

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~~disclosure 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.

~~In one aspect, the present disclosure provides~~ 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 ~~embodiment~~examples, 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 ~~embodiment~~examples, the spacer domains are rich in amino acid residues G, S and P.

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

In some [embodimentexamples](#), SD1 comprises an amino acid sequence comprising one of SEQ ID NOs: 24-32

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

5 In some [embodimentexamples](#), SD2 comprises an amino acid sequence comprising one of SEQ ID NOs: 35-40.

In some [embodimentexamples](#), the masked cytokine comprises a first polypeptide chain comprising:

N' **HL1** C'

and a second polypeptide chain comprising:

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

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

In some [embodimentexamples](#), the masked cytokine comprises a first polypeptide chain comprising:

N' **HL1** C'

15 and a second polypeptide chain comprising:

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

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

20 In some [embodimentexamples](#), the 'IL-15R α domain' comprises the wild-type sushi domain sIL-15R α or an sIL-15R α variant.

In some [embodimentexamples](#), the IL-15R α subunit or a fragment thereof comprises an amino acid sequence comprising one of SEQ ID NOs: 55, 56, 57, and 58.

25 In some [embodimentexamples](#), the 'IL-15R α domain' comprises an amino acid sequence of SEQ ID NO: 56.

30 In some [embodimentexamples](#), the IL-15R α comprises an amino acid substitution at position R26. In some [embodimentexamples](#), the IL-15R α comprises amino acid substitution R26N. In some [embodimentexamples](#), the IL-15R α comprises amino acid substitution R26S. In some [embodimentexamples](#), the IL-15R α comprises an amino acid substitution at position R35. In some [embodimentexamples](#), the IL-15R α comprises amino acid substitution R35Q. In some [embodimentexamples](#), the IL-15R α comprises amino acid substitution R35S. In some [embodimentexamples](#), the IL-15R α comprises an amino acid substitution at positions R26 and R35. In some [embodimentexamples](#), the IL-15R α comprises amino acid substitutions R26S or R26N, and R35Q or R35S. In some [embodimentexamples](#), the IL-15R α comprises amino acid substitutions R26N and R35Q.

35 In some [embodimentexamples](#), the IL-15R α 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 [embodimentexamples](#), the IL-15R α comprises an amino acid sequence of SEQ ID NOs: 55, 56, 57 or 58, with additional amino acid substitution R26N.

40

In some [embodimentexamples](#), the IL-15R α comprises an amino acid sequence of SEQ ID NOs: 55, 56, 57 or 58, with additional amino acid substitution R26S. In some [embodimentexamples](#), the IL-15R α 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 [embodimentexamples](#), the IL-15R α comprises an amino acid sequence of SEQ ID NOs: 55, 56, 57 or 58, with additional amino acid substitution R35Q. In some [embodimentexamples](#), the IL-15R α comprises an amino acid sequence of SEQ ID NOs: 55, 56, 57 or 58, with additional amino acid substitution R35S. In some [embodimentexamples](#), the IL-15R α 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 [embodimentexamples](#), the IL-15R α 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 [embodimentexamples](#), the IL-15R α comprises an amino acid sequence of SEQ ID NOs: 55, 56, 57 or 58, with additional amino acid substitutions R26N and R35Q.

In some [embodimentexamples](#), the IL-15R α comprises an amino acid sequence of SEQ ID NO: 56, with an additional amino acid substitution at position R26. In some [embodimentexamples](#), the IL-15R α comprises an amino acid sequence of SEQ ID NO: 56, with additional amino acid substitution R26N. In some [embodimentexamples](#), the IL-15R α comprises an amino acid sequence of SEQ ID NO: 56, with additional amino acid substitution R26S. In some [embodimentexamples](#), the IL-15R α comprises an amino acid sequence of SEQ ID NO: 56, with an additional amino acid substitution at position R35. In some [embodimentexamples](#), the IL-15R α comprises an amino acid sequence of SEQ ID NO: 56, with additional amino acid substitution R35Q. In some [embodimentexamples](#), the IL-15R α comprises an amino acid sequence of SEQ ID NO: 56, with additional amino acid substitution R35S. In some [embodimentexamples](#), the IL-15R α comprises an amino acid sequence of SEQ ID NO: 56, with additional amino acid substitutions at positions R26 and R35. In some [embodimentexamples](#), the IL-15R α comprises an amino acid sequence of SEQ ID NO: 56, with additional amino acid substitutions R26S or R26N, and R35Q or R35S. In some [embodimentexamples](#), the IL-15R α 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 [embodimentexamples](#), 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.

