CLAIMS

What is claimed is:

1. An IL-18 variant polypeptide comprising:

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

at least two mutations in at least two 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 WT IL-18 (SEQ ID NO:30);

wherein the IL-18 variant polypeptide specifically binds to IL-18 receptor (IL-18R) and exhibits substantially reduced binding to IL-18 binding protein (IL-18BP).

2. The polypeptide of claim 1, wherein the IL-18 variant polypeptide comprises mutations in at least five positions selected from the group consisting of M51, K53, Q56, P57, M60, S105, D110, and N111, relative to SEQ ID NO: 30.

3. The polypeptide of claim 1 or claim 2, wherein the IL-18 variant polypeptide comprises mutations at positions M51, K53, Q56, D110, and N111, relative to SEQ ID NO: 30.

4. The polypeptide of claim 3, wherein the IL-18 variant polypeptide further comprises mutations at positions P57, M60, and S105 relative to SEQ ID NO: 30.

5. An IL-18 variant polypeptide comprising at least one mutation relative to human wild-type (WT) IL-18 (SEQ ID NO: 30), wherein:

(a) the at least one mutation is selected from the group consisting of Y1H, Y1R, L5H, L5I, L5Y, K8Q, K8R, M51T, M51K, M51D, M51N, M51E, M51R, K53R, K53G, K53S, K53T, S55K, S55R, Q56E, Q56A, Q56R, Q56V, Q56G, Q56K, Q56L, P57L, P57G, P57A, P57K, G59T, G59A, M60K, M60Q, M60R, M60L, E77D, Q103E, Q103K, Q103P, Q103A, Q103R, S105R, S105D, S105K, S105N, S105A, D110H, D110K, D110N, D110Q, D110E, D110S,

D110G, N111H, N111Y, N111D, N111R, N111S, N111G, M113V, M113R, M113T, M113K, V153I, V153T, V153A, N155K, and N155H, relative to SEQ ID NO: 30; and

(b) the IL-18 variant polypeptide specifically binds to IL-18 receptor (IL-18R) and exhibits substantially reduced binding to IL-18 binding protein (IL-18BP).

6. The polypeptide of any one of claims 1-5, wherein the IL-18 variant polypeptide comprises an amino acid sequence with at least 90% sequence identity to the amino acid sequence set forth in SEQ ID NO: 30.

7. The polypeptide of any one of claims 1-6, wherein the IL-18 variant polypeptide comprises at least one mutation selected from the group consisting of:

(a) an M51T, M51K, M51D, M51N, M51E, or M51R mutation relative to SEQ ID NO: 30;

(b) a K53R, K53G, K53S, or K53T mutation relative to SEQ ID NO: 30;

(c) a Q56E, Q56A, Q56R, Q56V, Q56G, Q56K, or Q56L mutation relative to SEQ ID NO: 30;

(d) a P57L, P57G, P57A, or P57K mutation relative to SEQ ID NO: 30;

(e) an M60K, M60Q, M60R, or M60L mutation relative to SEQ ID NO: 30;

(f) an S105R, S105D, S105K, S105N, or S105A mutation relative to SEQ ID NO: 30;

(g) a D110H, D110K, D110N, D110Q, D110E, D110S, or D110G mutation relative to SEQ ID NO: 30; and

(h) an N111H, N111Y, N111D, N111R, N111S, or N111G mutation relative to SEQ ID NO: 30.

8. The polypeptide of any one of claims 1-7, wherein the IL-18 variant polypeptide comprises at least three of:

(a) an M51T, M51K, M51D, M51N, M51E, or M51R mutation relative to SEQ ID NO: 30;

(b) a K53R, K53G, K53S, or K53T mutation relative to SEQ ID NO: 30;

(c) a Q56E, Q56A, Q56R, Q56V, Q56G, Q56K, or Q56L mutation relative to SEQ ID NO: 30;

(d) a P57L, P57G, P57A, or P57K mutation relative to SEQ ID NO: 30;

(e) an M60K, M60Q, M60R, or M60L mutation relative to SEQ ID NO: 30;

(f) an S105R, S105D, S105K, S105N, or S105A mutation relative to SEQ ID NO: 30;

(g) a D110H, D110K, D110N, D110Q, D110E, D110S, or D110G mutation relative to SEQ ID NO: 30; and

(h) an N111H, N111Y, N111D, N111R, N111S, or N111G mutation relative to SEQ ID NO: 30.

