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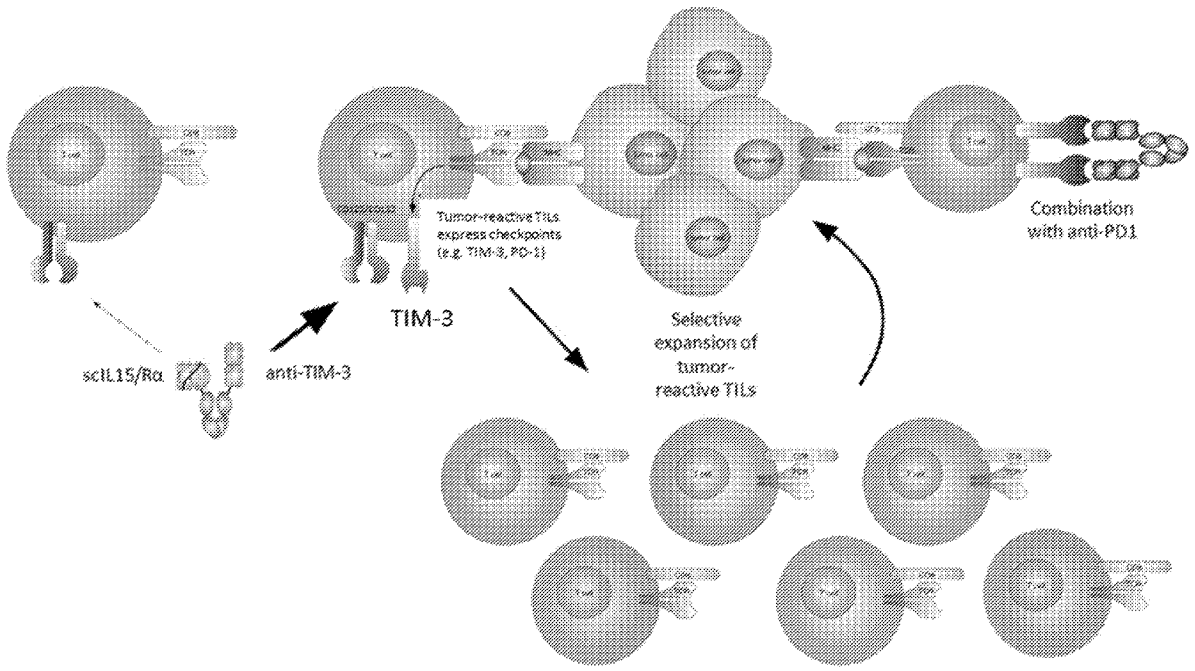
Figure 1



(57) Abstract: The present invention is directed to novel targeted heterodimeric fusion proteins comprising an IL-15/IL-15Rα Fc-fusion protein and a TIM-3 antibody fragment-Fc fusion protein.

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[Continued on next page]

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TIM-3 TARGETED HETERODIMERIC FUSION PROTEINS CONTAINING IL-15/IL-15RA Fc-FUSION PROTEINS AND TIM-3 ANTIGEN BINDING DOMAINS

CROSS-REFERENCE TO RELATED APPLICATIONS

[0001] This application claims priority to U.S. Provisional Patent Application Nos. 62/659,626, filed April 18, 2018 and 62/783,110, filed December 20, 2018, which are expressly incorporated herein by reference in their entirety, with particular reference to the figures, legends, and claims therein.

BACKGROUND OF THE INVENTION

[0002] Two very promising approaches in cancer immunotherapy include cytokine-based treatments and blockade of immune checkpoint proteins such as PD-1.

[0003] Cytokines such as IL-2 and IL-15 function in aiding the proliferation and differentiation of B cells, T cells, and NK cells. Both cytokines exert their cell signaling function through binding to a trimeric complex consisting of two shared receptors, the common gamma chain (γc; CD132) and IL-2 receptor beta-chain (IL-2Rß; CD122), as well as an alpha chain receptor unique to each cytokine: IL-2 receptor alpha (IL-2Rα; CD25) or IL-15 receptor alpha (IL-15Rα; CD215). Both cytokines are considered as potentially valuable therapeutics in oncology, and IL-2 has been approved for use in patients with metastatic renal-cell carcinoma and malignant melanoma. Currently, there are no approved uses of recombinant IL-15, although several clinical trials are ongoing. However, as potential drugs, both cytokines suffer from a very fast clearance, with half-lives measured in minutes. IL-2 immunotherapy has been associated with systemic toxicity when administered in high doses to overcome fast clearance. Such systemic toxicity has also been reported with IL-15 immunotherapy in recent clinical trials (Guo et al., J Immunol, 2015, 195(5):2353-64).

[0004] Immune checkpoint proteins such as PD-1 are up-regulated following T cell activation to preclude autoimmunity by exhausting activated T cells upon binding to immune checkpoint ligands such as PD-L1. However, immune checkpoint proteins are also up-regulated in tumor-infiltrating lymphocytes (TILs), and immune checkpoint ligands are

overexpressed on tumor cells, contributing to immune escape by tumor cells. De-repression of TILs by blockade of immune checkpoint interactions by drugs such as Opdivo® (nivolumab) and Keytruda® (pembrolizumab) have proven highly effective in treatment of cancer. Despite the promise of checkpoint blockade therapies such as nivolumab and pembrolizumab, many patients still fail to achieve sufficient response to checkpoint blockade alone.

[0005] Therefore, there remains an unmet need in oncology treatment for therapeutic strategies with cytokines that do not require high doses and are targeted to tumors to avoid systemic toxicity. Further, there is a need to identify additional therapeutic modalities to stack with checkpoint blockade that could increase patient response rate.

[0006] The present invention addresses these needs and caveats by providing TIM-3-targeted IL-15 heterodimeric fusion proteins with enhanced half-life and more selective targeted of TILs to improve safety profile, and which synergistically combine with checkpoint blockade antibodies (Figure 1).

BRIEF SUMMARY OF THE INVENTION

[0007] In one aspect, the present invention provides a targeted IL-15/IL-15Rα heterodimeric protein comprising: (a) a first monomer comprising, from N-to C-terminal: i) an IL-15 sushi domain; ii) a first domain linker; iii) a variant IL-15 domain; iv) a second domain linker; v) a first variant Fc domain comprising CH2-CH3; and (b) a second monomer comprising, from N-to C-terminal: i) a scFv domain; ii) a third domain linker; iii) a second variant Fc domain comprising CH2-CH3; wherein the scFv domain comprises a first variable heavy domain, an scFv linker and a first variable light domain, wherein the scFv domain binds human TIM-3.

[0008] In other aspects of the present invention, provided herein is a targeted IL-15/IL-15Rα heterodimeric protein comprising: (a) a first monomer comprising, from N-to C-terminal: i) an IL-15 sushi domain; ii) a first domain linker; iii) a first variant Fc domain comprising CH2-CH3; (b) a second monomer comprising, from N-to C-terminal: i) a scFv domain; ii) a third domain linker; iii) a second variant Fc domain comprising CH2-CH3; wherein the scFv domain comprises a first variable heavy domain, an scFv linker and a first variable light

domain; and (c) a third monomer comprising a variant IL-15 domain; wherein the scFv domain binds human TIM-3.

