKSLSLSPG	GGSSPP MPYDLYHP

	Hole:	DKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMASRTPEVTCVVVDVSHED
		PEVKFNWYVDGVEVHNAKTKPREEQYASTYRVVSVLTVLHQDWLNGK
VA625	hFc(N297A,	EYKCKVSNKALPAPIEKTISKAKGQPREPQVCTLPPSRDELTKNQVSLSCA
	I253A)	VKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLVSKLTVDKSRW
		QQGNVFSCSVMHEALHNHYTQKSLSLSPG
		ESKYGPPCPPCPAPEFLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSQE
		DPEVQFNWYVDGVEVHNAKTKPREEQFNSTYRVVSVLTVLHQDWLNG
VA626	Hole: hFcIgG4	KEYKCKVSNKGLPSSIEKTISKAKGQPREPQVCTLPPSQEEMTKNQVSLSC
		AVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSRLTVDKSR
		WQEGNVFSCSVMHEALHNHYTQKSLSLSLGPG
		DKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHED
		PEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGK
VA669	Hole: hFc-hCD122	EYKCKVSNKALPAPIEKTISKAKGQPREPQVCTLPPSRDELTKNQVSLSCA PGSGS
		VKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLVSKLTVDKSRW
		QQGNVFSCSVMHEALHNHYTQKSLSLSPG
		AVNGTSQFTCFYNSRANISCVWSQDGALQDTSCQVHAWPDRRRWI
		TCELLPVSQASWACNLILGAPDSQKLTTVDIVTLRVLCREGVRWRVM
		QDFKPFENLRLMAPISLQVVHVETHRCNISWEISQASHYFERHLEFEA
		LSPGHTWEEAPLLTLKQKQEWICLETLTPDTQYEFQVRVKPLQGEFTT
		SPWSQPLAFRTKPAALGKD

VA670	Knob: hFc-hIL2(R38A, F42A, Y45A, E62A, C125A)	DKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHED PEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGK EYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPCRDELTKNQVSLWC LVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSR WQQGNVFSCSVMHEALHNHYTQKSLSLSPG	GGSSPPGGGSSG GGSGP	APTSSSTKKTQLQLEHLLLDLQMILNGINNYKNPKLTAMLTAKFAMP ATELKHLQCLEEALKPLEEVLNLAQSKNFHLRPRDLISNINVIVLELKGS TFMCEYADETATIVEFLNRWITFAQSIISTLT
VA671	Knob: hFc-[VPLSLY]-hIL2(R38A, F42A, Y45A, E62A, C125A)	DKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHED PEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGK EYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPCRDELTKNQVSLWC LVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSR WQQGNVFSCSVMHEALHNHYTQKSLSLSPG	GSPG	VPLSLY
VA672	Hole: hFcIgG4-[VPLSLY]-hCD122	DKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHED PEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGK EYKCKVSNKALPAPIEKTISKAKGQPREPQVCTLPPSRDELTKNQVSLSCA VKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLVSKLTVDKSRW QQGNVFSCSVMHEALHNHYTQKSLSLSPG	GPPSGSSPG	VPLSLY

me	newnames	Component4Sequence	Component5Sequence	FullSequence
IA158	Hole: hFc(N297A)			DKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYA STYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVCTLPPSRDELTKNQVSLSCAVKGFYPSD IAVEWESNGQPENNYKTTPPVLDSDGSFFLVSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPG
IA187	Hole: hFc(N297A)-hCD122			DKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYA STYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVCTLPPSRDELTKNQVSLSCAVKGFYPSD IAVEWESNGQPENNYKTTPPVLDSDGSFFLVSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGPGSGSA VNGTSQFTCFYNSRANISCVWSQDGALQDTSCQVHAWPDRRRWNQTCELLPVSQASWACNLILGAPDSQKLTTV DIVTLRVLCREGVRWRVMAIQDFKPFENLRLMAPISLQVVHVETHRCNISWEISQASHYFERHLEFEARTLSPGHTW EEAPLLTLKQKQEWICLETLTPDTQYEFQVRVKPLQGEFTTWSPWSQPLAFRTKPAALGKD
IA255	Knob: hFc(N297A)-hIL2(R38A, F42A, Y45A, E62A, C125A)			DKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYA STYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPCRDELTKNQVSLWCLVKGFYPS DIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGGGSSP PGGGSSGGGSGPAPTSSSTKKTQLQLEHLLLDLQMILNGINNYKNPKLTAMLTAKFAMPKKATELKHLQCLEEALKPL EEVLNLAQSKNFHLRPRDLISNINVIVLELKGSETTFMCEYADETATIVEFLNRWITFAQSIISTLT
IA263	Knob: hFc(N297A)-[VPLSLY]-hIL2(R38A, F42A, Y45A, E62A, C125A)	SGP	APTSSSTKKTQLQLEHLLLDLQMILNGI NNYKNPKLTAMLTAKFAMPKKATELKH LQCLEEALKPLEEVLNLAQSKNFHLRPR DLISNINVIVLELKGSETTFMCEYADETA TIVEFLNRWITFAQSIISTLT	DKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYA STYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPCRDELTKNQVSLWCLVKGFYPS DIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGGSPGV PLSLYSGPAPTSSSTKKTQLQLEHLLLDLQMILNGINNYKNPKLTAMLTAKFAMPKKATELKHLQCLEEALKPLEEVLNL AQSKNFHLRPRDLISNINVIVLELKGSETTFMCEYADETATIVEFLNRWITFAQSIISTLT
IA278	Knob: hFc(N297A)-[DSGGFMLT]-hIL2(C125A)	SGP	APTSSSTKKTQLQLEHLLLDLQMILNGI NNYKNPKLTRMLTFKFYMPKKATELKH LQCLEEELKPLEEVLNLAQSKNFHLRPR DLISNINVIVLELKGSETTFMCEYADETA TIVEFLNRWITFAQSIISTLT	DKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYA STYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPCRDELTKNQVSLWCLVKGFYPS DIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGGSGPD SGGFMLTSGPAPTSSSTKKTQLQLEHLLLDLQMILNGINNYKNPKLTRMLTFKFYMPKKATELKHLQCLEEELKPLEEV LNLAQSKNFHLRPRDLISNINVIVLELKGSETTFMCEYADETATIVEFLNRWITFAQSIISTLT
IA281	Knob: hFc(N297A)-[DSGGFMLT]-hIL2(R38A, F42A, Y45A, E62A, C125A)	SGP	APTSSSTKKTQLQLEHLLLDLQMILNGI NNYKNPKLTAMLTAKFAMPKKATELKH LQCLEEALKPLEEVLNLAQSKNFHLRPR DLISNINVIVLELKGSETTFMCEYADETA TIVEFLNRWITFAQSIISTLT	DKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYA STYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPCRDELTKNQVSLWCLVKGFYPS DIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGGSGPD SGGFMLTSGPAPTSSSTKKTQLQLEHLLLDLQMILNGINNYKNPKLTAMLTAKFAMPKKATELKHLQCLEEALKPLEE VLNLAQSKNFHLRPRDLISNINVIVLELKGSETTFMCEYADETATIVEFLNRWITFAQSIISTLT
IA440	Hole: hFc(N297A)-hCD122(C122S, C168S)			DKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYA STYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVCTLPPSRDELTKNQVSLSCAVKGFYPSD IAVEWESNGQPENNYKTTPPVLDSDGSFFLVSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGPGSGSA VNGTSQFTCFYNSRANISCVWSQDGALQDTSCQVHAWPDRRRWNQTCELLPVSQASWACNLILGAPDSQKLTTV DIVTLRVLCREGVRWRVMAIQDFKPFENLRLMAPISLQVVHVETHRSNISWEISQASHYFERHLEFEARTLSPGHTWE EAPLLTLKQKQEWISLETLTPDTQYEFQVRVKPLQGEFTTWSPWSQPLAFRTKPAALGKD

IA476	Knob: hFc(N297A)-[NPMGSDPVNFKLLRVVNG]-hIL2(F42S, E62S, C125A)	GP	APTSSSTKKTQLQLEHLLLDLQMILNGINNYKNPKLTRMLTSKFYMPKKATELKHLQCLEESLKPLEEVLNLAQSKNFHLRPRDLISNINVIVLELKGSETTFMCEYADETATIVEFLNRWITFAQSIISTLT	DKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYASTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPCRDELTKNQVSLWCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGGNPMGSDPVNFKLLRVVNGGPAPTSSSTKKTQLQLEHLLLDLQMILNGINNYKNPKLTRMLTSKFYMPKKATELKHLQCLEESLKPLEEVLNLAQSKNFHLRPRDLISNINVIVLELKGSETTFMCEYADETATIVEFLNRWITFAQSIISTLT
IA477	Knob: mFcIgG2a(LALAPG)-hIL2(R38A, F42A, Y45A, E62A, C125A)			TIKPCPPCKCPAPNAAGGPSVFIFPPKIKDVLMISLSPIVTCVVVDVSEDDPDVQISWFVNNVEVHTAQTQTHREDYNSTLRVVSALPIQHQDWMSGKEFKCKVNNKDLGAPIERTISKPKGSVRAPQVYVLPPCEEEMTKKQVTLWCMVTDFMPEDIYVEWTNNGKTELNYKNTEPVLDSDGSYFMYSKLRVEKKNWVERNSYSCSVVHEGLHNHHTTKSFSRTPGGGSSPPGGGSSGGGSGPAPTSSSTKKTQLQLEHLLLDLQMILNGINNYKNPKLTAMLTAKFAMPKKATELKHLQCLEEALKPLEEVLNLAQSKNFHLRPRDLISNINVIVLELKGSETTFMCEYADETATIVEFLNRWITFAQSIISTLT
IA478	Knob: mFcIgG2a(LALAPG)-[VPLSLY]-hIL2(R38A, F42A, Y45A, E62A, C125A)	SGP	APTSSSTKKTQLQLEHLLLDLQMILNGINNYKNPKLTAMLTAKFAMPKKATELKHLQCLEEALKPLEEVLNLAQSKNFHLRPRDLISNINVIVLELKGSETTFMCEYADETATIVEFLNRWITFAQSIISTLT	TIKPCPPCKCPAPNAAGGPSVFIFPPKIKDVLMISLSPIVTCVVVDVSEDDPDVQISWFVNNVEVHTAQTQTHREDYNSTLRVVSALPIQHQDWMSGKEFKCKVNNKDLGAPIERTISKPKGSVRAPQVYVLPPCEEEMTKKQVTLWCMVTDFMPEDIYVEWTNNGKTELNYKNTEPVLDSDGSYFMYSKLRVEKKNWVERNSYSCSVVHEGLHNHHTTKSFSRTPGGSPGVPLSLYSGPAPTSSSTKKTQLQLEHLLLDLQMILNGINNYKNPKLTAMLTAKFAMPKKATELKHLQCLEEALKPLEEVLNLAQSKNFHLRPRDLISNINVIVLELKGSETTFMCEYADETATIVEFLNRWITFAQSIISTLT
IA479	Hole: mFcIgG2a(LALAPG)			TIKPCPPCKCPAPNAAGGPSVFIFPPKIKDVLMISLSPIVTCVVVDVSEDDPDVQISWFVNNVEVHTAQTQTHREDYNSTLRVVSALPIQHQDWMSGKEFKCKVNNKDLGAPIERTISKPKGSVRAPQVCVLPPPEEEMTKKQVTLSCAVTDFMPEDIYVEWTNNGKTELNYKNTEPVLDSDGSYFMVSKLRVEKKNWVERNSYSCSVVHEGLHNHHTTKSFSRTPG
IA480	Hole: mFcIgG2a(LALAPG)-hCD122			TIKPCPPCKCPAPNAAGGPSVFIFPPKIKDVLMISLSPIVTCVVVDVSEDDPDVQISWFVNNVEVHTAQTQTHREDYNSTLRVVSALPIQHQDWMSGKEFKCKVNNKDLGAPIERTISKPKGSVRAPQVCVLPPPEEEMTKKQVTLSCAVTDFMPEDIYVEWTNNGKTELNYKNTEPVLDSDGSYFMVSKLRVEKKNWVERNSYSCSVVHEGLHNHHTTKSFSRTPGPGSGSAVNGTSQFTCFYNSRANISCVWSQDGALQDTSCQVHAWPDRRRWNQTCELLPVSQASWACNLILGAPDSQKLTTVDIVTLRVLCREGVRWRVMAIQDFKPFENLRLMAPISLQVVHVETHRCNISWEISQASHYFERHLEFEARTLSPGHTWEEAPLLTLKQKQEWICLETLTPDTQYEFQVRVKPLQGEFTTWSPWSQPLAFRTKPAALGKD
IA516	F8ScFvVersion1-Hole: hFc(N297A)-hCD122	PGSGS	AVNGTSQFTCFYNSRANISCVWSQDGALQDTSCQVHAWPDRRRWNQTCELLPVSQASWACNLILGAPDSQKLTTVDIVTLRVLCREGVRWRVMAIQDFKPFENLRLMAPISLQVVHVETHRCNISWEISQASHYFERHLEFEARTLSPGHTWEEAPLLTLKQKQEWICLETLTPDTQYEFQVRVKPLQGEFTTWSPWSQPLAFRTKPAALGKD	EVQLLESGGGLVQPGGSLRLSCAASGFTFSLFTMSVVVRQAPGKGLEVVVSAISGSGGGSTYYADSVKGRFTISRDNSKNTLYLQMNSLRAEDTAVYYCAKSTHLYLFDYWGQGTLVTVSSGGGGSGGGGSGGGGSGGGGSEIVLTQSPGTLSLSPGERATLSCRASQSVSMPFLAWYQQKPGQAPRLLIYGASSRATGIPDRFSGSGSGTDFTLTISRLEPEDFAVYYCQQMRGRPPTFGQGTKVEIKGGSDKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYASTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVCTLPPSRDELTKNQVSLSCAVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLVSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGPGSGSAVNGTSQFTCFYNSRANISCVWSQDGALQDTSCQVHAWPDRRRWNQTCELLPVSQASWACNLILGAPDSQKLTTVDIVTLRVLCREGVRWRVMAIQDFKPFENLRLMAPISLQVVHVETHRCNISWEISQASHYFERHLEFEARTLSPGHTWEEAPLLTLKQKQEWICLETLTPDTQYEFQVRVKPLQGEFTTWSPWSQPLAFRTKPAALGKD

