CLAIMS

WHAT IS CLAIMED IS:

1. A modified interleukin-18 (IL-18) polypeptide that preferentially binds IL-18 receptor (IL-18R) over IL-18 binding protein (IL-18BP) comprising:

(i) at least five mutations in at least five of the following positions: Y1, L5, K8, M51, K53, S55, Q56, P57, G59, M60, E77, Q103, S105, D110, N111, M113, V153, and N155 relative to wild type (WT) IL-18 as set forth in SEQ ID NO: 30; and

(ii) mutations at amino acid positions C38 and C68 relative to SEQ ID NO: 30, wherein said modified IL-18 preferentially binds IL-18R over IL-18BP, wherein the modified IL-18 polypeptide comprises cysteine residues at positions C76 and C127 relative to SEQ ID NO: 30.

2. The modified IL-18 polypeptide of claim 1, wherein the mutation at position C38 is C38S and the mutation at position C68 is:

(a) C68S, C68G, C68A, C68V, C68D, C68E, or C68N;

(b) C68S, C68G, C68A, C68D, or C68N;

(c) C68G, C68A, C68V, C68D, C68E, or C68N;

(d) C68G, C68A, C68D, or C68N;

(e) C68S;

(f) C68G; or

(g) C68D.

3. The modified IL-18 polypeptide of claim 1, wherein the mutation at position C38 is C38S and the mutation at position C68 is C68D.

4. The modified IL-18 polypeptide of any one of claims 1-3, wherein the modified IL-18 polypeptide comprises:

(a) mutations in at least two of the following positions: M51, K53, Q56, P57, M60, Q103, S105, D110, N111, and M113, relative to SEQ ID NO: 30;

(b) mutations in at least five of the following positions: M51, K53, Q56, P57, M60, Q103, S105, D110, N111, and M113, relative to SEQ ID NO: 30;

(c) at least 5 of the following 18 mutations: (1) Y1H, or Y1R, (2) L5H, L5I, or L5Y, (3) K8Q, or K8R, (4) M51T, M51K, M51D, M51N, M51E, or M51R, (5) K53R, K53G,

K53S, or K53T, (6) S55K, or S55R, (7) Q56E, Q56A, Q56R, Q56V, Q56G, Q56K, or Q56L, (8) P57L, P57G, P57A, or P57K, (9) G59T, or G59A, (10) M60K, M60Q, M60R, or M60L, (11) E77D, (12) Q103E, Q103K, Q103P, Q103A, or Q103R, (13) S105R, S105D, S105K, S105N, or S105A, (14) D110H, D110K, D110N, D110Q, D110E, D110S, or D110G, (15) N111H, N111Y, N111D, N111R, N111S, or N111G, (16) M113V, M113R, M113T, or M113K, (17) V153I, V153T, or V153A, and (18) N155K, or N155H, relative to SEQ ID NO: 30; or

(d) at least 5 of the following 10 mutations: (1) M51T, M51K, M51D, M51N, M51E, or M51R, (2) K53R, K53G, K53S, or K53T, (3) Q56E, Q56A, Q56R, Q56V, Q56G, Q56K, or Q56L, (4) P57L, P57G, P57A, or P57K, (5) M60K, M60Q, M60R, or M60L, (6) Q103E, Q103K, Q103P, Q103A, or Q103R, (7) S105R, S105D, S105K, S105N, or S105A, (8) D110H, D110K, D110N, D110Q, D110E, D110S, or D110G, (9) N111H, N111Y, N111D, N111R, N111S, or N111G, and (10) M113V, M113R, M113T, or M113K, relative to SEQ ID NO: 30.

5. The modified IL-18 polypeptide of claim 4, wherein the modified IL-18 polypeptide comprises mutations at positions M51, K53, Q56, D110, and N111, relative to SEQ ID NO: 30.

6. The modified IL-18 polypeptide of claim 5, wherein the modified IL-18 polypeptide further comprises mutations at positions P57 and M60 relative to SEQ ID NO: 30.

7. The modified IL-18 polypeptide of claim 6, wherein the modified IL-18 polypeptide further comprises a mutation at position S105, relative to SEQ ID NO: 30.

