

INTERLEUKIN-18 VARIANTS AND METHODS OF USE

CROSS-REFERENCE

This application claims the benefit of U.S. Provisional Patent Application Nos.
5 62/554,605 filed September 6, 2017, and 62/652,279 filed April 3, 2018, each of which
application is incorporated herein by reference in its entirety.

INTRODUCTION

Interleukin 18 (IL-18) is a pro-inflammatory cytokine that can stimulate T cells,
10 NK cells, and myeloid cells. IL-18 has been proposed as an immunotherapeutic agent for
the treatment of cancer, given its ability to stimulate anti-tumor immune cells. However,
the clinical efficacy of IL-18 has been limited.

Thus, there is a need for compositions and methods that provide effective IL-18
signaling activity to treat and prevent cancer and other diseases and disorders. The present
15 invention addresses this unmet need.

SUMMARY

In one aspect, the disclosure relates to a composition comprising an IL-18 variant
polypeptide. In some embodiments, 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).

In some embodiments, the IL-18 variant polypeptide comprises at least one
mutation relative to wild-type (WT) IL-18. In some embodiments, the WT IL-18 is human
IL-18 comprising the amino acid sequence of SEQ ID NO: 30. In some embodiments, the
WT IL-18 is murine IL-18 comprising the amino acid sequence of SEQ ID NO: 31.

In some embodiments, the IL-18 variant polypeptide comprises at least one
mutation 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, relative to SEQ ID NO: 30.

In some embodiments, the IL-18 variant polypeptide comprises at least one
mutation 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.

In some embodiments, the IL-18 variant polypeptide comprises the mutations M51X, M60X, S105X, D110X, and N111X, relative to SEQ ID NO: 30.

In some embodiments, the IL-18 variant polypeptide comprises the mutations M51X, K53X, Q56X, S105X, and N111X, relative to SEQ ID NO: 30.

In some embodiments, 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.

In some embodiments, 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.

In some embodiments, the IL-18 variant polypeptide comprises at least one mutation selected from 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.

In some embodiments, the IL-18 variant polypeptide comprises the amino acid sequence set forth in any one of SEQ ID NOs.: 60-72, or a fragment thereof.

In one aspect, the disclosure relates to a composition comprising a nucleic acid encoding the IL-18 variant polypeptide.

In some embodiments, the composition further comprises 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 $\alpha_v\beta_3$, 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.

In one aspect, the disclosure relates to a method of treating or preventing a disease or disorder in a subject in need thereof, comprising administering to the subject a composition comprising the IL-18 variant polypeptide or the nucleic acid encoding the IL-18 variant polypeptide.

In some embodiments, the disease or disorder is cancer. In some embodiments, the cancer is a cancer that is resistant to immune checkpoint inhibitors (ICIs). In some embodiments, the cancer is associated with a tumor that has lost expression of MHC class I.

In some embodiments, the disease or disorder is a metabolic disease or disorder. In some embodiments, the disease or disorder is an infectious disease.

In some embodiments, the method comprises administering to the subject the IL-18 variant polypeptide and at least one other agent. In some embodiments, the at least one other agent comprises an immune checkpoint inhibitor. In some embodiments, 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. In some embodiments, the at least one other agent comprises a cancer cell opsonizing agent. In some embodiments, the at least one other agent 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. In some embodiments, the at least one other agent is conjugated to the IL-18 variant polypeptide. In some embodiments, the at least one other agent is an altered T-cell or NK cell. In some embodiments, the at least one other agent is an oncolytic virus.

In one aspect, the disclosure relates to a composition comprising an IL-18 binding protein (IL-18BP) inhibitor or IL-18BP antagonist, wherein the inhibitor or antagonist inhibits the ability of IL-18BP to neutralize endogenous IL-18. In some embodiments, the inhibitor or antagonist comprises at least one selected from the group consisting of: a chemical compound, a polypeptide, a peptide, a peptidomimetic, an antibody, a ribozyme, a small molecule chemical compound, and an antisense nucleic acid molecule.

In some embodiments, the composition comprises an IL-18 variant polypeptide, wherein the IL-18 variant polypeptide specifically binds to IL-18BP and wherein the IL-18 variant polypeptide exhibits substantially reduced binding to IL-18 receptor (IL-18R).

In some embodiments, the IL-18 variant polypeptide comprises at least one mutation relative to wild-type (WT) IL-18. In some embodiments, the WT IL-18 is human IL-18 comprising the amino acid sequence of SEQ ID NO: 30. In some embodiments, the WT IL-18 is murine IL-18 comprising the amino acid sequence of SEQ ID NO: 31.

In some embodiments, the IL-18 variant polypeptide comprises at least one mutation selected from the group consisting of Y1X, L5X, D17X, E31X, T34X, D35X, S36X, D37X, D40X, N41X, M51X, Q56X, M60X, Q103X, H109X, M113X, and R131X, relative to SEQ ID NO: 30.

In some embodiments, the IL-18 variant polypeptide comprises at least one mutation selected from the group consisting of Y1D, Y1F, Y1H, Y1L, L5F, L5H, D17A, D17G, D17R, D17H, E31A, E31T, E31G, E31K, E31R, T34A, T34K, T34E, D35S, D35A,

D35Y, S36N, S36K, S36R, D37P, D37A, D37R, D37H, D37L, D37V, D40Y, D40S, D40A, N41K, N41S, N41R, M51F, M51L, M51I, Q56H, M60L, M60F, M60I, Q103L, Q103I, H109A, H109P, H109D, M113L, M113I, M113F, and R131S, relative to SEQ ID NO: 30.

In some embodiments, the IL-18 variant polypeptide comprises the amino acid sequence set forth in any one of SEQ ID NOs.: 92-125, or a fragment thereof.

In some embodiments, the IL-18 variant polypeptide comprises the mutations D17X, E30X, and Q103X, relative to SEQ ID NO: 30.

In some embodiments, the IL-18 variant polypeptide comprises the mutations D17X, E30X, D35X, M51X, and Q103X, relative to SEQ ID NO: 30.

In some embodiments, the IL-18 variant polypeptide comprises at least one mutation selected from the group consisting of N1X, L5X, D17X, E30X, T33X, D34X, I35X, D36X, M50X, Q102X, R104, H108X, N109X, M111X, D129X, and D130X, relative to SEQ ID NO: 31.

In some embodiments, the IL-18 variant polypeptide comprises at least one mutation selected from the group consisting of N1Y, N1D, N1H, N1L, N1F, N1V, N1I, L5Y, L5H, D17Q, D17G, D17A, D17E, D17S, D17N, E30A, E30R, E30K, E30T, E30G, T33G, T33A, T33E, T33R, T33K, D34Y, D34S, D34A, I35T, I35K, I35R, D36V, D36A, D36G, D36H, D36P, D36R, D36L, M50F, M50L, Q102L, Q102I, R104E, R104A, R104P, R104G, R104Q, R104H, H108D, H108A, N109R, N109S, N109T, N109I, M111L, M111I, D129A, D129F, D129V, D129Y, D129S, D130E, D130T, D130G, D130N, D130R, D130S, D130Q, and D130H, relative to SEQ ID NO: 31.

In some embodiments, the IL-18 variant polypeptide comprises the amino acid sequence set forth in any one of SEQ ID NOs.: 126-190, or a fragment thereof.

In one aspect, the disclosure relates to a composition comprising a nucleic acid encoding the IL-18 variant polypeptide.

In one aspect, the composition further comprises 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 $\alpha_v\beta_3$, 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.

In one aspect, the disclosure relates to a method of treating or preventing a disease or disorder in a subject in need thereof, comprising administering to the subject a composition comprising the IL-18 binding protein (IL-18BP) inhibitor or IL-18BP antagonist.

In some embodiments, the disease or disorder is cancer. In some embodiments, the cancer is a cancer that is resistant to immune checkpoint inhibitors (ICIs). In some embodiments, the cancer is associated with a tumor that has lost expression of MHC class I.

In some embodiments, the disease or disorder is a metabolic disease or disorder. In some embodiments, the disease or disorder is an infectious disease.

In some embodiments, the method comprises administering to the subject at least one other agent in addition to the IL-18BP inhibitor or IL-18BP antagonist. In some embodiments, the at least one other agent comprises an immune checkpoint inhibitor. In some embodiments, 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. In some embodiments, the at least one other agent comprises a cancer cell opsonizing agent. In some embodiments, the at least one other agent 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. In some embodiments, the inhibitor or antagonist is an IL-18 variant polypeptide, and the at least one other agent is conjugated to the IL-18 variant polypeptide. In some embodiments, the at least one other agent is an altered T-cell or NK cell. In some embodiments, the at least one other agent is an oncolytic virus.

BRIEF DESCRIPTION OF THE DRAWINGS

The following detailed description of embodiments of the invention will be better understood when read in conjunction with the appended drawings. It should be understood that the invention is not limited to the precise arrangements and instrumentalities of the
5 embodiments shown in the drawings.

Figure 1A and Figure 1B depict results from example experiments, demonstrating the IL-18 pathway is a target for tumor immunotherapy. (Figure 1A) The IL-18 pathway (including IL-18 and its receptor subunits) is upregulated in both activated and
10 dysfunctional tumor T cell programs, as seen in RNAseq expression analysis for cytokines and receptors in CD8⁺ TILs. Genes are assigned “activation” and “dysfunction” scores in comparison to naïve T cells. Yellow highlights indicate IL-18 cytokine, IL-18R1 ($R\alpha$), and IL-18RAP ($R\beta$). Data are adapted from Singer et al. (Singer, M. et al., 2016, Cell, 166:1500-1511, e1509). (Figure 1B) The IL-18 receptor subunits IL-18R α and IL-18R β
15 are part of a gene expression program associated with chronic antigen exposure, as seen after infection with LCMV (left; CD4) or VSV-OVA (right; CD8). Data are from the ImmGen database.

Figure 2A through Figure 2C depict results from example experiments, demonstrating IL-18BP has features of a “soluble immune checkpoint”. (Figure 2A) IL-
20 18BP mediates Interferon- γ (IFN- γ) driven negative feedback of IL-18, reminiscent of the immune checkpoint PD-L1. A schematic of the IL-18/IFN- γ /IL-18BP feedback loop is

depicted. Black arrows indicate stimulation, red circuits indicate inhibition. (Figure 2B) IL-18BP is upregulated in gastric and breast cancer, as seen in data from the TCGA and Oncomine databases. (Figure 2C) PD-1 and IL-18BP expression is strongly correlated in bulk breast and gastric cancer samples (from TCGA database). $R=0.78$ and 0.65 ,
 5 respectively.

Figure 3A through Figure 3C depict results from example experiments, demonstrating engineering human IL-18 variants for independence to IL-18BP using yeast display. (Figure 3A) A structure-guided library to randomize residues on the IL-18:IL-18BP interface was designed and introduced into a yeast-display system. Yeast clones were
 10 selected using magnetic and fluorescence cell sorting for binding to IL-18R α and counter-selected against IL-18BP. (Figure 3B) Summary of directed evolution to generate IL-18BP resistant IL-18 variants. Blue text indicates positive selection conditions, red text shows counterselection. (Figure 3C) Flow cytometric analysis of yeast-displayed WT IL-18 (left) or variants after directed evolution (right). Y-axes show IL-18BP binding, x-axes show IL-
 15 18R α binding. After 5 rounds of directed evolution, the remaining clones greatly preferred IL-18R α to IL-18BP.

Figure 4 depicts results from example experiments, demonstrating a summary of the sequences of decoy-resistant human IL-18 (“DR-IL-18”, also called “DR-18”) variants. The position of each mutated position and the corresponding residue in the mature form of
 20 wild-type human IL-18 is indicated at the top of the table. hC4 through hE12 represent sequences obtained after selection with directed evolution. hCS1-hCS4 are consensus sequences derived from the selected sequences. Shaded residues represent the five most conserved mutations observed.

Figure 5A and Figure 5B depict results from example experiments, demonstrating
 25 biophysical characterization of human DR-IL-18 variants. (Figure 5A) Yeast-displayed DR-IL-18 variants hCS1-hCS4 and A8 are capable of binding hIL-18R α with comparable binding isotherms as WT human IL-18 (left). By contrast, very little binding is observed with the same variants and hIL-18BP (right). (Figure 5B) Representative surface plasmon resonance sensorgrams between immobilized biotinylated hIL-18BP and the DR-IL-18
 30 variants. Recombinant hIL-18 (left) binds IL-18BP with exquisitely high affinity, $K_D=2.0$

pM, whereas hCS1 (right) shows greatly attenuated binding, with a much faster off-rate and $K_D = 15.2$ nM. This data is summarized in Tables 6 and 7.

Figure 6A and Figure 6B depict results from example experiments, demonstrating human DR-IL-18 variants are not inhibited by IL-18BP. (Figure 6A) Recombinant IL-18BP inhibits biotinylated IL-18R α from binding yeast-displayed WT IL-18, but does not affect the DR-IL-18 variants hCS1-4 and A8 (left). By contrast, IL-18BP effectively neutralizes the IL-18 E42A, K89A and E42A/K89A previously described (Kim et al., 2001, Proc. Natl. Acad. Sci., 98(6):3304-3309) (right) [E42 and K89 of Kim et al. are E6 and K53 of SEQ ID NO: 30, respectively]. Biotinylated IL-18R α was kept at a fixed concentration of 100 nM for all samples. (Figure 6B) WT IL-18 and hCS1, hCS3, and hCS4 stimulate IL-18 HEK-Blue reporter cells with comparable potency and efficacy (left). Wild-type IL-18 is highly sensitive to application of recombinant IL-18BP in this assay ($IC_{50} = 3$ nM), whereas hCS1 and hCS3 are not inhibited by recombinant IL-18BP, even at IL-18BP concentrations of 1 μ M (Right). hIL-18 was kept at a fixed concentration of 5 nM and hCS1 and hCS3 at 2.5 nM.

Figure 7A through Figure 7C depict results from example experiments demonstrating engineering additional human IL-18 variants for independence to IL-18BP (version 2 variants) using yeast display. (Figure 7A) Summary of the positions in human IL-18 randomized in the version 2.0 library. Degenerate codons and the set of encoded amino acids are given for each position. (Figure 7B) Summary of directed evolution to generate version 2.0 IL-18BP resistant IL-18 variants. Blue text indicates positive selection conditions, red text shows counterselection. (Figure 7C) Flow cytometric analysis of progress in creating version 2.0 DR-IL-18 variants. Yeast obtained after rounds 1, 4, and 6 were stained simultaneously with 250 nM IL-18BP streptavidin-PE tetramers or 100 nM IL-18R α directly labeled with AlexaFluor647. Y-axes show IL-18BP binding, x-axes show IL-18R α binding. After 6 rounds of directed evolution, the remaining clones greatly preferred IL-18R α to IL-18BP.

Figure 8 depicts results from example experiments, demonstrating a summary of the sequences of version 2.0 decoy-resistant human IL-18 (DR-IL-18) variants. The position of each mutated position and the corresponding residue in the mature form of wild-

type human IL-18 is indicated at the top of the table. Shaded rows indicate recurrent sequence variants obtained in both round 5 and round 6.

Figure 9 depicts results from example experiments, demonstrating biophysical characterization of version 2.0 human DR-IL-18 variants. (Figure 9A) Yeast-displayed version 2.0 DR-IL-18 variants are capable of binding hIL-18R α with comparable binding isotherms as WT human IL-18. (Figure 9B) By contrast, very little binding is observed with the same variants and hIL-18BP. (Figure 9C) Thermal stability of the version 2.0 DR-IL-18 variants was assessed by heating the yeast-displayed variants across a range of temperatures for 15 minutes, followed by staining with hIL-18R α . The version 2.0 DR-IL-18 variants were more thermostable than WT IL-18 ($T_m = 47.6^\circ\text{C}$) and the first-generation consensus sequences ($T_m = 50.9$ and 40.2 for hCS1 and hCS2, respectively). (Figure 9D) Summary of the receptor binding properties and thermal stability of the second-generation DR-IL-18 variants. NBD = no binding detected. N.D. = value not determined.

Figure 10A through Figure 10C depict results from example experiments, demonstrating engineering murine IL-18 variants for independence to IL-18BP using yeast display. (Figure 10A) Summary of directed evolution to generate IL-18BP resistant murine IL-18 variants. Blue text indicates positive selection conditions, red text shows counterselection. (Figure 10B) Flow cytometric analysis of yeast-displayed murine IL-18 variants after 5 rounds of directed evolution. Y-axes show IL-18BP binding, x-axes show IL-18R α binding. (Figure 10C) Summary of the sequences of decoy-resistant murine IL-18 (DR-IL-18) variants. The position of each mutated position and the corresponding residue in the mature form of wild-type murine IL-18 is indicated at the top of the table. mC1 through mH3 represent sequences obtained after selection with directed evolution. mCS1 and mCS2 are consensus sequences derived from the selected sequences. Shaded residues represent the five most conserved mutations observed.

Figure 11A and Figure 11B depict results from example experiments, demonstrating biophysical characterization of murine DR-IL-18 variants. (Figure 11A) Yeast-displayed DR-IL-18 variants mA7, mB1, mC1, mE8, mCS1, and mCS2 are capable of binding mIL-18R α with comparable binding isotherms as WT murine IL-18 (left). By contrast, very little binding is observed with the same variants and mIL-18BP (right). (Figure 11B) Representative surface plasmon resonance sensorgrams between

immobilized biotinylated mIL-18BP and the murine DR-IL-18 variants. Recombinant mIL-18 (left) binds mIL-18BP with high affinity, $K_D=0.8$ pM, whereas mCS2 (right) shows greatly decreased binding with a K_D value greater than 10 μ M. This data is summarized in Tables 8 and 9.

5 Figure 12A through Figure 12D depict results from example experiments, demonstrating pharmacodynamics of DR-IL-18 administered to mice. (Figure 12A) Schematic of study design. Mice were administered vehicle (PBS), mIL-18 (1 mg/kg), or the DR-IL-18 variant mCS2 (1 mg/kg) once daily for seven total doses (depicted as syringes). Blood samples were taken five hours post-injection two days prior to the
10 experiment, and on days 0, 3, and 6. (Figure 12B) Peripheral blood cell counts for CD4, CD8, NK cells, and monocytes at day 0, day 3, and day 6. Both IL-18 and mCS2 expanded NK cells and monocytes to a similar degree by day 3. For each time point (day), left bar is PBS, middle bar is IL-18, and right bar is mCS2. (Figure 12C) CD69 expression on peripheral CD4, CD8, and NK cells. mCS2, but not IL-18 stimulated CD69 expression on
15 CD4 and CD8 cells. Both IL-18 and mCS2 increased CD69 on NK cells, but mCS2 treatment caused sustained CD69 expression evident at day 6, in comparison to IL-18, which reverted to baseline CD69 levels. For each time point (day), left bar is PBS, middle bar is IL-18, and right bar is mCS2. (Figure 12D) Serum cytokine levels for interferon- γ (IFN- γ), MIP-1b, and G-CSF. mCS2 treatment yielded higher levels of IFN- γ , MIP-1b, and
20 G-CSF than mIL-18 treatment.

Figure 13 depicts results from example experiments, demonstrating DR-IL-18 treatment decreases body fat composition in mice. Body fat and lean mass composition were measured in mice treated with 0.01, 0.1, or 1 mg/kg of the DR-IL-18 variant mCS2 or 1 mg/kg WT mIL-18 every three days. mCS2 treatment produced a significant decrease
25 in body fat as a total percentage of body mass (top panel). This was manifested by decreases or stable fat mass (left panel), with concordant increases in lean mass (right panel). Vehicle treated and mIL-18 treated mice showed increases in body fat mass and stable lean mass over the same treatment period.

Figure 14A through 14B depict results from example experiments, demonstrating
30 DR-IL-18 is an effective immunotherapeutic in a melanoma model. (Figure 14A) Tumor growth spider plots for mice bearing Yummer1.7 melanoma tumors treated with saline

(control), WT IL-18 (0.32 mg/kg), the DR-IL-18 variant mCS2 (0.32 mg/kg), anti-PD1 (8 mg/kg), IL-18 + anti-PD1, or mCS2 + anti-PD-1 twice per week. (Figure 14B) Survival curves from the same groups as in (Figure 11A). mCS2 was effective as a monotherapy and synergized in combination with anti-PD1 in this model.

5 Figure 15A and Figure 15B depict results from example experiments that demonstrate that the effectiveness of DR-IL-18 in the melanoma model of Figure 14 is dependent on CD4 and CD8 lymphocytes and interferon gamma. (Figure 15A) Tumor growth spider plots for mice bearing Yummer1.7 melanoma tumors treated with saline (control), or the DR-IL-18 variant mCS2 (0.32 mg/kg) alone, or in combination with
10 depleting antibodies against CD8, CD4, interferon gamma, or NK1.1. (Figure 15B) Survival curves from the same groups as in (Figure 15A).

 Figure 16 depicts results from example experiments, demonstrating dose-dependent efficacy of DR-IL-18 in the MC38 tumor model. Tumor growth spider plots from mice bearing MC38 colon cancer tumors treated with PBS (control), 1.0 mg/kg WT
15 IL-18, 1.0 mg/kg mCS2, 0.1 mg/kg mCS2, or 0.01 mg/kg mCS2 every three days. WT IL-18 was not efficacious at 1 mg/kg, whereas mCS2 showed partial efficacy at 0.1 mg/kg and maximal efficacy at 1.0 mg/kg.

 Figure 17 depicts results from example experiments demonstrating the efficacy of DR-IL-18 alone in combination with the immune checkpoint inhibitor anti-PD1 in the
20 MC38 tumor model. Tumor growth spider plots are shown from mice bearing MC38 colon cancer tumors treated with PBS (control), 0.32 mg/kg WT IL-18, 0.32 mg/kg of the DR-IL-18 variant mCS2, 5mg/kg anti-PD1, the combinations of anti-PD1 with WT IL-18, or the combination of anti-PD1 with mCS2. All agents were dosed intraperitoneally twice per week for up to 6 total doses.

25 Figure 18A and Figure 18B depict results from example experiments that investigate the anti-tumor mechanism of DR-IL-18 in mice bearing MC38 tumors. (Figure 18A) Tumor immunophenotyping experiments from mice treated twice weekly for four doses with saline, WT IL-18, or the DR-IL-18 variant mCS2. DR-IL-18 treatment resulted in increased numbers of CD8 and NK cells per mg of tumor (upper left two panels) and
30 increased expression of activation markers granzyme B and KLRG1 on CD8 and NK cells (upper right two panels). DR-IL-18 treatment did not improve the CD8:Treg ratio

compared to saline treatment, whereas WT IL-18 made the ratio less favorable. However, DR-IL-18 treatment increased the ratio of CD8 cells to inhibitory myeloid populations including tumor associated macrophages (TAM), and monocytic and granulocytic myeloid derived suppressor cells (mMDSCs and gMDSCs). (Figure 18B) Serum Luminex cytokine measurements from the same mice as (Figure 18A) taken 24 hours after the 4th treatment dose. DR-IL-18 shows a dramatically altered secondary cytokine release profile from treatment with WT IL-18, notably increasing Interferon-gamma, IL-7, and IL-15 levels by more than 100-fold.

Figure 19A through Figure 19C depicts results from example experiments demonstrating the capability of DR-IL-18 to effectively treat tumors that are refractory to immune checkpoint inhibitors through loss of surface MHC class I expression. (Figure 19A) Tumor growth spider plots from mice bearing B2m-deficient Yummer1.7 tumors treated with saline, anti-PD1 + anti-CTLA4, the DR-IL-18 variant mCS2, or mCS2 with depletion of NK cells with anti-NK1.1 antibodies. DR-IL-18 demonstrated strong efficacy in terms of tumor growth and survival (Figure 19B), curing 60% of treated mice in this model that is completely resistant to even combination treatment with anti-CTLA4 + anti-PD1. This efficacy is NK cell dependent since administration of anti-NK1.1 abrogates the mCS2 treatment effect. (Figure 19C) NK cells isolated from B2m-deficient Yummer1.7 are dysfunctional and show diminished proliferation (Ki67 staining) and function (Interferon-gamma secretion). However, treatment with DR-IL-18 reverses this phenotype to enable robust proliferation and cytokine secretion.

Figure 20A through Figure 20C depicts example experiments demonstrating engineering of human IL-18 variants as IL-18BP antagonists (or “decoys-to-the-decoy”, D2D) using yeast display. These variants bind IL-18BP but do not signal, thereby antagonizing the effect of IL-18BP on endogenous IL-18 (Figure 20A) Summary of the positions in human IL-18 randomized in the D2D library. Degenerate codons and the set of encoded amino acids are given for each position. (Figure 20B) Summary of directed evolution to generate D2D IL-18 variants that bind and neutralize IL-18BP, but do not signal through the IL-18R. Blue text indicates positive selection conditions, red text shows counter-selection. (Figure 20C) Flow cytometric analysis of progress in creating D2D hIL-18 variants. Yeast obtained after rounds 1-4 were stained with 1 nM of mouse IL-18BP

(left panel), 1 nM human IL-18BP (middle panel), or 1 μ M IL18R α plus 1 μ M IL18R β . Selected variants show enhanced IL-18BP binding across rounds of selection without increases in IL18R α or IL18R β binding.

Figure 21 depicts results from example experiments demonstrating a summary of the sequences of D2D human IL-18 variants. The position of each mutated position and the corresponding residue in the mature form of wild-type human IL-18 is indicated at the top of the table.

Figure 22A through Figure 22C depict results from example experiments demonstrating biophysical characterization of the human decoy-to-the-decoy (D2D) IL-28 variants. (Figure 22A) Yeast-displayed D2D IL-8 variants 5-B02, 5-E08, 5-F10, 5-F02, 5-F01, hD2D-CS1, hD2D-CS2, and hD2D-CS3 are capable of binding hIL-18RBP with comparable binding isotherms as WT human IL-18. (Figure 22b) By contrast, very little binding is observed with the same variants and hIL-18R α . (Figure 11C) Summary of the receptor binding properties of the D2D IL-18 variants. NBD = no binding detected.

Figure 23 depicts results from example experiments demonstrating a summary of the sequences of D2D murine IL-18 variants. The position of each mutated position and the corresponding residue in the mature form of wild-type murine IL-18 is indicated at the top of the table.

Figure 24 depicts results from biophysical affinity measurements (sensograms) of the second-generation DR-IL-18 variants for binding to IL-18Ra and IL-18BP using Surface Plasmon Resonance (SPR). Top row: representative sensograms of the indicated IL-18 variants (soluble analytes) for hIL-18Ra (immobilized ligand). Bottom row: representative sensograms of the indicated IL-18 variants for human (hIL-18BP). The x axis is time in seconds and the y axis is Response Units (RU). The curves are the observed data over time for different concentrations (2-fold dilutions starting at 1nM), superimposed with curves of best fit assuming a 1:1 langmuir binding model.

Figure 25A and 25B depict data demonstrating efficacy of DR-IL-18 on the CT26 colorectal tumor model. 250,000 CT26 cells were implanted subcutaneously and treatment initiated at day 7 once tumors were ~60 mm³ on average. WT IL-18 and mCS2 were dosed at 0.32mg/kg twice weekly for a total of 5 doses. Anti-PD1 was given at 10mg/kg at the same schedule. (A) Overlay of spider plots showing tumor growth of saline (PBS) treated

animals in black lines (circles), WT IL-18 in blue lines (squares), and DR-IL-18 (mCS2) in pink (triangles). Only treatment with DR-IL-18, but not WT IL-18, resulted in tumor growth inhibition and tumor clearance in a subset of animals. (B) Survival curves for mice treated with anti-PD-1, WT IL-18, and DR-IL-18 (mCS2). Numbers of complete responses are indicated in parentheses. DR-IL-18, but not WT IL-18 resulted in prolonged survival and tumor clearance in 40% of mice, an improvement over the checkpoint inhibitor anti-PD-1.

Figure 26A and 26B depict data demonstrating efficacy of DR-IL-18 in the 4T1 breast cancer model and B16-F10 melanoma model. (A) Tumor growth curves of 4T1 tumors engrafted into BALB/C mice after treatment with saline (PBS; black), WT IL-18 (blue), or the DR-IL-18 variant CS2 (pink). (B) Tumor growth curves of B16-F10 tumors engrafted into C57BL/6 mice after treatment with saline (PBS; black), WT IL-18 (blue), or the DR-IL-18 variant CS2 (pink). In both models, only DR-IL-18, but not WT IL-18 resulted in tumor growth inhibition. Treatments were administered after tumors exceeded an average volume 50 mm³ as indicated by the boxes marked with “t”.

Figure 27A and 27B depict data that extend the data of Figure 19A through 19C. Depicted is data demonstrating efficacy of DR-IL-18 in the treatment of additional MHC class I deficient tumor models that are resistant to immune checkpoint inhibitors. (A) B2m deficient MC38 cells were prepared using CRISPR/Cas9 mediated deletion as described for B2m deficient YUMMER cells. B2m^{-/-} MC38 cells were implanted subcutaneously and treatment initiated at day 7 once tumors were ~65 mm³ on average. mCS2 was dosed at 0.32mg/kg twice weekly for 5 doses. Anti-PD1 and anti-CTLA4 were given at 8mg/kg at the same schedule. (B) RMA/S is a variant of the RMA lymphoma line that contains a spontaneous mutation in Tapasin. The result is a defect in antigen loading and therefore decreased MHC class I surface expression. It is congenic to C57BL/6 and refractory to immune checkpoint inhibitors. Mice were implanted with 1,000,000 RMA/S cells subcutaneously and treatment initiated at day 7. mCS2 was dosed at 0.32mg/kg twice weekly. Anti-PD1 was given at 8mg/kg at the same schedule. In both studies, only treatment with the DR-18 variant mCS2 exhibited anti-tumor efficacy in the form of tumor growth inhibition (B2m^{-/-} MC38) or tumor clearance (RMA/S).

Figure 28 depicts data demonstrating efficacy of DR-IL-18 to enhance anti-tumor antibody-dependent cell mediated cytotoxicity (ADCC). *Ex vivo* cytotoxicity studies used CFSE labeled Raji (B cell lymphoma) cells and isolated human peripheral blood mononuclear cells (PBMCs). PBMCs and labeled Raji cells were incubated together at an effector:target (E:T) ratio of 1:10 for 25 hours. The human DR-IL-18 variant hCS-1 (1uM), rituximab (10 ug/mL), or the combination of both agents were applied to the samples as indicated. Cytotoxicity was measured by flow cytometry and calculated as the fraction of CFSE cells that became DAPI positive. DR-18 stimulated significant tumor cell killing as a single agent and significantly enhanced the killing by the therapeutic antibody rituximab. * p<0.05 by two-way ANOVA with Tukey's correction for multiple comparisons.

Figure 29A and 29B depict data demonstrating anti-viral efficacy of DR-18 variant mCS2 for the treatment viral infections (e.g., in this case in the treatment of systemic vaccinia virus infection). (A) Experimental design scheme. C57BL/6 mice were infected with 10⁶ PFU of Vaccinia virus (VACV) intraperitoneally (IP) and administered 1 mg/kg WT mIL-18 or mCS2 IP. Mice were sacrificed and viral titers were measured in the blood and ovaries by RT-PCR on day 3 post-infection. (B) Quantification of VACV viral copies in ovaries and blood of treated mice at day 3 post infection. Treatment with CS2 showed a significant reduction of viral titers, whereas WT IL-18 was not effective. * p<0.05, ** p<0.01, *** p<0.001.

Figure 30A depicts data demonstrating that the second generation human DR-IL-18 variants are active. (Figure 30A) WT IL-18 and h6-12, h6-27, h6-29, and h6-31 stimulate IL-18 HEK-Blue reporter cells. h6-12, h6-27, and h6-29 show enhanced potency compared to WT hIL-18, whereas h6-31 has equivalent potency as WT hIL-18. The data demonstrate, therefore, that all tested second generation human DR-IL-18 variants actively signal through IL-18R.

DETAILED DESCRIPTION

The present invention relates to the variants of IL-18 to induce or enhance IL-18 signaling. In one aspect, the invention relates to variants of IL-18 that can bind to IL-18 receptor (IL-18R) but do not bind to IL-18 binding protein (IL-18BP), thereby providing IL-18 signaling activity while not capable of being inhibited by IL-18BP. In one aspect,

the invention relates to variants of IL-18 that binds to IL-18BP, thereby reducing or preventing IL-18BP from binding to and inhibiting endogenous IL-18 and thereby providing IL-18 signaling activity. Thus, the invention provides compositions and methods for providing IL-18 signaling activity even in the presence of IL-18BP.

5 In various embodiments, the invention is an IL-18 variant polypeptide, or a fragment thereof, that specifically binds to IL-18R, and exhibits substantially reduced binding to IL-18BP. In some embodiments, an IL-18 variant polypeptide, or a fragment thereof, that binds to IL-18R, but does not bind substantially to IL-18BP, is useful for providing IL-18 signaling activity that is uninhibited by the presence and activity of IL-
10 18BP. In various embodiments, the invention relates to IL-18 variant polypeptide, or fragment thereof, that specifically binds to IL-18BP and reducing or preventing IL-18BP from inhibiting endogenous IL-18. In some embodiments, an IL-18 variant polypeptide, or a fragment thereof, that binds to IL-18BP, is useful for inhibiting IL-18BP activity thereby inducing, enhancing, or promoting IL-18 signaling activity.

15 In some embodiments, the IL-18 variant polypeptides are useful for the treatment and prevention of a disease or disorder. In various embodiments, the disease or disorder is cancer or an infectious disease, such as poxviruses that encode an IL-18BP ortholog, a metabolic disease or disorder (including obesity and diabetes), or macular degeneration (e.g., wet macular degeneration, e.g., wet age-related macular degeneration, e.g., the IL-18
20 variant can be used as an anti-angiogenic -as an illustrative example in some cases a subject IL-18 variant polypeptide can attenuate Choroidal neovascularization). Thus, in some embodiments, the invention is a composition comprising at least one IL-18 variant polypeptide, or a fragment thereof. In other embodiments, the invention is a method of administering at least one IL-18 variant polypeptide, or a fragment thereof, to treat or
25 prevent a disease or disorder, such as, but not limited to, cancer, or infectious disease, a metabolic disease or disorder, or macular degeneration (e.g., wet macular degeneration such as wet age-related macular degeneration).

 In various embodiments, the IL-18 variant polypeptide comprises a mutation relative to a wild-type (WT) IL-18 polypeptide. In some embodiments, the WT IL-18

polypeptide comprises the amino acid sequence of SEQ ID NO: 30. In other embodiments, the WT IL-18 polypeptide comprises the amino acid sequence of SEQ ID NO: 31.

In some embodiments, the IL-18 variant polypeptides of the invention exhibit decreased binding affinity to IL-18BP, as compared with the WT IL-18 polypeptide. In some embodiments, the IL-18 variant polypeptides of the invention exhibit increased binding affinity to IL-18BP, as compared with the WT IL-18 polypeptide. In some embodiments, the IL-18 variant polypeptides of the invention exhibit similar binding affinity to IL-18BP, as compared with the WT IL-18 polypeptide.

In some embodiments, the IL-18 variant polypeptide exhibits increased binding affinity to IL-18R, as compared with the WT IL-18 polypeptide. In some embodiments, the IL-18 variant polypeptide exhibits similar binding affinity to IL-18R, as compared with the WT IL-18 polypeptide. In some embodiments, the IL-18 variant polypeptide exhibits decreased binding affinity to IL-18R, as compared with the WT IL-18 polypeptide.

In some embodiments, the IL-18 variant polypeptide is a mammalian IL-18 variant polypeptide. In some embodiments, the IL-18 variant polypeptide is a human IL-18 variant polypeptide. In some embodiments, the IL-18 variant polypeptide is a murine IL-18 variant polypeptide.

In various embodiments, the compositions and methods of the invention include compositions and methods for treating and preventing disease and disorders, such as cancer, infectious disease, and metabolic diseases and disorders. In some embodiments, a method comprises administering to a subject in need thereof a composition comprising at least one IL-18 variant polypeptide. In some embodiments, a method comprises administering to a subject in need thereof a composition comprising at least one IL-18 variant polypeptide, and administering to the subject a composition comprising an additional agent.

In one such embodiment, the additional agent comprises an immunotherapeutic agent comprising at least one selected from the group including, but not limited to an altered T-cell, a chimeric antigen receptor T-cell (CAR-T), an armored CAR-T cell, a virus, an antigen, a vaccine, an antibody, an immune checkpoint inhibitor, a small molecule, a chemotherapeutic agent, and a stem cell. In some embodiments, a composition comprising at least one IL-18 variant polypeptide is used in a method to increase immune system

activity before, during, or after infection by a bacterium, virus, or other pathogen. In some embodiments, a composition comprising at least one IL-18 variant polypeptide is used in a method to increase the number and/or activity of immune cells in vitro, in vivo or ex vivo, such as the number and/or activity of T cells, NK cells, and/or myeloid cells.

5

Definitions

Unless defined otherwise, all technical and scientific terms used herein have the same meaning as commonly understood by one of ordinary skill in the art to which the invention pertains. In describing and claiming the present invention, the following
10 terminology will be used.

It is also to be understood that the terminology used herein is for the purpose of describing particular embodiments only, and is not intended to be limiting.

The articles “a” and “an” are used herein to refer to one or to more than one (i.e., to at least one) of the grammatical object of the article. By way of example, “an element”
15 means one element or more than one element.

“About” as used herein when referring to a measurable value such as an amount, a temporal duration, and the like, is meant to encompass non-limiting variations of $\pm 40\%$ or $\pm 20\%$ or $\pm 10\%$, $\pm 5\%$, $\pm 1\%$, or $\pm 0.1\%$ from the specified value, as such variations are appropriate.

20 The term “abnormal” when used in the context of organisms, tissues, cells or components thereof, refers to those organisms, tissues, cells or components thereof that differ in at least one observable or detectable characteristic (e.g., age, treatment, time of day, etc.) from those organisms, tissues, cells or components thereof that display the “normal” (expected) respective characteristic. Characteristics which are normal or
25 expected for one cell or tissue type, might be abnormal for a different cell or tissue type.

The term “antibody,” as used herein, refers to an immunoglobulin molecule which is able to specifically bind to a specific epitope on an antigen. Antibodies can be intact immunoglobulins derived from natural sources or from recombinant sources and can be immunoreactive portions of intact immunoglobulins. The antibodies in the present
30 invention may exist in a variety of forms including, for example, polyclonal antibodies, monoclonal antibodies, intracellular antibodies (“intrabodies”), Fv, Fab and F(ab)₂, as well

as single chain antibodies (scFv), heavy chain antibodies, such as camelid antibodies, synthetic antibodies, chimeric antibodies, and a humanized antibodies (Harlow et al., 1999, Using Antibodies: A Laboratory Manual, Cold Spring Harbor Laboratory Press, NY; Harlow et al., 1989, Antibodies: A Laboratory Manual, Cold Spring Harbor, New York; 5 Houston et al., 1988, Proc. Natl. Acad. Sci. USA 85:5879-5883; Bird et al., 1988, Science 242:423-426).

An “antibody heavy chain,” as used herein, refers to the larger of the two types of polypeptide chains present in all antibody molecules in their naturally occurring conformations.

10 An “antibody light chain,” as used herein, refers to the smaller of the two types of polypeptide chains present in all antibody molecules in their naturally occurring conformations. κ and λ light chains refer to the two major antibody light chain isotypes.

By the term “synthetic antibody” as used herein, is meant an antibody which is generated using recombinant DNA technology, such as, for example, an antibody 15 expressed by a bacteriophage as described herein. The term should also be construed to mean an antibody which has been generated by the synthesis of a DNA molecule encoding the antibody and which DNA molecule expresses an antibody protein, or an amino acid sequence specifying the antibody, wherein the DNA or amino acid sequence has been obtained using synthetic DNA or amino acid sequence technology which is available and 20 well known in the art.

As used herein, an “immunoassay” refers to any binding assay that uses an antibody capable of binding specifically to a target molecule to detect and quantify the target molecule.

By the term “specifically binds,” as used herein with respect to an IL-18 variant 25 polypeptide, is meant an IL-18 variant polypeptide that recognizes and binds to a specific receptor, such as IL-18R, or to IL-18BP. In some instances, the IL-18 variant polypeptide substantially reduced binding to IL-18BP. For example, an IL-18 variant polypeptide that specifically binds to a receptor from one species may also bind to that receptor from one or more species. But, such cross-species reactivity does not itself alter the classification of 30 an IL-18 variant polypeptide as specific. In another example, an IL-18 variant polypeptide that specifically binds to a receptor may also bind to different allelic forms of the receptor.

However, such cross reactivity does not itself alter the classification of an IL-18 variant polypeptide as specific. In some instances, the terms “specific binding” or “specifically binding,” can be used in reference to the interaction of an antibody, a protein, or a peptide with a second chemical species, to mean that the interaction is dependent upon the presence of a particular structure (e.g., an antigenic determinant or epitope) on the chemical species; for example, an IL-18 variant polypeptide recognizes and binds to a specific protein structure rather than to proteins generally.

By the term “applicator,” as the term is used herein, is meant any device including, but not limited to, a hypodermic syringe, a pipette, an iontophoresis device, a patch, and the like, for administering the compositions of the invention to a subject.

“Cancer,” as used herein, refers to the abnormal growth or division of cells. Generally, the growth and/or life span of a cancer cell exceeds, and is not coordinated with, that of the normal cells and tissues around it. Cancers may be benign, pre-malignant or malignant. Cancer occurs in a variety of cells and tissues, including the oral cavity (e.g., mouth, tongue, pharynx, etc.), digestive system (e.g., esophagus, stomach, small intestine, colon, rectum, liver, bile duct, gall bladder, pancreas, etc.), respiratory system (e.g., larynx, lung, bronchus, etc.), bones, joints, skin (e.g., basal cell, squamous cell, meningioma, etc.), breast, genital system, (e.g., uterus, ovary, prostate, testis, etc.), urinary system (e.g., bladder, kidney, ureter, etc.), eye, nervous system (e.g., brain, etc.), endocrine system (e.g., thyroid, etc.), and hematopoietic system (e.g., lymphoma, myeloma, leukemia, acute lymphocytic leukemia, chronic lymphocytic leukemia, acute myeloid leukemia, chronic myeloid leukemia, etc.).

The term “coding sequence,” as used herein, means a sequence of a nucleic acid or its complement, or a part thereof, that can be transcribed and/or translated to produce the mRNA and/or the polypeptide or a fragment thereof. Coding sequences include exons in a genomic DNA or immature primary RNA transcripts, which are joined together by the cell's biochemical machinery to provide a mature mRNA. The anti-sense strand is the complement of such a nucleic acid, and the coding sequence can be deduced therefrom. In contrast, the term “non-coding sequence,” as used herein, means a sequence of a nucleic acid or its complement, or a part thereof, that is not translated into amino acid in vivo, or where tRNA does not interact to place or attempt to place an amino acid. Non-coding

sequences include both intron sequences in genomic DNA or immature primary RNA transcripts, and gene-associated sequences such as promoters, enhancers, silencers, and the like.

As used herein, the terms “complementary” or “complementarity” are used in reference to polynucleotides (i.e., a sequence of nucleotides) related by the base-pairing rules. For example, the sequence “A-G-T,” is complementary to the sequence “T-C-A.” Complementarity may be “partial,” in which only some of the nucleic acids' bases are matched according to the base pairing rules. Or, there may be “complete” or “total” complementarity between the nucleic acids. The degree of complementarity between nucleic acid strands has significant effects on the efficiency and strength of hybridization between nucleic acid strands. This is of particular importance in amplification reactions, as well as detection methods that depend upon binding between nucleic acids.

A “disease” is a state of health of an animal wherein the animal cannot maintain homeostasis, and wherein if the disease is not ameliorated then the animal's health continues to deteriorate. In contrast, a “disorder” in an animal is a state of health in which the animal is able to maintain homeostasis, but in which the animal's state of health is less favorable than it would be in the absence of the disorder. Left untreated, a disorder does not necessarily cause a further decrease in the animal's state of health.

An “effective amount” as used herein, means an amount which provides a therapeutic, prophylactic, or other desired benefit.

“Encoding” refers to the inherent property of specific sequences of nucleotides in a polynucleotide, such as a gene, a cDNA, or an mRNA, to serve as templates for synthesis of other polymers and macromolecules in biological processes having either a defined sequence of nucleotides (i.e., rRNA, tRNA and mRNA) or a defined sequence of amino acids and the biological properties resulting therefrom. Thus, a gene encodes a protein if transcription and translation of mRNA corresponding to that gene produces the protein in a cell or other biological system. Both the coding strand, the nucleotide sequence of which is identical to the mRNA sequence and is usually provided in sequence listings, and the non-coding strand, used as the template for transcription of a gene or cDNA, can be referred to as encoding the protein or other product of that gene or cDNA.

As used herein, the term “fragment,” as applied to a nucleic acid, refers to a subsequence of a larger nucleic acid. A “fragment” of a nucleic acid can be at least about 15 nucleotides in length; for example, at least about 50 nucleotides to about 100 nucleotides; at least about 100 to about 500 nucleotides, at least about 500 to about 1000 nucleotides; at least about 1000 nucleotides to about 1500 nucleotides; about 1500 nucleotides to about 2500 nucleotides; or about 2500 nucleotides (and any integer value in between). As used herein, the term “fragment,” as applied to a protein, polypeptide or peptide, refers to a subsequence of a larger protein, polypeptide or peptide. A “fragment” of a protein, polypeptide, or peptide can be at least about 5 amino acids in length; for example, at least about 10 amino acids in length; at least about 20 amino acids in length; at least about 50 amino acids in length; at least about 100 amino acids in length; at least about 200 amino acids in length; or at least about 300 amino acids in length (and any integer value in between).

The term “gene” refers to a nucleic acid (e.g., DNA) sequence that includes coding sequences necessary for the production of a polypeptide, precursor, or RNA (e.g., mRNA). The polypeptide may be encoded by a full-length coding sequence or by any portion of the coding sequence so long as the desired activity or functional property (e.g., enzymatic activity, receptor binding, signal transduction, immunogenicity, etc.) of the full-length or fragment is retained. The term also encompasses the coding region of a structural gene and the sequences located adjacent to the coding region on both the 5' and 3' ends for a distance of about 2 kb or more on either end such that the gene corresponds to the length of the full-length mRNA and 5' regulatory sequences which influence the transcriptional properties of the gene. Sequences located 5' of the coding region and present on the mRNA are referred to as 5'-untranslated sequences. The 5'-untranslated sequences usually contain the regulatory sequences. Sequences located 3' or downstream of the coding region and present on the mRNA are referred to as 3'-untranslated sequences. The term “gene” encompasses both cDNA and genomic forms of a gene. A genomic form or clone of a gene contains the coding region interrupted with non-coding sequences termed “introns” or “intervening regions” or “intervening sequences.” Introns are segments of a gene that are transcribed into nuclear RNA (hnRNA); introns may contain regulatory elements such as enhancers. Introns are removed or “spliced out” from the nuclear or primary transcript; introns

therefore are absent in the messenger RNA (mRNA) transcript. The mRNA functions during translation to specify the sequence or order of amino acids in a nascent polypeptide.

“Homologous”, “identical,” or “identity” as used herein in the context of two or more nucleic acids or polypeptide sequences means that the sequences have a specified percentage of residues that are the same over a specified region. The percentage can be calculated by optimally aligning the two sequences, comparing the two sequences over the specified region, determining the number of positions at which the identical residue occurs in both sequences to yield the number of matched positions, dividing the number of matched positions by the total number of positions in the specified region, and multiplying the result by 100 to yield the percentage of sequence identity. In cases where the two sequences are of different lengths or the alignment produces one or more staggered ends and the specified region of comparison includes only a single sequence, the residues of the single sequence are included in the denominator but not the numerator of the calculation. When comparing DNA and RNA, thymine (T) and uracil (U) can be considered equivalent. Identity can be performed manually or by using a computer sequence algorithm such as BLAST or BLAST 2.0.

“Instructional material,” as that term is used herein, includes a publication, a recording, a diagram, or any other medium of expression which can be used to communicate the usefulness of the nucleic acid, peptide, polypeptide, and/or compound of the invention in the kit for identifying or alleviating or treating the various diseases or disorders recited herein. Optionally, or alternately, the instructional material may describe one or more methods of identifying or alleviating the diseases or disorders in a cell or a tissue of a subject. The instructional material of the kit may, for example, be affixed to a container that contains the nucleic acid, polypeptide, and/or compound of the invention or be shipped together with a container that contains the nucleic acid, polypeptide, and/or compound. Alternatively, the instructional material may be shipped separately from the container with the intention that the recipient uses the instructional material and the compound cooperatively.

“Isolated” means altered or removed from the natural state. For example, a nucleic acid or a polypeptide naturally present in a living animal is not “isolated,” but the same nucleic acid or polypeptide partially or completely separated from the coexisting materials

of its natural state is “isolated.” An isolated nucleic acid or protein can exist in substantially purified form, or can exist in a non-native environment such as, for example, a host cell.

An “isolated nucleic acid” refers to a nucleic acid segment or fragment which has been separated from sequences which flank it in a naturally occurring state, e.g., a DNA
5 fragment which has been removed from the sequences which are normally adjacent to the fragment, e.g., the sequences adjacent to the fragment in a genome in which it naturally occurs. The term also applies to nucleic acids which have been substantially purified from other components which naturally accompany the nucleic acid, e.g., RNA or DNA or proteins, which naturally accompany it in the cell. The term therefore includes, for
10 example, a recombinant DNA which is incorporated into a vector, into an autonomously replicating plasmid or virus, or into the genomic DNA of a prokaryote or eukaryote, or which exists as a separate molecule (e.g., as a cDNA or a genomic or cDNA fragment produced by PCR or restriction enzyme digestion) independent of other sequences. It also includes a recombinant DNA which is part of a hybrid gene encoding additional
15 polypeptide sequence.

The term “label” when used herein refers to a detectable compound or composition that is conjugated directly or indirectly to a probe to generate a “labeled” probe. The label may be detectable by itself (e.g., radioisotope labels or fluorescent labels) or, in the case of an enzymatic label, may catalyze chemical alteration of a substrate compound or
20 composition that is detectable (e.g., avidin-biotin). In some instances, primers can be labeled to detect a PCR product.

By the term “modulating,” as used herein, is meant mediating a detectable increase or decrease in the activity and/or level of a mRNA, polypeptide, or a response in a subject compared with the activity and/or level of a mRNA, polypeptide or a response in the subject
25 in the absence of a treatment or compound, and/or compared with the activity and/or level of a mRNA, polypeptide, or a response in an otherwise identical but untreated subject. The term encompasses activating, inhibiting and/or otherwise affecting a native signal or response thereby mediating a beneficial therapeutic, prophylactic, or other desired response in a subject, for example, a human.

30 A “mutation,” “mutant,” or “variant,” as used herein, refers to a change in nucleic acid or polypeptide sequence relative to a reference sequence (which may be a naturally-

occurring normal or the “wild-type” sequence), and includes translocations, deletions, insertions, and substitutions/point mutations. A “mutant” or “variant” as used herein, refers to either a nucleic acid or protein comprising a mutation.

A “nucleic acid” refers to a polynucleotide and includes poly-ribonucleotides and
5 poly-deoxyribonucleotides. Nucleic acids according to the present invention may include any polymer or oligomer of pyrimidine and purine bases, preferably cytosine, thymine, and uracil, and adenine and guanine, respectively. (See Albert L. Lehninger, Principles of Biochemistry, at 793-800 (Worth Pub. 1982) which is herein incorporated in its entirety for all purposes). Indeed, the present invention contemplates any deoxyribonucleotide,
10 ribonucleotide or peptide nucleic acid component, and any chemical variants thereof, such as methylated, hydroxymethylated or glucosylated forms of these bases, and the like. The polymers or oligomers may be heterogeneous or homogeneous in composition, and may be isolated from naturally occurring sources or may be artificially or synthetically produced. In addition, the nucleic acids may be DNA or RNA, or a mixture thereof, and
15 may exist permanently or transitionally in single-stranded or double-stranded form, including homoduplex, heteroduplex, and hybrid states.