9. The polypeptide of any one of claims 1-7, wherein the IL-18 variant polypeptide comprises:

(a) an M51T, M51K, M51D, M51N, M51E, or M51R mutation relative to SEQ ID NO: 30;

(b) a K53R, K53G, K53S, or K53T mutation relative to SEQ ID NO: 30;

(c) a Q56E, Q56A, Q56R, Q56V, Q56G, Q56K, or Q56L mutation relative to SEQ ID NO: 30;

(d) a D110H, D110K, D110N, D110Q, D110E, D110S, or D110G mutation relative to SEQ ID NO: 30; and

(e) an N111H, N111Y, N111D, N111R, N111S, or N111G mutation relative to SEQ ID NO: 30.

10. The polypeptide of any one of claims 1-7, wherein the IL-18 variant polypeptide comprises:

(a) an M51T, M51K, M51D, M51N, M51E, or M51R mutation relative to SEQ ID NO: 30;

(b) a K53R, K53G, K53S, or K53T mutation relative to SEQ ID NO: 30;

(c) a Q56E, Q56A, Q56R, Q56V, Q56G, Q56K, or Q56L mutation relative to SEQ ID NO: 30;

(d) a P57L, P57G, P57A, or P57K mutation relative to SEQ ID NO: 30;

(e) an M60K, M60Q, M60R, or M60L mutation relative to SEQ ID NO: 30;

(f) a D110H, D110K, D110N, D110Q, D110E, D110S, or D110G mutation relative to SEQ ID NO: 30; and

(g) an N111H, N111Y, N111D, N111R, N111S, or N111G mutation relative to SEQ ID NO: 30.

11. The polypeptide of any one of claims 1-7, wherein the IL-18 variant polypeptide comprises:

(a) an M51K or M51R mutation relative to SEQ ID NO: 30;

(b) a K53G or K53S mutation relative to SEQ ID NO: 30;

(c) a Q56R, Q56G, or Q56L mutation relative to SEQ ID NO: 30;

(d) a P57A mutation relative to SEQ ID NO: 30;

(e) an M60L mutation relative to SEQ ID NO: 30;

(f) an S105D, S105N, or S105A mutation relative to SEQ ID NO: 30;

(g) a D110N, D110S, or D110G mutation relative to SEQ ID NO: 30; or

(h) an N111R or N111G mutation relative to SEQ ID NO: 30.

12. The polypeptide of claim 11, wherein the IL-18 variant polypeptide comprises at least five of the mutations recited in (a) to (h).

13. The polypeptide of any one of claims 1-12, wherein the IL-18 variant polypeptide comprises:
(a) an M51K mutation relative to SEQ ID NO: 30;
(b) a K53S mutation relative to SEQ ID NO: 30;
(c) a Q56L mutation relative to SEQ ID NO: 30; and
(d) an N111R mutation relative to SEQ ID NO: 30.

14. The polypeptide of claim 13, wherein the IL-18 variant polypeptide further comprises:
(e) a P57A mutation relative to SEQ ID NO: 30;
(f) a M60L mutation relative to SEQ ID NO: 30;
(g) an S105D mutation relative to SEQ ID NO: 30; and
(h) a D110S mutation relative to SEQ ID NO: 30.

15. The polypeptide of any one of claims 1, 2 or 5-8, wherein the IL-18 variant polypeptide comprises:
(a) mutations at positions M51, M60, S105, D110, and N111, relative to SEQ ID NO: 30; or
(b) mutations at positions M51, K53, Q56, S105, and N111, relative to SEQ ID NO: 30.

16. The polypeptide of claim 1, wherein the IL-18 variant polypeptide comprises the amino acid sequence set forth in any one of SEQ ID NOs: 34-59, 73-91, 191-193, or a fragment thereof.

17. The polypeptide of any one of claims 1-16, wherein the IL-18 variant polypeptide has an EC50 for IL-18BP that is at least 10-fold higher than the EC50 of WT IL-18 to IL-18BP.

18. A nucleic acid molecule encoding the IL-18 variant polypeptide of any one of claims 1-17.

19. An engineered immune cell comprising a nucleic acid encoding the IL-18 variant polypeptide of any one of claims 1-17.

