# MASKED IL-15 CYTOKINES AND THEIR CLEAVAGE PRODUCTS

## CROSS-REFERENCE TO RELATED APPLICATIONS

This application claims the priority benefit of U.S. Provisional Application Serial Nos. 63/003,845, filed April 1, 2020; 63/113,751, filed November 13, 2020; and 63/118,582, 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: 737762002940SEQLIST.TXT, date recorded: March 25, 2021, size: 2,000 KB).

## FIELD

This invention relates to masked IL-15 cytokines and methods related to the use and manufacture of the same. This invention also relates to cleavage products of said masked IL-15 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 cytokine therapeutics that effectively target tumors without the side effects associated with systemic immune activation. Provided herein are masked IL-15 cytokines, cleavage products of said masked IL-15 cytokines, and compositions thereof and methods of use thereof for addressing this need.

## SUMMARY

The disclosed invention relates to IL-15 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-15 cytokine or functional fragment thereof. The IL-15 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-15 cytokine or functional fragment thereof to its cognate receptor. Upon proteolytic cleavage of the cleavable linker at the target site, the IL-15 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-15 cytokine comprising a first polypeptide chain comprising:

N' **HL1** C'

and a second polypeptide chain comprising:

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

where HL is a half life extension domain, L1 is the first linker, MM is a masking moiety, L2 is a second linker, and C is a IL-15 cytokine or functional fragment thereof, wherein the first linker comprises a proteolytically cleavable peptide (CP) and the second linker does not comprise a proteolytically cleavable peptide.

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

N' **HL1** C'

and a second polypeptide chain comprising:

N' **HL2-non cleavable L2-MM- SD1-CP-SD2 -C** C'

where CP is the cleavable peptide, SD1 is a first spacer domain and SD2 is a second spacer domain.

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

In some embodiments, the first spacer domain (SD1) is between 3 and 18 amino acids in length.

In some embodiments, SD1 comprises an amino acid sequence comprising one of SEQ ID NOs: 24-32

In some embodiments, the second spacer domain (SD2) is between 2 and 10 amino acids in length.

In some embodiments, SD2 comprises an amino acid sequence comprising one of SEQ ID NOs: 35-40.

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

N' **HL1** C'

and a second polypeptide chain comprising:

N' **HL2-non cleavable L2-MM- ['IL-15Rα domain']-SD1-CP-SD2 -C** C'

where 'IL-15Rα domain' is a domain comprising an IL-15Rα subunit or a fragment thereof.

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

N' **HL1** C'

and a second polypeptide chain comprising:

N' **HL2-non cleavable L2-MM- SD1-CP-SD2-['IL-15Rα domain']-C** C'

where 'IL-15Rα domain' is a domain comprising an IL-15Rα subunit or a fragment thereof.

In some embodiments, the 'IL-15Rα domain' comprises the wild-type sushi domain sIL-15Rα or an sIL-15Rα variant.

In some embodiments, the IL-15Rα subunit or a fragment thereof comprises an amino acid sequence comprising one of SEQ ID NOs: 55, 56, 57, and 58.

In some embodiments, the 'IL-15Rα domain' comprises an amino acid sequence of SEQ ID NO: 56.

In some embodiments, the IL-15Rα comprises an amino acid substitution at position R26. In some embodiments, the IL-15Rα comprises amino acid substitution R26N. In some embodiments, the IL-15Rα comprises amino acid substitution R26S. In some embodiments, the IL-15Rα comprises an amino acid substitution at position R35. In some embodiments, the IL-15Rα comprises amino acid substitution R35Q. In some embodiments, the IL-15Rα comprises amino acid substitution R35S. In some embodiments, the IL-15Rα comprises an amino acid substitution at positions R26 and R35. In some embodiments, the IL-15Rα comprises amino acid substitutions R26S or R26N, and R35Q or R35S. In some embodiments, the IL-15Rα comprises amino acid substitutions R26N and R35Q.

In some embodiments, the IL-15Rα comprises an amino acid sequence of SEQ ID NOs: 55, 56, 57 or 58, with an additional amino acid substitution at position R26. In some embodiments, the IL-15Rα comprises an amino acid sequence of SEQ ID NOs: 55, 56, 57 or 58, with additional amino acid substitution R26N.

In some embodiments, the IL-15Rα comprises an amino acid sequence of SEQ ID NOs: 55, 56, 57 or 58, with additional amino acid substitution R26S. In some embodiments, the IL-15Rα comprises an amino acid sequence of SEQ ID NOs: 55, 56, 57 or 58, with an additional amino acid substitution at position R35. In some embodiments, the IL-15Rα comprises an amino acid sequence of SEQ ID NOs: 55, 56, 57 or 58, with additional amino acid substitution R35Q. In some embodiments, the IL-15Rα comprises an amino acid sequence of SEQ ID NOs: 55, 56, 57 or 58, with additional amino acid substitution R35S. In some embodiments, the IL-15Rα comprises an amino acid sequence of SEQ ID NOs: 55, 56, 57 or 58, with additional amino acid substitutions at positions R26 and R35. In some embodiments, the IL-15Rα comprises an amino acid sequence of SEQ ID NOs: 55, 56, 57 or 58, with additional amino acid substitutions R26S or R26N, and R35Q or R35S. In some embodiments, the IL-15Rα comprises an amino acid sequence of SEQ ID NOs: 55, 56, 57 or 58, with additional amino acid substitutions R26N and R35Q.

In some embodiments, the IL-15Rα comprises an amino acid sequence of SEQ ID NO: 56, with an additional amino acid substitution at position R26. In some embodiments, the IL-15Rα comprises an amino acid sequence of SEQ ID NO: 56, with additional amino acid substitution R26N. In some embodiments, the IL-15Rα comprises an amino acid sequence of SEQ ID NO: 56, with additional amino acid substitution R26S. In some embodiments, the IL-15Rα comprises an amino acid sequence of SEQ ID NO: 56, with an additional amino acid substitution at position R35. In some embodiments, the IL-15Rα comprises an amino acid sequence of SEQ ID NO: 56, with additional amino acid substitution R35Q. In some embodiments, the IL-15Rα comprises an amino acid sequence of SEQ ID NO: 56, with additional amino acid substitution R35S. In some embodiments, the IL-15Rα comprises an amino acid sequence of SEQ ID NO: 56, with additional amino acid substitutions at positions R26 and R35. In some embodiments, the IL-15Rα comprises an amino acid sequence of SEQ ID NO: 56, with additional amino acid substitutions R26S or R26N, and R35Q or R35S. In some embodiments, the IL-15Rα comprises an amino acid sequence of SEQ ID NO: 56, with additional amino acid substitutions R26N and R35Q. (See SEQ ID NO: 172, 173, 174, 175 and 176.)

Provided herein is a masked IL-15 cytokine comprising a first polypeptide chain comprising:

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

and the second polypeptide chain comprising:

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

where HL1 is a first half life extension domain, L1 is a first linker, MM is the masking moiety, HL2 is a second half life extension domain, L2 is a second linker, and C is an IL-15 cytokine or functional fragment thereof, wherein the second linker comprises a proteolytically cleavable peptide (CP) and the first linker does not comprise a proteolytically cleavable peptide.

In some embodiments, the first polypeptide chain comprises:

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

and the second polypeptide chain comprises:

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

where CP is the cleavable peptide, SD1 is a first spacer domain and SD2 is a second spacer domain.

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

In some embodiments, the first spacer domain (SD1) is between 3 and 18 amino acids in length.

In some embodiments, SD1 comprises an amino acid sequence comprising one of SEQ ID NOs: 24-32

In some embodiments, the second spacer domain (SD2) is between 2 and 10 amino acids in length.

In some embodiments, SD2 comprises an amino acid sequence comprising one of SEQ ID NOs: 35-40.

In some embodiments, the masked cytokine further comprises a third polypeptide chain, comprising:

**N' IL-15Rα domain C'**

where the 'IL-15Rα domain' is a domain comprising an IL-15Rα subunit or a fragment thereof and wherein the 'IL-15Rα domain' is non-covalently bonded to the IL-15 cytokine (C).

In some embodiments, the second polypeptide chain further comprises a domain comprising an IL-15Rα subunit or fragment thereof that is covalently attached to the C' terminal of the IL-15 cytokine or fragment thereof, such that the first polypeptide chain comprises:

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

and the second polypeptide chain comprises:

N' **HL2- SD1-CP-SD2 –C-['IL-15Rα domain']** C'

where the IL-15Rα domain comprises the IL-15Rα subunit or a fragment thereof.

In some embodiments, the first polypeptide chain comprises:

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

and the second polypeptide chain comprises:

N' **HL2–['IL-15Rα domain']- SD1-CP-SD2 - C** C'

where the IL-15Rα domain comprises the IL-15Rα subunit or a fragment thereof.

In some embodiments, the 'IL-15Rα domain' comprises the wild-type sushi domain sIL-15Rα or an sIL-15Rα variant.

In some embodiments, the IL-15Rα subunit or a fragment thereof comprises an amino acid sequence comprising one of SEQ ID NOs: 55, 56, 57, and 58.

In some embodiments, the 'IL-15Rα domain' comprises an amino acid sequence of SEQ ID NO: 56.

In some embodiments, the IL-15Rα comprises an amino acid substitution at position R26. In some embodiments, the IL-15Rα comprises amino acid substitution R26N. In some embodiments, the IL-15Rα comprises amino acid substitution R26S. In some embodiments, the IL-15Rα comprises an amino acid substitution at position R35. In some embodiments, the IL-15Rα comprises amino acid substitution R35Q. In some embodiments, the IL-15Rα comprises amino acid substitution R35S. In some embodiments, the IL-15Rα comprises an amino acid substitution at positions R26 and R35. In some embodiments, the IL-15Rα comprises amino acid substitutions R26S or R26N, and R35Q or R35S. In some embodiments, the IL-15Rα comprises amino acid substitutions R26N and R35Q.

In some embodiments, the IL-15Rα comprises an amino acid sequence of SEQ ID NOs: 55, 56, 57 or 58, with an additional amino acid substitution at position R26. In some embodiments, the IL-15Rα comprises an amino acid sequence of SEQ ID NOs: 55, 56, 57 or 58, with additional amino acid substitution R26N. In some embodiments, the IL-15Rα comprises an amino acid sequence of SEQ ID NOs: 55, 56, 57 or 58, with additional amino acid substitution R26S. In some embodiments, the IL-15Rα comprises an amino acid sequence of SEQ ID NOs: 55, 56, 57 or 58, with an additional amino acid substitution at position R35. In some embodiments, the IL-15Rα comprises an amino acid sequence of SEQ ID NOs: 55, 56, 57 or 58, with additional amino acid substitution R35Q. In some embodiments, the IL-15Rα comprises an amino acid sequence of SEQ ID NOs: 55, 56, 57 or 58, with additional amino acid substitution R35S. In some embodiments, the IL-15Rα comprises an amino acid sequence of SEQ ID NOs: 55, 56, 57 or 58, with additional amino acid substitutions at positions R26 and R35. In some embodiments, the IL-15Rα comprises an amino acid sequence of SEQ ID NOs: 55, 56, 57 or 58, with additional amino acid substitutions R26S or R26N, and R35Q or R35S. In some embodiments, the IL-15Rα comprises an amino acid sequence of SEQ ID NOs: 55, 56, 57 or 58, with additional amino acid substitutions R26N and R35Q.

In some embodiments, the IL-15Rα comprises an amino acid sequence of SEQ ID NO: 56, with an additional amino acid substitution at position R26. In some embodiments, the IL-15Rα comprises an amino acid sequence of SEQ ID NO: 56, with additional amino acid substitution R26N. In some embodiments, the IL-15Rα comprises an amino acid sequence of SEQ ID NO: 56, with additional amino acid substitution R26S. In some embodiments, the IL-15Rα comprises an amino acid sequence of SEQ ID NO: 56, with an additional amino acid substitution at position R35. In some embodiments, the IL-15Rα comprises an amino acid sequence of SEQ ID NO: 56, with additional amino acid substitution R35Q. In some embodiments, the IL-15Rα comprises an amino acid sequence of SEQ ID NO: 56, with additional amino acid substitution R35S. In some embodiments, the IL-15Rα comprises an amino acid sequence of SEQ ID NO: 56, with additional amino acid substitutions at positions R26 and R35. In some embodiments, the IL-15Rα comprises an amino acid sequence of SEQ ID NO: 56, with additional amino acid substitutions R26S or R26N, and R35Q or R35S. In some embodiments, the IL-15Rα comprises an amino acid sequence of SEQ

ID NO: 56, with additional amino acid substitutions R26N and R35Q. (See SEQ ID NO: 172, 173, 174, 175 and 176.)

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

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

In some embodiments, the non-cleavable linker comprises an amino acid sequence as shown in SEQ ID NO: 22.

Provided herein is a masked IL-15 cytokine comprising a first polypeptide chain comprising:

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

and the second polypeptide chain comprising:

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

where HL1 is a first half life extension domain, L1 is a first linker, MM is the masking moiety, HL2 is a second half life extension domain, L2 is a second linker, and C is an IL-15 cytokine or functional fragment thereof, wherein the first linker comprises a proteolytically cleavable peptide (CP) and the second linker does not comprise a proteolytically cleavable peptide .

In some embodiments, the first polypeptide chain comprises:

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

and the second polypeptide chain comprises:

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

where CP is the cleavable peptide, SD1 is a first spacer domain and SD2 is a second spacer domain.

In some embodiments, the masked cytokine further comprises a third polypeptide chain, comprising:

**N' IL-15Rα domain C'**

where the 'IL-15Rα domain' is a domain comprising an IL-15Rα subunit or a fragment thereof and wherein the 'IL-15Rα domain' is non-covalently bonded to the IL-15 cytokine (C).

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

In some embodiments, the first spacer domain (SD1) is between 3 and 18 amino acids in length.

In some embodiments, SD1 comprises an amino acid sequence comprising one of SEQ ID NOs: 24-32

In some embodiments, the second spacer domain (SD2) is between 2 and 10 amino acids in length.

In some embodiments, SD2 comprises an amino acid sequence comprising one of SEQ ID NOs: 35-40.

In some embodiments, the non-cleavable linker is between 18 and 22 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 comprises an amino acid sequence as shown in SEQ ID NO: 21.

In some embodiments, the IL-15 cytokine or fragment thereof comprises SEQ ID NO: 2 or a functional fragment thereof.

In some embodiments, the IL-15 cytokine or functional fragment thereof comprises an amino acid sequence of SEQ ID NO: 2.

In some embodiments, the IL-15 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.

In some embodiments, the IL-15 cytokine or functional fragment thereof comprises an amino acid sequence having one or more amino acid substitutions at positions D22, E46, E53 as compared to the amino acid sequence of SEQ ID NO: 2.

In some embodiments, the IL-15 cytokine or functional fragment thereof comprises an amino acid sequence having one or more amino acid substitutions at positions D22, E46, E53, N71, N79, or N112 as compared to the amino acid sequence of SEQ ID NO: 2.

In some embodiments, the IL-15 cytokine or functional fragment thereof comprises an amino acid sequence having an amino acid substitution at position N71 and N79 as compared to the amino acid sequence of SEQ ID NO: 2.

In some embodiments, wherein the IL-15 cytokine or functional fragment thereof comprises an amino acid sequence having an amino acid substitution at position N71 and N112 as compared to the amino acid sequence of SEQ ID NO: 2.

In some embodiments, wherein the IL-15 cytokine or functional fragment thereof comprises an amino acid sequence having an amino acid substitution at position N79 and N112 as compared to the amino acid sequence of SEQ ID NO: 2.

In some embodiments, wherein the IL-15 cytokine or functional fragment thereof comprises an amino acid sequence having an amino acid substitution at position N71, N79 and N112 as compared to the amino acid sequence of SEQ ID NO: 2.

In some embodiments, the IL-15 cytokine or functional fragment thereof comprises an amino acid sequence of SEQ ID NO: 3.

In some embodiments, the IL-15 cytokine or functional fragment thereof comprises an amino acid sequence of SEQ ID NO: 4.

In some embodiments, the IL-15 cytokine or functional fragment thereof comprises an amino acid sequence of SEQ ID NO: 5.

In some embodiments, the IL-15 cytokine or functional fragment thereof comprises an amino acid sequence of SEQ ID NO: 6.

In some embodiments, the IL-15 cytokine or functional fragment thereof comprises an amino acid sequence of SEQ ID NO: 7.

In some embodiments, the IL-15 cytokine or functional fragment thereof comprises an amino acid sequence of SEQ ID NO: 8.

In some embodiments, the IL-15 cytokine or functional fragment thereof comprises an amino acid sequence of SEQ ID NO: 127.

In some embodiments, the IL-15 cytokine or functional fragment thereof comprises an amino acid sequence of SEQ ID NO: 128.

In some embodiments, the IL-15 cytokine or functional fragment thereof comprises an amino acid sequence of SEQ ID NO: 129.

In some embodiments, the IL-15 cytokine or functional fragment thereof comprises an amino acid sequence of SEQ ID NO: 130.

In some embodiments, the IL-15 cytokine or functional fragment thereof comprises an amino acid sequence of SEQ ID NO: 131.

In some embodiments, the IL-15 cytokine or functional fragment thereof comprises an amino acid sequence of SEQ ID NO: 132.

In some embodiments, the IL-15 cytokine or functional fragment thereof comprises an amino acid sequence of SEQ ID NO: 133.

In some embodiments, the IL-15 cytokine or functional fragment thereof comprises an additional mutation at position N71.

In some embodiments, the IL-15 cytokine or functional fragment thereof comprises an additional mutation at position S73.

In some embodiments, the IL-15 cytokine or functional fragment thereof comprises an additional mutation at one or more of amino acid positions N72, N79, V80, T81, and N112.

In some embodiments, the first polypeptide chain comprises:

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

and the second polypeptide chain comprises:

N' **HL2- SD1-CP-SD2 – [N' terminal domain of C]-['IL-15Rα domain']-[C' terminal domain of C]** C'

where the IL-15 cytokine or functional fragment thereof comprises an N' terminal domain and a C' terminal domain and where the IL-15Rα domain comprises the IL-15Rα subunit or a fragment thereof flanked on either side by a non-cleavable 'α subunit linker'.

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

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

In some embodiments, the non-cleavable linker comprises an amino acid sequence as shown in SEQ ID NO: 22.

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

In some embodiments, the first spacer domain (SD1) is between 3 and 18 amino acids in length.

In some embodiments, SD1 comprises an amino acid sequence comprising one of SEQ ID NOs: 24-32.

In some embodiments, the second spacer domain (SD2) is between 2 and 10 amino acids in length.

In some embodiments, SD2 comprises an amino acid sequence comprising one of SEQ ID NOs: 35-40.

In some embodiments, the 'IL-15Rα domain' comprises the wild-type sushi domain sIL-15Rα or an sIL-15Rα variant.

In some embodiments, the IL-15Rα subunit or a fragment thereof comprises an amino acid sequence comprising one of SEQ ID NOs: 55, 56, 57, and 58.

In some embodiments, the 'IL-15Rα domain' comprises an amino acid sequence of SEQ ID NO: 56.

In some embodiments, the IL-15Rα comprises an amino acid substitution at position R26. In some embodiments, the IL-15Rα comprises amino acid substitution R26N. In some embodiments, the IL-15Rα comprises amino acid substitution R26S. In some embodiments, the IL-15Rα comprises an amino acid substitution at position R35. In some embodiments, the IL-15Rα comprises amino acid substitution R35Q. In some embodiments, the IL-15Rα comprises amino acid substitution R35S. In some embodiments, the IL-15Rα comprises an amino acid substitution at positions R26 and R35. In some embodiments, the IL-15Rα comprises amino acid substitutions R26S or R26N, and R35Q or R35S. In some embodiments, the IL-15Rα comprises amino acid substitutions R26N and R35Q.

In some embodiments, the IL-15Rα comprises an amino acid sequence of SEQ ID NOs: 55, 56, 57 or 58, with an additional amino acid substitution at position R26. In some embodiments, the IL-15Rα comprises an amino acid sequence of SEQ ID NOs: 55, 56, 57 or 58, with additional amino acid substitution R26N. In some embodiments, the IL-15Rα comprises an amino acid sequence of SEQ ID NOs: 55, 56, 57 or 58, with additional amino acid substitution R26S. In some embodiments, the IL-15Rα comprises an amino acid sequence of SEQ ID NOs: 55, 56, 57 or 58, with an additional amino acid substitution at position R35. In some embodiments, the IL-15Rα comprises an amino acid sequence of SEQ ID NOs: 55, 56, 57 or 58, with additional amino acid substitution R35Q. In some embodiments, the IL-15Rα comprises an amino acid sequence of SEQ ID NOs: 55, 56, 57 or 58, with additional amino acid substitution R35S. In some embodiments, the IL-15Rα comprises an amino acid sequence of SEQ ID NOs: 55, 56, 57 or 58, with additional amino acid substitutions at positions R26 and R35. In some embodiments, the IL-15Rα comprises an amino acid sequence of SEQ ID NOs: 55, 56, 57 or 58, with additional amino acid substitutions R26S or R26N, and R35Q or R35S. In some embodiments, the IL-15Rα comprises an amino acid sequence of SEQ ID NOs: 55, 56, 57 or 58, with additional amino acid substitutions R26N and R35Q.

In some embodiments, the IL-15Rα comprises an amino acid sequence of SEQ ID NO: 56, with an additional amino acid substitution at position R26. In some embodiments, the IL-15Rα comprises an amino acid sequence of SEQ ID NO: 56, with additional amino acid substitution R26N. In some embodiments, the IL-15Rα comprises an amino acid sequence of SEQ ID NO: 56, with additional amino acid substitution R26S. In some embodiments, the IL-15Rα comprises an amino acid sequence of SEQ ID NO: 56, with an additional amino acid substitution at position R35. In some embodiments, the IL-15Rα comprises an amino acid sequence of SEQ ID NO: 56, with additional amino acid substitution R35Q. In some embodiments, the IL-15Rα comprises an amino acid sequence of SEQ ID NO: 56, with additional amino acid substitution R35S. In some embodiments, the IL-15Rα comprises an amino acid sequence of SEQ ID NO: 56, with additional amino acid substitutions at positions R26 and R35. In some embodiments, the IL-15Rα comprises an amino acid sequence of SEQ ID NO: 56, with additional amino acid substitutions R26S or R26N, and R35Q or R35S. In some embodiments, the IL-15Rα comprises an amino acid sequence of SEQ ID NO: 56, with additional amino acid substitutions R26N and R35Q. (See SEQ ID NO: 172, 173, 174, 175 and 176.)

In some embodiments, the N' terminal domain and C' terminal domain of the IL-15 cytokine or functional fragment thereof comprise an amino acid sequence having one or more amino acid substitutions at positions D22, E46, E53 as compared to the amino acid sequence of SEQ ID NO: 2.

In some embodiments, the N' terminal domain and C' terminal domain of the IL-15 cytokine or functional fragment thereof comprises an additional mutation at position N71.

In some embodiments, the N' terminal domain and C' terminal domain of the IL-15 cytokine or functional fragment thereof comprises an additional mutation at position S73.

In some embodiments, the N' terminal domain and C' terminal domain of the IL-15 cytokine or functional fragment thereof comprises an additional mutation at one or more of amino acid positions N72, N79, V80, T81, and N112.

In some embodiments, the N' terminal domain of the IL-15 cytokine or functional fragment thereof, comprises an amino acid sequence of SEQ ID NO: 9 and the C' terminal domain of the IL-115 cytokine or functional fragment thereof comprises an amino acid sequence of SEQ ID NO: 10.

In some embodiments, the cleavable peptide comprises an amino acid sequence of SEQ ID NO: 33.

In some embodiments, the cleavable peptide comprises an amino acid sequence of SEQ ID NO: 34.

In some embodiments, the cleavable peptide within the proteolytically cleavable linker comprises SEQ ID

NO: 155.

In some embodiments, the cleavable peptide within the proteolytically cleavable linker comprises SEQ ID NO: 156.

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: 155.

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: 156.

In some embodiments, the cleavable linker comprises an amino acid sequence comprising one of SEQ ID NOs: 41-54, 162, 163, 164, 165, 166, or 167.

In some embodiments, the cleavable linker comprises an amino acid sequence comprising one of SEQ ID NOs: 41-54, 162, 163, 164, 165, 166, 167, 168, 169, 170 or 171.

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

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 half-life extension domain comprises the amino acid sequence of SEQ ID NO: 63, and the second half-life extension domain thereof comprises the amino acid sequence of SEQ ID NO: 64.

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 acid 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: 65 and the second half-life extension domain thereof comprises the amino acid sequence of SEQ ID NO: 66.

In some embodiments, the masking moiety comprises IL-15Rβ or a fragment or variant thereof.

In some embodiments, the masking moiety comprises the amino acid sequence of SEQ ID NO: 11.

In some embodiments, the masking moiety comprises IL-15Rβ variant or a fragment thereof having an amino acid substitution at position C122.

In some embodiments, the masking moiety comprises IL-15Rβ variant or a fragment thereof having amino acid substitution C122S.

In some embodiments, the masking moiety comprises IL-15Rβ variant or a fragment thereof having an amino acid substitution at position C168.

In some embodiments, the masking moiety comprises IL-15Rβ variant or a fragment thereof having amino acid substitution C168S.

In some embodiments, the masking moiety comprises IL-15Rβ variant or a fragment thereof having an amino acid substitution at positions C122 and C168.

In some embodiments, the masking moiety comprises an amino acid sequence of SEQ ID NO: 12.

Provided here is a masked IL-15 cytokine comprising a first polypeptide chain comprising:

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

and a second polypeptide chain comprising:

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

and a third polypeptide chain, comprising:

**N' IL-15Rα domain C'**

where HL1 is a first half life extension domain, L1 is a first linker, MM is a masking moiety, HL2 is a second half life extension domain, L2 is a second linker, and C is an IL-15 cytokine or functional fragment thereof, wherein the second linker comprises a proteolytically cleavable peptide (CP) and the first linker does not comprise a proteolytically cleavable peptide,

wherein the 'IL-15Rα domain' is a domain comprising an IL-15Rα subunit or a fragment thereof and the 'IL-15Rα domain' is non-covalently bonded to the IL-15 cytokine or functional fragment thereof (C) in the second polypeptide chain.

In some embodiments, the first polypeptide chain comprises:

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

and the second polypeptide chain comprises:

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

and the third polypeptide chain, comprises:

**N' IL-15Rα domain C'**

where CP is the cleavable peptide, SD1 is a first spacer domain,SD2 is a second spacer domain, and the 'IL-15Rα domain' is non-covalently bonded to the IL-15 cytokine or functional fragment thereof (C) in the second polypeptide chain..

Provided herein is a masked IL-15 cytokine comprising a first polypeptide chain comprising:

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

and a second polypeptide chain comprising:

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

and a third polypeptide chain comprising:

**N' IL-15Rα domain C'**

where HL1 is a first half life extension domain, L1 is a first linker, MM is a masking moiety, HL2 is a second half life extension domain, L2 is a second linker, and C is an IL-15 cytokine or functional fragment thereof, wherein the first linker comprises a proteolytically cleavable peptide (CP) and the second linker does not comprise a proteolytically cleavable peptide,

wherein the 'IL-15Rα domain' is a domain comprising an IL-15Rα subunit or a fragment thereof and the 'IL-15Rα domain' is non-covalently bonded to the IL-15 cytokine or functional fragment thereof (C) in the second polypeptide chain.

In some embodiments, the first polypeptide chain comprises:

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

and the second polypeptide chain comprises:

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

and the third polypeptide chain comprises:

**N' IL-15Rα domain C'**

where CP is the cleavable peptide, SD1 is a first spacer domain, SD2 is a second spacer domain and the 'IL-15Rα domain' is non-covalently bonded to the IL-15 cytokine or functional fragment thereof (C) in the second polypeptide chain.

In some embodiments, the 'IL-15Rα domain' is only non-covalently bonded to the IL-15 cytokine, i.e. no covalent bonds are present between the second and third polypeptide chains.

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

In some embodiments, the first half-life extension domain comprises the amino acid sequence of SEQ ID NO: 63, and the second half-life extension domain thereof comprises the amino acid sequence of SEQ ID NO: 64.

In some embodiments, the masking moiety comprises IL-15Rβ or a fragment or variant thereof.

In some embodiments, the masking moiety comprises the amino acid sequence of SEQ ID NO: 12.

In some embodiments, the first polypeptide chain comprises an amino acid sequence of SEQ ID NO: 177.

In some embodiments, the second polypeptide chain comprises an amino acid sequence of SEQ ID NO: 183.

In some embodiments, the IL-15 cytokine or functional fragment thereof comprises an amino acid sequence having an amino acid substitution N71Q and N79Q as compared to the amino acid sequence of SEQ ID NO: 2.

In some embodiments, the IL-15 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: 130.

In some embodiments, the IL-15 cytokine or functional fragment thereof comprises an amino acid sequence of SEQ ID NO: 130.

In some embodiments, the 'IL-15Rα domain' comprises the wild-type sushi domain sIL-15Rα or a variant thereof.

In some embodiments, the 'IL-15Rα domain' comprises an amino acid sequence of SEQ ID NO: 56.

In some embodiments, the first polypeptide chain comprises an amino acid sequence of SEQ ID NO: 68 and the second polypeptide chain comprises an amino acid sequence of SEQ ID NO: 83.

In some embodiments, the first polypeptide chain comprises an amino acid sequence of SEQ ID NO: 177 and the second polypeptide chain comprises an amino acid sequence of SEQ ID NO: 193.

In some embodiments, the first polypeptide chain comprises an amino acid sequence of SEQ ID NO: 177 and the second polypeptide chain comprises an amino acid sequence of SEQ ID NO: 194.

Provided herein is a cleavage product of a masked IL-15 cytokine, wherein said cleavage product is capable of binding to its cognate receptor, the cleavage product comprising an IL-15 cytokine or functional fragment thereof, preparable by proteolytic cleavage of the cleavable peptide in the masked IL-15 cytokine as described herein.

Provided herein is a cleavage product of a masked IL-15 cytokine, where the cleavage product is capable of binding to its cognate receptor, the cleavage product comprising a polypeptide comprising:

**PCP-SD-C**

wherein PCP is a portion of a proteolytically cleavable peptide; SD is a spacer domain; and C is an IL-15 cytokine or functional fragment thereof.

In some embodiments, SD is SD2 described herein.

In some embodiments, the proteolytically cleavable peptide is described herein.

In some embodiments, the IL-15 cytokine or a fragment thereof is described herein.

In some embodiments, the cleavage product further comprises a domain comprising an IL-15Rα subunit or a fragment thereof.

In some embodiments, the IL-15Rα subunit or a fragment thereof is non-covalently bonded to the IL-15 cytokine (C).

In some embodiments, the cleavage product comprises:

**PCP-SD-['IL-15Rα domain']-C**

In some embodiments, the cleavage product comprises:

**PCP-SD-C-['IL-15Rα domain']**

In some embodiments, the IL-15Rα subunit or a fragment thereof comprises an amino acid sequence comprising one of SEQ ID NOs: 55, 56, 57, and 58.

In some embodiments, the IL-15Rα comprises an amino acid substitution at position R26. In some embodiments, the IL-15Rα comprises amino acid substitution R26N. In some embodiments, the IL-15Rα comprises amino acid substitution R26S. In some embodiments, the IL-15Rα comprises an amino acid substitution at position R35. In some embodiments, the IL-15Rα comprises amino acid substitution R35Q. In some embodiments, the IL-15Rα comprises amino acid substitution R35S. In some embodiments, the IL-15Rα comprises an amino acid substitution at positions R26 and R35. In some embodiments, the IL-15Rα comprises amino acid substitutions R26S or R26N, and R35Q or R35S. In some embodiments, the IL-15Rα comprises amino acid substitutions R26N and R35Q.

In some embodiments, the IL-15Rα comprises an amino acid sequence of SEQ ID NOs: 55, 56, 57 or 58, with an additional amino acid substitution at position R26. In some embodiments, the IL-15Rα comprises an amino acid sequence of SEQ ID NOs: 55, 56, 57 or 58, with additional amino acid substitution R26N. In some embodiments, the IL-15Rα comprises an amino acid sequence of SEQ ID NOs: 55, 56, 57 or 58, with additional amino acid substitution R26S. In some embodiments, the IL-15Rα comprises an amino acid sequence of SEQ ID NOs: 55, 56, 57 or 58, with an additional amino acid substitution at position R35. In some embodiments, the IL-15Rα comprises an amino acid sequence of SEQ ID NOs: 55, 56, 57 or 58, with additional amino acid substitution R35Q. In some embodiments, the IL-15Rα comprises an amino acid sequence of SEQ ID NOs: 55, 56, 57 or 58, with additional amino acid substitution R35S. In some embodiments, the IL-15Rα comprises an amino acid sequence of SEQ ID NOs: 55, 56, 57 or 58, with additional amino acid substitutions at positions R26 and R35. In some embodiments, the IL-15Rα comprises an amino acid sequence of SEQ ID NOs: 55, 56, 57 or 58, with additional amino acid substitutions R26S or R26N, and R35Q or R35S. In some embodiments, the IL-15Rα comprises an amino acid sequence of SEQ ID NOs: 55, 56, 57 or 58, with additional amino acid substitutions R26N and R35Q.

In some embodiments, the IL-15Rα comprises an amino acid sequence of SEQ ID NO: 56, with an additional amino acid substitution at position R26. In some embodiments, the IL-15Rα comprises an amino acid sequence of SEQ ID NO: 56, with additional amino acid substitution R26N. In some embodiments, the IL-15Rα comprises an amino acid sequence of SEQ ID NO: 56, with additional amino acid substitution R26S. In some embodiments, the IL-15Rα comprises an amino acid sequence of SEQ ID NO: 56, with an additional amino acid substitution at position R35. In some embodiments, the IL-15Rα comprises an amino acid sequence of SEQ ID NO: 56, with additional amino acid substitution R35Q. In some embodiments, the IL-15Rα comprises an amino acid sequence of SEQ ID NO: 56, with additional amino acid substitution R35S. In some embodiments, the IL-15Rα comprises an amino acid sequence of SEQ ID NO: 56, with additional amino acid substitutions at positions R26 and R35. In some embodiments, the IL-15Rα comprises an amino acid sequence of SEQ ID NO: 56, with additional amino acid substitutions R26S or R26N, and R35Q or R35S. In some embodiments, the IL-15Rα comprises an amino acid sequence of SEQ ID NO: 56, with additional amino acid substitutions R26N and R35Q. (See SEQ ID NO: 172, 173, 174, 175 and 176.)

Provided herein is a cleavage product of a masked IL-15 cytokine, where the cleavage product is capable of binding to its cognate receptor, the cleavage product comprising:

**PCP-SD2 – [N' terminal domain of C]-['IL-15Rα domain']-[C' terminal domain of C]**

where the IL-15Rα domain comprises an IL-15Rα subunit or a fragment thereof flanked on either side by a non-cleavable 'α subunit linker'.

Provided herein is a cleavage product of a masked IL-15 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:

**HL1-SD-PCP**

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

and a second polypeptide chain comprising a polypeptide comprising:

**HL2-L2-C**

wherein HL2 is a second half-life extension domain; L2 is a linker; and C is an IL-15 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, SD is SD1 described herein.

In some embodiments, the proteolytically cleavable peptide is described herein.

In some embodiments, the IL-15 cytokine or a fragment thereof is described herein.

In some embodiments, the first and second half life extension domains are described herein.

In some embodiments, the non-cleavable linker L2 is described herein.

In some embodiments, the cleavage product further comprises a IL-15Rα subunit or a fragment thereof non-covalently bonded to the IL-15 cytokine (C).

Provided herein is a cleavage product comprising an amino acid sequence selected from SEQ ID NOs: 104, 105, 106, 107, 108, 109, 110, 111, 112, 113, 114, 115, 116, 117, 118, 119, 120, 121, 122, 123, 124, 125,126, 136, 137, 138, 139, 140, 141, 142 and 143, and 144 to 147, and 199 to 228, and 307.

Provided herein is a nucleic acid encoding a masked IL-15 cytokine described herein.

Provided herein is a nucleic acid encoding one of the chains of the masked IL-15 cytokine 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-15 cytokine described herein.

Provided herein is a vector comprising a nucleic acid encoding one of the chains of a masked IL-15 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 a masked IL-15 cytokine described herein.

Provided herein is a pharmaceutical composition comprising a masked IL-15 cytokine described herein, and a pharmaceutically acceptable carrier.

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

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

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

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

Provided herein is a kit comprising a masked IL-15 cytokine described herein, or a composition described herein, or a pharmaceutical composition described herein.

Provided herein is a method of producing a masked IL-15 cytokine described herein comprising culturing a host cell described herein under a condition that produces the masked IL-15 cytokine.

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

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

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

Provided herein is a masked IL-15 cytokine described herein for use in medicine.

Provided herein is a cleavage product 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-15 cytokine described herein.

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

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

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-15 cytokine described herein, whereby the masked cytokine is proteolytically cleaved *in vivo* to produce a cleavage product described herein.

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

In some embodiments, the cancer is a solid tumor.

Provided herein is a masked IL-15 cytokine described herein for use in treating or preventing cancer.

Provided herein is a masked IL-15 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-15 cytokine, whereby the masked cytokine is proteolytically cleaved *in vivo* to produce a cleavage product described herein.

In some embodiments, the cancer is a solid tumor.

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

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

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

In some embodiments, the cancer is a solid tumor.

## BREIF 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-B** show exemplary embodiments of a masked IL-2 cytokine. Cleavage by a protease releases a masking moiety (e.g., IL-2Rβ, as shown in **FIGs. 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** shows results from an in vivo study that assessed vascular leakage using the exemplary masked IL-2 polypeptide construct AK111 or AK168, or the non-masked IL-2 polypeptide construct AK081 or AK167, or an anti-RSV control. **FIG.25A** shows the percentage (%) of body weight loss, and **FIGs. 25B, 25C**, and **25D** shows the weight in grams of the liver, lung, and spleen, respectively, for each.

**FIGs. 26A** and **26B** show results from an in vivo study that assessed vascular leakage as indicated by measuring the extent of dye leakage into liver and lung tissue following administration of the AK081, AK111, AK167, or AK168 construct, or an anti-RSV control. The extent of dye leakage into liver (**FIG. 26A**) and lung (**FIG. 26B**) was measured based on absorbance at 650nm.

**FIGs. 27A** and **27B** show results from an in vivo study that assessed vascular leakage as indicated by measuring the extent of monomuclear 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** show 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 Example 6ix. **FIGs. 43A and 43B** show results of Example 6x.

**FIGs. 44A and 44B** show the results from reporter bioassays on an exemplary masked IL-15 polypeptide construct, AK248, with (+ MMP) or without prior exposure to an activating protease, or a rhIL-15 as a control. **FIG. 44A** shows results from a reporter bioassay using a HEK-Blue IL2 reporter cell line, and **FIG. 44B** shows results from a reporter bioassay using an IL-15 bioassay with a mouse CTLL2 cell line.

**FIGs. 45A and 45B** show the results of Example 7.

**FIGs. 46A and 46B, FIGs. 47A-47D, FIGs. 48A-48D, FIGs. 49A-49B, FIGs. 50A-50D and FIGs. 51A-51D** show the results of Example 8.

**FIGs. 52A-52D** show results from studies testing the in vivo responses of CD8 T cells and NK cells in a B16F10 mouse model, in the spleen and tumor, using the AK244, AK507 or XTX200 construct. For spleen tissue, CD8 T cells (**FIG. 52A**), NK cell (**FIG. 52B**) is shown. For tumor, CD8 T cells (**FIG. 52C**), NK cell (**FIG. 52D**) is shown.

**FIG. 53** shows results from pharmacokinetic studies carried out in tumor-bearing mice using the construct AK244, AK507 and XTX200 at dosages 0.5mpk, 1.5mpk and 5.0mpk. **FIG. 53** shows Fc levels in plasma (μg/mL) by detecting human IgG.

**FIG 54A and 54B** show the results of an in vivo study that assessed spleen weight and lung weight, using exemplary constructs AK244, AK507, AK443 and XTX200. **FIG 54A** shows the spleen weight in grams (g). **FIG 54B** shows the lung weight in grams (g).

**FIGs 55A and 55B** show the results from a study evaluating the effect of adding a masking moiety to exemplary IL-15 constructs, using AK507, AK564, AK595 and AK596. AK253 is and IL-2, unasked positive control. Fig 55A shows the effect on the half-life, measured in days. Fig 55B shows the effect on drug exposure, measured in day µg/ml.

**FIGs. 56A and 56B** showsresults from an in vivo study that assessed the percentage (%) of body weight loss using the exemplary non-cleavable masked IL-15 constructs AK564 and AK596, non-masked IL-15 construct AK507 and AK595, and XTX200 and a vehicle as controls. **FIG. 56A** shows the percentage (%) of body weight loss for constructs AK507 and AK564, which comprise a wild-type IL-15 domain. **FIG. 56B** shows the percentage (%) of body weight loss for constructs AK595 and AK596, which comprise a single mutation (N71Q) in the IL-15 domain.

**FIGs. 57A and 57B** show the results from an in vivo study that assessed the %Tregs (of CD4+) and %CD8+ (of CD45+) in tumor, using exemplary non-cleavable masked IL-15 constructs AK564 and AK596, non-masked IL-15 constructs AK507 and AK595, and AK253 and a vehicle as controls. **FIG. 57A** shows the %Tregs. **FIG. 57B** shows the %CD8+.

**FIG. 58** shows the results from an in vivo study that assessed the CD8:Treg ratio in tumor, using exemplary non-cleavable masked IL-15 constructs AK564 or AK596, non-masked IL-15 constructs AK507 or AK595, and AK253 and a vehicle as controls.

**FIG. 59** shows the results from an in vivo study that assessed the % NK cells (of CD45+) in tumor, using exemplary non-cleavable masked IL-15 constructs AK564 or AK596, non-masked IL-15 constructs AK507 or AK595, and AK253 and a vehicle as controls.

**FIGs. 60A and 60B** show the results from an in vivo study that assessed the % Ki-67+ NK cells and %ICOS+ NK cells in tumor, using exemplary non-cleavable masked IL-15 constructs AK564 or AK596, non-masked IL-15 constructs AK507 or AK595, and AK253 and a vehicle as controls. **FIG 60A** shows the results for the % Ki-67+ NK cells. **FIG 60B** shows the results for %ICOS+ NK cells.

**FIGs. 61A-61D** show the results from an in vivo study that assessed the % Ki-67+ Tregs, %Ki-67+ Tconv, %Ki-67+ CD8+ and %Ki-67+ NK cells in tumor, using exemplary non-cleavable masked IL-15 constructs AK564 or AK596, non-masked IL-15 constructs AK507 or AK595, and AK253 and a vehicle as controls. **FIG 61A** shows the results for the % Ki-67+ Tregs. **FIG 61B** shows the results for % Ki-67+ Tconv. **FIG 61C** shows the results for the % Ki-67+ CD8+. **FIG 61D** shows the results for the % Ki-67+ NK cells.

**FIG. 62** shows the gel image results from an SDS-PAGE analysis of AK564, AK596, AK906, AK907, AK908, AK909, AK910 and AK911.

**FIG. 63** shows the results of a HEK-Blue IL-2 bioassay was used to test activation of masked and unmasked IL-15 constructs in CHO-K1 cells.

**FIGs. 64A-64D** and **FIGs. 65A-65J** 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, AK929, AK935, AK931, AK937, AK934, AK933, AK939 and AK940 that do include a peptide substrate. **FIGs 64A-64D** show the SDS-PAGE gel results. **FIGs. 65A-65J** show the HEK-Blue IL-2 bioassay results.

**FIGs. 66A-66D** and **FIGs. 67A-67F** shows 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 66A-66D** show the SDS-PAGE gel results. **FIGs. 67A-67F** show the HEK-Blue IL-2 bioassay results.

**FIGs. 68A-68D** and **69A-69D** show the results of SPR studies of Example 16, using exemplary constructs rhIL-15, AK507, AK564 and AK564.

## DETAILED DESCRIPTION

By using a masking moiety, the systemic side effects of an administered IL-15 cytokine or functional fragment thereof can be reduced by interfering with the binding capability of the IL-15 cytokine or functional fragment thereof to its cognate receptor.

The Interleukin-15 receptor is a type I cytokine receptor, binding IL-15. It consists of an IL-15Rα subunit and shares common β and γ subunits with the IL-2 receptor (the IL-2Rβ chain is also referred to as CD122 and the IL-2Rγ chain is also referred to as CD132). The shared subunits form the basis for many overlapping biological effects of IL-15 and IL-2. IL-15Rα specifically binds IL15 with very high affinity and is capable of binding IL-15 independently of the β and γ subunits. The term "IL-15R" as used herein refers to the high-affinity IL-15 receptor complex that comprises three separate and non-covalently linked chains: the IL-15Rα chain, the IL-2Rβ chain, and the IL-2Rγ chain. IL-15 is capable of binding to the IL-15Rα chain, to the heterodimer of the IL-2Rβ chain and the IL-2Rγ chain, and to the IL-15Rα chain in combination with the IL-2Rβ chain and the IL-2Rγ chain. In some cases, the term also encompasses the IL-15Rα chain, the IL-2Rβ chain, or the IL-2Rγ chain, or combinations thereof.

Thus, in some embodiments, a masking moiety is employed that reduces or prevents binding of the IL-15 cytokine or functional fragment thereof to IL-15Rα. To further reduce systemic effects, in some embodiments, binding of the IL-15 cytokine or fragment thereof to the IL-15Rβ and/or IL-15Rγ subunits of the IL-15 receptor may also be reduced or prevented by the masking moiety in the masked cytokine.

By masking the IL-15 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-15 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-15 cytokine or functional fragment thereof in the form of an IL-15 cleavage product. Activation of the IL-15 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-15 cytokines of the invention may be viewed as a pro-drug.

Masked IL-15 cytokines described herein have been found to show various advantageous properties. Masked IL-15 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-15 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-15 cytokines described anywhere herein have been found to demonstrate advantageous prolonged drug exposure. Masked IL-15 cytokines described herein have been found to demonstrate advantageous stability. Masked IL-15 cytokines described herein have been found to demonstrate advantageous tolerability. Further, masked IL-15 cytokines described herein have been found to demonstrate advantageous potency.

## 1. MASKED CYTOKINES

Provided herein, are masked cytokines.

In some embodiments, a masked IL-15 cytokine is provided comprising a masking moiety and an IL-15 cytokine or functional fragment thereof, wherein the masking moiety masks the IL-15 cytokine or functional fragment thereof thereby reducing or preventing binding of the IL-cytokine or functional fragment thereof to its cognate receptor, and where a proteolytically cleavable peptide is present between the IL-15 cytokine or functional fragment thereof and the masking moiety.

### 1.1 'Linear' masked Cytokines

In some embodiments, the masked IL-15 cytokine comprises a masking moiety and an IL-15 cytokine or functional fragment thereof linked in a single polypeptide chain.

In some embodiments, the masked IL-15 cytokine comprises a polypeptide chain comprising:

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

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-15 cytokine or functional fragment thereof, wherein at least the first linker comprises a proteolytically cleavable peptide.

In some embodiments, the masked IL-15 cytokine comprises a polypeptide chain comprising:

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

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-15 cytokine or functional fragment thereof, wherein at least the first linker comprises a proteolytically cleavable peptide.

In some embodiments, the masking moiety is as described anywhere herein.

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 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 thereto such that:

a first polypeptide chain comprises:

N' **HL1** C'

and a second polypeptide chain comprises:

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

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-non-cleavable L2-MM-cleavable L1-C** C'

In some embodiments, the IL-15 cytokine or functional fragment thereof is as described anywhere herein.

1.2 'Heterodimeric' masked cytokines

Provided herein, in some embodiments, is a masked cytokine comprising a masking moiety in a first polypeptide chain and an IL-15 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-15 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 comprises a proteolytically cleavable peptide.

The masking moiety, half-life extension domains, IL-15 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-15 cytokine or functional fragment thereof. In some embodiments, the first polypeptide chain comprises:

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

and the second polypeptide chain comprises:

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

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-15 cytokine or functional fragment thereof.

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'). This arrangement is described herein as 'Structure A'. In some embodiments, the first polypeptide chain comprises:

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

and the second polypeptide chain comprises:

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

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'). This arrangement is described herein as 'Structure B' (and represents a 'flipped' version of Structure A). In some embodiments, the first polypeptide chain comprises:

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

and the second polypeptide chain comprises:

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

### 1.3 IL-15 Cytokines

Provided herein is an IL-15 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-15 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, IL-15 is synthesized as a precursor polypeptide of 162 amino acids (SEQ ID NO: 1), which is then processed into mature IL-15 by the removal of amino acid residues 1-48. This results in a mature form of IL-15 consisting of 114 amino acids (amino acid residues 49-162) that is secreted in a mature, active form (*see* SEQ ID NO: 2).

IL-15 precursor polypeptide (SEQ ID NO: 1):
MRISKPHLRSISIQCYLCLLLNSHFLTEAGIHVFILGCFSAGLPKTEANWVNVISDLKKIEDLIQSM
HIDATLYTESDVHPSCKVTAMKCFLLELQVISLESGDASIHDTVENLIILANNSLSSNGNVTESGC
KECEELEEKNIKEFLQSFVHIVQMFINTS

IL-15 mature polypeptide (SEQ ID NO: 2):
NWVNVISDLKKIEDLIQSMHIDATLYTESDVHPSCKVTAMKCFLLELQVISLESGDASIHDTVENL
IILANNSLSSNGNVTESGCKECEELEEKNIKEFLQSFVHIVQMFINTS

The term "IL-15" or "IL-15 polypeptide" as used herein refers to any interleukin-15 (IL-15) protein, or a functional fragment or variant thereof. The term encompasses any native IL-15 from any vertebrate source, including mammals such as primates (e.g., humans) and rodents (e.g., rats and mice).The term encompasses unprocessed IL-15 (e.g., a full length, precursor form of IL-15 that consists of amino acid residues 1-162) as well as any form of IL-15 that results from processing in the cell (e.g., a mature form of IL-15 that consists of amino acid residues 49-162). As such, the term encompasses a protein encoded by the amino acid sequence of SEQ ID NO: 2, as well as sequence variants thereof. The term also encompasses naturally occurring variants of IL-15. The term also encompasses non-naturally occurring variants of IL-15, such as truncations, deletions, forms where IL-15 is linked to another molecule, and variants caused by at least one amino acid change to the amino acid sequence (e.g., by substitution, addition, or deletion). In some aspects, the variants or homologs have at least 90%, 95%, 96%, 97%, 98%, 99% or 100% amino acid sequence identity across the whole sequence or a portion of the sequence (e.g., a 50, 100, or 114 continuous amino acid portion) compared to a naturally occurring IL-15 polypeptide, such as an IL-15 polypeptide encoded by the amino acid sequence of SEQ ID NO: 1 or 2. As such, the term "IL-15" or "IL-15 polypeptide" includes an IL- 15 protein comprising the amino acid sequence of SEQ ID NO: 1 or 2, including variants thereof, such as variants created by one or more amino acid substitutions to the amino acid sequence of SEQ ID NO: 1 or 2.

"Functional fragments" of an IL-15 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-15 cytokine or functional fragment thereof is any naturally occurring interleukin-2 (IL-15) protein or modified variant thereof capable of binding to an interleukin-2 receptor, particularly the IL-15Rα chain.

In some embodiments, the IL-15 cytokine or fragment thereof comprises SEQ ID NO: 2 or a functional fragment thereof.

In some embodiments, the IL-15 cytokine or functional fragment thereof comprises an amino acid sequence of SEQ ID NO: 2.

In some embodiments, the IL-15 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-15 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-15 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-15 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: 2.

In some embodiments, the IL-15 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. In some embodiments, the IL-15 cytokine or functional fragment thereof comprises an amino acid sequence having one or more amino acid substitutions at positions D22, E46, E53 as compared to the amino acid sequence of SEQ ID NO: 2. In some embodiments, the IL-15 cytokine or functional fragment thereof comprises an amino acid sequence having one or more amino acid substitutions at positions D22, E46, E53, N71, N79, N112 as compared to the amino acid sequence of SEQ ID NO: 2.

In some embodiments, the IL-15 cytokine or functional fragment thereof comprises an amino acid sequence having an amino acid substitution at position D22 as compared to the amino acid sequence of SEQ ID NO: 2.

In some embodiments, the IL-15 cytokine or functional fragment thereof comprises an amino acid sequence having an amino acid substitution at position E46 as compared to the amino acid sequence of SEQ ID NO: 2.

In some embodiments, the IL-15 cytokine or functional fragment thereof comprises an amino acid sequence having an amino acid substitution at position E53 as compared to the amino acid sequence of SEQ ID NO: 2.

In some embodiments, the IL-15 cytokine or functional fragment thereof comprises an amino acid sequence having an amino acid substitution at position N71 as compared to the amino acid sequence of SEQ ID NO: 2.

In some embodiments, the IL-15 cytokine or functional fragment thereof comprises an amino acid sequence having an amino acid substitution at position N79 as compared to the amino acid sequence of SEQ ID NO: 2.

In some embodiments, the IL-15 cytokine or functional fragment thereof comprises an amino acid sequence having an amino acid substitution at position N112 as compared to the amino acid sequence of SEQ ID NO: 2.

In some embodiments, the IL-15 cytokine or functional fragment thereof comprises an amino acid sequence having amino acid substitutions at positions E46 and E53 as compared to the amino acid sequence of SEQ ID NO: 2.

In some embodiments, the IL-15 cytokine or functional fragment thereof comprises an amino acid sequence having an amino acid substitution at position N71 and N79 as compared to the amino acid sequence of SEQ ID NO: 2.

In some embodiments, the IL-15 cytokine or functional fragment thereof comprises an amino acid sequence having an amino acid substitution at position N71 and N112 as compared to the amino acid sequence of SEQ ID NO: 2.

In some embodiments, the IL-15 cytokine or functional fragment thereof comprises an amino acid sequence having an amino acid substitution at position N79 and N112 as compared to the amino acid sequence of SEQ ID NO: 2.

In some embodiments, the IL-15 cytokine or functional fragment thereof comprises an amino acid sequence having an amino acid substitution at position N71, N79 and N112 as compared to the amino acid sequence of SEQ ID NO: 2.

In some embodiments, the amino acid substitution at position D22 is D22A.
In some embodiments, the amino acid substitution at position E46 is E46A.
In some embodiments, the amino acid substitution at position E46 is E46R.
In some embodiments, the amino acid substitution at position E46 is E46S.
In some embodiments, the amino acid substitution at position E53 is E53A.
In some embodiments, the amino acid substitution at position E53 is E53R.
In some embodiments, the amino acid substitution at position E53 is E53S.
In some embodiments, the amino acid substitution at position N71 is N71Q.
In some embodiments, the amino acid substitution at position N79 is N79Q.
In some embodiments, the amino acid substitution at position N112 is N112Q.

In some embodiments, the IL-15 cytokine or functional fragment thereof comprises an amino acid sequence having an amino acid substitution D22A as compared to the amino acid sequence of SEQ ID NO: 2. In some embodiments, the IL-15 cytokine or functional fragment thereof comprises an amino acid sequence

of SEQ ID NO: 3. In some embodiments, the IL-15 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-15 cytokine or functional fragment thereof comprises an amino acid sequence having an amino acid substitution E46A as compared to the amino acid sequence of SEQ ID NO: 2. In some embodiments, the IL-15 cytokine or functional fragment thereof comprises an amino acid sequence of SEQ ID NO: 4. In some embodiments, the IL-15 cytokine or functional fragment thereof comprises an amino acid sequence having about or at least about 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% sequence identity to the amino acid sequence of SEQ ID NO: 4.

In some embodiments, the IL-15 cytokine or functional fragment thereof comprises an amino acid sequence having amino acid substitutions E46A and E53A as compared to the amino acid sequence of SEQ ID NO: 2. In some embodiments, the IL-15 cytokine or functional fragment thereof comprises an amino acid sequence of SEQ ID NO: 5. In some embodiments, the IL-15 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: 5.

In some embodiments, the IL-15 cytokine or functional fragment thereof comprises an amino acid sequence having amino acid substitutions E46R and E53R as compared to the amino acid sequence of SEQ ID NO: 2. In some embodiments, the IL-15 cytokine or functional fragment thereof comprises an amino acid sequence of SEQ ID NO: 6. In some embodiments, the IL-15 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: 6.

In some embodiments, the IL-15 cytokine or functional fragment thereof comprises an amino acid sequence having amino acid substitutions E46S and E53S as compared to the amino acid sequence of SEQ ID NO: 2. In some embodiments, the IL-15 cytokine or functional fragment thereof comprises an amino acid sequence of SEQ ID NO: 7. In some embodiments, the IL-15 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: 7.

In some embodiments, the IL-15 cytokine or functional fragment thereof comprises an amino acid sequence having an amino acid substitution E53A as compared to the amino acid sequence of SEQ ID NO: 2. In some embodiments, the IL-15 cytokine or functional fragment thereof comprises an amino acid sequence of SEQ ID NO: 8. In some embodiments, the IL-15 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: 8.

In some embodiments, the IL-15 cytokine or functional fragment thereof comprises an amino acid sequence of SEQ ID NO: 9 and an amino acid sequence of SEQ ID NO: 10. In some embodiments, the IL-15 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: 9 and 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: 10.

In some embodiments, the IL-15 cytokine or functional fragment thereof comprises an amino acid sequence having an amino acid substitution N71Q as compared to the amino acid sequence of SEQ ID NO: 2. In some embodiments, the IL-15 cytokine or functional fragment thereof comprises an amino acid sequence of SEQ ID NO: 127. In some embodiments, the IL-15 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: 127.

In some embodiments, the IL-15 cytokine or functional fragment thereof comprises an amino acid sequence having an amino acid substitution N79Q as compared to the amino acid sequence of SEQ ID NO: 2. In some embodiments, the IL-15 cytokine or functional fragment thereof comprises an amino acid sequence of SEQ ID NO: 128. In some embodiments, the IL-15 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: 128.

In some embodiments, the IL-15 cytokine or functional fragment thereof comprises an amino acid sequence having an amino acid substitution N112Q as compared to the amino acid sequence of SEQ ID NO: 2. In some embodiments, the IL-15 cytokine or functional fragment thereof comprises an amino acid sequence of SEQ ID NO: 129. In some embodiments, the IL-15 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: 129.

In some embodiments, the IL-15 cytokine or functional fragment thereof comprises an amino acid sequence having an amino acid substitution N71Q and N79Q as compared to the amino acid sequence of SEQ ID NO: 2. In some embodiments, the IL-15 cytokine or functional fragment thereof comprises an amino acid sequence of SEQ ID NO: 130. In some embodiments, the IL-15 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: 130.

In some embodiments, the IL-15 cytokine or functional fragment thereof comprises an amino acid sequence having an amino acid substitution N71Q and N112Q as compared to the amino acid sequence of SEQ ID NO: 2. In some embodiments, the IL-15 cytokine or functional fragment thereof comprises an amino acid sequence of SEQ ID NO: 131. In some embodiments, the IL-15 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: 131.

In some embodiments, the IL-15 cytokine or functional fragment thereof comprises an amino acid sequence having an amino acid substitution N79Q and N112Q as compared to the amino acid sequence of SEQ ID NO: 2. In some embodiments, the IL-15 cytokine or functional fragment thereof comprises an amino acid sequence of SEQ ID NO: 132. In some embodiments, the IL-15 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: 132.

In some embodiments, the IL-15 cytokine or functional fragment thereof comprises an amino acid sequence having an amino acid substitution N71Q, N79Q and N112Q as compared to the amino acid sequence of SEQ ID NO: 2. In some embodiments, the IL-15 cytokine or functional fragment thereof comprises an amino acid sequence of SEQ ID NO: 133. In some embodiments, the IL-15 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: 133.

Sequences are shown in Table 1 below.

In some embodiments, an additional mutation may be included in any of the sequences above at position N71. In some embodiments, the mutation is N71A, N71R, N71W, N71F, N71P, N71M, N71L, N71T, N71S, or N71Y.

In some embodiments, an additional mutation may be included in any of the sequences above at position S73. In some embodiments, the mutation is S73A, S73W, S73V, or S73M.

In some embodiments, an additional mutation may be included in any of the sequences above at one or more of amino acid positions N72, N79, V80, T81, and N112. In some embodiments, one or more additional

mutations selected from N72A, N79A, V80A, T81A and N112R may be included in any of the sequences above.

In some embodiments, an additional mutation may be included in any of the sequences above at one or more of amino acid positions N72, S73, N79, V80, T81, and N112. In some embodiments, one or more additional mutations N72A, S73A, N79A, V80A, T81A, and N112 may be included in any of the sequences above.

In some embodiments, the IL-15 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-15 of SEQ ID 2, for the purpose of removing an O-glycosylation site. In some embodiments, the IL-15 cytokine or functional fragment thereof has one or more amino acid residues substituted as compared to the amino acid sequence of the mature IL-15 of SEQ ID 2, for the purpose of removing an O-glycosylation site. In some embodiments, the IL-15 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-15 of SEQ ID 2, for the purpose of removing an O-glycosylation site. In some embodiments, the IL-15 cytokine or functional fragment thereof does not have an O-glycosylation site within residues 1-3.

### 1.4 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-15 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-15 cytokine or functional fragment thereof to IL-15Rα. 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-15 cytokine or functional fragment thereof, such as an anti-IL-15 antibody or IL-15 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-15 cytokine receptor, or a subunit or functional fragment thereof.

In some embodiments, the masking moiety comprises IL-15Rβ (also referred to as CD122) or a fragment, portion, or variant thereof that retains or otherwise demonstrates an affinity to IL-15.

The wild type sequence of IL-15Rβ is shown in SEQ ID NO: 11.

In some embodiments, the masking moiety comprises the amino acid sequence of SEQ ID NO: 11. In some embodiments, the masking moiety comprises an amino acid sequence having about or at least about 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% sequence identity to the amino acid sequence of SEQ ID NO: 11. In some embodiments, the masking moiety comprises an amino acid sequence having the amino acid sequence of SEQ ID NO: 11 with one to four amino acid substitutions In some embodiments, the masking moiety comprises an amino acid sequence having the amino acid sequence of SEQ ID NO: 11 with one or two amino acid substitutions.

In some embodiments, the masking moiety comprises IL-15Rβ (or a functional fragment, portion, or variant thereof), where the IL-15Rβ has an amino acid substitution at position C122.

In some embodiments, the masking moiety comprises IL-15Rβ (or a functional fragment, portion, or variant thereof), where the IL-15Rβ has amino acid substitution C122S.

In some embodiments, the IL-15Rβ or a fragment, portion or variant thereof has an amino acid substitution at position C122 as compared to IL-15Rβ of SEQ ID NO: 11.

In some embodiments, the IL-15Rβ or a fragment, portion or variant thereof has mutation C122S at amino acid position 122 as compared to IL-15Rβ of SEQ ID NO: 11.

In some embodiments, the masking moiety comprises an amino acid sequence of SEQ ID NO: 11 with a C122 mutation.

In some embodiments, the masking moiety comprises an amino acid sequence of SEQ ID NO: 11 with a C122S mutation.

In some embodiments, the masking moiety comprises IL-15Rβ (or a functional fragment, portion, or variant thereof), where the IL-15Rβ has an amino acid substitution at position C168.

In some embodiments, the masking moiety comprises IL-15Rβ (or a functional fragment, portion, or variant thereof), where the IL-15Rβ has amino acid substitution C168S.

In some embodiments, the IL-15Rβ or a fragment, portion or variant thereof has mutation at amino acid position C168 as compared to IL-15Rβ of SEQ ID NO: 11.

In some embodiments, the IL-15Rβ or a fragment, portion or variant thereof has mutation C168S at amino acid position 168 as compared to IL-15Rβ of SEQ ID NO: 11.

In some embodiments, the masking moiety comprises an amino acid sequence of SEQ ID NO: 11 with a C168 mutation.

In some embodiments, the masking moiety comprises an amino acid sequence of SEQ ID NO: 11 with a C168S mutation.

The masked cytokine of any one of claims, wherein the IL-15Rβ or a fragment, portion or variant thereof has mutation at amino acid positions C122 and C168 as compared to IL-15Rβ of SEQ ID NO: 11.

The masked cytokine of any one of claims, wherein the IL-15Rβ or a fragment, portion or variant thereof has mutation C122S and C168S as compared to IL-15Rβ of SEQ ID NO: 11.

In some embodiments, the masking moiety comprises an amino acid sequence of SEQ ID NO: 12.

Sequences are shown in Table 2.

### 1.5 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 non-cleavable linkers and cleavable linkers of some embodiments are described in more detail below.

#### 1.5.1 Non-Proteolytically Cleavable Linkers

In some embodiments, the non-cleavable linker is between 3 and 25 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.

***Non-Proteolytically Cleavable Linkers in 'Linear' Masked Cytokines and 'Heterodimeric' Masked Cytokines of Structure A***

In some embodiments of Structure A of the heterodimeric masked cytokine, 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 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: 22 (PGSGS).

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 25 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: 22 (PGSGS).

Sequences are shown in Table 3.

*Non-Proteolytically Cleavable Linkers in 'Heterodimeric' Masked Cytokine of Structure B*

In some embodiments of Structure B of the heterodimeric masked cytokine, the non-cleavable linker is between 15 and 25 amino acids in length. In some embodiments, the non-cleavable linker is between 18 and 22 amino acids in length. In some embodiments, the non-cleavable linker comprises an amino acid sequence as shown in SEQ ID NO: 21 (GGSSPPGGGSSGGGSGPSGSPG).

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 25 amino acids in length. In some embodiments, the non-cleavable linker comprises an amino acid sequence as shown in SEQ ID NO: 21 (GGSSPPGGGSSGGGSGP).

Sequences are shown in Table 3.

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.

### 1.5.2 Proteolytically Cleavable Linkers

*Proteolytically 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-15 cytokine receptor, particularly IL-15Rα.

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 as shown below:

| Sequence (* indicates a cleavage site within the cleavable peptide) |
|---|
| MPYD*LYHP |
| DSGG*FMLT |
| HEQ*LTV |
| RAAA*VKSP |
| VPLS*LY |
| DLLA*VVAAS |
| 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: 33 (MPYD*LYHP).

In some embodiments, the cleavable peptide comprises an amino acid sequence of SEQ ID NO: 34 (VPLS*LY) , for example the cleavable peptide may comprise an amino acid sequence of SEQ ID NO: 320 (VPLSLYSG).

In some embodiments, the cleavable peptide comprises an amino acid sequence of SEQ ID NO: 27 (RAAA*VKSP).

In some embodiments, the cleavable peptide comprises an amino acid sequence of SEQ ID NO: 134 (DSGG*FMLT).

In some embodiments, the cleavable peptide comprises an amino acid sequence of SEQ ID NO: 135 (HEQ*LTV).

In some embodiments, the cleavable peptide comprises an amino acid sequence of SEQ ID NO: 155 (ISSGLL*SG*RS), for example the cleavable peptide may comprise an amino acid sequence of SEQ ID NO: 321 (ISSGLLSGRSDQP).

In some embodiments, the cleavable peptide comprises an amino acid sequence of SEQ ID NO: 156 (DLLA*VVAAS).

In some embodiments, the cleavable peptide consists of an amino acid sequence of SEQ ID NO: 33 (MPYD*LYHP).

In some embodiments, the cleavable peptide consists of an amino acid sequence of SEQ ID NO: 34 (VPLS*LY). In some embodiments, the cleavable peptide consists of an amino acid sequence of SEQ ID NO: 320 (VPLSLYSG).

In some embodiments, the cleavable peptide consists of an amino acid sequence of SEQ ID NO: 27 (RAAA*VKSP).

In some embodiments, the cleavable peptide consists of an amino acid sequence of SEQ ID NO: 134 (DSGG*FMLT).

In some embodiments, the cleavable peptide consists of an amino acid sequence of SEQ ID NO: 135 (HEQ*LTV).

In some embodiments, the cleavable peptide consists of an amino acid sequence of SEQ ID NO: 155 (ISSGLL*SG*RS). In some embodiments, the cleavable peptide consists of an amino acid sequence of SEQ ID NO: 321 (ISSGLLSGRSDQP).

In some embodiments, the cleavable peptide consists of an amino acid sequence of SEQ ID NO: 156 (DLLA*VVAAS).

Cleavable peptides having an amino acid sequence as shown in SEQ ID NOs: 155 or 156 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-15 cytokine as disclosed anywhere herein, any systemic side effects of the administered IL-15 cytokine or functional fragment thereof may be further reduced.

***Other Linker Domains***

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):

SD-CP-SD

In some embodiments, the cleavable linker comprises:

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

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

In some embodiments, the masked cytokine further comprises a domain comprising an IL-15Rα subunit or a functional fragment thereof ('IL-15Rα domain'). Incorporating an 'IL-15Rα domain' into a masked cytokine construct has been demonstrated to increase the potency of said cytokine in activating CD8 T cell and NK cells.

The IL-15Rα subunit (also referred to as CD215) is structurally similar to IL-2Rα; the ectodomain of IL-15Rα consists of a single protein-binding Sushi domain, a membrane-proximal proline-threonine-rich (PT) region, and a linker/hinge region that connects the sushi domain and the PT region. The IL-15Rα subunit specifically binds IL-15 with very high affinity and is capable of binding IL-15 independently of the β and γ subunits.

Interleukin (IL)-15 is a cytokine that acts on a wide range of cell types but is most crucial for the development, homeostasis, and function of a specific group of immune cells that includes CD8 T cells, NK cells, NKT cells, and CD8αα intraepithelial lymphocytes. IL-15 signals are transmitted through the IL-2/15Rβ and common γ (γC) chains; however, it is the delivery of IL-15 to these signalling components that is quite unique. As opposed to other cytokines that are secreted, IL-15 primarily exists bound to the high affinity IL-15Rα. When IL-15/IL-15Rα complexes are shuttled to the cell surface, they can stimulate opposing cells through the β/γC receptor complex. This novel mechanism of IL-15 delivery has been called trans-presentation (S. W. Stonier and K. S. Schluns, 'Trans-presentation: a novel mechanism regulating IL-15 delivery and responses', Immunol Lett Jan 4 2010; 127(2): 85-92, the contents of which is incorporated herein by reference).

The IL-15Rα subunit comprises a conserved protein binding motif called a sushi domain. The sushi domain sIL-15Rα, which comprises amino acids 31 to 95 of the IL-15Rα subunit, is responsible for interacting with IL-15 and is essential for IL-15/ IL-15Rα function (Wei X *et al.* 'The Sushi Domain of Soluble IL-15 Receptor α Is Essential for Binding IL-15 and Inhibiting Inflammatory and Allogenic Responses In Vitro and In Vivo', J Immunol July 1 2001; 167(1) 277-282, the contents of which is incorporated herein by reference).

The sequence of the wild-type IL-15Rα subunit is shown below, along with a breakdown of the main domains:

```
         10          20          30          40          50
MAPRRARGCR TLGLPALLLL LLLRPPATRG ITCPPPMSVE HADIWVKSYS
         60          70          80          90          100
LYSRERYICN SGFKRKAGTS SLTECVLNKA TNVAHWTTPS LKCIRDPALV
         110         120         130         140         150
HQRPAPPSTV TTAGVTPQPE SLSPSGKEPA ASSPSSNNTA ATTAAIVPGS
         160         170         180         190         200
QLMPSKSPST GTTEISSHES SHGTPSQTTA KNWELTASAS HQPPGVYPQG
         210         220         230         240         250
HSDTTVAIST STVLLCGLSA VSLLACYLKS RQTPPLASVE MEAMEALPVT
         260
WGTSSRDEDL ENCSHHL
```

>sp|Q13261|1-30 (signal peptide)
MAPRRARGCRTLGLPALLLLLLLRPPATRG

>sp|Q13261|31-205 (Extracellular domain) [Note: 31-95 is canonical "Sushi domain"]
ITCPPPMSVEHADIWVKSYSLYSRERYICNSGFKRKAGTSSLTECVLNKATNVAHWTTPS
LKCIRDPALVHQRPAPPSTVTTAGVTPQPESLSPSGKEPAASSPSSNNTAATTAAIVPGS
QLMPSKSPSTGTTEISSHESSHGTPSQTTAKNWELTASASHQPPGVYPQGHSDTT

>sp|Q13261|206-228 (Transmembrane domain)
VAISTSTVLLCGLSAVSLLACYL

>sp|Q13261|229-267 (Cytoplasmic domain)
KSRQTPPLASVEMEAMEALPVTWGTSSRDEDLENCSHHL

The 'IL-15Rα domain' herein can comprise of the sequence of the extracellular domain of the wild-type IL-15Rα subunit or a variant thereof, such as the sequence of the extracellular domain of the wild-type IL-

15Rα subunit with one or more e.g. 1, 2, 3 or 4 amino acid substitutions. In some embodiments, the IL-15Rα subunit comprises an amino acid sequence of SEQ ID NO: 55.

The 'IL-15Rα domain' herein can comprise the sequence of the wild-type sushi domain sIL-15Rα or a variant thereof, such as the sequence of the wild-type sushi domain sIL-15Rα with one or more e.g. 1, 2, 3 or 4 amino acid substitutions.

In some embodiments, the IL-15Rα subunit comprises an amino acid sequence of SEQ ID NO: 56. In some embodiments, the IL-15Rα subunit comprises an amino acid sequence of SEQ ID NO: 57. In some embodiments, the IL-15Rα subunit comprises an amino acid sequence of SEQ ID NO: 58.

The 'IL-15Rα domain' herein can consist of the sequence of the wild-type sushi domain sIL-15Rα or a variant thereof, such as the sequence of the wild-type sushi domain sIL-15Rα with one or more e.g. 1, 2, 3 or 4 amino acid substitutions. In some embodiments, the IL-15Rα subunit consists of an amino acid sequence of SEQ ID NO: 56. In some embodiments, the IL-15Rα subunit consists of an amino acid sequence of SEQ ID NO: 56 with one or more amino acid substitutions, such as one, two or three substitutions.

In some embodiments, the IL-15Rα comprises an amino acid substitution at position R26. In some embodiments, the IL-15Rα comprises amino acid substitution R26N. In some embodiments, the IL-15Rα comprises amino acid substitution R26S. In some embodiments, the IL-15Rα comprises an amino acid substitution at position R35. In some embodiments, the IL-15Rα comprises amino acid substitution R35Q. In some embodiments, the IL-15Rα comprises amino acid substitution R35S. In some embodiments, the IL-15Rα comprises an amino acid substitution at positions R26 and R35. In some embodiments, the IL-15Rα comprises amino acid substitutions R26S or R26N, and R35Q or R35S. In some embodiments, the IL-15Rα comprises amino acid substitutions R26N and R35Q.

In some embodiments, the IL-15Rα comprises an amino acid sequence of SEQ ID NOs: 55, 56, 57 or 58, with an additional amino acid substitution at position R26. In some embodiments, the IL-15Rα comprises an amino acid sequence of SEQ ID NOs: 55, 56, 57 or 58, with additional amino acid substitution R26N. In some embodiments, the IL-15Rα comprises an amino acid sequence of SEQ ID NOs: 55, 56, 57 or 58, with additional amino acid substitution R26S. In some embodiments, the IL-15Rα comprises an amino acid sequence of SEQ ID NOs: 55, 56, 57 or 58, with an additional amino acid substitution at position R35. In some embodiments, the IL-15Rα comprises an amino acid sequence of SEQ ID NOs: 55, 56, 57 or 58, with additional amino acid substitution R35Q. In some embodiments, the IL-15Rα comprises an amino acid sequence of SEQ ID NOs: 55, 56, 57 or 58, with additional amino acid substitution R35S. In some embodiments, the IL-15Rα comprises an amino acid sequence of SEQ ID NOs: 55, 56, 57 or 58, with additional amino acid substitutions at positions R26 and R35. In some embodiments, the IL-15Rα

comprises an amino acid sequence of SEQ ID NOs: 55, 56, 57 or 58, with additional amino acid substitutions R26S or R26N, and R35Q or R35S. In some embodiments, the IL-15Rα comprises an amino acid sequence of SEQ ID NOs: 55, 56, 57 or 58, with additional amino acid substitutions R26N and R35Q.

In some embodiments, the IL-15Rα comprises an amino acid sequence of SEQ ID NO: 56, with an additional amino acid substitution at position R26. In some embodiments, the IL-15Rα comprises an amino acid sequence of SEQ ID NO: 56, with additional amino acid substitution R26N. In some embodiments, the IL-15Rα comprises an amino acid sequence of SEQ ID NO: 56, with additional amino acid substitution R26S. In some embodiments, the IL-15Rα comprises an amino acid sequence of SEQ ID NO: 56, with an additional amino acid substitution at position R35. In some embodiments, the IL-15Rα comprises an amino acid sequence of SEQ ID NO: 56, with additional amino acid substitution R35Q. In some embodiments, the IL-15Rα comprises an amino acid sequence of SEQ ID NO: 56, with additional amino acid substitution R35S. In some embodiments, the IL-15Rα comprises an amino acid sequence of SEQ ID NO: 56, with additional amino acid substitutions at positions R26 and R35. In some embodiments, the IL-15Rα comprises an amino acid sequence of SEQ ID NO: 56, with additional amino acid substitutions R26S or R26N, and R35Q or R35S. In some embodiments, the IL-15Rα comprises an amino acid sequence of SEQ ID NO: 56, with additional amino acid substitutions R26N and R35Q. (See SEQ ID NO: 172, 173, 174, 175 and 176.)

In one embodiment, the sequence of the 'IL-15Rα domain' is the wild type sequence.

In some embodiments, the IL-15Rα domain comprises an IL-15Rα subunit or a fragment thereof and a 'α subunit linker'.

Sequences are shown in Table 5.

In some embodiments of the 'linear' masked cytokine, the masked cytokine comprises a polypeptide chain comprising:

N' **HL-non cleavable L2-MM- SD1-CP-SD2 -C** C'

In some embodiments of the 'linear' masked cytokine, the masked cytokine comprises a first polypeptide chain comprising:

N' **HL1** C'

and a second polypeptide chain comprising:

N' **HL2-non cleavable L2-MM- SD1-CP-SD2 -C** C'

In some embodiments of the 'linear' masked cytokine, the masked cytokine comprises a first polypeptide chain comprising:

N' **HL1** C'

and a second polypeptide chain comprising:

N' **HL2-non cleavable L2-MM- ['IL-15Rα domain']-SD1-CP-SD2 -C** C'

This is shown schematically below:




In some embodiments of the 'linear' masked cytokine, the masked cytokine comprises a first polypeptide chain comprising:

N' **HL1** C'

and a second polypeptide chain comprising:

N' **HL2-non cleavable L2-MM- SD1-CP-SD2-['IL-15Rα domain']-C** C'

This is shown schematically below:




In some embodiments of the 'heterodimeric' masked cytokine of Structure A, the first polypeptide chain comprises:

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

and the second polypeptide chain comprises:

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

This is shown schematically below:



In some embodiments of the 'heterodimeric' masked cytokine, the domain comprising an IL-15Rα subunit or fragment thereof is covalently attached to the IL-15 cytokine or fragment thereof such that:
the first polypeptide chain comprises:

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

and the second polypeptide chain comprises:

N' **HL2- SD1-CP-SD2 –[C(-['IL-15Rα domain']) ]** C'

In some embodiments of the 'heterodimeric' masked cytokine of Structure A, the domain comprising an IL-15Rα subunit or fragment thereof is inserted in an IL-15 cytokine or fragment thereof such that:
the first polypeptide chain comprises:

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

and the second polypeptide chain comprises:

N' **HL2- SD1-CP-SD2 – [N' terminal domain of C]-['IL-15Rα domain']-[C' terminal domain of C]** C'

where the IL-15Rα domain comprises an IL-15Rα subunit or a fragment thereof flanked on either side by a non-cleavable 'α subunit linker'.
This is shown schematically below:




In some embodiments, the N' terminal domain of C comprises an amino acid sequence of SEQ ID NO: 9.
In some embodiments, the C' terminal domain of C comprises an amino acid sequence of SEQ ID NO: 10.

In some embodiments of the 'heterodimeric' masked cytokine of Structure A, the domain comprising an IL-15Rα subunit or fragment thereof is covalently attached to the C' terminal of the IL-15 cytokine or fragment thereof, such that the first polypeptide chain comprises:

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

and the second polypeptide chain comprises:

N' **HL2- SD1-CP-SD2 –C-['IL-15Rα domain']** C'

This is shown schematically below:




In some embodiments of the 'heterodimeric' masked cytokine of Structure A, the first polypeptide chain comprises:

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

and the second polypeptide chain comprises:

N' **HL2--['IL-15Rα domain']- SD1-CP-SD2 - C** C'

This is shown schematically below:




In some embodiments of the 'heterodimeric' masked cytokine of Structure B, the first polypeptide chain comprises:

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

and the second polypeptide chain comprises:

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

This is shown schematically below:




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 N-terminus of SD1 is a glycine (G).

In some embodiments, the first spacer domain (SD1) is between 3 and 18 amino acids in length. In some embodiments, the first spacer domain (SD1) is between 6 and 14 amino acids in length. In some embodiments, the first spacer domain (SD1) is between 6 and 9 amino acids in length.

In some embodiments, SD1 comprises SEQ ID NO: 23.
In some embodiments, SD1 comprises SEQ ID NO: 24.
In some embodiments, SD1 comprises SEQ ID NO: 25.
In some embodiments, SD1 comprises SEQ ID NO: 26.
In some embodiments, SD1 comprises SEQ ID NO: 197.
In some embodiments, SD1 comprises SEQ ID NO: 28.
In some embodiments, SD1 comprises SEQ ID NO: 29.
In some embodiments, SD1 comprises SEQ ID NO: 30.
In some embodiments, SD1 comprises SEQ ID NO: 31.
In some embodiments, SD1 comprises SEQ ID NO: 32.
In some embodiments, SD1 comprises SEQ ID NO: 148.
In some embodiments, SD1 comprises SEQ ID NO: 149.
In some embodiments, SD1 comprises SEQ ID NO: 150.
In some embodiments, SD1 comprises SEQ ID NO: 151.
In some embodiments, SD1 comprises SEQ ID NO: 152.
In some embodiments, SD1 comprises SEQ ID NO: 153.
In some embodiments, SD1 comprises SEQ ID NO: 154.

In some embodiments, SD1 consists of SEQ ID NO: 23.
In some embodiments, SD1 consists of SEQ ID NO: 24.
In some embodiments, SD1 consists of SEQ ID NO: 25.
In some embodiments, SD1 consists of SEQ ID NO: 26.
In some embodiments, SD1 consists of SEQ ID NO: 197.
In some embodiments, SD1 consists of SEQ ID NO: 28.

In some embodiments, SD1 consists of SEQ ID NO: 29.
In some embodiments, SD1 consists of SEQ ID NO: 30.
In some embodiments, SD1 consists of SEQ ID NO: 31.
In some embodiments, SD1 consists of SEQ ID NO: 32.
In some embodiments, SD1 consists of SEQ ID NO: 148.
In some embodiments, SD1 consists of SEQ ID NO: 149.
In some embodiments, SD1 consists of SEQ ID NO: 150.
In some embodiments, SD1 consists of SEQ ID NO: 151.
In some embodiments, SD1 consists of SEQ ID NO: 152.
In some embodiments, SD1 consists of SEQ ID NO: 153.
In some embodiments, SD1 consists of SEQ ID NO: 154.

In some embodiments, the second spacer domain (SD2) is between 2 and 10 amino acids in length. In some embodiments, the second spacer domain (SD2) is between 3 and 8 amino acids in length.

In some embodiments, SD2 comprises SEQ ID NO: 35.
In some embodiments, SD2 comprises SEQ ID NO: 36.
In some embodiments, SD2 comprises SEQ ID NO: 37.
In some embodiments, SD2 comprises SEQ ID NO: 38.
In some embodiments, SD2 comprises SEQ ID NO: 39.
In some embodiments, SD2 comprises SEQ ID NO: 40.
In some embodiments, SD2 comprises SEQ ID NO: 157.
In some embodiments, SD2 comprises SEQ ID NO: 158.
In some embodiments, SD2 comprises SEQ ID NO: 159.
In some embodiments, SD2 comprises SEQ ID NO: 160.
In some embodiments, SD2 comprises SEQ ID NO: 161.

In some embodiments, SD2 consists of SEQ ID NO: 35.
In some embodiments, SD2 consists of SEQ ID NO: 36.
In some embodiments, SD2 consists of SEQ ID NO: 37.
In some embodiments, SD2 consists of SEQ ID NO: 38.
In some embodiments, SD2 consists of SEQ ID NO: 39.
In some embodiments, SD2 consists of SEQ ID NO: 40.
In some embodiments, SD2 consists of SEQ ID NO: 157.
In some embodiments, SD2 consists of SEQ ID NO: 158.
In some embodiments, SD2 consists of SEQ ID NO: 159.
In some embodiments, SD2 consists of SEQ ID NO: 160.
In some embodiments, SD2 consists of SEQ ID NO: 161.

In some embodiments, the cleavable linker comprises an SD1 comprising an amino acid sequence of SEQ ID NO: 23 and an SD2 comprising an amino acid sequence of SEQ ID NO: 38.

In some embodiments, the cleavable linker comprises an SD1 comprising an amino acid sequence of SEQ ID NO: 24 and an SD2 comprising an amino acid sequence of SEQ ID NO: 36.

In some embodiments, the cleavable linker comprises an SD1 comprising an amino acid sequence of SEQ ID NO: 24 and an SD2 comprising an amino acid sequence of SEQ ID NO: 40.

In some embodiments, the cleavable linker comprises an SD1 comprising an amino acid sequence of SEQ ID NO: 24 and an SD2 comprising an amino acid sequence of SEQ ID NO: 39.

In some embodiments, the cleavable linker comprises an SD1 comprising an amino acid sequence of SEQ ID NO: 25 and an SD2 comprising an amino acid sequence of SEQ ID NO: 35.

In some embodiments, the cleavable linker comprises an SD1 comprising an amino acid sequence of SEQ ID NO: 26 and an SD2 comprising an amino acid sequence of SEQ ID NO: 37.

In some embodiments, the cleavable linker comprises an SD1 comprising an amino acid sequence of SEQ ID NO: 26 and an SD2 comprising an amino acid sequence of SEQ ID NO: 36.

In some embodiments, the cleavable linker comprises an SD1 comprising an amino acid sequence of SEQ ID NO: 197 and an SD2 comprising an amino acid sequence of SEQ ID NO: 38.

In some embodiments, the cleavable linker comprises an SD1 comprising an amino acid sequence of SEQ ID NO: 28 and an SD2 comprising an amino acid sequence of SEQ ID NO: 38.

In some embodiments, the cleavable linker comprises an SD1 comprising an amino acid sequence of SEQ ID NO: 29 and an SD2 comprising an amino acid sequence of SEQ ID NO: 36.

In some embodiments, the cleavable linker comprises an SD1 comprising an amino acid sequence of SEQ ID NO: 30 and an SD2 comprising an amino acid sequence of SEQ ID NO: 36.

In some embodiments, the cleavable linker comprises an SD1 comprising an amino acid sequence of SEQ ID NO: 31 and an SD2 comprising an amino acid sequence of SEQ ID NO: 36.

In some embodiments, the cleavable linker comprises an SD1 comprising an amino acid sequence of SEQ ID NO: 32 and an SD2 comprising an amino acid sequence of SEQ ID NO: 36.

In some embodiments, the cleavable linker comprises an SD1 comprising an amino acid sequence of SEQ ID NO: 148 and an SD2 comprising an amino acid sequence of SEQ ID NO: 39.

In some embodiments, the cleavable linker comprises an SD1 comprising an amino acid sequence of SEQ ID NO: 149 and an SD2 comprising an amino acid sequence of SEQ ID NO: 39.

In some embodiments, the cleavable linker comprises an SD1 comprising an amino acid sequence of SEQ ID NO: 150 and an SD2 comprising an amino acid sequence of SEQ ID NO: 157.

In some embodiments, the cleavable linker comprises an SD1 comprising an amino acid sequence of SEQ ID NO: 151 and an SD2 comprising an amino acid sequence of SEQ ID NO: 159.

In some embodiments, the cleavable linker comprises an SD1 comprising an amino acid sequence of SEQ ID NO: 150 and an SD2 comprising an amino acid sequence of SEQ ID NO: 158.

In some embodiments, the cleavable linker comprises an SD1 comprising an amino acid sequence of SEQ

ID NO: 152 and an SD2 comprising an amino acid sequence of SEQ ID NO: 39.

In some embodiments, the cleavable linker comprises an SD1 comprising an amino acid sequence of SEQ ID NO: 153 and an SD2 comprising an amino acid sequence of SEQ ID NO: 160.

In some embodiments, the cleavable linker comprises an SD1 comprising an amino acid sequence of SEQ ID NO: 154 and an SD2 comprising an amino acid sequence of SEQ ID NO: 37.

In some embodiments, the cleavable linker comprises an SD1 comprising an amino acid sequence of SEQ ID NO: 26 and an SD2 comprising an amino acid sequence of SEQ ID NO: 161.

In some embodiments, the cleavable linker comprises an SD1 consisting of an amino acid sequence of SEQ ID NO: 23 and an SD2 consisting of an amino acid sequence of SEQ ID NO: 38.

In some embodiments, the cleavable linker comprises an SD1 consisting of an amino acid sequence of SEQ ID NO: 24 and an SD2 consisting of an amino acid sequence of SEQ ID NO: 36.

In some embodiments, the cleavable linker comprises an SD1 consisting of an amino acid sequence of SEQ ID NO: 24 and an SD2 consisting of an amino acid sequence of SEQ ID NO: 40.

In some embodiments, the cleavable linker comprises an SD1 consisting of an amino acid sequence of SEQ ID NO: 24 and an SD2 consisting of an amino acid sequence of SEQ ID NO: 39.

In some embodiments, the cleavable linker comprises an SD1 consisting of an amino acid sequence of SEQ ID NO: 25 and an SD2 consisting of an amino acid sequence of SEQ ID NO: 35.

In some embodiments, the cleavable linker comprises an SD1 consisting of an amino acid sequence of SEQ ID NO: 26 and an SD2 consisting of an amino acid sequence of SEQ ID NO: 37.

In some embodiments, the cleavable linker comprises an SD1 consisting of an amino acid sequence of SEQ ID NO: 26 and an SD2 consisting of an amino acid sequence of SEQ ID NO: 36.

In some embodiments, the cleavable linker comprises an SD1 consisting of an amino acid sequence of SEQ ID NO: 197 and an SD2 consisting of an amino acid sequence of SEQ ID NO: 38.

In some embodiments, the cleavable linker comprises an SD1 consisting of an amino acid sequence of SEQ ID NO: 28 and an SD2 consisting of an amino acid sequence of SEQ ID NO: 38.

In some embodiments, the cleavable linker comprises an SD1 consisting of an amino acid sequence of SEQ ID NO: 29 and an SD2 consisting of an amino acid sequence of SEQ ID NO: 36.

In some embodiments, the cleavable linker comprises an SD1 consisting of an amino acid sequence of SEQ ID NO: 30 and an SD2 consisting of an amino acid sequence of SEQ ID NO: 36.

In some embodiments, the cleavable linker comprises an SD1 consisting of an amino acid sequence of SEQ ID NO: 31 and an SD2 consisting of an amino acid sequence of SEQ ID NO: 36.

In some embodiments, the cleavable linker comprises an SD1 consisting of an amino acid sequence of SEQ ID NO: 32 and an SD2 consisting of an amino acid sequence of SEQ ID NO: 36.

In some embodiments, the cleavable linker comprises an SD1 consisting of an amino acid sequence of SEQ ID NO: 148 and an SD2 consisting of an amino acid sequence of SEQ ID NO: 39.

In some embodiments, the cleavable linker comprises an SD1 consisting of an amino acid sequence of SEQ ID NO: 149 and an SD2 consisting of an amino acid sequence of SEQ ID NO: 39.

In some embodiments, the cleavable linker comprises an SD1 consisting of an amino acid sequence of SEQ ID NO: 150 and an SD2 consisting of an amino acid sequence of SEQ ID NO: 157.
In some embodiments, the cleavable linker comprises an SD1 consisting of an amino acid sequence of SEQ ID NO: 151 and an SD2 consisting of an amino acid sequence of SEQ ID NO: 159.
In some embodiments, the cleavable linker comprises an SD1 consisting of an amino acid sequence of SEQ ID NO: 150 and an SD2 consisting of an amino acid sequence of SEQ ID NO: 158.
In some embodiments, the cleavable linker comprises an SD1 consisting of an amino acid sequence of SEQ ID NO: 152 and an SD2 consisting of an amino acid sequence of SEQ ID NO: 39.
In some embodiments, the cleavable linker comprises an SD1 consisting of an amino acid sequence of SEQ ID NO: 153 and an SD2 consisting of an amino acid sequence of SEQ ID NO: 160.
In some embodiments, the cleavable linker comprises an SD1 consisting of an amino acid sequence of SEQ ID NO: 154 and an SD2 consisting of an amino acid sequence of SEQ ID NO: 37.
In some embodiments, the cleavable linker comprises an SD1 consisting of an amino acid sequence of SEQ ID NO: 26 and an SD2 consisting of an amino acid sequence of SEQ ID NO: 161.

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: 155. 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: 156. 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 cleavable linker comprises an amino acid sequence of SEQ ID NO: 41.
In some embodiments, the cleavable linker comprises an amino acid sequence of SEQ ID NO: 42.
In some embodiments, the cleavable linker comprises an amino acid sequence of SEQ ID NO: 43.
In some embodiments, the cleavable linker comprises an amino acid sequence of SEQ ID NO: 44.
In some embodiments, the cleavable linker comprises an amino acid sequence of SEQ ID NO: 45.
In some embodiments, the cleavable linker comprises an amino acid sequence of SEQ ID NO: 46.
In some embodiments, the cleavable linker comprises an amino acid sequence of SEQ ID NO: 47.
In some embodiments, the cleavable linker comprises an amino acid sequence of SEQ ID NO: 48.
In some embodiments, the cleavable linker comprises an amino acid sequence of SEQ ID NO: 49.
In some embodiments, the cleavable linker comprises an amino acid sequence of SEQ ID NO: 50.
In some embodiments, the cleavable linker comprises an amino acid sequence of SEQ ID NO: 51.

In some embodiments, the cleavable linker comprises an amino acid sequence of SEQ ID NO: 52.
In some embodiments, the cleavable linker comprises an amino acid sequence of SEQ ID NO: 53.
In some embodiments, the cleavable linker comprises an amino acid sequence of SEQ ID NO: 54.
In some embodiments, the cleavable linker comprises an amino acid sequence of SEQ ID NO: 162.
In some embodiments, the cleavable linker comprises an amino acid sequence of SEQ ID NO: 163.
In some embodiments, the cleavable linker comprises an amino acid sequence of SEQ ID NO: 164.
In some embodiments, the cleavable linker comprises an amino acid sequence of SEQ ID NO: 165.
In some embodiments, the cleavable linker comprises an amino acid sequence of SEQ ID NO: 166.
In some embodiments, the cleavable linker comprises an amino acid sequence of SEQ ID NO: 167.
In some embodiments, the cleavable linker comprises an amino acid sequence of SEQ ID NO: 168.
In some embodiments, the cleavable linker comprises an amino acid sequence of SEQ ID NO: 169.
In some embodiments, the cleavable linker comprises an amino acid sequence of SEQ ID NO: 170.
In some embodiments, the cleavable linker comprises an amino acid sequence of SEQ ID NO: 171.

In some embodiments, the cleavable linker consists of an amino acid sequence of SEQ ID NO: 41.
In some embodiments, the cleavable linker consists of an amino acid sequence of SEQ ID NO: 42.
In some embodiments, the cleavable linker consists of an amino acid sequence of SEQ ID NO: 43.
In some embodiments, the cleavable linker consists of an amino acid sequence of SEQ ID NO: 44.
In some embodiments, the cleavable linker consists of an amino acid sequence of SEQ ID NO: 45.
In some embodiments, the cleavable linker consists of an amino acid sequence of SEQ ID NO: 46.
In some embodiments, the cleavable linker consists of an amino acid sequence of SEQ ID NO: 47.
In some embodiments, the cleavable linker consists of an amino acid sequence of SEQ ID NO: 48.
In some embodiments, the cleavable linker consists of an amino acid sequence of SEQ ID NO: 49.
In some embodiments, the cleavable linker consists of an amino acid sequence of SEQ ID NO: 50.
In some embodiments, the cleavable linker consists of an amino acid sequence of SEQ ID NO: 51.
In some embodiments, the cleavable linker consists of an amino acid sequence of SEQ ID NO: 52.
In some embodiments, the cleavable linker consists of an amino acid sequence of SEQ ID NO: 53.
In some embodiments, the cleavable linker consists of an amino acid sequence of SEQ ID NO: 54.
In some embodiments, the cleavable linker consists of an amino acid sequence of SEQ ID NO: 162.
In some embodiments, the cleavable linker consists of an amino acid sequence of SEQ ID NO: 163.
In some embodiments, the cleavable linker consists of an amino acid sequence of SEQ ID NO: 164.
In some embodiments, the cleavable linker consists of an amino acid sequence of SEQ ID NO: 165.
In some embodiments, the cleavable linker consists of an amino acid sequence of SEQ ID NO: 166.
In some embodiments, the cleavable linker consists of an amino acid sequence of SEQ ID NO: 167.
In some embodiments, the cleavable linker consists of an amino acid sequence of SEQ ID NO: 168.
In some embodiments, the cleavable linker consists of an amino acid sequence of SEQ ID NO: 169.
In some embodiments, the cleavable linker consists of an amino acid sequence of SEQ ID NO: 170.
In some embodiments, the cleavable linker consists of an amino acid sequence of SEQ ID NO: 171.

In some embodiments, the cleavable linker comprises SEQ ID NO: 168 and the non-cleavable linker comprises SEQ ID NO: 22.

In some embodiments, the cleavable linker comprises SEQ ID NO: 169 and the non-cleavable linker comprises SEQ ID NO: 22.

In some embodiments, the cleavable linker comprises SEQ ID NO: 170 and the non-cleavable linker comprises SEQ ID NO: 21.

In some embodiments, the cleavable linker comprises SEQ ID NO: 171 and the non-cleavable linker comprises SEQ ID NO: 21.

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: 168 and the non-cleavable linker comprises SEQ ID NO: 22. In some embodiments, the cleavable linker comprises SEQ ID NO: 169 and the non-cleavable linker comprises SEQ ID NO: 22.

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: 170 and the non-cleavable linker comprises SEQ ID NO: 21. In some embodiments, the cleavable linker comprises SEQ ID NO: 171 and the non-cleavable linker comprises SEQ ID NO: 21.

In some embodiments, the proteolytically cleavable linker comprises a cleavable peptide consisting of an amino acid sequence of SEQ ID NO: 155. (ISSGLL*SGRS).

In some embodiments, the proteolytically cleavable linker comprises a cleavable peptide consisting of an amino acid sequence of SEQ ID NO: 156. (DLLA*VVAAS).

#### 1.5.3 Non-Cleavable 'α Subunit Linkers'

In some embodiments, the non-cleavable α subunit linker is between 8 and 30 amino acids in length. In some embodiments, the non-cleavable linker α subunit linker is between 10 and 25 amino acids in length.

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

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

In some embodiments, the non-cleavable α subunit linker comprises an amino acid sequence as shown in SEQ ID NO: 13. In some embodiments, the non-cleavable α subunit linker comprises an amino acid sequence as shown in SEQ ID NO: 14. In some embodiments, the non-cleavable α subunit linker comprises an amino acid sequence as shown in SEQ ID NO: 15. In some embodiments, the non-cleavable α subunit linker comprises an amino acid sequence as shown in SEQ ID NO: 16. In some embodiments, the non-cleavable α subunit linker comprises an amino acid sequence as shown in SEQ ID NO: 17. In some embodiments, the non-cleavable α subunit linker comprises an amino acid sequence as shown in SEQ ID NO: 18. In some embodiments, the non-cleavable α subunit linker comprises an amino acid sequence as shown in SEQ ID NO: 19.

Exemplary non-cleavable linkers, cleavage peptides, spacer domains, cleavable linkers, and α subunit linkers are shown in Tables 3 and 4. Linker combinations disclosed in exemplary AK molecules may be used with any IL-15 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 disclosed herein. In other words, linker combinations disclosed in exemplary AK molecules may be used with any combination of IL-15 cytokine or fragment thereof disclosed herein, masking moiety disclosed herein and/or half-life extension domains disclosed herein.

### 1.6 Three Chain Construct

In some embodiments, the masked IL-15 construct comprises a first polypeptide chain, a second polypeptide chain and a third polypeptide chain.

In some embodiments, the third polypeptide chain comprises a 'IL-15Rα domain'. Incorporating an 'IL-15Rα domain' into a masked cytokine construct has been demonstrated to increase the potency of said cytokine in activating CD8 T cell and NK cells.

In some embodiments, the 'IL-15Rα domain' is associated with the IL-15 cytokine.

In some embodiments, the 'IL-15Rα domain' is non-covalently bonded to the IL-15 cytokine.

In some embodiments of the 'heterodimeric' masked cytokine of Structure A, the first polypeptide chain comprises:

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

and the second polypeptide chain comprises:

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

wherein the masked cytokine further comprises a third polypeptide chain, comprising:

N' **IL-15Rα domain** C'

wherein the 'IL-15Rα domain' is non-covalently bonded to the IL-15 cytokine or functional fragment thereof (C) in the second polypeptide chain.

In some embodiments of the 'heterodimeric' masked cytokine of Structure A, the first polypeptide chain comprises:

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

and the second polypeptide chain comprises:

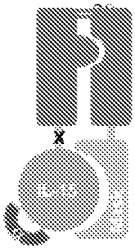
N' **HL2- SD1-CP-SD2 -C** C'

wherein the masked cytokine further comprises a third polypeptide chain, comprising:

N' **IL-15Rα domain** C'

wherein the 'IL-15Rα domain' is non-covalently bonded to the IL-15 cytokine or functional fragment thereof (C) in the second polypeptide chain.

This is shown schematically below:



In some embodiments of the 'heterodimeric' masked cytokine of Structure B, the first polypeptide chain comprises:

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

and the second polypeptide chain comprises:

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

wherein the masked cytokine further comprises a third polypeptide chain, comprising:

N' **IL-15Rα domain** C'

wherein the 'IL-15Rα domain' is non-covalently bonded to the IL-15 cytokine or functional fragment thereof (C) in the second polypeptide chain.

In some embodiments of the 'heterodimeric' masked cytokine of Structure B, the first polypeptide chain comprises:

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

and the second polypeptide chain comprises:

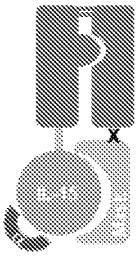
N' **HL2- non-cleavable L2-C** C'

wherein the masked cytokine further comprises a third polypeptide chain, comprising:

N' **IL-15Rα domain** C'

wherein the 'IL-15Rα domain' is non-covalently bonded to the IL-15 cytokine or functional fragment thereof (C) in the second polypeptide chain.

This is shown schematically below:



It will be understood that the non-covalent bond between the IL-15 cytokine and the 'IL-15Rα domain' arises from the biochemical cytokine-receptor relationship of these polypeptide sequences, in forming a cytokine-receptor complex. This natural interaction between the two sequences is purely non-covalent in nature. Thus, in some embodiments, the 'IL-15Rα domain' is only non-covalently bonded to the IL-15 cytokine, i.e. no covalent bonds are present between the second and third polypeptide chains.

The HL1, HL2, MM, C, L1, L2 and 'IL-15Rα domain' can be as defined anywhere herein.

In one embodiment, the sequence of the 'IL-15Rα domain' is the wild type sequence.

Exemplary combinations of sequences for HL1, HL2, MM, C, L1, L2 and 'IL-15Rα domain' that may be used in the three chain constructs are disclosed in 'Exemplary masked cytokine' section below.

In some embodiments of the three chain construct, the IL-15 cytokine or functional fragment thereof comprises the amino acid sequence of SEQ ID NO: 130 and the masking moiety comprises the amino acid sequence of SEQ ID NO: 12 and the first half-life extension domain comprises SEQ ID NO: 63 (Y349C; T366S; L38A; Y407V; and N297A) and the second half-life extension domain comprises SEQ ID NO 64 (S354C, T366W and N297A) and the non-cleavable linker comprises an amino acid sequence as shown in

SEQ ID NO: 22, and the cleavable peptide comprises an amino acid sequence as shown in SEQ ID NO: 27 and the IL-15Rα domain comprises an amino acid sequence as shown in SEQ ID NO: 56.

In some embodiments, the IL-15 cytokine or functional fragment thereof comprises the amino acid sequence of SEQ ID NO: 130 and the masking moiety comprises the amino acid sequence of SEQ ID NO: 12 and the first half-life extension domain comprises SEQ ID NO: 63 (Y349C; T366S; L38A; Y407V; and N297A) and the second half-life extension domain comprises SEQ ID NO 64 (S354C, T366W and N297A) and the non-cleavable linker comprises an amino acid sequence as shown in SEQ ID NO: 22, and the cleavable peptide comprises an amino acid sequence as shown in SEQ ID NO: 34 and the IL-15Rα domain comprises an amino acid sequence as shown in SEQ ID NO: 56.

In some embodiments, the IL-15 cytokine or functional fragment thereof comprises the amino acid sequence of SEQ ID NO: 130 and the masking moiety comprises the amino acid sequence of SEQ ID NO: 12 and the first half-life extension domain comprises SEQ ID NO: 63 (Y349C; T366S; L38A; Y407V; and N297A) and the second half-life extension domain comprises SEQ ID NO 64 (S354C, T366W and N297A) and the non-cleavable linker comprises an amino acid sequence as shown in SEQ ID NO: 22 and the cleavable peptide comprises an amino acid sequence as shown in SEQ ID NO: 33, and the IL-15Rα domain comprises an amino acid sequence as shown in SEQ ID NO: 56.

In some embodiments, the IL-15 cytokine or functional fragment thereof comprises the amino acid sequence of SEQ ID NO: 130 and the masking moiety comprises the amino acid sequence of SEQ ID NO: 12 and the first half-life extension domain comprises SEQ ID NO: 63 (Y349C; T366S; L38A; Y407V; and N297A) and the second half-life extension domain comprises SEQ ID NO 64 (S354C, T366W and N297A) and the non-cleavable linker comprises an amino acid sequence as shown in SEQ ID NO: 22 and the cleavable peptide comprises an amino acid sequence as shown in SEQ ID NO: 134 and the IL-15Rα domain comprises an amino acid sequence as shown in SEQ ID NO: 56.

In some embodiments of the three chain construct, the IL-15 cytokine or functional fragment thereof comprises the amino acid sequence of SEQ ID NO: 130 and the masking moiety comprises the amino acid sequence of SEQ ID NO: 12 and the first half-life extension domain comprises SEQ ID NO: 63 (Y349C; T366S; L38A; Y407V; and N297A) and the second half-life extension domain comprises SEQ ID NO 64 (S354C, T366W and N297A) and the non-cleavable linker comprises an amino acid sequence as shown in SEQ ID NO: 22, and the cleavable linker comprises an amino acid sequence as shown in SEQ ID NO: 54b and the IL-15Rα domain comprises an amino acid sequence as shown in SEQ ID NO: 56.

In some embodiments, the IL-15 cytokine or functional fragment thereof comprises the amino acid sequence of SEQ ID NO: 130 and the masking moiety comprises the amino acid sequence of SEQ ID NO: 12 and the first half-life extension domain comprises SEQ ID NO: 63 (Y349C; T366S; L38A; Y407V; and

N297A) and the second half-life extension domain comprises SEQ ID NO 64 (S354C, T366W and N297A) and the non-cleavable linker comprises an amino acid sequence as shown in SEQ ID NO: 22, and the cleavable linker comprises an amino acid sequence as shown in SEQ ID NO: 166 and the IL-15Rα domain comprises an amino acid sequence as shown in SEQ ID NO: 56.

In some embodiments, the IL-15 cytokine or functional fragment thereof comprises the amino acid sequence of SEQ ID NO: 130 and the masking moiety comprises the amino acid sequence of SEQ ID NO: 12 and the first half-life extension domain comprises SEQ ID NO: 63 (Y349C; T366S; L38A; Y407V; and N297A) and the second half-life extension domain comprises SEQ ID NO 64 (S354C, T366W and N297A) and the non-cleavable linker comprises an amino acid sequence as shown in SEQ ID NO: 22 and the cleavable linker comprises an amino acid sequence as shown in SEQ ID NO: 42, and the IL-15Rα domain comprises an amino acid sequence as shown in SEQ ID NO: 56.

In some embodiments, the IL-15 cytokine or functional fragment thereof comprises the amino acid sequence of SEQ ID NO: 130 and the masking moiety comprises the amino acid sequence of SEQ ID NO: 12 and the first half-life extension domain comprises SEQ ID NO: 63 (Y349C; T366S; L38A; Y407V; and N297A) and the second half-life extension domain comprises SEQ ID NO 64 (S354C, T366W and N297A) and the non-cleavable linker comprises an amino acid sequence as shown in SEQ ID NO: 22 and the cleavable linker comprises an amino acid sequence as shown in SEQ ID NO: 162 and the IL-15Rα domain comprises an amino acid sequence as shown in SEQ ID NO: 56.

In some embodiments of the three chain construct, the IL-15 cytokine or functional fragment thereof comprises the amino acid sequence of SEQ ID NO: 130 and the masking moiety comprises the amino acid sequence of SEQ ID NO: 12 and the first half-life extension domain comprises SEQ ID NO: 63 (Y349C; T366S; L38A; Y407V; and N297A) and the second half-life extension domain comprises SEQ ID NO 64 (S354C, T366W and N297A) and the non-cleavable linker comprises an amino acid sequence as shown in SEQ ID NO: 21, and the cleavable peptide comprises an amino acid sequence as shown in SEQ ID NO: 27 and the IL-15Rα domain comprises an amino acid sequence as shown in SEQ ID NO: 56.

In some embodiments, the IL-15 cytokine or functional fragment thereof comprises the amino acid sequence of SEQ ID NO: 130 and the masking moiety comprises the amino acid sequence of SEQ ID NO: 12 and the first half-life extension domain comprises SEQ ID NO: 63 (Y349C; T366S; L38A; Y407V; and N297A) and the second half-life extension domain comprises SEQ ID NO 64 (S354C, T366W and N297A) and the non-cleavable linker comprises an amino acid sequence as shown in SEQ ID NO: 21, and the cleavable peptide comprises an amino acid sequence as shown in SEQ ID NO: 34 and the IL-15Rα domain comprises an amino acid sequence as shown in SEQ ID NO: 56.

In some embodiments, the IL-15 cytokine or functional fragment thereof comprises the amino acid sequence of SEQ ID NO: 130 and the masking moiety comprises the amino acid sequence of SEQ ID NO: 12 and the first half-life extension domain comprises SEQ ID NO: 63 (Y349C; T366S; L38A; Y407V; and N297A) and the second half-life extension domain comprises SEQ ID NO 64 (S354C, T366W and N297A) and the non-cleavable linker comprises an amino acid sequence as shown in SEQ ID NO: 21 and the cleavable peptide comprises an amino acid sequence as shown in SEQ ID NO: 33, and the IL-15Rα domain comprises an amino acid sequence as shown in SEQ ID NO: 56.

In some embodiments, the IL-15 cytokine or functional fragment thereof comprises the amino acid sequence of SEQ ID NO: 130 and the masking moiety comprises the amino acid sequence of SEQ ID NO: 12 and the first half-life extension domain comprises SEQ ID NO: 63 (Y349C; T366S; L38A; Y407V; and N297A) and the second half-life extension domain comprises SEQ ID NO 64 (S354C, T366W and N297A) and the non-cleavable linker comprises an amino acid sequence as shown in SEQ ID NO: 21 and the cleavable peptide comprises an amino acid sequence as shown in SEQ ID NO: 134 and the IL-15Rα domain comprises an amino acid sequence as shown in SEQ ID NO: 56.

In some embodiments of the three chain construct, the IL-15 cytokine or functional fragment thereof comprises the amino acid sequence of SEQ ID NO: 130 and the masking moiety comprises the amino acid sequence of SEQ ID NO: 12 and the first half-life extension domain comprises SEQ ID NO: 63 (Y349C; T366S; L38A; Y407V; and N297A) and the second half-life extension domain comprises SEQ ID NO 64 (S354C, T366W and N297A) and the non-cleavable linker comprises an amino acid sequence as shown in SEQ ID NO: 21, and the cleavable linker comprises an amino acid sequence as shown in SEQ ID NO: 165 and the IL-15Rα domain comprises an amino acid sequence as shown in SEQ ID NO: 56.

In some embodiments, the IL-15 cytokine or functional fragment thereof comprises the amino acid sequence of SEQ ID NO: 130 and the masking moiety comprises the amino acid sequence of SEQ ID NO: 12 and the first half-life extension domain comprises SEQ ID NO: 63 (Y349C; T366S; L38A; Y407V; and N297A) and the second half-life extension domain comprises SEQ ID NO 64 (S354C, T366W and N297A) and the non-cleavable linker comprises an amino acid sequence as shown in SEQ ID NO: 21, and the cleavable linker comprises an amino acid sequence as shown in SEQ ID NO: 167 and the IL-15Rα domain comprises an amino acid sequence as shown in SEQ ID NO: 56.

In some embodiments, the IL-15 cytokine or functional fragment thereof comprises the amino acid sequence of SEQ ID NO: 130 and the masking moiety comprises the amino acid sequence of SEQ ID NO: 12 and the first half-life extension domain comprises SEQ ID NO: 63 (Y349C; T366S; L38A; Y407V; and N297A) and the second half-life extension domain comprises SEQ ID NO 64 (S354C, T366W and N297A) and the non-cleavable linker comprises an amino acid sequence as shown in SEQ ID NO: 21 and the

cleavable linker comprises an amino acid sequence as shown in SEQ ID NO: 47, and the IL-15Rα domain comprises an amino acid sequence as shown in SEQ ID NO: 56.

In some embodiments, the IL-15 cytokine or functional fragment thereof comprises the amino acid sequence of SEQ ID NO: 130 and the masking moiety comprises the amino acid sequence of SEQ ID NO: 12 and the first half-life extension domain comprises SEQ ID NO: 63 (Y349C; T366S; L38A; Y407V; and N297A) and the second half-life extension domain comprises SEQ ID NO 64 (S354C, T366W and N297A) and the non-cleavable linker comprises an amino acid sequence as shown in SEQ ID NO: 21 and the cleavable linker comprises an amino acid sequence as shown in SEQ ID NO: 163 and the IL-15Rα domain comprises an amino acid sequence as shown in SEQ ID NO: 56.

In some embodiments of the three chain construct, the first polypeptide chain comprises the amino acid sequence of SEQ ID NO: 177.

In some embodiments of the three chain construct, the first polypeptide chain comprises the amino acid sequence of SEQ ID NO: 177, the second polypeptide chain comprises the amino acid sequence of SEQ ID NO: 185, and the third polypeptide chain comprises the amino acid sequence of SEQ ID NO: 56.

In some embodiments of the three chain construct, the first polypeptide chain comprises the amino acid sequence of SEQ ID NO: 177, the second polypeptide chain comprises the amino acid sequence of SEQ ID NO: 186, and the third polypeptide chain comprises the amino acid sequence of SEQ ID NO: 56.

In some embodiments of the three chain construct, the first polypeptide chain comprises the amino acid sequence of SEQ ID NO: 177, the second polypeptide chain comprises the amino acid sequence of SEQ ID NO: 187, and the third polypeptide chain comprises the amino acid sequence of SEQ ID NO: 56.

In some embodiments of the three chain construct, the first polypeptide chain comprises the amino acid sequence of SEQ ID NO: 177, the second polypeptide chain comprises the amino acid sequence of SEQ ID NO: 191, and the third polypeptide chain comprises the amino acid sequence of SEQ ID NO: 56.

In some embodiments of the three chain construct, the second polypeptide chain comprises the amino acid sequence of SEQ ID NO: 183.

In some embodiments of the three chain construct, the first polypeptide chain comprises the amino acid sequence of SEQ ID NO: 188, the second polypeptide chain comprises the amino acid sequence of SEQ ID NO: 183, and the third polypeptide chain comprises the amino acid sequence of SEQ ID NO: 56.

In some embodiments of the three chain construct, the first polypeptide chain comprises the amino acid sequence of SEQ ID NO: 189, the second polypeptide chain comprises the amino acid sequence of SEQ ID NO: 183, and the third polypeptide chain comprises the amino acid sequence of SEQ ID NO: 56.

In some embodiments of the three chain construct, the first polypeptide chain comprises the amino acid sequence of SEQ ID NO: 190n, the second polypeptide chain comprises the amino acid sequence of SEQ ID NO: 183, and the third polypeptide chain comprises the amino acid sequence of SEQ ID NO: 56.

In some embodiments of the three chain construct, the first polypeptide chain comprises the amino acid sequence of SEQ ID NO: 192, the second polypeptide chain comprises the amino acid sequence of SEQ ID NO: 183, and the third polypeptide chain comprises the amino acid sequence of SEQ ID NO: 56.

### 1.7 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**
**QQGNVFSCSVMHEALHNHYTQKSLSLSPG**K
(SEQ ID NO: 59)

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: 59 (the 'parent sequence'), such that the first and second half-life extension domains each comprise SEQ ID NO: 59 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: 59 shown in bold above, optionally with one or more amino acid modifications, i.e.:

DKTHTCPPCPAPELLGG
PSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYN
STYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSRDE
LTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRW

QQGNVFSCSVMHEALHNHYTQKSLSLSPG
(SEQ ID NO: 60)

In some embodiments, the first and second half-life extension domains comprise SEQ ID NO: 60with 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: 60 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 61 and 62 shown below:

First half-life extension domain (Y349C; T366S; L38A; and Y407V) SEQ ID NO 61:

DKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHE
DPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWL
NGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVCTLPPSRDELTKNQ
VSLSCAVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLVSKL
TVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPG

Second half-life extension domain (S354C and T366W) SEQ ID NO 62:

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 63:

DKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHE
DPEVKFNWYVDGVEVHNAKTKPREEQYASTYRVVSVLTVLHQDWL
NGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVCTLPPSRDELTKNQ
VSLSCAVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLVSKL
TVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPG

Second half-life extension domain (S354C, T366W and N297A) SEQ ID NO 64:

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 65:

DKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMASRTPEVTCVVVDVSHEDP
EVKFNWYVDGVEVHNAKTKPREEQYASTYRVVSVLTVLHQDWLNGKE
YKCKVSNKALPAPIEKTISKAKGQPREPQVCTLPPSRDELTKNQVSLSCA
VKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLVSKLTVDKSRW
QQGNVFSCSVMHEALHNHYTQKSLSLSPG

Second half-life extension domain (S354C, T366W, N297A and I253A) SEQ ID NO 66:

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: 60, 61, 63 and 65.