An “oligonucleotide” or “polynucleotide” is a nucleic acid ranging from at least 2, preferably at least 8, 15 or 25 nucleotides in length, but may be up to 50, 100, 1000, or 5000 nucleotides long or a compound that specifically hybridizes to a polynucleotide.
20 Polynucleotides include sequences of deoxyribonucleic acid (DNA) or ribonucleic acid (RNA) or mimetics thereof which may be isolated from natural sources, recombinantly produced or artificially synthesized. A further example of a polynucleotide of the present invention may be a peptide nucleic acid (PNA). (See U.S. Pat. No. 6,156,501 which is hereby incorporated by reference in its entirety.) The invention also encompasses situations
25 in which there is a nontraditional base pairing such as Hoogsteen base pairing which has been identified in certain tRNA molecules and postulated to exist in a triple helix. “Polynucleotide” and “oligonucleotide” are used interchangeably in this disclosure. It will be understood that when a nucleotide sequence is represented herein by a DNA sequence (e.g., A, T, G, and C), this also includes the corresponding RNA sequence (e.g., A, U, G,
30 C) in which “U” replaces “T”.

The terms “patient,” “subject,” “individual,” and the like are used interchangeably herein, and refer to any animal, or cells thereof whether in vivo, in vitro or in situ, amenable to the methods described herein. In certain non-limiting embodiments, the patient, subject or individual is a human.

5 As used herein, the terms “peptide,” “polypeptide,” and “protein” are used interchangeably, and refer to a compound comprised of amino acid residues covalently linked by peptide bonds. A protein or peptide must contain at least two amino acids, and no limitation is placed on the maximum number of amino acids that can comprise a protein’s or peptide’s sequence. Polypeptides include any peptide or protein comprising
10 two or more amino acids joined to each other by peptide bonds. As used herein, the term refers to both short chains, which also commonly are referred to in the art as peptides, oligopeptides and oligomers, for example, and to longer chains, which generally are referred to in the art as proteins, of which there are many types. “Polypeptides” include, for example, biologically active fragments, substantially homologous polypeptides,
15 oligopeptides, homodimers, heterodimers, variants of polypeptides, modified polypeptides, derivatives, analogs, fusion proteins, among others. The polypeptides include natural peptides, recombinant peptides, synthetic peptides, mutant polypeptides, variant polypeptides, or a combination thereof.

 As used herein, “polynucleotide” includes cDNA, RNA, DNA/RNA hybrid,
20 antisense RNA, ribozyme, genomic DNA, synthetic forms, and mixed polymers, both sense and antisense strands, and may be chemically or biochemically modified to exhibit non-natural or derivatized, synthetic, or semi-synthetic nucleotide bases. Also, contemplated are alterations of a wild type or synthetic gene, including but not limited to deletion, insertion, substitution of one or more nucleotides, or fusion to other
25 polynucleotide sequences.

To “prevent” a disease or disorder as the term is used herein, means to reduce the severity or frequency of at least one sign or symptom of a disease or disorder that is to be experienced by a subject.

 “Sample” or “biological sample” as used herein means a biological material
30 isolated from a subject. The biological sample may contain any biological material suitable for detecting a mRNA, polypeptide or other marker of a physiologic or pathologic process

in a subject, and may comprise fluid, tissue, cellular and/or non-cellular material obtained from the individual.

As used herein, “substantially purified” refers to being essentially free of other components. For example, a substantially purified polypeptide is a polypeptide which has
5 been separated from other components with which it is normally associated in its naturally occurring state.

As used herein, the terms “therapy” or “therapeutic regimen” refer to those activities taken to prevent, treat or alter a disease or disorder, e.g., a course of treatment intended to reduce or eliminate at least one sign or symptom of a disease or disorder using
10 pharmacological, surgical, dietary and/or other techniques. A therapeutic regimen may include a prescribed dosage of one or more compounds or surgery. Therapies will most often be beneficial and reduce or eliminate at least one sign or symptom of the disorder or disease state, but in some instances the effect of a therapy will have non-desirable or side-effects. The effect of therapy will also be impacted by the physiological state of the subject,
15 e.g., age, gender, genetics, weight, other disease conditions, etc.

The term “therapeutically effective amount” refers to the amount of the subject compound or composition that will elicit the biological, physiologic, clinical or medical response of a cell, tissue, organ, system, or subject that is being sought by the researcher, veterinarian, medical doctor or other clinician. The term “therapeutically effective amount”
20 includes that amount of a compound or composition that, when administered, is sufficient to prevent development of, or treat to some extent, one or more of the signs or symptoms of the disorder or disease being treated. The therapeutically effective amount will vary depending on the compound or composition, the disease and its severity and the age, weight, etc., of the subject to be treated.

25 To “treat” a disease or disorder as the term is used herein, means to reduce the frequency or severity of at least one sign or symptom of a disease or disorder experienced by a subject. The terms “treatment”, “treating”, “treat” and the like are used herein to generally refer to obtaining a desired pharmacologic and/or physiologic effect. The effect can be prophylactic in terms of completely or partially preventing a disease or symptom(s)
30 thereof and/or may be therapeutic in terms of a partial or complete stabilization or cure for a disease and/or adverse effect attributable to the disease. The term “treatment”

encompasses any treatment of a disease in a mammal, particularly a human, and includes: (a) preventing the disease and/or symptom(s) from occurring in a subject who may be predisposed to the disease or symptom but has not yet been diagnosed as having it; (b) inhibiting the disease and/or symptom(s), e.g., slowing or arresting their development (e.g., halting the growth of tumors, slowing the rate of tumor growth, halting the rate of cancer cell proliferation, and the like); or (c) relieving the disease symptom(s), i.e., causing regression of the disease and/or symptom(s) (e.g., causing decrease in tumor size, reducing the number of cancer cells present, and the like). Those in need of treatment include those already inflicted (e.g., those with cancer, those with an infection, those with a metabolic disorder, those with macular degeneration, etc.) as well as those in which prevention is desired (e.g., those with increased susceptibility to cancer, those with an increased likelihood of infection, those suspected of having cancer, those suspected of harboring an infection, those with increased susceptibility for metabolic disease, those with increased susceptibility for macular degeneration, etc.).

As used herein, the term “wild-type” refers to a gene or gene product isolated from a naturally occurring source. A wild-type gene is that which is most frequently observed in a population and is thus arbitrarily designated the “normal” or “wild-type” form of the gene. In contrast, the term “modified,” “variant,” or “mutant” refers to a gene or gene product that possesses modifications in sequence and/or functional properties (i.e., altered characteristics) when compared to the wild-type gene or gene product.

Ranges: throughout this disclosure, various aspects of the invention can be presented in a range format. It should be understood that the description in range format is merely for convenience and brevity and should not be construed as an inflexible limitation on the scope of the invention. Accordingly, the description of a range should be considered to have specifically disclosed all the possible subranges as well as individual numerical values within that range. For example, description of a range such as from 1 to 6 should be considered to have specifically disclosed subranges such as from 1 to 3, from 1 to 4, from 1 to 5, from 2 to 4, from 2 to 6, from 3 to 6 etc., as well as individual numbers within that range, for example, 1, 2, 2.7, 3, 4, 5, 5.3, and 6. This applies regardless of the breadth of the range.

Description

In some embodiments, the compositions and methods of the invention comprise an activator of IL-18 activity, such as signaling activity through IL-18R. In some embodiments, the activator is an IL-18 variant polypeptide. In some embodiments, the
5 activator is a molecule that is able to bind and to signal through the IL-18R. In some embodiments, the activator is a molecule that inhibits IL-18BP, thereby promoting IL-18 signaling.

In some embodiments, the invention is an IL-18 variant polypeptide, or a fragment thereof, that specifically binds to IL-18R, and exhibits substantially reduced binding IL-
10 18BP. In some embodiments, an IL-18 variant polypeptide, or a fragment thereof, that binds to IL-18R, but does not bind substantially to IL-18BP, is useful for providing IL-18 signaling activity that is uninhibited by the presence and activity of IL-18BP.

In some embodiments, the IL-18 variant polypeptide is resistant to or independent of negative regulation by IL-18BP polypeptide. In some embodiments, IL-18BP
15 polypeptide is unable to substantially bind to the IL-18 variant polypeptide. The IL-18 variant polypeptides of the invention exhibit decreased binding affinity to IL-18BP, as compared with the WT IL-18 polypeptide. In some embodiments, the IL-18 variant polypeptide exhibits increased binding affinity to IL-18R, as compared with the WT IL-18 polypeptide. In some embodiments, the IL-18 variant polypeptide exhibits similar binding
20 affinity to IL-18R, as compared with the WT IL-18 polypeptide. In some embodiments, the IL-18 variant polypeptide exhibits decreased binding affinity to IL-18R, as compared with the WT IL-18 polypeptide.

In some embodiments, the invention provides a composition comprising an inhibitor of IL-18BP, wherein the inhibitor inhibits or reduces IL-18BP expression,
25 activity, or both. Exemplary inhibitors of IL-18BP include, but are not limited to, a chemical compound, a protein, a peptidomimetic, an antibody, a ribozyme, and an antisense nucleic acid molecule. In certain embodiments, the inhibitor of IL-18BP comprises a IL-18 variant that binds IL-18BP, thereby reducing or preventing IL-18BP from inhibiting IL-18 and IL-18 signaling.

30 In some embodiments, the IL-18 variant polypeptides are useful for the treatment or prevention of a disease or disorder. In various embodiments, the disease or disorder is

cancer or a metabolic disease or disorder, including obesity and diabetes (e.g., a subject method can cause a decrease in body fat). Thus, in some embodiments, the invention is a composition comprising at least one IL-18 variant polypeptide, or a fragment thereof. In other embodiments, the invention is a method of administering at least one IL-18 variant polypeptide, or a fragment thereof, to treat or prevent a disease or disorder, such as, but not limited to, cancer or a metabolic disease or disorder.

In some embodiments, the IL-18 variant polypeptide binds to IL-18R and exhibits substantially reduced binding to IL-18BP. In some embodiments, the IL-18 variant polypeptide that binds to IL-18R and exhibits substantially reduced binding to IL-18BP binds to IL-18BP with a binding affinity that is about 0.000000000001% to about 95% of the binding affinity of wild-type IL-18 to IL-18BP.

In some embodiments, the IL-18 variant polypeptide that binds to IL-18R and exhibits substantially reduced binding to IL-18BP binds to IL-18BP with a binding affinity that is about 95% of the binding affinity of wild-type IL-18 to IL-18BP. In some embodiments, the IL-18 variant polypeptide that binds to IL-18R and exhibits substantially reduced binding to IL-18BP binds to IL-18BP with a binding affinity that is about 90% of the binding affinity of wild-type IL-18 to IL-18BP. In some embodiments, the IL-18 variant polypeptide that binds to IL-18R and exhibits substantially reduced binding to IL-18BP binds to IL-18BP with a binding affinity that is about 85% of the binding affinity of wild-type IL-18 to IL-18BP. In some embodiments, the IL-18 variant polypeptide that binds to IL-18R and exhibits substantially reduced binding to IL-18BP binds to IL-18BP with a binding affinity that is about 80% of the binding affinity of wild-type IL-18 to IL-18BP. In some embodiments, the IL-18 variant polypeptide that binds to IL-18R and exhibits substantially reduced binding to IL-18BP binds to IL-18BP with a binding affinity that is about 75% of the binding affinity of wild-type IL-18 to IL-18BP. In some embodiments, the IL-18 variant polypeptide that binds to IL-18R and exhibits substantially reduced binding to IL-18BP binds to IL-18BP with a binding affinity that is about 70% of the binding affinity of wild-type IL-18 to IL-18BP. In some embodiments, the IL-18 variant polypeptide that binds to IL-18R and exhibits substantially reduced binding to IL-18BP binds to IL-18BP with a binding affinity that is about 65% of the binding affinity of wild-type IL-18 to IL-18BP. In some embodiments, the IL-18 variant polypeptide that binds to

IL-18R and exhibits substantially reduced binding to IL-18BP binds to IL-18BP with a binding affinity that is about 60% of the binding affinity of wild-type IL-18 to IL-18BP. In some embodiments, the IL-18 variant polypeptide that binds to IL-18R and exhibits substantially reduced binding to IL-18BP binds to IL-18BP with a binding affinity that is about 55% of the binding affinity of wild-type IL-18 to IL-18BP. In some embodiments, the IL-18 variant polypeptide that binds to IL-18R and exhibits substantially reduced binding to IL-18BP binds to IL-18BP with a binding affinity that is about 50% of the binding affinity of wild-type IL-18 to IL-18BP. In some embodiments, the IL-18 variant polypeptide that binds to IL-18R and exhibits substantially reduced binding to IL-18BP binds to IL-18BP with a binding affinity that is about 45% of the binding affinity of wild-type IL-18 to IL-18BP. In some embodiments, the IL-18 variant polypeptide that binds to IL-18R and exhibits substantially reduced binding to IL-18BP binds to IL-18BP with a binding affinity that is about 40% of the binding affinity of wild-type IL-18 to IL-18BP. In some embodiments, the IL-18 variant polypeptide that binds to IL-18R and exhibits substantially reduced binding to IL-18BP binds to IL-18BP with a binding affinity that is about 35% of the binding affinity of wild-type IL-18 to IL-18BP. In some embodiments, the IL-18 variant polypeptide that binds to IL-18R and exhibits substantially reduced binding to IL-18BP binds to IL-18BP with a binding affinity that is about 30% of the binding affinity of wild-type IL-18 to IL-18BP. In some embodiments, the IL-18 variant polypeptide that binds to IL-18R and exhibits substantially reduced binding to IL-18BP binds to IL-18BP with a binding affinity that is about 25% of the binding affinity of wild-type IL-18 to IL-18BP. In some embodiments, the IL-18 variant polypeptide that binds to IL-18R and exhibits substantially reduced binding to IL-18BP binds to IL-18BP with a binding affinity that is about 20% of the binding affinity of wild-type IL-18 to IL-18BP. In some embodiments, the IL-18 variant polypeptide that binds to IL-18R and exhibits substantially reduced binding to IL-18BP binds to IL-18BP with a binding affinity that is about 15% of the binding affinity of wild-type IL-18 to IL-18BP. In some embodiments, the IL-18 variant polypeptide that binds to IL-18R and exhibits substantially reduced

binding to IL-18BP binds to IL-18BP with a binding affinity that is about 10% of the binding affinity of wild-type IL-18 to IL-18BP.

In some embodiments, the IL-18 variant polypeptide that binds to IL-18R and exhibits substantially reduced binding to IL-18BP binds to IL-18BP with a binding affinity that is about 5% of the binding affinity of wild-type IL-18 to IL-18BP. In some
5 embodiments, the IL-18 variant polypeptide that binds to IL-18R and exhibits substantially reduced binding to IL-18BP binds to IL-18BP with a binding affinity that is about 4% of the binding affinity of wild-type IL-18 to IL-18BP. In some embodiments, the IL-18 variant polypeptide that binds to IL-18R and exhibits substantially reduced binding to IL-18BP
10 binds to IL-18BP with a binding affinity that is about 3% of the binding affinity of wild-type IL-18 to IL-18BP. In some embodiments, the IL-18 variant polypeptide that binds to IL-18R and exhibits substantially reduced binding to IL-18BP binds to IL-18BP with a binding affinity that is about 2% of the binding affinity of wild-type IL-18 to IL-18BP. In some embodiments, the IL-18 variant polypeptide that binds to IL-18R and exhibits
15 substantially reduced binding to IL-18BP binds to IL-18BP with a binding affinity that is about 1% of the binding affinity of wild-type IL-18 to IL-18BP.

In some embodiments, the IL-18 variant polypeptide that binds to IL-18R and exhibits substantially reduced binding to IL-18BP binds to IL-18BP with a binding affinity that is about 0.1% of the binding affinity of wild-type IL-18 to IL-18BP. In some
20 embodiments, the IL-18 variant polypeptide that binds to IL-18R and exhibits substantially reduced binding to IL-18BP binds to IL-18BP with a binding affinity that is about 0.01% of the binding affinity of wild-type IL-18 to IL-18BP. In some embodiments, the IL-18 variant polypeptide that binds to IL-18R and exhibits substantially reduced binding to IL-18BP binds to IL-18BP with a binding affinity that is about 0.001% of the binding affinity
25 of wild-type IL-18 to IL-18BP. In some embodiments, the IL-18 variant polypeptide that binds to IL-18R and exhibits substantially reduced binding to IL-18BP binds to IL-18BP with a binding affinity that is about 0.0001% of the binding affinity of wild-type IL-18 to IL-18BP. In some embodiments, the IL-18 variant polypeptide that binds to IL-18R and exhibits substantially reduced binding to IL-18BP binds to IL-18BP with a binding affinity
30 that is about 0.00001% of the binding affinity of wild-type IL-18 to IL-18BP. In some embodiments, the IL-18 variant polypeptide that binds to IL-18R and exhibits substantially

- reduced binding to IL-18BP binds to IL-18BP with a binding affinity that is about 0.000001% of the binding affinity of wild-type IL-18 to IL-18BP. In some embodiments, the IL-18 variant polypeptide that binds to IL-18R and exhibits substantially reduced binding to IL-18BP binds to IL-18BP with a binding affinity that is about 0.0000001% of the binding affinity of wild-type IL-18 to IL-18BP. In some embodiments, the IL-18 variant polypeptide that binds to IL-18R and exhibits substantially reduced binding to IL-18BP binds to IL-18BP with a binding affinity that is about 0.00000001% of the binding affinity of wild-type IL-18 to IL-18BP. In some embodiments, the IL-18 variant polypeptide that binds to IL-18R and exhibits substantially reduced binding to IL-18BP binds to IL-18BP with a binding affinity that is about 0.000000001% of the binding affinity of wild-type IL-18 to IL-18BP. In some embodiments, the IL-18 variant polypeptide that binds to IL-18R and exhibits substantially reduced binding to IL-18BP binds to IL-18BP with a binding affinity that is about 0.0000000001% of the binding affinity of wild-type IL-18 to IL-18BP. In some embodiments, the IL-18 variant polypeptide that binds to IL-18R and exhibits substantially reduced binding to IL-18BP binds to IL-18BP with a binding affinity that is about 0.00000000001% of the binding affinity of wild-type IL-18 to IL-18BP. In some embodiments, the IL-18 variant polypeptide that binds to IL-18R and exhibits substantially reduced binding to IL-18BP binds to IL-18BP with a binding affinity that is about 0.000000000001% of the binding affinity of wild-type IL-18 to IL-18BP.
- 20 In some embodiments, a subject IL-18 variant polypeptide (a DR-IL-18) that binds to IL-18R and exhibits substantially reduced binding to IL-18BP has a K_D for IL-18BP that is 10nM or greater (higher K_D means lower binding affinity). In some embodiments, a subject DR-IL-18 variant polypeptide has a K_D for IL-18BP that is 20nM or greater (e.g., 50 nM or greater, 100nM or greater, 500nM or greater, or 1 μ M or greater).
- 25 In some embodiments, the IL-18 variant polypeptide binds to IL-18R and exhibits substantially reduced binding to IL-18BP. In some embodiments, the variant polypeptide that binds to IL-18R and exhibits substantially reduced binding to IL-18BP has an IL-18BP/IL-18R dissociation constant ratio that is about at least 2-fold higher than the IL-18BP/IL-18R dissociation constant ratio of wild-type IL-18 (note that an increased dissociation constant ratio implies a relative decrease in IL-18BP binding relative to IL-18R binding). In some embodiments, the variant polypeptide that binds to IL-18R and
- 30

exhibits substantially reduced binding to IL-18BP has an IL-18BP/IL-18R dissociation constant ratio that is about at least 20-fold higher than the IL-18BP/IL-18R dissociation constant ratio of wild-type IL-18. In some embodiments, the variant polypeptide that binds to IL-18R and exhibits substantially reduced binding to IL-18BP has an IL-18BP/IL-18R dissociation constant ratio that is about at least 200-fold higher than the IL-18BP/IL-18R dissociation constant ratio of wild-type IL-18. In some embodiments, the variant polypeptide that binds to IL-18R and exhibits substantially reduced binding to IL-18BP has an IL-18BP/IL-18R dissociation constant ratio that is about at least 2,000-fold higher than the IL-18BP/IL-18R dissociation constant ratio of wild-type IL-18. In some embodiments, the variant polypeptide that binds to IL-18R and exhibits substantially reduced binding to IL-18BP has an IL-18BP/IL-18R dissociation constant ratio that is about at least 20,000-fold higher than the IL-18BP/IL-18R dissociation constant ratio of wild-type IL-18. In some embodiments, the variant polypeptide that binds to IL-18R and exhibits substantially reduced binding to IL-18BP has an IL-18BP/IL-18R dissociation constant ratio that is about at least 200,000-fold higher than the IL-18BP/IL-18R dissociation constant ratio of wild-type IL-18. In some embodiments, the variant polypeptide that binds to IL-18R and exhibits substantially reduced binding to IL-18BP has an IL-18BP/IL-18R dissociation constant ratio that is about at least 2,000,000-fold higher than the IL-18BP/IL-18R dissociation constant ratio of wild-type IL-18. In some embodiments, the variant polypeptide that binds to IL-18R and exhibits substantially reduced binding to IL-18BP has an IL-18BP/IL-18R dissociation constant ratio that is about at least 20,000,000-fold higher than the IL-18BP/IL-18R dissociation constant ratio of wild-type IL-18.

In some embodiments, the IL-18 variant polypeptide binds to IL-18R and exhibits substantially reduced binding to IL-18BP. In some embodiments, the variant polypeptide that binds to IL-18R and exhibits substantially reduced binding to IL-18BP has an IL-18BP/IL-18R dissociation constant ratio that is about at least 3-fold higher than the IL-18BP/IL-18R dissociation constant ratio of wild-type IL-18. In some embodiments, the variant polypeptide that binds to IL-18R and exhibits substantially reduced binding to IL-18BP has an IL-18BP/IL-18R dissociation constant ratio that is about at least 30-fold higher than the IL-18BP/IL-18R dissociation constant ratio of wild-type IL-18. In some

embodiments, the variant polypeptide that binds to IL-18R and exhibits substantially reduced binding to IL-18BP has an IL-18BP/IL-18R dissociation constant ratio that is about at least 300-fold higher than the IL-18BP/IL-18R dissociation constant ratio of wild-type IL-18. In some embodiments, the variant polypeptide that binds to IL-18R and exhibits substantially reduced binding to IL-18BP has an IL-18BP/IL-18R dissociation constant ratio that is about at least 3,000-fold higher than the IL-18BP/IL-18R dissociation constant ratio of wild-type IL-18. In some embodiments, the variant polypeptide that binds to IL-18R and exhibits substantially reduced binding to IL-18BP has an IL-18BP/IL-18R dissociation constant ratio that is about at least 30,000-fold higher than the IL-18BP/IL-18R dissociation constant ratio of wild-type IL-18. In some embodiments, the variant polypeptide that binds to IL-18R and exhibits substantially reduced binding to IL-18BP has an IL-18BP/IL-18R dissociation constant ratio that is about at least 300,000-fold higher than the IL-18BP/IL-18R dissociation constant ratio of wild-type IL-18. In some embodiments, the variant polypeptide that binds to IL-18R and exhibits substantially reduced binding to IL-18BP has an IL-18BP/IL-18R dissociation constant ratio that is about at least 3,000,000-fold higher than the IL-18BP/IL-18R dissociation constant ratio of wild-type IL-18. In some embodiments, the variant polypeptide that binds to IL-18R and exhibits substantially reduced binding to IL-18BP has an IL-18BP/IL-18R dissociation constant ratio that is about at least 30,000,000-fold higher than the IL-18BP/IL-18R dissociation constant ratio of wild-type IL-18.

In some embodiments, the IL-18 variant polypeptide binds to IL-18R and exhibits substantially reduced binding to IL-18BP. In some embodiments, the variant polypeptide that binds to IL-18R and exhibits substantially reduced binding to IL-18BP has an IL-18BP/IL-18R dissociation constant ratio that is about at least 5-fold higher than the IL-18BP/IL-18R dissociation constant ratio of wild-type IL-18. In some embodiments, the variant polypeptide that binds to IL-18R and exhibits substantially reduced binding to IL-18BP has an IL-18BP/IL-18R dissociation constant ratio that is about at least 50-fold higher than the IL-18BP/IL-18R dissociation constant ratio of wild-type IL-18. In some embodiments, the variant polypeptide that binds to IL-18R and exhibits substantially reduced binding to IL-18BP has an IL-18BP/IL-18R dissociation constant ratio that is about at least 500-fold higher than the IL-18BP/IL-18R dissociation constant ratio of wild-

type IL-18. In some embodiments, the variant polypeptide that binds to IL-18R and exhibits substantially reduced binding to IL-18BP has an IL-18BP/IL-18R dissociation constant ratio that is about at least 5,000-fold higher than the IL-18BP/IL-18R dissociation constant ratio of wild-type IL-18. In some embodiments, the variant polypeptide that binds to IL-18R and exhibits substantially reduced binding to IL-18BP has an IL-18BP/IL-18R dissociation constant ratio that is about at least 50,000-fold higher than the IL-18BP/IL-18R dissociation constant ratio of wild-type IL-18. In some embodiments, the variant polypeptide that binds to IL-18R and exhibits substantially reduced binding to IL-18BP has an IL-18BP/IL-18R dissociation constant ratio that is about at least 500,000-fold higher than the IL-18BP/IL-18R dissociation constant ratio of wild-type IL-18. In some embodiments, the variant polypeptide that binds to IL-18R and exhibits substantially reduced binding to IL-18BP has an IL-18BP/IL-18R dissociation constant ratio that is about at least 5,000,000-fold higher than the IL-18BP/IL-18R dissociation constant ratio of wild-type IL-18. In some embodiments, the variant polypeptide that binds to IL-18R and exhibits substantially reduced binding to IL-18BP has an IL-18BP/IL-18R dissociation constant ratio that is about at least 50,000,000-fold higher than the IL-18BP/IL-18R dissociation constant ratio of wild-type IL-18.

In some embodiments, the IL-18 variant polypeptide binds to IL-18R and exhibits substantially reduced binding to IL-18BP. In some embodiments, the variant polypeptide that binds to IL-18R and exhibits substantially reduced binding to IL-18BP has an IL-18BP/IL-18R dissociation constant ratio that is about at least 10-fold higher than the IL-18BP/IL-18R dissociation constant ratio of wild-type IL-18. In some embodiments, the variant polypeptide that binds to IL-18R and exhibits substantially reduced binding to IL-18BP has an IL-18BP/IL-18R dissociation constant ratio that is about at least 100-fold higher than the IL-18BP/IL-18R dissociation constant ratio of wild-type IL-18. In some embodiments, the variant polypeptide that binds to IL-18R and exhibits substantially reduced binding to IL-18BP has an IL-18BP/IL-18R dissociation constant ratio that is about at least 1000-fold higher than the IL-18BP/IL-18R dissociation constant ratio of wild-type IL-18. In some embodiments, the variant polypeptide that binds to IL-18R and exhibits substantially reduced binding to IL-18BP has an IL-18BP/IL-18R dissociation constant ratio that is about at least 10,000-fold higher than the IL-18BP/IL-18R

dissociation constant ratio of wild-type IL-18. In some embodiments, the variant polypeptide that binds to IL-18R and exhibits substantially reduced binding to IL-18BP has an IL-18BP/IL-18R dissociation constant ratio that is about at least 100,000-fold higher than the IL-18BP/IL-18R dissociation constant ratio of wild-type IL-18. In some
5 embodiments, the variant polypeptide that binds to IL-18R and exhibits substantially reduced binding to IL-18BP has an IL-18BP/IL-18R dissociation constant ratio that is about at least 1,000,000-fold higher than the IL-18BP/IL-18R dissociation constant ratio of wild-type IL-18. In some embodiments, the variant polypeptide that binds to IL-18R and exhibits substantially reduced binding to IL-18BP has an IL-18BP/IL-18R dissociation
10 constant ratio that is about at least 10,000,000-fold higher than the IL-18BP/IL-18R dissociation constant ratio of wild-type IL-18. In some embodiments, the variant polypeptide that binds to IL-18R and exhibits substantially reduced binding to IL-18BP has an IL-18BP/IL-18R dissociation constant ratio that is about at least 100,000,000-fold higher than the IL-18BP/IL-18R dissociation constant ratio of wild-type IL-18.

15 In some embodiments, a subject IL-18 variant polypeptide (a DR-IL-18) that binds to IL-18R and exhibits substantially reduced binding to IL-18BP has an inhibitor constant (K_i) for IL-18BP that is greater than 3 nM (e.g., 5nM or more, 10nM or more, 50 nM or more, 100nM or more, 500nM or more, 750 nM or more, or 1 μ M or more). In some embodiments, a subject DR-IL-18 variant polypeptide has a K_i for IL-18BP that is 500nM
20 or more. In some embodiments, a subject DR-IL-18 variant polypeptide has a K_i for IL-18BP that is 1 μ M or more.

 In some embodiments, a subject IL-18 variant polypeptide (a DR-IL-18) that binds to IL-18R and exhibits substantially reduced binding to IL-18BP has a K_i for IL-18BP that is greater than 200 nM (e.g., 500nM or more, 750 nM or more, or 1 μ M or more). In some
25 embodiments, a subject DR-IL-18 variant polypeptide has a K_i for IL-18BP that is 1 μ M or more.

 In some embodiments, a subject IL-18 variant polypeptide (a DR-IL-18) that binds to IL-18R and exhibits substantially reduced binding to IL-18BP has an inhibitor constant (K_i) for IL-18BP that is at least 2-fold higher than the K_i of wild type IL-18 for IL-18BP
30 (i.e., the K_i of the subject IL-18 variant polypeptide for IL-18BP is at least 2-fold relative to the K_i of WT IL-18 for IL-18BP). For example, in some cases a subject DR-IL-18 variant

polypeptide has a K_i for IL-18BP that is at least 5-fold higher (e.g., at least 10-fold, at least 50-fold, at least 100-fold, at least 200-fold, at least 500-fold, or at least 1000-fold higher) than the K_i of wild type IL-18 for IL-18BP.

In some embodiments, a subject IL-18 variant polypeptide (a DR-IL-18) that binds
5 to IL-18R and exhibits substantially reduced binding to IL-18BP has an EC_{50} for IL-18BP that is at least 2-fold higher than the EC_{50} of wild type IL-18 for IL-18BP (i.e., the EC_{50} of the subject IL-18 variant polypeptide for IL-18BP is at least 2-fold relative to the EC_{50} of WT IL-18 for IL-18BP). For example, in some cases a subject DR-IL-18 variant polypeptide has a EC_{50} for IL-18BP that is at least 5-fold higher (e.g., at least 10-fold, at
10 least 50-fold, at least 100-fold, at least 200-fold, at least 500-fold, or at least 1000-fold higher) than the EC_{50} of wild type IL-18 for IL-18BP.

In various embodiments, the IL-18 variant polypeptide comprises a mutation relative to a wild-type (WT) IL-18 polypeptide. In some embodiments, the WT IL-18 polypeptide comprises the amino acid sequence of SEQ ID NO: 30. In other embodiments,
15 the WT IL-18 polypeptide comprises the amino acid sequence of SEQ ID NO: 31. Unless otherwise specified, the term "X" is used below to represent any amino acid.

In various embodiments, the human IL-18 variant polypeptide, or fragment thereof, comprises at least one mutation (e.g., at least 2, at least 3, at least 4, at least 5, or at least 6 mutations) selected from the group consisting of Y1X, L5X, K8X, M51X, K53X, S55X,
20 Q56X, P57X, G59X, M60X, E77X, Q103X, S105X, D110X, N111X, M113X, V153X, and N155X, wherein X denotes any amino acid. In various embodiments, the human IL-18 variant polypeptide, or fragment thereof, comprises at least 4 mutations 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. In various
25 embodiments, the human IL-18 variant polypeptide, or fragment thereof, comprises at least 6 mutations 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. In various embodiments, the human IL-18 variant polypeptide, or fragment thereof, comprises at least one mutation (e.g., at least 2, at least 3, at least 4, at least 5, or
30 at least 6 mutations) selected from the group consisting of Y1X, L5X, K8X, S55X, Q56X, P57X, G59X, E77X, Q103X, S105X, D110X, N111X, M113X, V153X, and N155X. In

some embodiments, a human IL-18 variant polypeptide, or fragment thereof, comprises at least one mutation (e.g., at least 2, at least 3, at least 4, at least 5, or at least 6 mutations) 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. In some embodiments, a human IL-18 variant polypeptide comprises at least one IL-18 variant polypeptide, or fragment thereof, selected from the group consisting of hCS1 (SEQ ID NO: 34), hCS2 (SEQ ID NO: 35), hCS3 (SEQ ID NO: 36), hCS4 (SEQ ID NO: 37), hC4 (SEQ ID NO: 38), hA8 (SEQ ID NO: 39), hD6 (SEQ ID NO: 40), hH12 (SEQ ID NO: 41), hB11 (SEQ ID NO: 42), hC3 (SEQ ID NO: 43), hC2 (SEQ ID NO: 44), hG10 (SEQ ID NO: 45), hG1 (SEQ ID NO: 46), hF1 (SEQ ID NO: 47), hD2 (SEQ ID NO: 48), hA1 (SEQ ID NO: 49), hB3 (SEQ ID NO: 50), hB4 (SEQ ID NO: 51), hH3 (SEQ ID NO: 52), hH5 (SEQ ID NO: 53), hH4 (SEQ ID NO: 54), hE1 (SEQ ID NO: 55), hG2 (SEQ ID NO: 56), hB9 (SEQ ID NO: 57), hE12 (SEQ ID NO: 58), hC5 (SEQ ID NO: 59), 5-18 (SEQ ID NO: 73), 5-29 (SEQ ID NO: 74), 5-8 (SEQ ID NO: 75), 5-6 (SEQ ID NO: 76), 5-27 (SEQ ID NO: 77), 5-20 (SEQ ID NO: 78), 5-2 (SEQ ID NO: 79), 5-9 (SEQ ID NO: 80), 5-42 (SEQ ID NO: 81), 5-13 (SEQ ID NO: 82), 5-12 (SEQ ID NO: 83), 5-1 (SEQ ID NO: 84), 5-33 (SEQ ID NO: 85), 5-21 (SEQ ID NO: 86), 6-31 (SEQ ID NO: 87), 6-20 (SEQ ID NO: 88), 6-12 (SEQ ID NO: 89), 6-27 (SEQ ID NO: 90), 6-29 (SEQ ID NO: 91), 5-26 (SEQ ID NO: 191), 5-17 (SEQ ID NO: 192), 5-41 (SEQ ID NO: 193), or a fragment thereof.

In some cases a subject DR-IL-18 variant, or fragment thereof, includes at least one mutation (e.g., at least 2, at least 3, or at least 4 mutations) selected from the group consisting of M51X, M60X, S105X, D110X, and N111X, relative to SEQ ID NO: 30. In some cases a subject DR-IL-18 variant, or fragment thereof, includes at least 3 mutations selected from the group consisting of M51X, M60X, S105X, D110X, and N111X, relative to SEQ ID NO: 30. In some cases a subject DR-IL-18 variant, or fragment thereof, includes at least one mutation (e.g., at least 2, at least 3, or at least 4 mutations) selected from the

group consisting of M51X₁, M60X₂, S105X₃, D110X₄, and N111X₅, relative to SEQ ID NO: 30, where X₁ is T, K, D, E, R, or N; X₂ is K, Q, L, or R; X₃ is R, D, K, A, or N; X₄ is H, K, N, Q, E, N, S, or G; and X₅ is H, D, Y, R, S, or G. In some cases a subject DR-IL-18 variant, or fragment thereof, includes at least 3 mutations (e.g., at least 2, at least 3, or at least 4 mutations) selected from the group consisting of M51X₁, M60X₂, S105X₃, D110X₄, and N111X₅, relative to SEQ ID NO: 30, where X₁ is T, K, D, E, R, or N; X₂ is K, Q, L, or R; X₃ is R, D, K, A, or N; X₄ is H, K, N, Q, E, N, S, or G; and X₅ is H, D, Y, R, S, or G. In some cases a subject DR-IL-18 variant, or fragment thereof, includes at least one mutation (e.g., at least 2, at least 3, or at least 4 mutations) selected from the group consisting of M51X₁, M60X₂, S105X₃, D110X₄, and N111X₅, relative to SEQ ID NO: 30, where X₁ is T or K; X₂ is K or L; X₃ is D, N, or A; X₄ is K, N, S, or G; and X₅ is H, Y, G, or R.

In some cases a subject DR-IL-18 variant, or fragment thereof, includes the mutations M51X, M60X, S105X, D110X, and N111X, relative to SEQ ID NO: 30. For example, in some cases a subject DR-IL-18 variant, or fragment thereof, includes the mutations M51X₁, M60X₂, S105X₃, D110X₄, and N111X₅, relative to SEQ ID NO: 30, where X₁ is T, K, D, E, R, or N; X₂ is K, Q, L, or R; X₃ is R, D, K, A, or N; X₄ is H, K, N, Q, E, N, S, or G; and X₅ is H, D, Y, R, S, or G. In some cases a subject DR-IL-18 variant, or fragment thereof, includes the mutations M51X₁, M60X₂, S105X₃, D110X₄, and N111X₅, relative to SEQ ID NO: 30, where X₁ is T or K; X₂ is K or L; X₃ is D, N, or A; X₄ is K, N, S, or G; and X₅ is H, Y, G, or R. In other words, in some cases a subject DR-IL-18 variant, or fragment thereof, includes the mutations {M51T or M51K}; {M60K or M60L}; {S105D, S105N, S105A}; {D110K, D110N, D110S, or D110G}; and {N111H, N111Y, N111R, or N111G}, relative to SEQ ID NO: 30.

In some cases a subject DR-IL-18 variant, or fragment thereof, includes at least one mutation (e.g., at least 2, at least 3, or at least 4 mutations) selected from the group consisting of M51X, K53X, Q56X, S105X, and N111X, relative to SEQ ID NO: 30. In some cases a subject DR-IL-18 variant, or fragment thereof, includes at least 3 mutations selected from the group consisting of M51X, K53X, Q56X, S105X, and N111X, relative to SEQ ID NO: 30. In some cases a subject DR-IL-18 variant, or fragment thereof, includes at least one mutation (e.g., at least 2, at least 3, or at least 4 mutations) selected from the group consisting of M51X₁, K53X₂, Q56X₃, S105X₄, and N111X₅, relative to SEQ ID

NO: 30, where X_1 is E, R, or K; X_2 is G, S, or T; X_3 is E, A, R, V, G, K, or L; X_4 is N, S, K, or G; and X_5 is R, S, G, or D. In some cases a subject DR-IL-18 variant, or fragment thereof, includes at least 3 mutations selected from the group consisting of M51 X_1 , K53 X_2 , Q56 X_3 , S105 X_4 , and N111 X_5 , relative to SEQ ID NO: 30, where X_1 is E, R, or K; X_2 is G, S, or T; X_3 is E, A, R, V, G, K, or L; X_4 is N, S, K, or G; and X_5 is R, S, G, or D. In some cases a subject DR-IL-18 variant, or fragment thereof, includes at least one mutation (e.g., at least 2, at least 3, or at least 4 mutations) selected from the group consisting of M51 X_1 , K53 X_2 , Q56 X_3 , S105 X_4 , and N111 X_5 , relative to SEQ ID NO: 30, where X_1 is K; X_2 is G or S; X_3 is G, R, or L; X_4 is S, N, or G; and X_5 is G or R.

10 In some cases a subject DR-IL-18 variant, or fragment thereof, includes the mutations M51X, K53X, Q56X, S105X, and N111X, relative to SEQ ID NO: 30. For example, in some cases a subject DR-IL-18 variant, or fragment thereof, includes the mutations M51 X_1 , K53 X_2 , Q56 X_3 , S105 X_4 , and N111 X_5 , relative to SEQ ID NO: 30, where X_1 is E, R, or K; X_2 is G, S, or T; X_3 is E, A, R, V, G, K, or L; X_4 is N, S, K, or G; and X_5 is R, S, G, or D. In some cases a subject DR-IL-18 variant, or fragment thereof, includes the mutations M51 X_1 , K53 X_2 , Q56 X_3 , S105 X_4 , and N111 X_5 , relative to SEQ ID NO: 30, where X_1 is K; X_2 is G or S; X_3 is G, R, or L; X_4 is S, N, or G; and X_5 is G or R. In other words, in some cases a subject DR-IL-18 variant, or fragment thereof, includes the mutations {M51K}; {K53G or K53S}; {Q56G, Q56R, or Q56L}; {D110S, D110N, or D110G}; and {N111R, or N111G}, relative to SEQ ID NO: 30.

25 In some cases a subject DR-IL-18 variant, or fragment thereof, comprises an amino acid sequence having 85% or more sequence identity (e.g., 90% or more, 92% or more, 93% or more, 94% or more, 95% or more, 96% or more, 97% or more, 98% or more, or 99% or more sequence identity) with the wild type human IL-18 amino acid sequence set forth as SEQ ID NO: 30. As such in some cases a subject DR-IL-18 variant, or fragment thereof, comprises an amino acid sequence that (i) has 85% or more sequence identity (e.g., 90% or more, 92% or more, 93% or more, 94% or more, 95% or more, 96% or more, 97% or more, 98% or more, or 99% or more sequence identity) with the wild type human IL-18 amino acid sequence set forth as SEQ ID NO: 30; and (ii) includes at least one mutation (e.g., at least 2, at least 3, at least 4, at least 5, or at least 6 mutations) relative to wild type IL-18 (e.g., human IL-18).

In some cases a subject DR-IL-18 variant, or fragment thereof, comprises an amino acid sequence having 85% or more sequence identity (e.g., 90% or more, 92% or more, 93% or more, 94% or more, 95% or more, 96% or more, 97% or more, 98% or more, or 99% or more sequence identity) with the amino acid sequence set forth in any one of SEQ ID NOs: 34-59, 73-91, and 191-193. As such in some cases a subject DR-IL-18 variant, or fragment thereof, comprises an amino acid sequence that (i) has 85% or more sequence identity (e.g., 90% or more, 92% or more, 93% or more, 94% or more, 95% or more, 96% or more, 97% or more, 98% or more, or 99% or more sequence identity) with the amino acid sequence set forth in any one of SEQ ID NOs: 34-59, 73-91, and 191-193; and (ii) includes at least one mutation (e.g., at least 2, at least 3, at least 4, at least 5, or at least 6 mutations) relative to wild type IL-18 (e.g., human IL-18).

In some cases a subject DR-IL-18 variant, or fragment thereof, comprises an amino acid sequence that (i) has 85% or more sequence identity (e.g., 90% or more, 92% or more, 93% or more, 94% or more, 95% or more, 96% or more, 97% or more, 98% or more, or 99% or more sequence identity) with the wild type human IL-18 amino acid sequence set forth as SEQ ID NO: 30; and (ii) includes at least one mutation (e.g., at least 2, at least 3, at least 4, at least 5, or at least 6 mutations) 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, relative to SEQ ID NO: 30. In some cases a subject DR-IL-18 variant, or fragment thereof, comprises an amino acid sequence that (i) has 85% or more sequence identity (e.g., 90% or more, 92% or more, 93% or more, 94% or more, 95% or more, 96% or more, 97% or more, 98% or more, or 99% or more sequence identity) with the wild type human IL-18 amino acid sequence set forth as SEQ ID NO: 30; and (ii) includes at least 4 mutations 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, relative to SEQ ID NO: 30. In some cases a subject DR-IL-18 variant, or fragment thereof, comprises an amino acid sequence that (i) has 85% or more sequence identity (e.g., 90% or more, 92% or more, 93% or more, 94% or more, 95% or more, 96% or more, 97% or more, 98% or more, or 99% or more sequence identity) with the wild type human IL-18 amino acid sequence set forth as SEQ ID NO: 30; and (ii) includes at least 6 mutations 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, relative to SEQ ID NO: 30. In some cases a subject DR-IL-18 variant, or fragment thereof, comprises an amino acid sequence that (i) has 85% or more sequence identity (e.g., 90% or more, 92% or more, 93% or more, 94% or more, 95% or more, 96% or more, 97% or more, 98% or more, or 99% or more sequence identity) with the wild type human IL-18 amino acid sequence set forth as SEQ ID NO: 30; and (ii) includes at least one mutation (e.g., at least 2, at least 3, at least 4, at least 5, or at least 6 mutations) selected from the group consisting of Y1X, L5X, K8X, S55X, Q56X, P57X, G59X, E77X, Q103X, S105X, D110X, N111X, M113X, V153X, and N155X, relative to SEQ ID NO: 30.

In some cases a subject DR-IL-18 variant, or fragment thereof, comprises an amino acid sequence that (i) has 85% or more sequence identity (e.g., 90% or more, 92% or more, 93% or more, 94% or more, 95% or more, 96% or more, 97% or more, 98% or more, or 99% or more sequence identity) with the wild type human IL-18 amino acid sequence set forth as SEQ ID NO: 30; and (ii) includes at least one mutation (e.g., at least 2, at least 3, or at least 4 mutations) selected from the group consisting of M51X, M60X, S105X, D110X, and N111X, relative to SEQ ID NO: 30. In some cases a subject DR-IL-18 variant, or fragment thereof, comprises an amino acid sequence that (i) has 85% or more sequence identity (e.g., 90% or more, 92% or more, 93% or more, 94% or more, 95% or more, 96% or more, 97% or more, 98% or more, or 99% or more sequence identity) with the wild type human IL-18 amino acid sequence set forth as SEQ ID NO: 30; and (ii) includes at least 3 mutations selected from the group consisting of M51X, M60X, S105X, D110X, and N111X, relative to SEQ ID NO: 30. In some cases a subject DR-IL-18 variant, or fragment thereof, comprises an amino acid sequence that (i) has 85% or more sequence identity (e.g., 90% or more, 92% or more, 93% or more, 94% or more, 95% or more, 96% or more, 97% or more, 98% or more, or 99% or more sequence identity) with the wild type human IL-18 amino acid sequence set forth as SEQ ID NO: 30; and (ii) includes at least one mutation (e.g., at least 2, at least 3, or at least 4 mutations) selected from the group consisting of M51X₁, M60X₂, S105X₃, D110X₄, and N111X₅, relative to SEQ ID NO: 30, where X₁ is T, K, D, E, R, or N; X₂ is K, Q, L, or R; X₃ is R, D, K, A, or N; X₄ is H, K, N, Q, E, N, S, or G; and X₅ is H, D, Y, R, S, or G. In some cases a subject DR-IL-18 variant, or fragment

thereof, comprises an amino acid sequence that (i) has 85% or more sequence identity (e.g., 90% or more, 92% or more, 93% or more, 94% or more, 95% or more, 96% or more, 97% or more, 98% or more, or 99% or more sequence identity) with the wild type human IL-18 amino acid sequence set forth as SEQ ID NO: 30; and (ii) includes at least 3 mutations selected from the group consisting of M51X₁, M60X₂, S105X₃, D110X₄, and N111X₅, relative to SEQ ID NO: 30, where X₁ is T, K, D, E, R, or N; X₂ is K, Q, L, or R; X₃ is R, D, K, A, or N; X₄ is H, K, N, Q, E, N, S, or G; and X₅ is H, D, Y, R, S, or G. In some cases a subject DR-IL-18 variant, or fragment thereof, comprises an amino acid sequence that (i) has 85% or more sequence identity (e.g., 90% or more, 92% or more, 93% or more, 94% or more, 95% or more, 96% or more, 97% or more, 98% or more, or 99% or more sequence identity) with the wild type human IL-18 amino acid sequence set forth as SEQ ID NO: 30; and (ii) includes at least one mutation (e.g., at least 2, at least 3, or at least 4 mutations) selected from the group consisting of M51X₁, M60X₂, S105X₃, D110X₄, and N111X₅, relative to SEQ ID NO: 30, where X₁ is T or K; X₂ is K or L; X₃ is D, N, or A; X₄ is K, N, S, or G; and X₅ is H, Y, G, or R.

In some cases a subject DR-IL-18 variant, or fragment thereof, comprises an amino acid sequence that (i) has 85% or more sequence identity (e.g., 90% or more, 92% or more, 93% or more, 94% or more, 95% or more, 96% or more, 97% or more, 98% or more, or 99% or more sequence identity) with the wild type human IL-18 amino acid sequence set forth as SEQ ID NO: 30; and (ii) includes the mutations M51X, M60X, S105X, D110X, and N111X, relative to SEQ ID NO: 30. In some cases a subject DR-IL-18 variant, or fragment thereof, comprises an amino acid sequence that (i) has 85% or more sequence identity (e.g., 90% or more, 92% or more, 93% or more, 94% or more, 95% or more, 96% or more, 97% or more, 98% or more, or 99% or more sequence identity) with the wild type human IL-18 amino acid sequence set forth as SEQ ID NO: 30; and (ii) includes the mutations M51X₁, M60X₂, S105X₃, D110X₄, and N111X₅, relative to SEQ ID NO: 30, where X₁ is T, K, D, E, R, or N; X₂ is K, Q, L, or R; X₃ is R, D, K, A, or N; X₄ is H, K, N, Q, E, N, S, or G; and X₅ is H, D, Y, R, S, or G. In some cases a subject DR-IL-18 variant, or fragment thereof, comprises an amino acid sequence that (i) has 85% or more sequence identity (e.g., 90% or more, 92% or more, 93% or more, 94% or more, 95% or more, 96% or more, 97% or more, 98% or more, or 99% or more sequence identity) with the wild

type human IL-18 amino acid sequence set forth as SEQ ID NO: 30; and (ii) includes the mutations M51X₁, M60X₂, S105X₃, D110X₄, and N111X₅, relative to SEQ ID NO: 30, where X₁ is T or K; X₂ is K or L; X₃ is D, N, or A; X₄ is K, N, S, or G; and X₅ is H, Y, G, or R.

- 5 In some cases a subject DR-IL-18 variant, or fragment thereof, comprises an amino acid sequence that (i) has 85% or more sequence identity (e.g., 90% or more, 92% or more, 93% or more, 94% or more, 95% or more, 96% or more, 97% or more, 98% or more, or 99% or more sequence identity) with the wild type human IL-18 amino acid sequence set forth as SEQ ID NO: 30; and (ii) includes at least one mutation (e.g., at least 2, at least 3,
- 10 or at least 4 mutations) selected from the group consisting of M51X, K53X, Q56X, S105X, and N111X, relative to SEQ ID NO: 30. In some cases a subject DR-IL-18 variant, or fragment thereof, comprises an amino acid sequence that (i) has 85% or more sequence identity (e.g., 90% or more, 92% or more, 93% or more, 94% or more, 95% or more, 96% or more, 97% or more, 98% or more, or 99% or more sequence identity) with the wild
- 15 type human IL-18 amino acid sequence set forth as SEQ ID NO: 30; and (ii) includes at least 3 mutations selected from the group consisting of M51X, K53X, Q56X, S105X, and N111X, relative to SEQ ID NO: 30. In some cases a subject DR-IL-18 variant, or fragment thereof, comprises an amino acid sequence that (i) has 85% or more sequence identity (e.g., 90% or more, 92% or more, 93% or more, 94% or more, 95% or more, 96% or more, 97%
- 20 or more, 98% or more, or 99% or more sequence identity) with the wild type human IL-18 amino acid sequence set forth as SEQ ID NO: 30; and (ii) includes at least one mutation (e.g., at least 2, at least 3, or at least 4 mutations) selected from the group consisting of M51X₁, K53X₂, Q56X₃, S105X₄, and N111X₅, relative to SEQ ID NO: 30, where X₁ is E, R, or K; X₂ is G, S, or T; X₃ is E, A, R, V, G, K, or L; X₄ is N, S, K, or G; and X₅ is R, S,
- 25 G, or D. In some cases a subject DR-IL-18 variant, or fragment thereof, comprises an amino acid sequence that (i) has 85% or more sequence identity (e.g., 90% or more, 92% or more, 93% or more, 94% or more, 95% or more, 96% or more, 97% or more, 98% or more, or 99% or more sequence identity) with the wild type human IL-18 amino acid sequence set forth as SEQ ID NO: 30; and (ii) includes at least 3 mutations selected from the group
- 30 consisting of M51X₁, K53X₂, Q56X₃, S105X₄, and N111X₅, relative to SEQ ID NO: 30, where X₁ is E, R, or K; X₂ is G, S, or T; X₃ is E, A, R, V, G, K, or L; X₄ is N, S, K, or G;

and X₅ is R, S, G, or D. In some cases a subject DR-IL-18 variant, or fragment thereof, comprises an amino acid sequence that (i) has 85% or more sequence identity (e.g., 90% or more, 92% or more, 93% or more, 94% or more, 95% or more, 96% or more, 97% or more, 98% or more, or 99% or more sequence identity) with the wild type human IL-18 amino acid sequence set forth as SEQ ID NO: 30; and (ii) includes at least one mutation (e.g., at least 2, at least 3, or at least 4 mutations) selected from the group consisting of M51X₁, K53X₂, Q56X₃, S105X₄, and N111X₅, relative to SEQ ID NO: 30, where X₁ is K; X₂ is G or S; X₃ is G, R, or L; X₄ is S, N, or G; and X₅ is G or R.