5 In some [embodimentexamples](#), the spacer domains are rich in amino acid residues G, S and P.

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

10 In some [embodimentexamples](#), SD1 comprises an amino acid sequence comprising one of SEQ ID NOs: 24-32

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

In some [embodimentexamples](#), SD2 comprises an amino acid sequence comprising one of SEQ ID NOs: 35-40.

15 In some [embodimentexamples](#), the masked cytokine further comprises a third polypeptide chain, comprising:

N' IL-15R α domain C'

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

20 In some [embodimentexamples](#), the second polypeptide chain further comprises a domain comprising an IL-15R α 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:

25 **N' HL2- SD1-CP-SD2 -C-['IL-15R α domain'] C'**

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

In some [embodimentexamples](#), the first polypeptide chain comprises:

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

30 and the second polypeptide chain comprises:

N' HL2-['IL-15R α domain']- SD1-CP-SD2 - C C'

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

35 In some [embodimentexamples](#), the 'IL-15R α domain' comprises the wild-type sushi domain sIL-15R α or an sIL-15R α variant.

In some [embodimentexamples](#), the IL-15R α subunit or a fragment thereof comprises an amino acid sequence comprising one of SEQ ID NOs: 55, 56, 57, and 58.

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

In some [embodimentexamples](#), the non-cleavable linker is between 4 and 6 amino acids in length.

In some [embodimentexamples](#), the non-cleavable linker includes a 'GS' repeat.

In some [embodimentexamples](#), 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 [embodimentexamples](#), 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 [embodimentexamples](#), the masked cytokine further comprises a third polypeptide chain, comprising:

N' IL-15R α domain C'

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

In some [embodimentexamples](#), the spacer domains are rich in amino acid residues G, S and P.

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

In some [embodimentexamples](#), SD1 comprises an amino acid sequence comprising one of SEQ ID NOs: 24-32

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

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

In some embodimentexamples, the non-cleavable linker is between 18 and 22 amino acids in length.

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

In some embodimentexamples, the non-cleavable linker comprises an amino acid sequence as shown in SEQ ID NO: 21.

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

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

15 In some embodimentexamples, 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.

20 In some embodimentexamples, 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.

25 In some embodimentexamples, 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.

30 In some embodimentexamples, 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.

35 In some embodimentexamples, 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 embodimentexamples, 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 embodimentexamples, 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.

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

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

10

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

15

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

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

20

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

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

25

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

30

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

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

35

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

In some [embodimentexamples](#), the IL-15 cytokine or functional fragment thereof comprises an amino acid sequence of SEQ ID NO: 132.

In some [embodimentexamples](#), the IL-15 cytokine or functional fragment thereof comprises an amino acid sequence of SEQ ID NO: 133.

In some [embodimentexamples](#), the IL-15 cytokine or functional fragment thereof comprises an additional mutation at position N71.

In some [embodimentexamples](#), the IL-15 cytokine or functional fragment thereof comprises an additional mutation at position S73.

In some [embodimentexamples](#), 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 [embodimentexamples](#), 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-15R α 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-15R α domain comprises the IL-15R α subunit or a fragment thereof flanked on either side by a non-cleavable ' α subunit linker'.

In some [embodimentexamples](#), the non-cleavable linker is between 4 and 6 amino acids in length.

In some [embodimentexamples](#), the non-cleavable linker includes a 'GS' repeat.

In some [embodimentexamples](#), the non-cleavable linker comprises an amino acid sequence as shown in SEQ ID NO: 22.

In some [embodimentexamples](#), the spacer domains are rich in amino acid residues G, S and P.

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

In some [embodimentexamples](#), SD1 comprises an amino acid sequence comprising one of SEQ ID NOs: 24-32.

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

In some [embodimentexamples](#), SD2 comprises an amino acid sequence comprising one of SEQ ID NOs: 35-40.