8. The modified IL-18 polypeptide of claim 4, wherein the modified IL-18 polypeptide comprises at least five of the following seven mutations:

(1) M51E, M51R, M51K, M51T, M51D, or M51N;

(2) K53G, K53S, K53T, or K53R;

(3) Q56G, Q56R, Q56L, Q56E, Q56A, Q56V, or Q56K;

(4) D110S, D110N, D110G, D110K, D110H, D110Q, or D110E;

(5) N111G, N111R, N111S, N111D, N111H, or N111Y;

(6) P57A, P57L, P57G, or P57K; and

(7) M60L, M60R, M60K, or M60Q,

relative to SEQ ID NO: 30.

9. The modified IL-18 polypeptide of claim 8, wherein the modified IL-18 polypeptide further comprises the mutation S105D, S105A, S105N, S105R, S105D, or S105K relative to SEQ ID NO: 30.

10. The modified IL-18 polypeptide of any one of claims 1-3, wherein the modified IL-18 polypeptide comprises the mutations M51K, K53S, Q56L, D110S, and N111R, relative to SEQ ID NO: 30.

11. The modified IL-18 polypeptide of claim 10, wherein the modified IL-18 polypeptide further comprises the mutations P57A, M60L, and S105D.

12. The modified IL-18 polypeptide of claim 11, wherein the modified IL-18 polypeptide comprises the amino acid sequence set forth in any one of: (a) SEQ ID NOs: 1-21; (b) SEQ ID NOs: 6 and 16-21; or (c) SEQ ID NOs: 6, 16, 17, 19, and 21.

13. The modified IL-18 polypeptide of claim 11, wherein the modified IL-18 polypeptide comprises: (a) an amino acid sequence having 85% or more sequence identity with the amino acid sequence set forth in SEQ ID NO: 19; or (b) comprises the amino acid sequence set forth in SEQ ID NO: 19.

14. The modified IL-18 polypeptide of any one of claims 1-13, wherein the modified IL-18 polypeptide comprises mutations, relative to wild-type IL-18 as set forth in SEQ ID NO:30, at positions:

(i) M51, M60, S105, D110, and N111;
(ii) M51, S55, G59, M60, S105, D110, N111, and V153;
(iii) Y1, M51, M60, S105, D110, and N111;
(iv) Y1, M51, K53, M60, S105, D110, and N111;
(v) K8, M51, S55, G59, M60, S105, D110, and N155;
(vi) K8, M51, S55, G59, M60, S105, D110, N111, and V153;
(vii) L5, M51, K53, M60, S105, D110, and V153;
(viii) L5, M51, S55, G59, M60, S105, D110, N111, and N155;
(ix) L5, M51, S55, M60, Q103, S105, D110, N111, and V153;

(x) L5, M51, S55, M60, S105, D110, N111, V153, and N155;

(xi) L5, M51, S55, G59, M60, S105, D110, N111, V153, and N155;

(xii) L5, K8, M51, S55, M60, S105, N111, V153, and N155;

(xiii) L5, K8, M51, K53, M60, S105, D110, N111, and N155;

(xiv) Y1, L5, M51, K53, M60, S105, D110, and N155;

(xv) Y1, M51, K53, G59, M60, S105, D110, N111, V153, and N155;

(xvi) Y1, K8, M51, K53, M60, Q103, S105, D110, N111, and N155;

(xvii) Y1, K8, M51, M60, S105, D110, and N111;

(xviii) Y1, L5, M51, K53, M60, Q103, S105, D110, and N111;

(xix) Y1, K8, M51, K53, G59, M60, Q103, S105, D110, N111, V153, and N155;

(xx) Y1, K8, M51, K53, G59, M60, S105, D110, N111, and N155;

(xxi) Y1, K8, M51, G59, M60, Q103, S105, D110, N111, V153, and N155;

(xxii) Y1, L5, M51, G59, M60, E77, S105, D110, and N111;

(xxiii) M51, Q56, P57, M60, Q103, S105, D110, N111, and M113;

(xxiv) M51, Q56, P57, M60, Q103, S105, D110, and M113;