IA520	Hole: mFcIgG2a(LALAPG)-NoAnnotationFound		TIKPCPPCKCPAPNAAGGPSVFIFPPKIKDVLMISLSPIVTCVVVDVSEDDPDVQISWFVNNVEVHTAQTQTHREDYNSTLRVVSALPIQHQDWMSGKEFKCKVNNKDLGAPIERTISKPKGSVRAPQVCVLPPPEEEMTKKQVTLSCAVTDFMPEDIYVEWTNNGKTELNYKNTEPVLDSDGSYFMVSKLRVEKKNWVERNSYSCSVVHEGLHNHHTTKSFSRTPGHHHHHHHH
IA521	Hole: mFcIgG2a(LALAPG)-hCD122-NoAnnotationFound	GHHHHHHHH	TIKPCPPCKCPAPNAAGGPSVFIFPPKIKDVLMISLSPIVTCVVVDVSEDDPDVQISWFVNNVEVHTAQTQTHREDYNSTLRVVSALPIQHQDWMSGKEFKCKVNNKDLGAPIERTISKPKGSVRAPQVCVLPPPEEEMTKKQVTLSCAVTDFMPEDIYVEWTNNGKTELNYKNTEPVLDSDGSYFMVSKLRVEKKNWVERNSYSCSVVHEGLHNHHTTKSFSRTPGPGSGSAVNGTSQFTCFYNSRANISCVWSQDGALQDTSCQVHAWPDRRRWNQTCELLPVSQASWACNLILGAPDSQKLTTVDIVTLRVLCREGVRWRVMAIQDFKPFENLRLMAPISLQVVHVETHRCNISWEISQASHYFERHLEFEARTLSPGHTWEEAPLLTLKQKQEWICLETLTPDTQYEFQVRVKPLQGEFTTWSPWSQPLAFRTKPAALGKDGHHHHHHHH
IA522	Hole: mFcIgG2a(LALAPG)-mCD122-NoAnnotationFound	GHHHHHHHH	TIKPCPPCKCPAPNAAGGPSVFIFPPKIKDVLMISLSPIVTCVVVDVSEDDPDVQISWFVNNVEVHTAQTQTHREDYNSTLRVVSALPIQHQDWMSGKEFKCKVNNKDLGAPIERTISKPKGSVRAPQVCVLPPPEEEMTKKQVTLSCAVTDFMPEDIYVEWTNNGKTELNYKNTEPVLDSDGSYFMVSKLRVEKKNWVERNSYSCSVVHEGLHNHHTTKSFSRTPGPGSGSAVKNCSHLECFYNSRANVSCMWSHEEALNVTTCHVHAKSNLRHWNKTCELTLVRQASWACNLILGSFPESQSLTSVDLLDINVVCWEEKGWRRVKTCDFHPFDNLRLVAPHSLQVLHIDTQRCNISWKVSQVSHYIEPYLEFEARRRLLGHSWEDASVLSLKQRQQWLFLEMLIPSTSYEVQVRVKAQRNNTGTWSPWSQPLTFRTRPADPMKEGHHHHHHHH
IA528	Hole: hFc(N297A)-hCD122(C168S)		DKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYASTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVCTLPPSRDELTKNQVSLSCAVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLVSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGPGSGSAVNGTSQFTCFYNSRANISCVWSQDGALQDTSCQVHAWPDRRRWNQTCELLPVSQASWACNLILGAPDSQKLTTVDIVTLRVLCREGVRWRVMAIQDFKPFENLRLMAPISLQVVHVETHRCNISWEISQASHYFERHLEFEARTLSPGHTWEEAPLLTLKQKQEWISLETLTPDTQYEFQVRVKPLQGEFTTWSPWSQPLAFRTKPAALGKD
IA530	Knob: mFcIgG1(DAPG)-hIL2(R38A, F42A, Y45A, E62A, C125A)		VRSGCKPCICTVPEVSSVFIFPPKPKDVLTITLTPKVTCVVVAISKDDPEVQFSWFVDDVEVHTAQTQPREEQFNSTFRSVSELPIMHQDWLNGKEFKCRVNSAAFGAPIEKTISKTKGRPKAPQVYTIPPPKEQMAKDKVSLTCMITDFFPEDITVEWQWNGQPAENYDNTQPIMDTDGSYFVYSDLNVQKSNWEAGNTFTCSVLHEGLHNHHTEKSLSHSPGGGSSPPGGGSSGGGSGPAPTSSSTKKTQLQLEHLLLDLQMILNGINNYKNPKLTAMLTAKFAMPKKATELKHLQCLEEALKPLEEVLNLAQSKNFHLRPRDLISNINVIVLELKGSETTFMCEYADETATIVEFLNRWITFAQSIISTLT
IA531	Knob: mFcIgG1(DAPG)-[VPLSLY]-hIL2(R38A, F42A, Y45A, E62A, C125A)	SGP	APTSSSTKKTQLQLEHLLLDLQMILNGINNYKNPKLTAMLTAKFAMPKKATELKHLQCLEEALKPLEEVLNLAQSKNFHLRPRDLISNINVIVLELKGSETTFMCEYADETATIVEFLNRWITFAQSIISTLT VRSGCKPCICTVPEVSSVFIFPPKPKDVLTITLTPKVTCVVVAISKDDPEVQFSWFVDDVEVHTAQTQPREEQFNSTFRSVSELPIMHQDWLNGKEFKCRVNSAAFGAPIEKTISKTKGRPKAPQVYTIPPPKEQMAKDKVSLTCMITDFFPEDITVEWQWNGQPAENYDNTQPIMDTDGSYFVYSDLNVQKSNWEAGNTFTCSVLHEGLHNHHTEKSLSHSPGGSPGVPLSLYSGPAPTSSSTKKTQLQLEHLLLDLQMILNGINNYKNPKLTAMLTAKFAMPKKATELKHLQCLEEALKPLEEVLNLAQSKNFHLRPRDLISNINVIVLELKGSETTFMCEYADETATIVEFLNRWITFAQSIISTLT
IA532	Hole: mFcIgG1(DAPG)		VRSGCKPCICTVPEVSSVFIFPPKPKDVLTITLTPKVTCVVVAISKDDPEVQFSWFVDDVEVHTAQTQPREEQFNSTFRSVSELPIMHQDWLNGKEFKCRVNSAAFGAPIEKTISKTKGRPKAPQVYTIPPPKKQMAKDKVSLTCMITDFFPEDITVEWQWNGQPAENYKNTQPIMKTDGSYFVYSKLNVQKSNWEAGNTFTCSVLHEGLHNHHTEKSLSHSPG