In some cases a subject DR-IL-18 variant, or fragment thereof, comprises an amino acid sequence that (i) has 85% or more sequence identity (e.g., 90% or more, 92% or more, 93% or more, 94% or more, 95% or more, 96% or more, 97% or more, 98% or more, or 99% or more sequence identity) with the wild type human IL-18 amino acid sequence set forth as SEQ ID NO: 30; and (ii) includes the mutations M51X, K53X, Q56X, S105X, and N111X, relative to SEQ ID NO: 30. In some cases a subject DR-IL-18 variant, or fragment thereof, comprises an amino acid sequence that (i) has 85% or more sequence identity (e.g., 90% or more, 92% or more, 93% or more, 94% or more, 95% or more, 96% or more, 97% or more, 98% or more, or 99% or more sequence identity) with the wild type human IL-18 amino acid sequence set forth as SEQ ID NO: 30; and (ii) includes the mutations M51X₁, K53X₂, Q56X₃, S105X₄, and N111X₅, relative to SEQ ID NO: 30, where X₁ is E, R, or K; X₂ is G, S, or T; X₃ is E, A, R, V, G, K, or L; X₄ is N, S, K, or G; and X₅ is R, S, G, or D. In some cases a subject DR-IL-18 variant, or fragment thereof, comprises an amino acid sequence that (i) has 85% or more sequence identity (e.g., 90% or more, 92% or more, 93% or more, 94% or more, 95% or more, 96% or more, 97% or more, 98% or more, or 99% or more sequence identity) with the wild type human IL-18 amino acid sequence set forth as SEQ ID NO: 30; and (ii) includes the mutations M51X₁, K53X₂, Q56X₃, S105X₄, and N111X₅, relative to SEQ ID NO: 30, where X₁ is K; X₂ is G or S; X₃ is G, R, or L; X₄ is S, N, or G; and X₅ is G or R.

In some embodiments, the murine IL-18 variant polypeptide comprises at least one mutation (e.g., at least 2, at least 3, at least 4, at least 5, or at least 6 mutations) selected from the group consisting of N1X, M50X, Y51X, K52X, S54X, E55X, V56X, R57X, G58X, L59X, R104X, N109X, and L151X, wherein X denotes any amino acid. In some

embodiments, a murine IL-18 variant polypeptide, or fragment thereof, comprises at least one mutation (e.g., at least 2, at least 3, at least 4, at least 5, or at least 6 mutations) selected from 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. In some embodiments, a murine IL-18 variant polypeptide comprises at least one variant selected from the group consisting of mCS1 (SEQ ID NO: 60), mCS2 (SEQ ID NO: 61), mC1 (SEQ ID NO: 62), mA12 (SEQ ID NO: 63), mE8 (SEQ ID NO: 64), mC10 (SEQ ID NO: 65), mB7 (SEQ ID NO: 66), mB1 (SEQ ID NO: 67), mD1 (SEQ ID NO: 68), mH7 (SEQ ID NO: 69), mA7 (SEQ ID NO: 70), mE1 (SEQ ID NO: 71), and mH3 (SEQ ID NO: 72), or a fragment thereof.

In some embodiments, the invention is a nucleic acid (e.g., DNA, cDNA, mRNA, etc.) that encodes at least one IL-18 variant polypeptide.

Therapeutic Inhibitor Compositions and Methods

In various embodiments, the present invention includes IL-18BP inhibitor compositions and methods of treating or preventing a disease or disorder where a diminished activity or level of IL-18BP is desired. The indications for such an agent are encompassed by the indications elaborated for a DR-IL-18 variant above. Non-limiting examples of diseases or disorders where a diminished activity or level of IL-18BP is desired which can be treated or prevented with the compositions and methods of the invention include cancer, infectious disease, metabolic diseases or disorders, and macular degeneration. In various embodiments, the IL-18BP inhibitor compositions and methods of treatment or prevention of the invention diminish the amount of IL-18BP polypeptide, the amount of IL-18BP mRNA, the amount of IL-18BP enzymatic activity, the amount of IL-18BP substrate binding activity, or a combination thereof.

It will be understood by one skilled in the art, based upon the disclosure provided herein, that a decrease in the level of IL-18BP encompasses a decrease in IL-18BP expression, including transcription, translation, or both. The skilled artisan will also appreciate, once armed with the teachings of the present invention, that a decrease in the

level of IL-18BP includes a decrease in IL-18BP activity (e.g., enzymatic activity, substrate binding activity, etc.). Thus, decreasing the level or activity of IL-18BP includes, but is not limited to, decreasing transcription, translation, or both, of a nucleic acid encoding IL-18BP; and it also includes decreasing any activity of an IL-18BP polypeptide as well. The
5 IL-18BP inhibitor compositions and methods of the invention can selectively inhibit IL-18BP, or can inhibit both IL-18BP and another molecule.

Inhibition of IL-18BP can be assessed using a wide variety of methods, including those disclosed herein, as well as methods known in the art or to be developed in the future. That is, the routineer would appreciate, based upon the disclosure provided herein, that
10 decreasing the level or activity of IL-18BP can be readily assessed using methods that assess the level of a nucleic acid encoding IL-18BP (e.g., mRNA), the level of an IL-18BP polypeptide present in a biological sample, the level of IL-18BP activity (e.g., enzymatic activity, substrate binding activity, etc.), or combinations thereof.

One skilled in the art, based upon the disclosure provided herein, would understand
15 that the invention is useful in treating or preventing a disease or disorder in a subject in need thereof, whether or not the subject is also being treated with another medication or therapy. Further, the skilled artisan would appreciate, based upon the teachings provided herein, that the disease or disorder treatable by the compositions and methods described herein encompass any disease or disorder where IL-18BP plays a role and where
20 diminished IL-18BP level or activity promotes a positive therapeutic outcome.

The IL-18BP inhibitor compositions and methods of the invention that decrease the level or activity (e.g., enzymatic activity, ligand binding activity, etc.) of IL-18BP include, but should not be construed as being limited to, a chemical compound, a polypeptide, a peptide, a peptidomimetic, an antibody, a ribozyme, a small molecule chemical compound,
25 an antisense nucleic acid molecule (e.g., siRNA, miRNA, etc.), or combinations thereof. One of skill in the art would readily appreciate, based on the disclosure provided herein, that an IL-18BP inhibitor composition encompasses a chemical compound that decreases the level or activity of IL-18BP. Additionally, an IL-18BP inhibitor composition encompasses a chemically modified compound, and derivatives, as is well known to one
30 of skill in the chemical arts.

The IL-18BP inhibitor compositions and methods of the invention that decrease the level or activity (e.g., enzymatic activity, ligand binding activity, etc.) of IL-18BP include antibodies. The antibodies of the invention include a variety of forms of antibodies including, for example, polyclonal antibodies, monoclonal antibodies, intracellular
5 antibodies (“intrabodies”), Fv, Fab and F(ab)₂, single chain antibodies (scFv), heavy chain antibodies (such as camelid antibodies), synthetic antibodies, chimeric antibodies, and humanized antibodies. In some embodiments, the antibody of the invention is an antibody that specifically binds to IL-18BP.

In some embodiments, the IL-18BP inhibitor comprises an engineered IL-18
10 variant that is designed to bind to IL-18BP, but does not substantially bind to or interact with the IL-18R (e.g., IL-18R α and IL-18R β). This variant, a “decoy-to-the-decoy” (D2D), serves to bind to IL-18BP and thus prevent IL-18BP from inhibiting endogenously-produced or therapeutically-introduced IL-18. The “decoy-to-the-decoy” could be engineered using nearly the same yeast display selection strategies described elsewhere
15 herein, but instead positively selecting for IL-18BP binding, and counter-selected against substantially binding to IL-18R α .

In various embodiments, the IL-18 variant polypeptide that binds to and inhibits IL-18BP, comprises a mutation relative to a wild-type (WT) IL-18 polypeptide. In some
20 embodiments, the WT IL-18 polypeptide comprises the amino acid sequence of SEQ ID NO: 30. In other embodiments, the WT IL-18 polypeptide comprises the amino acid sequence of SEQ ID NO: 31.

In various embodiments, the IL-18 variant polypeptide that binds to and inhibits IL-18BP comprises a human IL-18 variant polypeptide, or fragment thereof, comprising at
25 least one mutation (e.g., at least 2, at least 3, at least 4, at least 5, or at least 6 mutations) selected from the group consisting of Y1X, L5X, D17X, E31X, T34X, D35X, S36X, D37X, D40X, N41X, M51X, Q56X, M60X, Q103X, H109X, M113X, and R131X, wherein X denotes any amino acid. In some embodiments, the IL-18 variant polypeptide that binds to and inhibits IL-18BP comprises a human IL-18 variant polypeptide, or
30 fragment thereof, comprising at least one mutation (e.g., at least 2, at least 3, at least 4, at least 5, or at least 6 mutations) selected from the group consisting of Y1D, Y1F, Y1H, Y1L, L5F, L5H, D17A, D17G, D17R, D17H, E31A, E31T, E31G, E31K, E31R, T34A,

T34K T34E, D35S, D35A, D35Y, S36N, S36K, S36R, D37P, D37A, D37R, D37H, D37L, D37V, D40Y D40S, D40A, N41K, N41S, N41R, M51F, M51L, M51I, Q56H, M60L, M60F, M60I, Q103L, Q103I, H109A, H109P, H109D, M113L, M113I, M113F, and R131S. In some embodiments, the IL-18 variant polypeptide that binds to and inhibits IL-18BP comprises a human IL-18 variant polypeptide, or fragment thereof, selected from the group consisting of hD2D-5F12 (SEQ ID NO: 92), hD2D-5F11 (SEQ ID NO: 93), hD2D-5F10 (SEQ ID NO: 94), hD2D-5F08 (SEQ ID NO: 95), hD2D-5F06 (SEQ ID NO: 96), hD2D-5F04 (SEQ ID NO: 97), hD2D-5F02 (SEQ ID NO: 98), hD2D-5F01 (SEQ ID NO: 99), hD2D-5E10 (SEQ ID NO: 100), hD2D-5E08 (SEQ ID NO: 101), hD2D-5E03 (SEQ ID NO: 102), hD2D-5E02 (SEQ ID NO: 103), hD2D-5D10 (SEQ ID NO: 104), hD2D-5D08 (SEQ ID NO: 105), hD2D-5D06 (SEQ ID NO: 106), hD2D-5D05 (SEQ ID NO: 107), hD2D-5D03 (SEQ ID NO: 108), hD2D-5D02 (SEQ ID NO: 109), hD2D-5C10 (SEQ ID NO: 110), hD2D-5C09 (SEQ ID NO: 111), hD2D-5C08 (SEQ ID NO: 112), hD2D-5C05 (SEQ ID NO: 113), hD2D-5C04 (SEQ ID NO: 114), hD2D-5C03 (SEQ ID NO: 115), hD2D-5B11 (SEQ ID NO: 116), hD2D-5B10 (SEQ ID NO: 117), hD2D-5B06 (SEQ ID NO: 118), hD2D-5B05 (SEQ ID NO: 119), hD2D-5B02 (SEQ ID NO: 120), hD2D-5A09 (SEQ ID NO: 121), hD2D-5A02 (SEQ ID NO: 122), hD2D-CS1 (SEQ ID NO: 123), hD2D-CS2 (SEQ ID NO: 124), hD2D-CS3 (SEQ ID NO: 125), or a fragment thereof.

In some cases a subject D2D-IL-18 variant, or fragment thereof, includes at least one mutation (e.g., at least 2, at least 3, or at least 4 mutations) selected from the group consisting of D17X, E30X, and Q103X, relative to SEQ ID NO: 30. In some cases a subject D2D-IL-18 variant, or fragment thereof, includes at least one mutation (e.g., at least 2, at least 3, or at least 4 mutations) selected from the group consisting of D17X₁, E30X₂, and Q103X₃, relative to SEQ ID NO: 30, where X₁ is G, H, R, or A; X₂ is A, T, G, K, or R; and X₃ is I or L. In some cases a subject D2D-IL-18 variant, or fragment thereof, includes at least one mutation (e.g., at least 2, at least 3, or at least 4 mutations) selected from the group consisting of D17G, E30A, and (Q103L or Q103I).

In some cases a subject D2D-IL-18 variant, or fragment thereof, includes the mutations D17X, E30X, and Q103X, relative to SEQ ID NO: 30. For example, in some cases a subject D2D-IL-18 variant, or fragment thereof, includes the mutations D17X₁, E30X₂, and Q103X₃, relative to SEQ ID NO: 30, where X₁ is G, H, R, or A; X₂ is A, T, G,

K, or R; and X₃ is I or L. In some cases a subject D2D-IL-18 variant, or fragment thereof, includes the mutations D17G, E30A, and (Q103L or Q103I).

In some cases a subject D2D-IL-18 variant, or fragment thereof, includes at least one mutation (e.g., at least 2, at least 3, or at least 4 mutations) selected from the group consisting of D17X, E30X, D35X, M51X, and Q103X, relative to SEQ ID NO: 30. In some cases a subject D2D-IL-18 variant, or fragment thereof, includes at least one mutation (e.g., at least 2, at least 3, or at least 4 mutations) selected from the group consisting of D17X₁, E30X₂, D35X₃, M51X₄, and Q103X₅, relative to SEQ ID NO: 30, where X₁ is G, H, R, or A; X₂ is A, T, G, K, or R; X₃ is S, A, or Y; X₄ is F, I, or L; and X₅ is I or L. In some cases a subject D2D-IL-18 variant, or fragment thereof, includes at least one mutation (e.g., at least 2, at least 3, or at least 4 mutations) selected from the group consisting of D17G, E30A, D35S, M51F, and (Q103L or Q103I), relative to SEQ ID NO: 30.

In some cases a subject D2D-IL-18 variant, or fragment thereof, includes the mutations D17X, E30X, D35X, M51X, and Q103X, relative to SEQ ID NO: 30. For example, in some cases a subject D2D-IL-18 variant, or fragment thereof, includes the mutations D17X₁, E30X₂, D35X₃, M51X₄, and Q103X₅, relative to SEQ ID NO: 30, where X₁ is G, H, R, or A; X₂ is A, T, G, K, or R; X₃ is S, A, or Y; X₄ is F, I, or L; and X₅ is I or L. In some cases a subject D2D-IL-18 variant, or fragment thereof, includes the mutations D17G, E30A, D35S, M51F, and (Q103L or Q103I), relative to SEQ ID NO: 30.

In some cases a subject D2D-IL-18 variant, or fragment thereof, comprises an amino acid sequence having 85% or more sequence identity (e.g., 90% or more, 92% or more, 93% or more, 94% or more, 95% or more, 96% or more, 97% or more, 98% or more, or 99% or more sequence identity) with the wild type human IL-18 amino acid sequence set forth as SEQ ID NO: 30. As such in some cases a subject D2D-IL-18 variant, or fragment thereof, comprises an amino acid sequence that (i) has 85% or more sequence identity (e.g., 90% or more, 92% or more, 93% or more, 94% or more, 95% or more, 96% or more, 97% or more, 98% or more, or 99% or more sequence identity) with the wild type human IL-18 amino acid sequence set forth as SEQ ID NO: 30; and (ii) includes at least one mutation (e.g., at least 2, at least 3, at least 4, at least 5, or at least 6 mutations) relative to wild type IL-18 (e.g., human IL-18).

In some cases a subject D2D-IL-18 variant, or fragment thereof, comprises an amino acid sequence having 85% or more sequence identity (e.g., 90% or more, 92% or more, 93% or more, 94% or more, 95% or more, 96% or more, 97% or more, 98% or more, or 99% or more sequence identity) with the amino acid sequence set forth in any one of SEQ ID NOs: 126-190. As such in some cases a subject D2D-IL-18 variant, or fragment thereof, comprises an amino acid sequence that (i) has 85% or more sequence identity (e.g., 90% or more, 92% or more, 93% or more, 94% or more, 95% or more, 96% or more, 97% or more, 98% or more, or 99% or more sequence identity) with the amino acid sequence set forth in any one of SEQ ID NOs: 126-190; and (ii) includes at least one mutation (e.g., at least 2, at least 3, at least 4, at least 5, or at least 6 mutations) relative to wild type IL-18 (e.g., human IL-18).

In some cases a subject DR-IL-18 variant, or fragment thereof, comprises an amino acid sequence that (i) has 85% or more sequence identity (e.g., 90% or more, 92% or more, 93% or more, 94% or more, 95% or more, 96% or more, 97% or more, 98% or more, or 99% or more sequence identity) with the wild type human IL-18 amino acid sequence set forth as SEQ ID NO: 30; and (ii) includes at least one mutation (e.g., at least 2, at least 3, at least 4, at least 5, or at least 6 mutations) selected from the group consisting of Y1X, L5X, D17X, E31X, T34X, D35X, S36X, D37X, D40X, N41X, M51X, Q56X, M60X, Q103X, H109X, M113X, and R131X.

In some cases a subject DR-IL-18 variant, or fragment thereof, comprises an amino acid sequence that (i) has 85% or more sequence identity (e.g., 90% or more, 92% or more, 93% or more, 94% or more, 95% or more, 96% or more, 97% or more, 98% or more, or 99% or more sequence identity) with the wild type human IL-18 amino acid sequence set forth as SEQ ID NO: 30; and (ii) includes at least one mutation (e.g., at least 2, at least 3, at least 4, at least 5, or at least 6 mutations) selected from the group consisting of D17X, E30X, and Q103X, relative to SEQ ID NO: 30. In some cases a subject DR-IL-18 variant, or fragment thereof, comprises an amino acid sequence that (i) has 85% or more sequence identity (e.g., 90% or more, 92% or more, 93% or more, 94% or more, 95% or more, 96% or more, 97% or more, 98% or more, or 99% or more sequence identity) with the wild type human IL-18 amino acid sequence set forth as SEQ ID NO: 30; and (ii) includes at least one mutation (e.g., at least 2, at least 3, at least 4, at least 5, or at least 6 mutations)

selected from the group consisting of D17X₁, E30X₂, and Q103X₃, relative to SEQ ID NO: 30, where X₁ is G, H, R, or A; X₂ is A, T. In some cases a subject DR-IL-18 variant, or fragment thereof, comprises an amino acid sequence that (i) has 85% or more sequence identity (e.g., 90% or more, 92% or more, 93% or more, 94% or more, 95% or more, 96% or more, 97% or more, 98% or more, or 99% or more sequence identity) with the wild type human IL-18 amino acid sequence set forth as SEQ ID NO: 30; and (ii) includes at least one mutation (e.g., at least 2, at least 3, at least 4, at least 5, or at least 6 mutations) selected from the group consisting of D17G, E30A, and (Q103L or Q103I).

In some cases a subject DR-IL-18 variant, or fragment thereof, comprises an amino acid sequence that (i) has 85% or more sequence identity (e.g., 90% or more, 92% or more, 93% or more, 94% or more, 95% or more, 96% or more, 97% or more, 98% or more, or 99% or more sequence identity) with the wild type human IL-18 amino acid sequence set forth as SEQ ID NO: 30; and (ii) includes the mutations D17X, E30X, and Q103X, relative to SEQ ID NO: 30. In some cases a subject DR-IL-18 variant, or fragment thereof, comprises an amino acid sequence that (i) has 85% or more sequence identity (e.g., 90% or more, 92% or more, 93% or more, 94% or more, 95% or more, 96% or more, 97% or more, 98% or more, or 99% or more sequence identity) with the wild type human IL-18 amino acid sequence set forth as SEQ ID NO: 30; and (ii) includes the mutations D17X₁, E30X₂, and Q103X₃, relative to SEQ ID NO: 30, where X₁ is G, H, R, or A; X₂ is A, T. In some cases a subject DR-IL-18 variant, or fragment thereof, comprises an amino acid sequence that (i) has 85% or more sequence identity (e.g., 90% or more, 92% or more, 93% or more, 94% or more, 95% or more, 96% or more, 97% or more, 98% or more, or 99% or more sequence identity) with the wild type human IL-18 amino acid sequence set forth as SEQ ID NO: 30; and (ii) includes the mutations D17G, E30A, and (Q103L or Q103I).

In some cases a subject DR-IL-18 variant, or fragment thereof, comprises an amino acid sequence that (i) has 85% or more sequence identity (e.g., 90% or more, 92% or more, 93% or more, 94% or more, 95% or more, 96% or more, 97% or more, 98% or more, or 99% or more sequence identity) with the wild type human IL-18 amino acid sequence set forth as SEQ ID NO: 30; and (ii) includes at least one mutation (e.g., at least 2, at least 3, at least 4, at least 5, or at least 6 mutations) selected from the group consisting of D17X, E30X, D35X, M51X, and Q103X, relative to SEQ ID NO: 30. In some cases a subject DR-

IL-18 variant, or fragment thereof, comprises an amino acid sequence that (i) has 85% or more sequence identity (e.g., 90% or more, 92% or more, 93% or more, 94% or more, 95% or more, 96% or more, 97% or more, 98% or more, or 99% or more sequence identity) with the wild type human IL-18 amino acid sequence set forth as SEQ ID NO: 30; and (ii) includes at least one mutation (e.g., at least 2, at least 3, at least 4, at least 5, or at least 6 mutations) selected from the group consisting of D17X₁, E30X₂, D35X₃, M51X₄, and Q103X₃, relative to SEQ ID NO: 30, where X₁ is G, H, R, or A; X₂ is A, T, G, K, or R; X₃ is S, A, or Y; X₄ is F, I, or L; and X₅ is I or L. In some cases a subject DR-IL-18 variant, or fragment thereof, comprises an amino acid sequence that (i) has 85% or more sequence identity (e.g., 90% or more, 92% or more, 93% or more, 94% or more, 95% or more, 96% or more, 97% or more, 98% or more, or 99% or more sequence identity) with the wild type human IL-18 amino acid sequence set forth as SEQ ID NO: 30; and (ii) includes at least one mutation (e.g., at least 2, at least 3, at least 4, at least 5, or at least 6 mutations) selected from the group consisting of D17G, E30A, D35S, M51F, and (Q103L or Q103I), relative to SEQ ID NO: 30.

In some cases a subject DR-IL-18 variant, or fragment thereof, comprises an amino acid sequence that (i) has 85% or more sequence identity (e.g., 90% or more, 92% or more, 93% or more, 94% or more, 95% or more, 96% or more, 97% or more, 98% or more, or 99% or more sequence identity) with the wild type human IL-18 amino acid sequence set forth as SEQ ID NO: 30; and (ii) includes the mutations D17X, E30X, D35X, M51X, and Q103X, relative to SEQ ID NO: 30. In some cases a subject DR-IL-18 variant, or fragment thereof, comprises an amino acid sequence that (i) has 85% or more sequence identity (e.g., 90% or more, 92% or more, 93% or more, 94% or more, 95% or more, 96% or more, 97% or more, 98% or more, or 99% or more sequence identity) with the wild type human IL-18 amino acid sequence set forth as SEQ ID NO: 30; and (ii) includes the mutations D17X₁, E30X₂, D35X₃, M51X₄, and Q103X₃, relative to SEQ ID NO: 30, where X₁ is G, H, R, or A; X₂ is A, T, G, K, or R; X₃ is S, A, or Y; X₄ is F, I, or L; and X₅ is I or L. In some cases a subject DR-IL-18 variant, or fragment thereof, comprises an amino acid sequence that (i) has 85% or more sequence identity (e.g., 90% or more, 92% or more, 93% or more, 94% or more, 95% or more, 96% or more, 97% or more, 98% or more, or 99% or more sequence identity) with the wild type human IL-18 amino acid sequence set forth as SEQ ID NO:

30; and (ii) includes the mutations D17G, E30A, D35S, M51F, and (Q103L or Q103I), relative to SEQ ID NO: 30.

In some embodiments, the IL-18 variant polypeptide that binds to and inhibits IL-18BP comprises a murine IL-18 variant polypeptide comprising at least one mutation (e.g.,
 5 at least 2, at least 3, at least 4, at least 5, or at least 6 mutations) selected from the group consisting of N1X, L5X, D17X, E30X, T33X, D34X, I35X, D36X, M50X, Q102X, R104, H108X, N109X, M111X, D129X, and D130X, wherein X denotes any amino acid. In some embodiments, the IL-18 variant polypeptide that binds to and inhibits IL-18BP comprises a murine IL-18 variant polypeptide, or fragment thereof, comprising at least one mutation
 10 (e.g., at least 2, at least 3, at least 4, at least 5, or at least 6 mutations) selected from the group consisting of N1Y, N1D, N1H, N1L, N1F, N1V, N1I, L5Y, L5H, D17Q, D17G, D17A, D17E, D17S, D17N, E30A, E30R, E30K, E30T, E30G, T33G, T33A, T33E, T33R, T33K, D34Y, D34S, D34A, I35T, I35K, I35R, D36V, D36A, D36G, D36H, D36P, D36R, D36L, M50F, M50L, Q102L, Q102I, R104E, R104A, R104P, R104G, R104Q, R104H,
 15 H108D, H108A, N109R, N109S, N109T, N109I, M111L, M111I, D129A, D129F, D129V, D129Y, D129S, D130E, D130T, D130G, D130N, D130R, D130S, D130Q, and D130H. In some embodiments, the IL-18 variant polypeptide that binds to and inhibits IL-18BP comprises a murine IL-18 variant polypeptide, or fragment thereof, selected from the group consisting of mD2D-A5 (SEQ ID NO: 126), mD2D-A6 (SEQ ID NO: 127), mD2D-A7
 20 (SEQ ID NO: 128), mD2D-A8 (SEQ ID NO: 129), mD2D-A9 (SEQ ID NO: 130), mD2D-A11 (SEQ ID NO: 131), mD2D-A12 (SEQ ID NO: 132), mD2D-B4 (SEQ ID NO: 133), mD2D-B7 (SEQ ID NO: 134), mD2D-B11 (SEQ ID NO: 135), mD2D-B12 (SEQ ID NO: 136), mD2D-C1 (SEQ ID NO: 137), mD2D-C3 (SEQ ID NO: 138), mD2D-C5 (SEQ ID NO: 139), mD2D-C6 (SEQ ID NO: 140), mD2D-C9 (SEQ ID NO: 141), mD2D-C10 (SEQ
 25 ID NO: 142), mD2D-C11 (SEQ ID NO: 143), mD2D-D1 (SEQ ID NO: 144), mD2D-D9 (SEQ ID NO: 145), mD2D-D12 (SEQ ID NO: 146), mD2D-E3 (SEQ ID NO: 147), mD2D-E4 (SEQ ID NO: 148), mD2D-E5 (SEQ ID NO: 149), mD2D-E7 (SEQ ID NO: 150), mD2D-E8 (SEQ ID NO: 151), mD2D-E9 (SEQ ID NO: 152), mD2D-E10 (SEQ ID NO: 153), mD2D-E11 (SEQ ID NO: 154), mD2D-E12 (SEQ ID NO: 155), mD2D-F3 (SEQ ID
 30 NO: 156), mD2D-F4 (SEQ ID NO: 157), mD2D-F5 (SEQ ID NO: 158), mD2D-F7 (SEQ ID NO: 159), mD2D-F8 (SEQ ID NO: 160), mD2D-F9 (SEQ ID NO: 161), mD2D-G1

(SEQ ID NO: 162), mD2D-G7 (SEQ ID NO: 163), mD2D-G9 (SEQ ID NO: 164), mD2D-H7 (SEQ ID NO: 165), mD2D-E1 (SEQ ID NO: 166), mD2D-G8 (SEQ ID NO: 167), mD2D-H3 (SEQ ID NO: 168), mD2D-A10 (SEQ ID NO: 169), mD2D-H1 (SEQ ID NO: 170), mD2D-F12 (SEQ ID NO: 171), mD2D-G10 (SEQ ID NO: 172), mD2D-G12 (SEQ ID NO: 173), mD2D-E2 (SEQ ID NO: 174), mD2D-G11 (SEQ ID NO: 175), mD2D-C4 (SEQ ID NO: 176), mD2D-F11 (SEQ ID NO: 177), mD2D-C2 (SEQ ID NO: 178), mD2D-F10 (SEQ ID NO: 179), mD2D-A2 (SEQ ID NO: 180), mD2D-F6 (SEQ ID NO: 181), mD2D-A1 (SEQ ID NO: 182), mD2D-E6 (SEQ ID NO: 183), mD2D-D4 (SEQ ID NO: 184), mD2D-D6 (SEQ ID NO: 185), mD2D-A3 (SEQ ID NO: 186), mD2D-A4 (SEQ ID NO: 187), mD2D-B10 (SEQ ID NO: 188), mD2D-B8 (SEQ ID NO: 189), mD2D-B9 (SEQ ID NO: 190), or a fragment thereof.

In some embodiments, the invention is a nucleic acid (e.g., DNA, cDNA, mRNA, etc.) that encodes at least one IL-18 variant polypeptide.

In some embodiments, the IL-18BP inhibitor is an IL-18 variant polypeptide that binds to IL-18BP and exhibits substantially reduced binding to IL-18R. In some embodiments, IL-18BP inhibitor that binds to IL-18BP and exhibits substantially reduced binding to IL-18R binds to IL-18R with a binding affinity that is about 0.000000000001% to about 95% of the binding affinity of wild-type IL-18 to IL-18R.

In some embodiments, the IL-18 variant polypeptide that binds to IL-18BP and exhibits substantially reduced binding to IL-18R binds to IL-18R with a binding affinity that is about 95% of the binding affinity of wild-type IL-18 to IL-18R. In some embodiments, the IL-18 variant polypeptide that binds to IL-18BP and exhibits substantially reduced binding to IL-18R binds to IL-18R with a binding affinity that is about 90% of the binding affinity of wild-type IL-18 to IL-18R. In some embodiments, the IL-18 variant polypeptide that binds to IL-18BP and exhibits substantially reduced binding to IL-18R binds to IL-18R with a binding affinity that is about 85% of the binding affinity of wild-type IL-18 to IL-18R. In some embodiments, the IL-18 variant polypeptide that binds to IL-18BP and exhibits substantially reduced binding to IL-18R binds to IL-18R with a binding affinity that is about 80% of the binding affinity of wild-type IL-18 to IL-18R. In some embodiments, the IL-18 variant polypeptide that binds to IL-18BP and

exhibits substantially reduced binding to IL-18R binds to IL-18R with a binding affinity that is about 75% of the binding affinity of wild-type IL-18 to IL-18R. In some embodiments, the IL-18 variant polypeptide that binds to IL-18BP and exhibits substantially reduced binding to IL-18R binds to IL-18R with a binding affinity that is about 70% of the binding affinity of wild-type IL-18 to IL-18R. In some embodiments, the IL-18 variant polypeptide that binds to IL-18BP and exhibits substantially reduced binding to IL-18R binds to IL-18R with a binding affinity that is about 65% of the binding affinity of wild-type IL-18 to IL-18R. In some embodiments, the IL-18 variant polypeptide that binds to IL-18BP and exhibits substantially reduced binding to IL-18R binds to IL-18R with a binding affinity that is about 60% of the binding affinity of wild-type IL-18 to IL-18R. In some embodiments, the IL-18 variant polypeptide that binds to IL-18BP and exhibits substantially reduced binding to IL-18R binds to IL-18R with a binding affinity that is about 55% of the binding affinity of wild-type IL-18 to IL-18R. In some embodiments, the IL-18 variant polypeptide that binds to IL-18BP and exhibits substantially reduced binding to IL-18R binds to IL-18R with a binding affinity that is about 50% of the binding affinity of wild-type IL-18 to IL-18R. In some embodiments, the IL-18 variant polypeptide that binds to IL-18BP and exhibits substantially reduced binding to IL-18R binds to IL-18R with a binding affinity that is about 45% of the binding affinity of wild-type IL-18 to IL-18R. In some embodiments, the IL-18 variant polypeptide that binds to IL-18BP and exhibits substantially reduced binding to IL-18R binds to IL-18R with a binding affinity that is about 40% of the binding affinity of wild-type IL-18 to IL-18R. In some embodiments, the IL-18 variant polypeptide that binds to IL-18BP and exhibits substantially reduced binding to IL-18R binds to IL-18R with a binding affinity that is about 35% of the binding affinity of wild-type IL-18 to IL-18R. In some embodiments, the IL-18 variant polypeptide that binds to IL-18BP and exhibits substantially reduced binding to IL-18R binds to IL-18R with a binding affinity that is about 30% of the binding affinity of wild-type IL-18 to IL-18R. In some embodiments, the IL-18 variant polypeptide that binds to IL-18BP and exhibits substantially reduced binding to IL-18R binds to IL-18R with a binding affinity that is about 25% of the binding affinity of wild-type IL-18 to IL-18R. In some embodiments, the IL-18 variant polypeptide that binds to IL-18BP and exhibits substantially reduced binding to IL-18R binds to IL-18R

with a binding affinity that is about 20% of the binding affinity of wild-type IL-18 to IL-18R. In some embodiments, the IL-18 variant polypeptide that binds to IL-18BP and exhibits substantially reduced binding to IL-18R binds to IL-18R with a binding affinity that is about 15% of the binding affinity of wild-type IL-18 to IL-18R. In some
5 embodiments, the IL-18 variant polypeptide that binds to IL-18BP and exhibits substantially reduced binding to IL-18R binds to IL-18R with a binding affinity that is about 10% of the binding affinity of wild-type IL-18 to IL-18R.

 In some embodiments, the IL-18 variant polypeptide that binds to IL-18BP and exhibits substantially reduced binding to IL-18R binds to IL-18R with a binding affinity
10 that is about 5% of the binding affinity of wild-type IL-18 to IL-18R. In some embodiments, the IL-18 variant polypeptide that binds to IL-18BP and exhibits substantially reduced binding to IL-18R binds to IL-18R with a binding affinity that is about 4% of the binding affinity of wild-type IL-18 to IL-18R. In some embodiments, the IL-18 variant polypeptide that binds to IL-18BP and exhibits substantially reduced binding
15 to IL-18R binds to IL-18R with a binding affinity that is about 3% of the binding affinity of wild-type IL-18 to IL-18R. In some embodiments, the IL-18 variant polypeptide that binds to IL-18BP and exhibits substantially reduced binding to IL-18R binds to IL-18R with a binding affinity that is about 2% of the binding affinity of wild-type IL-18 to IL-18R. In some embodiments, the IL-18 variant polypeptide that binds to IL-18BP and
20 exhibits substantially reduced binding to IL-18R binds to IL-18R with a binding affinity that is about 1% of the binding affinity of wild-type IL-18 to IL-18R.

 In some embodiments, the IL-18 variant polypeptide that binds to IL-18BP and exhibits substantially reduced binding to IL-18R binds to IL-18R with a binding affinity that is about 0.1% of the binding affinity of wild-type IL-18 to IL-18R. In some
25 embodiments, the IL-18 variant polypeptide that binds to IL-18BP and exhibits substantially reduced binding to IL-18R binds to IL-18R with a binding affinity that is about 0.01% of the binding affinity of wild-type IL-18 to IL-18R. In some embodiments, the IL-18 variant polypeptide that binds to IL-18BP and exhibits substantially reduced binding to IL-18R binds to IL-18R with a binding affinity that is about 0.001% of the
30 binding affinity of wild-type IL-18 to IL-18R. In some embodiments, the IL-18 variant polypeptide that binds to IL-18BP and exhibits substantially reduced binding to IL-18R

binds to IL-18R with a binding affinity that is about 0.0001% of the binding affinity of wild-type IL-18 to IL-18R. In some embodiments, the IL-18 variant polypeptide that binds to IL-18BP and exhibits substantially reduced binding to IL-18R binds to IL-18R with a binding affinity that is about 0.00001% of the binding affinity of wild-type IL-18 to IL-18R. In some embodiments, the IL-18 variant polypeptide that binds to IL-18BP and exhibits substantially reduced binding to IL-18R binds to IL-18R with a binding affinity that is about 0.000001% of the binding affinity of wild-type IL-18 to IL-18R. In some embodiments, the IL-18 variant polypeptide that binds to IL-18BP and exhibits substantially reduced binding to IL-18R binds to IL-18R with a binding affinity that is about 0.0000001% of the binding affinity of wild-type IL-18 to IL-18R. In some embodiments, the IL-18 variant polypeptide that binds to IL-18BP and exhibits substantially reduced binding to IL-18R binds to IL-18R with a binding affinity that is about 0.00000001% of the binding affinity of wild-type IL-18 to IL-18R. In some embodiments, the IL-18 variant polypeptide that binds to IL-18BP and exhibits substantially reduced binding to IL-18R binds to IL-18R with a binding affinity that is about 0.000000001% of the binding affinity of wild-type IL-18 to IL-18R. In some embodiments, the IL-18 variant polypeptide that binds to IL-18BP and exhibits substantially reduced binding to IL-18R binds to IL-18R with a binding affinity that is about 0.0000000001% of the binding affinity of wild-type IL-18 to IL-18R. In some embodiments, the IL-18 variant polypeptide that binds to IL-18BP and exhibits substantially reduced binding to IL-18R binds to IL-18R with a binding affinity that is about 0.00000000001% of the binding affinity of wild-type IL-18 to IL-18R.

Further, one of skill in the art, when equipped with this disclosure and the methods exemplified herein, would appreciate that an IL-18BP inhibitor composition includes such inhibitors as discovered in the future, as can be identified by well-known criteria in the art of pharmacology, such as the physiological results of inhibition of IL-18BP as described in detail herein and/or as known in the art. Therefore, the present invention is not limited in any way to any particular IL-18BP inhibitor composition as exemplified or disclosed

herein; rather, the invention encompasses those inhibitor compositions that would be understood by the routineer to be useful as are known in the art and as are discovered in the future.

Further methods of identifying and producing IL-18BP inhibitor compositions are well known to those of ordinary skill in the art, including, but not limited to, obtaining an inhibitor from a naturally occurring source (e.g., *Streptomyces* sp., *Pseudomonas* sp., *Stylorella aurantium*, etc.). Alternatively, an IL-18BP inhibitor can be synthesized chemically. Further, the routineer would appreciate, based upon the teachings provided herein, that an IL-18BP inhibitor composition can be obtained from a recombinant organism. Compositions and methods for chemically synthesizing IL-18BP inhibitors and for obtaining them from natural sources are well known in the art and are described in the art.

One of skill in the art will appreciate that an inhibitor can be administered as a small molecule chemical, a protein, an antibody, a nucleic acid construct encoding a protein, an antisense nucleic acid, a nucleic acid construct encoding an antisense nucleic acid, or combinations thereof. Numerous vectors and other compositions and methods are well known for administering a protein or a nucleic acid construct encoding a protein to cells or tissues. Therefore, the invention includes a method of administering a protein or a nucleic acid encoding a protein that is an inhibitor of IL-18BP. (Sambrook et al., 2012, *Molecular Cloning: A Laboratory Manual*, Cold Spring Harbor Laboratory, New York; Ausubel et al., 1997, *Current Protocols in Molecular Biology*, John Wiley & Sons, New York).

One of skill in the art will realize that diminishing the amount or activity of a molecule that itself increases the amount or activity of IL-18BP can serve in the compositions and methods of the present invention to decrease the amount or activity of IL-18BP.

Antisense oligonucleotides are DNA or RNA molecules that are complementary to some portion of an RNA molecule. When present in a cell, antisense oligonucleotides hybridize to an existing RNA molecule and inhibit translation into a gene product. Inhibiting the expression of a gene using an antisense oligonucleotide is well known in the art (Marcus-Sekura, 1988, *Anal. Biochem.* 172:289), as are methods of expressing an antisense oligonucleotide in a cell (Inoue, U.S. Pat. No. 5,190,931). The methods of the

invention include the use of an antisense oligonucleotide to diminish the amount of IL-18BP, or to diminish the amount of a molecule that causes an increase in the amount or activity of IL-18BP, thereby decreasing the amount or activity of IL-18BP.

5 Contemplated in the present invention are antisense oligonucleotides that are synthesized and provided to the cell by way of methods well known to those of ordinary skill in the art. As an example, an antisense oligonucleotide can be synthesized to be between about 10 and about 100, more exemplary between about 15 and about 50 nucleotides long. The synthesis of nucleic acid molecules is well known in the art, as is the synthesis of modified antisense oligonucleotides to improve biological activity in
10 comparison to unmodified antisense oligonucleotides (Tullis, 1991, U.S. Pat. No. 5,023,243).

Similarly, the expression of a gene may be inhibited by the hybridization of an antisense molecule to a promoter or other regulatory element of a gene, thereby affecting the transcription of the gene. Methods for the identification of a promoter or other
15 regulatory element that interacts with a gene of interest are well known in the art, and include such methods as the yeast two hybrid system (Bartel and Fields, eds., In: The Yeast Two Hybrid System, Oxford University Press, Cary, N.C.).

Alternatively, inhibition of a gene expressing IL-18BP, or of a gene expressing a protein that increases the level or activity of IL-18BP, can be accomplished through the
20 use of a ribozyme. Using ribozymes for inhibiting gene expression is well known to those of skill in the art (see, e.g., Cech et al., 1992, J. Biol. Chem. 267:17479; Hampel et al., 1989, Biochemistry 28: 4929; Altman et al., U.S. Pat. No. 5,168,053). Ribozymes are catalytic RNA molecules with the ability to cleave other single-stranded RNA molecules. Ribozymes are known to be sequence specific, and can therefore be modified to recognize
25 a specific nucleotide sequence (Cech, 1988, J. Amer. Med. Assn. 260:3030), allowing the selective cleavage of specific mRNA molecules. Given the nucleotide sequence of the molecule, one of ordinary skill in the art could synthesize an antisense oligonucleotide or ribozyme without undue experimentation, provided with the disclosure and references incorporated herein.

30 One of skill in the art will appreciate that inhibitors of IL-18BP can be administered acutely (e.g., over a short period of time, such as a day, a week or a month) or chronically

(e.g., over a long period of time, such as several months or a year or more). One of skill in the art will appreciate that inhibitors of IL-18BP can be administered singly or in any combination with other agents. Further, IL-18BP inhibitors can be administered singly or in any combination in a temporal sense, in that they may be administered concurrently, and/or before, and/or after each other. One of ordinary skill in the art will appreciate, based on the disclosure provided herein, that IL-18BP inhibitor compositions can be used to treat or prevent a disease or disorder in a subject in need thereof, and that an inhibitor composition can be used alone or in any combination with another agent to affect a therapeutic result.

10 In various embodiments, any of the inhibitors of IL-18BP of the invention described herein can be administered alone or in combination with other inhibitors of other molecules associated with a disease or disorder disclosed herein or known in the art.

 It will be appreciated by one of skill in the art, when armed with the present disclosure including the methods detailed herein, that the invention is not limited to treatment of a disease or disorder that is already established. Particularly, the disease or disorder need not have manifested to the point of detriment to the subject; indeed, the disease or disorder need not be detected in a subject before treatment is administered. That is, significant disease or disorder does not have to occur before the present invention may provide benefit. Therefore, the present invention includes a method for preventing a disease or disorder in a subject, in that an IL-18BP inhibitor composition, as discussed previously elsewhere herein, can be administered to a subject prior to the onset of the disease or disorder, thereby preventing the disease or disorder from developing. The preventive methods described herein also include the treatment of a subject that is in remission for the prevention of a recurrence of a disease or disorder.

25 One of skill in the art, when armed with the disclosure herein, would appreciate that the prevention of a disease or disorder encompasses administering to a subject an IL-18BP inhibitor composition as a preventative measure against the disease or disorder. As more fully discussed elsewhere herein, methods of decreasing the level or activity of IL-18BP encompass a wide plethora of techniques for decreasing not only IL-18BP activity, but also for decreasing expression of a nucleic acid encoding IL-18BP, including either a decrease in transcription, a decrease in translation, or both.

Additionally, as disclosed elsewhere herein, one skilled in the art would understand, once armed with the teaching provided herein, that the present invention encompasses a method of preventing a wide variety of diseases, disorders and pathologies where a decrease in expression and/or activity of IL-18BP mediates, treats or prevents the disease,
5 disorder or pathology. Methods for assessing whether a disease relates to the levels or activity of IL-18BP are known in the art. Further, the invention encompasses treatment or prevention of such diseases discovered in the future.

The invention encompasses administration of an inhibitor of IL-18BP to practice the methods of the invention; the skilled artisan would understand, based on the disclosure
10 provided herein, how to formulate and administer the appropriate IL-18BP inhibitor to a subject. However, the present invention is not limited to any particular method of administration or treatment regimen.

Cytokine Inhibitors

15 In some embodiments, the composition of the present invention comprises an inhibitor of one or more cytokines. In some embodiments, the inhibitor of one or more cytokines comprises a chemical compound, a protein, a peptide, a peptidomimetic, an antibody, a ribozyme, a small molecule chemical compound, or an antisense nucleic acid molecule (e.g., siRNA, miRNA, etc.) that inhibits the expression, activity, or both of one
20 or more cytokines. In some embodiments, the inhibitor inhibits the expression, activity, or both of IL-17, IL-5, or IL-3. In some embodiments, the cytokine inhibitor decreases toxicity. In some embodiments, the cytokine inhibitor increases efficacy of an administered IL-18 variant polypeptide or IL-18BP inhibitor.

25 Compositions and Methods of Treatment and Prevention

In various embodiments, the present invention includes compositions comprising an activator of IL-18 activity, such as signaling activity through at least one IL-18R, and methods of increasing IL-18 activity, such as signaling through at least one IL-18R, in a cell, tissue, organ, system, or subject in need thereof. In various embodiments, the activator
30 of IL-18 activity compositions, and methods of treatment of the invention, increase the amount of IL-18R signaling, the amount of immune cell activity, or both. In various

embodiments, the diseases and disorders in which an increase in IL-18R signaling may improve therapeutic outcomes include, but are not limited to cancer, infectious diseases, macular degeneration, and metabolic diseases or disorders.

The following are non-limiting examples of cancers that can be treated or prevented by the methods and compositions of the invention: acute lymphoblastic leukemia, acute myeloid leukemia, adrenocortical carcinoma, appendix cancer, basal cell carcinoma, bile duct cancer, bladder cancer, bone cancer, brain and spinal cord tumors, brain stem glioma, brain tumor, breast cancer, bronchial tumors, burkitt lymphoma, carcinoid tumor, central nervous system atypical teratoid/rhabdoid tumor, central nervous system embryonal tumors, central nervous system lymphoma, cerebellar astrocytoma, cerebral astrocytoma/malignant glioma, cerebral astrocytoma/malignant glioma, cervical cancer, childhood visual pathway tumor, chordoma, chronic lymphocytic leukemia, chronic myelogenous leukemia, chronic myeloproliferative disorders, colon cancer, colorectal cancer, craniopharyngioma, cutaneous cancer, cutaneous t-cell lymphoma, endometrial cancer, ependymoblastoma, ependymoma, esophageal cancer, ewing family of tumors, extracranial cancer, extragonadal germ cell tumor, extrahepatic bile duct cancer, extrahepatic cancer, eye cancer, fungoides, gallbladder cancer, gastric (stomach) cancer, gastrointestinal cancer, gastrointestinal carcinoid tumor, gastrointestinal stromal tumor (gist), germ cell tumor, gestational cancer, gestational trophoblastic tumor, glioblastoma, glioma, hairy cell leukemia, head and neck cancer, hepatocellular (liver) cancer, histiocytosis, hodgkin lymphoma, hypopharyngeal cancer, hypothalamic and visual pathway glioma, hypothalamic tumor, intraocular (eye) cancer, intraocular melanoma, islet cell tumors, kaposi sarcoma, kidney (renal cell) cancer, langerhans cell cancer, langerhans cell histiocytosis, laryngeal cancer, leukemia, lip and oral cavity cancer, liver cancer, lung cancer, lymphoma, macroglobulinemia, malignant fibrous histiocytoma of bone and osteosarcoma, medulloblastoma, medulloepithelioma, melanoma, merkel cell carcinoma, mesothelioma, metastatic squamous neck cancer with occult primary, mouth cancer, multiple endocrine neoplasia syndrome, multiple myeloma, mycosis, myelodysplastic syndromes, myelodysplastic/myeloproliferative diseases, myelogenous leukemia, myeloid leukemia, myeloma, myeloproliferative disorders, nasal cavity and paranasal sinus cancer, nasopharyngeal cancer, neuroblastoma, non-hodgkin lymphoma, non-small cell lung

cancer, oral cancer, oral cavity cancer, oropharyngeal cancer, osteosarcoma and malignant fibrous histiocytoma, osteosarcoma and malignant fibrous histiocytoma of bone, ovarian, ovarian cancer, ovarian epithelial cancer, ovarian germ cell tumor, ovarian low malignant potential tumor, pancreatic cancer, papillomatosis, paraganglioma, parathyroid cancer, penile cancer, pharyngeal cancer, pheochromocytoma, pineal parenchymal tumors of intermediate differentiation, pineoblastoma and supratentorial primitive neuroectodermal tumors, pituitary tumor, plasma cell neoplasm, plasma cell neoplasm/multiple myeloma, pleuropulmonary blastoma, primary central nervous system cancer, primary central nervous system lymphoma, prostate cancer, rectal cancer, renal cell (kidney) cancer, renal pelvis and ureter cancer, respiratory tract carcinoma involving the nut gene on chromosome 15, retinoblastoma, rhabdomyosarcoma, salivary gland cancer, sarcoma, sezary syndrome, skin cancer (melanoma), skin cancer (nonmelanoma), skin carcinoma, small cell lung cancer, small intestine cancer, soft tissue cancer, soft tissue sarcoma, squamous cell carcinoma, squamous neck cancer, stomach (gastric) cancer, supratentorial primitive neuroectodermal tumors, supratentorial primitive neuroectodermal tumors and pineoblastoma, T-cell lymphoma, testicular cancer, throat cancer, thymoma and thymic carcinoma, thyroid cancer, transitional cell cancer, transitional cell cancer of the renal pelvis and ureter, trophoblastic tumor, urethral cancer, uterine cancer, uterine sarcoma, vaginal cancer, visual pathway and hypothalamic glioma, vulvar cancer, waldenstrom macroglobulinemia, and Wilms Tumor.

Thus, non-limiting examples of cancers that can be treated or prevented by the methods and compositions of the disclosure include solid tumor cancers, liquid cancers, blood cancers, teratomas, sarcomas, and carcinomas.