[0009] In one aspect, provided are "scIL-15/Rα X Fab" format heterodimeric proteins. Such "scIL-15/Rα X Fab" format heterodimeric proteins include: a) a first monomer comprising, from N-to C-terminal: i) an IL-15Rα(sushi) domain; ii) a first domain linker; iii) an IL-15 variant; iv) a second domain linker; v) a first variant Fc domain comprising CH2-CH3; b) a second monomer comprising, from N-to C-terminal, VH-CH1-hinge-CH2-CH3, wherein CH2-CH3 is a second variant Fc domain; and c) a third monomer comprising a VL-CL. The VH and VL are a variable heavy domain and a variable light domain, respectively, that form a human TIM-3 antigen binding domain. In some embodiments, the second domain linker is an antibody hinge.

[0010] In certain embodiments of the "scIL-15/Rα X Fab" format heterodimeric protein, the first variant Fc domain the second variant Fc domain comprises one of the following skew variant sets: S267K/L368D/K370S : S267K/S364K/E357Q; S364K/E357Q : L368D/K370S; L368D/K370S : S364K; L368E/K370S : S364K; T411E/K360E/Q362E : D401K; L368D/K370S : S364K/E357L and K370S : S364K/E357Q, according to EU numbering. In an exemplary embodiment, the skew variant set is S364K/E357Q : L368D/K370S.

[0011] In an exemplary embodiment of the "scIL-15/Rα X Fab" format heterodimeric protein, the "scIL-15/Rα X Fab" format heterodimeric protein includes: a) a first monomer comprising, from N-to C-terminal: i) an IL-15Rα(sushi) domain; ii) a first domain linker; iii) an IL-15 variant; iv) a hinge; v) a first variant Fc domain comprising CH2-CH3; b) a second monomer comprising, from N-to C-terminal, VH-CH1-hinge-CH2-CH3, wherein the CH2-CH3 is a second variant Fc domain; and c) a third monomer comprising a VL-CL. The VH and VL are a variable heavy domain and a variable light domain, respectively, that form a human TIM-3 antigen binding domain. In such embodiments, the first variant Fc domain comprises skew variants L368D/K370S and the second variant Fc domain comprises skew variants S364K/E357Q, the first and second variant Fc domains each comprise FcKO variants E233P/L234V/L235A/G236del/S267K, the first variant Fc domain comprises pI variants Q295E/N384D/Q418E/N421D, wherein numbering is according to EU numbering. In some embodiments, the hinge of the first monomer comprises amino acid substitution C220S,

wherein numbering is according to EU numbering. In an exemplary embodiment, the first and second variant Fc domains each further comprise half-life extension variants M428:/N434S.

[0012] In some embodiments of the "scIL-15/Rα X Fab" format heterodimeric protein, the IL-15 variant of the heterodimeric protein provided herein comprises an amino acid substitution(s) selected from the group consisting of N1D, N4D, D8N, D30N, D61N, E64Q, N65D, Q108E, N4D/N65D, D30N/N65D, and D30N/E64Q/N65D. In an exemplary embodiment, the IL-15 variant comprises amino acid substitutions N4D/N65D, D30N/N65D, or D30N/E64Q/N65D

[0013] In an exemplary embodiment, the "scIL-15/Rα X Fab" format heterodimeric protein is XENP27974, XENP27979, XENC1000, XENC1001, XENC1002, or XENC1003.

[0014] In certain embodiment, the VH and VL of the scIL-15/Rα X Fab" format heterodimeric proteins provided herein are the variable heavy domain and variable domain of any of the TIM-3 antigen binding domains in Figures 12 and 13. In an exemplary embodiment, the TIM-3 antigen binding domain is 3H3_H1_L2.1.

[0015] In one aspect, provided herein is a heterodimeric protein having the "scIL-15/Rα X scFv" format. In one embodiment, the heterodimeric protein includes: a) a first monomer comprising, from N-to C-terminal: i) an IL-15Rα(sushi) domain; ii) a first domain linker; iii) an IL-15 variant; iv) a second domain linker; and v) a first variant Fc domain comprising CH2-CH3; and b) a second monomer comprising, from N-to C-terminal: i) a scFv domain; ii) a third domain linker; and iii) a second variant Fc domain comprising CH2-CH3. In some embodiments, the scFv domain comprises a variable heavy domain (VH), an scFv linker and a variable light domain (VL), and the scFv domain binds human TIM-3. In some embodiments of the "scIL-15/Rα X scFv" format heterodimeric protein, the second domain linker and the third domain linker are each an antibody hinge.

[0016] In certain embodiments, the first variant Fc domain the second variant Fc domain comprises one of the following skew variant sets: S267K/L368D/K370S : S267K/S364K/E357Q; S364K/E357Q : L368D/K370S; L368D/K370S : S364K; L368E/K370S : S364K; T411E/K360E/Q362E : D401K; L368D/K370S : S364K/E357L and K370S : S364K/E357Q,

according to EU numbering. In an exemplary embodiment, the skew variant set is S364K/E357Q : L368D/K370S.

[0017] In some embodiments of the "scIL-15/Rα X scFv" format, the heterodimeric protein includes: a) a first monomer comprising, from N-to C-terminal: i) an IL-15Rα(sushi) domain; ii) a first domain linker; iii) an IL-15 variant; iv) a hinge; and v) a first variant Fc domain comprising CH2-CH3; and b) a second monomer comprising, from N-to C-terminal: i) a scFv domain; ii) a hinge; and iii) a second variant Fc domain comprising CH2-CH3. In some embodiments, the scFv domain comprises a variable heavy domain (VH), an scFv linker and a variable light domain (VL), and the scFv domain binds human TIM-3. In such embodiments, the first variant Fc domain comprises skew variants L368D/K370S and the second variant Fc domain comprises skew variants S364K/E357Q, the first and second variant Fc domains each comprise FcKO variants E233P/L234V/L235A/G236del/S267K, the first variant Fc domain comprises pI variants Q295E/N384D/Q418E/N421D, and the numbering is according to EU numbering. In certain embodiments, the first and second hinges each comprise amino acid substitution C220S, wherein numbering is according to EU numbering. In one embodiment, the first and second variant Fc domains each further comprise half-life extension variants M428:/N434S.

[0018] In another aspect, provided herein are "scFv X ncIL-15/Rα" format heterodimeric proteins. Such heterodimeric proteins include: a) a first monomer comprising, from N-to C-terminal: i) a scFv domain; ii) a first domain linker; and iii) a first variant Fc domain comprising CH2-CH3; b) a second monomer comprising, from N-to C-terminal: i) an IL-15Rα(sushi) domain; ii) a second domain linker; and iii) a second variant Fc domain comprising CH2-CH3; and c) a third monomer comprising an IL-15 variant. The scFv domain comprises a variable heavy domain (VH), an scFv linker and a variable light domain (VL), and the scFv domain binds human TIM-3. In one embodiment, the first domain linker and the second domain linker are each an antibody hinge.

[0019] In some embodiments of the "scFv X ncIL-15/Rα" format heterodimeric protein, the first variant Fc domain the second variant Fc domain comprises one of the following skew variant sets: S267K/L368D/K370S : S267K/S364K/E357Q; S364K/E357Q : L368D/K370S; L368D/K370S : S364K; L368E/K370S : S364K; T411E/K360E/Q362E : D401K; L368D/K370S :

S364K/E357L and K370S : S364K/E357Q, according to EU numbering. In an exemplary embodiment, the skew variant set is S364K/E357Q : L368D/K370S.