			VRSGCKPCICTVPEVSSVFIFPPKPKDVLTITLTPKVTCVVVAISKDDPEVQFSWFVDDVEVHTAQTQPREEQFNSTFR
			SVSELPIMHQDWLNGKEFKCRVNSAAFGAPIEKTISKTKGRPKAPQVYTIPPPKKQMAKDKVSLTCMITDFFPEDITV
	Hole:		EWQWNGQPAENYKNTQPIMKTDGSYFVYSKLNVQKSNWEAGNTFTCSVLHEGLHNHHTEKSLSHSPGPGSGSAV
IA533	mFclgG1(DAPG)-hCD122		NGTSQFTCFYNSRANISCVWSQDGALQDTSCQVHAWPDRRRWNQTCELLPVSQASWACNLILGAPDSQKLTTVDI
			VTLRVLCREGVRWRVMAIQDFKPFENLRLMAPISLQVVHVETHRCNISWEISQASHYFERHLEFEARTLSPGHTWEE
			APLLTLKQKQEWICLETLTPDTQYEFQVRVKPLQGEFTTWSPWSQPLAFRTKPAALGKD
			VRSGCKPCICTVPEVSSVFIFPPKPKDVLTITLTPKVTCVVVAISKDDPEVQFSWFVDDVEVHTAQTQPREEQFNSTFR
	Hole:		SVSELPIMHQDWLNGKEFKCRVNSAAFGAPIEKTISKTKGRPKAPQVYTIPPPKKQMAKDKVSLTCMITDFFPEDITV
IA534	mFclgG1(DAPG)-mCD122		EWQWNGQPAENYKNTQPIMKTDGSYFVYSKLNVQKSNWEAGNTFTCSVLHEGLHNHHTEKSLSHSPGPGSGSAV
			KNCSHLECFYNSRANVSCMWSHEEALNVTTCHVHAKSNLRHWNKTCELTLVRQASWACNLILGSFPESQSLTSVDLL
			DINVVCWEEKGWRRVKTCDFHPFDNLRLVAPHSLQVLHIDTQRCNISWKVSQVSHYIEPYLEFEARRRLLGHSWEDA
			SVLSLKQRQQWLFLEMLIPSTSYEVQVRVKAQRNNTGTWSPWSQPLTFRTRPADPMKE
	Knob:		DKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYA
	hFc(N297A)-		STYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPCRDELTKNQVSLWCLVKGFYPS
IA542	hIL2(R38A, F42A,		DIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGGISSGL
	Y45A, E62A,		LSGRSDQPSGPAPTSSSTKKTQLQLEHLLLDLQMILNGINNYKNPKLTAMLTAKFAMPKKATELKHLQCLEEALKPLEE
	C125A)		VLNLAQSKNFHLRPRDLISNINVIVLELKGSETTFMCEYADETATIVEFLNRWITFAQSIISTLT
			AVNGTSQFTCFYNSRANISCVWSQDG
	Hole:		ALQDTSCQVHAWPDRRRWNQTCELL DKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYA
	hFc(N297A)-		PVSQASWACNLILGAPDSQKLTTVDIVT STYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVCTLPPSRDELTKNQVSLSCAVKGFYPSD
IA543	[VPLSLY]- GSGGG		LRVLCREGVRWRVMAIQDFKPFENLRL IAVEWESNGQPENNYKTTPPVLDSDGSFFLVSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGGPPSGS
	hCD122		MAPISLQVVHVETHRCNISWEISQASH SPGVPLSLYGSGGGAVNGTSQFTCFYNSRANISCVWSQDGALQDTSCQVHAWPDRRRWNQTCELLPVSQASWAC
			YFERHLEFEARTLSPGHTWEEAPLLTLK NLILGAPDSQKLTTVDIVTLRVLCREGVRWRVMAIQDFKPFENLRLMAPISLQVVHVETHRCNISWEISQASHYFERH
			QKQEWICLETLTPDTQYEFQVRVKPLQ LEFEARTLSPGHTWEEAPLLTLKQKQEWICLETLTPDTQYEFQVRVKPLQGEFTTWSPWSQPLAFRTKPAALGKD
			GEFTTWSPWSQPLAFRTKPAALGKD
	Knob:		
	hFc(N297A)-		APTSSSTKKTQLQLEHLLLDLQMILNGI DKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYA
	[VPLSLY]-		NNYKNPKLTAMLTAKFAMPKKATELKH STYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPCRDELTKNQVSLWCLVKGFYPS
IA544	hIL2(R38A, F42A, SGP		LQCLEEALKPLEEVLNLAQSKNFHFDPR DIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGGSPGV
	Y45A, E62A,		DVVSNINVFVLELKGSETTFMCEYADET PLSLYSGPAPTSSSTKKTQLQLEHLLLDLQMILNGINNYKNPKLTAMLTAKFAMPKKATELKHLQCLEEALKPLEEVLNL
	L80F, R81D,		ATIVEFLNRWITFAQSIISTLT AQSKNFHFDPRDVVSNINVFVLELKGSETTFMCEYADETATIVEFLNRWITFAQSIISTLT
	L85V, I86V, I92F,		
	C125A)		

	Knob:		DKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYA
	hFc(N297A)-		STYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPCRDELTKNQVSLWCLVKGFYPS
IA545	hIL2(R38A, F42A,		DIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGGISSGL
	Y45A, E62A,		LSGRSSGPAPTSSSTKKTQLQLEHLLLDLQMILNGINNYKNPKLTAMLTAKFAMPKKATELKHLQCLEEALKPLEEVLN
	C125A)		LAQSKNFHLRPRDLISNINVIVLELKGSETTFMCEYADETATIVEFLNRWITFAQSIISTLT
	Knob:		
	hFc(N297A)-		DKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYA
	hIL2(R38A, F42A,		STYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPCRDELTKNQVSLWCLVKGFYPS
IA546	Y45A, E62A,		DIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGGGSSP
	L80F, R81D,		PGGGSSGGGSGPAPTSSSTKKTQLQLEHLLLDLQMILNGINNYKNPKLTAMLTAKFAMPKKATELKHLQCLEEALKPL
	L85V, I86V, I92F,		EEVLNLAQSKNFHFDPRDVVSNINVFVLELKGSETTFMCEYADETATIVEFLNRWITFAQSIISTLT
	C125A)		
		AVNGTSQFTCFYNSRANISCVWSQDG	
		ALQDTSCQVHAWPDRRRWNQTCELL	EPKSSDKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPRE
	Hole:	PVSQASWACNLILGAPDSQKLTTVDIV	EQYASTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVCTLPPSRDELTKNQVSLSCAVKG
	hFcIgG1(N297A	TLRVLCREGVRWRVMAIQDFKPFENL	FYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLVSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGP
IA547	+ EPKSS)-Hole:	RLMAPISLQVVHVETHRCNISWEISQA	GSGSAVNGTSQFTCFYNSRANISCVWSQDGALQDTSCQVHAWPDRRRWNQTCELLPVSQASWACNLILGAPDSQ
	hFc(N297A)-	SHYFERHLEFEARTLSPGHTWEEAPLLT	KLTTVDIVTLRVLCREGVRWRVMAIQDFKPFENLRLMAPISLQVVHVETHRCNISWEISQASHYFERHLEFEARTLSP
	hCD122	LKQKQEWICLETLTPDTQYEFQVRVKP	GHTWEEAPLLTLKQKQEWICLETLTPDTQYEFQVRVKPLQGEFTTWSPWSQPLAFRTKPAALGKD
		LQGEFTTWSPWSQPLAFRTKPAALGK	
		D	
		AVNGTSQFTCFYNSRANISCVWSQDG	
		ALQDTSCQVHAWPDRRRWNQTCELL	AKTDKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREE
	Hole:	PVSQASWACNLILGAPDSQKLTTVDIV	QYASTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVCTLPPSRDELTKNQVSLSCAVKGF
	hFcIgG1(N297A	TLRVLCREGVRWRVMAIQDFKPFENL	YPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLVSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGPG
IA548	+ AKT)-Hole:	RLMAPISLQVVHVETHRCNISWEISQA	SGSAVNGTSQFTCFYNSRANISCVWSQDGALQDTSCQVHAWPDRRRWNQTCELLPVSQASWACNLILGAPDSQK
	hFc(N297A)-	SHYFERHLEFEARTLSPGHTWEEAPLLT	LTTVDIVTLRVLCREGVRWRVMAIQDFKPFENLRLMAPISLQVVHVETHRCNISWEISQASHYFERHLEFEARTLSPG
	hCD122	LKQKQEWICLETLTPDTQYEFQVRVKP	HTWEEAPLLTLKQKQEWICLETLTPDTQYEFQVRVKPLQGEFTTWSPWSQPLAFRTKPAALGKD
		LQGEFTTWSPWSQPLAFRTKPAALGK	
		D	
			AVNGTSQFTCFYNSRANISCVWSQDG
	Hole:		ALQDTSCQVHAWPDRRRWNQTCELL
	hFcIgG1(N297A		PVSQASWACNLILGAPDSQKLTTVDIVT
	+AKTEPKSS)-		LRVLCREGVRWRVMAIQDFKPFENLRL
	Hole:	PGSGS	MAPISLQVVHVETHRCNISWEISQASH
IA549	hFcIgG1(N297A		YFERHLEFEARTLSPGHTWEEAPLLTLK
	+ EPKSS)-Hole:		QKQEWICLETLTPDTQYEFQVRVKPLQ
	hFc(N297A)-		GEFTTWSPWSQPLAFRTKPAALGKD
	hCD122		

IA550	Knob: hFcIgG1(N297A + EPKSS)-[VPLSLY]-hIL2(R38A, F42A, Y45A, E62A, C125A)	SGP	APTSSSTKKTQLQLEHLLLDLQMILNGI NNYKNPKLTAMLTAKFAMPKKATELKH LQCLEEALKPLEEVLNLAQSKNFHLRPR DLISNINVIVLELKGSETTFMCEYADETA TIVEFLNRWITFAQSIISTLT	EPKSSDKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPRE EQYASTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPCRDELTKNQVSLWCLVKG FYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGG SPGVPLSLYSGPAPTSSSTKKTQLQLEHLLLDLQMILNGINNYKNPKLTAMLTAKFAMPKKATELKHLQCLEEALKPLE EVLNLAQSKNFHLRPRDLISNINVIVLELKGSETTFMCEYADETATIVEFLNRWITFAQSIISTLT
IA551	Knob: hFcIgG1(N297A + AKT)-[VPLSLY]-hIL2(R38A, F42A, Y45A, E62A, C125A)	SGP	APTSSSTKKTQLQLEHLLLDLQMILNGI NNYKNPKLTAMLTAKFAMPKKATELKH LQCLEEALKPLEEVLNLAQSKNFHLRPR DLISNINVIVLELKGSETTFMCEYADETA TIVEFLNRWITFAQSIISTLT	AKTDKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREE QYASTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPCRDELTKNQVSLWCLVKGF YPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGGS PGVPLSLYSGPAPTSSSTKKTQLQLEHLLLDLQMILNGINNYKNPKLTAMLTAKFAMPKKATELKHLQCLEEALKPLEE VLNLAQSKNFHLRPRDLISNINVIVLELKGSETTFMCEYADETATIVEFLNRWITFAQSIISTLT
IA552	Knob : hFcIgG1(N297A + AKTEPKSS)-Knob: -[VPLSLY]-hIL2(R38A, F42A, Y45A, E62A, C125A)	SGP	APTSSSTKKTQLQLEHLLLDLQMILNGI NNYKNPKLTAMLTAKFAMPKKATELKH LQCLEEALKPLEEVLNLAQSKNFHLRPR DLISNINVIVLELKGSETTFMCEYADETA TIVEFLNRWITFAQSIISTLT	AKTEPKSSDKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTK PREEQYASTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPCRDELTKNQVSLWCL VKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLS PGGSPGVPLSLYSGPAPTSSSTKKTQLQLEHLLLDLQMILNGINNYKNPKLTAMLTAKFAMPKKATELKHLQCLEEALK PLEEVLNLAQSKNFHLRPRDLISNINVIVLELKGSETTFMCEYADETATIVEFLNRWITFAQSIISTLT
IA553	Hole: hFc(N297A)-[DSGGFMLT]-hCD122	SGGG	AVNGTSQFTCFYNSRANISCVWSQDG ALQDTSCQVHAWPDRRRWNQTCELL PVSQASWACNLILGAPDSQKLTTVDIVT LRVLCREGVRWRVMAIQDFKPFENLRL MAPISLQVVHVETHRCNISWEISQASH YFERHLEFEARTLSPGHTWEEAPLLTLK QKQEWICLETLTPDTQYEFQVRVKPLQ GEFTTWSPWSQPLAFRTKPAALGKD	DKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYA STYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVCTLPPSRDELTKNQVSLSCAVKGFYPSD IAVEWESNGQPENNYKTTPPVLDSDGSFFLVSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGGPPSGS SPGDSGGFMLTSGGGAVNGTSQFTCFYNSRANISCVWSQDGALQDTSCQVHAWPDRRRWNQTCELLPVSQASW ACNLILGAPDSQKLTTVDIVTLRVLCREGVRWRVMAIQDFKPFENLRLMAPISLQVVHVETHRCNISWEISQASHYFE RHLEFEARTLSPGHTWEEAPLLTLKQKQEWICLETLTPDTQYEFQVRVKPLQGEFTTWSPWSQPLAFRTKPAALGKD
IA554	Knob: hFc(N297A)-[VPLSLY]-hIL2(E15R, L18C, D20R, R38A, F42A, Y45A, E62A)	SGP	APTSSSTKKTQLQLRHLCLRLQMILNGI NNYKNPKLTAMLTAKFAMPKKATELKH LQCLEEALKPLEEVLNLAQSKNFHLRPR DLISNINVIVLELKGSETTFMCEYADETA TIVEFLNRWITFCQSIISTLT	DKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYA STYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPCRDELTKNQVSLWCLVKGFYPS DIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGGSPGV PLSLYSGPAPTSSSTKKTQLQLRHLCLRLQMILNGINNYKNPKLTAMLTAKFAMPKKATELKHLQCLEEALKPLEEVLN LAQSKNFHLRPRDLISNINVIVLELKGSETTFMCEYADETATIVEFLNRWITFCQSIISTLT

