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 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 ~~an amino acid sequence having 85% or more sequence identity with~~ wild type (WT) IL-18 as set forth in SEQ ID NO: 30; or

(ii) 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 wild type (WT) IL-18 as set forth in SEQ ID NO: 30~~with the IL-18 variant amino sequence set forth in any one of SEQ ID NOs: 6, 87-91, and 16-21~~; and

(iii) 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.

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)

~~3. The modified IL-18 polypeptide of claim 1, wherein the mutation at position C38 is C38S and the mutation at position C68 is~~ C68S, C68G, C68A, C68D, or C68N;~~.~~

(c)

~~4. The modified IL-18 polypeptide of claim 1, wherein the mutation at position C38 is C38S and the mutation at position C68 is~~ C68G, C68A, C68V, C68D, C68E, or C68N;~~.~~

(d)

~~5. The modified IL-18 polypeptide of claim 1, wherein the mutation at position C38 is C38S and the mutation at position C68 is~~ C68G, C68A, C68D, or C68N;~~.~~

(e) C68S;

(f) C68G; or

(g) C68D.

~~6~~3. The modified IL-18 polypeptide of claim 1, wherein the mutation at position C38 is C38S and the mutation at position C68 is ~~C68S~~C68D.

~~7. The modified IL-18 polypeptide of claim 1, wherein the mutation at position C38 is C38S and the mutation at position C68 is C68G.~~

~~8. The modified IL-18 polypeptide of any one of claims 1-7, wherein the IL-18 polypeptide is a decoy resistant (DR) IL-18 variant or a decoy-to-the-decoy (D2D) IL-18 variant.~~

~~4~~9. The modified IL-18 polypeptide of any one of claims 1-~~3~~7, wherein the modified IL-18 polypeptide ~~is a DR IL-18 variant, wherein the DR IL-18 variant~~ comprises 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 SEQ ID NO: 30.

~~5~~10. The modified IL-18 polypeptide of claim ~~4~~9, wherein the modified IL-18 polypeptide ~~DR IL-18 variant~~ 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; ~~.~~

~~11. The modified IL-18 polypeptide of claim 9, wherein the DR IL-18 variant comprises~~

(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.

6~~12~~. The modified IL-18 polypeptide of claim 4~~9~~, wherein the modified IL-18 polypeptide ~~DR IL-18 variant~~ comprises mutations at positions M51, K53, Q56, D110, and N111, relative to SEQ ID NO: 30.

7~~13~~. The modified IL-18 polypeptide of claim 6~~12~~, wherein the modified IL-18 polypeptide ~~DR IL-18 variant~~ further comprises mutations at positions P57 and M60 relative to SEQ ID NO: 30.

~~14~~8. The modified IL-18 polypeptide of claim 7~~13~~, wherein the modified IL-18 polypeptide ~~DR IL-18 variant~~ further comprises a mutation at position S10~~5~~, relative to SEQ ID NO: 30.

~~15. The modified IL-18 polypeptide of claim 9, wherein the DR IL-18 variant comprises 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.~~

~~16. The modified IL-18 polypeptide of claim 9, wherein the DR IL-18 variant comprises 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.~~

9~~17~~. The modified IL-18 polypeptide of claim 4~~9~~, wherein the modified IL-18 polypeptide ~~DR IL-18 variant~~ 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.

~~18~~10. The modified IL-18 polypeptide of claim 9~~17~~, wherein the modified IL-18 polypeptide ~~DR IL-18 variant~~ further comprises the mutation S105D, S105A, S105N, S105R, S105D, or S105K relative to SEQ ID NO: 30.

~~19~~11. The modified IL-18 polypeptide ~~-~~of any one of claims 1-3~~7~~, ~~wherein the IL-18 polypeptide is a DR IL-18 variant that specifically binds to IL-18 receptor (IL-18R) but compared to said WT IL-18 exhibits reduced binding to IL-18 binding protein (IL-18BP),~~ wherein the modified IL-18 polypeptide ~~DR IL-18 variant~~ comprises the mutations M51K, K53S, Q56L, D110S, and N111R, relative to SEQ ID NO: 30.

12~~20~~. The modified IL-18 polypeptide of claim 11~~19~~, wherein the modified IL-18 polypeptide ~~DR IL-18 variant~~ further comprises the mutations P57A, M60L, and S105D.

~~21. The modified IL-18 polypeptide of claim 20, wherein the mutation at position C38 is C38S and the mutation at position C68 is C68G, C68A, C68D, or C68N.~~

~~22. The modified IL-18 polypeptide of claim 21, wherein the mutation at position C38 is C38S and the mutation at position C68 is C68G.~~

13~~23~~. The modified IL-18 polypeptide of claim 12~~21~~, wherein the modified IL-18 polypeptide ~~DR IL-18 variant~~ 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.