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: 60, 62, 64 and 66.

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: 60, 61, 63 and 65. 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: 60, 62, 64 and 66. 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 Q311I; N434A; N434W; M428F and N434S; V259I and V308F; M252Y, S254T, and T256E; V259I, V308F and M428F; T307Q and N434A; T307Q and N434S; T307Q, E380A, and N434A; V308P and N434A; N434H; and V308P.

For manufacturing purposes, a signal peptide may be engineered upstream of the half-life domain to improve secretion of the protein. The signal peptide is selected according to the cell line's requirements as

is known in the art. It will be understood that the signal peptide is not expressed as part of the protein that will be purified and formulated as drug product.

#### 1.7.1 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.

### 1.8 Exemplary masked cytokines

Masked cytokines according to the disclosure can combine a IL-15 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.

Masked cytokines according to the disclosure can combine a IL-15 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; cleavable and non-cleavable linkers as described anywhere herein; and IL-15Rα domains 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-15 cytokine or functional fragment thereof comprises the amino acid sequence of SEQ ID NO: 2 and the masking moiety comprises the amino acid sequence of SEQ ID NO: 11.

In some embodiments, the IL-15 cytokine or functional fragment thereof comprises the amino acid sequence of SEQ ID NO: 2 and the masking moiety comprises the amino acid sequence of SEQ ID NO: 12.

In some embodiments, the IL-15 cytokine or functional fragment thereof comprises the amino acid sequence of SEQ ID NO: 127 and the masking moiety comprises the amino acid sequence of SEQ ID NO: 12.

In some embodiments, the IL-15 cytokine or functional fragment thereof comprises the amino acid sequence of SEQ ID NO: 128 and the masking moiety comprises the amino acid sequence of SEQ ID NO: 12.

In some embodiments, the IL-15 cytokine or functional fragment thereof comprises the amino acid sequence of SEQ ID NO: 129 and the masking moiety comprises the amino acid sequence of SEQ ID NO: 12.

In some embodiments, the IL-15 cytokine or functional fragment thereof comprises the amino acid sequence of SEQ ID NO: 130 and the masking moiety comprises the amino acid sequence of SEQ ID NO: 12.

In some embodiments, the IL-15 cytokine or functional fragment thereof comprises the amino acid sequence of SEQ ID NO: 131 and the masking moiety comprises the amino acid sequence of SEQ ID NO: 12.

In some embodiments, the IL-15 cytokine or functional fragment thereof comprises the amino acid sequence of SEQ ID NO: 132 and the masking moiety comprises the amino acid sequence of SEQ ID NO: 12.

In some embodiments, the IL-15 cytokine or functional fragment thereof comprises the amino acid sequence of SEQ ID NO: 133 and the masking moiety comprises the amino acid sequence of SEQ ID NO: 12.

In some embodiments, the IL-15 cytokine or functional fragment thereof comprises the amino acid sequence of SEQ ID NO: 2 and the first half-life extension domain comprises SEQ ID NO: 63 (Y349C; T366S; L38A; Y407V; and N297A) and the second half-life extension domain comprises SEQ ID NO 64 (S354C, T366W and N297A).

In some embodiments, the IL-15 cytokine or functional fragment thereof comprises the amino acid sequence of SEQ ID NO: 127 and the first half-life extension domain comprises SEQ ID NO: 63 (Y349C; T366S; L38A; Y407V; and N297A) and the second half-life extension domain comprises SEQ ID NO 64 (S354C, T366W and N297A).

In some embodiments, the IL-15 cytokine or functional fragment thereof comprises the amino acid sequence of SEQ ID NO: 128 and the first half-life extension domain comprises SEQ ID NO: 63 (Y349C; T366S; L38A; Y407V; and N297A) and the second half-life extension domain comprises SEQ ID NO 64 (S354C, T366W and N297A).

In some embodiments, the IL-15 cytokine or functional fragment thereof comprises the amino acid sequence of SEQ ID NO: 129 and the first half-life extension domain comprises SEQ ID NO: 63 (Y349C; T366S; L38A; Y407V; and N297A) and the second half-life extension domain comprises SEQ ID NO 64 (S354C, T366W and N297A).

In some embodiments, the IL-15 cytokine or functional fragment thereof comprises the amino acid sequence of SEQ ID NO: 130 and the first half-life extension domain comprises SEQ ID NO: 63 (Y349C; T366S; L38A; Y407V; and N297A) and the second half-life extension domain comprises SEQ ID NO 64 (S354C, T366W and N297A).

In some embodiments, the IL-15 cytokine or functional fragment thereof comprises the amino acid sequence of SEQ ID NO: 131 and the first half-life extension domain comprises SEQ ID NO: 63 (Y349C; T366S; L38A; Y407V; and N297A) and the second half-life extension domain comprises SEQ ID NO 64 (S354C, T366W and N297A).

In some embodiments, the IL-15 cytokine or functional fragment thereof comprises the amino acid sequence of SEQ ID NO: 132 and the first half-life extension domain comprises SEQ ID NO: 63 (Y349C; T366S; L38A; Y407V; and N297A) and the second half-life extension domain comprises SEQ ID NO 64 (S354C, T366W and N297A).

In some embodiments, the IL-15 cytokine or functional fragment thereof comprises the amino acid sequence of SEQ ID NO: 133 and the first half-life extension domain comprises SEQ ID NO: 63 (Y349C; T366S; L38A; Y407V; and N297A) and the second half-life extension domain comprises SEQ ID NO 64 (S354C, T366W and N297A).

In some embodiments, the IL-15 cytokine or functional fragment thereof comprises the amino acid sequence of SEQ ID NO: 2 and the first half-life extension domain comprises SEQ ID NO: 65 (Y349C; T366S; L38A; Y407V, N297A and I253A) and the second half-life extension domain comprises SEQ ID NO: 66 (S354C, T366W, N297A and I253A).

In some embodiments, the IL-15 cytokine or functional fragment thereof comprises the amino acid sequence of SEQ ID NO: 2 and the non-cleavable linker comprises an amino acid sequence as shown in SEQ ID NO: 22.

In some embodiments, the IL-15 cytokine or functional fragment thereof comprises the amino acid sequence of SEQ ID NO: 127 and the non-cleavable linker comprises an amino acid sequence as shown in SEQ ID NO: 22.

In some embodiments, the IL-15 cytokine or functional fragment thereof comprises the amino acid sequence of SEQ ID NO: 128 and the non-cleavable linker comprises an amino acid sequence as shown in SEQ ID NO: 22.

In some embodiments, the IL-15 cytokine or functional fragment thereof comprises the amino acid sequence of SEQ ID NO: 129 and the non-cleavable linker comprises an amino acid sequence as shown in SEQ ID NO: 22.

In some embodiments, the IL-15 cytokine or functional fragment thereof comprises the amino acid sequence of SEQ ID NO: 130 and the non-cleavable linker comprises an amino acid sequence as shown in SEQ ID NO: 22.

In some embodiments, the IL-15 cytokine or functional fragment thereof comprises the amino acid sequence of SEQ ID NO: 131 and the non-cleavable linker comprises an amino acid sequence as shown in SEQ ID NO: 22.

In some embodiments, the IL-15 cytokine or functional fragment thereof comprises the amino acid sequence of SEQ ID NO: 132 and the non-cleavable linker comprises an amino acid sequence as shown in SEQ ID NO: 22.

In some embodiments, the IL-15 cytokine or functional fragment thereof comprises the amino acid sequence of SEQ ID NO: 133 and the non-cleavable linker comprises an amino acid sequence as shown in SEQ ID NO: 22.

In some embodiments, the IL-15 cytokine or functional fragment thereof comprises the amino acid sequence of SEQ ID NO: 2 and the cleavable peptide comprises an amino acid sequence as shown in SEQ ID NO: 33.

In some embodiments, the IL-15 cytokine or functional fragment thereof comprises the amino acid sequence of SEQ ID NO: 2 and the cleavable peptide comprises an amino acid sequence as shown in SEQ ID NO: 34.

In some embodiments, the IL-15 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: 11.

In some embodiments, the IL-15 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: 12.

In some embodiments, the IL-15 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: 63 (Y349C; T366S; L38A; Y407V; and N297A) and the second half-life extension domain comprises SEQ ID NO 64 (S354C, T366W and N297A).

In some embodiments, the IL-15 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: 65 (Y349C; T366S; L38A; Y407V, N297A and I253A) and the second half-life extension domain comprises SEQ ID NO: 66 (S354C, T366W, N297A and I253A).

In some embodiments, the IL-15 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: 22.

In some embodiments, the IL-15 cytokine or functional fragment thereof comprises the amino acid sequence of SEQ ID NO: 3 and the cleavable peptide comprises an amino acid sequence as shown in SEQ ID NO: 33.

In some embodiments, the IL-15 cytokine or functional fragment thereof comprises the amino acid sequence of SEQ ID NO: 3 and the cleavable peptide comprises an amino acid sequence as shown in SEQ ID NO: 34.

In some embodiments, the IL-15 cytokine or functional fragment thereof comprises the amino acid sequence of SEQ ID NO: 4 and the masking moiety comprises the amino acid sequence of SEQ ID NO: 11.

In some embodiments, the IL-15 cytokine or functional fragment thereof comprises the amino acid sequence of SEQ ID NO: 4 and the masking moiety comprises the amino acid sequence of SEQ ID NO: 12.

In some embodiments, the IL-15 cytokine or functional fragment thereof comprises the amino acid sequence of SEQ ID NO: 4 and the first half-life extension domain comprises SEQ ID NO: 63 (Y349C; T366S; L38A; Y407V; and N297A) and the second half-life extension domain comprises SEQ ID NO 64 (S354C, T366W and N297A).

In some embodiments, the IL-15 cytokine or functional fragment thereof comprises the amino acid sequence of SEQ ID NO: 4 and the first half-life extension domain comprises SEQ ID NO: 65 (Y349C; T366S; L38A; Y407V, N297A and I253A) and the second half-life extension domain comprises SEQ ID NO: 66 (S354C, T366W, N297A and I253A).

In some embodiments, the IL-15 cytokine or functional fragment thereof 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: 22.

In some embodiments, the IL-15 cytokine or functional fragment thereof comprises the amino acid sequence of SEQ ID NO: 4 and the cleavable peptide comprises an amino acid sequence as shown in SEQ ID NO: 33.

In some embodiments, the IL-15 cytokine or functional fragment thereof comprises the amino acid sequence of SEQ ID NO: 4 and the cleavable peptide comprises an amino acid sequence as shown in SEQ ID NO: 34.

In some embodiments, the IL-15 cytokine or functional fragment thereof comprises the amino acid sequence of SEQ ID NO: 5 and the masking moiety comprises the amino acid sequence of SEQ ID NO: 11.

In some embodiments, the IL-15 cytokine or functional fragment thereof comprises the amino acid sequence of SEQ ID NO: 5 and the masking moiety comprises the amino acid sequence of SEQ ID NO: 12.

In some embodiments, the IL-15 cytokine or functional fragment thereof comprises the amino acid sequence of SEQ ID NO: 5 and the first half-life extension domain comprises SEQ ID NO: 63 (Y349C; T366S; L38A; Y407V; and N297A) and the second half-life extension domain comprises SEQ ID NO 64 (S354C, T366W and N297A).

In some embodiments, the IL-15 cytokine or functional fragment thereof comprises the amino acid sequence of SEQ ID NO: 5 and the first half-life extension domain comprises SEQ ID NO: 65 (Y349C; T366S; L38A; Y407V, N297A and I253A) and the second half-life extension domain comprises SEQ ID NO: 66 (S354C, T366W, N297A and I253A).

In some embodiments, the IL-15 cytokine or functional fragment thereof 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: 22.

In some embodiments, the IL-15 cytokine or functional fragment thereof comprises the amino acid sequence of SEQ ID NO: 5 and the cleavable peptide comprises an amino acid sequence as shown in SEQ ID NO: 33.

In some embodiments, the IL-15 cytokine or functional fragment thereof comprises the amino acid sequence of SEQ ID NO: 5 and the cleavable peptide comprises an amino acid sequence as shown in SEQ ID NO: 34.

In some embodiments, the IL-15 cytokine or functional fragment thereof comprises the amino acid sequence of SEQ ID NO: 6 and the masking moiety comprises the amino acid sequence of SEQ ID NO: 11.

In some embodiments, the IL-15 cytokine or functional fragment thereof comprises the amino acid sequence of SEQ ID NO: 6 and the masking moiety comprises the amino acid sequence of SEQ ID NO: 12.

In some embodiments, the IL-15 cytokine or functional fragment thereof comprises the amino acid sequence of SEQ ID NO: 6 and the first half-life extension domain comprises SEQ ID NO: 63 (Y349C; T366S; L38A; Y407V; and N297A) and the second half-life extension domain comprises SEQ ID NO 64 (S354C, T366W and N297A).

In some embodiments, the IL-15 cytokine or functional fragment thereof comprises the amino acid sequence of SEQ ID NO: 6 and the first half-life extension domain comprises SEQ ID NO: 65 (Y349C; T366S; L38A; Y407V, N297A and I253A) and the second half-life extension domain comprises SEQ ID NO: 66 (S354C, T366W, N297A and I253A).

In some embodiments, the IL-15 cytokine or functional fragment thereof comprises the amino acid sequence of SEQ ID NO: 6 and the non-cleavable linker comprises an amino acid sequence as shown in SEQ ID NO: 22.

In some embodiments, the IL-15 cytokine or functional fragment thereof comprises the amino acid sequence of SEQ ID NO: 6 and the cleavable peptide comprises an amino acid sequence as shown in SEQ ID NO: 33.

In some embodiments, the IL-15 cytokine or functional fragment thereof comprises the amino acid sequence of SEQ ID NO: 6 and the cleavable peptide comprises an amino acid sequence as shown in SEQ ID NO: 34.

In some embodiments, the IL-15 cytokine or functional fragment thereof comprises the amino acid sequence of SEQ ID NO: 7 and the masking moiety comprises the amino acid sequence of SEQ ID NO: 11.

In some embodiments, the IL-15 cytokine or functional fragment thereof comprises the amino acid sequence of SEQ ID NO: 7 and the masking moiety comprises the amino acid sequence of SEQ ID NO: 12.

In some embodiments, the IL-15 cytokine or functional fragment thereof comprises the amino acid sequence of SEQ ID NO: 7 and the first half-life extension domain comprises SEQ ID NO: 63 (Y349C; T366S; L38A; Y407V; and N297A) and the second half-life extension domain comprises SEQ ID NO 64 (S354C, T366W and N297A).

In some embodiments, the IL-15 cytokine or functional fragment thereof comprises the amino acid sequence of SEQ ID NO: 7 and the first half-life extension domain comprises SEQ ID NO: 65 (Y349C; T366S; L38A; Y407V, N297A and I253A) and the second half-life extension domain comprises SEQ ID NO: 66 (S354C, T366W, N297A and I253A).

In some embodiments, the IL-15 cytokine or functional fragment thereof comprises the amino acid sequence of SEQ ID NO: 7 and the non-cleavable linker comprises an amino acid sequence as shown in SEQ ID NO: 22.

In some embodiments, the IL-15 cytokine or functional fragment thereof comprises the amino acid sequence of SEQ ID NO: 7 and the cleavable peptide comprises an amino acid sequence as shown in SEQ ID NO: 33.

In some embodiments, the IL-15 cytokine or functional fragment thereof comprises the amino acid sequence of SEQ ID NO: 7 and the cleavable peptide comprises an amino acid sequence as shown in SEQ ID NO: 34.

In some embodiments, the IL-15 cytokine or functional fragment thereof comprises the amino acid sequence of SEQ ID NO: 8 and the masking moiety comprises the amino acid sequence of SEQ ID NO: 11.

In some embodiments, the IL-15 cytokine or functional fragment thereof comprises the amino acid sequence of SEQ ID NO: 8 and the masking moiety comprises the amino acid sequence of SEQ ID NO: 12.

In some embodiments, the IL-15 cytokine or functional fragment thereof comprises the amino acid sequence of SEQ ID NO: 8 and the first half-life extension domain comprises SEQ ID NO: 63 (Y349C; T366S; L38A; Y407V; and N297A) and the second half-life extension domain comprises SEQ ID NO 64 (S354C, T366W and N297A).

In some embodiments, the IL-15 cytokine or functional fragment thereof comprises the amino acid sequence of SEQ ID NO: 8 and the first half-life extension domain comprises SEQ ID NO: 65 (Y349C; T366S; L38A; Y407V, N297A and I253A) and the second half-life extension domain comprises SEQ ID NO: 66 (S354C, T366W, N297A and I253A).

In some embodiments, the IL-15 cytokine or functional fragment thereof comprises the amino acid sequence of SEQ ID NO: 8 and the non-cleavable linker comprises an amino acid sequence as shown in SEQ ID NO: 22.

In some embodiments, the IL-15 cytokine or functional fragment thereof comprises the amino acid sequence of SEQ ID NO: 8 and the cleavable peptide comprises an amino acid sequence as shown in SEQ ID NO: 33.

In some embodiments, the IL-15 cytokine or functional fragment thereof comprises the amino acid sequence of SEQ ID NO: 8 and the cleavable peptide comprises an amino acid sequence as shown in SEQ ID NO: 34.

In some embodiments, the IL-15 cytokine or functional fragment thereof comprises the amino acid sequence of SEQ ID NO: 127 and the cleavable peptide comprises an amino acid sequence as shown in SEQ ID NO: 27.

In some embodiments, the IL-15 cytokine or functional fragment thereof comprises the amino acid sequence of SEQ ID NO: 127 and the cleavable peptide comprises an amino acid sequence as shown in SEQ ID NO: 33.

In some embodiments, the IL-15 cytokine or functional fragment thereof comprises the amino acid sequence of SEQ ID NO: 127 and the cleavable peptide comprises an amino acid sequence as shown in SEQ ID NO: 134.

In some embodiments, the IL-15 cytokine or functional fragment thereof comprises the amino acid sequence of SEQ ID NO: 128 and the cleavable peptide comprises an amino acid sequence as shown in SEQ ID NO: 27.

In some embodiments, the IL-15 cytokine or functional fragment thereof comprises the amino acid sequence of SEQ ID NO: 128 and the cleavable peptide comprises an amino acid sequence as shown in SEQ ID NO: 33.

In some embodiments, the IL-15 cytokine or functional fragment thereof comprises the amino acid sequence of SEQ ID NO: 128 and the cleavable peptide comprises an amino acid sequence as shown in SEQ ID NO: 134.

In some embodiments, the IL-15 cytokine or functional fragment thereof comprises the amino acid sequence of SEQ ID NO: 129 and the cleavable peptide comprises an amino acid sequence as shown in SEQ ID NO: 27.

In some embodiments, the IL-15 cytokine or functional fragment thereof comprises the amino acid sequence of SEQ ID NO: 129 and the cleavable peptide comprises an amino acid sequence as shown in SEQ ID NO: 33.

In some embodiments, the IL-15 cytokine or functional fragment thereof comprises the amino acid sequence of SEQ ID NO: 129 and the cleavable peptide comprises an amino acid sequence as shown in SEQ ID NO: 134.

In some embodiments, the IL-15 cytokine or functional fragment thereof comprises the amino acid sequence of SEQ ID NO: 130 and the cleavable peptide comprises an amino acid sequence as shown in SEQ ID NO: 27.

In some embodiments, the IL-15 cytokine or functional fragment thereof comprises the amino acid sequence of SEQ ID NO: 130 and the cleavable peptide comprises an amino acid sequence as shown in SEQ ID NO: 33.

In some embodiments, the IL-15 cytokine or functional fragment thereof comprises the amino acid sequence of SEQ ID NO: 130 and the cleavable peptide comprises an amino acid sequence as shown in SEQ ID NO: 134.

In some embodiments, the IL-15 cytokine or functional fragment thereof comprises the amino acid sequence of SEQ ID NO: 131 and the cleavable peptide comprises an amino acid sequence as shown in SEQ ID NO: 27.

In some embodiments, the IL-15 cytokine or functional fragment thereof comprises the amino acid sequence of SEQ ID NO: 131 and the cleavable peptide comprises an amino acid sequence as shown in SEQ ID NO: 33.

In some embodiments, the IL-15 cytokine or functional fragment thereof comprises the amino acid sequence of SEQ ID NO: 131 and the cleavable peptide comprises an amino acid sequence as shown in SEQ ID NO: 134.

In some embodiments, the IL-15 cytokine or functional fragment thereof comprises the amino acid sequence of SEQ ID NO: 132 and the cleavable peptide comprises an amino acid sequence as shown in SEQ ID NO: 27.

In some embodiments, the IL-15 cytokine or functional fragment thereof comprises the amino acid sequence of SEQ ID NO: 132 and the cleavable peptide comprises an amino acid sequence as shown in SEQ ID NO: 33.

In some embodiments, the IL-15 cytokine or functional fragment thereof comprises the amino acid sequence of SEQ ID NO: 132 and the cleavable peptide comprises an amino acid sequence as shown in SEQ ID NO: 134.

In some embodiments, the IL-15 cytokine or functional fragment thereof comprises the amino acid sequence of SEQ ID NO: 133 and the cleavable peptide comprises an amino acid sequence as shown in SEQ ID NO: 27.

In some embodiments, the IL-15 cytokine or functional fragment thereof comprises the amino acid sequence of SEQ ID NO: 133 and the cleavable peptide comprises an amino acid sequence as shown in SEQ ID NO: 33.

In some embodiments, the IL-15 cytokine or functional fragment thereof comprises the amino acid sequence of SEQ ID NO: 133 and the cleavable peptide comprises an amino acid sequence as shown in SEQ ID NO: 134.

In some embodiments, the masking moiety comprises the amino acid sequence of SEQ ID NO: 11 and the first half-life extension domain comprises SEQ ID NO: 63 (Y349C; T366S; L38A; Y407V; and N297A) and the second half-life extension domain comprises SEQ ID NO: 64 (S354C, T366W and N297A).

In some embodiments, the masking moiety comprises the amino acid sequence of SEQ ID NO: 11 and the first half-life extension domain comprises SEQ ID NO: 65 (Y349C; T366S; L38A; Y407V, N297A and I253A) and the second half-life extension domain comprises SEQ ID NO: 66 (S354C, T366W, N297A and I253A).

In some embodiments, the masking moiety comprises the amino acid sequence of SEQ ID NO: 11 and the non-cleavable linker comprises an amino acid sequence as shown in SEQ ID NO: 22.

In some embodiments, the masking moiety comprises the amino acid sequence of SEQ ID NO: 11 and the cleavable peptide comprises an amino acid sequence as shown in SEQ ID NO: 33.

In some embodiments, the masking moiety comprises the amino acid sequence of SEQ ID NO: 11 and the cleavable peptide comprises an amino acid sequence as shown in SEQ ID NO: 34.

In some embodiments, the masking moiety comprises the amino acid sequence of SEQ ID NO: 12 and the first half-life extension domain comprises SEQ ID NO: 63 (Y349C; T366S; L38A; Y407V; and N297A) and the second half-life extension domain comprises SEQ ID NO: 64 (S354C, T366W and N297A).

In some embodiments, the masking moiety comprises the amino acid sequence of SEQ ID NO: 12 and the first half-life extension domain comprises SEQ ID NO: 65 (Y349C; T366S; L38A; Y407V, N297A and I253A) and the second half-life extension domain comprises SEQ ID NO: 66 (S354C, T366W, N297A and I253A).

In some embodiments, the masking moiety comprises the amino acid sequence of SEQ ID NO: 12 and the non-cleavable linker comprises an amino acid sequence as shown in SEQ ID NO: 22.

In some embodiments, the masking moiety comprises the amino acid sequence of SEQ ID NO: 12 and the cleavable peptide comprises an amino acid sequence as shown in SEQ ID NO: 33.

In some embodiments, the masking moiety comprises the amino acid sequence of SEQ ID NO: 12 and the cleavable peptide comprises an amino acid sequence as shown in SEQ ID NO: 34.

In some embodiments, the masking moiety comprises the amino acid sequence of SEQ ID NO: 12 and the cleavable peptide comprises an amino acid sequence as shown in SEQ ID NO: 27.

In some embodiments, the masking moiety comprises the amino acid sequence of SEQ ID NO: 12 and the cleavable peptide comprises an amino acid sequence as shown in SEQ ID NO: 134.

In some embodiments, the first half-life extension domain comprises SEQ ID NO: 63 (Y349C; T366S; L38A; Y407V; and N297A) and the second half-life extension domain comprises SEQ ID NO 64 (S354C, T366W and N297A) and the non-cleavable linker comprises an amino acid sequence as shown in SEQ ID NO: 22.

In some embodiments, the first half-life extension domain comprises SEQ ID NO: 63 (Y349C; T366S; L38A; Y407V; and N297A) and the second half-life extension domain comprises SEQ ID NO 64 (S354C, T366W and N297A) and the cleavable peptide comprises an amino acid sequence as shown in SEQ ID NO: 33.

In some embodiments, the first half-life extension domain comprises SEQ ID NO: 63 (Y349C; T366S; L38A; Y407V; and N297A) and the second half-life extension domain comprises SEQ ID NO 64 (S354C, T366W and N297A) and the cleavable peptide comprises an amino acid sequence as shown in SEQ ID NO: 34.

In some embodiments, the first half-life extension domain comprises SEQ ID NO: 63 (Y349C; T366S; L38A; Y407V; and N297A) and the second half-life extension domain comprises SEQ ID NO 64 (S354C, T366W and N297A) and the cleavable peptide comprises an amino acid sequence as shown in SEQ ID NO: 27.

In some embodiments, the first half-life extension domain comprises SEQ ID NO: 63 (Y349C; T366S; L38A; Y407V; and N297A) and the second half-life extension domain comprises SEQ ID NO 64 (S354C, T366W and N297A) and the cleavable peptide comprises an amino acid sequence as shown in SEQ ID NO: 134.

In some embodiments, the first half-life extension domain comprises SEQ ID NO: 65 (Y349C; T366S; L38A; Y407V; and N297A) and the second half-life extension domain comprises SEQ ID NO 66 (S354C, T366W and N297A) and the non-cleavable linker comprises an amino acid sequence as shown in SEQ ID NO: 22.

In some embodiments, the first half-life extension domain comprises SEQ ID NO: 65 (Y349C; T366S; L38A; Y407V; and N297A) and the second half-life extension domain comprises SEQ ID NO 66 (S354C, T366W and N297A) and the cleavable peptide comprises an amino acid sequence as shown in SEQ ID NO: 33.

In some embodiments, the first half-life extension domain comprises SEQ ID NO: 65 (Y349C; T366S; L38A; Y407V; and N297A) and the second half-life extension domain comprises SEQ ID NO 66 (S354C, T366W and N297A) and the cleavable peptide comprises an amino acid sequence as shown in SEQ ID NO: 34.

In some embodiments, the non-cleavable linker comprises an amino acid sequence as shown in SEQ ID NO: 22 and the cleavable peptide comprises an amino acid sequence as shown in SEQ ID NO: 33.

In some embodiments, the non-cleavable linker comprises an amino acid sequence as shown in SEQ ID NO: 22 and the cleavable peptide comprises an amino acid sequence as shown in SEQ ID NO: 34.

In some embodiments, the non-cleavable linker comprises an amino acid sequence as shown in SEQ ID NO: 22 and the cleavable peptide comprises an amino acid sequence as shown in SEQ ID NO: 27.

In some embodiments, the non-cleavable linker comprises an amino acid sequence as shown in SEQ ID NO: 22 and the cleavable peptide comprises an amino acid sequence as shown in SEQ ID NO: 134.

In some embodiments, the IL-15 cytokine or functional fragment thereof comprises the amino acid sequence of SEQ ID NO: 2 and the masking moiety comprises the amino acid sequence of SEQ ID NO: 11 and the first half-life extension domain comprises SEQ ID NO: 63 (Y349C; T366S; L38A; Y407V; and N297A) and the second half-life extension domain comprises SEQ ID NO 64 (S354C, T366W and N297A).

In some embodiments, the IL-15 cytokine or functional fragment thereof comprises the amino acid sequence of SEQ ID NO: 2 and the masking moiety comprises the amino acid sequence of SEQ ID NO: 12 and the first half-life extension domain comprises SEQ ID NO: 63 (Y349C; T366S; L38A; Y407V; and N297A) and the second half-life extension domain comprises SEQ ID NO 64 (S354C, T366W and N297A).

In some embodiments, the IL-15 cytokine or functional fragment thereof comprises the amino acid sequence of SEQ ID NO: 127 and the masking moiety comprises the amino acid sequence of SEQ ID NO: 12 and the first half-life extension domain comprises SEQ ID NO: 63 (Y349C; T366S; L38A; Y407V; and N297A) and the second half-life extension domain comprises SEQ ID NO 64 (S354C, T366W and N297A).

In some embodiments, the IL-15 cytokine or functional fragment thereof comprises the amino acid sequence of SEQ ID NO: 128 and the masking moiety comprises the amino acid sequence of SEQ ID NO: 12 and the first half-life extension domain comprises SEQ ID NO: 63 (Y349C; T366S; L38A; Y407V; and N297A) and the second half-life extension domain comprises SEQ ID NO 64 (S354C, T366W and N297A).

In some embodiments, the IL-15 cytokine or functional fragment thereof comprises the amino acid sequence of SEQ ID NO: 129 and the masking moiety comprises the amino acid sequence of SEQ ID NO: 12 and the first half-life extension domain comprises SEQ ID NO: 63 (Y349C; T366S; L38A; Y407V; and N297A) and the second half-life extension domain comprises SEQ ID NO 64 (S354C, T366W and N297A).

In some embodiments, the IL-15 cytokine or functional fragment thereof comprises the amino acid sequence of SEQ ID NO: 130 and the masking moiety comprises the amino acid sequence of SEQ ID NO: 12 and the first half-life extension domain comprises SEQ ID NO: 63 (Y349C; T366S; L38A; Y407V; and N297A) and the second half-life extension domain comprises SEQ ID NO 64 (S354C, T366W and N297A).

In some embodiments, the IL-15 cytokine or functional fragment thereof comprises the amino acid sequence of SEQ ID NO: 131 and the masking moiety comprises the amino acid sequence of SEQ ID NO: 12 and the first half-life extension domain comprises SEQ ID NO: 63 (Y349C; T366S; L38A; Y407V; and N297A) and the second half-life extension domain comprises SEQ ID NO 64 (S354C, T366W and N297A).

In some embodiments, the IL-15 cytokine or functional fragment thereof comprises the amino acid sequence of SEQ ID NO: 132 and the masking moiety comprises the amino acid sequence of SEQ ID NO: 12 and the first half-life extension domain comprises SEQ ID NO: 63 (Y349C; T366S; L38A; Y407V; and N297A) and the second half-life extension domain comprises SEQ ID NO 64 (S354C, T366W and N297A).

In some embodiments, the IL-15 cytokine or functional fragment thereof comprises the amino acid sequence of SEQ ID NO: 133 and the masking moiety comprises the amino acid sequence of SEQ ID NO: 12 and the first half-life extension domain comprises SEQ ID NO: 63 (Y349C; T366S; L38A; Y407V; and N297A) and the second half-life extension domain comprises SEQ ID NO 64 (S354C, T366W and N297A).

In some embodiments, the IL-15 cytokine or functional fragment thereof comprises the amino acid sequence of SEQ ID NO: 2 and the masking moiety comprises the amino acid sequence of SEQ ID NO: 11 and the first half-life extension domain comprises SEQ ID NO: 65 (Y349C; T366S; L38A; Y407V, N297A and I253A) and the second half-life extension domain comprises SEQ ID NO: 66 (S354C, T366W, N297A and I253A)

In some embodiments, the IL-15 cytokine or functional fragment thereof comprises the amino acid sequence of SEQ ID NO: 2 and the masking moiety comprises the amino acid sequence of SEQ ID NO: 12 and the first half-life extension domain comprises SEQ ID NO: 65 (Y349C; T366S; L38A; Y407V, N297A and I253A) and the second half-life extension domain comprises SEQ ID NO:66 (S354C, T366W, N297A and I253A).

In some embodiments, the IL-15 cytokine or functional fragment thereof comprises the amino acid sequence of SEQ ID NO: 2 and the masking moiety comprises the amino acid sequence of SEQ ID NO: 11 and the non-cleavable linker comprises an amino acid sequence as shown in SEQ ID NO: 22.

In some embodiments, the IL-15 cytokine or functional fragment thereof comprises the amino acid sequence of SEQ ID NO: 2 and the masking moiety comprises the amino acid sequence of SEQ ID NO: 12 and the non-cleavable linker comprises an amino acid sequence as shown in SEQ ID NO: 22.

In some embodiments, the IL-15 cytokine or functional fragment thereof comprises the amino acid sequence of SEQ ID NO: 127 and the masking moiety comprises the amino acid sequence of SEQ ID NO: 12 and the non-cleavable linker comprises an amino acid sequence as shown in SEQ ID NO: 22.

In some embodiments, the IL-15 cytokine or functional fragment thereof comprises the amino acid sequence of SEQ ID NO: 128 and the masking moiety comprises the amino acid sequence of SEQ ID NO: 12 and the non-cleavable linker comprises an amino acid sequence as shown in SEQ ID NO: 22.

In some embodiments, the IL-15 cytokine or functional fragment thereof comprises the amino acid sequence of SEQ ID NO: 129 and the masking moiety comprises the amino acid sequence of SEQ ID NO: 12 and the non-cleavable linker comprises an amino acid sequence as shown in SEQ ID NO: 22.

In some embodiments, the IL-15 cytokine or functional fragment thereof comprises the amino acid sequence of SEQ ID NO: 130 and the masking moiety comprises the amino acid sequence of SEQ ID NO: 12 and the non-cleavable linker comprises an amino acid sequence as shown in SEQ ID NO: 22.

In some embodiments, the IL-15 cytokine or functional fragment thereof comprises the amino acid sequence of SEQ ID NO: 131 and the masking moiety comprises the amino acid sequence of SEQ ID NO: 12 and the non-cleavable linker comprises an amino acid sequence as shown in SEQ ID NO: 22.

In some embodiments, the IL-15 cytokine or functional fragment thereof comprises the amino acid sequence of SEQ ID NO: 132 and the masking moiety comprises the amino acid sequence of SEQ ID NO: 12 and the non-cleavable linker comprises an amino acid sequence as shown in SEQ ID NO: 22.

In some embodiments, the IL-15 cytokine or functional fragment thereof comprises the amino acid sequence of SEQ ID NO: 133 and the masking moiety comprises the amino acid sequence of SEQ ID NO: 12 and the non-cleavable linker comprises an amino acid sequence as shown in SEQ ID NO: 22.

In some embodiments, the IL-15 cytokine or functional fragment thereof comprises the amino acid sequence of SEQ ID NO: 2 and the masking moiety comprises the amino acid sequence of SEQ ID NO: 11 and the cleavable peptide comprises an amino acid sequence as shown in SEQ ID NO: 33.

In some embodiments, the IL-15 cytokine or functional fragment thereof comprises the amino acid sequence of SEQ ID NO: 2 and the masking moiety comprises the amino acid sequence of SEQ ID NO: 12 and the cleavable peptide comprises an amino acid sequence as shown in SEQ ID NO: 33.

In some embodiments, the IL-15 cytokine or functional fragment thereof comprises the amino acid sequence of SEQ ID NO: 127 and the masking moiety comprises the amino acid sequence of SEQ ID NO: 12 and the cleavable peptide comprises an amino acid sequence as shown in SEQ ID NO: 33.

In some embodiments, the IL-15 cytokine or functional fragment thereof comprises the amino acid sequence of SEQ ID NO: 128 and the masking moiety comprises the amino acid sequence of SEQ ID NO: 12 and the cleavable peptide comprises an amino acid sequence as shown in SEQ ID NO: 33.

In some embodiments, the IL-15 cytokine or functional fragment thereof comprises the amino acid sequence of SEQ ID NO: 129 and the masking moiety comprises the amino acid sequence of SEQ ID NO: 12 and the cleavable peptide comprises an amino acid sequence as shown in SEQ ID NO: 33.

In some embodiments, the IL-15 cytokine or functional fragment thereof comprises the amino acid sequence of SEQ ID NO: 130 and the masking moiety comprises the amino acid sequence of SEQ ID NO: 12 and the cleavable peptide comprises an amino acid sequence as shown in SEQ ID NO: 33.

In some embodiments, the IL-15 cytokine or functional fragment thereof comprises the amino acid sequence of SEQ ID NO: 131 and the masking moiety comprises the amino acid sequence of SEQ ID NO: 12 and the cleavable peptide comprises an amino acid sequence as shown in SEQ ID NO: 33.

In some embodiments, the IL-15 cytokine or functional fragment thereof comprises the amino acid sequence of SEQ ID NO: 132 and the masking moiety comprises the amino acid sequence of SEQ ID NO: 12 and the cleavable peptide comprises an amino acid sequence as shown in SEQ ID NO: 33.

In some embodiments, the IL-15 cytokine or functional fragment thereof comprises the amino acid sequence of SEQ ID NO: 133 and the masking moiety comprises the amino acid sequence of SEQ ID NO: 12 and the cleavable peptide comprises an amino acid sequence as shown in SEQ ID NO: 33.

In some embodiments, the IL-15 cytokine or functional fragment thereof comprises the amino acid sequence of SEQ ID NO: 2 and the masking moiety comprises the amino acid sequence of SEQ ID NO: 11 and the cleavable peptide comprises an amino acid sequence as shown in SEQ ID NO: 34.

In some embodiments, the IL-15 cytokine or functional fragment thereof comprises the amino acid sequence of SEQ ID NO: 2 and the masking moiety comprises the amino acid sequence of SEQ ID NO: 12 and the cleavable peptide comprises an amino acid sequence as shown in SEQ ID NO: 34.

In some embodiments, the IL-15 cytokine or functional fragment thereof comprises the amino acid sequence of SEQ ID NO: 127 and the masking moiety comprises the amino acid sequence of SEQ ID NO: 12 and the cleavable peptide comprises an amino acid sequence as shown in SEQ ID NO: 27.
In some embodiments, the IL-15 cytokine or functional fragment thereof comprises the amino acid sequence of SEQ ID NO: 128 and the masking moiety comprises the amino acid sequence of SEQ ID NO: 12 and the cleavable peptide comprises an amino acid sequence as shown in SEQ ID NO: 27.

In some embodiments, the IL-15 cytokine or functional fragment thereof comprises the amino acid sequence of SEQ ID NO: 129 and the masking moiety comprises the amino acid sequence of SEQ ID NO: 12 and the cleavable peptide comprises an amino acid sequence as shown in SEQ ID NO: 27.

In some embodiments, the IL-15 cytokine or functional fragment thereof comprises the amino acid sequence of SEQ ID NO: 130 and the masking moiety comprises the amino acid sequence of SEQ ID NO: 12 and the cleavable peptide comprises an amino acid sequence as shown in SEQ ID NO: 27.

In some embodiments, the IL-15 cytokine or functional fragment thereof comprises the amino acid sequence of SEQ ID NO: 131 and the masking moiety comprises the amino acid sequence of SEQ ID NO: 12 and the cleavable peptide comprises an amino acid sequence as shown in SEQ ID NO: 27.

In some embodiments, the IL-15 cytokine or functional fragment thereof comprises the amino acid sequence of SEQ ID NO: 132 and the masking moiety comprises the amino acid sequence of SEQ ID NO: 12 and the cleavable peptide comprises an amino acid sequence as shown in SEQ ID NO: 27.

In some embodiments, the IL-15 cytokine or functional fragment thereof comprises the amino acid sequence of SEQ ID NO: 133 and the masking moiety comprises the amino acid sequence of SEQ ID NO: 12 and the cleavable peptide comprises an amino acid sequence as shown in SEQ ID NO: 27.

In some embodiments, the IL-15 cytokine or functional fragment thereof comprises the amino acid sequence of SEQ ID NO: 127 and the masking moiety comprises the amino acid sequence of SEQ ID NO: 12 and the cleavable peptide comprises an amino acid sequence as shown in SEQ ID NO: 134.

In some embodiments, the IL-15 cytokine or functional fragment thereof comprises the amino acid sequence of SEQ ID NO: 128 and the masking moiety comprises the amino acid sequence of SEQ ID NO: 12 and the cleavable peptide comprises an amino acid sequence as shown in SEQ ID NO: 134.

In some embodiments, the IL-15 cytokine or functional fragment thereof comprises the amino acid sequence of SEQ ID NO: 129 and the masking moiety comprises the amino acid sequence of SEQ ID NO: 12 and the cleavable peptide comprises an amino acid sequence as shown in SEQ ID NO: 134.

In some embodiments, the IL-15 cytokine or functional fragment thereof comprises the amino acid sequence of SEQ ID NO: 130 and the masking moiety comprises the amino acid sequence of SEQ ID NO: 12 and the cleavable peptide comprises an amino acid sequence as shown in SEQ ID NO: 134.

In some embodiments, the IL-15 cytokine or functional fragment thereof comprises the amino acid sequence of SEQ ID NO: 131 and the masking moiety comprises the amino acid sequence of SEQ ID NO: 12 and the cleavable peptide comprises an amino acid sequence as shown in SEQ ID NO: 134.

In some embodiments, the IL-15 cytokine or functional fragment thereof comprises the amino acid sequence of SEQ ID NO: 132 and the masking moiety comprises the amino acid sequence of SEQ ID NO: 12 and the cleavable peptide comprises an amino acid sequence as shown in SEQ ID NO: 134.

In some embodiments, the IL-15 cytokine or functional fragment thereof comprises the amino acid sequence of SEQ ID NO: 133 and the masking moiety comprises the amino acid sequence of SEQ ID NO: 12 and the cleavable peptide comprises an amino acid sequence as shown in SEQ ID NO: 134.

In some embodiments, the IL-15 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: 11 and the first half-life extension domain comprises SEQ ID NO: 63 (Y349C; T366S; L38A; Y407V; and N297A) and the second half-life extension domain comprises SEQ ID NO 64 (S354C, T366W and N297A).

In some embodiments, the IL-15 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: 12 and the first half-life extension domain comprises SEQ ID NO: 63 (Y349C; T366S; L38A; Y407V; and N297A) and the second half-life extension domain comprises SEQ ID NO 64 (S354C, T366W and N297A).

In some embodiments, the IL-15 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: 11 and the first half-life extension domain comprises SEQ ID NO: 65 (Y349C; T366S; L38A; Y407V, N297A

and I253A) and the second half-life extension domain comprises SEQ ID NO: 66 (S354C, T366W, N297A and I253A)

In some embodiments, the IL-15 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: 12 and the first half-life extension domain comprises SEQ ID NO: 65 (Y349C; T366S; L38A; Y407V, N297A and I253A) and the second half-life extension domain comprises SEQ ID NO:66 (S354C, T366W, N297A and I253A).

In some embodiments, the IL-15 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: 11 and the non-cleavable linker comprises an amino acid sequence as shown in SEQ ID NO: 22.

In some embodiments, the IL-15 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: 12 and the non-cleavable linker comprises an amino acid sequence as shown in SEQ ID NO: 22.

In some embodiments, the IL-15 cytokine or functional fragment thereof comprises the amino acid sequence of SEQ ID NO: 4 and the masking moiety comprises the amino acid sequence of SEQ ID NO: 11 and the cleavable peptide comprises an amino acid sequence as shown in SEQ ID NO: 33.

In some embodiments, the IL-15 cytokine or functional fragment thereof comprises the amino acid sequence of SEQ ID NO: 4 and the masking moiety comprises the amino acid sequence of SEQ ID NO: 12 and the cleavable peptide comprises an amino acid sequence as shown in SEQ ID NO: 33.

In some embodiments, the IL-15 cytokine or functional fragment thereof comprises the amino acid sequence of SEQ ID NO: 4 and the masking moiety comprises the amino acid sequence of SEQ ID NO: 11 and the cleavable peptide comprises an amino acid sequence as shown in SEQ ID NO: 34.

In some embodiments, the IL-15 cytokine or functional fragment thereof comprises the amino acid sequence of SEQ ID NO: 4 and the masking moiety comprises the amino acid sequence of SEQ ID NO: 12 and the cleavable peptide comprises an amino acid sequence as shown in SEQ ID NO: 34.

In some embodiments, the IL-15 cytokine or functional fragment thereof comprises the amino acid sequence of SEQ ID NO: 4 and the masking moiety comprises the amino acid sequence of SEQ ID NO: 11 and the first half-life extension domain comprises SEQ ID NO: 63 (Y349C; T366S; L38A; Y407V; and N297A) and the second half-life extension domain comprises SEQ ID NO 64 (S354C, T366W and N297A).

In some embodiments, the IL-15 cytokine or functional fragment thereof comprises the amino acid sequence of SEQ ID NO: 4 and the masking moiety comprises the amino acid sequence of SEQ ID NO: 12 and the first half-life extension domain comprises SEQ ID NO: 63 (Y349C; T366S; L38A; Y407V; and N297A) and the second half-life extension domain comprises SEQ ID NO 64 (S354C, T366W and N297A).

In some embodiments, the IL-15 cytokine or functional fragment thereof comprises the amino acid sequence of SEQ ID NO: 4 and the masking moiety comprises the amino acid sequence of SEQ ID NO: 11 and the first half-life extension domain comprises SEQ ID NO: 65 (Y349C; T366S; L38A; Y407V, N297A and I253A) and the second half-life extension domain comprises SEQ ID NO: 66 (S354C, T366W, N297A and I253A)

In some embodiments, the IL-15 cytokine or functional fragment thereof comprises the amino acid sequence of SEQ ID NO: 4 and the masking moiety comprises the amino acid sequence of SEQ ID NO: 12 and the first half-life extension domain comprises SEQ ID NO: 65 (Y349C; T366S; L38A; Y407V, N297A and I253A) and the second half-life extension domain comprises SEQ ID NO:66 (S354C, T366W, N297A and I253A).

In some embodiments, the IL-15 cytokine or functional fragment thereof comprises the amino acid sequence of SEQ ID NO: 4 and the masking moiety comprises the amino acid sequence of SEQ ID NO: 11 and the non-cleavable linker comprises an amino acid sequence as shown in SEQ ID NO: 22.

In some embodiments, the IL-15 cytokine or functional fragment thereof comprises the amino acid sequence of SEQ ID NO: 4 and the masking moiety comprises the amino acid sequence of SEQ ID NO: 12 and the non-cleavable linker comprises an amino acid sequence as shown in SEQ ID NO: 22.

In some embodiments, the IL-15 cytokine or functional fragment thereof comprises the amino acid sequence of SEQ ID NO: 4 and the masking moiety comprises the amino acid sequence of SEQ ID NO: 11 and the cleavable peptide comprises an amino acid sequence as shown in SEQ ID NO: 33.

In some embodiments, the IL-15 cytokine or functional fragment thereof comprises the amino acid sequence of SEQ ID NO: 4 and the masking moiety comprises the amino acid sequence of SEQ ID NO: 12 and the cleavable peptide comprises an amino acid sequence as shown in SEQ ID NO: 33.

In some embodiments, the IL-15 cytokine or functional fragment thereof comprises the amino acid sequence of SEQ ID NO: 4 and the masking moiety comprises the amino acid sequence of SEQ ID NO: 11 and the cleavable peptide comprises an amino acid sequence as shown in SEQ ID NO: 34.

In some embodiments, the IL-15 cytokine or functional fragment thereof comprises the amino acid sequence of SEQ ID NO: 4 and the masking moiety comprises the amino acid sequence of SEQ ID NO: 12 and the cleavable peptide comprises an amino acid sequence as shown in SEQ ID NO: 34.

In some embodiments, the IL-15 cytokine or functional fragment thereof comprises the amino acid sequence of SEQ ID NO: 5 and the masking moiety comprises the amino acid sequence of SEQ ID NO: 11 and the first half-life extension domain comprises SEQ ID NO: 63 (Y349C; T366S; L38A; Y407V; and N297A) and the second half-life extension domain comprises SEQ ID NO 64 (S354C, T366W and N297A).

In some embodiments, the IL-15 cytokine or functional fragment thereof comprises the amino acid sequence of SEQ ID NO: 5 and the masking moiety comprises the amino acid sequence of SEQ ID NO: 12 and the first half-life extension domain comprises SEQ ID NO: 63 (Y349C; T366S; L38A; Y407V; and N297A) and the second half-life extension domain comprises SEQ ID NO 64 (S354C, T366W and N297A).

In some embodiments, the IL-15 cytokine or functional fragment thereof comprises the amino acid sequence of SEQ ID NO: 5 and the masking moiety comprises the amino acid sequence of SEQ ID NO: 11 and the first half-life extension domain comprises SEQ ID NO: 65 (Y349C; T366S; L38A; Y407V, N297A and I253A) and the second half-life extension domain comprises SEQ ID NO: 66 (S354C, T366W, N297A and I253A)

In some embodiments, the IL-15 cytokine or functional fragment thereof comprises the amino acid sequence of SEQ ID NO: 5 and the masking moiety comprises the amino acid sequence of SEQ ID NO: 12 and the first half-life extension domain comprises SEQ ID NO: 65 (Y349C; T366S; L38A; Y407V, N297A and I253A) and the second half-life extension domain comprises SEQ ID NO:66 (S354C, T366W, N297A and I253A).

In some embodiments, the IL-15 cytokine or functional fragment thereof comprises the amino acid sequence of SEQ ID NO: 5 and the masking moiety comprises the amino acid sequence of SEQ ID NO: 11 and the non-cleavable linker comprises an amino acid sequence as shown in SEQ ID NO: 22.

In some embodiments, the IL-15 cytokine or functional fragment thereof comprises the amino acid sequence of SEQ ID NO: 5 and the masking moiety comprises the amino acid sequence of SEQ ID NO: 12 and the non-cleavable linker comprises an amino acid sequence as shown in SEQ ID NO: 22.

In some embodiments, the IL-15 cytokine or functional fragment thereof comprises the amino acid sequence of SEQ ID NO: 5 and the masking moiety comprises the amino acid sequence of SEQ ID NO: 11 and the cleavable peptide comprises an amino acid sequence as shown in SEQ ID NO: 33.

In some embodiments, the IL-15 cytokine or functional fragment thereof comprises the amino acid sequence of SEQ ID NO: 5 and the masking moiety comprises the amino acid sequence of SEQ ID NO: 12 and the cleavable peptide comprises an amino acid sequence as shown in SEQ ID NO: 33.

In some embodiments, the IL-15 cytokine or functional fragment thereof comprises the amino acid sequence of SEQ ID NO: 5 and the masking moiety comprises the amino acid sequence of SEQ ID NO: 11 and the cleavable peptide comprises an amino acid sequence as shown in SEQ ID NO: 34.

In some embodiments, the IL-15 cytokine or functional fragment thereof comprises the amino acid sequence of SEQ ID NO: 5 and the masking moiety comprises the amino acid sequence of SEQ ID NO: 12 and the cleavable peptide comprises an amino acid sequence as shown in SEQ ID NO: 34.

In some embodiments, the IL-15 cytokine or functional fragment thereof comprises the amino acid sequence of SEQ ID NO: 6 and the masking moiety comprises the amino acid sequence of SEQ ID NO: 11 and the first half-life extension domain comprises SEQ ID NO: 63 (Y349C; T366S; L38A; Y407V; and N297A) and the second half-life extension domain comprises SEQ ID NO 64 (S354C, T366W and N297A).

In some embodiments, the IL-15 cytokine or functional fragment thereof comprises the amino acid sequence of SEQ ID NO: 6 and the masking moiety comprises the amino acid sequence of SEQ ID NO: 12 and the first half-life extension domain comprises SEQ ID NO: 63 (Y349C; T366S; L38A; Y407V; and N297A) and the second half-life extension domain comprises SEQ ID NO 64 (S354C, T366W and N297A).

In some embodiments, the IL-15 cytokine or functional fragment thereof comprises the amino acid sequence of SEQ ID NO: 6 and the masking moiety comprises the amino acid sequence of SEQ ID NO: 11 and the first half-life extension domain comprises SEQ ID NO: 65 (Y349C; T366S; L38A; Y407V, N297A and I253A) and the second half-life extension domain comprises SEQ ID NO: 66 (S354C, T366W, N297A and I253A)

In some embodiments, the IL-15 cytokine or functional fragment thereof comprises the amino acid sequence of SEQ ID NO: 6 and the masking moiety comprises the amino acid sequence of SEQ ID NO: 12 and the first half-life extension domain comprises SEQ ID NO: 65 (Y349C; T366S; L38A; Y407V, N297A and I253A) and the second half-life extension domain comprises SEQ ID NO:66 (S354C, T366W, N297A and I253A).

In some embodiments, the IL-15 cytokine or functional fragment thereof comprises the amino acid sequence of SEQ ID NO: 6 and the masking moiety comprises the amino acid sequence of SEQ ID NO: 11 and the non-cleavable linker comprises an amino acid sequence as shown in SEQ ID NO: 22.

In some embodiments, the IL-15 cytokine or functional fragment thereof comprises the amino acid sequence of SEQ ID NO: 6 and the masking moiety comprises the amino acid sequence of SEQ ID NO: 12 and the non-cleavable linker comprises an amino acid sequence as shown in SEQ ID NO: 22.

In some embodiments, the IL-15 cytokine or functional fragment thereof comprises the amino acid sequence of SEQ ID NO: 6 and the masking moiety comprises the amino acid sequence of SEQ ID NO: 11 and the cleavable peptide comprises an amino acid sequence as shown in SEQ ID NO: 33.

In some embodiments, the IL-15 cytokine or functional fragment thereof comprises the amino acid sequence of SEQ ID NO: 6 and the masking moiety comprises the amino acid sequence of SEQ ID NO: 12 and the cleavable peptide comprises an amino acid sequence as shown in SEQ ID NO: 33.

In some embodiments, the IL-15 cytokine or functional fragment thereof comprises the amino acid sequence of SEQ ID NO: 6 and the masking moiety comprises the amino acid sequence of SEQ ID NO: 11 and the cleavable peptide comprises an amino acid sequence as shown in SEQ ID NO: 34.

In some embodiments, the IL-15 cytokine or functional fragment thereof comprises the amino acid sequence of SEQ ID NO: 7 and the masking moiety comprises the amino acid sequence of SEQ ID NO: 12 and the cleavable peptide comprises an amino acid sequence as shown in SEQ ID NO: 34.

In some embodiments, the IL-15 cytokine or functional fragment thereof comprises the amino acid sequence of SEQ ID NO: 7 and the masking moiety comprises the amino acid sequence of SEQ ID NO: 11 and the first half-life extension domain comprises SEQ ID NO: 63 (Y349C; T366S; L38A; Y407V; and N297A) and the second half-life extension domain comprises SEQ ID NO 64 (S354C, T366W and N297A).

In some embodiments, the IL-15 cytokine or functional fragment thereof comprises the amino acid sequence of SEQ ID NO: 7 and the masking moiety comprises the amino acid sequence of SEQ ID NO: 12 and the first half-life extension domain comprises SEQ ID NO: 63 (Y349C; T366S; L38A; Y407V; and N297A) and the second half-life extension domain comprises SEQ ID NO 64 (S354C, T366W and N297A).

In some embodiments, the IL-15 cytokine or functional fragment thereof comprises the amino acid sequence of SEQ ID NO: 7 and the masking moiety comprises the amino acid sequence of SEQ ID NO: 11 and the first half-life extension domain comprises SEQ ID NO: 65 (Y349C; T366S; L38A; Y407V, N297A and I253A) and the second half-life extension domain comprises SEQ ID NO: 66 (S354C, T366W, N297A and I253A)

In some embodiments, the IL-15 cytokine or functional fragment thereof comprises the amino acid sequence of SEQ ID NO: 7 and the masking moiety comprises the amino acid sequence of SEQ ID NO: 12

and the first half-life extension domain comprises SEQ ID NO: 65 (Y349C; T366S; L38A; Y407V, N297A and I253A) and the second half-life extension domain comprises SEQ ID NO:66 (S354C, T366W, N297A and I253A).

In some embodiments, the IL-15 cytokine or functional fragment thereof comprises the amino acid sequence of SEQ ID NO: 7 and the masking moiety comprises the amino acid sequence of SEQ ID NO: 11 and the non-cleavable linker comprises an amino acid sequence as shown in SEQ ID NO: 22.

In some embodiments, the IL-15 cytokine or functional fragment thereof comprises the amino acid sequence of SEQ ID NO: 7 and the masking moiety comprises the amino acid sequence of SEQ ID NO: 12 and the non-cleavable linker comprises an amino acid sequence as shown in SEQ ID NO: 22.

In some embodiments, the IL-15 cytokine or functional fragment thereof comprises the amino acid sequence of SEQ ID NO: 7 and the masking moiety comprises the amino acid sequence of SEQ ID NO: 11 and the cleavable peptide comprises an amino acid sequence as shown in SEQ ID NO: 33.

In some embodiments, the IL-15 cytokine or functional fragment thereof comprises the amino acid sequence of SEQ ID NO: 7 and the masking moiety comprises the amino acid sequence of SEQ ID NO: 12 and the cleavable peptide comprises an amino acid sequence as shown in SEQ ID NO: 33.

In some embodiments, the IL-15 cytokine or functional fragment thereof comprises the amino acid sequence of SEQ ID NO: 7 and the masking moiety comprises the amino acid sequence of SEQ ID NO: 11 and the cleavable peptide comprises an amino acid sequence as shown in SEQ ID NO: 34.

In some embodiments, the IL-15 cytokine or functional fragment thereof comprises the amino acid sequence of SEQ ID NO: 7 and the masking moiety comprises the amino acid sequence of SEQ ID NO: 12 and the cleavable peptide comprises an amino acid sequence as shown in SEQ ID NO: 34.

In some embodiments, the IL-15 cytokine or functional fragment thereof comprises the amino acid sequence of SEQ ID NO: 8 and the masking moiety comprises the amino acid sequence of SEQ ID NO: 11 and the first half-life extension domain comprises SEQ ID NO: 63 (Y349C; T366S; L38A; Y407V; and N297A) and the second half-life extension domain comprises SEQ ID NO 64 (S354C, T366W and N297A).

In some embodiments, the IL-15 cytokine or functional fragment thereof comprises the amino acid sequence of SEQ ID NO: 8 and the masking moiety comprises the amino acid sequence of SEQ ID NO: 12 and the first half-life extension domain comprises SEQ ID NO: 63 (Y349C; T366S; L38A; Y407V; and N297A) and the second half-life extension domain comprises SEQ ID NO 64 (S354C, T366W and N297A).

In some embodiments, the IL-15 cytokine or functional fragment thereof comprises the amino acid sequence of SEQ ID NO: 8 and the masking moiety comprises the amino acid sequence of SEQ ID NO: 11 and the first half-life extension domain comprises SEQ ID NO: 65 (Y349C; T366S; L38A; Y407V, N297A and I253A) and the second half-life extension domain comprises SEQ ID NO: 66 (S354C, T366W, N297A and I253A)

In some embodiments, the IL-15 cytokine or functional fragment thereof comprises the amino acid sequence of SEQ ID NO: 8 and the masking moiety comprises the amino acid sequence of SEQ ID NO: 12 and the first half-life extension domain comprises SEQ ID NO: 65 (Y349C; T366S; L38A; Y407V, N297A and I253A) and the second half-life extension domain comprises SEQ ID NO:66 (S354C, T366W, N297A and I253A).

In some embodiments, the IL-15 cytokine or functional fragment thereof comprises the amino acid sequence of SEQ ID NO: 8 and the masking moiety comprises the amino acid sequence of SEQ ID NO: 11 and the non-cleavable linker comprises an amino acid sequence as shown in SEQ ID NO: 22.

In some embodiments, the IL-15 cytokine or functional fragment thereof comprises the amino acid sequence of SEQ ID NO: 8 and the masking moiety comprises the amino acid sequence of SEQ ID NO: 12 and the non-cleavable linker comprises an amino acid sequence as shown in SEQ ID NO: 22.

In some embodiments, the IL-15 cytokine or functional fragment thereof comprises the amino acid sequence of SEQ ID NO: 8 and the masking moiety comprises the amino acid sequence of SEQ ID NO: 11 and the cleavable peptide comprises an amino acid sequence as shown in SEQ ID NO: 33.

In some embodiments, the IL-15 cytokine or functional fragment thereof comprises the amino acid sequence of SEQ ID NO: 8 and the masking moiety comprises the amino acid sequence of SEQ ID NO: 12 and the cleavable peptide comprises an amino acid sequence as shown in SEQ ID NO: 33.

In some embodiments, the IL-15 cytokine or functional fragment thereof comprises the amino acid sequence of SEQ ID NO: 8 and the masking moiety comprises the amino acid sequence of SEQ ID NO: 11 and the cleavable peptide comprises an amino acid sequence as shown in SEQ ID NO: 34.

In some embodiments, the IL-15 cytokine or functional fragment thereof comprises the amino acid sequence of SEQ ID NO: 8 and the masking moiety comprises the amino acid sequence of SEQ ID NO: 12 and the cleavable peptide comprises an amino acid sequence as shown in SEQ ID NO: 34.

In some embodiments, the IL-15 cytokine or functional fragment thereof comprises the amino acid sequence of SEQ ID NO: 2 and the first half-life extension domain comprises SEQ ID NO: 63 (Y349C;

T366S; L38A; Y407V; and N297A) and the second half-life extension domain comprises SEQ ID NO 64 (S354C, T366W and N297A) and the non-cleavable linker comprises an amino acid sequence as shown in SEQ ID NO: 22.

In some embodiments, the IL-15 cytokine or functional fragment thereof comprises the amino acid sequence of SEQ ID NO: 127 and the first half-life extension domain comprises SEQ ID NO: 63 (Y349C; T366S; L38A; Y407V; and N297A) and the second half-life extension domain comprises SEQ ID NO 64 (S354C, T366W and N297A) and the non-cleavable linker comprises an amino acid sequence as shown in SEQ ID NO: 22.

In some embodiments, the IL-15 cytokine or functional fragment thereof comprises the amino acid sequence of SEQ ID NO: 128 and the first half-life extension domain comprises SEQ ID NO: 63 (Y349C; T366S; L38A; Y407V; and N297A) and the second half-life extension domain comprises SEQ ID NO 64 (S354C, T366W and N297A) and the non-cleavable linker comprises an amino acid sequence as shown in SEQ ID NO: 22.

In some embodiments, the IL-15 cytokine or functional fragment thereof comprises the amino acid sequence of SEQ ID NO: 129 and the first half-life extension domain comprises SEQ ID NO: 63 (Y349C; T366S; L38A; Y407V; and N297A) and the second half-life extension domain comprises SEQ ID NO 64 (S354C, T366W and N297A) and the non-cleavable linker comprises an amino acid sequence as shown in SEQ ID NO: 22.

In some embodiments, the IL-15 cytokine or functional fragment thereof comprises the amino acid sequence of SEQ ID NO: 130 and the first half-life extension domain comprises SEQ ID NO: 63 (Y349C; T366S; L38A; Y407V; and N297A) and the second half-life extension domain comprises SEQ ID NO 64 (S354C, T366W and N297A) and the non-cleavable linker comprises an amino acid sequence as shown in SEQ ID NO: 22.

In some embodiments, the IL-15 cytokine or functional fragment thereof comprises the amino acid sequence of SEQ ID NO: 131 and the first half-life extension domain comprises SEQ ID NO: 63 (Y349C; T366S; L38A; Y407V; and N297A) and the second half-life extension domain comprises SEQ ID NO 64 (S354C, T366W and N297A) and the non-cleavable linker comprises an amino acid sequence as shown in SEQ ID NO: 22.

In some embodiments, the IL-15 cytokine or functional fragment thereof comprises the amino acid sequence of SEQ ID NO: 132 and the first half-life extension domain comprises SEQ ID NO: 63 (Y349C; T366S; L38A; Y407V; and N297A) and the second half-life extension domain comprises SEQ ID NO 64

(S354C, T366W and N297A) and the non-cleavable linker comprises an amino acid sequence as shown in SEQ ID NO: 22.

In some embodiments, the IL-15 cytokine or functional fragment thereof comprises the amino acid sequence of SEQ ID NO: 133 and the first half-life extension domain comprises SEQ ID NO: 63 (Y349C; T366S; L38A; Y407V; and N297A) and the second half-life extension domain comprises SEQ ID NO 64 (S354C, T366W and N297A) and the non-cleavable linker comprises an amino acid sequence as shown in SEQ ID NO: 22.

In some embodiments, the IL-15 cytokine or functional fragment thereof comprises the amino acid sequence of SEQ ID NO: 2 and the first half-life extension domain comprises SEQ ID NO: 63 (Y349C; T366S; L38A; Y407V; and N297A) and the second half-life extension domain comprises SEQ ID NO 64 (S354C, T366W and N297A) and the cleavable peptide comprises an amino acid sequence as shown in SEQ ID NO: 33.

In some embodiments, the IL-15 cytokine or functional fragment thereof comprises the amino acid sequence of SEQ ID NO: 2 and the first half-life extension domain comprises SEQ ID NO: 63 (Y349C; T366S; L38A; Y407V; and N297A) and the second half-life extension domain comprises SEQ ID NO 64 (S354C, T366W and N297A) and the cleavable peptide comprises an amino acid sequence as shown in SEQ ID NO: 34.

In some embodiments, the IL-15 cytokine or functional fragment thereof comprises the amino acid sequence of SEQ ID NO: 127 and the first half-life extension domain comprises SEQ ID NO: 63 (Y349C; T366S; L38A; Y407V; and N297A) and the second half-life extension domain comprises SEQ ID NO 64 (S354C, T366W and N297A) and the cleavable peptide comprises an amino acid sequence as shown in SEQ ID NO: 33.

In some embodiments, the IL-15 cytokine or functional fragment thereof comprises the amino acid sequence of SEQ ID NO: 128 and the first half-life extension domain comprises SEQ ID NO: 63 (Y349C; T366S; L38A; Y407V; and N297A) and the second half-life extension domain comprises SEQ ID NO 64 (S354C, T366W and N297A) and the cleavable peptide comprises an amino acid sequence as shown in SEQ ID NO: 33.

In some embodiments, the IL-15 cytokine or functional fragment thereof comprises the amino acid sequence of SEQ ID NO: 129 and the first half-life extension domain comprises SEQ ID NO: 63 (Y349C; T366S; L38A; Y407V; and N297A) and the second half-life extension domain comprises SEQ ID NO 64 (S354C, T366W and N297A) and the cleavable peptide comprises an amino acid sequence as shown in SEQ ID NO: 33.

In some embodiments, the IL-15 cytokine or functional fragment thereof comprises the amino acid sequence of SEQ ID NO: 130 and the first half-life extension domain comprises SEQ ID NO: 63 (Y349C; T366S; L38A; Y407V; and N297A) and the second half-life extension domain comprises SEQ ID NO 64 (S354C, T366W and N297A) and the cleavable peptide comprises an amino acid sequence as shown in SEQ ID NO: 33.

In some embodiments, the IL-15 cytokine or functional fragment thereof comprises the amino acid sequence of SEQ ID NO: 131 and the first half-life extension domain comprises SEQ ID NO: 63 (Y349C; T366S; L38A; Y407V; and N297A) and the second half-life extension domain comprises SEQ ID NO 64 (S354C, T366W and N297A) and the cleavable peptide comprises an amino acid sequence as shown in SEQ ID NO: 33.

In some embodiments, the IL-15 cytokine or functional fragment thereof comprises the amino acid sequence of SEQ ID NO: 132 and the first half-life extension domain comprises SEQ ID NO: 63 (Y349C; T366S; L38A; Y407V; and N297A) and the second half-life extension domain comprises SEQ ID NO 64 (S354C, T366W and N297A) and the cleavable peptide comprises an amino acid sequence as shown in SEQ ID NO: 33.

In some embodiments, the IL-15 cytokine or functional fragment thereof comprises the amino acid sequence of SEQ ID NO: 133 and the first half-life extension domain comprises SEQ ID NO: 63 (Y349C; T366S; L38A; Y407V; and N297A) and the second half-life extension domain comprises SEQ ID NO 64 (S354C, T366W and N297A) and the cleavable peptide comprises an amino acid sequence as shown in SEQ ID NO: 33.

In some embodiments, the IL-15 cytokine or functional fragment thereof comprises the amino acid sequence of SEQ ID NO: 127 and the first half-life extension domain comprises SEQ ID NO: 63 (Y349C; T366S; L38A; Y407V; and N297A) and the second half-life extension domain comprises SEQ ID NO 64 (S354C, T366W and N297A) and the cleavable peptide comprises an amino acid sequence as shown in SEQ ID NO: 27.

In some embodiments, the IL-15 cytokine or functional fragment thereof comprises the amino acid sequence of SEQ ID NO: 128 and the first half-life extension domain comprises SEQ ID NO: 63 (Y349C; T366S; L38A; Y407V; and N297A) and the second half-life extension domain comprises SEQ ID NO 64 (S354C, T366W and N297A) and the cleavable peptide comprises an amino acid sequence as shown in SEQ ID NO: 27.

In some embodiments, the IL-15 cytokine or functional fragment thereof comprises the amino acid sequence of SEQ ID NO: 129 and the first half-life extension domain comprises SEQ ID NO: 63 (Y349C; T366S; L38A; Y407V; and N297A) and the second half-life extension domain comprises SEQ ID NO 64 (S354C, T366W and N297A) and the cleavable peptide comprises an amino acid sequence as shown in SEQ ID NO: 27.

In some embodiments, the IL-15 cytokine or functional fragment thereof comprises the amino acid sequence of SEQ ID NO: 130 and the first half-life extension domain comprises SEQ ID NO: 63 (Y349C; T366S; L38A; Y407V; and N297A) and the second half-life extension domain comprises SEQ ID NO 64 (S354C, T366W and N297A) and the cleavable peptide comprises an amino acid sequence as shown in SEQ ID NO: 27.

In some embodiments, the IL-15 cytokine or functional fragment thereof comprises the amino acid sequence of SEQ ID NO: 131 and the first half-life extension domain comprises SEQ ID NO: 63 (Y349C; T366S; L38A; Y407V; and N297A) and the second half-life extension domain comprises SEQ ID NO 64 (S354C, T366W and N297A) and the cleavable peptide comprises an amino acid sequence as shown in SEQ ID NO: 27.

In some embodiments, the IL-15 cytokine or functional fragment thereof comprises the amino acid sequence of SEQ ID NO: 132 and the first half-life extension domain comprises SEQ ID NO: 63 (Y349C; T366S; L38A; Y407V; and N297A) and the second half-life extension domain comprises SEQ ID NO 64 (S354C, T366W and N297A) and the cleavable peptide comprises an amino acid sequence as shown in SEQ ID NO: 27.

In some embodiments, the IL-15 cytokine or functional fragment thereof comprises the amino acid sequence of SEQ ID NO: 133 and the first half-life extension domain comprises SEQ ID NO: 63 (Y349C; T366S; L38A; Y407V; and N297A) and the second half-life extension domain comprises SEQ ID NO 64 (S354C, T366W and N297A) and the cleavable peptide comprises an amino acid sequence as shown in SEQ ID NO: 27.

In some embodiments, the IL-15 cytokine or functional fragment thereof comprises the amino acid sequence of SEQ ID NO: 127 and the first half-life extension domain comprises SEQ ID NO: 63 (Y349C; T366S; L38A; Y407V; and N297A) and the second half-life extension domain comprises SEQ ID NO 64 (S354C, T366W and N297A) and the cleavable peptide comprises an amino acid sequence as shown in SEQ ID NO: 134.

In some embodiments, the IL-15 cytokine or functional fragment thereof comprises the amino acid sequence of SEQ ID NO: 128 and the first half-life extension domain comprises SEQ ID NO: 63 (Y349C;

T366S; L38A; Y407V; and N297A) and the second half-life extension domain comprises SEQ ID NO 64 (S354C, T366W and N297A) and the cleavable peptide comprises an amino acid sequence as shown in SEQ ID NO: 134.

In some embodiments, the IL-15 cytokine or functional fragment thereof comprises the amino acid sequence of SEQ ID NO: 129 and the first half-life extension domain comprises SEQ ID NO: 63 (Y349C; T366S; L38A; Y407V; and N297A) and the second half-life extension domain comprises SEQ ID NO 64 (S354C, T366W and N297A) and the cleavable peptide comprises an amino acid sequence as shown in SEQ ID NO: 134.

In some embodiments, the IL-15 cytokine or functional fragment thereof comprises the amino acid sequence of SEQ ID NO: 130 and the first half-life extension domain comprises SEQ ID NO: 63 (Y349C; T366S; L38A; Y407V; and N297A) and the second half-life extension domain comprises SEQ ID NO 64 (S354C, T366W and N297A) and the cleavable peptide comprises an amino acid sequence as shown in SEQ ID NO: 134.

In some embodiments, the IL-15 cytokine or functional fragment thereof comprises the amino acid sequence of SEQ ID NO: 131 and the first half-life extension domain comprises SEQ ID NO: 63 (Y349C; T366S; L38A; Y407V; and N297A) and the second half-life extension domain comprises SEQ ID NO 64 (S354C, T366W and N297A) and the cleavable peptide comprises an amino acid sequence as shown in SEQ ID NO: 134.

In some embodiments, the IL-15 cytokine or functional fragment thereof comprises the amino acid sequence of SEQ ID NO: 132 and the first half-life extension domain comprises SEQ ID NO: 63 (Y349C; T366S; L38A; Y407V; and N297A) and the second half-life extension domain comprises SEQ ID NO 64 (S354C, T366W and N297A) and the cleavable peptide comprises an amino acid sequence as shown in SEQ ID NO: 134.

In some embodiments, the IL-15 cytokine or functional fragment thereof comprises the amino acid sequence of SEQ ID NO: 133 and the first half-life extension domain comprises SEQ ID NO: 63 (Y349C; T366S; L38A; Y407V; and N297A) and the second half-life extension domain comprises SEQ ID NO 64 (S354C, T366W and N297A) and the cleavable peptide comprises an amino acid sequence as shown in SEQ ID NO: 134.

In some embodiments, the IL-15 cytokine or functional fragment thereof comprises the amino acid sequence of SEQ ID NO: 2 and the first half-life extension domain comprises SEQ ID NO: 65 (Y349C; T366S; L38A; Y407V; and N297A) and the second half-life extension domain comprises SEQ ID NO 66

(S354C, T366W and N297A) and the non-cleavable linker comprises an amino acid sequence as shown in SEQ ID NO: 22.

In some embodiments, the IL-15 cytokine or functional fragment thereof comprises the amino acid sequence of SEQ ID NO: 2 and the first half-life extension domain comprises SEQ ID NO: 65 (Y349C; T366S; L38A; Y407V; and N297A) and the second half-life extension domain comprises SEQ ID NO 66 (S354C, T366W and N297A) and the cleavable peptide comprises an amino acid sequence as shown in SEQ ID NO: 33.

In some embodiments, the IL-15 cytokine or functional fragment thereof comprises the amino acid sequence of SEQ ID NO: 2 and the first half-life extension domain comprises SEQ ID NO: 65 (Y349C; T366S; L38A; Y407V; and N297A) and the second half-life extension domain comprises SEQ ID NO 66 (S354C, T366W and N297A) and the cleavable peptide comprises an amino acid sequence as shown in SEQ ID NO: 34.

In some embodiments, the IL-15 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: 63 (Y349C; T366S; L38A; Y407V; and N297A) and the second half-life extension domain comprises SEQ ID NO 64 (S354C, T366W and N297A) and the non-cleavable linker comprises an amino acid sequence as shown in SEQ ID NO: 22.

In some embodiments, the IL-15 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: 63 (Y349C; T366S; L38A; Y407V; and N297A) and the second half-life extension domain comprises SEQ ID NO 64 (S354C, T366W and N297A) and the cleavable peptide comprises an amino acid sequence as shown in SEQ ID NO: 33.

In some embodiments, the IL-15 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: 63 (Y349C; T366S; L38A; Y407V; and N297A) and the second half-life extension domain comprises SEQ ID NO 64 (S354C, T366W and N297A) and the cleavable peptide comprises an amino acid sequence as shown in SEQ ID NO: 34.

In some embodiments, the IL-15 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: 65 (Y349C; T366S; L38A; Y407V; and N297A) and the second half-life extension domain comprises SEQ ID NO 66 (S354C, T366W and N297A) and the non-cleavable linker comprises an amino acid sequence as shown in SEQ ID NO: 22.

In some embodiments, the IL-15 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: 65 (Y349C; T366S; L38A; Y407V; and N297A) and the second half-life extension domain comprises SEQ ID NO 66 (S354C, T366W and N297A) and the cleavable peptide comprises an amino acid sequence as shown in SEQ ID NO: 33.

In some embodiments, the IL-15 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: 65 (Y349C; T366S; L38A; Y407V; and N297A) and the second half-life extension domain comprises SEQ ID NO 66 (S354C, T366W and N297A) and the cleavable peptide comprises an amino acid sequence as shown in SEQ ID NO: 34.

In some embodiments, the IL-15 cytokine or functional fragment thereof comprises the amino acid sequence of SEQ ID NO: 4 and the first half-life extension domain comprises SEQ ID NO: 63 (Y349C; T366S; L38A; Y407V; and N297A) and the second half-life extension domain comprises SEQ ID NO 64 (S354C, T366W and N297A) and the non-cleavable linker comprises an amino acid sequence as shown in SEQ ID NO: 22.

In some embodiments, the IL-15 cytokine or functional fragment thereof comprises the amino acid sequence of SEQ ID NO: 4 and the first half-life extension domain comprises SEQ ID NO: 63 (Y349C; T366S; L38A; Y407V; and N297A) and the second half-life extension domain comprises SEQ ID NO 64 (S354C, T366W and N297A) and the cleavable peptide comprises an amino acid sequence as shown in SEQ ID NO: 33.

In some embodiments, the IL-15 cytokine or functional fragment thereof comprises the amino acid sequence of SEQ ID NO: 4 and the first half-life extension domain comprises SEQ ID NO: 63 (Y349C; T366S; L38A; Y407V; and N297A) and the second half-life extension domain comprises SEQ ID NO 64 (S354C, T366W and N297A) and the cleavable peptide comprises an amino acid sequence as shown in SEQ ID NO: 34.

In some embodiments, the IL-15 cytokine or functional fragment thereof comprises the amino acid sequence of SEQ ID NO: 4 and the first half-life extension domain comprises SEQ ID NO: 65 (Y349C; T366S; L38A; Y407V; and N297A) and the second half-life extension domain comprises SEQ ID NO 66 (S354C, T366W and N297A) and the non-cleavable linker comprises an amino acid sequence as shown in SEQ ID NO: 22.

In some embodiments, the IL-15 cytokine or functional fragment thereof comprises the amino acid sequence of SEQ ID NO: 4 and the first half-life extension domain comprises SEQ ID NO: 65 (Y349C; T366S; L38A; Y407V; and N297A) and the second half-life extension domain comprises SEQ ID NO 66 (S354C, T366W and N297A) and the cleavable peptide comprises an amino acid sequence as shown in SEQ ID NO: 33.

In some embodiments, the IL-15 cytokine or functional fragment thereof comprises the amino acid sequence of SEQ ID NO: 4 and the first half-life extension domain comprises SEQ ID NO: 65 (Y349C; T366S; L38A; Y407V; and N297A) and the second half-life extension domain comprises SEQ ID NO 66 (S354C, T366W and N297A) and the cleavable peptide comprises an amino acid sequence as shown in SEQ ID NO: 34.

In some embodiments, the IL-15 cytokine or functional fragment thereof comprises the amino acid sequence of SEQ ID NO: 5 and the first half-life extension domain comprises SEQ ID NO: 63 (Y349C; T366S; L38A; Y407V; and N297A) and the second half-life extension domain comprises SEQ ID NO 64 (S354C, T366W and N297A) and the non-cleavable linker comprises an amino acid sequence as shown in SEQ ID NO: 22.

In some embodiments, the IL-15 cytokine or functional fragment thereof comprises the amino acid sequence of SEQ ID NO: 5 and the first half-life extension domain comprises SEQ ID NO: 63 (Y349C; T366S; L38A; Y407V; and N297A) and the second half-life extension domain comprises SEQ ID NO 64 (S354C, T366W and N297A) and the cleavable peptide comprises an amino acid sequence as shown in SEQ ID NO: 33.

In some embodiments, the IL-15 cytokine or functional fragment thereof comprises the amino acid sequence of SEQ ID NO: 5 and the first half-life extension domain comprises SEQ ID NO: 63 (Y349C; T366S; L38A; Y407V; and N297A) and the second half-life extension domain comprises SEQ ID NO 64 (S354C, T366W and N297A) and the cleavable peptide comprises an amino acid sequence as shown in SEQ ID NO: 34.

In some embodiments, the IL-15 cytokine or functional fragment thereof comprises the amino acid sequence of SEQ ID NO: 5 and the first half-life extension domain comprises SEQ ID NO: 65 (Y349C; T366S; L38A; Y407V; and N297A) and the second half-life extension domain comprises SEQ ID NO 66 (S354C, T366W and N297A) and the non-cleavable linker comprises an amino acid sequence as shown in SEQ ID NO: 22.

In some embodiments, the IL-15 cytokine or functional fragment thereof comprises the amino acid sequence of SEQ ID NO: 5 and the first half-life extension domain comprises SEQ ID NO: 65 (Y349C;

T366S; L38A; Y407V; and N297A) and the second half-life extension domain comprises SEQ ID NO 66 (S354C, T366W and N297A) and the cleavable peptide comprises an amino acid sequence as shown in SEQ ID NO: 33.

In some embodiments, the IL-15 cytokine or functional fragment thereof comprises the amino acid sequence of SEQ ID NO: 5 and the first half-life extension domain comprises SEQ ID NO: 65 (Y349C; T366S; L38A; Y407V; and N297A) and the second half-life extension domain comprises SEQ ID NO 66 (S354C, T366W and N297A) and the cleavable peptide comprises an amino acid sequence as shown in SEQ ID NO: 34.

In some embodiments, the IL-15 cytokine or functional fragment thereof comprises the amino acid sequence of SEQ ID NO: 6 and the first half-life extension domain comprises SEQ ID NO: 63 (Y349C; T366S; L38A; Y407V; and N297A) and the second half-life extension domain comprises SEQ ID NO 64 (S354C, T366W and N297A) and the non-cleavable linker comprises an amino acid sequence as shown in SEQ ID NO: 22.

In some embodiments, the IL-15 cytokine or functional fragment thereof comprises the amino acid sequence of SEQ ID NO: 6 and the first half-life extension domain comprises SEQ ID NO: 63 (Y349C; T366S; L38A; Y407V; and N297A) and the second half-life extension domain comprises SEQ ID NO 64 (S354C, T366W and N297A) and the cleavable peptide comprises an amino acid sequence as shown in SEQ ID NO: 33.

In some embodiments, the IL-15 cytokine or functional fragment thereof comprises the amino acid sequence of SEQ ID NO: 6 and the first half-life extension domain comprises SEQ ID NO: 63 (Y349C; T366S; L38A; Y407V; and N297A) and the second half-life extension domain comprises SEQ ID NO 64 (S354C, T366W and N297A) and the cleavable peptide comprises an amino acid sequence as shown in SEQ ID NO: 34.

In some embodiments, the IL-15 cytokine or functional fragment thereof comprises the amino acid sequence of SEQ ID NO: 6 and the first half-life extension domain comprises SEQ ID NO: 65 (Y349C; T366S; L38A; Y407V; and N297A) and the second half-life extension domain comprises SEQ ID NO 66 (S354C, T366W and N297A) and the non-cleavable linker comprises an amino acid sequence as shown in SEQ ID NO: 22.

In some embodiments, the IL-15 cytokine or functional fragment thereof comprises the amino acid sequence of SEQ ID NO: 6 and the first half-life extension domain comprises SEQ ID NO: 65 (Y349C; T366S; L38A; Y407V; and N297A) and the second half-life extension domain comprises SEQ ID NO 66

(S354C, T366W and N297A) and the cleavable peptide comprises an amino acid sequence as shown in SEQ ID NO: 33.

In some embodiments, the IL-15 cytokine or functional fragment thereof comprises the amino acid sequence of SEQ ID NO: 6 and the first half-life extension domain comprises SEQ ID NO: 65 (Y349C; T366S; L38A; Y407V; and N297A) and the second half-life extension domain comprises SEQ ID NO 66 (S354C, T366W and N297A) and the cleavable peptide comprises an amino acid sequence as shown in SEQ ID NO: 34.

In some embodiments, the IL-15 cytokine or functional fragment thereof comprises the amino acid sequence of SEQ ID NO: 7 and the first half-life extension domain comprises SEQ ID NO: 63 (Y349C; T366S; L38A; Y407V; and N297A) and the second half-life extension domain comprises SEQ ID NO 64 (S354C, T366W and N297A) and the non-cleavable linker comprises an amino acid sequence as shown in SEQ ID NO: 22.

In some embodiments, the IL-15 cytokine or functional fragment thereof comprises the amino acid sequence of SEQ ID NO: 7 and the first half-life extension domain comprises SEQ ID NO: 63 (Y349C; T366S; L38A; Y407V; and N297A) and the second half-life extension domain comprises SEQ ID NO 64 (S354C, T366W and N297A) and the cleavable peptide comprises an amino acid sequence as shown in SEQ ID NO: 33.

In some embodiments, the IL-15 cytokine or functional fragment thereof comprises the amino acid sequence of SEQ ID NO: 7 and the first half-life extension domain comprises SEQ ID NO: 63 (Y349C; T366S; L38A; Y407V; and N297A) and the second half-life extension domain comprises SEQ ID NO 64 (S354C, T366W and N297A) and the cleavable peptide comprises an amino acid sequence as shown in SEQ ID NO: 34.

In some embodiments, the IL-15 cytokine or functional fragment thereof comprises the amino acid sequence of SEQ ID NO: 7 and the first half-life extension domain comprises SEQ ID NO: 65 (Y349C; T366S; L38A; Y407V; and N297A) and the second half-life extension domain comprises SEQ ID NO 66 (S354C, T366W and N297A) and the non-cleavable linker comprises an amino acid sequence as shown in SEQ ID NO: 22.

In some embodiments, the IL-15 cytokine or functional fragment thereof comprises the amino acid sequence of SEQ ID NO: 7 and the first half-life extension domain comprises SEQ ID NO: 65 (Y349C; T366S; L38A; Y407V; and N297A) and the second half-life extension domain comprises SEQ ID NO 66 (S354C, T366W and N297A) and the cleavable peptide comprises an amino acid sequence as shown in SEQ ID NO: 33.

In some embodiments, the IL-15 cytokine or functional fragment thereof comprises the amino acid sequence of SEQ ID NO: 7 and the first half-life extension domain comprises SEQ ID NO: 65 (Y349C; T366S; L38A; Y407V; and N297A) and the second half-life extension domain comprises SEQ ID NO 66 (S354C, T366W and N297A) and the cleavable peptide comprises an amino acid sequence as shown in SEQ ID NO: 34.

In some embodiments, the IL-15 cytokine or functional fragment thereof comprises the amino acid sequence of SEQ ID NO: 8 and the first half-life extension domain comprises SEQ ID NO: 63 (Y349C; T366S; L38A; Y407V; and N297A) and the second half-life extension domain comprises SEQ ID NO 64 (S354C, T366W and N297A) and the non-cleavable linker comprises an amino acid sequence as shown in SEQ ID NO: 22.

In some embodiments, the IL-15 cytokine or functional fragment thereof comprises the amino acid sequence of SEQ ID NO: 8 and the first half-life extension domain comprises SEQ ID NO: 63 (Y349C; T366S; L38A; Y407V; and N297A) and the second half-life extension domain comprises SEQ ID NO 64 (S354C, T366W and N297A) and the cleavable peptide comprises an amino acid sequence as shown in SEQ ID NO: 33.

In some embodiments, the IL-15 cytokine or functional fragment thereof comprises the amino acid sequence of SEQ ID NO: 8 and the first half-life extension domain comprises SEQ ID NO: 63 (Y349C; T366S; L38A; Y407V; and N297A) and the second half-life extension domain comprises SEQ ID NO 64 (S354C, T366W and N297A) and the cleavable peptide comprises an amino acid sequence as shown in SEQ ID NO: 34.

In some embodiments, the IL-15 cytokine or functional fragment thereof comprises the amino acid sequence of SEQ ID NO: 8 and the first half-life extension domain comprises SEQ ID NO: 65 (Y349C; T366S; L38A; Y407V; and N297A) and the second half-life extension domain comprises SEQ ID NO 66 (S354C, T366W and N297A) and the non-cleavable linker comprises an amino acid sequence as shown in SEQ ID NO: 22.

In some embodiments, the IL-15 cytokine or functional fragment thereof comprises the amino acid sequence of SEQ ID NO: 8 and the first half-life extension domain comprises SEQ ID NO: 65 (Y349C; T366S; L38A; Y407V; and N297A) and the second half-life extension domain comprises SEQ ID NO 66 (S354C, T366W and N297A) and the cleavable peptide comprises an amino acid sequence as shown in SEQ ID NO: 33.

In some embodiments, the IL-15 cytokine or functional fragment thereof comprises the amino acid sequence of SEQ ID NO: 8 and the first half-life extension domain comprises SEQ ID NO: 65 (Y349C; T366S; L38A; Y407V; and N297A) and the second half-life extension domain comprises SEQ ID NO 66 (S354C, T366W and N297A) and the cleavable peptide comprises an amino acid sequence as shown in SEQ ID NO: 34.

In some embodiments, the IL-15 cytokine or functional fragment thereof comprises the amino acid sequence of SEQ ID NO: 2 and the non-cleavable linker comprises an amino acid sequence as shown in SEQ ID NO: 22 and the cleavable peptide comprises an amino acid sequence as shown in SEQ ID NO: 33.

In some embodiments, the IL-15 cytokine or functional fragment thereof comprises the amino acid sequence of SEQ ID NO: 2 and the non-cleavable linker comprises an amino acid sequence as shown in SEQ ID NO: 22 and the cleavable peptide comprises an amino acid sequence as shown in SEQ ID NO: 34.

In some embodiments, the IL-15 cytokine or functional fragment thereof comprises the amino acid sequence of SEQ ID NO: 127 and the non-cleavable linker comprises an amino acid sequence as shown in SEQ ID NO: 22 and the cleavable peptide comprises an amino acid sequence as shown in SEQ ID NO: 27.

In some embodiments, the IL-15 cytokine or functional fragment thereof comprises the amino acid sequence of SEQ ID NO: 128 and the non-cleavable linker comprises an amino acid sequence as shown in SEQ ID NO: 22 and the cleavable peptide comprises an amino acid sequence as shown in SEQ ID NO: 27.

In some embodiments, the IL-15 cytokine or functional fragment thereof comprises the amino acid sequence of SEQ ID NO: 129 and the non-cleavable linker comprises an amino acid sequence as shown in SEQ ID NO: 22 and the cleavable peptide comprises an amino acid sequence as shown in SEQ ID NO: 27.

In some embodiments, the IL-15 cytokine or functional fragment thereof comprises the amino acid sequence of SEQ ID NO: 130 and the non-cleavable linker comprises an amino acid sequence as shown in SEQ ID NO: 22 and the cleavable peptide comprises an amino acid sequence as shown in SEQ ID NO: 27.

In some embodiments, the IL-15 cytokine or functional fragment thereof comprises the amino acid sequence of SEQ ID NO: 131 and the non-cleavable linker comprises an amino acid sequence as shown in SEQ ID NO: 22 and the cleavable peptide comprises an amino acid sequence as shown in SEQ ID NO: 27.

In some embodiments, the IL-15 cytokine or functional fragment thereof comprises the amino acid sequence of SEQ ID NO: 132 and the non-cleavable linker comprises an amino acid sequence as shown in SEQ ID NO: 22 and the cleavable peptide comprises an amino acid sequence as shown in SEQ ID NO: 27.

In some embodiments, the IL-15 cytokine or functional fragment thereof comprises the amino acid sequence of SEQ ID NO: 133 and the non-cleavable linker comprises an amino acid sequence as shown in SEQ ID NO: 22 and the cleavable peptide comprises an amino acid sequence as shown in SEQ ID NO: 27.

In some embodiments, the IL-15 cytokine or functional fragment thereof comprises the amino acid sequence of SEQ ID NO: 127 and the non-cleavable linker comprises an amino acid sequence as shown in SEQ ID NO: 22 and the cleavable peptide comprises an amino acid sequence as shown in SEQ ID NO: 33.

In some embodiments, the IL-15 cytokine or functional fragment thereof comprises the amino acid sequence of SEQ ID NO: 128 and the non-cleavable linker comprises an amino acid sequence as shown in SEQ ID NO: 22 and the cleavable peptide comprises an amino acid sequence as shown in SEQ ID NO: 33.

In some embodiments, the IL-15 cytokine or functional fragment thereof comprises the amino acid sequence of SEQ ID NO: 129 and the non-cleavable linker comprises an amino acid sequence as shown in SEQ ID NO: 22 and the cleavable peptide comprises an amino acid sequence as shown in SEQ ID NO: 33.

In some embodiments, the IL-15 cytokine or functional fragment thereof comprises the amino acid sequence of SEQ ID NO: 130 and the non-cleavable linker comprises an amino acid sequence as shown in SEQ ID NO: 22 and the cleavable peptide comprises an amino acid sequence as shown in SEQ ID NO: 33.

In some embodiments, the IL-15 cytokine or functional fragment thereof comprises the amino acid sequence of SEQ ID NO: 131 and the non-cleavable linker comprises an amino acid sequence as shown in SEQ ID NO: 22 and the cleavable peptide comprises an amino acid sequence as shown in SEQ ID NO: 33.

In some embodiments, the IL-15 cytokine or functional fragment thereof comprises the amino acid sequence of SEQ ID NO: 132 and the non-cleavable linker comprises an amino acid sequence as shown in SEQ ID NO: 22 and the cleavable peptide comprises an amino acid sequence as shown in SEQ ID NO: 33.

In some embodiments, the IL-15 cytokine or functional fragment thereof comprises the amino acid sequence of SEQ ID NO: 133 and the non-cleavable linker comprises an amino acid sequence as shown in SEQ ID NO: 22 and the cleavable peptide comprises an amino acid sequence as shown in SEQ ID NO: 33.

In some embodiments, the IL-15 cytokine or functional fragment thereof comprises the amino acid sequence of SEQ ID NO: 127 and the non-cleavable linker comprises an amino acid sequence as shown in SEQ ID NO: 22 and the cleavable peptide comprises an amino acid sequence as shown in SEQ ID NO: 134.

In some embodiments, the IL-15 cytokine or functional fragment thereof comprises the amino acid sequence of SEQ ID NO: 128 and the non-cleavable linker comprises an amino acid sequence as shown in SEQ ID NO: 22 and the cleavable peptide comprises an amino acid sequence as shown in SEQ ID NO: 134.

In some embodiments, the IL-15 cytokine or functional fragment thereof comprises the amino acid sequence of SEQ ID NO: 129 and the non-cleavable linker comprises an amino acid sequence as shown in SEQ ID NO: 22 and the cleavable peptide comprises an amino acid sequence as shown in SEQ ID NO: 134.

In some embodiments, the IL-15 cytokine or functional fragment thereof comprises the amino acid sequence of SEQ ID NO: 130 and the non-cleavable linker comprises an amino acid sequence as shown in SEQ ID NO: 22 and the cleavable peptide comprises an amino acid sequence as shown in SEQ ID NO: 134.

In some embodiments, the IL-15 cytokine or functional fragment thereof comprises the amino acid sequence of SEQ ID NO: 131 and the non-cleavable linker comprises an amino acid sequence as shown in SEQ ID NO: 22 and the cleavable peptide comprises an amino acid sequence as shown in SEQ ID NO: 134.

In some embodiments, the IL-15 cytokine or functional fragment thereof comprises the amino acid sequence of SEQ ID NO: 132 and the non-cleavable linker comprises an amino acid sequence as shown in SEQ ID NO: 22 and the cleavable peptide comprises an amino acid sequence as shown in SEQ ID NO: 134.

In some embodiments, the IL-15 cytokine or functional fragment thereof comprises the amino acid sequence of SEQ ID NO: 133 and the non-cleavable linker comprises an amino acid sequence as shown in SEQ ID NO: 22 and the cleavable peptide comprises an amino acid sequence as shown in SEQ ID NO: 134.

In some embodiments, the IL-15 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: 22 and the cleavable peptide comprises an amino acid sequence as shown in SEQ ID NO: 33.

In some embodiments, the IL-15 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: 22 and the cleavable peptide comprises an amino acid sequence as shown in SEQ ID NO: 34.

In some embodiments, the IL-15 cytokine or functional fragment thereof 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: 22 and the cleavable peptide comprises an amino acid sequence as shown in SEQ ID NO: 33.

In some embodiments, the IL-15 cytokine or functional fragment thereof 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: 22 and the cleavable peptide comprises an amino acid sequence as shown in SEQ ID NO: 34.

In some embodiments, the IL-15 cytokine or functional fragment thereof 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: 22 and the cleavable peptide comprises an amino acid sequence as shown in SEQ ID NO: 33.

In some embodiments, the IL-15 cytokine or functional fragment thereof 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: 22 and the cleavable peptide comprises an amino acid sequence as shown in SEQ ID NO: 34.

In some embodiments, the IL-15 cytokine or functional fragment thereof comprises the amino acid sequence of SEQ ID NO: 6 and the non-cleavable linker comprises an amino acid sequence as shown in SEQ ID NO: 22 and the cleavable peptide comprises an amino acid sequence as shown in SEQ ID NO: 33.

In some embodiments, the IL-15 cytokine or functional fragment thereof comprises the amino acid sequence of SEQ ID NO: 6 and the non-cleavable linker comprises an amino acid sequence as shown in SEQ ID NO: 22 and the cleavable peptide comprises an amino acid sequence as shown in SEQ ID NO: 34.

In some embodiments, the IL-15 cytokine or functional fragment thereof comprises the amino acid sequence of SEQ ID NO: 7 and the non-cleavable linker comprises an amino acid sequence as shown in SEQ ID NO: 22 and the cleavable peptide comprises an amino acid sequence as shown in SEQ ID NO: 33.

In some embodiments, the IL-15 cytokine or functional fragment thereof comprises the amino acid sequence of SEQ ID NO: 7 and the non-cleavable linker comprises an amino acid sequence as shown in SEQ ID NO: 22 and the cleavable peptide comprises an amino acid sequence as shown in SEQ ID NO: 34.

In some embodiments, the IL-15 cytokine or functional fragment thereof comprises the amino acid sequence of SEQ ID NO: 8 and the non-cleavable linker comprises an amino acid sequence as shown in SEQ ID NO: 22 and the cleavable peptide comprises an amino acid sequence as shown in SEQ ID NO: 33.

In some embodiments, the IL-15 cytokine or functional fragment thereof comprises the amino acid sequence of SEQ ID NO: 8 and the non-cleavable linker comprises an amino acid sequence as shown in SEQ ID NO: 22 and the cleavable peptide comprises an amino acid sequence as shown in SEQ ID NO: 34.

In some embodiments, the masking moiety comprises the amino acid sequence of SEQ ID NO: 11 and the first half-life extension domain comprises SEQ ID NO: 63 (Y349C; T366S; L38A; Y407V; and N297A) and the second half-life extension domain comprises SEQ ID NO 64 (S354C, T366W and N297A) and the non-cleavable linker comprises an amino acid sequence as shown in SEQ ID NO: 22.

In some embodiments, the masking moiety comprises the amino acid sequence of SEQ ID NO: 12 and the first half-life extension domain comprises SEQ ID NO: 63 (Y349C; T366S; L38A; Y407V; and N297A) and the second half-life extension domain comprises SEQ ID NO 64 (S354C, T366W and N297A) and the non-cleavable linker comprises an amino acid sequence as shown in SEQ ID NO: 22.

In some embodiments, the masking moiety comprises the amino acid sequence of SEQ ID NO: 11 and the first half-life extension domain comprises SEQ ID NO: 65 (Y349C; T366S; L38A; Y407V, N297A and I253A) and the second half-life extension domain comprises SEQ ID NO: 66 (S354C, T366W, N297A and I253A) and the non-cleavable linker comprises an amino acid sequence as shown in SEQ ID NO: 22.

In some embodiments, the masking moiety comprises the amino acid sequence of SEQ ID NO: 12 and the first half-life extension domain comprises SEQ ID NO: 65 (Y349C; T366S; L38A; Y407V, N297A and I253A) and the second half-life extension domain comprises SEQ ID NO: 66 (S354C, T366W, N297A and I253A) and the non-cleavable linker comprises an amino acid sequence as shown in SEQ ID NO: 22.

In some embodiments, the masking moiety comprises the amino acid sequence of SEQ ID NO: 11 and the non-cleavable linker comprises an amino acid sequence as shown in SEQ ID NO: 22 and the cleavable peptide comprises an amino acid sequence as shown in SEQ ID NO: 33.

In some embodiments, the masking moiety comprises the amino acid sequence of SEQ ID NO: 12 and the non-cleavable linker comprises an amino acid sequence as shown in SEQ ID NO: 22 and the cleavable peptide comprises an amino acid sequence as shown in SEQ ID NO: 33.

In some embodiments, the masking moiety comprises the amino acid sequence of SEQ ID NO: 12 and the non-cleavable linker comprises an amino acid sequence as shown in SEQ ID NO: 22 and the cleavable peptide comprises an amino acid sequence as shown in SEQ ID NO: 27.

In some embodiments, the masking moiety comprises the amino acid sequence of SEQ ID NO: 12 and the non-cleavable linker comprises an amino acid sequence as shown in SEQ ID NO: 22 and the cleavable peptide comprises an amino acid sequence as shown in SEQ ID NO: 134

In some embodiments, the masking moiety comprises the amino acid sequence of SEQ ID NO: 11 and the non-cleavable linker comprises an amino acid sequence as shown in SEQ ID NO: 22 and the cleavable peptide comprises an amino acid sequence as shown in SEQ ID NO: 34.

In some embodiments, the masking moiety comprises the amino acid sequence of SEQ ID NO: 12 and the non-cleavable linker comprises an amino acid sequence as shown in SEQ ID NO: 22 and the cleavable peptide comprises an amino acid sequence as shown in SEQ ID NO: 34.

In some embodiments, the first half-life extension domain comprises SEQ ID NO: 63 (Y349C; T366S; L38A; Y407V; and N297A) and the second half-life extension domain comprises SEQ ID NO 64 (S354C, T366W and N297A) and the non-cleavable linker comprises an amino acid sequence as shown in SEQ ID NO: 22 and the cleavable peptide comprises an amino acid sequence as shown in SEQ ID NO: 33.

In some embodiments, the first half-life extension domain comprises SEQ ID NO: 63 (Y349C; T366S; L38A; Y407V; and N297A) and the second half-life extension domain comprises SEQ ID NO 64 (S354C, T366W and N297A) and the non-cleavable linker comprises an amino acid sequence as shown in SEQ ID NO: 22 and the cleavable peptide comprises an amino acid sequence as shown in SEQ ID NO: 34.

In some embodiments, the first half-life extension domain comprises SEQ ID NO: 63 (Y349C; T366S; L38A; Y407V; and N297A) and the second half-life extension domain comprises SEQ ID NO 64 (S354C, T366W and N297A) and the non-cleavable linker comprises an amino acid sequence as shown in SEQ ID NO: 22 and the cleavable peptide comprises an amino acid sequence as shown in SEQ ID NO: 27.

In some embodiments, the first half-life extension domain comprises SEQ ID NO: 63 (Y349C; T366S; L38A; Y407V; and N297A) and the second half-life extension domain comprises SEQ ID NO 64 (S354C, T366W and N297A) and the non-cleavable linker comprises an amino acid sequence as shown in SEQ ID NO: 22 and the cleavable peptide comprises an amino acid sequence as shown in SEQ ID NO: 134.

In some embodiments, the first half-life extension domain comprises SEQ ID NO: 65 (Y349C; T366S; L38A; Y407V; and N297A) and the second half-life extension domain comprises SEQ ID NO 65 (S354C, T366W and N297A) and the non-cleavable linker comprises an amino acid sequence as shown in SEQ ID NO: 22 and the cleavable peptide comprises an amino acid sequence as shown in SEQ ID NO: 33.

In some embodiments, the first half-life extension domain comprises SEQ ID NO: 65 (Y349C; T366S; L38A; Y407V; and N297A) and the second half-life extension domain comprises SEQ ID NO 66 (S354C, T366W and N297A) and the non-cleavable linker comprises an amino acid sequence as shown in SEQ ID NO: 22 and the cleavable peptide comprises an amino acid sequence as shown in SEQ ID NO: 34.

In some embodiments, the IL-15 cytokine or functional fragment thereof comprises the amino acid sequence of SEQ ID NO: 2 and the masking moiety comprises the amino acid sequence of SEQ ID NO: 11 and the first half-life extension domain comprises SEQ ID NO: 63 (Y349C; T366S; L38A; Y407V; and N297A) and the second half-life extension domain comprises SEQ ID NO 64 (S354C, T366W and N297A) and the non-cleavable linker comprises an amino acid sequence as shown in SEQ ID NO: 22.

In some embodiments, the IL-15 cytokine or functional fragment thereof comprises the amino acid sequence of SEQ ID NO: 2 and the masking moiety comprises the amino acid sequence of SEQ ID NO: 12 and the first half-life extension domain comprises SEQ ID NO: 63 (Y349C; T366S; L38A; Y407V; and N297A) and the second half-life extension domain comprises SEQ ID NO 64 (S354C, T366W and N297A) and the non-cleavable linker comprises an amino acid sequence as shown in SEQ ID NO: 22.

In some embodiments, the IL-15 cytokine or functional fragment thereof comprises the amino acid sequence of SEQ ID NO: 127 and the masking moiety comprises the amino acid sequence of SEQ ID NO: 12 and the first half-life extension domain comprises SEQ ID NO: 63 (Y349C; T366S; L38A; Y407V; and N297A) and the second half-life extension domain comprises SEQ ID NO 64 (S354C, T366W and N297A) and the non-cleavable linker comprises an amino acid sequence as shown in SEQ ID NO: 22.

In some embodiments, the IL-15 cytokine or functional fragment thereof comprises the amino acid sequence of SEQ ID NO: 128 and the masking moiety comprises the amino acid sequence of SEQ ID NO: 12 and the first half-life extension domain comprises SEQ ID NO: 63 (Y349C; T366S; L38A; Y407V; and N297A) and the second half-life extension domain comprises SEQ ID NO 64 (S354C, T366W and N297A) and the non-cleavable linker comprises an amino acid sequence as shown in SEQ ID NO: 22.

In some embodiments, the IL-15 cytokine or functional fragment thereof comprises the amino acid sequence of SEQ ID NO: 129 and the masking moiety comprises the amino acid sequence of SEQ ID NO: 12 and the first half-life extension domain comprises SEQ ID NO: 63 (Y349C; T366S; L38A; Y407V; and N297A) and the second half-life extension domain comprises SEQ ID NO 64 (S354C, T366W and N297A) and the non-cleavable linker comprises an amino acid sequence as shown in SEQ ID NO: 22.

In some embodiments, the IL-15 cytokine or functional fragment thereof comprises the amino acid sequence of SEQ ID NO: 130 and the masking moiety comprises the amino acid sequence of SEQ ID NO: 12 and the first half-life extension domain comprises SEQ ID NO: 63 (Y349C; T366S; L38A; Y407V; and

N297A) and the second half-life extension domain comprises SEQ ID NO 64 (S354C, T366W and N297A) and the non-cleavable linker comprises an amino acid sequence as shown in SEQ ID NO: 22.

In some embodiments, the IL-15 cytokine or functional fragment thereof comprises the amino acid sequence of SEQ ID NO: 131 and the masking moiety comprises the amino acid sequence of SEQ ID NO: 12 and the first half-life extension domain comprises SEQ ID NO: 63 (Y349C; T366S; L38A; Y407V; and N297A) and the second half-life extension domain comprises SEQ ID NO 64 (S354C, T366W and N297A) and the non-cleavable linker comprises an amino acid sequence as shown in SEQ ID NO: 22.

In some embodiments, the IL-15 cytokine or functional fragment thereof comprises the amino acid sequence of SEQ ID NO: 132 and the masking moiety comprises the amino acid sequence of SEQ ID NO: 12 and the first half-life extension domain comprises SEQ ID NO: 63 (Y349C; T366S; L38A; Y407V; and N297A) and the second half-life extension domain comprises SEQ ID NO 64 (S354C, T366W and N297A) and the non-cleavable linker comprises an amino acid sequence as shown in SEQ ID NO: 22.

In some embodiments, the IL-15 cytokine or functional fragment thereof comprises the amino acid sequence of SEQ ID NO: 133 and the masking moiety comprises the amino acid sequence of SEQ ID NO: 12 and the first half-life extension domain comprises SEQ ID NO: 63 (Y349C; T366S; L38A; Y407V; and N297A) and the second half-life extension domain comprises SEQ ID NO 64 (S354C, T366W and N297A) and the non-cleavable linker comprises an amino acid sequence as shown in SEQ ID NO: 22.

In some embodiments, the IL-15 cytokine or functional fragment thereof comprises the amino acid sequence of SEQ ID NO: 2 and the masking moiety comprises the amino acid sequence of SEQ ID NO: 11 and the first half-life extension domain comprises SEQ ID NO: 65 (Y349C; T366S; L38A; Y407V, N297A and I253A) and the second half-life extension domain comprises SEQ ID NO: 66 (S354C, T366W, N297A and I253A) and the non-cleavable linker comprises an amino acid sequence as shown in SEQ ID NO: 22.

In some embodiments, the IL-15 cytokine or functional fragment thereof comprises the amino acid sequence of SEQ ID NO: 2 and the masking moiety comprises the amino acid sequence of SEQ ID NO: 12 and the first half-life extension domain comprises SEQ ID NO: 65 (Y349C; T366S; L38A; Y407V, N297A and I253A) and the second half-life extension domain comprises SEQ ID NO: 66 (S354C, T366W, N297A and I253A) and the non-cleavable linker comprises an amino acid sequence as shown in SEQ ID NO: 22.

In some embodiments, the IL-15 cytokine or functional fragment thereof comprises the amino acid sequence of SEQ ID NO: 2 and the masking moiety comprises the amino acid sequence of SEQ ID NO: 11 and the first half-life extension domain comprises SEQ ID NO: 63 (Y349C; T366S; L38A; Y407V; and N297A) and the second half-life extension domain comprises SEQ ID NO 64 (S354C, T366W and N297A) and the cleavable peptide comprises an amino acid sequence as shown in SEQ ID NO: 33.

In some embodiments, the IL-15 cytokine or functional fragment thereof comprises the amino acid sequence of SEQ ID NO: 2 and the masking moiety comprises the amino acid sequence of SEQ ID NO: 12 and the first half-life extension domain comprises SEQ ID NO: 63 (Y349C; T366S; L38A; Y407V; and N297A) and the second half-life extension domain comprises SEQ ID NO 64 (S354C, T366W and N297A) and the cleavable peptide comprises an amino acid sequence as shown in SEQ ID NO: 33.

In some embodiments, the IL-15 cytokine or functional fragment thereof comprises the amino acid sequence of SEQ ID NO: 2 and the masking moiety comprises the amino acid sequence of SEQ ID NO: 11 and the first half-life extension domain comprises SEQ ID NO: 65 (Y349C; T366S; L38A; Y407V; and N297A) and the second half-life extension domain comprises SEQ ID NO 66 (S354C, T366W and N297A) and the cleavable peptide comprises an amino acid sequence as shown in SEQ ID NO: 33.

In some embodiments, the IL-15 cytokine or functional fragment thereof comprises the amino acid sequence of SEQ ID NO: 2 and the masking moiety comprises the amino acid sequence of SEQ ID NO: 12 and the first half-life extension domain comprises SEQ ID NO: 65 (Y349C; T366S; L38A; Y407V; and N297A) and the second half-life extension domain comprises SEQ ID NO 66 (S354C, T366W and N297A) and the cleavable peptide comprises an amino acid sequence as shown in SEQ ID NO: 33.

In some embodiments, the IL-15 cytokine or functional fragment thereof comprises the amino acid sequence of SEQ ID NO: 2 and the masking moiety comprises the amino acid sequence of SEQ ID NO: 11 and the first half-life extension domain comprises SEQ ID NO: 63 (Y349C; T366S; L38A; Y407V; and N297A) and the second half-life extension domain comprises SEQ ID NO 64 (S354C, T366W and N297A) and the cleavable peptide comprises an amino acid sequence as shown in SEQ ID NO: 34.

In some embodiments, the IL-15 cytokine or functional fragment thereof comprises the amino acid sequence of SEQ ID NO: 2 and the masking moiety comprises the amino acid sequence of SEQ ID NO: 12 and the first half-life extension domain comprises SEQ ID NO: 63 (Y349C; T366S; L38A; Y407V; and N297A) and the second half-life extension domain comprises SEQ ID NO 64 (S354C, T366W and N297A) and the cleavable peptide comprises an amino acid sequence as shown in SEQ ID NO: 34.

In some embodiments, the IL-15 cytokine or functional fragment thereof comprises the amino acid sequence of SEQ ID NO: 127 and the masking moiety comprises the amino acid sequence of SEQ ID NO: 12 and the first half-life extension domain comprises SEQ ID NO: 63 (Y349C; T366S; L38A; Y407V; and N297A) and the second half-life extension domain comprises SEQ ID NO 64 (S354C, T366W and N297A) and the cleavable peptide comprises an amino acid sequence as shown in SEQ ID NO: 27.

In some embodiments, the IL-15 cytokine or functional fragment thereof comprises the amino acid sequence of SEQ ID NO: 128 and the masking moiety comprises the amino acid sequence of SEQ ID NO: 12 and the first half-life extension domain comprises SEQ ID NO: 63 (Y349C; T366S; L38A; Y407V; and N297A) and the second half-life extension domain comprises SEQ ID NO 64 (S354C, T366W and N297A) and the cleavable peptide comprises an amino acid sequence as shown in SEQ ID NO: 27.

In some embodiments, the IL-15 cytokine or functional fragment thereof comprises the amino acid sequence of SEQ ID NO: 129 and the masking moiety comprises the amino acid sequence of SEQ ID NO: 12 and the first half-life extension domain comprises SEQ ID NO: 63 (Y349C; T366S; L38A; Y407V; and N297A) and the second half-life extension domain comprises SEQ ID NO 64 (S354C, T366W and N297A) and the cleavable peptide comprises an amino acid sequence as shown in SEQ ID NO: 27.

In some embodiments, the IL-15 cytokine or functional fragment thereof comprises the amino acid sequence of SEQ ID NO: 130 and the masking moiety comprises the amino acid sequence of SEQ ID NO: 12 and the first half-life extension domain comprises SEQ ID NO: 63 (Y349C; T366S; L38A; Y407V; and N297A) and the second half-life extension domain comprises SEQ ID NO 64 (S354C, T366W and N297A) and the cleavable peptide comprises an amino acid sequence as shown in SEQ ID NO: 27.

In some embodiments, the IL-15 cytokine or functional fragment thereof comprises the amino acid sequence of SEQ ID NO: 132 and the masking moiety comprises the amino acid sequence of SEQ ID NO: 12 and the first half-life extension domain comprises SEQ ID NO: 63 (Y349C; T366S; L38A; Y407V; and N297A) and the second half-life extension domain comprises SEQ ID NO 64 (S354C, T366W and N297A) and the cleavable peptide comprises an amino acid sequence as shown in SEQ ID NO: 27.

In some embodiments, the IL-15 cytokine or functional fragment thereof comprises the amino acid sequence of SEQ ID NO: 133 and the masking moiety comprises the amino acid sequence of SEQ ID NO: 12 and the first half-life extension domain comprises SEQ ID NO: 63 (Y349C; T366S; L38A; Y407V; and N297A) and the second half-life extension domain comprises SEQ ID NO 64 (S354C, T366W and N297A) and the cleavable peptide comprises an amino acid sequence as shown in SEQ ID NO: 27.

In some embodiments, the IL-15 cytokine or functional fragment thereof comprises the amino acid sequence of SEQ ID NO: 127 and the masking moiety comprises the amino acid sequence of SEQ ID NO: 12 and the first half-life extension domain comprises SEQ ID NO: 63 (Y349C; T366S; L38A; Y407V; and N297A) and the second half-life extension domain comprises SEQ ID NO 64 (S354C, T366W and N297A) and the cleavable peptide comprises an amino acid sequence as shown in SEQ ID NO: 33.

In some embodiments, the IL-15 cytokine or functional fragment thereof comprises the amino acid sequence of SEQ ID NO: 128 and the masking moiety comprises the amino acid sequence of SEQ ID NO:

12 and the first half-life extension domain comprises SEQ ID NO: 63 (Y349C; T366S; L38A; Y407V; and N297A) and the second half-life extension domain comprises SEQ ID NO 64 (S354C, T366W and N297A) and the cleavable peptide comprises an amino acid sequence as shown in SEQ ID NO: 33.

In some embodiments, the IL-15 cytokine or functional fragment thereof comprises the amino acid sequence of SEQ ID NO: 129 and the masking moiety comprises the amino acid sequence of SEQ ID NO: 12 and the first half-life extension domain comprises SEQ ID NO: 63 (Y349C; T366S; L38A; Y407V; and N297A) and the second half-life extension domain comprises SEQ ID NO 64 (S354C, T366W and N297A) and the cleavable peptide comprises an amino acid sequence as shown in SEQ ID NO: 33.