In some embodiments, the method of the present invention is useful for treating or preventing a tumor or cancer that is resistant to immune checkpoint inhibitors (ICIs). Exemplary immune checkpoint inhibitors include, but is not limited to, anti-PD1 (e.g., nivolumab), anti-CTLA4 (e.g., ipilimumab), anti-TIM3, anti-TIGIT, anti-LAG3, anti-B7H3, anti-B7H4, anti-VISTA, anti-ICOS, anti-GITR, anti-41BB, anti-OX40, and anti-CD40. Examples of targets of immune checkpoint inhibitors include but are not limited to: PD-L1, PD1, CTLA4, TIM3, TIGIT, LAG3, B7H3, B7H4, VISTA, ICOS, GITR, 41BB, OX40, and CD40. Thus, examples of immune checkpoint inhibitors include agents that

inhibit proteins such as: PD-L1, PD1, CTLA4, TIM3, TIGIT, LAG3, B7H3, B7H4, VISTA, ICOS, GITR, 41BB, OX40, or CD40. In some cases, a subject IL-18 variant polypeptide (e.g., a DR-IL-18 variant, a D2D-IL-18 variant) is co-administered with an immune checkpoint inhibitor (e.g., an agent that inhibits PD-L1, PD1, CTLA4, TIM3, 5 TIGIT, LAG3, B7H3, B7H4, VISTA, ICOS, GITR, 41BB, OX40, or CD40, or any combination thereof).

Fusions/conjugations

In some embodiments, an IL-18 variant polypeptide of the present disclosure is 10 fused to another protein, *i.e.*, an IL-18 variant polypeptide or a fragment thereof can be fused in frame with a second polypeptide (a fusion partner). In some embodiments, the second polypeptide (the fusion partner) is capable of increasing the overall size of the fusion protein, *e.g.*, so that the fusion protein will not be cleared from the circulation rapidly. In some cases, a IL-18 variant polypeptide or a fragment thereof is not fused to a 15 second polypeptide.

In some embodiments, the second polypeptide (the fusion partner for a IL-18 variant polypeptide or a fragment thereof) is part or whole of an immunoglobulin Fc region (*i.e.*, an antibody Fc sequence). In other embodiments, the second polypeptide is any suitable polypeptide that is substantially similar to Fc, *e.g.*, providing increased size, 20 multimerization domains, and/or additional binding or interaction with Ig molecules. In some embodiments, the second polypeptide is part or whole of Human Serum Albumin (HSA). In some embodiments, the second polypeptide is part or whole of an antibody, antibody fragment, camelid antibody or “nanobody” or other affinity reagent that binds to or interacts with HSA. These fusion proteins can facilitate purification, multimerization, 25 and show an increased half-life *in vivo*. Fusion proteins having disulfide-linked multimeric structures can also, in some cases, be more efficient in binding and neutralizing other molecules.

When fused to a heterologous polypeptide, the portion corresponding to the IL-18 variant polypeptide or a fragment thereof can be referred to as the “IL-18 variant 30 polypeptide portion” of a subject IL-18 variant polypeptide. In some cases, the “IL-18 variant polypeptide portion” can be 100 amino acids or more in length (*e.g.*, 110 amino

acids or more, 125 amino acids or more, 150 amino acids or more, 90 amino acids or more, 95 amino acids or more, 100 amino acids or more, 105 amino acids or more, 110 amino acids or more, 115 amino acids or more, 120 amino acids or more, 125 amino acids or more, 130 amino acids or more, 140 amino acids or more, or 150 amino acids or more), up to full-length IL-18, and can further be fused to a heterologous polypeptide.

In some cases, IL-18 variant polypeptide portion of a IL-18 variant polypeptide has a length in a range of from 100 amino acids to 157 amino acids (e.g., from 100 amino acids to 150 amino acids, from 100 amino acids to 140 amino acids, from 140 amino acids to 157 amino acids, from 140 amino acids to 150 amino acids, from 145 amino acids to 157 amino acids, or from 150 amino acids to 157 amino acids).

In some cases, the second polypeptide is a marker sequence (e.g., an affinity tag), such as a peptide that facilitates purification of the fused polypeptide. For example, the marker amino acid sequence can be a hexa-histidine peptide, such as the tag provided in a pQE vector (QIAGEN, Inc., 9259 Eton Avenue, Chatsworth, Calif., 91311), among others, many of which are commercially available. As described in *Gentz et al.*, Proc. Natl. Acad. Sci. USA 86: 821-824, 1989, for instance, hexa-histidine provides for convenient purification of the fusion protein. Another peptide tag useful for purification, the "HA" tag, corresponds to an epitope derived from the influenza hemagglutinin protein. *Wilson et al.*, Cell 37: 767, 1984. The addition of peptide moieties to facilitate handling of polypeptides are familiar and routine techniques in the art.

A subject IL-18 variant polypeptide can be modified, e.g., joined/conjugated to a wide variety of other oligopeptides, proteins, and/or non-protein moieties for a variety of purposes. For example, post-translationally modified, for example by prenylation, acetylation, amidation, carboxylation, glycosylation, PEGylation (covalent attachment of polyethylene glycol (PEG) polymer chains), etc. Such modifications can also include modifications of glycosylation, e.g. those made by modifying the glycosylation patterns of a polypeptide during its synthesis and processing or in further processing steps; e.g. by exposing the polypeptide to enzymes which affect glycosylation, such as mammalian glycosylating or deglycosylating enzymes. In some embodiments, a subject IL-18 variant polypeptide has one or more phosphorylated amino acid residues, e.g. phosphotyrosine, phosphoserine, or phosphothreonine.

In some other embodiments, IL-18 variant polypeptides of the disclosure include reagents further modified to improve their resistance to proteolytic degradation or to optimize solubility properties or to render them more suitable as a therapeutic agent. For example, variants of the present disclosure further include analogs containing residues
5 other than naturally occurring L-amino acids, *e.g.* D-amino acids or non-naturally occurring synthetic amino acids. D-amino acids may be substituted for some or all of the amino acid residues.

Co-administration and Multispecific IL-18 variant polypeptides

10 As noted elsewhere in this disclosure, in some cases a subject IL-18 variant polypeptide is administered with an additional agent. The terms "co-administration", "co-administer", and "in combination with" include the administration of two or more therapeutic agents (*e.g.*, a subject IL-18 variant such as DR-IL-18 or D2D in combination with an additional agent) either simultaneously, concurrently or sequentially within no
15 specific time limits. In some embodiments, the agents are present in the cell or in the subject's body at the same time or exert their biological or therapeutic effect at the same time. In some embodiments, the therapeutic agents are in the same composition or unit dosage form. In other embodiments, the therapeutic agents are in separate compositions or unit dosage forms. In certain embodiments, a first agent can be administered prior to
20 (*e.g.*, minutes, 15 minutes, 30 minutes, 45 minutes, 1 hour, 2 hours, 4 hours, 6 hours, 12 hours, 24 hours, 48 hours, 72 hours, 96 hours, 1 week, 2 weeks, 3 weeks, 4 weeks, 5 weeks, 6 weeks, 8 weeks, or 12 weeks before), concomitantly with, or subsequent to (*e.g.*, 5 minutes, 15 minutes, 30 minutes, 45 minutes, 1 hour, 2 hours, 4 hours, 6 hours, 12 hours, 24 hours, 48 hours, 72 hours, 96 hours, 1 week, 2 weeks, 3 weeks, 4 weeks, 5
25 weeks, 6 weeks, 8 weeks, or 12 weeks after) the administration of a second therapeutic agent.

In some cases, a subject IL-18 variant (*e.g.*, a DR-IL-18 variant or a D2D variant) (*e.g.*, formulated as a pharmaceutical composition) is co-administered with a cancer therapeutic drug, therapeutic drug to treat an infection, or cancer-directed antibody. Such
30 administration may involve concurrent (*i.e.* at the same time), prior, or subsequent administration of the drug/antibody with respect to the administration of an agent or

agents of the disclosure. A person of ordinary skill in the art would have no difficulty determining the appropriate timing, sequence and dosages of administration for particular drugs and compositions of the present disclosure.

In some embodiments, treatment is accomplished by administering a combination
5 (co-administration) of a subject IL-18 variant (e.g., a DR-IL-18 variant or a D2D variant)
with another agent (e.g., an immune stimulant, an agent to treat chronic infection, a
cytotoxic agent, an anti-cancer agent, etc.). One example class of cytotoxic agents that
can be used are chemotherapeutic agents. Exemplary chemotherapeutic agents include,
but are not limited to, aldesleukin, altretamine, amifostine, asparaginase, bleomycin,
10 capecitabine, carboplatin, carmustine, cladribine, cisapride, cisplatin, cyclophosphamide,
cytarabine, dacarbazine (DTIC), dactinomycin, docetaxel, doxorubicin, dronabinol,
duocarmycin, etoposide, filgrastim, fludarabine, fluorouracil, gemcitabine, granisetron,
hydroxyurea, idarubicin, ifosfamide, interferon alpha, irinotecan, lansoprazole,
levamisole, leucovorin, megestrol, mesna, methotrexate, metoclopramide, mitomycin,
15 mitotane, mitoxantrone, omeprazole, ondansetron, paclitaxel (TaxolTM), pilocarpine,
prochloroperazine, rituximab, saproin, tamoxifen, taxol, topotecan hydrochloride,
trastuzumab, vinblastine, vincristine and vinorelbine tartrate.

A subject IL-18 variant (e.g., a DR-IL-18 variant or a D2D variant) need not be,
but is optionally formulated with one or more agents that potentiate activity, or that
20 otherwise increase the therapeutic effect. In some embodiments, treatment is
accomplished by administering a combination (co-administration) of a subject IL-18
variant (e.g., a DR-IL-18 variant) and an agent that opsonizes a target cell. Thus, also
envisioned herein are compositions (and methods that use the compositions) that include:
(a) a subject IL-18 variant (e.g., a DR-IL-18 variant); and (b) an agent that opsonizes the
25 target cell. In some cases, that agent that opsonizes the target cell is Rituximab. In some
cases, that agent that opsonizes the target cell is Cetuximab.

An “agent that opsonizes a target cell” (an “opsonizing agent”) is any agent that
can bind to a target cell (e.g., a cancer cell, a cell harboring an intracellular pathogen,
etc.) and opsonize the target cell (e.g., mark the target cell for phagocytosis and/or for
30 antibody-dependent cell mediated cytotoxicity (ADCC)). For example, any antibody that
can bind to a target cell (e.g., a cancer cell such as a tumor cell), where the antibody has

an FC region, is considered to be an agent that opsonizes a target cell. In some cases, the agent that opsonizes a target cell is an antibody that binds to a target cell (e.g., an anti-tumor antibody, an anti-cancer antibody, an anti-infection antibody, and the like).

For example antibodies selective for tumor cell markers, radiation, surgery, and/or
5 hormone deprivation, see *Kwon et al.*, Proc. Natl. Acad. Sci U.S.A., 96: 15074-9, 1999. Angiogenesis inhibitors can also be combined with the methods of the invention. A number of antibodies are currently in clinical use for the treatment of cancer, and others are in varying stages of clinical development. For example, there are a number of antigens and corresponding monoclonal antibodies for the treatment of B cell
10 malignancies. One target antigen is CD20. Rituximab is a chimeric unconjugated monoclonal antibody directed at the CD20 antigen. CD20 has an important functional role in B cell activation, proliferation, and differentiation. The CD52 antigen is targeted by the monoclonal antibody alemtuzumab, which is indicated for treatment of chronic lymphocytic leukemia. CD22 is targeted by a number of antibodies, and has recently
15 demonstrated efficacy combined with toxin in chemotherapy-resistant hairy cell leukemia. Two new monoclonal antibodies targeting CD20, tositumomab and ibritumomab, have been submitted to the Food and Drug Administration (FDA). These antibodies are conjugated with radioisotopes. Alemtuzumab (Campath) is used in the treatment of chronic lymphocytic leukemia; Gemtuzumab (Mylotarg) finds use in the
20 treatment of acute myelogenous leukemia; Ibritumomab (Zevalin) finds use in the treatment of non-Hodgkin's lymphoma; Panitumumab (Vectibix) finds use in the treatment of colon cancer.

Monoclonal antibodies useful in the methods of the disclosure that have been used in solid tumors include, without limitation, edrecolomab and trastuzumab (herceptin).
25 Edrecolomab targets the 17-1A antigen seen in colon and rectal cancer, and has been approved for use in Europe for these indications. Trastuzumab targets the HER-2/neu antigen. Cetuximab (Erbix) is also of interest for use in the methods of the disclosure. The antibody binds to the EGF receptor (EGFR), and has been used in the treatment of solid tumors including colon cancer and squamous cell carcinoma of the head and neck
30 (SCCHN).

A subject IL-18 variant polypeptide (e.g., a DR-IL-18 variant, a D2D-IL-18 variant) can be combined with any of the above mentioned agents (e.g., agents such as antibodies that opsonize a target cell). Thus, in some cases, a subject IL-18 variant polypeptide (e.g., a DR-IL-18 variant, a D2D-IL-18 variant) is used in a combination therapy (is co-administered) with one or more opsonizing agents selective for cancer cells, e.g., tumor cells. In some cases, a subject IL-18 variant polypeptide (e.g., a DR-IL-18 variant, a D2D-IL-18 variant) is used in a combination therapy (is co-administered) with one or more of: cetuximab (binds EGFR), panitumumab (binds EGFR), rituximab (binds CD20), trastuzumab (binds HER2), pertuzumab (binds HER2),
 5 alemtuzumab (binds CD52), brentuximab (binds CD30), tositumomab, ibritumomab, gemtuzumab, ibritumomab, and edrecolomab (binds 17-1A), or a combination thereof.

In some cases, a subject IL-18 variant polypeptide (e.g., a DR-IL-18 variant, a D2D-IL-18 variant) is co-administered with a cancer cell opsonizing agent (e.g., one that comprises an antigen binding region that targets CD19, CD20, CD22, CD24, CD25,
 15 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,
 20 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, or
 25 NRP1, or any combination thereof).

In some cases, a subject IL-18 variant polypeptide (e.g., a DR-IL-18 variant, a D2D-IL-18 variant) is co-administered with and agent that targets one or more antigens selected from: CD19, CD20, CD22, CD24, CD25, CD30, CD33, CD38, CD44, CD47, SIRPA, CD52, CD56, CD70, CD96, CD97, CD99, CD123, CD279 (PD-1), CD274 (PD-
 30 L1), EGFR, 17-1A, HER2, CD117, C-Met, PTHR2, and HAVCR2 (TIM3).

In some cases, a subject IL-18 variant polypeptide (e.g., a DR-IL-18 variant, a D2D-IL-18 variant) is used in a combination therapy (is co-administered) with any convenient immunomodulatory agent (e.g., an anti-CTLA4 antibody, an anti-PD-1 antibody, an anti-PD-L1 antibody, a TIGIT antibody, a TIM3 antibody, a LAG3 antibody, a VISTA antibody, a B7H3 antibody, a B7H4 antibody, a CD40 agonist, a 4-1BB modulator (e.g., a 41BB-agonist), an OX-40 modulator (e.g., an OX-40 agonist), a GITR modulator (e.g., a GITR agonist), a CD47 binding agent such as an anti-CD47 antibody or a high affinity CD47 binding agent, a SIRPA binding agent such as an anti-SIRPA antibody or high affinity SIRPA binding agent, and the like), a TGFbeta antagonist such as an anti-TGFbeta antibody, a cytokine or a cytokine variant including IL-1, IL-2, IL-10, IL-12, IL-15, IL-18, IL-21, IL-33, Interferon alpha, Interferon beta, Interferon gamma, TNF, TRAIL, lymphotoxin, LIGHT/TNSF14, or an agonist of a Toll Like Receptor including TLR2, TLR4, TLR5, TLR7, TLR9, an agonist of an inflammasome, an agonist of the STING/cGAS pathway, or an agonist of the RIG-I pathway, an antagonist of the adenosine receptors A2aR/A2bR, an antagonist of the Aryl hydrocarbon receptor, an antagonist of IDO and/or TDO, or an oncolytic virus.

In some cases, a subject IL-18 variant polypeptide (e.g., a DR-IL-18 variant, a D2D-IL-18 variant) is used in a combination therapy (is co-administered) with an inhibitor of BTLA and/or CD160. In some cases, a subject IL-18 variant polypeptide (e.g., a DR-IL-18 variant, a D2D-IL-18 variant) is used in a combination therapy (is co-administered) with an anti-CD47/SIRPA agent (e.g., anti-CD47, anti-SIRPA, a high affinity CD47 binding agent, a high affinity SIRPA binding agent, and the like). In some cases, a subject IL-18 variant polypeptide (e.g., a DR-IL-18 variant, a D2D-IL-18 variant) is used in a combination therapy (is co-administered) with an inhibitor of TIM3 and/or CEACAM1.

As noted above, in some cases a subject IL-18 variant polypeptide is fused to another protein (i.e., a “fusion partner”, a “second polypeptide”). In some embodiments, the second polypeptide (the fusion partner for a subject IL-18 variant polypeptide) specifically binds to a target molecule other than the target molecule bound by the IL-18 variant polypeptide portion of the fusion protein (e.g., other than IL-18R for variants that bind IL-18R; or other than IL-18BP for variants that bind to IL-18BP).

Thus, in some embodiments, a subject IL-18 variant polypeptide is multispecific (e.g., bispecific). The terms “multispecific” or “bispecific” are commonly used when referring to agents (e.g., ligands or antibodies) that recognize two or more different antigens by virtue of possessing at least one region (e.g., a ligand or a Fab of a first antibody) that is specific for a first target, and at least a second region (e.g., a ligand or a Fab of a second antibody) that is specific for a second target. A bispecific agent specifically binds to two targets and is thus one type of multispecific agent.

In some embodiments, a subject IL-18 variant polypeptide is multispecific (e.g., bispecific), such that a first region of the polypeptide includes a subject IL-18 variant polypeptide sequence (i.e., the first region includes a IL-18 variant polypeptide), and a second region that specifically binds to another target molecule (e.g., an antigen). For example, in some cases, a IL-18 variant polypeptide is fused to a second polypeptide that binds specifically to a target molecule other than the target molecule bound by the IL-18 variant polypeptide.

Any one of the agents discussed above in the context of co-administration can be conjugated to a subject IL-18 variant polypeptide. The term “co-administration” as used herein is meant to encompass such conjugated compounds. For example, when agent 1 is co-administered with agent 2, the term is meant to encompass embodiments where agent 1 and agent 2 are not conjugated to one another, and is also meant to encompass embodiments where agent 1 and agent 2 are conjugated to one another (e.g., where agent 1 and agent 2 are both proteins and agent 1 is fused to agent 2).

In some cases, the second region of a multispecific IL-18 variant polypeptide is a checkpoint inhibitor. In some cases, the second region of a multispecific IL-18 variant polypeptide inhibits one or more proteins selected from: PD-L1, PD1, CTLA4, TIM3, TIGIT, LAG3, B7H3, B7H4, VISTA, ICOS, GITR, 41BB, OX40, and CD40.

In some cases, the second region of a multispecific IL-18 variant polypeptide is a cancer cell opsonizing agent. In some cases, the second region of a multispecific IL-18 variant polypeptide targets one or more proteins selected from: CD19, CD20, CD22, CD24, CD25, CD30, CD33, CD38, CD44, CD47, SIRPA, CD52, CD56, CD70, CD96, CD97, CD99, CD123, CD279 (PD-1), CD274 (PD-L1), EGFR, 17-1A, HER2, CD117, C-Met, PTHR2, and HAVCR2 (TIM3). In some cases, the second region of a

multispecific IL-18 variant polypeptide is an opsonizing agent that targets one or more proteins selected from: CD19, CD20, CD22, CD24, CD25, CD30, CD33, CD38, CD44, CD47, SIRPA, CD52, CD56, CD70, CD96, CD97, CD99, CD123, CD279 (PD-1), CD274 (PD-L1), EGFR, 17-1A, HER2, CD117, C-Met, PTHR2, and HAVCR2 (TIM3).

- 5 For example, in some cases, the second region of a multispecific IL-18 variant polypeptide includes an ectodomain, e.g., an ectodomain from PD-1, PD-L1, CD47 (e.g., a high affinity CD47 variant / polypeptide), or SIRPA. (e.g., a high affinity SIRPA variant / polypeptide). In some cases, the second region of a multispecific IL-18 variant polypeptide specifically binds an antigen selected from: CTLA-4, Lag-3, BTLA, Tim-3, CD244, CD40, CD40L, CD47, SIRPA, PD-1, and PD-L1.

- 10 In some embodiments, a subject IL-18 variant polypeptide includes a linker (e.g., a linker polypeptide). For example, in some embodiments, a subject IL-18 variant polypeptide and a fusion partner are separated by a linker (e.g., a linker polypeptide). A linker polypeptide may have any of a variety of amino acid sequences. Proteins can be joined by a linker polypeptide can be of a flexible nature (e.g., a flexible linker polypeptide), although other chemical linkages are not excluded. Suitable linkers include polypeptides of between about 6 amino acids and about 40 amino acids in length, or between about 6 amino acids and about 25 amino acids in length. These linkers can be produced by using synthetic, linker-encoding oligonucleotides to couple the proteins.
- 15 Peptide linkers with a degree of flexibility can be used. The linking peptides may have virtually any amino acid sequence, bearing in mind that the in some case, linkers will have a sequence that results in a generally flexible peptide. The use of small amino acids, such as glycine and alanine, are of use in creating a flexible peptide. The creation of such sequences is routine to those of skill in the art. A variety of different linkers are commercially available and are considered suitable for use.

20 In some embodiments the IL-18 variant polypeptide is co-administered with an engineered immune cell such as a CAR-T or CAR-NK cell or T or NK cell transduced with an engineered T cell receptor. In other embodiments, the IL-18 variant polypeptide is co-administered with an oncolytic virus.

- 30 In some embodiments, a nucleic acid encoding an IL-18 variant polypeptide is included within an engineered ("altered") immune cell such as a CAR-T or CAR-NK cell

or T or NK cell transduced with an engineered T cell receptor. In this instance, the engineered cell (e.g., altered T cell, altered NK cell) would secrete the IL-18 variant polypeptide. The ability to secrete the IL-18 variant peptide can be regulated in a contextual manner (e.g., turned on within the tumor microenvironment), for instance, by a synthetic
5 NOTCH receptor.

In some embodiments, a nucleic acid encoding an IL-18 variant polypeptide is included within an oncolytic virus. In this instance, cells infected by the oncolytic virus would secrete the IL-18 variant polypeptide.

In some embodiments, the method of the present invention is useful for treating or
10 preventing a tumor or cancer tumors that have lost surface expression of MHC class I; such as a tumor that has lost B2m, the MHC locus, or has mutations in other members of the antigen presentation and/or antigen loading complex, such as tapasin.

Metabolic diseases and disorders include various metabolic and endocrine-related diseases and disorders. The following are non-limiting examples of metabolic and
15 endocrine-related diseases and disorders that can be treated or prevented by the methods and compositions of the invention: obesity, diabetes, prediabetes, type II diabetes, mature onset diabetes of the young (MODY), hyperglycemia, metabolic syndrome, dyslipidemia, hypertriglyceridemia, and hypercholesterolemia.

Non-limiting examples of other diseases and disorders that can be treated or
20 prevented using the compositions and methods of the invention include viral infections, bacterial infections, parasitic infections, and low immune activity. In some embodiments, the viral infection is at least one of a pox virus, a smallpox virus, molluscum contagiosum, HPV infection, and warts caused by a virus. In some embodiments, the infection is a systemic infection. In some embodiments, the viral infection is a vaccinia virus infection.
25 In some embodiments, the viral infection is a systemic vaccinia virus infection. In some embodiments, the bacterial infection is sepsis. In some embodiments, the low immune activity is neutropenia, for example, as may occur with chemotherapy.

Non-limiting examples of other diseases and disorders that can be treated or prevented using the compositions and methods of the invention include macular
30 degeneration. For example, in some cases the disease or disorder is wet macular degeneration, and in some cases the disease or disorder is wet age-related macular

degeneration. In some such cases, the IL-18 variant can be used as an anti-angiogenic. For example, a subject IL-18 variant polypeptide can in some cases attenuate choroidal neovascularization.

Thus, the present invention relates to the prevention and treatment of a disease or disorder by administration of a therapeutically effective amount of an IL-18 variant polypeptide, a recombinant IL-18 variant polypeptide, an active IL-18 variant polypeptide fragment (e.g., IL-18 variant peptide, etc.), an activator of IL-18 variant expression or activity, or a nucleic acid (e.g., DNA, cDNA, mRNA, etc.) that encodes at least one IL-18 variant polypeptide, to a cell, tissue, organ, or subject in need thereof, for the treatment or prevention of a disease or disorder, or its associated signs, symptoms or pathologies.

In some embodiments, a composition of the invention is administered to a cell, tissue, organ, system, or subject to treat or prevent a disease or disorder. In some embodiments, a human IL-18 variant polypeptide is administered to a cell, tissue, organ, system, or subject. In some embodiments, a nucleic acid (e.g., DNA, cDNA, mRNA, etc.) encoding at least one human IL-18 variant polypeptide is administered to a cell, tissue, organ, system, or subject.

In various embodiments, the human IL-18 variant polypeptide, or fragment thereof, comprises at least one mutation (e.g., at least 2, at least 3, at least 4, at least 5, or at least 6 mutations) 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, wherein X denotes any amino acid. In various embodiments, the human IL-18 variant polypeptide, or fragment thereof, comprises at least 4 mutations 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, wherein X denotes any amino acid. In various embodiments, the human IL-18 variant polypeptide, or fragment thereof, comprises at least 6 mutations 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. In various embodiments, the human IL-18 variant polypeptide, or fragment thereof, comprises at least one mutation (e.g., at least 2, at least 3, at least 4, at least 5, or at least 6 mutations) selected from the group consisting

of Y1X, L5X, K8X, S55X, Q56X, P57X, G59X, E77X, Q103X, S105X, D110X, N111X, M113X, V153X, and N155X.

In some embodiments, a human IL-18 variant polypeptide, or fragment thereof, comprises at least one mutation (e.g., at least 2, at least 3, at least 4, at least 5, or at least 6 mutations) 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. In some embodiments, a human IL-18 variant polypeptide comprises at least one IL-18 variant polypeptide, or fragment thereof, selected from the group consisting of hCS1 (SEQ ID NO: 34), hCS2 (SEQ ID NO: 35), hCS3 (SEQ ID NO: 36), hCS4 (SEQ ID NO: 37), hC4 (SEQ ID NO: 38), hA8 (SEQ ID NO: 39), hD6 (SEQ ID NO: 40), hH12 (SEQ ID NO: 41), hB11 (SEQ ID NO: 42), hC3 (SEQ ID NO: 43), hC2 (SEQ ID NO: 44), hG10 (SEQ ID NO: 45), hG1 (SEQ ID NO: 46), hF1 (SEQ ID NO: 47), hD2 (SEQ ID NO: 48), hA1 (SEQ ID NO: 49), hB3 (SEQ ID NO: 50), hB4 (SEQ ID NO: 51), hH3 (SEQ ID NO: 52), hH5 (SEQ ID NO: 53), hH4 (SEQ ID NO: 54), hE1 (SEQ ID NO: 55), hG2 (SEQ ID NO: 56), hB9 (SEQ ID NO: 57), hE12 (SEQ ID NO: 58), hC5 (SEQ ID NO: 59), 5-18 (SEQ ID NO: 73), 5-29 (SEQ ID NO: 74), 5-8 (SEQ ID NO: 75), 5-6 (SEQ ID NO: 76), 5-27 (SEQ ID NO: 77), 5-20 (SEQ ID NO: 78), 5-2 (SEQ ID NO: 79), 5-9 (SEQ ID NO: 80), 5-42 (SEQ ID NO: 81), 5-13 (SEQ ID NO: 82), 5-12 (SEQ ID NO: 83), 5-1 (SEQ ID NO: 84), 5-33 (SEQ ID NO: 85), 5-21 (SEQ ID NO: 86), 6-31 (SEQ ID NO: 87), 6-20 (SEQ ID NO: 88), 6-12 (SEQ ID NO: 89), 6-27 (SEQ ID NO: 90), 6-29 (SEQ ID NO: 91), 5-26 (SEQ ID NO: 191), 5-17 (SEQ ID NO: 192), 5-41 (SEQ ID NO: 193), or a fragment thereof.

In some cases a subject DR-IL-18 variant, or fragment thereof, includes at least one mutation (e.g., at least 2, at least 3, or at least 4 mutations) selected from the group consisting of M51X, M60X, S105X, D110X, and N111X, relative to SEQ ID NO: 30. In some cases a subject DR-IL-18 variant, or fragment thereof, includes at least 3 mutations selected from the group consisting of M51X, M60X, S105X, D110X, and N111X, relative

to SEQ ID NO: 30. In some cases a subject DR-IL-18 variant, or fragment thereof, includes at least one mutation (e.g., at least 2, at least 3, or at least 4 mutations) selected from the group consisting of M51X₁, M60X₂, S105X₃, D110X₄, and N111X₅, relative to SEQ ID NO: 30, where X₁ is T, K, D, E, R, or N; X₂ is K, Q, L, or R; X₃ is R, D, K, A, or N; X₄ is H, K, N, Q, E, N, S, or G; and X₅ is H, D, Y, R, S, or G. In some cases a subject DR-IL-18 variant, or fragment thereof, includes at least 3 mutations selected from the group consisting of M51X₁, M60X₂, S105X₃, D110X₄, and N111X₅, relative to SEQ ID NO: 30, where X₁ is T, K, D, E, R, or N; X₂ is K, Q, L, or R; X₃ is R, D, K, A, or N; X₄ is H, K, N, Q, E, N, S, or G; and X₅ is H, D, Y, R, S, or G. In some cases a subject DR-IL-18 variant, or fragment thereof, includes at least one mutation (e.g., at least 2, at least 3, or at least 4 mutations) selected from the group consisting of M51X₁, M60X₂, S105X₃, D110X₄, and N111X₅, relative to SEQ ID NO: 30, where X₁ is T or K; X₂ is K or L; X₃ is D, N, or A; X₄ is K, N, S, or G; and X₅ is H, Y, G, or R.

In some cases a subject DR-IL-18 variant, or fragment thereof, includes the mutations M51X, M60X, S105X, D110X, and N111X, relative to SEQ ID NO: 30. For example, in some cases a subject DR-IL-18 variant, or fragment thereof, includes the mutations M51X₁, M60X₂, S105X₃, D110X₄, and N111X₅, relative to SEQ ID NO: 30, where X₁ is T, K, D, E, R, or N; X₂ is K, Q, L, or R; X₃ is R, D, K, A, or N; X₄ is H, K, N, Q, E, N, S, or G; and X₅ is H, D, Y, R, S, or G. In some cases a subject DR-IL-18 variant, or fragment thereof, includes the mutations M51X₁, M60X₂, S105X₃, D110X₄, and N111X₅, relative to SEQ ID NO: 30, where X₁ is T or K; X₂ is K or L; X₃ is D, N, or A; X₄ is K, N, S, or G; and X₅ is H, Y, G, or R. In other words, in some cases a subject DR-IL-18 variant, or fragment thereof, includes the mutations {M51T or M51K}; {M60K or M60L}; {S105D, S105N, S105A}; {D110K, D110N, D110S, or D110G}; and {N111H, N111Y, N111R, or N111G}, relative to SEQ ID NO: 30.

In some cases a subject DR-IL-18 variant, or fragment thereof, includes at least one mutation (e.g., at least 2, at least 3, or at least 4 mutations) selected from the group consisting of M51X, K53X, Q56X, S105X, and N111X, relative to SEQ ID NO: 30. In some cases a subject DR-IL-18 variant, or fragment thereof, includes at least 3 mutations selected from the group consisting of M51X, K53X, Q56X, S105X, and N111X, relative to SEQ ID NO: 30. In some cases a subject DR-IL-18 variant, or fragment thereof, includes

at least one mutation (e.g., at least 2, at least 3, or at least 4 mutations) selected from the group consisting of M51X₁, K53X₂, Q56X₃, S105X₄, and N111X₅, relative to SEQ ID NO: 30, where X₁ is E, R, or K; X₂ is G, S, or T; X₃ is E, A, R, V, G, K, or L; X₄ is N, S, K, or G; and X₅ is R, S, G, or D. In some cases a subject DR-IL-18 variant, or fragment thereof, includes at least 3 mutations selected from the group consisting of M51X₁, K53X₂, Q56X₃, S105X₄, and N111X₅, relative to SEQ ID NO: 30, where X₁ is E, R, or K; X₂ is G, S, or T; X₃ is E, A, R, V, G, K, or L; X₄ is N, S, K, or G; and X₅ is R, S, G, or D. In some cases a subject DR-IL-18 variant, or fragment thereof, includes at least one mutation (e.g., at least 2, at least 3, or at least 4 mutations) selected from the group consisting of M51X₁, K53X₂, Q56X₃, S105X₄, and N111X₅, relative to SEQ ID NO: 30, where X₁ is K; X₂ is G or S; X₃ is G, R, or L; X₄ is S, N, or G; and X₅ is G or R.

In some cases a subject DR-IL-18 variant, or fragment thereof, includes the mutations M51X, K53X, Q56X, S105X, and N111X, relative to SEQ ID NO: 30. For example, in some cases a subject DR-IL-18 variant, or fragment thereof, includes the mutations M51X₁, K53X₂, Q56X₃, S105X₄, and N111X₅, relative to SEQ ID NO: 30, where X₁ is E, R, or K; X₂ is G, S, or T; X₃ is E, A, R, V, G, K, or L; X₄ is N, S, K, or G; and X₅ is R, S, G, or D. In some cases a subject DR-IL-18 variant, or fragment thereof, includes the mutations M51X₁, K53X₂, Q56X₃, S105X₄, and N111X₅, relative to SEQ ID NO: 30, where X₁ is K; X₂ is G or S; X₃ is G, R, or L; X₄ is S, N, or G; and X₅ is G or R. In other words, in some cases a subject DR-IL-18 variant, or fragment thereof, includes the mutations {M51K}; {K53G or K53S}; {Q56G, Q56R, or Q56L}; {D110S, D110N, or D110G}; and {N111R, or N111G}, relative to SEQ ID NO: 30.

In some cases a subject DR-IL-18 variant, or fragment thereof, comprises an amino acid sequence having 85% or more sequence identity (e.g., 90% or more, 92% or more, 93% or more, 94% or more, 95% or more, 96% or more, 97% or more, 98% or more, or 99% or more sequence identity) with the wild type human IL-18 amino acid sequence set forth as SEQ ID NO: 30. As such in some cases a subject DR-IL-18 variant, or fragment thereof, comprises an amino acid sequence that (i) has 85% or more sequence identity (e.g., 90% or more, 92% or more, 93% or more, 94% or more, 95% or more, 96% or more, 97% or more, 98% or more, or 99% or more sequence identity) with the wild type human IL-18 amino acid sequence set forth as SEQ ID NO: 30; and (ii) includes at least one mutation

(e.g., at least 2, at least 3, at least 4, at least 5, or at least 6 mutations) relative to wild type IL-18 (e.g., human IL-18).

In some cases a subject DR-IL-18 variant, or fragment thereof, comprises an amino acid sequence that (i) has 85% or more sequence identity (e.g., 90% or more, 92% or more, 93% or more, 94% or more, 95% or more, 96% or more, 97% or more, 98% or more, or 99% or more sequence identity) with the wild type human IL-18 amino acid sequence set forth as SEQ ID NO: 30; and (ii) includes at least one mutation (e.g., at least 2, at least 3, at least 4, at least 5, or at least 6 mutations) 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, relative to SEQ ID NO: 30. In some cases a subject DR-IL-18 variant, or fragment thereof, comprises an amino acid sequence that (i) has 85% or more sequence identity (e.g., 90% or more, 92% or more, 93% or more, 94% or more, 95% or more, 96% or more, 97% or more, 98% or more, or 99% or more sequence identity) with the wild type human IL-18 amino acid sequence set forth as SEQ ID NO: 30; and (ii) includes at least 4 mutations 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, relative to SEQ ID NO: 30. In some cases a subject DR-IL-18 variant, or fragment thereof, comprises an amino acid sequence that (i) has 85% or more sequence identity (e.g., 90% or more, 92% or more, 93% or more, 94% or more, 95% or more, 96% or more, 97% or more, 98% or more, or 99% or more sequence identity) with the wild type human IL-18 amino acid sequence set forth as SEQ ID NO: 30; and (ii) includes at least 6 mutations 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, relative to SEQ ID NO: 30. In some cases a subject DR-IL-18 variant, or fragment thereof, comprises an amino acid sequence that (i) has 85% or more sequence identity (e.g., 90% or more, 92% or more, 93% or more, 94% or more, 95% or more, 96% or more, 97% or more, 98% or more, or 99% or more sequence identity) with the wild type human IL-18 amino acid sequence set forth as SEQ ID NO: 30; and (ii) includes at least one mutation (e.g., at least 2, at least 3, at least 4, at least 5, or at least 6 mutations) selected from the group consisting of Y1X, L5X, K8X, S55X, Q56X, P57X,

G59X, E77X, Q103X, S105X, D110X, N111X, M113X, V153X, and N155X, relative to SEQ ID NO: 30.

In some cases a subject DR-IL-18 variant, or fragment thereof, comprises an amino acid sequence that (i) has 85% or more sequence identity (e.g., 90% or more, 92% or more, 93% or more, 94% or more, 95% or more, 96% or more, 97% or more, 98% or more, or 99% or more sequence identity) with the wild type human IL-18 amino acid sequence set forth as SEQ ID NO: 30; and (ii) includes at least one mutation (e.g., at least 2, at least 3, or at least 4 mutations) selected from the group consisting of M51X, M60X, S105X, D110X, and N111X, relative to SEQ ID NO: 30. In some cases a subject DR-IL-18 variant, or fragment thereof, comprises an amino acid sequence that (i) has 85% or more sequence identity (e.g., 90% or more, 92% or more, 93% or more, 94% or more, 95% or more, 96% or more, 97% or more, 98% or more, or 99% or more sequence identity) with the wild type human IL-18 amino acid sequence set forth as SEQ ID NO: 30; and (ii) includes at least 3 mutations (e.g., at least 2, at least 3, or at least 4 mutations) selected from the group consisting of M51X, M60X, S105X, D110X, and N111X, relative to SEQ ID NO: 30. In some cases a subject DR-IL-18 variant, or fragment thereof, comprises an amino acid sequence that (i) has 85% or more sequence identity (e.g., 90% or more, 92% or more, 93% or more, 94% or more, 95% or more, 96% or more, 97% or more, 98% or more, or 99% or more sequence identity) with the wild type human IL-18 amino acid sequence set forth as SEQ ID NO: 30; and (ii) includes at least one mutation (e.g., at least 2, at least 3, or at least 4 mutations) selected from the group consisting of M51X₁, M60X₂, S105X₃, D110X₄, and N111X₅, relative to SEQ ID NO: 30, where X₁ is T, K, D, E, R, or N; X₂ is K, Q, L, or R; X₃ is R, D, K, A, or N; X₄ is H, K, N, Q, E, N, S, or G; and X₅ is H, D, Y, R, S, or G. In some cases a subject DR-IL-18 variant, or fragment thereof, comprises an amino acid sequence that (i) has 85% or more sequence identity (e.g., 90% or more, 92% or more, 93% or more, 94% or more, 95% or more, 96% or more, 97% or more, 98% or more, or 99% or more sequence identity) with the wild type human IL-18 amino acid sequence set forth as SEQ ID NO: 30; and (ii) includes at least 3 mutations selected from the group consisting of M51X₁, M60X₂, S105X₃, D110X₄, and N111X₅, relative to SEQ ID NO: 30, where X₁ is T, K, D, E, R, or N; X₂ is K, Q, L, or R; X₃ is R, D, K, A, or N; X₄ is H, K, N, Q, E, N, S, or G; and X₅ is H, D, Y, R, S, or G. In some cases a subject DR-IL-18 variant, or fragment

thereof, comprises an amino acid sequence that (i) has 85% or more sequence identity (e.g., 90% or more, 92% or more, 93% or more, 94% or more, 95% or more, 96% or more, 97% or more, 98% or more, or 99% or more sequence identity) with the wild type human IL-18 amino acid sequence set forth as SEQ ID NO: 30; and (ii) includes at least one mutation
 5 (e.g., at least 2, at least 3, or at least 4 mutations) selected from the group consisting of M51X₁, M60X₂, S105X₃, D110X₄, and N111X₅, relative to SEQ ID NO: 30, where X₁ is T or K; X₂ is K or L; X₃ is D, N, or A; X₄ is K, N, S, or G; and X₅ is H, Y, G, or R.

In some cases a subject DR-IL-18 variant, or fragment thereof, comprises an amino acid sequence that (i) has 85% or more sequence identity (e.g., 90% or more, 92% or more, 93% or more, 94% or more, 95% or more, 96% or more, 97% or more, 98% or more, or 99% or more sequence identity) with the wild type human IL-18 amino acid sequence set forth as SEQ ID NO: 30; and (ii) includes the mutations M51X, M60X, S105X, D110X, and N111X, relative to SEQ ID NO: 30. In some cases a subject DR-IL-18 variant, or fragment thereof, comprises an amino acid sequence that (i) has 85% or more sequence
 10 identity (e.g., 90% or more, 92% or more, 93% or more, 94% or more, 95% or more, 96% or more, 97% or more, 98% or more, or 99% or more sequence identity) with the wild type human IL-18 amino acid sequence set forth as SEQ ID NO: 30; and (ii) includes the mutations M51X₁, M60X₂, S105X₃, D110X₄, and N111X₅, relative to SEQ ID NO: 30, where X₁ is T, K, D, E, R, or N; X₂ is K, Q, L, or R; X₃ is R, D, K, A, or N; X₄ is H, K, N, Q, E, N, S, or G; and X₅ is H, D, Y, R, S, or G. In some cases a subject DR-IL-18 variant, or fragment thereof, comprises an amino acid sequence that (i) has 85% or more sequence
 15 identity (e.g., 90% or more, 92% or more, 93% or more, 94% or more, 95% or more, 96% or more, 97% or more, 98% or more, or 99% or more sequence identity) with the wild type human IL-18 amino acid sequence set forth as SEQ ID NO: 30; and (ii) includes the mutations M51X₁, M60X₂, S105X₃, D110X₄, and N111X₅, relative to SEQ ID NO: 30, where X₁ is T or K; X₂ is K or L; X₃ is D, N, or A; X₄ is K, N, S, or G; and X₅ is H, Y, G, or R.
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In some cases a subject DR-IL-18 variant, or fragment thereof, comprises an amino acid sequence that (i) has 85% or more sequence identity (e.g., 90% or more, 92% or more, 93% or more, 94% or more, 95% or more, 96% or more, 97% or more, 98% or more, or 99% or more sequence identity) with the wild type human IL-18 amino acid sequence set
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forth as SEQ ID NO: 30; and (ii) includes at least one mutation (e.g., at least 2, at least 3, or at least 4 mutations) selected from the group consisting of M51X, K53X, Q56X, S105X, and N111X, relative to SEQ ID NO: 30. In some cases a subject DR-IL-18 variant, or fragment thereof, comprises an amino acid sequence that (i) has 85% or more sequence identity (e.g., 90% or more, 92% or more, 93% or more, 94% or more, 95% or more, 96% or more, 97% or more, 98% or more, or 99% or more sequence identity) with the wild type human IL-18 amino acid sequence set forth as SEQ ID NO: 30; and (ii) includes at least 3 mutations selected from the group consisting of M51X, K53X, Q56X, S105X, and N111X, relative to SEQ ID NO: 30. In some cases a subject DR-IL-18 variant, or fragment thereof, comprises an amino acid sequence that (i) has 85% or more sequence identity (e.g., 90% or more, 92% or more, 93% or more, 94% or more, 95% or more, 96% or more, 97% or more, 98% or more, or 99% or more sequence identity) with the wild type human IL-18 amino acid sequence set forth as SEQ ID NO: 30; and (ii) includes at least one mutation (e.g., at least 2, at least 3, or at least 4 mutations) selected from the group consisting of M51X₁, K53X₂, Q56X₃, S105X₄, and N111X₅, relative to SEQ ID NO: 30, where X₁ is E, R, or K; X₂ is G, S, or T; X₃ is E, A, R, V, G, K, or L; X₄ is N, S, K, or G; and X₅ is R, S, G, or D. In some cases a subject DR-IL-18 variant, or fragment thereof, comprises an amino acid sequence that (i) has 85% or more sequence identity (e.g., 90% or more, 92% or more, 93% or more, 94% or more, 95% or more, 96% or more, 97% or more, 98% or more, or 99% or more sequence identity) with the wild type human IL-18 amino acid sequence set forth as SEQ ID NO: 30; and (ii) includes at least 3 mutations selected from the group consisting of M51X₁, K53X₂, Q56X₃, S105X₄, and N111X₅, relative to SEQ ID NO: 30, where X₁ is E, R, or K; X₂ is G, S, or T; X₃ is E, A, R, V, G, K, or L; X₄ is N, S, K, or G; and X₅ is R, S, G, or D. In some cases a subject DR-IL-18 variant, or fragment thereof, comprises an amino acid sequence that (i) has 85% or more sequence identity (e.g., 90% or more, 92% or more, 93% or more, 94% or more, 95% or more, 96% or more, 97% or more, 98% or more, or 99% or more sequence identity) with the wild type human IL-18 amino acid sequence set forth as SEQ ID NO: 30; and (ii) includes at least one mutation (e.g., at least 2, at least 3, or at least 4 mutations) selected from the group consisting of M51X₁, K53X₂, Q56X₃, S105X₄, and N111X₅, relative to SEQ ID NO: 30, where X₁ is K; X₂ is G or S; X₃ is G, R, or L; X₄ is S, N, or G; and X₅ is G or R.

- In some cases a subject DR-IL-18 variant, or fragment thereof, comprises an amino acid sequence that (i) has 85% or more sequence identity (e.g., 90% or more, 92% or more, 93% or more, 94% or more, 95% or more, 96% or more, 97% or more, 98% or more, or 99% or more sequence identity) with the wild type human IL-18 amino acid sequence set forth as SEQ ID NO: 30; and (ii) includes the mutations M51X, K53X, Q56X, S105X, and N111X, relative to SEQ ID NO: 30. In some cases a subject DR-IL-18 variant, or fragment thereof, comprises an amino acid sequence that (i) has 85% or more sequence identity (e.g., 90% or more, 92% or more, 93% or more, 94% or more, 95% or more, 96% or more, 97% or more, 98% or more, or 99% or more sequence identity) with the wild type human IL-18 amino acid sequence set forth as SEQ ID NO: 30; and (ii) includes the mutations M51X₁, K53X₂, Q56X₃, S105X₄, and N111X₅, relative to SEQ ID NO: 30, where X₁ is E, R, or K; X₂ is G, S, or T; X₃ is E, A, R, V, G, K, or L; X₄ is N, S, K, or G; and X₅ is R, S, G, or D.
- In some cases a subject DR-IL-18 variant, or fragment thereof, comprises an amino acid sequence that (i) has 85% or more sequence identity (e.g., 90% or more, 92% or more, 93% or more, 94% or more, 95% or more, 96% or more, 97% or more, 98% or more, or 99% or more sequence identity) with the wild type human IL-18 amino acid sequence set forth as SEQ ID NO: 30; and (ii) includes the mutations M51X₁, K53X₂, Q56X₃, S105X₄, and N111X₅, relative to SEQ ID NO: 30, where X₁ is K; X₂ is G or S; X₃ is G, R, or L; X₄ is S, N, or G; and X₅ is G or R.
- In various embodiments, the IL-18 variant polypeptide that binds to and inhibits IL-18BP comprises a human IL-18 variant polypeptide, or fragment thereof, comprising at least one mutation (e.g., at least 2, at least 3, at least 4, at least 5, or at least 6 mutations) selected from the group consisting of Y1X, L5X, D17X, E31X, T34X, D35X, S36X, D37X, D40X, N41X, M51X, Q56X, M60X, Q103X, H109X, M113X, and R131X, wherein X denotes any amino acid. In some embodiments, the IL-18 variant polypeptide that binds to and inhibits IL-18BP comprises a human IL-18 variant polypeptide, or fragment thereof, comprising at least one mutation (e.g., at least 2, at least 3, at least 4, at least 5, or at least 6 mutations) selected from the group consisting of Y1D, Y1F, Y1H, Y1L, L5F, L5H, D17A, D17G, D17R, D17H, E31A, E31T, E31G, E31K, E31R, T34A, T34K T34E, D35S, D35A, D35Y, S36N, S36K, S36R, D37P, D37A, D37R, D37H, D37L, D37V, D40Y D40S, D40A, N41K, N41S, N41R, M51F, M51L, M51I, Q56H, M60L,

M60F, M60I, Q103L, Q103I, H109A, H109P, H109D, M113L, M113I, M113F, and R131S. In some embodiments, the IL-18 variant polypeptide that binds to and inhibits IL-18BP comprises a human IL-18 variant polypeptide, or fragment thereof, selected from the group consisting of hD2D-5F12 (SEQ ID NO: 92), hD2D-5F11 (SEQ ID NO: 93), hD2D-5F10 (SEQ ID NO: 94), hD2D-5F08 (SEQ ID NO: 95), hD2D-5F06 (SEQ ID NO: 96), hD2D-5F04 (SEQ ID NO: 97), hD2D-5F02 (SEQ ID NO: 98), hD2D-5F01 (SEQ ID NO: 99), hD2D-5E10 (SEQ ID NO: 100), hD2D-5E08 (SEQ ID NO: 101), hD2D-5E03 (SEQ ID NO: 102), hD2D-5E02 (SEQ ID NO: 103), hD2D-5D10 (SEQ ID NO: 104), hD2D-5D08 (SEQ ID NO: 105), hD2D-5D06 (SEQ ID NO: 106), hD2D-5D05 (SEQ ID NO: 107), hD2D-5D03 (SEQ ID NO: 108), hD2D-5D02 (SEQ ID NO: 109), hD2D-5C10 (SEQ ID NO: 110), hD2D-5C09 (SEQ ID NO: 111), hD2D-5C08 (SEQ ID NO: 112), hD2D-5C05 (SEQ ID NO: 113), hD2D-5C04 (SEQ ID NO: 114), hD2D-5C03 (SEQ ID NO: 115), hD2D-5B11 (SEQ ID NO: 116), hD2D-5B10 (SEQ ID NO: 117), hD2D-5B06 (SEQ ID NO: 118), hD2D-5B05 (SEQ ID NO: 119), hD2D-5B02 (SEQ ID NO: 120), hD2D-5A09 (SEQ ID NO: 121), hD2D-5A02 (SEQ ID NO: 122), hD2D-CS1 (SEQ ID NO: 123), hD2D-CS2 (SEQ ID NO: 124), hD2D-CS3 (SEQ ID NO: 125), or a fragment thereof.

In some cases a subject D2D-IL-18 variant, or fragment thereof, includes at least one mutation (e.g., at least 2, at least 3, or at least 4 mutations) selected from the group consisting of D17X, E30X, and Q103X, relative to SEQ ID NO: 30. In some cases a subject D2D-IL-18 variant, or fragment thereof, includes at least one mutation (e.g., at least 2, at least 3, or at least 4 mutations) selected from the group consisting of D17X₁, E30X₂, and Q103X₃, relative to SEQ ID NO: 30, where X₁ is G, H, R, or A; X₂ is A, T, G, K, or R; and X₃ is I or L. In some cases a subject D2D-IL-18 variant, or fragment thereof, includes at least one mutation (e.g., at least 2, at least 3, or at least 4 mutations) selected from the group consisting of D17G, E30A, and (Q103L or Q103I).

In some cases a subject D2D-IL-18 variant, or fragment thereof, includes the mutations D17X, E30X, and Q103X, relative to SEQ ID NO: 30. For example, in some cases a subject D2D-IL-18 variant, or fragment thereof, includes the mutations D17X₁, E30X₂, and Q103X₃, relative to SEQ ID NO: 30, where X₁ is G, H, R, or A; X₂ is A, T, G, K, or R; and X₃ is I or L. In some cases a subject D2D-IL-18 variant, or fragment thereof, includes the mutations D17G, E30A, and (Q103L or Q103I).