~~24. The modified IL-18 polypeptide of claim 21, wherein the DR IL-18 variant comprises the amino acid sequence set forth in any one of SEQ ID NOs: 6 and 16-21.~~

~~25. The modified IL-18 polypeptide of claim 21, wherein the DR IL-18 variant comprises the amino acid sequence set forth in any one of SEQ ID NOs: 6, 16, 17, 19, and 21.~~

14~~26~~. The modified IL-18 polypeptide of claim 12~~21~~, wherein the modified IL-18 polypeptide ~~DR IL-18 variant~~ 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 ~~.~~

~~27. The modified IL-18 polypeptide of claim 21, wherein the DR IL-18 variant~~ (b) comprises the amino acid sequence set forth in SEQ ID NO: 19.

~~28. The modified IL-18 polypeptide of claim 1, wherein the modified IL-18 polypeptide does not comprise cysteine residues that are not native to the IL-18 polypeptide.~~

~~29. The modified IL-18 polypeptide of claim 1, wherein the IL-18 polypeptide has binding affinity for IL-18BP that is less than 0.6 nM.~~

~~30. The modified IL-18 polypeptide of claim 1, wherein the modified IL-18 polypeptide exhibits greater stability compared to an IL-18 polypeptide with a different sequence.~~

~~31. The modified IL-18 polypeptide of claim 30, wherein the modified IL-18 polypeptide demonstrates less dimer, multimer, aggregate, and/or degradation product formation after exposure to a stability assay, freeze-thaw cycles, or agitation.~~

32. The modified IL-18 polypeptide of claim 1, wherein the amino acid sequence having 85% or more sequence identity with WT IL-18 does not comprise pegylated amino acids.

33. The modified IL-18 polypeptide of claim 1, wherein the amino acid sequence having 85% or more sequence identity with WT IL-18 does not comprise non-native cysteine residues.

34. The modified IL-18 polypeptide of claim 1, wherein the amino acid sequence having 85% or more sequence identity with WT IL-18 does not comprise pegylated cysteine residues.

3515. A pharmaceutical composition comprising the modified IL-18 polypeptide of any one of claims 1-1434, and a pharmaceutically acceptable excipient.

36. The composition of claim 35, further comprising an immune checkpoint inhibitor that inhibits PD-L1, PD1, CTLA4, TIM3, TIGIT, LAG3, B7H3, B7H4, VISTA, BTLA, CD47, SIRP alpha, CD48, CD155, CD160, TREM2, IDO1, Adenosine 2A receptor, Aryl hydrocarbon receptor, KIR, LILRB2, or any combination thereof.

37. The composition of claim 35, further comprising an immune agonist selected from: an agent that agonizes a tumor necrosis factor receptor superfamily (TNFRSF) protein (e.g., GITR, 41BB, OX40, CD27, CD40, HVEM); an agent that agonizes an immunoglobulin superfamily (IgSF) protein (e.g., CD28, ICOS, CD226, NKG2D); an agent that agonizes a TLR (e.g., TLR2, TLR4, TLR5, TLR7, TLR9); an agent that agonizes a nucleic acid sensor (e.g., STING, cGAS, RIG-I (DDX58)); an inflammasome activator; a T cell engager; a cytokine or cytokine variant (e.g., IL-2, IL-7, IL-10, IL-12, IL-15, IL-21, IL-23, IL-27, IL-33, TNF,TL1A,IFNA,IFNB,IFNG); or any combination thereof.

38. The composition of claim 35, further comprising a cancer cell opsonizing 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 $\alpha_v\beta_3$, 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.~~

~~39. The composition of claim 35, wherein the interleukin-18 (IL-18) polypeptide is conjugated to the immune checkpoint inhibitor, to the immune agonist, or to the cancer cell opsonizing agent.~~

~~40. The composition of any one of claims 35-39, further comprising 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).~~

~~41. A pharmaceutical formulation comprising the modified IL-18 polypeptide of any one of claims 1-40.~~

~~42. The pharmaceutical formulation of claim 41, further comprising the IL-18 polypeptide at a concentration of from 15-100 mg/mL.~~

16~~43~~. The ~~pharmaceutical formulation~~composition of claim 15~~claim 41 or claim 42~~, wherein the pharmaceutical ~~formulation~~ composition is formulated for subcutaneous injection.