In some embodiments, the IL-15 cytokine or functional fragment thereof comprises the amino acid sequence of SEQ ID NO: 130 and the masking moiety comprises the amino acid sequence of SEQ ID NO: 12 and the first half-life extension domain comprises SEQ ID NO: 63 (Y349C; T366S; L38A; Y407V; and N297A) and the second half-life extension domain comprises SEQ ID NO 64 (S354C, T366W and N297A) and the cleavable peptide comprises an amino acid sequence as shown in SEQ ID NO: 33.

In some embodiments, the IL-15 cytokine or functional fragment thereof comprises the amino acid sequence of SEQ ID NO: 131 and the masking moiety comprises the amino acid sequence of SEQ ID NO: 12 and the first half-life extension domain comprises SEQ ID NO: 63 (Y349C; T366S; L38A; Y407V; and N297A) and the second half-life extension domain comprises SEQ ID NO 64 (S354C, T366W and N297A) and the cleavable peptide comprises an amino acid sequence as shown in SEQ ID NO: 33.

In some embodiments, the IL-15 cytokine or functional fragment thereof comprises the amino acid sequence of SEQ ID NO: 132 and the masking moiety comprises the amino acid sequence of SEQ ID NO: 12 and the first half-life extension domain comprises SEQ ID NO: 63 (Y349C; T366S; L38A; Y407V; and N297A) and the second half-life extension domain comprises SEQ ID NO 64 (S354C, T366W and N297A) and the cleavable peptide comprises an amino acid sequence as shown in SEQ ID NO: 33.

In some embodiments, the IL-15 cytokine or functional fragment thereof comprises the amino acid sequence of SEQ ID NO: 133 and the masking moiety comprises the amino acid sequence of SEQ ID NO: 12 and the first half-life extension domain comprises SEQ ID NO: 63 (Y349C; T366S; L38A; Y407V; and N297A) and the second half-life extension domain comprises SEQ ID NO 64 (S354C, T366W and N297A) and the cleavable peptide comprises an amino acid sequence as shown in SEQ ID NO: 33.

In some embodiments, the IL-15 cytokine or functional fragment thereof comprises the amino acid sequence of SEQ ID NO: 127 and the masking moiety comprises the amino acid sequence of SEQ ID NO: 12 and the first half-life extension domain comprises SEQ ID NO: 63 (Y349C; T366S; L38A; Y407V; and

N297A) and the second half-life extension domain comprises SEQ ID NO 64 (S354C, T366W and N297A) and the cleavable peptide comprises an amino acid sequence as shown in SEQ ID NO: 134.

In some embodiments, the IL-15 cytokine or functional fragment thereof comprises the amino acid sequence of SEQ ID NO: 128 and the masking moiety comprises the amino acid sequence of SEQ ID NO: 12 and the first half-life extension domain comprises SEQ ID NO: 63 (Y349C; T366S; L38A; Y407V; and N297A) and the second half-life extension domain comprises SEQ ID NO 64 (S354C, T366W and N297A) and the cleavable peptide comprises an amino acid sequence as shown in SEQ ID NO: 134.

In some embodiments, the IL-15 cytokine or functional fragment thereof comprises the amino acid sequence of SEQ ID NO: 129 and the masking moiety comprises the amino acid sequence of SEQ ID NO: 12 and the first half-life extension domain comprises SEQ ID NO: 63 (Y349C; T366S; L38A; Y407V; and N297A) and the second half-life extension domain comprises SEQ ID NO 64 (S354C, T366W and N297A) and the cleavable peptide comprises an amino acid sequence as shown in SEQ ID NO: 134.

In some embodiments, the IL-15 cytokine or functional fragment thereof comprises the amino acid sequence of SEQ ID NO: 130 and the masking moiety comprises the amino acid sequence of SEQ ID NO: 12 and the first half-life extension domain comprises SEQ ID NO: 63 (Y349C; T366S; L38A; Y407V; and N297A) and the second half-life extension domain comprises SEQ ID NO 64 (S354C, T366W and N297A) and the cleavable peptide comprises an amino acid sequence as shown in SEQ ID NO: 134.

In some embodiments, the IL-15 cytokine or functional fragment thereof comprises the amino acid sequence of SEQ ID NO: 131 and the masking moiety comprises the amino acid sequence of SEQ ID NO: 12 and the first half-life extension domain comprises SEQ ID NO: 63 (Y349C; T366S; L38A; Y407V; and N297A) and the second half-life extension domain comprises SEQ ID NO 64 (S354C, T366W and N297A) and the cleavable peptide comprises an amino acid sequence as shown in SEQ ID NO: 134.

In some embodiments, the IL-15 cytokine or functional fragment thereof comprises the amino acid sequence of SEQ ID NO: 132 and the masking moiety comprises the amino acid sequence of SEQ ID NO: 12 and the first half-life extension domain comprises SEQ ID NO: 63 (Y349C; T366S; L38A; Y407V; and N297A) and the second half-life extension domain comprises SEQ ID NO 64 (S354C, T366W and N297A) and the cleavable peptide comprises an amino acid sequence as shown in SEQ ID NO: 134.

In some embodiments, the IL-15 cytokine or functional fragment thereof comprises the amino acid sequence of SEQ ID NO: 133 and the masking moiety comprises the amino acid sequence of SEQ ID NO: 12 and the first half-life extension domain comprises SEQ ID NO: 63 (Y349C; T366S; L38A; Y407V; and N297A) and the second half-life extension domain comprises SEQ ID NO 64 (S354C, T366W and N297A) and the cleavable peptide comprises an amino acid sequence as shown in SEQ ID NO: 134.

In some embodiments, the IL-15 cytokine or functional fragment thereof comprises the amino acid sequence of SEQ ID NO: 2 and the masking moiety comprises the amino acid sequence of SEQ ID NO: 11 and the first half-life extension domain comprises SEQ ID NO: 65 (Y349C; T366S; L38A; Y407V; and N297A) and the second half-life extension domain comprises SEQ ID NO 66 (S354C, T366W and N297A) and the cleavable peptide comprises an amino acid sequence as shown in SEQ ID NO: 34.

In some embodiments, the IL-15 cytokine or functional fragment thereof comprises the amino acid sequence of SEQ ID NO: 2 and the masking moiety comprises the amino acid sequence of SEQ ID NO: 12 and the first half-life extension domain comprises SEQ ID NO: 65 (Y349C; T366S; L38A; Y407V; and N297A) and the second half-life extension domain comprises SEQ ID NO 66 (S354C, T366W and N297A) and the cleavable peptide comprises an amino acid sequence as shown in SEQ ID NO: 34.

In some embodiments, the IL-15 cytokine or functional fragment thereof comprises the amino acid sequence of SEQ ID NO: 2 and the masking moiety comprises the amino acid sequence of SEQ ID NO: 11 and the non-cleavable linker comprises an amino acid sequence as shown in SEQ ID NO: 22 and the cleavable peptide comprises an amino acid sequence as shown in SEQ ID NO: 33.

In some embodiments, the IL-15 cytokine or functional fragment thereof comprises the amino acid sequence of SEQ ID NO: 2 and the masking moiety comprises the amino acid sequence of SEQ ID NO: 12 and the non-cleavable linker comprises an amino acid sequence as shown in SEQ ID NO: 22 and the cleavable peptide comprises an amino acid sequence as shown in SEQ ID NO: 33.

In some embodiments, the IL-15 cytokine or functional fragment thereof comprises the amino acid sequence of SEQ ID NO: 127 and the masking moiety comprises the amino acid sequence of SEQ ID NO: 12 and the non-cleavable linker comprises an amino acid sequence as shown in SEQ ID NO: 22 and the cleavable peptide comprises an amino acid sequence as shown in SEQ ID NO: 27.

In some embodiments, the IL-15 cytokine or functional fragment thereof comprises the amino acid sequence of SEQ ID NO: 128 and the masking moiety comprises the amino acid sequence of SEQ ID NO: 12 and the non-cleavable linker comprises an amino acid sequence as shown in SEQ ID NO: 22 and the cleavable peptide comprises an amino acid sequence as shown in SEQ ID NO: 27.

In some embodiments, the IL-15 cytokine or functional fragment thereof comprises the amino acid sequence of SEQ ID NO: 129 and the masking moiety comprises the amino acid sequence of SEQ ID NO: 12 and the non-cleavable linker comprises an amino acid sequence as shown in SEQ ID NO: 22 and the cleavable peptide comprises an amino acid sequence as shown in SEQ ID NO: 27.

In some embodiments, the IL-15 cytokine or functional fragment thereof comprises the amino acid sequence of SEQ ID NO: 130 and the masking moiety comprises the amino acid sequence of SEQ ID NO: 12 and the non-cleavable linker comprises an amino acid sequence as shown in SEQ ID NO: 22 and the cleavable peptide comprises an amino acid sequence as shown in SEQ ID NO: 27.

In some embodiments, the IL-15 cytokine or functional fragment thereof comprises the amino acid sequence of SEQ ID NO: 131 and the masking moiety comprises the amino acid sequence of SEQ ID NO: 12 and the non-cleavable linker comprises an amino acid sequence as shown in SEQ ID NO: 22 and the cleavable peptide comprises an amino acid sequence as shown in SEQ ID NO: 27.

In some embodiments, the IL-15 cytokine or functional fragment thereof comprises the amino acid sequence of SEQ ID NO: 132 and the masking moiety comprises the amino acid sequence of SEQ ID NO: 12 and the non-cleavable linker comprises an amino acid sequence as shown in SEQ ID NO: 22 and the cleavable peptide comprises an amino acid sequence as shown in SEQ ID NO: 27.

In some embodiments, the IL-15 cytokine or functional fragment thereof comprises the amino acid sequence of SEQ ID NO: 133 and the masking moiety comprises the amino acid sequence of SEQ ID NO: 12 and the non-cleavable linker comprises an amino acid sequence as shown in SEQ ID NO: 22 and the cleavable peptide comprises an amino acid sequence as shown in SEQ ID NO: 27.

In some embodiments, the IL-15 cytokine or functional fragment thereof comprises the amino acid sequence of SEQ ID NO: 127 and the masking moiety comprises the amino acid sequence of SEQ ID NO: 12 and the non-cleavable linker comprises an amino acid sequence as shown in SEQ ID NO: 22 and the cleavable peptide comprises an amino acid sequence as shown in SEQ ID NO: 33.

In some embodiments, the IL-15 cytokine or functional fragment thereof comprises the amino acid sequence of SEQ ID NO: 128 and the masking moiety comprises the amino acid sequence of SEQ ID NO: 12 and the non-cleavable linker comprises an amino acid sequence as shown in SEQ ID NO: 22 and the cleavable peptide comprises an amino acid sequence as shown in SEQ ID NO: 33.

In some embodiments, the IL-15 cytokine or functional fragment thereof comprises the amino acid sequence of SEQ ID NO: 129 and the masking moiety comprises the amino acid sequence of SEQ ID NO: 12 and the non-cleavable linker comprises an amino acid sequence as shown in SEQ ID NO: 22 and the cleavable peptide comprises an amino acid sequence as shown in SEQ ID NO: 33.

In some embodiments, the IL-15 cytokine or functional fragment thereof comprises the amino acid sequence of SEQ ID NO: 130 and the masking moiety comprises the amino acid sequence of SEQ ID NO:

12 and the non-cleavable linker comprises an amino acid sequence as shown in SEQ ID NO: 22 and the cleavable peptide comprises an amino acid sequence as shown in SEQ ID NO: 33.

In some embodiments, the IL-15 cytokine or functional fragment thereof comprises the amino acid sequence of SEQ ID NO: 131 and the masking moiety comprises the amino acid sequence of SEQ ID NO: 12 and the non-cleavable linker comprises an amino acid sequence as shown in SEQ ID NO: 22 and the cleavable peptide comprises an amino acid sequence as shown in SEQ ID NO: 33.

In some embodiments, the IL-15 cytokine or functional fragment thereof comprises the amino acid sequence of SEQ ID NO: 132 and the masking moiety comprises the amino acid sequence of SEQ ID NO: 12 and the non-cleavable linker comprises an amino acid sequence as shown in SEQ ID NO: 22 and the cleavable peptide comprises an amino acid sequence as shown in SEQ ID NO: 33.

In some embodiments, the IL-15 cytokine or functional fragment thereof comprises the amino acid sequence of SEQ ID NO: 133 and the masking moiety comprises the amino acid sequence of SEQ ID NO: 12 and the non-cleavable linker comprises an amino acid sequence as shown in SEQ ID NO: 22 and the cleavable peptide comprises an amino acid sequence as shown in SEQ ID NO: 33.

In some embodiments, the IL-15 cytokine or functional fragment thereof comprises the amino acid sequence of SEQ ID NO: 127 and the masking moiety comprises the amino acid sequence of SEQ ID NO: 12 and the non-cleavable linker comprises an amino acid sequence as shown in SEQ ID NO: 22 and the cleavable peptide comprises an amino acid sequence as shown in SEQ ID NO: 134.

In some embodiments, the IL-15 cytokine or functional fragment thereof comprises the amino acid sequence of SEQ ID NO: 128 and the masking moiety comprises the amino acid sequence of SEQ ID NO: 12 and the non-cleavable linker comprises an amino acid sequence as shown in SEQ ID NO: 22 and the cleavable peptide comprises an amino acid sequence as shown in SEQ ID NO: 134.

In some embodiments, the IL-15 cytokine or functional fragment thereof comprises the amino acid sequence of SEQ ID NO: 129 and the masking moiety comprises the amino acid sequence of SEQ ID NO: 12 and the non-cleavable linker comprises an amino acid sequence as shown in SEQ ID NO: 22 and the cleavable peptide comprises an amino acid sequence as shown in SEQ ID NO: 134.

In some embodiments, the IL-15 cytokine or functional fragment thereof comprises the amino acid sequence of SEQ ID NO: 130 and the masking moiety comprises the amino acid sequence of SEQ ID NO: 12 and the non-cleavable linker comprises an amino acid sequence as shown in SEQ ID NO: 22 and the cleavable peptide comprises an amino acid sequence as shown in SEQ ID NO: 134.

In some embodiments, the IL-15 cytokine or functional fragment thereof comprises the amino acid sequence of SEQ ID NO: 131 and the masking moiety comprises the amino acid sequence of SEQ ID NO: 12 and the non-cleavable linker comprises an amino acid sequence as shown in SEQ ID NO: 22 and the cleavable peptide comprises an amino acid sequence as shown in SEQ ID NO: 134.

In some embodiments, the IL-15 cytokine or functional fragment thereof comprises the amino acid sequence of SEQ ID NO: 132 and the masking moiety comprises the amino acid sequence of SEQ ID NO: 12 and the non-cleavable linker comprises an amino acid sequence as shown in SEQ ID NO: 22 and the cleavable peptide comprises an amino acid sequence as shown in SEQ ID NO: 134.

In some embodiments, the IL-15 cytokine or functional fragment thereof comprises the amino acid sequence of SEQ ID NO: 133 and the masking moiety comprises the amino acid sequence of SEQ ID NO: 12 and the non-cleavable linker comprises an amino acid sequence as shown in SEQ ID NO: 22 and the cleavable peptide comprises an amino acid sequence as shown in SEQ ID NO: 134.

In some embodiments, the IL-15 cytokine or functional fragment thereof comprises the amino acid sequence of SEQ ID NO: 2 and the masking moiety comprises the amino acid sequence of SEQ ID NO: 11 and the non-cleavable linker comprises an amino acid sequence as shown in SEQ ID NO: 22 and the cleavable peptide comprises an amino acid sequence as shown in SEQ ID NO: 34.

In some embodiments, the IL-15 cytokine or functional fragment thereof comprises the amino acid sequence of SEQ ID NO: 2 and the masking moiety comprises the amino acid sequence of SEQ ID NO: 12 and the non-cleavable linker comprises an amino acid sequence as shown in SEQ ID NO: 22 and the cleavable peptide comprises an amino acid sequence as shown in SEQ ID NO: 34.

In some embodiments, the IL-15 cytokine or functional fragment thereof comprises the amino acid sequence of SEQ ID NO: 2 and the first half-life extension domain comprises SEQ ID NO: 63 (Y349C; T366S; L38A; Y407V; and N297A) and the second half-life extension domain comprises SEQ ID NO 64 (S354C, T366W and N297A) and the non-cleavable linker comprises an amino acid sequence as shown in SEQ ID NO: 22 and the cleavable peptide comprises an amino acid sequence as shown in SEQ ID NO: 33.

In some embodiments, the IL-15 cytokine or functional fragment thereof comprises the amino acid sequence of SEQ ID NO: 127 and the first half-life extension domain comprises SEQ ID NO: 63 (Y349C; T366S; L38A; Y407V; and N297A) and the second half-life extension domain comprises SEQ ID NO 64 (S354C, T366W and N297A) and the non-cleavable linker comprises an amino acid sequence as shown in SEQ ID NO: 22 and the cleavable peptide comprises an amino acid sequence as shown in SEQ ID NO: 27.

In some embodiments, the IL-15 cytokine or functional fragment thereof comprises the amino acid sequence of SEQ ID NO: 128 and the first half-life extension domain comprises SEQ ID NO: 63 (Y349C; T366S; L38A; Y407V; and N297A) and the second half-life extension domain comprises SEQ ID NO 64 (S354C, T366W and N297A) and the non-cleavable linker comprises an amino acid sequence as shown in SEQ ID NO: 22 and the cleavable peptide comprises an amino acid sequence as shown in SEQ ID NO: 27.

In some embodiments, the IL-15 cytokine or functional fragment thereof comprises the amino acid sequence of SEQ ID NO: 129 and the first half-life extension domain comprises SEQ ID NO: 63 (Y349C; T366S; L38A; Y407V; and N297A) and the second half-life extension domain comprises SEQ ID NO 64 (S354C, T366W and N297A) and the non-cleavable linker comprises an amino acid sequence as shown in SEQ ID NO: 22 and the cleavable peptide comprises an amino acid sequence as shown in SEQ ID NO: 27.

In some embodiments, the IL-15 cytokine or functional fragment thereof comprises the amino acid sequence of SEQ ID NO: 130 and the first half-life extension domain comprises SEQ ID NO: 63 (Y349C; T366S; L38A; Y407V; and N297A) and the second half-life extension domain comprises SEQ ID NO 64 (S354C, T366W and N297A) and the non-cleavable linker comprises an amino acid sequence as shown in SEQ ID NO: 22 and the cleavable peptide comprises an amino acid sequence as shown in SEQ ID NO: 27.

In some embodiments, the IL-15 cytokine or functional fragment thereof comprises the amino acid sequence of SEQ ID NO: 131 and the first half-life extension domain comprises SEQ ID NO: 63 (Y349C; T366S; L38A; Y407V; and N297A) and the second half-life extension domain comprises SEQ ID NO 64 (S354C, T366W and N297A) and the non-cleavable linker comprises an amino acid sequence as shown in SEQ ID NO: 22 and the cleavable peptide comprises an amino acid sequence as shown in SEQ ID NO: 27.

In some embodiments, the IL-15 cytokine or functional fragment thereof comprises the amino acid sequence of SEQ ID NO: 132 and the first half-life extension domain comprises SEQ ID NO: 63 (Y349C; T366S; L38A; Y407V; and N297A) and the second half-life extension domain comprises SEQ ID NO 64 (S354C, T366W and N297A) and the non-cleavable linker comprises an amino acid sequence as shown in SEQ ID NO: 22 and the cleavable peptide comprises an amino acid sequence as shown in SEQ ID NO: 27.

In some embodiments, the IL-15 cytokine or functional fragment thereof comprises the amino acid sequence of SEQ ID NO: 133 and the first half-life extension domain comprises SEQ ID NO: 63 (Y349C; T366S; L38A; Y407V; and N297A) and the second half-life extension domain comprises SEQ ID NO 64 (S354C, T366W and N297A) and the non-cleavable linker comprises an amino acid sequence as shown in SEQ ID NO: 22 and the cleavable peptide comprises an amino acid sequence as shown in SEQ ID NO: 27.

In some embodiments, the IL-15 cytokine or functional fragment thereof comprises the amino acid sequence of SEQ ID NO: 127 and the first half-life extension domain comprises SEQ ID NO: 63 (Y349C; T366S; L38A; Y407V; and N297A) and the second half-life extension domain comprises SEQ ID NO 64 (S354C, T366W and N297A) and the non-cleavable linker comprises an amino acid sequence as shown in SEQ ID NO: 22 and the cleavable peptide comprises an amino acid sequence as shown in SEQ ID NO: 33.

In some embodiments, the IL-15 cytokine or functional fragment thereof comprises the amino acid sequence of SEQ ID NO: 127 and the first half-life extension domain comprises SEQ ID NO: 63 (Y349C; T366S; L38A; Y407V; and N297A) and the second half-life extension domain comprises SEQ ID NO 64 (S354C, T366W and N297A) and the non-cleavable linker comprises an amino acid sequence as shown in SEQ ID NO: 22 and the cleavable peptide comprises an amino acid sequence as shown in SEQ ID NO: 33.

In some embodiments, the IL-15 cytokine or functional fragment thereof comprises the amino acid sequence of SEQ ID NO: 128 and the first half-life extension domain comprises SEQ ID NO: 63 (Y349C; T366S; L38A; Y407V; and N297A) and the second half-life extension domain comprises SEQ ID NO 64 (S354C, T366W and N297A) and the non-cleavable linker comprises an amino acid sequence as shown in SEQ ID NO: 22 and the cleavable peptide comprises an amino acid sequence as shown in SEQ ID NO: 33.

In some embodiments, the IL-15 cytokine or functional fragment thereof comprises the amino acid sequence of SEQ ID NO: 129 and the first half-life extension domain comprises SEQ ID NO: 63 (Y349C; T366S; L38A; Y407V; and N297A) and the second half-life extension domain comprises SEQ ID NO 64 (S354C, T366W and N297A) and the non-cleavable linker comprises an amino acid sequence as shown in SEQ ID NO: 22 and the cleavable peptide comprises an amino acid sequence as shown in SEQ ID NO: 33.

In some embodiments, the IL-15 cytokine or functional fragment thereof comprises the amino acid sequence of SEQ ID NO: 130 and the first half-life extension domain comprises SEQ ID NO: 63 (Y349C; T366S; L38A; Y407V; and N297A) and the second half-life extension domain comprises SEQ ID NO 64 (S354C, T366W and N297A) and the non-cleavable linker comprises an amino acid sequence as shown in SEQ ID NO: 22 and the cleavable peptide comprises an amino acid sequence as shown in SEQ ID NO: 33.

In some embodiments, the IL-15 cytokine or functional fragment thereof comprises the amino acid sequence of SEQ ID NO: 132 and the first half-life extension domain comprises SEQ ID NO: 63 (Y349C; T366S; L38A; Y407V; and N297A) and the second half-life extension domain comprises SEQ ID NO 64 (S354C, T366W and N297A) and the non-cleavable linker comprises an amino acid sequence as shown in SEQ ID NO: 22 and the cleavable peptide comprises an amino acid sequence as shown in SEQ ID NO: 33.

In some embodiments, the IL-15 cytokine or functional fragment thereof comprises the amino acid sequence of SEQ ID NO: 133 and the first half-life extension domain comprises SEQ ID NO: 63 (Y349C;

T366S; L38A; Y407V; and N297A) and the second half-life extension domain comprises SEQ ID NO 64 (S354C, T366W and N297A) and the non-cleavable linker comprises an amino acid sequence as shown in SEQ ID NO: 22 and the cleavable peptide comprises an amino acid sequence as shown in SEQ ID NO: 33.

In some embodiments, the IL-15 cytokine or functional fragment thereof comprises the amino acid sequence of SEQ ID NO: 127 and the first half-life extension domain comprises SEQ ID NO: 63 (Y349C; T366S; L38A; Y407V; and N297A) and the second half-life extension domain comprises SEQ ID NO 64 (S354C, T366W and N297A) and the non-cleavable linker comprises an amino acid sequence as shown in SEQ ID NO: 22 and the cleavable peptide comprises an amino acid sequence as shown in SEQ ID NO: 134.

In some embodiments, the IL-15 cytokine or functional fragment thereof comprises the amino acid sequence of SEQ ID NO: 128 and the first half-life extension domain comprises SEQ ID NO: 63 (Y349C; T366S; L38A; Y407V; and N297A) and the second half-life extension domain comprises SEQ ID NO 64 (S354C, T366W and N297A) and the non-cleavable linker comprises an amino acid sequence as shown in SEQ ID NO: 22 and the cleavable peptide comprises an amino acid sequence as shown in SEQ ID NO: 134.

In some embodiments, the IL-15 cytokine or functional fragment thereof comprises the amino acid sequence of SEQ ID NO: 129 and the first half-life extension domain comprises SEQ ID NO: 63 (Y349C; T366S; L38A; Y407V; and N297A) and the second half-life extension domain comprises SEQ ID NO 64 (S354C, T366W and N297A) and the non-cleavable linker comprises an amino acid sequence as shown in SEQ ID NO: 22 and the cleavable peptide comprises an amino acid sequence as shown in SEQ ID NO: 134.

In some embodiments, the IL-15 cytokine or functional fragment thereof comprises the amino acid sequence of SEQ ID NO: 130 and the first half-life extension domain comprises SEQ ID NO: 63 (Y349C; T366S; L38A; Y407V; and N297A) and the second half-life extension domain comprises SEQ ID NO 64 (S354C, T366W and N297A) and the non-cleavable linker comprises an amino acid sequence as shown in SEQ ID NO: 22 and the cleavable peptide comprises an amino acid sequence as shown in SEQ ID NO: 134.

In some embodiments, the IL-15 cytokine or functional fragment thereof comprises the amino acid sequence of SEQ ID NO: 131 and the first half-life extension domain comprises SEQ ID NO: 63 (Y349C; T366S; L38A; Y407V; and N297A) and the second half-life extension domain comprises SEQ ID NO 64 (S354C, T366W and N297A) and the non-cleavable linker comprises an amino acid sequence as shown in SEQ ID NO: 22 and the cleavable peptide comprises an amino acid sequence as shown in SEQ ID NO: 134.

In some embodiments, the IL-15 cytokine or functional fragment thereof comprises the amino acid sequence of SEQ ID NO: 132 and the first half-life extension domain comprises SEQ ID NO: 63 (Y349C; T366S; L38A; Y407V; and N297A) and the second half-life extension domain comprises SEQ ID NO 64 (S354C, T366W and N297A) and the non-cleavable linker comprises an amino acid sequence as shown in SEQ ID NO: 22 and the cleavable peptide comprises an amino acid sequence as shown in SEQ ID NO: 134.

In some embodiments, the IL-15 cytokine or functional fragment thereof comprises the amino acid sequence of SEQ ID NO: 133 and the first half-life extension domain comprises SEQ ID NO: 63 (Y349C; T366S; L38A; Y407V; and N297A) and the second half-life extension domain comprises SEQ ID NO 64 (S354C, T366W and N297A) and the non-cleavable linker comprises an amino acid sequence as shown in SEQ ID NO: 22 and the cleavable peptide comprises an amino acid sequence as shown in SEQ ID NO: 134.

In some embodiments, the IL-15 cytokine or functional fragment thereof comprises the amino acid sequence of SEQ ID NO: 2 and the first half-life extension domain comprises SEQ ID NO: 63 (Y349C; T366S; L38A; Y407V; and N297A) and the second half-life extension domain comprises SEQ ID NO 64 (S354C, T366W and N297A) and the non-cleavable linker comprises an amino acid sequence as shown in SEQ ID NO: 22 and the cleavable peptide comprises an amino acid sequence as shown in SEQ ID NO: 34.

In some embodiments, the IL-15 cytokine or functional fragment thereof comprises the amino acid sequence of SEQ ID NO: 2 and the first half-life extension domain comprises SEQ ID NO: 65 (Y349C; T366S; L38A; Y407V; and N297A) and the second half-life extension domain comprises SEQ ID NO 66 (S354C, T366W and N297A) and the non-cleavable linker comprises an amino acid sequence as shown in SEQ ID NO: 22 and the cleavable peptide comprises an amino acid sequence as shown in SEQ ID NO: 33.

In some embodiments, the IL-15 cytokine or functional fragment thereof comprises the amino acid sequence of SEQ ID NO: 2 and the first half-life extension domain comprises SEQ ID NO: 65 (Y349C; T366S; L38A; Y407V; and N297A) and the second half-life extension domain comprises SEQ ID NO 66 (S354C, T366W and N297A) and the non-cleavable linker comprises an amino acid sequence as shown in SEQ ID NO: 22 and the cleavable peptide comprises an amino acid sequence as shown in SEQ ID NO: 34.

In some embodiments, the IL-15 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: 11 and the first half-life extension domain comprises SEQ ID NO: 63 (Y349C; T366S; L38A; Y407V; and N297A) and the second half-life extension domain comprises SEQ ID NO 64 (S354C, T366W and N297A) and the non-cleavable linker comprises an amino acid sequence as shown in SEQ ID NO: 22.

In some embodiments, the IL-15 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: 12 and the first half-life extension domain comprises SEQ ID NO: 63 (Y349C; T366S; L38A; Y407V; and N297A) and the second half-life extension domain comprises SEQ ID NO 64 (S354C, T366W and N297A) and the non-cleavable linker comprises an amino acid sequence as shown in SEQ ID NO: 22.

In some embodiments, the IL-15 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: 11 and the first half-life extension domain comprises SEQ ID NO: 65 (Y349C; T366S; L38A; Y407V, N297A and I253A) and the second half-life extension domain comprises SEQ ID NO: 66 (S354C, T366W, N297A and I253A) and the non-cleavable linker comprises an amino acid sequence as shown in SEQ ID NO: 22.

In some embodiments, the IL-15 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: 12 and the first half-life extension domain comprises SEQ ID NO: 65 (Y349C; T366S; L38A; Y407V, N297A and I253A) and the second half-life extension domain comprises SEQ ID NO: 66 (S354C, T366W, N297A and I253A) and the non-cleavable linker comprises an amino acid sequence as shown in SEQ ID NO: 22.

In some embodiments, the IL-15 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: 11 and the first half-life extension domain comprises SEQ ID NO: 63 (Y349C; T366S; L38A; Y407V; and N297A) and the second half-life extension domain comprises SEQ ID NO 64 (S354C, T366W and N297A) and the cleavable peptide comprises an amino acid sequence as shown in SEQ ID NO: 33.

In some embodiments, the IL-15 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: 12 and the first half-life extension domain comprises SEQ ID NO: 63 (Y349C; T366S; L38A; Y407V; and N297A) and the second half-life extension domain comprises SEQ ID NO 64 (S354C, T366W and N297A) and the cleavable peptide comprises an amino acid sequence as shown in SEQ ID NO: 33.

In some embodiments, the IL-15 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: 11 and the first half-life extension domain comprises SEQ ID NO: 65 (Y349C; T366S; L38A; Y407V; and N297A) and the second half-life extension domain comprises SEQ ID NO 66 (S354C, T366W and N297A) and the cleavable peptide comprises an amino acid sequence as shown in SEQ ID NO: 33.

In some embodiments, the IL-15 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: 12 and the first half-life extension domain comprises SEQ ID NO: 65 (Y349C; T366S; L38A; Y407V; and N297A) and the second half-life extension domain comprises SEQ ID NO 66 (S354C, T366W and N297A) and the cleavable peptide comprises an amino acid sequence as shown in SEQ ID NO: 33.

In some embodiments, the IL-15 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: 11 and the first half-life extension domain comprises SEQ ID NO: 63 (Y349C; T366S; L38A; Y407V; and N297A) and the second half-life extension domain comprises SEQ ID NO 64 (S354C, T366W and N297A) and the cleavable peptide comprises an amino acid sequence as shown in SEQ ID NO: 34.

In some embodiments, the IL-15 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: 12 and the first half-life extension domain comprises SEQ ID NO: 63 (Y349C; T366S; L38A; Y407V; and N297A) and the second half-life extension domain comprises SEQ ID NO 64 (S354C, T366W and N297A) and the cleavable peptide comprises an amino acid sequence as shown in SEQ ID NO: 34.

In some embodiments, the IL-15 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: 11 and the first half-life extension domain comprises SEQ ID NO: 65 (Y349C; T366S; L38A; Y407V; and N297A) and the second half-life extension domain comprises SEQ ID NO 66 (S354C, T366W and N297A) and the cleavable peptide comprises an amino acid sequence as shown in SEQ ID NO: 34.

In some embodiments, the IL-15 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: 12 and the first half-life extension domain comprises SEQ ID NO: 65 (Y349C; T366S; L38A; Y407V; and N297A) and the second half-life extension domain comprises SEQ ID NO 66 (S354C, T366W and N297A) and the cleavable peptide comprises an amino acid sequence as shown in SEQ ID NO: 34.

In some embodiments, the IL-15 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: 11 and the non-cleavable linker comprises an amino acid sequence as shown in SEQ ID NO: 22 and the cleavable peptide comprises an amino acid sequence as shown in SEQ ID NO: 33.

In some embodiments, the IL-15 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: 12

and the non-cleavable linker comprises an amino acid sequence as shown in SEQ ID NO: 22 and the cleavable peptide comprises an amino acid sequence as shown in SEQ ID NO: 33.

In some embodiments, the IL-15 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: 11 and the non-cleavable linker comprises an amino acid sequence as shown in SEQ ID NO: 22 and the cleavable peptide comprises an amino acid sequence as shown in SEQ ID NO: 34.

In some embodiments, the IL-15 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: 12 and the non-cleavable linker comprises an amino acid sequence as shown in SEQ ID NO: 22 and the cleavable peptide comprises an amino acid sequence as shown in SEQ ID NO: 34.

In some embodiments, the IL-15 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: 63 (Y349C; T366S; L38A; Y407V; and N297A) and the second half-life extension domain comprises SEQ ID NO 64 (S354C, T366W and N297A) and the non-cleavable linker comprises an amino acid sequence as shown in SEQ ID NO: 22 and the cleavable peptide comprises an amino acid sequence as shown in SEQ ID NO: 33.

In some embodiments, the IL-15 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: 63 (Y349C; T366S; L38A; Y407V; and N297A) and the second half-life extension domain comprises SEQ ID NO 64 (S354C, T366W and N297A) and the non-cleavable linker comprises an amino acid sequence as shown in SEQ ID NO: 22 and the cleavable peptide comprises an amino acid sequence as shown in SEQ ID NO: 34.

In some embodiments, the IL-15 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: 65 (Y349C; T366S; L38A; Y407V; and N297A) and the second half-life extension domain comprises SEQ ID NO 66 (S354C, T366W and N297A) and the non-cleavable linker comprises an amino acid sequence as shown in SEQ ID NO: 22 and the cleavable peptide comprises an amino acid sequence as shown in SEQ ID NO: 33.

In some embodiments, the IL-15 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: 65 (Y349C; T366S; L38A; Y407V; and N297A) and the second half-life extension domain comprises SEQ ID NO 66 (S354C, T366W and N297A) and the non-cleavable linker comprises an amino acid sequence as shown in SEQ ID NO: 22 and the cleavable peptide comprises an amino acid sequence as shown in SEQ ID NO: 34.

In some embodiments, the IL-15 cytokine or functional fragment thereof comprises the amino acid sequence of SEQ ID NO: 4 and the masking moiety comprises the amino acid sequence of SEQ ID NO: 11 and the first half-life extension domain comprises SEQ ID NO: 63 (Y349C; T366S; L38A; Y407V; and N297A) and the second half-life extension domain comprises SEQ ID NO 64 (S354C, T366W and N297A) and the non-cleavable linker comprises an amino acid sequence as shown in SEQ ID NO: 22.

In some embodiments, the IL-15 cytokine or functional fragment thereof comprises the amino acid sequence of SEQ ID NO: 4 and the masking moiety comprises the amino acid sequence of SEQ ID NO: 12 and the first half-life extension domain comprises SEQ ID NO: 63 (Y349C; T366S; L38A; Y407V; and N297A) and the second half-life extension domain comprises SEQ ID NO 64 (S354C, T366W and N297A) and the non-cleavable linker comprises an amino acid sequence as shown in SEQ ID NO: 22.

In some embodiments, the IL-15 cytokine or functional fragment thereof comprises the amino acid sequence of SEQ ID NO: 4 and the masking moiety comprises the amino acid sequence of SEQ ID NO: 11 and the first half-life extension domain comprises SEQ ID NO: 65 (Y349C; T366S; L38A; Y407V, N297A and I253A) and the second half-life extension domain comprises SEQ ID NO: 66 (S354C, T366W, N297A and I253A) and the non-cleavable linker comprises an amino acid sequence as shown in SEQ ID NO: 22.

In some embodiments, the IL-15 cytokine or functional fragment thereof comprises the amino acid sequence of SEQ ID NO: 4 and the masking moiety comprises the amino acid sequence of SEQ ID NO: 12 and the first half-life extension domain comprises SEQ ID NO: 65 (Y349C; T366S; L38A; Y407V, N297A and I253A) and the second half-life extension domain comprises SEQ ID NO: 66 (S354C, T366W, N297A and I253A) and the non-cleavable linker comprises an amino acid sequence as shown in SEQ ID NO: 22.

In some embodiments, the IL-15 cytokine or functional fragment thereof comprises the amino acid sequence of SEQ ID NO: 4 and the masking moiety comprises the amino acid sequence of SEQ ID NO: 11 and the first half-life extension domain comprises SEQ ID NO: 63 (Y349C; T366S; L38A; Y407V; and N297A) and the second half-life extension domain comprises SEQ ID NO 64 (S354C, T366W and N297A) and the cleavable peptide comprises an amino acid sequence as shown in SEQ ID NO: 33.

In some embodiments, the IL-15 cytokine or functional fragment thereof comprises the amino acid sequence of SEQ ID NO: 4 and the masking moiety comprises the amino acid sequence of SEQ ID NO: 12 and the first half-life extension domain comprises SEQ ID NO: 63 (Y349C; T366S; L38A; Y407V; and N297A) and the second half-life extension domain comprises SEQ ID NO 64 (S354C, T366W and N297A) and the cleavable peptide comprises an amino acid sequence as shown in SEQ ID NO: 33.

In some embodiments, the IL-15 cytokine or functional fragment thereof comprises the amino acid sequence of SEQ ID NO: 4 and the masking moiety comprises the amino acid sequence of SEQ ID NO: 11

and the first half-life extension domain comprises SEQ ID NO: 65 (Y349C; T366S; L38A; Y407V; and N297A) and the second half-life extension domain comprises SEQ ID NO 66 (S354C, T366W and N297A) and the cleavable peptide comprises an amino acid sequence as shown in SEQ ID NO: 33.

In some embodiments, the IL-15 cytokine or functional fragment thereof comprises the amino acid sequence of SEQ ID NO: 4 and the masking moiety comprises the amino acid sequence of SEQ ID NO: 12 and the first half-life extension domain comprises SEQ ID NO: 65 (Y349C; T366S; L38A; Y407V; and N297A) and the second half-life extension domain comprises SEQ ID NO 66 (S354C, T366W and N297A) and the cleavable peptide comprises an amino acid sequence as shown in SEQ ID NO: 33.

In some embodiments, the IL-15 cytokine or functional fragment thereof comprises the amino acid sequence of SEQ ID NO: 4 and the masking moiety comprises the amino acid sequence of SEQ ID NO: 11 and the first half-life extension domain comprises SEQ ID NO: 63 (Y349C; T366S; L38A; Y407V; and N297A) and the second half-life extension domain comprises SEQ ID NO 64 (S354C, T366W and N297A) and the cleavable peptide comprises an amino acid sequence as shown in SEQ ID NO: 34.

In some embodiments, the IL-15 cytokine or functional fragment thereof comprises the amino acid sequence of SEQ ID NO: 4 and the masking moiety comprises the amino acid sequence of SEQ ID NO: 12 and the first half-life extension domain comprises SEQ ID NO: 63 (Y349C; T366S; L38A; Y407V; and N297A) and the second half-life extension domain comprises SEQ ID NO 64 (S354C, T366W and N297A) and the cleavable peptide comprises an amino acid sequence as shown in SEQ ID NO: 34.

In some embodiments, the IL-15 cytokine or functional fragment thereof comprises the amino acid sequence of SEQ ID NO: 4 and the masking moiety comprises the amino acid sequence of SEQ ID NO: 11 and the first half-life extension domain comprises SEQ ID NO: 65 (Y349C; T366S; L38A; Y407V; and N297A) and the second half-life extension domain comprises SEQ ID NO 66 (S354C, T366W and N297A) and the cleavable peptide comprises an amino acid sequence as shown in SEQ ID NO: 34.

In some embodiments, the IL-15 cytokine or functional fragment thereof comprises the amino acid sequence of SEQ ID NO: 4 and the masking moiety comprises the amino acid sequence of SEQ ID NO: 12 and the first half-life extension domain comprises SEQ ID NO: 65 (Y349C; T366S; L38A; Y407V; and N297A) and the second half-life extension domain comprises SEQ ID NO 66 (S354C, T366W and N297A) and the cleavable peptide comprises an amino acid sequence as shown in SEQ ID NO: 34.

In some embodiments, the IL-15 cytokine or functional fragment thereof comprises the amino acid sequence of SEQ ID NO: 4 and the masking moiety comprises the amino acid sequence of SEQ ID NO: 11 and the non-cleavable linker comprises an amino acid sequence as shown in SEQ ID NO: 22 and the cleavable peptide comprises an amino acid sequence as shown in SEQ ID NO: 33.

In some embodiments, the IL-15 cytokine or functional fragment thereof comprises the amino acid sequence of SEQ ID NO: 4 and the masking moiety comprises the amino acid sequence of SEQ ID NO: 12 and the non-cleavable linker comprises an amino acid sequence as shown in SEQ ID NO: 22 and the cleavable peptide comprises an amino acid sequence as shown in SEQ ID NO: 33.

In some embodiments, the IL-15 cytokine or functional fragment thereof comprises the amino acid sequence of SEQ ID NO: 4 and the masking moiety comprises the amino acid sequence of SEQ ID NO: 11 and the non-cleavable linker comprises an amino acid sequence as shown in SEQ ID NO: 22 and the cleavable peptide comprises an amino acid sequence as shown in SEQ ID NO: 34.

In some embodiments, the IL-15 cytokine or functional fragment thereof comprises the amino acid sequence of SEQ ID NO: 4 and the masking moiety comprises the amino acid sequence of SEQ ID NO: 12 and the non-cleavable linker comprises an amino acid sequence as shown in SEQ ID NO: 22 and the cleavable peptide comprises an amino acid sequence as shown in SEQ ID NO: 34.

In some embodiments, the IL-15 cytokine or functional fragment thereof comprises the amino acid sequence of SEQ ID NO: 4 and the first half-life extension domain comprises SEQ ID NO: 63 (Y349C; T366S; L38A; Y407V; and N297A) and the second half-life extension domain comprises SEQ ID NO 64 (S354C, T366W and N297A) and the non-cleavable linker comprises an amino acid sequence as shown in SEQ ID NO: 22 and the cleavable peptide comprises an amino acid sequence as shown in SEQ ID NO: 33.

In some embodiments, the IL-15 cytokine or functional fragment thereof comprises the amino acid sequence of SEQ ID NO: 4 and the first half-life extension domain comprises SEQ ID NO: 63 (Y349C; T366S; L38A; Y407V; and N297A) and the second half-life extension domain comprises SEQ ID NO 64 (S354C, T366W and N297A) and the non-cleavable linker comprises an amino acid sequence as shown in SEQ ID NO: 22 and the cleavable peptide comprises an amino acid sequence as shown in SEQ ID NO: 34.

In some embodiments, the IL-15 cytokine or functional fragment thereof comprises the amino acid sequence of SEQ ID NO: 4 and the first half-life extension domain comprises SEQ ID NO: 65 (Y349C; T366S; L38A; Y407V; and N297A) and the second half-life extension domain comprises SEQ ID NO 66 (S354C, T366W and N297A) and the non-cleavable linker comprises an amino acid sequence as shown in SEQ ID NO: 22 and the cleavable peptide comprises an amino acid sequence as shown in SEQ ID NO: 33.

In some embodiments, the IL-15 cytokine or functional fragment thereof comprises the amino acid sequence of SEQ ID NO: 4 and the first half-life extension domain comprises SEQ ID NO: 65 (Y349C; T366S; L38A; Y407V; and N297A) and the second half-life extension domain comprises SEQ ID NO 66

(S354C, T366W and N297A) and the non-cleavable linker comprises an amino acid sequence as shown in SEQ ID NO: 22 and the cleavable peptide comprises an amino acid sequence as shown in SEQ ID NO: 34.

In some embodiments, the IL-15 cytokine or functional fragment thereof comprises the amino acid sequence of SEQ ID NO: 5 and the masking moiety comprises the amino acid sequence of SEQ ID NO: 11 and the first half-life extension domain comprises SEQ ID NO: 63 (Y349C; T366S; L38A; Y407V; and N297A) and the second half-life extension domain comprises SEQ ID NO 64 (S354C, T366W and N297A) and the non-cleavable linker comprises an amino acid sequence as shown in SEQ ID NO: 22.

In some embodiments, the IL-15 cytokine or functional fragment thereof comprises the amino acid sequence of SEQ ID NO: 5 and the masking moiety comprises the amino acid sequence of SEQ ID NO: 12 and the first half-life extension domain comprises SEQ ID NO: 63 (Y349C; T366S; L38A; Y407V; and N297A) and the second half-life extension domain comprises SEQ ID NO 64 (S354C, T366W and N297A) and the non-cleavable linker comprises an amino acid sequence as shown in SEQ ID NO: 22.

In some embodiments, the IL-15 cytokine or functional fragment thereof comprises the amino acid sequence of SEQ ID NO: 5 and the masking moiety comprises the amino acid sequence of SEQ ID NO: 11 and the first half-life extension domain comprises SEQ ID NO: 65 (Y349C; T366S; L38A; Y407V, N297A and I253A) and the second half-life extension domain comprises SEQ ID NO: 66 (S354C, T366W, N297A and I253A) and the non-cleavable linker comprises an amino acid sequence as shown in SEQ ID NO: 22.

In some embodiments, the IL-15 cytokine or functional fragment thereof comprises the amino acid sequence of SEQ ID NO: 5 and the masking moiety comprises the amino acid sequence of SEQ ID NO: 12 and the first half-life extension domain comprises SEQ ID NO: 65 (Y349C; T366S; L38A; Y407V, N297A and I253A) and the second half-life extension domain comprises SEQ ID NO: 66 (S354C, T366W, N297A and I253A) and the non-cleavable linker comprises an amino acid sequence as shown in SEQ ID NO: 22.

In some embodiments, the IL-15 cytokine or functional fragment thereof comprises the amino acid sequence of SEQ ID NO: 5 and the masking moiety comprises the amino acid sequence of SEQ ID NO: 11 and the first half-life extension domain comprises SEQ ID NO: 63 (Y349C; T366S; L38A; Y407V; and N297A) and the second half-life extension domain comprises SEQ ID NO 64 (S354C, T366W and N297A) and the cleavable peptide comprises an amino acid sequence as shown in SEQ ID NO: 33.

In some embodiments, the IL-15 cytokine or functional fragment thereof comprises the amino acid sequence of SEQ ID NO: 5 and the masking moiety comprises the amino acid sequence of SEQ ID NO: 12 and the first half-life extension domain comprises SEQ ID NO: 63 (Y349C; T366S; L38A; Y407V; and N297A) and the second half-life extension domain comprises SEQ ID NO 64 (S354C, T366W and N297A) and the cleavable peptide comprises an amino acid sequence as shown in SEQ ID NO: 33.

In some embodiments, the IL-15 cytokine or functional fragment thereof comprises the amino acid sequence of SEQ ID NO: 5 and the masking moiety comprises the amino acid sequence of SEQ ID NO: 11 and the first half-life extension domain comprises SEQ ID NO: 65 (Y349C; T366S; L38A; Y407V; and N297A) and the second half-life extension domain comprises SEQ ID NO 66 (S354C, T366W and N297A) and the cleavable peptide comprises an amino acid sequence as shown in SEQ ID NO: 33.

In some embodiments, the IL-15 cytokine or functional fragment thereof comprises the amino acid sequence of SEQ ID NO: 5 and the masking moiety comprises the amino acid sequence of SEQ ID NO: 12 and the first half-life extension domain comprises SEQ ID NO: 65 (Y349C; T366S; L38A; Y407V; and N297A) and the second half-life extension domain comprises SEQ ID NO 66 (S354C, T366W and N297A) and the cleavable peptide comprises an amino acid sequence as shown in SEQ ID NO: 33.

In some embodiments, the IL-15 cytokine or functional fragment thereof comprises the amino acid sequence of SEQ ID NO: 5 and the masking moiety comprises the amino acid sequence of SEQ ID NO: 11 and the first half-life extension domain comprises SEQ ID NO: 63 (Y349C; T366S; L38A; Y407V; and N297A) and the second half-life extension domain comprises SEQ ID NO 64 (S354C, T366W and N297A) and the cleavable peptide comprises an amino acid sequence as shown in SEQ ID NO: 34.

In some embodiments, the IL-15 cytokine or functional fragment thereof comprises the amino acid sequence of SEQ ID NO: 5 and the masking moiety comprises the amino acid sequence of SEQ ID NO: 12 and the first half-life extension domain comprises SEQ ID NO: 63 (Y349C; T366S; L38A; Y407V; and N297A) and the second half-life extension domain comprises SEQ ID NO 64 (S354C, T366W and N297A) and the cleavable peptide comprises an amino acid sequence as shown in SEQ ID NO: 34.

In some embodiments, the IL-15 cytokine or functional fragment thereof comprises the amino acid sequence of SEQ ID NO: 5 and the masking moiety comprises the amino acid sequence of SEQ ID NO: 11 and the first half-life extension domain comprises SEQ ID NO: 65 (Y349C; T366S; L38A; Y407V; and N297A) and the second half-life extension domain comprises SEQ ID NO 66 (S354C, T366W and N297A) and the cleavable peptide comprises an amino acid sequence as shown in SEQ ID NO: 34.

In some embodiments, the IL-15 cytokine or functional fragment thereof comprises the amino acid sequence of SEQ ID NO: 5 and the masking moiety comprises the amino acid sequence of SEQ ID NO: 12 and the first half-life extension domain comprises SEQ ID NO: 65 (Y349C; T366S; L38A; Y407V; and N297A) and the second half-life extension domain comprises SEQ ID NO 66 (S354C, T366W and N297A) and the cleavable peptide comprises an amino acid sequence as shown in SEQ ID NO: 34.

In some embodiments, the IL-15 cytokine or functional fragment thereof comprises the amino acid sequence of SEQ ID NO: 5 and the masking moiety comprises the amino acid sequence of SEQ ID NO: 11 and the non-cleavable linker comprises an amino acid sequence as shown in SEQ ID NO: 22 and the cleavable peptide comprises an amino acid sequence as shown in SEQ ID NO: 33.

In some embodiments, the IL-15 cytokine or functional fragment thereof comprises the amino acid sequence of SEQ ID NO: 5 and the masking moiety comprises the amino acid sequence of SEQ ID NO: 12 and the non-cleavable linker comprises an amino acid sequence as shown in SEQ ID NO: 22 and the cleavable peptide comprises an amino acid sequence as shown in SEQ ID NO: 33.

In some embodiments, the IL-15 cytokine or functional fragment thereof comprises the amino acid sequence of SEQ ID NO: 5 and the masking moiety comprises the amino acid sequence of SEQ ID NO: 11 and the non-cleavable linker comprises an amino acid sequence as shown in SEQ ID NO: 22 and the cleavable peptide comprises an amino acid sequence as shown in SEQ ID NO: 34.

In some embodiments, the IL-15 cytokine or functional fragment thereof comprises the amino acid sequence of SEQ ID NO: 5 and the masking moiety comprises the amino acid sequence of SEQ ID NO: 12 and the non-cleavable linker comprises an amino acid sequence as shown in SEQ ID NO: 22 and the cleavable peptide comprises an amino acid sequence as shown in SEQ ID NO: 34.

In some embodiments, the IL-15 cytokine or functional fragment thereof comprises the amino acid sequence of SEQ ID NO: 5 and the first half-life extension domain comprises SEQ ID NO: 63 (Y349C; T366S; L38A; Y407V; and N297A) and the second half-life extension domain comprises SEQ ID NO 64 (S354C, T366W and N297A) and the non-cleavable linker comprises an amino acid sequence as shown in SEQ ID NO: 22 and the cleavable peptide comprises an amino acid sequence as shown in SEQ ID NO: 33.

In some embodiments, the IL-15 cytokine or functional fragment thereof comprises the amino acid sequence of SEQ ID NO: 5 and the first half-life extension domain comprises SEQ ID NO: 63 (Y349C; T366S; L38A; Y407V; and N297A) and the second half-life extension domain comprises SEQ ID NO 64 (S354C, T366W and N297A) and the non-cleavable linker comprises an amino acid sequence as shown in SEQ ID NO: 22 and the cleavable peptide comprises an amino acid sequence as shown in SEQ ID NO: 34.

In some embodiments, the IL-15 cytokine or functional fragment thereof comprises the amino acid sequence of SEQ ID NO: 5 and the first half-life extension domain comprises SEQ ID NO: 65 (Y349C; T366S; L38A; Y407V; and N297A) and the second half-life extension domain comprises SEQ ID NO 66 (S354C, T366W and N297A) and the non-cleavable linker comprises an amino acid sequence as shown in SEQ ID NO: 22 and the cleavable peptide comprises an amino acid sequence as shown in SEQ ID NO: 33.

In some embodiments, the IL-15 cytokine or functional fragment thereof comprises the amino acid sequence of SEQ ID NO: 5 and the first half-life extension domain comprises SEQ ID NO: 65 (Y349C; T366S; L38A; Y407V; and N297A) and the second half-life extension domain comprises SEQ ID NO 66 (S354C, T366W and N297A) and the non-cleavable linker comprises an amino acid sequence as shown in SEQ ID NO: 22 and the cleavable peptide comprises an amino acid sequence as shown in SEQ ID NO: 34.

In some embodiments, the IL-15 cytokine or functional fragment thereof comprises the amino acid sequence of SEQ ID NO: 6 and the masking moiety comprises the amino acid sequence of SEQ ID NO: 11 and the first half-life extension domain comprises SEQ ID NO: 63 (Y349C; T366S; L38A; Y407V; and N297A) and the second half-life extension domain comprises SEQ ID NO 64 (S354C, T366W and N297A) and the non-cleavable linker comprises an amino acid sequence as shown in SEQ ID NO: 22.

In some embodiments, the IL-15 cytokine or functional fragment thereof comprises the amino acid sequence of SEQ ID NO: 6 and the masking moiety comprises the amino acid sequence of SEQ ID NO: 12 and the first half-life extension domain comprises SEQ ID NO: 63 (Y349C; T366S; L38A; Y407V; and N297A) and the second half-life extension domain comprises SEQ ID NO 64 (S354C, T366W and N297A) and the non-cleavable linker comprises an amino acid sequence as shown in SEQ ID NO: 22.

In some embodiments, the IL-15 cytokine or functional fragment thereof comprises the amino acid sequence of SEQ ID NO: 6 and the masking moiety comprises the amino acid sequence of SEQ ID NO: 11 and the first half-life extension domain comprises SEQ ID NO: 65 (Y349C; T366S; L38A; Y407V, N297A and I253A) and the second half-life extension domain comprises SEQ ID NO: 66 (S354C, T366W, N297A and I253A) and the non-cleavable linker comprises an amino acid sequence as shown in SEQ ID NO: 22.

In some embodiments, the IL-15 cytokine or functional fragment thereof comprises the amino acid sequence of SEQ ID NO: 6 and the masking moiety comprises the amino acid sequence of SEQ ID NO: 12 and the first half-life extension domain comprises SEQ ID NO: 65 (Y349C; T366S; L38A; Y407V, N297A and I253A) and the second half-life extension domain comprises SEQ ID NO: 66 (S354C, T366W, N297A and I253A) and the non-cleavable linker comprises an amino acid sequence as shown in SEQ ID NO: 22.

In some embodiments, the IL-15 cytokine or functional fragment thereof comprises the amino acid sequence of SEQ ID NO: 6 and the masking moiety comprises the amino acid sequence of SEQ ID NO: 11 and the first half-life extension domain comprises SEQ ID NO: 63 (Y349C; T366S; L38A; Y407V; and N297A) and the second half-life extension domain comprises SEQ ID NO 64 (S354C, T366W and N297A) and the cleavable peptide comprises an amino acid sequence as shown in SEQ ID NO: 33.

In some embodiments, the IL-15 cytokine or functional fragment thereof comprises the amino acid sequence of SEQ ID NO: 6 and the masking moiety comprises the amino acid sequence of SEQ ID NO: 12

and the first half-life extension domain comprises SEQ ID NO: 63 (Y349C; T366S; L38A; Y407V; and N297A) and the second half-life extension domain comprises SEQ ID NO 64 (S354C, T366W and N297A) and the cleavable peptide comprises an amino acid sequence as shown in SEQ ID NO: 33.

In some embodiments, the IL-15 cytokine or functional fragment thereof comprises the amino acid sequence of SEQ ID NO: 6 and the masking moiety comprises the amino acid sequence of SEQ ID NO: 11 and the first half-life extension domain comprises SEQ ID NO: 65 (Y349C; T366S; L38A; Y407V; and N297A) and the second half-life extension domain comprises SEQ ID NO 66 (S354C, T366W and N297A) and the cleavable peptide comprises an amino acid sequence as shown in SEQ ID NO: 33.

In some embodiments, the IL-15 cytokine or functional fragment thereof comprises the amino acid sequence of SEQ ID NO: 6 and the masking moiety comprises the amino acid sequence of SEQ ID NO: 12 and the first half-life extension domain comprises SEQ ID NO: 65 (Y349C; T366S; L38A; Y407V; and N297A) and the second half-life extension domain comprises SEQ ID NO 66 (S354C, T366W and N297A) and the cleavable peptide comprises an amino acid sequence as shown in SEQ ID NO: 33.

In some embodiments, the IL-15 cytokine or functional fragment thereof comprises the amino acid sequence of SEQ ID NO: 6 and the masking moiety comprises the amino acid sequence of SEQ ID NO: 11 and the first half-life extension domain comprises SEQ ID NO: 63 (Y349C; T366S; L38A; Y407V; and N297A) and the second half-life extension domain comprises SEQ ID NO 64 (S354C, T366W and N297A) and the cleavable peptide comprises an amino acid sequence as shown in SEQ ID NO: 34.

In some embodiments, the IL-15 cytokine or functional fragment thereof comprises the amino acid sequence of SEQ ID NO: 6 and the masking moiety comprises the amino acid sequence of SEQ ID NO: 12 and the first half-life extension domain comprises SEQ ID NO: 63 (Y349C; T366S; L38A; Y407V; and N297A) and the second half-life extension domain comprises SEQ ID NO 64 (S354C, T366W and N297A) and the cleavable peptide comprises an amino acid sequence as shown in SEQ ID NO: 34.

In some embodiments, the IL-15 cytokine or functional fragment thereof comprises the amino acid sequence of SEQ ID NO: 6 and the masking moiety comprises the amino acid sequence of SEQ ID NO: 11 and the first half-life extension domain comprises SEQ ID NO: 65 (Y349C; T366S; L38A; Y407V; and N297A) and the second half-life extension domain comprises SEQ ID NO 66 (S354C, T366W and N297A) and the cleavable peptide comprises an amino acid sequence as shown in SEQ ID NO: 34.

In some embodiments, the IL-15 cytokine or functional fragment thereof comprises the amino acid sequence of SEQ ID NO: 6 and the masking moiety comprises the amino acid sequence of SEQ ID NO: 12 and the first half-life extension domain comprises SEQ ID NO: 65 (Y349C; T366S; L38A; Y407V; and

N297A) and the second half-life extension domain comprises SEQ ID NO 66 (S354C, T366W and N297A) and the cleavable peptide comprises an amino acid sequence as shown in SEQ ID NO: 34.

In some embodiments, the IL-15 cytokine or functional fragment thereof comprises the amino acid sequence of SEQ ID NO: 6 and the masking moiety comprises the amino acid sequence of SEQ ID NO: 11 and the non-cleavable linker comprises an amino acid sequence as shown in SEQ ID NO: 22 and the cleavable peptide comprises an amino acid sequence as shown in SEQ ID NO: 33.

In some embodiments, the IL-15 cytokine or functional fragment thereof comprises the amino acid sequence of SEQ ID NO: 6 and the masking moiety comprises the amino acid sequence of SEQ ID NO: 12 and the non-cleavable linker comprises an amino acid sequence as shown in SEQ ID NO: 22 and the cleavable peptide comprises an amino acid sequence as shown in SEQ ID NO: 33.

In some embodiments, the IL-15 cytokine or functional fragment thereof comprises the amino acid sequence of SEQ ID NO: 6 and the masking moiety comprises the amino acid sequence of SEQ ID NO: 11 and the non-cleavable linker comprises an amino acid sequence as shown in SEQ ID NO: 22 and the cleavable peptide comprises an amino acid sequence as shown in SEQ ID NO: 34.

In some embodiments, the IL-15 cytokine or functional fragment thereof comprises the amino acid sequence of SEQ ID NO: 6 and the masking moiety comprises the amino acid sequence of SEQ ID NO: 12 and the non-cleavable linker comprises an amino acid sequence as shown in SEQ ID NO: 22 and the cleavable peptide comprises an amino acid sequence as shown in SEQ ID NO: 34.

In some embodiments, the IL-15 cytokine or functional fragment thereof comprises the amino acid sequence of SEQ ID NO: 6 and the first half-life extension domain comprises SEQ ID NO: 63 (Y349C; T366S; L38A; Y407V; and N297A) and the second half-life extension domain comprises SEQ ID NO 64 (S354C, T366W and N297A) and the non-cleavable linker comprises an amino acid sequence as shown in SEQ ID NO: 22 and the cleavable peptide comprises an amino acid sequence as shown in SEQ ID NO: 33.

In some embodiments, the IL-15 cytokine or functional fragment thereof comprises the amino acid sequence of SEQ ID NO: 6 and the first half-life extension domain comprises SEQ ID NO: 63 (Y349C; T366S; L38A; Y407V; and N297A) and the second half-life extension domain comprises SEQ ID NO 64 (S354C, T366W and N297A) and the non-cleavable linker comprises an amino acid sequence as shown in SEQ ID NO: 22 and the cleavable peptide comprises an amino acid sequence as shown in SEQ ID NO: 34.

In some embodiments, the IL-15 cytokine or functional fragment thereof comprises the amino acid sequence of SEQ ID NO: 6 and the first half-life extension domain comprises SEQ ID NO: 65 (Y349C; T366S; L38A; Y407V; and N297A) and the second half-life extension domain comprises SEQ ID NO 66

(S354C, T366W and N297A) and the non-cleavable linker comprises an amino acid sequence as shown in SEQ ID NO: 22 and the cleavable peptide comprises an amino acid sequence as shown in SEQ ID NO: 33.

In some embodiments, the IL-15 cytokine or functional fragment thereof comprises the amino acid sequence of SEQ ID NO: 6 and the first half-life extension domain comprises SEQ ID NO: 65 (Y349C; T366S; L38A; Y407V; and N297A) and the second half-life extension domain comprises SEQ ID NO 66 (S354C, T366W and N297A) and the non-cleavable linker comprises an amino acid sequence as shown in SEQ ID NO: 22 and the cleavable peptide comprises an amino acid sequence as shown in SEQ ID NO: 34.

In some embodiments, the IL-15 cytokine or functional fragment thereof comprises the amino acid sequence of SEQ ID NO: 7 and the masking moiety comprises the amino acid sequence of SEQ ID NO: 11 and the first half-life extension domain comprises SEQ ID NO: 63 (Y349C; T366S; L38A; Y407V; and N297A) and the second half-life extension domain comprises SEQ ID NO 64 (S354C, T366W and N297A) and the non-cleavable linker comprises an amino acid sequence as shown in SEQ ID NO: 22.

In some embodiments, the IL-15 cytokine or functional fragment thereof comprises the amino acid sequence of SEQ ID NO: 7 and the masking moiety comprises the amino acid sequence of SEQ ID NO: 12 and the first half-life extension domain comprises SEQ ID NO: 63 (Y349C; T366S; L38A; Y407V; and N297A) and the second half-life extension domain comprises SEQ ID NO 64 (S354C, T366W and N297A) and the non-cleavable linker comprises an amino acid sequence as shown in SEQ ID NO: 22.

In some embodiments, the IL-15 cytokine or functional fragment thereof comprises the amino acid sequence of SEQ ID NO: 7 and the masking moiety comprises the amino acid sequence of SEQ ID NO: 11 and the first half-life extension domain comprises SEQ ID NO: 65 (Y349C; T366S; L38A; Y407V, N297A and I253A) and the second half-life extension domain comprises SEQ ID NO: 66 (S354C, T366W, N297A and I253A) and the non-cleavable linker comprises an amino acid sequence as shown in SEQ ID NO: 22.

In some embodiments, the IL-15 cytokine or functional fragment thereof comprises the amino acid sequence of SEQ ID NO: 7 and the masking moiety comprises the amino acid sequence of SEQ ID NO: 12 and the first half-life extension domain comprises SEQ ID NO: 65 (Y349C; T366S; L38A; Y407V, N297A and I253A) and the second half-life extension domain comprises SEQ ID NO: 66 (S354C, T366W, N297A and I253A) and the non-cleavable linker comprises an amino acid sequence as shown in SEQ ID NO: 22.

In some embodiments, the IL-15 cytokine or functional fragment thereof comprises the amino acid sequence of SEQ ID NO: 7 and the masking moiety comprises the amino acid sequence of SEQ ID NO: 11 and the first half-life extension domain comprises SEQ ID NO: 63 (Y349C; T366S; L38A; Y407V; and N297A) and the second half-life extension domain comprises SEQ ID NO 64 (S354C, T366W and N297A) and the cleavable peptide comprises an amino acid sequence as shown in SEQ ID NO: 33.

In some embodiments, the IL-15 cytokine or functional fragment thereof comprises the amino acid sequence of SEQ ID NO: 7 and the masking moiety comprises the amino acid sequence of SEQ ID NO: 12 and the first half-life extension domain comprises SEQ ID NO: 63 (Y349C; T366S; L38A; Y407V; and N297A) and the second half-life extension domain comprises SEQ ID NO 64 (S354C, T366W and N297A) and the cleavable peptide comprises an amino acid sequence as shown in SEQ ID NO: 33.

In some embodiments, the IL-15 cytokine or functional fragment thereof comprises the amino acid sequence of SEQ ID NO: 7 and the masking moiety comprises the amino acid sequence of SEQ ID NO: 11 and the first half-life extension domain comprises SEQ ID NO: 65 (Y349C; T366S; L38A; Y407V; and N297A) and the second half-life extension domain comprises SEQ ID NO 66 (S354C, T366W and N297A) and the cleavable peptide comprises an amino acid sequence as shown in SEQ ID NO: 33.

In some embodiments, the IL-15 cytokine or functional fragment thereof comprises the amino acid sequence of SEQ ID NO: 7 and the masking moiety comprises the amino acid sequence of SEQ ID NO: 12 and the first half-life extension domain comprises SEQ ID NO: 65 (Y349C; T366S; L38A; Y407V; and N297A) and the second half-life extension domain comprises SEQ ID NO 66 (S354C, T366W and N297A) and the cleavable peptide comprises an amino acid sequence as shown in SEQ ID NO: 33.

In some embodiments, the IL-15 cytokine or functional fragment thereof comprises the amino acid sequence of SEQ ID NO: 7 and the masking moiety comprises the amino acid sequence of SEQ ID NO: 11 and the first half-life extension domain comprises SEQ ID NO: 63 (Y349C; T366S; L38A; Y407V; and N297A) and the second half-life extension domain comprises SEQ ID NO 64 (S354C, T366W and N297A) and the cleavable peptide comprises an amino acid sequence as shown in SEQ ID NO: 34.

In some embodiments, the IL-15 cytokine or functional fragment thereof comprises the amino acid sequence of SEQ ID NO: 7 and the masking moiety comprises the amino acid sequence of SEQ ID NO: 12 and the first half-life extension domain comprises SEQ ID NO: 63 (Y349C; T366S; L38A; Y407V; and N297A) and the second half-life extension domain comprises SEQ ID NO 64 (S354C, T366W and N297A) and the cleavable peptide comprises an amino acid sequence as shown in SEQ ID NO: 34.

In some embodiments, the IL-15 cytokine or functional fragment thereof comprises the amino acid sequence of SEQ ID NO: 7 and the masking moiety comprises the amino acid sequence of SEQ ID NO: 11 and the first half-life extension domain comprises SEQ ID NO: 65 (Y349C; T366S; L38A; Y407V; and N297A) and the second half-life extension domain comprises SEQ ID NO 66 (S354C, T366W and N297A) and the cleavable peptide comprises an amino acid sequence as shown in SEQ ID NO: 34.

In some embodiments, the IL-15 cytokine or functional fragment thereof comprises the amino acid sequence of SEQ ID NO: 7 and the masking moiety comprises the amino acid sequence of SEQ ID NO: 12 and the first half-life extension domain comprises SEQ ID NO: 65 (Y349C; T366S; L38A; Y407V; and N297A) and the second half-life extension domain comprises SEQ ID NO 66 (S354C, T366W and N297A) and the cleavable peptide comprises an amino acid sequence as shown in SEQ ID NO: 34.

In some embodiments, the IL-15 cytokine or functional fragment thereof comprises the amino acid sequence of SEQ ID NO: 7 and the masking moiety comprises the amino acid sequence of SEQ ID NO: 11 and the non-cleavable linker comprises an amino acid sequence as shown in SEQ ID NO: 22 and the cleavable peptide comprises an amino acid sequence as shown in SEQ ID NO: 33.

In some embodiments, the IL-15 cytokine or functional fragment thereof comprises the amino acid sequence of SEQ ID NO: 7 and the masking moiety comprises the amino acid sequence of SEQ ID NO: 12 and the non-cleavable linker comprises an amino acid sequence as shown in SEQ ID NO: 22 and the cleavable peptide comprises an amino acid sequence as shown in SEQ ID NO: 33.

In some embodiments, the IL-15 cytokine or functional fragment thereof comprises the amino acid sequence of SEQ ID NO: 7 and the masking moiety comprises the amino acid sequence of SEQ ID NO: 11 and the non-cleavable linker comprises an amino acid sequence as shown in SEQ ID NO: 22 and the cleavable peptide comprises an amino acid sequence as shown in SEQ ID NO: 34.

In some embodiments, the IL-15 cytokine or functional fragment thereof comprises the amino acid sequence of SEQ ID NO: 7 and the masking moiety comprises the amino acid sequence of SEQ ID NO: 12 and the non-cleavable linker comprises an amino acid sequence as shown in SEQ ID NO: 22 and the cleavable peptide comprises an amino acid sequence as shown in SEQ ID NO: 34.

In some embodiments, the IL-15 cytokine or functional fragment thereof comprises the amino acid sequence of SEQ ID NO: 7 and the first half-life extension domain comprises SEQ ID NO: 63 (Y349C; T366S; L38A; Y407V; and N297A) and the second half-life extension domain comprises SEQ ID NO 64 (S354C, T366W and N297A) and the non-cleavable linker comprises an amino acid sequence as shown in SEQ ID NO: 22 and the cleavable peptide comprises an amino acid sequence as shown in SEQ ID NO: 33.

In some embodiments, the IL-15 cytokine or functional fragment thereof comprises the amino acid sequence of SEQ ID NO: 7 and the first half-life extension domain comprises SEQ ID NO: 63 (Y349C; T366S; L38A; Y407V; and N297A) and the second half-life extension domain comprises SEQ ID NO 64 (S354C, T366W and N297A) and the non-cleavable linker comprises an amino acid sequence as shown in SEQ ID NO: 22 and the cleavable peptide comprises an amino acid sequence as shown in SEQ ID NO: 34.

In some embodiments, the IL-15 cytokine or functional fragment thereof comprises the amino acid sequence of SEQ ID NO: 7 and the first half-life extension domain comprises SEQ ID NO: 65 (Y349C; T366S; L38A; Y407V; and N297A) and the second half-life extension domain comprises SEQ ID NO 66 (S354C, T366W and N297A) and the non-cleavable linker comprises an amino acid sequence as shown in SEQ ID NO: 22 and the cleavable peptide comprises an amino acid sequence as shown in SEQ ID NO: 33.

In some embodiments, the IL-15 cytokine or functional fragment thereof comprises the amino acid sequence of SEQ ID NO: 7 and the first half-life extension domain comprises SEQ ID NO: 65 (Y349C; T366S; L38A; Y407V; and N297A) and the second half-life extension domain comprises SEQ ID NO 66 (S354C, T366W and N297A) and the non-cleavable linker comprises an amino acid sequence as shown in SEQ ID NO: 22 and the cleavable peptide comprises an amino acid sequence as shown in SEQ ID NO: 34.

In some embodiments, the IL-15 cytokine or functional fragment thereof comprises the amino acid sequence of SEQ ID NO: 8 and the masking moiety comprises the amino acid sequence of SEQ ID NO: 11 and the first half-life extension domain comprises SEQ ID NO: 63 (Y349C; T366S; L38A; Y407V; and N297A) and the second half-life extension domain comprises SEQ ID NO 64 (S354C, T366W and N297A) and the non-cleavable linker comprises an amino acid sequence as shown in SEQ ID NO: 22.

In some embodiments, the IL-15 cytokine or functional fragment thereof comprises the amino acid sequence of SEQ ID NO: 8 and the masking moiety comprises the amino acid sequence of SEQ ID NO: 12 and the first half-life extension domain comprises SEQ ID NO: 63 (Y349C; T366S; L38A; Y407V; and N297A) and the second half-life extension domain comprises SEQ ID NO 64 (S354C, T366W and N297A) and the non-cleavable linker comprises an amino acid sequence as shown in SEQ ID NO: 22.

In some embodiments, the IL-15 cytokine or functional fragment thereof comprises the amino acid sequence of SEQ ID NO: 8 and the masking moiety comprises the amino acid sequence of SEQ ID NO: 11 and the first half-life extension domain comprises SEQ ID NO: 65 (Y349C; T366S; L38A; Y407V, N297A and I253A) and the second half-life extension domain comprises SEQ ID NO: 66 (S354C, T366W, N297A and I253A) and the non-cleavable linker comprises an amino acid sequence as shown in SEQ ID NO: 22.

In some embodiments, the IL-15 cytokine or functional fragment thereof comprises the amino acid sequence of SEQ ID NO: 8 and the masking moiety comprises the amino acid sequence of SEQ ID NO: 12 and the first half-life extension domain comprises SEQ ID NO: 65 (Y349C; T366S; L38A; Y407V, N297A and I253A) and the second half-life extension domain comprises SEQ ID NO: 66 (S354C, T366W, N297A and I253A) and the non-cleavable linker comprises an amino acid sequence as shown in SEQ ID NO: 22.

In some embodiments, the IL-15 cytokine or functional fragment thereof comprises the amino acid sequence of SEQ ID NO: 8 and the masking moiety comprises the amino acid sequence of SEQ ID NO: 11

and the first half-life extension domain comprises SEQ ID NO: 63 (Y349C; T366S; L38A; Y407V; and N297A) and the second half-life extension domain comprises SEQ ID NO 64 (S354C, T366W and N297A) and the cleavable peptide comprises an amino acid sequence as shown in SEQ ID NO: 33.

In some embodiments, the IL-15 cytokine or functional fragment thereof comprises the amino acid sequence of SEQ ID NO: 8 and the masking moiety comprises the amino acid sequence of SEQ ID NO: 12 and the first half-life extension domain comprises SEQ ID NO: 63 (Y349C; T366S; L38A; Y407V; and N297A) and the second half-life extension domain comprises SEQ ID NO 64 (S354C, T366W and N297A) and the cleavable peptide comprises an amino acid sequence as shown in SEQ ID NO: 33.

In some embodiments, the IL-15 cytokine or functional fragment thereof comprises the amino acid sequence of SEQ ID NO: 8 and the masking moiety comprises the amino acid sequence of SEQ ID NO: 11 and the first half-life extension domain comprises SEQ ID NO: 65 (Y349C; T366S; L38A; Y407V; and N297A) and the second half-life extension domain comprises SEQ ID NO 66 (S354C, T366W and N297A) and the cleavable peptide comprises an amino acid sequence as shown in SEQ ID NO: 33.

In some embodiments, the IL-15 cytokine or functional fragment thereof comprises the amino acid sequence of SEQ ID NO: 8 and the masking moiety comprises the amino acid sequence of SEQ ID NO: 12 and the first half-life extension domain comprises SEQ ID NO: 65 (Y349C; T366S; L38A; Y407V; and N297A) and the second half-life extension domain comprises SEQ ID NO 66 (S354C, T366W and N297A) and the cleavable peptide comprises an amino acid sequence as shown in SEQ ID NO: 33.

In some embodiments, the IL-15 cytokine or functional fragment thereof comprises the amino acid sequence of SEQ ID NO: 8 and the masking moiety comprises the amino acid sequence of SEQ ID NO: 11 and the first half-life extension domain comprises SEQ ID NO: 63 (Y349C; T366S; L38A; Y407V; and N297A) and the second half-life extension domain comprises SEQ ID NO 64 (S354C, T366W and N297A) and the cleavable peptide comprises an amino acid sequence as shown in SEQ ID NO: 34.

In some embodiments, the IL-15 cytokine or functional fragment thereof comprises the amino acid sequence of SEQ ID NO: 8 and the masking moiety comprises the amino acid sequence of SEQ ID NO: 12 and the first half-life extension domain comprises SEQ ID NO: 63 (Y349C; T366S; L38A; Y407V; and N297A) and the second half-life extension domain comprises SEQ ID NO 64 (S354C, T366W and N297A) and the cleavable peptide comprises an amino acid sequence as shown in SEQ ID NO: 34.

In some embodiments, the IL-15 cytokine or functional fragment thereof comprises the amino acid sequence of SEQ ID NO: 8 and the masking moiety comprises the amino acid sequence of SEQ ID NO: 11 and the first half-life extension domain comprises SEQ ID NO: 65 (Y349C; T366S; L38A; Y407V; and

N297A) and the second half-life extension domain comprises SEQ ID NO 66 (S354C, T366W and N297A) and the cleavable peptide comprises an amino acid sequence as shown in SEQ ID NO: 34.

In some embodiments, the IL-15 cytokine or functional fragment thereof comprises the amino acid sequence of SEQ ID NO: 8 and the masking moiety comprises the amino acid sequence of SEQ ID NO: 12 and the first half-life extension domain comprises SEQ ID NO: 65 (Y349C; T366S; L38A; Y407V; and N297A) and the second half-life extension domain comprises SEQ ID NO 66 (S354C, T366W and N297A) and the cleavable peptide comprises an amino acid sequence as shown in SEQ ID NO: 34.

In some embodiments, the IL-15 cytokine or functional fragment thereof comprises the amino acid sequence of SEQ ID NO: 8 and the masking moiety comprises the amino acid sequence of SEQ ID NO: 11 and the non-cleavable linker comprises an amino acid sequence as shown in SEQ ID NO: 22 and the cleavable peptide comprises an amino acid sequence as shown in SEQ ID NO: 33.

In some embodiments, the IL-15 cytokine or functional fragment thereof comprises the amino acid sequence of SEQ ID NO: 8 and the masking moiety comprises the amino acid sequence of SEQ ID NO: 12 and the non-cleavable linker comprises an amino acid sequence as shown in SEQ ID NO: 22 and the cleavable peptide comprises an amino acid sequence as shown in SEQ ID NO: 33.

In some embodiments, the IL-15 cytokine or functional fragment thereof comprises the amino acid sequence of SEQ ID NO: 8 and the masking moiety comprises the amino acid sequence of SEQ ID NO: 11 and the non-cleavable linker comprises an amino acid sequence as shown in SEQ ID NO: 22 and the cleavable peptide comprises an amino acid sequence as shown in SEQ ID NO: 34.

In some embodiments, the IL-15 cytokine or functional fragment thereof comprises the amino acid sequence of SEQ ID NO: 8 and the masking moiety comprises the amino acid sequence of SEQ ID NO: 12 and the non-cleavable linker comprises an amino acid sequence as shown in SEQ ID NO: 22 and the cleavable peptide comprises an amino acid sequence as shown in SEQ ID NO: 34.

In some embodiments, the IL-15 cytokine or functional fragment thereof comprises the amino acid sequence of SEQ ID NO: 8 and the first half-life extension domain comprises SEQ ID NO: 63 (Y349C; T366S; L38A; Y407V; and N297A) and the second half-life extension domain comprises SEQ ID NO 64 (S354C, T366W and N297A) and the non-cleavable linker comprises an amino acid sequence as shown in SEQ ID NO: 22 and the cleavable peptide comprises an amino acid sequence as shown in SEQ ID NO: 33.

In some embodiments, the IL-15 cytokine or functional fragment thereof comprises the amino acid sequence of SEQ ID NO: 8 and the first half-life extension domain comprises SEQ ID NO: 63 (Y349C; T366S; L38A; Y407V; and N297A) and the second half-life extension domain comprises SEQ ID NO 64

(S354C, T366W and N297A) and the non-cleavable linker comprises an amino acid sequence as shown in SEQ ID NO: 22 and the cleavable peptide comprises an amino acid sequence as shown in SEQ ID NO: 34.

In some embodiments, the IL-15 cytokine or functional fragment thereof comprises the amino acid sequence of SEQ ID NO: 8 and the first half-life extension domain comprises SEQ ID NO: 65 (Y349C; T366S; L38A; Y407V; and N297A) and the second half-life extension domain comprises SEQ ID NO 66 (S354C, T366W and N297A) and the non-cleavable linker comprises an amino acid sequence as shown in SEQ ID NO: 22 and the cleavable peptide comprises an amino acid sequence as shown in SEQ ID NO: 33.

In some embodiments, the IL-15 cytokine or functional fragment thereof comprises the amino acid sequence of SEQ ID NO: 8 and the first half-life extension domain comprises SEQ ID NO: 65 (Y349C; T366S; L38A; Y407V; and N297A) and the second half-life extension domain comprises SEQ ID NO 66 (S354C, T366W and N297A) and the non-cleavable linker comprises an amino acid sequence as shown in SEQ ID NO: 22 and the cleavable peptide comprises an amino acid sequence as shown in SEQ ID NO: 34.

In some embodiments, the IL-15 cytokine or functional fragment thereof comprises the amino acid sequence of SEQ ID NO: 2 and the masking moiety comprises the amino acid sequence of SEQ ID NO: 11 and the first half-life extension domain comprises SEQ ID NO: 63 (Y349C; T366S; L38A; Y407V; and N297A) and the second half-life extension domain comprises SEQ ID NO 64 (S354C, T366W and N297A) and the non-cleavable linker comprises an amino acid sequence as shown in SEQ ID NO: 22 and the cleavable peptide comprises an amino acid sequence as shown in SEQ ID NO: 33.

In some embodiments, the IL-15 cytokine or functional fragment thereof comprises the amino acid sequence of SEQ ID NO: 2 and the masking moiety comprises the amino acid sequence of SEQ ID NO: 12 and the first half-life extension domain comprises SEQ ID NO: 63 (Y349C; T366S; L38A; Y407V; and N297A) and the second half-life extension domain comprises SEQ ID NO 64 (S354C, T366W and N297A) and the non-cleavable linker comprises an amino acid sequence as shown in SEQ ID NO: 22 and the cleavable peptide comprises an amino acid sequence as shown in SEQ ID NO: 33.

In some embodiments, the IL-15 cytokine or functional fragment thereof comprises the amino acid sequence of SEQ ID NO: 127 and the masking moiety comprises the amino acid sequence of SEQ ID NO: 12 and the first half-life extension domain comprises SEQ ID NO: 63 (Y349C; T366S; L38A; Y407V; and N297A) and the second half-life extension domain comprises SEQ ID NO 64 (S354C, T366W and N297A) and the non-cleavable linker comprises an amino acid sequence as shown in SEQ ID NO: 22 and the cleavable peptide comprises an amino acid sequence as shown in SEQ ID NO: 27.

In some embodiments, the IL-15 cytokine or functional fragment thereof comprises the amino acid sequence of SEQ ID NO: 128 and the masking moiety comprises the amino acid sequence of SEQ ID NO:

12 and the first half-life extension domain comprises SEQ ID NO: 63 (Y349C; T366S; L38A; Y407V; and N297A) and the second half-life extension domain comprises SEQ ID NO 64 (S354C, T366W and N297A) and the non-cleavable linker comprises an amino acid sequence as shown in SEQ ID NO: 22 and the cleavable peptide comprises an amino acid sequence as shown in SEQ ID NO: 27.

In some embodiments, the IL-15 cytokine or functional fragment thereof comprises the amino acid sequence of SEQ ID NO: 129 and the masking moiety comprises the amino acid sequence of SEQ ID NO: 12 and the first half-life extension domain comprises SEQ ID NO: 63 (Y349C; T366S; L38A; Y407V; and N297A) and the second half-life extension domain comprises SEQ ID NO 64 (S354C, T366W and N297A) and the non-cleavable linker comprises an amino acid sequence as shown in SEQ ID NO: 22 and the cleavable peptide comprises an amino acid sequence as shown in SEQ ID NO: 27.

In some embodiments, the IL-15 cytokine or functional fragment thereof comprises the amino acid sequence of SEQ ID NO: 130 and the masking moiety comprises the amino acid sequence of SEQ ID NO: 12 and the first half-life extension domain comprises SEQ ID NO: 63 (Y349C; T366S; L38A; Y407V; and N297A) and the second half-life extension domain comprises SEQ ID NO 64 (S354C, T366W and N297A) and the non-cleavable linker comprises an amino acid sequence as shown in SEQ ID NO: 22 and the cleavable peptide comprises an amino acid sequence as shown in SEQ ID NO: 27.

In some embodiments, the IL-15 cytokine or functional fragment thereof comprises the amino acid sequence of SEQ ID NO: 131 and the masking moiety comprises the amino acid sequence of SEQ ID NO: 12 and the first half-life extension domain comprises SEQ ID NO: 63 (Y349C; T366S; L38A; Y407V; and N297A) and the second half-life extension domain comprises SEQ ID NO 64 (S354C, T366W and N297A) and the non-cleavable linker comprises an amino acid sequence as shown in SEQ ID NO: 22 and the cleavable peptide comprises an amino acid sequence as shown in SEQ ID NO: 27.

In some embodiments, the IL-15 cytokine or functional fragment thereof comprises the amino acid sequence of SEQ ID NO: 132 and the masking moiety comprises the amino acid sequence of SEQ ID NO: 12 and the first half-life extension domain comprises SEQ ID NO: 63 (Y349C; T366S; L38A; Y407V; and N297A) and the second half-life extension domain comprises SEQ ID NO 64 (S354C, T366W and N297A) and the non-cleavable linker comprises an amino acid sequence as shown in SEQ ID NO: 22 and the cleavable peptide comprises an amino acid sequence as shown in SEQ ID NO: 27.

In some embodiments, the IL-15 cytokine or functional fragment thereof comprises the amino acid sequence of SEQ ID NO: 133 and the masking moiety comprises the amino acid sequence of SEQ ID NO: 12 and the first half-life extension domain comprises SEQ ID NO: 63 (Y349C; T366S; L38A; Y407V; and N297A) and the second half-life extension domain comprises SEQ ID NO 64 (S354C, T366W and N297A)

and the non-cleavable linker comprises an amino acid sequence as shown in SEQ ID NO: 22 and the cleavable peptide comprises an amino acid sequence as shown in SEQ ID NO: 27.

In some embodiments, the IL-15 cytokine or functional fragment thereof comprises the amino acid sequence of SEQ ID NO: 127 and the masking moiety comprises the amino acid sequence of SEQ ID NO: 12 and the first half-life extension domain comprises SEQ ID NO: 63 (Y349C; T366S; L38A; Y407V; and N297A) and the second half-life extension domain comprises SEQ ID NO 64 (S354C, T366W and N297A) and the non-cleavable linker comprises an amino acid sequence as shown in SEQ ID NO: 22 and the cleavable peptide comprises an amino acid sequence as shown in SEQ ID NO: 33.

In some embodiments, the IL-15 cytokine or functional fragment thereof comprises the amino acid sequence of SEQ ID NO: 128 and the masking moiety comprises the amino acid sequence of SEQ ID NO: 12 and the first half-life extension domain comprises SEQ ID NO: 63 (Y349C; T366S; L38A; Y407V; and N297A) and the second half-life extension domain comprises SEQ ID NO 64 (S354C, T366W and N297A) and the non-cleavable linker comprises an amino acid sequence as shown in SEQ ID NO: 22 and the cleavable peptide comprises an amino acid sequence as shown in SEQ ID NO: 33.

In some embodiments, the IL-15 cytokine or functional fragment thereof comprises the amino acid sequence of SEQ ID NO: 129 and the masking moiety comprises the amino acid sequence of SEQ ID NO: 12 and the first half-life extension domain comprises SEQ ID NO: 63 (Y349C; T366S; L38A; Y407V; and N297A) and the second half-life extension domain comprises SEQ ID NO 64 (S354C, T366W and N297A) and the non-cleavable linker comprises an amino acid sequence as shown in SEQ ID NO: 22 and the cleavable peptide comprises an amino acid sequence as shown in SEQ ID NO: 33.

In some embodiments, the IL-15 cytokine or functional fragment thereof comprises the amino acid sequence of SEQ ID NO: 130 and the masking moiety comprises the amino acid sequence of SEQ ID NO: 12 and the first half-life extension domain comprises SEQ ID NO: 63 (Y349C; T366S; L38A; Y407V; and N297A) and the second half-life extension domain comprises SEQ ID NO 64 (S354C, T366W and N297A) and the non-cleavable linker comprises an amino acid sequence as shown in SEQ ID NO: 22 and the cleavable peptide comprises an amino acid sequence as shown in SEQ ID NO: 33.

In some embodiments, the IL-15 cytokine or functional fragment thereof comprises the amino acid sequence of SEQ ID NO: 131 and the masking moiety comprises the amino acid sequence of SEQ ID NO: 12 and the first half-life extension domain comprises SEQ ID NO: 63 (Y349C; T366S; L38A; Y407V; and N297A) and the second half-life extension domain comprises SEQ ID NO 64 (S354C, T366W and N297A) and the non-cleavable linker comprises an amino acid sequence as shown in SEQ ID NO: 22 and the cleavable peptide comprises an amino acid sequence as shown in SEQ ID NO: 33.

In some embodiments, the IL-15 cytokine or functional fragment thereof comprises the amino acid sequence of SEQ ID NO: 132 and the masking moiety comprises the amino acid sequence of SEQ ID NO: 12 and the first half-life extension domain comprises SEQ ID NO: 63 (Y349C; T366S; L38A; Y407V; and N297A) and the second half-life extension domain comprises SEQ ID NO 64 (S354C, T366W and N297A) and the non-cleavable linker comprises an amino acid sequence as shown in SEQ ID NO: 22 and the cleavable peptide comprises an amino acid sequence as shown in SEQ ID NO: 33.

In some embodiments, the IL-15 cytokine or functional fragment thereof comprises the amino acid sequence of SEQ ID NO: 133 and the masking moiety comprises the amino acid sequence of SEQ ID NO: 12 and the first half-life extension domain comprises SEQ ID NO: 63 (Y349C; T366S; L38A; Y407V; and N297A) and the second half-life extension domain comprises SEQ ID NO 64 (S354C, T366W and N297A) and the non-cleavable linker comprises an amino acid sequence as shown in SEQ ID NO: 22 and the cleavable peptide comprises an amino acid sequence as shown in SEQ ID NO: 33.

In some embodiments, the IL-15 cytokine or functional fragment thereof comprises the amino acid sequence of SEQ ID NO: 127 and the masking moiety comprises the amino acid sequence of SEQ ID NO: 12 and the first half-life extension domain comprises SEQ ID NO: 63 (Y349C; T366S; L38A; Y407V; and N297A) and the second half-life extension domain comprises SEQ ID NO 64 (S354C, T366W and N297A) and the non-cleavable linker comprises an amino acid sequence as shown in SEQ ID NO: 22 and the cleavable peptide comprises an amino acid sequence as shown in SEQ ID NO: 134.

In some embodiments, the IL-15 cytokine or functional fragment thereof comprises the amino acid sequence of SEQ ID NO: 128 and the masking moiety comprises the amino acid sequence of SEQ ID NO: 12 and the first half-life extension domain comprises SEQ ID NO: 63 (Y349C; T366S; L38A; Y407V; and N297A) and the second half-life extension domain comprises SEQ ID NO 64 (S354C, T366W and N297A) and the non-cleavable linker comprises an amino acid sequence as shown in SEQ ID NO: 22 and the cleavable peptide comprises an amino acid sequence as shown in SEQ ID NO: 134.

In some embodiments, the IL-15 cytokine or functional fragment thereof comprises the amino acid sequence of SEQ ID NO: 129 and the masking moiety comprises the amino acid sequence of SEQ ID NO: 12 and the first half-life extension domain comprises SEQ ID NO: 63 (Y349C; T366S; L38A; Y407V; and N297A) and the second half-life extension domain comprises SEQ ID NO 64 (S354C, T366W and N297A) and the non-cleavable linker comprises an amino acid sequence as shown in SEQ ID NO: 22 and the cleavable peptide comprises an amino acid sequence as shown in SEQ ID NO: 134.

In some embodiments, the IL-15 cytokine or functional fragment thereof comprises the amino acid sequence of SEQ ID NO: 130 and the masking moiety comprises the amino acid sequence of SEQ ID NO: 12 and the first half-life extension domain comprises SEQ ID NO: 63 (Y349C; T366S; L38A; Y407V; and

N297A) and the second half-life extension domain comprises SEQ ID NO 64 (S354C, T366W and N297A) and the non-cleavable linker comprises an amino acid sequence as shown in SEQ ID NO: 22 and the cleavable peptide comprises an amino acid sequence as shown in SEQ ID NO: 134.

In some embodiments, the IL-15 cytokine or functional fragment thereof comprises the amino acid sequence of SEQ ID NO: 131 and the masking moiety comprises the amino acid sequence of SEQ ID NO: 12 and the first half-life extension domain comprises SEQ ID NO: 63 (Y349C; T366S; L38A; Y407V; and N297A) and the second half-life extension domain comprises SEQ ID NO 64 (S354C, T366W and N297A) and the non-cleavable linker comprises an amino acid sequence as shown in SEQ ID NO: 22 and the cleavable peptide comprises an amino acid sequence as shown in SEQ ID NO: 134.

In some embodiments, the IL-15 cytokine or functional fragment thereof comprises the amino acid sequence of SEQ ID NO: 132 and the masking moiety comprises the amino acid sequence of SEQ ID NO: 12 and the first half-life extension domain comprises SEQ ID NO: 63 (Y349C; T366S; L38A; Y407V; and N297A) and the second half-life extension domain comprises SEQ ID NO 64 (S354C, T366W and N297A) and the non-cleavable linker comprises an amino acid sequence as shown in SEQ ID NO: 22 and the cleavable peptide comprises an amino acid sequence as shown in SEQ ID NO: 134.

In some embodiments, the IL-15 cytokine or functional fragment thereof comprises the amino acid sequence of SEQ ID NO: 133 and the masking moiety comprises the amino acid sequence of SEQ ID NO: 12 and the first half-life extension domain comprises SEQ ID NO: 63 (Y349C; T366S; L38A; Y407V; and N297A) and the second half-life extension domain comprises SEQ ID NO 64 (S354C, T366W and N297A) and the non-cleavable linker comprises an amino acid sequence as shown in SEQ ID NO: 22 and the cleavable peptide comprises an amino acid sequence as shown in SEQ ID NO: 134.

In some embodiments, the IL-15 cytokine or functional fragment thereof comprises the amino acid sequence of SEQ ID NO: 2 and the masking moiety comprises the amino acid sequence of SEQ ID NO: 11 and the first half-life extension domain comprises SEQ ID NO: 65 (Y349C; T366S; L38A; Y407V; and N297A) and the second half-life extension domain comprises SEQ ID NO 66 (S354C, T366W and N297A) and the non-cleavable linker comprises an amino acid sequence as shown in SEQ ID NO: 22 and the cleavable peptide comprises an amino acid sequence as shown in SEQ ID NO: 33.

In some embodiments, the IL-15 cytokine or functional fragment thereof comprises the amino acid sequence of SEQ ID NO: 2 and the masking moiety comprises the amino acid sequence of SEQ ID NO: 12 and the first half-life extension domain comprises SEQ ID NO: 65 (Y349C; T366S; L38A; Y407V; and N297A) and the second half-life extension domain comprises SEQ ID NO 66 (S354C, T366W and N297A) and the non-cleavable linker comprises an amino acid sequence as shown in SEQ ID NO: 22 and the cleavable peptide comprises an amino acid sequence as shown in SEQ ID NO: 33.

In some embodiments, the IL-15 cytokine or functional fragment thereof comprises the amino acid sequence of SEQ ID NO: 2 and the masking moiety comprises the amino acid sequence of SEQ ID NO: 11 and the first half-life extension domain comprises SEQ ID NO: 63 (Y349C; T366S; L38A; Y407V; and N297A) and the second half-life extension domain comprises SEQ ID NO 64 (S354C, T366W and N297A) and the non-cleavable linker comprises an amino acid sequence as shown in SEQ ID NO: 22 and the cleavable peptide comprises an amino acid sequence as shown in SEQ ID NO: 34.

In some embodiments, the IL-15 cytokine or functional fragment thereof comprises the amino acid sequence of SEQ ID NO: 2 and the masking moiety comprises the amino acid sequence of SEQ ID NO: 12 and the first half-life extension domain comprises SEQ ID NO: 63 (Y349C; T366S; L38A; Y407V; and N297A) and the second half-life extension domain comprises SEQ ID NO 64 (S354C, T366W and N297A) and the non-cleavable linker comprises an amino acid sequence as shown in SEQ ID NO: 22 and the cleavable peptide comprises an amino acid sequence as shown in SEQ ID NO: 34.

In some embodiments, the IL-15 cytokine or functional fragment thereof comprises the amino acid sequence of SEQ ID NO: 2 and the masking moiety comprises the amino acid sequence of SEQ ID NO: 11 and the first half-life extension domain comprises SEQ ID NO: 65 (Y349C; T366S; L38A; Y407V; and N297A) and the second half-life extension domain comprises SEQ ID NO 66 (S354C, T366W and N297A) and the non-cleavable linker comprises an amino acid sequence as shown in SEQ ID NO: 22 and the cleavable peptide comprises an amino acid sequence as shown in SEQ ID NO: 34.

In some embodiments, the IL-15 cytokine or functional fragment thereof comprises the amino acid sequence of SEQ ID NO: 2 and the masking moiety comprises the amino acid sequence of SEQ ID NO: 12 and the first half-life extension domain comprises SEQ ID NO: 65 (Y349C; T366S; L38A; Y407V; and N297A) and the second half-life extension domain comprises SEQ ID NO 66 (S354C, T366W and N297A) and the non-cleavable linker comprises an amino acid sequence as shown in SEQ ID NO: 22 and the cleavable peptide comprises an amino acid sequence as shown in SEQ ID NO: 34.

In some embodiments, the IL-15 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: 11 and the first half-life extension domain comprises SEQ ID NO: 63 (Y349C; T366S; L38A; Y407V; and N297A) and the second half-life extension domain comprises SEQ ID NO 64 (S354C, T366W and N297A) and the non-cleavable linker comprises an amino acid sequence as shown in SEQ ID NO: 22 and the cleavable peptide comprises an amino acid sequence as shown in SEQ ID NO: 33.

In some embodiments, the IL-15 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: 12

and the first half-life extension domain comprises SEQ ID NO: 63 (Y349C; T366S; L38A; Y407V; and N297A) and the second half-life extension domain comprises SEQ ID NO 64 (S354C, T366W and N297A) and the non-cleavable linker comprises an amino acid sequence as shown in SEQ ID NO: 22 and the cleavable peptide comprises an amino acid sequence as shown in SEQ ID NO: 33.

In some embodiments, the IL-15 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: 11 and the first half-life extension domain comprises SEQ ID NO: 65 (Y349C; T366S; L38A; Y407V; and N297A) and the second half-life extension domain comprises SEQ ID NO 66 (S354C, T366W and N297A) and the non-cleavable linker comprises an amino acid sequence as shown in SEQ ID NO: 22 and the cleavable peptide comprises an amino acid sequence as shown in SEQ ID NO: 33.

In some embodiments, the IL-15 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: 12 and the first half-life extension domain comprises SEQ ID NO: 65 (Y349C; T366S; L38A; Y407V; and N297A) and the second half-life extension domain comprises SEQ ID NO 66 (S354C, T366W and N297A) and the non-cleavable linker comprises an amino acid sequence as shown in SEQ ID NO: 22 and the cleavable peptide comprises an amino acid sequence as shown in SEQ ID NO: 33.

In some embodiments, the IL-15 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: 11 and the first half-life extension domain comprises SEQ ID NO: 63 (Y349C; T366S; L38A; Y407V; and N297A) and the second half-life extension domain comprises SEQ ID NO 64 (S354C, T366W and N297A) and the non-cleavable linker comprises an amino acid sequence as shown in SEQ ID NO: 22 and the cleavable peptide comprises an amino acid sequence as shown in SEQ ID NO: 34.

In some embodiments, the IL-15 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: 12 and the first half-life extension domain comprises SEQ ID NO: 63 (Y349C; T366S; L38A; Y407V; and N297A) and the second half-life extension domain comprises SEQ ID NO 64 (S354C, T366W and N297A) and the non-cleavable linker comprises an amino acid sequence as shown in SEQ ID NO: 22 and the cleavable peptide comprises an amino acid sequence as shown in SEQ ID NO: 34.

In some embodiments, the IL-15 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: 11 and the first half-life extension domain comprises SEQ ID NO: 65 (Y349C; T366S; L38A; Y407V; and N297A) and the second half-life extension domain comprises SEQ ID NO 66 (S354C, T366W and N297A)

and the non-cleavable linker comprises an amino acid sequence as shown in SEQ ID NO: 22 and the cleavable peptide comprises an amino acid sequence as shown in SEQ ID NO: 34.

In some embodiments, the IL-15 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: 12 and the first half-life extension domain comprises SEQ ID NO: 65 (Y349C; T366S; L38A; Y407V; and N297A) and the second half-life extension domain comprises SEQ ID NO 66 (S354C, T366W and N297A) and the non-cleavable linker comprises an amino acid sequence as shown in SEQ ID NO: 22 and the cleavable peptide comprises an amino acid sequence as shown in SEQ ID NO: 34.

In some embodiments, the IL-15 cytokine or functional fragment thereof comprises the amino acid sequence of SEQ ID NO: 4 and the masking moiety comprises the amino acid sequence of SEQ ID NO: 11 and the first half-life extension domain comprises SEQ ID NO: 63 (Y349C; T366S; L38A; Y407V; and N297A) and the second half-life extension domain comprises SEQ ID NO 64 (S354C, T366W and N297A) and the non-cleavable linker comprises an amino acid sequence as shown in SEQ ID NO: 22 and the cleavable peptide comprises an amino acid sequence as shown in SEQ ID NO: 33.

In some embodiments, the IL-15 cytokine or functional fragment thereof comprises the amino acid sequence of SEQ ID NO: 4 and the masking moiety comprises the amino acid sequence of SEQ ID NO: 12 and the first half-life extension domain comprises SEQ ID NO: 63 (Y349C; T366S; L38A; Y407V; and N297A) and the second half-life extension domain comprises SEQ ID NO 64 (S354C, T366W and N297A) and the non-cleavable linker comprises an amino acid sequence as shown in SEQ ID NO: 22 and the cleavable peptide comprises an amino acid sequence as shown in SEQ ID NO: 33.

In some embodiments, the IL-15 cytokine or functional fragment thereof comprises the amino acid sequence of SEQ ID NO: 4 and the masking moiety comprises the amino acid sequence of SEQ ID NO: 11 and the first half-life extension domain comprises SEQ ID NO: 65 (Y349C; T366S; L38A; Y407V; and N297A) and the second half-life extension domain comprises SEQ ID NO 66 (S354C, T366W and N297A) and the non-cleavable linker comprises an amino acid sequence as shown in SEQ ID NO: 22 and the cleavable peptide comprises an amino acid sequence as shown in SEQ ID NO: 33.

In some embodiments, the IL-15 cytokine or functional fragment thereof comprises the amino acid sequence of SEQ ID NO: 4 and the masking moiety comprises the amino acid sequence of SEQ ID NO: 12 and the first half-life extension domain comprises SEQ ID NO: 65 (Y349C; T366S; L38A; Y407V; and N297A) and the second half-life extension domain comprises SEQ ID NO 66 (S354C, T366W and N297A) and the non-cleavable linker comprises an amino acid sequence as shown in SEQ ID NO: 22 and the cleavable peptide comprises an amino acid sequence as shown in SEQ ID NO: 33.

In some embodiments, the IL-15 cytokine or functional fragment thereof comprises the amino acid sequence of SEQ ID NO: 4 and the masking moiety comprises the amino acid sequence of SEQ ID NO: 11 and the first half-life extension domain comprises SEQ ID NO: 63 (Y349C; T366S; L38A; Y407V; and N297A) and the second half-life extension domain comprises SEQ ID NO 64 (S354C, T366W and N297A) and the non-cleavable linker comprises an amino acid sequence as shown in SEQ ID NO: 22 and the cleavable peptide comprises an amino acid sequence as shown in SEQ ID NO: 34.

In some embodiments, the IL-15 cytokine or functional fragment thereof comprises the amino acid sequence of SEQ ID NO: 4 and the masking moiety comprises the amino acid sequence of SEQ ID NO: 12 and the first half-life extension domain comprises SEQ ID NO: 63 (Y349C; T366S; L38A; Y407V; and N297A) and the second half-life extension domain comprises SEQ ID NO 64 (S354C, T366W and N297A) and the non-cleavable linker comprises an amino acid sequence as shown in SEQ ID NO: 22 and the cleavable peptide comprises an amino acid sequence as shown in SEQ ID NO: 34.

In some embodiments, the IL-15 cytokine or functional fragment thereof comprises the amino acid sequence of SEQ ID NO: 4 and the masking moiety comprises the amino acid sequence of SEQ ID NO: 11 and the first half-life extension domain comprises SEQ ID NO: 65 (Y349C; T366S; L38A; Y407V; and N297A) and the second half-life extension domain comprises SEQ ID NO 66 (S354C, T366W and N297A) and the non-cleavable linker comprises an amino acid sequence as shown in SEQ ID NO: 22 and the cleavable peptide comprises an amino acid sequence as shown in SEQ ID NO: 34.

In some embodiments, the IL-15 cytokine or functional fragment thereof comprises the amino acid sequence of SEQ ID NO: 4 and the masking moiety comprises the amino acid sequence of SEQ ID NO: 12 and the first half-life extension domain comprises SEQ ID NO: 65 (Y349C; T366S; L38A; Y407V; and N297A) and the second half-life extension domain comprises SEQ ID NO 66 (S354C, T366W and N297A) and the non-cleavable linker comprises an amino acid sequence as shown in SEQ ID NO: 22 and the cleavable peptide comprises an amino acid sequence as shown in SEQ ID NO: 34.

In some embodiments, the IL-15 cytokine or functional fragment thereof comprises the amino acid sequence of SEQ ID NO: 5 and the masking moiety comprises the amino acid sequence of SEQ ID NO: 11 and the first half-life extension domain comprises SEQ ID NO: 63 (Y349C; T366S; L38A; Y407V; and N297A) and the second half-life extension domain comprises SEQ ID NO 64 (S354C, T366W and N297A) and the non-cleavable linker comprises an amino acid sequence as shown in SEQ ID NO: 22 and the cleavable peptide comprises an amino acid sequence as shown in SEQ ID NO: 33.

In some embodiments, the IL-15 cytokine or functional fragment thereof comprises the amino acid sequence of SEQ ID NO: 5 and the masking moiety comprises the amino acid sequence of SEQ ID NO: 12 and the first half-life extension domain comprises SEQ ID NO: 63 (Y349C; T366S; L38A; Y407V; and

N297A) and the second half-life extension domain comprises SEQ ID NO 64 (S354C, T366W and N297A) and the non-cleavable linker comprises an amino acid sequence as shown in SEQ ID NO: 22 and the cleavable peptide comprises an amino acid sequence as shown in SEQ ID NO: 33.

In some embodiments, the IL-15 cytokine or functional fragment thereof comprises the amino acid sequence of SEQ ID NO: 5 and the masking moiety comprises the amino acid sequence of SEQ ID NO: 11 and the first half-life extension domain comprises SEQ ID NO: 65 (Y349C; T366S; L38A; Y407V; and N297A) and the second half-life extension domain comprises SEQ ID NO 66 (S354C, T366W and N297A) and the non-cleavable linker comprises an amino acid sequence as shown in SEQ ID NO: 22 and the cleavable peptide comprises an amino acid sequence as shown in SEQ ID NO: 33.

In some embodiments, the IL-15 cytokine or functional fragment thereof comprises the amino acid sequence of SEQ ID NO: 5 and the masking moiety comprises the amino acid sequence of SEQ ID NO: 12 and the first half-life extension domain comprises SEQ ID NO: 65 (Y349C; T366S; L38A; Y407V; and N297A) and the second half-life extension domain comprises SEQ ID NO 66 (S354C, T366W and N297A) and the non-cleavable linker comprises an amino acid sequence as shown in SEQ ID NO: 22 and the cleavable peptide comprises an amino acid sequence as shown in SEQ ID NO: 33.

In some embodiments, the IL-15 cytokine or functional fragment thereof comprises the amino acid sequence of SEQ ID NO: 5 and the masking moiety comprises the amino acid sequence of SEQ ID NO: 11 and the first half-life extension domain comprises SEQ ID NO: 63 (Y349C; T366S; L38A; Y407V; and N297A) and the second half-life extension domain comprises SEQ ID NO 64 (S354C, T366W and N297A) and the non-cleavable linker comprises an amino acid sequence as shown in SEQ ID NO: 22 and the cleavable peptide comprises an amino acid sequence as shown in SEQ ID NO: 34.

In some embodiments, the IL-15 cytokine or functional fragment thereof comprises the amino acid sequence of SEQ ID NO: 5 and the masking moiety comprises the amino acid sequence of SEQ ID NO: 12 and the first half-life extension domain comprises SEQ ID NO: 63 (Y349C; T366S; L38A; Y407V; and N297A) and the second half-life extension domain comprises SEQ ID NO 64 (S354C, T366W and N297A) and the non-cleavable linker comprises an amino acid sequence as shown in SEQ ID NO: 22 and the cleavable peptide comprises an amino acid sequence as shown in SEQ ID NO: 34.

In some embodiments, the IL-15 cytokine or functional fragment thereof comprises the amino acid sequence of SEQ ID NO: 5 and the masking moiety comprises the amino acid sequence of SEQ ID NO: 11 and the first half-life extension domain comprises SEQ ID NO: 65 (Y349C; T366S; L38A; Y407V; and N297A) and the second half-life extension domain comprises SEQ ID NO 66 (S354C, T366W and N297A) and the non-cleavable linker comprises an amino acid sequence as shown in SEQ ID NO: 22 and the cleavable peptide comprises an amino acid sequence as shown in SEQ ID NO: 34.

In some embodiments, the IL-15 cytokine or functional fragment thereof comprises the amino acid sequence of SEQ ID NO: 5 and the masking moiety comprises the amino acid sequence of SEQ ID NO: 12 and the first half-life extension domain comprises SEQ ID NO: 65 (Y349C; T366S; L38A; Y407V; and N297A) and the second half-life extension domain comprises SEQ ID NO 66 (S354C, T366W and N297A) and the non-cleavable linker comprises an amino acid sequence as shown in SEQ ID NO: 22 and the cleavable peptide comprises an amino acid sequence as shown in SEQ ID NO: 34.

In some embodiments, the IL-15 cytokine or functional fragment thereof comprises the amino acid sequence of SEQ ID NO: 6 and the masking moiety comprises the amino acid sequence of SEQ ID NO: 11 and the first half-life extension domain comprises SEQ ID NO: 63 (Y349C; T366S; L38A; Y407V; and N297A) and the second half-life extension domain comprises SEQ ID NO 64 (S354C, T366W and N297A) and the non-cleavable linker comprises an amino acid sequence as shown in SEQ ID NO: 22 and the cleavable peptide comprises an amino acid sequence as shown in SEQ ID NO: 33.

In some embodiments, the IL-15 cytokine or functional fragment thereof comprises the amino acid sequence of SEQ ID NO: 6 and the masking moiety comprises the amino acid sequence of SEQ ID NO: 12 and the first half-life extension domain comprises SEQ ID NO: 63 (Y349C; T366S; L38A; Y407V; and N297A) and the second half-life extension domain comprises SEQ ID NO 64 (S354C, T366W and N297A) and the non-cleavable linker comprises an amino acid sequence as shown in SEQ ID NO: 22 and the cleavable peptide comprises an amino acid sequence as shown in SEQ ID NO: 33.

In some embodiments, the IL-15 cytokine or functional fragment thereof comprises the amino acid sequence of SEQ ID NO: 6 and the masking moiety comprises the amino acid sequence of SEQ ID NO: 11 and the first half-life extension domain comprises SEQ ID NO: 65 (Y349C; T366S; L38A; Y407V; and N297A) and the second half-life extension domain comprises SEQ ID NO 66 (S354C, T366W and N297A) and the non-cleavable linker comprises an amino acid sequence as shown in SEQ ID NO: 22 and the cleavable peptide comprises an amino acid sequence as shown in SEQ ID NO: 33.

In some embodiments, the IL-15 cytokine or functional fragment thereof comprises the amino acid sequence of SEQ ID NO: 6 and the masking moiety comprises the amino acid sequence of SEQ ID NO: 12 and the first half-life extension domain comprises SEQ ID NO: 65 (Y349C; T366S; L38A; Y407V; and N297A) and the second half-life extension domain comprises SEQ ID NO 66 (S354C, T366W and N297A) and the non-cleavable linker comprises an amino acid sequence as shown in SEQ ID NO: 22 and the cleavable peptide comprises an amino acid sequence as shown in SEQ ID NO: 33.

In some embodiments, the IL-15 cytokine or functional fragment thereof comprises the amino acid sequence of SEQ ID NO: 6 and the masking moiety comprises the amino acid sequence of SEQ ID NO: 11

and the first half-life extension domain comprises SEQ ID NO: 63 (Y349C; T366S; L38A; Y407V; and N297A) and the second half-life extension domain comprises SEQ ID NO 64 (S354C, T366W and N297A) and the non-cleavable linker comprises an amino acid sequence as shown in SEQ ID NO: 22 and the cleavable peptide comprises an amino acid sequence as shown in SEQ ID NO: 34.

In some embodiments, the IL-15 cytokine or functional fragment thereof comprises the amino acid sequence of SEQ ID NO: 6 and the masking moiety comprises the amino acid sequence of SEQ ID NO: 12 and the first half-life extension domain comprises SEQ ID NO: 63 (Y349C; T366S; L38A; Y407V; and N297A) and the second half-life extension domain comprises SEQ ID NO 64 (S354C, T366W and N297A) and the non-cleavable linker comprises an amino acid sequence as shown in SEQ ID NO: 22 and the cleavable peptide comprises an amino acid sequence as shown in SEQ ID NO: 34.

In some embodiments, the IL-15 cytokine or functional fragment thereof comprises the amino acid sequence of SEQ ID NO: 6 and the masking moiety comprises the amino acid sequence of SEQ ID NO: 11 and the first half-life extension domain comprises SEQ ID NO: 65 (Y349C; T366S; L38A; Y407V; and N297A) and the second half-life extension domain comprises SEQ ID NO 66 (S354C, T366W and N297A) and the non-cleavable linker comprises an amino acid sequence as shown in SEQ ID NO: 22 and the cleavable peptide comprises an amino acid sequence as shown in SEQ ID NO: 34.

In some embodiments, the IL-15 cytokine or functional fragment thereof comprises the amino acid sequence of SEQ ID NO: 6 and the masking moiety comprises the amino acid sequence of SEQ ID NO: 12 and the first half-life extension domain comprises SEQ ID NO: 65 (Y349C; T366S; L38A; Y407V; and N297A) and the second half-life extension domain comprises SEQ ID NO 66 (S354C, T366W and N297A) and the non-cleavable linker comprises an amino acid sequence as shown in SEQ ID NO: 22 and the cleavable peptide comprises an amino acid sequence as shown in SEQ ID NO: 34.

In some embodiments, the IL-15 cytokine or functional fragment thereof comprises the amino acid sequence of SEQ ID NO: 7 and the masking moiety comprises the amino acid sequence of SEQ ID NO: 11 and the first half-life extension domain comprises SEQ ID NO: 63 (Y349C; T366S; L38A; Y407V; and N297A) and the second half-life extension domain comprises SEQ ID NO 64 (S354C, T366W and N297A) and the non-cleavable linker comprises an amino acid sequence as shown in SEQ ID NO: 22 and the cleavable peptide comprises an amino acid sequence as shown in SEQ ID NO: 33.

In some embodiments, the IL-15 cytokine or functional fragment thereof comprises the amino acid sequence of SEQ ID NO: 7 and the masking moiety comprises the amino acid sequence of SEQ ID NO: 12 and the first half-life extension domain comprises SEQ ID NO: 63 (Y349C; T366S; L38A; Y407V; and N297A) and the second half-life extension domain comprises SEQ ID NO 64 (S354C, T366W and N297A)

and the non-cleavable linker comprises an amino acid sequence as shown in SEQ ID NO: 22 and the cleavable peptide comprises an amino acid sequence as shown in SEQ ID NO: 33.

In some embodiments, the IL-15 cytokine or functional fragment thereof comprises the amino acid sequence of SEQ ID NO: 7 and the masking moiety comprises the amino acid sequence of SEQ ID NO: 11 and the first half-life extension domain comprises SEQ ID NO: 65 (Y349C; T366S; L38A; Y407V; and N297A) and the second half-life extension domain comprises SEQ ID NO 66 (S354C, T366W and N297A) and the non-cleavable linker comprises an amino acid sequence as shown in SEQ ID NO: 22 and the cleavable peptide comprises an amino acid sequence as shown in SEQ ID NO: 33.