IA563	Knob: hFc(N297A)-[VPLSLY]- hIL2(E15R, L18C, D20R, R38A, F42A, Y45A, E62A, N88L) SGP	APTSSSTKKTQLQLRHLCLRLQMILNGI NNYKNPKLTAMLTAKFAMPKKATELKH LQCLEEALKPLEEVLNLAQSKNFHLRPR DLISLINVIVLELKGSETTFMCEYADETA TIVEFLNRWITFCQSIISTLT	DKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYA STYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPCRDELTKNQVSLWCLVKGFYPS DIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGGSPGV PLSLYSGPAPTSSSTKKTQLQLRHLCLRLQMILNGINNYKNPKLTAMLTAKFAMPKKATELKHLQCLEEALKPLEEVLN LAQSKNFHLRPRDLISLINVIVLELKGSETTFMCEYADETATIVEFLNRWITFCQSIISTLT
IA565	Knob: hFc(N297A)-[VPLSLY]- hIL2(E15L, L18C, D20R, R38A, F42A, Y45A, E62A, N88L) SGP	APTSSSTKKTQLQLLHLCLRLQMILNGI NNYKNPKLTAMLTAKFAMPKKATELKH LQCLEEALKPLEEVLNLAQSKNFHLRPR DLISLINVIVLELKGSETTFMCEYADETA TIVEFLNRWITFCQSIISTLT	DKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYA STYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPCRDELTKNQVSLWCLVKGFYPS DIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGGSPGV PLSLYSGPAPTSSSTKKTQLQLLHLCLRLQMILNGINNYKNPKLTAMLTAKFAMPKKATELKHLQCLEEALKPLEEVLNL AQSKNFHLRPRDLISLINVIVLELKGSETTFMCEYADETATIVEFLNRWITFCQSIISTLT
IA566	Knob: hFc(N297A)-[VPLSLY]- hIL2(E15R, L18C, R38A, F42A, Y45A, E62A, N88L) SGP	APTSSSTKKTQLQLRHLCLDLQMILNGI NNYKNPKLTAMLTAKFAMPKKATELKH LQCLEEALKPLEEVLNLAQSKNFHLRPR DLISLINVIVLELKGSETTFMCEYADETA TIVEFLNRWITFCQSIISTLT	DKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYA STYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPCRDELTKNQVSLWCLVKGFYPS DIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGGSPGV PLSLYSGPAPTSSSTKKTQLQLRHLCLDLQMILNGINNYKNPKLTAMLTAKFAMPKKATELKHLQCLEEALKPLEEVLN LAQSKNFHLRPRDLISLINVIVLELKGSETTFMCEYADETATIVEFLNRWITFCQSIISTLT
IA567	Knob: hFc(N297A)-[VPLSLY]- hIL2(L18C, D20R, R38A, F42A, Y45A, E62A, N88L) SGP	APTSSSTKKTQLQLEHLCLRLQMILNGI NNYKNPKLTAMLTAKFAMPKKATELKH LQCLEEALKPLEEVLNLAQSKNFHLRPR DLISLINVIVLELKGSETTFMCEYADETA TIVEFLNRWITFCQSIISTLT	DKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYA STYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPCRDELTKNQVSLWCLVKGFYPS DIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGGSPGV PLSLYSGPAPTSSSTKKTQLQLEHLCLRLQMILNGINNYKNPKLTAMLTAKFAMPKKATELKHLQCLEEALKPLEEVLNL AQSKNFHLRPRDLISLINVIVLELKGSETTFMCEYADETATIVEFLNRWITFCQSIISTLT
IA568	Knob: hFc(N297A)-[VPLSLY]- hIL2(E15F, L18C, D20R, R38A, F42A, Y45A, E62A, N88L) SGP	APTSSSTKKTQLQLFHLCLRLQMILNGI NNYKNPKLTAMLTAKFAMPKKATELKH LQCLEEALKPLEEVLNLAQSKNFHLRPR DLISLINVIVLELKGSETTFMCEYADETA TIVEFLNRWITFCQSIISTLT	DKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYA STYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPCRDELTKNQVSLWCLVKGFYPS DIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGGSPGV PLSLYSGPAPTSSSTKKTQLQLFHLCLRLQMILNGINNYKNPKLTAMLTAKFAMPKKATELKHLQCLEEALKPLEEVLNL AQSKNFHLRPRDLISLINVIVLELKGSETTFMCEYADETATIVEFLNRWITFCQSIISTLT

IA575	Hole: hFc(N297A, I253A)-hCD122		DKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMASRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQY ASTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVCTLPPSRDELTKNQVSLSCAVKGFYPS DIAVEWESNGQPENNYKTTPPVLDSDGSFFLVSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGPGSGS AVNGTSQFTCFYNSRANISCVWSQDGALQDTSCQVHAWPDRRRWNQTCELLPVSQASWACNLILGAPDSQKLTT VDIVTLRVLCREGVRWRVMAIQDFKPFENLRLMAPISLQVVHVETHRCNISWEISQASHYFERHLEFEARTLSPGHT WEEAPLLTLKQKQEWICLETLTPDTQYEFQVRVKPLQGEFTTWSPWSQPLAFRTKPAALGKD
IA576	Hole: hFc(N297A, M252Y, S254T, T256E)-hCD122		DKTHTCPPCPAPELLGGPSVFLFPPKPKDTLYITREPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYAS TYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVCTLPPSRDELTKNQVSLSCAVKGFYPSDI AVEWESNGQPENNYKTTPPVLDSDGSFFLVSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGPGSGSA VNGTSQFTCFYNSRANISCVWSQDGALQDTSCQVHAWPDRRRWNQTCELLPVSQASWACNLILGAPDSQKLTTV DIVTLRVLCREGVRWRVMAIQDFKPFENLRLMAPISLQVVHVETHRCNISWEISQASHYFERHLEFEARTLSPGHTW EEAPLLTLKQKQEWICLETLTPDTQYEFQVRVKPLQGEFTTWSPWSQPLAFRTKPAALGKD
IA577	Knob: hFc(N297, I253A)-hIL2(R38A, F42A, Y45A, E62A, C125A)		DKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMASRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQY ASTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPCRDELTKNQVSLWCLVKGFYP SDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGGGSS PPGGGSSGGGSGPAPTSSSTKKTQLQLEHLLLDLQMILNGINNYKNPKLTAMLTAKFAMPKKATELKHLQCLEEALKP LEEVLNLAQSKNFHLRPRDLISNINVIVLELKGSETTFMCEYADETATIVEFLNRWITFAQSIISTLT
IA578	Knob: hFc(N297A, M252Y, S254T, T256E)-hIL2(R38A, F42A, Y45A, E62A, C125A)		DKTHTCPPCPAPELLGGPSVFLFPPKPKDTLYITREPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYAS TYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPCRDELTKNQVSLWCLVKGFYPSD IAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGGGSSPP GGGSSGGGSGPAPTSSSTKKTQLQLEHLLLDLQMILNGINNYKNPKLTAMLTAKFAMPKKATELKHLQCLEEALKPLE EVLNLAQSKNFHLRPRDLISNINVIVLELKGSETTFMCEYADETATIVEFLNRWITFAQSIISTLT
IA579	Knob: hFc(N297, I253A)-[VPLSLY]-hIL2(R38A, F42A, Y45A, E62A, C125A)	APTSSSTKKTQLQLEHLLLDLQMILNGI NNYKNPKLTAMLTAKFAMPKKATELKH LQCLEEALKPLEEVLNLAQSKNFHLRPR DLISNINVIVLELKGSETTFMCEYADETA TIVEFLNRWITFAQSIISTLT	DKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMASRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQY ASTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPCRDELTKNQVSLWCLVKGFYP SDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGGSPG VPLSLYSGPAPTSSSTKKTQLQLEHLLLDLQMILNGINNYKNPKLTAMLTAKFAMPKKATELKHLQCLEEALKPLEEVL NLAQSKNFHLRPRDLISNINVIVLELKGSETTFMCEYADETATIVEFLNRWITFAQSIISTLT
IA580	Knob: hFc(N297A, M252Y, S254T, T256E)-[VPLSLY]- SGP hIL2(R38A, F42A, Y45A, E62A, C125A)	APTSSSTKKTQLQLEHLLLDLQMILNGI NNYKNPKLTAMLTAKFAMPKKATELKH LQCLEEALKPLEEVLNLAQSKNFHLRPR DLISNINVIVLELKGSETTFMCEYADETA TIVEFLNRWITFAQSIISTLT	DKTHTCPPCPAPELLGGPSVFLFPPKPKDTLYITREPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYAS TYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPCRDELTKNQVSLWCLVKGFYPSD IAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGGSPGVP LSLYSGPAPTSSSTKKTQLQLEHLLLDLQMILNGINNYKNPKLTAMLTAKFAMPKKATELKHLQCLEEALKPLEEVLNLA QSKNFHLRPRDLISNINVIVLELKGSETTFMCEYADETATIVEFLNRWITFAQSIISTLT

	Knob:		APTSSSTKKTQLQLEHLCLDLQMILNGI	DKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYA
	hFc(N297A)-		NNYKNPKLTAMLTAKFAMPKKATELKH	STYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPCRDELTKNQVSLWCLVKGFYPS
IA581	[VPLSLY]-hIL2(L18C, R38A, F42A, Y45A, E62A)	SGP	LQCLEEALKPLEEVLNLAQSKNFHLRPR	DIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGGSPGV
			DLISNINVIVLELKGSETTFMCEYADETA	PLSLYSGPAPTSSSTKKTQLQLEHLCLDLQMILNGINNYKNPKLTAMLTAKFAMPKKATELKHLQCLEEALKPLEEVLN
			TIVEFLNRWITFCQSIISTLT	LAQSKNFHLRPRDLISNINVIVLELKGSETTFMCEYADETATIVEFLNRWITFCQSIISTLT
	Knob:			
	hFc(N297A)-		APTSSSTKKTQLQLEYLLDLQMILNGIN	DKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYA
	[VPLSLY]-		NYKNPKLTAMLTAKFAMPKKATELKHL	STYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPCRDELTKNQVSLWCLVKGFYPS
IA582	hIL2(H16Y, R38A, F42A, Y45A, E62A, C125A)	SGP	QCLEEALKPLEEVLNLAQSKNFHLRPRD	DIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGGSPGV
			LISNINVIVLELKGSETTFMCEYADETATI	PLSLYSGPAPTSSSTKKTQLQLEYLLLDLQMILNGINNYKNPKLTAMLTAKFAMPKKATELKHLQCLEEALKPLEEVLNL
			VEFLNRWITFAQSIISTLT	AQSKNFHLRPRDLISNINVIVLELKGSETTFMCEYADETATIVEFLNRWITFAQSIISTLT
	Knob:			
	hFc(N297A)-		APTSSSTKKTQLQLEELLLDLQMILNGIN	DKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYA
	[VPLSLY]-		NYKNPKLTAMLTAKFAMPKKATELKHL	STYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPCRDELTKNQVSLWCLVKGFYPS
IA583	hIL2(H16E, R38A, F42A, Y45A, E62A, C125A)	SGP	QCLEEALKPLEEVLNLAQSKNFHLRPRD	DIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGGSPGV
			LISNINVIVLELKGSETTFMCEYADETATI	PLSLYSGPAPTSSSTKKTQLQLEELLLDLQMILNGINNYKNPKLTAMLTAKFAMPKKATELKHLQCLEEALKPLEEVLNL
			VEFLNRWITFAQSIISTLT	AQSKNFHLRPRDLISNINVIVLELKGSETTFMCEYADETATIVEFLNRWITFAQSIISTLT
	Knob:			
	hFc(N297A)-		APTSSSTKKTQLQLEHLLLLLLQMILNGIN	DKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYA
	[VPLSLY]-		NYKNPKLTAMLTAKFAMPKKATELKHL	STYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPCRDELTKNQVSLWCLVKGFYPS
IA584	hIL2(D20L, R38A, F42A, Y45A, E62A, C125A)	SGP	QCLEEALKPLEEVLNLAQSKNFHLRPRD	DIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGGSPGV
			LISNINVIVLELKGSETTFMCEYADETATI	PLSLYSGPAPTSSSTKKTQLQLEHLLLLLLQMILNGINNYKNPKLTAMLTAKFAMPKKATELKHLQCLEEALKPLEEVLNL
			VEFLNRWITFAQSIISTLT	AQSKNFHLRPRDLISNINVIVLELKGSETTFMCEYADETATIVEFLNRWITFAQSIISTLT
	Knob:			
	hFc(N297A)-		APTSSSTKKTQLQLEYLCLDLQMILNGI	DKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYA
	[VPLSLY]-		NNYKNPKLTAMLTAKFAMPKKATELKH	STYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPCRDELTKNQVSLWCLVKGFYPS
IA585	hIL2(H16Y, L18C, R38A, F42A, Y45A, E62A)	SGP	LQCLEEALKPLEEVLNLAQSKNFHLRPR	DIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGGSPGV
			DLISNINVIVLELKGSETTFMCEYADETA	PLSLYSGPAPTSSSTKKTQLQLEYLCLDLQMILNGINNYKNPKLTAMLTAKFAMPKKATELKHLQCLEEALKPLEEVLNL
			TIVEFLNRWITFCQSIISTLT	AQSKNFHLRPRDLISNINVIVLELKGSETTFMCEYADETATIVEFLNRWITFCQSIISTLT
	Knob:			
	hFc(N297A)-		APTSSSTKKTQLQLEELCLDLQMILNGI	DKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYA
	[VPLSLY]-		NNYKNPKLTAMLTAKFAMPKKATELKH	STYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPCRDELTKNQVSLWCLVKGFYPS
IA586	hIL2(H16E, L18C, R38A, F42A, Y45A, E62A)	SGP	LQCLEEALKPLEEVLNLAQSKNFHLRPR	DIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGGSPGV
			DLISNINVIVLELKGSETTFMCEYADETA	PLSLYSGPAPTSSSTKKTQLQLEELCLDLQMILNGINNYKNPKLTAMLTAKFAMPKKATELKHLQCLEEALKPLEEVLNL
			TIVEFLNRWITFCQSIISTLT	AQSKNFHLRPRDLISNINVIVLELKGSETTFMCEYADETATIVEFLNRWITFCQSIISTLT