17~~44~~. The ~~pharmaceutical formulation~~composition of ~~any one of claims~~ claim 15 or claim 16~~41-43~~, 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~~45~~. A nucleic acid comprising a nucleotide sequence encoding the modified IL-18 polypeptide of any one of claims 1-14~~34~~.

1946. The nucleic acid of claim 1845, wherein the nucleic acid is a plasmid or a viral vector.

2047. The nucleic acid of claim 1845 or claim 1946, 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.

2148. The nucleic acid of claim 2047, wherein the SUMO protease is ULP1.

2249. The nucleic acid of claim 2148, wherein the SUMO tag comprises the amino acid sequence set forth in SEQ ID NO: 26 or 27.

2350. A cell comprising the nucleic acid of any one of claims 1845-2249.

2451. The cell of claim 2350, comprising a nucleic acid comprising a nucleotide sequence encoding a SUMO protease.

2552. The cell of claim 2350 or claim 2451, wherein the cell is a bacterial cell, ~~.~~

~~53. The cell of claim 50, wherein the cell is~~ a yeast cell, ~~.~~

~~54. The cell of claim 50, wherein the cell is~~ 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.

2655. ~~A method of treatment, comprising administering the~~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 2534 ~~to an individual in need thereof, thereby treating a disease or disorder in the individual~~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 promoting IL-18 signaling activity in an individual in need thereof.

2856. The method of claim 55, wherein 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 the treatment of the disease or disorder is selected from the group consisting of: a cancer, 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), a cancer associated with a tumor in which IL-18R is expressed on infiltrating T cells or NK cells, a metabolic disease or disorder, and or an infectious disease in an individual.

29. The use of claim 28, 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.

57. The method of claim 55 or claim 56, wherein the method comprises administering an immune checkpoint inhibitor, and immune agonist, or a cancer cell opsonizing agent to the individual.

58. The method of claim 55 or claim 56, wherein the method comprises administering to the individual an immune checkpoint inhibitor that inhibits PD-L1, PD1, CTLA4, TIM3, TIGIT, LAG3, B7H3, B7H4, VISTA, BTLA, CD47, SIRP alpha, CD48, CD155, CD160, TREM2, IDO1, Adenosine 2A receptor, Aryl hydrocarbon receptor, KIR, LILRB2, or any combination thereof.

~~59. The method of claim 55 or claim 56, wherein the method comprises administering to the individual an immune agonist selected from: an agent that agonizes a tumor necrosis factor receptor superfamily (TNFRSF) protein (e.g., GITR, 41BB, OX40, CD27, CD40, HVEM); an agent that agonizes an immunoglobulin superfamily (IgSF) protein (e.g., CD28, ICOS, CD226, NKG2D); an agent that agonizes a TLR (e.g., TLR2, TLR4, TLR5, TLR7, TLR9); an agent that agonizes a nucleic acid sensor (e.g., STING, cGAS, RIG-I (DDX58)); an inflammasome activator; a T cell engager; a cytokine or cytokine variant (e.g., IL-2, IL-7, IL-10, IL-12, IL-15, IL-21, IL-23, IL-27, IL-33, TNF,TL1A,IFNA,IFNB,IFNG); or any combination thereof.~~

~~60. The method of any one of claims 55-59, wherein the method comprises administering to the individual a cancer cell opsonizing 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 $\alpha_v\beta_3$, 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.~~

~~61. The method of any one of claims 55-60, wherein the method comprises administering to the individual 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).~~

~~62. The method of any one of claims 55-61, wherein the method comprises administering an oncolytic virus to the individual.~~

30~~63~~. 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~~34~~ that is activated by the removal of the SUMO tag.

~~64. The method of claim 63, wherein the N-terminal amino acid of the IL-18 polypeptide after cleavage by the SUMO protease is tyrosine.~~

~~65. The method of claim 64, wherein the IL-18 polypeptide is a decoy resistant (DR) IL-18 variant or a decoy-to-the-decoy (D2D) IL-18 variant.~~

~~66. The method of any one of claims 63-65, wherein the SUMO protease is ULP1.~~

~~67. The method of any one of claims 63-66, wherein the SUMO tag comprises the amino acid sequence set forth in SEQ ID NO: 26 or 27.~~

~~68. The method of any one of claims 63-67, wherein said contacting comprises introducing into the bacterial cell a nucleic acid encoding the fusion protein.~~

~~69. The method of any one of claims 63-68, wherein said contacting comprises introducing into the bacterial cell a nucleic acid encoding the SUMO protease.~~

~~70. The method of any one of claims 63-69, wherein said contacting comprises inducing expression of the SUMO protease in the bacterial cell from an inducible promoter.~~

~~71. The method of claim 70, wherein the inducible promoter is a rhamnose-inducible promoter.~~

~~72. The method of any one of claims 63-71, further comprising purifying the polypeptide of interest after removal of the SUMO tag.~~

73. The method of any one of claims 64-65, further comprising purifying the IL-18 polypeptide after removal of the SUMO tag, wherein said purifying comprises ion exchange chromatography, immobilized metal affinity chromatography (IMAC), multimodal chromatography (MMC) and hydrophobic interaction chromatography (HIC).