In some cases a subject D2D-IL-18 variant, or fragment thereof, includes at least one mutation (e.g., at least 2, at least 3, or at least 4 mutations) selected from the group consisting of D17X, E30X, D35X, M51X, and Q103X, relative to SEQ ID NO: 30. In some cases a subject D2D-IL-18 variant, or fragment thereof, includes at least one mutation (e.g.,
 5 at least 2, at least 3, or at least 4 mutations) selected from the group consisting of D17X₁, E30X₂, D35X₃, M51X₄, and Q103X₅, relative to SEQ ID NO: 30, where X₁ is G, H, R, or A; X₂ is A, T, G, K, or R; X₃ is S, A, or Y; X₄ is F, I, or L; and X₅ is I or L. In some cases a subject D2D-IL-18 variant, or fragment thereof, includes at least one mutation (e.g., at least 2, at least 3, or at least 4 mutations) selected from the group consisting of D17G,
 10 E30A, D35S, M51F, and (Q103L or Q103I), relative to SEQ ID NO: 30.

In some cases a subject D2D-IL-18 variant, or fragment thereof, includes the mutations D17X, E30X, D35X, M51X, and Q103X, relative to SEQ ID NO: 30. For example, in some cases a subject D2D-IL-18 variant, or fragment thereof, includes the mutations D17X₁, E30X₂, D35X₃, M51X₄, and Q103X₅, relative to SEQ ID NO: 30, where
 15 X₁ is G, H, R, or A; X₂ is A, T, G, K, or R; X₃ is S, A, or Y; X₄ is F, I, or L; and X₅ is I or L. In some cases a subject D2D-IL-18 variant, or fragment thereof, includes the mutations D17G, E30A, D35S, M51F, and (Q103L or Q103I), relative to SEQ ID NO: 30.

In some cases a subject D2D-IL-18 variant, or fragment thereof, comprises an amino acid sequence having 85% or more sequence identity (e.g., 90% or more, 92% or more,
 20 or more, 93% or more, 94% or more, 95% or more, 96% or more, 97% or more, 98% or more, or 99% or more sequence identity) with the wild type human IL-18 amino acid sequence set forth as SEQ ID NO: 30. As such in some cases a subject D2D-IL-18 variant, or fragment thereof, comprises an amino acid sequence that (i) has 85% or more sequence identity (e.g., 90% or more, 92% or more, 93% or more, 94% or more, 95% or more, 96%
 25 or more, 97% or more, 98% or more, or 99% or more sequence identity) with the wild type human IL-18 amino acid sequence set forth as SEQ ID NO: 30; and (ii) includes at least one mutation (e.g., at least 2, at least 3, at least 4, at least 5, or at least 6 mutations) relative to wild type IL-18 (e.g., human IL-18).

In some cases a subject DR-IL-18 variant, or fragment thereof, comprises an amino
 30 acid sequence that (i) has 85% or more sequence identity (e.g., 90% or more, 92% or more, 93% or more, 94% or more, 95% or more, 96% or more, 97% or more, 98% or more, or

99% or more sequence identity) with the wild type human IL-18 amino acid sequence set forth as SEQ ID NO: 30; and (ii) includes at least one mutation (e.g., at least 2, at least 3, at least 4, at least 5, or at least 6 mutations) selected from the group consisting of Y1X, L5X, D17X, E31X, T34X, D35X, S36X, D37X, D40X, N41X, M51X, Q56X, M60X, Q103X, H109X, M113X, and R131X.

In some cases a subject DR-IL-18 variant, or fragment thereof, comprises an amino acid sequence that (i) has 85% or more sequence identity (e.g., 90% or more, 92% or more, 93% or more, 94% or more, 95% or more, 96% or more, 97% or more, 98% or more, or 99% or more sequence identity) with the wild type human IL-18 amino acid sequence set forth as SEQ ID NO: 30; and (ii) includes at least one mutation (e.g., at least 2, at least 3, at least 4, at least 5, or at least 6 mutations) selected from the group consisting of D17X, E30X, and Q103X, relative to SEQ ID NO: 30. In some cases a subject DR-IL-18 variant, or fragment thereof, comprises an amino acid sequence that (i) has 85% or more sequence identity (e.g., 90% or more, 92% or more, 93% or more, 94% or more, 95% or more, 96% or more, 97% or more, 98% or more, or 99% or more sequence identity) with the wild type human IL-18 amino acid sequence set forth as SEQ ID NO: 30; and (ii) includes at least one mutation (e.g., at least 2, at least 3, at least 4, at least 5, or at least 6 mutations) selected from the group consisting of D17X₁, E30X₂, and Q103X₃, relative to SEQ ID NO: 30, where X₁ is G, H, R, or A; X₂ is A, T. In some cases a subject DR-IL-18 variant, or fragment thereof, comprises an amino acid sequence that (i) has 85% or more sequence identity (e.g., 90% or more, 92% or more, 93% or more, 94% or more, 95% or more, 96% or more, 97% or more, 98% or more, or 99% or more sequence identity) with the wild type human IL-18 amino acid sequence set forth as SEQ ID NO: 30; and (ii) includes at least one mutation (e.g., at least 2, at least 3, at least 4, at least 5, or at least 6 mutations) selected from the group consisting of D17G, E30A, and (Q103L or Q103I).

In some cases a subject DR-IL-18 variant, or fragment thereof, comprises an amino acid sequence that (i) has 85% or more sequence identity (e.g., 90% or more, 92% or more, 93% or more, 94% or more, 95% or more, 96% or more, 97% or more, 98% or more, or 99% or more sequence identity) with the wild type human IL-18 amino acid sequence set forth as SEQ ID NO: 30; and (ii) includes the mutations D17X, E30X, and Q103X, relative to SEQ ID NO: 30. In some cases a subject DR-IL-18 variant, or fragment thereof,

comprises an amino acid sequence that (i) has 85% or more sequence identity (e.g., 90% or more, 92% or more, 93% or more, 94% or more, 95% or more, 96% or more, 97% or more, 98% or more, or 99% or more sequence identity) with the wild type human IL-18 amino acid sequence set forth as SEQ ID NO: 30; and (ii) includes the mutations D17X₁, E30X₂, and Q103X₃, relative to SEQ ID NO: 30, where X₁ is G, H, R, or A; X₂ is A, T. In some cases a subject DR-IL-18 variant, or fragment thereof, comprises an amino acid sequence that (i) has 85% or more sequence identity (e.g., 90% or more, 92% or more, 93% or more, 94% or more, 95% or more, 96% or more, 97% or more, 98% or more, or 99% or more sequence identity) with the wild type human IL-18 amino acid sequence set forth as SEQ ID NO: 30; and (ii) includes the mutations D17G, E30A, and (Q103L or Q103I).

In some cases a subject DR-IL-18 variant, or fragment thereof, comprises an amino acid sequence that (i) has 85% or more sequence identity (e.g., 90% or more, 92% or more, 93% or more, 94% or more, 95% or more, 96% or more, 97% or more, 98% or more, or 99% or more sequence identity) with the wild type human IL-18 amino acid sequence set forth as SEQ ID NO: 30; and (ii) includes at least one mutation (e.g., at least 2, at least 3, at least 4, at least 5, or at least 6 mutations) selected from the group consisting of D17X, E30X, D35X, M51X, and Q103X, relative to SEQ ID NO: 30. In some cases a subject DR-IL-18 variant, or fragment thereof, comprises an amino acid sequence that (i) has 85% or more sequence identity (e.g., 90% or more, 92% or more, 93% or more, 94% or more, 95% or more, 96% or more, 97% or more, 98% or more, or 99% or more sequence identity) with the wild type human IL-18 amino acid sequence set forth as SEQ ID NO: 30; and (ii) includes at least one mutation (e.g., at least 2, at least 3, at least 4, at least 5, or at least 6 mutations) selected from the group consisting of D17X₁, E30X₂, D35X₃, M51X₄, and Q103X₃, relative to SEQ ID NO: 30, where X₁ is G, H, R, or A; X₂ is A, T, G, K, or R; X₃ is S, A, or Y; X₄ is F, I, or L; and X₅ is I or L. In some cases a subject DR-IL-18 variant, or fragment thereof, comprises an amino acid sequence that (i) has 85% or more sequence identity (e.g., 90% or more, 92% or more, 93% or more, 94% or more, 95% or more, 96% or more, 97% or more, 98% or more, or 99% or more sequence identity) with the wild type human IL-18 amino acid sequence set forth as SEQ ID NO: 30; and (ii) includes at least one mutation (e.g., at least 2, at least 3, at least 4, at least 5, or at least 6 mutations)

selected from the group consisting of D17G, E30A, D35S, M51F, and (Q103L or Q103I), relative to SEQ ID NO: 30.

In some cases a subject DR-IL-18 variant, or fragment thereof, comprises an amino acid sequence that (i) has 85% or more sequence identity (e.g., 90% or more, 92% or more, 93% or more, 94% or more, 95% or more, 96% or more, 97% or more, 98% or more, or 99% or more sequence identity) with the wild type human IL-18 amino acid sequence set forth as SEQ ID NO: 30; and (ii) includes the mutations D17X, E30X, D35X, M51X, and Q103X, relative to SEQ ID NO: 30. In some cases a subject DR-IL-18 variant, or fragment thereof, comprises an amino acid sequence that (i) has 85% or more sequence identity (e.g., 90% or more, 92% or more, 93% or more, 94% or more, 95% or more, 96% or more, 97% or more, 98% or more, or 99% or more sequence identity) with the wild type human IL-18 amino acid sequence set forth as SEQ ID NO: 30; and (ii) includes the mutations D17X₁, E30X₂, D35X₃, M51X₄, and Q103X₅, relative to SEQ ID NO: 30, where X₁ is G, H, R, or A; X₂ is A, T, G, K, or R; X₃ is S, A, or Y; X₄ is F, I, or L; and X₅ is I or L. In some cases a subject DR-IL-18 variant, or fragment thereof, comprises an amino acid sequence that (i) has 85% or more sequence identity (e.g., 90% or more, 92% or more, 93% or more, 94% or more, 95% or more, 96% or more, 97% or more, 98% or more, or 99% or more sequence identity) with the wild type human IL-18 amino acid sequence set forth as SEQ ID NO: 30; and (ii) includes the mutations D17G, E30A, D35S, M51F, and (Q103L or Q103I), relative to SEQ ID NO: 30.

In some embodiments, a composition of the invention is administered to a murine cell, tissue, organ, system, or subject to treat or prevent a disease or disorder. In some embodiments, a murine IL-18 variant polypeptide, or a fragment thereof, is administered to a cell, tissue, organ, system, or subject (e.g., a human cell, tissue, organ, system, or subject). In some embodiments, a nucleic acid (e.g., DNA, cDNA, mRNA, etc.) encoding at least one murine IL-18 variant polypeptide is administered to a cell, tissue, organ, system, or subject.

In some embodiments, a murine IL-18 variant polypeptide, or a fragment thereof, comprises at least one mutation (e.g., at least 2, at least 3, at least 4, at least 5, or at least 6 mutations) selected from the group consisting of N1X, M50X, Y51X, K52X, S54X, E55X, V56X, R57X, G58X, L59X, R104X, N109X, and L151X, wherein X denotes any amino

acid. In some embodiments, a murine IL-18 variant polypeptide, or fragment thereof, comprises at least one mutation (e.g., at least 2, at least 3, at least 4, at least 5, or at least 6 mutations) selected from 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. In some embodiments, a murine IL-18 variant polypeptide comprises at least one variant selected from the group consisting of mCS1 (SEQ ID NO: 60), mCS2 (SEQ ID NO: 61), mC1 (SEQ ID NO: 62), mA12 (SEQ ID NO: 63), mE8 (SEQ ID NO: 64), mC10 (SEQ ID NO: 65), mB7 (SEQ ID NO: 66), mB1 (SEQ ID NO: 67), mD1 (SEQ ID NO: 68), mH7 (SEQ ID NO: 69), mA7 (SEQ ID NO: 70), mE1 (SEQ ID NO: 71), and mH3 (SEQ ID NO: 72), or a fragment thereof.

In some embodiments, the IL-18 variant polypeptide that binds to and inhibits IL-18BP comprises a murine IL-18 variant polypeptide comprising at least one mutation (e.g., at least 2, at least 3, at least 4, at least 5, or at least 6 mutations) selected from the group consisting of N1X, L5X, D17X, E30X, T33X, D34X, I35X, D36X, M50X, Q102X, R104, H108X, N109X, M111X, D129X, and D130X, wherein X denotes any amino acid. In some embodiments, the IL-18 variant polypeptide that binds to and inhibits IL-18BP comprises a murine IL-18 variant polypeptide, or fragment thereof, comprising at least one mutation (e.g., at least 2, at least 3, at least 4, at least 5, or at least 6 mutations) selected from the group consisting of N1Y, N1D, N1H, N1L, N1F, N1V, N1I, L5Y, L5H, D17Q, D17G, D17A, D17E, D17S, D17N, E30A, E30R, E30K, E30T, E30G, T33G, T33A, T33E, T33R, T33K, D34Y, D34S, D34A, I35T, I35K, I35R, D36V, D36A, D36G, D36H, D36P, D36R, D36L, M50F, M50L, Q102L, Q102I, R104E, R104A, R104P, R104G, R104Q, R104H, H108D, H108A, N109R, N109S, N109T, N109I, M111L, M111I, D129A, D129F, D129V, D129Y, D129S, D130E, D130T, D130G, D130N, D130R, D130S, D130Q, and D130H. the IL-18 variant polypeptide that binds to and inhibits IL-18BP comprises a murine IL-18 variant polypeptide, or fragment thereof, selected from the group consisting of mD2D-A5 (SEQ ID NO: 126), mD2D-A6 (SEQ ID NO: 127), mD2D-A7 (SEQ ID NO: 128), mD2D-A8 (SEQ ID NO: 129), mD2D-A9 (SEQ ID NO: 130), mD2D-A11 (SEQ ID NO: 131), mD2D-A12 (SEQ ID NO: 132), mD2D-B4 (SEQ ID NO: 133), mD2D-B7 (SEQ ID NO:

134), mD2D-B11 (SEQ ID NO: 135), mD2D-B12 (SEQ ID NO: 136), mD2D-C1 (SEQ ID NO: 137), mD2D-C3 (SEQ ID NO: 138), mD2D-C5 (SEQ ID NO: 139), mD2D-C6 (SEQ ID NO: 140), mD2D-C9 (SEQ ID NO: 141), mD2D-C10 (SEQ ID NO: 142), mD2D-C11 (SEQ ID NO: 143), mD2D-D1 (SEQ ID NO: 144), mD2D-D9 (SEQ ID NO: 145), mD2D-D12 (SEQ ID NO: 146), mD2D-E3 (SEQ ID NO: 147), mD2D-E4 (SEQ ID NO: 148), mD2D-E5 (SEQ ID NO: 149), mD2D-E7 (SEQ ID NO: 150), mD2D-E8 (SEQ ID NO: 151), mD2D-E9 (SEQ ID NO: 152), mD2D-E10 (SEQ ID NO: 153), mD2D-E11 (SEQ ID NO: 154), mD2D-E12 (SEQ ID NO: 155), mD2D-F3 (SEQ ID NO: 156), mD2D-F4 (SEQ ID NO: 157), mD2D-F5 (SEQ ID NO: 158), mD2D-F7 (SEQ ID NO: 159), mD2D-F8 (SEQ ID NO: 160), mD2D-F9 (SEQ ID NO: 161), mD2D-G1 (SEQ ID NO: 162), mD2D-G7 (SEQ ID NO: 163), mD2D-G9 (SEQ ID NO: 164), mD2D-H7 (SEQ ID NO: 165), mD2D-E1 (SEQ ID NO: 166), mD2D-G8 (SEQ ID NO: 167), mD2D-H3 (SEQ ID NO: 168), mD2D-A10 (SEQ ID NO: 169), mD2D-H1 (SEQ ID NO: 170), mD2D-F12 (SEQ ID NO: 171), mD2D-G10 (SEQ ID NO: 172), mD2D-G12 (SEQ ID NO: 173), mD2D-E2 (SEQ ID NO: 174), mD2D-G11 (SEQ ID NO: 175), mD2D-C4 (SEQ ID NO: 176), mD2D-F11 (SEQ ID NO: 177), mD2D-C2 (SEQ ID NO: 178), mD2D-F10 (SEQ ID NO: 179), mD2D-A2 (SEQ ID NO: 180), mD2D-F6 (SEQ ID NO: 181), mD2D-A1 (SEQ ID NO: 182), mD2D-E6 (SEQ ID NO: 183), mD2D-D4 (SEQ ID NO: 184), mD2D-D6 (SEQ ID NO: 185), mD2D-A3 (SEQ ID NO: 186), mD2D-A4 (SEQ ID NO: 187), mD2D-B10 (SEQ ID NO: 188), mD2D-B8 (SEQ ID NO: 189), mD2D-B9 (SEQ ID NO: 190), or a fragment thereof.

In some embodiments, the method comprises administering to a subject, cell, or tissue, an isolated nucleic acid molecule encoding on or more of the IL-18 variant peptides described herein.

It will be understood by one skilled in the art that an increase in the level of IL-18 signaling through the IL-18R encompasses an increase in the amount of IL-18 or IL-18 variant polypeptide available for binding to and activating IL-18R. This can be accomplished by increasing the level or activity of IL-18 which includes, but is not limited to, the direct or indirect administration of IL-18, the direct or indirect administration of an IL-18 variant polypeptide, and the direct or indirect administration of an inhibitor of IL-18BP, as well as increasing transcription, translation, or both, of a nucleic acid encoding

IL-18 or an IL-18 variant polypeptide; and it also includes increasing any activity of IL-18 or IL-18 variant polypeptide as well.

The increased level of IL-18 signaling, including by using an IL-18 variant polypeptide, can be assessed using a wide variety of methods, including those disclosed
5 herein, as well as methods known in the art or to be developed in the future. That is, the routineer would appreciate, based upon the disclosure provided herein, that increasing the level or activity of IL-18 signaling can be readily assessed using methods that assess the level of a nucleic acid (e.g., mRNA) encoding IL-18 or an IL-18 variant polypeptide or
10 fragment thereof, the level of IL-18 or an IL-18 variant polypeptide or fragment polypeptide, and/or the level of IL-18 or an IL-18 variant polypeptide or fragment activity in a biological sample obtained from a subject.

One skilled in the art, based upon the disclosure provided herein, would understand that the invention is useful in subjects who, in whole (e.g., systemically) or in part (e.g., locally, cell, tissue, organ), are being or will be, treated for a disease or disorder where an
15 increase in IL-18 signaling activity would be beneficial. The skilled artisan will appreciate, based upon the teachings provided herein, that the diseases and disorders treatable by the compositions and methods described herein encompass any disease or disorder wherein an increase in IL-18 signaling will promote a positive biologic, physiologic, clinical or therapeutic outcome.

20 One of skill in the art will realize that in addition to increasing IL-18 signaling directly, diminishing the amount or activity of a molecule that itself diminishes the amount or activity of IL-18 signaling can also serve to increase the amount or activity of IL-18 signaling. Thus, an activator of IL-18 activity can include, but should not be construed as being limited to, a chemical compound, a protein, a peptidomimetic, an antibody, a
25 ribozyme, and an antisense nucleic acid molecule. One of skill in the art would readily appreciate, based on the disclosure provided herein, that an activator of IL-18 activity encompasses a compound that increases the level of IL-18 signaling. Additionally, an activator of IL-18 activity encompasses a compound that inhibits the level or activity of a molecule that itself diminishes the amount or activity of IL-18 signaling (i.e., IL-18BP).
30 Contemplated in the present inventions are IL-18BP antagonists that include (but are not limited to) monoclonal antibodies, small molecular therapeutics that neutralize IL-18BP,

and an engineered IL-18 variant that binds to IL-18BP but does not substantially bind to or interact with the IL-18R by inhibiting IL-18BP in this manner, the activity of endogenously-produced IL-18 is enhanced through disinhibition.

The skilled artisan will also appreciate, once armed with the teachings of the present invention, that an increase in the level of IL-18 signaling includes an increase in IL-18 level or IL-18 activity (e.g., receptor binding activity, receptor signaling activity, etc.). Thus, increasing the level or activity of IL-18 signaling includes, but is not limited to, increasing the amount of available IL-18 polypeptide or IL-18 variant polypeptide, increasing transcription, translation, or both, of a nucleic acid encoding IL-18 polypeptide or an IL-18 variant polypeptide; and it also includes increasing any activity of an IL-18 polypeptide or IL-18 variant polypeptide as well. The activator of IL-18 activity compositions and methods of the invention can selectively activate IL-18 signaling, or can activate both IL-18 signaling and another molecule or pathway. Thus, the present invention relates to administration of an activator of IL-18 activity, a recombinant activator of IL-18 activity polypeptide, an active activator of IL-18 activity polypeptide fragment, or an activator of IL-18 signaling pathway component expression or activity.

Further, one of skill in the art would, when equipped with this disclosure and the methods exemplified herein, appreciate that an activator of IL-18 activity includes such activators as discovered in the future, as can be identified by well-known criteria in the art of pharmacology, such as the physiological results of activation of IL-18 signaling as described in detail herein and/or as known in the art. Therefore, the present invention is not limited in any way to any particular activator of IL-18 activity as exemplified or disclosed herein; rather, the invention encompasses those activators that would be understood by the routineer to be useful as are known in the art and as are discovered in the future.

Further methods of identifying and producing an activator of IL-18 activity are well known to those of ordinary skill in the art, including, but not limited, obtaining an activator from a naturally occurring source (e.g., *Streptomyces* sp., *Pseudomonas* sp., *Stylorella aurantium*, etc.). Alternatively, an activator of IL-18 activity can be synthesized chemically. Further, the routineer would appreciate, based upon the teachings provided herein, that an activator of IL-18 activity can be obtained from a recombinant organism.

Compositions and methods for chemically synthesizing an activator of IL-18 activity, and for obtaining them from natural sources, are well known and are described in the art.

One of skill in the art will appreciate that an activator can be administered as a small molecule chemical, a polypeptide, a nucleic acid construct encoding a polypeptide, or combinations thereof. Numerous vectors and other compositions and methods are well known for administering a polypeptide or a nucleic acid encoding a polypeptide to cells or tissues. Therefore, the invention includes a method of administering a polypeptide or a nucleic acid encoding a polypeptide that is an activator of IL-18 signaling. (Sambrook et al., 2012, *Molecular Cloning: A Laboratory Manual*, Cold Spring Harbor Laboratory, New York; Ausubel et al., 1997, *Current Protocols in Molecular Biology*, John Wiley & Sons, New York).

One of skill in the art will realize that diminishing the amount or activity of a molecule that itself diminishes the amount or activity of IL-18 signaling can serve to increase the amount or activity of IL-18 signaling (e.g., IL-18BP). Antisense oligonucleotides are DNA or RNA molecules that are complementary to some portion of a mRNA molecule. When present in a cell, antisense oligonucleotides hybridize to an existing mRNA molecule and inhibit translation into a gene product. Inhibiting the expression of a gene using an antisense oligonucleotide is well known in the art (Marcus-Sekura, 1988, *Anal. Biochem.* 172:289), as are methods of expressing an antisense oligonucleotide in a cell (Inoue, U.S. Pat. No. 5,190,931). The methods of the invention include the use of an antisense oligonucleotide to diminish the amount of a molecule that causes a decrease in the amount or activity of IL-18 signaling, thereby increasing the amount or activity of IL-18 signaling. Contemplated in the present invention are antisense oligonucleotides that are synthesized and provided to the cell by way of methods well known to those of ordinary skill in the art. As an example, an antisense oligonucleotide can be synthesized to be between about 10 and about 100, more exemplary between about 15 and about 50 nucleotides long. The synthesis of nucleic acid molecules is well known in the art, as is the synthesis of modified antisense oligonucleotides to improve biological activity in comparison to unmodified antisense oligonucleotides (Tullis, 1991, U.S. Pat. No. 5,023,243).

Similarly, the expression of a gene may be inhibited by the hybridization of an antisense molecule to a promoter or other regulatory element of a gene, thereby affecting the transcription of the gene. Methods for the identification of a promoter or other regulatory element that interacts with a gene of interest are well known in the art, and
5 include such methods as the yeast two hybrid system (Bartel and Fields, eds., In: The Yeast Two Hybrid System, Oxford University Press, Cary, N.C.).

Alternatively, inhibition of a gene expressing a protein that diminishes the level or activity of IL-18 signaling can be accomplished through the use of a ribozyme. Using ribozymes for inhibiting gene expression is well known to those of skill in the art (see, e.g.,
10 Cech et al., 1992, J. Biol. Chem. 267:17479; Hampel et al., 1989, Biochemistry 28: 4929; Altman et al., U.S. Pat. No. 5,168,053). Ribozymes are catalytic RNA molecules with the ability to cleave other single-stranded RNA molecules. Ribozymes are known to be sequence specific, and can therefore be modified to recognize a specific nucleotide sequence (Cech, 1988, J. Amer. Med. Assn. 260:3030), allowing the selective cleavage of
15 specific mRNA molecules. Given the nucleotide sequence of the molecule, one of ordinary skill in the art could synthesize an antisense oligonucleotide or ribozyme without undue experimentation, provided with the disclosure and references incorporated herein.

One of skill in the art will appreciate that an activator of IL-18 activity, such as an IL-18 variant polypeptide, or fragment thereof, or a nucleic acid (e.g., DNA, cDNA,
20 mRNA, etc.) encoding an IL-18 variant polypeptide, or fragment thereof, can be administered singly or in any combination thereof.

One of skill in the art will also appreciate administration can be acute (e.g., over a short period of time, such as a day, a week or a month) or chronic (e.g., over a long period of time, such as several months or a year or more). Further, an activator of IL-18 activity,
25 such as an IL-18 variant polypeptide or fragment thereof, or a nucleic acid (e.g., DNA, cDNA, mRNA, etc.) encoding an IL-18 variant polypeptide, or fragment thereof, can be administered singly or in any combination thereof, in a temporal sense, in that they may be administered simultaneously, before, and/or after each other. One of ordinary skill in the art will appreciate, based on the disclosure provided herein, that a activator of IL-18
30 activity, a activator of IL-18 activity polypeptide, a recombinant activator of IL-18 activity polypeptide, or an active activator of IL-18 activity polypeptide fragment can be used alone

or in any combination with another activator of IL-18 activity, activator of IL-18 activity polypeptide, recombinant activator of IL-18 activity polypeptide, or active activator of IL-18 activity polypeptide fragment to effect a therapeutic result.

It will be appreciated by one of skill in the art, when armed with the present disclosure including the methods detailed herein, that the invention is not limited to treatment of a disease or disorder once it is established. Particularly, the symptoms of the disease or disorder need not have manifested to the point of detriment to the subject; indeed, the disease or disorder need not be detected in a subject before treatment is administered. That is, significant pathology from disease or disorder does not have to occur before the present invention may provide benefit. Therefore, the present invention, as described more fully herein, includes a method for preventing diseases and disorders in a subject, in that an activator of IL-18 activity molecule (e.g., polypeptide, peptide, etc.), or an activator of IL-18 activity, as discussed elsewhere herein, can be administered to a subject prior to the onset of the disease or disorder, thereby preventing the disease or disorder from developing.

The invention encompasses administration of an activator of IL-18 activity, an activator of IL-18 activity polypeptide, a recombinant IL-18 signaling polypeptide, or an active IL-18 signaling polypeptide fragment to practice the methods of the invention; the skilled artisan would understand, based on the disclosure provided herein, how to formulate and administer the appropriate activator of IL-18 activity, activator of IL-18 activity polypeptide, recombinant IL-18 signaling polypeptide, or active IL-18 signaling polypeptide fragment to a subject. However, the present invention is not limited to any particular method of administration or treatment regimen. This is especially true where it would be appreciated by one skilled in the art, equipped with the disclosure provided herein, including the reduction to practice using an art-recognized model of disease, that methods of administering a activator of IL-18 activity, IL-18 signaling polypeptide, a recombinant IL-18 signaling polypeptide, or an active IL-18 signaling polypeptide fragment can be determined by one of skill in the pharmacological arts.

In some embodiments, a method comprises administering to a subject in need thereof a composition comprising at least one IL-18 variant polypeptide, and administering to the subject a composition comprising an additional agent. In one such embodiment, the additional agent comprises an immunotherapeutic agent comprising at least one selected

from the group including, but not limited to an altered T-cell, a chimeric antigen receptor T-cell (CAR-T), an armored CAR-T cell, a virus, an antigen, a vaccine, an antibody, an immune checkpoint inhibitor, a small molecule, a chemotherapeutic agent, and a stem cell. In some embodiments, a composition comprising at least one IL-18 variant polypeptide is used in a method to increase immune system activity before, during, or after infection by a bacterium, virus, or other pathogen. In some embodiments, a composition comprising at least one IL-18 variant polypeptide is used in a method to increase the number and/or activity of immune cells in vitro, in vivo or ex vivo, such as the number and/or activity of T cells, NK cells, and/or myeloid cells.

In some embodiments, the additional agent comprises an inhibitor of one or more cytokines. In some embodiments, the inhibitor of one or more cytokines comprises a chemical compound, a protein, a peptide, a peptidomimetic, an antibody, a ribozyme, a small molecule chemical compound, or an antisense nucleic acid molecule (e.g., siRNA, miRNA, etc.) that inhibits the expression, activity, or both of one or more cytokines. In some embodiments, the inhibitor inhibits the expression, activity, or both of IL-17, IL-5, or IL-3. In some embodiments, the administration of a cytokine inhibitor decreases toxicity. In some embodiments, the administration of a cytokine inhibitor increases efficacy of an administered IL-18 variant polypeptide or IL-18BP inhibitor.

As used herein, the term “pharmaceutically-acceptable carrier” means a chemical composition with which an appropriate IL-18 signaling modulator may be combined and which, following the combination, can be used to administer the appropriate IL-18 signaling modulator thereof, to a subject.

Kits

The present invention also pertains to kits useful in the methods of the invention. Such kits comprise various combinations of components useful in any of the methods described elsewhere herein, including for example, an activator of IL-18 activity, such as an IL-18 variant polypeptide, and/or an IL-18BP inhibitor, and/or materials for quantitatively analyzing IL-18 variant polypeptide or IL-18 variant nucleic acid, and/or materials for assessing the activity of an IL-18 variant polypeptide or an IL-18 variant nucleic acid, and/or instructional material. For example, In some embodiments, the kit

comprises components useful for the quantification of IL-18 variant nucleic acid in a biological sample. In another embodiment, the kit comprises components useful for the quantification of IL-18 variant polypeptide in a biological sample. In a further embodiment, the kit comprises components useful for the assessment of the activity (e.g., enzymatic activity, ligand binding activity, etc.) of an IL-18 variant polypeptide in a biological sample.

In a further embodiment, the kit comprises the components of an assay for monitoring the effectiveness of a treatment administered to a subject in need thereof, containing instructional material and the components for determining whether the level of IL-18 signaling in a biological sample obtained from the subject is modulated during or after administration of the treatment. In various embodiments, to determine whether the level of IL-18 signaling is modulated in a biological sample obtained from the subject, the level of IL-18 signaling is compared with the level of at least one comparator control contained in the kit, such as a positive control, a negative control, a historical control, a historical norm, or the level of another reference molecule in the biological sample. In certain embodiments, the ratio of IL-18 signaling and a reference molecule is determined to aid in the monitoring of the treatment.

Pharmaceutical Compositions and Administration

Compositions comprising a polypeptide, a polypeptide fragment, an activator of IL-18 signaling level or activity, or an inhibitor of IL-18BP level or activity, as described elsewhere herein can be formulated and administered to a subject, as now described. By way of non-limiting examples, a composition identified as an activator of IL-18 activity, including IL-18 variant polypeptides, recombinant IL-18 variant polypeptides, and active IL-18 variant polypeptide fragments, for the treatment and/or prevention of a disease or disorder can be formulated and administered to a subject, as now described. By way of more non-limiting examples, a composition identified as a useful IL-18BP inhibitor, including a chemical compound, a protein, a peptide, a peptidomimetic, an antibody, a ribozyme, a small molecule chemical compound, an antisense nucleic acid molecule (e.g., siRNA, miRNA, etc.), for the treatment and/or prevention of a disease or disorder can be formulated and administered to a subject, as now described.

The invention encompasses the preparation and use of pharmaceutical compositions comprising a composition useful for the treatment or prevention of a disease or disorder, disclosed herein as an active ingredient. Such a pharmaceutical composition may consist of the active ingredient alone, in a form suitable for administration to a subject, 5 or the pharmaceutical composition may comprise the active ingredient and one or more pharmaceutically acceptable carriers, one or more additional ingredients, or some combination of these. The active ingredient may be present in the pharmaceutical composition in the form of a physiologically acceptable ester or salt, such as in combination with a physiologically acceptable cation or anion, as is well known in the art. In various 10 embodiments, the active ingredient is a polypeptide, a polypeptide fragment, an activator of IL-18 signaling level or activity, an inhibitor of IL-18BP level or activity, or a combination thereof, as elsewhere described herein.

As used herein, the term “pharmaceutically-acceptable carrier” means a chemical composition with which an appropriate IL-18 signaling modulator thereof, may be 15 combined and which, following the combination, can be used to administer the appropriate modulator (e.g., activator, inhibitor, etc.) thereof, to a subject.

In some embodiments, pharmaceutical compositions can include large, slowly metabolized macromolecules such as proteins, polysaccharides such as chitosan, polylactic acids, polyglycolic acids and copolymers (such as latex functionalized Sepharose™, 20 agarose, cellulose, and the like), polymeric amino acids, amino acid copolymers, and lipid aggregates (such as oil droplets or liposomes).

The pharmaceutical compositions useful for practicing the invention may be administered to deliver a dose of between about 0.1 ng/kg/day and 100 mg/kg/day, or more.

In various embodiments, the pharmaceutical compositions useful in the methods of 25 the invention may be administered, by way of example, systemically, parenterally, or topically, such as, in oral formulations, inhaled formulations, including solid or aerosol, and by topical or other similar formulations. In addition to the appropriate therapeutic composition, such pharmaceutical compositions may contain pharmaceutically acceptable carriers and other ingredients known to enhance and facilitate drug administration. Other 30 possible formulations, such as nanoparticles, liposomes, other preparations containing the

active ingredient, and immunologically based systems may also be used to administer an appropriate modulator thereof, according to the methods of the invention.

5 A carrier may bear a subject agent (e.g., IL-18 variant polypeptide) in a variety of ways, including covalent bonding either directly or via a linker group, and non-covalent associations. Suitable covalent-bond carriers include proteins such as albumins, peptides, and polysaccharides such as aminodextran, each of which have multiple sites for the attachment of moieties. A carrier may also bear a IL-18 variant polypeptide by non-covalent associations, such as non-covalent bonding or by encapsulation. The nature of the carrier can be either soluble or insoluble for purposes of the invention.

10 Acceptable carriers, excipients, or stabilizers are non-toxic to recipients at the dosages and concentrations employed, and include buffers such as phosphate, citrate, and other organic acids; antioxidants including ascorbic acid and methionine; preservatives (such as octadecyldimethylbenzyl ammonium chloride; hexamethonium chloride; benzalkonium chloride, benzethonium chloride; phenol, butyl or benzyl alcohol; alkyl parabens such as methyl or propyl paraben; catechol; resorcinol; cyclohexanol; 3-pentanol; and m-cresol); low molecular weight (less than about 10 residues) polypeptides; proteins, such as serum albumin, gelatin, or immunoglobulins; hydrophilic polymers such as polyvinylpyrrolidone; amino acids such as glycine, glutamine, asparagine, histidine, arginine, or lysine; monosaccharides, disaccharides, and other carbohydrates including
15 glucose, mannose, or dextrans; chelating agents such as EDTA; sugars such as sucrose, mannitol, trehalose or sorbitol; salt-forming counter-ions such as sodium; metal complexes (e.g., Zn-protein complexes); and/or non-ionic surfactants such as TWEEN™, PLURONICS™ or polyethylene glycol (PEG). Formulations to be used for *in vivo* administration must be sterile. This is readily accomplished by filtration through sterile
20 filtration membranes.

The active ingredients may also be entrapped in microcapsule prepared, for example, by coacervation techniques or by interfacial polymerization, for example, hydroxymethylcellulose or gelatin-microcapsule and poly-(methylmethacrylate) microcapsule, respectively, in colloidal drug delivery systems (for example, liposomes,
25 albumin microspheres, microemulsions, nano-particles and nanocapsules) or in

macroemulsions. Such techniques are disclosed in Remington's Pharmaceutical Sciences 16th edition, Osol, A. Ed. (1980).

5 Compositions can be prepared as injectables, either as liquid solutions or suspensions; solid forms suitable for solution in, or suspension in, liquid vehicles prior to injection can also be prepared. The preparation also can be emulsified or encapsulated in liposomes or micro particles such as polylactide, polyglycolide, or copolymer for enhanced adjuvant effect, as discussed above. Langer, Science 249: 1527, 1990 and Hanes, Advanced Drug Delivery Reviews 28: 97-119, 1997. The agents of this invention can be administered in the form of a depot injection or implant preparation which can be formulated in such a
10 manner as to permit a sustained or pulsatile release of the active ingredient. The pharmaceutical compositions are generally formulated as sterile, substantially isotonic and in full compliance with all Good Manufacturing Practice (GMP) regulations of the U.S. Food and Drug Administration.

As used herein, the term "physiologically acceptable" ester or salt means an ester
15 or salt form of the active ingredient which is compatible with any other ingredients of the pharmaceutical composition, which is not deleterious to the subject to which the composition is to be administered.

The formulations of the pharmaceutical compositions described herein may be prepared by any method known or hereafter developed in the art of pharmacology. In
20 general, such preparatory methods include the step of bringing the active ingredient into association with a carrier or one or more other accessory ingredients, and then, if necessary or desirable, shaping or packaging the product into a desired single- or multi-dose unit.

Although the descriptions of pharmaceutical compositions provided herein are principally directed to pharmaceutical compositions which are suitable for ethical
25 administration to humans, it will be understood by the skilled artisan that such compositions are generally suitable for administration to animals of all sorts. Modification of pharmaceutical compositions suitable for administration to humans in order to render the compositions suitable for administration to various animals is well understood, and the ordinarily skilled veterinary pharmacologist can design and perform such modification
30 with merely ordinary, if any, experimentation.

Pharmaceutical compositions that are useful in the methods of the invention may be prepared, packaged, or sold in formulations suitable for oral, rectal, vaginal, parenteral, topical, pulmonary, intranasal, buccal, intravenous, transdermal, intralesional, subcutaneous, intramuscular, ophthalmic, intrathecal and other known routes of administration. Other contemplated formulations include projected nanoparticles, liposomal preparations, other preparations containing the active ingredient, and immunologically-based formulations.

A pharmaceutical composition of the invention may be prepared, packaged, or sold in bulk, as a single unit dose, or as a plurality of single unit doses. As used herein, a “unit dose” is discrete amount of the pharmaceutical composition comprising a predetermined amount of the active ingredient. The amount of the active ingredient is generally equal to the dosage of the active ingredient which would be administered to a subject or a convenient fraction of such a dosage such as, for example, one-half or one-third of such a dosage.

The relative amounts of the active ingredient, the pharmaceutically acceptable carrier, and any additional ingredients in a pharmaceutical composition of the invention will vary, depending upon the identity, size, and condition of the subject treated and further depending upon the route by which the composition is to be administered. By way of example, the composition may comprise between 0.1% and 100% (w/w) active ingredient.

In addition to the active ingredient, a pharmaceutical composition of the invention may further comprise one or more additional pharmaceutically active agents.

Controlled- or sustained-release formulations of a pharmaceutical composition of the invention may be made using conventional technology.

A formulation of a pharmaceutical composition of the invention suitable for oral administration may be prepared, packaged, or sold in the form of a discrete solid dose unit including, but not limited to, a tablet, a hard or soft capsule, a cachet, a troche, or a lozenge, each containing a predetermined amount of the active ingredient. Other formulations suitable for oral administration include, but are not limited to, a powdered or granular formulation, an aqueous or oily suspension, an aqueous or oily solution, or an emulsion.

Pharmaceutically acceptable excipients used in the manufacture of pharmaceutical compositions include, but are not limited to, inert diluents, granulating and disintegrating

agents, binding agents, and lubricating agents. Known dispersing agents include, but are not limited to, potato starch and sodium starch glycolate. Known surface active agents include, but are not limited to, sodium lauryl sulphate. Known diluents include, but are not limited to, calcium carbonate, sodium carbonate, lactose, microcrystalline cellulose, calcium phosphate, calcium hydrogen phosphate, and sodium phosphate. Known granulating and disintegrating agents include, but are not limited to, corn starch and alginic acid. Known binding agents include, but are not limited to, gelatin, acacia, pre-gelatinized maize starch, polyvinylpyrrolidone, and hydroxypropyl methylcellulose. Known lubricating agents include, but are not limited to, magnesium stearate, stearic acid, silica, and talc.

Liquid formulations of a pharmaceutical composition of the invention may be prepared, packaged, and sold either in liquid form or in the form of a dry product intended for reconstitution with water or another suitable vehicle prior to use.

Liquid suspensions may be prepared using conventional methods to achieve suspension of the active ingredient in an aqueous or oily vehicle. Aqueous vehicles include, for example, water and isotonic saline. Oily vehicles include, for example, almond oil, oily esters, ethyl alcohol, vegetable oils such as arachis, olive, sesame, or coconut oil, fractionated vegetable oils, and mineral oils such as liquid paraffin. Liquid suspensions may further comprise one or more additional ingredients including, but not limited to, suspending agents, dispersing or wetting agents, emulsifying agents, demulcents, preservatives, buffers, salts, flavorings, coloring agents, and sweetening agents. Oily suspensions may further comprise a thickening agent.

Known suspending agents include, but are not limited to, sorbitol syrup, hydrogenated edible fats, sodium alginate, polyvinylpyrrolidone, gum tragacanth, gum acacia, and cellulose derivatives such as sodium carboxymethylcellulose, methylcellulose, and hydroxypropylmethylcellulose. Known dispersing or wetting agents include, but are not limited to, naturally-occurring phosphatides such as lecithin, condensation products of an alkylene oxide with a fatty acid, with a long chain aliphatic alcohol, with a partial ester derived from a fatty acid and a hexitol, or with a partial ester derived from a fatty acid and a hexitol anhydride (e.g. polyoxyethylene stearate, heptadecaethyleneoxycetanol, polyoxyethylene sorbitol monooleate, and polyoxyethylene sorbitan monooleate,

respectively). Known emulsifying agents include, but are not limited to, lecithin and acacia. Known preservatives include, but are not limited to, methyl, ethyl, or n-propyl-parahydroxybenzoates, ascorbic acid, and sorbic acid. Known sweetening agents include, for example, glycerol, propylene glycol, sorbitol, sucrose, and saccharin. Known thickening agents for oily suspensions include, for example, beeswax, hard paraffin, and cetyl alcohol.

Liquid solutions of the active ingredient in aqueous or oily solvents may be prepared in substantially the same manner as liquid suspensions, the primary difference being that the active ingredient is dissolved, rather than suspended in the solvent. Liquid solutions of the pharmaceutical composition of the invention may comprise each of the components described with regard to liquid suspensions, it being understood that suspending agents will not necessarily aid dissolution of the active ingredient in the solvent. Aqueous solvents include, for example, water and isotonic saline. Oily solvents include, for example, almond oil, oily esters, ethyl alcohol, vegetable oils such as arachis, olive, sesame, or coconut oil, fractionated vegetable oils, and mineral oils such as liquid paraffin.

Powdered and granular formulations of a pharmaceutical preparation of the invention may be prepared using known methods. Such formulations may be administered directly to a subject, used, for example, to form tablets, to fill capsules, or to prepare an aqueous or oily suspension or solution by addition of an aqueous or oily vehicle thereto. Each of these formulations may further comprise one or more of dispersing or wetting agent, a suspending agent, and a preservative. Additional excipients, such as fillers and sweetening, flavoring, or coloring agents, may also be included in these formulations.

A pharmaceutical composition of the invention may also be prepared, packaged, or sold in the form of oil-in-water emulsion or a water-in-oil emulsion. The oily phase may be a vegetable oil such as olive or arachis oil, a mineral oil such as liquid paraffin, or a combination of these. Such compositions may further comprise one or more emulsifying agents such as naturally occurring gums such as gum acacia or gum tragacanth, naturally-occurring phosphatides such as soybean or lecithin phosphatide, esters or partial esters derived from combinations of fatty acids and hexitol anhydrides such as sorbitan monooleate, and condensation products of such partial esters with ethylene oxide such as

polyoxyethylene sorbitan monooleate. These emulsions may also contain additional ingredients including, for example, sweetening or flavoring agents.

Methods for impregnating or coating a material with a chemical composition are known in the art, and include, but are not limited to methods of depositing or binding a
5 chemical composition onto a surface, methods of incorporating a chemical composition into the structure of a material during the synthesis of the material (i.e., such as with a physiologically degradable material), and methods of absorbing an aqueous or oily solution or suspension into an absorbent material, with or without subsequent drying.

As used herein, "parenteral administration" of a pharmaceutical composition
10 includes any route of administration characterized by physical breaching of a tissue of a subject and administration of the pharmaceutical composition through the breach in the tissue. Parenteral administration thus includes, but is not limited to, administration of a pharmaceutical composition by injection of the composition, by application of the composition through a surgical incision, by application of the composition through a tissue-
15 penetrating non-surgical wound, and the like. In particular, parenteral administration is contemplated to include, but is not limited to, cutaneous, subcutaneous, intraperitoneal, intravenous, intramuscular, intracisternal injection, and kidney dialytic infusion techniques.

Formulations of a pharmaceutical composition suitable for parenteral
20 administration comprise the active ingredient combined with a pharmaceutically acceptable carrier, such as sterile water or sterile isotonic saline. Such formulations may be prepared, packaged, or sold in a form suitable for bolus administration or for continuous administration. Injectable formulations may be prepared, packaged, or sold in unit dosage form, such as in ampules or in multi-dose containers containing a preservative.
25 Formulations for parenteral administration include, but are not limited to, suspensions, solutions, emulsions in oily or aqueous vehicles, pastes, and implantable sustained-release or biodegradable formulations. Such formulations may further comprise one or more additional ingredients including, but not limited to, suspending, stabilizing, or dispersing agents. In some embodiments of a formulation for parenteral administration, the active
30 ingredient is provided in dry (i.e., powder or granular) form for reconstitution with a

suitable vehicle (e.g., sterile pyrogen-free water) prior to parenteral administration of the reconstituted composition.

The pharmaceutical compositions may be prepared, packaged, or sold in the form of a sterile injectable aqueous or oily suspension or solution. This suspension or solution
5 may be formulated according to the known art, and may comprise, in addition to the active ingredient, additional ingredients such as the dispersing agents, wetting agents, or suspending agents described herein. Such sterile injectable formulations may be prepared using a non-toxic parenterally-acceptable diluent or solvent, such as water or 1,3-butane diol, for example. Other acceptable diluents and solvents include, but are not limited to,
10 Ringer's solution, isotonic sodium chloride solution, and fixed oils such as synthetic mono- or di-glycerides. Other parentally-administrable formulations which are useful include those which comprise the active ingredient in microcrystalline form, in a liposomal preparation, or as a component of a biodegradable polymer systems. Compositions for sustained release or implantation may comprise pharmaceutically acceptable polymeric or
15 hydrophobic materials such as an emulsion, an ion exchange resin, a sparingly soluble polymer, or a sparingly soluble salt.

Formulations suitable for topical administration include, but are not limited to, liquid or semi-liquid preparations such as liniments, lotions, oil-in-water or water-in-oil emulsions such as creams, ointments or pastes, and solutions or suspensions. Topically-
20 administrable formulations may, for example, comprise from about 1% to about 10% (w/w) active ingredient, although the concentration of the active ingredient may be as high as the solubility limit of the active ingredient in the solvent. Formulations for topical administration may further comprise one or more of the additional ingredients described herein.

25 A pharmaceutical composition of the invention may be prepared, packaged, or sold in a formulation suitable for pulmonary administration via the buccal cavity. Such a formulation may comprise dry particles which comprise the active ingredient and which have a diameter in the range from about 0.5 to about 7 nanometers, and preferably from about 1 to about 6 nanometers. Such compositions are conveniently in the form of dry
30 powders for administration using a device comprising a dry powder reservoir to which a stream of propellant may be directed to disperse the powder or using a self-propelling

solvent/powder-dispensing container such as a device comprising the active ingredient dissolved or suspended in a low-boiling propellant in a sealed container. Preferably, such powders comprise particles wherein at least 98% of the particles by weight have a diameter greater than 0.5 nanometers and at least 95% of the particles by number have a diameter
5 less than 7 nanometers. More preferably, at least 95% of the particles by weight have a diameter greater than 1 nanometer and at least 90% of the particles by number have a diameter less than 6 nanometers. Dry powder compositions preferably include a solid fine powder diluent such as sugar and are conveniently provided in a unit dose form.

Low boiling propellants generally include liquid propellants having a boiling point
10 of below 65 °F at atmospheric pressure. Generally the propellant may constitute 50 to 99.9% (w/w) of the composition, and the active ingredient may constitute 0.1 to 20% (w/w) of the composition. The propellant may further comprise additional ingredients such as a liquid non-ionic or solid anionic surfactant or a solid diluent (preferably having a particle size of the same order as particles comprising the active ingredient).

15 Pharmaceutical compositions of the invention formulated for pulmonary delivery may also provide the active ingredient in the form of droplets of a solution or suspension. Such formulations may be prepared, packaged, or sold as aqueous or dilute alcoholic solutions or suspensions, optionally sterile, comprising the active ingredient, and may conveniently be administered using any nebulization or atomization device. Such
20 formulations may further comprise one or more additional ingredients including, but not limited to, a flavoring agent such as saccharin sodium, a volatile oil, a buffering agent, a surface active agent, or a preservative such as methylhydroxybenzoate. The droplets provided by this route of administration preferably have an average diameter in the range from about 0.1 to about 200 nanometers. The formulations described herein as being useful
25 for pulmonary delivery are also useful for intranasal delivery of a pharmaceutical composition of the invention. Another formulation suitable for intranasal administration is a coarse powder comprising the active ingredient and having an average particle from about 0.2 to 500 micrometers.

Such a formulation is administered in the manner in which snuff is taken i.e. by
30 rapid inhalation through the nasal passage from a container of the powder held close to the nares. Formulations suitable for nasal administration may, for example, comprise from

about as little as 0.1% (w/w) and as much as 100% (w/w) of the active ingredient, and may further comprise one or more of the additional ingredients described herein.

A pharmaceutical composition of the invention may be prepared, packaged, or sold in a formulation suitable for buccal administration. Such formulations may, for example, be in the form of tablets or lozenges made using conventional methods, and may, for example, contain 0.1 to 20% (w/w) active ingredient, the balance comprising an orally dissolvable or degradable composition and, optionally, one or more of the additional ingredients described herein. Alternately, formulations suitable for buccal administration may comprise a powder or an aerosolized or atomized solution or suspension comprising the active ingredient. Such powdered, aerosolized, or aerosolized formulations, when dispersed, preferably have an average particle or droplet size in the range from about 0.1 to about 200 nanometers, and may further comprise one or more of the additional ingredients described herein.

A pharmaceutical composition of the invention may be prepared, packaged, or sold in a formulation suitable for ophthalmic administration. Such formulations may, for example, be in the form of eye drops including, for example, a 0.1-1.0% (w/w) solution or suspension of the active ingredient in an aqueous or oily liquid carrier. Such drops may further comprise buffering agents, salts, or one or more other of the additional ingredients described herein. Other ophthalmically-administrable formulations which are useful include those which comprise the active ingredient in microcrystalline form or in a liposomal preparation.