IA587	Knob: hFc(N297A)-[VPLSLY]-hIL2(L18C, D20L, R38A, F42A, Y45A, E62A)	SGP	APTSSSTKKTQLQLEHLCLLLQMILNGIN NYKNPKLTAMLTAKFAMPKKATELKHL QCLEEALKPLEEVLNLAQSKNFHLRPRD LISNINVIVLELKGSETTFMCEYADETATI VEFLNRWITFCQSIISTLT
IA588	Knob: hFc(N297A)-[VPLSLY]-hIL2(H16Y, L18C, D20L, R38A, F42A, Y45A, E62A)	SGP	APTSSSTKKTQLQLEYLCLLLQMILNGIN NYKNPKLTAMLTAKFAMPKKATELKHL QCLEEALKPLEEVLNLAQSKNFHLRPRD LISNINVIVLELKGSETTFMCEYADETATI VEFLNRWITFCQSIISTLT
IA589	Knob: hFc(N297A)-[VPLSLY]-hIL2(H16E, L18C, D20L, R38A, F42A, Y45A, E62A)	SGP	APTSSSTKKTQLQLEELCLLLQMILNGIN NYKNPKLTAMLTAKFAMPKKATELKHL QCLEEALKPLEEVLNLAQSKNFHLRPRD LISNINVIVLELKGSETTFMCEYADETATI VEFLNRWITFCQSIISTLT
IA603	Hole: hFcIgG4-hCD122		ESKYGPPCPPCPAPEFLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSQEDPEVQFNWYVDGVEVHNAKTKPREEQ FNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKGLPSSIEKTISKAKGQPREPQVCTLPPSQEEMTKNQVSLSCAVKGFY PSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSRLTVDKSRWQEGNVFSCSVMHEALHNHYTQKSLSLSLGPGS GSAVNGTSQFTCFYNSRANISCVWSQDGALQDTSCQVHAWPDRRRWNQTCELLPVSQASWACNLILGAPDSQKL TTVDIVTLRVLCREGVRWRVMAIQDFKPFENLRLMAPISLQVVHVETHRCNISWEISQASHYFERHLEFEARTLSPGH TWEEAPLLTLKQKQEWICLETLTPDTQYEFQVRVKPLQGEFTTWSPWSQPLAFRTKPAALGKD
IA604	Knob: IgG4 hFc-hIL2(R38A, F42A, Y45A, E62A, C125A)		ESKYGPPCPPCPAPEFLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSQEDPEVQFNWYVDGVEVHNAKTKPREEQ FNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKGLPSSIEKTISKAKGQPREPQVYTLPPCQEEMTKNQVSLWCLVKGF YPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSRLTVDKSRWQEGNVFSCSVMHEALHNHYTQKSLSLSLGGGS SPPGGGSSGGGSGGPAPTSSSTKKTQLQLEHLLLDLQMILNGININYKNPKLTAMLTAKFAMPKKATELKHLQCLEEALK PLEEVLNLAQSKNFHLRPRDLISNINVIVLELKGSETTFMCEYADETATIVEFLNRWITFAQSIISTLT
IA605	Knob: hFcIgG4-hIL2-[VPLSLY]-hIL2(R38A, F42A, Y45A, E62A, C125A)	SGP	APTSSSTKKTQLQLEHLLLDLQMILNGI NNYKNPKLTAMLTAKFAMPKKATELKH LQCLEEALKPLEEVLNLAQSKNFHLRPR DLISNINVIVLELKGSETTFMCEYADETA TIVEFLNRWITFAQSIISTLT	ESKYGPPCPPCPAPEFLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSQEDPEVQFNWYVDGVEVHNAKTKPREEQ FNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKGLPSSIEKTISKAKGQPREPQVYTLPPCQEEMTKNQVSLWCLVKGF YPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSRLTVDKSRWQEGNVFSCSVMHEALHNHYTQKSLSLSLGGGP GVPLSLYSGPAPTSSSTKKTQLQLEHLLLDLQMILNGINNYKNPKLTAMLTAKFAMPKKATELKHLQCLEEALKPLEEV LNLAQSKNFHLRPRDLISNINVIVLELKGSETTFMCEYADETATIVEFLNRWITFAQSIISTLT

		AVNGTSQFTCFYNSRANISCVWSQDG	
		ALQDTSCQVHAWPDRRRWNQTCELL	DKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYA
		PVSQASWACNLILGAPDSQKLTTVDIVT	STYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVCTLPPSRDELTKNQVSLSCAVKGFYPSD
		LRVLCREGVRWRVMAIQDFKPFENLRL	IAVEWESNGQPENNYKTTPPVLDSDGSFFLVSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGGPPSGS
IA606	hFc(N297A)-[RAAAVKSP]-hCD122	MAPISLQVVHVETHRCNISWEISQASH	SPRAAAVKSPSGGGAVNGTSQFTCFYNSRANISCVWSQDGALQDTSCQVHAWPDRRRWNQTCELLPVSQASWA
	SGGG	YFERHLEFEARTLSPGHTWEEAPLLTLK	CNLILGAPDSQKLTTVDIVTLRVLCREGVRWRVMAIQDFKPFENLRLMAPISLQVVHVETHRCNISWEISQASHYFER
		QKQEWICLETLTPDTQYEFQVRVKPLQ	HLEFEARTLSPGHTWEEAPLLTLKQKQEWICLETLTPDTQYEFQVRVKPLQGEFTTWSPWSQPLAFRTKPAALGKD
		GEFTTWSPWSQPLAFRTKPAALGKD	
		AVNGTSQFTCFYNSRANISCVWSQDG	
		ALQDTSCQVHAWPDRRRWNQTCELL	DKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMASRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQY
		PVSQASWACNLILGAPDSQKLTTVDIVT	ASTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVCTLPPSRDELTKNQVSLSCAVKGFYPS
	Hole: hFc(N297A,	LRVLCREGVRWRVMAIQDFKPFENLRL	DIAVEWESNGQPENNYKTTPPVLDSDGSFFLVSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGGPPSG
IA608	I253A)-[MPYDLYHP]-hCD122	MAPISLQVVHVETHRCNISWEISQASH	SSPMPYDLYHPSGGGAVNGTSQFTCFYNSRANISCVWSQDGALQDTSCQVHAWPDRRRWNQTCELLPVSQASW
	SGGG	YFERHLEFEARTLSPGHTWEEAPLLTLK	ACNLILGAPDSQKLTTVDIVTLRVLCREGVRWRVMAIQDFKPFENLRLMAPISLQVVHVETHRCNISWEISQASHYFE
		QKQEWICLETLTPDTQYEFQVRVKPLQ	RHLEFEARTLSPGHTWEEAPLLTLKQKQEWICLETLTPDTQYEFQVRVKPLQGEFTTWSPWSQPLAFRTKPAALGKD
		GEFTTWSPWSQPLAFRTKPAALGKD	
		AVNGTSQFTCFYNSRANISCVWSQDG	
		ALQDTSCQVHAWPDRRRWNQTCELL	DKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMASRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQY
		PVSQASWACNLILGAPDSQKLTTVDIVT	ASTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVCTLPPSRDELTKNQVSLSCAVKGFYPS
	Hole: hFc(N297A,	LRVLCREGVRWRVMAIQDFKPFENLRL	DIAVEWESNGQPENNYKTTPPVLDSDGSFFLVSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGGPPSG
IA609	I253A)-[VPLSLY]-hCD122	MAPISLQVVHVETHRCNISWEISQASH	SSPGVPLSLYGSGGGAVNGTSQFTCFYNSRANISCVWSQDGALQDTSCQVHAWPDRRRWNQTCELLPVSQASWA
	GSGGG	YFERHLEFEARTLSPGHTWEEAPLLTLK	CNLILGAPDSQKLTTVDIVTLRVLCREGVRWRVMAIQDFKPFENLRLMAPISLQVVHVETHRCNISWEISQASHYFER
		QKQEWICLETLTPDTQYEFQVRVKPLQ	HLEFEARTLSPGHTWEEAPLLTLKQKQEWICLETLTPDTQYEFQVRVKPLQGEFTTWSPWSQPLAFRTKPAALGKD
		GEFTTWSPWSQPLAFRTKPAALGKD	
		AVNGTSQFTCFYNSRANISCVWSQDG	
		ALQDTSCQVHAWPDRRRWNQTCELL	DKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYA
		PVSQASWACNLILGAPDSQKLTTVDIVT	STYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVCTLPPSRDELTKNQVSLSCAVKGFYPSD
	Hole: hFc(N297A)-[MPYDLYHP]-hCD122(C122S, C168S)	LRVLCREGVRWRVMAIQDFKPFENLRL	IAVEWESNGQPENNYKTTPPVLDSDGSFFLVSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGGPPSGS
IA612	SGGG	MAPISLQVVHVETHRSNISWEISQASH	SPMPYDLYHPSGGGAVNGTSQFTCFYNSRANISCVWSQDGALQDTSCQVHAWPDRRRWNQTCELLPVSQASWA
		YFERHLEFEARTLSPGHTWEEAPLLTLK	CNLILGAPDSQKLTTVDIVTLRVLCREGVRWRVMAIQDFKPFENLRLMAPISLQVVHVETHRSNISWEISQASHYFER
		QKQEWISLETLTPDTQYEFQVRVKPLQ	HLEFEARTLSPGHTWEEAPLLTLKQKQEWISLETLTPDTQYEFQVRVKPLQGEFTTWSPWSQPLAFRTKPAALGKD
		GEFTTWSPWSQPLAFRTKPAALGKD	
		AVNGTSQFTCFYNSRANISCVWSQDG	
		ALQDTSCQVHAWPDRRRWNQTCELL	DKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYA
		PVSQASWACNLILGAPDSQKLTTVDIVT	STYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVCTLPPSRDELTKNQVSLSCAVKGFYPSD
	Hole: hFc(N297A)-[MPYDLYHP]-hCD122(C122S, C168S)	LRVLCREGVRWRVMAIQDFKPFENLRL	IAVEWESNGQPENNYKTTPPVLDSDGSFFLVSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGGPPSGS
IA612	SGGG	MAPISLQVVHVETHRSNISWEISQASH	SPMPYDLYHPSGGGAVNGTSQFTCFYNSRANISCVWSQDGALQDTSCQVHAWPDRRRWNQTCELLPVSQASWA
		YFERHLEFEARTLSPGHTWEEAPLLTLK	CNLILGAPDSQKLTTVDIVTLRVLCREGVRWRVMAIQDFKPFENLRLMAPISLQVVHVETHRSNISWEISQASHYFER
		QKQEWISLETLTPDTQYEFQVRVKPLQ	HLEFEARTLSPGHTWEEAPLLTLKQKQEWISLETLTPDTQYEFQVRVKPLQGEFTTWSPWSQPLAFRTKPAALGKD
		GEFTTWSPWSQPLAFRTKPAALGKD	