5 In some [embodimentexamples](#), the 'IL-15R α domain' comprises the wild-type sushi domain sIL-15R α or an sIL-15R α variant.

In some [embodimentexamples](#), the IL-15R α subunit or a fragment thereof comprises an amino acid sequence comprising one of SEQ ID NOs: 55, 56, 57, and 58.

10

In some [embodimentexamples](#), the 'IL-15R α domain' comprises an amino acid sequence of SEQ ID NO: 56.

15 In some [embodimentexamples](#), the IL-15R α comprises an amino acid substitution at position R26. In some [embodimentexamples](#), the IL-15R α comprises amino acid substitution R26N. In some [embodimentexamples](#), the IL-15R α comprises amino acid substitution R26S. In some [embodimentexamples](#), the IL-15R α comprises an amino acid substitution at position R35. In some [embodimentexamples](#), the IL-15R α comprises amino acid substitution R35Q. In some [embodimentexamples](#), the IL-15R α comprises amino acid substitution R35S. In some [embodimentexamples](#), the IL-15R α comprises an amino acid substitution at positions R26 and R35. In some [embodimentexamples](#), the IL-15R α comprises amino acid substitutions R26S or R26N, and R35Q or R35S. In some [embodimentexamples](#), the IL-15R α comprises amino acid substitutions R26N and R35Q.

20

In some [embodimentexamples](#), the IL-15R α 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 [embodimentexamples](#), the IL-15R α comprises an amino acid sequence of SEQ ID NOs: 55, 56, 57 or 58, with additional amino acid substitution R26N. In some [embodimentexamples](#), the IL-15R α comprises an amino acid sequence of SEQ ID NOs: 55, 56, 57 or 58, with additional amino acid substitution R26S. In some [embodimentexamples](#), the IL-15R α 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 [embodimentexamples](#), the IL-15R α comprises an amino acid sequence of SEQ ID NOs: 55, 56, 57 or 58, with additional amino acid substitution R35Q. In some [embodimentexamples](#), the IL-15R α comprises an amino acid sequence of SEQ ID NOs: 55, 56, 57 or 58, with additional amino acid substitution R35S. In some [embodimentexamples](#), the IL-15R α 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 [embodimentexamples](#), the IL-15R α 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 [embodimentexamples](#), the IL-15R α comprises an amino acid sequence of SEQ ID NOs: 55, 56, 57 or 58, with additional amino acid substitutions R26N and R35Q.

25

30

35

In some [embodimentexamples](#), the IL-15R α comprises an amino acid sequence of SEQ ID NO: 56, with an additional amino acid substitution at position R26. In some [embodimentexamples](#), the IL-15R α comprises an amino acid sequence of SEQ ID NO: 56, with additional amino acid substitution R26N. In some [embodimentexamples](#), the IL-15R α comprises an amino acid sequence of SEQ ID NO: 56, with additional amino acid substitution R26S. In some [embodimentexamples](#), the IL-15R α comprises an amino acid sequence of SEQ ID NO: 56, with an additional amino acid substitution at position R35. In some [embodimentexamples](#), the IL-15R α comprises an amino acid sequence of SEQ ID NO: 56, with additional amino acid substitution R35Q. In some [embodimentexamples](#), the IL-15R α comprises an amino acid sequence of SEQ ID NO: 56, with additional amino acid substitution R35S. In some [embodimentexamples](#), the IL-15R α comprises an amino acid sequence of SEQ ID NO: 56, with additional amino acid substitutions at positions R26 and R35. In some [embodimentexamples](#), the IL-15R α comprises an amino acid sequence of SEQ ID NO: 56, with additional amino acid substitutions R26S or R26N, and R35Q or R35S. In some [embodimentexamples](#), the IL-15R α 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 [embodimentexamples](#), 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 [embodimentexamples](#), 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 [embodimentexamples](#), 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 [embodimentexamples](#), 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 [embodimentexamples](#), 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 [embodimentexamples](#), the cleavable peptide comprises an amino acid sequence of SEQ ID NO: 33.

In some [embodimentexamples](#), the cleavable peptide comprises an amino acid sequence of SEQ ID NO: 34.

In some [embodimentexamples](#), the cleavable peptide within the proteolytically cleavable linker comprises SEQ ID

NO: 155.