In some embodiments, the IL-15 cytokine or functional fragment thereof comprises the amino acid sequence of SEQ ID NO: 7 and the masking moiety comprises the amino acid sequence of SEQ ID NO: 12 and the first half-life extension domain comprises SEQ ID NO: 65 (Y349C; T366S; L38A; Y407V; and N297A) and the second half-life extension domain comprises SEQ ID NO 66 (S354C, T366W and N297A) and the non-cleavable linker comprises an amino acid sequence as shown in SEQ ID NO: 22 and the cleavable peptide comprises an amino acid sequence as shown in SEQ ID NO: 33.

In some embodiments, the IL-15 cytokine or functional fragment thereof comprises the amino acid sequence of SEQ ID NO: 7 and the masking moiety comprises the amino acid sequence of SEQ ID NO: 11 and the first half-life extension domain comprises SEQ ID NO: 63 (Y349C; T366S; L38A; Y407V; and N297A) and the second half-life extension domain comprises SEQ ID NO 64 (S354C, T366W and N297A) and the non-cleavable linker comprises an amino acid sequence as shown in SEQ ID NO: 22 and the cleavable peptide comprises an amino acid sequence as shown in SEQ ID NO: 34.

In some embodiments, the IL-15 cytokine or functional fragment thereof comprises the amino acid sequence of SEQ ID NO: 7 and the masking moiety comprises the amino acid sequence of SEQ ID NO: 12 and the first half-life extension domain comprises SEQ ID NO: 63 (Y349C; T366S; L38A; Y407V; and N297A) and the second half-life extension domain comprises SEQ ID NO 64 (S354C, T366W and N297A) and the non-cleavable linker comprises an amino acid sequence as shown in SEQ ID NO: 22 and the cleavable peptide comprises an amino acid sequence as shown in SEQ ID NO: 34.

In some embodiments, the IL-15 cytokine or functional fragment thereof comprises the amino acid sequence of SEQ ID NO: 7 and the masking moiety comprises the amino acid sequence of SEQ ID NO: 11 and the first half-life extension domain comprises SEQ ID NO: 65 (Y349C; T366S; L38A; Y407V; and N297A) and the second half-life extension domain comprises SEQ ID NO 66 (S354C, T366W and N297A) and the non-cleavable linker comprises an amino acid sequence as shown in SEQ ID NO: 22 and the cleavable peptide comprises an amino acid sequence as shown in SEQ ID NO: 34.

In some embodiments, the IL-15 cytokine or functional fragment thereof comprises the amino acid sequence of SEQ ID NO: 7 and the masking moiety comprises the amino acid sequence of SEQ ID NO: 12 and the first half-life extension domain comprises SEQ ID NO: 65 (Y349C; T366S; L38A; Y407V; and N297A) and the second half-life extension domain comprises SEQ ID NO 66 (S354C, T366W and N297A) and the non-cleavable linker comprises an amino acid sequence as shown in SEQ ID NO: 22 and the cleavable peptide comprises an amino acid sequence as shown in SEQ ID NO: 34.

In some embodiments, the IL-15 cytokine or functional fragment thereof comprises the amino acid sequence of SEQ ID NO: 8 and the masking moiety comprises the amino acid sequence of SEQ ID NO: 11 and the first half-life extension domain comprises SEQ ID NO: 63 (Y349C; T366S; L38A; Y407V; and N297A) and the second half-life extension domain comprises SEQ ID NO 64 (S354C, T366W and N297A) and the non-cleavable linker comprises an amino acid sequence as shown in SEQ ID NO: 22 and the cleavable peptide comprises an amino acid sequence as shown in SEQ ID NO: 33.

In some embodiments, the IL-15 cytokine or functional fragment thereof comprises the amino acid sequence of SEQ ID NO: 8 and the masking moiety comprises the amino acid sequence of SEQ ID NO: 12 and the first half-life extension domain comprises SEQ ID NO: 63 (Y349C; T366S; L38A; Y407V; and N297A) and the second half-life extension domain comprises SEQ ID NO 64 (S354C, T366W and N297A) and the non-cleavable linker comprises an amino acid sequence as shown in SEQ ID NO: 22 and the cleavable peptide comprises an amino acid sequence as shown in SEQ ID NO: 33.

In some embodiments, the IL-15 cytokine or functional fragment thereof comprises the amino acid sequence of SEQ ID NO: 8 and the masking moiety comprises the amino acid sequence of SEQ ID NO: 11 and the first half-life extension domain comprises SEQ ID NO: 65 (Y349C; T366S; L38A; Y407V; and N297A) and the second half-life extension domain comprises SEQ ID NO 66 (S354C, T366W and N297A) and the non-cleavable linker comprises an amino acid sequence as shown in SEQ ID NO: 22 and the cleavable peptide comprises an amino acid sequence as shown in SEQ ID NO: 33.

In some embodiments, the IL-15 cytokine or functional fragment thereof comprises the amino acid sequence of SEQ ID NO: 8 and the masking moiety comprises the amino acid sequence of SEQ ID NO: 12 and the first half-life extension domain comprises SEQ ID NO: 65 (Y349C; T366S; L38A; Y407V; and N297A) and the second half-life extension domain comprises SEQ ID NO 66 (S354C, T366W and N297A) and the non-cleavable linker comprises an amino acid sequence as shown in SEQ ID NO: 22 and the cleavable peptide comprises an amino acid sequence as shown in SEQ ID NO: 33.

In some embodiments, the IL-15 cytokine or functional fragment thereof comprises the amino acid sequence of SEQ ID NO: 8 and the masking moiety comprises the amino acid sequence of SEQ ID NO: 11 and the first half-life extension domain comprises SEQ ID NO: 63 (Y349C; T366S; L38A; Y407V; and

N297A) and the second half-life extension domain comprises SEQ ID NO 64 (S354C, T366W and N297A) and the non-cleavable linker comprises an amino acid sequence as shown in SEQ ID NO: 22 and the cleavable peptide comprises an amino acid sequence as shown in SEQ ID NO: 34.

In some embodiments, the IL-15 cytokine or functional fragment thereof comprises the amino acid sequence of SEQ ID NO: 8 and the masking moiety comprises the amino acid sequence of SEQ ID NO: 12 and the first half-life extension domain comprises SEQ ID NO: 63 (Y349C; T366S; L38A; Y407V; and N297A) and the second half-life extension domain comprises SEQ ID NO 64 (S354C, T366W and N297A) and the non-cleavable linker comprises an amino acid sequence as shown in SEQ ID NO: 22 and the cleavable peptide comprises an amino acid sequence as shown in SEQ ID NO: 34.

In some embodiments, the IL-15 cytokine or functional fragment thereof comprises the amino acid sequence of SEQ ID NO: 8 and the masking moiety comprises the amino acid sequence of SEQ ID NO: 11 and the first half-life extension domain comprises SEQ ID NO: 65 (Y349C; T366S; L38A; Y407V; and N297A) and the second half-life extension domain comprises SEQ ID NO 66 (S354C, T366W and N297A) and the non-cleavable linker comprises an amino acid sequence as shown in SEQ ID NO: 22 and the cleavable peptide comprises an amino acid sequence as shown in SEQ ID NO: 34.

In some embodiments, the IL-15 cytokine or functional fragment thereof comprises the amino acid sequence of SEQ ID NO: 8 and the masking moiety comprises the amino acid sequence of SEQ ID NO: 12 and the first half-life extension domain comprises SEQ ID NO: 65 (Y349C; T366S; L38A; Y407V; and N297A) and the second half-life extension domain comprises SEQ ID NO 66 (S354C, T366W and N297A) and the non-cleavable linker comprises an amino acid sequence as shown in SEQ ID NO: 22 and the cleavable peptide comprises an amino acid sequence as shown in SEQ ID NO: 34.

In some embodiments, the IL-15 cytokine or functional fragment thereof comprises the amino acid sequence of SEQ ID NO: 127 and the IL-15Rα domain comprises an amino acid sequence as shown in SEQ ID NO: 56.

In some embodiments, the IL-15 cytokine or functional fragment thereof comprises the amino acid sequence of SEQ ID NO: 128 and the IL-15Rα domain comprises an amino acid sequence as shown in SEQ ID NO: 56.

In some embodiments, the IL-15 cytokine or functional fragment thereof comprises the amino acid sequence of SEQ ID NO: 129 and the IL-15Rα domain comprises an amino acid sequence as shown in SEQ ID NO: 56.

In some embodiments, the IL-15 cytokine or functional fragment thereof comprises the amino acid sequence of SEQ ID NO: 130 and the IL-15Rα domain comprises an amino acid sequence as shown in SEQ ID NO: 56.

In some embodiments, the IL-15 cytokine or functional fragment thereof comprises the amino acid sequence of SEQ ID NO: 131 and the IL-15Rα domain comprises an amino acid sequence as shown in SEQ ID NO: 56.

In some embodiments, the IL-15 cytokine or functional fragment thereof comprises the amino acid sequence of SEQ ID NO: 132 and the IL-15Rα domain comprises an amino acid sequence as shown in SEQ ID NO: 56.

In some embodiments, the IL-15 cytokine or functional fragment thereof comprises the amino acid sequence of SEQ ID NO: 133 and the IL-15Rα domain comprises an amino acid sequence as shown in SEQ ID NO: 56.

In some embodiments, the IL-15 cytokine or functional fragment thereof comprises the amino acid sequence of SEQ ID NO: 127 and the masking moiety comprises the amino acid sequence of SEQ ID NO: 12, and the IL-15Rα domain comprises an amino acid sequence as shown in SEQ ID NO: 56.

In some embodiments, the IL-15 cytokine or functional fragment thereof comprises the amino acid sequence of SEQ ID NO: 128 and the masking moiety comprises the amino acid sequence of SEQ ID NO: 12, and the IL-15Rα domain comprises an amino acid sequence as shown in SEQ ID NO: 56.

In some embodiments, the IL-15 cytokine or functional fragment thereof comprises the amino acid sequence of SEQ ID NO: 129 and the masking moiety comprises the amino acid sequence of SEQ ID NO: 12, and the IL-15Rα domain comprises an amino acid sequence as shown in SEQ ID NO: 56.

In some embodiments, the IL-15 cytokine or functional fragment thereof comprises the amino acid sequence of SEQ ID NO: 130 and the masking moiety comprises the amino acid sequence of SEQ ID NO: 12, and the IL-15Rα domain comprises an amino acid sequence as shown in SEQ ID NO: 56.

In some embodiments, the IL-15 cytokine or functional fragment thereof comprises the amino acid sequence of SEQ ID NO: 131 and the masking moiety comprises the amino acid sequence of SEQ ID NO: 12, and the IL-15Rα domain comprises an amino acid sequence as shown in SEQ ID NO: 56.

In some embodiments, the IL-15 cytokine or functional fragment thereof comprises the amino acid sequence of SEQ ID NO: 132 and the masking moiety comprises the amino acid sequence of SEQ ID NO: 12, and the IL-15Rα domain comprises an amino acid sequence as shown in SEQ ID NO: 56.

In some embodiments, the IL-15 cytokine or functional fragment thereof comprises the amino acid sequence of SEQ ID NO: 133 and the masking moiety comprises the amino acid sequence of SEQ ID NO: 12, and the IL-15Rα domain comprises an amino acid sequence as shown in SEQ ID NO: 56.

In some embodiments, the IL-15 cytokine or functional fragment thereof comprises the amino acid sequence of SEQ ID NO: 127 and the masking moiety comprises the amino acid sequence of SEQ ID NO: 12 and the first half-life extension domain comprises SEQ ID NO: 63 (Y349C; T366S; L38A; Y407V; and N297A) and the second half-life extension domain comprises SEQ ID NO 64 (S354C, T366W and N297A), and the IL-15Rα domain comprises an amino acid sequence as shown in SEQ ID NO: 56.

In some embodiments, the IL-15 cytokine or functional fragment thereof comprises the amino acid sequence of SEQ ID NO: 128 and the masking moiety comprises the amino acid sequence of SEQ ID NO: 12 and the first half-life extension domain comprises SEQ ID NO: 63 (Y349C; T366S; L38A; Y407V; and N297A) and the second half-life extension domain comprises SEQ ID NO 64 (S354C, T366W and N297A), and the IL-15Rα domain comprises an amino acid sequence as shown in SEQ ID NO: 56.

In some embodiments, the IL-15 cytokine or functional fragment thereof comprises the amino acid sequence of SEQ ID NO: 129 and the masking moiety comprises the amino acid sequence of SEQ ID NO: 12 and the first half-life extension domain comprises SEQ ID NO: 63 (Y349C; T366S; L38A; Y407V; and N297A) and the second half-life extension domain comprises SEQ ID NO 64 (S354C, T366W and N297A), and the IL-15Rα domain comprises an amino acid sequence as shown in SEQ ID NO: 56.

In some embodiments, the IL-15 cytokine or functional fragment thereof comprises the amino acid sequence of SEQ ID NO: 130 and the masking moiety comprises the amino acid sequence of SEQ ID NO: 12 and the first half-life extension domain comprises SEQ ID NO: 63 (Y349C; T366S; L38A; Y407V; and N297A) and the second half-life extension domain comprises SEQ ID NO 64 (S354C, T366W and N297A), and the IL-15Rα domain comprises an amino acid sequence as shown in SEQ ID NO: 56.

In some embodiments, the IL-15 cytokine or functional fragment thereof comprises the amino acid sequence of SEQ ID NO: 131 and the masking moiety comprises the amino acid sequence of SEQ ID NO: 12 and the first half-life extension domain comprises SEQ ID NO: 63 (Y349C; T366S; L38A; Y407V; and N297A) and the second half-life extension domain comprises SEQ ID NO 64 (S354C, T366W and N297A), and the IL-15Rα domain comprises an amino acid sequence as shown in SEQ ID NO: 56.

In some embodiments, the IL-15 cytokine or functional fragment thereof comprises the amino acid sequence of SEQ ID NO: 132 and the masking moiety comprises the amino acid sequence of SEQ ID NO: 12 and the first half-life extension domain comprises SEQ ID NO: 63 (Y349C; T366S; L38A; Y407V; and N297A) and the second half-life extension domain comprises SEQ ID NO 64 (S354C, T366W and N297A), and the IL-15Rα domain comprises an amino acid sequence as shown in SEQ ID NO: 56.

In some embodiments, the IL-15 cytokine or functional fragment thereof comprises the amino acid sequence of SEQ ID NO: 133 and the masking moiety comprises the amino acid sequence of SEQ ID NO: 12 and the first half-life extension domain comprises SEQ ID NO: 63 (Y349C; T366S; L38A; Y407V; and N297A) and the second half-life extension domain comprises SEQ ID NO 64 (S354C, T366W and N297A), and the IL-15Rα domain comprises an amino acid sequence as shown in SEQ ID NO: 56.

In some embodiments, the IL-15 cytokine or functional fragment thereof comprises the amino acid sequence of SEQ ID NO: 127 and the masking moiety comprises the amino acid sequence of SEQ ID NO: 12 and the first half-life extension domain comprises SEQ ID NO: 63 (Y349C; T366S; L38A; Y407V; and N297A) and the second half-life extension domain comprises SEQ ID NO 64 (S354C, T366W and N297A) and the non-cleavable linker comprises an amino acid sequence as shown in SEQ ID NO: 22, and the IL-15Rα domain comprises an amino acid sequence as shown in SEQ ID NO: 56.

In some embodiments, the IL-15 cytokine or functional fragment thereof comprises the amino acid sequence of SEQ ID NO: 128 and the masking moiety comprises the amino acid sequence of SEQ ID NO: 12 and the first half-life extension domain comprises SEQ ID NO: 63 (Y349C; T366S; L38A; Y407V; and N297A) and the second half-life extension domain comprises SEQ ID NO 64 (S354C, T366W and N297A) and the non-cleavable linker comprises an amino acid sequence as shown in SEQ ID NO: 22, and the IL-15Rα domain comprises an amino acid sequence as shown in SEQ ID NO: 56

In some embodiments, the IL-15 cytokine or functional fragment thereof comprises the amino acid sequence of SEQ ID NO: 129 and the masking moiety comprises the amino acid sequence of SEQ ID NO: 12 and the first half-life extension domain comprises SEQ ID NO: 63 (Y349C; T366S; L38A; Y407V; and N297A) and the second half-life extension domain comprises SEQ ID NO 64 (S354C, T366W and N297A) and the non-cleavable linker comprises an amino acid sequence as shown in SEQ ID NO: 22, and the IL-15Rα domain comprises an amino acid sequence as shown in SEQ ID NO: 56

In some embodiments, the IL-15 cytokine or functional fragment thereof comprises the amino acid sequence of SEQ ID NO: 130 and the masking moiety comprises the amino acid sequence of SEQ ID NO: 12 and the first half-life extension domain comprises SEQ ID NO: 63 (Y349C; T366S; L38A; Y407V; and N297A) and the second half-life extension domain comprises SEQ ID NO 64 (S354C, T366W and N297A)

and the non-cleavable linker comprises an amino acid sequence as shown in SEQ ID NO: 22, and the IL-15Rα domain comprises an amino acid sequence as shown in SEQ ID NO: 56

In some embodiments, the IL-15 cytokine or functional fragment thereof comprises the amino acid sequence of SEQ ID NO: 131 and the masking moiety comprises the amino acid sequence of SEQ ID NO: 12 and the first half-life extension domain comprises SEQ ID NO: 63 (Y349C; T366S; L38A; Y407V; and N297A) and the second half-life extension domain comprises SEQ ID NO 64 (S354C, T366W and N297A) and the non-cleavable linker comprises an amino acid sequence as shown in SEQ ID NO: 22, and the IL-15Rα domain comprises an amino acid sequence as shown in SEQ ID NO: 56

In some embodiments, the IL-15 cytokine or functional fragment thereof comprises the amino acid sequence of SEQ ID NO: 132 and the masking moiety comprises the amino acid sequence of SEQ ID NO: 12 and the first half-life extension domain comprises SEQ ID NO: 63 (Y349C; T366S; L38A; Y407V; and N297A) and the second half-life extension domain comprises SEQ ID NO 64 (S354C, T366W and N297A) and the non-cleavable linker comprises an amino acid sequence as shown in SEQ ID NO: 22, and the IL-15Rα domain comprises an amino acid sequence as shown in SEQ ID NO: 56

In some embodiments, the IL-15 cytokine or functional fragment thereof comprises the amino acid sequence of SEQ ID NO: 133 and the masking moiety comprises the amino acid sequence of SEQ ID NO: 12 and the first half-life extension domain comprises SEQ ID NO: 63 (Y349C; T366S; L38A; Y407V; and N297A) and the second half-life extension domain comprises SEQ ID NO 64 (S354C, T366W and N297A) and the non-cleavable linker comprises an amino acid sequence as shown in SEQ ID NO: 22, and the IL-15Rα domain comprises an amino acid sequence as shown in SEQ ID NO: 56

In some embodiments, the IL-15 cytokine or functional fragment thereof comprises the amino acid sequence of SEQ ID NO: 127 and the masking moiety comprises the amino acid sequence of SEQ ID NO: 12 and the first half-life extension domain comprises SEQ ID NO: 63 (Y349C; T366S; L38A; Y407V; and N297A) and the second half-life extension domain comprises SEQ ID NO 64 (S354C, T366W and N297A) and the non-cleavable linker comprises an amino acid sequence as shown in SEQ ID NO: 22, and the cleavable peptide comprises an amino acid sequence as shown in SEQ ID NO: 27 and the IL-15Rα domain comprises an amino acid sequence as shown in SEQ ID NO: 56.

In some embodiments, the IL-15 cytokine or functional fragment thereof comprises the amino acid sequence of SEQ ID NO: 128 and the masking moiety comprises the amino acid sequence of SEQ ID NO: 12 and the first half-life extension domain comprises SEQ ID NO: 63 (Y349C; T366S; L38A; Y407V; and N297A) and the second half-life extension domain comprises SEQ ID NO 64 (S354C, T366W and N297A) and the non-cleavable linker comprises an amino acid sequence as shown in SEQ ID NO: 22, and the

cleavable peptide comprises an amino acid sequence as shown in SEQ ID NO: 27 and the IL-15Rα domain comprises an amino acid sequence as shown in SEQ ID NO: 56.

In some embodiments, the IL-15 cytokine or functional fragment thereof comprises the amino acid sequence of SEQ ID NO: 129 and the masking moiety comprises the amino acid sequence of SEQ ID NO: 12 and the first half-life extension domain comprises SEQ ID NO: 63 (Y349C; T366S; L38A; Y407V; and N297A) and the second half-life extension domain comprises SEQ ID NO 64 (S354C, T366W and N297A) and the non-cleavable linker comprises an amino acid sequence as shown in SEQ ID NO: 22, and the cleavable peptide comprises an amino acid sequence as shown in SEQ ID NO: 27 and the IL-15Rα domain comprises an amino acid sequence as shown in SEQ ID NO: 56.

In some embodiments, the IL-15 cytokine or functional fragment thereof comprises the amino acid sequence of SEQ ID NO: 130 and the masking moiety comprises the amino acid sequence of SEQ ID NO: 12 and the first half-life extension domain comprises SEQ ID NO: 63 (Y349C; T366S; L38A; Y407V; and N297A) and the second half-life extension domain comprises SEQ ID NO 64 (S354C, T366W and N297A) and the non-cleavable linker comprises an amino acid sequence as shown in SEQ ID NO: 22, and the cleavable peptide comprises an amino acid sequence as shown in SEQ ID NO: 27 and the IL-15Rα domain comprises an amino acid sequence as shown in SEQ ID NO: 56.

In some embodiments, the IL-15 cytokine or functional fragment thereof comprises the amino acid sequence of SEQ ID NO: 130 and the masking moiety comprises the amino acid sequence of SEQ ID NO: 12 and the first half-life extension domain comprises SEQ ID NO: 63 (Y349C; T366S; L38A; Y407V; and N297A) and the second half-life extension domain comprises SEQ ID NO 64 (S354C, T366W and N297A) and the non-cleavable linker comprises an amino acid sequence as shown in SEQ ID NO: 22, and the cleavable peptide comprises an amino acid sequence as shown in SEQ ID NO: 155 and the IL-15Rα domain comprises an amino acid sequence as shown in SEQ ID NO: 56.

In some embodiments, the IL-15 cytokine or functional fragment thereof comprises the amino acid sequence of SEQ ID NO: 130 and the masking moiety comprises the amino acid sequence of SEQ ID NO: 12 and the first half-life extension domain comprises SEQ ID NO: 63 (Y349C; T366S; L38A; Y407V; and N297A) and the second half-life extension domain comprises SEQ ID NO 64 (S354C, T366W and N297A) and the non-cleavable linker comprises an amino acid sequence as shown in SEQ ID NO: 22, and the cleavable peptide comprises an amino acid sequence as shown in SEQ ID NO: 156 and the IL-15Rα domain comprises an amino acid sequence as shown in SEQ ID NO: 56.

In some embodiments, the IL-15 cytokine or functional fragment thereof comprises the amino acid sequence of SEQ ID NO: 131 and the masking moiety comprises the amino acid sequence of SEQ ID NO: 12 and the first half-life extension domain comprises SEQ ID NO: 63 (Y349C; T366S; L38A; Y407V; and

N297A) and the second half-life extension domain comprises SEQ ID NO 64 (S354C, T366W and N297A) and the non-cleavable linker comprises an amino acid sequence as shown in SEQ ID NO: 22, and the cleavable peptide comprises an amino acid sequence as shown in SEQ ID NO: 27 and the IL-15Rα domain comprises an amino acid sequence as shown in SEQ ID NO: 56.

In some embodiments, the IL-15 cytokine or functional fragment thereof comprises the amino acid sequence of SEQ ID NO: 132 and the masking moiety comprises the amino acid sequence of SEQ ID NO: 12 and the first half-life extension domain comprises SEQ ID NO: 63 (Y349C; T366S; L38A; Y407V; and N297A) and the second half-life extension domain comprises SEQ ID NO 64 (S354C, T366W and N297A) and the non-cleavable linker comprises an amino acid sequence as shown in SEQ ID NO: 22, and the cleavable peptide comprises an amino acid sequence as shown in SEQ ID NO: 27 and the IL-15Rα domain comprises an amino acid sequence as shown in SEQ ID NO: 56.

In some embodiments, the IL-15 cytokine or functional fragment thereof comprises the amino acid sequence of SEQ ID NO: 133 and the masking moiety comprises the amino acid sequence of SEQ ID NO: 12 and the first half-life extension domain comprises SEQ ID NO: 63 (Y349C; T366S; L38A; Y407V; and N297A) and the second half-life extension domain comprises SEQ ID NO 64 (S354C, T366W and N297A) and the non-cleavable linker comprises an amino acid sequence as shown in SEQ ID NO: 22, and the cleavable peptide comprises an amino acid sequence as shown in SEQ ID NO: 27 and the IL-15Rα domain comprises an amino acid sequence as shown in SEQ ID NO: 56.

In some embodiments, the IL-15 cytokine or functional fragment thereof comprises the amino acid sequence of SEQ ID NO: 127 and the masking moiety comprises the amino acid sequence of SEQ ID NO: 12 and the first half-life extension domain comprises SEQ ID NO: 63 (Y349C; T366S; L38A; Y407V; and N297A) and the second half-life extension domain comprises SEQ ID NO 64 (S354C, T366W and N297A) and the non-cleavable linker comprises an amino acid sequence as shown in SEQ ID NO: 22, and the cleavable peptide comprises an amino acid sequence as shown in SEQ ID NO: 34 and the IL-15Rα domain comprises an amino acid sequence as shown in SEQ ID NO: 56.

In some embodiments, the IL-15 cytokine or functional fragment thereof comprises the amino acid sequence of SEQ ID NO: 128 and the masking moiety comprises the amino acid sequence of SEQ ID NO: 12 and the first half-life extension domain comprises SEQ ID NO: 63 (Y349C; T366S; L38A; Y407V; and N297A) and the second half-life extension domain comprises SEQ ID NO 64 (S354C, T366W and N297A) and the non-cleavable linker comprises an amino acid sequence as shown in SEQ ID NO: 22, and the cleavable peptide comprises an amino acid sequence as shown in SEQ ID NO: 34 and the IL-15Rα domain comprises an amino acid sequence as shown in SEQ ID NO: 56.

In some embodiments, the IL-15 cytokine or functional fragment thereof comprises the amino acid sequence of SEQ ID NO: 129 and the masking moiety comprises the amino acid sequence of SEQ ID NO: 12 and the first half-life extension domain comprises SEQ ID NO: 63 (Y349C; T366S; L38A; Y407V; and N297A) and the second half-life extension domain comprises SEQ ID NO 64 (S354C, T366W and N297A) and the non-cleavable linker comprises an amino acid sequence as shown in SEQ ID NO: 22, and the cleavable peptide comprises an amino acid sequence as shown in SEQ ID NO: 34 and the IL-15Rα domain comprises an amino acid sequence as shown in SEQ ID NO: 56.

In some embodiments, the IL-15 cytokine or functional fragment thereof comprises the amino acid sequence of SEQ ID NO: 130 and the masking moiety comprises the amino acid sequence of SEQ ID NO: 12 and the first half-life extension domain comprises SEQ ID NO: 63 (Y349C; T366S; L38A; Y407V; and N297A) and the second half-life extension domain comprises SEQ ID NO 64 (S354C, T366W and N297A) and the non-cleavable linker comprises an amino acid sequence as shown in SEQ ID NO: 22, and the cleavable peptide comprises an amino acid sequence as shown in SEQ ID NO: 34 and the IL-15Rα domain comprises an amino acid sequence as shown in SEQ ID NO: 56.

In some embodiments, the IL-15 cytokine or functional fragment thereof comprises the amino acid sequence of SEQ ID NO: 131 and the masking moiety comprises the amino acid sequence of SEQ ID NO: 12 and the first half-life extension domain comprises SEQ ID NO: 63 (Y349C; T366S; L38A; Y407V; and N297A) and the second half-life extension domain comprises SEQ ID NO 64 (S354C, T366W and N297A) and the non-cleavable linker comprises an amino acid sequence as shown in SEQ ID NO: 22, and the cleavable peptide comprises an amino acid sequence as shown in SEQ ID NO: 34 and the IL-15Rα domain comprises an amino acid sequence as shown in SEQ ID NO: 56.

In some embodiments, the IL-15 cytokine or functional fragment thereof comprises the amino acid sequence of SEQ ID NO: 132 and the masking moiety comprises the amino acid sequence of SEQ ID NO: 12 and the first half-life extension domain comprises SEQ ID NO: 63 (Y349C; T366S; L38A; Y407V; and N297A) and the second half-life extension domain comprises SEQ ID NO 64 (S354C, T366W and N297A) and the non-cleavable linker comprises an amino acid sequence as shown in SEQ ID NO: 22, and the cleavable peptide comprises an amino acid sequence as shown in SEQ ID NO: 34 and the IL-15Rα domain comprises an amino acid sequence as shown in SEQ ID NO: 56.

In some embodiments, the IL-15 cytokine or functional fragment thereof comprises the amino acid sequence of SEQ ID NO: 133 and the masking moiety comprises the amino acid sequence of SEQ ID NO: 12 and the first half-life extension domain comprises SEQ ID NO: 63 (Y349C; T366S; L38A; Y407V; and N297A) and the second half-life extension domain comprises SEQ ID NO 64 (S354C, T366W and N297A) and the non-cleavable linker comprises an amino acid sequence as shown in SEQ ID NO: 22, and the

cleavable peptide comprises an amino acid sequence as shown in SEQ ID NO: 34 and the IL-15Rα domain comprises an amino acid sequence as shown in SEQ ID NO: 56.

In some embodiments, the IL-15 cytokine or functional fragment thereof comprises the amino acid sequence of SEQ ID NO: 127 and the masking moiety comprises the amino acid sequence of SEQ ID NO: 12 and the first half-life extension domain comprises SEQ ID NO: 63 (Y349C; T366S; L38A; Y407V; and N297A) and the second half-life extension domain comprises SEQ ID NO 64 (S354C, T366W and N297A) and the non-cleavable linker comprises an amino acid sequence as shown in SEQ ID NO: 22 and the cleavable peptide comprises an amino acid sequence as shown in SEQ ID NO: 33, and the IL-15Rα domain comprises an amino acid sequence as shown in SEQ ID NO: 56.

In some embodiments, the IL-15 cytokine or functional fragment thereof comprises the amino acid sequence of SEQ ID NO: 128 and the masking moiety comprises the amino acid sequence of SEQ ID NO: 12 and the first half-life extension domain comprises SEQ ID NO: 63 (Y349C; T366S; L38A; Y407V; and N297A) and the second half-life extension domain comprises SEQ ID NO 64 (S354C, T366W and N297A) and the non-cleavable linker comprises an amino acid sequence as shown in SEQ ID NO: 22 and the cleavable peptide comprises an amino acid sequence as shown in SEQ ID NO: 33, and the IL-15Rα domain comprises an amino acid sequence as shown in SEQ ID NO: 56.

In some embodiments, the IL-15 cytokine or functional fragment thereof comprises the amino acid sequence of SEQ ID NO: 129 and the masking moiety comprises the amino acid sequence of SEQ ID NO: 12 and the first half-life extension domain comprises SEQ ID NO: 63 (Y349C; T366S; L38A; Y407V; and N297A) and the second half-life extension domain comprises SEQ ID NO 64 (S354C, T366W and N297A) and the non-cleavable linker comprises an amino acid sequence as shown in SEQ ID NO: 22 and the cleavable peptide comprises an amino acid sequence as shown in SEQ ID NO: 33, and the IL-15Rα domain comprises an amino acid sequence as shown in SEQ ID NO: 56.

In some embodiments, the IL-15 cytokine or functional fragment thereof comprises the amino acid sequence of SEQ ID NO: 130 and the masking moiety comprises the amino acid sequence of SEQ ID NO: 12 and the first half-life extension domain comprises SEQ ID NO: 63 (Y349C; T366S; L38A; Y407V; and N297A) and the second half-life extension domain comprises SEQ ID NO 64 (S354C, T366W and N297A) and the non-cleavable linker comprises an amino acid sequence as shown in SEQ ID NO: 22 and the cleavable peptide comprises an amino acid sequence as shown in SEQ ID NO: 33, and the IL-15Rα domain comprises an amino acid sequence as shown in SEQ ID NO: 56.

In some embodiments, the IL-15 cytokine or functional fragment thereof comprises the amino acid sequence of SEQ ID NO: 131 and the masking moiety comprises the amino acid sequence of SEQ ID NO: 12 and the first half-life extension domain comprises SEQ ID NO: 63 (Y349C; T366S; L38A; Y407V; and

N297A) and the second half-life extension domain comprises SEQ ID NO 64 (S354C, T366W and N297A) and the non-cleavable linker comprises an amino acid sequence as shown in SEQ ID NO: 22 and the cleavable peptide comprises an amino acid sequence as shown in SEQ ID NO: 33, and the IL-15Rα domain comprises an amino acid sequence as shown in SEQ ID NO: 56.

In some embodiments, the IL-15 cytokine or functional fragment thereof comprises the amino acid sequence of SEQ ID NO: 132 and the masking moiety comprises the amino acid sequence of SEQ ID NO: 12 and the first half-life extension domain comprises SEQ ID NO: 63 (Y349C; T366S; L38A; Y407V; and N297A) and the second half-life extension domain comprises SEQ ID NO 64 (S354C, T366W and N297A) and the non-cleavable linker comprises an amino acid sequence as shown in SEQ ID NO: 22 and the cleavable peptide comprises an amino acid sequence as shown in SEQ ID NO: 33, and the IL-15Rα domain comprises an amino acid sequence as shown in SEQ ID NO: 56.

In some embodiments, the IL-15 cytokine or functional fragment thereof comprises the amino acid sequence of SEQ ID NO: 133 and the masking moiety comprises the amino acid sequence of SEQ ID NO: 12 and the first half-life extension domain comprises SEQ ID NO: 63 (Y349C; T366S; L38A; Y407V; and N297A) and the second half-life extension domain comprises SEQ ID NO 64 (S354C, T366W and N297A) and the non-cleavable linker comprises an amino acid sequence as shown in SEQ ID NO: 22 and the cleavable peptide comprises an amino acid sequence as shown in SEQ ID NO: 33, and the IL-15Rα domain comprises an amino acid sequence as shown in SEQ ID NO: 56.

In some embodiments, the IL-15 cytokine or functional fragment thereof comprises the amino acid sequence of SEQ ID NO: 127 and the masking moiety comprises the amino acid sequence of SEQ ID NO: 12 and the first half-life extension domain comprises SEQ ID NO: 63 (Y349C; T366S; L38A; Y407V; and N297A) and the second half-life extension domain comprises SEQ ID NO 64 (S354C, T366W and N297A) and the non-cleavable linker comprises an amino acid sequence as shown in SEQ ID NO: 22 and the cleavable peptide comprises an amino acid sequence as shown in SEQ ID NO: 134 and the IL-15Rα domain comprises an amino acid sequence as shown in SEQ ID NO: 56.

In some embodiments, the IL-15 cytokine or functional fragment thereof comprises the amino acid sequence of SEQ ID NO: 128 and the masking moiety comprises the amino acid sequence of SEQ ID NO: 12 and the first half-life extension domain comprises SEQ ID NO: 63 (Y349C; T366S; L38A; Y407V; and N297A) and the second half-life extension domain comprises SEQ ID NO 64 (S354C, T366W and N297A) and the non-cleavable linker comprises an amino acid sequence as shown in SEQ ID NO: 22 and the cleavable peptide comprises an amino acid sequence as shown in SEQ ID NO: 134 and the IL-15Rα domain comprises an amino acid sequence as shown in SEQ ID NO: 56.

In some embodiments, the IL-15 cytokine or functional fragment thereof comprises the amino acid sequence of SEQ ID NO: 129 and the masking moiety comprises the amino acid sequence of SEQ ID NO: 12 and the first half-life extension domain comprises SEQ ID NO: 63 (Y349C; T366S; L38A; Y407V; and N297A) and the second half-life extension domain comprises SEQ ID NO 64 (S354C, T366W and N297A) and the non-cleavable linker comprises an amino acid sequence as shown in SEQ ID NO: 22 and the cleavable peptide comprises an amino acid sequence as shown in SEQ ID NO: 134 and the IL-15Rα domain comprises an amino acid sequence as shown in SEQ ID NO: 56.

In some embodiments, the IL-15 cytokine or functional fragment thereof comprises the amino acid sequence of SEQ ID NO: 130 and the masking moiety comprises the amino acid sequence of SEQ ID NO: 12 and the first half-life extension domain comprises SEQ ID NO: 63 (Y349C; T366S; L38A; Y407V; and N297A) and the second half-life extension domain comprises SEQ ID NO 64 (S354C, T366W and N297A) and the non-cleavable linker comprises an amino acid sequence as shown in SEQ ID NO: 22 and the cleavable peptide comprises an amino acid sequence as shown in SEQ ID NO: 134 and the IL-15Rα domain comprises an amino acid sequence as shown in SEQ ID NO: 56.

In some embodiments, the IL-15 cytokine or functional fragment thereof comprises the amino acid sequence of SEQ ID NO: 131 and the masking moiety comprises the amino acid sequence of SEQ ID NO: 12 and the first half-life extension domain comprises SEQ ID NO: 63 (Y349C; T366S; L38A; Y407V; and N297A) and the second half-life extension domain comprises SEQ ID NO 64 (S354C, T366W and N297A) and the non-cleavable linker comprises an amino acid sequence as shown in SEQ ID NO: 22 and the cleavable peptide comprises an amino acid sequence as shown in SEQ ID NO: 134 and the IL-15Rα domain comprises an amino acid sequence as shown in SEQ ID NO: 56.

In some embodiments, the IL-15 cytokine or functional fragment thereof comprises the amino acid sequence of SEQ ID NO: 132 and the masking moiety comprises the amino acid sequence of SEQ ID NO: 12 and the first half-life extension domain comprises SEQ ID NO: 63 (Y349C; T366S; L38A; Y407V; and N297A) and the second half-life extension domain comprises SEQ ID NO 64 (S354C, T366W and N297A) and the non-cleavable linker comprises an amino acid sequence as shown in SEQ ID NO: 22 and the cleavable peptide comprises an amino acid sequence as shown in SEQ ID NO: 134 and the IL-15Rα domain comprises an amino acid sequence as shown in SEQ ID NO: 56.

In some embodiments, the IL-15 cytokine or functional fragment thereof comprises the amino acid sequence of SEQ ID NO: 133 and the masking moiety comprises the amino acid sequence of SEQ ID NO: 12 and the first half-life extension domain comprises SEQ ID NO: 63 (Y349C; T366S; L38A; Y407V; and N297A) and the second half-life extension domain comprises SEQ ID NO 64 (S354C, T366W and N297A)

and the non-cleavable linker comprises an amino acid sequence as shown in SEQ ID NO: 22 and the cleavable peptide comprises an amino acid sequence as shown in SEQ ID NO: 134 and the IL-15Rα domain comprises an amino acid sequence as shown in SEQ ID NO: 56.

In some embodiments, the IL-15 cytokine or functional fragment thereof comprises the amino acid sequence of SEQ ID NO: 127 and the masking moiety comprises the amino acid sequence of SEQ ID NO: 12 and the first half-life extension domain comprises SEQ ID NO: 63 (Y349C; T366S; L38A; Y407V; and N297A) and the second half-life extension domain comprises SEQ ID NO 64 (S354C, T366W and N297A) and the non-cleavable linker comprises an amino acid sequence as shown in SEQ ID NO: 21, and the IL-15Rα domain comprises an amino acid sequence as shown in SEQ ID NO: 56.

In some embodiments, the IL-15 cytokine or functional fragment thereof comprises the amino acid sequence of SEQ ID NO: 128 and the masking moiety comprises the amino acid sequence of SEQ ID NO: 12 and the first half-life extension domain comprises SEQ ID NO: 63 (Y349C; T366S; L38A; Y407V; and N297A) and the second half-life extension domain comprises SEQ ID NO 64 (S354C, T366W and N297A) and the non-cleavable linker comprises an amino acid sequence as shown in SEQ ID NO: 21, and the IL-15Rα domain comprises an amino acid sequence as shown in SEQ ID NO: 56

In some embodiments, the IL-15 cytokine or functional fragment thereof comprises the amino acid sequence of SEQ ID NO: 129 and the masking moiety comprises the amino acid sequence of SEQ ID NO: 12 and the first half-life extension domain comprises SEQ ID NO: 63 (Y349C; T366S; L38A; Y407V; and N297A) and the second half-life extension domain comprises SEQ ID NO 64 (S354C, T366W and N297A) and the non-cleavable linker comprises an amino acid sequence as shown in SEQ ID NO: 21, and the IL-15Rα domain comprises an amino acid sequence as shown in SEQ ID NO: 56

In some embodiments, the IL-15 cytokine or functional fragment thereof comprises the amino acid sequence of SEQ ID NO: 130 and the masking moiety comprises the amino acid sequence of SEQ ID NO: 12 and the first half-life extension domain comprises SEQ ID NO: 63 (Y349C; T366S; L38A; Y407V; and N297A) and the second half-life extension domain comprises SEQ ID NO 64 (S354C, T366W and N297A) and the non-cleavable linker comprises an amino acid sequence as shown in SEQ ID NO: 21, and the IL-15Rα domain comprises an amino acid sequence as shown in SEQ ID NO: 56

In some embodiments, the IL-15 cytokine or functional fragment thereof comprises the amino acid sequence of SEQ ID NO: 131 and the masking moiety comprises the amino acid sequence of SEQ ID NO: 12 and the first half-life extension domain comprises SEQ ID NO: 63 (Y349C; T366S; L38A; Y407V; and N297A) and the second half-life extension domain comprises SEQ ID NO 64 (S354C, T366W and N297A) and the non-cleavable linker comprises an amino acid sequence as shown in SEQ ID NO: 21, and the IL-15Rα domain comprises an amino acid sequence as shown in SEQ ID NO: 56

In some embodiments, the IL-15 cytokine or functional fragment thereof comprises the amino acid sequence of SEQ ID NO: 132 and the masking moiety comprises the amino acid sequence of SEQ ID NO: 12 and the first half-life extension domain comprises SEQ ID NO: 63 (Y349C; T366S; L38A; Y407V; and N297A) and the second half-life extension domain comprises SEQ ID NO 64 (S354C, T366W and N297A) and the non-cleavable linker comprises an amino acid sequence as shown in SEQ ID NO: 21, and the IL-15Rα domain comprises an amino acid sequence as shown in SEQ ID NO: 56

In some embodiments, the IL-15 cytokine or functional fragment thereof comprises the amino acid sequence of SEQ ID NO: 133 and the masking moiety comprises the amino acid sequence of SEQ ID NO: 12 and the first half-life extension domain comprises SEQ ID NO: 63 (Y349C; T366S; L38A; Y407V; and N297A) and the second half-life extension domain comprises SEQ ID NO 64 (S354C, T366W and N297A) and the non-cleavable linker comprises an amino acid sequence as shown in SEQ ID NO: 21, and the IL-15Rα domain comprises an amino acid sequence as shown in SEQ ID NO: 56

In some embodiments, the IL-15 cytokine or functional fragment thereof comprises the amino acid sequence of SEQ ID NO: 127 and the masking moiety comprises the amino acid sequence of SEQ ID NO: 12 and the first half-life extension domain comprises SEQ ID NO: 63 (Y349C; T366S; L38A; Y407V; and N297A) and the second half-life extension domain comprises SEQ ID NO 64 (S354C, T366W and N297A) and the non-cleavable linker comprises an amino acid sequence as shown in SEQ ID NO: 21, and the cleavable peptide comprises an amino acid sequence as shown in SEQ ID NO: 27 and the IL-15Rα domain comprises an amino acid sequence as shown in SEQ ID NO: 56.

In some embodiments, the IL-15 cytokine or functional fragment thereof comprises the amino acid sequence of SEQ ID NO: 127 and the masking moiety comprises the amino acid sequence of SEQ ID NO: 12 and the first half-life extension domain comprises SEQ ID NO: 63 (Y349C; T366S; L38A; Y407V; and N297A) and the second half-life extension domain comprises SEQ ID NO 64 (S354C, T366W and N297A) and the non-cleavable linker comprises an amino acid sequence as shown in SEQ ID NO: 21, and the cleavable peptide comprises an amino acid sequence as shown in SEQ ID NO: 155 and the IL-15Rα domain comprises an amino acid sequence as shown in SEQ ID NO: 56.

In some embodiments, the IL-15 cytokine or functional fragment thereof comprises the amino acid sequence of SEQ ID NO: 127 and the masking moiety comprises the amino acid sequence of SEQ ID NO: 12 and the first half-life extension domain comprises SEQ ID NO: 63 (Y349C; T366S; L38A; Y407V; and N297A) and the second half-life extension domain comprises SEQ ID NO 64 (S354C, T366W and N297A) and the non-cleavable linker comprises an amino acid sequence as shown in SEQ ID NO: 21, and the cleavable peptide comprises an amino acid sequence as shown in SEQ ID NO: 156 and the IL-15Rα domain comprises an amino acid sequence as shown in SEQ ID NO: 56.

In some embodiments, the IL-15 cytokine or functional fragment thereof comprises the amino acid sequence of SEQ ID NO: 128 and the masking moiety comprises the amino acid sequence of SEQ ID NO: 12 and the first half-life extension domain comprises SEQ ID NO: 63 (Y349C; T366S; L38A; Y407V; and N297A) and the second half-life extension domain comprises SEQ ID NO 64 (S354C, T366W and N297A) and the non-cleavable linker comprises an amino acid sequence as shown in SEQ ID NO: 21, and the cleavable peptide comprises an amino acid sequence as shown in SEQ ID NO: 27 and the IL-15Rα domain comprises an amino acid sequence as shown in SEQ ID NO: 56.

In some embodiments, the IL-15 cytokine or functional fragment thereof comprises the amino acid sequence of SEQ ID NO: 129 and the masking moiety comprises the amino acid sequence of SEQ ID NO: 12 and the first half-life extension domain comprises SEQ ID NO: 63 (Y349C; T366S; L38A; Y407V; and N297A) and the second half-life extension domain comprises SEQ ID NO 64 (S354C, T366W and N297A) and the non-cleavable linker comprises an amino acid sequence as shown in SEQ ID NO: 21, and the cleavable peptide comprises an amino acid sequence as shown in SEQ ID NO: 27 and the IL-15Rα domain comprises an amino acid sequence as shown in SEQ ID NO: 56.

In some embodiments, the IL-15 cytokine or functional fragment thereof comprises the amino acid sequence of SEQ ID NO: 130 and the masking moiety comprises the amino acid sequence of SEQ ID NO: 12 and the first half-life extension domain comprises SEQ ID NO: 63 (Y349C; T366S; L38A; Y407V; and N297A) and the second half-life extension domain comprises SEQ ID NO 64 (S354C, T366W and N297A) and the non-cleavable linker comprises an amino acid sequence as shown in SEQ ID NO: 21, and the cleavable peptide comprises an amino acid sequence as shown in SEQ ID NO: 27 and the IL-15Rα domain comprises an amino acid sequence as shown in SEQ ID NO: 56.

In some embodiments, the IL-15 cytokine or functional fragment thereof comprises the amino acid sequence of SEQ ID NO: 131 and the masking moiety comprises the amino acid sequence of SEQ ID NO: 12 and the first half-life extension domain comprises SEQ ID NO: 63 (Y349C; T366S; L38A; Y407V; and N297A) and the second half-life extension domain comprises SEQ ID NO 64 (S354C, T366W and N297A) and the non-cleavable linker comprises an amino acid sequence as shown in SEQ ID NO: 21, and the cleavable peptide comprises an amino acid sequence as shown in SEQ ID NO: 27 and the IL-15Rα domain comprises an amino acid sequence as shown in SEQ ID NO: 56.

In some embodiments, the IL-15 cytokine or functional fragment thereof comprises the amino acid sequence of SEQ ID NO: 132 and the masking moiety comprises the amino acid sequence of SEQ ID NO: 12 and the first half-life extension domain comprises SEQ ID NO: 63 (Y349C; T366S; L38A; Y407V; and N297A) and the second half-life extension domain comprises SEQ ID NO 64 (S354C, T366W and N297A) and the non-cleavable linker comprises an amino acid sequence as shown in SEQ ID NO: 21, and the

cleavable peptide comprises an amino acid sequence as shown in SEQ ID NO: 27 and the IL-15Rα domain comprises an amino acid sequence as shown in SEQ ID NO: 56.

In some embodiments, the IL-15 cytokine or functional fragment thereof comprises the amino acid sequence of SEQ ID NO: 133 and the masking moiety comprises the amino acid sequence of SEQ ID NO: 12 and the first half-life extension domain comprises SEQ ID NO: 63 (Y349C; T366S; L38A; Y407V; and N297A) and the second half-life extension domain comprises SEQ ID NO 64 (S354C, T366W and N297A) and the non-cleavable linker comprises an amino acid sequence as shown in SEQ ID NO: 21, and the cleavable peptide comprises an amino acid sequence as shown in SEQ ID NO: 27 and the IL-15Rα domain comprises an amino acid sequence as shown in SEQ ID NO: 56.

In some embodiments, the IL-15 cytokine or functional fragment thereof comprises the amino acid sequence of SEQ ID NO: 127 and the masking moiety comprises the amino acid sequence of SEQ ID NO: 12 and the first half-life extension domain comprises SEQ ID NO: 63 (Y349C; T366S; L38A; Y407V; and N297A) and the second half-life extension domain comprises SEQ ID NO 64 (S354C, T366W and N297A) and the non-cleavable linker comprises an amino acid sequence as shown in SEQ ID NO: 21, and the cleavable peptide comprises an amino acid sequence as shown in SEQ ID NO: 34 and the IL-15Rα domain comprises an amino acid sequence as shown in SEQ ID NO: 56.

In some embodiments, the IL-15 cytokine or functional fragment thereof comprises the amino acid sequence of SEQ ID NO: 128 and the masking moiety comprises the amino acid sequence of SEQ ID NO: 12 and the first half-life extension domain comprises SEQ ID NO: 63 (Y349C; T366S; L38A; Y407V; and N297A) and the second half-life extension domain comprises SEQ ID NO 64 (S354C, T366W and N297A) and the non-cleavable linker comprises an amino acid sequence as shown in SEQ ID NO: 21, and the cleavable peptide comprises an amino acid sequence as shown in SEQ ID NO: 34 and the IL-15Rα domain comprises an amino acid sequence as shown in SEQ ID NO: 56.

In some embodiments, the IL-15 cytokine or functional fragment thereof comprises the amino acid sequence of SEQ ID NO: 129 and the masking moiety comprises the amino acid sequence of SEQ ID NO: 12 and the first half-life extension domain comprises SEQ ID NO: 63 (Y349C; T366S; L38A; Y407V; and N297A) and the second half-life extension domain comprises SEQ ID NO 64 (S354C, T366W and N297A) and the non-cleavable linker comprises an amino acid sequence as shown in SEQ ID NO: 21, and the cleavable peptide comprises an amino acid sequence as shown in SEQ ID NO: 34 and the IL-15Rα domain comprises an amino acid sequence as shown in SEQ ID NO: 56.

In some embodiments, the IL-15 cytokine or functional fragment thereof comprises the amino acid sequence of SEQ ID NO: 130 and the masking moiety comprises the amino acid sequence of SEQ ID NO: 12 and the first half-life extension domain comprises SEQ ID NO: 63 (Y349C; T366S; L38A; Y407V; and

N297A) and the second half-life extension domain comprises SEQ ID NO 64 (S354C, T366W and N297A) and the non-cleavable linker comprises an amino acid sequence as shown in SEQ ID NO: 21, and the cleavable peptide comprises an amino acid sequence as shown in SEQ ID NO: 34 and the IL-15Rα domain comprises an amino acid sequence as shown in SEQ ID NO: 56.

In some embodiments, the IL-15 cytokine or functional fragment thereof comprises the amino acid sequence of SEQ ID NO: 131 and the masking moiety comprises the amino acid sequence of SEQ ID NO: 12 and the first half-life extension domain comprises SEQ ID NO: 63 (Y349C; T366S; L38A; Y407V; and N297A) and the second half-life extension domain comprises SEQ ID NO 64 (S354C, T366W and N297A) and the non-cleavable linker comprises an amino acid sequence as shown in SEQ ID NO: 21, and the cleavable peptide comprises an amino acid sequence as shown in SEQ ID NO: 34 and the IL-15Rα domain comprises an amino acid sequence as shown in SEQ ID NO: 56.

In some embodiments, the IL-15 cytokine or functional fragment thereof comprises the amino acid sequence of SEQ ID NO: 132 and the masking moiety comprises the amino acid sequence of SEQ ID NO: 12 and the first half-life extension domain comprises SEQ ID NO: 63 (Y349C; T366S; L38A; Y407V; and N297A) and the second half-life extension domain comprises SEQ ID NO 64 (S354C, T366W and N297A) and the non-cleavable linker comprises an amino acid sequence as shown in SEQ ID NO: 21, and the cleavable peptide comprises an amino acid sequence as shown in SEQ ID NO: 34 and the IL-15Rα domain comprises an amino acid sequence as shown in SEQ ID NO: 56.

In some embodiments, the IL-15 cytokine or functional fragment thereof comprises the amino acid sequence of SEQ ID NO: 133 and the masking moiety comprises the amino acid sequence of SEQ ID NO: 12 and the first half-life extension domain comprises SEQ ID NO: 63 (Y349C; T366S; L38A; Y407V; and N297A) and the second half-life extension domain comprises SEQ ID NO 64 (S354C, T366W and N297A) and the non-cleavable linker comprises an amino acid sequence as shown in SEQ ID NO: 21, and the cleavable peptide comprises an amino acid sequence as shown in SEQ ID NO: 34 and the IL-15Rα domain comprises an amino acid sequence as shown in SEQ ID NO: 56.

In some embodiments, the IL-15 cytokine or functional fragment thereof comprises the amino acid sequence of SEQ ID NO: 127 and the masking moiety comprises the amino acid sequence of SEQ ID NO: 12 and the first half-life extension domain comprises SEQ ID NO: 63 (Y349C; T366S; L38A; Y407V; and N297A) and the second half-life extension domain comprises SEQ ID NO 64 (S354C, T366W and N297A) and the non-cleavable linker comprises an amino acid sequence as shown in SEQ ID NO: 21 and the cleavable peptide comprises an amino acid sequence as shown in SEQ ID NO: 33, and the IL-15Rα domain comprises an amino acid sequence as shown in SEQ ID NO: 56.

In some embodiments, the IL-15 cytokine or functional fragment thereof comprises the amino acid sequence of SEQ ID NO: 128 and the masking moiety comprises the amino acid sequence of SEQ ID NO: 12 and the first half-life extension domain comprises SEQ ID NO: 63 (Y349C; T366S; L38A; Y407V; and N297A) and the second half-life extension domain comprises SEQ ID NO 64 (S354C, T366W and N297A) and the non-cleavable linker comprises an amino acid sequence as shown in SEQ ID NO: 21 and the cleavable peptide comprises an amino acid sequence as shown in SEQ ID NO: 33, and the IL-15Rα domain comprises an amino acid sequence as shown in SEQ ID NO: 56.

In some embodiments, the IL-15 cytokine or functional fragment thereof comprises the amino acid sequence of SEQ ID NO: 129 and the masking moiety comprises the amino acid sequence of SEQ ID NO: 12 and the first half-life extension domain comprises SEQ ID NO: 63 (Y349C; T366S; L38A; Y407V; and N297A) and the second half-life extension domain comprises SEQ ID NO 64 (S354C, T366W and N297A) and the non-cleavable linker comprises an amino acid sequence as shown in SEQ ID NO: 21 and the cleavable peptide comprises an amino acid sequence as shown in SEQ ID NO: 33, and the IL-15Rα domain comprises an amino acid sequence as shown in SEQ ID NO: 56.

In some embodiments, the IL-15 cytokine or functional fragment thereof comprises the amino acid sequence of SEQ ID NO: 130 and the masking moiety comprises the amino acid sequence of SEQ ID NO: 12 and the first half-life extension domain comprises SEQ ID NO: 63 (Y349C; T366S; L38A; Y407V; and N297A) and the second half-life extension domain comprises SEQ ID NO 64 (S354C, T366W and N297A) and the non-cleavable linker comprises an amino acid sequence as shown in SEQ ID NO: 21 and the cleavable peptide comprises an amino acid sequence as shown in SEQ ID NO: 33, and the IL-15Rα domain comprises an amino acid sequence as shown in SEQ ID NO: 56.

In some embodiments, the IL-15 cytokine or functional fragment thereof comprises the amino acid sequence of SEQ ID NO: 131 and the masking moiety comprises the amino acid sequence of SEQ ID NO: 12 and the first half-life extension domain comprises SEQ ID NO: 63 (Y349C; T366S; L38A; Y407V; and N297A) and the second half-life extension domain comprises SEQ ID NO 64 (S354C, T366W and N297A) and the non-cleavable linker comprises an amino acid sequence as shown in SEQ ID NO: 21 and the cleavable peptide comprises an amino acid sequence as shown in SEQ ID NO: 33, and the IL-15Rα domain comprises an amino acid sequence as shown in SEQ ID NO: 56.

In some embodiments, the IL-15 cytokine or functional fragment thereof comprises the amino acid sequence of SEQ ID NO: 132 and the masking moiety comprises the amino acid sequence of SEQ ID NO: 12 and the first half-life extension domain comprises SEQ ID NO: 63 (Y349C; T366S; L38A; Y407V; and N297A) and the second half-life extension domain comprises SEQ ID NO 64 (S354C, T366W and N297A) and the non-cleavable linker comprises an amino acid sequence as shown in SEQ ID NO: 21 and the

cleavable peptide comprises an amino acid sequence as shown in SEQ ID NO: 33, and the IL-15Rα domain comprises an amino acid sequence as shown in SEQ ID NO: 56.

In some embodiments, the IL-15 cytokine or functional fragment thereof comprises the amino acid sequence of SEQ ID NO: 133 and the masking moiety comprises the amino acid sequence of SEQ ID NO: 12 and the first half-life extension domain comprises SEQ ID NO: 63 (Y349C; T366S; L38A; Y407V; and N297A) and the second half-life extension domain comprises SEQ ID NO 64 (S354C, T366W and N297A) and the non-cleavable linker comprises an amino acid sequence as shown in SEQ ID NO: 21 and the cleavable peptide comprises an amino acid sequence as shown in SEQ ID NO: 33, and the IL-15Rα domain comprises an amino acid sequence as shown in SEQ ID NO: 56.

In some embodiments, the IL-15 cytokine or functional fragment thereof comprises the amino acid sequence of SEQ ID NO: 127 and the masking moiety comprises the amino acid sequence of SEQ ID NO: 12 and the first half-life extension domain comprises SEQ ID NO: 63 (Y349C; T366S; L38A; Y407V; and N297A) and the second half-life extension domain comprises SEQ ID NO 64 (S354C, T366W and N297A) and the non-cleavable linker comprises an amino acid sequence as shown in SEQ ID NO: 21 and the cleavable peptide comprises an amino acid sequence as shown in SEQ ID NO: 134 and the IL-15Rα domain comprises an amino acid sequence as shown in SEQ ID NO: 56.

In some embodiments, the IL-15 cytokine or functional fragment thereof comprises the amino acid sequence of SEQ ID NO: 128 and the masking moiety comprises the amino acid sequence of SEQ ID NO: 12 and the first half-life extension domain comprises SEQ ID NO: 63 (Y349C; T366S; L38A; Y407V; and N297A) and the second half-life extension domain comprises SEQ ID NO 64 (S354C, T366W and N297A) and the non-cleavable linker comprises an amino acid sequence as shown in SEQ ID NO: 21 and the cleavable peptide comprises an amino acid sequence as shown in SEQ ID NO: 134 and the IL-15Rα domain comprises an amino acid sequence as shown in SEQ ID NO: 56.

In some embodiments, the IL-15 cytokine or functional fragment thereof comprises the amino acid sequence of SEQ ID NO: 129 and the masking moiety comprises the amino acid sequence of SEQ ID NO: 12 and the first half-life extension domain comprises SEQ ID NO: 63 (Y349C; T366S; L38A; Y407V; and N297A) and the second half-life extension domain comprises SEQ ID NO 64 (S354C, T366W and N297A) and the non-cleavable linker comprises an amino acid sequence as shown in SEQ ID NO: 21 and the cleavable peptide comprises an amino acid sequence as shown in SEQ ID NO: 134 and the IL-15Rα domain comprises an amino acid sequence as shown in SEQ ID NO: 56.

In some embodiments, the IL-15 cytokine or functional fragment thereof comprises the amino acid sequence of SEQ ID NO: 130 and the masking moiety comprises the amino acid sequence of SEQ ID NO:

12 and the first half-life extension domain comprises SEQ ID NO: 63 (Y349C; T366S; L38A; Y407V; and N297A) and the second half-life extension domain comprises SEQ ID NO 64 (S354C, T366W and N297A) and the non-cleavable linker comprises an amino acid sequence as shown in SEQ ID NO: 21 and the cleavable peptide comprises an amino acid sequence as shown in SEQ ID NO: 134 and the IL-15Rα domain comprises an amino acid sequence as shown in SEQ ID NO: 56.

In some embodiments, the IL-15 cytokine or functional fragment thereof comprises the amino acid sequence of SEQ ID NO: 131 and the masking moiety comprises the amino acid sequence of SEQ ID NO: 12 and the first half-life extension domain comprises SEQ ID NO: 63 (Y349C; T366S; L38A; Y407V; and N297A) and the second half-life extension domain comprises SEQ ID NO 64 (S354C, T366W and N297A) and the non-cleavable linker comprises an amino acid sequence as shown in SEQ ID NO: 21 and the cleavable peptide comprises an amino acid sequence as shown in SEQ ID NO: 134 and the IL-15Rα domain comprises an amino acid sequence as shown in SEQ ID NO: 56.

In some embodiments, the IL-15 cytokine or functional fragment thereof comprises the amino acid sequence of SEQ ID NO: 132 and the masking moiety comprises the amino acid sequence of SEQ ID NO: 12 and the first half-life extension domain comprises SEQ ID NO: 63 (Y349C; T366S; L38A; Y407V; and N297A) and the second half-life extension domain comprises SEQ ID NO 64 (S354C, T366W and N297A) and the non-cleavable linker comprises an amino acid sequence as shown in SEQ ID NO: 21 and the cleavable peptide comprises an amino acid sequence as shown in SEQ ID NO: 134 and the IL-15Rα domain comprises an amino acid sequence as shown in SEQ ID NO: 56.

In some embodiments, the IL-15 cytokine or functional fragment thereof comprises the amino acid sequence of SEQ ID NO: 133 and the masking moiety comprises the amino acid sequence of SEQ ID NO: 12 and the first half-life extension domain comprises SEQ ID NO: 63 (Y349C; T366S; L38A; Y407V; and N297A) and the second half-life extension domain comprises SEQ ID NO 64 (S354C, T366W and N297A) and the non-cleavable linker comprises an amino acid sequence as shown in SEQ ID NO: 21 and the cleavable peptide comprises an amino acid sequence as shown in SEQ ID NO: 134 and the IL-15Rα domain comprises an amino acid sequence as shown in SEQ ID NO: 56.

### 1.9 Masked cytokine with variant masking moiety

An IL-15Rβ polypeptide, or a functional fragment thereof, is provided herein, where the IL-15Rβ polypeptide has an amino acid substitution at position C122.

An IL-15Rβ polypeptide, or a functional fragment thereof, is provided herein, where the IL-15Rβ polypeptide has amino acid substitution C122S.

An IL-15Rβ polypeptide, or a functional fragment thereof, is provided herein, where the IL-15Rβ polypeptide has an amino acid substitution at position C122 as compared to IL-15Rβ of SEQ ID NO: 11.

An IL-15Rβ polypeptide, or a functional fragment thereof, is provided herein, where the IL-15Rβ polypeptide has amino acid substitution C122S as compared to IL-15Rβ of SEQ ID NO: 11.

An IL-15Rβ polypeptide is provided herein, comprising the amino acid sequence of SEQ ID NO: 11 with a C122 mutation.

An IL-15Rβ polypeptide is provided herein, comprising the amino acid sequence of SEQ ID NO: 11 with a C122S mutation.

An IL-15Rβ polypeptide, or a functional fragment thereof, is provided herein, where the IL-15Rβ polypeptide has an amino acid substitution at position C168.

An IL-15Rβ polypeptide, or a functional fragment thereof, is provided herein, where the IL-15Rβ polypeptide has amino acid substitution C168S.

An IL-15Rβ polypeptide, or a functional fragment thereof, is provided herein, where the IL-15Rβ polypeptide has an amino acid substitution at position C168 as compared to IL-15Rβ of SEQ ID NO: 11.

An IL-15Rβ polypeptide, or a functional fragment thereof, is provided herein, where the IL-15Rβ polypeptide has amino acid substitution C168S as compared to IL-15Rβ of SEQ ID NO: 11.

An IL-15Rβ polypeptide is provided herein, comprising the amino acid sequence of SEQ ID NO: 11 with a C168 mutation.

An IL-15Rβ polypeptide is provided herein, comprising the amino acid sequence of SEQ ID NO: 11 with a C68S mutation.

An IL-15Rβ polypeptide, or a functional fragment thereof, is provided herein, where the IL-15Rβ polypeptide has amino acid substitutions at positions C122 and C168.

An IL-15Rβ polypeptide, or a functional fragment thereof, is provided herein, where the IL-15Rβ polypeptide has amino acid substitutions C122S and C168S.

An IL-15Rβ polypeptide, or a functional fragment thereof, is provided herein, where the IL-15Rβ polypeptide has amino acid substitutions at positions C122 and C168 as compared to IL-15Rβ of SEQ ID NO: 11.

An IL-15Rβ polypeptide, or a functional fragment thereof, is provided herein, where the IL-15Rβ polypeptide has amino acid substitutions C122S and C168S as compared to IL-15Rβ of SEQ ID NO: 11.

An IL-15Rβ polypeptide, or a functional fragment thereof, is provided herein, where the IL-15Rβ polypeptide comprises an amino acid of SEQ ID NO: 12.

A masked cytokine comprising a masking moiety and an IL-15 cytokine or functional fragment thereof, is provided herein, wherein the masking moiety masks the IL-15 cytokine or functional fragment thereof thereby reducing or preventing binding of the IL-15 cytokine or functional fragment thereof to its cognate receptor, and where a proteolytically cleavable peptide is present between the IL-15 cytokine or functional fragment thereof and the masking moiety, and the masking moiety is an IL-15Rβ polypeptide, or a functional fragment thereof, as defined anywhere herein.

### 1.10 Masked cytokine with variant half-life extension domain

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: 65 and a second polypeptide sequence comprising SEQ ID NO: 66.

A masked cytokine comprising a masking moiety, an IL-15 cytokine or functional fragment thereof, and a half life extension domain is provided herein wherein the masking moiety masks the IL-15 cytokine or functional fragment thereof thereby reducing or preventing binding of the IL-15 cytokine or functional fragment thereof to its cognate receptor, and where a proteolytically cleavable peptide is present between the IL-15 cytokine or functional fragment thereof and the masking moiety, and half life extension domain comprises dimerized IgG1 Fc domains, as defined in anywhere herein.

## 2. CLEAVAGE PRODUCT

Provided herein is a cleavage product of a masked IL-15 cytokine described herein.

The masked IL-15 cytokines described herein comprise a cleavable linker. Upon proteolytic cleavage of the cleavable linker at the cleavage site, a cleavage product comprising the IL-15 cytokine or functional fragment thereof is formed. The IL-15 cytokine or functional fragment thereof in the cleavage product is activated since it is no longer masked by the masking moiety. The IL-15 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-15 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 an IL-15 cleavage product capable of binding to its cognate receptor, the cleavage product comprising an IL-15 cytokine or functional fragment thereof, preparable by proteolytic cleavage of the cleavable peptide in the masked IL-15 cytokine as defined in of any one of the statements or embodiments described herein.

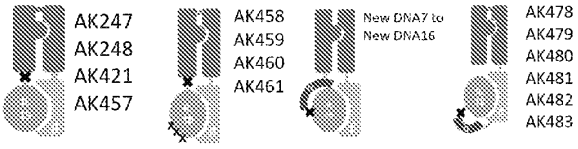
Also provided herein is a cleavage product of a masked IL-15 cytokine, where the cleavage product is capable of binding to IL-15R, the cleavage product comprising an IL-15 cytokine or functional fragment thereof as defined anywhere herein. Also provided herein is a distribution of cleavage products obtained or obtainable from a single structure of a masked IL-15 cytokine, where each cleavage product within the distribution of cleavage products (i) is capable of binding to IL-15R and (ii) comprises an IL-15 cytokine or functional fragment thereof as defined anywhere herein.

Provided herein is a cleavage product of a masked IL-15 cytokine as described herein, where the cleavage product is capable of binding to its cognate receptor, the cleavage product comprising a polypeptide comprising:

**PCP-SD-C**

wherein PCP is a portion of a proteolytically cleavable peptide as described herein; SD is a spacer domain as described herein; and C is an IL-15 cytokine or functional fragment thereof as described herein.

For example, such a cleavage product might be derived from masked cytokines:




In some embodiments, the cleavage product has an amino acid sequence with at least 90% homology to the mature IL-15 of SEQ ID NO: 2. In some embodiments, the cleavage product has an amino acid sequence with at least 90% homology to the mature IL-15 of SEQ ID NO: 3. In some embodiments, the cleavage product has an amino acid sequence with at least 90% homology to the mature IL-15 of SEQ ID NO: 4. In some embodiments, the cleavage product has an amino acid sequence with at least 90% homology to the mature IL-15 of SEQ ID NO: 5. In some embodiments, the cleavage product has an amino acid sequence with at least 90% homology to the mature IL-15 of SEQ ID NO: 8. In some embodiments, the cleavage product has an amino acid sequence with at least 90% homology to the mature IL-15 of SEQ ID NO: 127. In some embodiments, the cleavage product has an amino acid sequence with at least 90% homology to the mature IL-15 of SEQ ID NO: 128. In some embodiments, the cleavage product has an amino acid sequence with at least 90% homology to the mature IL-15 of SEQ ID NO: 129. In some embodiments, the cleavage product has an amino acid sequence with at least 90% homology to the mature IL-15 of SEQ ID NO: 130. In some embodiments, the cleavage product has an amino acid sequence with at least 90% homology to the mature IL-15 of SEQ ID NO: 131. In some embodiments, the cleavage product has an amino acid sequence with at least 90% homology to the mature IL-15 of SEQ ID NO: 132. In some embodiments, the cleavage product has an amino acid sequence with at least 90% homology to the mature IL-15 of SEQ ID NO: 133.

In some embodiments, the cleavage product further comprises a domain comprising an IL-15Rα subunit or a fragment thereof ('IL-15Rα domain') as described herein. In some embodiments, the IL-15Rα domain comprises an IL-15Rα subunit or a fragment thereof and an 'α subunit linker' as described herein.

In some embodiments, the domain comprising an IL-15Rα subunit or fragment thereof is covalently attached to the IL-15 cytokine or fragment thereof as described herein.

In some embodiments, the cleavage product comprises the amino acid sequence of SEQ ID NO: 104.
In some embodiments, the cleavage product comprises the amino acid sequence of SEQ ID NO: 105.
In some embodiments, the cleavage product comprises the amino acid sequence of SEQ ID NO: 106.
In some embodiments, the cleavage product comprises the amino acid sequence of SEQ ID NO: 107.
In some embodiments, the cleavage product comprises the amino acid sequence of SEQ ID NO: 117.
In some embodiments, the cleavage product comprises the amino acid sequence of SEQ ID NO: 118.
In some embodiments, the cleavage product comprises the amino acid sequence of SEQ ID NO: 119.
In some embodiments, the cleavage product comprises the amino acid sequence of SEQ ID NO: 120.
In some embodiments, the cleavage product comprises the amino acid sequence of SEQ ID NO: 121.
In some embodiments, the cleavage product comprises the amino acid sequence of SEQ ID NO: 122.
In some embodiments, the cleavage product comprises the amino acid sequence of SEQ ID NO: 307.

In some embodiments, the cleavage product consists of the amino acid sequence of SEQ ID NO: 104.
In some embodiments, the cleavage product consists of the amino acid sequence of SEQ ID NO: 105.
In some embodiments, the cleavage product consists of the amino acid sequence of SEQ ID NO: 106.
In some embodiments, the cleavage product consists of the amino acid sequence of SEQ ID NO: 107.
In some embodiments, the cleavage product consists of the amino acid sequence of SEQ ID NO: 117.
In some embodiments, the cleavage product consists of the amino acid sequence of SEQ ID NO: 118.
In some embodiments, the cleavage product consists of the amino acid sequence of SEQ ID NO: 119.
In some embodiments, the cleavage product consists of the amino acid sequence of SEQ ID NO: 120.
In some embodiments, the cleavage product consists of the amino acid sequence of SEQ ID NO: 121.
In some embodiments, the cleavage product consists of the amino acid sequence of SEQ ID NO: 122.
In some embodiments, the cleavage product consists of the amino acid sequence of SEQ ID NO: 307.

In some embodiments, the cleavage product comprises:

**PCP-SD-['IL-15Rα domain']-C**

For example, such a cleavage product might be derived from masked cytokine:




In some embodiments, the cleavage product comprises the amino acid sequence of SEQ ID NO: 116.
In some embodiments, the cleavage product consists of the amino acid sequence of SEQ ID NO: 116.

In some embodiments, the cleavage product comprises:

**PCP-SD-C-['IL-15Rα domain']**

For example, such a cleavage product might be derived from masked cytokine:




In some embodiments, the cleavage product comprises the amino acid sequence of SEQ ID NO: 108.
In some embodiments, the cleavage product comprises the amino acid sequence of SEQ ID NO: 109.
In some embodiments, the cleavage product comprises the amino acid sequence of SEQ ID NO: 110.
In some embodiments, the cleavage product comprises the amino acid sequence of SEQ ID NO: 111.
In some embodiments, the cleavage product comprises the amino acid sequence of SEQ ID NO: 112.
In some embodiments, the cleavage product comprises the amino acid sequence of SEQ ID NO: 113.
In some embodiments, the cleavage product comprises the amino acid sequence of SEQ ID NO: 114.
In some embodiments, the cleavage product comprises the amino acid sequence of SEQ ID NO: 115.
In some embodiments, the cleavage product comprises the amino acid sequence of SEQ ID NO: 126.

In some embodiments, the cleavage product consists of the amino acid sequence of SEQ ID NO: 108.
In some embodiments, the cleavage product consists of the amino acid sequence of SEQ ID NO: 109.
In some embodiments, the cleavage product consists of the amino acid sequence of SEQ ID NO: 110.
In some embodiments, the cleavage product consists of the amino acid sequence of SEQ ID NO: 111.
In some embodiments, the cleavage product consists of the amino acid sequence of SEQ ID NO: 112.
In some embodiments, the cleavage product consists of the amino acid sequence of SEQ ID NO: 113.
In some embodiments, the cleavage product consists of the amino acid sequence of SEQ ID NO: 114.
In some embodiments, the cleavage product consists of the amino acid sequence of SEQ ID NO: 115.
In some embodiments, the cleavage product consists of the amino acid sequence of SEQ ID NO: 126.

In some embodiments, the cleavage product comprises:

**PCP–SD-[C(-['IL-15Rα domain'])]**

In some embodiments, the cleavage product comprises:

**PCP-SD2 – [N' terminal domain of C]-['IL-15Rα domain']-[C' terminal domain of C]**

where the IL-15Rα domain as described herein comprises an IL-15Rα subunit or a fragment thereof flanked on either side by a non-cleavable 'α subunit linker'.

For example, such a cleavage product might be derived from masked cytokine:




In some embodiments, the cleavage product comprises the amino acid sequence of SEQ ID NO: 123.

In some embodiments, the cleavage product comprises the amino acid sequence of SEQ ID NO: 124.

In some embodiments, the cleavage product consists of the amino acid sequence of SEQ ID NO: 123.
In some embodiments, the cleavage product consists of the amino acid sequence of SEQ ID NO: 124.

In some embodiments, the domain comprising an IL-15Rα subunit or fragment thereof is non-covalently bonded to the IL-15 cytokine or fragment thereof as described herein. Thus, in some embodiments, the cleavage product comprises a polypeptide comprising:

**PCP-SD-C**

wherein PCP is a portion of a proteolytically cleavable peptide as described herein; SD is a spacer domain as described herein; and C is an IL-15 cytokine or functional fragment thereof as described herein, and a polypeptide chain comprising:

**IL-15Rα domain**

For example, such a cleavage product might be derived from masked cytokine:



In some embodiments, the cleavage product comprises the amino acid sequence of SEQ ID NO: 136.
In some embodiments, the cleavage product comprises the amino acid sequence of SEQ ID NO: 137.
In some embodiments, the cleavage product comprises the amino acid sequence of SEQ ID NO: 138.
In some embodiments, the cleavage product comprises the amino acid sequence of SEQ ID NO: 142.

In some embodiments, the cleavage product comprises the amino acid sequence of SEQ ID NO: 144.
In some embodiments, the cleavage product comprises the amino acid sequence of SEQ ID NO: 145.

In some embodiments, the cleavage product comprises a first polypeptide chain having an amino acid sequence of SEQ ID NO: 200 and a second polypeptide chain having an amino acid sequence of SEQ ID NO: 199.

In some embodiments, the cleavage product comprises a first polypeptide chain having an amino acid sequence of SEQ ID NO: 202 and a second polypeptide chain having an amino acid sequence of SEQ ID NO: 201.

In some embodiments, the cleavage product comprises a first polypeptide chain having an amino acid sequence of SEQ ID NO: 204 and a second polypeptide chain having an amino acid sequence of SEQ ID NO: 203.

In some embodiments, the cleavage product comprises a first polypeptide chain having an amino acid sequence of SEQ ID NO: 215 and a second polypeptide chain having an amino acid sequence of SEQ ID NO: 214.

In some embodiments, the cleavage product comprises a first polypeptide chain having an amino acid sequence of SEQ ID NO: 220 and a second polypeptide chain having an amino acid sequence of SEQ ID NO: 219.

In some embodiments, the cleavage product comprises a first polypeptide chain having an amino acid sequence of SEQ ID NO: 222 and a second polypeptide chain having an amino acid sequence of SEQ ID NO: 221.

In some embodiments, the cleavage product consists of the amino acid sequence of SEQ ID NO: 136.
In some embodiments, the cleavage product consists of the amino acid sequence of SEQ ID NO: 137.
In some embodiments, the cleavage product consists of the amino acid sequence of SEQ ID NO: 138.
In some embodiments, the cleavage product consists of the amino acid sequence of SEQ ID NO: 142.
In some embodiments, the cleavage product consists of the amino acid sequence of SEQ ID NO: 144.
In some embodiments, the cleavage product consists of the amino acid sequence of SEQ ID NO: 145.

In some embodiments, the cleavage product consists of a first polypeptide chain having an amino acid sequence of SEQ ID NO: 200 and a second polypeptide chain having an amino acid sequence of SEQ ID NO: 199.

In some embodiments, the cleavage product consists of a first polypeptide chain having an amino acid sequence of SEQ ID NO: 202 and a second polypeptide chain having an amino acid sequence of SEQ ID NO: 201.

In some embodiments, the cleavage product consists of a first polypeptide chain having an amino acid sequence of SEQ ID NO: 204 and a second polypeptide chain having an amino acid sequence of SEQ ID NO: 203.

In some embodiments, the cleavage product consists of a first polypeptide chain having an amino acid sequence of SEQ ID NO: 215 and a second polypeptide chain having an amino acid sequence of SEQ ID NO: 214.

In some embodiments, the cleavage product consists of a first polypeptide chain having an amino acid sequence of SEQ ID NO: 220 and a second polypeptide chain having an amino acid sequence of SEQ ID NO: 219.

In some embodiments, the cleavage product consists of a first polypeptide chain having an amino acid sequence of SEQ ID NO: 222 and a second polypeptide chain having an amino acid sequence of SEQ ID NO: 221.

It will be understood that for the above embodiments, the first polypeptide chain of the cleavage product is produced from the second polypeptide chain of the masked IL-15 cytokine and the second polypeptide chain of the cleavage product is the third polypeptide chain of the masked IL-15 cytokine.

Further provided herein is a cleavage product of a masked IL-15 cytokine, where the cleavage product is capable of binding to IL-15R, 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-15 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-15 cytokine, where each cleavage product within the distribution of cleavage products (i) is capable of binding to IL-15R 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-15 cytokine or functional fragment thereof; and wherein the first half-life extension domain is associated with the second half-life extension domain.

Provided herein is a cleavage product of a masked IL-15 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:

**HL1-SD-PCP**

wherein HL1 is a first half-life extension domain as described herein; SD is a spacer domain as described herein; and PCP is a portion of a proteolytically cleavable peptide as described herein; and a second polypeptide chain comprising a polypeptide comprising:

**HL2-L2-C**

wherein HL2 is a second half-life extension domain as described herein; L2 is a non-cleavable linker as described herein; and C is an IL-15 cytokine or functional fragment thereof as described herein; and wherein the first half-life extension domain is associated with the second half-life extension domain.

For example, such a cleavage product might be derived from masked cytokine:




In some embodiments, the cleavage product comprises the amino acid sequence of SEQ ID NO: 125.

In some embodiments, the cleavage product consists of the amino acid sequence of SEQ ID NO: 125.

Provided herein is a cleavage product of a masked IL-15 cytokine, where the cleavage product is capable of binding to its cognate receptor, the cleavage product comprising a first polypeptide chain comprising:

**HL1-SD-PCP**

wherein HL1 is a first half-life extension domain as described herein; SD is a spacer domain as described herein; and PCP is a portion of a proteolytically cleavable peptide as described herein; and a second polypeptide chain comprising a polypeptide comprising:

**HL2-L2-C**

wherein HL2 is a second half-life extension domain as described herein; L2 is a linker as described herein; and C is an IL-15 cytokine or functional fragment thereof as described herein; and wherein the first half-life extension domain is associated with the second half-life extension domain. and a third polypeptide chain comprising:

**IL-15Rα domain**

For example, such a cleavage product might be derived from masked cytokine:



In some embodiments, the cleavage product comprises the amino acid sequence of SEQ ID NO: 139.

In some embodiments, the cleavage product comprises the amino acid sequence of SEQ ID NO: 140.

In some embodiments, the cleavage product comprises the amino acid sequence of SEQ ID NO: 141.

In some embodiments, the cleavage product comprises the amino acid sequence of SEQ ID NO: 143.

In some embodiments, the cleavage product comprises the amino acid sequence of SEQ ID NO: 146.
In some embodiments, the cleavage product comprises the amino acid sequence of SEQ ID NO: 147.

In some embodiments, the cleavage product comprises a first polypeptide chain having an amino acid sequence of SEQ ID NO: 207, a second polypeptide chain having an amino acid sequence of SEQ ID NO: 206 and a third polypeptide chain having an amino acid sequence of SEQ ID NO: 205.
In some embodiments, the cleavage product comprises a first polypeptide chain having an amino acid sequence of SEQ ID NO: 210, a second polypeptide chain having an amino acid sequence of SEQ ID NO: 209 and a third polypeptide chain having an amino acid sequence of SEQ ID NO: 208.
In some embodiments, the cleavage product comprises a first polypeptide chain having an amino acid sequence of SEQ ID NO: 213, a second polypeptide chain having an amino acid sequence of SEQ ID NO: 212 and a third polypeptide chain having an amino acid sequence of SEQ ID NO: 211.
In some embodiments, the cleavage product comprises a first polypeptide chain having an amino acid sequence of SEQ ID NO: 218, a second polypeptide chain having an amino acid sequence of SEQ ID NO: 217 and a third polypeptide chain having an amino acid sequence of SEQ ID NO: 216.
In some embodiments, the cleavage product comprises a first polypeptide chain having an amino acid sequence of SEQ ID NO: 225, a second polypeptide chain having an amino acid sequence of SEQ ID NO: 224 and a third polypeptide chain having an amino acid sequence of SEQ ID NO: 223.
In some embodiments, the cleavage product comprises a first polypeptide chain having an amino acid sequence of SEQ ID NO: 228, a second polypeptide chain having an amino acid sequence of SEQ ID NO: 227 and a third polypeptide chain having an amino acid sequence of SEQ ID NO: 226.

In some embodiments, the cleavage product consists of the amino acid sequence of SEQ ID NO: 139.
In some embodiments, the cleavage product consists of the amino acid sequence of SEQ ID NO: 140.
In some embodiments, the cleavage product consists of the amino acid sequence of SEQ ID NO: 141.
In some embodiments, the cleavage product consists of the amino acid sequence of SEQ ID NO: 143.
In some embodiments, the cleavage product comprises the amino acid sequence of SEQ ID NO: 146.
In some embodiments, the cleavage product comprises the amino acid sequence of SEQ ID NO: 147.

In some embodiments, the cleavage product consists of a first polypeptide chain having an amino acid sequence of SEQ ID NO: 207, a second polypeptide chain having an amino acid sequence of SEQ ID NO: 206 and a third polypeptide chain having an amino acid sequence of SEQ ID NO: 205.
In some embodiments, the cleavage product consists of a first polypeptide chain having an amino acid sequence of SEQ ID NO: 210, a second polypeptide chain having an amino acid sequence of SEQ ID NO: 209 and a third polypeptide chain having an amino acid sequence of SEQ ID NO: 208.
In some embodiments, the cleavage product consists of a first polypeptide chain having an amino acid sequence of SEQ ID NO: 213, a second polypeptide chain having an amino acid sequence of SEQ ID NO: 212 and a third polypeptide chain having an amino acid sequence of SEQ ID NO: 211.

In some embodiments, the cleavage product consists of a first polypeptide chain having an amino acid sequence of SEQ ID NO: 218, a second polypeptide chain having an amino acid sequence of SEQ ID NO: 217 and a third polypeptide chain having an amino acid sequence of SEQ ID NO: 216.

In some embodiments, the cleavage product consists of a first polypeptide chain having an amino acid sequence of SEQ ID NO: 225, a second polypeptide chain having an amino acid sequence of SEQ ID NO: 224 and a third polypeptide chain having an amino acid sequence of SEQ ID NO: 223.

In some embodiments, the cleavage product consists of a first polypeptide chain having an amino acid sequence of SEQ ID NO: 228, a second polypeptide chain having an amino acid sequence of SEQ ID NO: 227 and a third polypeptide chain having an amino acid sequence of SEQ ID NO: 226.

Within the cleavage products, the masking moiety, half-life extension domains, IL-15 cytokine or functional fragment thereof, linkers, spacer 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-15 cytokine.

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

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

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

Cleavage sites for cleavable linkers disclosed herein are disclosed below:

| Sequence |
|---|

| (* indicates a cleavage site within the cleavable peptide) |
|---|
| MPYD*LYHP |
| DSGG*FMLT |
| HEQ*LTV |
| RAAA*VKSP |
| VPLS*LY |
| DLLA*VVAAS |
| 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.

## 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-15 cytokine to the IL-15 cytokine receptor (e.g., IL-15R or a component thereof, such as IL-15Rα, IL-15Rβ, IL-15Rγ, 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-15 cytokine binding, the target protein could be IL-15R (comprising the IL-15Rα, IL-15Rβ, and IL-15Rγ chains), the IL-15Rα chain, the IL-15Rβ chain, or the IL-15Rα/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-15R)/cytokine (e.g., IL-15) 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-15R (comprising the IL-15Rα, IL-15Rβ, and IL-15Rγ chains). In some embodiments, the target protein is IL-15Rα. In some embodiments, the target protein is IL-15Rβ. In some embodiments, the target protein is the IL-15Rα/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 ≤ 1M, ≤150 nM, ≤100 nM, ≤50 nM, ≤ 10 nM, ≤1 nM, ≤0.1 nM, ≤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. In some embodiments, a masked cytokine provided herein that comprises a cleavable peptide in the linker has a dissociation constant (Kd) of ≤1M, ≤150 nM, ≤ 100 nM, ≤50 nM, ≤10 nM, ≤1 nM, ≤0.1 nM, ≤ 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 ≤1M, ≤150 nM, ≤ 100 nM, ≤50 nM, ≤ 10 nM, ≤1 nM, ≤0.1 nM, ≤0.01nM, 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 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 ≥ 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 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-15 polypeptide, the occlusion ratio refers to the ratio of (a) a maximum detected level of target protein (e.g., IL-15R protein) binding to the masked IL-15 polypeptide in the presence of at least one protease capable of cleaving the cleavable peptide of the masked IL-15 polypeptide to (b) a minimum detected level of target protein (e.g., IL-15R protein) binding to the masked IL-15 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-15 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 IL-15 CYTOKINE PRODUCTION

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

### 4.1 Antibody Production

Some embodiments of the masked IL-15 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-15 cytokine provided herein. In some embodiments, the masked cytokine is in the form of a dimer produced by two copies of a masked IL-15 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 IgG1 according to the EU index as in Kabat, but, functionally, the flexible portion of the chain may be considered to include additional residues termed the upper and lower hinge regions, such as from E216 to G237 (Roux et al., 1998 J Immunol 161:4083) and the lower hinge has been referred to as residues 233 to 239 of the Fc region where FcγR binding was generally attributed. Modifications to any of the masked cytokines described herein, can be performed, for example, according to the methods described in US 20150139984A1, which is incorporated herein by reference, as well as by incorporating any of the modifications described therein.

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

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 (ManII). In some of the embodiments herein, the masked cytokine may comprise at least one amino acid substitution in the Fc region that improves ADCC activity.

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

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), Iie (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 orientation: Gly, Pro;

(6) aromatic: Trp, Tyr, Phe.

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

### 4.2 Masked IL-15 Cytokine-Drug Conjugates

The invention also provides masked IL-15 cytokine-drug conjugates (MCDCs) comprising a masked IL-15 cytokine provided herein, which can be any IL-15 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-15 cytokine includes, but is not limited to, a maytansinoid (see U.S. Patent Nos. 5,208,020, 5,416,064 and European Patent EP 0 425 235 B1); an auristatin such as monomethylauristatin drug moieties DE and DF (MMAE and MMAF) (see U.S. Patent Nos. 5,635,483 and 5,780,588, and 7,498,298); a dolastatin; a calicheamicin or derivative thereof (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-15 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-15 cytokine-drug conjugate comprises a masked IL-15 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-15 cytokine-drug conjugate comprises a masked IL-15 cytokine as described herein conjugated to a radioactive atom to form a radioconjugate. A variety of radioactive isotopes are available for the production of radioconjugates. Examples include At211,1131,1125, Y90, Re186, Re188, Sm153, 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-15 cytokine-drug conjugate comprises a masked IL-15 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): e1005324.

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-15 cytokine and a cytotoxic agent may be made using a variety of bifunctional protein coupling agents such as N-succinimidyl-3-(2-pyridyldithio) propionate (SPDP), succinimidyl-4-(N-maleimidomethyl) cyclohexane-1-carboxylate (SMCC), iminothiolane (IT), bifunctional derivatives of imidoesters (such as dimethyl adipimidate HCl), active esters (such as disuccinimidyl suberate), aldehydes (such as glutaraldehyde), bis-azido compounds (such as bis (p- azidobenzoyl) hexanediamine), bis-diazonium derivatives (such as bis-(p-diazoniumbenzoyl)- ethylenediamine), diisocyanates (such as toluene 2,6-diisocyanate), and bis-active fluorine compounds (such as 1,5-difluoro-2,4-dinitrobenzene). For example, a ricin immunotoxin can be prepared as described in Vitetta et ah, Science 238:1098 (1987). Carbon-14-labeled 1-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).

### 4.3 Vectors, Host Cells, and Recombinant Methods

For recombinant production of a IL-15 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-15 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-15 masked cytokine. In some embodiments, more than one vector is used to encode the masked IL-15 cytokine.

#### *1. Generating Masked IL-15 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 β-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, lpp, 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, hepahtis-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 restrichon fragment that also contains the SV40 viral origin of replication. The immediate early promoter of the human cytomegalovirus is conveniently obtained as a HindIII E restriction fragment. A system for expressing DNA in mammalian hosts using the bovine papilloma virus as a vector is disclosed in U.S. Pat. No. 4,419,446. A modification of this system is described in U.S. Pat. No. 4,601,978. See also Reyes et 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 invenhon 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 replicahon 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 terminahon 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 fractionahon 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.

## 5. COMPOSITIONS

In some aspects, also provided herein are compositions comprising any of the IL-15 masked cytokines described herein. In some embodiments, the composition comprises any of the exemplary embodiments of masked IL-15 cytokine described herein. In some embodiments, the composition comprises a dimer of any

of the masked IL-15 cytokines described herein. In some embodiments, the composition is a pharmaceutical composition. In some embodiments, the composition comprises a masked IL-15 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-15 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-15 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-15 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-15 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-15 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-15 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.

## 6. 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-15 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-15 cytokine described herein or compositions thereof, wherein the masked IL-15 cytokine is activated upon cleavage by an enzyme. In some embodiments, the masked IL-15 cytokine is activated at a tumor microenvironment. The masked IL-15 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-15 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-15 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-15 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-15 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-15 cytokine and the next administration of the masked IL-15 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-15 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-15 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-15 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-15 polypeptide described herein is administered at a flat dose. In some embodiments, a masked IL-15 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-15 polypeptide is administered to a subject at a dosage of about 25mg to about 50mg, about 50mg to about 75mg, about 75mg to about 100mg, about 100mg to about 125mg, about 125mg to about 150mg, about 150mg to about 175mg, about 175mg to about 200mg, about 200mg to about 225mg, about 225mg to about 250mg, about 250mg to about 275mg, about 275mg to about 300mg, about 300mg to about 325mg, about 325mg to about 350mg, about 350mg to about 375mg, about 375mg to about 400mg, about 400mg to about 425mg, about 425mg to about 450mg, about 450mg, to about 475mg, or about 475mg to about 500mg per dose.

In some embodiments, a masked IL-15 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. 0.1mg/kg-10mg/kg) of masked IL-15 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. 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-15 polypeptide described herein is administered to a subject at a dosage from about 25 mg to about 500 mg per dose. In some embodiments, a masked IL-15 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-15 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-15 polypeptide described herein is administered to a subject at a dosage of about or at least about 0.1 mg/kg, about or at least about 0.5 mg/kg, about or at least about 1.0 mg/kg, about or at least about 1.5 mg/kg, about or at least about 2.0 mg/kg, about or at least about 2.5 mg/kg, about or at least about 3.0 mg/kg, about or at least about 3.5 mg/kg, about or at least about 4.0 mg/kg, about or at least about 4.5 mg/kg, about or at least about 5.0 mg/kg, about or at least about 5.5 mg/kg, about or at least about 6.0 mg/kg, about or at least about 6.5 mg/kg, about or at least about 7.0 mg/kg, about or at least about 7.5 mg/kg, about or at least about 8.0 mg/kg, about or at least about 8.5 mg/kg, about or at least about 9.0 mg/kg, about or at least about 9.5 mg/kg, about or at least about 10.0 mg/kg, about or at least about 15.0 mg/kg, about or at least about 20mg/kg, about or at least about 30mg/kg, about or at least about 40mg/kg, about or at least about 50mg/kg, about or at least about 60mg/kg, about

or at least about 70mg/kg, about or at least about 80mg/kg, about or at least about 90mg/kg, or about or at least about 100mg/kg. Any of the dosing frequencies described above may be used.

A method of treatment contemplated herein is the treatment of a disorder or disease with any of the masked cytokines or compositions described herein. In some embodiments, the method of treatment uses any masked IL-2 polypeptide described herein, or any masked IL-15 polypeptide described herein, or any of the compositions comprising a masked cytokine 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 cytokines or compositions described herein. In some embodiments, provided herein is a method of treatment or prevention of a cancer by administration of a masked IL-2 polypeptide, or a masked IL-15 polypeptide, or any of the compositions comprising a masked cytokine described herein. As used herein, the term "cancer" refers to all types of cancer, neoplasm or malignant tumors found in mammals, including leukemias, lymphomas, melanomas, neuroendocrine tumors, carcinomas and sarcomas. Exemplary cancers that may be treated with a masked cytokine, pharmaceutical composition, or method provided herein, include lymphoma, sarcoma, bladder cancer, bone cancer, brain tumor, cervical cancer, colon cancer, esophageal cancer, gastric cancer, head and neck cancer, kidney cancer, myeloma, thyroid cancer, leukemia, prostate cancer, breast cancer (e.g. triple negative, ER positive, ER negative, chemotherapy resistant, Herceptin resistant, HER2 positive, doxorubicin resistant, tamoxifen resistant, ductal carcinoma, lobular carcinoma, primary, metastatic), ovarian cancer, pancreatic cancer, liver cancer (e.g. hepatocellular carcinoma), lung cancer (e.g. nonsmall cell lung carcinoma, squamous cell lung carcinoma, adenocarcinoma, large cell lung carcinoma, small cell lung carcinoma, carcinoid, sarcoma), glioblastoma multiforme, glioma, melanoma, prostate cancer, castration-resistant prostate cancer, breast cancer, triple negative breast cancer, glioblastoma, ovarian cancer, lung cancer, squamous cell carcinoma (e.g., head, neck, or esophagus), colorectal cancer, leukemia, acute myeloid leukemia, lymphoma, B cell lymphoma, or multiple myeloma. Additional examples include, cancer of the thyroid, endocrine system, brain, breast, cervix, colon, head & neck, esophagus, liver, kidney, lung, non-small cell lung, melanoma, mesothelioma, ovary, sarcoma, stomach, uterus or Medulloblastoma, Hodgkin's Disease, Non-Hodgkin's Lymphoma, multiple myeloma, neuroblastoma, glioma, glioblastoma multiforme, ovarian cancer, rhabdomyosarcoma, primary thrombocytosis, primary macroglobulinemia, primary brain tumors, cancer, malignant pancreatic insulanoma, malignant carcinoid, urinary bladder cancer, premalignant skin lesions, testicular cancer, lymphomas, thyroid cancer, neuroblastoma, esophageal cancer, genitourinary tract cancer, malignant hypercalcemia, endometrial cancer, adrenal cortical cancer, neoplasms of the endocrine or exocrine

pancreas, medullary thyroid cancer, medullary thyroid carcinoma, melanoma, colorectal cancer, papillary thyroid cancer, hepatocellular carcinoma, Paget's Disease of the Nipple, Phyllodes Tumors, Lobular Carcinoma, Ductal Carcinoma, cancer of the pancreatic stellate cells, cancer of the hepatic stellate cells, or prostate cancer.

In some embodiments, provided herein is a method of treatment or prevention of a cancer by administration of any masked IL-15 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-15 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-15 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-15 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-15 cytokine or composition described herein in combination with CAR-NK (Natural Killer) cells.

## 7. ARTICLES OF MANUFACTURE OR KITS

In another aspect, an article of manufacture or kit is provided which comprises any masked IL-15 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-15 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-15 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.

## 8. DEFINITIONS

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

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-15 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 $\alpha$ 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 $\alpha$ 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, IgA1 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.

## 9. 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 or IL-15 polypeptide construct, some examples also employ parental "non-masked" versions of the IL-2 polypeptide construct or IL-15 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 and Masked IL-15 Polypeptides**

Masked IL-2 polypeptide and masked IL-15 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 # | Structure (N- to C-terminal direction) |
|---|---|
| AK032 | H-C |
| AK035 | H-C |

Also generated are masked IL-2 polypeptide constructs that include an IL-2 polypeptide or functional fragment thereof, a first masking moiety, a second masking moiety, and a half-life extension domain, such as albumin, an antibody or fragment thereof (e.g., an Fc region, heavy chain, and/or light chain), an albumin-binding peptide, an IgG-binding peptide, or a polyamino acid sequence. Some of the constructs also include a linker that links the first masking moiety to the IL-2 polypeptide or functional fragment thereof. Some of the constructs also include a linker that links the second masking moiety to the IL-2 polypeptide or functional fragment thereof. Some of the constructs include a cleavable peptide in the linker linking the first masking moiety to the IL-2 polypeptide or functional fragment thereof and/or the linker linking the second masking moiety to the IL-2 polypeptide or functional fragment thereof, thereby 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 # | Structure (N- to C- terminal direction) |
|---|---|
| AK041 | H-L1-MM1-L2-C-L3-MM2 |

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.

Also generated are masked IL-15 polypeptide constructs that include an IL-15 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-15 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. 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-15 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-15 polypeptide or functional fragment thereof to the second half-life extension domain, thereby resulting in an activatable masked IL-15 polypeptide construct. The masked IL-15 polypeptide constructs that do not include a cleavable peptide in either the linker that links the IL-15 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-15 polypeptide constructs or non-activatable IL-15 polypeptide constructs because they do not include a cleavable peptide. The structure and composition of exemplary IL-15 polypeptide constructs are provided in Table 5.

Table 5

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

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

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

| Construct # | Structure (N- to C-terminal direction) |
|---|---|
| AK310 | H-L1-C |
| | H |
| AK311 | H-L1-C |
| | H-L1-MM |
| AK312 | H-L1-C |
| | H |
| AK313 | H-L1-C |
| | H-L1-MM |
| AK314 | H-L1-C |
| | H |
| AK315 | H-L1-C |
| | H-L1-MM |
| AK316 | H-L1-C |
| | H-L1-MM |
| AK248 | H-L1-C |
| | H-L1-MM |

**Example 2: In vitro characterization of masked IL-2 and IL-15 polypeptides**

The masked IL-2 polypeptide constructs and masked IL-15 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 and masked IL-15 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 | | | |

| Construct ID | Titer (mg/L) | | Construct ID | Titer (mg/L) |
|---|---|---|---|---|
| AK235 | 35.9 | | | |
| 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 and masked IL-15 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 one study, the exemplary masked IL-15 polypeptide construct AK248 was tested in accordance with this protocol, with or without prior exposure to an MMP10 protease, along with recombinant human IL-15 (rhIL-15) as a positive control. As shown in FIGs. 45A and 45B there was no detectable activation of the JAK- STAT pathway when using the masked IL-15 polypeptide construct AK248 (without protease cleavage), but following cleavage by an activating protease, strong levels of activation of the JAK-STAT pathway were observed. This demonstrates that an exemplary IL-15 polypeptide can be effectively masked and also activated through protease cleavage.

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 (e.g., the masked IL-15 polypeptide construct AK248, with or without prior exposure to an MMP protease, or a positive control such as rhIL-15) 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 and the masked IL-15 polypeptide constructs generated in Example 1 is assessed. For the masked IL-2 polypeptide constructs, in some experiments, ELISA plates are coated with a receptor subunit, such as IL-2Rα (also referred to as CD25), IL- 2Rβ (also referred to as CD122), or IL-2Rγ (also referred to as CD132), or combinations thereof. For the masked IL-15 polypeptide constructs, in some experiments, ELISA plates are coated with a receptor subunit, such as IL-15Rα, 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 or masked IL-15 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 and masked IL-15 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 and the masked IL-15 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 and masked IL-15 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 of the masked IL-15 polypeptide constructs is assessed by conducting receptor- binding assays, as described above, after incubation of the masked IL-15 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 or the masked IL-15 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 (e.g., masked IL-2 polypeptide constructs) and incubated at 37 degrees for 2 hours. Cleavage was assessed by SDS-PAGE using AnykD™ Criterion™ TGX Stain-Free™ Protein Gels. A similar approach is taken to test cleavage by other proteases.

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

**Proliferation**

Proliferation of IL-2 and IL-15 responsive tissue culture cell lines, such as CTLL2, YT, TF1B, LGL, HH, and CT6, following treatment with the masked IL-2 polypeptide constructs or the masked IL-15 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. For experiments involving the masked IL-15 polypeptide constructs, cells are plated in 96 well tissue culture plates in media lacking IL-15 for 2-4 hours and then treated with the masked IL-15 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 or the masked IL-15 polypeptide constructs generated in Example 1 is also assessed. Human, mouse, or cynomolgus peripheral blood mononuclear cells (PBMCs) are treated with the constructs at various concentrations, and the proliferation of cell types, such as Natural Killer (NK) cells, CD8+ T cells, CD4+ T cells, and/or Treg cells, is determined by staining for the particular cell type and analysis via fluorescence activated cell sorting (FACS). In some experiments, some PBMCs are treated with controls for comparison. In some experiments, some PBMCs are treated with aldesleukin as a control for the masked IL-2 polypeptide treatment. In some experiments, the masked IL-2 polypeptide constructs and the masked IL-15 polypeptide constructs are tested in conditions with and without protease cleavage (e.g., activation). In some experiments, the NK cells are stained as CD45+ CD3- CD56+, the CD8+ T cells are stained as CD45+ CD3+ CD8+, the CD4+ T cells are stained as CD45+ CD3+ CD4+ CD25-, and the Treg cells are stained as CD45+ CD3+ CD4+ CD25+ FOXP3+. In some experiments, the PBMCs are treated for a period of five days. In some experiments, the PBMCs are also stained with Ki67, a marker of cell proliferation. In some experiments, the PBMCs are labeled with CFSE (Sigma-Aldrich) prior to treatment and proliferation is measured by determining the extent of CFSE dilution. In some experiments, each construct, as well as aldesleukin and/or other controls, is administered at one or more concentrations, such as one or more concentrations ranging from 0.0001 nM to 500 nM.

**STAT5 Activation**

The activation of Signal Transducer and Activator of Transcription 5 (STAT5) following treatment with the masked IL-2 polypeptide constructs or the masked IL-15 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 or the masked IL-15 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 or the masked IL-15 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 or the masked IL-15 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 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 and IL-15 polypeptides**

**Pharmacokinetics**

The pharmacokinetics of the masked IL-2 polypeptide constructs and or the masked IL-15 polypeptide constructs generated in Example 1 is assessed in vivo using mouse models.

Mice are treated intravenously or subcutaneously with the constructs and the concentration of the construct in the plasma is measured over time. In some experiments, some mice are treated with controls for comparison. In some experiments, some mice are treated with aldesleukin as a control for masked IL-2 polypeptide treatment. In some experiments, the mice that are treated have tumors. In some experiments, the mice that are treated are tumor-free. In some experiments, mice are treated with the constructs and blood is drawn at various times over the course of treatment, which may include drawing blood prior to the initiation of treatment and processing it to obtain plasma. In some experiments, blood is drawn at various time points over the course of two weeks, three weeks, or four weeks or more of treatment. In some experiments, the mean plasma concentration of the administered constructs, as well as aldesleukin and/or other controls, is measured. Masked IL-2 polypeptide constructs are detected in the plasma samples after dilution into PBS Tween with IL-2- and human Fc-specific ELISAs and are quantified using a standard curve generated for each construct. Masked IL-15 polypeptide constructs are detected in the plasma samples after dilution into PBS Tween with IL-15- 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 and the masked IL-15 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 ($5 \times 10^5$ 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 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 constucts 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 constucts 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 and the masked IL-15 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 or the masked IL-15 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 x$10^5$ 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 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 (e.g., masked IL-2 polypeptide constructs or

masked IL-15 polypeptide constructs) is assessed. In some studies, a control antibody is administered for comparison. In some studies, in vivo cleavage is assessed by administering the construct of interest in a mouse and, after a certain period of time, capturing human IgG and then measuring the levels of, e.g., human IgG, CD122, and IL-2 or IL-15.

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 and the masked IL-15 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 and the masked IL-15 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 or the masked IL-15 polypeptide 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 and the masked IL-15 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 and the masked IL-15 polypeptide constructs are determined by ELISA, PAGE, and mass spectrometry.

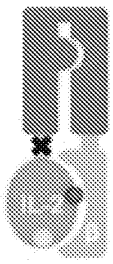
To test the safety profile of exemplary masked IL-2 polypeptide constructs in non-human primates, a dose ranging study is performed in accordance with the following method. Groups of 3 healthy male cynomolgus monkeys (Macaca fascicularis) are randomly assigned to receive a single intravenous bolus

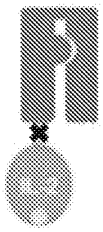
dose of 2 mL/kg of activatable (i.e., cleavable) masked IL-2 polypeptide proteins or non-cleavable masked IL-2 polypeptide proteins at 10, 30 and 100 nmol/kg in 100 mM sodium citrate buffer (pH 5.5). A third group receives the parental non-masked, cleavable protein at 3, 10 and 30 nmol/kg as a positive 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 ($5 \times 10^5$ 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**<br>(VPLSLY)<br>IL-2 dead |  |

| | |
|---|---|
| AK211 |  |
| AK235<br>(VPLSLY |  |
| AK209<br>(VPLSLY) |  |
| AK471<br>(VPLSLY)<br>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 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). 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 33A and 33B. 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 x$10^5$ cells per mouse) were injected subcutaneously into the right flank of each mouse. Upon reaching ~100 $mm^3$ sized tumors (day 0), the mice 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 and 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. 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 B.

**iv. Peripheral (spleen) vs tumor CD8 T cell expansion - AK252 and 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 B.

**v. Anti-tumor activity - AK252 and 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 B.

**vi. Peripheral (spleen) vs tumor CD8 T cell expansion - AK252 and 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 B.

**vii. Anti-tumor activity - AK252, AK508, AK509, AK510, and 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 B.

**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.

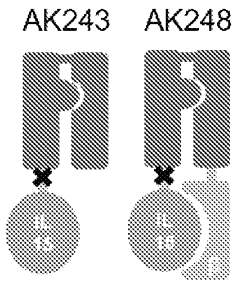
**x. Splenomegaly and lung edema - AK252, AK438, AK442, AK209, AK341**

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: IL-15 Heteroaklusion

The following constructs were used in this example:




| | AK243 | AK248 |
|---|---|---|
| Plasmids | DNA363/DNA158 | DNA363/DNA187 |
| Cleavage site | MPYDLYHP | MPYDLYHP |
| Linker sequence | GGSSPPMPYDLYHPSG PGSGS | GGSSPPMPYDLYHPSG PGSGS |
| Active molecule | Wild type IL-15 | Wild type IL-15 |
| Akluding molecule | N.A. | CD122 |

IL-15 Reporter Bioassay: Interleukin-15 (IL-15) is secreted by a variety of cell types including monocytes, macrophages, dendritic and epithelial cells. IL-15 promotes activation and expansion of natural killer (NK), natural killer T (NKT) and CD8+ memory T-cells, and unlike IL-2, doesn't impact CD4+ regulatory T cells (Treg) or induce activation-induced cell death (AICD). Multiple pathways can be activated by IL-15 signaling to impact cell proliferation, anti-apoptotic survival and cytotoxic effector functions. An IL-15 bioluminescent cell-based assay was used to quantify IL-15 using the cytokine's mechanism of action pathway. The assay was performed according to manufacturer's specifications. Briefly, a vial of IL-15 Bioassay cells (Promega) was thawed and added to warm assay buffer. Cell suspension was mixed and plated. Serial dilutions of the IL-15 constructs were plated on cells and incubated for 6 hours. The bioluminescent reagent was added to wells, incubated, and luminescence measured. Background was subtracted from raw data and the data were fit nonlinearly: [Agonist] vs. response – Variable slope (four parameters). The EC50 value of each construct is shown.

The results are shown in Figure 45A

Testing IL-15 molecules with HEK-Blue IL-2 cells :HEK-Blue IL-2 reporter cells developed by Invivogen have been specifically designed to monitor the activation of the JAK-STAT pathway. These cells were generated by stable transfection of HEK293 cells with the human IL-2Rα, IL-2Rβ, and IL-2Rγ genes, along with the human JAK3 and STAT5 genes to obtain a fully functional IL-2 signaling pathway. In addition, a STAT5-inducible SEAP reporter gene was also introduced. Upon stimulation, HEK-Blue™ IL-2 cells trigger the activation of STAT5 and the subsequent secretion of SEAP. The levels of STAT5-

induced SEAP can be readily monitored using QUANTI-Blue™. HEK-BlueIL-2 cells can be used to validate the functionality, toxicity, and variable dosage effects of human or murine IL-2. HEK Blue IL-2 cells were grown in passage media until ~80% confluent. Washed single-cell suspension in assay media was plated and serial dilutions of IL-15 molecules in assay media were added to cells. Plate was incubated at 37 oC for 24 hours. After 24 h, Quanti-Blue solution (Invivogen) was prepared and cell supernatant was added to the Quanti-Blue solution and incubated for 1-2 h at 37 oC. Absorbance at 625 nm measured. Data analysis was performed in Graphpad Prism, version 8.3. Background was subtracted from raw data and the data were fit nonlinearly: [Agonist] vs. response – Variable slope (four parameters). EC50 value of each IL-15 construct was reported.

The results are shown in Figure 45B.

**Example 8:**

**Cleavage of IL-15 molecules and analysis by SDS-PAGE and anti-IL-15 WB:**

**Cleavage with recombinant MMP**

- IL-15 molecules were buffer exchanged into MMP buffer (50 mM Tris pH 8.0, 150 mM NaCl, 10 mM CaCl2). Concentrations were measured by A280 absorbance.
- Each construct was diluted to 1 uM and split into two 100 uL aliquots.
- Pre-activated 200 ng MMP9 or MMP10 was added to one tube per construct. The other tube per construct had same amount of buffer only added (no protease samples).
- Samples were incubated at 37 oC for 16-18 h.

**Analysis by SDS-PAGE**

- Samples were prepared in Sample buffer for analysis by SDS-PAGE.
    - 12 uL construct + 4 uL 4x Lameli buffer (with BME for reduced samples, without BME for non-reduced samples).
    - Samples were boiled at 95 oC for 10 min.
- 15 uL of each sample was applied per well to an 18 well Stain Free SDS-PAGE (BioRad).
- Current was applied, 200 V for 35 min.
- Protein was detected on gel by using BioRad imaging instrument, stain free detection with 45 s activation.

**Analysis by anti-IL-15 Western blot**

- SDS-PAGE above was transferred to a nitrocellulose membrane using a semi-dry blotting system.
    - Transfer buffer: 200 mL 5x transfer buffer (BioRad), 600 mL H2O, and 200 mL ethanol.
    - Turbo transfer program: 2.5 A, 25 V for 7 min.

- Membrane was blocked with blocking solution (1x TBS + 1% casein blocker, BioRad) for 1-2 h, shaking at room temperature.
- Membrane was probed with rabbit pAb anti-IL-15 (ab7213, Abcam) diluted 1:500 in blocking solution for 2 h, shaking at room temperature.
- Membrane was washed 3 times for 5 min each wash with TBS-Tween, shaking at room temperature.
- Secondary HRP-conjugated anti-rabbit IgG (CST #7074) diluted 1:2000 in blocking solution was added to the membrane for 2 h, shaking at room temperature.
- Membrane was washed 3 times for 5 min each wash with TBS-Tween, shaking at room temperature.
- Membrane was developed by adding freshly prepared 12 mL of chemiluminescent substrate (Thermo #37069) for 1 min, shaking at room temperature.
- Chemiluminescent signal was measured using BioRad imaging instrument, chemiluminescent blot setting.
- Images were analyzed using BioRad Image Lab software.

**Molecules containing MPYDLYP:**

Constructs:

| Construct | Site | @ Benchling info |
|---|---|---|
| AK243 | MPYDLYHP | DNA363/DNA158 |
| AK244 | NC | DNA364/DNA158 |
| AK245 | MPYDLYHP | DNA365/DNA158 |
| AK246 | MPYDLYHP | DNA366/DNA158 |
| AK248 | MPYDLYHP | AK248 DNA363/DNA187 |
| AK249 | NC | DNA364/DNA187 |
| AK250 | MPYDLYHP | DNA365/DNA187 |
| AK251 | MPYDLYHP | DNA366/DNA187 |

i) **Molecules containing MPYDLYHP are cleaved by MMP10** - ***NC controls AK244 and AK249 are not cleaved***

Results are shown in Figures 46A ( stain-free SDS-PAGE) and B (Anti-IL-15 Western blot).

ii) **Potency of AK245 is reduced 40-fold compared to rhIL-15** - ***Does not return to full potency after enzymatic activation***

Results are show in in Figures 47A, B, C and D.

iii) **Potency of AK248 cannot be calculated using the current dilution series - *Activity is fully restored after enzymatic activation***

Results are show in in Figures 48A, B, C and D.

**Molecules containing VPLSLY:**

| Construct | Site | @ Benchling info |
|---|---|---|
| AK401 | VPLSLY | CD122, IL15RaSushi, IL15 V1 with plain Fc Knob |
| AK402 | VPLSLY | CD122, IL15RaSushi, IL15 V2 with plain Fc Knob |
| AK403 | VPLSLY | CD122, IL15RaSushi, IL15 V3 with plain Fc Knob |
| AK457 | VPLSLY | Knob: Fc-IL15(WT)_VPLSLY DNA558 + Hole: hFc(N297A)-hCD122 (WT) DNA187 |
| AK458 | VPLSLY | Knob: Fc-IL15(D22A)_VPLSLY DNA557 + Hole: hFc(N297A)-hCD122 (WT) DNA187 |
| AK459 | VPLSLY | Knob: Fc-IL15(E46A)_VPLSLY DNA556 + Hole: hFc(N297A)-hCD122 (WT) DNA187 |
| AK460 | VPLSLY | Knob: Fc-IL15(E53A)_VPLSLY DNA559 + Hole: hFc(N297A)-hCD122 (WT) DNA187 |
| AK461 | VPLSLY | Knob: Fc-IL15(E46A, E53A)_VPLSLY DNA560 + Hole: hFc(N297A)-hCD122 (WT) DNA187 |

i) **Molecules containing VPLSLY are partially cleaved by MMP9**

Results are shown in Figures 49A ( stain-free SDS-PAGE) and B (Anti-IL-15 Western blot

ii) **Potency of AK457 cannot be calculated using the current dilution series – *Does not return to full potency after enzymatic activation***

Results are show in in Figures 50A, B, C and D.

iii) **Potency of AK458, AK459, and AK461 cannot be calculated using the current dilution series**

Results are show in in Figures 51A, B, C and D.

The results of 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.

**Example 9:**

The following constructs were used in this example:




AK244 is an unmasked IL-15 construct.
AK507 is an unmasked IL-15 construct comprising a non-covalently bound Il-15Rα (sushi) domain.
XTX200 is an unmasked, IL-2 construct.
AK443 is a cleavable, masked, IL-2 dead construct.