IA614	Hole: hFc(N297A)-[DSGGFMLT]-hCD122(C122S, C168S)	SGGG	AVNGTSQFTCFYNSRANISCVWSQDGALQDTSCQVHAWPDRRRWNQTCELLPVSQASWACNLILGAPDSQKLTTVDIVTLRVLCREGVRWRVMAIQDFKPFENLRLMAPISLQVVHVETHRSNISWEISQASHYFERHLEFEARTLSPGHTWEEAPLLTLKQKQEWISLETLTPDTQYEFQVRVKPLQGEFTTWSPWSQPLAFRTKPAALGKD	DKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYASTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVCTLPPSRDELTKNQVSLSCAVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLVSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGGPPSGSSPGDSGGFMLTSGGGAVNGTSQFTCFYNSRANISCVWSQDGALQDTSCQVHAWPDRRRWNQTCELLPVSQASWACNLILGAPDSQKLTTVDIVTLRVLCREGVRWRVMAIQDFKPFENLRLMAPISLQVVHVETHRSNISWEISQASHYFERHLEFEARTLSPGHTWEEAPLLTLKQKQEWISLETLTPDTQYEFQVRVKPLQGEFTTWSPWSQPLAFRTKPAALGKD
IA621	Hole: hFcIgG4-[VPLSLY]-hCD122	GSGGG	AVNGTSQFTCFYNSRANISCVWSQDGALQDTSCQVHAWPDRRRWNQTCELLPVSQASWACNLILGAPDSQKLTTVDIVTLRVLCREGVRWRVMAIQDFKPFENLRLMAPISLQVVHVETHRCNISWEISQASHYFERHLEFEARTLSPGHTWEEAPLLTLKQKQEWICLETLTPDTQYEFQVRVKPLQGEFTTWSPWSQPLAFRTKPAALGKD	ESKYGPPCPPCPAPEFLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSQEDPEVQFNWYVDGVEVHNAKTKPREEQFNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKGLPSSIEKTISKAKGQPREPQVCTLPPSQEEMTKNQVSLSCAVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSRLTVDKSRWQEGNVFSCSVMHEALHNHYTQKSLSLSLGGPPSGSSPGVPLSLYGSGGGAVNGTSQFTCFYNSRANISCVWSQDGALQDTSCQVHAWPDRRRWNQTCELLPVSQASWACNLILGAPDSQKLTTVDIVTLRVLCREGVRWRVMAIQDFKPFENLRLMAPISLQVVHVETHRCNISWEISQASHYFERHLEFEARTLSPGHTWEEAPLLTLKQKQEWICLETLTPDTQYEFQVRVKPLQGEFTTWSPWSQPLAFRTKPAALGKD
IA623	Knob: hFc(N297, I253A)-[MPYDLYHP]-hIL2(R38A, F42A, Y45A, E62A, C125A)	SGP	APTSSSTKKTQLQLEHLLLDLQMILNGINNYKNPKLTAMLTAKFAMPKKATELKHLQCLEEALKPLEEVLNLAQSKNFHLRPRDLISNINVIVLELKGSETTFMCEYADETATIVEFLNRWITFAQSIISTLT	DKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMASRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYASTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPCRDELTKNQVSLWCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGGGSSPPMPYDLYHPSGPAPTSSSTKKTQLQLEHLLLDLQMILNGINNYKNPKLTAMLTAKFAMPKKATELKHLQCLEEALKPLEEVLNLAQSKNFHLRPRDLISNINVIVLELKGSETTFMCEYADETATIVEFLNRWITFAQSIISTLT
IA625	Hole: hFc(N297A, I253A)		DKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMASRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYASTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVCTLPPSRDELTKNQVSLSCAVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLVSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPG
IA626	Hole: hFcIgG4		ESKYGPPCPPCPAPEFLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSQEDPEVQFNWYVDGVEVHNAKTKPREEQFNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKGLPSSIEKTISKAKGQPREPQVCTLPPSQEEMTKNQVSLSCAVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSRLTVDKSRWQEGNVFSCSVMHEALHNHYTQKSLSLSLGPG
IA669	Hole: hFc-hCD122		DKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVCTLPPSRDELTKNQVSLSCAVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLVSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGPGSGSAVNGTSQFTCFYNSRANISCVWSQDGALQDTSCQVHAWPDRRRWNQTCELLPVSQASWACNLILGAPDSQKLTTVDIVTLRVLCREGVRWRVMAIQDFKPFENLRLMAPISLQVVHVETHRCNISWEISQASHYFERHLEFEARTLSPGHTWEEAPLLTLKQKQEWICLETLTPDTQYEFQVRVKPLQGEFTTWSPWSQPLAFRTKPAALGKD

			DKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYN
			STYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPCRDELTKNQVSLWCLVKGFYPS
IA670	Knob: hFc-hIL2(R38A, F42A, Y45A, E62A, C125A)		DIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGGSSP
			PGGGSSGGGSGPAPTSSSTKKTQLQLEHLLLDLQMILNGINNYKNPKLTAMLTAKFAMPKKATELKHLQCLEEALKPL
			EEVLNLAQSKNFHLRPRDLISNINVIVLELKGSETTFMCEYADETATIVEFLNRWITFAQSIISTLT
			APTSSSTKKTQLQLEHLLLDLQMILNGI DKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYN
	Knob: hFc-[VPLSLY]-hIL2(R38A, F42A, Y45A, E62A, C125A)		NNYKNPKLTAMLTAKFAMPKKATELKH STYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPCRDELTKNQVSLWCLVKGFYPS
IA671		SGP	LQCLEEALKPLEEVLNLAQSKNFHLRPR DIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGGSPGV
			DLISNINVIVLELKGSETTFMCEYADETA PLSLYSGPAPTSSSTKKTQLQLEHLLLDLQMILNGINNYKNPKLTAMLTAKFAMPKKATELKHLQCLEEALKPLEEVLNL
			TIVEFLNRWITFAQSIISTLT AQSKNFHLRPRDLISNINVIVLELKGSETTFMCEYADETATIVEFLNRWITFAQSIISTLT
			AVNGTSQFTCFYNSRANISCVWSQDG
			ALQDTSCQVHAWPDRRRWNQTCELL DKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYN
	Hole: hFclgG4-[VPLSLY]-hCD122		PVSQASWACNLILGAPDSQKLTTVDIVT STYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVCTLPPSRDELTKNQVSLSCAVKGFYPSD
			LRVLCREGVRWRVMAIQDFKPFENLRL IAVEWESNGQPENNYKTTPPVLDSDGSFFLVSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGGPPSGS
IA672		GSGGG	MAPISLQVVHVETHRCNISWEISQASH SPGVPLSLYGSGGGAVNGTSQFTCFYNSRANISCVWSQDGALQDTSCQVHAWPDRRRWNQTCELLPVSQASWAC
			YFERHLEFEARTLSPGHTWEEAPLLTLK NLILGAPDSQKLTTVDIVTLRVLCREGVRWRVMAIQDFKPFENLRLMAPISLQVVHVETHRCNISWEISQASHYFERH
			QKQEWICLETLTPDTQYEFQVRVKPLQ LEFEARTLSPGHTWEEAPLLTLKQKQEWICLETLTPDTQYEFQVRVKPLQGEFTTWSPWSQPLAFRTKPAALGKD
			GEFTTWSPWSQPLAFRTKPAALGKD

CLAIMS

What is claimed is:

1. A masked IL-2 cytokine comprising 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-2 cytokine or functional fragment thereof linked to a second half-life extension domain via a second linker,

   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 is a proteolytically cleavable linker comprising a proteolytically cleavable peptide.

2. The masked IL-2 cytokine of claim 1, wherein the first polypeptide chain comprises formula 6:

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

   (6)

   and the second polypeptide chain comprises formula 5:

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

   (5)

   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-2 cytokine or functional fragment thereof.

3. The masked IL-2 cytokine of claim 1 or claim 2, wherein the first half-life extension domain comprises a first Fc domain or a fragment thereof and the second half-life extension domain comprises an Fc domain or a fragment thereof.

4. The masked IL-2 cytokine of claim 3, 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.

5. The masked IL-2 cytokine of claim 3 or claim 4, wherein the first and second half-life extension domains are each an IgG1 Fc domain or fragment thereof.

6. The masked IL-2 cytokine of claims 5, wherein 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, numbered according to the Kabat EU numbering system.

7. The masked IL-2 cytokine of claims 5 or 6, wherein the first and second half-life extension domains are each an IgG1 Fc domain or fragment thereof and each comprise the amino substitution N297A, numbered according to the Kabat EU numbering system.

8. The masked IL-2 cytokine of any one of claims 5-7, wherein the first and second half-life extension domains are each an IgG1 Fc domain or fragment thereof and each comprise the amino substitution I253A, numbered according to the Kabat EU numbering system.

9. The masked IL-2 cytokine of claim 1 or claim 2, wherein the first half-life extension domain comprises the amino acid sequence of SEQ ID NO: 9, and the second half-life extension domain thereof comprises the amino acid sequence of SEQ ID NO: 12.

10. The masked IL-2 cytokine of claim 1 or claim 2, wherein the first half-life extension domain comprises the amino acid sequence of SEQ ID NO: 10 and the second half-life extension domain thereof comprises the amino acid sequence of SEQ ID NO: 13.

11. The masked IL-2 cytokine of any one of claims 1 to 10, wherein the IL-2 cytokine or functional fragment thereof is modified compared to the sequence of a mature IL-2 having SEQ ID NO: 2.

12. The masked IL-2 cytokine of claim 11, wherein the modified IL-2 cytokine or functional fragment thereof comprises modifications R38A, F42A, Y45A, and E62A relative to the sequence of a mature IL-2 having SEQ ID NO: 2.

13. The masked IL-2 cytokine of claim 11 or claim 12, wherein the modified IL-2 cytokine or functional fragment thereof comprises the modification C125A relative to the sequence of a mature IL-2 having SEQ ID NO: 2.