[0020] In an exemplary embodiment, the "scFv X ncIL-15/Rα" format heterodimeric protein includes: a) a first monomer comprising, from N-to C-terminal: i) a scFv domain; ii) a hinge; and iii) a first variant Fc domain comprising CH2-CH3; b) a second monomer comprising, from N-to C-terminal: i) an IL-15Rα(sushi) domain; ii) a hinge; and iii) a second variant Fc domain comprising CH2-CH3; and c) a third monomer comprising an IL-15 variant. Further, the scFv domain comprises a variable heavy domain (VH), an scFv linker and a variable light domain (VL), and the scFv domain binds human TIM-3. In such embodiments, the first variant Fc domain comprises skew variants L368D/K370S and the second variant Fc domain comprises skew variants S364K/E357Q, the first and second variant Fc domains each comprise FcKO variants E233P/L234V/L235A/G236del/S267K, the first variant Fc domain comprises pI variants Q295E/N384D/Q418E/N421D, and wherein numbering is according to EU numbering. In certain embodiments, the first and second hinges each comprise amino acid substitution C220S, wherein numbering is according to EU numbering. In one embodiment, the first and second variant Fc domains each further comprise half-life extension variants M428:/N434S.

[0021] In another aspect, provided herein are "scFv x dsIL-15/Rα" format heterodimeric proteins. The "scFv x dsIL-15/Rα" format heterodimeric protein includes: a) a first monomer comprising, from N-to C-terminal: i) a variant IL-15Rα(sushi) domain comprising an amino acid substituted for a cysteine residue; ii) a first domain linker; and iii) a first variant Fc domain comprising CH2-CH3; b) a second monomer comprising, from N-to C-terminal: i) a scFv domain; ii) a second domain linker; iii) a second variant Fc domain comprising CH2-CH3; an c) a third monomer comprising an IL-15 variant comprising an amino acid substituted for a cysteine residue. The scFv domain comprises a variable heavy domain (VH), an scFv linker and a variable light domain (VL), wherein the cysteine residue of the variant IL-15Rα(sushi) domain and the cysteine residue of the IL-15 variant form a disulfide bond and the scFv domain binds human TIM-3. In certain embodiments, the first domain linker and the second domain linker are each an antibody hinge.

[0022] In some embodiments of the "scFv x dsIL-15/Rα" format heterodimeric protein, the first variant Fc domain the second variant Fc domain comprises one of the following skew variant sets: S267K/L368D/K370S : S267K/S364K/E357Q; S364K/E357Q : L368D/K370S; L368D/K370S : S364K; L368E/K370S : S364K; T411E/K360E/Q362E : D401K; L368D/K370S : S364K/E357L and K370S : S364K/E357Q, according to EU numbering. In an exemplary embodiment, the skew variant set is S364K/E357Q : L368D/K370S.

[0023] In an exemplary embodiment, the "scFv x dsIL-15/Rα" format heterodimeric protein includes: a) a first monomer comprising, from N-to C-terminal: i) a variant IL-15Rα(sushi) domain comprising an amino acid substituted for a cysteine residue; ii) a hinge; and iii) a first variant Fc domain comprising CH2-CH3; b) a second monomer comprising, from N-to C-terminal: i) a scFv domain; ii) a hinge; iii) a second variant Fc domain comprising CH2-CH3; an c) a third monomer comprising an IL-15 variant comprising an amino acid substituted for a cysteine residue. The scFv domain comprises a variable heavy domain (VH), an scFv linker and a variable light domain (VL), wherein the cysteine residue of the variant IL-15Rα(sushi) domain and the cysteine residue of the IL-15 variant form a disulfide bond and the scFv domain binds human TIM-3. In such embodiments, the first variant Fc domain comprises skew variants L368D/K370S and the second variant Fc domain comprises skew variants S364K/E357Q, the first and second variant Fc domains each comprise FcKO variants E233P/L234V/L235A/G236del/S267K, the first variant Fc domain comprises pI variants Q295E/N384D/Q418E/N421D, wherein numbering is according to EU numbering. In certain embodiments, the hinges of the first and second monomers each comprise amino acid substitution C220S, wherein numbering is according to EU numbering. In some embodiments, the first and second variant Fc domains each comprise half-life extension variants M428:/N434S.

[0024] In one aspect, provided herein are "Fab X ncIL-15/Rα" format heterodimeric proteins. Such heterodimeric proteins include: a) a first monomer comprising, from N-to C-terminal, VH-CH1-hinge-CH2-CH3, wherein the CH2-CH3 is a first variant Fc domain; b) a second monomer comprising, from N-to C-terminal: i) an IL-15Rα(sushi) domain; ii) a first domain linker; iii) a first variant Fc domain comprising CH2-CH3; c) a third monomer comprising a light chain comprising VL-CL; and d) a fourth monomer comprising an IL-15

variant. The VH and VL are a variable heavy domain and a variable light domain, respectively, that form a human TIM-3 antigen binding domain. In some embodiments, the first domain linker is an antibody hinge.

[0025] In some embodiments of the "Fab X ncIL-15/Rα" format heterodimeric protein, the first variant Fc domain the second variant Fc domain comprises one of the following skew variant sets: S267K/L368D/K370S : S267K/S364K/E357Q; S364K/E357Q : L368D/K370S; L368D/K370S : S364K; L368E/K370S : S364K; T411E/K360E/Q362E : D401K; L368D/K370S : S364K/E357L and K370S : S364K/E357Q, according to EU numbering. In an exemplary embodiment, the skew variant set is S364K/E357Q : L368D/K370S.

[0026] In exemplary embodiments, the "Fab X ncIL-15/Rα" format heterodimeric protein includes: a) a first monomer comprising, from N-to C-terminal, VH-CH1-hinge-CH2-CH3, wherein the CH2-CH3 is a first variant Fc domain; b) a second monomer comprising, from N-to C-terminal: i) an IL-15Rα(sushi) domain; ii) a hinge; iii) a first variant Fc domain comprising CH2-CH3; c) a third monomer comprising a light chain comprising VL-CL; and d) a fourth monomer comprising an IL-15 variant. The VH and VL are a variable heavy domain and a variable light domain, respectively, that form a human TIM-3 antigen binding domain. In such embodiments, the first variant Fc domain comprises skew variants L368D/K370S and the second variant Fc domain comprises skew variants S364K/E357Q, the first and second variant Fc domains each comprise FcKO variants E233P/L234V/L235A/G236del/S267K, and the hinge-first variant Fc domain of the first monomer comprises pI variants N208D/Q295E/N384D/Q418E/N421D, wherein numbering is according to EU numbering. In some embodiments, the hinge of the second monomer comprises amino acid substitution C220S, wherein numbering is according to EU numbering. In certain embodiments, the first and second variant Fc domains each further comprise half-life extension variants M428:/N434S.

[0027] In another aspect, provided herein are "Fab X dsIL-15/Rα" format heterodimeric proteins. Such "Fab X dsIL-15/Rα" format heterodimeric proteins include: a) a first monomer comprising, from N-to C-terminal, VH-CH1-hinge-CH2-CH3, wherein CH2-CH3 is a first variant Fc domain; b) a second monomer comprising, from N-to C-terminal: i) a variant IL-15Rα(sushi) domain comprising an amino acid substituted for a cysteine residue;

ii) a first domain linker; and iii) a first variant Fc domain comprising CH2-CH3; c) a third monomer comprising, from N- to C- terminal, VL-CL; and d) a fourth monomer comprising an IL-15 variant comprising an amino acid substituted for a cysteine residue. Further, the cysteine residue of the variant IL-15Rα(sushi) domain and the cysteine residue of the IL-15 variant form a disulfide bond, and the VH and VL are a variable heavy domain and a variable light domain, respectively, that form a human TIM-3 antigen binding domain. In some embodiments, the first domain linker is an antibody hinge.