As used herein, "additional ingredients" include, but are not limited to, one or more of the following: excipients; surface active agents; dispersing agents; inert diluents; granulating and disintegrating agents; binding agents; lubricating agents; sweetening agents; flavoring agents; coloring agents; preservatives; physiologically degradable compositions such as gelatin; aqueous vehicles and solvents; oily vehicles and solvents; suspending agents; dispersing or wetting agents; emulsifying agents, demulcents; buffers; salts; thickening agents; fillers; emulsifying agents; antioxidants; antibiotics; antifungal agents; stabilizing agents; and pharmaceutically acceptable polymeric or hydrophobic materials. Other "additional ingredients" which may be included in the pharmaceutical compositions of the invention are known in the art and described, for example in Genaro,

ed., 1985, Remington's Pharmaceutical Sciences, Mack Publishing Co., Easton, Pa., which is incorporated herein by reference.

Typically dosages of the compound of the invention which may be administered to an animal, preferably a human, range in amount from about 0.001 mg to about 1000 mg per kilogram of body weight of the animal. The precise dosage administered will vary depending upon any number of factors, including, but not limited to, the type of animal and type of disease or disorder being treated, the age of the animal and the route of administration. Preferably, the dosage of the compound will vary from about 0.1 mg to about 10 mg per kilogram of body weight of the animal. The compound can be administered to an animal as frequently as several times daily, or it can be administered less frequently, such as once a day, once a week, once every two weeks, once a month, or even less frequently, such as once every several months or even once a year or less. The frequency of the dose will be readily apparent to the skilled artisan and will depend upon any number of factors, such as, but not limited to, the type and severity of the disease or disorder being treated, the type and age of the animal, etc.

EXPERIMENTAL EXAMPLES

The invention is further described in detail by reference to the following experimental examples. These examples are provided for purposes of illustration only, and are not intended to be limiting unless otherwise specified. Thus, the invention should in no way be construed as being limited to the following examples, but rather should be construed to encompass any and all variations which become evident as a result of the teaching provided herein.

Without further description, it is believed that one of ordinary skill in the art can, using the preceding description and the following illustrative examples, make and utilize the present invention and practice the claimed methods. The following working examples therefore are not to be construed as limiting in any way the remainder of the disclosure.

Example 1: IL-18 variant polypeptides

IL-18 is a pro-inflammatory cytokine that can stimulate T, NK, and myeloid cells. It has been proposed as an immunotherapeutic agent for cancer given its ability to stimulate

anti-tumor immune cells. As demonstrated herein, the therapeutic efficacy of recombinant IL-18 treatment is greatly limited by upregulation of its natural endogenous soluble inhibitor IL-18BP. The present invention is based, in part, on the development of variants of both human and mouse IL-18 that are almost entirely independent of IL-18BP. The cytokine variants exhibit altered relative preference for the receptors (IL-18R α and IL-18BP) by hundreds of thousands to over a million-fold. These variants have potent anti-tumor activity in preclinical tumor models, both as monotherapies and in combination with immune checkpoint inhibitors such as anti-PD-1. As an additional application, IL-18 also has a well-established anti-obesity role and it is demonstrated herein that administration of the variants greatly reduces body fat composition compared to WT IL-18 treatment. The new variants thus have indications in endocrinology/metabolism/obesity in addition to tumor immunotherapies.

Also described herein are an additional set of IL-18 variants that act as IL-18BP antagonists by exclusively binding IL-18BP with absent or greatly reduced binding IL-18R α . It is envisaged that these proteins could be used to enhance the activity of endogenous IL-18 by neutralizing IL-18BP.

The materials and methods employed in these experiments are now described.

Protein expression and purification

Human IL-18, mouse IL-18 (amino acids 1–157) and variants thereof, were assembled as gBlocks (Integrated DNA Technologies, IDT) and cloned into a pET28a-smt vector for expression of N-terminal sumo-tagged and C-terminal hexahistidine-tagged proteins in *E. coli* BL21 (DE3) Rosetta strain. Protein expression was induced with 0.5 mM IPTG at 16°C for 20 hours. The fusion proteins were first purified using Ni-chelating resins, followed by cleavage of the sumo tag with sumo protease. Proteins were then separated from aggregates by successive ammonium sulfate cuts, with aggregates precipitating at 20% ammonium sulfate and the target proteins at 70% ammonium sulfate. Protein pellets were resuspended and applied to Ni-chelating resins again to remove sumo tags, and were subjected to an endotoxin removal wash with 0.1% Triton X-114. Finally, eluted protein was buffer exchanged to PBS by PD-10 column (GE Healthcare). Protein

sample was tested for monodispersity by size exclusion chromatography using an FPLC (Bio-Rad) and SEC650 column (Bio-Rad).

Human IL-18R α ectodomain (amino acids 19–329), IL-18R β ectodomain (amino acids 15–356), and IL-18BP (amino acids 31–194), were secreted and purified via a baculovirus expression system. In brief, all construct sequences were cloned into the pAcBN-BH3 vector (BD Biosciences) with an N-terminal gp67 signal peptide and a C-terminal AviTagTM and hexahistidine tag. *Spodoptera frugiperda* (Sf9) insect cells cultured at 27°C in SF900 II SFM medium (Invitrogen) were transfected with the plasmid constructs to establish high-titer recombinant virus, which was subsequently amplified. *Trichopulsia ni* (High-Five) insect cells (Invitrogen) grown in Insect Xpress medium (Lonza) at 27°C were infected with the viruses to express recombinant protein. Three days after infection, proteins were extracted via Ni-NTA (QIAGEN) affinity chromatography, concentrated, and purified to >98% homogeneity with SEC650 sizing column (Bio-Rad) equilibrated in 10 mM HEPES (pH 7.5) and 150 mM NaCl.

Mouse IL-18R α ectodomain (amino acids 19–329) and IL-18BP (amino acids 31–194) were produced as secreted proteins using the Expi293 expression system (Thermo Fisher). In brief, all construct sequences were cloned into the BacMam expression vector pEZT_D_Lux with an N-terminal H7 signal peptide and a C-terminal AviTagTM and hexahistidine tag. Expi293 cells cultured at 37°C in Expi293 expression medium (Thermo Fisher) were transfected with plasmids using the ExpiFectamine 293 Transfection Kit (Thermo Fisher) according to the manufacturer's instructions. Cells were harvested 3–5 days after transfection. Protein purification procedures were the same as with the human proteins.

For protein biotinylation, a C-terminal biotin acceptor peptide (AviTag)-GLNDIFEAQKIEWHE was fused to all IL-18 receptor constructs. Protein biotinylation was carried out with soluble BirA ligase enzyme in 0.1 mM Bicine (pH 8.3), 10 mM ATP, 10 mM magnesium acetate, and 0.5 mM biotin (Sigma). Proteins were purified by size exclusion on a SEC650 column, as described above.

Yeast display of IL-18

Human and mouse IL-18 gene block (IDT) were synthesized and cloned into the vector pYAL and displayed on the *Saccharomyces cerevisiae* strain EBY100. Individual colonies of IL-18 yeast were grown overnight at 30 °C in SDCAA liquid media and induced in SGCAA liquid media for 1 day at 20 °C. IL-18 display levels on yeast were verified by
 5 flow cytometry using an anti-cMyc tag antibody (anti-myc-PE; Cell Signaling Technologies). Receptor staining with biotinylated IL-18R α (with or without IL-18R β) or biotinylated IL-18BP was performed in PBS supplemented with 0.5% BSA and 2 mM EDTA (PBE) on ice. All analysis was performed on a Sony SA3800 flow cytometer.

10 Human IL-18 library construction and selection

For the first human decoy-resistant IL-18 library, fourteen hIL-18R α and hIL-18BP contact residues in hIL-18 (Table 1) were identified from homologous positions by aligning the structure of hIL-18/hIL-18R α /hIL-18R β complex (Protein Data Bank (PDB ID) code 3OW4) to the structure of IL-18/IL-18BP (PDB ID 3F62). A library randomizing these
 15 residues was constructed using assembly PCR with the degenerate primers listed in Table 2. The library had a theoretical diversity of $\sim 1.96 \times 10^{11}$ unique protein sequences. The PCR products were further amplified with primers having homology to the pYAL vector and co-electroporated together with linearized pYAL into EBY100 yeast. The resulting library contained 2.5×10^8 transformants.

20 For the second V2.0 human decoy-resistant IL-18 library, eleven hIL-18R α and hIL-18BP contact residues in hIL-18 were selected to randomize, with a theoretical diversity of 3.44×10^9 variants (described in Figure 7A). A library randomizing these residues was constructed using assembly PCR with the degenerate primers and co-electroporated with pYAL into EBY100 yeast. The resulting library had a diversity of $6 \times$
 25 10^8 transformants.

Table 1: First Human IL-18 library design

Residue	Codon	Potential residues
1Y	YNT	Y, F, S, C, L, P, H, R
L5	NWT	L, F, I, Y, H, N, V, D
8K	MRA	K, R, R, Q

51M	RNS	M, I, T, N, K, S, R, V, A, D, E, G
53K	ARA	K, R
55S	RRW	S, R, G, G, N, K, D, E
59G	RNA	G, E, A, V, I, T, K, R
60M	VDG	M, K, R, L, Q, R, V, E, G
103Q	VAW	Q, K, E, D, N, H
105S	RRW	S, K, R, N, D, E, G, G
110D	VAW	D, E, K, N, Q, H
111N	NAT	N, D, H, Y
153V	RHT	V, A, D, I, T, N
155N	VAW	N, K, D, E, Q, H

Table 2: First human IL-18 library assembly primers

Primer	Sequence (5' to 3')
hIL18Lib1	CATTTTCATTAAGATGCAGTTACTTCGCTGTTTTTCAAT ATTTTCTGTTATTGCTAGC (SEQ ID NO: 1)
hIL18Lib2	AATTACGGATGACCGAAAGTYKGGATTCAWNCTTGCC GAAANRTGCTAAAACGCTAGCAATAACAGAAAATATT GAAAAA (SEQ ID NO: 2)
hIL18Lib3	ACTTTCGGTCATCCGTAATTTGAACGACCAAGTCCTTTT TATTGACCAGGG (SEQ ID NO: 3)
hIL18Lib4	ACTATCCGTCATATCCTCGAATAAGGGACGATTGCCCT GGTCAATAAAAAGGACT (SEQ ID NO: 4)
hIL18Lib5	CTTATTCGAGGATATGACGGATAGTGATTGCCGTGACA ACGCCC (SEQ ID NO: 5)
hIL18Lib6	ACTGAGATTGTTACCGCCHBTNYACGGGGTTGWYYATC TYTATASNYAGAGATGATGAAAATTGTACGAGGGGCGT TGTCACGG (SEQ ID NO: 6)
hIL18Lib7	GGCGGTAACAATCTCAGTTAAGTGCGAAAAAATCTCGA CACTTTCTTGTGAA (SEQ ID NO: 7)

hIL18Lib8	GGTTCATTTCTTGAACGAAATGATCTTGTTTTTCACAAG AAAGTGTCGAGATT (SEQ ID NO: 8)
hIL18Lib9	CATTTTCGTTCAAGGAAATGAACCCGCCGGATAATATCA AGGATACAAAATCAGATATTATTT (SEQ ID NO: 9)
hIL18Lib10	TGATGAGCTCTCGAATTGCATCTTATNWTBGTGTCCAG GCACWYYACGWTBGAAGAAAATAATATCTGATTTTGT ATCCTTGATATTA (SEQ ID NO: 10)
hIL18Lib11	ATAAGATGCAATTCGAGAGCTCATCATACGAAGGTTAC TTTTTAGCCTGCG (SEQ ID NO: 11)
hIL18Lib12	AATTAACCTTAAACAGGTCGCGCTCCTTCTCGCAGGCTA AAAAGTAACCTT (SEQ ID NO: 12)
hIL18Lib13	GCGACCTGTTTAAGTTAATTCTTAAGAAAGAAGATGAG TTGGGGGATCG (SEQ ID NO: 13)
hIL18Lib14	CCAGAACCACCGTCCTCWTBCTGADYGGTAAACATGAT GCTACGATCCCCCAACTCATCTT (SEQ ID NO: 14)
hIL18Lib15	GAGGACGGTGGTTCTGGATCCGAACAAAAGCTTATCTC CGAAGAAGACTTGG (SEQ ID NO: 15)
hIL18Lib16	CCACCAGATCCACCACCACCAAGTCTTCTTCGGAGAT AAG (SEQ ID NO: 16)

For both libraries, transformed yeast were recovered and expanded in liquid synthetic dextrose medium with casamino acids (SDCAA) medium at 30 °C and induced by dilution 1:10 into liquid synthetic galactose medium with casamino acids (SGCAA) medium and cultured at 20 °C for 24 hours. Appropriate numbers of induced yeast were used in each round to ensure at least 10-fold coverage of the expected diversity of the library at each step, and not less than 10⁸ cells. All selection steps were carried out at 4 °C using PBE buffer (PBS with 0.5% BSA and 2 mM EDTA). For the first generation library, each round's selection reagents are listed in Table 3. For round 1, yeast were counter-selected with anti-Cy5/AlexaFluor 647 microbeads (Miltenyi) and an LS MACS column (Miltenyi) to remove non-specific bead binders. Positive selection was performed by labeling yeast with 1 μM biotinylated hIL-18Rα for 1 hour at 4 °C, followed by magnetic

selection with SA/AlexaFluor 647 microbeads and an LS MACS column. For round 2, counter-selection was performed with 1 μ M biotinylated IL-18BP, with positive selection identical to round 1. For rounds 3-5, selection was performed by incubating yeast with 100 nM (rounds 3-4) or 10 nM (round 5) biotinylated IL-18R α and 250 nM pre-formed, biotin-capped hIL-18BP/SA-PE tetramers. After competition binding, yeast were washed and labeled with SA AlexaFluor 647 to detect IL-18R α . Display levels were determined by staining with AlexaFluor 488-conjugated anti-cMyc (Cell Signaling Technologies), and the top 1% of display-normalized IL-18R α binders (out of IL-18BP non-binders) were isolated using FACS with a Sony SA3800 cell sorter. After each round of selection, recovered yeast were expanded in SDCAA medium at 30 °C overnight and later induced at 20 °C by a 1:10 dilution into SGCAA medium for 24 hours.

The V2.0 human DR-IL-18 library was selected in a similar fashion, with specific selection steps elaborated in Figure 7B.

15 Mouse IL-18 library construction and selection

Construction and selection procedures are similar to human IL-18, with the following changes. Library construction was informed by an in-silico modeled mouse IL-18/receptor complex structure (predicted by Phyer2.0). Thirteen positions were chosen for randomization (Table 3) using primers described in Table 4. Co-electroporation with pYAL yielded a library of 4×10^8 transformants. Selection reagents used for each round are listed in Table 5.

Table 3: Mouse IL-18 library design

Residue	Codon	Potential residues
1N	NWT	F, Y, L, H, I, N, V, D
50M	RNS	M, I, T, N, K, S, R, V, A, D, E, G
51Y	NRN	Y, K, R, D, E
52K	VNS	L, P, H, Q, R, I, M, T, N, K, S, V, A, D, E, G
54S	RRW	S, R, G, G, N, K, D, E
55E	VRN	E, K, N, R, S, R, H
56V	VNV	V, S, P, T, A, K, R
57R	RVW	R, D, E, S, T
58G	RNA	G, E, A, V, I, T, K, R
59L	VDR	L, K, R, Q, R, V, E, G

104R	NDH	R, D, E, N, Y, F, I, L, V
109N	NAT	N, D, H, Y
151L	VHY	L, V, A, D, I, T, N

Table 4: Mouse IL-18 library assembly primers

Primer	Sequence (5' to 3')
mIL18lib1	CATTTTCATTAAGATGCAGTTACTTCGCTGTTTTTCAATATTTT CTGTTATTGCTAGCGTTT (SEQ ID NO: 17)
mIL18lib2	TTGTACAGTGAAGTCGGCCAAA WNTGCTAAAACGCTAGCAA TAACAGAAAATAT (SEQ ID NO: 18)
mIL18lib3	GCCGACTTCACTGTACAACCGCAGTAATACGGAATATAAATG ACCAAGTTCTCTTCGTT (SEQ ID NO: 19)
mIL18lib4	TTGATCAATATCAGTCATATCCTCGAACACAGGCTGTCTTTTGT CAACGAAGAGAACTTGGTCATTT (SEQ ID NO: 20)
mIL18lib5	GTGTTGAGGATATGACTGATATTGATCAAAGTGCCAGTGAAC CCCAGACCAGA (SEQ ID NO: 21)
mIL18lib6	TCACAGAGAGGGTCACAGCYHBTNYWBYBNBNYBWYYGTCS NBNYNSNYGTATATTATCAGTCTGGTCTGGGGTTCAC (SEQ ID NO: 22)
mIL18lib7	GCTGTGACCCTCTCTGTGAAGGATAGTAAAATGTCTACCCTCT CCTGTAAGAACAAGA (SEQ ID NO: 23)
mIL18lib8	GTATATCATCAATATTTTCAGGTGGATCCATTTCTCAAAGGA AATGATCTTGTTCTTACAGGAGAGGG (SEQ ID NO: 24)
mIL18lib9	AATGGATCCACCTGAAAATATTGATGATATACAAAGTGATCTC ATATTCTTTCAGAAANDHGTTCAGGACACNATAAGATGGAG TTTGAATCTTCACT (SEQ ID NO: 25)
mIL18lib10	CCTTTTGGCAAGCAAGAAAGTGTCTTCATACAGTGAAGATTC AAACTCCATCTTAT (SEQ ID NO: 26)
mIL18lib11	CTTCTTGCTTGCCAAAAGGAAGATGATGCTTTCAAATCATT CTGAAAAAAAAGGATGA (SEQ ID NO: 27)

mIL18lib12	CCACCACTTTGATGTAAGTTAGTRDBAGTGAACATTACAGATT TATCCCCATTTTCATCCTTTTTTTTCAGAATGAG (SEQ ID NO: 28)
mIL18lib13	ACTAACTTACATCAAAGTGGTGGTTCTGGATCCGAACAAAAGC TTATCTCCGAAGAAGA (SEQ ID NO: 29)

Table 5: Summary of library selection reagents

	Human IL-18 library selection		Mouse IL-18 library selection	
	Counter-selection	Positive Selection	Counter-selection	Positive Selection
Round1	SA-beads alone	1 μ M hIL-18R α -SA-beads	-----	1000nM IL-18R α -SA-beads
Round2	1 μ M IL-18BP	1 μ M IL-18R α -SA-beads	-----	1 μ M IL-18R α
Round3	1 μ M IL-18BP	100nM hIL-18R α	1 μ M IL-18BP	1 μ M IL-18R α
Round4	1 μ M IL-18BP	10nM hIL-18R α	1 μ M IL-18BP	100 nM IL-18R α
Round5	250nM IL-18BP tetramer	10nM hIL-18R α	1 μ M IL-18BP	10 nM IL-18R α
Round6	-----	-----	250nM IL-18BP tetramer	200 nM IL-18R α

Surface Plasmon Resonance

- 5 Experiments were conducted using a Biacore T100 and carried out at 25 °C. Biotinylated IL-18R α or IL-18BP were immobilized onto a Biacore biotin capture chip (Series S CAP sensor chip, GE Healthcare) to yield an R_{max} of ~50 RU (IL-18R α) or ~10 RU (IL-18BP). Measurements were made with serial dilutions of the IL-18 variants in HEPES buffered Saline-P⁺ buffer (10 mM HEPES pH 7.4, 150 mM NaCl, 0.005% surfactant P20). The surface was regenerated by three 60-sec injections of regeneration buffer (3/4 (v/v) 8M guanidine hydrochloride with 1/4 (v/v) 1M sodium hydroxide). Experiments were performed in multiple channels simultaneously for increased observations. All data were analyzed with the Biacore T100 evaluation software version 2.0 with a 1:1 Langmuir binding model.
- 10

Cell lines

HEK-Blue IL-18 sensor cells (InvivoGen) were maintained in complete media (DMEM containing 10% heat-inactivated FBS, 2 mM L-glutamine, 50 U/mL penicillin, and 50 µg/mL streptomycin) supplemented with 100 µg/mL Normocin, 30 µg/mL Blasticidin, 180 µg/mL Zeocin, and 200 µg/mL Hygromycin. YUMMER1.7 melanoma cells were cultured and prepared as previously described (Wang et al., 2017, Pigment Cell Melanoma Res., 30(4):428-435).

HEK-Blue cytokine activity assay

For cytokine activity measurements, 50,000 HEK-Blue IL-18 sensor cells per well of a flat-bottom 96-well plate were incubated with recombinant human IL-18 at successively decreasing concentrations in a total volume of 200 µL of complete media. After 20 – 24 hours of incubation at 37 °C and 5% CO₂, 30 µL of cell culture supernatant was mixed with 170 µL QUANTI-Blue detection media (InvivoGen) and incubated at 37 °C and 5% CO₂ until a color change from pink to blue was detectable (0.5 - 4 hours). Levels of alkaline phosphatase were quantified using a spectrophotometer at 655 nm wavelength. Cytokine activity was determined by calculating the relative absorbance value (percentage of the maximal absorbance value measured at 655 nm) for each cytokine in the assay.

For IL-18BP blockade experiments, a fixed concentration of recombinant human IL-18 was pre-incubated with recombinant human IL-18BP at successively decreasing concentrations for 1 hour at 4 °C. Subsequently, the protein mixture was added to the HEK-Blue IL-18 sensor cells and the assay was performed as described.

25

Mice

C57BL/6 wild type mice (6-9 weeks old) from Jackson Laboratory were used for in vivo mouse experiments. Experimental groups were matched by weight, sex, and age. All animal experiments were conducted in compliance with approval from the Yale Institutional Animal Care and Use Committee.

30

In vivo pharmacodynamic and pharmacokinetic studies

Mice (n=9 per group) received daily intraperitoneal (i.p.) injections of 1 mg/kg recombinant IL-18 (WT or variant mCS2), or PBS as vehicle control. On day 1, day 4, and day 7 of the experiment, 3 mice per group were sacrificed 5 hours post-injection for blood collection via cardiac puncture, and subsequent analysis of blood plasma or white blood cells (see mouse IL-18BP ELISA, Luminex-based multiplex immunoassay for mouse cytokine analysis, as well as immunophenotyping via flow cytometry) was performed. Throughout the 7 days of the experiment, body temperatures were monitored daily using the Rodent thermometer BIO-TK8851 (Bioseb) and the RET 3 rectal probe for mice (Braintree Scientific Inc.). Body weights were monitored daily.

Plasma preparation from whole blood

Plasma preparation from whole blood was performed using EDTA-coated Microtainer Plasma Separator Tubes (BD) according to manufacturer's instruction. Plasma samples were frozen once at -20 °C before being used for analytical assays.

IFN- γ and IL-18BP ELISA

To measure levels of human IFN- γ in cell culture supernatant, the Human IFN- γ ELISA MAX Deluxe Set (BioLegend) with a sensitivity of 4 pg/mL and a detection range of 7.8 – 500 pg/mL was used according to the manufacturer's instructions. For quantification of human IL-18BP in cell culture supernatant, the Quantikine Human IL-18BP Immunoassay (R&D Systems) with a sensitivity of 7.52 pg/mL and a detection range of 26.6 – 1,700 pg/mL was used. Mouse IL-18BP levels in blood plasma were quantified using the Mouse IL-18BP ELISA Kit (R&D systems) with a sensitivity of 0.156 ng/mL and a detection range of 0.156 – 10 ng/mL. All assays including sample preparation were performed according to manufacturer's instructions.

Luminex-based multiplex immunoassay for mouse cytokine analysis

To quantify a variety of mouse cytokine levels in blood plasma including IFN- γ and IL-12, the luminex-based Bio-Plex Pro multiplex immunoassay (Bio-Rad) was performed using the Bio-Plex 200 System (Bio-Rad). Cytokines of interest were analyzed

using the Bio-Plex Pro Mouse Cytokine Standard 23-Plex (Group I) reconstituted in DMEM, following the manufacturer's instructions.

Immunophenotyping via flow cytometry

5 For white blood cell analysis, 100 μ L of whole blood were collected into an EDTA-coated Microtainer Plasma Separator Tube (BD) additionally containing 50 μ L Heparin-solution, and mixed by inverting several times. Red blood cell lysis was performed by adding ACK Lysing Buffer (VWR) and incubating for 3 - 5 minutes at room temperature. After adding MACS buffer (2 mM EDTA, 2% FBS, in PBS), white blood cells were
10 collected by centrifugation (5 minutes, 400 $\times$ g, 4 $^{\circ}$ C) and aspiration of the supernatant. White blood cells were washed once with cold MACS buffer, and collected again as described. The cell pellet was resuspended in 200 μ L MACS buffer containing 10% (v/v) rat serum (STEMCELL Technologies Inc.) and specific fluorescently-labeled antibodies to stain for subsequent flow cytometric analysis. Staining was performed for 30 minutes at
15 4 $^{\circ}$ C using the following antibodies: α CD4-AF700 (BioLegend), α CD8-APC (BioLegend), B220-APC-Cy7 (BioLegend), CD11b-PB (BioLegend), NK1.1-PE (BioLegend), NKp46-PE (BioLegend), and CD69-FITC (BioLegend). Thereafter, white blood cells were washed twice with MACS buffer as described before. Finally, the cells were resuspended in 100 μ L MACS buffer and samples were acquired using the flow cytometer (Sony SA3800). An
20 aliquot of 10 μ L was taken to perform cell counting using the Invitrogen Countess II Automated Cell Counter (Thermo Fisher Scientific). FlowJo v10.3 software was used for data analysis, and cells were gated for leukocytes and single events using the forward and side scatter.

25 Tumor treatment experiments

0.5 $\times$ 10⁶ YUMMER1.7 cells were implanted subcutaneously into C57BL/6J mice. 7 days after implantation, when tumors were approximately 50 mg, treatment was initiated. Mice were divided into treatment cohorts which included: 1) vehicle (saline), 2) anti-PD1 (rat clone RMP1-14, Bio X Cell, West Lebanon, New Hampshire, US), 3) wildtype IL-18,
30 4) mCS2, 5) wild type IL-18 + anti-PD-1, and 6) CS2 IL-18 + anti-PD1. Anti PD-1, wild type IL-18, and mCS2 IL-18 were administered via intraperitoneal injection twice weekly

at 8 mg/kg, 0.32 mg/kg, and 0.32 mg/kg, respectively. Mice were monitored for signs of clinical toxicity, and tumor growth was tracked twice weekly using caliper measurements. Mice were euthanized when the tumor diameter reached or exceeded 1.5 cm in greatest dimension; this was considered the endpoint for survival analyses.

- 5 B2m-deficient YUMMER1.7 studies were conducted in a similar fashion, with the minor changes. 1.0×10^6 cells were engrafted, as tumors grew slower than the parental strain. Treatments consisted of saline, anti-PD1 plus anti-CTLA4, and mCS2 given at the same schedule and dose as the studies above.

The results of the experiments are now described.

10

The IL-18 axis as a target for cancer immunotherapy

- To identify potential signaling nodes for immunotherapeutic intervention, single cell RNAseq data from tumor infiltrating lymphocytes was analyzed for the expression of cytokine pathway components (Singer et al., 2016, Cell, 166:1500-1511, e1509). As seen
15 in Figure 1A, the receptor subunits for IL-18—IL-18R α (i.e., IL-18R1) and IL-18R β (i.e., IL-18RAP)—as well as IL-18 itself were upregulated in both activated and dysfunctional lymphocyte programs. Further analysis of the Immunological Genome (ImmGen) database revealed that expression of both IL-18 receptor subunits correlated with expression of T cell “exhaustion” markers in CD4 and CD8 cells including PD-1, Tim3, Lag3, and TIGIT
20 following chronic antigen exposure (Figure 1B). These expression features suggested that the IL-18 pathway could be used to selectively stimulate activated and dysfunctional/exhausted T cells within tumors as an immunotherapeutic paradigm.

- IL-18 is a Th1 cytokine initially termed “interferon-gamma-inducing-factor” (IGIF) for its ability to robustly stimulate release of interferon gamma (IFN- γ) by T and
25 NK cells. Feedback inhibition of IL-18 is achieved by IFN- γ -driven induction of IL-18BP, a high-affinity secreted decoy receptor for IL-18 that sterically hinders IL-18’s ability to bind and activate its receptor (Figure 2A). Without wishing to be bound by any particular theory, this mechanism is reminiscent of the induction of PD-L1 by IFN- γ , suggesting that IL-18BP may act as a “soluble immune checkpoint.” Consistent with this hypothesis, it
30 was found that IL-18BP is upregulated in several types of cancer, most notably breast, gastric, and brain cancer in the TCGA and Oncomine databases (Figure 2B). Furthermore,

IL-18BP expression strongly correlates with expression of the crucial immune checkpoint PD-1 in tumors ($r=0.65$ and 0.78 in gastric and breast cancer respectively, Figure 2C), suggesting that IL-18BP may also contribute to tumor immune evasion and lymphocyte exhaustion.

5 Recombinant IL-18 has been administered to cancer patients in multiple clinical trials. It was found to be well-tolerated even at high doses of 2 mg/kg, with robust pharmacodynamics outputs including expansion of activated CD69⁺ natural killer (NK) cells and dramatic increases in serum IFN- γ levels. However, a phase II trial of melanoma patients was discontinued due to lack of efficacy. Examination of the reported
10 pharmacodynamics results from these clinical trials reveals that the effectiveness of rIL-18 wanes with repeated dosing, with tachyphylaxis seen with respect to peripheral NK cell activation/expansion and cytokine release (including IFN- γ and GM-CSF). The waning effectiveness of rIL-18 coincides with a profound increase in the serum levels of IL-18BP, more than two orders of magnitude over pre-treatment levels and often exceeding 100
15 ng/mL. Without wishing to be bound by any particular theory, it was hypothesized that IL-18BP limits the effectiveness of rIL-18 therapy and that IL-18 variants that are impervious to IL-18BP inhibition could be effective tumor immunotherapies. Additionally, inhibitors of IL-18BP will likely be effective for tumor immunotherapy.

20 Engineering IL-18 variants that are resistant to IL-18BP inhibition (human DR-IL-18 variants)

To obtain variants of IL-18 that can signal through IL-18R α /IL-18R β , but are impervious to inhibition by IL-18BP, directed evolution with yeast surface display was utilized. The structure of the ternary signaling complex of human IL-18:IL-18R α :IL-18R β
25 (PDB = 3OW4) was first analyzed, and residues of IL-18 that have a shared interface with the signaling complex and IL-18BP were identified (Figure 3A). As the structure of hIL-18:hIL-18BP has not been determined, a related complex between IL-18 and a viral (ectromelia virus) orthologue of IL-18BP was utilized (PDB = 3F62). A combinatorial library randomizing this set of residues to a defined set of alternatives (see Table 1) was
30 created using degenerate oligonucleotide primers and assembly PCR. This library was electroporated into yeast together with the N-terminal yeast display vector pYAL to obtain

a library with 2.5×10^8 transformants. Using this library, directed evolution was performed by conducting successive rounds of selection using magnetic and fluorescent cell sorting (FACS) with recombinant hIL-18R α and counterselection with hIL-18BP, as summarized in Figure 3B. After five rounds of selection, the clear majority of the library clones had completely swapped their relative preference for hIL-18BP and hIL-18R α as compared to WT hIL-18 (Figure 3C). These clones were designated as “DR-hIL-18” variants, where “DR” stands for “decoy-resistant.”

Sequencing of 96 clones from the post-round five pool revealed 21 unique sequences, which were analyzed to create four “consensus sequences”, hCS1-4 (Figure 4). To estimate the binding affinities of these variants for hIL-18R α and hIL-18BP, binding isotherms were established for hIL-18R α and IL-18BP binding using yeast-displayed cytokine variants and flow cytometry. As seen in Figure 5A, the DR-hIL-18 variants bound hIL-18R α with comparable affinity to WT IL-18, but showed severely attenuated binding to hIL-18BP, with apparent binding EC₅₀ values significantly greater than 1 μ M. To additionally characterize the receptor binding activities of the DR-IL-18 variants, the cytokines were expressed recombinantly and surface plasmon resonance for IL-18R α and IL-18BP was performed (see Figure 5B for representative traces). These results are summarized in Tables 6 and 7 and demonstrate that the DR-hIL-18 variants have a dramatically decreased preference for IL-18BP compared to IL-18R α , by several orders of magnitude.

Table 6: IL-18R α and IL-18BP binding affinities of human IL-18 variants by on-yeast binding isotherms.

IL-18 Variant	K _D IL-18R α (M)	K _D IL-18BP (M)	K _D ratio: IL-18BP/IL-18R α	Dissociation Constant Ratio normalized to WT IL-18
hIL-18 WT	2.40E-08	7.08E-09	2.95E-01	1
hA8	5.77E-08	NBD	>3.47E+02	>1.17E+03
hH3	8.38E-08	NBD	>2.39E+02	>8.09E+02
hB9	1.27E-07	NBD	>1.57E+02	>5.34E+02
hCS1	6.44E-08	1.93E-05	3.00E+02	1.02E+03
hCS2	9.15E-08	NBD	>2.19E+02	>7.41E+02
hCS3	1.13E-07	1.16E-05	1.03E+02	3.48E+02

hCS4	1.60E-07	NBD	>1.25E+02	>4.24E+02
6-31	4.1E-08	NBD	4.9E+02	>7.2E+03
6-20	N.D.	3.4E-07	-----	-----
6-12	1.7E-08	NBD	1.2E+03	>1.7E+04
6-27	4.2E-08	NBD	4.8E+02	>7.0E+03
6-29	3.7E-08	NBD	5.4E+02	>8.0E+03

NBD, no binding detected (20 μ M used for ratio calculations)

-----, value not determined

Table 7: IL-18R α and IL-18BP binding affinities of human IL-18 variants by SPR

IL-18 Variant	K _D IL-18R α (M)	K _D IL-18BP (M)	K _D ratio: IL-18BP/IL-18R α	Dissociation Constant Ratio normalized to WT IL-18
hIL-18 WT	2.93E-09	1.90E-12	6.48E-04	1
hA8	-----	-----	-----	-----
hH3	-----	-----	-----	-----
hB9	-----	-----	-----	-----
hCS1	8.05E-09	1.94E-08	2.41E+00	3.72E+03
hCS2	1.31E-08	-----	-----	-----
hCS3	8.18E-09	1.86E-08	2.27E+00	3.50E+03
hCS4	4.38E-09	1.83E-07	4.18E+01	6.45E+04

5 -----, value not determined

Functional characterization of human DR-IL-18 variants

A previous report from Kim et al (Kim et al., 2001, Proc Natl Acad Sci U S A, 98(6):3304-9) described 3 hIL-18 variants with enhanced activity and purportedly decreased inhibition by IL-18BP: E42A, K89A, and E42A/K89A. These cytokine variants were displayed on yeast and IL-18BP inhibition of IL-18R α binding was assessed by flow cytometry. As seen in Figure 6A, while the DR-hIL-18 variants were impervious to inhibition of hIL-18R α binding by hIL-18BP, the Kim et al variants showed roughly equivalent hIL-18BP neutralization as compared to WT hIL-18. These results indicate that the DR-hIL-18 variants are IL-18BP independent, whereas the Kim et al variants are highly sensitive to IL-18BP inhibition, similar to WT hIL-18.

To confirm that the DR-hIL-18 could yield productive signaling through the IL-18 receptor in a cellular context, concentration-response experiments were performed using

the HEK-blue IL-18 reporter cell line. In this system, IL-18R signaling is read-out by expression of secreted alkaline phosphatase (SEAP) downstream of a NF κ B/AP1 promotor. In the absence of IL-18BP, DR-hIL-18 variants yielded signaling EC50 values commensurate with WT hIL-18. However, the DR-hIL-18 variants demonstrated virtually
5 no inhibition by hIL-18BP, with no detectable inhibition at 1 μ M IL-18BP (Figure 6B). Taken together, these studies establish that the DR-hIL-18 variants are biologically active and impervious to IL-18BP neutralization in a cell signaling context.

10 Engineering and characterization of second-generation human IL-18 variants that are resistant to IL-18BP inhibition (human v2.0 DR-IL-18 variants)

To obtain additional, potentially enhanced human DR-IL-18 variants, a second library of human IL-18 randomized at 11 positions (Figure 7A) was designed and yeast was transformed as described above. The resulting library of 6×10^8 transformants was selected as outlined in (Figure 7B), yielding a robust preference for IL-18R α compared to
15 IL-18BP with successive selection steps (Figure 7C). 17 unique sequences were recovered after 5-6 rounds of selection (Figure 8). (Figure 9A) Compared to WT IL-18, clones 6-12, 6-27, 6-29, and 6-31 had equal or somewhat stronger binding to IL18R α as measured by yeast-binding isotherms with biotinylated IL18R α . (Figure 9B) However, these clones did not show any appreciable binding to IL-18BP. (Figure 9C) Measurement of thermal
20 stability by applying a range of temperatures to the yeast-displayed clones showed that they were more thermal stable than WT IL-18 by 7-13°C. These results are summarized in (Figure 9D).

25 Engineering IL-18 variants that are resistant to IL-18BP inhibition (murine DR-IL-18 variants)

As the human and mouse interspecies cross-reactivity of IL-18 for IL-18R α is poor, murine equivalents of the DR-IL-18 variants that could be used for studies in mice were created. Similar to the approach taken for hIL-18 above, a combinatorial library of mIL-18 variants randomizing a similar set of mIL-18R α /mIL-18BP contact residues (Table 3) was
30 created, yielding a library of 4×10^8 transformants. Directed evolution was performed on this library similar to how it was performed with the human IL-18 library; the selection

strategy is summarized in Figure 10A. After the completion of six rounds of selection, the remaining clones had a near-complete preference for mIL-18R α over mIL-18BP (Figure 10B). Analysis of 96 clones revealed 11 unique sequences, from which were derived two consensus sequences mCS1 and mCS2 (Figure 10C). Yeast binding isotherms and surface plasmon resonance experiments confirmed these DR-IL-18 clones had an even greater independence for IL-18BP than the human IL-18 variants described herein, with the mIL-18BP binding KD's being well above 1 μ M, with mIL-18R α binding remaining roughly equal to WT mIL-18 (Figure 11A, Figure 11B, Tables 8 and 9).

Table 8: IL-18R α and IL-18BP binding affinities of mouse IL-18 variants by on-yeast binding isotherms

IL-18 Variant	K _D IL-18R α (M)	K _D IL-18BP (M)	K _D ratio: IL-18BP/IL-18R α	Dissociation Constant Ratio normalized to WT IL-18
mIL-18 WT	1.13E-08	2.13E-09	1.88E-01	1
mA7	1.35E-08	NBD	>7.41E+02	>3.93E+03
mB1	1.79E-08	NBD	>5.59E+02	>2.96E+03
mE8	4.20E-08	NBD	>2.38E+02	>1.26E+03
mC1	4.30E-08	NBD	>2.33E+02	>1.23E+03
mCS1	1.07E-08	NBD	>9.35E+02	>4.96E+03
mCS2	1.13E-08	NBD	>8.85E+02	>4.69E+03

NBD, no binding detected (10 μ M used for ratio calculations)

Table 9: IL-18R α and IL-18BP binding affinities of mouse IL-18 variants by SPR

IL-18 Variant	K _D IL-18R α (M)	K _D IL-18BP (M)	K _D ratio: IL-18BP/IL-18R α	Dissociation Constant Ratio normalized to WT IL-18
mIL-18 WT	6.00E-10	1.10E-12	1.83E-03	1
mA7	2.20E-10	1.39E-05	6.32E+04	3.45E+07
mB1	7.00E-10	1.47E-05	2.10E+04	1.15E+07
mE8	1.69E-09	NBD	>1.78E+04	>9.68E+06
mC1	1.09E-09	2.87E-05	2.63E+04	1.44E+07
mCS1	5.40E-10	3.80E-06	7.04E+03	3.84E+06
mCS2	7.90E-11	1.05E-05	1.33E+05	7.25E+07

NBD, no binding detected (30 μ M used for ratio calculations)

In vivo pharmacodynamic studies of DR-IL-18 variants

To assess the biologic effects of administration of the DR-IL-18 variants in vivo, pharmacodynamics studies were performed in mice, comparing WT mIL-18 to mCS2. In the first study, mice were treated with vehicle (PBS), mIL-18 (1 mg/kg/day), or mCS2 (1 mg/kg/day) for a total of seven injections (Figure 12A). Analysis of peripheral blood phenotypes by flow cytometry showed that both WT mIL-18 and mCS2 increased peripheral NK cell numbers by over ten-fold, and peripheral monocyte counts by over five-fold compared to vehicle treatment; total CD4 and CD8 cell counts were not significantly affected (Figure 12B). Examination of cellular activation status by CD69 induction revealed that mCS2 treatment dramatically increased CD69 levels on CD4 and CD8 cells compared to mIL-18 or vehicle treatment; reaching over 30% and over 50% positivity for CD4 and CD8 subsets, respectively (Figure 12C). While both mIL-18 and mCS2 stimulated CD69 expression on peripheral NK cells to over 20% positive by day 3, the CD69 levels decreased to non-significant levels for mIL-18 by day 6, but remained significantly elevated with mCS2 treatment (Figure 12C). Peripheral cytokine levels were also measured with a multiplexed Luminex panel. As seen in Figure 12D, both mIL-18 and mCS2 increased serum IFN-g, MIP1b, and G-CSF compared to vehicle treatment, but mCS2 achieved much higher levels than mIL-18 by day 6 for each of these cytokines, as mIL-18 exhibited tachyphylaxis with plateaued or decreasing induced cytokine levels with subsequent administration.

Effect of mCS2 on body fat composition

To assess the effect of the DR-IL-18 variants on body fat composition, we administered WT IL-18 at 1 mg/kg or 0.01, 0.1, or 1 mg/kg mCS2 by intraperitoneal injection to C57BL/6 mice every three days. Body fat and lean mass composition were monitored by echoMRI. All tested doses of mCS2 (1 mg/kg, 0.1 mg/kg, and 0.01 mg/kg) resulted in striking decreases in the overall percentage of body fat by day 12, while vehicle and mIL-18 treated mice did not have a significant change in total body fat composition (Figure 13, top). Specifically, mCS2-treated mice had either reduced or stable levels of total fat mass during the experiment (Figure 13, bottom left), but substantially increased

their total lean mass (Figure 13, bottom right). These results indicate that mCS2, and other variants disclosed herein, could be used to therapeutically decrease body fat composition (e.g., for treatment of obesity, diabetes, and/or metabolic syndrome).

5 Anti-tumor efficacy of DR-IL-18 variants

 The anti-tumor efficacy of DR-IL-18 (mCS2) was assessed using the transplantable, syngenic YUMMER1.7 malignant melanoma tumor model. WT mIL-18 and mCS2 were administered to mice bearing YUMMER1.7 tumors biweekly at a dose of 0.32 mg/kg, with or without co-administration of anti-PD1 antibodies (8 mg/kg/q3d).
10 Consistent with previous reports on its use in mice and humans, WT IL-18 did not affect tumor growth or survival compared to vehicle (saline), and only marginally improved the efficacy anti-PD1 when administered in combination. However, mCS2 cured 27% of treated mice as a monotherapy and produced a partial response in another 27%, an effect commensurate with anti-PD1 treatment. The combination of mCS2 with anti-PD1 cured
15 80% of treated mice (Figure 14A and Figure 14B).

 To establish the mechanism of action of DR-IL-18 on YUMMER1.7 tumors, cell depletion studies were performed using antibodies against CD8, CD4, NK1.1, and Interferon-gamma. As seen in Figure 15A and Figure 15B, depletion of CD8 cells or neutralization of Interferon-gamma completely abrogated the effectiveness of DR-IL-18.
20 Depletion of CD4 cells did not affect the initial activity of DR-IL-18 in terms of tumor growth, however, in CD4 treated mice, therapeutic responses are not sustained, suggesting a role of CD4 cells in supporting and sustaining anti-tumor immunity. Depletion of NK cells did not affect tumor growth or survival in YUMMER1.7 cells.

 The activity of DR-IL-18 was additionally assessed in the immunogenic MC38 colorectal tumor model. A dose-finding study was first performed, administering saline, WT IL-18 (1mg/kg twice weekly), or a range of DR-IL-18 doses from 0.01mg/kg, 0.1mg/kg, or 1mg/kg twice weekly. As seen in Figure 16, WT IL-18 had no effect on tumor growth, whereas DR-IL-18 (mCS2) showed dose-dependent efficacy, slowing tumor growth at 0.1mg/kg and producing tumor regression at 1mg/kg. The cohorts were then
25 expanded and potential synergism with immune checkpoint inhibition was assessed. Again, WT IL-18 had no effect as a monotherapy and showed no enhancement of anti-PD1
30

efficacy. By contrast, DR-IL-18 showed robust monotherapeutic activity commensurate with or superior to anti-PD1, and the two therapies given together showed exceptional synergism, producing complete regression in all treated mice (Figure 17).

To further characterize the mechanism of DR-IL-18, flow cytometric studies were performed on the immune infiltrate of MC38 tumors from mice treated with saline, WT IL-18, or DR-IL-18 (mCS2). Relative to saline or WT IL-18, DR-IL-18 treatment increased CD8 and NK cell infiltration per mg of tumor and additionally resulting in upregulation of activation markers of effector cells such as granzyme B and KLRG1 (Figure 18A, top row). Unlike other cytokine therapies such as IL-2 or IL-15, DR-IL-18 does not increase the CD8:Treg ratio within tumors compared to saline treatment. However, DR-IL-18 treatment leads to a more favorable tumor immune microenvironment, by increasing the ratio of CD8 cells to tumor associated macrophages (TAMs), and monocytic and granulocytic myeloids derived suppressor cells (MDSCs). The secondary cytokine release profile was also measured from serum of the same mice using a Luminex assay. As seen in Figure 18B, DR-IL-18 treatment increased systemic levels of Interferon-gamma, IL-7, and IL-15 by over 100-fold relative to WT IL-18 treatment. Taken in aggregate, these results indicate that DR-IL-18 produces anti-tumor efficacy through a unique mechanism of action distinct from IL-2, IL-15, or WT IL-18 treatment.

Some of the secondary cytokines induced by DR-IL-18 therapy would be predicted to potentially contribute to toxicity and/or decreased effectiveness. For instance, IL-17 which is upregulated >100-fold by DR-IL-18 contributes to colitis and psoriasis and additionally stimulates granulocytes that can become immunosuppressive myeloid derived suppressor cells. IL-5 and IL-13 are type 2 cytokines also upregulated by DR-IL-18 and could contribute to allergy, exacerbation of asthma, or anaphylaxis. Th2 T cells do not contribute to immunotherapeutics responses and may promote immunosuppressive Treg development. As such, in certain instances the effectiveness and safety of DR-IL-18 could be enhanced by selective inhibition of undesired secondary cytokines such as IL-17, IL-5, and IL-13, for instance by a neutralizing antibody.

Many tumors are resistant to immune checkpoint inhibition, either at initial presentation (primary resistance) or after an initial response to treatment (secondary resistance). The most prevalent cause of resistance of checkpoint inhibitors is loss of

antigen presentation through MHC class I. Loss of surface MHC class I is classically associated with NK-cell mediated cytotoxicity, however, NK cells can become exhausted within MHC I deficient tumors. As NK cells express the IL-18R and our previous results in MC38 indicated that NK cells are expanded and activated by DR-IL-18, we thus tested whether DR-IL-18 could stimulate NK cell attack against MHC I deficient tumors. We used CRISPR/cas9 to knockout B2m in the YummeR1.7 cell line and found that implanted B2m-deficient YUMMER1.7 tumors were refractory to even combined treatment with both anti-CTLA4 and anti-PD1 (Figure 19A and Figure 19B), a combination that routinely cures close to 100% of parental YummeR1.7 tumors. However, single-agent treatment with DR-IL-18 (mCS2) cured 60% of B2m-deficient YummeR1.7 tumors in an NK-cell dependent fashion, as depletion with anti-NK1.1 abrogated the effect (Figure 19A and Figure 19B). Experiments were conducted to understand the effect that DR-IL-18 had on intratumoral NK cells in the setting of an MHC class I deficient tumor. Immunophenotyping studies were performed with flow cytometry on B2m-deficient YummeR1.7 tumors from mice treated with saline or DR-IL-18. 24 hours after the 3rd dose of treatment, the mice were sacrificed, tumors were dissociated, and the cell suspension was treated with PMA/ionomycin for four hours. The proliferative index and functional capacity of the NK cells were then analyzed by intracellular flow cytometry with Ki67 and Interferon-gamma. As seen in Figure 19C, NK cells from saline-treated B2m-deficient YummeR1.7 tumors had scant Interferon-gamma production and Ki67 levels, indicating an exhausted phenotype. By contrast, NK cells from tumors treated with DR-IL-18 had robust Interferon-gamma production and Ki67 levels, with the majority of NK cells being positive for both markers. These results thus establish that DR-IL-18 is effective in the treatment of MHC class I deficient tumors that are refractory to immune checkpoint blockade in an NK cell-dependent manner.

These results establish DR-IL-18 as a highly promising tumor immunotherapeutic, and provide strong evidence that IL-18BP greatly limits the effectiveness of IL-18 therapy, given the greatly improved activity of the mCS2 DR-IL-18 variant. From these results, it is predicted that other strategies, such as blocking IL-18BP with an antibody, small protein, and/or small molecule could augment IL-18 therapy and other immunotherapeutic regimens.

Efforts were undertaken to engineer an IL-18BP antagonist by creating a “decoy-to-the-decoy” (D2D), or IL-18 variants that specifically bind IL-18BP, but do not bind IL-18R α and thus do not signal. The potential advantage of such an agent is that it would serve to neutralize IL-18BP and enhance the activity of endogenous IL-18, as opposed to driving IL-18R signaling systemically. IL-18 was thus randomized at contact positions for IL-18R α (Figure 20A) and a yeast-displayed library was prepared as described previously for human and mouse DR-IL-18. The resulting library of 3.9×10^8 transformants was selected for 3 rounds as indicated in (Figure 20B), selecting for retained IL-18BP binding, while counterselecting against IL-18R α . As seen in (Figure 20C), each round of selection conferred enrichment for binding to IL-18BP (human and mouse), but without acquisition of IL-18R α binding. 96 clones were sequenced, yielding 31 unique sequences, from which three consensus sequences hD2D-CS1, hD2D-CS2, and hD2D-CS3 were derived (Figure 21). Biophysical characterization of the resulting clones indicated that they showed similar binding isotherms to IL-18BP as WT IL-18 (Figure 22A), but with greatly decreased/absent binding to IL-18R α (Figure 22B). These data are summarized in (Figure 22C). An identical selection process was performed for murine IL-18, creating a library of 2.0×10^8 transformants, which we selected to obtain 51 unique sequences (summarized in Figure 23).

Example 2: Binding affinity measurements of second generation variants

Surface Plasmon Resonance (SPR) was used to perform biophysical affinity measurements of second generation DR-IL-18 variants (binding to IL-18R vs IL-18BP). See Figure 24 for the generated sensograms. Table 10 is a summary of the measured kinetics, Table 11 is a summary of the affinity measurements, and Table 12 is a general summary, including results for the dissociation constant ratios of the second generation DR-IL-18 variants.