**IL-15 constructs comprising a non-covalently bound Il-15Rα (sushi) domain are more potent than constructs without a sushi domain.**

Dose dependent activation of CD8 T cell and NK cell was observed by AK244 and AK507 in both periphery and TME. AK507 showed higher potency at lower dose level (0.5 mpk) in CD8 T cell activation. AK507 is more potent *in vivo* than AK244.

Results are shown in Figures 52A-D.

**IL-15 constructs comprising a non-covalently bound Il-15Rα (sushi) domain exhibit a similar PK profile compared to constructs without a sushi domain.**

Both AK244 and AK507 have a similar, linear PK profile compared to XTX200.

Results are shown in Figure 53.

**IL-15 constructs comprising a non-covalently bound Il-15Rα (sushi) domain show more severe side effects and thus greater potency compared to constructs without a sushi domain.**

AK507 shows more severe splenomegaly and pulmonary toxicity than AK244. AK507 showed 100% lethality on Day 5, and a dose dependent increase of lung weight.

| Treatment | Dose | Clinical Observation | Lethality before day 5 |
|---|---|---|---|
| XTX200 | 0.5 mpk | | 0/0 |
| AK244 | 0.5 mpk | | 0/0 |
| | 1.5 mpk | | 0/0 |
| | 5 mpk | | 0/0 |
| AK507 | 0.5 mpk | | 0/0 |
| | 1.5 mpk | weakness 3/4 | 0/0 |
| | 5 mpk | | 4/4 |
| AK443 | 5 mpk | | 0/0 |

Results are shown in Figures 54A and B.

**Example 10**

The following study design was used in this example:

| | | Test Article | M.W. (kDa) | Apprx. Dosing (mg/kg) | Actual Dosing (nMoles/kg) | Actual Dosing (mg/kg) |
|---|---|---|---|---|---|---|
| | 1 | AK253 (IL2 unmasked, positive control) | 67 | 2 | 22.2 | 1.49 |
| | 2 | vehicle only (negative control) | 92 | 6 | 66.6 | 6.13 |
| WT IL-15 + WT sushi | 3 | IL-15 unmasked AK507 | 65 | 0.6 | 6.7 | 0.43 |
| | 4 | IL-15 unmasked AK507 | 65 | 2 | 22.2 | 1.45 |
| | 5 | IL-15 masked non-cleavable AK564 | ~90 | 2 | 22.2 | 2 |
| | 6 | IL-15 masked non-cleavable AK564 | ~90 | 6 | 66.6 | 6 |
| | 7 | IL-15 masked non-cleavable AK564 | ~90 | 20 | 222 | 20 |
| N71Q IL-15 + WT sushi | 8 | IL-15 unmasked AK595 | 65 | 0.6 | 6.7 | 0.43 |
| | 9 | IL-15 unmasked AK595 | 65 | 2 | 22.2 | 1.45 |
| | 10 | IL-15 masked non-cleavable AK596 | ~90 | 2 | 22.2 | 2 |
| | 11 | IL-15 masked non-cleavable AK596 | ~90 | 6 | 66.6 | 6 |
| | 12 | IL-15 masked non-cleavable AK596 | ~90 | 20 | 222 | 20 |

**IL-15 constructs comprising a non-covalently bound sushi can be effectively 'masked' with a masking moiety, increasing half-life and drug exposure.**

Comparing AK507 (unmasked, sushi) with AK564 (masked, non-cleavable, sushi), and AK595 (unmasked, sushi + single IL-15 mutation N71Q) with AK596 (masked, non-cleavable, sushi + single IL-15 mutation N71Q), the masked constructs have longer half-lives compared to the unmasked counterpart. Dose proportionality is also achieved for masked constructs.

Results are shown in Figures 55A and B.

**Masked IL-15 constructs comprising a non-covalently bound sushi have a different clinical effect compared to unmasked IL-15 constructs comprising a non-covalently bound sushi.**

Comparing AK507 (unmasked, sushi) with AK564 (masked, non-cleavable, sushi), and AK595 (unmasked, sushi + single IL-15 mutation N71Q) with AK596 (masked, non-cleavable, sushi + single IL-15 mutation N71Q):

i) % BW loss was observed with unmasked constructs but not with masked constructs.

Results are show in in Figures 56A and B.

ii) The percentage of Tregs in tumor remained consistent between masked and unmasked constructs. The percentage of CD8+ T cells in tumor increased with unmasked constructs.

Results are shown in Figures 57A and B.

iii) The CD8:Treg ratio is not elevated with masked constructs. There is an improvement over AK253 at equivalent dose.

Results are shown in Figure 58.

iv) The percentage of NK cells of CD45+ population in tumor is elevated with unmasked constructs. This indicates increased recruitment or proliferation of NKs relative to other leukocyte populations.

Results are shown in Figure 59.

v) An increase in the percentage of proliferating and activated NK cells in tumor was observed with unmasked constructs.

Results are shown in Figures 60A and B.

vi) Masked constructs induced a lower percentage of Ki-67+ and ICOS+ NK cells in the blood. An increase in dose can overcome masking in the blood

Results are shown in Figures 61A-D.

**Example 11**

The following constructs were used in this example:

| AK molecule | Key construct features |
|---|---|
| AK507 | WT IL-15 domain<br>Unmasked<br>Non-covalent sushi |
| AK564 | WT IL-15 domain<br>Masked and non-cleavable<br>Non-covalent sushi |
| AK595 | N71Q single mutant IL-15 domain<br>Unmasked<br>Non-covalent sushi |
| AK596 | N71Q single mutant IL-15 domain<br>Masked and non-cleavable<br>Non-covalent sushi |
| AK900 | N112Q single mutant IL-15 domain<br>Unmasked<br>Non-covalent sushi |
| AK906 | N112Q single mutant IL-15 domain<br>Masked and non-cleavable<br>Non-covalent sushi |
| AK901 | N79Q single mutant IL-15 domain<br>Unmasked<br>Non-covalent sushi |
| AK907 | N79Q single mutant IL-15 domain<br>Masked and non-cleavable<br>Non-covalent sushi |
| AK902 | N79Q; N112Q double mutant IL-15 domain<br>Unmasked<br>Non-covalent sushi |
| AK908 | N79Q; N112Q double mutant IL-15 domain<br>Masked and non-cleavable<br>Non-covalent sushi |
| AK903 | N71Q; N112Q double mutant IL-15 domain<br>Unmasked<br>Non-covalent sushi |

| | |
|---|---|
| AK909 | N71Q; N112Q double mutant IL-15 domain<br>Masked and non-cleavable<br>Non-covalent sushi |
| AK904 | N71Q; N79Q double mutant IL-15 domain<br>Unmasked<br>Non-covalent sushi |
| AK910 | N71Q; N79Q double mutant IL-15 domain<br>Masked and non-cleavable<br>Non-covalent sushi |
| AK905 | N71Q; N79Q; N112Q triple mutant IL-15 domain<br>Unmasked<br>Non-covalent sushi |
| AK911 | N71Q; N79Q; N112Q triple mutant IL-15 domain<br>Masked and non-cleavable<br>Non-covalent sushi |

**Adding single, double or triple mutations to the IL-15 domain alters the glycosylation pattern of the molecules, producing a more homogenous mixture of proteins. This is useful for consistency in the manufacturing process of the constructs.**

An SDS-PAGE analysis was carried out using masked IL-15 constructs AK564, AK596, AK906, AK907, AK908, AK909, AK910 and AK911. AK564 (wild-type IL-15 domain) displayed a doubling of the band displaying the 'knob' domain of the construct (see labels in Figure 62). This doubling is removed in AK910 and AK911, which include a double and triple mutation of the IL-15 domains respectively.

The results as shown in Figure 62.

**Adding single, double or triple mutations to the IL-15 domain does not alter the potency or masking of the constructs.**

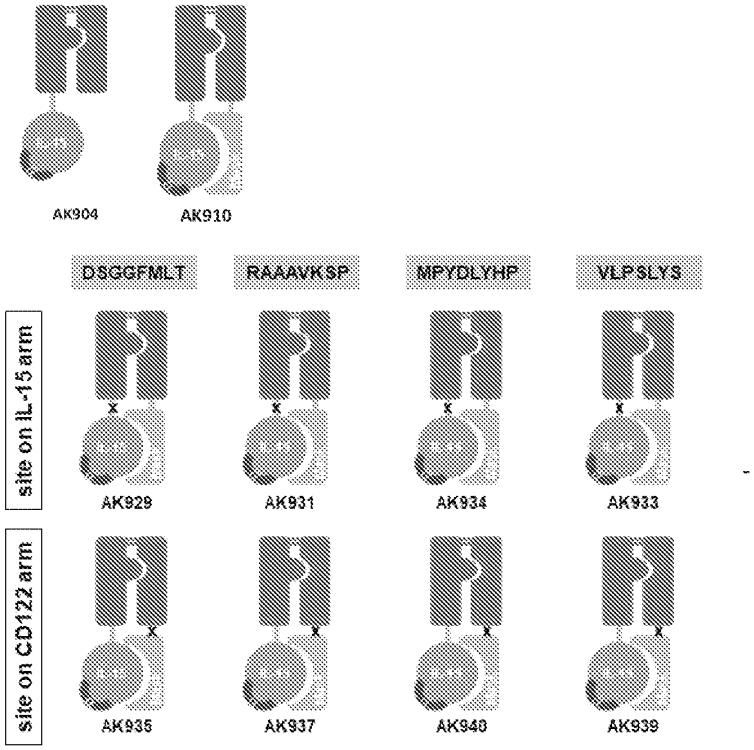
A HEK-Blue IL-2 bioassay was used to test JAK-STAT pathway in a reporter cell line engineered to monitor IL-2 and IL-15 activity. The extent of activation was measured by induction of a STAT5-SEAP reporter gene, which can be quantified with substrate solution by absorbance at 650nm. No significant difference in unmasked $EC_{50}$ between glycovariants was observed, compared to WT IL-15 (AK507). No significant loss of masking ability between glycovariants was observed, compared to Masked-WT IL-15

(AK564). Masked-Triple mutant (AK911) is 3.6-fold more masked than Masked-WT IL-15 (AK564).

Results are shown in Figure 63.

## Example 12

The following constructs were used in this example:




Details on the domain features and sequences of each AK molecule may be found in the sequence tables in Section 10.

Importantly, AK929, AK931, AK933, AK934, and their 'flipped' (Structure B) counterparts AK935, AK937m AK939 and AK940 include a peptide substrate (the sequence of which is depicted in the box above each molecule). AK904 is an non-cleavable unmasked construct, and AK910 is a non-cleavable masked construct, both acting as negative controls.

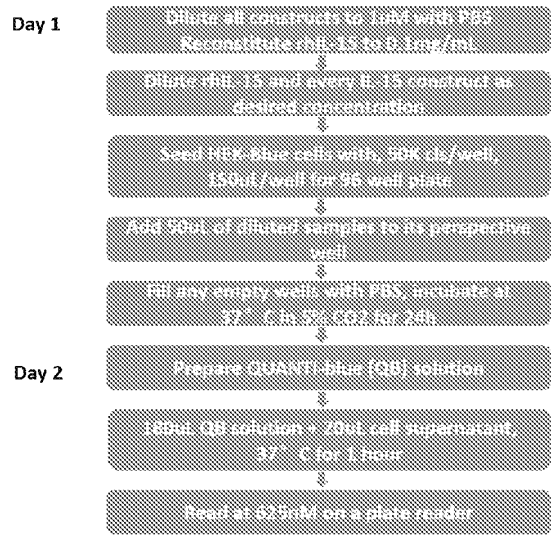
**Cleavage was achieved for masked IL-15 constructs including a peptide substrate.**

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 |
| AK929 | 7 (600ng) | √ |
| | 9 | - |
| | 10 | - |
| AK935 | 7 (600ng) | √ |
| | 9 | - |
| | 10 | - |
| AK931 | 7 | - |
| | 9 | - |
| | 10 | √ |
| AK937 | 7 | - |
| | 9 | - |
| | 10 | √ |
| AK934 | 7 | - |
| | 9 | - |

| | | |
|---|---|---|
| | 10 | √ |
| AK940 | 7 | - |
| | 9 | - |
| | 10 | √ |
| AK933 | 7 | - |
| | 9 | √ |
| | 10 | - |
| AK939 | 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 |
| AK929 (1:1:2) | - | 2486.47 | 1.38 |
| | 7 (600ng) | 9.87 | 1.44 |
| | 9 | - | - |
| | 10 | - | - |
| AK935 (1:1:2) | - | 753.71 | 1.41 |
| | 7 (600ng) | 15.54 | 1.40 |
| | 9 | - | - |
| | 10 | - | - |
| AK931 (1:1:2) | - | 662.98 | 1.35 |
| | 7 | - | - |
| | 9 | - | - |
| | 10 | 10.24 | 1.46 |
| AK937 (1:1:2) | - | 689.46 | 1.38 |
| | 7 | - | - |
| | 9 | - | - |
| | 10 | 11.96 | 1.41 |
| AK934 (1:1:2) | - | 1972.77 | 1.36 |
| | 7 | - | - |
| | 9 | - | - |
| | 10 | 8.46 | 1.44 |
| AK940 (1:1:2) | - | 853.59 | 1.30 |
| | 7 | - | - |
| | 9 | - | - |
| | 10 | 10.30 | 1.41 |
| AK933 (1:1:2) | - | 1981.55 | 1.39 |

| | | | |
|---|---|---|---|
| | 7 | - | - |
| | 9 | 8.01 | 1.40 |
| | 10 | - | - |
| AK939 | - | 872.84 | 1.39 |
| | 7 | - | - |
| | 9 | 15.41 | 1.38 |
| | 10 | - | - |

The SDS-PAGE gel results are shown in Figures 64A-D. The HEK-Blue IL-2 bioassay results are shown in Figures 65A-J.

**Example 13**

**Production of the three chain construct**

Plasmids encoding the constructs (e.g., masked IL-2 polypeptide constructs and masked IL-15 polypeptide constructs) were transfected into Expi293 cells (Life Technologies A14527). Transfections were performed using 1 mg of total DNA per liter culture using PEI MAX ( Polysciences, Inc, 24765-1) with 1:4 and 1:3 DNA ratios forIL-2 and IL-15, respectively. A DNA ratio of 1:3:2 was used for three chains IL-15 molecules (IL-15 with the Sushi domain). The DNA and PEI were each added to 50 mL of FreeStyle F17 Expression Medium (Life Technologies A1383502). The DNA and PEI mixture were incubated for 5-10 minutes and added to the Expi293 cells with a cell density of 4-6 x $10^6$ cells/mL and a viability of at least 95%. After 5-7 days, the cells were pelleted by centrifugation at 4000 x g and the supernatant was filtered through a 0.45 µm membrane. Filtered supernatant was passed through HiTrap MabSelect SuRe column (Cytiva, 11003494). The bound protein was eluted from the column with 20 mM citrate, 100 mM NaCl, pH 2.9. The eluted protein was further purified by passing through HiLoad 16/600 Superdex 200 pg column (Cytiva, 28989335).

**Example 14**

**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.

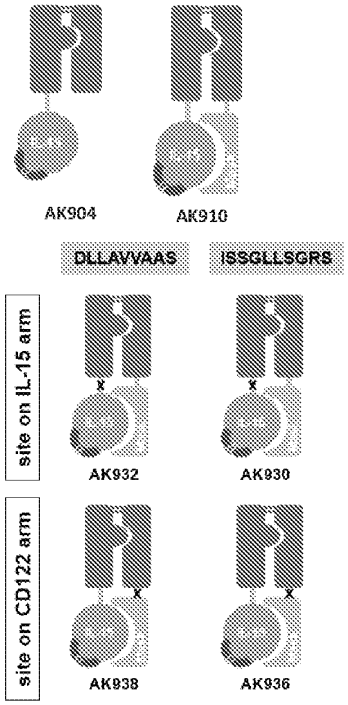
Results are as follows:

| Substrate | 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 15**

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) | DKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYASTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVCTLPPSRDELTKNQVSLSCAVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLVSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGK | DNA158 |
| | 2nd polypeptide chain: Fc(knob)-IL15 V1 Non-cleavable (N71Q, N79Q) | DKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYASTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPCRDELTKNQVSLWCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGGGSSPPGGGSSGGGSGPSGSPGNWVNVISDLKKIEDLIQSMHIDATLYTESDVHPSCKVTAMKCFLLELQVISLESGDASIHDTVENLIILAQNSLSSNGQVTESGCKECEELEEKNIKEFLQSFVHIVQMFINTS | AK904 |
| AK910 | 1st polypeptide chain: Fc(Hole) CD122(C122S, C168S) | DKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYASTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVCTLPPSRDELTKNQVSLSCAVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLVSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGPGSGSAVNGTSQFTCFYNSRANISCVWSQDGALQDTSCQVHAWPDRRRWNQTCELLPVSQASWACNLILGAPDSQKLTTVDIVTLRVLCREGVRWRVMAIQDFKPFENLRLMAPISLQVVHVETHRSNISWEISQASHYFERHLEFEARTLSPGHTWEEAPLLTLKQKQEWISLETLTPDTQYEFQVRVKPLQGEFTTWSPWSQPLAFRTKPAALGKD | DNA440 |
| | 2nd polypeptide chain: Fc(knob)-IL15 | DKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYASTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKA | DNA904 |

| | | | |
|---|---|---|---|
| | V1 Non-cleavable (N71Q, N79Q) | KGQPREPQVYTLPPCRDELTKNQVSLWCLV KGFYPSDIAVEWESNGQPENNYKTTPPVLD SDGSFFLYSKLTVDKSRWQQGNVFSCSVMHE ALHNHYTQKSLSLSPGGGSSPPGGGSSGGGSG PSGSPGNWVNVISDLKKIEDLIQSMHIDATLY TESDVHPSCKVTAMKCFLLELQVISLESGDASI HDTVENLIILAQNSLSSNGQVTESGCKECEEL EEKNIKEFLQSFVHIVQMFINTS | |
| AK932 | 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)-[DLLAVVAAS]-IL15 (N71Q,N79Q) *cleavable peptide bolded | DKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEV TCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREE QYASTYRVVSVLTVLHQDWLNGKEYKCKVSNKALP APIEKTISKAKGQPREPQVYTLPPCRDELTKNQVSLW CLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGS FFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQK S LSLSPGSGS**DLLAVVAAS**SGPGSGNWVNVISDLKKIE DLIQSMHIDATLYTESDVHPSCKVTAMKCFLLELQVIS L ESGDASIHDTVENLIILAQNSLSSNGQVTESGCKECEE LEEKNIKEFLQSFVHIVQMFINTS | DNA924 |
| AK938 | 1st polypeptide chain: Fc(hole)-[DLLAVVAAS] | DKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRT PEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAK TKPREEQYASTYRVVSVLTVLHQDWLNGKEYK CKVSNKALPAPIEKTISKAKGQPREPQVCTLPPS RDELTKNQVSLSCAVKGFYPSDIAVEWESNGQ | DNA822 |

| | | | |
|---|---|---|---|
| | -CD122<br><br>*cleavable peptide bolded | PENNYKTTPPVLDSDGSFFLVSKLTVDKSRWQQ<br>GNVFSCSVMHEALHNHYTQKSLSLSPGSGSPSG**D**<br>**LLAVVAAS**SGPGSGSPAVNGTSQFTCFYNSRANISC<br>VWSQDGALQDTSCQVHAWPDRRRWNQTCELLPV<br>SQASWACNLILGAPDSQKLTTVDIVTLRVLCREGV<br>RWRVMAIQDFKPFENLRLMAPISLQVVHVETHRSN<br>ISWEISQASHYFERHLEFEARTLSPGHTWEEAPLLTL<br>KQKQEWISLETLTPDTQYEFQVRVKPLQGEFTT<br>WSPWSQPLAFRTKPAALGKD | |
| | 2nd polypeptide chain:<br><br>Fc(knob)-IL15 V1 Non-cleavable (N71Q, N79Q) | DKTHTCPPCPAPELLGGPSVFLFPPKPKDTL<br>MISRTPEVTCVVVDVSHEDPEVKFNWYVD<br>GVEVHNAKTKPREEQYASTYRVVSVLTVL<br>HQDWLNGKEYKCKVSNKALPAPIEKTISKA<br>KGQPREPQVYTLPPCRDELTKNQVSLWCLV<br>KGFYPSDIAVEWESNGQPENNYKTTPPVLD<br>SDGSFFLYSKLTVDKSRWQQGNVFSCSVMHE<br>ALHNHYTQKSLSLSPGGGSSPPGGGSSGGGSG<br>PSGSPGNWVNVISDLKKIEDLIQSMHIDATLY<br>TESDVHPSCKVTAMKCFLLELQVISLESGDASI<br>HDTVENLIILAQNSLSSNGQVTESGCKECEEL<br>EEKNIKEFLQSFVHIVQMFINTS | DNA90<br>4 |
| AK93<br>0 | 1st polypeptide chain:<br>Fc(Hole) CD122(C122S, C168S) | DKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPE<br>VTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPRE<br>EQYASTYRVVSVLTVLHQDWLNGKEYKCKVSNKAL<br>PAPIEKTISKAKGQPREPQVCTLPPSRDELTKNQVSLS<br>CAVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDG<br>SFFLVSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQ<br>KSLSLSPGPGSGSAVNGTSQFTCFYNSRANISCVWSQ<br>DGALQDTSCQVHAWPDRRRWNQTCELLPVSQASW<br>ACNLILGAPDSQKLTTVDIVTLRVLCREGVRWRVMA<br>IQDFKPFENLRLMAPISLQVVHVETHRSNISWEISQAS<br>HYFERHLEFEARTLSPGHTWEEAPLLTLKQKQEWIS<br>LETLTPDTQYEFQVRVKPLQGEFTTWSPWSQPLAF<br>RTKPAALGKD | DNA44<br>0 |
| | 2nd polypeptide chain:<br><br>Fc(knob)- | DKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISR<br>TPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNA<br>KTKPREEQYASTYRVVSVLTVLHQDWLNGKEY<br>KCKVSNKALPAPIEKTISKAKGQPREPQVYTLPP | DNA92<br>2 |

| | | | |
|---|---|---|---|
| | [ISSGLLSGRS]-IL15 (N71Q,N79Q)<br><br>*cleavable peptide bolded | CRDELTKNQVSLWCLVKGFYPSDIAVEWESNGQ PENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQ GNVFSCSVMHEALHNHYTQKSLSLSPGGGSSGGS **PISSGLLSGRS**SGPGSGSNWVNVISDLKKIEDLIQSMH IDATLYTESDVHPSCKVTAMKCFLLELQVISLESGDA SIHDTVENLIILAQNSLSSNGQVTESGCKECEELE EKNIKEFLQSFVHIVQMFINTS | |
| AK936 | 1st polypeptide chain:<br><br>Fc(hole)-[ISSGLLSGRS]-CD122 | DKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTP EVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTK PREEQYASTYRVVSVLTVLHQDWLNGKEYKCKVS NKALPAPIEKTISKAKGQPREPQVCTLPPSRDELTKN QVSLSCAVKGFYPSDIAVEWESNGQPENNYKTTPPV LDSDGSFFLVSKLTVDKSRWQQGNVFSCSVMHEAL HNHYTQKSLSLSPGGPPSGSSP**ISSGLLSGRS**SGGGAV NGTSQFTCFYNSRANISCVWSQDGALQDTSCQVHAW PDRRRWNQTCELLPVSQASWACNLILGAPDSQKLT TVDIVTLRVLCREGVRWRVMAIQDFKPFENLRLMA PISLQVVHVETHRSNISWEISQASHYFERHLEFEAR TLSPGHTWEEAPLLTLKQKQEWISLETLTPDTQYE FQVRVKPLQGEFTTWSPWSQPLAFRTKPAALGKD | DNA823 |
| | 2nd polypeptide chain:<br><br>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.

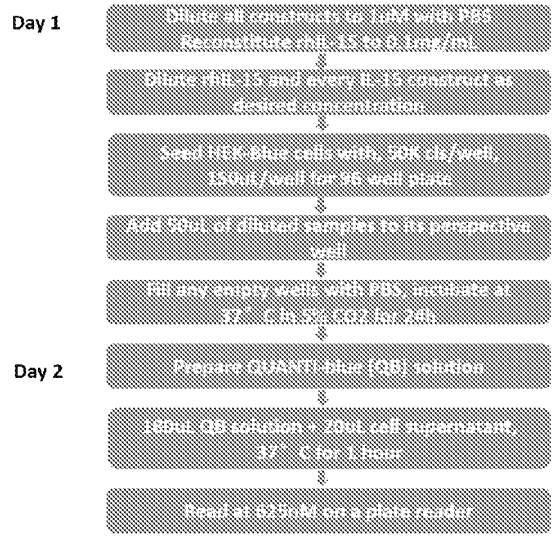
**Cleavage was achieved for masked constructs including a peptide substrate.**

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 66A-D. The HEK-Blue IL-2 bioassay results are shown in Figures 67A-F.

**Example 16**

The following molecules were used in this example:

| | |
|---|---|
| rhIL-15 | control |
| AK507 | unmasked, with non-covalently bound IL-15Rα (sushi) domain |
| AK563 | masked, no sushi |
| AK564 | masked, with non-covalently bound IL-15Rα (sushi) domain |

Sequence information for these molecules is found in Section 10.

Surface Plasmin Resonance (SPR) studies were carried out to determine i) masking of IL-15 binding to CD122 and ii) sushi domain attachment to IL-15 constructs. Details of the studies is set out below:

**Assay Principle:**

Surface Plasmon Resonance (SPR) assay is performed to test the kinetic feature of the IL15 constructs binding to CD215 and CD122. The multi-cycle kinetics assay is performed using amine coupling method.

**Reagent List:**

| Reagent | Cat No. | Brand |
|---|---|---|
| HEPES | V900477-500G | Sigma |
| NaCL | 71376-1KG | Sigma |
| Tween-20 | P9416-100ML | Sigma |
| TCEP | S16054-100G | Yuanye biology |
| 96-well plate | 650201 | Greiner |
| 96 Microplate foils | 28975816 | GE |
| 96-well plate-1ML | 780201 | Greiner |
| Series S Sensor Chip CM5 | 29-1496-03 | GE Healthcare |
| Glycine2.0 | BR-1003-55 | GE Healthcare |
| Amine coupling kit | BR-1006-33 | GE Healthcare |
| Acetate 5.5 | BR-1003-52 | GE Healthcare |
| Acetate 5.0 | BR-1003-51 | GE Healthcare |
| Recombinant Human IL-15R alpha Fc chimera Protein(CD215) | 147-IR-100 | R&D |
| Recombinant Human IL-15 Protein | 247-ILB-025 | R&D |
| Recombinant Human IL-2 R beta Protein(CD122) | 224-2B-025 | R&D |

**Instrument:** **biacore 8k**

**Procedure:**

**Immobilization**

1. Dilute the proteins at 20 ug/mL in 10 mM Sodium Acetate for immobilization.

2. Thaw out EDC and NHS aliquots from -20°C just before running. Use the 1:1 mix to activate chip surface of channel, flow rate10 ul/min, contact time 420s.
3. Inject the diluted proteins until the desired loading amounts are achieved.
4. The surface of the chip was blocked with Ethanolamine-HCL, contact time 420s、flow rate 10ul/min.

Example

| Step | Reagents | Flowcell | Conditions | Immobilization level (RU) |
|---|---|---|---|---|
| Activation | EDC+NHS | Fc1 &2 | 420s @10ul/min | |
| Immobilization | CD122 | Fc2 | 20 ug/ml in acetate5.5, 300s @2ul/min | 540-740 |
| | CD215 | Fc2 | 5ug/ml in acetate5.5, 300s @10ul/min | 1500-2000 |
| | IL15 | Fc2 | 30ug/ml in acetate5.0, 300s, @2ul/min | 3000 |
| Block | Ethanolamine-HCl | Fc1 &2 | 420s @10ul/min | |

**Binding test**

- Running buffer: 20mM HEPES, 300mM NaCL, 0.1% Tween 20, 1mM TCEP
- Dilute the analyte with running buffer to the top dose
- Make a minimum of 8 serial dilutions (2-fold) in running buffer
- Flow samples from low to high concentrations
- Run experiment at 25 °C, flow rate 60 ul/min
- Repeated one concentration in the middle concentration at the end of the run.

Example

| **Ligand** | **Assoc. Time(s)** | **Dissoc. Time(s)** | **Dose** | **Regen ID** | **Regen Time(s)** | **Wait Time(s)** |
|---|---|---|---|---|---|---|
| CD122 | 180 | 500 | Top 500 nM, 2x dilution | NA | NA | NA |
| CD215 | 180 | 600 | Top 40 nM, 2x dilution | 10 mM Glycine pH 2.0 | 30 | 90 |

**Data analysis:**

Data is analyzed using Biacore Insight Evaluation Software (GE Healthcare).

Results are shown in Figures 68A-D (i) and Figures 69A-D (ii).

*i) masking of IL-15 binding to CD122 (ligand = human CD122), where no binding to the ligand*

*indicates successful masking*

rhIL-15 showed binding of IL-15 to CD122
AK507 showed binding of IL-15 to CD122
AK563 showed no binding of IL-15 to CD122
AK564 showed no binding of IL-15 to CD122

*ii) sushi domain attachment to IL-15 constructs (ligand = human CD215), where no binding to the ligand indicates successful attachment of sushi domain*

rhIL-15 showed binding of IL-15 to CD215
AK507 showed no binding of IL-15 to CD215
AK563 showed binding of IL-15 to CD215
AK564 showed no binding of IL-15 to CD215

## 10. SEQUENCES

Table 1:

| Component | SEQ ID NO | Sequence | DC |
|---|---|---|---|
| hIL-15 (precursor) | 1 | MRISKPHLRSISIQCYLCLLLNSHFLTEAGIHVFILGCFSAGLPKTEANWVN<br>VISDLKKIEDLIQSMHIDATLYTESDVHPSCKVTAMKCFLLELQVISLESGD<br>ASIHDTVENLIILANNSLSSNGNVTESGCKECEELEEKNIKEFLQSFVHIVQM<br>FINTS | |
| hIL15 | 2 | NWVNVISDLKKIEDLIQSMHIDATLYTESDVHPSCKVTAMKCFLLELQVISL<br>ESGDASIHDTVENLIILANNSLSSNGNVTESGCKECEELEEKNIKEFLQSFVH<br>IVQMFINTS | AK401<br>AK402<br>AK403<br>AK481<br>AK482<br>AK483<br>AK478<br>AK479<br>AK480<br>AK242<br>AK243<br>AK247<br>AK248<br>AK245<br>AK250<br>AK419<br>AK246 |

| | | | |
|---|---|---|---|
| | | | AK251<br>AK420<br>AK421<br>AK457<br>AK399<br>AK404<br>AK405<br>AK400<br>AK244<br>AK249<br>AK418<br>AK507<br>AK564 |
| hIL15(D22A) | 3 | NWVNVISDLKKIEDLIQSMHIAATLYTESDVHPSCKVTAMKCFLLELQVISLESGD<br>ASIHDTVENLIILANNSLSSNGNVTESGCKECEELEEKNIKEFLQSFVHIVQMFINTS | AK458 |
| hIL15(E46A) | 4 | NWVNVISDLKKIEDLIQSMHIDATLYTESDVHPSCKVTAMKCFLLALQVISLESGDA<br>SIHDTVENLIILANNSLSSNGNVTESGCKECEELEEKNIKEFLQSFVHIVQMFINTS | AK459 |
| hIL15(E46A, E53A) | 5 | NWVNVISDLKKIEDLIQSMHIDATLYTESDVHPSCKVTAMKCFLLALQVISLASGD<br>ASIHDTVENLIILANNSLSSNGNVTESGCKECEELEEKNIKEFLQSFVHIVQMFINTS | AK461<br><br>AK527<br>AK506 |
| hIL15(E46R, E53R) | 6 | NWVNVISDLKKIEDLIQSMHIDATLYTESDVHPSCKVTAMKCFLLRLQVISLRSGD<br>ASIHDTVENLIILANNSLSSNGNVTESGCKECEELEEKNIKEFLQSFVHIVQMFINTS | not named yet-2 |
| hIL15(E46S, E53S) | 7 | NWVNVISDLKKIEDLIQSMHIDATLYTESDVHPSCKVTAMKCFLLSLQVISLSSGD<br>ASIHDTVENLIILANNSLSSNGNVTESGCKECEELEEKNIKEFLQSFVHIVQMFINTS | not named yet-1 |

| | | | |
|---|---|---|---|
| hIL15(E53A) | 8 | NWVNVISDLKKIEDLIQSMHIDATLYTESDVHPSCKVTAMKCFLLELQVISLASGD<br>ASIHDTVENLIILANNSLSSNGNVTESGCKECEELEEKNIKEFLQSFVHIVQMFINTS | AK460 |
| hIL-15 (N-ter) | 9 | NWVNVISDLKKIEDLIQS | not named yet-3<br>not named yet-4 |
| hIL15 (C-ter) | 10 | KVTAMKCFLLELQVISLESGDASIHDTVENLIILANNSLSSNGNVTESGCKECEELEE<br>KNIKEFLQSFVHIVQMFINTS | not named yet-3<br>not named yet-4 |
| hIL-15 (N71Q) | 127 | NWVNVISDLKKIEDLIQSMHIDATLYTESDVHPSCKVTAMKCFLLELQVISLESGD<br>ASIHDTVENLIILAQNSLSSNGNVTESGCKECEELEEKNIKEFLQSFVHIVQMFINTS | AK595<br>AK596 |
| hIL-15 (N79Q) | 128 | NWVNVISDLKKIEDLIQSMHIDATLYTESDVHPSCKVTAMKCFLLELQVISLESGD<br>ASIHDTVENLIILANNSLSSNGQVTESGCKECEELEEKNIKEFLQSFVHIVQMFINTS | AK901<br>AK907 |
| hIL-15 (N112Q) | 129 | NWVNVISDLKKIEDLIQSMHIDATLYTESDVHPSCKVTAMKCFLLELQVISLESGD<br>ASIHDTVENLIILANNSLSSNGNVTESGCKECEELEEKNIKEFLQSFVHIVQMFIQTS | AK900<br>AK906 |
| hIL-15 (N71Q, N79Q) | 130 | NWVNVISDLKKIEDLIQSMHIDATLYTESDVHPSCKVTAMKCFLLELQVISLESGD<br>ASIHDTVENLIILAQNSLSSNGQVTESGCKECEELEEKNIKEFLQSFVHIVQMFINTS | AK904<br>AK910<br>AK929<br>AK935<br>AK931<br>AK937<br>AK934<br>AK940<br>AK933<br>AK939<br>AK932<br>AK938 |

| | | | |
|---|---|---|---|
| | | | AK930<br>AK936 |
| hIL-15 (N71Q, N112Q) | 131 | NWVNVISDLKKIEDLIQSMHIDATLYTESDVHPSCKVTAMKCFLLELQVISLESGD<br>ASIHDTVENLIILAQNSLSSNGNVTESGCKECEELEEKNIKEFLQSFVHIVQMFIQTS | AK903<br>AK909 |
| hIL-15 (N79Q, N112Q) | 132 | NWVNVISDLKKIEDLIQSMHIDATLYTESDVHPSCKVTAMKCFLLELQVISLESGD<br>ASIHDTVENLIILANNSLSSNGQVTESGCKECEELEEKNIKEFLQSFVHIVQMFIQTS | AK902<br>AK908 |
| hIL-15 (N71Q, N79Q, N112Q) | 133 | NWVNVISDLKKIEDLIQSMHIDATLYTESDVHPSCKVTAMKCFLLELQVISLESGD<br>ASIHDTVENLIILAQNSLSSNGQVTESGCKECEELEEKNIKEFLQSFVHIVQMFIQTS | AK905<br>AK911 |

**Table 2:**

| Component | | SEQ ID NO | Sequence | DC |
|---|---|---|---|---|
| Masking moiety (MM) | hCD122 | 11 | AVNGTSQFTCFYNSRANISCVWSQDGALQDTSCQVHAWPDRRRWNQTCELLPVSQ<br>ASWACNLILGAPDSQKLTTVDIVTLRVLCREGVRWRVMAIQDFKPFENLRLMAPISLQ<br>VVHVETHRCNISWEISQASHYFERHLEFEARTLSPGHTWEEAPLLTLKQKQEWICLETLT<br>PDTQYEFQVRVKPLQGEFTTWSPWSQPLAFRTKPAALGKD | AK247<br>AK248<br>AK421<br>AK457<br>AK249<br>AK418<br>AK250<br>AK251 |

| | | | | |
|---|---|---|---|---|
| AK399 | | | | |
| AK400 | | | | |
| AK404 | | | | |
| AK405 | | | | |
| AK419 | | | | |
| AK420 | | | | |
| AK401 | | | | |
| AK402 | | | | |
| AK403 | | | | |
| AK458 | | | | |
| AK459 | | | | |
| AK460 | | | | |
| AK461 | | | | |
| AK478 | | | | |
| AK479 | | | | |
| AK480 | | | | |
| AK481 | | | | |
| AK482 | | | | |
| AK483 | | | | |
| AK527 | | | | |
| not named yet-3 | | | | |
| not named yet-4 | | | | |
| AK564 | AVNGTSQFTCFYNSRANISCVWSQDGALQDTSCQVHAWPDRRRWNQTCELLPVSQASW | 12 | hCD122 C122S, C168S) | |
| AK596 | ACNLILGAPDSQKLTTVDIVTLRVLCREGVRWRVMAIQDFKPFENLRLMAPISLQVVHVETH | | | |
| AK900 | RSNISWEISQASHYFERHLEFEARTLSPGHTWEEAPLLTLKQKQEWISLETLTPDTQYEFQVR | | | |
| AK901 | VKPLQGEFTTWSPWSQPLAFRTKPAALGKD | | | |
| AK902 | | | | |
| AK903 | | | | |

| | | | |
|---|---|---|---|
| | | | AK904 |
| | | | AK905 |
| | | | AK906 |
| | | | AK907 |
| | | | AK908 |
| | | | AK909 |
| | | | AK910 |
| | | | AK911 |
| | | | AK929 |
| | | | AK935 |
| | | | AK931 |
| | | | AK937 |
| | | | AK934 |
| | | | AK940 |
| | | | AK933 |
| | | | AK939 |
| | | | AK932 |
| | | | AK938 |
| | | | AK930 |
| | | | AK936 |

**Table 3:**

| Component | | SEQ ID NO | Sequence | Molecule |
|---|---|---|---|---|
| Non-cleavable linker | (a) | 13 | GGGGSGGGGS | not named yet-3 |
| | | 14 | GGGGSGGGGSGGGGSGGGGS | AK245<br>AK246<br>AK250<br>AK251 |

| | | | | |
|---|---|---|---|---|
| | | | | AK419<br>AK420<br>AK478<br>AK479<br>AK480<br>AK481<br>AK482<br>AK483 |
| | 15 | GGGGSGGGGSGGGGSGGGGSGGGGS | AK399 | |
| | | | AK400 | |
| | 16 | GGGGSGGGGSGGS | not named yet-4 | |
| | 17 | GGSGGGSGGGSGGGSGGGSG | AK404 | |
| | 18 | GGSGGGSGGGSGGGSGGGSGGGSG | AK405 | |
| | 19 | SGGGGSGGGSGGGGSGGGGS | AK401 | |
| | | | AK402 | |
| | | | AK403 | |
| (HL) | 20 | GGSSPPGGGSSGGSGGPGSGS | AK418 | |
| | 21 | GGSSPPGGGSSGGGSGGPSGSPG | AK244 | |
| | | | AK249 | |
| | | | AK506 | |
| | | | AK507 | |
| | | | AK527 | |
| | | | not named yet-1 | |
| | | | not named yet-2 | |
| | | | AK564 | |
| | | | AK595 | |
| | | | AK596 | |

| | | | | |
|---|---|---|---|---|
| | | | | AK900<br>AK901<br>AK902<br>AK903<br>AK904<br>AK905<br>AK906<br>AK907<br>AK908<br>AK909<br>AK910<br>AK911<br>AK935<br>AK937<br>AK939<br>AK940<br>AK938<br>AK936 |
| | | 22 | PGSGS | AK247<br>AK248<br>AK249<br>AK250<br>AK251<br>AK399<br>AK400<br>AK401<br>AK402<br>AK403<br>AK404<br>AK405<br>AK418<br>AK419<br>AK420 |

| | | | |
|---|---|---|---|
| AK421 | | | |
| AK457 | | | |
| AK458 | | | |
| AK459 | | | |
| AK460 | | | |
| AK461 | | | |
| AK478 | | | |
| AK479 | | | |
| AK480 | | | |
| AK481 | | | |
| AK482 | | | |
| AK483 | | | |
| not named yet-3 | | | |
| not named yet-4 | | | |
| AK564 | | | |
| AK596 | | | |
| AK906 | | | |
| AK907 | | | |
| AK908 | | | |
| AK909 | | | |
| AK910 | | | |
| AK911 | | | |
| AK929 | | | |
| AK931 | | | |
| AK934 | | | |
| AK933 | | | |
| AK932 | | | |
| AK930 | | | |

Table 4:

| Component | SEQ ID NO | Sequence | BC |
|---|---|---|---|
| N terminal spacer domain | 23 | GGGSSP | AK402 |
| | 24 | GGSSPP | AK481 |
| | | | AK242 |
| | | | AK243 |
| | | | AK247 |
| | | | AK248 |
| | | | AK245 |
| | | | AK250 |
| | | | AK419 |
| | | | AK246 |
| | | | AK251 |
| | | | AK420 |
| | | | AK421 |
| | | | AK399 |
| | | | AK404 |
| | | | AK405 |
| | | | AK400 |
| | | | not named yet-3 |
| | | | not named yet-4 |
| | | | AK934 |
| | 25 | GGSSPPG | AK457 |
| | | | AK458 |
| | | | AK459 |
| | | | AK461 |
| | | | AK460 |
| | 26 | GPPSGSSP | AK527 |

| | | | | |
|---|---|---|---|---|
| | | | | AK483<br>AK937<br>AK940<br>AK936 |
| | 197 | PGGPSP | AK401 | |
| | 28 | SGSGGSP | AK403 | |
| | 29 | SPSPPGPGSPP | AK478 | |
| | 30 | SPSSGSG | AK482 | |
| | 31 | SSGGPGSGPPSG | AK479 | |
| | 32 | SSSGSSPGGPSGPP | AK480 | |
| | 148 | PSGS | AK929 | |
| | 149 | PSGSP | AK931 | |
| | 150 | GPPSGSSPG | AK935<br>AK939 | |
| | 151 | PSSGSGSG | AK933 | |
| | 152 | GGSSGGSP | AK930 | |
| | 153 | SGS | AK932 | |
| | 154 | SGSPSG | AK938 | |
| Peptide substrate | [MPYDLYHP] | 33 | MPYD*LYHP<br>(* indicates cleavage site) | AK527<br>AK242<br>AK243<br>AK247<br>AK248<br>AK245<br>AK250<br>AK419<br>AK246<br>AK251<br>AK420<br>not named yet-3<br>not named yet-4 |

| | | | | |
|---|---|---|---|---|
| | | | | AK934<br>AK940 |
| [VPLSLY] | 34 | VPLS*LY<br><br>(* indicates cleavage site) | | AK401<br>AK402<br>AK403<br>AK481<br>AK482<br>AK483<br>AK478<br>AK479<br>AK480<br>AK421<br>AK457<br>AK458<br>AK459<br>AK461<br>AK460<br>AK399<br>AK404<br>AK405<br>AK400<br>AK933<br>AK939 |
| | | | [DSGGFMLT] | 134 | DSGG*FMLT<br><br>(*indicates cleavage site) | AK929<br>AK935 |
| | | | [HEQLTV] | 135 | HEQ*LTV<br><br>(*indicates cleavage site) | |
| | | | [RAAAVKSP] | 27 | RAAA*VKSP<br><br>(*indicates cleavage site) | AK931<br>AK937 |
| | | | [ISSGLLSGRS] | 155 | ISSGLL*SGRS | AK930 |

| | | | | (*indicates cleavage site) | AK936 |
|---|---|---|---|---|---|
| | [DLLAVVAAS] | 156 | DLLA*VVAAS<br>(*indicates cleavage site) | AK932<br>AK938 |
| C terminal spacer domain | | 35 | GSGPGSGS | AK457<br>AK458<br>AK459<br>AK461<br>AK460 |
| | | 36 | SGG | AK481<br>AK482<br>AK483<br>AK478<br>AK479<br>AK480 |
| | | 37 | SGGG | AK527<br>AK937<br>AK940<br>AK936 |
| | | 38 | SGGP | AK401<br>AK402<br>AK403 |
| | | 39 | SGPGSGS | AK243<br>AK248<br>AK419<br>AK420<br>AK421<br>AK399<br>AK400<br>not named yet-3<br>not named yet-4 |

| | | | |
|---|---|---|---|
| | | | AK929<br>AK931<br>AK934<br>AK930 |
| | 40 | SGPSGSPG | AK242<br>AK247<br>AK245<br>AK250<br>AK246<br>AK251<br>AK404<br>AK405 |
| | 157 | SGGSGPSGS | AK935 |
| | 158 | SGSGGG | AK939 |
| | 159 | SGSGPGSGS | AK933 |
| | 160 | SGPGSG | AK932 |
| | 161 | SGPGSGSP | AK938 |
| Cleavable linker | 41 | GGGSSPVPLSLYSGGP | AK402 |
| | 198 | GGSSPPGVPLSLYGSGPGSGS | AK457<br>AK458<br>AK459<br>AK461<br>AK460 |
| | 42 | GGSSPPMPYDLYHPSGPGSGS | AK243<br>AK248<br>AK419<br>AK420<br>not named yet-3<br>not named yet-4<br>AK934 |
| | 43 | GGSSPPMPYDLYHPSGPSGSPG | AK242 |

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| | | AK247<br>AK245<br>AK250<br>AK246<br>AK251 |
| 44 | GGSSPPVPLSLYSGG | AK481 |
| 45 | GGSSPPVPLSLYSGPGSGS | AK421<br>AK399<br>AK400 |
| 46 | GGSSPPVPLSLYSGPSGSPG | AK404<br>AK405 |
| 47 | GPPSGSSPMPYDLYHPSGGG | AK527<br>AK940 |
| 48 | GPPSGSSPVPLSLYSGG | AK483 |
| 49 | PGGPSPVPLSLYSGGP | AK401 |
| 50 | SGSGGSPVPLSLYSGGP | AK403 |
| 51 | SPSPPGPGSPPVPLSLYSGG | AK478 |
| 52 | SPSSGSGVPLSLYSGG | AK482 |
| 53 | SSGGPGSGPPSGVPLSLYSGG | AK479 |
| 54 | SSSGSSPGGPSGPPVPLSLYSGG | AK480 |
| 162 | PSGSDSGGFMLTSGPGSGS | AK929 |
| 163 | PSGSPRAAAVKSPSGPGSGS | AK931 |
| 164 | GPPSGSSPGDSGGFMLTSGGSGPSGS | AK935 |
| 165 | GPPSGSSPRAAAVKSPSGGG | AK937 |
| 166 | PSSGSGSGVPLSLYSGSGPGSGS | AK933 |
| 167 | GPPSGSSPGVPLSLYSGSGGG | AK939 |
| 168 | GGSSGGSPISSGLLSGRSSGPGSGS | AK930 |
| 169 | SGSDLLAVVAASSGPGSG | AK932 |
| 170 | GPPSGSSPISSGLLSGRSSGGG | AK936 |

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|---|---|---|
| 171 | SGSPSGDLLAVVAASSGPGSGSP | AK938 |

**Table 5:**

| Component | | SEQ ID NO | Sequence | DC |
|---|---|---|---|---|
| a subunit | hCD215 | 55 | ITCPPPMSVEHADIWVKSYSLYSRERYICNSGFKRKAGTSSLTECVLN<br>KATNVAHWTTPSLKCIRDPALVHQRPAPPSTVTTAGVTPQPESLSP<br>SGKEPAASSPSSNNTAATTAAIVPGSQLMPSKSPSTGTTEISSHESSH<br>GTPSQTTAKNWELTASASHQPPGVYPQGHSDTT | AK245<br>AK250<br>AK399<br>AK404<br>AK405<br>AK419 |
| | hCD215(1to66) | 56 | ITCPPPMSVEHADIWVKSYSLYSRERYICNSGFKRKAGTSSLTECVLNK<br>ATNVAHWTTPSLKCIRD | AK481<br>AK482<br>AK483<br>AK507<br>not named yet-3<br>not named yet-4<br>AK564<br>AK595<br>AK596<br>AK900<br>AK901<br>AK902<br>AK903 |

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| | | | | AK904 |
| | | | | AK905 |
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| | | | | AK931 |
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| | | | | AK937 |
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| | | | | AK933 |
| | | | | AK939 |
| | | | | AK930 |
| | | | | AK932 |
| | | | | AK936 |
| | | | | AK938 |
| hCD215(1to66) R26N | 172 | ITCPPPMSVEHADIWVKSYSLYSRENYICNSGFKRKAGTSSLTECVLNK ATNVAHWTTPSLKCIRD | | |
| hCD215(1to66) R35Q | 173 | ITCPPPMSVEHADIWVKSYSLYSRERYICNSGFKQKAGTSSLTECVLNK ATNVAHWTTPSLKCIRD | | |
| hCD215(1to66) R26S | 174 | ITCPPPMSVEHADIWVKSYSLYSRESYICNSGFKRKAGTSSLTECVLNK ATNVAHWTTPSLKCIRD | | |
| hCD215(1to66) R35S | 175 | ITCPPPMSVEHADIWVKSYSLYSRERYICNSGFKSKAGTSSLTECVLNK ATNVAHWTTPSLKCIRD | | |
| hCD215(1to66) R26N; R35Q | 176 | ITCPPPMSVEHADIWVKSYSLYSRENYICNSGFKQKAGTSSLTECVLNK ATNVAHWTTPSLKCIRD | | |
| hCD215(Sushi) | 57 | ITCPPPMSVEHADIWVKSYSLYSRERYICNSGFKRKAGTSSLTECVLNKATN VAHWTTPSLKCIRDPALVHQRPAPP | | AK401 AK402 AK403 |

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|---|---|---|---|---|
| | | | | AK478<br>AK479<br>AK480 |
| hCD215(Truncated) | 58 | ITCPPPMSVEHADIWVKSYSLYSRERYICNSGFKRKAGTSSLTECVLNKATNV<br>AHWTTPSLKCIRDPALVHQRPAPPSTVTTAGVTPQPESLSPSGKEPAAS | | AK246<br><br>AK251<br>AK400<br>AK420 |
| | | | | |

**Table 6:**

| Component | | SEQ ID NO | Sequence | Molecule |
|---|---|---|---|---|
| Human IgG1 Immunoglobulin heavy constant gamma 1 (parent sequence) | | 59 | ASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSG<br>VHTFPAVLQSSGLYSLSSVVTVPSSSLGTQTYICNVNHKPSNTKVDKKVE<br>PKSCDKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVS<br>HEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLN<br>GKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSRDELTKNQVSL<br>TCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKS<br>RWQQGNVFSCSVMHEALHNHYTQKSLSLSPGK | |
| Human IgG1 Immunoglobulin heavy constant gamma 1 | | 60 | DKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHED<br>PEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGK<br>EYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSRDELTKNQVSLTCL<br>VKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRW<br>QQGNVFSCSVMHEALHNHYTQKSLSLSPG | |

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|---|---|---|---|
| 1st Half-life extension domain (Y349C; T366S; L38A; and Y407V) | 61 | DKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHE DPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWL NGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVCTLPPSRDELTKNQ VSLSCAVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLVSKL TVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPG | |
| 2nd Half-life extension domain (S354C and T366W) | | 62 | DKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSH EDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDW LNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPCRDELTK NQVSLWCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFL YSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPG | |
| 1st Half-life extension domain (Y349C; T366S; L38A; Y407V and N297A) | Hole: hFc(N297A) | 63 | DKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHED PEVKFNWYVDGVEVHNAKTKPREEQYASTYRVVSVLTVLHQDWLNGK EYKCKVSNKALPAPIEKTISKAKGQPREPQVCTLPPSRDELTKNQVSLSCA VKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLVSKLTVDKSRWQ QGNVFSCSVMHEALHNHYTQKSLSLSPG | AK242<br>AK243<br>AK244<br>AK245<br>AK246<br>AK247<br>AK248<br>AK421<br>AK457<br>AK249<br>AK418<br>AK250<br>AK251<br>AK399<br>AK400<br>AK404<br>AK405<br>AK419<br>AK420 |

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|---|---|---|---|
| AK401 | | | |
| AK402 | | | |
| AK403 | | | |
| AK458 | | | |
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| AK478 | | | |
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| AK481 | | | |
| AK482 | | | |
| AK483 | | | |
| AK507 | | | |
| AK527 | | | |
| AK506 | | | |
| not named yet-3 | | | |
| not named yet-4 | | | |
| not named yet-1 | | | |
| not named yet-2 | | | |
| AK564 | | | |
| AK595 | | | |
| AK596 | | | |
| AK900 | | | |
| AK901 | | | |

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| | | | DKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHE<br>DPEVKFNWYVDGVEVHNAKTKPREEQYASTYRVVSVLTVLHQDWLN<br>GKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPCRDELTKNQVS<br>LWCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTV<br>DKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPG | AK902<br>AK903<br>AK904<br>AK905<br>AK906<br>AK907<br>AK908<br>AK909<br>AK910<br>AK911<br>AK929<br>AK931<br>AK934<br>AK935<br>AK937<br>AK940<br>AK933<br>AK939<br>AK930<br>AK932<br>AK936<br>AK938 |
| | | 64 | | |
| | Knob: hFc(N297A) | | | |
| 2nd Half-life extension domain (S354C, T366W and N297A) | | | | |

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|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| AK250 | AK251 | AK399 | AK400 | AK404 | AK405 | AK419 | AK420 | AK401 | AK402 | AK403 | AK458 | AK459 | AK460 | AK461 | AK478 | AK479 | AK480 | AK481 | AK482 | AK483 | AK507 | AK527 | AK506 | not named yet-3 | not named yet-4 |
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| | | | | not named yet-1<br>not named yet-2<br>AK564<br>AK595<br>AK596<br>AK900<br>AK901<br>AK902<br>AK903<br>AK904<br>AK905<br>AK906<br>AK907<br>AK908<br>AK909<br>AK910<br>AK911<br>AK929<br>AK931<br>AK934<br>AK935<br>AK937<br>AK940<br>AK933<br>AK939<br>AK930<br>AK932<br>AK936<br>AK938 |

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|---|---|---|---|
| 1st Half-life extension domain (Y349C; T366S; L38A, Y407V, N297A and I253A) | 65 | DKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMASRTPEVTCVVVDVSHEDP EVKFNWYVDGVEVHNAKTKPREEQYASTYRVVSVLTVLHQDWLNGKE YKCKVSNKALPAPIEKTISKAKGQPREPQVCTLPPSRDELTKNQVSLSCA VKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLVSKLTVDKSRW QQGNVFSCSVMHEALHNHYTQKSLSLSPG | |
| 2nd Half-life extension domain (S354C, T366W, N297A and I253A) | 66 | DKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMASRTPEVTCVVVDVSHEDP EVKFNWYVDGVEVHNAKTKPREEQYASTYRVVSVLTVLHQDWLNGKEY KCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPCRDELTKNQVSLWCLVK GFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRW QQGNVFSCSVMHEALHNHYTQKSLSLSPG | |

**Table 7:**

| Component | SEQ ID NO | Sequence | DC | |
|---|---|---|---|---|
| Hole: hFc(N297A) | 67 | DKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEV KFNWYVDGVEVHNAKTKPREEQYASTYRVVSVLTVLHQDWLNGKEYKCKV SNKALPAPIEKTISKAKGQPREPQVCTLPPSRDELTKNQVSLSCAVKGFYPSD IAVEWESNGQPENNYKTTPPVLDSDGSFFLVSKLTVDKSRWQQGNVFSCSV MHEALHNHYTQKSLSLSPGK | AK242 | DNA158 |
| | | | AK243 | |
| | | | AK244 | |
| | | | AK245 | |
| | | | AK246 | |
| | | | AK506 | |
| | | | AK507 | |
| | | | not named yet-1 | |
| | | | not named yet-2 AK595 | |

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|---|---|---|---|---|
| | | AK900<br>AK901<br>AK902<br>AK903<br>AK904<br>AK905 | | DNA187 |
| Hole: hFc(N297A)-hCD122 | 68 | AK247 | DKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNW<br>YVDGVEVHNAKTKPREEQYASTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEK<br>TISKAKGQPREPQVCTLPPSRDELTKNQVSLSCAVKGFYPSDIAVEWESNGQPENN<br>YKTTPPVLDSDGSFFLVSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPG<br>PGSGSAVNGTSQFTCFYNSRANISCVWSQDGALQDTSCQVHAWPDRRRWNQTCELL<br>PVSQASWACNLILGAPDSQKLTTVDIVTLRVLCREGVRWRVMAIQDFKPFENLRLM<br>APISLQVVHVETHRCNISWEISQASHYFERHLEFEARTLSPGHTWEEAPLLTLKQKQ<br>EWICLETLTPDTQYEFQVRVKPLQGEFTTWSPWSQPLAFRTKPAALGKD | |
| | | AK248 | | |
| | | AK249 | | |
| | | AK250 | | |
| | | AK251 | | |
| | | AK399 | | |
| | | AK400 | | |
| | | AK404 | | |
| | | AK405 | | |
| | | AK418 | | |
| | | AK419 | | |
| | | AK420 | | |
| | | AK421 | | |
| | | AK457 | | |
| | | AK458 | | |
| | | AK459 | | |
| | | AK460 | | |
| | | AK461 | | |
| | | not named yet-3 | | |
| | | not named yet-4 | | |

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|---|---|---|---|---|
| Hole: hFc(N297A)-[MPYDLYHP]-hCD122 | 69 | DKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFN WYVDGVEVHNAKTKPREEQYASTYRVVSVLTVLHQDWLNGKEYKCKVSNKALP APIEKTISKAKGQPREPQVCTLPPSRDELTKNQVSLSCAVKGFYPSDIAVEWESNG QPENNYKTTPPVLDSDGSFFLVSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQ KSLSLSPGGPPSGSPMPYDLYHPSGGGAVNGTSQFTCFYNSRANISCVWSQDGA LQDTSCQVHAWPDRRRWNQTCELLPVSQASWACNLILGAPDSQKLTTVDIVTLR VLCREGVRWRVMAIQDFKPFENLRLMAPISLQVVHVETHRCNISWEISQASHYFERH LEFEARTLSPGHTWEEAPLLTLKQKQEWICLETLTPDTQYEFQVRVKPLQGEFTTWSP WSQPLAFRTKPAALGKD | AK527 | DNA37 0 |
| Hole: hFc(N297A)-hCD122-[VPLSLY]-hCD215(Sushi)-hIL15 | 70 | DKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKF NWYVDGVEVHNAKTKPREEQYASTYRVVSVLTVLHQDWLNGKEYKCKVSNKAL PAPIEKTISKAKGQPREPQVCTLPPSRDELTKNQVSLSCAVKGFYPSDIAVEWESN GQPENNYKTTPPVLDSDGSFFLVSKLTVDKSRWQQGNVFSCSVMHEALHNH YTQKSLSLSPGPGSGSAVNGTSQFTCFYNSRANISCVWSQDGALQDTSCQVHA WPDRRRWNQTCELLPVSQASWACNLILGAPDSQKLTTVDIVTLRVLCREGVRW RVMAIQDFKPFENLRLMAPISLQVVHVETHRCNISWEISQASHYFERHLEFEARTLS PGHTWEEAPLLTLKQKQEWICLETLTPDTQYEFQVRVKPLQGEFTTWSPWSQPL AFRTKPAALGKDPGGPSPVPLSLYSGGPITCPPPMSVEHADIWVKSYSLYSRERYIC NSGFKRKAGTSSLTECVLNKATNVAHWTTPSLKCIRDPALVHQRPAPPSGGGGSG GGGSGGGGSGGGGSNWVNVISDLKKIEDLIQSMHIDATLYTESDVHPSCKVTAM KCFLLELQVISLESGDASIHDTVENLIILANNSLSSNGNVTESGCKECEELEEKNIKEFL QSFVHIVQMFINTS | AK401 | DNA52 3 |
| | 71 | DKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFN WYVDGVEVHNAKTKPREEQYASTYRVVSVLTVLHQDWLNGKEYKCKVSNKALP APIEKTISKAKGQPREPQVCTLPPSRDELTKNQVSLSCAVKGFYPSDIAVEWESN GQPENNYKTTPPVLDSDGSFFLVSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQ KSLSLSPGPGSGSAVNGTSQFTCFYNSRANISCVWSQDGALQDTSCQVHAWP DRRRWNQTCELLPVSQASWACNLILGAPDSQKLTTVDIVTLRVLCREGVRWRV MAIQDFKPFENLRLMAPISLQVVHVETHRCNISWEISQASHYFERHLEFEARTLSPG HTWEEAPLLTLKQKQEWICLETLTPDTQYEFQVRVKPLQGEFTTWSPWSQPLAFRT KPAALGKDPGGPSPVPLSLYSGGPITCPPPMSVEHADIWVKSYSLYSRERYICNSGFK RKAGTSSLTECVLNKATNVAHWTTPSLKCIRDPALVHQRPAPPSGGGGSGGGGS GGGSGGGGSNWVNVISDLKKIEDLIQSMHIDATLYTESDVHPSCKVTAMKCFLLELQ VISLESGDASIHDTVENLIILANNSLSSNGNVTESGCKECEELEEKNIKEFLQSFVHIVQMFINTS | AK402 | DNA52 4 |

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|---|---|---|---|---|
| | 72 | DKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFN<br>WYVDGVEVHNAKTKPREEQYASTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPA<br>PIEKTISKAKGQPREPQVCTLPPSRDELTKNQVSLSCAVKGFYPSDIAVEWESNGQP<br>ENNYKTTPPVLDSDGSFFLVSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSL<br>SLSPGPGSGSAVNGTSQFTCFYNSRANISCVWSQDGALQDTSCQVHAWPDRRR<br>WNQTCELLPVSQASWACNLILGAPDSQKLTTVDIVTLRVLCREGVRWRVMAIQDF<br>KPFENLRLMAPISLQVVHVETHRCNISWEISQASHYFERHLEFEARTLSPGHTWEEA<br>PLLTLKQKQEWICLETLTPDTQYEFQVRVKPLQGEFTTWSPWSQPLAFRTKPAAL<br>GKDSGSGGSPVPLSLYSGGPITCPPPMSVEHADIWVKSYSLYSRERYICNSGFKRKAG<br>TSSLTECVLNKATNVAHWTTPSLKCIRDPALVHQRPAPPSGGGSGGGSGGGSGG<br>GGGSNWVNVISDLKKIEDLIQSMHIDATLYTESDVHPSCKVTAMKCFLLELQVISLES<br>GDASIHDTVENLIILANNSLSSNGNVTESGCKECEELEEKNIKEFLQSFVHIVQMFINTS | AK403 | DNA525 |
| Hole:<br>hFc(N297A)-<br>hCD122-<br>hCD215(Sushi)-<br>[VPLSLY]-<br>hIL15 | 73 | DKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFN<br>WYVDGVEVHNAKTKPREEQYASTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAP<br>IEKTISKAKGQPREPQVCTLPPSRDELTKNQVSLSCAVKGFYPSDIAVEWESNGQP<br>ENNYKTTPPVLDSDGSFFLVSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSL<br>SLSPGPGSGSAVNGTSQFTCFYNSRANISCVWSQDGALQDTSCQVHAWPDRRR<br>WNQTCELLPVSQASWACNLILGAPDSQKLTTVDIVTLRVLCREGVRWRVMAIQD<br>FKPFENLRLMAPISLQVVHVETHRCNISWEISQASHYFERHLEFEARTLSPGHTWE<br>EAPLLTLKQKQEWICLETLTPDTQYEFQVRVKPLQGEFTTWSPWSQPLAFRTKPAA<br>LGKDGGGGSGGGGSGGGSGGGSITCPPPMSVEHADIWVKSYSLYSRERYICNS<br>GFKRKAGTSSLTECVLNKATNVAHWTTPSLKCIRDPALVHQRPAPPSPSPPGPGSPPV<br>PLSLYSGGNWVNVISDLKKIEDLIQSMHIDATLYTESDVHPSCKVTAMKCFLLELQVISL<br>ESGDASIHDTVENLIILANNSLSSNGNVTESGCKECEELEEKNIKEFLQSFVHIVQMFINTS | AK478 | DNA569 |

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| | 74 | DKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVD<br>GVEVHNAKTKPREEQYASTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKA<br>KGQPREPQVCTLPPSRDELTKNQVSLSCAVKGFYPSDIAVEWESNGQPENNYKTTPPV<br>LDSDGSFFLVSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGPGSGSAVN<br>GTSQFTCFYNSRANISCVWSQDGALQDTSCQVHAWPDRRRWNQTCELLPVSQASW<br>ACNLILGAPDSQKLTTVDIVTLRVLCREGVRWRVMAIQDFKPFENLRLMAPISLQVVHV<br>ETHRCNISWEISQASHYFERHLEFEARTLSPGHTWEEAPLLTLKQKQEWICLETLTPDTQ<br>YEFQVRVKPLQGEFTTWSPWSQPLAFRTKPAALGKDGGGSGGGGSGGGGSGGGGSI<br>TCPPPMSVEHADIWVKSYSLYSRERYICNSGFKRKAGTSSLTECVLNKATNVAHWTTPSL<br>KCIRDPALVHQRPAPPSSGGPGSGPPSGVPLSLYSGGNWVNVISDLKKIEDLIQSMHIDA<br>TLYTESDVHPSCKVTAMKCFLLELQVISLESGDASIHDTVENLILLANNSLSSNGNVTESGC<br>KECEELEEKNIKEFLQSFVHIVQMFINTS | AK479 | DNA570 |
| | 75 | DKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYV<br>DGVEVHNAKTKPREEQYASTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISK<br>AKGQPREPQVCTLPPSRDELTKNQVSLSCAVKGFYPSDIAVEWESNGQPENNYKTTPPV<br>LDSDGSFFLVSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGPGSGSAVNG<br>TSQFTCFYNSRANISCVWSQDGALQDTSCQVHAWPDRRRWNQTCELLPVSQASWAC<br>NLILGAPDSQKLTTVDIVTLRVLCREGVRWRVMAIQDFKPFENLRLMAPISLQVVHVET<br>HRCNISWEISQASHYFERHLEFEARTLSPGHTWEEAPLLTLKQKQEWICLETLTPDTQYE<br>FQVRVKPLQGEFTTWSPWSQPLAFRTKPAALGKDGGGSGGGGSGGGGSGGGGS<br>ITCPPPMSVEHADIWVKSYSLYSRERYICNSGFKRKAGTSSLTECVLNKATNVAHWTTP<br>SLKCIRDPALVHQRPAPPSSSGSSPGGPSGPPVPLSLYSGGNWVNVISDLKKIEDLIQSM<br>HIDATLYTESDVHPSCKVTAMKCFLLELQVISLESGDASIHDTVENLILLANNSLSSNGNVTE<br>SGCKECEELEEKNIKEFLQSFVHIVQMFINTS | AK480 | DNA571 |

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| Hole: hFc(N297A)-hCD122-hCD215(1to66)-[VPLSLY]-hIL15 | 76 | DKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNW YVDGVEVHNAKTKPREEQYASTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIE KTISKAKGQPREPQVCTLPPSRDELTKNQVSLSCAVKGFYPSDIAVEWESNGQPENN YKTTPPVLDSDGSFFLVSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPG PGSGSAVNGTSQFTCFYNSRANISCVWSQDGALQDTSCQVHAWPDRRRWNQTC ELLPVSQASWACNLILGAPDSQKLTTVDIVTLRVLCREGVRWRVMAIQDFKPFENLR LMAPISLQVVHVETHRCNISWEISQASHYFERHLEFEARTLSPGHTWEEAPLLTLKQK QEWICLETLTPDTQYEFQVRVKPLQGEFTTWSPWSQPLAFRTKPAALGKDGGGSG GGGSGGGGSGGGGSITCPPPMSVEHADIWVKSYSLYSRERYICNSGFKRKAGTSSLTE CVLNKATNVAHWTTPSLKCIRDGGSSPPVPLSLYSGGNWVNVISDLKKIEDLIQSMHIDA TLYTESDVHPSCKVTAMKCFLLELQVISLESGDASIHDTVENLIILANNSLSSNGNVTESGC KECEELEEKNIKEFLQSFVHIVQMFINTS | AK481 | DNA57 2 |
| | 77 | DKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVD GVEVHNAKTKPREEQYASTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAK GQPREPQVCTLPPSRDELTKNQVSLSCAVKGFYPSDIAVEWESNGQPENNYKTTPPVLDS DGSFFLVSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGPGSGSAVNGTSQF TCFYNSRANISCVWSQDGALQDTSCQVHAWPDRRRWNQTCELLPVSQASWACNLILG APDSQKLTTVDIVTLRVLCREGVRWRVMAIQDFKPFENLRLMAPISLQVVHVETHRCNIS WEISQASHYFERHLEFEARTLSPGHTWEEAPLLTLKQKQEWICLETLTPDTQYEFQVRVKP LQGEFTTWSPWSQPLAFRTKPAALGKDGGGSGGGGSGGGGSITCPPPMSVE HADIWVKSYSLYSRERYICNSGFKRKAGTSSLTECVLNKATNVAHWTTPSLKCIRDSPSSGSG VPLSLYSGGNWVNVISDLKKIEDLIQSMHIDATLYTESDVHPSCKVTAMKCFLLELQVISLES GDASIHDTVENLIILANNSLSSNGNVTESGCKECEELEEKNIKEFLQSFVHIVQMFINTS | AK482 | DNA57 3 |

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| | 78 | DKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFN<br>WYVDGVEVHNAKTKPREEQYASTYRVVSVLTVLHQDWLNGKEYKCKVSNKALP<br>APIEKTISKAKGQPREPQVCTLPPSRDELTKNQVSLSCAVKGFYPSDIAVEWESNG<br>QPENNYKTTPPVLDSDGSFFLVSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQK<br>SLSLSPGPGSGSAVNGTSQFTCFYNSRANISCVWSQDGALQDTSCQVHAWPDRR<br>RWNQTCELLPVSQASWACNLILGAPDSQKLTTVDIVTLRVLCREGVRWRVMAIQD<br>FKPFENLRLMAPISLQVVHVETHRCNISWEISQASHYFERHLEFEARTLSPGHTWEE<br>APLLTLKQKQEWICLETLTPDTQYEFQVRVKPLQGEFTTWSPWSQPLAFRTKPAAL<br>GKDGGGGSGGGGSGGGGSGGGGSITCPPPMSVEHADIWVKSYSLYSRERYICNSG<br>FKRKAGTSSLTECVLNKATNVAHWTTPSLKCIRDGPPSGSSPVPLSLYSGGNWVNVISD<br>LKKIEDLIQSMHIDATLYTESDVHPSCKVTAMKCFLLELQVISLESGDASIHDTVENLII<br>LANNSLSSNGNVTESGCKECEELEEKNIKEFLQSFVHIVQMFINTS | AK483 | DNA574 |
| Knob: hFc(N297A) | 79 | DKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNW<br>YVDGVEVHNAKTKPREEQYASTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIE<br>KTISKAKGQPREPQVYTLPPCRDELTKNQVSLWCLVKGFYPSDIAVEWESNGQPEN<br>NYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGK | AK401<br>AK402<br>AK403<br>AK478<br>AK479<br>AK480<br>AK481<br>AK482<br>AK483 | DNA161 |
| Knob: hFc(N297A)-hIL15 | 80 | DKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFN<br>WYVDGVEVHNAKTKPREEQYASTYRVVSVLTVLHQDWLNGKEYKCKVSNKAL<br>PAPIEKTISKAKGQPREPQVYTLPPCRDELTKNQVSLWCLVKGFYPSDIAVEWE<br>SNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHN<br>HYTQKSLSLSPGGGSSPPGGGSSGGGSGPSGSPGNWVNVISDLKKIEDLIQSMHI<br>DATLYTESDVHPSCKVTAMKCFLLELQVISLESGDASIHDTVENLIILANNSLSSNG<br>NVTESGCKECEELEEKNIKEFLQSFVHIVQMFINTS | AK244<br>AK249<br>AK507<br>AK564 | DNA364 |

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| | 81 | DKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKF<br>NWYVDGVEVHNAKTKPREEQYASTYRVVSVLTVLHQDWLNGKEYKCKVSNKAL<br>PAPIEKTISKAKGQPREPQVYTLPPCRDELTKNQVSLWCLVKGFYPSDIAVEWES<br>NGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYT<br>QKSLSLSPGGGSSPPGGGSSGGGSGPGSGSNWVNVISDLKKIEDLIQSMHIDATLY<br>TESDVHPSCKVTAMKCFLLELQVISLESGDASIHDTVENLIILANNSLSSNGNVTESG<br>CKECEELEEKNIKEFLQSFVHIVQMFINTS | AK418 | DNA36<br>7 |
| Knob:<br>hFc(N297A)-<br>[MPYDLYHP]-<br>hIL15 | 82 | DKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKF<br>NWYVDGVEVHNAKTKPREEQYASTYRVVSVLTVLHQDWLNGKEYKCKVSNK<br>ALPAPIEKTISKAKGQPREPQVYTLPPCRDELTKNQVSLWCLVKGFYPSDIAVEW<br>ESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHN<br>HYTQKSLSLSPGGGSSPPMPYDLYHPSGPSGSPGNWVNVISDLKKIEDLIQSMHIDA<br>TLYTESDVHPSCKVTAMKCFLLELQVISLESGDASIHDTVENLIILANNSLSSNGNVTE<br>SGCKECEELEEKNIKEFLQSFVHIVQMFINTS | AK242<br>AK247 | DNA36<br>2 |
| | 83 | DKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFN<br>WYVDGVEVHNAKTKPREEQYASTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPA<br>PIEKTISKAKGQPREPQVYTLPPCRDELTKNQVSLWCLVKGFYPSDIAVEWESNGQP<br>ENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSL<br>SPGGGSSPPMPYDLYHPSGPGSGSNWVNVISDLKKIEDLIQSMHIDATLYTESDVHP<br>SCKVTAMKCFLLELQVISLESGDASIHDTVENLIILANNSLSSNGNVTESGCKECEEL<br>EEKNIKEFLQSFVHIVQMFINTS | AK243<br>AK248 | DNA36<br>3 |
| Knob:<br>hFc(N297A)-<br>[MPYDLYHP]-<br>hIL15-hCD215 | 84 | DKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNW<br>YVDGVEVHNAKTKPREEQYASTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKT<br>ISKAKGQPREPQVYTLPPCRDELTKNQVSLWCLVKGFYPSDIAVEWESNGQPENNYK<br>TTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGGGS<br>SPPMPYDLYHPSGPSGSPGNWVNVISDLKKIEDLIQSMHIDATLYTESDVHPSCKVTA<br>MKCFLLELQVISLESGDASIHDTVENLIILANNSLSSNGNVTESGCKECEELEEKNIKEFL<br>QSFVHIVQMFINTSGGGGSGGGGSGGGGSGGGGSITCPPPMSVEHADIWVKSYSLY<br>SRERYICNSGFKRKAGTSSLTECVLNKATNVAHWTTPSLKCIRDPALVHQRPAPPSTVT<br>TAGVTPQPESLSPSGKEPAASSPSSNNTAATTAAIVPGSQLMPSKSPSTGTTEISSHESSH<br>GTPSQTTAKNWELTASASHQPPGVYPQGHSDTT | AK245<br>AK250 | DNA36<br>5 |

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| | 85 | DKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNW<br>YVDGVEVHNAKTKPREEQYASTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIE<br>KTISKAKGQPREPQVYTLPPCRDELTKNQVSLWCLVKGFYPSDIAVEWESNGQPEN<br>NYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSP<br>GGGSSPPMPYDLYHPSGPGSGSNWVNVISDLKKIEDLIQSMHIDATLYTESDVHPSC<br>KVTAMKCFLLELQVISLESGDASIHDTVENLIILANNSLSSNGNVTESGCKECEELEEK<br>NIKEFLQSFVHIVQMFINTSGGGGSGGGGSGGGGSGGGSITCPPPMSVEHADIW<br>VKSYSLYSRERYICNSGFKRKAGTSSLTECVLNKATNVAHWTTPSLKCIRDPALVHQR<br>PAPPSTVTTAGVTPQPESLSPSGKEPAASPSSNNTAATTAAIVPGSQLMPSKSPSTG<br>TTEISSHESSHGTPSQTTAKNWELTASASHQPPGVYPQGHSDTT | AK419 | DNA368 |
| Knob: hFc(N297A)-[MPYDLYHPJ-hIL15-hCD215(Truncated) | 86 | KLATMSVPTQVLGLLLLWLTDARCDKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRT<br>PEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYASTYRVVSVLTVLHQD<br>WLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPCRDELTKNQVSLWCLVKG<br>FYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMH<br>EALHNHYTQKSLSLSPGGGSSPPMPYDLYHPSGPSGSPGNWVNVISDLKKIEDLIQSM<br>HIDATLYTESDVHPSCKVTAMKCFLLELQVISLESGDASIHDTVENLIILANNSLSSNGN<br>VTESGCKECEELEEKNIKEFLQSFVHIVQMFINTSGGGGSGGGGSGGGGSGGGSITC<br>PPPMSVEHADIWVKSYSLYSRERYICNSGFKRKAGTSSLTECVLNKATNVAHWTTPSL<br>KCIRDPALVHQRPAPPSTVTTAGVTPQPESLSPSGKEPAAS*AADKTHTCPPCPAPELLG<br>GPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQ<br>YASTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPCRD<br>ELTKNQVSLWCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSR<br>WQQGNVFSCSVMHEALHNHYTQKSLSLSPGGGSSPPMPYDLYHPSGPSGSPGNWVN<br>VISDLKKIEDLIQSMHIDATLYTESDVHPSCKVTAMKCFLLELQVISLESGDASIHDTVENLIIL<br>ANNSLSSNGNVTESGCKECEELEEKNIKEFLQSFVHIVQMFINTSGGGGSGGGGSGGGGS<br>GGGGSITCPPPMSVEHADIWVKSYSLYSRERYICNSGFKRKAGTSSLTECVLNKATNVAH<br>WTTPSLKCIRDPALVHQRPAPPSTVTTAGVTPQPESLSPSGKEPAAS | AK246<br>AK251 | DNA366 |

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| | 87 | DKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKF<br>NWYVDGVEVHNAKTKPREEQYASTYRVVSVLTVLHQDWLNGKEYKCKVSNKA<br>LPAPIEKTISKAKGQPREPQVYTLPPCRDELTKNQVSLWCLVKGFYPSDIAVEWES<br>NGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHY<br>TQKSLSLSPGGGSSPPMPYDLYHPSGPGSGSNWVNVISDLKKIEDLIQSMHIDATL<br>YTESDVHPSCKVTAMKCFLLELQVISLESGDASIHDTVENLIILANNSLSSNGNVTES<br>GCKECEELEEKNIKEFLQSFVHIVQMFINTSGGGGSGGGGSGGGGSGGGGSITCP<br>PPMSVEHADIWVKSYSLYSRERYICNSGFKRKAGTSSLTECVLNKATNVAHWTTP<br>SLKCIRDPALVHQRPAPPSTVTTAGVTPQPESLSPSGKEPAAS | AK420 | DNA36<br>9 |
| Knob:<br>hFc(N297A)-<br>[VPLSLY]-<br>hIL15 | 88 | DKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFN<br>WYVDGVEVHNAKTKPREEQYASTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPA<br>PIEKTISKAKGQPREPQVYTLPPCRDELTKNQVSLWCLVKGFYPSDIAVEWESNGQ<br>PENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSL<br>SLSPGGGSSPPVPLSLYSGPGSGSNWVNVISDLKKIEDLIQSMHIDATLYTESDVHPS<br>CKVTAMKCFLLELQVISLESGDASIHDTVENLIILANNSLSSNGNVTESGCKECEELE<br>EKNIKEFLQSFVHIVQMFINTS | AK421 | DNA48<br>7 |
| | 89 | DKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFN<br>WYVDGVEVHNAKTKPREEQYASTYRVVSVLTVLHQDWLNGKEYKCKVSNKALP<br>APIEKTISKAKGQPREPQVYTLPPCRDELTKNQVSLWCLVKGFYPSDIAVEWESN<br>GQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYT<br>QKSLSLSPGGGSSPPGVPLSLYGSGPGSGSNWVNVISDLKKIEDLIQSMHIDATLY<br>TESDVHPSCKVTAMKCFLLELQVISLESGDASIHDTVENLIILANNSLSSNGNVTE<br>SGCKECEELEEKNIKEFLQSFVHIVQMFINTS | AK457 | DNA55<br>6 |
| Knob:<br>hFc(N297A)-<br>[VPLSLY]-<br>hIL15-hCD215 | 90 | DKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFN<br>WYVDGVEVHNAKTKPREEQYASTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPA<br>PIEKTISKAKGQPREPQVYTLPPCRDELTKNQVSLWCLVKGFYPSDIAVEWESNGQ<br>PENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKS<br>LSLSPGGGSSPPVPLSLYSGPGSGSNWVNVISDLKKIEDLIQSMHIDATLYTESDVHP<br>SCKVTAMKCFLLELQVISLESGDASIHDTVENLIILANNSLSSNGNVTESGCKECEE<br>LEEKNIKEFLQSFVHIVQMFINTSGGGGSGGGGSGGGGSGGGGSITCPPPM<br>SVEHADIWVKSYSLYSRERYICNSGFKRKAGTSSLTECVLNKATNVAHWTTPSLKC<br>IRDPALVHQRPAPPSTVTTAGVTPQPESLSPSGKEPAASPSSNNTAATTAAIVPG<br>SQLMPSKSPSTGTTEISSHESSHGTPSQTTAKNWELTASASHQPPGVYPQGHSDTT | AK399 | DNA51<br>7 |

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| | 91 | DKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKF<br>NWYVDGVEVHNAKTKPREEQYASTYRVVSVLTVLHQDWLNGKEYKCKVSNKA<br>LPAPIEKTISKAKGQPREPQVYTLPPCRDELTKNQVSLWCLVKGFYPSDIAVEW<br>ESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALH<br>NHYTQKSLSLSPGGGSSPPVPLSLYSGPSGSPGNWVNVISDLKKIEDLIQSMHID<br>ATLYTESDVHPSCKVTAMKCFLLELQVISLESGDASIHDTVENLIILANNSLSSNG<br>NVTESGCKECEELEEKNIKEFLQSFVHIVQMFINTSGGSGGGSGGGSGGGSGG<br>GSGITCPPPMSVEHADIWVKSYSLYSRERYICNSGFKRKAGTSSLTECVLNKAT<br>NVAHWTTPSLKCIRDPALVHQRPAPPSTVTTAGVTPQPESLSPSGKEPAASSPS<br>SNNTAATTAAIVPGSQLMPSKSPSTGTTEISSHESSHGTPSQTTAKNWELTASA<br>SHQPPGVYPQGHSDTT | AK404 | DNA52<br>6 |
| | 92 | DKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFN<br>WYVDGVEVHNAKTKPREEQYASTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPA<br>PIEKTISKAKGQPREPQVYTLPPCRDELTKNQVSLWCLVKGFYPSDIAVEWESNGQ<br>PENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLS<br>LSPGGGSSPPVPLSLYSGPSGSPGNWVNVISDLKKIEDLIQSMHIDATLYTESDVHPS<br>CKVTAMKCFLLELQVISLESGDASIHDTVENLIILANNSLSSNGNVTESGCKECEELE<br>EKNIKEFLQSFVHIVQMFINTSGGSGGGSGGGSGGGSGGGSGITCPPPMS<br>VEHADIWVKSYSLYSRERYICNSGFKRKAGTSSLTECVLNKATNVAHWTTPSLKCI<br>RDPALVHQRPAPPSTVTTAGVTPQPESLSPSGKEPAASSPSSNNTAATTAAIVPG<br>SQLMPSKSPSTGTTEISSHESSHGTPSQTTAKNWELTASASHQPPGVYPQGHSDTT | AK405 | DNA52<br>7 |
| Knob: hFc(N297A)-[VPLSLY]-hIL15-hCD215(Truncated) | 93 | DKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNW<br>YVDGVEVHNAKTKPREEQYASTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIE<br>KTISKAKGQPREPQVYTLPPCRDELTKNQVSLWCLVKGFYPSDIAVEWESNGQPEN<br>NYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSP<br>GGGSSPPVPLSLYSGPGSGSNWVNVISDLKKIEDLIQSMHIDATLYTESDVHPSCKV<br>TAMKCFLLELQVISLESGDASIHDTVENLIILANNSLSSNGNVTESGCKECEELEEKNI<br>KEFLQSFVHIVQMFINTSGGSGGGSGGGSGGGSGGGSGGGSITCPPPMSVE<br>HADIWVKSYSLYSRERYICNSGFKRKAGTSSLTECVLNKATNVAHWTTPSLKCIRDPA<br>LVHQRPAPPSTVTTAGVTPQPESLSPSGKEPAAS | AK400 | DNA51<br>8 |

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| Knob: hFc(N297A)-[VPLSLY]-hIL15(D22A) | 94 | DKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFN WYVDGVEVHNAKTKPREEQYASTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPA PIEKTISKAKGQPREPQVYTLPPCRDELTKNQVSLWCLVKGFYPSDIAVEWESNGQ PENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSL SLSPGGGSSPPGVPLSLYGSGPGSGSNWVNVISDLKKIEDLIQSMHIAATLYTESDV HPSCKVTAMKCFLLELQVISLESGDASIHDTVENLIILANNSLSSNGNVTESGCKEC EELEEKNIKEFLQSFVHIVQMFINTS | AK458 | DNA55 7 |
| Knob: hFc(N297A)-[VPLSLY]-hIL15(E46A) | 95 | DKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNW YVDGVEVHNAKTKPREEQYASTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIE KTISKAKGQPREPQVYTLPPCRDELTKNQVSLWCLVKGFYPSDIAVEWESNGQPEN NYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSP GGGSSPPGVPLSLYGSGPGSGSNWVNVISDLKKIEDLIQSMHIDATLYTESDVHPSC KVTAMKCFLLALQVISLESGDASIHDTVENLIILANNSLSSNGNVTESGCKECEELEEK NIKEFLQSFVHIVQMFINTS | AK459 | DNA55 8 |
| Knob: hFc(N297A)-[VPLSLY]-hIL15(E53A) | 96 | DKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNW YVDGVEVHNAKTKPREEQYASTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEK TISKAKGQPREPQVYTLPPCRDELTKNQVSLWCLVKGFYPSDIAVEWESNGQPENN YKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPG GGSSPPGVPLSLYGSGPGSGSNWVNVISDLKKIEDLIQSMHIDATLYTESDVHPSCK VTAMKCFLLELQVISLASGDASIHDTVENLIILANNSLSSNGNVTESGCKECEELEEK NIKEFLQSFVHIVQMFINTS | AK460 | DNA55 9 |
| Knob: hFc(N297A)-[VPLSLY]-hIL15(E46A, E53A) | 97 | DKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWY VDGVEVHNAKTKPREEQYASTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKT ISKAKGQPREPQVYTLPPCRDELTKNQVSLWCLVKGFYPSDIAVEWESNGQPENNYK TTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGGG SSPPGVPLSLYGSGPGSGSNWVNVISDLKKIEDLIQSMHIDATLYTESDVHPSCKVTA MKCFLLALQVISLASGDASIHDTVENLIILANNSLSSNGNVTESGCKECEELEEKNIKE FLQSFVHIVQMFINTS | AK461 | DNA56 0 |
| Knob: hFc(N297A)- | 98 | DKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNW YVDGVEVHNAKTKPREEQYASTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEK | AK506 | DNA59 6 |

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| hIL15(E46A, E53A) | | TISKAKGQPREPQVYTLPPCRDELTKNQVSLWCLVKGFYPSDIAVEWESNGQPENNY KTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGG GSSPPGGGSSGGGSGPSGSPGNWVNVISDLKKIEDLIQSMHIDATLYTESDVHPSCKV TAMKCFLLALQVISLASGDASIHDTVENLILLANNSLSSNGNVTESGCKECEELEEKNIK EFLQSFVHIVQMFINTS | AK527 | | |
| Knob: hFc(N297A)-hIL15(E46S, E53S) | 99 | DKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWY VDGVEVHNAKTKPREEQYASTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEK TISKAKGQPREPQVYTLPPCRDELTKNQVSLWCLVKGFYPSDIAVEWESNGQPENNY KTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGG SSPPGGGSSGGGSGPSGSPGNWVNVISDLKKIEDLIQSMHIDATLYTESDVHP TAMKCFLLALQVISLASGDASIHDTVENLILLANNSLSSNGNVTESGCKECEELEEKNIK EFLQSFVHIVQMFINTS | not named yet-1 | DNAtb d1 |
| Knob: hFc(N297A)-hIL15(E46R, E53R) | 100 | DKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNW YVDGVEVHNAKTKPREEQYASTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEK TISKAKGQPREPQVYTLPPCRDELTKNQVSLWCLVKGFYPSDIAVEWESNGQPENN YKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPG GGSSPPGGGSSGGGSGPSGSPGNWVNVISDLKKIEDLIQSMHIDATLYTESDVHP SCKVTAMKCFLLALQVISLASGDASIHDTVENLILLANNSLSSNGNVTESGCKECEEL EEKNIKEFLQSFVHIVQMFINTS | not named yet-2 | DNAtb d2 |
| knob:IL15-CD215(1-66)_Chimera_V1 | 101 | DKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNW YVDGVEVHNAKTKPREEQYASTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIE KTISKAKGQPREPQVYTLPPCRDELTKNQVSLWCLVKGFYPSDIAVEWESNGQPEN NYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSP GGGSSPPMPYDLYHPSGPGSGSNWVNVISDLKKIEDLIQSGGGGSGGGGSITCPP PMSVEHADIWVKSYSLYSRERYICNSGFKRKAGTSSLTECVLNKATNVAHWTTPSL KCIRDGGGSGGGGSKVTAMKCFLLELQVISLESGDASIHDTVENLILLANNSLSSNG NVTESGCKECEELEEKNIKEFLQSFVHIVQMFINTS | not named yet-3 | DNAtb d3 |

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| knob:IL15-CD215(1-66)_Chimera_V2 | 102 | DKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNW YVDGVEVHNAKTKPREEQYASTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIE KTISKAKGQPREPQVYTLPPCRDELTKNQVSLWCLVKGFYPSDIAVEWESNGQPEN NYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSP GGGSSPPMPYDLYHPSGPGSGSNWVNVISDLKKIEDLIQSGGGGSGGGGSGGGSITC PPPMSVEHADIWVKSYSLYSRERYICNSGFKRKAGTSSLTECVLNKATNVAHWTTPS LKCIRDGGGSGGGGSGGGSKVTAMKCFLLELQVISLESGDASIHDTVENLIILANNSL SSNGNVTESGCKECEELEEKNIKEFLQSFVHIVQMFINTS | not named yet-4 | AK507 | DNA595 |
| hCD215(1to66) | 103 | ITCPPPMSVEHADIWVKSYSLYSRERYICNSGFKRKAGTSSLTECVLNKATNVAH WTTPSLKCIRD | | | |
| Fc(Hole) (N297A) CD122 (C122S, C168S) | 177 | DKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFN WYVDGVEVHNAKTKPREEQYASTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPA PIEKTISKAKGQPREPQVCTLPPSRDELTKNQVSLSCAVKGFYPSDIAVEWESNGQP ENNYKTTPPVLDSDGSFFLVSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLS LSPGPGSGSAVNGTSQFTCFYNSRANISCVWSQDGALQDTSCQVHAWPDRRRWN QTCELLPVSQASWACNLILGAPDSQKLTTVDIVTLRVLCREGVRWRVMAIQDFKPF ENLRLMAPISLQVVHVETHRSNISWEISQASHYFERHLEFEARTLSPGHTWEEAPLL TLKQKQEWICLETLTPDTQYEFQVRVKPLQGEFTTWSPWSQPLAFRTKPAALGKD | AK564 | DNA440 |
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| | | | AK930<br>AK932 | |
| Fc(knob)-IL15 V1 Non-cleavable (N71Q) | 178 | DKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNW<br>YVDGVEVHNAKTKPREEQYASTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPI<br>EKTISKAKGQPREPQVYTLPPCRDELTKNQVSLWCLVKGFYPSDIAVEWESNGQPEN<br>NYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPG<br>GGSSPPGGGSSGGGSGPSGSPGNWVNVISDLKKIEDLIQSMHIDATLYTESDVHPSCKV<br>TAMKCFLLELQVISLESGDASIHDTVENLIILAQNSLSSNGNVTESGCKECEELEEKNIK<br>EFLQSFVHIVQMFINTS | AK595<br>AK596 | DNA736 |
| Fc(knob)-IL15 V1 Non-cleavable (N112Q) | 179 | DKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYV<br>DGVEVHNAKTKPREEQYASTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKT<br>ISKAKGQPREPQVYTLPPCRDELTKNQVSLWCLVKGFYPSDIAVEWESNGQPENNYKT<br>TPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGGGSSPP<br>G<br>GGSSGGGSGPSGSPGNWVNVISDLKKIEDLIQSMHIDATLYTESDVHPSCKVTAMKCFLL<br>ELQVISLESGDASIHDTVENLIILANNSLSSNGNVTESGCKECEELEEKNIKEFLQSFVHIV<br>QMFIQTS | AK900<br>AK901 | DNA900 |
| Fc(knob)-IL15 V1 Non-cleavable (N79Q) | 180 | DKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVD<br>GVEVHNAKTKPREEQYASTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISK<br>AKGQPREPQVYTLPPCRDELTKNQVSLWCLVKGFYPSDIAVEWESNGQPENNYKTTPPV<br>LDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGGGSSPPGGGS<br>SGGGSGPSGSPGNWVNVISDLKKIEDLIQSMHIDATLYTESDVHPSCKVTAMKCFLLEL<br>QVISLESGDASIHDTVENLIILANNSLSSNGQVTESGCKECEELEEKNIKEFLQSFVHIVQ<br>MFINTS | AK902<br>AK907 | DNA901 |
| Fc(knob)-IL15 V1 Non-cleavable (N79Q, N112Q) | 181 | DKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVD<br>G<br>VEVHNAKTKPREEQYASTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKA<br>KGQPR<br>EPQVYTLPPCRDELTKNQVSLWCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGS<br>FFLYS<br>KLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGGGSSPPGGGSSGGGSGPSGSP<br>GNW<br>VNVISDLKKIEDLIQSMHIDATLYTESDVHPSCKVTAMKCFLLELQVISLESGDASIHDTV<br>ENLIILAN | AK902<br>AK908 | DNA902 |

| | | NSLSSNGQVTESGCKECEELEEKNIKEFLQSFVHIVQMFIQTS | | |
|---|---|---|---|---|
| Fc(knob)-IL15 V1 Non-cleavable (N71Q, N112Q) | 182 | DKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVD GVEVHN AKTKPREEQYASTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPRE PQVYT LPPCRDELTKNQVSLWCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSK LTVDKSR WQQGNVFSCSVMHEALHNHYTQKSLSLSPGGGSSPPGGGSSGGGSGPSGSPGNWVNVIS DLKKIED LIQSMHIDATLYTESDVHPSCKVTAMKCFLLELQVISLESGDASIHDTVENLIILAQNSLSS NGNVTES GCKECEELEEKNIKEFLQSFVHIVQMFIQTS | AK90 3 AK90 9 | DNA9 03 |
| Fc(knob)-IL15 V1 Non-cleavable (N71Q, N79Q) | 183 | DKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVD GVEVHNAK TKPREEQYASTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQ VYTLPPCR DELTKNQVSLWCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDK SRWQQG NVFSCSVMHEALHNHYTQKSLSLSPGGGSSPPGGGSSGGPSGSPGNWVNVISDLKKI EDLIQS MHIDATLYTESDVHPSCKVTAMKCFLLELQVISLESGDASIHDTVENLIILAQNSLSSNGQ VTESGC KECEELEEKNIKEFLQSFVHIVQMFINTS | AK90 4 AK91 0 AK93 5 AK93 7 AK94 0 AK93 9 AK93 6 AK38 | DNA9 04 |
| Fc(knob)-IL15 V1 Non-cleavable (N71Q, N79Q, N112Q) | 184 | DKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVD GVEVHNA KTKPREEQYASTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREP QVYTLPPC RDELTKNQVSLWCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVD KSRWQQG NVFSCSVMHEALHNHYTQKSLSLSPGGGSSPPGGGSSGGPSGSPGNWVNVISDLKKI EDLIQSM | AK90 5 AK91 1 | DNA9 05 |

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| | | HIDATLYTESDVHPSCKVTAMKCFLLELQVISLESGDASIHDTVENLIILAQNSLSSNGQV<br>TESGCKECE<br>ELEEKNIKEFLQSFVHIVQMFIQTS | | |
| Fc(knob)-[DSGGFMLTI]-IL15 (N71Q,N79Q) | 185 | DKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVD<br>GVEVHNAKT<br>KPREEQYASTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQV<br>YTLPPCRDEL<br>TKNQVSLWCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRW<br>QQGNVFSC<br>SVMHEALHNHYTQKSLSLSPGPSGSDSGGFMLTSGPGSGSNWVNVISDLKKIEDLIQSM<br>HIDATLYTES<br>DVHPSCKVTAMKCFLLELQVISLESGDASIHDTVENLIILAQNSLSSNGQVTESGCKECEE<br>LEEKNIKE<br>FLQSFVHIVQMFINTS | AK929 | DNA921 |
| Fc(knob)-[RAAAVKSPJ-IL15 (N71Q,N79Q) | 186 | DKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVD<br>GVEVHNA<br>KTKPREEQYASTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREP<br>QVYTLPPC<br>RDELTKNQVSLWCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVD<br>KSRWQQG<br>NVFSCSVMHEALHNHYTQKSLSLSPGPSGSPRAAAVKSPSGPGSGSNWVNVISDLKKIED<br>LIQSMH<br>IDATLYTESDVHPSCKVTAMKCFLLELQVISLESGDASIHDTVENLIILAQNSLSSNGQVT<br>ESGCKECE<br>ELEEKNIKEFLQSFVHIVQMFINTS | AK931 | DNA923 |
| Fc(knob)-[MPYDLYHPJ-IL15 (N71Q,N79Q) | 187 | DKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVD<br>GVEVHNA<br>KTKPREEQYASTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREP<br>QVYTLPP<br>CRDELTKNQVSLWCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTV<br>DKSRWQQ<br>GNVFSCSVMHEALHNHYTQKSLSLSPGGGSSPPMPYDLYHPSGPGSGSNWVNVISDLKK<br>IEDLIQS<br>MHIDATLYTESDVHPSCKVTAMKCFLLELQVISLESGDASIHDTVENLIILAQNSLSSNGQ<br>VTESGCK<br>ECEELEEKNIKEFLQSFVHIVQMFINTS | AK934 | DNA926 |

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| Fc(hole)-[DSGGFMLT]-CD122 | 188 | DKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVD<br>GVEVHNAKTKP<br>REEQYASTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVCT<br>LPPSRDELTK<br>NQVSLSCAVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLVSKLTVDKSRWQQ<br>GNVFSCSVM<br>HEALHNHYTQKSLSLSPGGPPSGSSPGDSGGFMLTSGGSGPSGSAVNGTSQFTCFYNSRA<br>NISCVWSQD<br>GALQDTSCQVHAWPDRRRWNQTCELLPVSQASWACNLILGAPDSQKLTTVDIVTLRVL<br>CREGVRW<br>RVMAIQDFKPFENLRLMAPISLQVVHVETHRSNISWEISQASHYFERHLEFEARTLSPGHT<br>WEEAPLL<br>TLKQKQEWISLETLTPDTQYEFQVRVKPLQGEFTTWSPWSQPLAFRTKPAALGKD | AK935 | DNA820 |
| Fc(hole)-[RAAAVKSP]-CD122 | 189 | DKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVD<br>GVEVHNAK<br>TKPREEQYASTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQ<br>VCTLPPSR<br>DELTKNQVSLSCAVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLVSKLTVDK<br>SRWQQGNVF<br>SCSVMHEALHNHYTQKSLSLSPGGPPSGSSPRAAAVKSPSGGGAVNGTSQFTCFYNSRA<br>NISCVWSQDG<br>ALQDTSCQVHAWPDRRRWNQTCELLPVSQASWACNLILGAPDSQKLTTVDIVTLRVLC<br>REGVRWRVM<br>AIQDFKPFENLRLMAPISLQVVHVETHRSNISWEISQASHYFERHLEFEARTLSPGHTWEE<br>APLLTLKQ<br>KQEWISLETLTPDTQYEFQVRVKPLQGEFTTWSPWSQPLAFRTKPAALGKD | AK937 | DNA927 |
| Fc(hole)-[MPYDLYHP]-CD122 | 190 | DKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVD<br>GVEVHNAKTKP<br>REEQYASTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVCT<br>LPPSRDELTKNQ<br>VSLSCAVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLVSKLTVDKSRWQQGN<br>VFSCSVMHEALH<br>NHYTQKSLSLSPGGPPSGSSPMPYDLYHPSGGGAVNGTSQFTCFYNSRANISCVWSQDG<br>ALQDTSCQVHA<br>WPDRRRWNQTCELLPVSQASWACNLILGAPDSQKLTTVDIVTLRVLCREGVRWRVMAI<br>QDFKPFENLR | AK940 | DNA928 |

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| | | LMAPISLQVVHVETHRSNISWEISQASHYFERHLEFEARTLSPGHTWEEAPLLTLKQKQE<br>WISLETLTPD<br>TQYEFQVRVKPLQGEFTTWSPWSQPLAFRTKPAALGKD | | | |
| Fc(knob)-[VPLSLYSG]-IL15 (N71Q,N79Q) | 191 | DKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVD<br>GVEVHNAKT<br>KPREEQYASTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQV<br>YTLPPCRDEL<br>TKNQVSLWCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRW<br>QQGNVFSCSV<br>MHEALHNHYTQKSLSLSPGPSSGSGSGVPLSLYSGSGPGSGSNWVNVISDLKKIEDLIQS<br>MHIDATLYTES<br>DVHPSCKVTAMKCFLLELQVISLESGDASIHDTVENLIILAQNSLSSNGQVTESGCKECEE<br>LEEKNIKEFLQ<br>SFVHIVQMFINTS | AK933 | DNA925 |
| Fc(hole)-[VPLSLYSG]-CD122 | 192 | DKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVD<br>GVEVHNAKTK<br>PREEQYASTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVC<br>TLPPSRDELTK<br>NQVSLSCAVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLVSKLTVDKSRWQQ<br>GNVFSCSVMH<br>EALHNHYTQKSLSLSPGGPPSGSSPGVPLSLYSGSGGGAVNGTSQFTCFYNSRANISCVW<br>SQDGALQDTSC<br>QVHAWPDRRRWNQTCELLPVSQASWACNLILGAPDSQKLTTVDIVTLRVLCREGVRWR<br>VMAIQDFKPFE<br>NLRLMAPISLQVVHVETHRSNISWEISQASHYFERHLEFEARTLSPGHTWEEAPLLTLKQ<br>KQEWISLETLT<br>PDTQYEFQVRVKPLQGEFTTWSPWSQPLAFRTKPAALGKD | AK939 | DNA817 |
| Fc(knob)-[SSGLLSGR]-IL15 (N71Q,N79Q) | 193 | DKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWY<br>VDGVEVHNAKTKPREEQYASTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIE<br>KTISKAKGQPREPQVYTLPPCRDELTKNQVSLWCLVKGFYPSDIAVEWESNGQPENN<br>YKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGG<br>GSSGGSPISSGLLSGRSSGPGSGSNWVNVISDLKKIEDLIQSMHIDATLYTESDVHPSC<br>KVTAMKCFLLELQVISLESGDASIHDTVENLIILAQNSLSSNGQVTESGCKECEELEEK<br>NIKEFLQSFVHIVQMFINTS | AK930 | DNA922 |
| Fc(knob)-[DLLAVVAA]- | 194 | DKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWY<br>VDGVEVHNAKTKPREEQYASTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIE | AK932 | AK924 |

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| IL15 (N71Q,N79Q) | | KTISKAKGQPREPQVYTLPPCRDELTKNQVSLWCLVKGFYPSDIAVEWESNGQPENN YKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGG GSSGGSPISSGLLSGRSSGPGSGSNWVNVISDLKKIEDLIQSMHIDATLYTESDVHPSCK VTAMKCFLLELQVISLESGDASIHDTVENLIILAQNSLSSNGQVTESGCKECEELEEK NIKEFLQSFVHIVQMFINTS | | |
| Fc(hole)-[ISSGLLSGR]-CD122 | 195 | DKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWY VDGVEVHNAKTKPREEQYASTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEK TISKAKGQPREPQVCTLPPSRDELTKNQVSLSCAVKGFYPSDIAVEWESNGQPENNY KTTPPVLDSDGSFFLVSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGGP PSGSSPISSGLLSGRSSGGGAVNGTSQFTCFYNSRANISCVWSQDGALQDTSCQVHAW PDRRRWNQTCELLPVSQASWACNLILGAPDSQKLTTVDIVTLRVLCREGVRWRVMAIQ IQDFKPFENLRLMAPISLQVVHVETHRSNISWEISQASHYFERHLEFEARTLSPGHTWE EAPLLTLKQKQEWISLETLTPDTQYEFQVRVKPLQGEFTTWSPWSQPLAFRTKPAALGK D | AK936 | DNA823 |
| Fc(hole)-[DLLAVVAA]-CD122 | 196 | DKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVD GVEVHNAKTKPREEQYASTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISK A KGQPREPQVCTLPPSRDELTKNQVSLSCAVKGFYPSDIAVEWESNGQPENNYKTTPPVL D SDGSFFLVSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGSGSPSGDLLAVV AASSGPGSGSPAVNGTSQFTCFYNSRANISCVWSQDGALQDTSCQVHAWPDRRRWNQT CELLPVSQASWACNLILGAPDSQKLTTVDIVTLRVLCREGVRWRVMAIQDFKPFENLRL M APISLQVVHVETHRSNISWEISQASHYFERHLEFEARTLSPGHTWEEAPLLTLKQKQEWIS L ETLTPDTQYEFQVRVKPLQGEFTTWSPWSQPLAFRTKPAALGKD | AK938 | DNA822 |

Table 8:

| DC | Polypeptide number | Description | Components |
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| AK242 | DNA158 | 1 | Hole: hFc(N297A) | | | | | | | | |
| | DNA362 | 2 | Knob: hFc(N297A)-[MPYDLYHP]-hIL15 | Knob: hFc(N297A) | N termina l spacer domain | [MPYDLYHP] | C termina l spacer domain | hIL15 | | | |
| AK243 | DNA158 | 1 | Hole: hFc(N297A) | Hole: hFc(N297A) | | | | | | | |
| | DNA363 | 2 | Knob: hFc(N297A)-[MPYDLYHP]-hIL15 | Knob: hFc(N297A) | N termina l spacer domain | [MPYDLYHP] | C termina l spacer domain | hIL15 | | | |
| AK244 | DNA158 | 1 | Hole: hFc(N297A) | Hole: hFc(N297A) | | | | | | | |
| | DNA364 | 2 | Knob: hFc(N297A)-hIL15 | Knob: hFc(N297A) | Non-cleavable linker (HL) | hIL15 | | | | | |
| AK245 | DNA158 | 1 | Hole: hFc(N297A) | Hole: hFc(N297A) | | | | | | | |
| | DNA365 | 2 | Knob: hFc(N297A)-[MPYDLYHP]-hIL15-hCD215 | Knob: hFc(N297A) | N termina l spacer domain | [MPYDLYHP] | C termina l spacer domain | hIL15 | Non-cleavable linker (a) | hCD215 | |
| AK246 | DNA158 | 1 | Hole: hFc(N297A) | Hole: hFc(N297A) | | | | | | | |
| | DNA366 | 2 | Knob: hFc(N297A)-[MPYDLYHP]-hIL15-hCD215(Truncated) | Knob: hFc(N297A) | N termina l spacer domain | [MPYDLYHP] | C termina l spacer domain | hIL15 | Non-cleavable linker (a) | hCD215 (Truncated) | |

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|---|---|---|---|---|---|---|---|---|---|
| AK247 | DNA187 | 1 | Hole: hFc(N297A)-hCD122 | Hole: hFc(N297A) | Non-cleavable linker (HL) | hCD122 | | | |
| | DNA362 | 2 | Knob: hFc(N297A)-[MPYDLYHP]-hIL15 | Knob: hFc(N297A) | N terminal spacer domain | [MPYDLYHP] | C terminal spacer domain | hIL15 | |
| AK248 | DNA187 | 1 | Hole: hFc(N297A)-hCD122 | Hole: hFc(N297A) | Non-cleavable linker (HL) | hCD122 | | | |
| | DNA363 | 2 | Knob: hFc(N297A)-[MPYDLYHP]-hIL15 | Knob: hFc(N297A) | N terminal spacer domain | [MPYDLYHP] | C terminal spacer domain | hIL15 | |
| AK421 | DNA187 | 1 | Hole: hFc(N297A)-hCD122 | Hole: hFc(N297A) | Non-cleavable linker (HL) | hCD122 | | | |
| | DNA487 | 2 | Knob: hFc(N297A)-[VPLSLY]-hIL15 | Knob: hFc(N297A) | N terminal spacer domain | [VPLSLY] | C terminal spacer domain | hIL15 | |
| AK457 | DNA187 | 1 | Hole: hFc(N297A)-hCD122 | Hole: hFc(N297A) | Non-cleavable linker (HL) | hCD122 | | | |
| | DNA556 | 2 | Knob: hFc(N297A)-[VPLSLY]-hIL15 | Knob: hFc(N297A) | N terminal spacer domain | [VPLSLY] | C terminal spacer domain | hIL15 | |

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|---|---|---|---|---|---|---|---|---|---|---|---|---|
| AK249 | DNA187 | 1 | Hole: hFc(N297A)-hCD122 | Hole: hFc(N297A) | Non-cleavable linker (HL) | hCD122 | | | | | | |
| | DNA364 | 2 | Knob: hFc(N297A)-hIL15 | Knob: hFc(N297A) | Non-cleavable linker (HL) | hIL15 | | | | | | |
| AK418 | DNA187 | 1 | Hole: hFc(N297A)-hCD122 | Hole: hFc(N297A) | Non-cleavable linker (HL) | hCD122 | | | | | | |
| | DNA367 | 2 | Knob: hFc(N297A)-hIL15 | Knob: hFc(N297A) | Non-cleavable linker (HL) | hIL15 | | | | | | |
| AK250 | DNA187 | 1 | Hole: hFc(N297A)-hCD122 | Hole: hFc(N297A) | Non-cleavable linker (HL) | hCD122 | | | | | | |
| | DNA365 | 2 | Knob: hFc(N297A)-[MPYDLYHP]-hIL15-hCD215 | Knob: hFc(N297A) | N terminal spacer domain | [MPYDLYHP] | C terminal spacer domain | hIL15 | Non-cleavable linker (a) | hCD215 | | |
| AK251 | DNA187 | 1 | Hole: hFc(N297A)-hCD122 | Hole: hFc(N297A) | Non-cleavable linker (HL) | hCD122 | | | | | | |

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|---|---|---|---|---|---|---|---|---|---|---|---|
| | DNA366 | 2 | Knob: hFc(N297A)-[MPYDLYHP]-hIL15-hCD215(Truncated) | Knob: hFc(N297A) | N terminal spacer domain | [MPYDLYHP] | C terminal spacer domain | hIL15 | Non-cleavable linker (a) | hCD215 (Truncated) | |
| AK399 | DNA187 | 1 | Hole: hFc(N297A)-hCD122 | Hole: hFc(N297A) | Non-cleavable linker (HL) | hCD122 | | | | | |
| | DNA517 | 2 | Knob: hFc(N297A)-[VPLSLY]-hIL15-hCD215 | Knob: hFc(N297A) | N terminal spacer domain | [VPLSLY] | C terminal spacer domain | hIL15 | Non-cleavable linker (a) | hCD215 | |
| AK400 | DNA187 | 1 | Hole: hFc(N297A)-hCD122 | Hole: hFc(N297A) | Non-cleavable linker (HL) | hCD122 | | | | | |
| | DNA518 | 2 | Knob: hFc(N297A)-[VPLSLY]-hIL15-hCD215(Truncated) | Knob: hFc(N297A) | N terminal spacer domain | [VPLSLY] | C terminal spacer domain | hIL15 | Non-cleavable linker (a) | hCD215 (Truncated) | |
| AK404 | DNA187 | 1 | Hole: hFc(N297A)-hCD122 | Hole: hFc(N297A) | Non-cleavable linker (HL) | hCD122 | | | | | |
| | DNA526 | 2 | Knob: hFc(N297A)-[VPLSLY]-hIL15-hCD215 | Knob: hFc(N297A) | N terminal spacer domain | [VPLSLY] | C terminal spacer domain | hIL15 | Non-cleavable | hCD215 | |

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|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| AK405 | DNA187 | 1 | Hole: hFc(N297A)-hCD122 | Hole: hFc(N297A) | Non-cleavable linker (HL) | hCD122 | | | | | linker (a) | | | |
| | DNA527 | 2 | Knob: hFc(N297A)-[VPLSLY]-hIL15-hCD215 | Knob: hFc(N297A) | N termina l spacer domain | [VPLSLY] | C termina l spacer domain | hIL15 | | | Non-cleavable linker (a) | hCD215 | | |
| AK419 | DNA187 | 1 | Hole: hFc(N297A)-hCD122 | Hole: hFc(N297A) | Non-cleavable linker (HL) | hCD122 | | | | | | | | |
| | DNA368 | 2 | Knob: hFc(N297A)-[MPYDLYHP]-hIL15-hCD215 | Knob: hFc(N297A) | N termina l spacer domain | [MPYDLYHP] | C termina l spacer domain | hIL15 | | | Non-cleavable linker (a) | hCD215 | | |
| AK420 | DNA187 | 1 | Hole: hFc(N297A)-hCD122 | Hole: hFc(N297A) | Non-cleavable linker (HL) | hCD122 | | | | | | | | |
| | DNA369 | 2 | Knob: hFc(N297A)-[MPYDLYHP]-hIL15-hCD215(Truncated) | Knob: hFc(N297A) | N termina l spacer domain | [MPYDLYHP] | C termina l spacer domain | hIL15 | | | Non-cleavable linker (a) | hCD215 (Truncated) | | |

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|---|---|---|---|---|---|---|---|---|---|---|---|---|
| AK401 | DNA161 | 2 | Knob: hFc(N297A) | | | | | | | | | |
| | DNA523 | 1 | Hole: hFc(N297A)-hCD122-[VPLSLY]-hCD215(Sushi)-hIL15 | Knob: hFc(N297A) | Non-cleavable linker (HL) | hCD122 | N terminal spacer domain | [VPLSLY] | C terminal spacer domain | hCD215 (Sushi) | Non-cleavable linker (a) | hIL15 |
| AK402 | DNA161 | 2 | Knob: hFc(N297A) | | | | | | | | | |
| | DNA524 | 1 | Hole: hFc(N297A)-hCD122-[VPLSLY]-hCD215(Sushi)-hIL15 | Knob: hFc(N297A) | Non-cleavable linker (HL) | hCD122 | N terminal spacer domain | [VPLSLY] | C terminal spacer domain | hCD215 (Sushi) | Non-cleavable linker (a) | hIL15 |
| AK403 | DNA161 | 2 | Knob: hFc(N297A) | | | | | | | | | |
| | DNA525 | 1 | Hole: hFc(N297A)-hCD122-[VPLSLY]-hCD215(Sushi)-hIL15 | Knob: hFc(N297A) | Non-cleavable linker (HL) | hCD122 | N terminal spacer domain | [VPLSLY] | C terminal spacer domain | hCD215 (Sushi) | Non-cleavable linker (a) | hIL15 |
| AK506 | DNA158 | 1 | Hole: hFc(N297A) | | | | | | | | | |
| | DNA596 | 2 | Knob: hFc(N297A)-hIL15(E46A, E53A) | Hole: hFc(N297A) | Non-cleavable linker (HL) | hIL15(E46A, E53A) | | | | | | |
| not named yet-1 | DNAtbd1 | 2 | Knob: hFc(N297A)-hIL15(E46S, E53S) | Knob: hFc(N297A) | Non-cleavable | hIL15(E46S, E53S) | | | | | | |

| | | | | | | linker (HL) | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | DNA158 | 1 | Hole: hFc(N297A) | Hole: hFc(N297A) | | | | | | |
| not named yet-2 | DNAbd2 | 2 | Knob: hFc(N297A)-hIL15(E46R, E53R) | Knob: hFc(N297A) | Non-cleavable linker (HL) | hIL15(E46R, E53R) | | | | |
| | DNA158 | 1 | Hole: hFc(N297A) | Hole: hFc(N297A) | | | | | | |
| AK507 | DNA158 | 1 | Hole: hFc(N297A) | Hole: hFc(N297A) | | | | | | |
| | DNA364 | 2 | Knob: hFc(N297A)-hIL15 | Knob: hFc(N297A) | Non-cleavable linker (HL) | hIL15 | | | | |
| | DNA595 | 3 | hCD215(1to66) | hCD215(1to66) | | | | | | |
| AK458 | DNA187 | 1 | Hole: hFc(N297A)-hCD122 | Hole: hFc(N297A) | Non-cleavable linker (HL) | hCD122 | | | | |
| | DNA557 | 2 | Knob: hFc(N297A)-[VPLSLY]-hIL15(D22A) | Knob: hFc(N297A) | N terminal spacer domain | [VPLSLY] | C terminal spacer domain | hIL15(D22A) | | |
| AK459 | DNA187 | 1 | Hole: hFc(N297A)-hCD122 | Hole: hFc(N297A) | Non-cleavable | hCD122 | | | | |

| | | | | | | linker (HL) | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | DNA558 | 2 | Knob: hFc(N297A)-[VPLSLY]-hIL15(E46A) | Knob: hFc(N297A) | N termina l spacer domain | [VPLSLY] | C termina l spacer domain | hIL15(E46A) | | | | | | |
| AK460 | DNA187 | 1 | Hole: hFc(N297A)-hCD122 | Hole: hFc(N297A) | Non-cleavable linker (HL) | hCD122 | | | | | | | | |
| | DNA559 | 2 | Knob: hFc(N297A)-[VPLSLY]-hIL15(E53A) | Knob: hFc(N297A) | N termina l spacer domain | [VPLSLY] | C termina l spacer domain | hIL15(E53A) | | | | | | |
| AK461 | DNA187 | 1 | Hole: hFc(N297A)-hCD122 | Hole: hFc(N297A) | Non-cleavable linker (HL) | hCD122 | | | | | | | | |
| | DNA560 | 2 | Knob: hFc(N297A)-[VPLSLY]-hIL15(E46A, E53A) | Knob: hFc(N297A) | N termina l spacer domain | [VPLSLY] | C termina l spacer domain | hIL15(E46A, E53A) | | | | | | |
| AK478 | DNA161 | 2 | Knob: hFc(N297A) | Knob: hFc(N297A) | | | | | | | | | | |
| | DNA569 | 1 | Hole: hFc(N297A)-hCD122-hCD215(Sushi)-[VPLSLY]-hIL15 | Hole: hFc(N297A) | Non-cleavable linker (HL) | hCD122 | Non-cleavable linker (a) | hCD215 (Sushi) | N termina l spacer domain | [VPLSLY] | C termina l spacer domain | hIL15 | | |