[0028] In some embodiments of the "Fab X dsIL-15/Rα" format heterodimeric protein, the first variant Fc domain the second variant Fc domain comprises one of the following skew variant sets: S267K/L368D/K370S : S267K/S364K/E357Q; S364K/E357Q : L368D/K370S; L368D/K370S : S364K; L368E/K370S : S364K; T411E/K360E/Q362E : D401K; L368D/K370S : S364K/E357L and K370S : S364K/E357Q, according to EU numbering. In an exemplary embodiment, the skew variant set is S364K/E357Q : L368D/K370S.

[0029] In an exemplary embodiment, the "Fab X dsIL-15/Rα" format heterodimeric protein includes: a) a first monomer comprising, from N-to C-terminal, VH-CH1-hinge-CH2-CH3, wherein CH2-CH3 is a first variant Fc domain; b) a second monomer comprising, from N-to C-terminal: i) a variant IL-15Rα(sushi) domain comprising an amino acid substituted for a cysteine residue; ii) a hinge; and iii) a first variant Fc domain comprising CH2-CH3; c) a third monomer comprising, from N- to C- terminal, VL-CL; and d) a fourth monomer comprising an IL-15 variant comprising an amino acid substituted for a cysteine residue. Further, the cysteine residue of the variant IL-15Rα(sushi) domain and the cysteine residue of the IL-15 variant form a disulfide bond, and the VH and VL are a variable heavy domain and a variable light domain, respectively, that form a humanTIM-3 antigen binding domain. In such embodiments, the first variant Fc domain comprises skew variants L368D/K370S and the second variant Fc domain comprises skew variants S364K/E357Q, the first and second variant Fc domains each comprise FcKO variants E233P/L234V/L235A/G236del/S267K, and the hinge-first variant Fc domain of the first monomer comprises pI variants N208D/Q295E/N384D/Q418E/N421D, wherein numbering is according to EU numbering. In certain embodiments, the hinge of the second monomer comprises amino acid substitution C220S, wherein numbering is according to EU

numbering. In some embodiments, the first and second variant Fc domains each further comprise half-life extension variants M428:/N434S.

[0030] In one aspect, provided herein are "mAb-scIL-15/Rα" format heterodimeric proteins. The "mAb-scIL-15/Rα" format heterodimeric proteins include: a) a first monomer comprising, from N-to C-terminal, VH-CH1-hinge-CH2-CH3, wherein the CH2-CH3 is a first variant Fc domain; b) a second monomer comprising, from N-to C-terminal, VH-CH1-hinge-CH2-CH3-domain linker-IL-15Rα(sushi) domain-domain linker-IL-15 variant, wherein the CH2-CH3 is a second variant Fc domain; and c) a third monomer and fourth monomer that each comprises, from N- to C- terminal, VL-CL. Further, the VH of the first monomer and the VL of the third monomer form a first human TIM-3 binding domain, and the VH of the second monomer and the VL of the fourth monomer form a second human TIM-3 binding domain.

[0031] In some embodiments of the "mAb-scIL-15/Rα" format heterodimeric protein, the first variant Fc domain the second variant Fc domain comprises one of the following skew variant sets: S267K/L368D/K370S : S267K/S364K/E357Q; S364K/E357Q : L368D/K370S; L368D/K370S : S364K; L368E/K370S : S364K; T411E/K360E/Q362E : D401K; L368D/K370S : S364K/E357L and K370S : S364K/E357Q, according to EU numbering. In an exemplary embodiment, the skew variant set is S364K/E357Q : L368D/K370S.

[0032] In some embodiments of the "mAb-scIL-15/Rα" format heterodimeric protein, the first variant Fc domain comprises skew variants L368D/K370S and the second variant Fc domain comprises skew variants S364K/E357Q, and the first and second variant Fc domains each comprise FcKO variants E233P/L234V/L235A/G236del/S267K, wherein numbering is according to EU numbering. In certain embodiments, a) the hinge-first variant Fc domain of the first monomer further comprises pI substitutions N208D/Q295E/N384D/Q418D/N421D and the hinge-second variant Fc domain of the second monomer further comprises pI variants Q196K/I199T/P271R/P228R/N276K; b) the hinge-first variant Fc domain of the first monomer further comprises pI substitutions N208D/Q295E/N384D/Q418D/N421D; or c) the hinge-second variant Fc domain of the second monomer further comprises pI variants Q196K/I199T/P271R/P228R/N276K, wherein numbering is according to EU numbering.

[0033] In some embodiments of the "mAb-scIL-15/Rα" format heterodimeric protein, the first variant Fc domain comprises skew variants S364K/E357Q and the second variant Fc domain comprises skew variants L368D/K370S, and the first and second variant Fc domains each comprise FcKO variants E233P/L234V/L235A/G236del/S267K, wherein numbering is according to EU numbering. In such embodiments, a) the hinge-first variant Fc domain of the first monomer further comprises pI substitutions Q196K/I199T/P271R/P228R/N276K and the hinge-second variant Fc domain of the second monomer further comprises pI variants N208D/Q295E/N384D/Q418D/N421D; b) the hinge-first variant Fc domain of the first monomer further comprises pI substitutions Q196K/I199T/P271R/P228R/N276K; or c) the hinge-second variant Fc domain of the second monomer further comprises pI variants N208D/Q295E/N384D/Q418D/N421D, wherein numbering is according to EU numbering.

[0034] In some embodiments of the "mAb-scIL-15/Rα" format heterodimeric protein, the first and second variant Fc domains each further comprise half-life extension variants M428:/N434S.

[0035] In another aspect, provided herein are "mAb-ncIL-15/Rα" format heterodimeric proteins. Such heterodimeric protein include: a) a first monomer comprising, from N-to C-terminal, VH-CH1-hinge-CH2-CH3, wherein the CH2-CH3 is a first variant Fc domain; b) a second monomer comprising, from N-to C-terminal, VH-CH1-hinge-CH2-CH3-domain linker-IL-15Rα(sushi) domain, wherein the CH2-CH3 is a second variant Fc domain; c) a third monomer comprising an IL-15 variant; and d) a fourth and fifth monomer that each comprises, from N- to C- terminal, VL-CL. The VH of the first monomer and the VL of the fourth monomer form a first human TIM-3 binding domain, and the VH of the second monomer and the VL of the fifth monomer form a second human TIM-3 binding domain.

[0036] In some embodiments of the "mAb-ncIL-15/Rα" format heterodimeric protein, the first variant Fc domain the second variant Fc domain comprises one of the following skew variant sets: S267K/L368D/K370S : S267K/S364K/E357Q; S364K/E357Q : L368D/K370S; L368D/K370S : S364K; L368E/K370S : S364K; T411E/K360E/Q362E : D401K; L368D/K370S : S364K/E357L and K370S : S364K/E357Q, according to EU numbering. In an exemplary embodiment, the skew variant set is S364K/E357Q : L368D/K370S.