14. The masked IL-2 cytokine of any one of claims 11 to 13, wherein the modified IL-2 cytokine or functional fragment thereof comprises R38A, F42A, Y45A, E62A and C125A relative to the sequence of a mature IL-2 having SEQ ID NO: 2.

15. The masked IL-2 cytokine of any one of claims 1 to 14, wherein the IL-2 cytokine or functional fragment thereof comprises an amino acid sequence of SEQ ID NO: 3.

16. The masked IL-2 cytokine of any one of claims 1 to 15, wherein the masking moiety comprises IL-2Rβ or a fragment, portion or variant thereof.

17. The masked IL-2 cytokine of claim 16, wherein the IL-2Rβ or a fragment, portion or variant thereof comprises an amino acid sequence of SEQ ID NO: 4.

18. The masked IL-2 cytokine of claim 16, wherein the IL-2Rβ or a fragment, portion or variant thereof comprises an amino acid sequence of SEQ ID NO: 5.

19. The masked IL-2 cytokine of any one of claims 1 to 18, 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.

20. The masked IL-2 cytokine of any one of claims 1 to 18, wherein the first linker comprises a proteolytically cleavable peptide such that the first linker is a proteolytically cleavable linker and the second linker does not comprise a proteolytically cleavable peptide such that the second linker is a non- proteolytically cleavable linker.

21. The masked IL-2 cytokine of claim 19 or claim 20, wherein the non-proteolytically cleavable linker is between 3 and 18 amino acids in length.

22. The masked IL-2 cytokine of claim 21, wherein the non-proteolytically cleavable linker is between 3 and 8 amino acids in length.

23. The masked IL-2 cytokine of any one of claims 19 to 22, wherein the non-proteolytically cleavable linker is rich in amino acid residues G, S and P.

24. The masked IL-2 cytokine of any one of claims 19 to 23, wherein the non-proteolytically cleavable linker comprises an amino acid sequence of SEQ ID NO: 14.

25. The masked IL-2 cytokine of any one of claims 19 to 23, wherein the non-proteolytically cleavable linker comprises an amino acid sequence of SEQ ID NO: 23.

26. The masked IL-2 cytokine of any one of claims 1 to 25, wherein the proteolytically cleavable linker is from 10 to 25 amino acids in length.

27. The masked IL-2 cytokine of any one of claims 1 to 26, wherein the cleavable peptide within the proteolytically cleavable linker comprises an amino acid sequence selected from the group consisting of SEQ ID NOs: 24, 25, 26, 27 and 28.

28. The masked IL-2 cytokine of any one of claims 1 to 26, wherein the cleavable peptide within the proteolytically cleavable linker comprises SEQ ID NO: 118.

29. The masked IL-2 cytokine of any one of claims 1 to 26, wherein the cleavable peptide within the proteolytically cleavable linker comprises SEQ ID NO: 119.

30. The masked IL-2 cytokine of any one of claims 1 to 29, wherein the proteolytically cleavable linker comprises a proteolytically cleavable peptide flanked on both sides by a spacer domain.

31. The masked IL-2 cytokine of claim 30, wherein the spacer domains are rich in amino acid residues G, S and P.

32. The masked IL-2 cytokine of claim 30 or claim 31 wherein the spacer domains only include amino acid residue types selected from the group consisting of G, S and P.

33. The masked IL-2 cytokine of any one of claims 1 to 25, wherein the proteolytically cleavable linker comprises an amino acid sequence selected from the group consisting of SEQ ID NOs: 16, 17, 18, 19, 20, 21 and 22.

34. The masked IL-2 cytokine of claim 33, wherein the proteolytically cleavable linker comprises an amino acid sequence selected from the group consisting of SEQ ID NO: 19.

35. The masked IL-2 cytokine of claim 33, wherein the proteolytically cleavable linker comprises an amino acid sequence selected from the group consisting of SEQ ID NO: 17.

36. The masked IL-2 cytokine of claim 30, 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: 118 and SD2 has an amino acid sequence as shown in SEQ ID NO: 29.

37. The masked IL-2 cytokine of claim 30, 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: 119 and SD2 has an amino acid sequence as shown in SEQ ID NO: 29.

38. The masked IL-2 cytokine of claim 36 or claim 37, wherein SD2 is from 3 to 6 amino acids in length.

39. The masked IL-2 cytokine of claim 25, wherein the proteolytically cleavable linker comprises an amino acid sequence selected from the group consisting of SEQ ID NO: 115.

40. The masked IL-2 cytokine of claim 25, wherein the proteolytically cleavable linker comprises an amino acid sequence selected from the group consisting of SEQ ID NO: 116 and 117.

41. The masked IL-2 cytokine of claim 25, wherein the proteolytically cleavable linker comprises an amino acid sequence selected from the group consisting of SEQ ID NO: 112.

42. The masked IL-2 cytokine of claim 25, wherein the proteolytically cleavable linker comprises an amino acid sequence selected from the group consisting of SEQ ID NO: 113.

43. The masked IL-2 cytokine of claim 25, wherein the proteolytically cleavable linker comprises an amino acid sequence selected from the group consisting of SEQ ID NO: 114.

44. The masked IL-2 cytokine of claim 1 or claim 19, wherein the masked IL-2 cytokine comprises a first polypeptide chain of SEQ ID NO: 38.

45. The masked IL-2 cytokine of claim 1 or claim 19, wherein the masked IL-2 cytokine comprises a first polypeptide chain of SEQ ID NO: 39.

46. The masked IL-2 cytokine of claim 1 or claim 20, wherein the masked IL-2 cytokine comprises a first polypeptide chain of SEQ ID NO: 125.

47. The masked IL-2 cytokine of claim 1 or claim 20, wherein the masked IL-2 cytokine comprises a first polypeptide chain of SEQ ID NO: 126.

48. The masked IL-2 cytokine of claim 1 or claim 20, wherein the masked IL-2 cytokine comprises a first polypeptide chain of SEQ ID NO: 127.

49. The masked IL-2 cytokine of claim 1, wherein the masked IL-2 cytokine comprises a first polypeptide chain of SEQ ID NO: 39 and a second polypeptide chain of SEQ ID NO: 49.

50. The masked IL-2 cytokine of claim 1, wherein the masked IL-2 cytokine comprises a first polypeptide chain of SEQ ID NO: 40 and a second polypeptide chain of SEQ ID NO: 51.

51. The masked IL-2 cytokine of claim 1, wherein the masked IL-2 cytokine comprises a first polypeptide chain of SEQ ID NO: 38 and a second polypeptide chain of SEQ ID NO: 128.

52. The masked IL-2 cytokine of claim 1, wherein the masked IL-2 cytokine comprises a first polypeptide chain of SEQ ID NO: 38 and a second polypeptide chain of SEQ ID NO: 129.

53. The masked IL-2 cytokine of claim 1, wherein the masked IL-2 cytokine comprises a first polypeptide chain of SEQ ID NO: 38 and a second polypeptide chain of SEQ ID NO: 130.

54. The masked IL-2 cytokine of claim 1, wherein the masked IL-2 cytokine comprises a first polypeptide chain of SEQ ID NO: 125 and a second polypeptide chain of SEQ ID NO: 51.

55. The masked IL-2 cytokine of claim 1, wherein the masked IL-2 cytokine comprises a first polypeptide chain of SEQ ID NO: 126 and a second polypeptide chain of SEQ ID NO: 51.

56. The masked IL-2 cytokine of claim 1, wherein the masked IL-2 cytokine comprises a first polypeptide chain of SEQ ID NO: 127 and a second polypeptide chain of SEQ ID NO: 51.

57. A masked IL-2 cytokine comprising a masking moiety and an IL-2 cytokine or functional fragment thereof, wherein the masking moiety masks the IL-2 cytokine or functional fragment thereof thereby reducing or preventing binding of the IL-cytokine or functional fragment thereof to its cognate receptor, and wherein a proteolytically cleavable peptide is present between the IL-2 fragment or functional fragment thereof and masking moiety.

58. A masked IL-2 cytokine according to claim 57, wherein the masking moiety and IL-2 cytokine or functional fragment thereof are linked in a single polypeptide chain.

59. A masked IL-2 cytokine according to claim 57 or claim 58, wherein the masked IL-2 cytokine comprises a polypeptide chain comprising formula 1:

N' **HL-L2-C-L1-MM** C'

(1)

where HL is the half life extension domain, L1 is the first linker, MM is the masking moiety, L2 is the second linker, and C is the IL-2 cytokine or functional fragment thereof, wherein at least the first linker comprises a proteolytically cleavable peptide.

60. A masked IL-2 cytokine according to claim 57 or claim 58, wherein the masked IL-2 cytokine comprises a polypeptide chain comprising formula 2:

N' **HL-L2-MM-L1-C** C'

(2)

where HL is the half life extension domain, L1 is the first linker, MM is the masking moiety, L2 is the second linker, and C is the IL-2 cytokine or functional fragment thereof, wherein at least the first linker comprises a proteolytically cleavable peptide.

61. The masked IL-2 cytokine of any one of claims 57 to 60, wherein the masking moiety comprises IL-2Rβ or a fragment, portion or variant thereof.

62. The masked cytokine of claim 61, wherein the IL-2Rβ or a fragment, portion or variant thereof has mutations at amino acid positions C122 and C168 as compared to IL-2β of SEQ ID NO: 4.

63. The masked cytokine of claim 61 or claim 62, wherein the IL-2Rβ or a fragment, portion or variant thereof has mutations C122S and C168S as compared to IL-2β of SEQ ID NO: 4.

64. The masked IL-2 cytokine of any one of claims 57 to 63, wherein the half life extension domain (HL) comprises first and second half-life extension domains which are each an IgG1 Fc domain or fragment thereof.

65. The masked IL-2 cytokine of claim 64, 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.

66. The masked IL-2 cytokine of claim 64 or claim 65, wherein 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, numbered according to the Kabat EU numbering system.

67. The masked IL-2 cytokine of any one of claims 64 to 66, wherein the first and second half-life extension domains are each an IgG1 Fc domain or fragment thereof and each comprise the amino substitution N297A, numbered according to the Kabat EU numbering system.

68. The masked IL-2 cytokine of any one of claims 64 to 67, the first and second half-life extension domains are each an IgG1 Fc domain or fragment thereof and each comprise the amino substitution I253A, numbered according to the Kabat EU numbering system.

69. The masked IL-2 cytokine of any one of claims 64 to 67, wherein the first half-life extension domain

comprises the amino acid sequence of SEQ ID NO: 9, and the second half-life extension domain thereof comprises the amino acid sequence of SEQ ID NO: 12.

70. The masked IL-2 cytokine of any one of claims 64 to 68, wherein the first half-life extension domain comprises the amino acid sequence of SEQ ID NO: 10 and the second half-life extension domain thereof comprises the amino acid sequence of SEQ ID NO: 13.

71. The masked IL-2 cytokine of any one of claims 57 to 70, wherein the cleavable peptide within the proteolytically cleavable linker comprises SEQ ID NO: 118.

72. The masked IL-2 cytokine of any one of claims 57 to 70, wherein the cleavable peptide within the proteolytically cleavable linker comprises SEQ ID NO: 119.

73. The masked IL-2 cytokine of claim 71, 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: 118 and SD2 has an amino acid sequence as shown in SEQ ID NO: 29.

74. The masked IL-2 cytokine of claim 72, 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: 118 and SD2 has an amino acid sequence as shown in SEQ ID NO: 29.

75. The masked IL-2 cytokine of claim 73 or claim 74, wherein SD2 is from 3 to 6 amino acids in length.

76. The masked IL-2 cytokine of any one of claims 57 to 70, wherein the proteolytically cleavable linker comprises an amino acid sequence selected from the group consisting of SEQ ID NO: 115.