Table 10: Summary of SPR data for second generation hDR-IL-18 variants (kinetics)

Surface Ligand	Analyte	ka (1/Ms)	kd (1/s)	KD (M) Exp 2	KD (M) Exp 1	% Rmax
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hIL-18Ra	hIL-18	5.55E+05	2.97E-03	5.36E-09	5.35E-09	32
hIL-18Ra	6-12	4.95E+05	9.10E-04	1.84E-09	2.24E-09	35
hIL-18Ra	6-27	6.31E+05	2.43E-03	3.85E-09	3.48E-09	35
hIL-18Ra	6-29	5.75E+05	1.19E-03	2.07E-09	2.65E-09	36
hIL-18Ra	6-31	2.18E+05	3.32E-03	1.52E-08	1.94E-08	19
hIL-18BP	hIL-18	5.18E+05	2.23E-07	4.30E-13	6.94E-13	48
hIL-18BP	6-12	Too weak to measure				-1
hIL-18BP	6-27	Too weak to measure				2
hIL-18BP	6-29	Too weak to measure				0
hIL-18BP	6-31	Too weak to measure				-1

Table 11: Summary of SPR data for second generation hDR-IL-18 variants (affinity)

Sample	KD _{apparent} hIL-18Ra (nM)	KD _{apparent} hIL-18BP (nM)
hIL-18	5.4, 5.4	<0.1
6-12	1.8, 2.2	too weak
6-27	3.9, 3.5	too weak
6-29	2.1, 2.7	too weak
6-31	15.2, 19.4	too weak

Table 12: Summary of SPR affinity measurements. Summary of the SPR affinity measurements of second generation hDR-IL-18 variants for IL-18Ra and IL-18BP. The IL-18 BP:Ra Dissociation Constant Ratio is the ratio of the KD for IL-18BP to the KD for IL-18Ra normalized to the same ratio of WT IL-18. A higher number for this ratio indicates that the IL-18 variant has an enhanced preference for binding IL-18Ra over IL-18BP compared to WT IL-18. *Average of 2 studies. k is a multiple of 1,000. m is a multiple of 1,000,000.

Protein	SPR: K _D Ra (nM)	SPR: K _D BP (nM)	IL-18 BP:Ra Dissociation Constant Ratio
WT hIL-18	4.1*	0.002	1
hCS1	8.0*	11.8*	3,024
hCS3	9.1*	19.3*	4,348
hCS4	7.7*	121*	32,215
6-12	2.2	>10,000	>9,318,275
6-27	3.5	>10,000	>5,857,201
6-29	2.7	>10,000	>7,592,669

6-31	19.4	>10,000	>1,056,712
WT mL-18	0.60	0.0011	1
mCS2	0.08	11,000	>75,000,136
A7, B1, C1, E8	0.22 - 1.7	14k - 29k	9.3m - 35m

Example 3: Efficacy for cancer treatment

Efficacy of DR-IL-18 variants was tested using multiple different cancer models, including models of colorectal tumors, breast cancer, melanoma, and MHC class I deficient tumors that are resistant to immune checkpoint inhibitors. The results show that DR-IL-18 variants with a bias to bind IL-18R and not IL-18BP can be used to treat a broad range of cancers (not limited to just those that were tested).

Figure 25A and 25B: data demonstrating efficacy of DR-IL-18 on the CT26 colorectal tumor model. 250,000 CT26 cells were implanted subcutaneously and treatment initiated at day 7 once tumors were ~60 mm³ on average. WT IL-18 and mCS2 were dosed at 0.32mg/kg twice weekly for a total of 5 doses. Anti-PD1 was given at 10mg/kg at the same schedule. (A) Overlay of spider plots showing tumor growth of saline (PBS) treated animals in black lines (circles), WT IL-18 in blue lines (squares), and DR-IL-18 (mCS2) in pink (triangles). Only treatment with DR-IL-18, but not WT IL-18, resulted in tumor growth inhibition and tumor clearance in a subset of animals. (B) Survival curves for mice treated with anti-PD-1, WT IL-18, and DR-IL-18 (mCS2). Numbers of complete responses are indicated in parentheses. DR-IL-18, but not WT IL-18 resulted in prolonged survival and tumor clearance in 40% of mice, an improvement over the checkpoint inhibitor anti-PD-1.

Figure 26A and 26B: data demonstrating efficacy of DR-IL-18 in the 4T1 breast cancer model and B16-F10 melanoma model. (A) Tumor growth curves of 4T1 tumors engrafted into BALB/C mice after treatment with saline (PBS; black), WT IL-18 (blue), or the DR-IL-18 variant CS2 (pink). (B) Tumor growth curves of B16-F10 tumors engrafted into C57BL/6 mice after treatment with saline (PBS; black), WT IL-18 (blue), or the DR-IL-18 variant CS2 (pink). In both models, only DR-IL-18, but not WT IL-18 resulted in tumor growth inhibition. Treatments were administered after tumors exceeded an average volume 50 mm³ as indicated by the boxes marked with “t”.

Figure 27A and 27B: These data extend those of Figure 19A through 19C. Depicted is data demonstrating efficacy of DR-IL-18 in the treatment of additional MHC class I deficient tumor models that are resistant to immune checkpoint inhibitors. (A) B2m deficient MC38 cells were prepared using CRISPR/Cas9 mediated deletion as described for B2m deficient YUMMER cells. B2m^{-/-} MC38 cells were implanted subcutaneously and treatment initiated at day 7 once tumors were ~65 mm³ on average. mCS2 was dosed at 0.32mg/kg twice weekly for 5 doses. Anti-PD1 and anti-CTLA4 were given at 8mg/kg at the same schedule. (B) RMA/S is a variant of the RMA lymphoma line that contains a spontaneous mutation in Tapasin. The result is a defect in antigen loading and therefore decreased MHC class I surface expression. It is congenic to C57BL/6 and refractory to immune checkpoint inhibitors. Mice were implanted with 1,000,000 RMA/S cells subcutaneously and treatment initiated at day 7. mCS2 was dosed at 0.32mg/kg twice weekly. Anti-PD1 was given at 8mg/kg at the same schedule.

15 Example 4: Combination therapy

Figure 28: data demonstrating efficacy of DR-IL-18 variants to enhance anti-tumor antibody-dependent cell mediated cytotoxicity (ADCC) (supporting combination therapy with opsonizing agents such as tumor-targeting antibodies). *Ex vivo* cytotoxicity studies used CFSE labeled Raji (B cell lymphoma) cells and isolated human peripheral blood mononuclear cells (PBMCs). PBMCs and labeled Raji cells were incubated together at an effector:target (E:T) ratio of 1:10 for 25 hours. The human DR-IL-18 variant hCS-1 (1uM), rituximab (10 ug/mL), or the combination of both agents were applied to the samples as indicated. Cytotoxicity was measured by flow cytometry and calculated as the fraction of CFSE cells that became DAPI positive. * p<0.05 by two-way ANOVA with Tukey's correction for multiple comparisons.

Example 5: Efficacy against viral infections

Figure 29A and 29B: data demonstrating anti-viral efficacy of DR-18 variants for the treatment of infection disease (e.g., for viral infections, e.g., in this illustrative example, mCS2 was used for treatment of systemic vaccinia virus infection). (A) Experimental design scheme. C57BL/6 mice were infected with 10⁶ PFU of Vaccinia virus (VACV)

intraperitoneally (IP) and administered 1 mg/kg WT mIL-18 or mCS2 IP. Mice were sacrificed and viral titers were measured in the blood and ovaries by RT-PCR on day 3 post-infection. (B) Quantification of VACV viral copies in ovaries and blood of treated mice at day 3 post infection. Treatment with CS2 showed a significant reduction of viral titers, whereas WT IL-18 was not effective. * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$.

Example 6: Second generation human DR-IL-18 variants

Figure 30A depicts data demonstrating that the second generation human DR-IL-18 variants are active. (Figure 30A) WT IL-18 and h6-12, h6-27, h6-29, and h6-31 stimulate IL-18 HEK-Blue reporter cells. h6-12, h6-27, and h6-29 show enhanced potency compared to WT hIL-18, whereas h6-31 has equivalent potency as WT hIL-18. The data demonstrate, therefore, that all tested second generation human DR-IL-18 variants actively signal through IL-18R.

Wild-type IL-18 amino acid sequences

HUMAN Interleukin-18 (mature form)

YFGKLESKLSVIRNLNDQVLFIDQGNRPLFEDMTDSDCRDNAPRTIFIISMYKDSQ
PRGMAVTISVKCEKISTLSCENKIISFKEMNPPDNIKDTKSDIIFQRSVPGHDNKM
QFESSYEGYFLACEKERDLFKLILKKKEDELGDRSIMFTVQNED (SEQ ID NO: 30)

MOUSE Interleukin-18 (mature form)

NFGRLHCTTAVIRNINDQVLFVDKRQPVFEDMTDIDQSASEPQTRLIIYMYKDSE
VRGLAVTILSVKDSKMSTLSCKNKIISFEEMDPPENIDDIQSDLIFFQKRVPGHNKM
EFESSLYEGHFLACQKEDDAFKLILKKKDENGDKSVMFTLTNLHQS (SEQ ID NO:
31)

Q14116|IL18_HUMAN Interleukin-18 (uncleaved precursor)

MAAEPVEDNCINFVAMKFIDNTLYFIAEDDENLESDYFGKLESKLSVIRNLNDQV
LFIDQGNRPLFEDMTDSDCRDNAPRTIFIISMYKDSQPRGMAVTISVKCEKISTLSC
ENKIISFKEMNPPDNIKDTKSDIIFQRSVPGHDNKMQFESSYEGYFLACEKERD
LFKLILKKKEDELGDRSIMFTVQNED (SEQ ID NO: 32)

P70380|IL18_MOUSE Interleukin-18 (uncleaved precursor)

MAAMSEDCVNFKEMMFIDNTLYFIPEENGDLSDNFGRLHCTTAVIRNINDQVL
FVDKRQPVFEDMTDIDQSASEPQTRLIIYMYKDSEVRGLAVTILSVKDSKMSTLSC
KNKIISFEEMDPPENIDDIQSDLIFFQKRVPGHNKMFEFESSLYEGHFLACQKEDDA
FKLILKKKDENGDKSVMFTLTNLHQS (SEQ ID NO: 33)

Generation 1 Human Interleukin-18 Decoy-Resistant variants amino acid sequences

hCS1	YFGKLESKLSVIRNLNDQVLFIDQGNRPLFEDMTDSDCRDNAPRTIFIIS TYKDSQPRGKAVTISVKCEKISTLSCENKIISFKEMNPPDNIKDTKSDIIF FQRDVPGHKHKMQFESSSYEGYFLACEKERDLFKLILKKEDELGDRSI MFTVQNED (SEQ ID NO: 34)
hCS2	YFGKLESKLSVIRNLNDQVLFIDQGNRPLFEDMTDSDCRDNAPRTIFIIS TYKDKQPRAKAVTISVKCEKISTLSCENKIISFKEMNPPDNIKDTKSDIIF FQRDVPGHKHKMQFESSSYEGYFLACEKERDLFKLILKKEDELGDRSI MFTIQNED (SEQ ID NO: 35)
hCS3	RFGKLESKLSVIRNLNDQVLFIDQGNRPLFEDMTDSDCRDNAPRTIFIIS TYKDSQPRGKAVTISVKCEKISTLSCENKIISFKEMNPPDNIKDTKSDIIF FQRDVPGHKHKMQFESSSYEGYFLACEKERDLFKLILKKEDELGDRSI MFTVQNED (SEQ ID NO: 36)
hCS4	RFGKLESKLSVIRNLNDQVLFIDQGNRPLFEDMTDSDCRDNAPRTIFIIS TYRDSQPRGKAVTISVKCEKISTLSCENKIISFKEMNPPDNIKDTKSDIIF FQRNVPGHKYKMQFESSSYEGYFLACEKERDLFKLILKKEDELGDRSI MFTVQNED (SEQ ID NO: 37)
hC4	YFGKLESQLSVIRNLNDQVLFIDQGNRPLFEDMTDSDCRDNAPRTIFIIS TYKDKQPRTKAVTISVKCEKISTLSCENKIISFKEMNPPDNIKDTKSDIIF FQRRVPGHHNKMQFESSSYEGYFLACEKERDLFKLILKKEDELGDRSI MFTVQKED (SEQ ID NO: 38)
hA8	YFGKLESRLSVIRNLNDQVLFIDQGNRPLFEDMTDSDCRDNAPRTIFIIS KYKDKQPRAQAVTISVKCEKISTLSCENKIISFKEMNPPDNIKDTKSDIIF FQRDVPGHKHKMQFESSSYEGYFLACEKERDLFKLILKKEDELGDRSI MFTIQNED (SEQ ID NO: 39)
hD6	YFGKLESRLSVIRNLNDQVLFIDQGNRPLFEDMTDSDCRDNAPRTIFIIS DYKDKQPRAQAVTISVKCEKISTLSCENKIISFKEMNPPDNIKDTKSDIIF FQRDVPGHKHKMQFESSSYEGYFLACEKERDLFKLILKKEDELGDRSI MFTIQNED (SEQ ID NO: 40)
hH12	YFGKHESKLSVIRNLNDQVLFIDQGNRPLFEDMTDSDCRDNAPRTIFIIS TYRDSQPRGKAVTISVKCEKISTLSCENKIISFKEMNPPDNIKDTKSDIIF FQRDVPGHNNKMQFESSSYEGYFLACEKERDLFKLILKKEDELGDRSI MFTTQNED (SEQ ID NO: 41)
hB11	YFGKIESKLSVIRNLNDQVLFIDQGNRPLFEDMTDSDCRDNAPRTIFIIS KYKDKQPRAQAVTISVKCEKISTLSCENKIISFKEMNPPDNIKDTKSDIIF FQRKVPGHQHKMQFESSSYEGYFLACEKERDLFKLILKKEDELGDRSI MFTVQKED (SEQ ID NO: 42)
hC3	YFGKIESKLSVIRNLNDQVLFIDQGNRPLFEDMTDSDCRDNAPRTIFIIST YKDRQPRGKAVTISVKCEKISTLSCENKIISFKEMNPPDNIKDTKSDIIF ERDVPGHHHKMQFESSSYEGYFLACEKERDLFKLILKKEDELGDRSIM FTIQNED (SEQ ID NO: 43)
hC2	YFGKIESKLSVIRNLNDQVLFIDQGNRPLFEDMTDSDCRDNAPRTIFIIST YKDKQPRGKAVTISVKCEKISTLSCENKIISFKEMNPPDNIKDTKSDIIF QRDVPGHKHKMQFESSSYEGYFLACEKERDLFKLILKKEDELGDRSIM FTTQHED (SEQ ID NO: 44)

hG10	YFGKIESKLSVIRNLNDQVLFIDQGNRPLFEDMTDSDCRDNAPRTIFIIST YKDKQPRAKAVTISVKCEKISTLSCENKIISFKEMNPPDNIKDTKSDIIF QRRVPGHHHKMQFESSYEGYFLACEKERDLFKLILKKEDELGDRSIM FTIQKED (SEQ ID NO: 45)
hG1	YFGKIESRSLSVIRNLNDQVLFIDQGNRPLFEDMTDSDCRDNAPRTIFIIST YKDKQPRGKAVTISVKCEKISTLSCENKIISFKEMNPPDNIKDTKSDIIF QRDVPGHDYKMQFESSYEGYFLACEKERDLFKLILKKEDELGDRSIM FTIQKED (SEQ ID NO: 46)
hF1	YFGKYESRSLSVIRNLNDQVLFIDQGNRPLFEDMTDSDCRDNAPRTIFIIS TYRDSQPRGKAVTISVKCEKISTLSCENKIISFKEMNPPDNIKDTKSDIIF FQRDVPGHEHKMQFESSYEGYFLACEKERDLFKLILKKEDELGDRSI MFTVQKED (SEQ ID NO: 47)
hD2	HFGKYESKLSVIRNLNDQVLFIDQGNRPLFEDMTDSDCRDNAPRTIFIIS TYRDSQPRGKAVTISVKCEKISTLSCENKIISFKEMNPPDNIKDTKSDIIF FQRDVPGHHNKMQLFESSYEGYFLACEKERDLFKLILKKEDELGDRSI MFTVQKED (SEQ ID NO: 48)
hA1	RFGKLESKLSVIRNLNDQVLFIDQGNRPLFEDMTDSDCRDNAPRTIFIIS TYRDSQPRAKAVTISVKCEKISTLSCENKIISFKEMNPPDNIKDTKSDIIF FQRDVPGHQHKMQFESSYEGYFLACEKERDLFKLILKKEDELGDRSI MFTAQKED (SEQ ID NO: 49)
hB3	RFGKLESRSLSVIRNLNDQVLFIDQGNRPLFEDMTDSDCRDNAPRTIFIIS DYRDSQPRGRAVTISVKCEKISTLSCENKIISFKEMNPPDNIKDTKSDIIF FKRNVPGHKYKMQFESSYEGYFLACEKERDLFKLILKKEDELGDRSI MFTVQHED (SEQ ID NO: 50)
hB4	RFGKLESRSLSVIRNLNDQVLFIDQGNRPLFEDMTDSDCRDNAPRTIFIIS NYRDSQPRGQAVTISVKCEKISTLSCENKIISFKEMNPPDNIKDTKSDIIF FKRRVPGHNHKMQFESSYEGYFLACEKERDLFKLILKKEDELGDRSI MFTVQKED (SEQ ID NO: 51)
hH3	RFGKLESRSLSVIRNLNDQVLFIDQGNRPLFEDMTDSDCRDNAPRTIFIIS TYKDSQPRGKAVTISVKCEKISTLSCENKIISFKEMNPPDNIKDTKSDIIF FQRDVPGHKKHKMQFESSYEGYFLACEKERDLFKLILKKEDELGDRSI MFTVQNED (SEQ ID NO: 52)
hH5	RFGKHESKLSVIRNLNDQVLFIDQGNRPLFEDMTDSDCRDNAPRTIFIIS TYRDSQPRGKAVTISVKCEKISTLSCENKIISFKEMNPPDNIKDTKSDIIF FERNVPGHKYKMQFESSYEGYFLACEKERDLFKLILKKEDELGDRSI MFTVQNED (SEQ ID NO: 53)
hH4	RFGKLESRSLSVIRNLNDQVLFIDQGNRPLFEDMTDSDCRDNAPRTIFIIS TYRDSQPRAKAVTISVKCEKISTLSCENKIISFKEMNPPDNIKDTKSDIIF FERDVPGHQHKMQFESSYEGYFLACEKERDLFKLILKKEDELGDRSI MFTIQXED (SEQ ID NO: 54)
hE1	RFGKLESRSLSVIRNLNDQVLFIDQGNRPLFEDMTDSDCRDNAPRTIFIIS TYRDSQPRTKAVTISVKCEKISTLSCENKIISFKEMNPPDNIKDTKSDIIF FQRNVPGHHDKMQFESSYEGYFLACEKERDLFKLILKKEDELGDRSI MFTVQHED (SEQ ID NO: 55)

hG2	RFGKLESRLSVIRNLNDQVLFIDQGNRPLFEDMTDSDCRDNAPRTIFIIS TYKDSQPRAKAVTISVKCEKISTLSCENKIISFKEMNPPDNIKDTKSDIIF FERDVPGHQHKMQFESSSYEGYFLACEKERDLFKLILKKEDELGDRSI MFTIQKED (SEQ ID NO: 56)
hB9	RFGKHESKLSVIRNLNDQVLFIDQGNRPLFEDMTDSDCRDNAPRTIFIIS TYRDSQPRGKAVTISVKCEKISTLSCENKIISFKEMNPPDNIKDTKSDIIF FERNVPGHKYKMQFESSSYEGYFLACEKERDLFKLILKKEDELGDRSI MFTVQNED (SEQ ID NO: 57)
hE12	RFGKYESKLSVIRNLNDQVLFIDQGNRPLFEDMTDSDCRDNAPRTIFIIS TYKDSQPRTKAVTISVKCEKISTLSCDNKIISFKEMNPPDNIKDTKSDIIF FQRDVPGHKHKMQFESSSYEGYFLACEKERDLFKLILKKEDELGDRSI MFTVQNED (SEQ ID NO: 58)
hC5	RFGKLESRLSVIRNLNDQVLFIDQGNRPLFEDMTDSDCRDNAPRTIFIIS TYRDSQPRTKAVTISVKCEKISTLSCENKIISFKEMNPPDNIKDTKSDIIF FQRKVPGHNHKMQFESSSYEGYFLACEKERDLFKLILKKEDELGDRSI MFTVQKED (SEQ ID NO: 59)

Generation 2 Human Interleukin-18 Decoy-Resistant variants amino acid sequences

5-18	YFGKLESKLSVIRNLNDQVLFIDQGNRPLFEDMTDSDCRDNAPRTIFIIS EYKDSSELRGRAVTISVKCEKISTLSCENKIISFKEMNPPDNIKDTKSDIIF FPRAVPGHNRKVQFESSSYEGYFLACEKERDLFKLILKKEDELGDRSIM FTVQNED (SEQ ID NO: 73)
5-29	YFGKLESKLSVIRNLNDQVLFIDQGNRPLFEDMTDSDCRDNAPRTIFIIS KYKDSAGRGLAVTISVKCEKISTLSCENKIISFKEMNPPDNIKDTKSDIIF FERDVPGHNSNKVQFESSSYEGYFLACEKERDLFKLILKKEDELGDRSIM FTVQNED (SEQ ID NO: 74)
5-8	YFGKLESKLSVIRNLNDQVLFIDQGNRPLFEDMTDSDCRDNAPRTIFIIS KYGDSAARGLAVTISVKCEKISTLSCENKIISFKEMNPPDNIKDTKSDIIF FQRSVPGHKRKMVQFESSSYEGYFLACEKERDLFKLILKKEDELGDRSI MFTVQNED (SEQ ID NO: 75)
5-6	YFGKLESKLSVIRNLNDQVLFIDQGNRPLFEDMTDSDCRDNAPRTIFIIS KYGDSRGRGLAVTISVKCEKISTLSCENKIISFKEMNPPDNIKDTKSDIIF FERDVPGHNSKRQFESSSYEGYFLACEKERDLFKLILKKEDELGDRSIM FTVQNED (SEQ ID NO: 76)
5-27	YFGKLESKLSVIRNLNDQVLFIDQGNRPLFEDMTDSDCRDNAPRTIFIIS KYGDSVPRGLAVTISVKCEKISTLSCENKIISFKEMNPPDNIKDTKSDIIF FARAVPGHSRKTQFESSSYEGYFLACEKERDLFKLILKKEDELGDRSIM FTVQNED (SEQ ID NO: 77)
5-20	YFGKLESKLSVIRNLNDQVLFIDQGNRPLFEDMTDSDCRDNAPRTIFIIS KYSDSGARGLAVTISVKCEKISTLSCENKIISFKEMNPPDNIKDTKSDIIF FARAVPGHGRKTQFESSSYEGYFLACEKERDLFKLILKKEDELGDRSIM FTVQNED (SEQ ID NO: 78)
5-2	YFGKLESKLSVIRNLNDQVLFIDQGNRPLFEDMTDSDCRDNAPRTIFIIS KYSDSKARGMAVTISVKCEKISTLSCENKIISFKEMNPPDNIKDTKSDIIF

	FARDVPGHSSKRQFESSSYEGYFLACEKERDLFKLILKKEDELGDRSIM FTVQNE (SEQ ID NO: 79)
5-9	YFGKLESKLSVIRNLNDQVLFIDQGNRPLFEDMTDSDCRDNAPRTIFIIS KYSDSLARGLAVTISVKCEKISTLSCENKIISFKEMNPPDNIKDTKSDIIF FQRDVPGHRSRKMQFESSSYEGYFLACEKERDLFKLILKKEDELGDRSI MFTVQNE (SEQ ID NO: 80)
5-42	YFGKLESKLSVIRNLNDQVLFIDQGNRPLFEDMTDSDCRDNAPRTIFIIS KYSDSRARGLAVTISVKCEKISTLSCENKIISFKEMNPPDNIKDTKSDIIF FQRNVPGHGRKMQFESSSYEGYFLACEKERDLFKLILKKEDELGDRSI MFTVQNE (SEQ ID NO: 81)
5-13	YFGKLESKLSVIRNLNDQVLFIDQGNRPLFEDMTDSDCRDNAPRTIFIIS KYSDSRARGLAVTISVKCEKISTLSCENKIISFKEMNPPDNIKDTKSDIIF FARSVPGHGRKTQFESSSYEGYFLACEKERDLFKLILKKEDELGDRSIM FTVQNE (SEQ ID NO: 82)
5-12	YFGKLESKLSVIRNLNDQVLFIDQGNRPLFEDMTDSDCRDNAPRTIFIIS KYSDSRARGLAVTISVKCEKISTLSCENKIISFKEMNPPDNIKDTKSDIIF FARDVPGHSGKRQFESSSYEGYFLACEKERDLFKLILKKEDELGDRSIM FTVQNE (SEQ ID NO: 83)
5-1	YFGKLESKLSVIRNLNDQVLFIDQGNRPLFEDMTDSDCRDNAPRTIFIIS KYTDSRPRGLAVTISVKCEKISTLSCENKIISFKEMNPPDNIKDTKSDIIF FERDVPGHSSKKQFESSSYEGYFLACEKERDLFKLILKKEDELGDRSIM FTVQNE (SEQ ID NO: 84)
5-33	YFGKLESKLSVIRNLNDQVLFIDQGNRPLFEDMTDSDCRDNAPRTIFIIS KYTDSRARGMAVTISVKCEKISTLSCENKIISFKEMNPPDNIKDTKSDIIF FERDVPGHNDKRQFESSSYEGYFLACEKERDLFKLILKKEDELGDRSIM FTVQNE (SEQ ID NO: 85)
5-21	YFGKLESKLSVIRNLNDQVLFIDQGNRPLFEDMTDSDCRDNAPRTIFIIS RYKDSGKRGLAVTISVKCEKISTLSCENKIISFKEMNPPDNIKDTKSDIIF FRRSVPGHSRKVQFESSSYEGYFLACEKERDLFKLILKKEDELGDRSIM FTVQNE (SEQ ID NO: 86)
6-31	YFGKLESKLSVIRNLNDQVLFIDQGNRPLFEDMTDSDCRDNAPRTIFIIS KYGDSGARGLAVTISVKCEKISTLSCENKIISFKEMNPPDNIKDTKSDIIF FERDVPGHSGKVQFESSSYEGYFLACEKERDLFKLILKKEDELGDRSIM FTVQNE (SEQ ID NO: 87)
6-20	YFGKLESKLSVIRNLNDQVLFIDQGNRPLFEDMTDSDCRDNAPRTIFIIS KYGDSRPRGMAVTISVKCEKISTLSCENKIISFKEMNPPDNIKDTKSDIIF FQRAVPGHNRKMQFESSSYEGYFLACEKERDLFKLILKKEDELGDRSI MFTVQNE (SEQ ID NO: 88)
6-12	YFGKLESKLSVIRNLNDQVLFIDQGNRPLFEDMTDSDCRDNAPRTIFIIS KYSDSLARGLAVTISVKCEKISTLSCENKIISFKEMNPPDNIKDTKSDIIF FQRDVPGHRSRKMQFESSSYEGYFLACEKERDLFKLILKKEDELGDRSI MFTVQNE (SEQ ID NO: 89)
6-27	YFGKLESKLSVIRNLNDQVLFIDQGNRPLFEDMTDSDCRDNAPRTIFIIS KYSDSRARGLAVTISVKCEKISTLSCENKIISFKEMNPPDNIKDTKSDIIF

	FARVPGHGRKTQFESSYEGYFLACEKERDLFKLILKKEDELGDRSIM FTVQNE (SEQ ID NO: 90)
6-29	YFGKLESKLSVIRNLNDQVLFIDQGNRPLFEDMTDSDCRDNAPRTIFIIS KYSDSRARGLAVTISVKCEKISTLSCENKISFKEMNPPDNIKDTKSDIIF FQRNVPGHGRKMQFESSYEGYFLACEKERDLFKLILKKEDELGDRSI MFTVQNE (SEQ ID NO: 91)
5-26	YFGKLESKLSVIRNLNDQVLFIDQGNRPLFEDMTDSDCRDNAPRTIFIIS KYGDSVPRGLAVTISVKCEKISTLSCENKISFKEMNPPDNIKDTKSDIIF FARVPGHGRKTQFESSYEGYFLACEKERDLFKLILKKEDELGDRSIM FTVQNE (SEQ ID NO: 191)
5-17	YFGKLESKLSVIRNLNDQVLFIDQGNRPLFEDMTDSDCRDNAPRTIFIIS KYSDSRARGLAVTISVKCEKISTLSCENKISFKEMNPPDNIKDTKSDIIF FARVPGHGRKTQFESSYEGYFLACEKERDLFKLILKKEDELGDRSIM FTVQNE (SEQ ID NO: 192)
5-41	YFGKLESKLSVIRNLNDQVLFIDQGNRPLFEDMTDSDCRDNAPRTIFIIS KYSDSRARGLAVTISVKCEKISTLSCENKISFKEMNPPDNIKDTKSDIIF FARDVPGHSGKRQFESSYEGYFLACEKERDLFKLILKKEDELGDRSIM FTVQNE (SEQ ID NO: 193)

Mouse Interleukin-18 Decoy-Resistant variants amino acid sequences

mCS1	NFGRLHCTTAVIRNINDQVLFVDRQPVFEDMTDIDQSASEPQTRLIIY GYADSRVRGKAVTL SVKDSKMSTL SCKNKIISFEEMDPPENIDDIQSDL IFFQKRVP GHNKMEFESSLYEGHFLACQKEDDAFKLILKKKDENGDKS VMFTLTNLHQS (SEQ ID NO: 60)
mCS2	HFGRLHCTTAVIRNINDQVLFVDRQPVFEDMTDIDQSASEPQTRLIIY AYGDSRARGKAVTL SVKDSKMSTL SCKNKIISFEEMDPPENIDDIQSDL IFFQKRVP GHNKMEFESSLYEGHFLACQKEDDAFKLILKKKDENGDKS VMFTLTNLHQS (SEQ ID NO: 61)
mC1	NFGRLHCTTAVIRNINDQVLFVDRQPVFEDMTDIDQSASEPQTRLIIY AYVDRRLRGKAVTL SVKDSKMSTL SCKNKIISFEEMDPPENIDDIQSDL IFFQKKVP GHNKMEFESSLYEGHFLACQKEDDAFKLILKKKDENGDKS VMFTLTNLHQS (SEQ ID NO: 62)
mA12	NFGRLHCTTAVIRNINDQVLFVDRQPVFEDMTDIDQSASEPQTRLIIY YSDKHMRGKAVTL SVKDSKMSTL SCKNKIISFEEMDPPENIDDIQSDLI FFQKLVP GHNKMEFESSLYEGHFLACQKEDDAFKLILKKKDENGDKS VMFTLTNLHQS (SEQ ID NO: 63)
mE8	NFGRLHCTTAVIRNINDQVLFVDRQPVFEDMTDIDQSASEPQTRLIIY VYTDGRRRGKAVTL SVKDSKMSTL SCKNKIISFEEMDPPENIDDIQSDL IFFQKKVP GHDKMEFESSLYEGHFLACQKEDDAFKLILKKKDENGDKS VMFTLTNLHQS (SEQ ID NO: 64)

mC10	HFGRLHCTTAVIRNINDQVLFFVDRQPVFEDMTDIDQSASEPQTRLIIY AYGDSHMRGKAVTL SVKDSKMSTLSCKNKIISFEEMDPPENIDDIQSDL IFFQKQVPGHNKMEFESSLYEGHFLACQKEDDAFKLILKKKDENGDKS VMFTVTNLHQS (SEQ ID NO: 65)
mB7	HFGRLHCTTAVIRNINDQVLFFVDRQPVFEDMTDIDQSASEPQTRLIIY AYGDSNAGGRAVTL SVKDSKMSTLSCKNKIISFEEMDPPENIDDIQSDL IFFQKQVPGHNKMEFESSLYEGHFLACQKEDDAFKLILKKKDENGDKS VMFTLTNLHQS (SEQ ID NO: 66)
mB1	HFGRLHCTTAVIRNINDQVLFFVDRQPVFEDMTDIDQSASEPQTRLIIY GYADSDARAKAVTL SVKDSKMSTLSCKNKIISFEEMDPPENIDDIQSDL IFFQKSVPGHNKMEFESSLYEGHFLACQKEDDAFKLILKKKDENGDKS VMFTVTNLHQS (SEQ ID NO: 67)
mD1	HFGRLHCTTAVIRNINDQVLFFVDRQPVFEDMTDIDQSASEPQTRLIIY GYSDRGSKGKAVTL SVKDSKMSTLSCKNKIISFEEMDPPENIDDIQSDLI FFQKQVPGHNKMEFESSLYEGHFLACQKEDDAFKLILKKKDENGDKS VMFTLTNLHQS (SEQ ID NO: 68)
mH7	YFGR LHCTTAVIRNINDQVLFFVDRQPVFEDMTDIDQSASEPQTRLIIY MYADRRARGKAVTL SVKDSKMSTLSCKNKIISFEEMDPPENIDDIQSD L IFFQKQVPGHDKMEFESSLYEGHFLACQKEDDAFKLILKKKDENGDK SVMFTVTNLHQS (SEQ ID NO: 69)
mA7	YFGR LHCTTAVIRNINDQVLFFVDRQPVFEDMTDIDQSASEPQTRLIIY AYGDNVRGKAVTL SVKDSKMSTLSCKNKIISFEEMDPPENIDDIQSDL IFFQKRVP GHNKMEFESSLYEGHFLACQKEDDAFKLILKKKDENGDKS VMFTLTNLHQS (SEQ ID NO: 70)
mE1	YFGR LHCTTAVIRNINDQVLFFVDRQPVFEDMTDIDQSASEPQTRLIIY GYGDSERGGRAVTL SVKDSKMSTLSCKNKIISFEEMDPPENIDDIQSDLI FFQKRVP GHDKMEFESSLYEGHFLACQKEDDAFKLILKKKDENGDKS VMFTLTNLHQS (SEQ ID NO: 71)
mH3	YFGR LHCTTAVIRNINDQVLFFVDRQPVFEDMTDIDQSASEPQTRLIIY TRTDGGQKGVAVTL SVKDSKMSTLSCKNKIISFEEMDPPENIDDIQSDL IFFQKRVP GHDKMEFESSLYEGHFLACQKEDDAFKLILKKKDENGDKS VMFTLTNLHQS (SEQ ID NO: 72)

Human Decoy-to-the-Decoy (D2D) variants amino acid sequences

hD2D-5F12	HFGKLESKLSVIRNLNGQVLFDQGNRPLFKDMTASDCRANAPR TIFIISFYKDSQPRGMAVTISVKCEKISTLSCENKIISFKEMNPPDNI KDTKSDIIFIRSVPGADNKFQFESSSYEGYFLACEKERDLFKLIL KKEDELGDRSIMFTVQNE (SEQ ID NO: 92)
hD2D-5F11	DFGKLESKLSVIRNLNDQVLFDQGNRPLFADMTDNPCRSNAPR TIFIISFYKDSQPRGIAVTISVKCEKISTLSCENKIISFKEMNPPDNIK DTKSDIIFLRSVPGPDNKMQFESSSYEGYFLACEKERDLFKLILK KEDELGDRSIMFTVQNE (SEQ ID NO: 93)
hD2D-5F10	HFGKLESKLSVIRNLNGQVLFDQGNRPLFADMEASPCRDNAPR TIFIISFYKDSQPRGLAVTISVKCEKISTLSCENKIISFKEMNPPDNI

	KDTKSDIIFLRSVPGHDNKMQFESSSYEGYFLACEKERDLFKLIL KKEDELGDRSIMFTVQNE (SEQ ID NO: 94)
hD2D-5F08	LFGKLESKLSVIRNLNGQVLFIDQGNRPLFADMTSSPCRSRAPRTI FIISFYKDSQPRGFAVTISVKCEKISTLSCENKIISFKEMNPPDNIK TKSDIIFIRSVPGHDNKIQFESSSYEGYFLACEKERDLFKLILKKE DELGDRSIMFTVQNE (SEQ ID NO: 95)
hD2D-5F06	HFGKLESKLSVIRNLNDQVLFIDQGNRPLFTDMESKPCRDSAPRT IFIISMYKDSQPRGIAVTISVKCEKISTLSCENKIISFKEMNPPDNIK DTKSDIIFIRSVPGHDNKFQFESSSYEGYFLACEKERDLFKLILK KEDELGDRSIMFTVQNE (SEQ ID NO: 96)
hD2D-5F04	YFGKLESKLSVIRNLNRQVLFIDQGNRPLFTDMTYKDCRDNAPR TIFIISFYKDSQPRGFAVTISVKCEKISTLSCENKIISFKEMNPPDNI KDTKSDIIFIRSVPGADNKIQFESSSYEGYFLACEKERDLFKLILK KEDELGDRSIMFTVQNE (SEQ ID NO: 97)
hD2D-5F02	HFGKLESKLSVIRNLNGQVLFIDQGNRPLFGDMEASPCRDNAPR TIFIISFYKDSQPRGMAVTISVKCEKISTLSCENKIISFKEMNPPDNI KDTKSDIIFIRSVPGADNKLQFESSSYEGYFLACEKERDLFKLIL KKEDELGDRSIMFTVQNE (SEQ ID NO: 98)
hD2D-5F01	HFGKLESKLSVIRNLNGQVLFIDQGNRPLFTDMTSSDCRDKAPRT IFIISFYKDSQPRGLAVTISVKCEKISTLSCENKIISFKEMNPPDNIK DTKSDIIFLRSVPGPDNKFQFESSSYEGYFLACEKERDLFKLILK KEDELGDRSIMFTVQNE (SEQ ID NO: 99)
hD2D-5E10	HFGKLESKLSVIRNLNGQVLFIDQGNRPLFADMESNRCRDSAPRT IFIISMYKDSQPRGFAVTISVKCEKISTLSCENKIISFKEMNPPDNIK DTKSDIIFLRSVPGHDNKIQFESSSYEGYFLACEKERDLFKLILK KEDELGDRSIMFTVQNE (SEQ ID NO: 100)
hD2D-5E08	YFGKLESKLSVIRNLNGQVLFIDQGNRPLFTDMTASPCRDNAPRT IFIISFYKDSQPRGLAVTISVKCEKISTLSCENKIISFKEMNPPDNIK DTKSDIIFLRSVPGHDNKIQFESSSYEGYFLACEKERSLFLKLILKK EDELGDRSIMFTVQNE (SEQ ID NO: 101)
hD2D-5E03	DFGKLESKLSVIRNLNDQVLFIDQGNRPLFADMKSNVCRANAPR TIFIISMYKDSQPRGMAVTISVKCEKISTLSCENKIISFKEMNPPDN IKDTKSDIIFIRSVPGPDNKLQFESSSYEGYFLACEKERDLFKLIL KKEDELGDRSIMFTVQNE (SEQ ID NO: 102)
hD2D-5E02	HFGKLESKLSVIRNLNGQVLFIDQGNRPLFGDMEASPCRAKAPR TIFIISFYKDSQPRGFAVTISVKCEKISTLSCENKIISFKEMNPPDNIK DTKSDIIFLRSVPGHDNKFQFESSSYEGYFLACEKERSLFLKLILK KEDELGDRSIMFTVQNE (SEQ ID NO: 103)
hD2D-5D10	HFGKLESKLSVIRNLNGQVLFIDQGNRPLFADMASNRCRANAPR TIFIISMYKDSQPRGFAVTISVKCEKISTLSCENKIISFKEMNPPDNI KDTKSDIIFIRSVPGPDNKFQFESSSYEGYFLACEKERDLFKLILK KEDELGDRSIMFTVQNE (SEQ ID NO: 104)
hD2D-5D08	YFGKLESKLSVIRNLNDQVLFIDQGNRPLFADMKAKACRSNAPR TIFIISFYKDSQPRGFAVTISVKCEKISTLSCENKIISFKEMNPPDNI

	KDTKSDIIFFLRSVPGADNKKIQFESSSYEGYFLACEKERDLFKLIL KKEDELGDRSIMFTVQNED (SEQ ID NO: 105)
hD2D-5D06	HFGKLESKLSVIRNLNHQVLFIDQGNRPLFTDMADNACRDNAPR TIFIISFYKDSQPRGLAVTISVKCEKISTLSCENKIISFKEMNPPDNI KDTKSDIIFFLRSVPGDDNKMVFESSSYEGYFLACEKERDLFKLIL KKEDELGDRSIMFTVQNED (SEQ ID NO: 106)
hD2D-5D05	YFGKLESKLSVIRNLNGQVLFIDQGNRPLFTDMKSNLCRSNAPRT IFIISFYKDSQPRGIAVTISVKCEKISTLSCENKIISFKEMNPPDNIK DTKSDIIFFLRSVPGDDNKKIQFESSSYEGYFLACEKERDLFKLILKK EDELGDRSIMFTVQNED (SEQ ID NO: 107)
hD2D-5D03	HFGKLESKLSVIRNLNGQVLFIDQGNRPLFRDMAASHCRDSAPR TIFIISYKDSQPRGFAVTISVKCEKISTLSCENKIISFKEMNPPDNIK DTKSDIIFFLRSVPGHDNKKIQFESSSYEGYFLACEKERDLFKLILK KEDELGDRSIMFTVQNED (SEQ ID NO: 108)
hD2D-5D02	YFGKLESKLSVIRNLNDQVLFIDQGNRPLFADMASNPCRYKAPR TIFIISMYKDSQPRGLAVTISVKCEKISTLSCENKIISFKEMNPPDNI KDTKSDIIFFLRSVPGADNKLQFESSSYEGYFLACEKERDLFKLIL KKEDELGDRSIMFTVQNED (SEQ ID NO: 109)
hD2D-5C10	HFGKLESKLSVIRNLNGQVLFIDQGNRPLFTDMASNHCRYNAPR TIFIISMYKDSQPRGLAVTISVKCEKISTLSCENKIISFKEMNPPDNI KDTKSDIIFFLRSVPGADNKKIQFESSSYEGYFLACEKERDLFKLIL KKEDELGDRSIMFTVQNED (SEQ ID NO: 110)
hD2D-5C09	HFGKLESKLSVIRNLNGQVLFIDQGNRPLFADMTDNPCRSRAPRT IFIISFYKDSQPRGMAVTISVKCEKISTLSCENKIISFKEMNPPDNIK DTKSDIIFFLRSVPGHDNKFQFESSSYEGYFLACEKERDLFKLILK KEDELGDRSIMFTVQNED (SEQ ID NO: 111)
hD2D-5C08	YFGKLESKLSVIRNLNGQVLFIDQGNRPLFTDMTASHCRSSAPRT IFIISLYKDSQPRGMAVTISVKCEKISTLSCENKIISFKEMNPPDNIK DTKSDIIFFLRSVPGHDNKFQFESSSYEGYFLACEKERDLFKLILK KEDELGDRSIMFTVQNED (SEQ ID NO: 112)
hD2D-5C05	YFGKLESKLSVIRNLNGQVLFIDQGNRPLFTDMEYRLCRANAPR TIFIISFYKDSHPRGLAVTISVKCEKISTLSCENKIISFKEMNPPDNI KDTKSDIIFFLRSVPGDDNKLQFESSSYEGYFLACEKERDLFKLIL KKEDELGDRSIMFTVQNED (SEQ ID NO: 113)
hD2D-5C04	YFGKLESKLSVIRNLNGQVLFIDQGNRPLFTDMESSLCRDNAPRT IFIISLYKDSQPRGMAVTISVKCEKISTLSCENKIISFKEMNPPDNIK DTKSDIIFFLRSVPGADNKFQFESSSYEGYFLACEKERSLFLKILK KEDELGDRSIMFTVQNED (SEQ ID NO: 114)
hD2D-5C03	YFGKLESKLSVIRNLNGQVLFIDQGNRPLFKDMEANDCRSSAPR TIFIISYKDSQPRGLAVTISVKCEKISTLSCENKIISFKEMNPPDNIK DTKSDIIFFLRSVPGADNKMVFESSSYEGYFLACEKERDLFKLILK KEDELGDRSIMFTVQNED (SEQ ID NO: 115)
hD2D-5B11	DFGKLESKLSVIRNLNDQVLFIDQGNRPLFADMKASACRANAPR TIFIISMYKDSQPRGLAVTISVKCEKISTLSCENKIISFKEMNPPDNI

	KDTKSDIIFFLRSVPGHDNKFQFESSSYEGYFLACEKERDLFKLIL KKEDELGDRSIMFTVQNED (SEQ ID NO: 116)
hD2D-5B10	YFGKLESKLSVIRNLNGQVLFIDQGNRPLFGDMTAKHCRARAPR TIFIISFYKDSQPRGMAVTISVKCEKISTLSCENKIISFKEMNPPDNI KDTKSDIIFFLRSVPGADNKFQFESSSYEGYFLACEKERDLFKLIL KKEDELGDRSIMFTVQNED (SEQ ID NO: 117)
hD2D-5B06	FFGKFESKLSVIRNLNGQVLFIDQGNRPLFTDMESKDCRDRAPRT IFIISFYKDSQPRGLAVTISVKCEKISTLSCENKIISFKEMNPPDNIK DTKSDIIFFLRSVPGHDNKLQFESSSYEGYFLACEKERDLFKLILK KEDELGDRSIMFTVQNED (SEQ ID NO: 118)
hD2D-5B05	FFGKLESKLSVIRNLNGQVLFIDQGNRPLFADMASNHCRANAPR TIFIISLYKDSQPRGLAVTISVKCEKISTLSCENKIISFKEMNPPDNI KDTKSDIIFFLRSVPGHDNKMQFESSSYEGYFLACEKERDLFKLIL KKEDELGDRSIMFTVQNED (SEQ ID NO: 119)
hD2D-5B02	YFGKLESKLSVIRNLNGQVLFIDQGNRPLFADMTSKRCRDNAPR TIFIISLYKDSQPRGFAVTISVKCEKISTLSCENKIISFKEMNPPDNI KDTKSDIIFFLRSVPGHDNKLQFESSSYEGYFLACEKERDLFKLILK KEDELGDRSIMFTVQNED (SEQ ID NO: 120)
hD2D-5A09	LFGKHESKLSVIRNLNGQVLFIDQGNRPLFGDMESSPCRYNAPRT IFIISFYKDSQPRGLAVTISVKCEKISTLSCENKIISFKEMNPPDNIK DTKSDIIFFLRSVPGHDNKMQFESSSYEGYFLACEKERDLFKLILK KEDELGDRSIMFTVQNED (SEQ ID NO: 121)
hD2D-5A02	YFGKLESKLSVIRNLNAQVLFIDQGNRPLFTDMTASPCRSSAPRTI FIISLYKDSQPRGLAVTISVKCEKISTLSCENKIISFKEMNPPDNIK DTKSDIIFFLRSVPGPDNKLQFESSSYEGYFLACEKERDLFKLILKK EDELGDRSIMFTVQNED (SEQ ID NO: 122)
hD2D-CS1	YFGKLESKLSVIRNLNGQVLFIDQGNRPLFADMTSDSCRDNAPR TIFIISMYKDSQPRGMAVTISVKCEKISTLSCENKIISFKEMNPPDN IKDTKSDIIFFLRSVPGHDNKMQFESSSYEGYFLACEKERDLFKLI LKKDELGDRSIMFTVQNED (SEQ ID NO: 123)
hD2D-CS2	YFGKLESKLSVIRNLNGQVLFIDQGNRPLFADMTSSDCRDNAPR TIFIISFYKDSQPRGMAVTISVKCEKISTLSCENKIISFKEMNPPDNI KDTKSDIIFFLRSVPGHDNKMQFESSSYEGYFLACEKERDLFKLIL KKEDELGDRSIMFTVQNED (SEQ ID NO: 124)
hD2D-CS3	YFGKLESKLSVIRNLNGQVLFIDQGNRPLFADMESSDCRDNAPR TIFIISFYKDSQPRGLAVTISVKCEKISTLSCENKIISFKEMNPPDNI KDTKSDIIFFLRSVPGHDNKMQFESSSYEGYFLACEKERDLFKLIL KKEDELGDRSIMFTVQNED (SEQ ID NO: 125)