[0037] In an exemplary embodiment of the "mAb-ncIL-15/Rα" format heterodimeric protein, the first variant Fc domain comprises skew variants L368D/K370S and the second variant Fc domain comprises skew variants S364K/E357Q, and the first and second variant Fc domains each comprise FcKO variants E233P/L234V/L235A/G236del/S267K, wherein numbering is according to EU numbering. In some embodiments, a) the hinge-first variant Fc domain of the first monomer further comprises pI substitutions N208D/Q295E/N384D/Q418D/N421D and the hinge-second variant Fc domain of the second monomer further comprises pI variants Q196K/I199T/P271R/P228R/N276K; b) the hinge-first variant Fc domain of the first monomer further comprises pI substitutions N208D/Q295E/N384D/Q418D/N421D; or c) the hinge-second variant Fc domain of the second monomer further comprises pI variants Q196K/I199T/P271R/P228R/N276K, wherein numbering is according to EU numbering.

[0038] In another exemplary embodiment of the "mAb-ncIL-15/Rα" format heterodimeric protein, the first variant Fc domain comprises skew variants S364K/E357Q and the second variant Fc domain comprises skew variants L368D/K370S, and the first and second variant Fc domains each comprise FcKO variants E233P/L234V/L235A/G236del/S267K, wherein numbering is according to EU numbering. In certain embodiments, a) the hinge-first variant Fc domain of the first monomer further comprises pI substitutions Q196K/I199T/P271R/P228R/N276K and the hinge-second variant Fc domain of the second monomer further comprises pI variants N208D/Q295E/N384D/Q418D/N421D; b) the hinge-first variant Fc domain of the first monomer further comprises pI substitutions Q196K/I199T/P271R/P228R/N276K; or c) the hinge-second variant Fc domain of the second monomer comprises pI variants N208D/Q295E/N384D/Q418D/N421D, wherein numbering is according to EU numbering.

[0039] In certain embodiments, the first and second variant Fc domains each further comprise half-life extension variants M428:/N434S.

[0040] In another aspect, provided herein are "mAb-dsIL-15/Rα" heterodimeric proteins. Such "mAb-dsIL-15/Rα" heterodimeric proteins include: a) a first monomer comprising, from N-to C-terminal, VH-CH1-hinge-CH2-CH3, wherein the CH2-CH3 is a first variant Fc domain; b) a second monomer comprising, from N-to C-terminal, VH-CH1-hinge-CH2-

CH3-domain linker-variant IL-15Rα(sushi) domain, wherein the variant IL-15Rα(sushi) domain an amino acid substituted for a cysteine residue and wherein the CH2-CH3 is a second variant Fc domain; c) a third monomer comprising an IL-15 variant comprising an amino acid substituted for a cysteine residue; and

[0041] d) a fourth and fifth monomer that each comprises, from N- to C- terminal, VL-CL. The cysteine residue of the variant IL-15Rα(sushi) domain and the cysteine residue of the IL-15 variant form a disulfide bond, the VH of the first monomer and the VL of the fourth monomer form a first human TIM-3 binding domain, and the VH of the second monomer and the VL of the fifth monomer form a second human TIM-3 binding domain.

[0042] In some embodiments, the first variant Fc domain the second variant Fc domain comprises one of the following skew variant sets: S267K/L368D/K370S : S267K/S364K/E357Q; S364K/E357Q : L368D/K370S; L368D/K370S : S364K; L368E/K370S : S364K; T411E/K360E/Q362E : D401K; L368D/K370S : S364K/E357L and K370S : S364K/E357Q, according to EU numbering. In an exemplary embodiment, the skew variant set is S364K/E357Q : L368D/K370S.

[0043] In an exemplary embodiment of the "mAb-dsIL-15/Rα" heterodimeric proteins, the first variant Fc domain comprises skew variants L368D/K370S and the second variant Fc domain comprises skew variants S364K/E357Q, and the first and second variant Fc domains each comprise FcKO variants E233P/L234V/L235A/G236del/S267K, wherein numbering is according to EU numbering. In some embodiments, a) the hinge-first variant Fc domain of the first monomer further comprises pI substitutions N208D/Q295E/N384D/Q418D/N421D and the hinge-second variant Fc domain of the second monomer further comprises pI variants Q196K/I199T/P271R/P228R/N276K; b) the hinge-first variant Fc domain of the first monomer further comprises pI substitutions N208D/Q295E/N384D/Q418D/N421D; or c) the hinge-second variant Fc domain of the second monomer further comprises pI variants Q196K/I199T/P271R/P228R/N276K, wherein numbering is according to EU numbering.

[0044] In another exemplary embodiment of the "mAb-dsIL-15/Rα" heterodimeric proteins, the first variant Fc domain comprises skew variants S364K/E357Q and the second variant Fc domain comprises skew variants L368D/K370S, and the first and second variant

Fc domains each comprise FcKO variants E233P/L234V/L235A/G236del/S267K, wherein numbering is according to EU numbering. In certain embodiments, a) the hinge-first variant Fc domain of the first monomer further comprises pI substitutions Q196K/I199T/P271R/P228R/N276K and the hinge-second variant Fc domain of the second monomer further comprises pI variants N208D/Q295E/N384D/Q418D/N421D; b) the hinge-first variant Fc domain of the first monomer further comprises pI substitutions Q196K/I199T/P271R/P228R/N276K; or c) the hinge-second variant Fc domain of the second monomer further comprises pI variants N208D/Q295E/N384D/Q418D/N421D, wherein numbering is according to EU numbering. In certain embodiments, the first and second variant Fc domains each further comprise half-life extension variants M428:/N434S.

[0045] In one aspect, provided herein are "central-IL-15/Rα" format heterodimeric proteins. Such "central-IL-15/Rα" format heterodimeric proteins include: a) a first monomer comprising, from N- to C-terminal, a VH-CH1-domain linker- IL-15 variant-hinge-CH2-CH3, wherein the CH2-CH3 is a first variant Fc domain; b) a second monomer comprising, from N- to C-terminal, a VH-CH1-domain linker- IL-15Rα(sushi) domain-hinge-CH2-CH3, wherein the CH2-CH3 is a second variant Fc domain; and c) a third and fourth monomer that each comprises, from N- to C- terminal, VL-CL. The VH of the first monomer and the VL of the third monomer form a first human TIM-3 binding domain, and the VH of the second monomer and the VL of the fourth monomer form a second human TIM-3 binding domain.

[0046] In some embodiments of the "central-IL-15/Rα" format heterodimeric protein, the first variant Fc domain the second variant Fc domain comprises one of the following skew variant sets: S267K/L368D/K370S : S267K/S364K/E357Q; S364K/E357Q : L368D/K370S; L368D/K370S : S364K; L368E/K370S : S364K; T411E/K360E/Q362E : D401K; L368D/K370S : S364K/E357L and K370S : S364K/E357Q, according to EU numbering. In an exemplary embodiment, the skew variant set is S364K/E357Q : L368D/K370S.

[0047] In an exemplary embodiment, the first variant Fc domain comprises skew variants L368D/K370S and the second variant Fc domain comprise the skew variant pair S364K/E357Q, the first and second variant Fc domains each comprise FcKO variants E233P/L234V/L235A/G236del/S267K, and the first variant Fc domain comprises pI

substitutions Q295E/N384D/Q418D/N421D, wherein numbering is according to EU numbering.