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|---|---|---|---|---|---|---|---|---|---|---|---|---|
| AK479 | DNA161 | 2 | Knob: hFc(N297A) | | | | | | | | | |
| | DNA570 | 1 | Hole: hFc(N297A)-hCD122-hCD215(Sushi)-[VPLSLY]-hIL15 | Knob: hFc(N297A) | Non-cleavable linker (HL) | hCD122 | Non-cleavable linker (a) | hCD215 (Sushi) | N terminal spacer domain | [VPLSLY] | C terminal spacer domain | hIL15 |
| AK480 | DNA161 | 2 | Knob: hFc(N297A) | | | | | | | | | |
| | DNA571 | 1 | Hole: hFc(N297A)-hCD122-hCD215(Sushi)-[VPLSLY]-hIL15 | Knob: hFc(N297A) | Non-cleavable linker (HL) | hCD122 | Non-cleavable linker (a) | hCD215 (Sushi) | N terminal spacer domain | [VPLSLY] | C terminal spacer domain | hIL15 |
| AK481 | DNA161 | 2 | Knob: hFc(N297A) | | | | | | | | | |
| | DNA572 | 1 | Hole: hFc(N297A)-hCD122-hCD215(1to66)-[VPLSLY]-hIL15 | Knob: hFc(N297A) | Non-cleavable linker (HL) | hCD122 | Non-cleavable linker (a) | hCD215 (1to66) | N terminal spacer domain | [VPLSLY] | C terminal spacer domain | hIL15 |
| AK482 | DNA161 | 2 | Knob: hFc(N297A) | | | | | | | | | |
| | DNA573 | 1 | Hole: hFc(N297A)-hCD122-hCD215(1to66)-[VPLSLY]-hIL15 | Knob: hFc(N297A) | Non-cleavable linker (HL) | hCD122 | Non-cleavable linker (a) | hCD215 (1to66) | N terminal spacer domain | [VPLSLY] | C terminal spacer domain | hIL15 |
| AK483 | DNA161 | 2 | Knob: hFc(N297A) | Hole: hFc(N297A) | | | | | | | | |

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|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | DNA574 | 1 | Hole: hFc(N297A)-hCD122-hCD215(1to66)-[VPLSLY]-hIL15 | Hole: hFc(N297A) | Non-cleavable linker (HL) | hCD122 | Non-cleavable linker (a) | hCD215 (1to66) | N terminal spacer domain | [VPLSLY] | C terminal spacer domain | hIL15 |
| not named yet-3 | DNAbd3 | 2 | knob:IL15-CD215(1-66)_Chimera_V1 | Knob: hFc(N297A) | N terminal spacer domain | [MPYDLYHP] | C terminal spacer domain | hIL-15 (N-ter) | Linker | hCD215 (1to66) | Linker | hIL15 (C-ter) |
| | DNA187 | 1 | Hole: hFc(N297A)-hCD122 | Hole: hFc(N297A) | Non-cleavable linker (HL) | hCD122 | | | | | | |
| not named yet-4 | DNAbd4 | 2 | knob:IL15-CD215(1-66)_Chimera_V2 | Knob: hFc(N297A) | N terminal spacer domain | [MPYDLYHP] | C terminal spacer domain | hIL-15 (N-ter) | Linker | hCD215(1to66) | Linker | hIL15 (C-ter) |
| | DNA187 | 1 | Hole: hFc(N297A)-hCD122 | Hole: hFc(N297A) | Non-cleavable linker (HL) | hCD122 | | | | | | |
| AK527 | DNA370 | 1 | Hole: hFc(N297A)-[MPYDLYHP]-hCD122 | Hole: hFc(N297A) | N terminal spacer domain | [MPYDLYHP] | C terminal spacer domain | hCD122 | | | | |
| | DNA596 | 2 | Knob: hFc(N297A)-hIL15(E46A, E53A) | Knob: hFc(N297A) | Non-cleavable linker (HL) | hIL15(E46A, E53A) | | | | | | |

Table 9:

| Released post cleavage | SEQ ID NO | DC |
|---|---|---|
| LYHPSGPSGSPGNWVNVISDLKKIEDLIQSMHIDATLYTESDVHPSCKVTAMKCFLL<br>ELQVISLESGDASIHDTVENLILANNSLSSNGNVTESGCKECEELEEKNIKEFLQSFVH<br>IVQMFINTS | 104 | AK242<br>AK247 |
| LYHPSGPGSGSNWVNVISDLKKIEDLIQSMHIDATLYTESDVHPSCKVTAMKCFLLELQV<br>ISLESGDASIHDTVENLILANNSLSSNGNVTESGCKECEELEEKNIKEFLQSFVHIVQMFINTS | 105 | AK243<br>AK248 |
| LYGSGPGSGSNWVNVISDLKKIEDLIQSMHIDATLYTESDVHPSCKVTAMKCFLLELQVIS<br>LESGDASIHDTVENLILANNSLSSNGNVTESGCKECEELEEKNIKEFLQSFVHIVQMFINTS | 106 | AK457 |
| LYSGPGSGSNWVNVISDLKKIEDLIQSMHIDATLYTESDVHPSCKVTAMKCFLLELQVISL<br>ESGDASIHDTVENLILANNSLSSNGNVTESGCKECEELEEKNIKEFLQSFVHIVQMFINTS | 107 | AK421 |
| LYHPSGPSGSPGNWVNVISDLKKIEDLIQSMHIDATLYTESDVHPSCKVTAMKCFLLEL<br>QVISLESGDASIHDTVENLILANNSLSSNGNVTESGCKECEELEEKNIKEFLQSFVHIVQM<br>FINTSGGGGSGGGGSGGGGSITCPPPMSVEHADIWVKSYSLYSRERYICNSGFK<br>RKAGTISSLTECVLNKATNVAHWTTPSLKCIRDPALVHQRPAPPSTVTTAGVTPQPESLS<br>PSGKEPAASSPSSNNTAATTAAIVPGSQLMPSKSPSTGTTEISSHESSHGTPSQTTAKN<br>WELTASASHQPPGVYPQGHSDTT | 108 | AK250 |
| LYHPSGPSGSPGNWVNVISDLKKIEDLIQSMHIDATLYTESDVHPSCKVTAMKCFLLELQV<br>ISLESGDASIHDTVENLILANNSLSSNGNVTESGCKECEELEEKNIKEFLQSFVHIVQMFINT<br>SGGGGSGGGGSGGGGSGGGGSITCPPPMSVEHADIWVKSYSLYSRERYICNSGFKRKAG<br>TSSLTECVLNKATNVAHWTTPSLKCIRDPALVHQRPAPPSTVTTAGVTPQPESLSPSGKEP<br>AAS | 109 | AK251 |
| LYSGPGSGSNWVNVISDLKKIEDLIQSMHIDATLYTESDVHPSCKVTAMKCFLLELQVISLESG<br>DASIHDTVENLILANNSLSSNGNVTESGCKECEELEEKNIKEFLQSFVHIVQMFINTSGGGGS<br>GGGGSGGGGSGGGGSGGGGSITCPPPMSVEHADIWVKSYSLYSRERYICNSGFKRKAGTSS<br>LTECVLNKATNVAHWTTPSLKCIRDPALVHQRPAPPSTVTTAGVTPQPESLSPSGKEPAAS | 110 | AK400 |

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|---|---|---|
| LYSGPGSGSNWVNVISDLKKIEDLIQSMHIDATLYTESDVHPSCKVTAMKCFLLELQVISLES<br>GDASIHDTVENLIILANNSLSSNGNVTESGCKECEELEEKNIKEFLQSFVHIVQMFINTSGGG<br>GSGGGGSGGGGSGGGGSGGGSITCPPPMSVEHADIWVKSYSLYSRERYICNSGFKRKAG<br>TSSLTECVLNKATNVAHWTTPSLKCIRDPALVHQRPAPPSTVTTAGVTPQPESLSPSGKEP<br>AASSPSSNNTAATTAAIVPGSQLMPSKSPSTGTTEISSHESSHGTPSQTTAKNWELTASAS<br>HQPPGVYPQGHSDTT | 111 | AK399 |
| LYHPSGPGSGSNWVNVISDLKKIEDLIQSMHIDATLYTESDVHPSCKVTAMKCFLLELQVISL<br>ESGDASIHDTVENLIILANNSLSSNGNVTESGCKECEELEEKNIKEFLQSFVHIVQMFINTSG<br>GGGSGGGGSGGGGSGGGSITCPPPMSVEHADIWVKSYSLYSRERYICNSGFKRKAGTSS<br>LTECVLNKATNVAHWTTPSLKCIRDPALVHQRPAPPSTVTTAGVTPQPESLSPSGKEPAAS | 112 | AK420 |
| LYHPSGPGSGSNWVNVISDLKKIEDLIQSMHIDATLYTESDVHPSCKVTAMKCFLLELQVIS<br>LESGDASIHDTVENLIILANNSLSSNGNVTESGCKECEELEEKNIKEFLQSFVHIVQMFINTS<br>GGGGSGGGGSGGGGSGGGSITCPPPMSVEHADIWVKSYSLYSRERYICNSGFKRKAGTS<br>SLTECVLNKATNVAHWTTPSLKCIRDPALVHQRPAPPSTVTTAGVTPQPESLSPSGKEPAAS<br>SPSSNNTAATTAAIVPGSQLMPSKSPSTGTTEISSHESSHGTPSQTTAKNWELTASASHQ<br>PPGVYPQGHSDTT | 113 | AK419 |
| LYSGPSGSPGNWVNVISDLKKIEDLIQSMHIDATLYTESDVHPSCKVTAMKCFLLELQVISLESG<br>DASIHDTVENLIILANNSLSSNGNVTESGCKECEELEEKNIKEFLQSFVHIVQMFINTSGGSGGG<br>SGGGSGGGSGGGSGGGSGITCPPPMSVEHADIWVKSYSLYSRERYICNSGFKRKAGTSSLTEC<br>VLNKATNVAHWTTPSLKCIRDPALVHQRPAPPSTVTTAGVTPQPESLSPSGKEPAASSPSSN<br>NTAATTAAIVPGSQLMPSKSPSTGTTEISSHESSHGTPSQTTAKNWELTASASHQPPGVYPQGHSDTT | 114 | AK405 |
| LYSGPSGSPGNWVNVISDLKKIEDLIQSMHIDATLYTESDVHPSCKVTAMKCFLLELQVISLE<br>SGDASIHDTVENLIILANNSLSSNGNVTESGCKECEELEEKNIKEFLQSFVHIVQMFINTSGGS<br>GGGSGGGSGGGSGGGSGITCPPPMSVEHADIWVKSYSLYSRERYICNSGFKRKAGTSSLTE<br>CVLNKATNVAHWTTPSLKCIRDPALVHQRPAPPSTVTTAGVTPQPESLSPSGKEPAASSPS<br>SNNTAATTAAIVPGSQLMPSKSPSTGTTEISSHESSHGTPSQTTAKNWELTASASHQPPGV<br>YPQGHSDTT | 115 | AK404 |
| LYSGGPITCPPPMSVEHADIWVKSYSLYSRERYICNSGFKRKAGTSSLTECVLNKATNVAH<br>WTTPSLKCIRDPALVHQRPAPPSGGGGSGGGGSGGGGSGGGGSNWVNVISDLKKIEDLI<br>QSMHIDATLYTESDVHPSCKVTAMKCFLLELQVISLESGDASIHDTVENLIILANNSLSSNG<br>NVTESGCKECEELEEKNIKEFLQSFVHIVQMFINTS | 116 | AK401<br>AK402<br>AK403 |
| LYGSGPGSGSNWVNVISDLKKIEDLIQSMHIAATLYTESDVHPSCKVTAMKCFLLELQVIS<br>LESGDASIHDTVENLIILANNSLSSNGNVTESGCKECEELEEKNIKEFLQSFVHIVQMFINTS | 117 | AK458 |

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|---|---|---|
| LYGSGPGSGSNWVNVISDLKKIEDLIQSMHIDATLYTESDVHPSCKVTAMKCFLLALQVISL<br>ASGDASIHDTVENLIILANNSLSSNGNVTESGCKECEELEEKNIKEFLQSFVHIVQMFINTS | 118 | AK461 |
| LYGSGPGSGSNWVNVISDLKKIEDLIQSMHIDATLYTESDVHPSCKVTAMKCFLLALQVI<br>SLESGDASIHDTVENLIILANNSLSSNGNVTESGCKECEELEEKNIKEFLQSFVHIVQMFINTS | 119 | AK459 |
| LYGSGPGSGSNWVNVISDLKKIEDLIQSMHIDATLYTESDVHPSCKVTAMKCFLLELQVIS<br>LASGDASIHDTVENLIILANNSLSSNGNVTESGCKECEELEEKNIKEFLQSFVHIVQMFINTS | 120 | AK460 |
| SLYSGGNWVNVISDLKKIEDLIQSMHIDATLYTESDVHPSCKVTAMKCFLLELQVISLESGDA<br>SIHDTVENLIILANNSLSSNGNVTESGCKECEELEEKNIKEFLQSFVHIVQMFINTS | 121 | AK482 |
| LYSGGNWVNVISDLKKIEDLIQSMHIDATLYTESDVHPSCKVTAMKCFLLELQVISLESGDA<br>SIHDTVENLIILANNSLSSNGNVTESGCKECEELEEKNIKEFLQSFVHIVQMFINTS | 307 | AK482 |
| LYSGGNWVNVISDLKKIEDLIQSMHIDATLYTESDVHPSCKVTAMKCFLLELQVISLESGDASI<br>HDTVENLIILANNSLSSNGNVTESGCKECEELEEKNIKEFLQSFVHIVQMFINTS | 122 | AK478<br>AK479<br>AK480<br>AK481<br>AK483 |
| LYHPSGPGSGSNWVNVISDLKKIEDLIQSGGGGSGGGGSGGSITCPPPMSVEHADIWVKSY<br>SLYSRERYICNSGFKRKAGTSSLTECVLNKATNVAHWTTPSLKCIRDGGGSGGGGSGGSKV<br>TAMKCFLLELQVISLESGDASIHDTVENLIILANNSLSSNGNVTESGCKECEELEEKNIKEFLQS<br>FVHIVQMFINTS | 123 | not named<br>yet-4 |
| LYHPSGPGSGSNWVNVISDLKKIEDLIQSGGGGSGGGGSITCPPPMSVEHADIWVKSYSLYSRER<br>YICNSGFKRKAGTSSLTECVLNKATNVAHWTTPSLKCIRDGGGGSGGGGSKVTAMKCFLLELQV<br>ISLESGDASIHDTVENLIILANNSLSSNGNVTESGCKECEELEEKNIKEFLQSFVHIVQMFINTS | 124 | not named<br>yet-3 |
| LYHPSGGGAVNGTSQFTCFYNSRANISCVWSQDGALQDTSCQVHAWPDRRRWNQTCELLPV<br>SQASWACNLILGAPDSQKLTTVDIVTLRVLCREGVRWRVMAIQDFKPFENLRLMAPISLQVVHV<br>ETHRCNISWEISQASHYFERHLEFEARTLSPGHTWEEAPLLTLKQKQEWICLETLTPDTQYEFQV<br>RVKPLQGEFTTWSPWSQPLAFRTKPAALGKD | 125 | AK527 |
| LYHPSGPSGSPGNWVNVISDLKKIEDLIQSMHIDATLYTESDVHPSCKVTAMKCFLLELQVISLES<br>GDASIHDTVENLIILANNSLSSNGNVTESGCKECEELEEKNIKEFLQSFVHIVQMFINTSGGGGS<br>GGGGSGGGGSGGGGSITCPPPMSVEHADIWVKSYSLYSRERYICNSGFKRKAGTSSLTECVLNK<br>ATNVAHWTTPSLKCIRDPALVHQRPAPPSTVTTAGVTPQPESLSPSGKEPAASSPSSNNTAATTA<br>AIVPGSQLMPSKSPSTGTTEISSHESSHGTPSQTTAKNWELTASASHQPPGVYPQGHSDTT | 126 | AK245 |

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|---|---|---|
| ITCPPPMSVEHADIWVKSYSLYSRERYICNSGFKRKAGTSSLTECVLNKATNVAHWTTPSLKCIRD (3rd polypeptide chain – SEQ ID NO: 199)<br>FMLTSGPGSGSNWVNVISDLKKIEDLIQSMHIDATLYTESDVHPSCKVTAMKCFLLELQVISLESG DASIHDTVENLIILAQNSLSSNGQVTESGCKECEELEEKNIKEFLQSFVHIVQMFINTS (2nd polypeptide chain – SEQ ID NO: 200) | 136 | AK929 |
| ITCPPPMSVEHADIWVKSYSLYSRERYICNSGFKRKAGTSSLTECVLNKATNVAHWTTPSLKCIRD (3rd polypeptide chain – SEQ ID NO: 201)<br>VKSPSGPGSGSNWVNVISDLKKIEDLIQSMHIDATLYTESDVHPSCKVTAMKCFLLELQVISLESGD ASIHDTVENLIILAQNSLSSNGQVTESGCKECEELEEKNIKEFLQSFVHIVQMFINTS (2nd polypeptide chain – SEQ ID NO: 202) | 137 | AK931 |
| ITCPPPMSVEHADIWVKSYSLYSRERYICNSGFKRKAGTSSLTECVLNKATNVAHWTTPSLKCIRD (3rd polypeptide chain – SEQ ID NO: 203)<br>LYHPSGPGSGSNWVNVISDLKKIEDLIQSMHIDATLYTESDVHPSCKVTAMKCFLLELQVISLESGDA SIHDTVENLIILAQNSLSSNGQVTESGCKECEELEEKNIKEFLQSFVHIVQMFINTS (2nd polypeptide chain – SEQ ID NO: 204) | 138 | AK934 |
| ITCPPPMSVEHADIWVKSYSLYSRERYICNSGFKRKAGTSSLTECVLNKATNVAHWTTPSLKCIRD (3rd polypeptide chain – SEQ ID NO: 205)<br>DKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHN AKTKPREEQYASTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLP PCRDELTKNQVSLWCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQ QGNVFSCSVMHEALHNHYTQKSLSLSPGGGSSPPGGGSSGGGSGPSGSPGNWVNVISDLKKIEDLIQ SMHIDATLYTESDVHPSCKVTAMKCFLLELQVISLESGDASIHDTVENLIILAQNSLSSNGQVTESGCK ECEELEEKNIKEFLQSFVHIVQMFINTS (2nd polypeptide chain – SEQ ID NO: 206)<br>DKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNA KTKPREEQYASTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVCTLPP SRDELTKNQVSLSCAVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLVSKLTVDKSRWQQ GNVFSCSVMHEALHNHYTQKSLSLSPGGPPSGSSPGDSGG (1st polypeptide chain – SEQ ID NO: 207) | 139 | AK935 |

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| ITCPPPMSVEHADIWVKSYSLYSRERYICNSGFKRKAGTSSLTECVLNKATNVAHWTTPSLKCIRD ($3^{rd}$ polypeptide chain – SEQ ID NO: 208)<br><br>DKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHN<br>AKTKPREEQYASTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLP<br>PCRDELTKNQVSLWCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQ<br>QGNVFSCSVMHEALHNHYTQKSLSLSPGGSSPPGGGSSGGGSGPSGGPGNWVNVISDLKKIEDLIQ<br>SMHIDATLYTESDVHPSCKVTAMKCFLLELQVISLESGDASIHDTVENLIILAQNSLSSNGQVTESGCK<br>ECEELEEKNIKEFLQSFVHIVQMFINTS ($2^{nd}$ polypeptide chain – SEQ ID NO: 209)<br><br>DKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNA<br>KTKPREEQYASTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVCTLPPSR<br>DELTKNQVSLSCAVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLVSKLTVDKSRWQQGNV<br>FSCSVMHEALHNHYTQKSLSLSPGGPPSGSSPRAAAA ($1^{st}$ polypeptide chain – SEQ ID NO: 210) | 140 | AK937 |
| ITCPPPMSVEHADIWVKSYSLYSRERYICNSGFKRKAGTSSLTECVLNKATNVAHWTTPSLKCIRD ($3^{rd}$ polypeptide chain – SEQ ID NO:211 )<br><br>DKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHN<br>AKTKPREEQYASTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLP<br>PCRDELTKNQVSLWCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQ<br>QGNVFSCSVMHEALHNHYTQKSLSLSPGGGSSPPGGGSSGGGSGPSGPGNWVNVISDLKKIEDLIQ<br>SMHIDATLYTESDVHPSCKVTAMKCFLLELQVISLESGDASIHDTVENLIILAQNSLSSNGQVTESGCK<br>ECEELEEKNIKEFLQSFVHIVQMFINTS ($2^{nd}$ polypeptide chain – SEQ ID NO: 212)<br><br>DKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNA<br>KTKPREEQYASTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVCTLPP<br>SRDELTKNQVSLSCAVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLVSKLTVDKSRWQQG<br>NVFSCSVMHEALHNHYTQKSLSLSPGGPPSGSSPMPYD ($1^{st}$ polypeptide chain – SEQ ID NO: 213) | 141 | AK940 |
| ITCPPPMSVEHADIWVKSYSLYSRERYICNSGFKRKAGTSSLTECVLNKATNVAHWTTPSLKCIRD ($3^{rd}$ polypeptide chain – SEQ ID NO: 214)<br><br>LYSGSGPGSGSNWVNVISDLKKIEDLIQSMHIDATLYTESDVHPSCKVTAMKCFLLELQVISLESG<br>DASIHDTVENLIILAQNSLSSNGQVTESGCKECEELEEKNIKEFLQSFVHIVQMFINTS ($2^{nd}$ polypeptide chain – SEQ ID NO: 215) | 142 | AK933 |

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| ITCPPPMSVEHADIWVKSYSLYSRERYICNSGFKRKAGTSSLTECVLNKATNVAHWTTPSLKCIRD (3rd polypeptide chain – SEQ ID NO: 216)<br><br>DKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVH NAKTKPREEQYASTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVY TLPPCRDELTKNQVSLWCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVD KSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGGGSSPPGGGSSGGGSPSGPGNWVNVISD LKKIEDLIQSMHIDATLYTESDVHPSCKVTAMKCFLLELQVISLESGDASIHDTVENLIILAQNSLSS NGQVTESGCKECEELEEKNIKEFLQSFVHIVQMFINTS (2nd polypeptide chain – SEQ ID NO: 217)<br><br>DKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVH NAKTKPREEQYASTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVCTL PPSRDELTKNQVSLSCAVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLVSKLTVDKSRWQQG NVFSCSVMHEALHNHYTQKSLSLSPGGPPSGSSPGVPLS (1st polypeptide chain – SEQ ID NO: 218) | 143 | AK939 |
| ITCPPPMSVEHADIWVKSYSLYSRERYICNSGFKRKAGTSSLTECVLNKATNVAHWTTPSLKCIRD (3rd polypeptide chain – SEQ ID NO: 219)<br><br>SGRSSGPGSGSNWVNVISDLKKIEDLIQSMHIDATLYTESDVHPSCKVTAMKCFLLELQVISLESG DASIHDTVENLIILAQNSLSSNGQVTESGCKECEELEEKNIKEFLQSFVHIVQMFINTS (2nd polypeptide chain – SEQ ID NO: 220) | 144 | AK930 |
| ITCPPPMSVEHADIWVKSYSLYSRERYICNSGFKRKAGTSSLTECVLNKATNVAHWTTPSLKCIRD (3rd polypeptide chain – SEQ ID NO: 221)<br><br>VVAASSGPGSGSNWVNVISDLKKIEDLIQSMHIDATLYTESDVHPSCKVTAMKCFLLELQVISLESGD ASIHDTVENLIILAQNSLSSNGQVTESGCKECEELEEKNIKEFLQSFVHIVQMFINTS (2nd polypeptide chain – SEQ ID NO: 222) | 145 | AK932 |
| ITCPPPMSVEHADIWVKSYSLYSRERYICNSGFKRKAGTSSLTECVLNKATNVAHWTTPSLKCIRD (3rd polypeptide chain – SEQ ID NO: 223)<br><br>DKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVH NAKTKPREEQYASTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVY TLPPCRDELTKNQVSLWCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVD KSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGGGSSPPGGGSSGGGSPSGPGNWVNVISD LKKIEDLIQSMHIDATLYTESDVHPSCKVTAMKCFLLELQVISLESGDASIHDTVENLIILAQNSLSS | 146 | AK936 |

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| NGQVTESGCKECEELEEKNIKEFLQSFVHIVQMFINTS (2nd polypeptide chain – SEQ ID NO: 224)<br><br>DKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHN<br>AKTKPREEQYASTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVCTLP<br>PSRDELTKNQVSLSCAVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLVSKLTVDKSRW<br>QQGNVFSCSVMHEALHNHYTQKSLSLSPGGPPSGSSPISSGLL (1st polypeptide chain – SEQ ID NO: 225) | | | |
| ITCPPPMSVEHADIWVKSYSLYSRERYICNSGFKRKAGTSSLTECVLNKATNVAHWTTPSLKCIRD<br>(3rd polypeptide chain – SEQ ID NO: 226)<br><br>DKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVH<br>NAKTKPREEQYASTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVY<br>TLPPCRDELTKNQVSLWCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVD<br>KSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGGGSSPPGGGSSGGGSGPSGPGNWVNVISD<br>LKKIEDLIQSMHIDATLYTESDVHPSCKVTAMKCFLLELQVISLESGDASIHDTVENLIILAQNSLSS<br>NGQVTESGCKECEELEEKNIKEFLQSFVHIVQMFINTS (2nd polypeptide chain – SEQ ID NO: 227)<br><br>DKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVH<br>NAKTKPREEQYASTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVCT<br>LPPSRDELTKNQVSLSCAVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLVSKLTVDKSRW<br>QQGNVFSCSVMHEALHNHYTQKSLSLSPGSGSPSGDLLA (1st polypeptide chain – SEQ ID NO: 228) | 147 | AK938 |

## 11. LIST OF CONSTRUCTS

The table below shows the full sequences for molecules labelled by 'AK' reference number. The component parts of the sequence are also shown as well as the order in which they are assembled in the chains of the molecules. Individual chains are labelled by a 'DNA' reference number:

| Molecule | name | newnames | Component1Sequence | Component2Sequence | Component3Sequence | Component4Sequence |
|---|---|---|---|---|---|---|
| AK242 | DNA158 | Hole: hFc(N297A) | DKTHTCPPCPAPELLGGPSVFLFPPKPKD TLMISRTPEVTCVVVDVSHEDPEVKFN WYVDGVEVHNAKTKPREEQYASTYRVV SVLTVLHQDWLNGKEYKCKVSNKALPA PIEKTISKAKGQPREPQVCTLPPSRDELT KNQVSLSCAVKGFYPSDIAVEWESNGQ PENNYKTTPPVLDSDGSFFLVSKLTVDKS RWQQGNVFSCSVMHEALHNHYTQKSL SLSPG | | | |
| AK242 | DNA362 | Knob: hFc(N297A)-[MPYDLYHP]-hIL15 | DKTHTCPPCPAPELLGGPSVFLFPPKPKD TLMISRTPEVTCVVVDVSHEDPEVKFN WYVDGVEVHNAKTKPREEQYASTYRVV SVLTVLHQDWLNGKEYKCKVSNKALPA PIEKTISKAKGQPREPQVYTLPPCRDELT KNQVSLWCLVKGFYPSDIAVEWESNGQ PENNYKTTPPVLDSDGSFFLYSKLTVDKS RWQQGNVFSCSVMHEALHNHYTQKSL SLSPG | GGSSPP | MPYDLYHP | SGPSGSPG |
| AK243 | DNA158 | Hole: hFc(N297A) | DKTHTCPPCPAPELLGGPSVFLFPPKPKD TLMISRTPEVTCVVVDVSHEDPEVKFN WYVDGVEVHNAKTKPREEQYASTYRVV SVLTVLHQDWLNGKEYKCKVSNKALPA PIEKTISKAKGQPREPQVCTLPPSRDELT KNQVSLSCAVKGFYPSDIAVEWESNGQ PENNYKTTPPVLDSDGSFFLVSKLTVDKS RWQQGNVFSCSVMHEALHNHYTQKSL SLSPG | | | |

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|---|---|---|---|---|---|---|
| AK243 | DNA363 | Knob: hFc(N297A)-[MPYDLYHP]-hIL15 | DKTHTCPPCPAPELLGGPSVFLFPPKPKD<br>TLMISRTPEVTCVVVDVSHEDPEVKFN<br>WYVDGVEVHNAKTKPREEQYASTYRVV<br>SVLTVLHQDWLNGKEYKCKVSNKALPA<br>PIEKTISKAKGQPREPQVYTLPPCRDELT<br>KNQVSLWCLVKGFYPSDIAVEWESNGQ<br>PENNYKTTPPVLDSDGSFFLYSKLTVDKS<br>RWQQGNVFSCSVMHEALHNHYTQKSL<br>SLSPG | GGSSPP | MPYDLYHP | SGPGSGS |
| AK244 | DNA158 | Hole: hFc(N297A) | DKTHTCPPCPAPELLGGPSVFLFPPKPKD<br>TLMISRTPEVTCVVVDVSHEDPEVKFN<br>WYVDGVEVHNAKTKPREEQYASTYRVV<br>SVLTVLHQDWLNGKEYKCKVSNKALPA<br>PIEKTISKAKGQPREPQVCTLPPSRDELT<br>KNQVSLSCAVKGFYPSDIAVEWESNGQ<br>PENNYKTTPPVLDSDGSFFLVSKLTVDKS<br>RWQQGNVFSCSVMHEALHNHYTQKSL<br>SLSPG | | | |
| AK244 | DNA364 | Knob: hFc(N297A)-hIL15 | DKTHTCPPCPAPELLGGPSVFLFPPKPKD<br>TLMISRTPEVTCVVVDVSHEDPEVKFN<br>WYVDGVEVHNAKTKPREEQYASTYRVV<br>SVLTVLHQDWLNGKEYKCKVSNKALPA<br>PIEKTISKAKGQPREPQVYTLPPCRDELT<br>KNQVSLWCLVKGFYPSDIAVEWESNGQ<br>PENNYKTTPPVLDSDGSFFLYSKLTVDKS<br>RWQQGNVFSCSVMHEALHNHYTQKSL<br>SLSPG | GGSSPPGGGS<br>SGGGSGPSGS<br>PG | NWVNVISDLKKIEDLIQSMHIDATLYTES<br>DVHPSCKVTAMKCFLLELQVISLESGDAS<br>IHDTVENLIILANNSLSSNGNVTESGCKEC<br>EELEEKNIKEFLQSFVHIVQMFINTS | |

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|---|---|---|---|---|---|---|
| AK245 | DNA158 | Hole: hFc(N297A) | DKTHTCPPCPAPELLGGPSVFLFPPKPKD TLMISRTPEVTCVVVDVSHEDPEVKFN WYVDGVEVHNAKTKPREEQYASTYRVV SVLTVLHQDWLNGKEYKCKVSNKALPA PIEKTISKAKGQPREPQVCTLPPSRDELT KNQVSLSCAVKGFYPSDIAVEWESNGQ PENNYKTTPPVLDSDGSFFLVSKLTVDKS RWQQGNVFSCSVMHEALHNHYTQKSL SLSPG | | | |
| AK245 | DNA365 | Knob: hFc(N297A)-[MPYDLYHP]-hIL15-hCD215 | DKTHTCPPCPAPELLGGPSVFLFPPKPKD TLMISRTPEVTCVVVDVSHEDPEVKFN WYVDGVEVHNAKTKPREEQYASTYRVV SVLTVLHQDWLNGKEYKCKVSNKALPA PIEKTISKAKGQPREPQVYTLPPCRDELT KNQVSLWCLVKGFYPSDIAVEWESNGQ PENNYKTTPPVLDSDGSFFLYSKLTVDKS RWQQGNVFSCSVMHEALHNHYTQKSL SLSPG | GGSSPP | MPYDLYHP | SGPSGSPG |
| AK246 | DNA158 | Hole: hFc(N297A) | DKTHTCPPCPAPELLGGPSVFLFPPKPKD TLMISRTPEVTCVVVDVSHEDPEVKFN WYVDGVEVHNAKTKPREEQYASTYRVV SVLTVLHQDWLNGKEYKCKVSNKALPA PIEKTISKAKGQPREPQVCTLPPSRDELT KNQVSLSCAVKGFYPSDIAVEWESNGQ PENNYKTTPPVLDSDGSFFLVSKLTVDKS RWQQGNVFSCSVMHEALHNHYTQKSL SLSPG | | | |

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|---|---|---|---|---|---|
| AK246 | DNA366 | Knob: hFc(N297A)-[MPYDLYHP]-hIL15-hCD215(Truncated) | DKTHTCPPCPAPELLGGPSVFLFPPKPKD GGSSPP<br>TLMISRTPEVTCVVVDVSHEDPEVKFN<br>WYVDGVEVHNAKTKPREEQYASTYRVV<br>SVLTVLHQDWLNGKEYKCKVSNKALPA<br>PIEKTISKAKGQPREPQVYTLPPCRDELT<br>KNQVSLWCLVKGFYPSDIAVEWESNGQ<br>PENNYKTTPPVLDSDGSFFLYSKLTVDKS<br>RWQQGNVFSCSVMHEALHNHYTQKSL<br>SLSPG | MPYDLYHP | SGPSGSPG |
| AK247 | DNA187 | Hole: hFc(N297A)-hCD122 | DKTHTCPPCPAPELLGGPSVFLFPPKPKD PGSGS<br>TLMISRTPEVTCVVVDVSHEDPEVKFN<br>WYVDGVEVHNAKTKPREEQYASTYRVV<br>SVLTVLHQDWLNGKEYKCKVSNKALPA<br>PIEKTISKAKGQPREPQVCTLPPSRDELT<br>KNQVSLSCAVKGFYPSDIAVEWESNGQ<br>PENNYKTTPPVLDSDGSFFLVSKLTVDKS<br>RWQQGNVFSCSVMHEALHNHYTQKSL<br>SLSPG | AVNGTSQFTCFYNSRANISCVWSQDGA<br>LQDTSCQVHAWPDRRRWNQTCELLPV<br>SQASWACNLILGAPDSQKLTTVDIVTILR<br>VLCREGVRWRVMAIQDFKPFENLRLMA<br>PISLQVVHVETHRCNISWEISQASHYFER<br>HLEFEARTLSPGHTWEEAPLLTLKQKQE<br>WICLETLTPDTQYEFQVRVKPLQGEFTT<br>WSPWSQPLAFRTKPAALGKD | |
| AK247 | DNA362 | Knob: hFc(N297A)-[MPYDLYHP]-hIL15 | DKTHTCPPCPAPELLGGPSVFLFPPKPKD GGSSPP<br>TLMISRTPEVTCVVVDVSHEDPEVKFN<br>WYVDGVEVHNAKTKPREEQYASTYRVV<br>SVLTVLHQDWLNGKEYKCKVSNKALPA<br>PIEKTISKAKGQPREPQVYTLPPCRDELT<br>KNQVSLWCLVKGFYPSDIAVEWESNGQ<br>PENNYKTTPPVLDSDGSFFLYSKLTVDKS<br>RWQQGNVFSCSVMHEALHNHYTQKSL<br>SLSPG | MPYDLYHP | SGPSGSPG |

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|---|---|---|---|---|
| AK248 | DNA187 | Hole: hFc(N297A)-hCD122 | DKTHTCPPCPAPELLGGPSVFLFPPKPKD PGSGS<br>TLMISRTPEVTCVVVDVSHEDPEVKFN<br>WYVDGVEVHNAKTKPREEQYASTYRVV<br>SVLTVLHQDWLNGKEYKCKVSNKALPA<br>PIEKTISKAKGQPREPQVCTLPPSRDELT<br>KNQVSLSCAVKGFYPSDIAVEWESNGQ<br>PENNYKTTPPVLDSDGSFFLVSKLTVDKS<br>RWQQGNVFSCSVMHEALHNHYTQKSL<br>SLSPG | AVNGTSQFTCFYNSRANISCVWSQDGA<br>LQDTSCQVHAWPDRRRWNQTCELLPV<br>SQASWACNLILGAPDSQKLTTVDIVTLR<br>VLCREGVRWRVMAIQDFKPFENLRLMA<br>PISLQVVHVETHRCNISWEISQASHYFER<br>HLEFEARTLSPGHTWEEAPLLTLKQKQE<br>WICLETLTPDTQYEFQVRVKPLQGEFTT<br>WSPWSQPLAFRTKPAALGKD |
| AK248 | DNA363 | Knob: hFc(N297A)-[MPYDLYHP]-hIL15 | DKTHTCPPCPAPELLGGPSVFLFPPKPKD GGSSPP<br>TLMISRTPEVTCVVVDVSHEDPEVKFN<br>WYVDGVEVHNAKTKPREEQYASTYRVV<br>SVLTVLHQDWLNGKEYKCKVSNKALPA<br>PIEKTISKAKGQPREPQVYTLPPCRDELT<br>KNQVSLWCLVKGFYPSDIAVEWESNGQ<br>PENNYKTTPPVLDSDGSFFLYSKLTVDKS<br>RWQQGNVFSCSVMHEALHNHYTQKSL<br>SLSPG | MPYDLYHP SGPGSGS |
| AK249 | DNA187 | Hole: hFc(N297A)-hCD122 | DKTHTCPPCPAPELLGGPSVFLFPPKPKD PGSGS<br>TLMISRTPEVTCVVVDVSHEDPEVKFN<br>WYVDGVEVHNAKTKPREEQYASTYRVV<br>SVLTVLHQDWLNGKEYKCKVSNKALPA<br>PIEKTISKAKGQPREPQVCTLPPSRDELT<br>KNQVSLSCAVKGFYPSDIAVEWESNGQ<br>PENNYKTTPPVLDSDGSFFLVSKLTVDKS<br>RWQQGNVFSCSVMHEALHNHYTQKSL<br>SLSPG | AVNGTSQFTCFYNSRANISCVWSQDGA<br>LQDTSCQVHAWPDRRRWNQTCELLPV<br>SQASWACNLILGAPDSQKLTTVDIVTLR<br>VLCREGVRWRVMAIQDFKPFENLRLMA<br>PISLQVVHVETHRCNISWEISQASHYFER<br>HLEFEARTLSPGHTWEEAPLLTLKQKQE<br>WICLETLTPDTQYEFQVRVKPLQGEFTT<br>WSPWSQPLAFRTKPAALGKD |

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|---|---|---|---|---|
| AK249 | DNA364 | Knob: hFc(N297A)-hIL15 | DKTHTCPPCPAPELLGGPSVFLFPPKPKD<br>TLMISRTPEVTCVVVDVSHEDPEVKFN<br>WYVDGVEVHNAKTKPREEQYASTYRVV<br>SVLTVLHQDWLNGKEYKCKVSNKALPA<br>PIEKTISKAKGQPREPQVYTLPPCRDELT<br>KNQVSLWCLVKGFYPSDIAVEWESNGQ<br>PENNYKTTPPVLDSDGSFFLYSKLTVDKS<br>RWQQGNVFSCSVMHEALHNHYTQKSL<br>SLSPG | GGSSPPGGGS<br>SGGGSGPSGS<br>PG | NWVNVISDLKKIEDLIQSMHIDATLYTES<br>DVHPSCKVTAMKCFLLELQVISLESGDAS<br>IHDTVENLIILANNSLSSNGNVTESGCKEC<br>EELEEKNIKEFLQSFVHIVQMFINTS |
| AK250 | DNA187 | Hole: hFc(N297A)-hCD122 | DKTHTCPPCPAPELLGGPSVFLFPPKPKD<br>TLMISRTPEVTCVVVDVSHEDPEVKFN<br>WYVDGVEVHNAKTKPREEQYASTYRVV<br>SVLTVLHQDWLNGKEYKCKVSNKALPA<br>PIEKTISKAKGQPREPQVCTLPPSRDELT<br>KNQVSLSCAVKGFYPSDIAVEWESNGQ<br>PENNYKTTPPVLDSDGSFFLVSKLTVDKS<br>RWQQGNVFSCSVMHEALHNHYTQKSL<br>SLSPG | PGSGS | AVNGTSQFTCFYNSRANISCVWSQDGA<br>LQDTSCQVHAWPDRRRWNQTCELLPV<br>SQASWACNLILGAPDSQKLTTVDIVTLR<br>VLCREGVRWRVMAIQDFKPFENLRLMA<br>PISLQVVHVETHRCNISWEISQASHYFER<br>HLEFEARTLSPGHTWEEAPLLTLKQKQE<br>WICLETLTPDTQYEFQVRVKPLQGEFTT<br>WSPWSQPLAFRTKPAALGKD |
| AK250 | DNA365 | Knob: hFc(N297A)-[MPYDLYHP]-hIL15-hCD215 | DKTHTCPPCPAPELLGGPSVFLFPPKPKD<br>TLMISRTPEVTCVVVDVSHEDPEVKFN<br>WYVDGVEVHNAKTKPREEQYASTYRVV<br>SVLTVLHQDWLNGKEYKCKVSNKALPA<br>PIEKTISKAKGQPREPQVYTLPPCRDELT<br>KNQVSLWCLVKGFYPSDIAVEWESNGQ<br>PENNYKTTPPVLDSDGSFFLYSKLTVDKS<br>RWQQGNVFSCSVMHEALHNHYTQKSL<br>SLSPG | GGSSPP | MPYDLYHP<br>SGPSGSPG |

| | | | | |
|---|---|---|---|---|
| AK251 | DNA187 | Hole: hFc(N297A)-hCD122 | DKTHTCPPCPAPELLGGPSVFLFPPKPKD PGSGS<br>TLMISRTPEVTCVVVDVSHEDPEVKFN<br>WYVDGVEVHNAKTKPREEQYASTYRVV<br>SVLTVLHQDWLNGKEYKCKVSNKALPA<br>PIEKTISKAKGQPREPQVCTLPPSRDELT<br>KNQVSLSCAVKGFYPSDIAVEWESNGQ<br>PENNYKTTPPVLDSDGSFFLVSKLTVDKS<br>RWQQGNVFSCSVMHEALHNHYTQKSL<br>SLSPG | AVNGTSQFTCFYNSRANISCVWSQDGA<br>LQDTSCQVHAWPDRRRWNQTCELLPV<br>SQASWACNLILGAPDSQKLTTVDIVTLR<br>VLCREGVRWRVMAIQDFKPFENLRLMA<br>PISLQVVHVETHRCNISWEISQASHYFER<br>HLEFEARTLSPGHTWEEAPLLTLKQKQE<br>WICLETLTPDTQYEFQVRVKPLQGEFTT<br>WSPWSQPLAFRTKPAALGKD |
| AK251 | DNA366 | Knob: hFc(N297A)-[MPYDLYHP]-hIL15-hCD215(Truncated) | DKTHTCPPCPAPELLGGPSVFLFPPKPKD GGSSPP<br>TLMISRTPEVTCVVVDVSHEDPEVKFN<br>WYVDGVEVHNAKTKPREEQYASTYRVV<br>SVLTVLHQDWLNGKEYKCKVSNKALPA<br>PIEKTISKAKGQPREPQVYTLPPCRDELT<br>KNQVSLWCLVKGFYPSDIAVEWESNGQ<br>PENNYKTTPPVLDSDGSFFLYSKLTVDKS<br>RWQQGNVFSCSVMHEALHNHYTQKSL<br>SLSPG | MPYDLYHP SGPSGSPG |
| AK399 | DNA187 | Hole: hFc(N297A)-hCD122 | DKTHTCPPCPAPELLGGPSVFLFPPKPKD PGSGS<br>TLMISRTPEVTCVVVDVSHEDPEVKFN<br>WYVDGVEVHNAKTKPREEQYASTYRVV<br>SVLTVLHQDWLNGKEYKCKVSNKALPA<br>PIEKTISKAKGQPREPQVCTLPPSRDELT<br>KNQVSLSCAVKGFYPSDIAVEWESNGQ<br>PENNYKTTPPVLDSDGSFFLVSKLTVDKS<br>RWQQGNVFSCSVMHEALHNHYTQKSL<br>SLSPG | AVNGTSQFTCFYNSRANISCVWSQDGA<br>LQDTSCQVHAWPDRRRWNQTCELLPV<br>SQASWACNLILGAPDSQKLTTVDIVTLR<br>VLCREGVRWRVMAIQDFKPFENLRLMA<br>PISLQVVHVETHRCNISWEISQASHYFER<br>HLEFEARTLSPGHTWEEAPLLTLKQKQE<br>WICLETLTPDTQYEFQVRVKPLQGEFTT<br>WSPWSQPLAFRTKPAALGKD |

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| AK399 | DNA517 | Knob: hFc(N297A)-[VPLSLY]-hIL15-hCD215 | DKTHTCPPCPAPELLGGPSVFLFPPKPKD GGSSPP<br>TLMISRTPEVTCVVVDVSHEDPEVKFN<br>WYVDGVEVHNAKTKPREEQYASTYRVV<br>SVLTVLHQDWLNGKEYKCKVSNKALPA<br>PIEKTISKAKGQPREPQVYTLPPCRDELT<br>KNQVSLWCLVKGFYPSDIAVEWESNGQ<br>PENNYKTTPPVLDSDGSFFLYSKLTVDKS<br>RWQQGNVFSCSVMHEALHNHYTQKSL<br>SLSPG | VPLSLY | SGPGSGS |
| AK400 | DNA187 | Hole: hFc(N297A)-hCD122 | DKTHTCPPCPAPELLGGPSVFLFPPKPKD PGSGS<br>TLMISRTPEVTCVVVDVSHEDPEVKFN<br>WYVDGVEVHNAKTKPREEQYASTYRVV<br>SVLTVLHQDWLNGKEYKCKVSNKALPA<br>PIEKTISKAKGQPREPQVCTLPPSRDELT<br>KNQVSLSCAVKGFYPSDIAVEWESNGQ<br>PENNYKTTPPVLDSDGSFFLVSKLTVDKS<br>RWQQGNVFSCSVMHEALHNHYTQKSL<br>SLSPG | AVNGTSQFTCFYNSRANISCVWSQDGA<br>LQDTSCQVHAWPDRRRWNQTCELLPV<br>SQASWACNLILGAPDSQKLTTVDIVTILR<br>VLCREGVRWRVMAIQDFKPFENLRLMA<br>PISLQVVHVETHRCNISWEISQASHYFER<br>HLEFEARTLSPGHTWEEAPLLTLKQKQE<br>WICLETLTPDTQYEFQVRVKPLQGEFTT<br>WSPWSQPLAFRTKPAALGKD | |
| AK400 | DNA518 | Knob: hFc(N297A)-[VPLSLY]-hIL15-hCD215(Truncated) | DKTHTCPPCPAPELLGGPSVFLFPPKPKD GGSSPP<br>TLMISRTPEVTCVVVDVSHEDPEVKFN<br>WYVDGVEVHNAKTKPREEQYASTYRVV<br>SVLTVLHQDWLNGKEYKCKVSNKALPA<br>PIEKTISKAKGQPREPQVYTLPPCRDELT<br>KNQVSLWCLVKGFYPSDIAVEWESNGQ<br>PENNYKTTPPVLDSDGSFFLYSKLTVDKS<br>RWQQGNVFSCSVMHEALHNHYTQKSL<br>SLSPG | VPLSLY | SGPGSGS |

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| AK401 | DNA161 | Knob: hFc(N297A) | DKTHTCPPCPAPELLGGPSVFLFPPKPKD<br>TLMISRTPEVTCVVVDVSHEDPEVKFN<br>WYVDGVEVHNAKTKPREEQYASTYRVV<br>SVLTVLHQDWLNGKEYKCKVSNKALPA<br>PIEKTISKAKGQPREPQVYTLPPCRDELT<br>KNQVSLWCLVKGFYPSDIAVEWESNGQ<br>PENNYKTTPPVLDSDGSFFLYSKLTVDKS<br>RWQQGNVFSCSVMHEALHNHYTQKSL<br>SLSPG |
| AK401 | DNA523 | Hole: hFc(N297A)-hCD122-[VPLSLY]-hCD215(Sushi)-hIL15 | DKTHTCPPCPAPELLGGPSVFLFPPKPKD PGSGS<br>TLMISRTPEVTCVVVDVSHEDPEVKFN<br>WYVDGVEVHNAKTKPREEQYASTYRVV<br>SVLTVLHQDWLNGKEYKCKVSNKALPA<br>PIEKTISKAKGQPREPQVCTLPPSRDELT<br>KNQVSLSCAVKGFYPSDIAVEWESNGQ<br>PENNYKTTPPVLDSDGSFFLVSKLTVDKS<br>RWQQGNVFSCSVMHEALHNHYTQKSL<br>SLSPG |
| AK402 | DNA161 | Knob: hFc(N297A) | DKTHTCPPCPAPELLGGPSVFLFPPKPKD<br>TLMISRTPEVTCVVVDVSHEDPEVKFN<br>WYVDGVEVHNAKTKPREEQYASTYRVV<br>SVLTVLHQDWLNGKEYKCKVSNKALPA<br>PIEKTISKAKGQPREPQVYTLPPCRDELT<br>KNQVSLWCLVKGFYPSDIAVEWESNGQ<br>PENNYKTTPPVLDSDGSFFLYSKLTVDKS<br>RWQQGNVFSCSVMHEALHNHYTQKSL<br>SLSPG |

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| AK401 | DNA161 | Knob: hFc(N297A) | AVNGTSQFTCFYNSRANISCVWSQDGA PGGPSP<br>LQDTSCQVHAWPDRRRWNQTCELLPV<br>SQASWACNLILGAPDSQKLTTVDIVTILR<br>VLCREGVRWRVMAIQDFKPFENLRLMA<br>PISLQVVHVETHRCNISWEISQASHYFER<br>HLEFEARTLSPGHTWEEAPLLTLKQKQE<br>WICLETLTPDTQYEFQVRVKPLQGEFTT<br>WSPWSQPLAFRTKPAALGKD |
| AK401 | DNA523 | Hole: hFc(N297A)-hCD122-[VPLSLY]-hCD215(Sushi)-hIL15 | |
| AK402 | DNA161 | Knob: hFc(N297A) | |

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| AK402 | DNA524 | Hole: hFc(N297A)-hCD122-[VPLSLY]-hCD215(Sushi)-hIL15 | DKTHTCPPCPAPELLGGPSVFLFPPKPKD PGSGS TLMISRTPEVTCVVVDVSHEDPEVKFN WYVDGVEVHNAKTKPREEQYASTYRVV SVLTVLHQDWLNGKEYKCKVSNKALPA PIEKTISKAKGQPREPQVCTLPPSRDELT KNQVSLSCAVKGFYPSDIAVEWESNGQ PENNYKTTPPVLDSDGSFFLVSKLTVDKS RWQQGNVFSCSVMHEALHNHYTQKSL SLSPG | AVNGTSQFTCFYNSRANISCVWSQDGA GGGSSP LQDTSCQVHAWPDRRRWNQTCELLPV SQASWACNLILGAPDSQKLTTVDIVTLR VLCREGVRWRVMAIQDFKPFENLRLMA PISLQVVHVETHRCNISWEISQASHYFER HLEFEARTLSPGHTWEEAPLLTLKQKQE WICLETLTPDTQYEFQVRVKPLQGEFTT WSPWSQPLAFRTKPAALGKD |
| AK403 | DNA161 | Knob: hFc(N297A) | DKTHTCPPCPAPELLGGPSVFLFPPKPKD TLMISRTPEVTCVVVDVSHEDPEVKFN WYVDGVEVHNAKTKPREEQYASTYRVV SVLTVLHQDWLNGKEYKCKVSNKALPA PIEKTISKAKGQPREPQVYTLPPCRDELT KNQVSLWCLVKGFYPSDIAVEWESNGQ PENNYKTTPPVLDSDGSFFLYSKLTVDKS RWQQGNVFSCSVMHEALHNHYTQKSL SLSPG | |
| AK403 | DNA525 | Hole: hFc(N297A)-hCD122-[VPLSLY]-hCD215(Sushi)-hIL15 | DKTHTCPPCPAPELLGGPSVFLFPPKPKD PGSGS TLMISRTPEVTCVVVDVSHEDPEVKFN WYVDGVEVHNAKTKPREEQYASTYRVV SVLTVLHQDWLNGKEYKCKVSNKALPA PIEKTISKAKGQPREPQVCTLPPSRDELT KNQVSLSCAVKGFYPSDIAVEWESNGQ PENNYKTTPPVLDSDGSFFLVSKLTVDKS RWQQGNVFSCSVMHEALHNHYTQKSL SLSPG | AVNGTSQFTCFYNSRANISCVWSQDGA SGSGGSP LQDTSCQVHAWPDRRRWNQTCELLPV SQASWACNLILGAPDSQKLTTVDIVTLR VLCREGVRWRVMAIQDFKPFENLRLMA PISLQVVHVETHRCNISWEISQASHYFER HLEFEARTLSPGHTWEEAPLLTLKQKQE WICLETLTPDTQYEFQVRVKPLQGEFTT WSPWSQPLAFRTKPAALGKD |

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| AK404 | DNA187 | Hole: hFc(N297A)-hCD122 | DKTHTCPPCPAPELLGGPSVFLFPPKPKD PGSGS<br>TLMISRTPEVTCVVVDVSHEDPEVKFN<br>WYVDGVEVHNAKTKPREEQYASTYRVV<br>SVLTVLHQDWLNGKEYKCKVSNKALPA<br>PIEKTISKAKGQPREPQVCTLPPSRDELT<br>KNQVSLSCAVKGFYPSDIAVEWESNGQ<br>PENNYKTTPPVLDSDGSFFLVSKLTVDKS<br>RWQQGNVFSCSVMHEALHNHYTQKSL<br>SLSPG | AVNGTSQFTCFYNSRANISCVWSQDGA<br>LQDTSCQVHAWPDRRRWNQTCELLPV<br>SQASWACNLILGAPDSQKLTTVDIVTLR<br>VLCREGVRWRVMAIQDFKPFENLRLMA<br>PISLQVVHVETHRCNISWEISQASHYFER<br>HLEFEARTLSPGHTWEEAPLLTLKQKQE<br>WICLETLTPDTQYEFQVRVKPLQGEFTT<br>WSPWSQPLAFRTKPAALGKD |
| AK404 | DNA526 | Knob: hFc(N297A)-[VPLSLY]-hIL15-hCD215 | DKTHTCPPCPAPELLGGPSVFLFPPKPKD GGSSPP<br>TLMISRTPEVTCVVVDVSHEDPEVKFN<br>WYVDGVEVHNAKTKPREEQYASTYRVV<br>SVLTVLHQDWLNGKEYKCKVSNKALPA<br>PIEKTISKAKGQPREPQVYTLPPCRDELT<br>KNQVSLWCLVKGFYPSDIAVEWESNGQ<br>PENNYKTTPPVLDSDGSFFLYSKLTVDKS<br>RWQQGNVFSCSVMHEALHNHYTQKSL<br>SLSPG | VPLSLY SGPSGSPG |
| AK405 | DNA187 | Hole: hFc(N297A)-hCD122 | DKTHTCPPCPAPELLGGPSVFLFPPKPKD PGSGS<br>TLMISRTPEVTCVVVDVSHEDPEVKFN<br>WYVDGVEVHNAKTKPREEQYASTYRVV<br>SVLTVLHQDWLNGKEYKCKVSNKALPA<br>PIEKTISKAKGQPREPQVCTLPPSRDELT<br>KNQVSLSCAVKGFYPSDIAVEWESNGQ<br>PENNYKTTPPVLDSDGSFFLVSKLTVDKS<br>RWQQGNVFSCSVMHEALHNHYTQKSL<br>SLSPG | AVNGTSQFTCFYNSRANISCVWSQDGA<br>LQDTSCQVHAWPDRRRWNQTCELLPV<br>SQASWACNLILGAPDSQKLTTVDIVTLR<br>VLCREGVRWRVMAIQDFKPFENLRLMA<br>PISLQVVHVETHRCNISWEISQASHYFER<br>HLEFEARTLSPGHTWEEAPLLTLKQKQE<br>WICLETLTPDTQYEFQVRVKPLQGEFTT<br>WSPWSQPLAFRTKPAALGKD |

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| AK405 | DNA527 | Knob: hFc(N297A)-[VPLSLY]-hIL15-hCD215 | DKTHTCPPCPAPELLGGPSVFLFPPKPKD<br>TLMISRTPEVTCVVVDVSHEDPEVKFN<br>WYVDGVEVHNAKTKPREEQYASTYRVV<br>SVLTVLHQDWLNGKEYKCKVSNKALPA<br>PIEKTISKAKGQPREPQVYTLPPCRDELT<br>KNQVSLWCLVKGFYPSDIAVEWESNGQ<br>PENNYKTTPPVLDSDGSFFLYSKLTVDKS<br>RWQQGNVFSCSVMHEALHNHYTQKSL<br>SLSPG | GGSSPP | VPLSLY | SGPSGSPG |
| AK418 | DNA187 | Hole: hFc(N297A)-hCD122 | DKTHTCPPCPAPELLGGPSVFLFPPKPKD<br>TLMISRTPEVTCVVVDVSHEDPEVKFN<br>WYVDGVEVHNAKTKPREEQYASTYRVV<br>SVLTVLHQDWLNGKEYKCKVSNKALPA<br>PIEKTISKAKGQPREPQVCTLPPSRDELT<br>KNQVSLSCAVKGFYPSDIAVEWESNGQ<br>PENNYKTTPPVLDSDGSFFLVSKLTVDKS<br>RWQQGNVFSCSVMHEALHNHYTQKSL<br>SLSPG | PGSGS | AVNGTSQFTCFYNSRANISCVWSQDGA<br>LQDTSCQVHAWPDRRRWNQTCELLPV<br>SQASWACNLILGAPDSQKLTTVDIVTLR<br>VLCREGVRWRVMAIQDFKPFENLRLMA<br>PISLQVVHVETHRCNISWEISQASHYFER<br>HLEFEARTLSPGHTWEEAPLLTLKQKQE<br>WICLETLTPDTQYEFQVRVKPLQGEFTT<br>WSPWSQPLAFRTKPAALGKD | |
| AK418 | DNA367 | Knob: hFc(N297A)-hIL15 | DKTHTCPPCPAPELLGGPSVFLFPPKPKD<br>TLMISRTPEVTCVVVDVSHEDPEVKFN<br>WYVDGVEVHNAKTKPREEQYASTYRVV<br>SVLTVLHQDWLNGKEYKCKVSNKALPA<br>PIEKTISKAKGQPREPQVYTLPPCRDELT<br>KNQVSLWCLVKGFYPSDIAVEWESNGQ<br>PENNYKTTPPVLDSDGSFFLYSKLTVDKS<br>RWQQGNVFSCSVMHEALHNHYTQKSL<br>SLSPG | GGSSPPGGGS<br>SGGGSGPGSG<br>S | NWVNVISDLKKIEDLIQSMHIDATLYTES<br>DVHPSCKVTAMKCFLLELQVISLESGDAS<br>IHDTVENLIILANNSLSSNGNVTESGCKEC<br>EELEEKNIKEFLQSFVHIVQMFINTS | |

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| AK419 | DNA187 | Hole: hFc(N297A)-hCD122 | DKTHTCPPCPAPELLGGPSVFLFPPKPKD PGSGS<br>TLMISRTPEVTCVVVDVSHEDPEVKFN<br>WYVDGVEVHNAKTKPREEQYASTYRVV<br>SVLTVLHQDWLNGKEYKCKVSNKALPA<br>PIEKTISKAKGQPREPQVCTLPPSRDELT<br>KNQVSLSCAVKGFYPSDIAVEWESNGQ<br>PENNYKTTPPVLDSDGSFFLVSKLTVDKS<br>RWQQGNVFSCSVMHEALHNHYTQKSL<br>SLSPG | AVNGTSQFTCFYNSRANISCVWSQDGA<br>LQDTSCQVHAWPDRRRWNQTCELLPV<br>SQASWACNLILGAPDSQKLTTVDIVTLR<br>VLCREGVRWRVMAIQDFKPFENLRLMA<br>PISLQVVHVETHRCNISWEISQASHYFER<br>HLEFEARTLSPGHTWEEAPLLTLKQKQE<br>WICLETLTPDTQYEFQVRVKPLQGEFTT<br>WSPWSQPLAFRTKPAALGKD |
| AK419 | DNA368 | Knob: hFc(N297A)-[MPYDLYHP]-hIL15-hCD215 | DKTHTCPPCPAPELLGGPSVFLFPPKPKD GGSSPP<br>TLMISRTPEVTCVVVDVSHEDPEVKFN<br>WYVDGVEVHNAKTKPREEQYASTYRVV<br>SVLTVLHQDWLNGKEYKCKVSNKALPA<br>PIEKTISKAKGQPREPQVYTLPPCRDELT<br>KNQVSLWCLVKGFYPSDIAVEWESNGQ<br>PENNYKTTPPVLDSDGSFFLYSKLTVDKS<br>RWQQGNVFSCSVMHEALHNHYTQKSL<br>SLSPG | MPYDLYHP SGPGSGS |
| AK420 | DNA187 | Hole: hFc(N297A)-hCD122 | DKTHTCPPCPAPELLGGPSVFLFPPKPKD PGSGS<br>TLMISRTPEVTCVVVDVSHEDPEVKFN<br>WYVDGVEVHNAKTKPREEQYASTYRVV<br>SVLTVLHQDWLNGKEYKCKVSNKALPA<br>PIEKTISKAKGQPREPQVCTLPPSRDELT<br>KNQVSLSCAVKGFYPSDIAVEWESNGQ<br>PENNYKTTPPVLDSDGSFFLVSKLTVDKS<br>RWQQGNVFSCSVMHEALHNHYTQKSL<br>SLSPG | AVNGTSQFTCFYNSRANISCVWSQDGA<br>LQDTSCQVHAWPDRRRWNQTCELLPV<br>SQASWACNLILGAPDSQKLTTVDIVTLR<br>VLCREGVRWRVMAIQDFKPFENLRLMA<br>PISLQVVHVETHRCNISWEISQASHYFER<br>HLEFEARTLSPGHTWEEAPLLTLKQKQE<br>WICLETLTPDTQYEFQVRVKPLQGEFTT<br>WSPWSQPLAFRTKPAALGKD |

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| AK420 | DNA369 | Knob: hFc(N297A)-[MPYDLYHP]-hIL15-hCD215(Truncated) | DKTHTCPPCPAPELLGGPSVFLFPPKPKD GGSSPP<br>TLMISRTPEVTCVVVDVSHEDPEVKFN<br>WYVDGVEVHNAKTKPREEQYASTYRVV<br>SVLTVLHQDWLNGKEYKCKVSNKALPA<br>PIEKTISKAKGQPREPQVYTLPPCRDELT<br>KNQVSLWCLVKGFYPSDIAVEWESNGQ<br>PENNYKTTPPVLDSDGSFFLYSKLTVDKS<br>RWQQGNVFSCSVMHEALHNHYTQKSL<br>SLSPG | MPYDLYHP | SGPGSGS |
| AK421 | DNA187 | Hole: hFc(N297A)-hCD122 | DKTHTCPPCPAPELLGGPSVFLFPPKPKD PGSGS<br>TLMISRTPEVTCVVVDVSHEDPEVKFN<br>WYVDGVEVHNAKTKPREEQYASTYRVV<br>SVLTVLHQDWLNGKEYKCKVSNKALPA<br>PIEKTISKAKGQPREPQVCTLPPSRDELT<br>KNQVSLSCAVKGFYPSDIAVEWESNGQ<br>PENNYKTTPPVLDSDGSFFLVSKLTVDKS<br>RWQQGNVFSCSVMHEALHNHYTQKSL<br>SLSPG | AVNGTSQFTCFYNSRANISCVWSQDGA<br>LQDTSCQVHAWPDRRRWNQTCELLPV<br>SQASWACNLILGAPDSQKLTTVDIVTLR<br>VLCREGVRWRVMAIQDFKPFENLRLMA<br>PISLQVVHVETHRCNISWEISQASHYFER<br>HLEFEARTLSPGHTWEEAPLLTLKQKQE<br>WICLETLTPDTQYEFQVRVKPLQGEFTT<br>WSPWSQPLAFRTKPAALGKD | |
| AK421 | DNA487 | Knob: hFc(N297A)-[VPLSLY]-hIL15 | DKTHTCPPCPAPELLGGPSVFLFPPKPKD GGSSPP<br>TLMISRTPEVTCVVVDVSHEDPEVKFN<br>WYVDGVEVHNAKTKPREEQYASTYRVV<br>SVLTVLHQDWLNGKEYKCKVSNKALPA<br>PIEKTISKAKGQPREPQVYTLPPCRDELT<br>KNQVSLWCLVKGFYPSDIAVEWESNGQ<br>PENNYKTTPPVLDSDGSFFLYSKLTVDKS<br>RWQQGNVFSCSVMHEALHNHYTQKSL<br>SLSPG | VPLSLY | SGPGSGS |

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| AK457 | DNA187 | Hole: hFc(N297A)-hCD122 | DKTHTCPPCPAPELLGGPSVFLFPPKPKD PGSGS<br>TLMISRTPEVTCVVVDVSHEDPEVKFN<br>WYVDGVEVHNAKTKPREEQYASTYRVV<br>SVLTVLHQDWLNGKEYKCKVSNKALPA<br>PIEKTISKAKGQPREPQVCTLPPSRDELT<br>KNQVSLSCAVKGFYPSDIAVEWESNGQ<br>PENNYKTTPPVLDSDGSFFLVSKLTVDKS<br>RWQQGNVFSCSVMHEALHNHYTQKSL<br>SLSPG | AVNGTSQFTCFYNSRANISCVWSQDGA<br>LQDTSCQVHAWPDRRRWNQTCELLPV<br>SQASWACNLILGAPDSQKLTTVDIVTLR<br>VLCREGVRWRVMAIQDFKPFENLRLMA<br>PISLQVVHVETHRCNISWEISQASHYFER<br>HLEFEARTLSPGHTWEEAPLLTLKQKQE<br>WICLETLTPDTQYEFQVRVKPLQGEFTT<br>WSPWSQPLAFRTKPAALGKD |
| AK457 | DNA556 | Knob: hFc(N297A)-[VPLSLY]-hIL15 | DKTHTCPPCPAPELLGGPSVFLFPPKPKD GGSSPPG<br>TLMISRTPEVTCVVVDVSHEDPEVKFN<br>WYVDGVEVHNAKTKPREEQYASTYRVV<br>SVLTVLHQDWLNGKEYKCKVSNKALPA<br>PIEKTISKAKGQPREPQVYTLPPCRDELT<br>KNQVSLWCLVKGFYPSDIAVEWESNGQ<br>PENNYKTTPPVLDSDGSFFLYSKLTVDKS<br>RWQQGNVFSCSVMHEALHNHYTQKSL<br>SLSPG | VPLSLY GSGPGSGS |
| AK458 | DNA187 | Hole: hFc(N297A)-hCD122 | DKTHTCPPCPAPELLGGPSVFLFPPKPKD PGSGS<br>TLMISRTPEVTCVVVDVSHEDPEVKFN<br>WYVDGVEVHNAKTKPREEQYASTYRVV<br>SVLTVLHQDWLNGKEYKCKVSNKALPA<br>PIEKTISKAKGQPREPQVCTLPPSRDELT<br>KNQVSLSCAVKGFYPSDIAVEWESNGQ<br>PENNYKTTPPVLDSDGSFFLVSKLTVDKS<br>RWQQGNVFSCSVMHEALHNHYTQKSL<br>SLSPG | AVNGTSQFTCFYNSRANISCVWSQDGA<br>LQDTSCQVHAWPDRRRWNQTCELLPV<br>SQASWACNLILGAPDSQKLTTVDIVTLR<br>VLCREGVRWRVMAIQDFKPFENLRLMA<br>PISLQVVHVETHRCNISWEISQASHYFER<br>HLEFEARTLSPGHTWEEAPLLTLKQKQE<br>WICLETLTPDTQYEFQVRVKPLQGEFTT<br>WSPWSQPLAFRTKPAALGKD |

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| AK458 | DNA557 | Knob: hFc(N297A)-[VPLSLY]-hIL15(D22A) | DKTHTCPPCPAPELLGGPSVFLFPPKPKD GGSSPPG<br>TLMISRTPEVTCVVVDVSHEDPEVKFN<br>WYVDGVEVHNAKTKPREEQYASTYRVV<br>SVLTVLHQDWLNGKEYKCKVSNKALPA<br>PIEKTISKAKGQPREPQVYTLPPCRDELT<br>KNQVSLWCLVKGFYPSDIAVEWESNGQ<br>PENNYKTTPPVLDSDGSFFLYSKLTVDKS<br>RWQQGNVFSCSVMHEALHNHYTQKSL<br>SLSPG | VPLSLY | | GSGPGSGS |
| AK459 | DNA187 | Hole: hFc(N297A)-hCD122 | DKTHTCPPCPAPELLGGPSVFLFPPKPKD PGSGS<br>TLMISRTPEVTCVVVDVSHEDPEVKFN<br>WYVDGVEVHNAKTKPREEQYASTYRVV<br>SVLTVLHQDWLNGKEYKCKVSNKALPA<br>PIEKTISKAKGQPREPQVCTLPPSRDELT<br>KNQVSLSCAVKGFYPSDIAVEWESNGQ<br>PENNYKTTPPVLDSDGSFFLVSKLTVDKS<br>RWQQGNVFSCSVMHEALHNHYTQKSL<br>SLSPG | AVNGTSQFTCFYNSRANISCVWSQDGA<br>LQDTSCQVHAWPDRRRWNQTCELLPV<br>SQASWACNLILGAPDSQKLTTVDIVTLR<br>VLCREGVRWRVMAIQDFKPFENLRLMA<br>PISLQVVHVETHRCNISWEISQASHYFER<br>HLEFEARTLSPGHTWEEAPLLTLKQKQE<br>WICLETLTPDTQYEFQVRVKPLQGEFTT<br>WSPWSQPLAFRTKPAALGKD | | |
| AK459 | DNA558 | Knob: hFc(N297A)-[VPLSLY]-hIL15(E46A) | DKTHTCPPCPAPELLGGPSVFLFPPKPKD GGSSPPG<br>TLMISRTPEVTCVVVDVSHEDPEVKFN<br>WYVDGVEVHNAKTKPREEQYASTYRVV<br>SVLTVLHQDWLNGKEYKCKVSNKALPA<br>PIEKTISKAKGQPREPQVYTLPPCRDELT<br>KNQVSLWCLVKGFYPSDIAVEWESNGQ<br>PENNYKTTPPVLDSDGSFFLYSKLTVDKS<br>RWQQGNVFSCSVMHEALHNHYTQKSL<br>SLSPG | VPLSLY | | GSGPGSGS |

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| AK460 | DNA187 | Hole: hFc(N297A)-hCD122 | DKTHTCPPCPAPELLGGPSVFLFPPKPKD PGSGS<br>TLMISRTPEVTCVVVDVSHEDPEVKFN<br>WYVDGVEVHNAKTKPREEQYASTYRVV<br>SVLTVLHQDWLNGKEYKCKVSNKALPA<br>PIEKTISKAKGQPREPQVCTLPPSRDELT<br>KNQVSLSCAVKGFYPSDIAVEWESNGQ<br>PENNYKTTPPVLDSDGSFFLVSKLTVDKS<br>RWQQGNVFSCSVMHEALHNHYTQKSL<br>SLSPG | AVNGTSQFTCFYNSRANISCVWSQDGA<br>LQDTSCQVHAWPDRRRWNQTCELLPV<br>SQASWACNLILGAPDSQKLTTVDIVTLR<br>VLCREGVRWRVMAIQDFKPFENLRLMA<br>PISLQVVHVETHRCNISWEISQASHYFER<br>HLEFEARTLSPGHTWEEAPLLTLKQKQE<br>WICLETLTPDTQYEFQVRVKPLQGEFTT<br>WSPWSQPLAFRTKPAALGKD |
| AK460 | DNA559 | Knob: hFc(N297A)-[VPLSLY]-hIL15(E53A) | DKTHTCPPCPAPELLGGPSVFLFPPKPKD GGSSPPG<br>TLMISRTPEVTCVVVDVSHEDPEVKFN<br>WYVDGVEVHNAKTKPREEQYASTYRVV<br>SVLTVLHQDWLNGKEYKCKVSNKALPA<br>PIEKTISKAKGQPREPQVYTLPPCRDELT<br>KNQVSLWCLVKGFYPSDIAVEWESNGQ<br>PENNYKTTPPVLDSDGSFFLYSKLTVDKS<br>RWQQGNVFSCSVMHEALHNHYTQKSL<br>SLSPG | VPLSLY GSGPGSGS |
| AK461 | DNA187 | Hole: hFc(N297A)-hCD122 | DKTHTCPPCPAPELLGGPSVFLFPPKPKD PGSGS<br>TLMISRTPEVTCVVVDVSHEDPEVKFN<br>WYVDGVEVHNAKTKPREEQYASTYRVV<br>SVLTVLHQDWLNGKEYKCKVSNKALPA<br>PIEKTISKAKGQPREPQVCTLPPSRDELT<br>KNQVSLSCAVKGFYPSDIAVEWESNGQ<br>PENNYKTTPPVLDSDGSFFLVSKLTVDKS<br>RWQQGNVFSCSVMHEALHNHYTQKSL<br>SLSPG | AVNGTSQFTCFYNSRANISCVWSQDGA<br>LQDTSCQVHAWPDRRRWNQTCELLPV<br>SQASWACNLILGAPDSQKLTTVDIVTLR<br>VLCREGVRWRVMAIQDFKPFENLRLMA<br>PISLQVVHVETHRCNISWEISQASHYFER<br>HLEFEARTLSPGHTWEEAPLLTLKQKQE<br>WICLETLTPDTQYEFQVRVKPLQGEFTT<br>WSPWSQPLAFRTKPAALGKD |

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| AK461 | DNA560 | Knob: hFc(N297A)-[VPLSLY]-hIL15(E46A, E53A) | DKTHTCPPCPAPELLGGPSVFLFPPKPKD GGSSPPG<br>TLMISRTPEVTCVVVDVSHEDPEVKFN<br>WYVDGVEVHNAKTKPREEQYASTYRVV<br>SVLTVLHQDWLNGKEYKCKVSNKALPA<br>PIEKTISKAKGQPREPQVYTLPPCRDELT<br>KNQVSLWCLVKGFYPSDIAVEWESNGQ<br>PENNYKTTPPVLDSDGSFFLYSKLTVDKS<br>RWQQGNVFSCSVMHEALHNHYTQKSL<br>SLSPG | VPLSLY | | GSGPGSGS |
| AK478 | DNA161 | Knob: hFc(N297A) | DKTHTCPPCPAPELLGGPSVFLFPPKPKD<br>TLMISRTPEVTCVVVDVSHEDPEVKFN<br>WYVDGVEVHNAKTKPREEQYASTYRVV<br>SVLTVLHQDWLNGKEYKCKVSNKALPA<br>PIEKTISKAKGQPREPQVYTLPPCRDELT<br>KNQVSLWCLVKGFYPSDIAVEWESNGQ<br>PENNYKTTPPVLDSDGSFFLYSKLTVDKS<br>RWQQGNVFSCSVMHEALHNHYTQKSL<br>SLSPG | | | |
| AK478 | DNA569 | Hole: hFc(N297A)-hCD122-hCD215(Sushi)-[VPLSLY]-hIL15 | DKTHTCPPCPAPELLGGPSVFLFPPKPKD PGSGS<br>TLMISRTPEVTCVVVDVSHEDPEVKFN<br>WYVDGVEVHNAKTKPREEQYASTYRVV<br>SVLTVLHQDWLNGKEYKCKVSNKALPA<br>PIEKTISKAKGQPREPQVCTLPPSRDELT<br>KNQVSLSCAVKGFYPSDIAVEWESNGQ<br>PENNYKTTPPVLDSDGSFFLVSKLTVDKS<br>RWQQGNVFSCSVMHEALHNHYTQKSL<br>SLSPG | AVNGTSQFTCFYNSRANISCVWSQDGA<br>LQDTSCQVHAWPDRRRWNQTCELLPV<br>SQASWACNLILGAPDSQKLTTVDIVTLR<br>VICREGVRWRVMAIQDFKPFENLRLMA<br>PISLQVVHVETHRCNISWEISQASHYFER<br>HLEFEARTLSPGHTWEEAPLLTLKQKQE<br>WICLETLTPDTQYEFQVRVKPLQGEFTT<br>WSPWSQPLAFRTKPAALGKD | GGGGSGGGGS<br>GGGGSGGGGS | |

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| AK479 | DNA161 | Knob: hFc(N297A) | DKTHTCPPCPAPELLGGPSVFLFPPKPKD<br>TLMISRTPEVTCVVVDVSHEDPEVKFN<br>WYVDGVEVHNAKTKPREEQYASTYRVV<br>SVLTVLHQDWLNGKEYKCKVSNKALPA<br>PIEKTISKAKGQPREPQVYTLPPCRDELT<br>KNQVSLWCLVKGFYPSDIAVEWESNGQ<br>PENNYKTTPPVLDSDGSFFLYSKLTVDKS<br>RWQQGNVFSCSVMHEALHNHYTQKSL<br>SLSPG |
| AK479 | DNA570 | Hole: hFc(N297A)-hCD122-hCD215(Sushi)-[VPLSLY]-hIL15 | DKTHTCPPCPAPELLGGPSVFLFPPKPKD PGSGS<br>TLMISRTPEVTCVVVDVSHEDPEVKFN<br>WYVDGVEVHNAKTKPREEQYASTYRVV<br>SVLTVLHQDWLNGKEYKCKVSNKALPA<br>PIEKTISKAKGQPREPQVCTLPPSRDELT<br>KNQVSLSCAVKGFYPSDIAVEWESNGQ<br>PENNYKTTPPVLDSDGSFFLVSKLTVDKS<br>RWQQGNVFSCSVMHEALHNHYTQKSL<br>SLSPG |
| AK480 | DNA161 | Knob: hFc(N297A) | DKTHTCPPCPAPELLGGPSVFLFPPKPKD<br>TLMISRTPEVTCVVVDVSHEDPEVKFN<br>WYVDGVEVHNAKTKPREEQYASTYRVV<br>SVLTVLHQDWLNGKEYKCKVSNKALPA<br>PIEKTISKAKGQPREPQVYTLPPCRDELT<br>KNQVSLWCLVKGFYPSDIAVEWESNGQ<br>PENNYKTTPPVLDSDGSFFLYSKLTVDKS<br>RWQQGNVFSCSVMHEALHNHYTQKSL<br>SLSPG |

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| | | | AVNGTSQFTCFYNSRANISCVWSQDGA GGGGSGGGGS<br>LQDTSCQVHAWPDRRRWNQTCELLPV GGGGSGGGGS<br>SQASWACNLILGAPDSQKLTTVDIVTLR<br>VLCREGVRWRVMAIQDFKPFENLRLMA<br>PISLQVVHVETHRCNISWEISQASHYFER<br>HLEFEARTLSPGHTWEEAPLLTLKQKQE<br>WICLETLTPDTQYEFQVRVKPLQGEFTT<br>WSPWSQPLAFRTKPAALGKD |

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| AK480 | DNA571 | Hole: hFc(N297A)-hCD122-hCD215(Sushi)-[VPLSLY]-hIL15 | DKTHTCPPCPAPELLGGPSVFLFPPKPKD PGSGS<br>TLMISRTPEVTCVVVDVSHEDPEVKFN<br>WYVDGVEVHNAKTKPREEQYASTYRVV<br>SVLTVLHQDWLNGKEYKCKVSNKALPA<br>PIEKTISKAKGQPREPQVCTLPPSRDELT<br>KNQVSLSCAVKGFYPSDIAVEWESNGQ<br>PENNYKTTPPVLDSDGSFFLVSKLTVDKS<br>RWQQGNVFSCSVMHEALHNHYTQKSL<br>SLSPG | AVNGTSQFTCFYNSRANISCVWSQDGA GGGGSGGGGS<br>LQDTSCQVHAWPDRRRWNQTCELLPV GGGGSGGGGS<br>SQASWACNLILGAPDSQKLTTVDIVTLR<br>VLCREGVRWRVMAIQDFKPFENLRLMA<br>PISLQVVHVETHRCNISWEISQASHYFER<br>HLEFEARTLSPGHTWEEAPLLTLKQKQE<br>WICLETLTPDTQYEFQVRVKPLQGEFTT<br>WSPWSQPLAFRTKPAALGKD |
| AK481 | DNA161 | Knob: hFc(N297A) | DKTHTCPPCPAPELLGGPSVFLFPPKPKD<br>TLMISRTPEVTCVVVDVSHEDPEVKFN<br>WYVDGVEVHNAKTKPREEQYASTYRVV<br>SVLTVLHQDWLNGKEYKCKVSNKALPA<br>PIEKTISKAKGQPREPQVYTLPPCRDELT<br>KNQVSLWCLVKGFYPSDIAVEWESNGQ<br>PENNYKTTPPVLDSDGSFFLYSKLTVDKS<br>RWQQGNVFSCSVMHEALHNHYTQKSL<br>SLSPG | |
| AK481 | DNA572 | Hole: hFc(N297A)-hCD122-hCD215(1to66)-[VPLSLY]-hIL15 | DKTHTCPPCPAPELLGGPSVFLFPPKPKD PGSGS<br>TLMISRTPEVTCVVVDVSHEDPEVKFN<br>WYVDGVEVHNAKTKPREEQYASTYRVV<br>SVLTVLHQDWLNGKEYKCKVSNKALPA<br>PIEKTISKAKGQPREPQVCTLPPSRDELT<br>KNQVSLSCAVKGFYPSDIAVEWESNGQ<br>PENNYKTTPPVLDSDGSFFLVSKLTVDKS<br>RWQQGNVFSCSVMHEALHNHYTQKSL<br>SLSPG | AVNGTSQFTCFYNSRANISCVWSQDGA GGGGSGGGGS<br>LQDTSCQVHAWPDRRRWNQTCELLPV GGGGSGGGGS<br>SQASWACNLILGAPDSQKLTTVDIVTLR<br>VLCREGVRWRVMAIQDFKPFENLRLMA<br>PISLQVVHVETHRCNISWEISQASHYFER<br>HLEFEARTLSPGHTWEEAPLLTLKQKQE<br>WICLETLTPDTQYEFQVRVKPLQGEFTT<br>WSPWSQPLAFRTKPAALGKD |

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| AK482 | DNA161 | Knob: hFc(N297A) | DKTHTCPPCPAPELLGGPSVFLFPPKPKD<br>TLMISRTPEVTCVVVDVSHEDPEVKFN<br>WYVDGVEVHNAKTKPREEQYASTYRVV<br>SVLTVLHQDWLNGKEYKCKVSNKALPA<br>PIEKTISKAKGQPREPQVYTLPPCRDELT<br>KNQVSLWCLVKGFYPSDIAVEWESNGQ<br>PENNYKTTPPVLDSDGSFFLYSKLTVDKS<br>RWQQGNVFSCSVMHEALHNHYTQKSL<br>SLSPG |
| AK482 | DNA573 | Hole: hFc(N297A)-hCD122-hCD215(1to66)-[VPLSLY]-hIL15 | DKTHTCPPCPAPELLGGPSVFLFPPKPKD PGSGS<br>TLMISRTPEVTCVVVDVSHEDPEVKFN<br>WYVDGVEVHNAKTKPREEQYASTYRVV<br>SVLTVLHQDWLNGKEYKCKVSNKALPA<br>PIEKTISKAKGQPREPQVCTLPPSRDELT<br>KNQVSLSCAVKGFYPSDIAVEWESNGQ<br>PENNYKTTPPVLDSDGSFFLVSKLTVDKS<br>RWQQGNVFSCSVMHEALHNHYTQKSL<br>SLSPG |
| AK483 | DNA161 | Knob: hFc(N297A) | DKTHTCPPCPAPELLGGPSVFLFPPKPKD<br>TLMISRTPEVTCVVVDVSHEDPEVKFN<br>WYVDGVEVHNAKTKPREEQYASTYRVV<br>SVLTVLHQDWLNGKEYKCKVSNKALPA<br>PIEKTISKAKGQPREPQVYTLPPCRDELT<br>KNQVSLWCLVKGFYPSDIAVEWESNGQ<br>PENNYKTTPPVLDSDGSFFLYSKLTVDKS<br>RWQQGNVFSCSVMHEALHNHYTQKSL<br>SLSPG |

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| | | | AVNGTSQFTCFYNSRANISCVWSQDGA GGGGSGGGGS<br>LQDTSCQVHAWPDRRRWNQTCELLPV GGGGSGGGGS<br>SQASWACNLILGAPDSQKLTTVDIVTLR<br>VLCREGVRWRVMAIQDFKPFENLRLMA<br>PISLQVVHVETHRCNISWEISQASHYFER<br>HLEFEARTLSPGHTWEEAPLLTLKQKQE<br>WICLETLTPDTQYEFQVRVKPLQGEFTT<br>WSPWSQPLAFRTKPAALGKD |

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| AK483 | DNA574 | Hole: hFc(N297A)-hCD122-hCD215(1to66)-[VPLSLY]-hIL15 | DKTHTCPPCPAPELLGGPSVFLFPPKPKD PGSGS<br>TLMISRTPEVTCVVVDVSHEDPEVKFN<br>WYVDGVEVHNAKTKPREEQYASTYRVV<br>SVLTVLHQDWLNGKEYKCKVSNKALPA<br>PIEKTISKAKGQPREPQVCTLPPSRDELT<br>KNQVSLSCAVKGFYPSDIAVEWESNGQ<br>PENNYKTTPPVLDSDGSFFLVSKLTVDKS<br>RWQQGNVFSCSVMHEALHNHYTQKSL<br>SLSPG | AVNGTSQFTCFYNSRANISCVWSQDGA GGGGSGGGGS<br>LQDTSCQVHAWPDRRRWNQTCELLPV GGGGSGGGGS<br>SQASWACNLILGAPDSQKLTTVDIVTLR<br>VLCREGVRWRVMAIQDFKPFENLRLMA<br>PISLQVVHVETHRCNISWEISQASHYFER<br>HLEFEARTLSPGHTWEEAPLLTLKQKQE<br>WICLETLTPDTQYEFQVRVKPLQGEFTT<br>WSPWSQPLAFRTKPAALGKD |
| AK506 | DNA158 | Hole: hFc(N297A) | DKTHTCPPCPAPELLGGPSVFLFPPKPKD<br>TLMISRTPEVTCVVVDVSHEDPEVKFN<br>WYVDGVEVHNAKTKPREEQYASTYRVV<br>SVLTVLHQDWLNGKEYKCKVSNKALPA<br>PIEKTISKAKGQPREPQVCTLPPSRDELT<br>KNQVSLSCAVKGFYPSDIAVEWESNGQ<br>PENNYKTTPPVLDSDGSFFLVSKLTVDKS<br>RWQQGNVFSCSVMHEALHNHYTQKSL<br>SLSPG | |
| AK506 | DNA596 | Knob: hFc(N297A)-hIL15(E46A, E53A) | DKTHTCPPCPAPELLGGPSVFLFPPKPKD GGSSPPGGGS<br>TLMISRTPEVTCVVVDVSHEDPEVKFN SGGGSGPSGS<br>WYVDGVEVHNAKTKPREEQYASTYRVV PG<br>SVLTVLHQDWLNGKEYKCKVSNKALPA<br>PIEKTISKAKGQPREPQVYTLPPCRDELT<br>KNQVSLWCLVKGFYPSDIAVEWESNGQ<br>PENNYKTTPPVLDSDGSFFLYSKLTVDKS<br>RWQQGNVFSCSVMHEALHNHYTQKSL<br>SLSPG | NWVNVISDLKKIEDLIQSMHIDATLYTES<br>DVHPSCKVTAMKCFLLALQVISLASGDA<br>SIHDTVENLIILANNSLSSNGNVTESGCKE<br>CEELEEKNIKEFLQSFVHIVQMFINTS |

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| AK507 | DNA158 | Hole: hFc(N297A) | DKTHTCPPCPAPELLGGPSVFLFPPKPKD<br>TLMISRTPEVTCVVVDVSHEDPEVKFN<br>WYVDGVEVHNAKTKPREEQYASTYRVV<br>SVLTVLHQDWLNGKEYKCKVSNKALPA<br>PIEKTISKAKGQPREPQVCTLPPSRDELT<br>KNQVSLSCAVKGFYPSDIAVEWESNGQ<br>PENNYKTTPPVLDSDGSFFLVSKLTVDKS<br>RWQQGNVFSCSVMHEALHNHYTQKSL<br>SLSPG | | | |
| AK507 | DNA364 | Knob: hFc(N297A)-hIL15 | DKTHTCPPCPAPELLGGPSVFLFPPKPKD<br>TLMISRTPEVTCVVVDVSHEDPEVKFN<br>WYVDGVEVHNAKTKPREEQYASTYRVV<br>SVLTVLHQDWLNGKEYKCKVSNKALPA<br>PIEKTISKAKGQPREPQVYTLPPCRDELT<br>KNQVSLWCLVKGFYPSDIAVEWESNGQ<br>PENNYKTTPPVLDSDGSFFLYSKLTVDKS<br>RWQQGNVFSCSVMHEALHNHYTQKSL<br>SLSPG | GGSSPPGGGS<br>SGGGSGPSGS<br>PG | NWVNVISDLKKIEDLIQSMHIDATLYTES<br>DVHPSCKVTAMKCFLLELQVISLESGDAS<br>IHDTVENLIILANNSLSSNGNVTESGCKEC<br>EELEEKNIKEFLQSFVHIVQMFINTS |
| AK507 | DNA595 | hCD215(1to66) | ITCPPPMSVEHADIWVKSYSLYSRERYIC<br>NSGFKRKAGTSSLTECVLNKATNVAHW<br>TTPSLKCIRD | | |

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| AK527 | DNA370 | Hole: hFc(N297A)-[MPYDLYHP]-hCD122 | DKTHTCPPCPAPELLGGPSVFLFPPKPKD<br>TLMISRTPEVTCVVVDVSHEDPEVKFN<br>WYVDGVEVHNAKTKPREEQYASTYRVV<br>SVLTVLHQDWLNGKEYKCKVSNKALPA<br>PIEKTISKAKGQPREPQVCTLPPSRDELT<br>KNQVSLSCAVKGFYPSDIAVEWESNGQ<br>PENNYKTTPPVLDSDGSFFLVSKLTVDKS<br>RWQQGNVFSCSVMHEALHNHYTQKSL<br>SLSPG | GPPSGSSP | MPYDLYHP | SGGG |
| AK527 | DNA596 | Knob: hFc(N297A)-hIL15(E46A, E53A) | DKTHTCPPCPAPELLGGPSVFLFPPKPKD<br>TLMISRTPEVTCVVVDVSHEDPEVKFN<br>WYVDGVEVHNAKTKPREEQYASTYRVV<br>SVLTVLHQDWLNGKEYKCKVSNKALPA<br>PIEKTISKAKGQPREPQVYTLPPCRDELT<br>KNQVSLWCLVKGFYPSDIAVEWESNGQ<br>PENNYKTTPPVLDSDGSFFLYSKLTVDKS<br>RWQQGNVFSCSVMHEALHNHYTQKSL<br>SLSPG | GGSSPPGGGS<br>SGGGSGPSGS<br>PG | NWVNVISDLKKIEDLIQSMHIDATLYTES<br>DVHPSCKVTAMKCFLLALQVISLASGDA<br>SIHDTVENLIILANNSLSSNGNVTESGCKE<br>CEELEEKNIKEFLQSFVHIVQMFINTS | |
| not named yet-1 | DNAtbd1 | Knob: hFc(N297A)-hIL15(E46S, E53S) | DKTHTCPPCPAPELLGGPSVFLFPPKPKD<br>TLMISRTPEVTCVVVDVSHEDPEVKFN<br>WYVDGVEVHNAKTKPREEQYASTYRVV<br>SVLTVLHQDWLNGKEYKCKVSNKALPA<br>PIEKTISKAKGQPREPQVYTLPPCRDELT<br>KNQVSLWCLVKGFYPSDIAVEWESNGQ<br>PENNYKTTPPVLDSDGSFFLYSKLTVDKS<br>RWQQGNVFSCSVMHEALHNHYTQKSL<br>SLSPG | GGSSPPGGGS<br>SGGGSGPSGS<br>PG | NWVNVISDLKKIEDLIQSMHIDATLYTES<br>DVHPSCKVTAMKCFLLSLQVISLSSGDAS<br>IHDTVENLIILANNSLSSNGNVTESGCKEC<br>EELEEKNIKEFLQSFVHIVQMFINTS | |

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| not named yet-1 | DNA158 | Hole: hFc(N297A) | DKTHTCPPCPAPELLGGPSVFLFPPKPKD<br>TLMISRTPEVTCVVVDVSHEDPEVKFN<br>WYVDGVEVHNAKTKPREEQYASTYRVV<br>SVLTVLHQDWLNGKEYKCKVSNKALPA<br>PIEKTISKAKGQPREPQVCTLPPSRDELT<br>KNQVSLSCAVKGFYPSDIAVEWESNGQ<br>PENNYKTTPPVLDSDGSFFLVSKLTVDKS<br>RWQQGNVFSCSVMHEALHNHYTQKSL<br>SLSPG |
| not named yet-2 | DNAtbd2 | Knob: hFc(N297A)-hIL15(E46R, E53R) | DKTHTCPPCPAPELLGGPSVFLFPPKPKD GGSSPPGGGS<br>TLMISRTPEVTCVVVDVSHEDPEVKFN SGGGSGPSGS<br>WYVDGVEVHNAKTKPREEQYASTYRVV PG<br>SVLTVLHQDWLNGKEYKCKVSNKALPA<br>PIEKTISKAKGQPREPQVYTLPPCRDELT<br>KNQVSLWCLVKGFYPSDIAVEWESNGQ<br>PENNYKTTPPVLDSDGSFFLYSKLTVDKS<br>RWQQGNVFSCSVMHEALHNHYTQKSL<br>SLSPG |
| not named yet-2 | DNA158 | Hole: hFc(N297A) | DKTHTCPPCPAPELLGGPSVFLFPPKPKD<br>TLMISRTPEVTCVVVDVSHEDPEVKFN<br>WYVDGVEVHNAKTKPREEQYASTYRVV<br>SVLTVLHQDWLNGKEYKCKVSNKALPA<br>PIEKTISKAKGQPREPQVCTLPPSRDELT<br>KNQVSLSCAVKGFYPSDIAVEWESNGQ<br>PENNYKTTPPVLDSDGSFFLVSKLTVDKS<br>RWQQGNVFSCSVMHEALHNHYTQKSL<br>SLSPG |

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| | | NWVNVISDLKKIEDLIQSMHIDATLYTES<br>DVHPSCKVTAMKCFLLRLQVISLRSGDA<br>SIHDTVENLIILANNSLSSNGNVTESGCKE<br>CEELEEKNIKEFLQSFVHIVQMFINTS |

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| not named yet-3 | DNAtbd3 | knob:IL15-CD215(1-66)_Chimera_V1 | DKTHTCPPCPAPELLGGPSVFLFPPKPKD GGSSPP TLMISRTPEVTCVVVDVSHEDPEVKFN WYVDGVEVHNAKTKPREEQYASTYRVV SVLTVLHQDWLNGKEYKCKVSNKALPA PIEKTISKAKGQPREPQVYTLPPCRDELT KNQVSLWCLVKGFYPSDIAVEWESNGQ PENNYKTTPPVLDSDGSFFLYSKLTVDKS RWQQGNVFSCSVMHEALHNHYTQKSL SLSPG | MPYDLYHP | SGPGSGS |
| not named yet-3 | DNA187 | Hole: hFc(N297A)-hCD122 | DKTHTCPPCPAPELLGGPSVFLFPPKPKD PGSGS TLMISRTPEVTCVVVDVSHEDPEVKFN WYVDGVEVHNAKTKPREEQYASTYRVV SVLTVLHQDWLNGKEYKCKVSNKALPA PIEKTISKAKGQPREPQVCTLPPSRDELT KNQVSLSCAVKGFYPSDIAVEWESNGQ PENNYKTTPPVLDSDGSFFLVSKLTVDKS RWQQGNVFSCSVMHEALHNHYTQKSL SLSPG | AVNGTSQFTCFYNSRANISCVWSQDGA LQDTSCQVHAWPDRRRWNQTCELLPV SQASWACNLILGAPDSQKLTTVDIVTLR VLCREGVRWRVMAIQDFKPFENLRLMA PISLQVVHVETHRCNISWEISQASHYFER HLEFEARTLSPGHTWEEAPLLTLKQKQE WICLETLTPDTQYEFQVRVKPLQGEFTT WSPWSQPLAFRTKPAALGKD | |
| not named yet-4 | DNAtbd4 | knob:IL15-CD215(1-66)_Chimera_V2 | DKTHTCPPCPAPELLGGPSVFLFPPKPKD GGSSPP TLMISRTPEVTCVVVDVSHEDPEVKFN WYVDGVEVHNAKTKPREEQYASTYRVV SVLTVLHQDWLNGKEYKCKVSNKALPA PIEKTISKAKGQPREPQVYTLPPCRDELT KNQVSLWCLVKGFYPSDIAVEWESNGQ PENNYKTTPPVLDSDGSFFLYSKLTVDKS RWQQGNVFSCSVMHEALHNHYTQKSL SLSPG | MPYDLYHP | SGPGSGS |

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|---|---|---|---|---|
| not named yet-4 | DNA187 | Hole: hFc(N297A)-hCD122 | DKTHTCPPCPAPELLGGPSVFLFPPKPKD PGSGS<br>TLMISRTPEVTCVVVDVSHEDPEVKFN<br>WYVDGVEVHNAKTKPREEQYASTYRVV<br>SVLTVLHQDWLNGKEYKCKVSNKALPA<br>PIEKTISKAKGQPREPQVCTLPPSRDELT<br>KNQVSLSCAVKGFYPSDIAVEWESNGQ<br>PENNYKTTPPVLDSDGSFFLVSKLTVDKS<br>RWQQGNVFSCSVMHEALHNHYTQKSL<br>SLSPG | AVNGTSQFTCFYNSRANISCVWSQDGA<br>LQDTSCQVHAWPDRRRWNQTCELLPV<br>SQASWACNLILGAPDSQKLTTVDIVTLR<br>VLCREGVRWRVMAIQDFKPFENLRLMA<br>PISLQVVHVETHRCNISWEISQASHYFER<br>HLEFEARTLSPGHTWEEAPLLTLKQKQE<br>WICLETLTPDTQYEFQVRVKPLQGEFTT<br>WSPWSQPLAFRTKPAALGKD |

| Molecule | name | newnames | Component5Sequence | Component6Sequence | Component7Sequence | Component8Sequence |
|---|---|---|---|---|---|---|
| AK242 | DNA158 | Hole: hFc(N297A) | | | | |
| AK242 | DNA362 | Knob: hFc(N297A)-[MPYDLYHPJ-hIL15 | NWVNVISDLKKIEDLIQSMHID ATLYTESDVHPSCKVTAMKCF LLELQVISLESGDASIHDTVENL IILANNSLSSNGNVTESGCKEC EELEEKNIKEFLQSFVHIVQMFI NTS | | | |
| AK243 | DNA158 | Hole: hFc(N297A) | | | | |

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|---|---|---|---|
| AK243 | DNA363 | Knob: hFc(N297A)-[MPYDLYHP]-hIL15 | NWVNVISDLKKIEDLIQSMHID<br>ATLYTESDVHPSCKVTAMKCF<br>LLELQVISLESGDASIHDTVENL<br>IILANNSLSSNGNVTESGCKEC<br>EELEEKNIKEFLQSFVHIVQMFI<br>NTS |
| AK244 | DNA158 | Hole: hFc(N297A) | |
| AK244 | DNA364 | Knob: hFc(N297A)-hIL15 | |

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|---|---|---|---|
| AK245 | DNA158 | Hole: hFc(N297A) | |
| AK245 | DNA365 | Knob: hFc(N297A)-[MPYDLYHP]-hIL15-hCD215 | NWVNVISDLKKIEDLIQSMHID GGGGSGGGGS ITCPPPMSVEHADIWVKSYSL<br>ATLYTESDVHPSCKVTAMKCF GGGGSGGGGS YSRERYICNSGFKRKAGTSSLT<br>LLELQVISLESGDASIHDTVENL ECVLNKATNVAHWTTPSLKCI<br>IILANNSLSSNGNVTESGCKEC RDPALVHQRPAPPSTVTTAG<br>EELEEKNIKEFLQSFVHIVQMFI VTPQPESLSPSGKEPAASSPSS<br>NTS NNTAATTAAIVPGSQLMPSK<br>SPSTGTTEISSHESSHGTPSQT<br>TAKNWELTASASHQPPGVYP<br>QGHSDTT |
| AK246 | DNA158 | Hole: hFc(N297A) | |

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|---|---|---|---|
| AK246 | DNA366 | Knob: hFc(N297A)-[MPYDLYHP]-hIL15-hCD215(Truncated) | NWVNVISDLKKIEDLIQSMHID ATLYTESDVHPSCKVTAMKCF LLELQVISLESGDASIHDTVENL IILANNSLSSNGNVTESGCKEC EELEEKNIKEFLQSFVHIVQMFI NTS |
| | | | GGGGSGGGGS GGGGSGGGGS |
| | | | ITCPPPMSVEHADIWVKSYSL YSRERYICNSGFKRKAGTSSLT ECVLNKATNVAHWTTPSLKCI RDPALVHQRPAPPSTVTTAG VTPQPESLSPSGKEPAAS |
| AK247 | DNA187 | Hole: hFc(N297A)-hCD122 | |
| AK247 | DNA362 | Knob: hFc(N297A)-[MPYDLYHP]-hIL15 | NWVNVISDLKKIEDLIQSMHID ATLYTESDVHPSCKVTAMKCF LLELQVISLESGDASIHDTVENL IILANNSLSSNGNVTESGCKEC EELEEKNIKEFLQSFVHIVQMFI NTS |

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|---|---|---|---|
| AK248 | DNA187 | Hole: hFc(N297A)-hCD122 | |
| AK248 | DNA363 | Knob: hFc(N297A)-[MPYDLYHP]-hIL15 | NWVNVISDLKKIEDLIQSMHID<br>ATLYTESDVHPSCKVTAMKCF<br>LLELQVISLESGDASIHDTVENL<br>IILANNSLSSNGNVTESGCKEC<br>EELEEKNIKEFLQSFVHIVQMFI<br>NTS |
| AK249 | DNA187 | Hole: hFc(N297A)-hCD122 | |

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|---|---|---|---|
| AK249 | DNA364 | Knob: hFc(N297A)-hIL15 | |
| AK250 | DNA187 | Hole: hFc(N297A)-hCD122 | |
| AK250 | DNA365 | Knob: hFc(N297A)-[MPYDLYHP]-hIL15-hCD215 | NWVNVISDLKKIEDLIQSMHID GGGGSGGGGS ITCPPPMSVEHADIWVKSYSL<br>ATLYTESDVHPSCKVTAMKCF GGGGSGGGGS YSRERYICNSGFKRKAGTSSLT<br>LLELQVISLESGDASIHDTVENL ECVLNKATNVAHWTTPSLKCI<br>IILANNSLSSNGNVTESGCKEC RDPALVHQRPAPPSTVTTAG<br>EELEEKNIKEFLQSFVHIVQMFI VTPQPESLSPSGKEPAASSPSS<br>NTS NNTAATTAAIVPGSQLMPSK<br>SPSTGTTEISSHESSHGTPSQT<br>TAKNWELTASASHQPPGVYP<br>QGHSDTT |

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|---|---|---|---|
| AK251 | DNA187 | Hole: hFc(N297A)-hCD122 | |
| AK251 | DNA366 | Knob: hFc(N297A)-[MPYDLYHP]-hIL15-hCD215(Truncated) | NWVNVISDLKKIEDLIQSMHID GGGGSGGGGS ITCPPPMSVEHADIWVKSYSL<br>ATLYTESDVHPSCKVTAMKCF GGGGSGGGGS YSRERYICNSGFKRKAGTSSLT<br>LLELQVISLESGDASIHDTVENL ECVLNKATNVAHWTTPSLKCI<br>IILANNSLSSNGNVTESGCKEC RDPALVHQRPAPPSTVTTAG<br>EELEEKNIKEFLQSFVHIVQMFI VTPQPESLSPSGKEPAAS<br>NTS |
| AK399 | DNA187 | Hole: hFc(N297A)-hCD122 | |

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|---|---|---|---|
| AK399 | DNA517 | Knob: hFc(N297A)-[VPLSLY]-hIL15-hCD215 | NWVNVISDLKKIEDLIQSMHID GGGGSGGGGS ITCPPPMSVEHADIWVKSYSL<br>ATLYTESDVHPSCKVTAMKCF GGGGSGGGGS YSRERYICNSGFKRKAGTSSLT<br>LLELQVISLESGDASIHDTVENL GGGGS ECVLNKATNVAHWTTPSLKCI<br>IILANNSLSSNGNVTESGCKEC RDPALVHQRPAPPSTVTTAG<br>EELEEKNIKEFLQSFVHIVQMFI VTPQPESLSPSGKEPAASPSS<br>NTS NNTAATTAAIVPGSQLMPSK<br>SPSTGTTEISSHESSHGTPSQT<br>TAKNWELTASASHQPPGVYP<br>QGHSDTT |
| AK400 | DNA187 | Hole: hFc(N297A)-hCD122 | |
| AK400 | DNA518 | Knob: hFc(N297A)-[VPLSLY]-hIL15-hCD215(Truncated) | NWVNVISDLKKIEDLIQSMHID GGGGSGGGGS ITCPPPMSVEHADIWVKSYSL<br>ATLYTESDVHPSCKVTAMKCF GGGGSGGGGS YSRERYICNSGFKRKAGTSSLT<br>LLELQVISLESGDASIHDTVENL GGGGS ECVLNKATNVAHWTTPSLKCI<br>IILANNSLSSNGNVTESGCKEC RDPALVHQRPAPPSTVTTAG<br>EELEEKNIKEFLQSFVHIVQMFI VTPQPESLSPSGKEPAAS<br>NTS |

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|---|---|---|---|---|---|
| AK401 | DNA161 | Knob: hFc(N297A) | | | |
| AK401 | DNA523 | Hole: hFc(N297A)-hCD122-[VPLSLY]-hCD215(Sushi)-hIL15 | VPLSLY | SGGP | ITCPPPMSVEHADIWVKSYSL SGGGGSGGGGSGGGGSG<br>YSRERYICNSGFKRKAGTSSLT GGGS<br>ECVLNKATNVAHWTTPSLKCI<br>RDPALVHQRPAPP |
| AK402 | DNA161 | Knob: hFc(N297A) | | | |

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|---|---|---|---|---|---|
| AK402 | DNA524 | Hole: hFc(N297A)-hCD122-[VPLSLY]-hCD215(Sushi)-hIL15 | VPLSLY | SGGP | ITCPPPMSVEHADIWVKSYSL SGGGGSGGGGSGGGGSG<br>YSRERYICNSGFKRKAGTSSLT GGGS<br>ECVLNKATNVAHWTTPSLKCI<br>RDPALVHQRPAPP |
| AK403 | DNA161 | Knob: hFc(N297A) | | | |
| AK403 | DNA525 | Hole: hFc(N297A)-hCD122-[VPLSLY]-hCD215(Sushi)-hIL15 | VPLSLY | SGGP | ITCPPPMSVEHADIWVKSYSL SGGGGSGGGGSGGGGSG<br>YSRERYICNSGFKRKAGTSSLT GGGS<br>ECVLNKATNVAHWTTPSLKCI<br>RDPALVHQRPAPP |

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|---|---|---|---|
| AK404 | DNA187 | Hole: hFc(N297A)-hCD122 | |
| AK404 | DNA526 | Knob: hFc(N297A)-[VPLSLY]-hIL15-hCD215 | NWVNVISDLKKIEDLIQSMHID GGSGGGSGGG ITCPPPMSVEHADIWVKSYSL<br>ATLYTESDVHPSCKVTAMKCF SGGGSGGGSG YSRERYICNSGFKRKAGTSSLT<br>LLELQVISLESGDASIHDTVENL ECVLNKATNVAHWTTPSLKCI<br>IILANNSLSSNGNVTESGCKEC RDPALVHQRPAPPSTVTTAG<br>EELEEKNIKEFLQSFVHIVQMFI VTPQPESLSPSGKEPAASPSS<br>NTS NNTAATTAAIVPGSQLMPSK<br>SPSTGTTEISSHESSHGTPSQT<br>TAKNWELTASASHQPPGVYP<br>QGHSDTT |
| AK405 | DNA187 | Hole: hFc(N297A)-hCD122 | |

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|---|---|---|---|---|---|
| AK405 | DNA527 | Knob: hFc(N297A)-[VPLSLYI-hIL15-hCD215 | NWVNVISDLKKIEDLIQSMHID<br>ATLYTESDVHPSCKVTAMKCF<br>LLELQVISLESGDASIHDTVENL<br>IILANNSLSSNGNVTESGCKEC<br>EELEEKNIKEFLQSFVHIVQMFI<br>NTS | GGSGGGSGGG<br>SGGGSGGGSG<br>GGSG | ITCPPPMSVEHADIWVKSYSL<br>YSRERYICNSGFKRKAGTSSLT<br>ECVLNKATNVAHWTTPSLKCI<br>RDPALVHQRPAPPSTVTTAG<br>VTPQPESLSPSGKEPAASSPSS<br>NNTAATTAAIVPGSQLMPSK<br>SPSTGTTEISSHESSHGTPSQT<br>TAKNWELTASASHQPPGVYP<br>QGHSDTT |
| AK418 | DNA187 | Hole: hFc(N297A)-hCD122 | | | |
| AK418 | DNA367 | Knob: hFc(N297A)-hIL15 | | | |

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|---|---|---|---|
| AK419 | DNA187 | Hole: hFc(N297A)-hCD122 | |
| AK419 | DNA368 | Knob: hFc(N297A)-[MPYDLYHP]-hIL15-hCD215 | NWVNVISDLKKIEDLIQSMHID GGGGSGGGGS ITCPPPMSVEHADIWVKSYSL<br>ATLYTESDVHPSCKVTAMKCF GGGGSGGGGS YSRERYICNSGFKRKAGTSSLT<br>LLELQVISLESGDASIHDTVENL ECVLNKATNVAHWTTPSLKCI<br>IILANNSLSSNGNVTESGCKEC RDPALVHQRPAPPSTVTTAG<br>EELEEKNIKEFLQSFVHIVQMFI VTPQPESLSPSGKEPAASPSS<br>NTS NNTAATTAAIVPGSQLMPSK<br>SPSTGTTEISSHESSHGTPSQT<br>TAKNWELTASASHQPPGVYP<br>QGHSDTT |
| AK420 | DNA187 | Hole: hFc(N297A)-hCD122 | |

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|---|---|---|---|
| AK420 | DNA369 | Knob: hFc(N297A)-[MPYDLYHP]-hIL15-hCD215(Truncated) | NWVNVISDLKKIEDLIQSMHID GGGGSGGGGS ITCPPPMSVEHADIWVKSYSL<br>ATLYTESDVHPSCKVTAMKCF GGGGSGGGGS YSRERYICNSGFKRKAGTSSLT<br>LLELQVISLESGDASIHDTVENL ECVLNKATNVAHWTTPSLKCI<br>IILANNSLSSNGNVTESGCKEC RDPALVHQRPAPPSTVTTAG<br>EELEEKNIKEFLQSFVHIVQMFI VTPQPESLSPSGKEPAAS<br>NTS |
| AK421 | DNA187 | Hole: hFc(N297A)-hCD122 | |
| AK421 | DNA487 | Knob: hFc(N297A)-[VPLSLY]-hIL15 | NWVNVISDLKKIEDLIQSMHID<br>ATLYTESDVHPSCKVTAMKCF<br>LLELQVISLESGDASIHDTVENL<br>IILANNSLSSNGNVTESGCKEC<br>EELEEKNIKEFLQSFVHIVQMFI<br>NTS |

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|---|---|---|
| AK457 | DNA187 | Hole: hFc(N297A)-hCD122 |
| AK457 | DNA556 | Knob: hFc(N297A)-[VPLSLY]-hIL15 |
| | | NWVNVISDLKKIEDLIQSMHID<br>ATLYTESDVHPSCKVTAMKCF<br>LLELQVISLESGDASIHDTVENL<br>IILANNSLSSNGNVTESGCKEC<br>EELEEKNIKEFLQSFVHIVQMFI<br>NTS |
| AK458 | DNA187 | Hole: hFc(N297A)-hCD122 |

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| AK458 | DNA557 | Knob: hFc(N297A)-[VPLSLY]-hIL15(D22A) | NWVNVISDLKKIEDLIQSMHIA<br>ATLYTESDVHPSCKVTAMKCF<br>LLELQVISLESGDASIHDTVENL<br>IILANNSLSSNGNVTESGCKEC<br>EELEEKNIKEFLQSFVHIVQMFI<br>NTS |
| AK459 | DNA187 | Hole: hFc(N297A)-hCD122 | |
| AK459 | DNA558 | Knob: hFc(N297A)-[VPLSLY]-hIL15(E46A) | NWVNVISDLKKIEDLIQSMHID<br>ATLYTESDVHPSCKVTAMKCF<br>LLALQVISLESGDASIHDTVENL<br>IILANNSLSSNGNVTESGCKEC<br>EELEEKNIKEFLQSFVHIVQMFI<br>NTS |

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|---|---|---|---|
| AK460 | DNA187 | Hole: hFc(N297A)-hCD122 | |
| AK460 | DNA559 | Knob: hFc(N297A)-[VPLSLY]-hIL15(E53A) | NWVNVISDLKKIEDLIQSMHID<br>ATLYTESDVHPSCKVTAMKCF<br>LLELQVISLASGDASIHDTVENL<br>IILANNSLSSNGNVTESGCKEC<br>EELEEKNIKEFLQSFVHIVQMFI<br>NTS |
| AK461 | DNA187 | Hole: hFc(N297A)-hCD122 | |

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|---|---|---|---|
| AK461 | DNA560 | Knob: hFc(N297A)-[VPLSLY]-hIL15(E46A, E53A) | NWVNVISDLKKIEDLIQSMHID<br>ATLYTESDVHPSCKVTAMKCF<br>LLALQVISLASGDASIHDTVENL<br>IILANNSLSSNGNVTESGCKEC<br>EELEEKNIKEFLQSFVHIVQMFI<br>NTS |
| AK478 | DNA161 | Knob: hFc(N297A) | |
| AK478 | DNA569 | Hole: hFc(N297A)-hCD122-hCD215(Sushi)-[VPLSLY]-hIL15 | ITCPPPMSVEHADIWVKSYSLY SPSPPGPGSPP VPLSLY<br>SRERYICNSGFKRKAGTSSLTEC<br>VLNKATNVAHWTTPSLKCIRD<br>PALVHQRPAPP<br>SGG |

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|---|---|---|---|---|---|
| AK479 | DNA161 | Knob: hFc(N297A) | | | |
| AK479 | DNA570 | Hole: hFc(N297A)-hCD122-hCD215(Sushi)-[VPLSLY]-hIL15 | ITCPPPMSVEHADIWVKSYSLY SSGGPGSGPPS VPLSLY<br>SRERYICNSGFKRKAGTSSLTEC G<br>VLNKATNVAHWTTPSLKCIRD<br>PALVHQRPAPP | SGG | |
| AK480 | DNA161 | Knob: hFc(N297A) | | | |

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|---|---|---|---|---|---|---|
| AK480 | DNA571 | Hole: hFc(N297A)-hCD122-hCD215(Sushi)-[VPLSLY]-hIL15 | ITCPPPMSVEHADIWVKSYSLY SRERYICNSGFKRKAGTSSLTEC VLNKATNVAHWTTPSLKCIRD PALVHQRPAPP | SSSGSSPGGPS GPP | VPLSLY | SGG |
| AK481 | DNA161 | Knob: hFc(N297A) | | | | |
| AK481 | DNA572 | Hole: hFc(N297A)-hCD122-hCD215(1to66)-[VPLSLY]-hIL15 | ITCPPPMSVEHADIWVKSYSLY SRERYICNSGFKRKAGTSSLTEC VLNKATNVAHWTTPSLKCIRD | GGSSPP | VPLSLY | SGG |

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|---|---|---|---|---|---|---|
| AK482 | DNA161 | Knob: hFc(N297A) | | | | |
| AK482 | DNA573 | Hole: hFc(N297A)-hCD122-hCD215(1to66)-[VPLSLY]-hIL15 | ITCPPPMSVEHADIWVKSYSLY SRERYICNSGFKRKAGTSSLTEC VLNKATNVAHWTTPSLKCIRD | SPSSGSG | VPLSLY | SGG |
| AK483 | DNA161 | Knob: hFc(N297A) | | | | |