In some [embodimentexamples](#), the cleavable peptide within the proteolytically cleavable linker comprises SEQ ID NO: 156.

5

In some [embodimentexamples](#), 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.

10 In some [embodimentexamples](#), 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.

15 In some [embodimentexamples](#), 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 [embodimentexamples](#), 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.

20 In some [embodimentexamples](#), 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.

25 In some [embodimentexamples](#), 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 [embodimentexamples](#), the first and/ or second Fc domains each contain one or more modifications that promote the non-covalent association of the first and the second half-life extension domains.

30

In some [embodimentexamples](#), the first and second half-life extension domains are each an IgG1 Fc domain or fragment thereof.

35 In some [embodimentexamples](#), 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 embodiment examples, 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.

5 In some embodiment examples, 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.

10 In some embodiment examples, 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.

15 In some embodiment examples, 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 embodiment examples, the masking moiety comprises IL-15R β or a fragment or variant thereof.

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

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

25 In some embodiment examples, the masking moiety comprises IL-15R β variant or a fragment thereof having amino acid substitution C122S.

30 In some embodiment examples, the masking moiety comprises IL-15R β variant or a fragment thereof having an amino acid substitution at position C168.

In some embodiment examples, the masking moiety comprises IL-15R β variant or a fragment thereof having amino acid substitution C168S.

35 In some embodiment examples, the masking moiety comprises IL-15R β variant or a fragment thereof having an amino acid substitution at positions C122 and C168.

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

| In another aspect, the present disclosure provides 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-15R α 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-15R α domain' is a domain comprising an IL-15R α subunit or a fragment thereof and the 'IL-15R α domain' is non-covalently bonded to the IL-15 cytokine or functional fragment thereof (C) in the second polypeptide chain.

In some ~~embodiment~~examples, 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-15R α domain C'

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

In some ~~embodiment~~examples, 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:

5 **N' IL-15R α domain C'**

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

10 In some [embodimentexamples](#), the 'IL-15R α 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.

15 In some [embodimentexamples](#), 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.

20 In some [embodimentexamples](#), 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 [embodimentexamples](#), the masking moiety comprises IL-15R β or a fragment or variant thereof.

25 In some [embodimentexamples](#), the masking moiety comprises the amino acid sequence of SEQ ID NO: 12.

In some [embodimentexamples](#), the first polypeptide chain comprises an amino acid sequence of SEQ ID NO: 177.

30 In some [embodimentexamples](#), the second polypeptide chain comprises an amino acid sequence of SEQ ID NO: 183.

35 In some [embodimentexamples](#), 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 [embodimentexamples](#), 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 embodiment examples, the IL-15 cytokine or functional fragment thereof comprises an amino acid sequence of SEQ ID NO: 130.

In some embodiment examples, the 'IL-15R α domain' comprises the wild-type sushi domain sIL-15R α or a variant thereof.

In some embodiment examples, the 'IL-15R α domain' comprises an amino acid sequence of SEQ ID NO: 56.

In some embodiment examples, 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 embodiment examples, 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 embodiment examples, 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.

In another aspect, the present disclosure provides 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 embodiment examples, SD is SD2 described herein.

In some embodiment examples, the proteolytically cleavable peptide is described herein.

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

In some embodiment examples, the cleavage product further comprises a domain comprising an IL-15R α subunit or a fragment thereof.

In some [embodimentexamples](#), the IL-15R α subunit or a fragment thereof is non-covalently bonded to the IL-15 cytokine (C).

5 In some [embodimentexamples](#), the cleavage product comprises:

PCP-SD-['IL-15R α domain']-C

In some [embodimentexamples](#), the cleavage product comprises:

PCP-SD-C-['IL-15R α domain']

10

In some [embodimentexamples](#), the IL-15R α subunit or a fragment thereof comprises an amino acid sequence comprising one of SEQ ID NOs: 55, 56, 57, and 58.