[0048] In an exemplary embodiment of the "central-IL-15/Rα" format heterodimeric protein, the first variant Fc domain comprises skew variants S364K/E357Q and the second variant Fc domain comprise the skew variant pair L368D/K370S, the first and second variant Fc domains each comprise FcKO variants E233P/L234V/L235A/G236del/S267K, and the second variant Fc domain of the second monomer comprises pI substitutions Q295E/N384D/Q418D/N421D, wherein numbering is according to EU numbering. In some embodiments of the "central-IL-15/Rα" format heterodimeric protein, the hinge of the first and second monomers each comprise amino acid substitution C220S, wherein numbering is according to EU numbering. In certain embodiments, the first and second variant Fc domains each further comprise half-life extension variants M428:/N434S.

[0049] In another aspect, provided herein are "central-scIL-15/Rα" format heterodimeric proteins. Such "central-scIL-15/Rα" format heterodimeric proteins include: a) a first monomer comprising, from N-to C-terminal, VH-CH1-domain linker- IL-15Rα(sushi) domain-domain linker-IL-15 variant-hinge-CH2-CH3, wherein the CH2-CH3 is a first variant Fc domain; b) a second monomer comprising, from N-to C-terminal, a VH-CH1-hinge-CH2-CH3, wherein the CH2-CH3 is a second variant Fc domain; and c) a third and fourth monomer that each comprises, from N-to C-terminal, VL-CL. The VH of the first monomer and the VL of the third monomer form a first human TIM-3 binding domain, and the VH of the second monomer and the VL of the fourth monomer form a second human TIM-3 binding domain.

[0050] In some embodiments of the "central-scIL-15/Rα" format heterodimeric protein, the first variant Fc domain the second variant Fc domain comprises one of the following skew variant sets: S267K/L368D/K370S : S267K/S364K/E357Q; S364K/E357Q : L368D/K370S; L368D/K370S : S364K; L368E/K370S : S364K; T411E/K360E/Q362E : D401K; L368D/K370S : S364K/E357L and K370S : S364K/E357Q, according to EU numbering. In an exemplary embodiment, the skew variant set is S364K/E357Q : L368D/K370S.

[0051] In an exemplary embodiment, the first variant Fc domain comprises skew variants L368D/K370S and the second variant Fc domain comprises skew variants S364K/E357Q, the first and second variant Fc domains each comprise FcKO variants E233P/L234V/L235A/G236del/S267K, and the first variant Fc domain comprises pI variants Q295E/N384D/Q418E/N421D, wherein numbering is according to EU numbering. In some embodiments, the hinge of the first monomer comprises amino acid substitution C220S, wherein numbering is according to EU numbering. In certain embodiments, the first and second variant Fc domains each further comprise half-life extension variants M428:/N434S.

[0052] In certain embodiment, the VH and VL of any of the heterodimeric proteins provided herein are the variable heavy domain and variable domain of any of the TIM-3 antigen binding domains in Figures 12 and 13. In an exemplary embodiment, the TIM-3 antigen binding domain is 3H3_H1_L2.1.

[0053] In some embodiments, the IL-15 variant of the heterodimeric protein provided herein comprises an amino acid substitution(s) selected from the group consisting of N1D, N4D, D8N, D30N, D61N, E64Q, N65D, Q108E, N4D/N65D, D30N/N65D, and D30N/E64Q/N65D. In an exemplary embodiment, the IL-15 variant comprises amino acid substitutions N4D/N65D, D30N/N65D, or D30N/E64Q/N65D

[0054] In one aspect, provided herein is a pharmaceutical composition that includes any of the heterodimeric proteins disclosed herein and a pharmaceutically acceptable carrier.

[0055] In another aspect, provided herein is a method of treating a patient in need thereof comprising administering to the patient any one of the heterodimeric proteins or pharmaceutical compositions disclosed herein. In some embodiments, the method further comprising administering an antibody, where the antibody is an anti-PD-1 antibody, an anti-PD-L1 antibody, an anti-CTLA-4 antibody, an anti-TIM-3 antibody or an-anti-TIGIT antibody.

[0056] In another aspect, provided herein are nucleic acid compositions that include one or more nucleic acids encoding any of the heterodimeric proteins disclosed herein, expression vectors that include the nucleic acids, host cells that include the nucleic acids or expression

vectors. Also provided herein are methods of making subject heterodimeric proteins by culturing host cells under suitable conditions and recovering the heterodimeric proteins.

BRIEF DESCRIPTION OF THE DRAWINGS

[0057] Figure 1 depicts selectivity of TIM-3-targeted IL-15/Rα-Fc fusion proteins for tumor-reactive tumor-infiltrating lymphocytes expressing PD-1, and its combination with PD-1 blockade antibody.

[0058] Figures 2A-2B depict the sequences for IL-15 and its receptors.

[0059] Figure 3 depicts the sequences for TIM-3, including both human and cyno (predicted), to facilitate the development of antigen binding domains that bind to both for ease of clinical development.

[0060] Figures 4A-4E depict useful pairs of Fc heterodimerization variant sets (including skew and pI variants). There are variants for which there are no corresponding "monomer 2" variants; these are pI variants which can be used alone on either monomer.

[0061] Figure 5 depicts a list of isosteric variant antibody constant regions and their respective substitutions. pI_(-) indicates lower pI variants, while pI_(+) indicates higher pI variants. These can be optionally and independently combined with other heterodimerization variants of the inventions (and other variant types as well, as outlined herein.)

[0062] Figure 6 depicts useful ablation variants that ablate FcγR binding (sometimes referred to as "knock outs" or "KO" variants). Generally, ablation variants are found on both monomers, although in some cases they may be on only one monomer.

[0063] Figures 7A-7F show particularly useful embodiments of "non-cytokine"/"non-Fv" components of the TIM-3-targeting IL-15/Rα-Fc fusion proteins of the invention.

[0064] Figure 8 depicts a number of exemplary variable length linkers for use in IL-15/Rα-Fc fusion proteins. In some embodiments, these linkers find use linking the C-terminus of IL-15 and/or IL-15Rα(sushi) to the N-terminus of the Fc region. In some embodiments, these linkers find use fusing IL-15 to the IL-15Rα(sushi).

[0065] Figure 9A-9C depict a number of charged scFv linkers that find use in increasing or decreasing the pI of heterodimeric antibodies that utilize one or more scFv as a component. The (+H) positive linker finds particular use herein. A single prior art scFv linker with single charge is referenced as "Whitlow", from Whitlow et al., Protein Engineering 6(8):989-995 (1993). It should be noted that this linker was used for reducing aggregation and enhancing proteolytic stability in scFvs.