20. The engineered immune cell of claim 19, wherein the cell is selected from the group consisting of a CAR-T cell, a CAR-NK cell, a T cell transduced with an engineered T cell receptor, and an NK cell transduced with an engineered T cell receptor.

21. A composition comprising one or more of the IL-18 variant polypeptide of any one of claims 1-17, the nucleic acid molecule of claim 18, and/or the engineered immune cell of claim 19 or 20, and a pharmaceutically-acceptable excipient, vehicle, carrier, or diluent.

22. The composition of claim 21, further comprising one or more agents selected from:

(i) an immune checkpoint inhibitor;

(ii) an agent that inhibits one or more proteins selected from PD-L1, PD1, CTLA4, TIM3, TIGIT, LAG3, B7H3, B7H4, VISTA, ICOS, GITR, 41BB, OX40, and CD40;

(iii) a cancer cell opsonizing agent; and

(iv) an agent that targets one or more antigens selected from: CD19, CD20, CD22, CD24, CD25, CD30, CD33, CD37, CD38, CD44, CD45, CD47, CD51, CD52, CD56, CD62L, CD70, CD74, CD79, CD80, CD96, CD97, CD99, CD123, CD134, CD138, CD152 (CTLA-4), CD200, CD213A2, CD221, CD248, CD276 (B7-H3), B7-H4, CD279 (PD-1), CD274 (PD-L1), CD319, EGFR, EPCAM, 17-1A, HER1, HER2, HER3, CD117, C-Met, HGFR, PDGFRA, AXL, TWEAKR, PTHR2, HAVCR2 (TIM3), GD2 ganglioside, MUC1, mucin CanAg, mesothelin, endoglin, Lewis-Y antigen, CEA, CEACAM1, CEACAM5, CA-125, PSMA, BAFF, FGFR2, TAG-72, gelatinase B, glypican 3, nectin-4, BCMA, CSF1R, SLAMF7, integrin αvβ3, TYRP1, GPNMB, CLDN18.2, FOLR1, CCR4, CXCR4, MICA, C242 antigen, DLL3, DLL4, EGFL7, vimentin, fibronectin extra domain-B, TROP-2, LRRC15, FAP, SLITRK6, NOTCH2, NOTCH3, Tenascin-3, STEAP1, and NRP1.

23. A method of treating or preventing a cancer, metabolic disease or disorder and/or an infectious disease in a human subject in need thereof, the method comprising administering to the

human subject the IL-18 variant polypeptide of any one of claims 1-17 or the nucleic acid molecule of claim 18 or the engineered immune cell of claim 19 or 20 or the composition of claim 21 or 22.

24. Use of the IL-18 variant polypeptide of any one of claims 1-17 or the nucleic acid molecule of claim 18 or the engineered immune cell of claim 19 or 20 or the composition of claim 21 or 22 in the manufacture of a medicament for preventing or treating a cancer, metabolic disease or disorder and/or an infectious disease in a human subject in need thereof.

25. The method of claim 23 or the use of claim 24, wherein the disease or disorder is cancer.

26. The method or use of claim 25, wherein the cancer is a cancer that is resistant to an immune checkpoint inhibitor (ICI).

27. The method or use of claim 25 or 26, wherein the cancer comprises a tumor that has lost expression of major histocompatibility complex (MHC) class I.

28. The method of claim 23 or any one of claims 25-27 or the use of any one of claims 24-27, wherein the treating or preventing comprises administering to the subject the IL-18 variant polypeptide, the nucleic acid molecule, the engineered immune cell and/or the composition and at least one other agent, wherein the at least one other agent comprises an immune checkpoint inhibitor and/or a cancer cell opsonizing agent.

29. The method or use of claim 28, wherein the immune checkpoint inhibitor is an agent that inhibits PD-L1, PD1, CTLA4, TIM3, TIGIT, LAG3, B7H3, B7H4, VISTA, ICOS, GITR, 41BB, OX40, or CD40, or any combination thereof.

30. The method of claim 23 or any one of claims 25-27 or the use of any one of claims 24-27, wherein the treating or preventing comprises administering to the subject the IL-18 variant polypeptide, the nucleic acid molecule, the engineered immune cell and/or the composition and at least one other agent, wherein the at least one other agent is an altered T-cell or NK cell.