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|---|---|---|---|---|---|
| AK483 | DNA574 | Hole: hFc(N297A)-hCD122-hCD215(1to66)-[VPLSLY]-hIL15 | ITCPPPMSVEHADIWVKSYSLY GPPSGSSP SRERYICNSGFKRKAGTSSLTEC VLNKATNVAHWTTPSLKCIRD | VPLSLY | SGG |
| AK506 | DNA158 | Hole: hFc(N297A) | | | |
| AK506 | DNA596 | Knob: hFc(N297A)-hIL15(E46A, E53A) | | | |

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| AK507 | DNA158 | Hole: hFc(N297A) |
| AK507 | DNA364 | Knob: hFc(N297A)-hIL15 |
| AK507 | DNA595 | hCD215(1to66) |

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|---|---|---|---|
| AK527 | DNA370 | Hole: hFc(N297A)-[MPYDLYHP]-hCD122 | AVNGTSQFTCFYNSRANISCV<br>WSQDGALQDTSCQVHAWPD<br>RRRWNQTCELLPVSQASWAC<br>NLILGAPDSQKLTTVDIVTILRVL<br>CREGVRWRVMAIQDFKPFEN<br>LRLMAPISLQVVHVETHRCNIS<br>WEISQASHYFERHLEFEARTLS<br>PGHTWEEAPLLTLKQKQEWIC<br>LETLTPDTQYEFQVRVKPLQG<br>EFTTWSPWSQPLAFRTKPAAL<br>GKD |
| AK527 | DNA596 | Knob: hFc(N297A)-hIL15(E46A, E53A) | |
| not named yet-1 | DNAtbd1 | Knob: hFc(N297A)-hIL15(E46S, E53S) | |

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|---|---|---|
| not named yet-1 | DNA158 | Hole: hFc(N297A) |
| not named yet-2 | DNAtbd2 | Knob: hFc(N297A)-hIL15(E46R, E53R) |
| not named yet-2 | DNA158 | Hole: hFc(N297A) |

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|---|---|---|---|---|---|
| not named yet-3 | DNAtbd3 | knob:IL15-CD215(1-66)_Chimera_V1 | NWVNVISDLKKIEDLIQS | GGGGSGGGGS | ITCPPPMSVEHADIWVKSYSL YSRERYICNSGFKRKAGTSSLT ECVLNKATNVAHWTTPSLKCI RD GGGGSGGGGS |
| not named yet-3 | DNA187 | Hole: hFc(N297A)-hCD122 | | | |
| not named yet-4 | DNAtbd4 | knob:IL15-CD215(1-66)_Chimera_V2 | NWVNVISDLKKIEDLIQS | GGGGSGGGGS GGS | ITCPPPMSVEHADIWVKSYSL YSRERYICNSGFKRKAGTSSLT ECVLNKATNVAHWTTPSLKCI RD GGGGSGGGGSGGGS |

not named DNA187 Hole: hFc(N297A)-
yet-4 hCD122

| Molecule | name | newnames | Component9Sequence | Released post cleavage |
|---|---|---|---|---|
| AK242 | DNA158 | Hole: hFc(N297A) | | |
| AK242 | DNA362 | Knob: hFc(N297A)-[MPYDLYHP]-hIL15 | | LYHPSGPSGSPGNWVNVISDLKKIEDLIQSMHIDATLYTESDVHPSC KVTAMKCFLLELQVISLESGDASIHDTVENLIILANNSLSSNGNVTES GCKECEELEEKNIKEFLQSFVHIVQMFINTS |
| AK243 | DNA158 | Hole: hFc(N297A) | | |

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|---|---|---|---|
| | | | LYHPSGPGSGSNWVNVISDLKKIEDLIQSMHIDATLYTESDVHPSCK VTAMKCFLLELQVISLESGDASIHDTVENLIILANNSLSSNGNVTESG CKECEELEEKNIKEFLQSFVHIVQMFINTS |
| AK243 | DNA363 | Knob: hFc(N297A)-[MPYDLYHP]-hIL15 | |
| AK244 | DNA158 | Hole: hFc(N297A) | |
| AK244 | DNA364 | Knob: hFc(N297A)-hIL15 | |

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|---|---|---|
| AK245 | DNA158 | Hole: hFc(N297A) |
| AK245 | DNA365 | Knob: hFc(N297A)-[MPYDLYHP]-hIL15-hCD215 |
| AK246 | DNA158 | Hole: hFc(N297A) |

LYHPSGPSGSPGNWVNVISDLKKIEDLIQSMHIDATLYTESDVHPSC
KVTAMKCFLLELQVISLESGDASIHDTVENLIILANNSLSSNGNVTES
GCKECEELEEKNIKEFLQSFVHIVQMFINTSGGGGSGGGGSGGGGS
GGGGSITCPPPMSVEHADIWVKSYSLYSRERYICNSGFKRKAGTSSL
TECVLNKATNVAHWTTPSLKCIRDPALVHQRPAPPSTVTTAGVTP
QPESLSPSGKEPAASSPSSNNTAATTAAIVPGSQLMPSKSPSTGTTE
ISSHESSHGTPSQTTAKNWELTASASHQPPGVYPQGHSDTT

| | | |
|---|---|---|
| AK246 | DNA366 | Knob: hFc(N297A)-[MPYDLYHP]-hIL15-hCD215(Truncated) |
| AK247 | DNA187 | Hole: hFc(N297A)-hCD122 |
| AK247 | DNA362 | Knob: hFc(N297A)-[MPYDLYHP]-hIL15 |

LYHPSGPSGSPGNWVNVISDLKKIEDLIQSMHIDATLYTESDVHPSC
KVTAMKCFLLELQVISLESGDASIHDTVENLIILANNSLSSNGNVTES
GCKECEELEEKNIKEFLQSFVHIVQMFINTS

| | | |
|---|---|---|
| AK248 | DNA187 | Hole: hFc(N297A)-hCD122 |
| AK248 | DNA363 | Knob: hFc(N297A)-[MPYDLYHP]-hIL15 |
| AK249 | DNA187 | Hole: hFc(N297A)-hCD122 |

LYHPSGPGSGSNWVNVISDLKKIEDLIQSMHIDATLYTESDVHPSCK
VTAMKCFLLELQVISLESGDASIHDTVENLIILANNSLSSNGNVTESG
CKECEELEEKNIKEFLQSFVHIVQMFINTS

| | | |
|---|---|---|
| AK249 | DNA364 | Knob: hFc(N297A)-hIL15 |
| AK250 | DNA187 | Hole: hFc(N297A)-hCD122 |
| AK250 | DNA365 | Knob: hFc(N297A)-[MPYDLYHP]-hIL15-hCD215 |

LYHPSGPSGSPGNWVNVISDLKKIEDLIQSMHIDATLYTESDVHPSC
KVTAMKCFLLELQVISLESGDASIHDTVENLIILANNSLSSNGNVTES
GCKECEELEEKNIKEFLQSFVHIVQMFINTSGGGGSGGGGSGGGGS
GGGGSITCPPPMSVEHADIWVKSYSLYSRERYICNSGFKRKAGTSSL
TECVLNKATNVAHWTTPSLKCIRDPALVHQRPAPPSTVTTAGVTP
QPESLSPSGKEPAASSPSSNNTAATTAAIVPGSQLMPSKSPSTGTTE
ISSHESSHGTPSQTTAKNWELTASASHQPPGVYPQGHSDTT

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|---|---|---|---|
| AK251 | DNA187 | Hole: hFc(N297A)-hCD122 | |
| AK251 | DNA366 | Knob: hFc(N297A)-[MPYDLYHP]-hIL15-hCD215(Truncated) | LYHPSGPSGSPGNWVNVISDLKKIEDLIQSMHIDATLYTESDVHPSC<br>KVTAMKCFLLELQVISLESGDASIHDTVENLIILANNSLSSNGNVTES<br>GCKECEELEEKNIKEFLQSFVHIVQMFINTSGGGGSGGGGSGGGGS<br>GGGGSITCPPPMSVEHADIWVKSYSLYSRERYICNSGFKRKAGTSSL<br>TECVLNKATNVAHWTTPSLKCIRDPALVHQRPAPPSTVTTAGVTP<br>QPESLSPSGKEPAAS |
| AK399 | DNA187 | Hole: hFc(N297A)-hCD122 | |

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|---|---|---|---|
| AK399 | DNA517 | Knob: hFc(N297A)-[VPLSLY]-hIL15-hCD215 | LYSGPGSGSNWVNVISDLKKIEDLIQSMHIDATLYTESDVHPSCKVT<br>AMKCFLLELQVISLESGDASIHDTVENLIILANNSLSSNGNVTESGCK<br>ECEELEEKNIKEFLQSFVHIVQMFINTSGGGGSGGGGSGGGGSGG<br>GGSGGGGSITCPPPMSVEHADIWVKSYSLYSRERYICNSGFKRKAG<br>TSSLTECVLNKATNVAHWTTPSLKCIRDPALVHQRPAPPSTVTTAG<br>VTPQPESLSPSGKEPAASSPSSNNTAATTAAIVPGSQLMPSKSPSTG<br>TTEISSHESSHGTPSQTTAKNWELTASASHQPPGVYPQGHSDTT |
| AK400 | DNA187 | Hole: hFc(N297A)-hCD122 | |
| AK400 | DNA518 | Knob: hFc(N297A)-[VPLSLY]-hIL15-hCD215(Truncated) | LYSGPGSGSNWVNVISDLKKIEDLIQSMHIDATLYTESDVHPSCKVT<br>AMKCFLLELQVISLESGDASIHDTVENLIILANNSLSSNGNVTESGCK<br>ECEELEEKNIKEFLQSFVHIVQMFINTSGGGGSGGGGSGGGGSGG<br>GGSGGGGSITCPPPMSVEHADIWVKSYSLYSRERYICNSGFKRKAG<br>TSSLTECVLNKATNVAHWTTPSLKCIRDPALVHQRPAPPSTVTTAG<br>VTPQPESLSPSGKEPAAS |

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|---|---|---|---|
| AK401 | DNA161 | Knob: hFc(N297A) | |
| AK401 | DNA523 | Hole: hFc(N297A)-hCD122-[VPLSLY]-hCD215(Sushi)-hIL15 | NWVNVISDLKKIEDLIQSMHIDATLYTESD<br>VHPSCKVTAMKCFLLELQVISLESGDASIH<br>DTVENLIILANNSLSSNGNVTESGCKECEEL<br>EEKNIKEFLQSFVHIVQMFINTS |
| AK402 | DNA161 | Knob: hFc(N297A) | |

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|---|---|---|---|
| AK402 | DNA524 | Hole: hFc(N297A)-hCD122-[VPLSLY]-hCD215(Sushi)-hIL15 | NWVNVISDLKKIEDLIQSMHIDATLYTESD VHPSCKVTAMKCFLLELQVISLESGDASIH DTVENLIILANNSLSSNGNVTESGCKECEEL EEKNIKEFLQSFVHIVQMFINTS |
| | | | LYSGGPITCPPPMSVEHADIWVKSYSLYSRERYICNSGFKRKAGTSSL TECVLNKATNVAHWTTPSLKCIRDPALVHQRPAPPSGGGSGGGG GSGGGGSGGGGSNWVNVISDLKKIEDLIQSMHIDATLYTESDVHPS CKVTAMKCFLLELQVISLESGDASIHDTVENLIILANNSLSSNGNVTE SGCKECEEELEEKNIKEFLQSFVHIVQMFINTS |
| AK403 | DNA161 | Knob: hFc(N297A) | |
| AK403 | DNA525 | Hole: hFc(N297A)-hCD122-[VPLSLY]-hCD215(Sushi)-hIL15 | NWVNVISDLKKIEDLIQSMHIDATLYTESD VHPSCKVTAMKCFLLELQVISLESGDASIH DTVENLIILANNSLSSNGNVTESGCKECEEL EEKNIKEFLQSFVHIVQMFINTS |
| | | | LYSGGPITCPPPMSVEHADIWVKSYSLYSRERYICNSGFKRKAGTSSL TECVLNKATNVAHWTTPSLKCIRDPALVHQRPAPPSGGGSGGGG GSGGGGSGGGGSNWVNVISDLKKIEDLIQSMHIDATLYTESDVHPS CKVTAMKCFLLELQVISLESGDASIHDTVENLIILANNSLSSNGNVTE SGCKECEEELEEKNIKEFLQSFVHIVQMFINTS |

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|---|---|---|---|
| AK404 | DNA187 | Hole: hFc(N297A)-hCD122 | |
| AK404 | DNA526 | Knob: hFc(N297A)-[VPLSLY]-hIL15-hCD215 | LYSGPSGSPGNWVNVISDLKKIEDLIQSMHIDATLYTESDVHPSCKV<br>TAMKCFLLELQVISLESGDASIHDTVENLIILANNSLSSNGNVTESGC<br>KECEELEEKNIKEFLQSFVHIVQMFINTSGGSGGGSGGGSGGGSGG<br>GSGITCPPPMSVEHADIWVKSYSLYSRERYICNSGFKRKAGTSSLTE<br>CVLNKATNVAHWTTPSLKCIRDPALVHQRPAPPSTVTTAGVTPQP<br>ESLSPSGKEPAASSPSSNNTAATTAAIVPGSQLMPSKSPSTGTTEISS<br>HESSHGTPSQTTAKNWELTASASHQPPGVYPQGHSDTT |
| AK405 | DNA187 | Hole: hFc(N297A)-hCD122 | |

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|---|---|---|---|
| AK405 | DNA527 | Knob: hFc(N297A)-[VPLSLY]-hIL15-hCD215 | LYSGPSGSPGNWVNVISDLKKIEDLIQSMHIDATLYTESDVHPSCKV<br>TAMKCFLLELQVISLESGDASIHDTVENLIILANNSLSSNGNVTESGC<br>KECEELEEKNIKEFLQSFVHIVQMFINTSGGSGGGSGGGSGGGSGG<br>GSGGGSGITCPPPMSVEHADIWVKSYSLYSRERYICNSGFKRKAGT<br>SSLTECVLNKATNVAHWTTPSLKCIRDPALVHQRPAPPSTVTTAGV<br>TPQPESLSPSGKEPAASSPSSNNTAATTAAIVPGSQLMPSKSPSTGT<br>TEISSHESSHGTPSQTTAKNWELTASASHQPPGVYPQGHSDTT |
| AK418 | DNA187 | Hole: hFc(N297A)-hCD122 | |
| AK418 | DNA367 | Knob: hFc(N297A)-hIL15 | |

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|---|---|---|---|
| AK419 | DNA187 | Hole: hFc(N297A)-hCD122 | |
| AK419 | DNA368 | Knob: hFc(N297A)-[MPYDLYHP]-hIL15-hCD215 | LYHPSGPGSGSNWVNVISDLKKIEDLIQSMHIDATLYTESDVHPSCK<br>VTAMKCFLLELQVISLESGDASIHDTVENLIILANNSLSSNGNVTESG<br>CKECEELEEKNIKEFLQSFVHIVQMFINTSGGGGSGGGGSGGGGSG<br>GGGSITCPPPMSVEHADIWVKSYSLYSRERYICNSGFKRKAGTSSLT<br>ECVLNKATNVAHWTTPSLKCIRDPALVHQRPAPPSTVTTAGVTPQ<br>PESLSPSGKEPAASSPSSNNTAATTAAIVPGSQLMPSKSPSTGTTEIS<br>SHESSHGTPSQTTAKNWELTASASHQPPGVYPQGHSDTT |
| AK420 | DNA187 | Hole: hFc(N297A)-hCD122 | |

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|---|---|---|---|
| AK420 | DNA369 | Knob: hFc(N297A)-[MPYDLYHP]-hIL15-hCD215(Truncated) | LYHPSGPGSGSNWVNVISDLKKIEDLIQSMHIDATLYTESDVHPSCK<br>VTAMKCFLLELQVISLESGDASIHDTVENLIILANNSLSSNGNVTESG<br>CKECEELEEKNIKEFLQSFVHIVQMFINTSGGGSGGGGSGGGGSG<br>GGGSITCPPPMSVEHADIWVKSYSLYSRERYICNSGFKRKAGTSSLT<br>ECVLNKATNVAHWTTPSLKCIRDPALVHQRPAPPSTVTTAGVTPQ<br>PESLSPSGKEPAAS |
| AK421 | DNA187 | Hole: hFc(N297A)-hCD122 | |
| AK421 | DNA487 | Knob: hFc(N297A)-[VPLSLY]-hIL15 | LYSGPGSGSNWVNVISDLKKIEDLIQSMHIDATLYTESDVHPSCKVT<br>AMKCFLLELQVISLESGDASIHDTVENLIILANNSLSSNGNVTESGCK<br>ECEELEEKNIKEFLQSFVHIVQMFINTS |

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| AK457 | DNA187 | Hole: hFc(N297A)-hCD122 |
| AK457 | DNA556 | Knob: hFc(N297A)-[VPLSLY]-hIL15 |
| AK458 | DNA187 | Hole: hFc(N297A)-hCD122 |

LYGSGPGSGSNWVNVISDLKKIEDLIQSMHIDATLYTESDVHPSCKV
TAMKCFLLELQVISLESGDASIHDTVENLIILANNSLSSNGNVTESGC
KECEELEEKNIKEFLQSFVHIVQMFINTS

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|---|---|---|---|
| AK458 | DNA557 | Knob: hFc(N297A)-[VPLSLY]-hIL15(D22A) | LYGSGPGSGSNWVNVISDLKKIEDLIQSMHIAATLYTESDVHPSCKV TAMKCFLLELQVISLESGDASIHDTVENLIILANNSLSSNGNVTESGC KECEELEEKNIKEFLQSFVHIVQMFINTS |
| AK459 | DNA187 | Hole: hFc(N297A)-hCD122 | |
| AK459 | DNA558 | Knob: hFc(N297A)-[VPLSLY]-hIL15(E46A) | LYGSGPGSGSNWVNVISDLKKIEDLIQSMHIDATLYTESDVHPSCKV TAMKCFLLALQVISLESGDASIHDTVENLIILANNSLSSNGNVTESGC KECEELEEKNIKEFLQSFVHIVQMFINTS |

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|---|---|---|---|
| AK460 | DNA187 | Hole: hFc(N297A)-hCD122 | |
| AK460 | DNA559 | Knob: hFc(N297A)-[VPLSLY]-hIL15(E53A) | LYGSGPGSGSNWVNVISDLKKIEDLIQSMHIDATLYTESDVHPSCKV<br>TAMKCFLLELQVISLASGDASIHDTVENLIILANNSLSSNGNVTESGC<br>KECEELEEKNIKEFLQSFVHIVQMFINTS |
| AK461 | DNA187 | Hole: hFc(N297A)-hCD122 | |

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|---|---|---|---|
| AK461 | DNA560 | Knob: hFc(N297A)-[VPLSLY]-hIL15(E46A, E53A) | LYGSGPGSGSNWVNVISDLKKIEDLIQSMHIDATLYTESDVHPSCKV<br>TAMKCFLLALQVISLASGDASIHDTVENLIILANNSLSSNGNVTESGC<br>KECEELEEKNIKEFLQSFVHIVQMFINTS |
| AK478 | DNA161 | Knob: hFc(N297A) | |
| AK478 | DNA569 | Hole: hFc(N297A)-hCD122-hCD215(Sushi)-[VPLSLY]-hIL15 | NWVNVISDLKKIEDLIQSMHIDATLYTESD<br>VHPSCKVTAMKCFLLELQVISLESGDASIH<br>DTVENLIILANNSLSSNGNVTESGCKECEEL<br>EEKNIKEFLQSFVHIVQMFINTS<br>LYSGGNWVNVISDLKKIEDLIQSMHIDATLYTESDVHPSCKVTAMK<br>CFLLELQVISLESGDASIHDTVENLIILANNSLSSNGNVTESGCKECEE<br>LEEKNIKEFLQSFVHIVQMFINTS |

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|---|---|---|---|---|
| AK479 | DNA161 | Knob: hFc(N297A) | | |
| AK479 | DNA570 | Hole: hFc(N297A)-hCD122-hCD215(Sushi)-[VPLSLY]-hIL15 | NWVNVISDLKKIEDLIQSMHIDATLYTESD<br>VHPSCKVTAMKCFLLELQVISLESGDASIH<br>DTVENLILANNSLSSNGNVTESGCKECEEL<br>EEKNIKEFLQSFVHIVQMFINTS | LYSGGNWVNVISDLKKIEDLIQSMHIDATLYTESDVHPSCKVTAMK<br>CFLLELQVISLESGDASIHDTVENLILANNSLSSNGNVTESGCKECEE<br>LEEKNIKEFLQSFVHIVQMFINTS |
| AK480 | DNA161 | Knob: hFc(N297A) | | |

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|---|---|---|---|
| AK480 | DNA571 | Hole: hFc(N297A)-hCD122-hCD215(Sushi)-[VPLSLY]-hIL15 | NWVNVISDLKKIEDLIQSMHIDATLYTESD LYSGGNWVNVISDLKKIEDLIQSMHIDATLYTESDVHPSCKVTAMK<br>VHPSCKVTAMKCFLLELQVISLESGDASIH CFLLELQVISLESGDASIHDTVENLIILANNSLSSNGNVTESGCKECEE<br>DTVENLIILANNSLSSNGNVTESGCKECEEL LEEKNIKEFLQSFVHIVQMFINTS<br>EEKNIKEFLQSFVHIVQMFINTS |
| AK481 | DNA161 | Knob: hFc(N297A) | |
| AK481 | DNA572 | Hole: hFc(N297A)-hCD122-hCD215(1to66)-[VPLSLY]-hIL15 | NWVNVISDLKKIEDLIQSMHIDATLYTESD LYSGGNWVNVISDLKKIEDLIQSMHIDATLYTESDVHPSCKVTAMK<br>VHPSCKVTAMKCFLLELQVISLESGDASIH CFLLELQVISLESGDASIHDTVENLIILANNSLSSNGNVTESGCKECEE<br>DTVENLIILANNSLSSNGNVTESGCKECEEL LEEKNIKEFLQSFVHIVQMFINTS<br>EEKNIKEFLQSFVHIVQMFINTS |

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| AK482 | DNA161 | Knob: hFc(N297A) | |
| AK482 | DNA573 | Hole: hFc(N297A)-hCD122-hCD215(1to66)-[VPLSLY]-hIL15 | NWVNVISDLKKIEDLIQSMHIDATLYTESD<br>VHPSCKVTAMKCFLLELQVISLESGDASIH<br>DTVENLILANNSLSSNGNVTESGCKECEEL<br>EEKNIKEFLQSFVHIVQMFINTS |
| AK483 | DNA161 | Knob: hFc(N297A) | |

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| SLYSGGNWVNVISDLKKIEDLIQSMHIDATLYTESDVHPSCKVTAM | | |
| KCFLLELQVISLESGDASIHDTVENLILANNSLSSNGNVTESGCKECE | | |
| ELEEKNIKEFLQSFVHIVQMFINTS | | |

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|---|---|---|---|
| AK483 | DNA574 | Hole: hFc(N297A)-hCD122-hCD215(1to66)-[VPLSLY]-hIL15 | NWVNVISDLKKIEDLIQSMHIDATLYTESD LYSGGNWVNVISDLKKIEDLIQSMHIDATLYTESDVHPSCKVTAMK<br>VHPSCKVTAMKCFLLELQVISLESGDASIH CFLLELQVISLESGDASIHDTVENLIILANNSLSSNGNVTESGCKECEE<br>DTVENLIILANNSLSSNGNVTESGCKECEEL LEEKNIKEFLQSFVHIVQMFINTS<br>EEKNIKEFLQSFVHIVQMFINTS |
| AK506 | DNA158 | Hole: hFc(N297A) | |
| AK506 | DNA596 | Knob: hFc(N297A)-hIL15(E46A, E53A) | |

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| AK507 | DNA158 | Hole: hFc(N297A) |
| AK507 | DNA364 | Knob: hFc(N297A)-hIL15 |
| AK507 | DNA595 | hCD215(1to66) |

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| AK527 | DNA370 | Hole: hFc(N297A)-[MPYDLYHP]-hCD122 | LYHPSGGGAVNGTSQFTCFYNSRANISCVWSQDGALQDTSCQVH<br>AWPDRRRWNQTCELLPVSQASWACNLILGAPDSQKLTTVDIVTLR<br>VLCREGVRWRVMAIQDFKPFENLRLMAPISLQVVHVETHRCNISW<br>EISQASHYFERHLEFEARTLSPGHTWEEAPLLTLKQKQEWICLETLTP<br>DTQYEFQVRVKPLQGEFTTWSPWSQPLAFRTKPAALGKD |
| AK527 | DNA596 | Knob: hFc(N297A)-hIL15(E46A, E53A) | |
| not named yet-1 | DNAtbd1 | Knob: hFc(N297A)-hIL15(E46S, E53S) | |

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| not named yet-1 | DNA158 | Hole: hFc(N297A) |
| not named yet-2 | DNAtbd2 | Knob: hFc(N297A)-hIL15(E46R, E53R) |
| not named yet-2 | DNA158 | Hole: hFc(N297A) |

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|---|---|---|---|
| not named yet-3 | DNAtbd3 | knob:IL15-CD215(1-66)_Chimera_V1 | KVTAMKCFLLELQVISLESGDASIHDTVENL LYHPSGPGSGSNWVNVISDLKKIEDLIQSGGGGSGGGGSITCPPPM<br>IILANNSLSSNGNVTESGCKECEELEEKNIKE SVEHADIWVKSYSLYSRERYICNSGFKRKAGTSSLTECVLNKATNVA<br>FLQSFVHIVQMFINTS HWTTPSLKCIRDGGGGSGGGGSKVTAMKCFLLELQVISLESGDASI<br>HDTVENLIILANNSLSSNGNVTESGCKECEELEEKNIKEFLQSFVHIV<br>QMFINTS |
| not named yet-3 | DNA187 | Hole: hFc(N297A)-hCD122 | |
| not named yet-4 | DNAtbd4 | knob:IL15-CD215(1-66)_Chimera_V2 | KVTAMKCFLLELQVISLESGDASIHDTVENL LYHPSGPGSGSNWVNVISDLKKIEDLIQSGGGGSGGGGSITCP<br>IILANNSLSSNGNVTESGCKECEELEEKNIKE PPMSVEHADIWVKSYSLYSRERYICNSGFKRKAGTSSLTECVLNKAT<br>FLQSFVHIVQMFINTS NVAHWTTPSLKCIRDGGGGSGGGGSGGGSKVTAMKCFLLELQVISL<br>ESGDASIHDTVENLIILANNSLSSNGNVTESGCKECEELEEKNIKEFL<br>QSFVHIVQMFINTS |

not named DNA187 Hole: hFc(N297A)-
yet-4 hCD122

| Molecule | name | newnames | FullSequence |
|---|---|---|---|
| AK242 | DNA158 | Hole: hFc(N297A) | DKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYASTY<br>RVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVCTLPPSRDELTKNQVSLSCAVKGFYPSDIAVEW<br>ESNGQPENNYKTTPPVLDSDGSFFLVSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGK |
| AK242 | DNA362 | Knob: hFc(N297A)-[MPYDLYHPJ-hIL15 | DKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYASTY<br>RVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPCRDELTKNQVSLWCLVKGFYPSDIAVE<br>WESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGGGSSPPMPYDLY<br>HPSGPSGSPGNWVNVISDLKKIEDLIQSMHIDATLYTESDVHPSCKVTAMKCFLLELQVISLESGDASIHDTVENLIILANNS<br>LSSNGNVTESGCKECEELEEKNIKEFLQSFVHIVQMFINTS |
| AK243 | DNA158 | Hole: hFc(N297A) | DKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYASTY<br>RVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVCTLPPSRDELTKNQVSLSCAVKGFYPSDIAVEW<br>ESNGQPENNYKTTPPVLDSDGSFFLVSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGK |

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| AK243 | DNA363 | Knob: hFc(N297A)-[MPYDLYHP]-hIL15 | DKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYASTY<br>RVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPCRDELTKNQVSLWCLVKGFYPSDIAVE<br>WESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGGGSSPPMPYDLY<br>HPSGPGSGSNWVNVISDLKKIEDLIQSMHIDATLYTESDVHPSCKVTAMKCFLLELQVISLESGDASIHDTVENLIILANNSL<br>SSNGNVTESGCKECEELEEKNIKEFLQSFVHIVQMFINTS |
| AK244 | DNA158 | Hole: hFc(N297A) | DKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYASTY<br>RVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVCTLPPSRDELTKNQVSLSCAVKGFYPSDIAVEW<br>ESNGQPENNYKTTPPVLDSDGSFFLVSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGK |
| AK244 | DNA364 | Knob: hFc(N297A)-hIL15 | DKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYASTY<br>RVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPCRDELTKNQVSLWCLVKGFYPSDIAVE<br>WESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGGGSSPPGGGSSG<br>GGSGPSGSPGNWVNVISDLKKIEDLIQSMHIDATLYTESDVHPSCKVTAMKCFLLELQVISLESGDASIHDTVENLIILANNS<br>LSSNGNVTESGCKECEELEEKNIKEFLQSFVHIVQMFINTS |

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| AK245 | DNA158 | Hole: hFc(N297A) | DKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYASTY<br>RVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVCTLPPSRDELTKNQVSLSCAVKGFYPSDIAVEW<br>ESNGQPENNYKTTPPVLDSDGSFFLVSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGK |
| AK245 | DNA365 | Knob: hFc(N297A)-[MPYDLYHP]-hIL15-hCD215 | DKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYASTY<br>RVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPCRDELTKNQVSLWCLVKGFYPSDIAVE<br>WESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGGGSSPPMPYDLY<br>HPSGPSGSPGNWVNVISDLKKIEDLIQSMHIDATLYTESDVHPSCKVTAMKCFLLELQVISLESGDASIHDTVENLIILANNS<br>LSSNGNVTESGCKECEELEEKNIKEFLQSFVHIVQMFINTSGGGSGGGGSGGGGSGGGGSITCPPPMSVEHADIWVKSY<br>SLYSRERYICNSGFKRKAGTSSLTECVLNKATNVAHWTTPSLKCIRDPALVHQRPAPPSTVTTAGVTPQPESLSPSGKEPAA<br>SSPSSNNTAATTAAIVPGSQLMPSKSPSTGTTEISSHESSHGTPSQTTAKNWELTASASHQPPGVYPQGHSDTT |
| AK246 | DNA158 | Hole: hFc(N297A) | DKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYASTY<br>RVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVCTLPPSRDELTKNQVSLSCAVKGFYPSDIAVEW<br>ESNGQPENNYKTTPPVLDSDGSFFLVSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGK |

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| AK246 | DNA366 | Knob: hFc(N297A)-[MPYDLYHP]-hIL15-hCD215(Truncated) | DKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYASTY<br>RVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPCRDELTKNQVSLWCLVKGFYPSDIAVE<br>WESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGGGSSPPMPYDLY<br>HPSGPSGSPGNWVNVISDLKKIEDLIQSMHIDATLYTESDVHPSCKVTAMKCFLLELQVISLESGDASIHDTVENLIILANNS<br>LSSNGNVTESGCKECEELEEKNIKEFLQSFVHIVQMFINTSGGGGSGGGGSGGGGSGGGGSITCPPPMSVEHADIWVKSY<br>SLYSRERYICNSGFKRKAGTSSLTECVLNKATNVAHWTTPSLKCIRDPALVHQRPAPPSTVTTAGVTPQPESLSPSGKEPAA<br>S |
| AK247 | DNA187 | Hole: hFc(N297A)-hCD122 | DKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYASTY<br>RVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVCTLPPSRDELTKNQVSLSCAVKGFYPSDIAVEW<br>ESNGQPENNYKTTPPVLDSDGSFFLVSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGPGSGSAVNGTSQFT<br>CFYNSRANISCVWSQDGALQDTSCQVHAWPDRRRWNQTCELLPVSQASWACNLILGAPDSQKLTTVDIVTLRVLCREG<br>VRWRVMAIQDFKPFENLRLMAPISLQVVHVETHRCNISWEISQASHYFERHLEFEARTLSPGHTWEEAPLLTLKQKQEWI<br>CLETLTPDTQYEFQVRVKPLQGEFTTWSPWSQPLAFRTKPAALGKD |
| AK247 | DNA362 | Knob: hFc(N297A)-[MPYDLYHP]-hIL15 | DKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYASTY<br>RVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPCRDELTKNQVSLWCLVKGFYPSDIAVE<br>WESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGGGSSPPMPYDLY<br>HPSGPSGSPGNWVNVISDLKKIEDLIQSMHIDATLYTESDVHPSCKVTAMKCFLLELQVISLESGDASIHDTVENLIILANNS<br>LSSNGNVTESGCKECEELEEKNIKEFLQSFVHIVQMFINTS |

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| AK248 | DNA187 | Hole: hFc(N297A)-hCD122 | DKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYASTY<br>RVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVCTLPPSRDELTKNQVSLSCAVKGFYPSDIAVEW<br>ESNGQPENNYKTTPPVLDSDGSFFLVSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGPGSGSAVNGTSQFT<br>CFYNSRANISCVWSQDGALQDTSCQVHAWPDRRRWNQTCELLPVSQASWACNLILGAPDSQKLTTVDIVTLRVLCREG<br>VRWRVMAIQDFKPFENLRLMAPISLQVVHVETHRCNISWEISQASHYFERHLEFEARTLSPGHTWEEAPLLTLKQKQEWI<br>CLETLTPDTQYEFQVRVKPLQGEFTTWSPWSQPLAFRTKPAALGKD |
| AK248 | DNA363 | Knob: hFc(N297A)-[MPYDLYHP]-hIL15 | DKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYASTY<br>RVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPCRDELTKNQVSLWCLVKGFYPSDIAVE<br>WESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGGGSSPPMPYDLY<br>HPSGPGSGSNWVNVISDLKKIEDLIQSMHIDATLYTESDVHPSCKVTAMKCFLLELQVISLESGDASIHDTVENLIILANNSL<br>SSNGNVTESGCKECEELEEKNIKEFLQSFVHIVQMFINTS |
| AK249 | DNA187 | Hole: hFc(N297A)-hCD122 | DKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYASTY<br>RVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVCTLPPSRDELTKNQVSLSCAVKGFYPSDIAVEW<br>ESNGQPENNYKTTPPVLDSDGSFFLVSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGPGSGSAVNGTSQFT<br>CFYNSRANISCVWSQDGALQDTSCQVHAWPDRRRWNQTCELLPVSQASWACNLILGAPDSQKLTTVDIVTLRVLCREG<br>VRWRVMAIQDFKPFENLRLMAPISLQVVHVETHRCNISWEISQASHYFERHLEFEARTLSPGHTWEEAPLLTLKQKQEWI<br>CLETLTPDTQYEFQVRVKPLQGEFTTWSPWSQPLAFRTKPAALGKD |

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| AK249 | DNA364 | Knob: hFc(N297A)-hIL15 | DKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYASTY<br>RVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPCRDELTKNQVSLWCLVKGFYPSDIAVE<br>WESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGGGSSPPGGGSSG<br>GGSGPSGSPGNWVNVISDLKKIEDLIQSMHIDATLYTESDVHPSCKVTAMKCFLLELQVISLESGDASIHDTVENLIILANNS<br>LSSNGNVTESGCKECEELEEKNIKEFLQSFVHIVQMFINTS |
| AK250 | DNA187 | Hole: hFc(N297A)-hCD122 | DKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYASTY<br>RVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVCTLPPSRDELTKNQVSLSCAVKGFYPSDIAVEW<br>ESNGQPENNYKTTPPVLDSDGSFFLVSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGPGSGSAVNGTSQFT<br>CFYNSRANISCVWSQDGALQDTSCQVHAWPDRRRWNQTCELLPVSQASWACNLILGAPDSQKLTTVDIVTLRVLCREG<br>VRWRVMAIQDFKPFENLRLMAPISLQVVHVETHRCNISWEISQASHYFERHLEFEARTLSPGHTWEEAPLLTLKQKQEWI<br>CLETLTPDTQYEFQVRVKPLQGEFTTWSPWSQPLAFRTKPAALGKD |
| AK250 | DNA365 | Knob: hFc(N297A)-[MPYDLYHPJ-hIL15-hCD215 | DKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYASTY<br>RVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPCRDELTKNQVSLWCLVKGFYPSDIAVE<br>WESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGGGSSPPMPYDLY<br>HPSGPSGSPGNWVNVISDLKKIEDLIQSMHIDATLYTESDVHPSCKVTAMKCFLLELQVISLESGDASIHDTVENLIILANNS<br>LSSNGNVTESGCKECEELEEKNIKEFLQSFVHIVQMFINTSGGGGSGGGGSGGGGSGGGGSITCPPPMSVEHADIWVKSY<br>SLYSRERYICNSGFKRKAGTSSLTECVLNKATNVAHWTTPSLKCIRDPALVHQRPAPPSTVTTAGVTPQPESLSPSGKEPAA<br>SSPSSNNTAATTAAIVPGSQLMPSKSPSTGTTEISSHESSHGTPSQTTAKNWELTASASHQPPGVYPQGHSDTT |

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| AK251 | DNA187 | Hole: hFc(N297A)-hCD122 | DKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYASTY<br>RVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVCTLPPSRDELTKNQVSLSCAVKGFYPSDIAVEW<br>ESNGQPENNYKTTPPVLDSDGSFFLVSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGPGSGSAVNGTSQFT<br>CFYNSRANISCVWSQDGALQDTSCQVHAWPDRRRWNQTCELLPVSQASWACNLILGAPDSQKLTTVDIVTLRVLCREG<br>VRWRVMAIQDFKPFENLRLMAPISLQVVHVETHRCNISWEISQASHYFERHLEFEARTLSPGHTWEEAPLLTLKQKQEWI<br>CLETILTPDTQYEFQVRVKPLQGEFTTWSPWSQPLAFRTKPAALGKD |
| AK251 | DNA366 | Knob: hFc(N297A)-[MPYDLYHP]-hIL15-hCD215(Truncated) | DKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYASTY<br>RVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPCRDELTKNQVSLWCLVKGFYPSDIAVE<br>WESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGGGSSPPMPYDLY<br>HPSGPSGSPGNWVNVISDLKKIEDLIQSMHIDATLYTESDVHPSCKVTAMKCFLLELQVISLESGDASIHDTVENLIILANNS<br>LSSNGNVTESGCKECEELEEKNIKEFLQSFVHIVQMFINTSGGGSGGGGSGGGGSGGGGSITCPPPMSVEHADIWVKSY<br>SLYSRERYICNSGFKRKAGTSSLTECVLNKATNVAHWTTPSLKCIRDPALVHQRPAPPSTVTTAGVTPQPESLSPSGKEPAA<br>S |
| AK399 | DNA187 | Hole: hFc(N297A)-hCD122 | DKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYASTY<br>RVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVCTLPPSRDELTKNQVSLSCAVKGFYPSDIAVEW<br>ESNGQPENNYKTTPPVLDSDGSFFLVSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGPGSGSAVNGTSQFT<br>CFYNSRANISCVWSQDGALQDTSCQVHAWPDRRRWNQTCELLPVSQASWACNLILGAPDSQKLTTVDIVTLRVLCREG<br>VRWRVMAIQDFKPFENLRLMAPISLQVVHVETHRCNISWEISQASHYFERHLEFEARTLSPGHTWEEAPLLTLKQKQEWI<br>CLETILTPDTQYEFQVRVKPLQGEFTTWSPWSQPLAFRTKPAALGKD |

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| AK399 | DNA517 | Knob: hFc(N297A)-[VPLSLY]-hIL15-hCD215 | DKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYASTY<br>RVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPCRDELTKNQVSLWCLVKGFYPSDIAVE<br>WESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGGGSSPPVPLSLYS<br>GPGSGSNWVNVISDLKKIEDLIQSMHIDATLYTESDVHPSCKVTAMKCFLLELQVISLESGDASIHDTVENLIILANNSLSSN<br>GNVTESGCKECEELEEKNIKEFLQSFVHIVQMFINTSGGGSGGGGSGGGGSGGGGSITCPPPMSVEHADIWVK<br>SYSLYSRERYICNSGFKRKAGTSSLTECVLNKATNVAHWTTPSLKCIRDPALVHQRPAPPSTVTTAGVTPQPESLSPSGKEP<br>AASSPSSNNTAATTAAIVPGSQLMPSKSPSTGTTEISSHESSHGTPSQTTAKNWELTASASHQPPGVYPQGHSDTT |
| AK400 | DNA187 | Hole: hFc(N297A)-hCD122 | DKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYASTY<br>RVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVCTLPPSRDELTKNQVSLSCAVKGFYPSDIAVEW<br>ESNGQPENNYKTTPPVLDSDGSFFLVSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGPGSGSAVNGTSQFT<br>CFYNSRANISCVWSQDGALQDTSCQVHAWPDRRRWNQTCELLPVSQASWACNLILGAPDSQKLTTVDIVTLRVLCREG<br>VRWRVMAIQDFKPFENLRLMAPISLQVVHVETHRCNISWEISQASHYFERHLEFEARTLSPGHTWEEAPLLTLKQKQEWI<br>CLETLTPDTQYEFQVRVKPLQGEFTTWSPWSQPLAFRTKPAALGKD |
| AK400 | DNA518 | Knob: hFc(N297A)-[VPLSLY]-hIL15-hCD215(Truncated) | DKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYASTY<br>RVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPCRDELTKNQVSLWCLVKGFYPSDIAVE<br>WESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGGGSSPPVPLSLYS<br>GPGSGSNWVNVISDLKKIEDLIQSMHIDATLYTESDVHPSCKVTAMKCFLLELQVISLESGDASIHDTVENLIILANNSLSSN<br>GNVTESGCKECEELEEKNIKEFLQSFVHIVQMFINTSGGGSGGGGSGGGGSGGGGSITCPPPMSVEHADIWVK<br>SYSLYSRERYICNSGFKRKAGTSSLTECVLNKATNVAHWTTPSLKCIRDPALVHQRPAPPSTVTTAGVTPQPESLSPSGKEP<br>AAS |

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| AK401 | DNA161 | Knob: hFc(N297A) | DKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYASTY<br>RVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPCRDELTKNQVSLWCLVKGFYPSDIAVE<br>WESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGK |
| AK401 | DNA523 | Hole: hFc(N297A)-hCD122-[VPLSLY]-hCD215(Sushi)-hIL15 | DKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYASTY<br>RVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVCTLPPSRDELTKNQVSLSCAVKGFYPSDIAVEW<br>ESNGQPENNYKTTPPVLDSDGSFFLVSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGPGSGSAVNGTSQFT<br>CFYNSRANISCVWSQDGALQDTSCQVHAWPDRRRWNQTCELLPVSQASWACNLILGAPDSQKLTTVDIVTLRVLCREG<br>VRWRVMAIQDFKPFENLRLMAPISLQVVHVETHRCNISWEISQASHYFERHLEFEARTLSPGHTWEEAPLLTLKQKQEWI<br>CLETLTPDTQYEFQVRVKPLQGEFTTWSPWSQPLAFRTKPAALGKDPGGPSPVPLSLYSGGPITCPPPMSVEHADIWVKS<br>YSLYSRERYICNSGFKRKAGTSSLTECVLNKATNVAHWTTPSLKCIRDPALVHQRPAPPSGGGGSGGGGSGGGGSGGGG<br>SNWVNVISDLKKIEDLIQSMHIDATLYTESDVHPSCKVTAMKCFLLELQVISLESGDASIHDTVENLIILANNSLSSNGNVTE<br>SGCKECEELEEKNIKEFLQSFVHIVQMFINTS |
| AK402 | DNA161 | Knob: hFc(N297A) | DKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYASTY<br>RVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPCRDELTKNQVSLWCLVKGFYPSDIAVE<br>WESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGK |

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| AK402 | DNA524 | Hole: hFc(N297A)-hCD122-[VPLSLY]-hCD215(Sushi)-hIL15 | DKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYASTY<br>RVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVCTLPPSRDELTKNQVSLSCAVKGFYPSDIAVEW<br>ESNGQPENNYKTTPPVLDSDGSFFLVSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGPGSGSAVNGTSQFT<br>CFYNSRANISCVWSQDGALQDTSCQVHAWPDRRRWNQTCELLPVSQASWACNLILGAPDSQKLTTVDIVTLRVLCREG<br>VRWRVMAIQDFKPFENLRLMAPISLQVVHVETHRCNISWEISQASHYFERHLEFEARTLSPGHTWEEAPLLTLKQKQEWI<br>CLETLTPDTQYEFQVRVKPLQGEFTTWSPWSQPLAFRTKPAALGKDGGSSPVPLSLYSGGPITCPPPMSVEHADIWVKS<br>YSLYSRERYICNSGFKRKAGTSSLTECVLNKATNVAHWTTPSLKCIRDPALVHQRPAPPSGGGGSGGGGSGGGGSGGGG<br>SNWVNVISDLKKIEDLIQSMHIDATLYTESDVHPSCKVTAMKCFLLELQVISLESGDASIHDTVENLIILANNSLSSNGNVTE<br>SGCKECEELEEKNIKEFLQSFVHIVQMFINTS |
| AK403 | DNA161 | Knob: hFc(N297A) | DKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYASTY<br>RVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPCRDELTKNQVSLWCLVKGFYPSDIAVE<br>WESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGK |

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| AK403 | DNA525 | Hole: hFc(N297A)-hCD122-[VPLSLY]-hCD215(Sushi)-hIL15 | DKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYASTY<br>RVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVCTLPPSRDELTKNQVSLSCAVKGFYPSDIAVEW<br>ESNGQPENNYKTTPPVLDSDGSFFLVSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGPGSGSAVNGTSQFT<br>CFYNSRANISCVWSQDGALQDTSCQVHAWPDRRRWNQTCELLPVSQASWACNLILGAPDSQKLTTVDIVTLRVLCREG<br>VRWRVMAIQDFKPFENLRLMAPISLQVVHVETHRCNISWEISQASHYFERHLEFEARTLSPGHTWEEAPLLTLKQKQEWI<br>CLETLTPDTQYEFQVRVKPLQGEFTTWSPWSQPLAFRTKPAALGKDSGSGGGSPVPLSLYSGGPITCPPPMSVEHADIWVK<br>SYSLYSRERYICNSGFKRKAGTSSLTECVLNKATNVAHWTTPSLKCIRDPALVHQRPAPPSGGGGSGGGGSGGGGSGGG<br>GSNWVNVISDLKKIEDLIQSMHIDATLYTESDVHPSCKVTAMKCFLLELQVISLESGDASIHDTVENLIILANNSLSSNGNVT<br>ESGCKECEEELEEKNIKEFLQSFVHIVQMFINTS |

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| AK404 | DNA187 | Hole: hFc(N297A)-hCD122 | DKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYASTY<br>RVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVCTLPPSRDELTKNQVSLSCAVKGFYPSDIAVEW<br>ESNGQPENNYKTTPPVLDSDGSFFLVSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGPGSGSAVNGTSQFT<br>CFYNSRANISCVWSQDGALQDTSCQVHAWPDRRRWNQTCELLPVSQASWACNLILGAPDSQKLTTVDIVTLRVLCREG<br>VRWRVMAIQDFKPFENLRLMAPISLQVVHVETHRCNISWEISQASHYFERHLEFEARTLSPGHTWEEAPLLTLKQKQEWI<br>CLETILTPDTQYEFQVRVKPLQGEFTTWSPWSQPLAFRTKPAALGKD |
| AK404 | DNA526 | Knob: hFc(N297A)-[VPLSLY]-hIL15-hCD215 | DKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYASTY<br>RVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPCRDELTKNQVSLWCLVKGFYPSDIAVE<br>WESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGGGSSPPVPLSLYS<br>GPSGSPGNWVNVISDLKKIEDLIQSMHIDATLYTESDVHPSCKVTAMKCFLLELQVISLESGDASIHDTVENLIILANNSLSS<br>NGNVTESGCKECEELEEKNIKEFLQSFVHIVQMFINTSGGSGGGSGGGSGGGSGGITCPPPMSVEHADIWVKSYSLY<br>SRERYICNSGFKRKAGTSSLTECVLNKATNVAHWTTPSLKCIRDPALVHQRPAPPSTVTTAGVTPQPESLSPSGKEPAASSP<br>SSNNTAATTAAIVPGSQLMPSKSPSTGTTEISSHESSHGTPSQTTAKNWELTASASHQPPGVYPQGHSDTT |
| AK405 | DNA187 | Hole: hFc(N297A)-hCD122 | DKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYASTY<br>RVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVCTLPPSRDELTKNQVSLSCAVKGFYPSDIAVEW<br>ESNGQPENNYKTTPPVLDSDGSFFLVSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGPGSGSAVNGTSQFT<br>CFYNSRANISCVWSQDGALQDTSCQVHAWPDRRRWNQTCELLPVSQASWACNLILGAPDSQKLTTVDIVTLRVLCREG<br>VRWRVMAIQDFKPFENLRLMAPISLQVVHVETHRCNISWEISQASHYFERHLEFEARTLSPGHTWEEAPLLTLKQKQEWI<br>CLETILTPDTQYEFQVRVKPLQGEFTTWSPWSQPLAFRTKPAALGKD |

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| AK405 | DNA527 | Knob: hFc(N297A)-[VPLSLY]-hIL15-hCD215 | DKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYASTY<br>RVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPCRDELTKNQVSLWCLVKGFYPSDIAVE<br>WESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGGGSSPPVPLSLYS<br>GPSGSPGNWVNVISDLKKIEDLIQSMHIDATLYTESDVHPSCKVTAMKCFLLELQVISLESGDASIHDTVENLIILANNSLSS<br>NGNVTESGCKECEELEEKNIKEFLQSFVHIVQMFINTSGGGGSGGGGSGGGGSGGGGSGGGSGITCPPPMSVEHADIWVK<br>SYSLYSRERYICNSGFKRKAGTSSLTECVLNKATNVAHWTTPSLKCIRDPALVHQRPAPPSTVTTAGVTPQPESLSPSGKEP<br>AASPSSNNTAATTAAIVPGSQLMPSKSPSTGTTEISSHESSHGTPSQTTAKNWELTASASHQPPGVYPQGHSDTT |
| AK418 | DNA187 | Hole: hFc(N297A)-hCD122 | DKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYASTY<br>RVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVCTLPPSRDELTKNQVSLSCAVKGFYPSDIAVEW<br>ESNGQPENNYKTTPPVLDSDGSFFLVSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGPGSGSAVNGTSQFT<br>CFYNSRANISCVWSQDGALQDTSCQVHAWPDRRRWNQTCELLPVSQASWACNLILGAPDSQKLTTVDIVTLRVLCREG<br>VRWRVMAIQDFKPFENLRLMAPISLQVVHVETHRCNISWEISQASHYFERHLEFEARTLSPGHTWEEAPLLTLKQKQEWI<br>CLETLTPDTQYEFQVRVKPLQGEFTTWSPWSQPLAFRTKPAALGKD |
| AK418 | DNA367 | Knob: hFc(N297A)-hIL15 | DKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYASTY<br>RVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPCRDELTKNQVSLWCLVKGFYPSDIAVE<br>WESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGGGSSPPGGGSSG<br>GGSGPGSGSNWVNVISDLKKIEDLIQSMHIDATLYTESDVHPSCKVTAMKCFLLELQVISLESGDASIHDTVENLIILANNSL<br>SSNGNVTESGCKECEELEEKNIKEFLQSFVHIVQMFINTS |

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| AK419 | DNA187 | Hole: hFc(N297A)-hCD122 | DKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYASTY<br>RVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVCTLPPSRDELTKNQVSLSCAVKGFYPSDIAVEW<br>ESNGQPENNYKTTPPVLDSDGSFFLVSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGPGSGSAVNGTSQFT<br>CFYNSRANISCVWSQDGALQDTSCQVHAWPDRRRWNQTCELLPVSQASWACNLILGAPDSQKLTTVDIVTLRVLCREG<br>VRWRVMAIQDFKPFENLRLMAPISLQVVHVETHRCNISWEISQASHYFERHLEFEARTLSPGHTWEEAPLLTLKQKQEWI<br>CLETLTPDTQYEFQVRVKPLQGEFTTWSPWSQPLAFRTKPAALGKD |
| AK419 | DNA368 | Knob: hFc(N297A)-[MPYDLYHP]-hIL15-hCD215 | DKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYASTY<br>RVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPCRDELTKNQVSLWCLVKGFYPSDIAVE<br>WESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGGGSSPPMPYDLY<br>HPSGPGSGSNWVNVISDLKKIEDLIQSMHIDATLYTESDVHPSCKVTAMKCFLLELQVISLESGDASIHDTVENLIILANNSL<br>SSNGNVTESGCKECEELEEKNIKEFLQSFVHIVQMFINTSGGGGSGGGGSGGGGSGGGGSITCPPPMSVEHADIWVKSYS<br>LYSRERYICNSGFKRKAGTSSLTECVLNKATNVAHWTTPSLKCIRDPALVHQRPAPPSTVTTAGVTPQPESLSPSGKEPAAS<br>SPSSNNTAATTAAIVPGSQLMPSKSPSTGTTEISSHESSHGTPSQTTAKNWELTASASHQPPGVYPQGHSDTT |
| AK420 | DNA187 | Hole: hFc(N297A)-hCD122 | DKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYASTY<br>RVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVCTLPPSRDELTKNQVSLSCAVKGFYPSDIAVEW<br>ESNGQPENNYKTTPPVLDSDGSFFLVSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGPGSGSAVNGTSQFT<br>CFYNSRANISCVWSQDGALQDTSCQVHAWPDRRRWNQTCELLPVSQASWACNLILGAPDSQKLTTVDIVTLRVLCREG<br>VRWRVMAIQDFKPFENLRLMAPISLQVVHVETHRCNISWEISQASHYFERHLEFEARTLSPGHTWEEAPLLTLKQKQEWI<br>CLETLTPDTQYEFQVRVKPLQGEFTTWSPWSQPLAFRTKPAALGKD |

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| AK420 | DNA369 | Knob: hFc(N297A)-[MPYDLYHP]-hIL15-hCD215(Truncated) | DKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYASTY<br>RVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPCRDELTKNQVSLWCLVKGFYPSDIAVE<br>WESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGGGSSPPMPYDLY<br>HPSGPGSGSNWVNVISDLKKIEDLIQSMHIDATLYTESDVHPSCKVTAMKCFLLELQVISLESGDASIHDTVENLIILANNSL<br>SSNGNVTESGCKECEELEEKNIKEFLQSFVHIVQMFINTSGGGGSGGGGSGGGGSGGGGSITCPPPMSVEHADIWVKSYS<br>LYSRERYICNSGFKRKAGTSSLTECVLNKATNVAHWTTPSLKCIRDPALVHQRPAPPSTVTTAGVTPQPESLSPSGKEPAAS |
| AK421 | DNA187 | Hole: hFc(N297A)-hCD122 | DKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYASTY<br>RVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVCTLPPSRDELTKNQVSLSCAVKGFYPSDIAVEW<br>ESNGQPENNYKTTPPVLDSDGSFFLVSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGPGSGSAVNGTSQFT<br>CFYNSRANISCVWSQDGALQDTSCQVHAWPDRRRWNQTCELLPVSQASWACNLILGAPDSQKLTTVDIVTLRVLCREG<br>VRWRVMAIQDFKPFENLRLMAPISLQVVHVETHRCNISWEISQASHYFERHLEFEARTLSPGHTWEEAPLLTLKQKQEWI<br>CLETLTPDTQYEFQVRVKPLQGEFTTWSPWSQPLAFRTKPAALGKD |
| AK421 | DNA487 | Knob: hFc(N297A)-[VPLSLY]-hIL15 | DKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYASTY<br>RVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPCRDELTKNQVSLWCLVKGFYPSDIAVE<br>WESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGGGSSPPVPLSLYS<br>GPGSGSNWVNVISDLKKIEDLIQSMHIDATLYTESDVHPSCKVTAMKCFLLELQVISLESGDASIHDTVENLIILANNSLSSN<br>GNVTESGCKECEELEEKNIKEFLQSFVHIVQMFINTS |

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| AK457 | DNA187 | Hole: hFc(N297A)-hCD122 | DKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYASTY<br>RVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVCTLPPSRDELTKNQVSLSCAVKGFYPSDIAVEW<br>ESNGQPENNYKTTPPVLDSDGSFFLVSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGPGSGSAVNGTSQFT<br>CFYNSRANISCVWSQDGALQDTSCQVHAWPDRRRWNQTCELLPVSQASWACNLILGAPDSQKLTTVDIVTLRVLCREG<br>VRWRVMAIQDFKPFENLRLMAPISLQVVHVETHRCNISWEISQASHYFERHLEFEARTLSPGHTWEEAPLLTLKQKQEWI<br>CLETILTPDTQYEFQVRVKPLQGEFTTWSPWSQPLAFRTKPAALGKD |
| AK457 | DNA556 | Knob: hFc(N297A)-[VPLSLY]-hIL15 | DKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYASTY<br>RVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPCRDELTKNQVSLWCLVKGFYPSDIAVE<br>WESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGGGSSPPGVPLSLY<br>GSGPGSGSNWVNVISDLKKIEDLIQSMHIDATLYTESDVHPSCKVTAMKCFLLELQVISLESGDASIHDTVENLIILANNSLS<br>SNGNVTESGCKECEELEEKNIKEFLQSFVHIVQMFINTS |
| AK458 | DNA187 | Hole: hFc(N297A)-hCD122 | DKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYASTY<br>RVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVCTLPPSRDELTKNQVSLSCAVKGFYPSDIAVEW<br>ESNGQPENNYKTTPPVLDSDGSFFLVSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGPGSGSAVNGTSQFT<br>CFYNSRANISCVWSQDGALQDTSCQVHAWPDRRRWNQTCELLPVSQASWACNLILGAPDSQKLTTVDIVTLRVLCREG<br>VRWRVMAIQDFKPFENLRLMAPISLQVVHVETHRCNISWEISQASHYFERHLEFEARTLSPGHTWEEAPLLTLKQKQEWI<br>CLETILTPDTQYEFQVRVKPLQGEFTTWSPWSQPLAFRTKPAALGKD |

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| AK458 | DNA557 | Knob: hFc(N297A)-[VPLSLY]-hIL15(D22A) | DKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYASTY<br>RVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPCRDELTKNQVSLWCLVKGFYPSDIAVE<br>WESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGGGSSPPGVPLSLY<br>GSGPGSGSNWVNVISDLKKIEDLIQSMHIAATLYTESDVHPSCKVTAMKCFLLELQVISLESGDASIHDTVENLIILANNSLS<br>SNGNVTESGCKECEELEEKNIKEFLQSFVHIVQMFINTS |
| AK459 | DNA187 | Hole: hFc(N297A)-hCD122 | DKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYASTY<br>RVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVCTLPPSRDELTKNQVSLSCAVKGFYPSDIAVEW<br>ESNGQPENNYKTTPPVLDSDGSFFLVSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGPGSGSAVNGTSQFT<br>CFYNSRANISCVWSQDGALQDTSCQVHAWPDRRRWNQTCELLPVSQASWACNLILGAPDSQKLTTVDIVTLRVLCREG<br>VRWRVMAIQDFKPFENLRLMAPISLQVVHVETHRCNISWEISQASHYFERHLEFEARTLSPGHTWEEAPLLTLKQKQEWI<br>CLETLTPDTQYEFQVRVKPLQGEFTTWSPWSQPLAFRTKPAALGKD |
| AK459 | DNA558 | Knob: hFc(N297A)-[VPLSLY]-hIL15(E46A) | DKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYASTY<br>RVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPCRDELTKNQVSLWCLVKGFYPSDIAVE<br>WESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGGGSSPPGVPLSLY<br>GSGPGSGSNWVNVISDLKKIEDLIQSMHIDATLYTESDVHPSCKVTAMKCFLLALQVISLESGDASIHDTVENLIILANNSLS<br>SNGNVTESGCKECEELEEKNIKEFLQSFVHIVQMFINTS |

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| AK460 | DNA187 | Hole: hFc(N297A)-hCD122 | DKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYASTY<br>RVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVCTLPPSRDELTKNQVSLSCAVKGFYPSDIAVEW<br>ESNGQPENNYKTTPPVLDSDGSFFLVSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGPGSGSAVNGTSQFT<br>CFYNSRANISCVWSQDGALQDTSCQVHAWPDRRRWNQTCELLPVSQASWACNLILGAPDSQKLTTVDIVTLRVLCREG<br>VRWRVMAIQDFKPFENLRLMAPISLQVVHVETHRCNISWEISQASHYFERHLEFEARTLSPGHTWEEAPLLTLKQKQEWI<br>CLETILTPDTQYEFQVRVKPLQGEFTTWSPWSQPLAFRTKPAALGKD |
| AK460 | DNA559 | Knob: hFc(N297A)-[VPLSLY]-hIL15(E53A) | DKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYASTY<br>RVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPCRDELTKNQVSLWCLVKGFYPSDIAVE<br>WESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGGGSSPPGVPLSLY<br>GSGPGSGSNWVNVISDLKKIEDLIQSMHIDATLYTESDVHPSCKVTAMKCFLLELQVISLASGDASIHDTVENLIILANNSLS<br>SNGNVTESGCKECEELEEKNIKEFLQSFVHIVQMFINTS |
| AK461 | DNA187 | Hole: hFc(N297A)-hCD122 | DKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYASTY<br>RVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVCTLPPSRDELTKNQVSLSCAVKGFYPSDIAVEW<br>ESNGQPENNYKTTPPVLDSDGSFFLVSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGPGSGSAVNGTSQFT<br>CFYNSRANISCVWSQDGALQDTSCQVHAWPDRRRWNQTCELLPVSQASWACNLILGAPDSQKLTTVDIVTLRVLCREG<br>VRWRVMAIQDFKPFENLRLMAPISLQVVHVETHRCNISWEISQASHYFERHLEFEARTLSPGHTWEEAPLLTLKQKQEWI<br>CLETILTPDTQYEFQVRVKPLQGEFTTWSPWSQPLAFRTKPAALGKD |

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| AK461 | DNA560 | Knob: hFc(N297A)-[VPLSLY]-hIL15(E46A, E53A) | DKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYASTY<br>RVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPCRDELTKNQVSLWCLVKGFYPSDIAVE<br>WESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGGGSSPPGVPLSLY<br>GSGPGSGSNWVNVISDLKKIEDLIQSMHIDATLYTESDVHPSCKVTAMKCFLLALQVISLASGDASIHDTVENLIILANNSLS<br>SNGNVTESGCKECEELEEKNIKEFLQSFVHIVQMFINTS |
| AK478 | DNA161 | Knob: hFc(N297A) | DKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYASTY<br>RVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPCRDELTKNQVSLWCLVKGFYPSDIAVE<br>WESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGK |
| AK478 | DNA569 | Hole: hFc(N297A)-hCD122-hCD215(Sushi)-[VPLSLY]-hIL15 | DKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYASTY<br>RVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVCTLPPSRDELTKNQVSLSCAVKGFYPSDIAVEW<br>ESNGQPENNYKTTPPVLDSDGSFFLVSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGPGSGSAVNGTSQFT<br>CFYNSRANISCVWSQDGALQDTSCQVHAWPDRRRWNQTCELLPVSQASWACNLILGAPDSQKLTTVDIVTLRVLCREG<br>VRWRVMAIQDFKPFENLRLMAPISLQVVHVETHRCNISWEISQASHYFERHLEFEARTLSPGHTWEEAPLLTLKQKQEWI<br>CLETLTPDTQYEFQVRVKPLQGEFTTWSPWSQPLAFRTKPAALGKDGGGSGGGGSGGGGSGGGGSITCPPPMSVEHA<br>DIWVKSYSLYSRERYICNSGFKRKAGTSSLTECVLNKATNVAHWTTPSLKCIRDPALVHQRPAPPSPSPPGPSPPVPLSLY<br>SGGNWVNVISDLKKIEDLIQSMHIDATLYTESDVHPSCKVTAMKCFLLELQVISLESGDASIHDTVENLIILANNSLSSNGNV<br>TESGCKECEELEEKNIKEFLQSFVHIVQMFINTS |

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| AK479 | DNA161 | Knob: hFc(N297A) | DKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYASTY<br>RVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPCRDELTKNQVSLWCLVKGFYPSDIAVE<br>WESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGK |
| AK479 | DNA570 | Hole: hFc(N297A)-hCD122-hCD215(Sushi)-[VPLSLY]-hIL15 | DKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYASTY<br>RVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVCTLPPSRDELTKNQVSLSCAVKGFYPSDIAVEW<br>ESNGQPENNYKTTPPVLDSDGSFFLVSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGPGSGSAVNGTSQFT<br>CFYNSRANISCVWSQDGALQDTSCQVHAWPDRRRWNQTCELLPVSQASWACNLILGAPDSQKLTTVDIVTLRVLCREG<br>VRWRVMAIQDFKPFENLRLMAPISLQVVHVETHRCNISWEISQASHYFERHLEFEARTLSPGHTWEEAPLLTLKQKQEWI<br>CLETLTPDTQYEFQVRVKPLQGEFTTWSPWSQPLAFRTKPAALGKDGGGGSGGGGSGGGGSITCPPPMSVEHA<br>DIWVKSYSLYSRERYICNSGFKRKAGTSSLTECVLNKATNVAHWTTPSLKCIRDPALVHQRPAPPSSGGPGSGPPSGVPLSL<br>YSGGNWVNVISDLKKIEDLIQSMHIDATLYTESDVHPSCKVTAMKCFLLELQVISLESGDASIHDTVENLIILANNSLSSNGN<br>VTESGCKECEELEEKNIKEFLQSFVHIVQMFINTS |
| AK480 | DNA161 | Knob: hFc(N297A) | DKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYASTY<br>RVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPCRDELTKNQVSLWCLVKGFYPSDIAVE<br>WESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGK |

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| AK480 | DNA571 | Hole: hFc(N297A)-hCD122-hCD215(Sushi)-[VPLSLY]-hIL15 | DKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYASTY<br>RVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVCTLPPSRDELTKNQVSLSCAVKGFYPSDIAVEW<br>ESNGQPENNYKTTPPVLDSDGSFFLVSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGPGSGSAVNGTSQFT<br>CFYNSRANISCVWSQDGALQDTSCQVHAWPDRRRWNQTCELLPVSQASWACNLILGAPDSQKLTTVDIVTLRVLCREG<br>VRWRVMAIQDFKPFENLRLMAPISLQVVHVETHRCNISWEISQASHYFERHLEFEARTLSPGHTWEEAPLLTLKQKQEWI<br>CLETLTPDTQYEFQVRVKPLQGEFTTWSPWSQPLAFRTKPAALGKDGGGSGGGGSGGGGSGGGGSITCPPPMSVEHA<br>DIWVKSYSLYSRERYICNSGFKRKAGTSSLTECVLNKATNVAHWTTPSLKCIRDPALVHQRPAPPSSGSSPGGPSGPPVPL<br>SLYSGGNWVNVISDLKKIEDLIQSMHIDATLYTESDVHPSCKVTAMKCFLLELQVISLESGDASIHDTVENLIILANNSLSSN<br>GNVTESGCKECEELEEKNIKEFLQSFVHIVQMFINTS |
| AK481 | DNA161 | Knob: hFc(N297A) | DKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYASTY<br>RVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPCRDELTKNQVSLWCLVKGFYPSDIAVE<br>WESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGK |
| AK481 | DNA572 | Hole: hFc(N297A)-hCD122-hCD215(1to66)-[VPLSLY]-hIL15 | DKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYASTY<br>RVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVCTLPPSRDELTKNQVSLSCAVKGFYPSDIAVEW<br>ESNGQPENNYKTTPPVLDSDGSFFLVSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGPGSGSAVNGTSQFT<br>CFYNSRANISCVWSQDGALQDTSCQVHAWPDRRRWNQTCELLPVSQASWACNLILGAPDSQKLTTVDIVTLRVLCREG<br>VRWRVMAIQDFKPFENLRLMAPISLQVVHVETHRCNISWEISQASHYFERHLEFEARTLSPGHTWEEAPLLTLKQKQEWI<br>CLETLTPDTQYEFQVRVKPLQGEFTTWSPWSQPLAFRTKPAALGKDGGGSGGGGSGGGGSGGGGSITCPPPMSVEHA<br>DIWVKSYSLYSRERYICNSGFKRKAGTSSLTECVLNKATNVAHWTTPSLKCIRDGGSSPPVPLSLYSGGNWVNVISDLKKIE<br>DLIQSMHIDATLYTESDVHPSCKVTAMKCFLLELQVISLESGDASIHDTVENLIILANNSLSSNGNVTESGCKECEELEEKNIK<br>EFLQSFVHIVQMFINTS |

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| AK482 | DNA161 | Knob: hFc(N297A) | DKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYASTY<br>RVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPCRDELTKNQVSLWCLVKGFYPSDIAVE<br>WESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGK |
| AK482 | DNA573 | Hole: hFc(N297A)-hCD122-hCD215(1to66)-[VPLSLY]-hIL15 | DKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYASTY<br>RVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVCTLPPSRDELTKNQVSLSCAVKGFYPSDIAVEW<br>ESNGQPENNYKTTPPVLDSDGSFFLVSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGPGSGSAVNGTSQFT<br>CFYNSRANISCVWSQDGALQDTSCQVHAWPDRRRWNQTCELLPVSQASWACNLILGAPDSQKLTTVDIVTLRVLCREG<br>VRWRVMAIQDFKPFENLRLMAPISLQVVHVETHRCNISWEISQASHYFERHLEFEARTLSPGHTWEEAPLLTLKQKQEWI<br>CLETLTPDTQYEFQVRVKPLQGEFTTWSPWSQPLAFRTKPAALGKDGGGGSGGGGSGGGGSITCPPPMSVEHA<br>DIWVKSYSLYSRERYICNSGFKRKAGTSSLTECVLNKATNVAHWTTPSLKCIRDSPSSGSGVPLSLYSGGNWVNVISDLKKIE<br>DLIQSMHIDATLYTESDVHPSCKVTAMKCFLLELQVISLESGDASIHDTVENLILLANNSLSSNGNVTESGCKECEELEEKNIK<br>EFLQSFVHIVQMFINTS |
| AK483 | DNA161 | Knob: hFc(N297A) | DKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYASTY<br>RVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPCRDELTKNQVSLWCLVKGFYPSDIAVE<br>WESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGK |

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| AK483 | DNA574 | Hole: hFc(N297A)-hCD122-hCD215(1to66)-[VPLSLY]-hIL15 | DKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYASTY<br>RVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVCTLPPSRDELTKNQVSLSCAVKGFYPSDIAVEW<br>ESNGQPENNYKTTPPVLDSDGSFFLVSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGPGSGSAVNGTSQFT<br>CFYNSRANISCVWSQDGALQDTSCQVHAWPDRRRWNQTCELLPVSQASWACNLILGAPDSQKLTTVDIVTLRVLCREG<br>VRWRVMAIQDFKPFENLRLMAPISLQVVHVETHRCNISWEISQASHYFERHLEFEARTLSPGHTWEEAPLLTLKQKQEWI<br>CLETLTPDTQYEFQVRVKPLQGEFTTWSPWSQPLAFRTKPAALGKDGGGSGGGGSGGGGSGGGGSITCPPPMSVEHA<br>DIWVKSYSLYSRERYICNSGFKRKAGTSSLTECVLNKATNVAHWTTPSLKCIRDGPPSGSSPVPLSLYSGGNWVNVISDLKK<br>IEDLIQSMHIDATLYTESDVHPSCKVTAMKCFLLELQVISLESGDASIHDTVENLIILANNSLSSNGNVTESGCKECEELEEKNI<br>KEFLQSFVHIVQMFINTS |
| AK506 | DNA158 | Hole: hFc(N297A) | DKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYASTY<br>RVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVCTLPPSRDELTKNQVSLSCAVKGFYPSDIAVEW<br>ESNGQPENNYKTTPPVLDSDGSFFLVSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGK |
| AK506 | DNA596 | Knob: hFc(N297A)-hIL15(E46A, E53A) | DKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYASTY<br>RVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPCRDELTKNQVSLWCLVKGFYPSDIAVE<br>WESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGGGSSPPGGGSSG<br>GGSGPSGSPGNWVNVISDLKKIEDLIQSMHIDATLYTESDVHPSCKVTAMKCFLLALQVISLASGDASIHDTVENLIILANN<br>SLSSNGNVTESGCKECEELEEKNIKEFLQSFVHIVQMFINTS |

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| AK507 | DNA158 | Hole: hFc(N297A) | DKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYASTY<br>RVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVCTLPPSRDELTKNQVSLSCAVKGFYPSDIAVEW<br>ESNGQPENNYKTTPPVLDSDGSFFLVSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGK |
| AK507 | DNA364 | Knob: hFc(N297A)-hIL15 | DKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYASTY<br>RVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPCRDELTKNQVSLWCLVKGFYPSDIAVE<br>WESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGGGSSPPGGGSSG<br>GGSGPSGSPGNWVNVISDLKKIEDLIQSMHIDATLYTESDVHPSCKVTAMKCFLLELQVISLESGDASIHDTVENLIILANNS<br>LSSNGNVTESGCKECEELEEKNIKEFLQSFVHIVQMFINTS |
| AK507 | DNA595 | hCD215(1to66) | ITCPPPMSVEHADIWVKSYSLYSRERYICNSGFKRKAGTSSLTECVLNKATNVAHWTTPSLKCIRD |

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| AK527 | DNA370 | Hole: hFc(N297A)-[MPYDLYHP]-hCD122 | DKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYASTY<br>RVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVCTLPPSRDELTKNQVSLSCAVKGFYPSDIAVEW<br>ESNGQPENNYKTTPPVLDSDGSFFLVSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGGPPSGSSPMPYDLY<br>HPSGGGAVNGTSQFTCFYNSRANISCVWSQDGALQDTSCQVHAWPDRRRWNQTCELLPVSQASWACNLILGAPDSQ<br>KLTTVDIVTLRVLCREGVRWRVMAIQDFKPFENLRLMAPISLQVVHVETHRCNISWEISQASHYFERHLEFEARTLSPGHT<br>WEEAPLLTLKQKQEWICLETILTPDTQYEFQVRVKPLQGEFTTWSPWSQPLAFRTKPAALGKD |
| AK527 | DNA596 | Knob: hFc(N297A)-hIL15(E46A, E53A) | DKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYASTY<br>RVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPCRDELTKNQVSLWCLVKGFYPSDIAVE<br>WESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGGGSSPPGGGSSG<br>GGSGPSGSPGNWVNVISDLKKIEDLIQSMHIDATLYTESDVHPSCKVTAMKCFLLALQVISLASGDASIHDTVENLIILANN<br>SLSSNGNVTESGCKECEELEEKNIKEFLQSFVHIVQMFINTS |
| not named yet-1 | DNAtbd1 | Knob: hFc(N297A)-hIL15(E46S, E53S) | DKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYASTY<br>RVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPCRDELTKNQVSLWCLVKGFYPSDIAVE<br>WESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGGGSSPPGGGSSG<br>GGSGPSGSPGNWVNVISDLKKIEDLIQSMHIDATLYTESDVHPSCKVTAMKCFLLALQVISLASGDASIHDTVENLIILANN<br>SLSSNGNVTESGCKECEELEEKNIKEFLQSFVHIVQMFINTS |

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| not named yet-1 | DNA158 | Hole: hFc(N297A) | DKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYASTY<br>RVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVCTLPPSRDELTKNQVSLSCAVKGFYPSDIAVEW<br>ESNGQPENNYKTTPPVLDSDGSFFLVSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGK |
| not named yet-2 | DNAtbd2 | Knob: hFc(N297A)-hIL15(E46R, E53R) | DKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYASTY<br>RVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPCRDELTKNQVSLWCLVKGFYPSDIAVE<br>WESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGGGSSPPGGGSSG<br>GGSGPSGSPGNWVNVISDLKKIEDLIQSMHIDATLYTESDVHPSCKVTAMKCFLLALQVISLASGDASIHDTVENLIILANN<br>SLSSNGNVTESGCKECEELEEKNIKEFLQSFVHIVQMFINTS |
| not named yet-2 | DNA158 | Hole: hFc(N297A) | DKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYASTY<br>RVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVCTLPPSRDELTKNQVSLSCAVKGFYPSDIAVEW<br>ESNGQPENNYKTTPPVLDSDGSFFLVSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGK |

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| not named yet-3 | DNAtbd3 | knob:IL15-CD215(1-66)_Chimera_V1 | DKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYASTY<br>RVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPCRDELTKNQVSLWCLVKGFYPSDIAVE<br>WESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGGGSSPPMPYDLY<br>HPSGPGSGSNWVNVISDLKKIEDLIQSGGGGSGGGGSITCPPPMSVEHADIWVKSYSLYSRERYICNSGFKRKAGTSSLTE<br>CVLNKATNVAHWTTPSLKCIRDGGGGSGGGGSKVTAMKCFLLELQVISLESGDASIHDTVENLIILANNSLSSNGNVTESG<br>CKECEELEEKNIKEFLQSFVHIVQMFINTS |
| not named yet-3 | DNA187 | Hole: hFc(N297A)-hCD122 | DKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYASTY<br>RVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVCTLPPSRDELTKNQVSLSCAVKGFYPSDIAVEW<br>ESNGQPENNYKTTPPVLDSDGSFFLVSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGPGSGSAVNGTSQFT<br>CFYNSRANISCVWSQDGALQDTSCQVHAWPDRRRWNQTCELLPVSQASWACNLILGAPDSQKLTTVDIVTLRVLCREG<br>VRWRVMAIQDFKPFENLRLMAPISLQVVHVETHRCNISWEISQASHYFERHLEFEARTLSPGHTWEEAPLLTLKQKQEWI<br>CLETLTPDTQYEFQVRVKPLQGEFTTWSPWSQPLAFRTKPAALGKD |
| not named yet-4 | DNAtbd4 | knob:IL15-CD215(1-66)_Chimera_V2 | DKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYASTY<br>RVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPCRDELTKNQVSLWCLVKGFYPSDIAVE<br>WESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGGGSSPPMPYDLY<br>HPSGPGSGSNWVNVISDLKKIEDLIQSGGGGSGGGGSGGSITCPPPMSVEHADIWVKSYSLYSRERYICNSGFKRKAGTSS<br>LTECVLNKATNVAHWTTPSLKCIRDGGGGSGGGGSGGSKVTAMKCFLLELQVISLESGDASIHDTVENLIILANNSLSSNG<br>NVTESGCKECEELEEKNIKEFLQSFVHIVQMFINTS |

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| not named yet-4 | DNA187 | Hole: hFc(N297A)-hCD122 | DKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYASTY<br>RVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVCTLPPSRDELTKNQVSLSCAVKGFYPSDIAVEW<br>ESNGQPENNYKTTPPVLDSDGSFFLVSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGPGSGSAVNGTSQFT<br>CFYNSRANISCVWSQDGALQDTSCQVHAWPDRRRWNQTCELLPVSQASWACNLILGAPDSQKLTTVDIVTLRVLCREG<br>VRWRVMAIQDFKPFENLRLMAPISLQVVHVETHRCNISWEISQASHYFERHLEFEARTLSPGHTWEEAPLLTLKQKQEWI<br>CLETLTPDTQYEFQVRVKPLQGEFTTWSPWSQPLAFRTKPAALGKD |

| name | newnames | Component1Sequence | Component2 Sequence | Component3Sequence |
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| DNA158 | Hole: hFc(N297A) | DKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMIS<br>RTPEVTCVVVDVSHEDPEVKFNWYVDGVEVH<br>NAKTKPREEQYASTYRVVSVLTVLHQDWLNGK<br>EYKCKVSNKALPAPIEKTISKAKGQPREPQVCTL<br>PPSRDELTKNQVSLSCAVKGFYPSDIAVEWESN<br>GQPENNYKTTPPVLDSDGSFFLVSKLTVDKSRW<br>QQGNVFSCSVMHEALHNHYTQKSLSLSPG | | |
| DNA161 | Knob: hFc(N297A) | DKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMIS<br>RTPEVTCVVVDVSHEDPEVKFNWYVDGVEVH<br>NAKTKPREEQYASTYRVVSVLTVLHQDWLNGK<br>EYKCKVSNKALPAPIEKTISKAKGQPREPQVYTL<br>PPCRDELTKNQVSLWCLVKGFYPSDIAVEWES<br>NGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSR<br>WQQGNVFSCSVMHEALHNHYTQKSLSLSPG | | |

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| | | DKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMIS | | AVNGTSQFTCFYNSRANISCVWSQDGALQ |
| | | RTPEVTCVVVDVSHEDPEVKFNWYVDGVEVH | | DTSCQVHAWPDRRRWNQTCELLPVSQAS |
| | | NAKTKPREEQYASTYRVVSVLTVLHQDWLNGK | | WACNLILGAPDSQKLTTVDIVTLRVLCREGV |
| DNA187 | Hole: hFc(N297A)-hCD122 | EYKCKVSNKALPAPIEKTISKAKGQPREPQVCTL | PGSGS | RWRVMAIQDFKPFENLRLMAPISLQVVHV |
| | | PPSRDELTKNQVSLSCAVKGFYPSDIAVEWESN | | ETHRCNISWEISQASHYFERHLEFEARTLSPG |
| | | GQPENNYKTTPPVLDSDGSFFLVSKLTVDKSRW | | HTWEEAPLLTLKQKQEWICLETLTPDTQYEF |
| | | QQGNVFSCSVMHEALHNHYTQKSLSLSPG | | QVRVKPLQGEFTTWSPWSQPLAFRTKPAA |
| | | | | LGKD |
| | | DKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMIS | | |
| | | RTPEVTCVVVDVSHEDPEVKFNWYVDGVEVH | | |
| | | NAKTKPREEQYASTYRVVSVLTVLHQDWLNGK | | |
| DNA362 | Knob: hFc(N297A)-[MPYDLYHP]-hIL15 | EYKCKVSNKALPAPIEKTISKAKGQPREPQVYTL | GGSSPP | MPYDLYHP |
| | | PPCRDELTKNQVSLWCLVKGFYPSDIAVEWES | | |
| | | NGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSR | | |
| | | WQQGNVFSCSVMHEALHNHYTQKSLSLSPG | | |

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| | | DKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMIS | | | |
| | | RTPEVTCVVVDVSHEDPEVKFNWYVDGVEVH | | | |
| | | NAKTKPREEQYASTYRVVSVLTVLHQDWLNGK | | | |
| DNA363 | Knob: hFc(N297A)-[MPYDLYHP]-hIL15 | EYKCKVSNKALPAPIEKTISKAKGQPREPQVYTL | GGSSPP | MPYDLYHP | |
| | | PPCRDELTKNQVSLWCLVKGFYPSDIAVEWES | | | |
| | | NGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSR | | | |
| | | WQQGNVFSCSVMHEALHNHYTQKSLSLSPG | | | |
| | | | | | |
| | | DKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMIS | | | |
| | | RTPEVTCVVVDVSHEDPEVKFNWYVDGVEVH | | | NWVNVISDLKKIEDLIQSMHIDATLYTESDV |
| | | NAKTKPREEQYASTYRVVSVLTVLHQDWLNGK | GGSSPPGGGS | | HPSCKVTAMKCFLLELQVISLESGDASIHDT |
| DNA364 | Knob: hFc(N297A)-hIL15 | EYKCKVSNKALPAPIEKTISKAKGQPREPQVYTL | SGGGSGPSGS | | VENLIILANNSLSSNGNVTESGCKECEELEEK |
| | | PPCRDELTKNQVSLWCLVKGFYPSDIAVEWES | PG | | NIKEFLQSFVHIVQMFINTS |
| | | NGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSR | | | |
| | | WQQGNVFSCSVMHEALHNHYTQKSLSLSPG | | | |

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| | | DKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMIS | | |
| | | RTPEVTCVVVDVSHEDPEVKFNWYVDGVEVH | | |
| | | NAKTKPREEQYASTYRVVSVLTVLHQDWLNGK | | |
| DNA365 | Knob: hFc(N297A)-[MPYDLYHP]-hIL15-hCD215 | EYKCKVSNKALPAPIEKTISKAKGQPREPQVYTL | GGSSPP | MPYDLYHP |
| | | PPCRDELTKNQVSLWCLVKGFYPSDIAVEWES | | |
| | | NGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSR | | |
| | | WQQGNVFSCSVMHEALHNHYTQKSLSLSPG | | |
| | | DKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMIS | | |
| | | RTPEVTCVVVDVSHEDPEVKFNWYVDGVEVH | | |
| | | NAKTKPREEQYASTYRVVSVLTVLHQDWLNGK | | |
| DNA366 | Knob: hFc(N297A)-[MPYDLYHP]-hIL15-hCD215(Truncated) | EYKCKVSNKALPAPIEKTISKAKGQPREPQVYTL | GGSSPP | MPYDLYHP |
| | | PPCRDELTKNQVSLWCLVKGFYPSDIAVEWES | | |
| | | NGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSR | | |
| | | WQQGNVFSCSVMHEALHNHYTQKSLSLSPG | | |

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| | | DKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMIS | | | |
| | | RTPEVTCVVVDVSHEDPEVKFNWYVDGVEVH | | | |
| | | NAKTKPREEQYASTYRVVSVLTVLHQDWLNGK | GGSSPPGGGS | | NWVNVISDLKKIEDLIQSMHIDATLYTESDV |
| DNA367 | Knob: hFc(N297A)-hIL15 | EYKCKVSNKALPAPIEKTISKAKGQPREPQVYTL | SGGGSGPGSG | | HPSCKVTAMKCFLLELQVISLESGDASIHDT |
| | | PPCRDELTKNQVSLWCLVKGFYPSDIAVEWES | S | | VENLIILANNSLSSNGNVTESGCKECEELEEK |
| | | NGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSR | | | NIKEFLQSFVHIVQMFINTS |
| | | WQQGNVFSCSVMHEALHNHYTQKSLSLSPG | | | |
| | | DKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMIS | | | |
| | | RTPEVTCVVVDVSHEDPEVKFNWYVDGVEVH | | | |
| | | NAKTKPREEQYASTYRVVSVLTVLHQDWLNGK | | | |
| DNA368 | Knob: hFc(N297A)-[MPYDLYHP]-hIL15-hCD215 | EYKCKVSNKALPAPIEKTISKAKGQPREPQVYTL | GGSSPP | | MPYDLYHP |
| | | PPCRDELTKNQVSLWCLVKGFYPSDIAVEWES | | | |
| | | NGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSR | | | |
| | | WQQGNVFSCSVMHEALHNHYTQKSLSLSPG | | | |

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| DNA369 | Knob: hFc(N297A)-[MPYDLYHP]-hIL15-hCD215(Truncated) | DKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMIS<br>RTPEVTCVVVDVSHEDPEVKFNWYVDGVEVH<br>NAKTKPREEQYASTYRVVSVLTVLHQDWLNGK<br>EYKCKVSNKALPAPIEKTISKAKGQPREPQVYTL<br>PPCRDELTKNQVSLWCLVKGFYPSDIAVEWES<br>NGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSR<br>WQQGNVFSCSVMHEALHNHYTQKSLSLSPG | GGSSPP | MPYDLYHP | |
| DNA370 | Hole: hFc(N297A)-[MPYDLYHP]-hCD122 | DKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMIS<br>RTPEVTCVVVDVSHEDPEVKFNWYVDGVEVH<br>NAKTKPREEQYASTYRVVSVLTVLHQDWLNGK<br>EYKCKVSNKALPAPIEKTISKAKGQPREPQVCTL<br>PPSRDELTKNQVSLSCAVKGFYPSDIAVEWESN<br>GQPENNYKTTPPVLDSDGSFFLVSKLTVDKSRW<br>QQGNVFSCSVMHEALHNHYTQKSLSLSPG | GPPSGSSP | MPYDLYHP | |

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| | | DKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMIS | | |
| | | RTPEVTCVVVDVSHEDPEVKFNWYVDGVEVH | | |
| | | NAKTKPREEQYASTYRVVSVLTVLHQDWLNGK | | |
| DNA487 | Knob: hFc(N297A)-[VPLSLY]-hIL15 | EYKCKVSNKALPAPIEKTISKAKGQPREPQVYTL | GGSSPP | VPLSLY |
| | | PPCRDELTKNQVSLWCLVKGFYPSDIAVEWES | | |
| | | NGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSR | | |
| | | WQQGNVFSCSVMHEALHNHYTQKSLSLSPG | | |
| | | DKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMIS | | |
| | | RTPEVTCVVVDVSHEDPEVKFNWYVDGVEVH | | |
| | | NAKTKPREEQYASTYRVVSVLTVLHQDWLNGK | | |
| DNA517 | Knob: hFc(N297A)-[VPLSLY]-hIL15-hCD215 | EYKCKVSNKALPAPIEKTISKAKGQPREPQVYTL | GGSSPP | VPLSLY |
| | | PPCRDELTKNQVSLWCLVKGFYPSDIAVEWES | | |
| | | NGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSR | | |
| | | WQQGNVFSCSVMHEALHNHYTQKSLSLSPG | | |

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| | | DKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMIS | | |
| | | RTPEVTCVVVDVSHEDPEVKFNWYVDGVEVH | | |
| | | NAKTKPREEQYASTYRVVSVLTVLHQDWLNGK | | |
| DNA518 | Knob: hFc(N297A)-[VPLSLY]-hIL15-hCD215(Truncated) | EYKCKVSNKALPAPIEKTISKAKGQPREPQVYTL | GGSSPP | VPLSLY |
| | | PPCRDELTKNQVSLWCLVKGFYPSDIAVEWES | | |
| | | NGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSR | | |
| | | WQQGNVFSCSVMHEALHNHYTQKSLSLSPG | | |
| | | | | AVNGTSQFTCFYNSRANISCVWSQDGALQ |
| | | | | DTSCQVHAWPDRRRWNQTCELLPVSQAS |
| | | | | WACNLILGAPDSQKLTTVDIVTLRVLCREGV |
| | | | | RWRVMAIQDFKPFENLRLMAPISLQVVHV |
| | | DKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMIS | | ETHRCNISWEISQASHYFERHLEFEARTLSPG |
| | | RTPEVTCVVVDVSHEDPEVKFNWYVDGVEVH | | HTWEEAPLLTLKQKQEWICLETLTPDTQYEF |
| | Hole: hFc(N297A)-hCD122-[VPLSLY]-hCD215(Sushi)-hIL15 | NAKTKPREEQYASTYRVVSVLTVLHQDWLNGK | | QVRVKPLQGEFTTWSPWSQPLAFRTKPAA |
| DNA523 | | EYKCKVSNKALPAPIEKTISKAKGQPREPQVCTL | PGSGS | LGKD |
| | | PPSRDELTKNQVSLSCAVKGFYPSDIAVEWESN | | |
| | | GQPENNYKTTPPVLDSDGSFFLVSKLTVDKSRW | | |
| | | QQGNVFSCSVMHEALHNHYTQKSLSLSPG | | |

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| | | DKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMIS | AVNGTSQFTCFYNSRANISCVWSQDGALQ |
| | | RTPEVTCVVVDVSHEDPEVKFNWYVDGVEVH | DTSCQVHAWPDRRRWNQTCELLPVSQAS |
| | Hole: hFc(N297A)-hCD122- | NAKTKPREEQYASTYRVVSVLTVLHQDWLNGK | WACNLILGAPDSQKLTTVDIVTLRVLCREGV |
| DNA524 | [VPLSLY]-hCD215(Sushi)- | EYKCKVSNKALPAPIEKTISKAKGQPREPQVCTL PGSGS | RWRVMAIQDFKPFENLRLMAPISLQVVHV |
| | hIL15 | PPSRDELTKNQVSLSCAVKGFYPSDIAVEWESN | ETHRCNISWEISQASHYFERHLEFEARTLSPG |
| | | GQPENNYKTTPPVLDSDGSFFLVSKLTVDKSRW | HTWEEAPLLTLKQKQEWICLETLTPDTQYEF |
| | | QQGNVFSCSVMHEALHNHYTQKSLSLSPG | QVRVKPLQGEFTTWSPWSQPLAFRTKPAA |
| | | | LGKD |

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| | | DKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMIS | AVNGTSQFTCFYNSRANISCVWSQDGALQ |
| | | RTPEVTCVVVDVSHEDPEVKFNWYVDGVEVH | DTSCQVHAWPDRRRWNQTCELLPVSQAS |
| | Hole: hFc(N297A)-hCD122- | NAKTKPREEQYASTYRVVSVLTVLHQDWLNGK | WACNLILGAPDSQKLTTVDIVTLRVLCREGV |
| DNA525 | [VPLSLY]-hCD215(Sushi)- | EYKCKVSNKALPAPIEKTISKAKGQPREPQVCTL PGSGS | RWRVMAIQDFKPFENLRLMAPISLQVVHV |
| | hIL15 | PPSRDELTKNQVSLSCAVKGFYPSDIAVEWESN | ETHRCNISWEISQASHYFERHLEFEARTLSPG |
| | | GQPENNYKTTPPVLDSDGSFFLVSKLTVDKSRW | HTWEEAPLLTLKQKQEWICLETLTPDTQYEF |
| | | QQGNVFSCSVMHEALHNHYTQKSLSLSPG | QVRVKPLQGEFTTWSPWSQPLAFRTKPAA |
| | | | LGKD |

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| | | DKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMIS | | |
| | | RTPEVTCVVVDVSHEDPEVKFNWYVDGVEVH | | |
| | | NAKTKPREEQYASTYRVVSVLTVLHQDWLNGK | | |
| DNA526 | Knob: hFc(N297A)-[VPLSLY]-hIL15-hCD215 | EYKCKVSNKALPAPIEKTISKAKGQPREPQVYTL | GGSSPP | VPLSLY |
| | | PPCRDELTKNQVSLWCLVKGFYPSDIAVEWES | | |
| | | NGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSR | | |
| | | WQQGNVFSCSVMHEALHNHYTQKSLSLSPG | | |
| | | DKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMIS | | |
| | | RTPEVTCVVVDVSHEDPEVKFNWYVDGVEVH | | |
| | | NAKTKPREEQYASTYRVVSVLTVLHQDWLNGK | | |
| DNA527 | Knob: hFc(N297A)-[VPLSLY]-hIL15-hCD215 | EYKCKVSNKALPAPIEKTISKAKGQPREPQVYTL | GGSSPP | VPLSLY |
| | | PPCRDELTKNQVSLWCLVKGFYPSDIAVEWES | | |
| | | NGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSR | | |
| | | WQQGNVFSCSVMHEALHNHYTQKSLSLSPG | | |

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| DNA556 | Knob: hFc(N297A)-[VPLSLY]-hIL15 | DKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMIS<br>RTPEVTCVVVDVSHEDPEVKFNWYVDGVEVH<br>NAKTKPREEQYASTYRVVSVLTVLHQDWLNGK<br>EYKCKVSNKALPAPIEKTISKAKGQPREPQVYTL<br>PPCRDELTKNQVSLWCLVKGFYPSDIAVEWES<br>NGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSR<br>WQQGNVFSCSVMHEALHNHYTQKSLSLSPG | GGSSPPG | VPLSLY |
| DNA557 | Knob: hFc(N297A)-[VPLSLY]-hIL15(D22A) | DKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMIS<br>RTPEVTCVVVDVSHEDPEVKFNWYVDGVEVH<br>NAKTKPREEQYASTYRVVSVLTVLHQDWLNGK<br>EYKCKVSNKALPAPIEKTISKAKGQPREPQVYTL<br>PPCRDELTKNQVSLWCLVKGFYPSDIAVEWES<br>NGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSR<br>WQQGNVFSCSVMHEALHNHYTQKSLSLSPG | GGSSPPG | VPLSLY |

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| DNA558 | Knob: hFc(N297A)-[VPLSLY]-hIL15(E46A) | DKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMIS<br>RTPEVTCVVVDVSHEDPEVKFNWYVDGVEVH<br>NAKTKPREEQYASTYRVVSVLTVLHQDWLNGK<br>EYKCKVSNKALPAPIEKTISKAKGQPREPQVYTL<br>PPCRDELTKNQVSLWCLVKGFYPSDIAVEWES<br>NGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSR<br>WQQGNVFSCSVMHEALHNHYTQKSLSLSPG | GGSSPPG | VPLSLY |
| DNA559 | Knob: hFc(N297A)-[VPLSLY]-hIL15(E53A) | DKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMIS<br>RTPEVTCVVVDVSHEDPEVKFNWYVDGVEVH<br>NAKTKPREEQYASTYRVVSVLTVLHQDWLNGK<br>EYKCKVSNKALPAPIEKTISKAKGQPREPQVYTL<br>PPCRDELTKNQVSLWCLVKGFYPSDIAVEWES<br>NGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSR<br>WQQGNVFSCSVMHEALHNHYTQKSLSLSPG | GGSSPPG | VPLSLY |

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| | | DKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMIS | | | |
| | | RTPEVTCVVVDVSHEDPEVKFNWYVDGVEVH | | | |
| | | NAKTKPREEQYASTYRVVSVLTVLHQDWLNGK | | | |
| DNA560 | Knob: hFc(N297A)-[VPLSLY]-hIL15(E46A, E53A) | EYKCKVSNKALPAPIEKTISKAKGQPREPQVYTL | GGSSPPG | VPLSLY | |
| | | PPCRDELTKNQVSLWCLVKGFYPSDIAVEWES | | | |
| | | NGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSR | | | |
| | | WQQGNVFSCSVMHEALHNHYTQKSLSLSPG | | | |
| | | | | | AVNGTSQFTCFYNSRANISCVWSQDGALQ |
| | | DKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMIS | | | DTSCQVHAWPDRRRWNQTCELLPVSQAS |
| | | RTPEVTCVVVDVSHEDPEVKFNWYVDGVEVH | | | WACNLILGAPDSQKLTTVDIVTLRVLCREGV |
| | Hole: hFc(N297A)-hCD122-hCD215(Sushi)-[VPLSLY]-hIL15 | NAKTKPREEQYASTYRVVSVLTVLHQDWLNGK | | | RWRVMAIQDFKPFENLRLMAPISLQVVHV |
| DNA569 | | EYKCKVSNKALPAPIEKTISKAKGQPREPQVCTL | PGSGS | | ETHRCNISWEISQASHYFERHLEFEARTLSPG |
| | | PPSRDELTKNQVSLSCAVKGFYPSDIAVEWESN | | | HTWEEAPLLTLKQKQEWICLETLTPDTQYEF |
| | | GQPENNYKTTPPVLDSDGSFFLVSKLTVDKSRW | | | QVRVKPLQGEFTTWSPWSQPLAFRTKPAA |
| | | QQGNVFSCSVMHEALHNHYTQKSLSLSPG | | | LGKD |

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| | | DKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMIS | AVNGTSQFTCFYNSRANISCVWSQDGALQ |
| | | RTPEVTCVVVDVSHEDPEVKFNWYVDGVEVH | DTSCQVHAWPDRRRWNQTCELLPVSQAS |
| | Hole: hFc(N297A)-hCD122- | NAKTKPREEQYASTYRVVSVLTVLHQDWLNGK | WACNLILGAPDSQKLTTVDIVTLRVLCREGV |
| DNA570 | hCD215(Sushi)-[VPLSLY]- | EYKCKVSNKALPAPIEKTISKAKGQPREPQVCTL PGSGS | RWRVMAIQDFKPFENLRLMAPISLQVVHV |
| | hIL15 | PPSRDELTKNQVSLSCAVKGFYPSDIAVEWESN | ETHRCNISWEISQASHYFERHLEFEARTLSPG |
| | | GQPENNYKTTPPVLDSDGSFFLVSKLTVDKSRW | HTWEEAPLLTLKQKQEWICLETLTPDTQYEF |
| | | QQGNVFSCSVMHEALHNHYTQKSLSLSPG | QVRVKPLQGEFTTWSPWSQPLAFRTKPAA |
| | | | LGKD |
| | | DKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMIS | AVNGTSQFTCFYNSRANISCVWSQDGALQ |
| | | RTPEVTCVVVDVSHEDPEVKFNWYVDGVEVH | DTSCQVHAWPDRRRWNQTCELLPVSQAS |
| | Hole: hFc(N297A)-hCD122- | NAKTKPREEQYASTYRVVSVLTVLHQDWLNGK | WACNLILGAPDSQKLTTVDIVTLRVLCREGV |
| DNA571 | hCD215(Sushi)-[VPLSLY]- | EYKCKVSNKALPAPIEKTISKAKGQPREPQVCTL PGSGS | RWRVMAIQDFKPFENLRLMAPISLQVVHV |
| | hIL15 | PPSRDELTKNQVSLSCAVKGFYPSDIAVEWESN | ETHRCNISWEISQASHYFERHLEFEARTLSPG |
| | | GQPENNYKTTPPVLDSDGSFFLVSKLTVDKSRW | HTWEEAPLLTLKQKQEWICLETLTPDTQYEF |
| | | QQGNVFSCSVMHEALHNHYTQKSLSLSPG | QVRVKPLQGEFTTWSPWSQPLAFRTKPAA |
| | | | LGKD |

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| | | DKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMIS | AVNGTSQFTCFYNSRANISCVWSQDGALQ |
| | | RTPEVTCVVVDVSHEDPEVKFNWYVDGVEVH | DTSCQVHAWPDRRRWNQTCELLPVSQAS |
| | Hole: hFc(N297A)-hCD122- | NAKTKPREEQYASTYRVVSVLTVLHQDWLNGK | WACNLILGAPDSQKLTTVDIVTLRVLCREGV |
| DNA572 | hCD215(1to66)-[VPLSLY]- | EYKCKVSNKALPAPIEKTISKAKGQPREPQVCTL PGSGS | RWRVMAIQDFKPFENLRLMAPISLQVVHV |
| | hIL15 | PPSRDELTKNQVSLSCAVKGFYPSDIAVEWESN | ETHRCNISWEISQASHYFERHLEFEARTLSPG |
| | | GQPENNYKTTPPVLDSDGSFFLVSKLTVDKSRW | HTWEEAPLLTLKQKQEWICLETLTPDTQYEF |
| | | QQGNVFSCSVMHEALHNHYTQKSLSLSPG | QVRVKPLQGEFTTWSPWSQPLAFRTKPAA |
| | | | LGKD |

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| | | DKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMIS | AVNGTSQFTCFYNSRANISCVWSQDGALQ |
| | | RTPEVTCVVVDVSHEDPEVKFNWYVDGVEVH | DTSCQVHAWPDRRRWNQTCELLPVSQAS |
| | Hole: hFc(N297A)-hCD122- | NAKTKPREEQYASTYRVVSVLTVLHQDWLNGK | WACNLILGAPDSQKLTTVDIVTLRVLCREGV |
| DNA573 | hCD215(1to66)-[VPLSLY]- | EYKCKVSNKALPAPIEKTISKAKGQPREPQVCTL PGSGS | RWRVMAIQDFKPFENLRLMAPISLQVVHV |
| | hIL15 | PPSRDELTKNQVSLSCAVKGFYPSDIAVEWESN | ETHRCNISWEISQASHYFERHLEFEARTLSPG |
| | | GQPENNYKTTPPVLDSDGSFFLVSKLTVDKSRW | HTWEEAPLLTLKQKQEWICLETLTPDTQYEF |
| | | QQGNVFSCSVMHEALHNHYTQKSLSLSPG | QVRVKPLQGEFTTWSPWSQPLAFRTKPAA |
| | | | LGKD |

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| | | DKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMIS | | AVNGTSQFTCFYNSRANISCVWSQDGALQ |
| | | RTPEVTCVVVDVSHEDPEVKFNWYVDGVEVH | | DTSCQVHAWPDRRRWNQTCELLPVSQAS |
| | Hole: hFc(N297A)-hCD122- | NAKTKPREEQYASTYRVVSVLTVLHQDWLNGK | | WACNLILGAPDSQKLTTVDIVTLRVLCREGV |
| DNA574 | hCD215(1to66)-[VPLSLY]- | EYKCKVSNKALPAPIEKTISKAKGQPREPQVCTL | PGSGS | RWRVMAIQDFKPFENLRLMAPISLQVVHV |
| | hIL15 | PPSRDELTKNQVSLSCAVKGFYPSDIAVEWESN | | ETHRCNISWEISQASHYFERHLEFEARTLSPG |
| | | GQPENNYKTTPPVLDSDGSFFLVSKLTVDKSRW | | HTWEEAPLLTLKQKQEWICLETLTPDTQYEF |
| | | QQGNVFSCSVMHEALHNHYTQKSLSLSPG | | QVRVKPLQGEFTTWSPWSQPLAFRTKPAA |
| | | | | LGKD |
| | | ITCPPPMSVEHADIWVKSYSLYSRERYICNSGFK | | |
| DNA595 | hCD215(1to66) | RKAGTSSLTECVLNKATNVAHWTTPSLKCIRD | | |

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| DNA596 | Knob: hFc(N297A)-hIL15(E46A, E53A) | DKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMIS<br>RTPEVTCVVVDVSHEDPEVKFNWYVDGVEVH<br>NAKTKPREEQYASTYRVVSVLTVLHQDWLNGK<br>EYKCKVSNKALPAPIEKTISKAKGQPREPQVYTL<br>PPCRDELTKNQVSLWCLVKGFYPSDIAVEWES<br>NGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSR<br>WQQGNVFSCSVMHEALHNHYTQKSLSLSPG | GGSSPPGGGS<br>SGGGSGPSGS<br>PG | NWVNVISDLKKIEDLIQSMHIDATLYTESDV<br>HPSCKVTAMKCFLLALQVISLASGDASIHDT<br>VENLIILANNSLSSNGNVTESGCKECEELEEK<br>NIKEFLQSFVHIVQMFINTS | |
| DNA561 | Knob: hFc(N297A)-[VPLSLY]-hIL15(D22A, E46A, E53A) | DKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMIS<br>RTPEVTCVVVDVSHEDPEVKFNWYVDGVEVH<br>NAKTKPREEQYASTYRVVSVLTVLHQDWLNGK<br>EYKCKVSNKALPAPIEKTISKAKGQPREPQVYTL<br>PPCRDELTKNQVSLWCLVKGFYPSDIAVEWES<br>NGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSR<br>WQQGNVFSCSVMHEALHNHYTQKSLSLSPG | GGSSPPG | VPLSLY | |

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| New DNA1 | Knob: hFc(N297A)-[VPLSLY]-hIL15 | DKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMIS<br>RTPEVTCVVVDVSHEDPEVKFNWYVDGVEVH<br>NAKTKPREEQYASTYRVVSVLTVLHQDWLNGK<br>EYKCKVSNKALPAPIEKTISKAKGQPREPQVYTL<br>PPCRDELTKNQVSLWCLVKGFYPSDIAVEWES<br>NGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSR<br>WQQGNVFSCSVMHEALHNHYTQKSLSLSPG | GGSGSSG | VPLSLY |
| New DNA2 | Knob: hFc(N297A)-[VPLSLY]-hIL15 | DKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMIS<br>RTPEVTCVVVDVSHEDPEVKFNWYVDGVEVH<br>NAKTKPREEQYASTYRVVSVLTVLHQDWLNGK<br>EYKCKVSNKALPAPIEKTISKAKGQPREPQVYTL<br>PPCRDELTKNQVSLWCLVKGFYPSDIAVEWES<br>NGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSR<br>WQQGNVFSCSVMHEALHNHYTQKSLSLSPG | GGSPGPP | VPLSLY |

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| New DNA3 | Knob: hFc(N297A)-[VPLSLY]-hIL15 | DKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMIS<br>RTPEVTCVVVDVSHEDPEVKFNWYVDGVEVH<br>NAKTKPREEQYASTYRVVSVLTVLHQDWLNGK<br>EYKCKVSNKALPAPIEKTISKAKGQPREPQVYTL<br>PPCRDELTKNQVSLWCLVKGFYPSDIAVEWES<br>NGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSR<br>WQQGNVFSCSVMHEALHNHYTQKSLSLSPG | PSSGSGSG | VPLSLY |
| New DNA7 | Knob: hFc(N297A)-hCD215(1-66)-[VPLSLY]-hIL15 | DKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMIS<br>RTPEVTCVVVDVSHEDPEVKFNWYVDGVEVH<br>NAKTKPREEQYASTYRVVSVLTVLHQDWLNGK<br>EYKCKVSNKALPAPIEKTISKAKGQPREPQVYTL<br>PPCRDELTKNQVSLWCLVKGFYPSDIAVEWES<br>NGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSR<br>WQQGNVFSCSVMHEALHNHYTQKSLSLSPG | SSGGSS | ITCPPPMSVEHADIWVKSYSLYSRERYICNS<br>GFKRKAGTSSLTECVLNKATNVAHWTTPSL<br>KCIRD |

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| New DNA8 | Knob: hFc(N297A)-hCD215(1-66)-[VPLSLY]-hIL15 | DKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMIS<br>RTPEVTCVVVDVSHEDPEVKFNWYVDGVEVH<br>NAKTKPREEQYASTYRVVSVLTVLHQDWLNGK<br>EYKCKVSNKALPAPIEKTISKAKGQPREPQVYTL<br>PPCRDELTKNQVSLWCLVKGFYPSDIAVEWES<br>NGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSR<br>WQQGNVFSCSVMHEALHNHYTQKSLSLSPG | SSGGSS | ITCPPPMSVEHADIWVKSYSLYSRERYICNS<br>GFKRKAGTSSLTECVLNKATNVAHWTTPSL<br>KCIRD |
| New DNA9 | Knob: hFc(N297A)-hCD215(1-66)-[VPLSLY]-hIL15 | DKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMIS<br>RTPEVTCVVVDVSHEDPEVKFNWYVDGVEVH<br>NAKTKPREEQYASTYRVVSVLTVLHQDWLNGK<br>EYKCKVSNKALPAPIEKTISKAKGQPREPQVYTL<br>PPCRDELTKNQVSLWCLVKGFYPSDIAVEWES<br>NGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSR<br>WQQGNVFSCSVMHEALHNHYTQKSLSLSPG | SSGGSS | ITCPPPMSVEHADIWVKSYSLYSRERYICNS<br>GFKRKAGTSSLTECVLNKATNVAHWTTPSL<br>KCIRD |

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| New DNA10 | Hole: hFc(N297A)-hCD122 | DKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMIS<br>RTPEVTCVVVDVSHEDPEVKFNWYVDGVEVH<br>NAKTKPREEQYASTYRVVSVLTVLHQDWLNGK<br>EYKCKVSNKALPAPIEKTISKAKGQPREPQVCTL<br>PPSRDELTKNQVSLSCAVKGFYPSDIAVEWESN<br>GQPENNYKTTPPVLDSDGSFFLVSKLTVDKSRW<br>QQGNVFSCSVMHEALHNHYTQKSLSLSPG | GGGGSGGGG<br>S | AVNGTSQFTCFYNSRANISCVWSQDGALQ<br>DTSCQVHAWPDRRRWNQTCELLPVSQAS<br>WACNLILGAPDSQKLTTVDIVTLRVLCREGV<br>RWRVMAIQDFKPFENLRLMAPISLQVVHV<br>ETHRCNISWEISQASHYFERHLEFEARTLSPG<br>HTWEEAPLLTLKQKQEWICLETLTPDTQYEF<br>QVRVKPLQGEFTTWSPWSQPLAFRTKPAA<br>LGKD |
| New DNA11 | Hole: hFc(N297A)-hCD122 | DKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMIS<br>RTPEVTCVVVDVSHEDPEVKFNWYVDGVEVH<br>NAKTKPREEQYASTYRVVSVLTVLHQDWLNGK<br>EYKCKVSNKALPAPIEKTISKAKGQPREPQVCTL<br>PPSRDELTKNQVSLSCAVKGFYPSDIAVEWESN<br>GQPENNYKTTPPVLDSDGSFFLVSKLTVDKSRW<br>QQGNVFSCSVMHEALHNHYTQKSLSLSPG | GGGGSGGGG<br>SGGGGS | AVNGTSQFTCFYNSRANISCVWSQDGALQ<br>DTSCQVHAWPDRRRWNQTCELLPVSQAS<br>WACNLILGAPDSQKLTTVDIVTLRVLCREGV<br>RWRVMAIQDFKPFENLRLMAPISLQVVHV<br>ETHRCNISWEISQASHYFERHLEFEARTLSPG<br>HTWEEAPLLTLKQKQEWICLETLTPDTQYEF<br>QVRVKPLQGEFTTWSPWSQPLAFRTKPAA<br>LGKD |

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|---|---|---|---|---|
| New DNA12 | Knob: hFc(N297A)-hCD215(1-66; R26N)-[VPLSLY]-hIL15 | DKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMIS<br>RTPEVTCVVVDVSHEDPEVKFNWYVDGVEVH<br>NAKTKPREEQYASTYRVVSVLTVLHQDWLNGK<br>EYKCKVSNKALPAPIEKTISKAKGQPREPQVYTL<br>PPCRDELTKNQVSLWCLVKGFYPSDIAVEWES<br>NGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSR<br>WQQGNVFSCSVMHEALHNHYTQKSLSLSPG | SSGGSS | ITCPPPMSVEHADIWVKSYSLYSRERYICNS<br>GFKRKAGTSSLTECVLNKATNVAHWTTPSL<br>KCIRD |
| New DNA13 | Knob: hFc(N297A)-hCD215(1-66; R35Q)-[VPLSLY]-hIL15 | DKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMIS<br>RTPEVTCVVVDVSHEDPEVKFNWYVDGVEVH<br>NAKTKPREEQYASTYRVVSVLTVLHQDWLNGK<br>EYKCKVSNKALPAPIEKTISKAKGQPREPQVYTL<br>PPCRDELTKNQVSLWCLVKGFYPSDIAVEWES<br>NGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSR<br>WQQGNVFSCSVMHEALHNHYTQKSLSLSPG | SSGGSS | ITCPPPMSVEHADIWVKSYSLYSRERYICNS<br>GFKQKAGTSSLTECVLNKATNVAHWTTPSL<br>KCIRD |

| | | | |
|---|---|---|---|
| New DNA14 | Knob: hFc(N297A)-hCD215(1-66; R26S)-[VPLSLY]-hIL15 | DKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMIS<br>RTPEVTCVVVDVSHEDPEVKFNWYVDGVEVH<br>NAKTKPREEQYASTYRVVSVLTVLHQDWLNGK<br>EYKCKVSNKALPAPIEKTISKAKGQPREPQVYTL<br>PPCRDELTKNQVSLWCLVKGFYPSDIAVEWES<br>NGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSR<br>WQQGNVFSCSVMHEALHNHYTQKSLSLSPG | SSGGSS<br>ITCPPPMSVEHADIWVKSYSLYSRESYICNSG<br>FKRKAGTSSLTECVLNKATNVAHWTTPSLKC<br>IRD |
| New DNA15 | Knob: hFc(N297A)-hCD215(1-66; R35S)-[VPLSLY]-hIL15 | DKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMIS<br>RTPEVTCVVVDVSHEDPEVKFNWYVDGVEVH<br>NAKTKPREEQYASTYRVVSVLTVLHQDWLNGK<br>EYKCKVSNKALPAPIEKTISKAKGQPREPQVYTL<br>PPCRDELTKNQVSLWCLVKGFYPSDIAVEWES<br>NGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSR<br>WQQGNVFSCSVMHEALHNHYTQKSLSLSPG | SSGGSS<br>ITCPPPMSVEHADIWVKSYSLYSRESYICNSG<br>FKRKAGTSSLTECVLNKATNVAHWTTPSLKC<br>IRD |

DKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMIS
RTPEVTCVVVDVSHEDPEVKFNWYVDGVEVH

Knob: hFc(N297A)-hCD215(1-66; R26N, R35Q)-[VPLSLY]-hIL15

NAKTKPREEQYASTYRVVSVLTVLHQDWLNGK
EYKCKVSNKALPAPIEKTISKAKGQPREPQVYTL
PPCRDELTKNQVSLWCLVKGFYPSDIAVEWES
NGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSR
WQQGNVFSCSVMHEALHNHYTQKSLSLSPG

SSGGSS

ITCPPPMSVEHADIWVKSYSLYSRENYICNS
GFKQKAGTSSLTECVLNKATNVAHWTTPSL
KCIRD

| name | newnames | Component4Sequence | Component5Sequence | Component6Sequence | Component7Sequence |
|---|---|---|---|---|---|
| DNA158 | Hole: hFc(N297A) | | | | |
| DNA161 | Knob: hFc(N297A) | | | | |

| | | | |
|---|---|---|---|
| | | | NWVNVISDLKKIEDLIQSMHIDATLY |
| | | | TESDVHPSCKVTAMKCFLLELQVISLE |
| DNA187 | Hole: hFc(N297A)-hCD122 | | SGDASIHDTVENLIILANNSLSSNGNV |
| DNA362 | Knob: hFc(N297A)-[MPYDLYHP]-hIL15 | SGPSGSPG | TESGCKECEELEEKNIKEFLQSFVHIV |
| | | | QMFINTS |

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|---|---|---|---|
| | | | NWVNVISDLKKIEDLIQSMHIDATLY |
| | | | TESDVHPSCKVTAMKCFLLELQVISLE |
| DNA363 | Knob: hFc(N297A)-[MPYDLYHP]-hIL15 | SGPGSGS | SGDASIHDTVENLIILANNSLSSNGNV |
| | | | TESGCKECEELEEKNIKEFLQSFVHIV |
| | | | QMFINTS |
| DNA364 | Knob: hFc(N297A)-hIL15 | | |