[0066] Figure 10 shows the sequences of several useful TIM-3-targeting IL-15/Rα-Fc fusion format backbones based on human IgG1, without the cytokine sequences (e.g., the Il-15 and/or IL-15Rα(sushi)) or VH, and further excluding light chain backbones which are depicted in Figure 11. Backbone 1 is based on human IgG1 (356E/358M allotype), and includes the S364K/E357Q : L368D/K370S skew variants, C220S and the Q295E/N384D/Q418E/N421D pI variants on the chain with L368D/K370S skew variants and the E233P/L234V/L235A/G236del/S267K ablation variants on both chains. Backbone 2 is based on human IgG1 (356E/358M allotype), and includes the S364K/E357Q : L368D/K370S skew variants, the N208D/Q295E/N384D/Q418E/N421D pI variants on the chain with L368D/K370S skew variants, C220S in the chain with S364K/E357Q variants, and the E233P/L234V/L235A/G236del/S267K ablation variants on both chains. Backbone 3 is based on human IgG1 (356E/358M allotype), and includes the S364K/E357Q : L368D/K370S skew variants, the N208D/Q295E/N384D/Q418E/N421D pI variants on the chains with L368D/K370S skew variants, the Q196K/I199T/P217R/P228R/N276K pI variants on the chains with S364K/E357Q variants, and the E233P/L234V/L235A/G236del/S267K ablation variants on both chains. Such backbone sequences can be included, for example, in the "scIL-15/Rα X Fab" format heterodimeric proteins described herein.). In some embodiments, the "scIL-15/Rα X Fab" format heterodimeric protein includes: a) a first monomer that includes, from N- to C-terminus, an IL-15Rα(sushi) domain-(domain linker)-IL-15 variant-(hinge)-CH2-CH3, where hinge-CH2-CH3 has the amino acid sequence of "Chain 2" of any of the backbone sequences in Figure 10 (SEQ ID NO: XXX-XXX); b) a second monomer that includes, from N- to C-terminus, a VH-CH1-hinge-CH2-CH3, where VH is a variable heavy domain and CH1-hinge-CH2-CH3 has the amino acid sequence of Chain 1 of any one of the backbone sequences in Figure 10 (SEQ ID NO: XXX-XXX), and c) a light chain that includes

from, N- to C-terminus, VL-VC, where VL is a variable light domain and VC has the sequence of "Constant Light Chain – Kappa" or "Constant Light Chain – Lambda "in Figure 11 (SEQ ID NO: XXX-XXX). In an exemplary embodiment, the IL-15 variant includes amino acid substitutions N4D/N65D, D30N/N65D, or D30N/E64Q/N65D. In exemplary embodiments, the VH and VL are the variable heavy domain and variable light domain, respectively, of any of the TIM-3 ABDs provided in Figures 12 and 13A-C.

[0067] In certain embodiments, these sequences can be of the 356D/358L allotype. In other embodiments, these sequences can include either the N297A or N297S substitutions. In some other embodiments, these sequences can include the M428L/N434S Xtend mutations. In yet other embodiments, these sequences can instead be based on human IgG4, and include a S228P (EU numbering, this is S241P in Kabat) variant on both chains that ablates Fab arm exchange as is known in the art. In yet further embodiments, these sequences can instead be based on human IgG2. Further, these sequences may instead utilize the other skew variants, pI variants, and ablation variants depicted in the Figures.

[0068] As will be appreciated by those in the art and outlined below, these sequences can be used with any IL-15 and IL-15Rα(sushi) pairs outlined herein, including but not limited to scIL-15/Rα, ncIL-15/Rα, and dsIL-15Rα, as schematically depicted in Figures 21. Further as will be appreciated by those in the art and outlined below, any IL-15 and/or IL-15Rα(sushi) variants can be incorporated in these backbones. Furthermore as will be appreciated by those in the art and outlined below, these sequences can be used with any VH and VL pairs outlined herein, including either a scFv or a Fab.

[0069] Included within each of these backbones are sequences that are 90, 95, 98 and 99% identical (as defined herein) to the recited sequences, and/or contain from 1, 2, 3, 4, 5, 6, 7, 8, 9 or 10 additional amino acid substitutions (as compared to the "parent" of the Figure, which, as will be appreciated by those in the art, already contain a number of amino acid modifications as compared to the parental human IgG1 (or IgG2 or IgG4, depending on the backbone). That is, the recited backbones may contain additional amino acid modifications (generally amino acid substitutions) in addition to the skew, pI and ablation variants contained within the backbones of this figure.

[0070] Figure 11 depicts the "non-Fv" backbone of light chains (i.e. constant light chain) which find use in TIM-3-targeting IL-15/Rα-Fc fusion proteins of the invention.

[0071] Figure 12 depicts the variable region sequences for a select number of anti-TIM-3 antibody binding domains. The CDRs are underlined. As noted herein and is true for every sequence herein containing CDRs, the exact identification of the CDR locations may be slightly different depending on the numbering used as is shown in Table 2, and thus included herein are not only the CDRs that are underlined but also CDRs included within the V_H and V_L domains using other numbering systems. Furthermore, as for all the sequences in the Figures, these V_H and V_L sequences can be used either in a scFv format or in a Fab format.

[0072] Figures 13A-13C depict the variable regions of additional TIM-3 ABDs which may find use in the TIM-3-targeting IL-15/Rα-Fc fusion proteins of the invention. The CDRs are underlined. As noted herein and is true for every sequence herein containing CDRs, the exact identification of the CDR locations may be slightly different depending on the numbering used as is shown in Table 2, and thus included herein are not only the CDRs that are underlined but also CDRs included within the V_H and V_L domains using other numbering systems. Furthermore, as for all the sequences in the Figures, these V_H and V_L sequences can be used either in a scFv format or in a Fab format.

[0073] Figure 14 depicts a structural model of the IL-15/Rα heterodimer showing locations of engineered disulfide bond pairs.

[0074] Figure 15 depicts sequences for illustrative IL-15Rα(sushi) variants engineered with additional residues at the C-terminus to serve as a scaffold for engineering cysteine residues.

[0075] Figure 16 depicts sequences for illustrative IL-15 variants engineered with cysteines in order to form covalent disulfide bonds with IL-15Rα(sushi) variants engineered with cysteines.

[0076] Figure 17 depicts sequences for illustrative IL-15Rα(sushi) variants engineered with cysteines in order to form covalent disulfide bonds with IL-15 variants engineered with cysteines.

[0077] Figure 18 depicts the structure of IL-15 complexed with IL-15Rα, IL-2Rß, and common gamma chain. Locations of substitutions designed to reduce potency are shown.

[0078] Figure 19A-19C depicts sequences for illustrative IL-15 variants engineered for reduced potency. Included within each of these variant IL-15 sequences are sequences that are 90, 95, 98 and 99% identical (as defined herein) to the recited sequences, and/or contain from 1, 2, 3, 4, 5, 6, 7, 8, 9 or 10 additional amino acid substitutions. In a non-limiting example, the recited sequences may contain additional amino acid modifications such as those contributing to formation of covalent disulfide bonds as shown in Figure 16 and Figure 17.

[0079] Figure 20 depicts EC50 for induction of NK and $CD8^+$ T cells proliferation by variant IL-15/Rα-Fc fusion proteins, and fold reduction in EC50 relative to XENP20818, the wild type. These fusion proteins do not contain a TIM-3 ABD.

[0080] Figures 21A- 21K depict several formats for the TIM-3-targeting IL-15/Rα-Fc fusion proteins of the present invention. The "scIL-15/Rα x scFv" format (Figures 21A) comprises IL-15Rα(sushi) fused to IL-15 by a variable length linker (termed "scIL-15/Rα") which is then fused to the N-terminus of a heterodimeric Fc-region, with an scFv fused to the other side of the heterodimeric Fc. The "scFv x ncIL-15/Rα" format (Figures 21B) comprises an scFv fused to the N-terminus of a heterodimeric Fc-region, with IL-15Rα(sushi) fused to the other side of the heterodimeric Fc, while IL-15 is transfected separately so that a non-covalent IL-15/Rα complex is formed. The "scFv x dsIL-15/Rα" format (Figures 21C) is the same as the "scFv x ncIL-15/Rα" format, but wherein IL-15Rα(sushi) and IL-15 are covalently linked as a result of engineered cysteines. The "scIL-15/Rα x Fab" format (Figures 21D) comprises IL-15Rα(sushi) fused to IL-15 by a variable length linker (termed "scIL-15/Rα") which is then fused to the N-terminus of a heterodimeric Fc-region, with a variable heavy chain (VH) fused to the other side of the heterodimeric Fc, while a corresponding light chain is transfected separately so as to form a Fab with the VH. The "ncIL-15/Rα x Fab" format (Figures 21E) comprises a VH fused to the N-terminus of a heterodimeric Fc-region, with IL-15Rα(sushi) fused to the other side of the heterodimeric Fc, while a corresponding light chain is transfected separately so as to form a Fab with the VH, and while IL-15 is transfected separately so that a non-covalent IL-15/Rα complex is formed.