(xxv) M51, K53, Q56, P57, M60, D110, and N111;

(xxvi) M51, K53, Q56, P57, M60, Q103, S105, D110, N111, and M113;

(xxvii) M51, K53, Q56, M60, Q103, S105, D110, N111, and M113;

(xxviii) M51, K53, Q56, P57, Q103, S105, D110, N111, and M113;

(xxix) M51, K53, Q56, P57, M60, S105, D110, and N111;

(xxx) M51, K53, Q56, P57, M60, Q103, D110, N111, and M113;

(xxxi) M51, Q56, P57, M60, Q103, D110, N111, and M113; or

(xxxii) M51, K53, Q56, S105, D110, and N111.

15. A pharmaceutical composition comprising the modified IL-18 polypeptide of any one of claims 1-14, and a pharmaceutically acceptable excipient.

16. The composition of claim 15, wherein the pharmaceutical composition is formulated for subcutaneous injection.

17. The composition of claim 15 or claim 16, comprising:

a buffering agent to maintain to pH between 6.2 and 6.8,

a sugar,

a chelating agent,

an agent to prevent oxidation, and

an agent to prevent protein absorption.

18. An isolated nucleic acid comprising a nucleotide sequence encoding the modified IL-18 polypeptide of any one of claims 1-14.

19. The isolated nucleic acid of claim 18, wherein the nucleic acid is a plasmid or a viral vector.

20. The isolated nucleic acid of claim 18 or claim 19, wherein the nucleotide sequence encoding the IL-18 polypeptide is fused immediately 3' of, and in frame with, a nucleotide sequence encoding a small ubiquitin-like modifier (SUMO) tag that is cleavable by a SUMO protease.

21. The isolated nucleic acid of claim 20, wherein the SUMO protease is ULP1.

22. The isolated nucleic acid of claim 21, wherein the SUMO tag comprises the amino acid sequence set forth in SEQ ID NO: 26 or 27.

23. An isolated cell comprising the nucleic acid of any one of claims 18-22.

24. The isolated cell of claim 23, comprising a nucleic acid comprising a nucleotide sequence encoding a SUMO protease.

25. The isolated cell of claim 23 or claim 24, wherein the cell is a bacterial cell, a yeast cell, an engineered T or NK cell, a chimeric antigen receptor T cell (CAR-T cell), an engineered TCR-T cell, a chimeric antigen receptor NK cell (CAR-NK cell), or a Tumor-infiltrating Lymphocyte (TIL), that expresses the encoded interleukin-18 (IL-18) polypeptide.

26. The modified IL-18 polypeptide of any one of claims 1-14, the composition of any one of claims 15 to 17, the nucleic acid of any one of claims 18 to 22 or the cell of any one of claims 23 to 25 for use in therapy.

27. Use of the modified IL-18 polypeptide of any one of claims 1-14, the composition of any one of claims 15 to 17, the nucleic acid of any one of claims 18 to 22 or the cell of any one of claims 23 to 25 in the manufacture of a medicament for:
(a) promoting IL-18 signaling activity in an individual in need thereof; and/or
(b) the treatment of a cancer or an infectious disease in an individual in need thereof.

28. The use of claim 27, wherein the cancer is a cancer that is resistant to immune checkpoint inhibitors (ICIs), a cancer that is associated with a tumor that has reduced or absent surface expression of MHC class I, a cancer associated with high levels of IL-18BP, a cancer with high tumor mutational burden (TMB), a cancer with tumors with microsatellite instability (MSI) or a cancer associated with a tumor in which IL-18R is expressed on infiltrating T cells or NK cells.

29. A method of making an active polypeptide, comprising:

contacting, inside of a bacterial cell, an exogenously provided fusion protein with an exogenously provided SUMO (small ubiquitin-like modifier) protease, wherein the fusion protein comprises a polypeptide of interest fused at the N-terminus to a SUMO tag,

wherein, within the bacterial cell, the SUMO protease cleaves the fusion protein, thus removing the SUMO tag from the polypeptide of interest, wherein the polypeptide of interest is an IL-18 polypeptide of any one of claims 1-14 that is activated by the removal of the SUMO tag.