Mouse Decoy-to-the-Decoy (D2D) variants amino acid sequences

mD2D-A5	YFGRYHCTTAVIRNINQVLFVDKRQPVFADMGYTVQSASEPQT RLIIYMYKDSEVRGLAVTL SVKDSKMSTLSCKNKIISFEEMDPPE NIDDIQSDLIFFLKEVPGHRKLEFESSLYEGHFLACQKEDEAFKLI LKKKDENGDKSVMFTLTNLHQS (SEQ ID NO: 126)
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mD2D-A6	DFGRLHCTTAVIRNINDQVLFVDKRQPVFADMGSIAQSASEPQT RLIIYFYKDSEVRGLAVTLSVKDSKMYTLSCKNKIISFEEMDPPE NIDDIQSDLIFFLKAVPGDNKIEFESSLYEGHFLACQKEATAFKLI LKKKDENGDKSVMFTLTNLHQS (SEQ ID NO: 127)
mD2D-A7	YFGRLHCTTAVIRNINGQVLFVDKRQPVFRDMADTVQSASEPQT RLIIYFYKDSEVRGLAVTLSVKDSKMSTLSCKNKIISFEEMDPPE IDDIQSDLIFFIKPVPGASKMEFESSLYEGHFLACQKEAGAFKLIL KKKDENGDKSVMFTLTNLHQS (SEQ ID NO: 128)
mD2D-A8	HFGRLHCTTAVIRNINDQVLFVDKRQPVFKDMEYTVQSASEPQT RLIIYMYKDSEVRGLAVTLSVKDSKMSTLSCKNKIISFEEMDPPE NIDDIQSDLIFFIKAVPGDRKIEFESSLYEGHFLACQKEDNAFKLIL KKKDENGDKSVMFTLTNLHQS (SEQ ID NO: 129)
mD2D-A9	YFGRLHCTTAVIRNINAQVLFVDKRQPVFADMADKGQSASEPQT RLIIYMYKDSEVRGLAVTLSVKDSKMSTLSCKNKIISFEEMDPPE NIDDIQSDLIFFLKPVPGDTKMEFESSLYEGHFLACQKEFGAFKLI LKKKDENGDKSVMFTLTNLHQS (SEQ ID NO: 130)
mD2D-A11	YFGRLHCTTAVIRNINEQVLFVDKRQPVFADMGDRHQSAASEPQT RLIIYMYKDSEVRGLAVTLSVKDSKMSTLSCKNKIISFEEMDPPE NIDDIQSDLIFFIKPVPGASKLEFESSLYEGHFLACQKEDDAFKLIL KKKDENGDKSVMFTLTNLHQS (SEQ ID NO: 131)
mD2D-A12	HFGRLHCTTAVIRNINDQVLFVDKRQPVFRDMGAIGQSASEPQT RLIIYFYKDSEVRGLAVTLSVKDSKMSTLSCKNKIISFEEMDPPE IDDIQSDLIFFIKPVPGDSKLEFESSLYEGHFLACQKEVDAFKLIL KKDENGDKSVMFTLTNLHQS (SEQ ID NO: 132)
mD2D-B4	HFGRLHCTTAVIRNINSQVLFVDKRQPVFTDMGSIVQSASEPQTR LIIYMYKDSEVRGLAVTLSVKDSKMSTLSCKNKIISFEEMDPPE DDIQSDLIFFIKGVPGDKNKIEFESSLYEGHFLACQKEDRAFKLIL KKDENGDKSVMFTLTNLHQS (SEQ ID NO: 133)
mD2D-B7	YFGRLHCTTAVIRNINSQVLFVDKRQPVFRDMEDTPQSASEPQTR LIIYFYKDSEVRGLAVTLSVKDSKMSTLSCKNKIISFEEMDPPE DDIQSDLIFFIKRVPGDSKLEFESSLYEGHFLACQKEFEAFKLIL KKDENGDKSVMFTLTNLHQS (SEQ ID NO: 134)
mD2D-B11	HFGRLHCTTAVIRNINAQVLFVDKRQPVFGDMTATVQSASEPQT RLIIYFYKDSEVRGLAVTLSVKDSKMSTLSCKNKIISFEEMDPPE IDDIQSDLIFFIKPVPGDSKLEFESSLYEGHFLACQKEDNAFKLIL KKDENGDKSVMFTLTNLHQS (SEQ ID NO: 135)
mD2D-B12	NFGRLHCTTAVIRNINNQVLFVDKRQPVFKDMEYTLQSASEPQT RLIIYFYKDSEVRGLAVTLSVKDSKMSTLSCKNKIISFEEMDPPE IDDIQSDLIFFIKPVPGDKNKLEFESSLYEGHFLACQKEYEAFKLIL KKDENGDKSVMFTLTNLHQS (SEQ ID NO: 136)
mD2D-C1	YFGRLHCTTAVIRNINGQVLFVDKRQPVFADMEATRQSASEPQT RLIIYFYKDSEVRGLAVTLSVKDSKMSTLSCKNKIISFEEMDPPE IDDIQSDLIFFIKGVPGANKMEFESSLYEGHFLACQKEDGAFKLIL KKKDENGDNVSMFTLTNLHQS (SEQ ID NO: 137)

mD2D-C3	NFGRLHCTTAVIRNINGQVLFVDKRQPVFADMRAILQSASEPQT RLIIYFYKDSEVRGLAVTL SVKDSKMSTLSCKNKIISFEEMDPPE IDDIQSDLIFFLKGVPGDNKLEFESSLYEGHFLACQKEDRAFKLIL KKKDENGDKSVMFTLTNLHQS (SEQ ID NO: 138)
mD2D-C5	YFGRHCTTAVIRNINAQVLFVDKRQPVFADMEATAQSASEPQT RLIIYFYKDSEVRGLAVTL SVKDSKMSTLSCKNKIISFEEMDPPE IDDIQSDLIFFIKGVPGASKMEFESSLYEGHFLACQKEDGAFAKLIL KKKDENGDKSVMFTLTNLHQS (SEQ ID NO: 139)
mD2D-C6	LFGRLHCTTAVIRNINGQVLFVDKRQPVFADMGATLQSASEPQT RLIIYMYKDSEVRGLAVTL SVKDSKMSTLSCKNKIISFEEMDPPE NIDDIQSDLIFFLKVPVPGDTKMEFESSLYEGHFLACQKEASAFKLI LKKKDENGDKSVMFTLTNLHQS (SEQ ID NO: 140)
mD2D-C9	NFGRLHCTTAVIRNINGQVLFVDKRQPVFEDMAYTVQSASEPQT RLIIYFYKDSEVRGLAVTL SVKDSKMSTLSCKNKIISFEEMDPPE IDDIQSDLIFFIKGVPGDSKMEFESSLYEGHFLACQKEYDAFAKLIL KKKDENGDKSVMFTLTNLHQS (SEQ ID NO: 141)
mD2D-C10	DFGRHCTTAVIRNINDQVLFVDKRQPVFKDMESKPQSASEPQT RLIIYFYKDSEVRGLAVTL SVKDSKMSTLSCKNKIISFEEMDPPE IDDIQSDLIFFLKAVPGASKLEFESSLYEGHFLACQKEANAFKLIL KKKDENGDKSVMFTLTNLHQS (SEQ ID NO: 142)
mD2D-C11	LFGRLHCTTAVIRNINGQVLFVDKRQPVFADMGDVQSASEPQT RLIIYMYKDSEVRGLAVTL SVKDSKMSTLSCKNKIISFEEMDPPE NIDDIQSDLIFFIKVPVPGDNKLEFESSLYEGHFLACQKEDEAFKLIL KTKDENGDKSVMFTLTNLHQS (SEQ ID NO: 143)
mD2D-D1	YFGRHHCTTAVIRNINQVLFVDKRQPVFRDMAATRQSASEPQT RLIIYMYKDSEVRGLAVTL SVKDSKMSTLSCKNKIISFEEMDPPE NIDDIQSDLIFFLKGVPGDNKMEFESSLYEGHFLACQKEDDAFAKL ILKKKDENGDKSVMFTLTNLHQS (SEQ ID NO: 144)
mD2D-D9	NFGRLHCTTAVIRNINQVLFVDKRQPVFTDMESIGQSASEPQTR LIIYFYKDSEVRGLAVTL SVKDSKMSTLSCKNKIISFEEMDPPE DDIQSDLIFFLKAVPGANKLEFESSLYEGHFLACQKEDSAFAKLIL KKDENGDKSVMFTLTNLHQS (SEQ ID NO: 145)
mD2D-D12	FFGRHHCTTAVIRNINGQVLFVDKRQPVFGDMGDRVQSASEPQT RLIIYMYKDSEVRGLAVTL SVKDSKMSTLSCKNKIISFEEMDPPE NIDDIQSDLIFFIKAVPGDSKIEFESSLYEGHFLACQKEDGAFAKLIL KKKDENGDKSVMFTLTNLHQS (SEQ ID NO: 146)
mD2D-E3	VFGRHHCTTAVIRNINGQVLFVDKRQPVFKDMTYIDQSASEPQT RLIIYMYKDSEVRGLAVTL SVKDSKMSTLSCKNKIISFEEMDPPE NIDDIQSDLIFFLKAVPGDTKMEFESSLYEGHFLACQKEAQAFAKLI LKKKDEIGDKSVMFTLTNLHQS (SEQ ID NO: 147)
mD2D-E4	NFGRLHCTTAVIRNINGQVLFVDKRQPVFADMTATRQSASEPQT RLIIYMYKDSEVRGLAVTL SVKDSKMSTLSCKNKIISFEEMDPPE NIDDIQSDLIFFIKQVPGANKIEFESSLYEGHFLACQKEFRAFAKLIL KKKDENGDKSVMFTLTNLHQS (SEQ ID NO: 148)

mD2D-E5	DFGRLHCTTAVIRNINGQVLFVDKRQPVFGDMAYIGQSASEPQT RLIIYFYKDSEVRGLAVTLSVKDSKMSTLSCKNKIISFEEMDPPEN IDDIQSDLIFFIKA VPGH斯基EFESSLYEGHFLACQKESGAFKLILK KKDENGDKSVMFTLTNLHQS (SEQ ID NO: 149)
mD2D-E7	YFGRLHCTTAVIRNINDQVLFVDKRQPVFRDMGSIAQSASEPQTR LIIYMYKDSEVRGLAVTLSVKDSKMSTLSCKNKIISFEEMDPPENI DDIQSDLIFFIKPVPGATKLEFESSLYEGHFLACQKEDGAFKLILK KKDENGDN SVMFTLTNLHQS (SEQ ID NO: 150)
mD2D-E8	YFGRLHCTTAVIRNINEQVLFVDKRQPVFTDMEAIQSASEPQTR LIIYFYKDSEVRGLAVTLSVKDSKMSTLSCKNKIISFEEMDPPENI DDIQSDLIFFIKGVPGDRKMEFESSLYEGHFLACQKEDGAFKLIL KKKDENGDKSVMFTLTNLHQS (SEQ ID NO: 151)
mD2D-E9	FFGRLHCTTAVIRNINNQVLFVDKRQPVFEDMEYRLQSASEPQT RLIIYMYKDSEVRGLAVTLSVKDSKMSTLSCKNKIISFEEMDPPE NIDDIQSDLIFFLKPVPGASKLEFESSLYEGHFLACQKESDAFKLIL KKKDENGDKSVMFTLTNLHQS (SEQ ID NO: 152)
mD2D-E10	NFGRLHCTTAVIRNINNQVLFVDKRQPVFADMEDRLQSASEPQT RLIIYMYKDSEVRGLAVTLSVKDSKMSTLSCKNKIISFEEMDPPE NIDDIQSDLIFFLKGVPGDNKMEFESSLYEGHFLACQKEDHAFKL ILKKKDENGDKSVMFTLTNLHQS (SEQ ID NO: 153)
mD2D-E11	YFGRLHCTTAVIRNINAQVLFVDKRQPVFRDMGYILQSASEPQT RLIIYLKDYKDSEVRGLAVTLSVKESKMSTLSCKNKIISFEEMDPPEN IDDIQSDLIFFLKPVPGDTKIEFESSLYEGHFLACQKEDNAFKLILK KKDENGDKSVMFTLTNLHQS (SEQ ID NO: 154)
mD2D-E12	YFGRLHCTTAVIRNINDQVLFVDKRQPVFGDMADTAQSASEPQT RLIIYFYKDSEVRGLAVTLSVKDSKMSTLSCKNKIISFEEMDPPEN IDDIQSDLIFFIKPVPGDSKMEFESSLYEGHFLACQKEADAFKLIL KKKDENGDKSVMFTLTNLHQS (SEQ ID NO: 155)
mD2D-F3	DFGRLHCTTAVIRNINGQVLFVDKRQPVFEDMAYIAQSASEPQT RLIIYFYKDSEVRGLAVTLSVKDSKMSTLSCKNKIISFEEMDPPEN IDDIQSDLIFFIKPVPGDSKIEFESSLYEGHFLACQKEADAFKLILK KKDENGDKSVMFTLTNLHQS (SEQ ID NO: 156)
mD2D-F4	NFGRLHCTTAVIRNINEQVLSVDKRQPVFRDMKYILQSASEPQTR LIIYFYKDSEVRGLAVTLSVKDSKMSTLSCKNKIISFEEMDPPENI DDIQSDLIFFLKGVPGDNKMEFESSLYEGHFLACQKEYGAFKLIL KKKDENGDKSVMFTLTNLHQS (SEQ ID NO: 157)
mD2D-F5	DFGRLHCTTAVIRNINEQVLFVDKRQPVFTDMAYILQSASEPQTR LIIYFYKDSEVRGLAVTLSVKESKMSTLSCKNKIISFEEMDPPENI DDIQSDLIFFIKA VPGDSKLEFESSLYEGHFLACQKEDTAFKLILK KKDENGDKSVMFTLTNLHQS (SEQ ID NO: 158)
mD2D-F7	DFGRLHCTTAVIRNINNQVLFVDKRQPVFKDMESTAQSASEPQT RLIIYMYKDSEVRGLAVTLSVKDSKMSTLSCKNKIISFEEMDPPE NIDDIQSDLIFFLKGVPGASKLEFESSLYEGHFLACQKEAGAFKLI LKKKDENGDKSVMFTLTNLHQS (SEQ ID NO: 159)

mD2D-F8	HFGR LHCTTAVIRNINEQVLFVDKRQPVFADMEAIQSSASEPQTR LIIYFYKDSEVRGLAVTL SVKESKMSTLSCKNKIISFEEMDPDENI DDIQSDLIFFIKGVPGDTKLEFESSLYAGHFLACQKEDGAFKLILK KKDENGDKSVMFTLTNLHQS (SEQ ID NO: 160)
mD2D-F9	IFGR LHCTTAVIRNINEQVLFVDKRQPVFKDMRYIVQSASEPQTR LIIYFYKDSEVRGLAVTL SVKDSKMSTLSCKNKIISFEEMDPDENI DDIQSDLIFFIKEVPGASKLEFESSLYEGHFLACQKEDAEAFKLILK KKDENGDKSVMFTLTNLHQS (SEQ ID NO: 161)
mD2D-G1	YFGR LHCTTAVIRNINAQVLFVDKRQPVFTDMGYTLQSASEPQT RLIIYLYKDSEVRGLAVTL SVKDSKMSTLSCKNKIISFEEMDPDENI IDDIQSDLIFFIKVPVGHNKIEFESSLYEGHFLACQKEDRAFKLILK KKDENGDKSVMFTLTNLHQS (SEQ ID NO: 162)
mD2D-G7	NFGR LHCTTAVIRNINNQVLFVDKRQPVFRDMASTAQSASEPQT RLIIYMYKDSEVRGLAVTL SVKDSKMSTLSCKNKIISFEEMDPPE NIDDIQSDLIFFIKGVPGANKIEFESSLYEGHFLACQKEDDAFKLIL KKDENGDKSVMFTLTNLHQS (SEQ ID NO: 163)
mD2D-G9	DFGR LHCTTAVIRNINGQVLFVDKRQPVFEDMKDRAQSASEPQT RLIIYFYKDSEVRGLAVTL SVKDSKMSTLSCKNKIISFEEMDPDENI IDDIQSDLIFFLKA VPGH SKMEFESSLYEGHFLACQKEDAEAFKLIL KKDENGDKSVMFTLTNLHQS (SEQ ID NO: 164)
mD2D-H7	NFGR LHCTTAVIRNINEQVLFVDKRQPVFADMTDIAQSASEPQTR LIIYMYKDSEVRGLAVTL SVKESKMSTLSCKNKIISFEEMDPDENI DDIQSDLIFFLKPVP GDIKMEFESSLYEGHFLACQKEYGAFKLILK KKDENGDN SVMFTLTNLHQS (SEQ ID NO: 165)
mD2D-E1	YFGR LHCTTAVIRNINEQVLFVDKRQPVFADMTDTLQSASEPQT RLIIYFYKDSEVRGLAVTL SVKDSKMSTLSCKNKIISFEEMDPDENI IDDIQSDLIFFLKGVP GDNKMEFESSLYEGHFLACQKEDTAFKLIL KKDENGDKSVMFTLTNLHQS (SEQ ID NO: 166)
mD2D-G8	YFGR LHCTTAVIRNINEQVLFVDKRQPVFADMTDTLQSASEPQT RLIIYFYKDSEVRGLAVTL SVKDSKMSTLSCKNKIISFEEMDPDENI IDDIQSDLIFFLKGVP GDNKMEFESSLYEGHFLACQKEDTAFKLIL KKDENGDKSVMFTLTNLHQS (SEQ ID NO: 167)
mD2D-H3	YFGR LHCTTAVIRNINEQVLFVDKRQPVFADMTDTLQSASEPQT RLIIYFYKDSEVRGLAVTL SVKDSKMSTLSCKNKIISFEEMDPDENI IDDIQSDLIFFLKGVP GDNKMEFESSLYEGHFLACQKEDTAFKLIL KKDENGDKSVMFTLTNLHQS (SEQ ID NO: 168)
mD2D-A10	HFGR LHCTTAVIRNINGQVLFVDKRQPVFKDMKYIVQSASEPQT RLIIYMYKDSEVRGLAVTL SVKDSKMSTLSCKNKIISFEEMDPPE NIDDIQSDLIFFLKA VPGH SKIEFESSLYEGHFLACQKEDSAFKLIL KKDENGDKSVMFTLTNLHQS (SEQ ID NO: 169)
mD2D-H1	HFGR LHCTTAVIRNINGQVLFVDKRQPVFKDMKYIVQSASEPQT RLIIYMYKDSEVRGLAVTL SVKDSKMSTLSCKNKIISFEEMDPPE NIDDIQSDLIFFLKA VPGH SKIEFESSLYEGHFLACQKEDSAFKLIL KKDENGDKSVMFTLTNLHQS (SEQ ID NO: 170)

mD2D-F12	YFGRLHCTTAVIRNINGQVLFVDKRQPVFEDMKAKAQSASEPQT RLIIYFYKDSEVRGLAVTLVKDSKMSTLSCKNKIISFEEMDPPEN IDDIQSDLIFFIKPVPKGASKMEFESSLYEGHFLACQKEDGAFKLIL KKKDENGDKSVMFTLTNLHQS (SEQ ID NO: 171)
mD2D-G10	YFGRLHCTTAVIRNINGQVLFVDKRQPVFEDMKAKAQSASEPQT RLIIYFYKDSEVRGLAVTLVKDSKMSTLSCKNKIISFEEMDPPEN IDDIQSDLIFFIKPVPKGASKMEFESSLYEGHFLACQKEDGAFKLIL KKKDENGDKSVMFTLTNLHQS (SEQ ID NO: 172)
mD2D-G12	YFGRLHCTTAVIRNINGQVLFVDKRQPVFEDMKAKAQSASEPQT RLIIYFYKDSEVRGLAVTLVKDSKMSTLSCKNKIISFEEMDPPEN IDDIQSDLIFFIKPVPKGASKMEFESSLYEGHFLACQKEDGAFKLIL KKKDENGDKSVMFTLTNLHQS (SEQ ID NO: 173)
mD2D-E2	LFGRHCTTAVIRNINGQVLFVDKRQPVFGDMGSIPQSASEPQTR LIIYFYKDSEVRGLAVTLVKDSKMSTLSCKNKIISFEEMDPPENI DDIQSDLIFFIKHVPKGATKMEFESSLYEGHFLACQKEDNAFKLILK KKDENGDKSVMFTLTNLHQS (SEQ ID NO: 174)
mD2D-G11	LFGRHCTTAVIRNINGQVLFVDKRQPVFGDMGSIPQSASEPQTR LIIYFYKDSEVRGLAVTLVKDSKMSTLSCKNKIISFEEMDPPENI DDIQSDLIFFIKHVPKGATKMEFESSLYEGHFLACQKEDNAFKLILK KKDENGDKSVMFTLTNLHQS (SEQ ID NO: 175)
mD2D-C4	YFGRLHCTTAVIRNINSQVLFVDKRQPVFTDMAYTVQSASEPQT RLIIYFYKDSEVRGLAVTLVKDSKMSTLSCKNKIISFEEMDPPEN IDDIQSDLIFFIKAVPGDSKLEFESSLYEGHFLACQKEDNAFKLILK KKDENGDKSVMFTLTNLHQS (SEQ ID NO: 176)
mD2D-F11	YFGRLHCTTAVIRNINSQVLFVDKRQPVFTDMAYTVQSASEPQT RLIIYFYKDSEVRGLAVTLVKDSKMSTLSCKNKIISFEEMDPPEN IDDIQSDLIFFIKAVPGDSKLEFESSLYEGHFLACQKEDNAFKLILK KKDENGDKSVMFTLTNLHQS (SEQ ID NO: 177)
mD2D-C2	YFGRLHCTTAVIRNINGQVLFVDKRQPVFTDMGARVQSASEPQT RLIIYFYKDSEVRGLAVTLVKDSKMYTSLCKNKIISFEEMDPPE NIDDIQSDLIFFLKPVPKGNKLEFESSLYEGHFLACQKESGAFKLI LKKKDENGDKSVMFTLTNLHQS (SEQ ID NO: 178)
mD2D-F10	YFGRLHCTTAVIRNINGQVLFVDKRQPVFTDMGARVQSASEPQT RLIIYFYKDSEVRGLAVTLVKDSKMYTSLCKNKIISFEEMDPPE NIDDIQSDLIFFLKPVPKGNKLEFESSLYEGHFLACQKESGAFKLI LKKKDENGDKSVMFTLTNLHQS (SEQ ID NO: 179)
mD2D-A2	DFGRHCTTAVIRNINGQVLFVDKRQPVFGDMKATGQSASEPQT RLIIYFYKDSEVRGLAVTLVKDSKMSTLSCKNKIISFEEMDPPEN IDDIQSDLIFFIKAVPGANKLEFESSLYEGHFLACQKEAGAFKLIL KKKDENGDKSVMFTLTNLHQS (SEQ ID NO: 180)
mD2D-F6	DFGRHCTTAVIRNINGQVLFVDKRQPVFGDMKATGQSASEPQT RLIIYFYKDSEVRGLAVTLVKDSKMSTLSCKNKIISFEEMDPPEN IDDIQSDLIFFIKAVPGANKLEFESSLYEGHFLACQKEAGAFKLIL KKKDENGDKSVMFTLTNLHQS (SEQ ID NO: 181)

mD2D-A1	DFGRLHCTTAVIRNINSQVLFVVDKRQPVFRDMGSIHQSASEPQTR LIIIFYKDSEVRGLAVTL SVKDSKMSTLSCKNKIISFEEMDPDEN DDIQSDLIFFLKA VPGANKLEFESSLYEGHFLACQKEDGAFLIL KKKDENGDKSVMFTLTNLHQS (SEQ ID NO: 182)
mD2D-E6	DFGRLHCTTAVIRNINSQVLFVVDKRQPVFRDMGSIHQSASEPQTR LIIIFYKDSEVRGLAVTL SVKDSKMSTLSCKNKIISFEEMDPDEN DDIQSDLIFFLKA VPGANKLEFESSLYEGHFLACQKEDGAFLIL KKKDENGDKSVMFTLTNLHQS (SEQ ID NO: 183)
mD2D-D4	YFGRLHCTTAVIRNINEQVLFVVDKRQPVFKDMKDKLQSASEPQT RLIIIFYKDSEVRGLAVTL SVKDSKMSTLSCKNKIISFEEMDPDEN IDDIQSDLIFFLKG VPGDNKLEFESSLYEGHFLACQKEFGAFLIL KKKDENGDKSVMFTLTNLHQS (SEQ ID NO: 184)
mD2D-D6	YFGRLHCTTAVIRNINEQVLFVVDKRQPVFKDMKDKLQSASEPQT RLIIIFYKDSEVRGLAVTL SVKDSKMSTLSCKNKIISFEEMDPDEN IDDIQSDLIFFLKG VPGDNKLEFESSLYEGHFLACQKEFGAFLIL KKKDENGDKSVMFTLTNLHQS (SEQ ID NO: 185)
mD2D-A3	YFGRLHCTTAVIRNINGQVLFVVDKRQPVFADMSTHQSASEPQT RLIIIFYKDSEVRGLAVTL SVKDSKMSTLSCKNKIISFEEMDPDEN IDDIQSDLIFFLKG VPGANKIEFESSLYEGHFLACQKEDDAFLIL KKKDENGDKSVMFTLTNLHQS (SEQ ID NO: 186)
mD2D-A4	YFGRLHCTTAVIRNINGQVLFVVDKRQPVFADMSTHQSASEPQT RLIIIFYKDSEVRGLAVTL SVKDSKMSTLSCKNKIISFEEMDPDEN IDDIQSDLIFFLKG VPGANKIEFESSLYEGHFLACQKEDDAFLIL KKKDENGDKSVMFTLTNLHQS (SEQ ID NO: 187)
mD2D-B10	YFGRLHCTTAVIRNINGQVLFVVDKRQPVFADMSTHQSASEPQT RLIIIFYKDSEVRGLAVTL SVKDSKMSTLSCKNKIISFEEMDPDEN IDDIQSDLIFFLKG VPGANKIEFESSLYEGHFLACQKEDDAFLIL KKKDENGDKSVMFTLTNLHQS (SEQ ID NO: 188)
mD2D-B8	YFGRLHCTTAVIRNINSQVLFVVDKRQPVFGDMKYIVQSASEPQT RLIIIFYKDSEVRGLAVTL SVKDSKMSTLSCKNKIISFEEMDPDEN IDDIQSDLIFFLKG VPGDTKMEFESSLYEGHFLACQKESGAFLIL KKKDENGDKSVMFTLTNLHQS (SEQ ID NO: 189)
mD2D-B9	YFGRLHCTTAVIRNINSQVLFVVDKRQPVFGDMKYIVQSASEPQT RLIIIFYKDSEVRGLAVTL SVKDSKMSTLSCKNKIISFEEMDPDEN IDDIQSDLIFFLKG VPGDTKMEFESSLYEGHFLACQKESGAFLIL KKKDENGDKSVMFTLTNLHQS (SEQ ID NO: 190)

Exemplary Non-Limiting Aspects of the Disclosure

- Aspects, including embodiments, of the present subject matter described above may be beneficial alone or in combination, with one or more other aspects or
- 5 embodiments. Without limiting the foregoing description, certain non-limiting aspects of the disclosure are provided below in SET A and SET B. As will be apparent to those of

ordinary skill in the art upon reading this disclosure, each of the individually numbered aspects may be used or combined with any of the preceding or following individually numbered aspects. This is intended to provide support for all such combinations of aspects and is not limited to combinations of aspects explicitly provided below. It will be
 5 apparent to one of ordinary skill in the art that various changes and modifications can be made without departing from the spirit or scope of the invention.

SET A

1. A composition comprising an IL-18 variant polypeptide, wherein the IL-
 10 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).
2. The composition of 1, wherein the IL-18 variant polypeptide comprises at least one mutation relative to wild-type (WT) IL-18.
- 15 3. The composition of 2, wherein the WT IL-18 is human IL-18 comprising the amino acid sequence of SEQ ID NO: 30.
4. The composition of 2, wherein the WT IL-18 is murine IL-18 comprising the amino acid sequence of SEQ ID NO: 31.
5. The composition of 3, wherein the IL-18 variant polypeptide comprises at
 20 least one mutation 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,, relative to SEQ ID NO: 30.
6. The composition of 3, wherein the IL-18 variant polypeptide comprises at least one mutation selected from the group consisting of Y1H, Y1R, L5H, L5I, L5Y,
 25 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,
 30 M113V, M113R, M113T, M113K, V153I, V153T, V153A, N155K, and N155H, relative to SEQ ID NO: 30.

7. The composition of 2, wherein the IL-18 variant polypeptide comprises at least one IL-18 variant polypeptide selected from the group consisting of SEQ ID NO: 34, SEQ ID NO: 35, SEQ ID NO: 36, SEQ ID NO: 37, SEQ ID NO: 38, SEQ ID NO: 39, SEQ ID NO: 40, SEQ ID NO: 41, SEQ ID NO: 42, SEQ ID NO: 43, SEQ ID NO: 44, SEQ ID NO: 45, SEQ ID NO: 46, SEQ ID NO: 47, SEQ ID NO: 48, SEQ ID NO: 49, SEQ ID NO: 50, SEQ ID NO: 51, SEQ ID NO: 52, SEQ ID NO: 53, SEQ ID NO: 54, SEQ ID NO: 55, SEQ ID NO: 56, SEQ ID NO: 57, SEQ ID NO: 58, SEQ ID NO: 59, SEQ ID NO: 73, SEQ ID NO: 74, SEQ ID NO: 75, SEQ ID NO: 76, SEQ ID NO: 77, SEQ ID NO: 78, SEQ ID NO: 79, SEQ ID NO: 80, SEQ ID NO: 81, SEQ ID NO: 82, SEQ ID NO: 83, SEQ ID NO: 84, SEQ ID NO: 85, SEQ ID NO: 86, SEQ ID NO: 87, SEQ ID NO: 88, SEQ ID NO: 89, SEQ ID NO: 90, SEQ ID NO: 91, or a fragment thereof.

8. The composition of 4, 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.

9. The composition of 4, wherein the IL-18 variant polypeptide comprises at least one mutation selected from 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.

10. The composition of 2, wherein the IL-18 variant polypeptide comprises at least one IL-18 variant polypeptide selected from the group consisting of SEQ ID NO: 60, SEQ ID NO: 61, SEQ ID NO: 62, SEQ ID NO: 63, SEQ ID NO: 64, SEQ ID NO: 65, SEQ ID NO: 66, SEQ ID NO: 67, SEQ ID NO: 68, SEQ ID NO: 69, SEQ ID NO: 70, SEQ ID NO: 71, SEQ ID NO: 72, or a fragment thereof.

11. A composition comprising a nucleic acid encoding at least one of the IL-18 variant polypeptides of 1-10.

12. A method of treating or preventing a disease or disorder in a subject in need thereof, comprising administering to the subject at least one composition of 1-11.

13. The method of 12, wherein the disease or disorder is cancer.
14. The method of 13, wherein the cancer is a cancer that is resistant to immune checkpoint inhibitors (ICIs).
15. The method of 13, wherein the cancer is associated with a tumor that has lost expression of MHC class I.
16. The method of 12, wherein the disease or disorder is a metabolic disease or disorder.
17. The method of 12, wherein the disease or disorder is an infectious disease.
18. The method of 12, further comprising administering to the subject at least one other agent.
19. The method of 18, wherein the at least one other agent comprises an inhibitor of one or more secondary cytokines.
20. The method of 19, wherein the one or more secondary cytokines are at least one selected from the group consisting of IL-17, IL-5, and IL-13.
21. The method of 19, wherein the inhibitor of one or more secondary cytokines comprises at least one selected from the group consisting of: a chemical compound, a polypeptide, a peptide, a peptidomimetic, an antibody, a ribozyme, a small molecule chemical compound, and an antisense nucleic acid molecule.
22. A composition comprising an IL-18BP inhibitor or IL-18BP antagonist, wherein the inhibitor or antagonist inhibits the ability of IL18BP to neutralize endogenous IL-18.
23. The composition of 22, wherein the inhibitor or antagonist comprises at least one selected from the group consisting of: a chemical compound, a polypeptide, a peptide, a peptidomimetic, an antibody, a ribozyme, a small molecule chemical compound, and an antisense nucleic acid molecule.
24. The composition of 22 comprising an IL-18 variant polypeptide, wherein the IL-18 variant polypeptide specifically binds to IL-18 binding protein (IL-18BP) and wherein the IL-18 variant polypeptide exhibits substantially reduced binding to IL-18 receptor (IL-18R).
25. The composition of 24, wherein the IL-18 variant polypeptide comprises at least one mutation relative to wild-type (WT) IL-18.

26. The composition of 24, wherein the WT IL-18 is human IL-18 comprising the amino acid sequence of SEQ ID NO: 30.
27. The composition of 24, wherein the WT IL-18 is murine IL-18 comprising the amino acid sequence of SEQ ID NO: 31.
- 5 28. The composition of 26, wherein the IL-18 variant polypeptide comprises at least one mutation selected from the group consisting of Y1X, L5X, D17X, E31X, T34X, D35X, S36X, D37X, D40X, N41X, M51X, Q56X, M60X, Q103X, H109X, M113X, and R131X, relative to SEQ ID NO: 30.
- 10 29. The composition of 26, wherein the IL-18 variant polypeptide comprises at least one mutation selected from the group consisting of Y1D, Y1F, Y1H, Y1L, L5F, L5H, D17A, D17G, D17R, D17H, E31A, E31T, E31G, E31K, E31R, T34A, T34K, T34E, D35S, D35A, D35Y, S36N, S36K, S36R, D37P, D37A, D37R, D37H, D37L, D37V, D40Y, D40S, D40A, N41K, N41S, N41R, M51F, M51L, M51I, Q56H, M60L, M60F, M60I, Q103L, Q103I, H109A, H109P, H109D, M113L, M113I, M113F, and R131S, relative to SEQ ID NO: 30.
- 15 30. The composition of 24, wherein the IL-18 variant polypeptide comprises at least one IL-18 variant polypeptide selected from the group consisting of SEQ ID NO: 92, SEQ ID NO: 93, SEQ ID NO: 94, SEQ ID NO: 95, SEQ ID NO: 96, SEQ ID NO: 97, SEQ ID NO: 98, SEQ ID NO: 99, SEQ ID NO: 100, SEQ ID NO: 101, SEQ ID NO: 102, SEQ ID NO: 103, SEQ ID NO: 104, SEQ ID NO: 105, SEQ ID NO: 106, SEQ ID NO: 107, SEQ ID NO: 108, SEQ ID NO: 109, SEQ ID NO: 110, SEQ ID NO: 111, SEQ ID NO: 112, SEQ ID NO: 113, SEQ ID NO: 114, SEQ ID NO: 115, SEQ ID NO: 116, SEQ ID NO: 117, SEQ ID NO: 118, SEQ ID NO: 119, SEQ ID NO: 120, SEQ ID NO: 121, SEQ ID NO: 122, SEQ ID NO: 123, SEQ ID NO: 124, SEQ ID NO: 125, or a fragment thereof.
- 25 31. The composition of 27, wherein the IL-18 variant polypeptide comprises at least one mutation selected from the group consisting of N1X, L5X, D17X, E30X, T33X, D34X, I35X, D36X, M50X, Q102X, R104, H108X, N109X, M111X, D129X, and D130X, relative to SEQ ID NO: 31.
- 30 32. The composition of 4, wherein the IL-18 variant polypeptide comprises at least one mutation selected from the group consisting of N1Y, N1D, N1H, N1L, N1F,

N1V, N1I, L5Y, L5H, D17Q, D17G, D17A, D17E, D17S, D17N, E30A, E30R, E30K, E30T, E30G, T33G, T33A, T33E, T33R, T33K, D34Y, D34S, D34A, I35T, I35K, I35R, D36V, D36A, D36G, D36H, D36P, D36R, D36L, M50F, M50L, Q102L, Q102I, R104E, R104A, R104P, R104G, R104Q, R104H, H108D, H108A, N109R, N109S, N109T,
 5 N109I, M111L, M111I, D129A, D129F, D129V, D129Y, D129S, D130E, D130T, D130G, D130N, D130R, D130S, D130Q, and D130H, relative to SEQ ID NO: 31.

33. The composition of 2, wherein the IL-18 variant polypeptide comprises at least one IL-18 variant polypeptide selected from the group consisting of SEQ ID NO: 126, SEQ ID NO: 127, SEQ ID NO: 128, SEQ ID NO: 129, SEQ ID NO: 130, SEQ ID
 10 NO: 131, SEQ ID NO: 132, SEQ ID NO: 133, SEQ ID NO: 134, SEQ ID NO: 135, SEQ ID NO: 136, SEQ ID NO: 137, SEQ ID NO: 138, SEQ ID NO: 139, SEQ ID NO: 140, SEQ ID NO: 141, SEQ ID NO: 142, SEQ ID NO: 143, SEQ ID NO: 144, SEQ ID NO: 145, SEQ ID NO: 146, SEQ ID NO: 147, SEQ ID NO: 148, SEQ ID NO: 149, SEQ ID NO: 150, SEQ ID NO: 151, SEQ ID NO: 152, SEQ ID NO: 153, SEQ ID NO: 154, SEQ
 15 ID NO: 155, SEQ ID NO: 156, SEQ ID NO: 157, SEQ ID NO: 158, SEQ ID NO: 159, SEQ ID NO: 160, SEQ ID NO: 161, SEQ ID NO: 162, SEQ ID NO: 163, SEQ ID NO: 164, SEQ ID NO: 165, SEQ ID NO: 166, SEQ ID NO: 167, SEQ ID NO: 168, SEQ ID NO: 169, SEQ ID NO: 170, SEQ ID NO: 171, SEQ ID NO: 172, SEQ ID NO: 173, SEQ ID NO: 174, SEQ ID NO: 175, SEQ ID NO: 176, SEQ ID NO: 177, SEQ ID NO: 178,
 20 SEQ ID NO: 179, SEQ ID NO: 180, SEQ ID NO: 181, SEQ ID NO: 182, SEQ ID NO: 183, SEQ ID NO: 184, SEQ ID NO: 185, SEQ ID NO: 186, SEQ ID NO: 187, SEQ ID NO: 188, SEQ ID NO: 189, SEQ ID NO: 190, or a fragment thereof.

34. A composition comprising a nucleic acid encoding at least one of the IL-18 variant polypeptides of 24-33.

25 35. A method of treating or preventing a disease or disorder in a subject in need thereof, comprising administering to the subject at least one composition of 22-34.

36. The method of 35, wherein the disease or disorder is cancer.

37. The method of 36, wherein the cancer is a cancer that is resistant to immune checkpoint inhibitors (ICIs).

30 38. The method of 36, wherein the cancer is associated with a tumor that has lost expression of MHC class I.

39. The method of 35, wherein the disease or disorder is a metabolic disease or disorder.
40. The method of 35, wherein the disease or disorder is an infectious disease.
41. The method of 35, further comprising administering to the subject at least one other agent.
42. The method of 41, wherein the at least one other agent comprises an inhibitor of one or more secondary cytokines.
43. The method of 42, wherein the one or more secondary cytokines are at least one selected from the group consisting of IL-17, IL-5, and IL-13.
44. The method of 42, wherein the inhibitor of one or more secondary cytokines comprises at least one selected from the group consisting of: a chemical compound, a polypeptide, a peptide, a peptidomimetic, an antibody, a ribozyme, a small molecule chemical compound, and an antisense nucleic acid molecule.

SET B

1. A composition comprising an IL-18 variant polypeptide, 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).
2. The composition of 1, wherein the IL-18 variant polypeptide comprises at least one mutation relative to wild-type (WT) IL-18.
3. The composition of 2, wherein the WT IL-18 is human IL-18 comprising the amino acid sequence of SEQ ID NO: 30.
4. The composition of 2, wherein the WT IL-18 is murine IL-18 comprising the amino acid sequence of SEQ ID NO: 31.
5. The composition of 3, wherein the IL-18 variant polypeptide comprises at least one mutation 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, relative to SEQ ID NO: 30.
6. The composition of 3, wherein the IL-18 variant polypeptide comprises at least one mutation 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,
 5 M113V, M113R, M113T, M113K, V153I, V153T, V153A, N155K, and N155H, relative to SEQ ID NO: 30.

7. The composition of 3, wherein the IL-18 variant polypeptide comprises the mutations M51X, M60X, S105X, D110X, and N111X, relative to SEQ ID NO: 30.

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

9. The composition of 2, wherein the IL-18 variant polypeptide comprises the amino acid sequence set forth in any one of SEQ ID NOs.: 34-59, 73-91, and 191-193, or a fragment thereof.

10. The composition of 4, wherein the IL-18 variant polypeptide comprises at
 15 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.

11. The composition of 4, wherein the IL-18 variant polypeptide comprises at least one mutation selected from the group consisting of N1H, N1Y, M50A, M50S,
 20 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.

12. The composition of 2, wherein the IL-18 variant polypeptide comprises
 25 the amino acid sequence set forth in any one of SEQ ID NOs.: 60-72, or a fragment thereof.

13. A composition comprising a nucleic acid encoding the IL-18 variant polypeptide of the composition of any one of 1-12.

14. The composition of any one of 1-13, further comprising one or more
 30 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
- 5 (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-
- 10 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.
- 15 15. A method of treating or preventing a disease or disorder in a subject in need thereof, comprising administering to the subject the composition of any one of 1-14.
16. The method of 15, wherein the disease or disorder is cancer.
17. The method of 16, wherein the cancer is a cancer that is resistant to immune checkpoint inhibitors (ICIs).
18. The method of 16, wherein the cancer is associated with a tumor that has
- 20 lost expression of MHC class I.
19. The method of 15, wherein the disease or disorder is a metabolic disease or disorder.
20. The method of 15, wherein the disease or disorder is an infectious disease.
21. The method of any one of 15-20, wherein the method comprises
- 25 administering to the subject the IL-18 variant polypeptide and at least one other agent.
22. The method of 21, wherein the at least one other agent comprises an immune checkpoint inhibitor.
23. The method of 22, wherein the immune checkpoint inhibitor is an agent that inhibits PD-L1, PD1, CTLA4, TIM3, TIGIT, LAG3, B7H3, B7H4, VISTA, ICOS,
- 30 GITR, 41BB, OX40, or CD40, or any combination thereof.

24. The method of 21, wherein the at least one other agent comprises a cancer cell opsonizing agent.
25. The method of 24, wherein the at least one other agent 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.
26. The method of any one of 21-25, wherein the at least one other agent is conjugated to the IL-18 variant polypeptide.
27. The method of 21, wherein the at least one other agent is an altered T-cell or NK cell.
28. The method of 21, wherein the at least one other agent is an oncolytic virus.
29. A composition comprising an IL-18BP inhibitor or IL-18BP antagonist, wherein the inhibitor or antagonist inhibits the ability of IL18BP to neutralize endogenous IL-18.
30. The composition of 29, wherein the inhibitor or antagonist comprises at least one selected from the group consisting of: a chemical compound, a polypeptide, a peptide, a peptidomimetic, an antibody, a ribozyme, a small molecule chemical compound, and an antisense nucleic acid molecule.
31. The composition of 30, comprising an IL-18 variant polypeptide, wherein the IL-18 variant polypeptide specifically binds to IL-18 binding protein (IL-18BP) and wherein the IL-18 variant polypeptide exhibits substantially reduced binding to IL-18 receptor (IL-18R).

32. The composition of 31, wherein the IL-18 variant polypeptide comprises at least one mutation relative to wild-type (WT) IL-18.
33. The composition of 32, wherein the WT IL-18 is human IL-18 comprising the amino acid sequence of SEQ ID NO: 30.
- 5 34. The composition of 32, wherein the WT IL-18 is murine IL-18 comprising the amino acid sequence of SEQ ID NO: 31.
35. The composition of 33, wherein the IL-18 variant polypeptide comprises at least one mutation selected from the group consisting of Y1X, L5X, D17X, E31X, T34X, D35X, S36X, D37X, D40X, N41X, M51X, Q56X, M60X, Q103X, H109X,
10 M113X, and R131X, relative to SEQ ID NO: 30.
36. The composition of 33, wherein the IL-18 variant polypeptide comprises at least one mutation selected from the group consisting of Y1D, Y1F, Y1H, Y1L, L5F, L5H, D17A, D17G, D17R, D17H, E31A, E31T, E31G, E31K, E31R, T34A, T34K, T34E, D35S, D35A, D35Y, S36N, S36K, S36R, D37P, D37A, D37R, D37H, D37L, D37V,
15 D40Y, D40S, D40A, N41K, N41S, N41R, M51F, M51L, M51I, Q56H, M60L, M60F, M60I, Q103L, Q103I, H109A, H109P, H109D, M113L, M113I, M113F, and R131S, relative to SEQ ID NO: 30.
37. The composition of 33, wherein the IL-18 variant polypeptide comprises the amino acid sequence set forth in any one of SEQ ID NOs.: 92-125, or a fragment
20 thereof.
38. The composition of 33, wherein the IL-18 variant polypeptide comprises the mutations D17X, E30X, and Q103X, relative to SEQ ID NO: 30.
39. The composition of 33, wherein the IL-18 variant polypeptide comprises the mutations D17X, E30X, D35X, M51X, and Q103X, relative to SEQ ID NO: 30.
- 25 40. The composition of 34, wherein the IL-18 variant polypeptide comprises at least one mutation selected from the group consisting of N1X, L5X, D17X, E30X, T33X, D34X, I35X, D36X, M50X, Q102X, R104, H108X, N109X, M111X, D129X, and D130X, relative to SEQ ID NO: 31.
41. The composition of 34, wherein the IL-18 variant polypeptide comprises at least one mutation selected from the group consisting of N1Y, N1D, N1H, N1L, N1F, N1V, N1I, L5Y, L5H, D17Q, D17G, D17A, D17E, D17S, D17N, E30A, E30R, E30K,
30

- E30T, E30G, T33G, T33A, T33E, T33R, T33K, D34Y, D34S, D34A, I35T, I35K, I35R, D36V, D36A, D36G, D36H, D36P, D36R, D36L, M50F, M50L, Q102L, Q102I, R104E, R104A, R104P, R104G, R104Q, R104H, H108D, H108A, N109R, N109S, N109T, N109I, M111L, M111I, D129A, D129F, D129V, D129Y, D129S, D130E, D130T, D130G, D130N, D130R, D130S, D130Q, and D130H, relative to SEQ ID NO: 31.
42. The composition of 34, wherein the IL-18 variant polypeptide comprises the amino acid sequence set forth in any one of SEQ ID NOs.: 126-190, or a fragment thereof.
43. A composition comprising a nucleic acid encoding the IL-18 variant polypeptide of the composition of any one of 31-42.
44. The composition of any one of 19-43, 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 $\alpha\beta$ 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.
45. A method of treating or preventing a disease or disorder in a subject in need thereof, comprising administering to the subject the composition of any one of 29-44.
46. The method of 45, wherein the disease or disorder is cancer.

47. The method of 46, wherein the cancer is a cancer that is resistant to immune checkpoint inhibitors (ICIs).
48. The method of 46, wherein the cancer is associated with a tumor that has lost expression of MHC class I.
- 5 49. The method of 45, wherein the disease or disorder is a metabolic disease or disorder.
50. The method of 45, wherein the disease or disorder is an infectious disease.
51. The method of any one of 45-50, wherein the method comprises administering to the subject the IL-18 variant polypeptide and at least one other agent.
- 10 52. The method of 51, wherein the at least one other agent comprises an immune checkpoint inhibitor.
53. The method of 52, 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.
- 15 54. The method of 51, wherein the at least one other agent comprises a cancer cell opsonizing agent.
55. The method of 51, wherein the at least one other agent 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.
- 25 56. The method of any one of 51-55, wherein the at least one other agent is conjugated to the IL-18 variant polypeptide.
- 30

57. The method of 51, wherein the at least one other agent is an altered T-cell or NK cell.

58. The method of 51, wherein the at least one other agent is an oncolytic virus.

5 The disclosures of each and every patent, patent application, and publication cited herein are hereby incorporated herein by reference in their entirety. While this invention has been disclosed with reference to specific embodiments, it is apparent that other embodiments and variations of this invention may be devised by others skilled in the art without departing from the true spirit and scope of the invention. The appended claims are
10 intended to be construed to include all such embodiments and equivalent variations.

CLAIMS

What is claimed is:

1. A composition comprising an IL-18 variant polypeptide, 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).
2. The composition of claim 1, wherein the IL-18 variant polypeptide comprises at least one mutation relative to wild-type (WT) IL-18.
3. The composition of claim 2, wherein the WT IL-18 is human IL-18 comprising the amino acid sequence of SEQ ID NO: 30.
4. The composition of claim 2, wherein the WT IL-18 is murine IL-18 comprising the amino acid sequence of SEQ ID NO: 31.
5. The composition of claim 3, wherein the IL-18 variant polypeptide comprises at least one mutation 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, relative to SEQ ID NO: 30.
6. The composition of claim 3, wherein the IL-18 variant polypeptide comprises at least one mutation 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.

7. The composition of claim 3, wherein the IL-18 variant polypeptide comprises the mutations M51X, M60X, S105X, D110X, and N111X, relative to SEQ ID NO: 30.

8. 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.

9. The composition of claim 2, 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.

10. The composition of claim 4, 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.

11. The composition of claim 4, wherein the IL-18 variant polypeptide comprises at least one mutation selected from 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.

12. The composition of claim 2, wherein the IL-18 variant polypeptide comprises the amino acid sequence set forth in any one of SEQ ID NOs.: 60-72, or a fragment thereof.

13. A composition comprising a nucleic acid encoding the IL-18 variant polypeptide of the composition of any one of claims 1-12.

14. The composition of any one of claims 1-13, 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 $\alpha_v\beta_3$, 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.

15. A method of treating or preventing a disease or disorder in a subject in need thereof, comprising administering to the subject the composition of any one of claims 1-14.

16. The method of claim 15, wherein the disease or disorder is cancer.

17. The method of claim 16, wherein the cancer is a cancer that is resistant to immune checkpoint inhibitors (ICIs).

18. The method of claim 16, wherein the cancer is associated with a tumor that has lost expression of MHC class I.

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

20. The method of claim 15, wherein the disease or disorder is an infectious disease.
21. The method of any one of claims 15-20, wherein the method comprises administering to the subject the IL-18 variant polypeptide and at least one other agent.
22. The method of claim 21, wherein the at least one other agent comprises an immune checkpoint inhibitor.
23. The method of claim 22, 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.
24. The method of claim 21, wherein the at least one other agent comprises a cancer cell opsonizing agent.
25. The method of claim 24, wherein the at least one other agent 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.
26. The method of any one of claims 21-25, wherein the at least one other agent is conjugated to the IL-18 variant polypeptide.

27. The method of claim 21, wherein the at least one other agent is an altered T-cell or NK cell.

28. The method of claim 21, wherein the at least one other agent is an oncolytic virus.

29. A composition comprising an IL-18BP inhibitor or IL-18BP antagonist, wherein the inhibitor or antagonist inhibits the ability of IL18BP to neutralize endogenous IL-18.

30. The composition of claim 29, wherein the inhibitor or antagonist comprises at least one selected from the group consisting of: a chemical compound, a polypeptide, a peptide, a peptidomimetic, an antibody, a ribozyme, a small molecule chemical compound, and an antisense nucleic acid molecule.

31. The composition of claim 30, comprising an IL-18 variant polypeptide, wherein the IL-18 variant polypeptide specifically binds to IL-18 binding protein (IL-18BP) and wherein the IL-18 variant polypeptide exhibits substantially reduced binding to IL-18 receptor (IL-18R).

32. The composition of claim 31, wherein the IL-18 variant polypeptide comprises at least one mutation relative to wild-type (WT) IL-18.

33. The composition of claim 32, wherein the WT IL-18 is human IL-18 comprising the amino acid sequence of SEQ ID NO: 30.

34. The composition of claim 32, wherein the WT IL-18 is murine IL-18 comprising the amino acid sequence of SEQ ID NO: 31.

35. The composition of claim 33, wherein the IL-18 variant polypeptide comprises at least one mutation selected from the group consisting of Y1X, L5X, D17X,

E31X, T34X, D35X, S36X, D37X, D40X, N41X, M51X, Q56X, M60X, Q103X, H109X, M113X, and R131X, relative to SEQ ID NO: 30.

36. The composition of claim 33, wherein the IL-18 variant polypeptide comprises at least one mutation selected from the group consisting of Y1D, Y1F, Y1H, Y1L, L5F, L5H, D17A, D17G, D17R, D17H, E31A, E31T, E31G, E31K, E31R, T34A, T34K, T34E, D35S, D35A, D35Y, S36N, S36K, S36R, D37P, D37A, D37R, D37H, D37L, D37V, D40Y, D40S, D40A, N41K, N41S, N41R, M51F, M51L, M51I, Q56H, M60L, M60F, M60I, Q103L, Q103I, H109A, H109P, H109D, M113L, M113I, M113F, and R131S, relative to SEQ ID NO: 30.

37. The composition of claim 33, wherein the IL-18 variant polypeptide comprises the amino acid sequence set forth in any one of SEQ ID NOs.: 92-125, or a fragment thereof.

38. The composition of claim 33, wherein the IL-18 variant polypeptide comprises the mutations D17X, E30X, and Q103X, relative to SEQ ID NO: 30.

39. The composition of claim 33, wherein the IL-18 variant polypeptide comprises the mutations D17X, E30X, D35X, M51X, and Q103X, relative to SEQ ID NO: 30.

40. The composition of claim 34, wherein the IL-18 variant polypeptide comprises at least one mutation selected from the group consisting of N1X, L5X, D17X, E30X, T33X, D34X, I35X, D36X, M50X, Q102X, R104, H108X, N109X, M111X, D129X, and D130X, relative to SEQ ID NO: 31.

41. The composition of claim 34, wherein the IL-18 variant polypeptide comprises at least one mutation selected from the group consisting of N1Y, N1D, N1H, N1L, N1F, N1V, N1I, L5Y, L5H, D17Q, D17G, D17A, D17E, D17S, D17N, E30A, E30R, E30K, E30T, E30G, T33G, T33A, T33E, T33R, T33K, D34Y, D34S, D34A,

I35T, I35K, I35R, D36V, D36A, D36G, D36H, D36P, D36R, D36L, M50F, M50L, Q102L, Q102I, R104E, R104A, R104P, R104G, R104Q, R104H, H108D, H108A, N109R, N109S, N109T, N109I, M111L, M111I, D129A, D129F, D129V, D129Y, D129S, D130E, D130T, D130G, D130N, D130R, D130S, D130Q, and D130H, relative to SEQ ID NO: 31.

42. The composition of claim 34, wherein the IL-18 variant polypeptide comprises the amino acid sequence set forth in any one of SEQ ID NOs.: 126-190, or a fragment thereof.

43. A composition comprising a nucleic acid encoding the IL-18 variant polypeptide of the composition of any one of claims 31-42.

44. The composition of any one of claims 19-43, 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 $\alpha_v\beta_3$, 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.

45. A method of treating or preventing a disease or disorder in a subject in need thereof, comprising administering to the subject the composition of any one of claims 29-44.

46. The method of claim 45, wherein the disease or disorder is cancer.

47. The method of claim 46, wherein the cancer is a cancer that is resistant to immune checkpoint inhibitors (ICIs).

48. The method of claim 46, wherein the cancer is associated with a tumor that has lost expression of MHC class I.

49. The method of claim 45, wherein the disease or disorder is a metabolic disease or disorder.

50. The method of claim 45, wherein the disease or disorder is an infectious disease.

51. The method of any one of claims 45-50, wherein the method comprises administering to the subject at least one other agent in addition to the IL-18BP inhibitor or IL-18BP antagonist.

52. The method of claim 51, wherein the at least one other agent comprises an immune checkpoint inhibitor.

53. The method of claim 52, 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.

54. The method of claim 51, wherein the at least one other agent comprises a cancer cell opsonizing agent.

55. The method of claim 51, wherein the at least one other agent 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 $\alpha\beta$ 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.

56. The method of any one of claims 51-55, wherein the inhibitor or antagonist is an IL-18 variant polypeptide, and the at least one other agent is conjugated to the IL-18 variant polypeptide.

57. The method of claim 51, wherein the at least one other agent is an altered T-cell or NK cell.

58. The method of claim 51, wherein the at least one other agent is an oncolytic virus.