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|---|---|---|---|---|---|
| | | | | | ITCPPPMSVEHADIWVKSYSLY |
| | | | | | SRERYICNSGFKRKAGTSSLTEC |
| | | | NWVNVISDLKKIEDLIQSMHIDATLY | | VLNKATNVAHWTTPSLKCIRD |
| | | | TESDVHPSCKVTAMKCFLLELQVISLE | | PALVHQRPAPPSTVTTAGVTP |
| DNA365 | Knob: hFc(N297A)-[MPYDLYHP]-hIL15-hCD215 | SGPSGSPG | SGDASIHDTVENLILLANNSLSSNGNV | GGGGSGGGGSG | QPESLSPSGKEPAASSPSSNNT |
| | | | TESGCKECEELEEKNIKEFLQSFVHIV | GGGSGGGGS | AATTAAIVPGSQLMPSKSPST |
| | | | QMFINTS | | GTTEISSHESSHGTPSQTTAKN |
| | | | | | WELTASASHQPPGVYPQGHS |
| | | | | | DTT |
| | | | | | |
| | | | NWVNVISDLKKIEDLIQSMHIDATLY | | ITCPPPMSVEHADIWVKSYSLY |
| | Knob: hFc(N297A)-[MPYDLYHP]-hIL15-hCD215(Truncated) | SGPSGSPG | TESDVHPSCKVTAMKCFLLELQVISLE | GGGGSGGGGSG | SRERYICNSGFKRKAGTSSLTEC |
| DNA366 | | | SGDASIHDTVENLILLANNSLSSNGNV | GGGSGGGGS | VLNKATNVAHWTTPSLKCIRD |
| | | | TESGCKECEELEEKNIKEFLQSFVHIV | | PALVHQRPAPPSTVTTAGVTP |
| | | | QMFINTS | | QPESLSPSGKEPAAS |

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|---|---|---|---|---|---|
| | | | | | ITCPPPMSVEHADIWVKSYSLY |
| | | | | | SRERYICNSGFKRKAGTSSLTEC |
| | | | | | VLNKATNVAHWTTPSLKCIRD |
| | | | | NWVNVISDLKKIEDLIQSMHIDATLY | |
| | | | | TESDVHPSCKVTAMKCFLLELQVISLE | GGGGSGGGGSG |
| DNA367 | Knob: hFc(N297A)-hIL15 | | | SGDASIHDTVENLIILANNSLSSNGNV | GGGSGGGGS |
| | | | | TESGCKECEELEEKNIKEFLQSFVHIV | |
| | | | | QMFINTS | |
| DNA368 | Knob: hFc(N297A)-[MPYDLYHP]-hIL15-hCD215 | SGPGSGS | | | |

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|---|---|---|---|---|---|---|
| | | | NWVNVISDLKKIEDLIQSMHIDATLY | | | ITCPPPMSVEHADIWVKSYSLY |
| | | | TESDVHPSCKVTAMKCFLLELQVISLE | | | SRERYICNSGFKRKAGTSSLTEC |
| DNA369 | Knob: hFc(N297A)-[MPYDLYHP]-hIL15-hCD215(Truncated) | SGPGSGS | SGDASIHDTVENLIILANNSLSSNGNV | GGGGSGGGGSG | | VLNKATNVAHWTTPSLKCIRD |
| | | | TESGCKECEELEEKNIKEFLQSFVHIV | GGGSGGGGS | | PALVHQRPAPPSTVTTAGVTP |
| | | | QMFINTS | | | QPESLSPSGKEPAAS |
| | | | | | | |
| | | | | AVNGTSQFTCFYNSRANISCVWSQD | | |
| | | | | GALQDTSCQVHAWPDRRRWNQTC | | |
| | | | | ELLPVSQASWACNLILGAPDSQKLTT | | |
| | | | | VDIVTLRVLCREGVRWRVMAIQDFK | | |
| DNA370 | Hole: hFc(N297A)-[MPYDLYHP]-hCD122 | SGGG | PFENLRLMAPISLQVVHVETHRCNIS | | | |
| | | | WEISQASHYFERHLEFEARTLSPGHT | | | |
| | | | WEEAPLLTLKQKQEWICLETLTPDTQ | | | |
| | | | YEFQVRVKPLQGEFTTWSPWSQPLA | | | |
| | | | FRTKPAALGKD | | | |

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|---|---|---|---|
| | | | NWVNVISDLKKIEDLIQSMHIDATLY |
| | | | TESDVHPSCKVTAMKCFLLELQVISLE |
| DNA487 | Knob: hFc(N297A)-[VPLSLY]-hIL15 | SGPGSGS | SGDASIHDTVENLILLANNSLSSNGNV |
| | | | TESGCKECEELEEKNIKEFLQSFVHIV |
| | | | QMFINTS |

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|---|---|---|---|
| | | | ITCPPPMSVEHADIWVKSYSLY |
| | | | SRERYICNSGFKRKAGTSSLTEC |
| | | | VLNKATNVAHWTTPSLKCIRD |
| | | | NWVNVISDLKKIEDLIQSMHIDATLY |
| | | | TESDVHPSCKVTAMKCFLLELQVISLE GGGGSGGGGSG |
| DNA517 | Knob: hFc(N297A)-[VPLSLY]-hIL15-hCD215 | SGPGSGS | SGDASIHDTVENLILLANNSLSSNGNV GGGSGGGGSGG |
| | | | TESGCKECEELEEKNIKEFLQSFVHIV GGS |
| | | | QMFINTS |

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|---|---|
| | PALVHQRPAPPSTVTTAGVTP |
| | QPESLSPSGKEPAASSPSSNNT |
| | AATTAAIVPGSQLMPSKSPST |
| | GTTEISSHESSHGTPSQTTAKN |
| | WELTASASHQPPGVYPQGHS |
| | DTT |

| | | | | | | |
|---|---|---|---|---|---|---|
| | | | | NWVNVISDLKKIEDLIQSMHIDATLY | | ITCPPPMSVEHADIWVKSYSLY |
| | | | | TESDVHPSCKVTAMKCFLLELQVISLE | GGGGSGGGGSG | SRERYICNSGFKRKAGTSSLTEC |
| DNA518 | Knob: hFc(N297A)-[VPLSLY]-hIL15-hCD215(Truncated) | SGPGSGS | | SGDASIHDTVENLILLANNSLSSNGNV | GGGSGGGGSGG | VLNKATNVAHWTTPSLKCIRD |
| | | | | TESGCKECEELEEKNIKEFLQSFVHIV | GGS | PALVHQRPAPPSTVTTAGVTP |
| | | | | QMFINTS | | QPESLSPSGKEPAAS |
| DNA523 | Hole: hFc(N297A)-hCD122-[VPLSLY]-hCD215(Sushi)-hIL15 | PGGPSP | VPLSLY | | SGGP | ITCPPPMSVEHADIWVKSYSLY<br>SRERYICNSGFKRKAGTSSLTEC<br>VLNKATNVAHWTTPSLKCIRD<br>PALVHQRPAPP |

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|---|---|---|---|---|---|
| | | | | | ITCPPPMSVEHADIWVKSYSLY |
| | | | | | SRERYICNSGFKRKAGTSSLTEC |
| DNA524 | Hole: hFc(N297A)-hCD122-[VPLSLY]-hCD215(Sushi)-hIL15 | GGGSSP | VPLSLY | SGGP | VLNKATNVAHWTTPSLKCIRD |
| | | | | | PALVHQRPAPP |
| | | | | | ITCPPPMSVEHADIWVKSYSLY |
| | | | | | SRERYICNSGFKRKAGTSSLTEC |
| DNA525 | Hole: hFc(N297A)-hCD122-[VPLSLY]-hCD215(Sushi)-hIL15 | SGSGGSP | VPLSLY | SGGP | VLNKATNVAHWTTPSLKCIRD |
| | | | | | PALVHQRPAPP |

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|---|---|---|---|---|---|---|
| | | | | | | ITCPPPMSVEHADIWVKSYSLY |
| | | | | | | SRERYICNSGFKRKAGTSSLTEC |
| | | | NWVNVISDLKKIEDLIQSMHIDATLY | | | VLNKATNVAHWTTPSLKCIRD |
| | | | TESDVHPSCKVTAMKCFLLELQVISLE | | | PALVHQRPAPPSTVTTAGVTP |
| DNA526 | Knob: hFc(N297A)-[VPLSLY]-hIL15-hCD215 | SGPSGSPG | SGDASIHDTVENLILLANNSLSSNGNV | GGSGGGSGGGS | | QPESLSPSGKEPAASSPSSNNT |
| | | | TESGCKECEELEEKNIKEFLQSFVHIV | GGGSGGGSG | | AATTAAIVPGSQLMPSKSPST |
| | | | QMFINTS | | | GTTEISSHESSHGTPSQTTAKN |
| | | | | | | WELTASASHQPPGVYPQGHS |
| | | | | | | DTT |
| | | | | | | ITCPPPMSVEHADIWVKSYSLY |
| | | | | | | SRERYICNSGFKRKAGTSSLTEC |
| | | | NWVNVISDLKKIEDLIQSMHIDATLY | | | VLNKATNVAHWTTPSLKCIRD |
| | | | TESDVHPSCKVTAMKCFLLELQVISLE | GGSGGGSGGGS | | PALVHQRPAPPSTVTTAGVTP |
| DNA527 | Knob: hFc(N297A)-[VPLSLY]-hIL15-hCD215 | SGPSGSPG | SGDASIHDTVENLILLANNSLSSNGNV | GGGSGGGSGGG | | QPESLSPSGKEPAASSPSSNNT |
| | | | TESGCKECEELEEKNIKEFLQSFVHIV | SG | | AATTAAIVPGSQLMPSKSPST |
| | | | QMFINTS | | | GTTEISSHESSHGTPSQTTAKN |
| | | | | | | WELTASASHQPPGVYPQGHS |
| | | | | | | DTT |

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|---|---|---|---|
| DNA556 | Knob: hFc(N297A)-[VPLSLY]-GSGPGSGS hIL15 | | NWVNVISDLKKIEDLIQSMHIDATLY<br>TESDVHPSCKVTAMKCFLLELQVISLE<br>SGDASIHDTVENLIILANNSLSSNGNV<br>TESGCKECEELEEKNIKEFLQSFVHIV<br>QMFINTS |
| DNA557 | Knob: hFc(N297A)-[VPLSLY]-GSGPGSGS hIL15(D22A) | | NWVNVISDLKKIEDLIQSMHIAATLY<br>TESDVHPSCKVTAMKCFLLELQVISLE<br>SGDASIHDTVENLIILANNSLSSNGNV<br>TESGCKECEELEEKNIKEFLQSFVHIV<br>QMFINTS |

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|---|---|---|---|
| | | | NWVNVISDLKKIEDLIQSMHIDATLY |
| | | | TESDVHPSCKVTAMKCFLLELQVISLE |
| DNA558 | Knob: hFc(N297A)-[VPLSLY]-hIL15(E46A) | GSGPGSGS | SGDASIHDTVENLIILANNSLSSNGNV |
| | | | TESGCKECEELEEKNIKEFLQSFVHIV |
| | | | QMFINTS |
| | | | NWVNVISDLKKIEDLIQSMHIDATLY |
| | | | TESDVHPSCKVTAMKCFLLELQVISLA |
| DNA559 | Knob: hFc(N297A)-[VPLSLY]-hIL15(E53A) | GSGPGSGS | SGDASIHDTVENLIILANNSLSSNGNV |
| | | | TESGCKECEELEEKNIKEFLQSFVHIV |
| | | | QMFINTS |

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|---|---|---|---|
| DNA560 | Knob: hFc(N297A)-[VPLSLY]-hIL15(E46A, E53A) | GSGPGSGS | NWVNVISDLKKIEDLIQSMHIDATLY<br>TESDVHPSCKVTAMKCFLLELQVISL<br>ASGDASIHDTVENLIILANNSLSSNGN<br>VTESGCKECEELEEKNIKEFLQSFVHIV<br>QMFINTS |
| DNA569 | Hole: hFc(N297A)-hCD122-hCD215(Sushi)-[VPLSLY]-hIL15 | GGGGSGGGGSG<br>GGGSGGGGS | ITCPPPMSVEHADIWVKSYSLYSRER<br>YICNSGFKRKAGTSSLTECVLNKATNV SPSPPGPGSPP<br>AHWTTPSLKCIRDPALVHQRPAPP VPLSLY |

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|---|---|---|---|
| DNA570 | Hole: hFc(N297A)-hCD122-hCD215(Sushi)-[VPLSLY]-hIL15 | GGGGSGGGGSG GGGSGGGGS | ITCPPPMSVEHADIWVKSYSLYSRER YICNSGFKRKAGTSSLTECVLNKATNV SSGGPGSGPPSG AHWTTPSLKCIRDPALVHQRPAPP VPLSLY |
| DNA571 | Hole: hFc(N297A)-hCD122-hCD215(Sushi)-[VPLSLY]-hIL15 | GGGGSGGGGSG GGGSGGGGS | ITCPPPMSVEHADIWVKSYSLYSRER YICNSGFKRKAGTSSLTECVLNKATNV SSSGSSPGGPSG AHWTTPSLKCIRDPALVHQRPAPP PP VPLSLY |

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|---|---|---|---|---|
| DNA572 | Hole: hFc(N297A)-hCD122-hCD215(1to66)-[VPLSLY]-hIL15 | GGGGSGGGGSG GGGSGGGGS | ITCPPPMSVEHADIWVKSYSLYSRER YICNSGFKRKAGTSSLTECVLNKATNV GGSSPP AHWTTPSLKCIRD | VPLSLY |
| DNA573 | Hole: hFc(N297A)-hCD122-hCD215(1to66)-[VPLSLY]-hIL15 | GGGGSGGGGSG GGGSGGGGS | ITCPPPMSVEHADIWVKSYSLYSRER YICNSGFKRKAGTSSLTECVLNKATNV SPSSGSG AHWTTPSLKCIRD | VPLSLY |

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|---|---|---|---|---|
| DNA574 | Hole: hFc(N297A)-hCD122-hCD215(1to66)-[VPLSLY]-hIL15 | GGGGSGGGGSG GGGSGGGGS | ITCPPPMSVEHADIWVKSYSLYSRER YICNSGFKRKAGTSSLTECVLNKATNV GPPSGSSP AHWTTPSLKCIRD | VPLSLY |
| DNA595 | hCD215(1to66) | | | |

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|---|---|---|---|
| DNA596 | Knob: hFc(N297A)-hIL15(E46A, E53A) | | NWVNVISDLKKIEDLIQSMHIAATLY<br>TESDVHPSCKVTAMKCFLLALQVISL<br>ASGDASIHDTVENLIILANNSLSSNGN<br>VTESGCKECEELEEKNIKEFLQSFVHIV<br>QMFINTS |
| DNA561 | Knob: hFc(N297A)-[VPLSLY]-hIL15(D22A, E46A, E53A) | GSGPGSGS | |

New DNA1 Knob: hFc(N297A)-[VPLSLY]-GSGPGSGS hIL15

NWVNVISDLKKIEDLIQSMHIDATLY
TESDVHPSCKVTAMKCFLLELQVISLE
SGDASIHDTVENLIILANNSLSSNGNV
TESGCKECEELEEKNIKEFLQSFVHIV
QMFINTS

New DNA2 Knob: hFc(N297A)-[VPLSLY]-GSGPGSGS hIL15

NWVNVISDLKKIEDLIQSMHIDATLY
TESDVHPSCKVTAMKCFLLELQVISLE
SGDASIHDTVENLIILANNSLSSNGNV
TESGCKECEELEEKNIKEFLQSFVHIV
QMFINTS

| | | | | | |
|---|---|---|---|---|---|
| New DNA3 | Knob: hFc(N297A)-[VPLSLY]-hIL15 | GSGPGSGS | | | NWVNVISDLKKIEDLIQSMHIDATLY<br>TESDVHPSCKVTAMKCFLLELQVISLE<br>SGDASIHDTVENLIILANNSLSSNGNV<br>TESGCKECEELEEKNIKEFLQSFVHIV<br>QMFINTS |
| New DNA7 | Knob: hFc(N297A)-hCD215(1-66)-[VPLSLY]-hIL15 | GGSSPP | VPLSLY | SGG | NWVNVISDLKKIEDLIQSMHI<br>DATLYTESDVHPSCKVTAMKC<br>FLLELQVISLESGDASIHDTVEN<br>LIILANNSLSSNGNVTESGCKE<br>CEELEEKNIKEFLQSFVHIVQM<br>FINTS |

| | | | | | |
|---|---|---|---|---|---|
| | | | | | NWVNVISDLKKIEDLIQSMHI |
| | | | | | DATLYTESDVHPSCKVTAMKC |
| | | | | | FLLELQVISLESGDASIHDTVEN |
| New DNA8 | Knob: hFc(N297A)-hCD215(1-66)-[VPLSLY]-hIL15 | SPSSGSG | VPLSLY | SGG | LIILANNSLSSNGNVTESGCKE |
| | | | | | CEELEEKNIKEFLQSFVHIVQM |
| | | | | | FINTS |
| | | | | | NWVNVISDLKKIEDLIQSMHI |
| | | | | | DATLYTESDVHPSCKVTAMKC |
| | | | | | FLLELQVISLESGDASIHDTVEN |
| New DNA9 | Knob: hFc(N297A)-hCD215(1-66)-[VPLSLY]-hIL15 | GPPSGSSP | VPLSLY | SGG | LIILANNSLSSNGNVTESGCKE |
| | | | | | CEELEEKNIKEFLQSFVHIVQM |
| | | | | | FINTS |

New DNA10 Hole: hFc(N297A)-hCD122

New DNA11 Hole: hFc(N297A)-hCD122

| | | | | | |
|---|---|---|---|---|---|
| | | | | | NWVNVISDLKKIEDLIQSMHI |
| | | | | | DATLYTESDVHPSCKVTAMKC |
| | | | | | FLLELQVISLESGDASIHDTVEN |
| New DNA12 | Knob: hFc(N297A)-hCD215(1-66; R26N)-[VPLSLY]-hIL15 | GGSSPP | VPLSLY | SGG | LIILANNSLSSNGNVTESGCKE |
| | | | | | CEELEEKNIKEFLQSFVHIVQM |
| | | | | | FINTS |
| | | | | | NWVNVISDLKKIEDLIQSMHI |
| | | | | | DATLYTESDVHPSCKVTAMKC |
| | | | | | FLLELQVISLESGDASIHDTVEN |
| New DNA13 | Knob: hFc(N297A)-hCD215(1-66; R35Q)-[VPLSLY]-hIL15 | GGSSPP | VPLSLY | SGG | LIILANNSLSSNGNVTESGCKE |
| | | | | | CEELEEKNIKEFLQSFVHIVQM |
| | | | | | FINTS |

| | | | | | |
|---|---|---|---|---|---|
| | | | | | NWVNVISDLKKIEDLIQSMHI |
| | | | | | DATLYTESDVHPSCKVTAMKC |
| | | | | | FLLELQVISLESGDASIHDTVEN |
| New DNA14 | Knob: hFc(N297A)-hCD215(1-66; R26S)-[VPLSLY]-hIL15 | GGSSPP | VPLSLY | SGG | LIILANNSLSSNGNVTESGCKE |
| | | | | | CEELEEKNIKEFLQSFVHIVQM |
| | | | | | FINTS |
| | | | | | NWVNVISDLKKIEDLIQSMHI |
| | | | | | DATLYTESDVHPSCKVTAMKC |
| | | | | | FLLELQVISLESGDASIHDTVEN |
| New DNA15 | Knob: hFc(N297A)-hCD215(1-66; R35S)-[VPLSLY]-hIL15 | GGSSPP | VPLSLY | SGG | LIILANNSLSSNGNVTESGCKE |
| | | | | | CEELEEKNIKEFLQSFVHIVQM |
| | | | | | FINTS |

| | | | | | |
|---|---|---|---|---|---|
| | | | | | NWVNVISDLKKIEDLIQSMHI |
| | | | | | DATLYTESDVHPSCKVTAMKC |
| | | | | | FLLELQVISLESGDASIHDTVEN |
| | | | | SGG | LIILANNSLSSNGNVTESGCKE |
| | | | | | CEELEEKNIKEFLQSFVHIVQM |
| | | | | | FINTS |
| New DNA16 | Knob: hFc(N297A)-hCD215(1-66; R26N, R35Q)-[VPLSLY]-hIL15 | GGSSPP | VPLSLY | | |

| name | newnames | Component8Sequence | Component9Sequence | Released post cleavage |
|---|---|---|---|---|
| DNA158 | Hole: hFc(N297A) | | | |
| DNA161 | Knob: hFc(N297A) | | | |

DNA187 Hole: hFc(N297A)-hCD122

DNA362 Knob: hFc(N297A)-[MPYDLYHP]-hIL15

LYHPSGPSGSPGNWVNVISDLKKIEDLIQSMHIDATLYT
ESDVHPSCKVTAMKCFLLELQVISLESGDASIHDTVENLII
LANNSLSSNGNVTESGCKECEELEEKNIKEFLQSFVHIVQ
MFINTS

LYHPSGPGSGSNWVNVISDLKKIEDLIQSMHIDATLYTE
SDVHPSCKVTAMKCFLLELQVISLESGDASIHDTVENLIIL
ANNSLSSNGNVTESGCKECEELEEKNIKEFLQSFVHIVQ
MFINTS

DNA363 Knob: hFc(N297A)-[MPYDLYHP]-hIL15

DNA364 Knob: hFc(N297A)-hIL15

| | | |
|---|---|---|
| | | LYHPSGPSGSPGNWVNVISDLKKIEDLIQSMHIDATLYT |
| | | ESDVHPSCKVTAMKCFLLELQVISLESGDASIHDTVENLII |
| | | LANNSLSSNGNVTESGCKECEELEEKNIKEFLQSFVHIVQ |
| | | MFINTSGGGGSGGGGSGGGGSGGGGSITCPPPMSVE |
| | | HADIWVKSYSLYSRERYICNSGFKRKAGTSSLTECVLNKA |
| | | TNVAHWTTPSLKCIRDPALVHQRPAPPSTVTTAGVTPQ |
| | | PESLSPSGKEPAASSPSSNNTAATTAAIVPGSQLMPSKS |
| | | PSTGTTEISSHESSHGTPSQTTAKNWELTASASHQPPGV |
| | | YPQGHSDTT |
| DNA365 | Knob: hFc(N297A)-[MPYDLYHP]-hIL15-hCD215 | |
| | | LYHPSGPSGSPGNWVNVISDLKKIEDLIQSMHIDATLYT |
| | | ESDVHPSCKVTAMKCFLLELQVISLESGDASIHDTVENLII |
| | | LANNSLSSNGNVTESGCKECEELEEKNIKEFLQSFVHIVQ |
| | | MFINTSGGGGSGGGGSGGGGSGGGGSITCPPPMSVE |
| | | HADIWVKSYSLYSRERYICNSGFKRKAGTSSLTECVLNKA |
| | | TNVAHWTTPSLKCIRDPALVHQRPAPPSTVTTAGVTPQ |
| | | PESLSPSGKEPAAS |
| DNA366 | Knob: hFc(N297A)-[MPYDLYHP]-hIL15-hCD215(Truncated) | |

DNA367 Knob: hFc(N297A)-hIL15

DNA368 Knob: hFc(N297A)-[MPYDLYHP]-hIL15-hCD215

LYHPSGPGSGSNWVNVISDLKKIEDLIQSMHIDATLYTE
SDVHPSCKVTAMKCFLLELQVISLESGDASIHDTVENLIIL
ANNSLSSNGNVTESGCKECEELEEKNIKEFLQSFVHIVQ
MFINTSGGGSGGGGSGGGGSGGGGSITCPPPMSVE
HADIWVKSYSLYSRERYICNSGFKRKAGTSSLTECVLNKA
TNVAHWTTPSLKCIRDPALVHQRPAPPSTVTTAGVTPQ
PESLSPSGKEPAASSPSSNNTAATTAAIVPGSQLMPSKS
PSTGTTEISSHESSHGTPSQTTAKNWELTASASHQPPGV
YPQGHSDTT

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|---|---|---|
| DNA369 | Knob: hFc(N297A)-[MPYDLYHP]-hIL15-hCD215(Truncated) | LYHPSGPGSGSNWVNVISDLKKIEDLIQSMHIDATLYTE<br>SDVHPSCKVTAMKCFLLELQVISLESGDASIHDTVENLIIL<br>ANNSLSSNGNVTESGCKECEELEEKNIKEFLQSFVHIVQ<br>MFINTSGGGGSGGGGSGGGGSGGGGSITCPPPMSVE<br>HADIWVKSYSLYSRERYICNSGFKRKAGTSSLTECVLNKA<br>TNVAHWTTPSLKCIRDPALVHQRPAPPSTVTTAGVTPQ<br>PESLSPSGKEPAAS |
| DNA370 | Hole: hFc(N297A)-[MPYDLYHP]-hCD122 | LYHPSGGGAVNGTSQFTCFYNSRANISCVWSQDGALQ<br>DTSCQVHAWPDRRRWNQTCELLPVSQASWACNLILG<br>APDSQKLTTVDIVTLRVLCREGVRWRVMAIQDFKPFEN<br>LRLMAPISLQVVHVETHRCNISWEISQASHYFERHLEFE<br>ARTLSPGHTWEEAPLLTLKQKQEWICLETLTPDTQYEFQ<br>VRVKPLQGEFTTWSPWSQPLAFRTKPAALGKD |

| | | |
|---|---|---|
| | | LYSGPGSGSNWVNVISDLKKIEDLIQSMHIDATLYTESD |
| | | VHPSCKVTAMKCFLLELQVISLESGDASIHDTVENLIILA |
| | | NNSLSSNGNVTESGCKECEELEEKNIKEFLQSFVHIVQM |
| | | FINTS |
| DNA487 | Knob: hFc(N297A)-[VPLSLY]-hIL15 | |
| | | LYSGPGSGSNWVNVISDLKKIEDLIQSMHIDATLYTESD |
| | | VHPSCKVTAMKCFLLELQVISLESGDASIHDTVENLIILA |
| | | NNSLSSNGNVTESGCKECEELEEKNIKEFLQSFVHIVQM |
| | | FINTSGGGGSGGGGSGGGGSGGGGSGGGGSITCPPP |
| | | MSVEHADIWVKSYSLYSRERYICNSGFKRKAGTSSLTEC |
| | | VLNKATNVAHWTTPSLKCIRDPALVHQRPAPPSTVTTA |
| | | GVTPQPESLSPSGKEPAASSPSSNNTAATTAAIVPGSQL |
| | | MPSKSPSTGTTEISSHESSHGTPSQTTAKNWELTASASH |
| | | QPPGVYPQGHSDTT |
| DNA517 | Knob: hFc(N297A)-[VPLSLY]-hIL15-hCD215 | |

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|---|---|---|---|
| | | | LYSGPGSGSNWVNVISDLKKIEDLIQSMHIDATLYTESD |
| | | | VHPSCKVTAMKCFLLELQVISLESGDASIHDTVENLIILA |
| | | | NNSLSSNGNVTESGCKECEELEEKNIKEFLQSFVHIVQM |
| DNA518 | Knob: hFc(N297A)-[VPLSLY]-hIL15-hCD215(Truncated) | | FINTSGGGGSGGGGSGGGGSGGGGSGGGGSITCPPP |
| | | | MSVEHADIWVKSYSLYSRERYICNSGFKRKAGTSSLTEC |
| | | | VLNKATNVAHWTTPSLKCIRDPALVHQRPAPPSTVTTA |
| | | | GVTPQPESLSPSGKEPAAS |
| | | | NWVNVISDLKKIED |
| | | | LIQSMHIDATLYTES LYSGGPITCPPPMSVEHADIWVKSYSLYSRERYICNSGFK |
| | | | DVHPSCKVTAMKCF RKAGTSSLTECVLNKATNVAHWTTPSLKCIRDPALVHQ |
| | Hole: hFc(N297A)-hCD122-[VPLSLY]-hCD215(Sushi)-hIL15 | SGGGGSGGGGSGGGGS | LLELQVISLESGDASI RPAPPSGGGGSGGGGSGGGGSGGGGSNWVNVISDLK |
| DNA523 | | GGGGS | HDTVENLIILANNSL KIEDLIQSMHIDATLYTESDVHPSCKVTAMKCFLLELQVI |
| | | | SSNGNVTESGCKEC SLESGDASIHDTVENLIILANNSLSSNGNVTESGCKECEE |
| | | | EELEEKNIKEFLQSFV LEEKNIKEFLQSFVHIVQMFINTS |
| | | | HIVQMFINTS |

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|---|---|---|---|
| DNA524 | Hole: hFc(N297A)-hCD122-[VPLSLY]-hCD215(Sushi)-hIL15 | SGGGGSGGGGSGGGGS GGGGS | NWVNVISDLKKIED<br>LIQSMHIDATLYTES LYSGGPITCPPPMSVFHADIWVKSYSLYSRERYICNSGFK<br>DVHPSCKVTAMKCF RKAGTSSLTECVLNKATNVAHWTTPSLKCIRDPALVHQ<br>LLELQVISLESGDASI RPAPPSGGGGSGGGGSGGGGSGGGGSNWVNVISDLK<br>HDTVENLIILANNSL KIEDLIQSMHIDATLYTESDVHPSCKVTAMKCFLLELQVI<br>SSNGNVTESGCKEC SLESGDASIHDTVENLIILANNSLSSNGNVTESGCKECEE<br>EELEEKNIKEFLQSFV LEEKNIKEFLQSFVHIVQMFINTS<br>HIVQMFINTS |
| DNA525 | Hole: hFc(N297A)-hCD122-[VPLSLY]-hCD215(Sushi)-hIL15 | SGGGGSGGGGSGGGGS GGGGS | NWVNVISDLKKIED<br>LIQSMHIDATLYTES LYSGGPITCPPPMSVFHADIWVKSYSLYSRERYICNSGFK<br>DVHPSCKVTAMKCF RKAGTSSLTECVLNKATNVAHWTTPSLKCIRDPALVHQ<br>LLELQVISLESGDASI RPAPPSGGGGSGGGGSGGGGSGGGGSNWVNVISDLK<br>HDTVENLIILANNSL KIEDLIQSMHIDATLYTESDVHPSCKVTAMKCFLLELQVI<br>SSNGNVTESGCKEC SLESGDASIHDTVENLIILANNSLSSNGNVTESGCKECEE<br>EELEEKNIKEFLQSFV LEEKNIKEFLQSFVHIVQMFINTS<br>HIVQMFINTS |

LYSGPSGSPGNWVNVISDLKKIEDLIQSMHIDATLYTES
DVHPSCKVTAMKCFLLELQVISLESGDASIHDTVENLIILA
NNSLSSNGNVTESGCKECEELEEKNIKEFLQSFVHIVQM
FINTSGGSGGGSGGGSGGGSGGGSGITCPPPMSVEHA
DIWVKSYSLYSRERYICNSGFKRKAGTSSLTECVLNKATN
VAHWTTPSLKCIRDPALVHQRPAPPSTVTTAGVTPQPE
SLSPSGKEPAASSPSSNNTAATTAAIVPGSQLMPSKSPS
TGTTEISSHESSHGTPSQTTAKNWELTASASHQPPGVYP
QGHSDTT

DNA526 Knob: hFc(N297A)-[VPLSLY]-hIL15-hCD215

LYSGPSGSPGNWVNVISDLKKIEDLIQSMHIDATLYTES
DVHPSCKVTAMKCFLLELQVISLESGDASIHDTVENLIILA
NNSLSSNGNVTESGCKECEELEEKNIKEFLQSFVHIVQM
FINTSGGSGGGSGGGSGGGSGGGSGGGSGITCPPPMS
VEHADIWVKSYSLYSRERYICNSGFKRKAGTSSLTECVLN
KATNVAHWTTPSLKCIRDPALVHQRPAPPSTVTTAGVT
PQPESLSPSGKEPAASSPSSNNTAATTAAIVPGSQLMPS
KSPSTGTTEISSHESSHGTPSQTTAKNWELTASASHQPP
GVYPQGHSDTT

DNA527 Knob: hFc(N297A)-[VPLSLY]-hIL15-hCD215

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|---|---|---|
| DNA556 | Knob: hFc(N297A)-[VPLSLY]-hIL15 | LYGSGPGSGSNWVNVISDLKKIEDLIQSMHIDATLYTES<br>DVHPSCKVTAMKCFLLELQVISLESGDASIHDTVENLIILA<br>NNSLSSNGNVTESGCKECEELEEKNIKEFLQSFVHIVQM<br>FINTS |
| DNA557 | Knob: hFc(N297A)-[VPLSLY]-hIL15(D22A) | LYGSGPGSGSNWVNVISDLKKIEDLIQSMHIAATLYTES<br>DVHPSCKVTAMKCFLLELQVISLESGDASIHDTVENLIILA<br>NNSLSSNGNVTESGCKECEELEEKNIKEFLQSFVHIVQM<br>FINTS |

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| DNA558 | Knob: hFc(N297A)-[VPLSLY]-hIL15(E46A) | LYGSGPGSGSNWVNVISDLKKIEDLIQSMHIDATLYTES<br>DVHPSCKVTAMKCFLLALQVISLESGDASIHDTVENLIIL<br>ANNSLSSNGNVTESGCKECEELEEKNIKEFLQSFVHIVQ<br>MFINTS |
| DNA559 | Knob: hFc(N297A)-[VPLSLY]-hIL15(E53A) | LYGSGPGSGSNWVNVISDLKKIEDLIQSMHIDATLYTES<br>DVHPSCKVTAMKCFLLELQVISLASGDASIHDTVENLIIL<br>ANNSLSSNGNVTESGCKECEELEEKNIKEFLQSFVHIVQ<br>MFINTS |

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|---|---|---|---|
| | | | LYGSGPGSGSNWVNVISDLKKIEDLIQSMHIDATLYTES |
| | | | DVHPSCKVTAMKCFLLELQVISLESGDASIHDTVENLIIL |
| | | | ANNSLSSNGNVTESGCKECEELEEKNIKEFLQSFVHIVQ |
| | | | MFINTS |
| DNA560 | Knob: hFc(N297A)-[VPLSLY]-hIL15(E46A, E53A) | | |
| | | | NWVNVISDLKKIED |
| | | | LIQSMHIDATLYTES |
| | | | DVHPSCKVTAMKCF |
| | | | LLELQVISLESGDASI |
| | | | HDTVENLIILANNSL |
| | | | SSNGNVTESGCKEC |
| | | | EELEEKNIKEFLQSFV |
| | | | HIVQMFINTS |
| DNA569 | Hole: hFc(N297A)-hCD122-hCD215(Sushi)-[VPLSLY]-hIL15 | SGG | LYSGGNWVNVISDLKKIEDLIQSMHIDATLYTESDVHPS CKVTAMKCFLLELQVISLESGDASIHDTVENLIILANNSLS SNGNVTESGCKECEELEEKNIKEFLQSFVHIVQMFINTS |

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|---|---|---|---|
| | | | NWVNVISDLKKIED |
| | | | LIQSMHIDATLYTES |
| | | | DVHPSCKVTAMKCF |
| | Hole: hFc(N297A)-hCD122- | | LLELQVISLESGDASI |
| DNA570 | hCD215(Sushi)-[VPLSLY]- | SGG | HDTVENLIILANNSL |
| | hIL15 | | SSNGNVTESGCKEC |
| | | | EELEEKNIKEFLQSFV |
| | | | HIVQMFINTS |

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|---|---|---|---|
| | | | LYSGGNWVNVISDLKKIEDLIQSMHIDATLYTESDVHPS |
| | | | CKVTAMKCFLLELQVISLESGDASIHDTVENLIILANNSLS |
| | | | SNGNVTESGCKECECELEEKNIKEFLQSFVHIVQMFINTS |

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|---|---|---|---|
| | | | NWVNVISDLKKIED |
| | | | LIQSMHIDATLYTES |
| | | | DVHPSCKVTAMKCF |
| | Hole: hFc(N297A)-hCD122- | | LLELQVISLESGDASI |
| DNA571 | hCD215(Sushi)-[VPLSLY]- | SGG | HDTVENLIILANNSL |
| | hIL15 | | SSNGNVTESGCKEC |
| | | | EELEEKNIKEFLQSFV |
| | | | HIVQMFINTS |

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|---|---|---|---|
| | | | LYSGGNWVNVISDLKKIEDLIQSMHIDATLYTESDVHPS |
| | | | CKVTAMKCFLLELQVISLESGDASIHDTVENLIILANNSLS |
| | | | SNGNVTESGCKECECELEEKNIKEFLQSFVHIVQMFINTS |

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|---|---|---|---|
| | | | NWVNVISDLKKIED |
| | | | LIQSMHIDATLYTES |
| | | | DVHPSCKVTAMKCF |
| | Hole: hFc(N297A)-hCD122- | | LLELQVISLESGDASI |
| DNA572 | hCD215(1to66)-[VPLSLY]- | SGG | HDTVENLIILANNSL |
| | hIL15 | | SSNGNVTESGCKEC |
| | | | EELEEKNIKEFLQSFV |
| | | | HIVQMFINTS |

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|---|---|---|---|
| | | | LYSGGNWVNVISDLKKIEDLIQSMHIDATLYTESDVHPS |
| | | | CKVTAMKCFLLELQVISLESGDASIHDTVENLIILANNSLS |
| | | | SNGNVTESGCKECEELEEKNIKEFLQSFVHIVQMFINTS |
| | | | NWVNVISDLKKIED |
| | | | LIQSMHIDATLYTES |
| | | | DVHPSCKVTAMKCF |
| | Hole: hFc(N297A)-hCD122- | | LLELQVISLESGDASI |
| DNA573 | hCD215(1to66)-[VPLSLY]- | SGG | HDTVENLIILANNSL |
| | hIL15 | | SSNGNVTESGCKEC |
| | | | EELEEKNIKEFLQSFV |
| | | | HIVQMFINTS |

NWVNVISDLKKIED
LIQSMHIDATLYTES
DVHPSCKVTAMKCF LYSGGNWVNVISDLKKIEDLIQSMHIDATLYTESDVHPS
LLELQVISLESGDASI CKVTAMKCFLLELQVISLESGDASIHDTVENLIILANNSLS
HDTVENLIILANNSL SNGNVTESGCKECEELEEKNIKEFLQSFVHIVQMFINTS
SSNGNVTESGCKEC
EELEEKNIKEFLQSFV
HIVQMFINTS

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|---|---|---|
| DNA574 | Hole: hFc(N297A)-hCD122-hCD215(1to66)-[VPLSLY]-hIL15 | SGG |
| DNA595 | hCD215(1to66) | |

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| DNA596 | Knob: hFc(N297A)-hIL15(E46A, E53A) | LYGSGPGSGSNWVNVISDLKKIEDLIQSMHIAATLYTES<br>DVHPSCKVTAMKCFLLALQVISLASGDASIHDTVENLIIL<br>ANNSLSSNGNVTESGCKECEELEEKNIKEFLQSFVHIVQ<br>MFINTS |
| DNA561 | Knob: hFc(N297A)-[VPLSLY]-hIL15(D22A, E46A, E53A) | |

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| New DNA1 | Knob: hFc(N297A)-[VPLSLY]-hIL15 | LYGSGPGSGSNWVNVISDLKKIEDLIQSMHIDATLYTES<br>DVHPSCKVTAMKCFLLELQVISLESGDASIHDTVENLIILA<br>NNSLSSNGNVTESGCKECEELEEKNIKEFLQSFVHIVQM<br>FINTS |
| New DNA2 | Knob: hFc(N297A)-[VPLSLY]-hIL15 | LYGSGPGSGSNWVNVISDLKKIEDLIQSMHIDATLYTES<br>DVHPSCKVTAMKCFLLELQVISLESGDASIHDTVENLIILA<br>NNSLSSNGNVTESGCKECEELEEKNIKEFLQSFVHIVQM<br>FINTS |

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| New DNA3 | Knob: hFc(N297A)-[VPLSLY]-hIL15 | LYGSGPGSGSNWVNVISDLKKIEDLIQSMHIDATLYTES<br>DVHPSCKVTAMKCFLLELQVISLESGDASIHDTVENLIILA<br>NNSLSSNGNVTESGCKECEELEEKNIKEFLQSFVHIVQM<br>FINTS |
| New DNA7 | Knob: hFc(N297A)-hCD215(1-66)-[VPLSLY]-hIL15 | LYSGGNWVNVISDLKKIEDLIQSMHIDATLYTESDVHPS<br>CKVTAMKCFLLELQVISLESGDASIHDTVENLIILANNSLS<br>SNGNVTESGCKECEELEEKNIKEFLQSFVHIVQMFINTS |

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| New DNA8 | Knob: hFc(N297A)-hCD215(1-66)-[VPLSLY]-hIL15 | LYSGGNWVNVISDLKKIEDLIQSMHIDATLYTESDVHPS<br>CKVTAMKCFLLELQVISLESGDASIHDTVENLIILANNSLS<br>SNGNVTESGCKECEELEEKNIKEFLQSFVHIVQMFINTS |

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|---|---|---|
| New DNA9 | Knob: hFc(N297A)-hCD215(1-66)-[VPLSLY]-hIL15 | LYSGGNWVNVISDLKKIEDLIQSMHIDATLYTESDVHPS<br>CKVTAMKCFLLELQVISLESGDASIHDTVENLIILANNSLS<br>SNGNVTESGCKECEELEEKNIKEFLQSFVHIVQMFINTS |

New DNA10 Hole: hFc(N297A)-hCD122

New DNA11 Hole: hFc(N297A)-hCD122

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| New DNA12 | Knob: hFc(N297A)-hCD215(1-66; R26N)-[VPLSLY]-hIL15 | LYSGGNWVNVISDLKKIEDLIQSMHIDATLYTESDVHPS<br>CKVTAMKCFLLELQVISLESGDASIHDTVENLIILANNSLS<br>SNGNVTESGCKECEELEEKNIKEFLQSFVHIVQMFINTS |
| New DNA13 | Knob: hFc(N297A)-hCD215(1-66; R35Q)-[VPLSLY]-hIL15 | LYSGGNWVNVISDLKKIEDLIQSMHIDATLYTESDVHPS<br>CKVTAMKCFLLELQVISLESGDASIHDTVENLIILANNSLS<br>SNGNVTESGCKECEELEEKNIKEFLQSFVHIVQMFINTS |

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| New DNA14 | Knob: hFc(N297A)-hCD215(1-66; R26S)-[VPLSLY]-hIL15 | LYSGGNWVNVISDLKKIEDLIQSMHIDATLYTESDVHPS<br>CKVTAMKCFLLELQVISLESGDASIHDTVENLIILANNSLS<br>SNGNVTESGCKECEELEEKNIKEFLQSFVHIVQMFINTS |
| New DNA15 | Knob: hFc(N297A)-hCD215(1-66; R35S)-[VPLSLY]-hIL15 | LYSGGNWVNVISDLKKIEDLIQSMHIDATLYTESDVHPS<br>CKVTAMKCFLLELQVISLESGDASIHDTVENLIILANNSLS<br>SNGNVTESGCKECEELEEKNIKEFLQSFVHIVQMFINTS |

LYSGGNWVNVISDLKKIEDLIQSMHIDATLYTESDVHPS
CKVTAMKCFLLELQVISLESGDASIHDTVENLIILANNSLS
SNGNVTESGCKECEELEEKNIKEFLQSFVHIVQMFINTS

Knob: hFc(N297A)-
New DNA16 hCD215(1-66; R26N, R35Q)-
[VPLSLY]-hIL15

| name | newnames | FullSequence |
|---|---|---|
| DNA158 | Hole: hFc(N297A) | DKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYASTYR<br>VVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVCTLPPSRDELTKNQVSLSCAVKGFYPSDIAVEWE<br>SNGQPENNYKTTPPVLDSDGSFFLVSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGK |
| DNA161 | Knob: hFc(N297A) | DKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYASTYR<br>VVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPCRDELTKNQVSLWCLVKGFYPSDIAVEWE<br>SNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGK |

| | | |
|---|---|---|
| | | DKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYASTYR |
| | | VVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVCTLPPSRDELTKNQVSLSCAVKGFYPSDIAVEWE |
| DNA187 | Hole: hFc(N297A)-hCD122 | SNGQPENNYKTTPPVLDSDGSFFLVSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGPGSGSAVNGTSQFTC |
| | | FYNSRANISCVWSQDGALQDTSCQVHAWPDRRRWNQTCELLPVSQASWACNLILGAPDSQKLTTVDIVTLRVLCREGV |
| | | RWRVMAIQDFKPFENLRLMAPISLQVVHVETHRCNISWEISQASHYFERHLEFEARTLSPGHTWEEAPLLTLKQKQEWICL |
| | | ETLTPDTQYEFQVRVKPLQGEFTTWSPWSQPLAFRTKPAALGKD |
| | | |
| | | DKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYASTYR |
| | Knob: hFc(N297A)-[MPYDLYHP]-hIL15 | VVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPCRDELTKNQVSLWCLVKGFYPSDIAVEWE |
| DNA362 | | SNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGGGSSPPMPYDLYHPS |
| | | GPSGSPGNWVNVISDLKKIEDLIQSMHIDATLYTESDVHPSCKVTAMKCFLLELQVISLESGDASIHDTVENLIILANNSLSS |
| | | NGNVTESGCKECEELEEKNIKEFLQSFVHIVQMFINTS |

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| DNA363 | Knob: hFc(N297A)-[MPYDLYHP]-hIL15 | DKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYASTYR<br>VVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPCRDELTKNQVSLWCLVKGFYPSDIAVEWE<br>SNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGGGSSPPMPYDLYHPS<br>GPGSGSNWVNVISDLKKIEDLIQSMHIDATLYTESDVHPSCKVTAMKCFLLELQVISLESGDASIHDTVENLIILANNSLSSN<br>GNVTESGCKECEELEEKNIKEFLQSFVHIVQMFINTS |
| DNA364 | Knob: hFc(N297A)-hIL15 | DKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYASTYR<br>VVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPCRDELTKNQVSLWCLVKGFYPSDIAVEWE<br>SNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGGGSSPPGGGSSGGGS<br>GPSGSPGNWVNVISDLKKIEDLIQSMHIDATLYTESDVHPSCKVTAMKCFLLELQVISLESGDASIHDTVENLIILANNSLSS<br>NGNVTESGCKECEELEEKNIKEFLQSFVHIVQMFINTS |

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|---|---|---|
| DNA365 | Knob: hFc(N297A)-[MPYDLYHP]-hIL15-hCD215 | DKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYASTYR<br>VVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPCRDELTKNQVSLWCLVKGFYPSDIAVEWE<br>SNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGGGSSPPMPYDLYHPS<br>GPSGSPGNWVNVISDLKKIEDLIQSMHIDATLYTESDVHPSCKVTAMKCFLLELQVISLESGDASIHDTVENLIILANNSLSS<br>NGNVTESGCKECEELEEKNIKEFLQSFVHIVQMFINTSGGGSGGGGSGGGGSGGGGSITCPPPMSVEHADIWVKSYSLY<br>SRERYICNSGFKRKAGTSSLTECVLNKATNVAHWTTPSLKCIRDPALVHQRPAPPSTVTTAGVTPQPESLSPSGKEPAASSP<br>SSNNTAATTAAIVPGSQLMPSKSPSTGTTEISSHESSHGTPSQTTAKNWELTASASHQPPGVYPQGHSDTT |
| DNA366 | Knob: hFc(N297A)-[MPYDLYHP]-hIL15-hCD215(Truncated) | DKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYASTYR<br>VVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPCRDELTKNQVSLWCLVKGFYPSDIAVEWE<br>SNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGGGSSPPMPYDLYHPS<br>GPSGSPGNWVNVISDLKKIEDLIQSMHIDATLYTESDVHPSCKVTAMKCFLLELQVISLESGDASIHDTVENLIILANNSLSS<br>NGNVTESGCKECEELEEKNIKEFLQSFVHIVQMFINTSGGGSGGGGSGGGGSGGGGSITCPPPMSVEHADIWVKSYSLY<br>SRERYICNSGFKRKAGTSSLTECVLNKATNVAHWTTPSLKCIRDPALVHQRPAPPSTVTTAGVTPQPESLSPSGKEPAAS |

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| | | DKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYASTYR |
| | | VVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPCRDELTKNQVSLWCLVKGFYPSDIAVEWE |
| DNA367 | Knob: hFc(N297A)-hIL15 | SNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGGGSSPPGGGSSGGGS |
| | | GPGSGSNWVNVISDLKKIEDLIQSMHIDATLYTESDVHPSCKVTAMKCFLLELQVISLESGDASIHDTVENLIILANNSLSSN |
| | | GNVTESGCKECEELEEKNIKEFLQSFVHIVQMFINTS |
| | | |
| | | DKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYASTYR |
| | | VVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPCRDELTKNQVSLWCLVKGFYPSDIAVEWE |
| | | SNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGGGSSPPMPYDLYHPS |
| DNA368 | Knob: hFc(N297A)-[MPYDLYHP]-hIL15-hCD215 | GPGSGSNWVNVISDLKKIEDLIQSMHIDATLYTESDVHPSCKVTAMKCFLLELQVISLESGDASIHDTVENLIILANNSLSSN |
| | | GNVTESGCKECEELEEKNIKEFLQSFVHIVQMFINTSGGGGSGGGGSGGGGSGGGGSITCPPPMSVEHADIWVKSYSLYS |
| | | RERYICNSGFKRKAGTSSLTECVLNKATNVAHWTTPSLKCIRDPALVHQRPAPPSTVTTAGVTPQPESLSPSGKEPAASSPS |
| | | SNNTAATTAAIVPGSQLMPSKSPSTGTTEISSHESSHGTPSQTTAKNWELTASASHQPPGVYPQGHSDTT |

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| DNA369 | Knob: hFc(N297A)-[MPYDLYHP]-hIL15-hCD215(Truncated) | DKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYASTYR<br>VVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPCRDELTKNQVSLWCLVKGFYPSDIAVEWE<br>SNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGGGSSPPMPYDLYHPS<br>GPGSGSNWVNVISDLKKIEDLIQSMHIDATLYTESDVHPSCKVTAMKCFLLELQVISLESGDASIHDTVENLIILANNSLSSN<br>GNVTESGCKECEELEEKNIKEFLQSFVHIVQMFINTSGGGGSGGGGSGGGGSGGGGSITCPPPMSVEHADIWVKSYSLYS<br>RERYICNSGFKRKAGTSSLTECVLNKATNVAHWTTPSLKCIRDPALVHQRPAPPSTVTTAGVTPQPESLSPSGKEPAAS |
| DNA370 | Hole: hFc(N297A)-[MPYDLYHP]-hCD122 | DKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYASTYR<br>VVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVCTLPPSRDELTKNQVSLSCAVKGFYPSDIAVEWE<br>SNGQPENNYKTTPPVLDSDGSFFLVSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGGPPSGSSPMPYDLYH<br>PSGGGAVNGTSQFTCFYNSRANISCVWSQDGALQDTSCQVHAWPDRRRWNQTCELLPVSQASWACNLILGAPDSQKL<br>TTVDIVTLRVLCREGVRWRVMAIQDFKPFENLRLMAPISLQVVHVETHRCNISWEISQASHYFERHLEFEARTLSPGHTWE<br>EAPLLTLKQKQEWICLETLTPDTQYEFQVRVKPLQGEFTTWSPWSQPLAFRTKPAALGKD |

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| DNA487 | Knob: hFc(N297A)-[VPLSLY]-hIL15 | DKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYASTYR<br>VVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPCRDELTKNQVSLWCLVKGFYPSDIAVEWE<br>SNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGGGSSPPVPLSLYSGPG<br>SGSNWVNVISDLKKIEDLIQSMHIDATLYTESDVHPSCKVTAMKCFLLELQVISLESGDASIHDTVENLIILANNSLSSNGNV<br>TESGCKECEELEEKNIKEFLQSFVHIVQMFINTS |
| DNA517 | Knob: hFc(N297A)-[VPLSLY]-hIL15-hCD215 | DKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYASTYR<br>VVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPCRDELTKNQVSLWCLVKGFYPSDIAVEWE<br>SNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGGGSSPPVPLSLYSGPG<br>SGSNWVNVISDLKKIEDLIQSMHIDATLYTESDVHPSCKVTAMKCFLLELQVISLESGDASIHDTVENLIILANNSLSSNGNV<br>TESGCKECEELEEKNIKEFLQSFVHIVQMFINTSGGGGSGGGGSGGGGSGGGGSITCPPPMSVEHADIWVKSYSL<br>YSRERYICNSGFKRKAGTSSLTECVLNKATNVAHWTTPSLKCIRDPALVHQRPAPPSTVTTAGVTPQPESLSPSGKEPAASS<br>PSSNNTAATTAAIVPGSQLMPSKSPSTGTTEISSHESSHGTPSQTTAKNWELTASASHQPPGVYPQGHSDTT |

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| | | DKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYASTYR |
| | | VVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPCRDELTKNQVSLWCLVKGFYPSDIAVEWE |
| DNA518 | Knob: hFc(N297A)-[VPLSLY]-hIL15-hCD215(Truncated) | SNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGGGSSPPVPLSLYSGPG |
| | | SGSNWVNVISDLKKIEDLIQSMHIDATLYTESDVHPSCKVTAMKCFLLELQVISLESGDASIHDTVENLIILANNSLSSNGNV |
| | | TESGCKECEELEEKNIKEFLQSFVHIVQMFINTSGGGGSGGGGSGGGGSGGGGSITCPPPMSVEHADIWVKSYSL |
| | | YSRERYICNSGFKRKAGTSSLTECVLNKATNVAHWTTPSLKCIRDPALVHQRPAPPSTVTTAGVTPQPESLSPSGKEPAAS |
| | | |
| | | DKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYASTYR |
| | | VVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVCTLPPSRDELTKNQVSLSCAVKGFYPSDIAVEWE |
| | | SNGQPENNYKTTPPVLDSDGSFFLVSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGPGSGSAVNGTSQFTC |
| DNA523 | Hole: hFc(N297A)-hCD122-[VPLSLY]-hCD215(Sushi)-hIL15 | FYNSRANISCVWSQDGALQDTSCQVHAWPDRRRWNQTCELLPVSQASWACNLILGAPDSQKLTTVDIVTLRVLCREGV |
| | | RWRVMAIQDFKPFENLRLMAPISLQVVHVETHRCNISWEISQASHYFERHLEFEARTLSPGHTWEEAPLLTLKQKQEWICL |
| | | ETLTPDTQYEFQVRVKPLQGEFTTWSPWSQPLAFRTKPAALGKDPGGPSPVPLSLYSGGPITCPPPMSVEHADIWVKSYSL |
| | | YSRERYICNSGFKRKAGTSSLTECVLNKATNVAHWTTPSLKCIRDPALVHQRPAPPSGGGGSGGGGSGGGGSGGGGSN |
| | | WVNVISDLKKIEDLIQSMHIDATLYTESDVHPSCKVTAMKCFLLELQVISLESGDASIHDTVENLIILANNSLSSNGNVTESGC |
| | | KECEELEEKNIKEFLQSFVHIVQMFINTS |

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| | | DKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYASTYR |
| | | VVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVCTLPPSRDELTKNQVSLSCAVKGFYPSDIAVEWE |
| | | SNGQPENNYKTTPPVLDSDGSFFLVSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGPGSGSAVNGTSQFTC |
| | Hole: hFc(N297A)-hCD122-[VPLSLY]-hCD215(Sushi)-hIL15 | FYNSRANISCVWSQDGALQDTSCQVHAWPDRRRWNQTCELLPVSQASWACNLILGAPDSQKLTTVDIVTLRVLCREGV |
| DNA524 | | RWRVMAIQDFKPFENLRLMAPISLQVVHVETHRCNISWEISQASHYFERHLEFEARTLSPGHTWEEAPLLTLKQKQEWICL |
| | | ETLTPDTQYEFQVRVKPLQGEFTTWSPWSQPLAFRTKPAALGKDGGGSSPVPLSLYSGGPITCPPPMSVEHADIWVKSYS |
| | | LYSRERYICNSGFKRKAGTSSLTECVLNKATNVAHWTTPSLKCIRDPALVHQRPAPPSGGGGSGGGGSGGGGSGGGGSN |
| | | WVVNVISDLKKIEDLIQSMHIDATLYTESDVHPSCKVTAMKCFLLELQVISLESGDASIHDTVENLIILANNSLSSNGNVTESGC |
| | | KECEELEEKNIKEFLQSFVHIVQMFINTS |
| | | |
| | | DKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYASTYR |
| | | VVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVCTLPPSRDELTKNQVSLSCAVKGFYPSDIAVEWE |
| | | SNGQPENNYKTTPPVLDSDGSFFLVSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGPGSGSAVNGTSQFTC |
| | Hole: hFc(N297A)-hCD122-[VPLSLY]-hCD215(Sushi)-hIL15 | FYNSRANISCVWSQDGALQDTSCQVHAWPDRRRWNQTCELLPVSQASWACNLILGAPDSQKLTTVDIVTLRVLCREGV |
| DNA525 | | RWRVMAIQDFKPFENLRLMAPISLQVVHVETHRCNISWEISQASHYFERHLEFEARTLSPGHTWEEAPLLTLKQKQEWICL |
| | | ETLTPDTQYEFQVRVKPLQGEFTTWSPWSQPLAFRTKPAALGKDSGSGGSPVPLSLYSGGPITCPPPMSVEHADIWVKSY |
| | | SLYSRERYICNSGFKRKAGTSSLTECVLNKATNVAHWTTPSLKCIRDPALVHQRPAPPSGGGGSGGGGSGGGGSGGGGS |
| | | NWVVNVISDLKKIEDLIQSMHIDATLYTESDVHPSCKVTAMKCFLLELQVISLESGDASIHDTVENLIILANNSLSSNGNVTES |
| | | GCKECEELEEKNIKEFLQSFVHIVQMFINTS |

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| DNA526 | Knob: hFc(N297A)-[VPLSLY]-hIL15-hCD215 | DKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYASTYR<br>VVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPCRDELTKNQVSLWCLVKGFYPSDIAVEWE<br>SNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGGGSSPPVPLSLYSGPS<br>GSPGNWVNVISDLKKIEDLIQSMHIDATLYTESDVHPSCKVTAMKCFLLELQVISLESGDASIHDTVENLIILANNSLSSNGN<br>VTESGCKECEELEEKNIKEFLQSFVHIVQMFINTSGGSGGGSGGGSGGGSGGGSGGITCPPPMSVEHADIWVKSYSLYSRER<br>YICNSGFKRKAGTSSLTECVLNKATNVAHWTTPSLKCIRDPALVHQRPAPPSTVTTAGVTPQPESLSPSGKEPAASSPSSNN<br>TAATTAAIVPGSQLMPSKSPSTGTTEISSHESSHGTPSQTTAKNWELTASASHQPPGVYPQGHSDTT |
| DNA527 | Knob: hFc(N297A)-[VPLSLY]-hIL15-hCD215 | DKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYASTYR<br>VVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPCRDELTKNQVSLWCLVKGFYPSDIAVEWE<br>SNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGGGSSPPVPLSLYSGPS<br>GSPGNWVNVISDLKKIEDLIQSMHIDATLYTESDVHPSCKVTAMKCFLLELQVISLESGDASIHDTVENLIILANNSLSSNGN<br>VTESGCKECEELEEKNIKEFLQSFVHIVQMFINTSGGSGGGSGGGSGGGSGGITCPPPMSVEHADIWVKSYSL<br>YSRERYICNSGFKRKAGTSSLTECVLNKATNVAHWTTPSLKCIRDPALVHQRPAPPSTVTTAGVTPQPESLSPSGKEPAASS<br>PSSNNTAATTAAIVPGSQLMPSKSPSTGTTEISSHESSHGTPSQTTAKNWELTASASHQPPGVYPQGHSDTT |

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| DNA556 | Knob: hFc(N297A)-[VPLSLY]-hIL15 | DKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYASTYR<br>VVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPCRDELTKNQVSLWCLVKGFYPSDIAVEWE<br>SNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGGGSSPPGVPLSLYGSG<br>PGSGSNWVNVISDLKKIEDLIQSMHIDATLYTESDVHPSCKVTAMKCFLLELQVISLESGDASIHDTVENLIILANNSLSSNG<br>NVTESGCKECEELEEKNIKEFLQSFVHIVQMFINTS |
| DNA557 | Knob: hFc(N297A)-[VPLSLY]-hIL15(D22A) | DKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYASTYR<br>VVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPCRDELTKNQVSLWCLVKGFYPSDIAVEWE<br>SNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGGGSSPPGVPLSLYGSG<br>PGSGSNWVNVISDLKKIEDLIQSMHIAATLYTESDVHPSCKVTAMKCFLLELQVISLESGDASIHDTVENLIILANNSLSSNG<br>NVTESGCKECEELEEKNIKEFLQSFVHIVQMFINTS |

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| DNA558 | Knob: hFc(N297A)-[VPLSLY]-hIL15(E46A) | DKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYASTYR<br>VVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPCRDELTKNQVSLWCLVKGFYPSDIAVEWE<br>SNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGGGSSPPGVPLSLYGSG<br>PGSGSNWVNVISDLKKIEDLIQSMHIDATLYTESDVHPSCKVTAMKCFLLELQVISLESGDASIHDTVENLIILANNSLSSNG<br>NVTESGCKECEELEEKNIKEFLQSFVHIVQMFINTS |
| DNA559 | Knob: hFc(N297A)-[VPLSLY]-hIL15(E53A) | DKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYASTYR<br>VVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPCRDELTKNQVSLWCLVKGFYPSDIAVEWE<br>SNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGGGSSPPGVPLSLYGSG<br>PGSGSNWVNVISDLKKIEDLIQSMHIDATLYTESDVHPSCKVTAMKCFLLELQVISLASGDASIHDTVENLIILANNSLSSNG<br>NVTESGCKECEELEEKNIKEFLQSFVHIVQMFINTS |

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| DNA560 | Knob: hFc(N297A)-[VPLSLY]-hIL15(E46A, E53A) | DKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYASTYR<br>VVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPCRDELTKNQVSLWCLVKGFYPSDIAVEWE<br>SNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGGGSSPPGVPLSLYGSG<br>PGSGSNWVNVISDLKKIEDLIQSMHIDATLYTESDVHPSCKVTAMKCFLLALQVISLASGDASIHDTVENLIILANNSLSSNG<br>NVTESGCKECEELEEKNIKEFLQSFVHIVQMFINTS |
| DNA569 | Hole: hFc(N297A)-hCD122-hCD215(Sushi)-[VPLSLY]-hIL15 | DKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYASTYR<br>VVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVCTLPPSRDELTKNQVSLSCAVKGFYPSDIAVEWE<br>SNGQPENNYKTTPPVLDSDGSFFLVSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGPGSGSAVNGTSQFTC<br>FYNSRANISCVWSQDGALQDTSCQVHAWPDRRRWNQTCELLPVSQASWACNLILGAPDSQKLTTVDIVTLRVLCREGV<br>RWRVMAIQDFKPFENLRLMAPISLQVVHVETHRCNISWEISQASHYFERHLEFEARTLSPGHTWEEAPLLTLKQKQEWICL<br>ETLTPDTQYEFQVRVKPLQGEFTTWSPWSQPLAFRTKPAALGKDGGGGSGGGGSGGGGSGGGGSITCPPPMSVEHADI<br>WVKSYSLYSRERYICNSGFKRKAGTSSLTECVLNKATNVAHWTTPSLKCIRDPALVHQRPAPPSPSPPGPGSPPVPLSLYSG<br>GNWVNVISDLKKIEDLIQSMHIDATLYTESDVHPSCKVTAMKCFLLELQVISLESGDASIHDTVENLIILANNSLSSNGNVTE<br>SGCKECEELEEKNIKEFLQSFVHIVQMFINTS |

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| | | DKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYASTYR |
| | | VVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVCTLPPSRDELTKNQVSLSCAVKGFYPSDIAVEWE |
| | | SNGQPENNYKTTPPVLDSDGSFFLVSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGPGSGSAVNGTSQFTC |
| | Hole: hFc(N297A)-hCD122- | FYNSRANISCVWSQDGALQDTSCQVHAWPDRRRWNQTCELLPVSQASWACNLILGAPDSQKLTTVDIVTLRVLCREGV |
| DNA570 | hCD215(Sushi)-[VPLSLY]- | RWRVMAIQDFKPFENLRLMAPISLQVVHVETHRCNISWEISQASHYFERHLEFEARTLSPGHTWEEAPLLTLKQKQEWICL |
| | hIL15 | ETLTPDTQYEFQVRVKPLQGEFTTWSPWSQPLAFRTKPAALGKDGGGGSGGGGSGGGGSGGGGSITCPPPMSVEHADI |
| | | WVKSYSLYSRERYICNSGFKRKAGTSSLTECVLNKATNVAHWTTPSLKCIRDPALVHQRPAPPSSGGPGSGPPSGVPLSLYS |
| | | GGNWVNVISDLKKIEDLIQSMHIDATLYTESDVHPSCKVTAMKCFLLELQVISLESGDASIHDTVENLIILANNSLSSNGNVT |
| | | ESGCKECEELEEKNIKEFLQSFVHIVQMFINTS |
| | | |
| | | DKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYASTYR |
| | | VVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVCTLPPSRDELTKNQVSLSCAVKGFYPSDIAVEWE |
| | | SNGQPENNYKTTPPVLDSDGSFFLVSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGPGSGSAVNGTSQFTC |
| | Hole: hFc(N297A)-hCD122- | FYNSRANISCVWSQDGALQDTSCQVHAWPDRRRWNQTCELLPVSQASWACNLILGAPDSQKLTTVDIVTLRVLCREGV |
| DNA571 | hCD215(Sushi)-[VPLSLY]- | RWRVMAIQDFKPFENLRLMAPISLQVVHVETHRCNISWEISQASHYFERHLEFEARTLSPGHTWEEAPLLTLKQKQEWICL |
| | hIL15 | ETLTPDTQYEFQVRVKPLQGEFTTWSPWSQPLAFRTKPAALGKDGGGGSGGGGSGGGGSGGGGSITCPPPMSVEHADI |
| | | WVKSYSLYSRERYICNSGFKRKAGTSSLTECVLNKATNVAHWTTPSLKCIRDPALVHQRPAPPSSSGSSPGGPSGPPVPLSL |
| | | YSGGNWVNVISDLKKIEDLIQSMHIDATLYTESDVHPSCKVTAMKCFLLELQVISLESGDASIHDTVENLIILANNSLSSNGN |
| | | VTESGCKECEELEEKNIKEFLQSFVHIVQMFINTS |

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| | | DKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYASTYR |
| | | VVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVCTLPPSRDELTKNQVSLSCAVKGFYPSDIAVEWE |
| | | SNGQPENNYKTTPPVLDSDGSFFLVSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGPGSGSAVNGTSQFTC |
| | Hole: hFc(N297A)-hCD122- | FYNSRANISCVWSQDGALQDTSCQVHAWPDRRRWNQTCELLPVSQASWACNLILGAPDSQKLTTVDIVTLRVLCREGV |
| DNA572 | hCD215(1to66)-[VPLSLY]- | RWRVMAIQDFKPFENLRLMAPISLQVVHVETHRCNISWEISQASHYFERHLEFEARTLSPGHTWEEAPLLTLKQKQEWICL |
| | hIL15 | ETLTPDTQYEFQVRVKPLQGEFTTWSPWSQPLAFRTKPAALGKDGGGSGGGGSGGGGSGGGGSITCPPPMSVEHADI |
| | | WVKSYSLYSRERYICNSGFKRKAGTSSLTECVLNKATNVAHWTTPSLKCIRDGGSSPPVPLSLYSGGNWVNVISDLKKIEDLI |
| | | QSMHIDATLYTESDVHPSCKVTAMKCFLLELQVISLESGDASIHDTVENLIILANNSLSSNGNVTESGCKECEELEEKNIKEFL |
| | | QSFVHIVQMFINTS |

| | | |
|---|---|---|
| | | DKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYASTYR |
| | | VVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVCTLPPSRDELTKNQVSLSCAVKGFYPSDIAVEWE |
| | | SNGQPENNYKTTPPVLDSDGSFFLVSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGPGSGSAVNGTSQFTC |
| | Hole: hFc(N297A)-hCD122- | FYNSRANISCVWSQDGALQDTSCQVHAWPDRRRWNQTCELLPVSQASWACNLILGAPDSQKLTTVDIVTLRVLCREGV |
| DNA573 | hCD215(1to66)-[VPLSLY]- | RWRVMAIQDFKPFENLRLMAPISLQVVHVETHRCNISWEISQASHYFERHLEFEARTLSPGHTWEEAPLLTLKQKQEWICL |
| | hIL15 | ETLTPDTQYEFQVRVKPLQGEFTTWSPWSQPLAFRTKPAALGKDGGGSGGGGSGGGGSGGGGSITCPPPMSVEHADI |
| | | WVKSYSLYSRERYICNSGFKRKAGTSSLTECVLNKATNVAHWTTPSLKCIRDSPSSGSGVPLSLYSGGNWVNVISDLKKIEDL |
| | | IQSMHIDATLYTESDVHPSCKVTAMKCFLLELQVISLESGDASIHDTVENLIILANNSLSSNGNVTESGCKECEELEEKNIKEFL |
| | | QSFVHIVQMFINTS |

| | | |
|---|---|---|
| | | DKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYASTYR |
| | | VVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVCTLPPSRDELTKNQVSLSCAVKGFYPSDIAVEWE |
| | | SNGQPENNYKTTPPVLDSDGSFFLVSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGPGSGSAVNGTSQFTC |
| | Hole: hFc(N297A)-hCD122- | FYNSRANISCVWSQDGALQDTSCQVHAWPDRRRWNQTCELLPVSQASWACNLILGAPDSQKLTTVDIVTLRVLCREGV |
| DNA574 | hCD215(1to66)-[VPLSLY]- | RWRVMAIQDFKPFENLRLMAPISLQVVHVETHRCNISWEISQASHYFERHLEFEARTLSPGHTWEEAPLLTLKQKQEWICL |
| | hIL15 | ETLTPDTQYEFQVRVKPLQGEFTTWSPWSQPLAFRTKPAALGKDGGGGSGGGGSGGGGSGGGGSITCPPPMSVEHADI |
| | | WVKSYSLYSRERYICNSGFKRKAGTSSLTECVLNKATNVAHWTTPSLKCIRD |
| | | DLIQSMHIDATLYTESDVHPSCKVTAMKCFLLELQVISLESGDASIHDTVENLIILANNSLSSNGNVTESGCKECEELEEKNIK |
| | | EFLQSFVHIVQMFINTS |
| | | |
| DNA595 | hCD215(1to66) | ITCPPPMSVEHADIWVKSYSLYSRERYICNSGFKRKAGTSSLTECVLNKATNVAHWTTPSLKCIRD |

| | | |
|---|---|---|
| DNA596 | Knob: hFc(N297A)-hIL15(E46A, E53A) | DKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYASTYR<br>VVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPCRDELTKNQVSLWCLVKGFYPSDIAVEWE<br>SNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGGGSSPPGGGSSGGGS<br>GPSGSPGNWVNVISDLKKIEDLIQSMHIDATLYTESDVHPSCKVTAMKCFLLALQVISLASGDASIHDTVENLIILANNSLSS<br>NGNVTESGCKECEELEEKNIKEFLQSFVHIVQMFINTS |
| DNA561 | Knob: hFc(N297A)-[VPLSLY]-hIL15(D22A, E46A, E53A) | DKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYASTYR<br>VVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPCRDELTKNQVSLWCLVKGFYPSDIAVEWE<br>SNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGGGSSPPGVPLSLYGSG<br>PGSGSNWVNVISDLKKIEDLIQSMHIAATLYTESDVHPSCKVTAMKCFLLALQVISLASGDASIHDTVENLIILANNSLSSNG<br>NVTESGCKECEELEEKNIKEFLQSFVHIVQMFINTS |

| | | |
|---|---|---|
| New DNA1 | Knob: hFc(N297A)-[VPLSLY]-hIL15 | DKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYASTYR<br>VVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPCRDELTKNQVSLWCLVKGFYPSDIAVEWE<br>SNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGGGSGSSGVPLSLYGS<br>GPGSGSNWVNVISDLKKIEDLIQSMHIDATLYTESDVHPSCKVTAMKCFLLELQVISLESGDASIHDTVENLIILANNSLSSN<br>GNVTESGCKECEELEEKNIKEFLQSFVHIVQMFINTS |
| New DNA2 | Knob: hFc(N297A)-[VPLSLY]-hIL15 | DKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYASTYR<br>VVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPCRDELTKNQVSLWCLVKGFYPSDIAVEWE<br>SNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGGGSPGPPVPLSLYGS<br>GPGSGSNWVNVISDLKKIEDLIQSMHIDATLYTESDVHPSCKVTAMKCFLLELQVISLESGDASIHDTVENLIILANNSLSSN<br>GNVTESGCKECEELEEKNIKEFLQSFVHIVQMFINTS |

| | | |
|---|---|---|
| New DNA3 | Knob: hFc(N297A)-[VPLSLY]-hIL15 | DKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYASTYR<br>VVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPCRDELTKNQVSLWCLVKGFYPSDIAVEWE<br>SNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGPSSGSGSGVPLSLYGS<br>GPGSGSNWVNVISDLKKIEDLIQSMHIDATLYTESDVHPSCKVTAMKCFLLELQVISLESGDASIHDTVENLIILANNSLSSN<br>GNVTESGCKECEELEEKNIKEFLQSFVHIVQMFINTS |
| New DNA7 | Knob: hFc(N297A)-hCD215(1-66)-[VPLSLY]-hIL15 | DKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYASTYR<br>VVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPCRDELTKNQVSLWCLVKGFYPSDIAVEWE<br>SNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGSSGGSSITCPPPMSVE<br>HADIWVKSYSLYSRERYICNSGFKRKAGTSSLTECVLNKATNVAHWTTPSLKCIRDGGSSPPVPLSLYSGGNWVNVISDLKK<br>IEDLIQSMHIDATLYTESDVHPSCKVTAMKCFLLELQVISLESGDASIHDTVENLIILANNSLSSNGNVTESGCKECEELEEKNI<br>KEFLQSFVHIVQMFINTS |

| | | |
|---|---|---|
| New DNA8 | Knob: hFc(N297A)-hCD215(1-66)-[VPLSLY]-hIL15 | DKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYASTYR<br>VVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPCRDELTKNQVSLWCLVKGFYPSDIAVEWE<br>SNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGSSGGSSITCPPPMSVE<br>HADIWVKSYSLYSRERYICNSGFKRKAGTSSLTECVLNKATNVAHWTTPSLKCIRDSPSSGSGVPLSLYSGGNWVNVISDLK<br>KIEDLIQSMHIDATLYTESDVHPSCKVTAMKCFLLELQVISLESGDASIHDTVENLIILANNSLSSNGNVTESGCKECEELEEK<br>NIKEFLQSFVHIVQMFINTS |
| New DNA9 | Knob: hFc(N297A)-hCD215(1-66)-[VPLSLY]-hIL15 | DKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYASTYR<br>VVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPCRDELTKNQVSLWCLVKGFYPSDIAVEWE<br>SNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGSSGGSSITCPPPMSVE<br>HADIWVKSYSLYSRERYICNSGFKRKAGTSSLTECVLNKATNVAHWTTPSLKCIRDGPPSGSSPVPLSLYSGGNWVNVISDL<br>KKIEDLIQSMHIDATLYTESDVHPSCKVTAMKCFLLELQVISLESGDASIHDTVENLIILANNSLSSNGNVTESGCKECEELEEK<br>NIKEFLQSFVHIVQMFINTS |

New DNA10 Hole: hFc(N297A)-hCD122

DKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYASTYR
VVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPCRDELTKNQVSLWCLVKGFYPSDIAVEWE
SNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGGGGSGGGGSGGGGSAVNG
TSQFTCFYNSRANISCVWSQDGALQDTSCQVHAWPDRRRWNQTCELLPVSQASWACNLILGAPDSQKLTTVDIVTLRVL
CREGVRWRVMAIQDFKPFENLRLMAPISLQVVHVETHRCNISWEISQASHYFERHLEFEARTLSPGHTWEEAPLLTLKQK
QEWICLETLTPDTQYEFQVRVKPLQGEFTTWSPWSQPLAFRTKPAALGKD

New DNA11 Hole: hFc(N297A)-hCD122

DKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYASTYR
VVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPCRDELTKNQVSLWCLVKGFYPSDIAVEWE
SNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGGGGSGGGGSGGGG
GSAVNGTSQFTCFYNSRANISCVWSQDGALQDTSCQVHAWPDRRRWNQTCELLPVSQASWACNLILGAPDSQKLTTV
DIVTLRVLCREGVRWRVMAIQDFKPFENLRLMAPISLQVVHVETHRCNISWEISQASHYFERHLEFEARTLSPGHTWEEAP
LLTLKQKQEWICLETLTPDTQYEFQVRVKPLQGEFTTWSPWSQPLAFRTKPAALGKD

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|---|---|
| New DNA12 | Knob: hFc(N297A)-hCD215(1-66; R26N)-[VPLSLY]-hIL15 |

DKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYASTYR
VVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPCRDELTKNQVSLWCLVKGFYPSDIAVEWE
SNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGSSGGSSITCPPPMSVE
HADIWVKSYSLYSRENYICNSGFKRKAGTSSLTECVLNKATNVAHWTTPSLKCIRDGGSSPPVPLSLYSGGNWVNVISDLKK
IEDLIQSMHIDATLYTESDVHPSCKVTAMKCFLLELQVISLESGDASIHDTVENLIILANNSLSSNGNVTESGCKECEELEEKNI
KEFLQSFVHIVQMFINTS

| | |
|---|---|
| New DNA13 | Knob: hFc(N297A)-hCD215(1-66; R35Q)-[VPLSLY]-hIL15 |

DKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYASTYR
VVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPCRDELTKNQVSLWCLVKGFYPSDIAVEWE
SNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGSSGGSSITCPPPMSVE
HADIWVKSYSLYSRERYICNSGFKQKAGTSSLTECVLNKATNVAHWTTPSLKCIRDGGSSPPVPLSLYSGGNWVNVISDLKK
IEDLIQSMHIDATLYTESDVHPSCKVTAMKCFLLELQVISLESGDASIHDTVENLIILANNSLSSNGNVTESGCKECEELEEKNI
KEFLQSFVHIVQMFINTS

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|---|---|---|
| New DNA14 | Knob: hFc(N297A)-hCD215(1-66; R26S)-[VPLSLY]-hIL15 | DKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYASTYR<br>VVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPCRDELTKNQVSLWCLVKGFYPSDIAVEWE<br>SNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGSSGGSSITCPPPMSVE<br>HADIWVKSYSLYSRESYICNSGFKRKAGTSSLTECVLNKATNVAHWTTPSLKCIRDGGSSPPVPLSLYSGGNWVNVISDLKK<br>IEDLIQSMHIDATLYTESDVHPSCKVTAMKCFLLELQVISLESGDASIHDTVENLIILANNSLSSNGNVTESGCKECEELEEKNI<br>KEFLQSFVHIVQMFINTS |
| New DNA15 | Knob: hFc(N297A)-hCD215(1-66; R35S)-[VPLSLY]-hIL15 | DKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYASTYR<br>VVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPCRDELTKNQVSLWCLVKGFYPSDIAVEWE<br>SNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGSSGGSSITCPPPMSVE<br>HADIWVKSYSLYSRERYICNSGFKSKAGTSSLTECVLNKATNVAHWTTPSLKCIRDGGSSPPVPLSLYSGGNWVNVISDLKK<br>IEDLIQSMHIDATLYTESDVHPSCKVTAMKCFLLELQVISLESGDASIHDTVENLIILANNSLSSNGNVTESGCKECEELEEKNI<br>KEFLQSFVHIVQMFINTS |

| | | |
|---|---|---|
| New DNA16 | Knob: hFc(N297A)-hCD215(1-66; R26N, R35Q)-[VPLSLY]-hIL15 | DKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYASTYR<br>VVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPCRDELTKNQVSLWCLVKGFYPSDIAVEWE<br>SNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGSSGGSSITCPPPMSVE<br>HADIWVKSYSLYSRENYICNSGFKQKAGTSSLTECVLNKATNVAHWTTPSLKCIRDGGSSPPVPLSLYSGGNWVNVISDLK<br>KIEDLIQSMHIDATLYTESDVHPSCKVTAMKCFLLELQVISLESGDASIHDTVENLIILANNSLSSNGNVTESGCKECEELEEK<br>NIKEFLQSFVHIVQMFINTS |

| Molecule | Unique sequences DNA | Unique sequences Name | Co-expression partner DNA | Co-expression partner Name |
|---|---|---|---|---|
| IL-15 | New DNA1 | Knob: hFc(N297A)-[VPLSLY]-hIL15 | DNA187 | Hole: hFc(N297A)-hCD122 |
| IL-15 | New DNA2 | Knob: hFc(N297A)-[VPLSLY]-hIL15 | DNA187 | Hole: hFc(N297A)-hCD122 |
| IL-15 | New DNA3 | Knob: hFc(N297A)-[VPLSLY]-hIL15 | DNA187 | Hole: hFc(N297A)-hCD122 |
| IL-15 | New DNA7 | Knob: hFc(N297A)-hCD215(1-66)-[VPLSLY]-hIL15 | New DNA10 | Hole: hFc(N297A)-hCD122 |
| IL-15 | New DNA8 | Knob: hFc(N297A)-hCD215(1-66)-[VPLSLY]-hIL15 | New DNA10 | Hole: hFc(N297A)-hCD122 |
| IL-15 | New DNA9 | Knob: hFc(N297A)-hCD215(1-66)-[VPLSLY]-hIL15 | New DNA10 | Hole: hFc(N297A)-hCD122 |
| IL-15 | New DNA12 | Knob: hFc(N297A)-hCD215(1-66; R26N)-[VPLSLY]-hIL15 | New DNA10 | Hole: hFc(N297A)-hCD122 |
| IL-15 | New DNA13 | Knob: hFc(N297A)-hCD215(1-66; R35Q)-[VPLSLY]-hIL15 | New DNA10 | Hole: hFc(N297A)-hCD122 |
| IL-15 | New DNA14 | Knob: hFc(N297A)-hCD215(1-66; R26S)-[VPLSLY]-hIL15 | New DNA10 | Hole: hFc(N297A)-hCD122 |
| IL-15 | New DNA15 | Knob: hFc(N297A)-hCD215(1-66; R35S)-[VPLSLY]-hIL15 | New DNA10 | Hole: hFc(N297A)-hCD122 |
| IL-15 | New DNA16 | Knob: hFc(N297A)-hCD215(1-66; R26N, R35Q)-[VPLSLY]-hIL15 | New DNA10 | Hole: hFc(N297A)-hCD122 |
| IL-15 | New DNA7 | Knob: hFc(N297A)-hCD215(1-66)-[VPLSLY]-hIL15 | New DNA11 | Hole: hFc(N297A)-hCD122 |
| IL-15 | New DNA8 | Knob: hFc(N297A)-hCD215(1-66)-[VPLSLY]-hIL15 | New DNA11 | Hole: hFc(N297A)-hCD122 |
| IL-15 | New DNA9 | Knob: hFc(N297A)-hCD215(1-66)-[VPLSLY]-hIL15 | New DNA11 | Hole: hFc(N297A)-hCD122 |
| IL-15 | New DNA12 | Knob: hFc(N297A)-hCD215(1-66; R26N)-[VPLSLY]-hIL15 | New DNA11 | Hole: hFc(N297A)-hCD122 |
| IL-15 | New DNA13 | Knob: hFc(N297A)-hCD215(1-66; R35Q)-[VPLSLY]-hIL15 | New DNA11 | Hole: hFc(N297A)-hCD122 |

| | | | | |
|---|---|---|---|---|
| IL-15 | New DNA14 | Knob: hFc(N297A)-hCD215(1-66; R26S)-[VPLSLY]-hIL15 | New DNA11 | Hole: hFc(N297A)-hCD122 |
| IL-15 | New DNA15 | Knob: hFc(N297A)-hCD215(1-66; R35S)-[VPLSLY]-hIL15 | New DNA11 | Hole: hFc(N297A)-hCD122 |
| IL-15 | New DNA16 | Knob: hFc(N297A)-hCD215(1-66; R26N, R35Q)-[VPLSLY]-hIL15 | New DNA11 | Hole: hFc(N297A)-hCD122 |
| IL-15 | New DNA1 | Knob: hFc(N297A)-[VPLSLY]-hIL15 | DNA158 | Hole: hFc(N297A) |
| IL-15 | New DNA2 | Knob: hFc(N297A)-[VPLSLY]-hIL15 | DNA158 | Hole: hFc(N297A) |
| IL-15 | New DNA3 | Knob: hFc(N297A)-[VPLSLY]-hIL15 | DNA158 | Hole: hFc(N297A) |
| IL-15 | New DNA7 | Knob: hFc(N297A)-hCD215(1-66)-[VPLSLY]-hIL15 | DNA158 | Hole: hFc(N297A) |
| IL-15 | New DNA8 | Knob: hFc(N297A)-hCD215(1-66)-[VPLSLY]-hIL15 | DNA158 | Hole: hFc(N297A) |
| IL-15 | New DNA9 | Knob: hFc(N297A)-hCD215(1-66)-[VPLSLY]-hIL15 | DNA158 | Hole: hFc(N297A) |
| IL-15 | New DNA12 | Knob: hFc(N297A)-hCD215(1-66; R26N)-[VPLSLY]-hIL15 | DNA158 | Hole: hFc(N297A) |
| IL-15 | New DNA13 | Knob: hFc(N297A)-hCD215(1-66; R35Q)-[VPLSLY]-hIL15 | DNA158 | Hole: hFc(N297A) |
| IL-15 | New DNA14 | Knob: hFc(N297A)-hCD215(1-66; R26S)-[VPLSLY]-hIL15 | DNA158 | Hole: hFc(N297A) |
| IL-15 | New DNA15 | Knob: hFc(N297A)-hCD215(1-66; R35S)-[VPLSLY]-hIL15 | DNA158 | Hole: hFc(N297A) |
| IL-15 | New DNA16 | Knob: hFc(N297A)-hCD215(1-66; R26N, R35Q)-[VPLSLY]-hIL15 | DNA158 | Hole: hFc(N297A) |

## CLAIMS

What is claimed is:

1. A masked IL-15 cytokine comprising a first polypeptide chain comprising:

N' **HL1** C'

and a second polypeptide chain comprising:

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

where HL is a half life extension domain, L1 is the first linker, MM is a masking moiety, L2 is a second linker, and C is a IL-15 cytokine or functional fragment thereof, wherein the first linker comprises a proteolytically cleavable peptide (CP) and the second linker does not comprise a proteolytically cleavable peptide .

2. The masked IL-15 cytokine of claim 1, wherein the masked cytokine comprises a first polypeptide chain comprising:

N' **HL1** C'

and a second polypeptide chain comprising:

N' **HL2-non cleavable L2-MM- SD1-CP-SD2 -C** C'

where CP is the cleavable peptide, SD1 is a first spacer domain and SD2 is a second spacer domain.

3. The masked IL-15 cytokine of claim 2, wherein the spacer domains are rich in amino acid residues G, S and P.

4. The masked IL-15 cytokine of claim 2 or claim 3, wherein the first spacer domain (SD1) is between 3 and 18 amino acids in length.

5. The masked IL-15 cytokine of any one of claims 2 to 4, wherein SD1 comprises an amino acid sequence comprising one of SEQ ID NOs: 24-32

6. The masked IL-15 cytokine of any one of claims 2 to 5, wherein the second spacer domain (SD2) is between 2 and 10 amino acids in length.

7. The masked IL-15 cytokine of any one of claims 2 to 6, wherein SD2 comprises an amino acid sequence comprising one of SEQ ID NOs: 35-40.

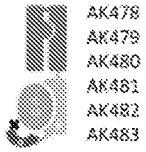
8. The masked IL-15 cytokine of any one of claims 2 to 7, wherein masked cytokine comprises a first polypeptide chain comprising:

N' **HL1** C'

and a second polypeptide chain comprising:

N' **HL2-non cleavable L2-MM- ['IL-15Rα domain']-SD1-CP-SD2 -C** C'

where 'IL-15Rα domain' is a domain comprising an IL-15Rα subunit or a fragment thereof.




9. The masked IL-15 cytokine of any one of claims 2 to 7, wherein the masked cytokine comprises a first polypeptide chain comprising:

N' **HL1** C'

and a second polypeptide chain comprising:

N' **HL2-non cleavable L2-MM- SD1-CP-SD2-['IL-15Rα domain']-C** C'

where 'IL-15Rα domain' is a domain comprising an IL-15Rα subunit or a fragment thereof.




10. The masked IL-15 cytokine of claim 8 or claim 9, wherein 'IL-15Rα domain' comprises the wild-type sushi domain sIL-15Rα or an sIL-15Rα variant.

11. The masked IL-15 cytokine of claim 8 or claim 9, wherein the IL-15Rα subunit or a fragment thereof comprises an amino acid sequence comprising one of SEQ ID NOs: 55, 56, 57, and 58, optionally with an additional amino acid substitution at position R26 and/or R35.

12. The masked IL-15 cytokine of claim 8 or claim 9, 'IL-15Rα domain' comprises an amino acid sequence of SEQ ID NO: 56.

13. A masked IL-15 cytokine comprising a first polypeptide chain comprising:

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

and the second polypeptide chain comprising:

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

where HL1 is a first half life extension domain, L1 is a first linker, MM is the masking moiety, HL2 is a second half life extension domain, L2 is a second linker, and C is an IL-15 cytokine or functional fragment thereof, wherein the second linker comprises a proteolytically cleavable peptide (CP) and the first linker does not comprise a proteolytically cleavable peptide.

14. The masked IL-15 cytokine of claim 13, wherein the first polypeptide chain comprises:

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

and the second polypeptide chain comprises:

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

where CP is the cleavable peptide, SD1 is a first spacer domain and SD2 is a second spacer domain.




15. The masked IL-15 cytokine of claim 14, wherein the spacer domains are rich in amino acid residues G, S and P.

16. The masked IL-15 cytokine of claim 14 or claim 15, wherein the first spacer domain (SD1) is between 3 and 18 amino acids in length.

17. The masked IL-15 cytokine of any one of claims 14 to 16, wherein SD1 comprises an amino acid sequence comprising one of SEQ ID NOs: 24-32

18. The masked IL-15 cytokine of any one of claims 14 to 17, wherein the second spacer domain (SD2) is between 2 and 10 amino acids in length.

19. The masked IL-15 cytokine of any one of claims 14 to 18, wherein SD2 comprises an amino acid sequence comprising one of SEQ ID NOs: 35-40.

20. The masked IL-15 cytokine of any one of claims 13 to 19, wherein the masked cytokine further comprises a third polypeptide chain, comprising:

N' **IL-15Rα domain** C'

where the 'IL-15Rα domain' is a domain comprising an IL-15Rα subunit or a fragment thereof and wherein the 'IL-15Rα domain' is non-covalently bonded to the IL-15 cytokine (C).

21. The masked IL-15 cytokine of any one of claims 13 to 19, wherein the second polypeptide further comprises a domain comprising an IL-15Rα subunit or fragment thereof that is covalently attached to the C' terminal of the IL-15 cytokine or fragment thereof, such that the first polypeptide chain comprises:

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

and the second polypeptide chain comprises:

N' **HL2- SD1-CP-SD2 –C-['IL-15Rα domain']** C'

where the IL-15Rα domain comprises the IL-15Rα subunit or a fragment thereof.



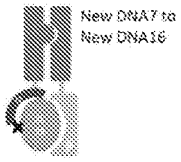

22. The masked IL-15 cytokine of any one of claims 13 to 19, wherein the first polypeptide chain comprises:

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

and the second polypeptide chain comprises:

N' **HL2–['IL-15Rα domain']- SD1-CP-SD2 - C** C'

where the IL-15Rα domain comprises the IL-15Rα subunit or a fragment thereof.




23. The masked IL-15 cytokine of any one of claims 20 to 22, wherein the 'IL-15Rα domain' comprises the wild-type sushi domain sIL-15Rα or an sIL-15Rα variant.

24. The masked IL-15 cytokine of any one of claims 20 to 22, wherein the IL-15Rα subunit or a fragment thereof comprises an amino acid sequence comprising one of SEQ ID NOs: 55, 56, 57, and 58, optionally with an additional amino acid substitution at position R26 and/or R35.

25. The masked IL-15 cytokine of any one of claims 20 to 22, wherein the 'IL-15Rα domain' comprises an amino acid sequence of SEQ ID NO: 56.

26. The masked IL-15 cytokine of any one of the preceding claims, wherein the non-cleavable linker is between 4 and 6 amino acids in length.

27. The masked IL-15 cytokine of any one of the preceding claims, wherein the non-cleavable linker includes a 'GS' repeat.

28. The masked IL-15 cytokine of any one of the preceding claims, wherein the non-cleavable linker comprises an amino acid sequence as shown in SEQ ID NO: 22 (PGSGS).

29. A masked IL-15 cytokine comprising a first polypeptide chain comprising:

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

and the second polypeptide chain comprising:

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

where HL1 is a first half life extension domain, L1 is a first linker, MM is the masking moiety, HL2 is a second half life extension domain, L2 is a second linker, and C is an IL-15 cytokine or functional fragment thereof, wherein the first linker comprises a proteolytically cleavable peptide (CP) and the second linker does not comprise a proteolytically cleavable peptide.

30. The masked IL-15 cytokine of claim 29, wherein the first polypeptide chain comprises:

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

and the second polypeptide chain comprises:

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

where CP is the cleavable peptide, SD1 is a first spacer domain and SD2 is a second spacer domain.




31. The masked IL-15 cytokine of claim 29 or claim 30, wherein the masked cytokine further comprises a third polypeptide chain, comprising:

N' IL-15Rα domain C'

where the 'IL-15Rα domain' is a domain comprising an IL-15Rα subunit or a fragment thereof and wherein the 'IL-15Rα domain' is non-covalently bonded to the IL-15 cytokine (C).

32. The masked IL-15 cytokine of any one of claims 29 to 31, wherein the spacer domains are rich in amino acid residues G, S and P.

33. The masked IL-15 cytokine of any of one claims 29 to 31, wherein the first spacer domain (SD1) is between 3 and 18 amino acids in length.

34. The masked IL-15 cytokine of any of one claims 29 to 31, wherein SD1 comprises an amino acid sequence comprising one of SEQ ID NOs: 24-32

35. The masked IL-15 cytokine of any one of claims 29 to 34, wherein the second spacer domain (SD2) is between 2 and 10 amino acids in length.

36. The masked IL-15 cytokine of any one of claims 29 to 35, wherein SD2 comprises an amino acid sequence comprising one of SEQ ID NOs: 35-40.

37. The masked IL-15 cytokine of any one of claims 29 to 36, the non-cleavable linker is between 18 and 22 amino acids in length.

38. The masked IL-15 cytokine of any one of claims 29 to 37, the non-cleavable linker is rich in amino acid residues G, S and P.

39. The masked IL-15 cytokine of any one of claims 29 to 38, the non-cleavable linker comprises an amino acid sequence as shown in SEQ ID NO: 21 (GGSSPPGGGSSGGGSGPSGSPG).

40. The masked IL-15 cytokine of any one of the preceding claims, wherein the IL-15 cytokine or functional fragment thereof comprises an amino acid sequence or SEQ ID NO: 2 or an amino acid sequence having at least one amino acid modification as compared to the amino acid sequence of SEQ ID NO: 2.

41. The masked IL-15 cytokine of claim 40, wherein the IL-15 cytokine or functional fragment thereof comprises an amino acid sequence having one or more amino acid substitutions at positions D22, E46, or E53 as compared to the amino acid sequence of SEQ ID NO: 2.

42. The masked IL-15 cytokine of claim 40, wherein the IL-15 cytokine or functional fragment thereof comprises an amino acid sequence having one or more amino acid substitutions at positions D22, E46, E53, N71, N79, or N112 as compared to the amino acid sequence of SEQ ID NO: 2.

43. The masked IL-15 cytokine of claim 40, wherein the IL-15 cytokine or functional fragment thereof comprises an amino acid sequence having an amino acid substitution at position N71 and N79 as compared to the amino acid sequence of SEQ ID NO: 2.

44. The masked IL-15 cytokine of claim 40, wherein the IL-15 cytokine or functional fragment thereof comprises an amino acid sequence having an amino acid substitution at position N71 and N112 as compared to the amino acid sequence of SEQ ID NO: 2.

45. The masked IL-15 cytokine of claim 40, wherein the IL-15 cytokine or functional fragment thereof comprises an amino acid sequence having an amino acid substitution at position N79 and N112 as compared to the amino acid sequence of SEQ ID NO: 2.

46. The masked IL-15 cytokine of claim 40, wherein the IL-15 cytokine or functional fragment thereof comprises an amino acid sequence having an amino acid substitution at position N71, N79 and N112 as compared to the amino acid sequence of SEQ ID NO: 2.

47. The masked IL-15 cytokine of claim 40, wherein the IL-15 cytokine or functional fragment thereof comprises an amino acid sequence of SEQ ID NO: 3.

48. The masked IL-15 cytokine of claim 40, wherein the IL-15 cytokine or functional fragment thereof comprises an amino acid sequence of SEQ ID NO: 4.

49. The masked IL-15 cytokine of claim 40, wherein the IL-15 cytokine or functional fragment thereof comprises an amino acid sequence of SEQ ID NO: 5.

50. The masked IL-15 cytokine of claim 40, wherein the IL-15 cytokine or functional fragment thereof comprises an amino acid sequence of SEQ ID NO: 6.

51. The masked IL-15 cytokine of claim 40, wherein the IL-15 cytokine or functional fragment thereof comprises an amino acid sequence of SEQ ID NO: 7.

52. The masked IL-15 cytokine of claim 40, wherein the IL-15 cytokine or functional fragment thereof comprises an amino acid sequence of SEQ ID NO: 8.

53. The masked IL-15 cytokine of claim 40, wherein the IL-15 cytokine or functional fragment thereof comprises an amino acid sequence of SEQ ID NO: 127.

54. The masked IL-15 cytokine of claim 40, wherein the IL-15 cytokine or functional fragment thereof comprises an amino acid sequence of SEQ ID NO: 128.

55. The masked IL-15 cytokine of claim 40, wherein the IL-15 cytokine or functional fragment thereof comprises an amino acid sequence of SEQ ID NO: 129.

56. The masked IL-15 cytokine of claim 40, wherein the IL-15 cytokine or functional fragment thereof comprises an amino acid sequence of SEQ ID NO: 130.

57. The masked IL-15 cytokine of claim 40, wherein the IL-15 cytokine or functional fragment thereof comprises an amino acid sequence of SEQ ID NO: 131.

58. The masked IL-15 cytokine of claim 40, wherein the IL-15 cytokine or functional fragment thereof comprises an amino acid sequence of SEQ ID NO: 132.

59. The masked IL-15 cytokine of claim 40, wherein the IL-15 cytokine or functional fragment thereof comprises an amino acid sequence of SEQ ID NO: 133.

60. The masked IL-15 cytokine of claim 40 or claim 41, wherein the IL-15 cytokine or functional fragment thereof comprises an additional mutation at position N71.

61. The masked IL-15 cytokine of any one of claims 40 to 60, wherein the IL-15 cytokine or functional fragment thereof comprises an additional mutation at position S73.

62. The masked IL-15 cytokine of any one of claims 40, 41, or 61, wherein the IL-15 cytokine or functional fragment thereof comprises an additional mutation at one or more of amino acid positions N72, N79, V80, T81, and N112.

63. The masked IL-15 cytokine of claim 62, wherein the first polypeptide chain comprises:

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

and the second polypeptide chain comprises:

N' **HL2- SD1-CP-SD2 – [N' terminal domain of C]-['IL-15Rα domain']-[C' terminal domain of C]** C'

where the IL-15 cytokine or functional fragment thereof comprises an N' terminal domain and a C' terminal domain and where the IL-15Rα domain comprises the IL-15Rα subunit or a fragment thereof flanked on either side by a non-cleavable 'α subunit linker'.




64. The masked IL-15 cytokine of claim 63, wherein the non-cleavable linker is between 4 and 6 amino acids in length.

65. The masked IL-15 cytokine of claim 63 or claim 64, wherein the non-cleavable linker includes a 'GS' repeat.

66. The masked IL-15 cytokine of any one of claims 63 to 65, wherein the non-cleavable linker comprises an amino acid sequence as shown in SEQ ID NO: 22 (PGSGS).

67. The masked IL-15 cytokine of any one of claims 63 to 66, wherein the spacer domains are rich in amino acid residues G, S and P.

68. The masked IL-15 cytokine of any one of claims 63 to 67, wherein the first spacer domain (SD1) is between 3 and 18 amino acids in length.

69. The masked IL-15 cytokine of any one of claims 63 to 68, wherein SD1 comprises an amino acid sequence comprising one of SEQ ID NOs: 24-32

70. The masked IL-15 cytokine of any one of claims 63 to 69, wherein the second spacer domain (SD2) is between 2 and 10 amino acids in length.

71. The masked IL-15 cytokine of any one of claims 63 to 70, wherein SD2 comprises an amino acid sequence comprising one of SEQ ID NOs: 35-40.

72. The masked IL-15 cytokine of any one of claims 63 to 71, wherein the 'IL-15Rα domain' comprises the wild-type sushi domain sIL-15Rα or an sIL-15Rα variant.

73. The masked IL-15 cytokine of any one of claims 63 to 71, wherein the IL-15Rα subunit or a fragment thereof comprises an amino acid sequence comprising one of SEQ ID NOs: 55, 56, 57, and 58, optionally with an additional amino acid substitution at position R26 and/or R35.

74. The masked IL-15 cytokine of any one of claims 63 to 71, wherein the 'IL-15Rα domain' comprises an amino acid sequence of SEQ ID NO: 56.

75. The masked IL-15 cytokine of any one of claims 63 to 74, wherein the N' terminal domain and C' terminal domain of the IL-15 cytokine or functional fragment thereof comprise an amino acid sequence having one or more amino acid substitutions at positions D22, E46, E53 as compared to the amino acid sequence of SEQ ID NO: 2.

76. The masked IL-15 cytokine of any one of claims 63 to 75, wherein the N' terminal domain and C' terminal domain of the IL-15 cytokine or functional fragment thereof comprises an additional mutation at position N71.

77. The masked IL-15 cytokine of any one of claims 63 to 76, wherein the N' terminal domain and C' terminal domain of the IL-15 cytokine or functional fragment thereof comprises an additional mutation at position S73.

78. The masked IL-15 cytokine of any one of claims 63 to 77, wherein the N' terminal domain and C' terminal domain of the IL-15 cytokine or functional fragment thereof comprises an additional mutation at one or more of amino acid positions N72, N79, V80, T81, and N112.

79. The masked IL-15 cytokine of any one of claims 63 to 74, wherein the N' terminal domain of the IL-15 cytokine or functional fragment thereof, comprises an amino acid sequence of SEQ ID NO: 9 and the C' terminal domain of the IL-15 cytokine or functional fragment thereof comprises an amino acid sequence of SEQ ID NO: 10.

80. The masked IL-15 cytokine of any one of the preceding claims, wherein the cleavable peptide comprises an amino acid sequence of SEQ ID NO: 33 (MPYD*LYHP).

81. The masked IL-15 cytokine of any one of the preceding claims, wherein the cleavable peptide comprises an amino acid sequence of SEQ ID NO: 34 (VPLS*LY).

82. The masked IL-15 cytokine of any one of the preceding claims, wherein the cleavable peptide comprises an amino acid sequence of SEQ ID NO: 155 (ISSGLL*SG*RS).

83. The masked IL-15 cytokine of any one of the preceding claims, wherein the cleavable peptide comprises an amino acid sequence of SEQ ID NO: 156 (DLLA*VVAAS).

84. The masked IL-15 cytokine of any one of the preceding claims, wherein the cleavable linker comprises an amino acid sequence comprising one of SEQ ID NOs: 41-54, 162, 163, 164, 165, 166, or 167.

85. The masked IL-15 cytokine of any one of the preceding claims, wherein the cleavable linker comprises an amino acid sequence comprising one of SEQ ID NOs: 41-54, 162, 163, 164, 165, 166, 167, 168, 169, 170 or 171.

86. The masked IL-15 cytokine of any one of the preceding claims, wherein the first half-life extension domain comprises a first antibody Fc domain or a fragment thereof and the second half-life extension domain comprises a second antibody Fc domain or a fragment thereof.

87. The masked IL-15 cytokine of claim 86, 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.

88. The masked IL-15 cytokine of claim 86 or claim 87, 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.

89. The masked IL-15 cytokine of any one of claims 86 to 88, wherein the first and second half-life extension domains are each an IgG1 Fc domain or fragment thereof.

90. The masked IL-15 cytokine of claim 89, 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.

91. The masked IL-15 cytokine of claim 89 or claim 90, 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.

92. The masked IL-15 cytokine of claim 86, wherein the first half-life extension domain comprises the amino acid sequence of SEQ ID NO: 63, and the second half-life extension domain thereof comprises the amino acid sequence of SEQ ID NO: 64.

93. The masked IL-15 cytokine of any one of claims 89 to 91, wherein the first and second half-life extension domains are each an IgG1 Fc domain or fragment thereof and each comprise the amino acid substitution I253A, numbered according to the Kabat EU numbering system.

94. The masked IL-15 cytokine of claim 93, wherein the first half-life extension domain comprises the amino acid sequence of SEQ ID NO: 65 and the second half-life extension domain thereof comprises the amino acid sequence of SEQ ID NO: 66.

95. The masked IL-15 cytokine of any one of the preceding claims, wherein the masking moiety comprises IL-15Rβ or a fragment or variant thereof.

96. The masked IL-15 cytokine of any one of the preceding claims, wherein the masking moiety comprises the amino acid sequence of SEQ ID NO: 11.

97. The masked IL-15 cytokine of any one of the preceding claims, wherein the masking moiety comprises an IL-15Rβ variant or a fragment thereof having an amino acid substitution at position C122.

98. The masked IL-15 cytokine of any one of the preceding claims, wherein the masking moiety comprises an IL-15Rβ variant or a fragment thereof having amino acid substitution C122S.

99. The masked IL-15 cytokine of any one of the preceding claims, wherein the masking moiety comprises an IL-15Rβ variant or a fragment thereof having an amino acid substitution at position C168.

100. The masked IL-15 cytokine of any one of the preceding claims, wherein the masking moiety comprises an IL-15Rβ variant or a fragment thereof having amino acid substitution C168S.

101. The masked IL-15 cytokine of any one of the preceding claims, wherein the masking moiety comprises an IL-15Rβ variant or a fragment thereof having an amino acid substitution at positions C122 and C168.

102. The masked IL-15 cytokine of claim 95, wherein the masking moiety comprises an amino acid sequence of SEQ ID NO: 12.

103. A masked IL-15 cytokine comprising a first polypeptide chain comprising:

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

and a second polypeptide chain comprising:

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

and a third polypeptide chain, comprising:

N' **IL-15Rα domain** C'

where HL1 is a first half life extension domain, L1 is a first linker, MM is a masking moiety, HL2 is a second half life extension domain, L2 is a second linker, and C is an IL-15 cytokine or functional fragment thereof, wherein the second linker comprises a proteolytically cleavable peptide (CP) and the first linker does not comprise a proteolytically cleavable peptide,

wherein the 'IL-15Rα domain' is a domain comprising an IL-15Rα subunit or a fragment thereof and the 'IL-15Rα domain' is non-covalently bonded to the IL-15 cytokine or functional fragment thereof (C) in the second polypeptide chain.

104. The masked IL-15 cytokine of claim 103, wherein the first polypeptide chain comprises:

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

and the second polypeptide chain comprises:

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

and the third polypeptide chain, comprises:

N' **IL-15Rα domain** C'

where CP is the cleavable peptide, SD1 is a first spacer domain and SD2 is a second spacer domain.

105. A masked IL-15 cytokine comprising a first polypeptide chain comprising:

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

and a second polypeptide chain comprising:

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

and a third polypeptide chain comprising:

N' **IL-15Rα domain** C'

where HL1 is a first half life extension domain, L1 is a first linker, MM is a masking moiety, HL2 is a second half life extension domain, L2 is a second linker, and C is an IL-15 cytokine or functional fragment thereof, wherein the first linker comprises a proteolytically cleavable peptide (CP) and the second linker does not comprise a proteolytically cleavable peptide,

wherein the 'IL-15Rα domain' is a domain comprising an IL-15Rα subunit or a fragment thereof and the 'IL-15Rα domain' is non-covalently bonded to the IL-15 cytokine or functional fragment thereof (C) in the second polypeptide chain.

106. The masked IL-15 cytokine of claim 105, wherein the first polypeptide chain comprises:

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

and the second polypeptide chain comprises:

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

and the third polypeptide chain comprises:

N' **IL-15Rα domain** C'

where CP is the cleavable peptide, SD1 is a first spacer domain and SD2 is a second spacer domain.

107. The masked IL-15 cytokine of any one of claims 103 to 106, wherein the first half-life extension domain comprises a first antibody Fc domain or a fragment thereof and the second half-life extension domain comprises a second antibody Fc domain or a fragment thereof.

108. The masked IL-15 cytokine of any one of claims 103 to 107, wherein the first half-life extension domain comprises the amino acid sequence of SEQ ID NO: 63, and the second half-life extension domain thereof comprises the amino acid sequence of SEQ ID NO: 64.

109. The masked IL-15 cytokine of any one of claims 103 to 108, wherein the masking moiety comprises IL-15Rβ or a fragment or variant thereof.

110. The masked IL-15 cytokine of any one of claims 103 to 109, wherein the masking moiety comprises the amino acid sequence of SEQ ID NO: 12.

111. The masked IL-15 cytokine of claim 103 or 104, wherein the first polypeptide chain comprises an amino acid sequence of SEQ ID NO: 177.

112. The masked IL-15 cytokine of claim 105 or 106, wherein the second polypeptide chain comprises an amino acid sequence of SEQ ID NO: 183.

113. The masked IL-15 cytokine of any one of claims 103 to 112, wherein the IL-15 cytokine or functional fragment thereof comprises an amino acid sequence having an amino acid substitution N71Q and N79Q as compared to the amino acid sequence of SEQ ID NO: 2.

114. The masked IL-15 cytokine of any one of claims 103 to 113, wherein the IL-15 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: 130.

115. The masked IL-15 cytokine of any one of claims 103 to 114, wherein the IL-15 cytokine or functional fragment thereof comprises an amino acid sequence of SEQ ID NO: 130.

116. The masked IL-15 cytokine of any one of claims 103 to 115, wherein the 'IL-15Rα domain' comprises the wild-type sushi domain sIL-15Rα or a variant thereof.

117. The masked IL-15 cytokine of any one of claims 103 to 116, wherein the 'IL-15Rα domain' comprises an amino acid sequence of SEQ ID NO: 56.

118. The masked IL-15 cytokine of claim 13, wherein the first polypeptide chain comprises an amino acid sequence of SEQ ID NO: 68 and the second polypeptide chain comprises an amino acid sequence of SEQ ID NO: 83.

119. The masked IL-15 cytokine of claim 13, wherein the first polypeptide chain comprises an amino acid sequence of SEQ ID NO: 177 and the second polypeptide chain comprises an amino acid sequence of SEQ ID NO: 193.

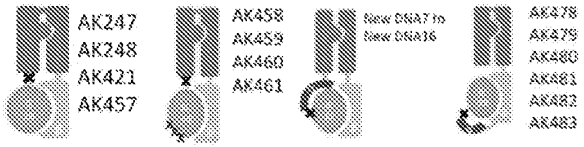
120. The masked IL-15 cytokine of claim 13, wherein the first polypeptide chain comprises an amino acid sequence of SEQ ID NO: 177 and the second polypeptide chain comprises an amino acid sequence of SEQ ID NO: 194.

121. A cleavage product of a masked IL-15 cytokine, wherein said cleavage product is capable of binding to its cognate receptor, the cleavage product comprising an IL-15 cytokine or functional fragment thereof, preparable by proteolytic cleavage of the cleavable peptide in the masked IL-15 cytokine as defined in of any one of claims 1 to 115.

122. A cleavage product of a masked IL-15 cytokine, where the cleavage product is capable of binding to its cognate receptor, the cleavage product comprising a polypeptide comprising:

**PCP-SD-C**

wherein PCP is a portion of a proteolytically cleavable peptide; SD is a spacer domain; and C is an IL-15 cytokine or functional fragment thereof.




123. The cleavage product of claim 122, wherein SD is SD2 as defined in any one of claims 15, 18 or 19.

124. The cleavage product of claim 122 or claim 123, wherein the proteolytically cleavable peptide is as defined in any one of claims 80 to 82.

125. The cleavage product of any one of claims 122 to 124, wherein the IL-15 cytokine or a fragment thereof is as defined in any one of claims 40 to 62.

126. The cleavage product of any one of claims 122 to 125, wherein the cleavage product further comprises a domain comprising an IL-15Rα subunit or a fragment thereof.

127. The cleavage product of claim 126, wherein the IL-15Rα subunit or a fragment thereof is non-covalently bonded to the IL-15 cytokine (C).

128. The cleavage product of claim 126, wherein the cleavage product comprises:

**PCP-SD-['IL-15Rα domain']-C**




129. The cleavage product of claim 126, wherein the cleavage product comprises:

**PCP-SD-C-['IL-15Rα domain']**

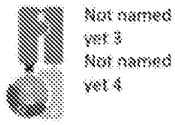



130. The cleavage product of any one of claims 126 to 129, wherein the IL-15Rα subunit or a fragment thereof comprises an amino acid sequence comprising one of SEQ ID NOs: 55, 56, 57, and 58, optionally with an additional amino acid substitution at position R26 and/or R35.

131. A cleavage product of a masked IL-15 cytokine, where the cleavage product is capable of binding to its cognate receptor, the cleavage product comprising:

**PCP-SD2 – [N' terminal domain of C]-['IL-15Rα domain']-[C' terminal domain of C]**

where the IL-15Rα domain comprises an IL-15Rα subunit or a fragment thereof flanked on either side by a non-cleavable 'α subunit linker'.




132. A cleavage product of a masked IL-15 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:

**HL1-SD-PCP**

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

and a second polypeptide chain comprising a polypeptide comprising:

**HL2-L2-C**

wherein HL2 is a second half-life extension domain; L2 is a linker; and C is an IL-15 cytokine or functional fragment thereof; and

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




133. The cleavage product of claim 132, wherein SD is SD1 as defined in any one of claims 32 to 34.

134. The cleavage product of claim 132 or claim 133, wherein the proteolytically cleavable peptide is as defined in any one of claims 80 to 85.

135. The cleavage product of any one of claims 132 to 134, wherein the IL-15 cytokine or a fragment thereof is as defined in any one of claims 40 to 62.

136. The cleavage product of any one of claims 132 to 135, wherein the first and second half life extension domains are as defined in any one of claims 86 to 94.

137. The cleavage product of any one of claims 132 to 136, wherein the non-cleavable linker L2 is as defined in any one of claims 26 to 28.

138. The cleavage product of any one of claims 132 to 137, wherein the cleavage product further comprises a IL-15Rα subunit or a fragment thereof non-covalently bonded to the IL-15 cytokine (C).

139. A cleavage product comprising an amino acid sequence selected from SEQ ID NOs: 104, 105, 106, 107, 108, 109, 110, 111, 112, 113, 114, 115, 116, 117, 118, 119, 120, 121, 122, 123, 124, 125, 126, and 136 to 143, and 144 to 147, and 199 to 228, and 307.

140. A nucleic acid encoding the masked IL-15 cytokine of any one of claims 1 to 120, or encoding one of the chains of the masked IL-15 cytokine.

141. A vector comprising the nucleic acid of claim 140.

142. A host cell comprising nucleic acid encoding the masked IL-15 cytokine of any one of claims 1 to 120.

143. A composition comprising the masked IL-15 cytokine of any one of claims 1 to 120.

144. A pharmaceutical composition comprising the masked IL-15 cytokine of any one of claims 1 to 120, and a pharmaceutically acceptable carrier.

145. The pharmaceutical composition of claim 144, wherein the pharmaceutical composition is in single unit dosage form.

146. The pharmaceutical composition of claim 144 or claim 145, wherein the pharmaceutical composition is formulated for intravenous administration and is in single unit dosage form.

147. The pharmaceutical composition of claim 144 or claim 145, wherein the pharmaceutical composition is formulated for injection and is in single unit dosage form.

148. The pharmaceutical composition of claim 144 or claim 145, wherein the pharmaceutical composition is a liquid and is in single unit dosage form.

149. A kit comprising the masked IL-15 cytokine of any one of claims 1 to 120, or the composition of claim 143, or the pharmaceutical composition of any one of claims 144 to 148.

150. A method of producing a masked IL-15 cytokine as defined in any one of claims 1-115 comprising culturing the host cell of claim 142 under a condition that produces the masked IL-15 cytokine.

151. A nucleic acid encoding the cleavage product of any one of claims 121 to 139.

152. A composition comprising the cleavage product of any one of claims 121 to 139.

153. A pharmaceutical composition comprising the cleavage product of any one of claims 121 to 139, and a pharmaceutically acceptable carrier.

154. A masked IL-15 cytokine as defined in any one of claims 1 to 120 for use in medicine.

155. A cleavage product as defined in any one of claims 121 to 139 for use in medicine.

156. A method of treating or preventing cancer in a subject, the method comprising administering to the subject an effective amount of a masked IL-15 cytokine according to any one of claims 1 to 120.

157. 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 143 or claim 152.

158. 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 144 to 148 or 153.

159. A method of treating or preventing cancer in a subject, the method comprising administering to the subject an effective amount of a masked IL-15 cytokine as defined in any one of claims 1-78, whereby the masked cytokine is proteolytically cleaved *in vivo* to produce a cleavage product as defined in any one of claims 121 to 139.

160. 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 121 to 139.

161. A method according to any one of claims 156 to 160, wherein the cancer is a solid tumor.

162. A masked IL-15 cytokine as defined in any one of claims 1 to 120 for use in treating or preventing cancer.

163. A masked IL-15 cytokine as defined in any one of claims 1 to 120 for use in a method of treating or preventing cancer, the method comprising administering to the subject an effective amount of the masked IL-15 cytokine, whereby the masked cytokine is proteolytically cleaved *in vivo* to produce a cleavage product as defined in any one of claims 121 to 139.

164. A masked IL-15 cytokine for use as defined in claim 162 or claim 163, wherein the cancer is a solid tumor.

165. A cleavage product as defined in any one of claims 121 to 139 for use in treating or preventing cancer.

166. A cleavage product as defined in any one of claims 121 to 139 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 120 to a patient, thereby producing the cleavage product by proteolytic cleavage of the masked cytokine *in vivo*.

167. A cleavage product as defined in any one of claims 121 to 139 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 120 that has been administered to the subject.

168. A cleavage product for use as defined in any one of claims 165 to 167, wherein the cancer is a solid tumor.