CLAIMS

What is claimed is:

~~A composition comprising an~~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 ~~wherein the IL-18 variant polypeptide~~ exhibits substantially reduced binding to IL-18 binding protein (IL-18BP).

~~1.~~ 2. The ~~composition~~polypeptide of claim 1, wherein the IL-18 variant polypeptide comprises mutations in at least ~~one mutation relative to wild-type (WT) IL-18.~~

~~2. The composition of claim 2, wherein the WT IL-18 is human IL-18 comprising the amino acid sequence of SEQ ID NO: 30.~~

~~3. The composition of claim 2, wherein the WT IL-18 is murine IL-18 comprising the amino acid sequence of SEQ ID NO: 31.~~

~~The composition of claim 3, wherein the IL-18 variant polypeptide comprises at least one mutation~~five positions selected from the group consisting of ~~Y1X, L5X, K8X, M51X, K53X, S55X, Q56X, P57X, G59X, M60X, E77X, Q103X, S105X, D110X, N111X, M113X, V153X, and N155X~~M51, K53, Q56, P57, M60, S105, D110, and N111, relative to SEQ ID NO: 30.

~~The composition~~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).

~~4.~~ 6. The ~~composition~~polypeptide of ~~claim 3~~any one of claims 1-5, wherein the IL-18 variant polypeptide comprises ~~the mutations M51X, M60X, S105X, D110X, and N111X, relative to SEQ ID NO: 30.~~

~~5. The composition of claim 3, wherein the IL-18 variant polypeptide comprises the mutations M51X, K53X, Q56X, S105X, and N111X, relative to SEQ ID NO: 30.~~

~~The composition of claim 2, 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.

~~6.~~ 7. The polypeptide of any one of ~~SEQ ID NOs.: 34-59, 73-91, 191-193, or a fragment thereof.~~

~~The composition of claim 4~~claims 1-6, wherein the IL-18 variant polypeptide comprises at least one mutation selected from the group consisting of ~~N1X, M50X, Y51X, K52X, S54X, E55X, V56X, R57X, G58X, L59X, R104X, N109X, and L151X, relative to SEQ ID NO: 31.~~

:

(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 ~~composition~~polypeptide of ~~claim 4~~any one of claims 1-7, wherein the IL-18 variant polypeptide comprises at least ~~one mutation selected from~~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 ~~group consisting of N1H, N1Y, M50A, M50S, M50V, M50G, M50T, Y51R, K52V, K52S, K52T, K52G, K52A, S54R, S54K, S54G, S54N, E55R, E55H, E55N, E55D, E55G, V56L, V56M, V56R, V56A, V56S, V56Q, R57G, R57K, G58A, L59K, L59R, L59V, R104K, R104L, R104Q, R104S, N109D, and L151V, relative to SEQ ID NO: 31.~~

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 ~~composition~~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~~.: 60-72~~: 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 ~~composition~~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 ~~the~~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.

~~7.~~ 22. The composition of ~~any one of claims 1-12.~~

~~The composition of any one of claims 1-13~~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 ~~composition~~IL-18 variant polypeptide of any one of claims 1-~~14.~~ 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 ~~15~~23 or the use of claim 24, wherein the disease or disorder is cancer.

26. The method or use of claim ~~16~~25, wherein the cancer is a cancer that is resistant to an immune checkpoint ~~inhibitors (ICIs~~inhibitor (ICI).

27. The method or use of claim ~~16~~25 or 26, wherein the cancer ~~is associated with~~ comprises a tumor that has lost expression of major histocompatibility complex (MHC) class I.

~~8. The method of claim 15, wherein the disease or disorder is a metabolic disease or disorder.~~

~~9.~~ 28. The method of claim ~~15, wherein the disease~~23 or ~~disorder is an infectious disease.~~

~~10. The method of~~ any one of claims ~~15-20~~25-27 or the use of any one of claims 24-27, wherein the ~~method~~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~~.~~

~~The method of claim 21~~, 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 ~~22~~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.

~~11.~~ 30. The method of claim ~~21, wherein the at least one other agent comprises a cancer cell opsonizing agent.~~

~~12. The method of claim 24, wherein the at least one other agent targets one~~23 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 $\alpha_v\beta_3$, 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.~~

~~13. The method~~ any one of claims 25-27 or the use of any one of claims ~~21-25~~24-27, wherein the ~~at least one other agent is conjugated~~treating or preventing comprises administering to the subject the IL-18 variant polypeptide~~.~~

~~The method of claim 21~~, 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.