77. The masked IL-2 cytokine of any one of claims 57 to 70, wherein the proteolytically cleavable linker comprises an amino acid sequence selected from the group consisting of SEQ ID NO: 116.

78. The masked IL-2 cytokine of any one of claims 57 to 70, wherein the proteolytically cleavable linker comprises an amino acid sequence selected from the group consisting of SEQ ID NO: 112.

79. The masked IL-2 cytokine of any one of claims 57 to 70, wherein the proteolytically cleavable linker comprises an amino acid sequence selected from the group consisting of SEQ ID NO: 113.

80. The masked IL-2 cytokine of any one of claims 57 to 70, wherein the proteolytically cleavable

linker comprises an amino acid sequence selected from the group consisting of SEQ ID NO: 114.

81. A cleavage product capable of binding to its cognate receptor, the cleavage product comprising an IL-2 cytokine or functional fragment thereof, preparable by proteolytic cleavage of the cleavable peptide in the masked IL-2 cytokine as defined in of any one of claims 1 to 80.

82. A cleavage product of a masked IL-2 cytokine, where the cleavage product is capable of binding to its cognate receptor, the cleavage product comprising a polypeptide comprising formula 3:

PCP-SD-C

(3)

wherein PCP is a portion of a proteolytically cleavable peptide; SD is a spacer domain; and C is an IL-2 cytokine or functional fragment thereof.

83. The cleavage product of claim 81 or claim 82, wherein the IL-2 cytokine or functional fragment thereof is modified compared to the sequence of a mature IL-2 polypeptide having SEQ ID NO: 2.

84. The cleavage product of claim 83, wherein the modified IL-2 cytokine or functional fragment thereof comprises modifications R38A, F42A, Y45A, and E62A relative to the sequence of a mature IL-2 having SEQ ID NO: 2.

85. The cleavage product of claim 83 or claim 84, wherein the modified IL-2 cytokine or functional fragment thereof comprises the modification C125A relative to the sequence of a mature IL-2 having SEQ ID NO: 2.

86. The cleavage product of any one of claims 83 to 85, wherein the modified IL-2 cytokine or functional fragment thereof comprises R38A, F42A, Y45A, E62A and C125A relative to the sequence of a mature IL-2 having SEQ ID NO: 2.

87. The cleavage product of any one of claims 83 to 86, wherein the IL-2 cytokine or functional fragment thereof comprises an amino acid sequence of SEQ ID NO: 3.

88. The cleavage product of any one of claims 82 to 87, wherein the spacer domain is rich in amino acid residues G, S and P.

89. The cleavage product of any one of claims 82 to 88, wherein the spacer domain only includes amino acid residue types selected from the group consisting of G, S and P.

90. The cleavage product of any one of claims 82 to 89, wherein the spacer domain comprises an amino

acid sequence of any one of SEQ ID NO: 29, 30 and 31.

91. The cleavage product of any one of claims 82 to 90, wherein the portion of the proteolytically cleavable peptide is a portion of an amino acid sequence of any one of SEQ ID NOs: 24, 25, 26, 27 and 28.

92. The cleavage product of any one of claims 82 to 90, wherein the portion of the proteolytically cleavable peptide is a portion of an amino acid sequence of SEQ ID NO: 118.

93. The cleavage product of any one of claims 82 to 90, wherein the portion of the proteolytically cleavable peptide is a portion of an amino acid sequence of SEQ ID NO: 119.

94. The cleavage product of claim 82, comprising the amino acid sequence of SEQ ID NO: 56.

95. The cleavage product of claim 82, comprising the amino acid sequence of SEQ ID NO: 137.

96. A cleavage product of a masked IL-2 cytokine, where the cleavage product is capable of binding to its cognate receptor, the cleavage product comprising a protein heterodimer comprising:

a) a first polypeptide chain comprising a polypeptide comprising formula 4:

HL1-SD-PCP

(4)

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 formula 5:

HL2-L2-C

(5)

wherein HL2 is a second half-life extension domain; L2 is a linker; and C is an IL-2 cytokine or functional fragment thereof; and

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

97. The cleavage product of claim 96, wherein the IL-2 cytokine or functional fragment thereof is modified compared to the sequence of a mature IL-2 having SEQ ID NO: 2.

98. The cleavage product of claim 97, wherein the modified IL-2 cytokine or functional fragment thereof comprises modifications R38A, F42A, Y45A, and E62A relative to the sequence of a mature IL-2 having SEQ ID NO: 2.

99. The cleavage product of claim 97 or claim 98, wherein the modified IL-2 cytokine or functional

fragment thereof comprises the modification C125A relative to the sequence of a mature IL-2 having SEQ ID NO: 2.

100. The cleavage product of any one of claims 97 to 99, wherein the modified IL-2 cytokine or functional fragment thereof comprises R38A, F42A, Y45A, E62A and C125A.

101. The cleavage product of any one of claims 97 to 100, wherein the IL-2 cytokine or functional fragment thereof comprises an amino acid sequence of SEQ ID NO: 3.

102. The cleavage product of any one of claims 96 to 101, wherein the first half-life extension domain comprises a first Fc domain or a fragment thereof and the second Fc domain comprises an Fc domain or a fragment thereof.

103. The cleavage product of claim 102, wherein the first Fc domain comprises a CH3 domain or a fragment thereof and the second Fc domain comprises a CH3 domain or a fragment thereof.

104. The cleavage product of claim 102 or claim 103, 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.

105. The cleavage product of any one of claims 96 to 104, wherein the first and second half-life extension domains are each an IgG1 Fc domain or fragment thereof.

106. The cleavage product of claim 105, wherein the first and second half-life extension domains are each an IgG1 Fc domain or fragment thereof and each comprise the amino substitution N297A, numbered according to the Kabat EU numbering system.

107. The cleavage product of claim 105 or claim 106, the first and second half-life extension domains are each an IgG1 Fc domain or fragment thereof and each comprise the amino substitutions N297A and I253A, numbered according to the Kabat EU numbering system.

108. The cleavage product of claim 105, wherein the first half-life extension domain comprises the amino acid sequence of SEQ ID NO: 9, and the second half-life extension domain thereof comprises the amino acid sequence of SEQ ID NO: 12.

109. The cleavage product of claim 105, wherein the first half-life extension domain comprises the amino acid sequence of SEQ ID NO: 10 and the second half-life extension domain thereof comprises

the amino acid sequence of SEQ ID NO: 13.

110. The cleavage product of any one of claims 96 to 109, wherein the second linker comprises an amino acid sequence of SEQ ID NO: 23.

111. The cleavage product of any one of claims 96 to 110, wherein the spacer domain is rich in amino acids residues G, S and P.

112. The cleavage product of any one of claims 96 to 111, wherein the spacer domain only includes amino acid residue types selected from the group consisting of G, S and P.

113. The cleavage product of any one of claims 96 to 112, wherein the spacer domain comprises an amino acid sequence of SEQ ID NOs: 32, 33, 34, 35, 36 and 37.

114. The cleavage product of any one of claims 96 to 113, wherein the portion of the proteolytically cleavable peptide is a portion of an amino acid sequence of any one of SEQ ID NOs: 24, 25, 26, 27 and 28.

115. The cleavage product of any one of claims 96 to 113, wherein the portion of the proteolytically cleavable peptide is a portion of an amino acid sequence of SEQ ID NO: 118.

116. The cleavage product of any one of claims 96 to 113, wherein the portion of the proteolytically cleavable peptide is a portion of an amino acid sequence of SEQ ID NO: 119.

117. The cleavage product of claim 96, comprising a first polypeptide chain having an amino acid sequence of SEQ ID NO: 59-B and a second polypeptide chain having an amino acid sequence of SEQ ID NO: 59-A.

118. The cleavage product of claim 96, comprising a first polypeptide chain having an amino acid sequence of SEQ ID NO: 139 and a second polypeptide chain having an amino acid sequence of SEQ ID NO: 138.

119. The cleavage product of claim 96, comprising a first polypeptide chain having an amino acid sequence of SEQ ID NO: 141 and a second polypeptide chain having an amino acid sequence of SEQ ID NO: 140.

120. The cleavage product of claim 96, comprising a first polypeptide chain having an amino acid sequence of SEQ ID NO: 143 and a second polypeptide chain having an amino acid sequence of

SEQ ID NO: 142.

121. A nucleic acid encoding the masked IL-2 cytokine of any one of claims 1 to 80 or encoding one of the polypeptide chains of a masked IL-2 cytokine of any one of claims 1 to 80.

122. A vector comprising the nucleic acid of claim 121.

123. A host cell comprising nucleic acid encoding the masked IL-2 cytokine of any one of claims 1 to 80.

124. A composition comprising the masked IL-2 cytokine of any one of claims 1 to 80.

125. A pharmaceutical composition comprising the masked IL-2 cytokine of any one of claims 1 to 80, and a pharmaceutically acceptable carrier.

126. A kit comprising the masked IL-2 cytokine of any one of claims 1 to 80, or the composition of claim 124, or the pharmaceutical composition of claim 125.

127. A method of producing a masked IL-2 cytokine as defined in any one of claims 1 to 80 comprising culturing the host cell of claim 123 under a condition that produces the masked IL-2 cytokine.

128. A nucleic acid encoding the cleavage product of any one of claims 81 to 120.

129. A composition comprising the cleavage product of any one of claims 81 to 120.

130. A pharmaceutical composition comprising the cleavage product of any one of claims 81 to 120, and a pharmaceutically acceptable carrier.

131. The pharmaceutical composition of claim 130, wherein the pharmaceutical composition is in single unit dosage form.

132. The pharmaceutical composition of claim 130, wherein the pharmaceutical composition is formulated for intravenous administration and is in single unit dosage form.

133. The pharmaceutical composition of claim 130, wherein the pharmaceutical composition is formulated for injection and is in single unit dosage form.

134. The pharmaceutical composition of claim 130, wherein the pharmaceutical composition is a liquid and is in single unit dosage form.

135. A masked IL-2 cytokine as defined in any one of claims 1 to 80 for use in medicine.

136. A cleavage product as defined in any one of claims 81 to 120 for use in medicine.

137. A method of treating or preventing cancer in a subject, the method comprising administering to the subject an effective amount of a masked IL-2 cytokine according to any one of claims 1 to 80.

138. 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 claim 124 or claim 129.

139. 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 125 and 130 to 134.

140. A method of treating or preventing cancer in a subject, the method comprising administering to the subject an effective amount of a masked IL-2 cytokine as defined in any one of claims 1 to 80, whereby the masked cytokine is proteolytically cleaved *in vivo* to produce a cleavage product as defined in one of claims 81 to 120.

141. 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 81 to 120.

142. A method according to any one of claims 137 to 141, wherein the cancer is a solid tumor.

143. A masked IL-2 cytokine as defined in any one of claims 1 to 80 for use in treating or preventing cancer.

144. A masked IL-2 cytokine as defined in any one of claims 1 to 80 for use in a method of treating or preventing cancer, the method comprising administering to the subject an effective amount of the masked IL-2 cytokine, whereby the masked cytokine is proteolytically cleaved *in vivo* to produce a cleavage product as defined in one of claims 81 to 120.

145. A masked IL-2 cytokine for use according to claim 143 or claim 144, wherein the cancer is a solid tumor.

146. A cleavage product as defined in any one of claims 81 to 120 for use in treating or preventing cancer.

147. A cleavage product as defined in any one of claims 81 to 120 for use in treating or preventing cancer, the method comprising a step of administering a masked cytokine as defined in any one of claims 1 to 80 to a patient, thereby producing the cleavage product by proteolytic cleavage of the masked cytokine *in vivo*.

148. A cleavage product as defined in any one of claims 81 to 120 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 80 that has been administered to the subject.

149. A cleavage product for use according to any one of claims 143 to 148, wherein the cancer is a solid tumor.