74. The method of any one of claims 63-73, wherein said contacting takes place inside of a cell.

75. The method of any one of claims 63-73, wherein said contacting takes place cell-free *in vitro*.

76. A method of treatment, comprising administering the modified IL-18 polypeptide of any one of claims 1-34 to an individual in need thereof, thereby treating a disease or disorder in the individual, wherein the frequency of said administering is once per week or less.

77. The method of claim 76, wherein the frequency of said administering is once per two weeks or less.

78. The method of claim 76, wherein the frequency of said administering is in a range of from once week to once per month.

79. The method of any one of claims 76-78, wherein the IL-18 polypeptide is an IL-18 polypeptide according to any one of claims 1-34.

80. The method of any one of claims 76-78, wherein the IL-18 polypeptide is a decoy resistant (DR) IL-18 variant or a decoy-to-the-decoy (D2D) IL-18 variant.

81. The method of any one of claims 76-80, wherein said administering comprises subcutaneous administration.

82. The method of any one of claims 76-80, wherein said administering comprises administration of the IL-18 polypeptide at a dose in a range of from 0.025-3.5 mg/kg.

83. The method of any one of claims 76-82, wherein the disease or disorder is selected from the group consisting of: a cancer, 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 associated with a tumor in which IL-18R is expressed on infiltrating T cells or NK cells, a metabolic disease or disorder, and an infectious disease.

84. The method of any one of claims 74-81, wherein the method comprises administering an immune checkpoint inhibitor or a cancer cell opsonizing agent to the individual.

85. The method of any one of claims 74-81, wherein the method comprises administering to the individual an immune checkpoint inhibitor that inhibits PD-L1, PD1, CTLA4, TIM3, TIGIT, LAG3, B7H3, B7H4, VISTA, BTLA, CD47, SIRP alpha, CD48, CD155, CD160, TREM2, IDO1, Adenosine 2A receptor, Aryl hydrocarbon receptor, KIR, LILRB2, or any combination thereof.

86. The method of any one of claims 74-81, wherein the method comprises administering to the individual an immune agonist selected from: an agent that agonizes a tumor necrosis factor receptor superfamily (TNFRSF) protein (e.g., GITR, 41BB, OX40, CD27, CD40, HVEM); an agent that agonizes an immunoglobulin superfamily (IgSF) protein (e.g., CD28, ICOS, CD226, NKG2D); an agent that agonizes a TLR (e.g., TLR2, TLR4, TLR5, TLR7, TLR9); an agent that agonizes a nucleic acid sensor (e.g., STING, cGAS, RIG-I (DDX58)); an inflammasome activator; a T cell engager; a cytokine or cytokine variant (e.g., IL-2, IL-7, IL-10, IL-12, IL-15, IL-21, IL-23, IL-27, IL-33, TNF,TL1A,IFNA,IFNB,IFNG); or any combination thereof.

87. The method of any one of claims 74-81, wherein the method comprises administering to the individual a cancer cell opsonizing 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 $\alpha_v\beta_3$, 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.

88. The method of any one of claims 74-85, wherein the method comprises administering to the individual 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).

89. The method of any one of claims 74-86, wherein the method comprises administering an oncolytic virus to the individual.

90. A pharmaceutical formulation comprising:

an IL-18 polypeptide of any one of claims 1-34,

a buffering agent,

a sugar,

a chelating agent,

an agent to prevent oxidation, and

an agent to prevent protein absorption.

91. The pharmaceutical formulation of claim 90, wherein the buffering agent comprises His/His-HCl.

92. The pharmaceutical formulation of claim 91, wherein the buffering agent maintains the pH between 6.2 and 6.8.

93. The pharmaceutical formulation of any one of claims 90 to 92 wherein the sugar comprises sucrose.

94. The pharmaceutical formulation of any one of claims 90 to 93, wherein the chelating agent comprises EDTA or a salt thereof.

95. The pharmaceutical formulation of any one of claims 90 to 94, wherein the agent to prevent oxidation is L-methionine.

96. The pharmaceutical formulation of any one of claims 90 to 95, wherein the agent to prevent protein absorption is polysorbate 80.