15 In some [embodimentexamples](#), the IL-15R α comprises an amino acid substitution at position R26. In some [embodimentexamples](#), the IL-15R α comprises amino acid substitution R26N. In some [embodimentexamples](#), the IL-15R α comprises amino acid substitution R26S. In some [embodimentexamples](#), the IL-15R α comprises an amino acid substitution at position R35. In some [embodimentexamples](#), the IL-15R α comprises amino acid substitution R35Q. In some [embodimentexamples](#), the IL-15R α comprises amino acid substitution R35S. In some [embodimentexamples](#), the IL-15R α comprises an amino acid substitution at positions R26 and R35. In some [embodimentexamples](#), the IL-15R α comprises amino acid substitutions R26S or R26N, and R35Q or R35S. In some [embodimentexamples](#), the IL-15R α comprises amino acid substitutions R26N and R35Q.

25 In some [embodimentexamples](#), the IL-15R α 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 [embodimentexamples](#), the IL-15R α comprises an amino acid sequence of SEQ ID NOs: 55, 56, 57 or 58, with additional amino acid substitution R26N. In some [embodimentexamples](#), the IL-15R α comprises an amino acid sequence of SEQ ID NOs: 55, 56, 57 or 58, with additional amino acid substitution R26S. In some [embodimentexamples](#), the IL-15R α 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 [embodimentexamples](#), the IL-15R α comprises an amino acid sequence of SEQ ID NOs: 55, 56, 57 or 58, with additional amino acid substitution R35Q. In some [embodimentexamples](#), the IL-15R α comprises an amino acid sequence of SEQ ID NOs: 55, 56, 57 or 58, with additional amino acid substitution R35S. In some [embodimentexamples](#), the IL-15R α 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 [embodimentexamples](#), the IL-15R α 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 [embodimentexamples](#), the IL-15R α comprises an amino acid sequence of SEQ ID NOs: 55, 56, 57 or 58, with additional amino acid substitutions R26N and R35Q.

In some [embodimentexamples](#), the IL-15R α comprises an amino acid sequence of SEQ ID NO: 56, with an additional amino acid substitution at position R26. In some [embodimentexamples](#), the IL-15R α comprises an amino acid sequence of SEQ ID NO: 56, with additional amino acid substitution R26N. In some [embodimentexamples](#), the IL-15R α comprises an amino acid sequence of SEQ ID NO: 56, with additional amino acid substitution R26S. In some [embodimentexamples](#), the IL-15R α comprises an amino acid sequence of SEQ ID NO: 56, with an additional amino acid substitution at position R35. In some [embodimentexamples](#), the IL-15R α comprises an amino acid sequence of SEQ ID NO: 56, with additional amino acid substitution R35Q. In some [embodimentexamples](#), the IL-15R α comprises an amino acid sequence of SEQ ID NO: 56, with additional amino acid substitution R35S. In some [embodimentexamples](#), the IL-15R α comprises an amino acid sequence of SEQ ID NO: 56, with additional amino acid substitutions at positions R26 and R35. In some [embodimentexamples](#), the IL-15R α comprises an amino acid sequence of SEQ ID NO: 56, with additional amino acid substitutions R26S or R26N, and R35Q or R35S. In some [embodimentexamples](#), the IL-15R α 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-15R α domain']-[C' terminal domain of C]

where the IL-15R α domain comprises an IL-15R α 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 [embodimentexamples](#), SD is SD1 described herein.

In some [embodimentexamples](#), the proteolytically cleavable peptide is described herein.

In some [embodimentexamples](#), the IL-15 cytokine or a fragment thereof is described herein.

In some ~~embodiment~~examples, the first and second half life extension domains are described herein.

In some ~~embodiment~~examples, the non-cleavable linker L2 is described herein.

- 5 In some ~~embodiment~~examples, the cleavage product further comprises a IL-15R α subunit or a fragment thereof non-covalently bonded to the IL-15 cytokine (C).

10 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.

~~In yet another aspect, the present disclosure p~~In yet another aspect, the present disclosure p~~Provides~~Provides herein ~~is~~ a nucleic acid encoding a masked IL-15 cytokine described herein.

- 15 ~~In yet another aspect, the present disclosure p~~In yet another aspect, the present disclosure p~~Provides~~Provides herein ~~is~~ a nucleic acid encoding one of the chains of the masked IL-15 cytokine described herein.

~~In yet another aspect, the present disclosure p~~In yet another aspect, the present disclosure p~~Provides~~Provides herein ~~is~~ a vector comprising a nucleic acid described herein.

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

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

~~In another aspect, the present disclosure provides a host cell comprising the nucleic acid encoding the masked IL-15 cytokine as described herein.~~In another aspect, the present disclosure provides a host cell comprising the nucleic acid encoding the masked IL-15 cytokine as described herein. Provided herein is a host cell comprising a nucleic acid described herein.

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

35 ~~In another aspect, the present disclosure p~~In another aspect, the present disclosure p~~Provides~~Provides herein ~~is~~ a composition comprising a masked IL-15 cytokine described herein.

~~In another aspect, the present disclosure p~~In another aspect, the present disclosure p~~Provides~~Provides herein ~~is~~ a pharmaceutical composition comprising a masked IL-15 cytokine described herein, and a pharmaceutically acceptable carrier.

| In some embodiment examples, the pharmaceutical composition is in single unit dosage form.

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

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

In some ~~embodiment~~examples, 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.

In another aspect, the present disclosure provides 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.

In yet another aspect, the present disclosure provides a use of a masked IL-15 cytokine as defined herein in the manufacture of a medicament for treating cancer.

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 embodiment examples, the cancer is a solid tumor.

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

5 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.

10 In some embodiment examples, the cancer is a solid tumor.

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

15 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*.

20 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 embodiment examples, the cancer is a solid tumor.

BREIF DESCRIPTION OF THE DRAWINGS

25 **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
30 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.

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-15R α domain']-SD1-CP-SD2-C-C'~~

~~where 'IL-15R α domain' is a domain comprising an IL-15R α 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-15R α domain']-C-C'~~

~~where 'IL-15R α domain' is a domain comprising an IL-15R α subunit or a fragment thereof.~~



10. ~~The masked IL-15 cytokine of claim 8 or claim 9, wherein 'IL-15R α domain' comprises the wild-type sushi domain sIL-15R α or an sIL-15R α variant.~~

11. ~~The masked IL-15 cytokine of claim 8 or claim 9, wherein the IL-15R α 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-15R α 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.~~

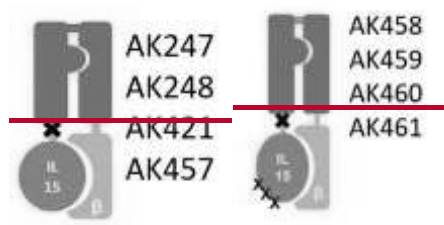
~~14. The masked IL-15 cytokine of claim 13, wherein the first polypeptide chain comprises:-~~

~~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-15R α domain C'

where the 'IL-15R α domain' is a domain comprising an IL-15R α subunit or a fragment thereof and wherein the 'IL-15R α 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-15R α 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-15R α domain']-C'

where the IL-15R α domain comprises the IL-15R α 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-15R α domain']-SD1-CP-SD2-C C'

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



23. The masked IL-15 cytokine of any one of claims 20 to 22, wherein the 'IL-15R α domain' comprises the wild-type sushi domain sIL-15R α or an sIL-15R α variant.

~~24. The masked IL-15 cytokine of any one of claims 20 to 22, wherein the IL-15R α 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-15R α 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-15R α domain-C'~~

~~where the 'IL-15R α domain' is a domain comprising an IL-15R α subunit or a fragment thereof and wherein the 'IL-15R α 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 one of 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 one of 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 (GGSSPPGGGSSGGSGPSGSPG).~~
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 of 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.~~

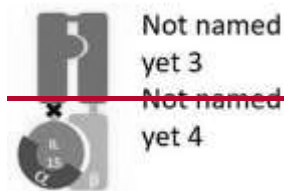
~~63. The masked IL-15 cytokine of claim 62, wherein the first polypeptide chain comprises:-~~

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

~~and the second polypeptide chain comprises:~~

~~N' HL2-SD1-CP-SD2-[N' terminal domain of C]-[IL-15R α 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-15R α domain comprises the IL-15R α 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-15R α domain' comprises the wild-type sushi domain sIL-15R α or an sIL-15R α variant.~~
- ~~73. The masked IL-15 cytokine of any one of claims 63 to 71, wherein the IL-15R α 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-15R α 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-15R β 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-15R β 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-15R β 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-15R β 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-15R β 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-15R β 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-15R α 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-15R α domain' is a domain comprising an IL-15R α subunit or a fragment thereof and the 'IL-15R α 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-15R α domain C'~~

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

~~105.2. 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-15R α 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-15R α domain' is a domain comprising an IL-15R α subunit or a fragment thereof and the 'IL-15R α 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-15R α -domain C'~~

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

~~107.3.~~ The masked IL-15 cytokine of ~~any one of claims 103 to 106~~claim 2, 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.

~~108.4.~~ The masked IL-15 cytokine of any one of claims ~~103 to 107~~2-3, 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.

~~109.5.~~ The masked IL-15 cytokine of any one of claims ~~103 to 108~~2-4, wherein the masking moiety comprises IL-15R β 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.~~

~~111.6.~~ The masked IL-15 cytokine of ~~any one of claims 103 or 104~~2-5, wherein the first polypeptide chain comprises an amino acid sequence of SEQ ID NO: 177.

~~112.7.~~ The masked IL-15 cytokine of ~~any one of claims 105 or 106~~2-6, 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-15R α domain' comprises the wild-type sushi domain sIL-15R α or a variant thereof.~~

~~117. The masked IL-15 cytokine of any one of claims 103 to 116, wherein the 'IL-15R α 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.~~

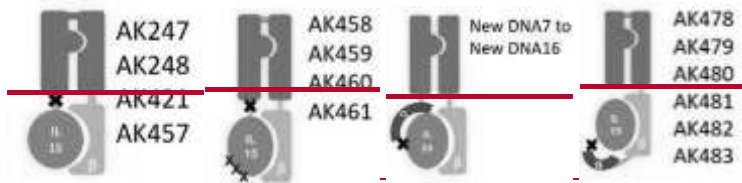
~~120. The masked IL-15 cytokine of claim 13, wherein the first polypeptide chain comprises an amino acid sequence of SEQ ID NO: 177 and the second polypeptide chain comprises an amino acid sequence of SEQ ID NO: 194.~~

~~121.8.~~ 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~~ 1-7.

~~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-15R α subunit or a fragment thereof.~~

127. ~~The cleavage product of claim 126, wherein the IL-15R α subunit or a fragment thereof is non-covalently bonded to the IL-15 cytokine (C).~~

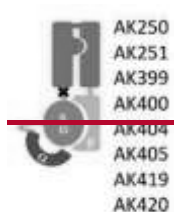
128. ~~The cleavage product of claim 126, wherein the cleavage product comprises:~~

PCP-SD-['IL-15R α domain']-C



129. ~~The cleavage product of claim 126, wherein the cleavage product comprises:~~

PCP-SD-C-['IL-15R α domain']

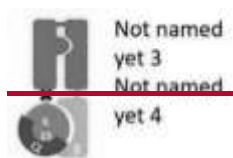


~~130. The cleavage product of any one of claims 126 to 129, wherein the IL-15R α subunit or a fragment thereof comprises an amino acid sequence comprising one of SEQ ID NOs: 55, 56, 57, and 58, optionally with an additional amino acid substitution at position R26 and/or R35.~~

~~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-15R α domain]-[C' terminal domain of C]**~~

~~where the IL-15R α domain comprises an IL-15R α 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-15 α 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.~~

~~140.9.~~ A nucleic acid encoding the masked IL-15 cytokine of any one of claims ~~1 to 120~~1-7, or encoding one of the chains of the masked IL-15 cytokine.

~~141.10.~~ A vector comprising the nucleic acid of claim ~~140~~9.

~~142.11.~~ A host cell comprising the nucleic acid encoding the masked IL-15 cytokine of any one of claims ~~1 to 120~~1-7.

~~143.12.~~ A composition comprising the masked IL-15 cytokine of any one of claims ~~1 to 120~~1-7.

~~144.13.~~ A pharmaceutical composition comprising the masked IL-15 cytokine of any one of claims ~~1 to 120~~1-7, 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~~.

~~150.14.~~ 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 ~~142-11~~ 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;15. — Use of aA masked IL-15 cytokine as defined in any one of claims 1 to 1201-7 for use in the manufacture of a medicament for~~ 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.~~