The "dsIL-15/Rα x Fab" format (Figures 21F) is the same as the "ncIL-15/Rα x Fab" format, but wherein IL-15Rα(sushi) and IL-15 are covalently linked as a result of engineered cysteines. The "mAb-scIL-15/Rα" format (Figures 21G) comprises VH fused to the N-terminus of a first and a second heterodimeric Fc, with IL-15 is fused to IL-15Rα(sushi) which is then further fused to the C-terminus of one of the heterodimeric Fc-region, while corresponding light chains are transfected separately so as to form a Fabs with the VHs. The "mAb-ncIL-15/Rα" format (Figures 21H) comprises VH fused to the N-terminus of a first and a second heterodimeric Fc, with IL-15Rα(sushi) fused to the C-terminus of one of the heterodimeric Fc-region, while corresponding light chains are transfected separately so as to form a Fabs with the VHs, and while and while IL-15 is transfected separately so that a non-covalent IL-15/Rα complex is formed. The "mAb-dsIL-15/Rα" format (Figures 21I) is the same as the "mAb-ncIL-15/Rα" format, but wherein IL-15Rα(sushi) and IL-15 are covalently linked as a result of engineered cysteines. The "central-IL-15/Rα" format (Figures 21J) comprises a VH recombinantly fused to the N-terminus of IL-15 which is then further fused to one side of a heterodimeric Fc and a VH recombinantly fused to the N-terminus of IL-15Rα(sushi) which is then further fused to the other side of the heterodimeric Fc, while corresponding light chains are transfected separately so as to form a Fabs with the VHs. The "central-scIL-15/Rα" format (Figures 21K) comprises a VH fused to the N-terminus of IL-15Rα(sushi) which is fused to IL-15 which is then further fused to one side of a heterodimeric Fc and a VH fused to the other side of the heterodimeric Fc, while corresponding light chains are transfected separately so as to form a Fabs with the VHs.

[0081] Figure 22 depicts sequences of XENP27974, an illustrative TIM-3-targeting IL-15/Rα-Fc fusion protein of the "scIL-15/Rα x Fab" format. The CDRs are in bold. As noted herein and is true for every sequence herein containing CDRs, the exact identification of the CDR locations may be slightly different depending on the numbering used as is shown in Table 2, and thus included herein are not only the CDRs that are underlined but also CDRs included within the V_H and V_L domains using other numbering systems. IL-15 and IL-15Rα(sushi) are underlined, linkers are double underlined (although as will be appreciated by those in the art, the linkers can be replaced by other linkers, some of which are depicted in the Figures,

and slashes (/) indicate the border(s) between IL-15, IL-15Rα, linkers, variable regions, and constant/Fc regions.

[0082] Figure 23 depicts the sequences for XENP16432, a bivalent anti-PD-1 mAb with an ablation variant (E233**P**/L234**V**/L235**A**/G236**del**/S267**K**, "IgG1_PVA_/S267k"). The CDRs are underlined. As noted herein and is true for every sequence herein containing CDRs, the exact identification of the CDR locations may be slightly different depending on the numbering used as is shown in Table 2, and thus included herein are not only the CDRs that are underlined but also CDRs included within the V_H and V_L domains using other numbering systems.

[0083] Figures 24A-24B depict $CD8^+$ T cell counts in whole blood of PBMC-engrafted NSG mice on Days A) 6 and B) 10 after first dose of the indicated test articles.

[0084] Figures 25A-25B depict $CD4^+$ T cell counts in whole blood of PBMC-engrafted NSG mice on Days A) 6 and B) 10 after first dose of the indicated test articles.

[0085] Figures 26A-26B depict $CD45^+$ T cell counts in whole blood of PBMC-engrafted NSG mice on Days A) 6 and B) 10 after first dose of the indicated test articles.

[0086] Figures 27A-27B depict $CD16^+CD56^+$ NK cell counts in whole blood of PBMC-engrafted NSG mice on Days A) 6 and B) 10 after first dose of the indicated test articles.

[0087] Figure 28 depicts the change in body weight (as percentage of initial body weight) of PBMC-engrafted NSG mice after dosing with the indicated test articles.

[0088] Figure 29 depicts the sequence of XENP27979 that include M428L/N434S variants in both Fc domains.

[0089] Figure 30 depicts induction of A) CD8+ T cells and B) CD4+ T cells proliferation by TIM-3-targeted IL-15/Rα-Fc fusions (and controls) as indicated by percentage proliferating cells (determined based on CFSE dilution). The data show that the TIM-3-targeted IL-15/Rα-Fc fusion is more potent in inducing proliferation of both CD8+ and CD4+ T cells in comparison to untargeted IL-15(D30N/E64Q/N65D)/Rα-Fc fusion (as well as control RSV-targeted IL-15/Rα-Fc fusion).

[0090] Figure 31 depicts induction of A) CD8 memory T cell and B) CD8 naive T cell proliferation by TIM-3-targeted IL-15/Rα-Fc fusions (and controls) as indicated by percentage proliferating cells (determined based on CFSE dilution). The data show that the TIM-3-targeted IL-15/Rα-Fc fusion is much more potent in inducing proliferation of CD8 memory T cells in comparison to untargeted IL-15(D30N/E64Q/N65D)/Rα-Fc fusion (as well as control RSV-targeted IL-15/Rα-Fc fusion). Notably, the TIM-3-targeted IL-15/Rα-Fc fusion is also more potent in inducing proliferation of CD8 memory T cells in comparison to CD8 naive T cells.

[0091] Figure 32 depicts induction of A) CD8 memory T cell and B) CD8 naive T cell proliferation by TIM-3-targeted IL-15/Rα-Fc fusions (and controls) as indicated by cell counts.

[0092] Figure 33 depicts induction of A) CD4 memory T cell and B) CD4 naive T cell proliferation by TIM-3-targeted IL-15/Rα-Fc fusions (and controls) as indicated by percentage proliferating cells (determined based on CFSE dilution). The data show that the TIM-3-targeted IL-15/Rα-Fc fusion is much more potent in inducing proliferation of CD4 memory T cells in comparison to untargeted IL-15(D30N/E64Q/N65D)/Rα-Fc fusion (as well as control RSV-targeted IL-15/Rα-Fc fusion). Notably, the TIM-3-targeted IL-15/Rα-Fc fusion is also more potent in inducing proliferation of CD4 memory T cells in comparison to CD4 naive T cells.

[0093] Figure 34 depicts induction of A) CD4 memory T cell and B) CD4 naive T cell proliferation by TIM-3-targeted IL-15/Rα-Fc fusions (and controls) as indicated by cell counts.

[0094] Figure 35 depicts induction of NK cells proliferation by TIM-3-targeted IL-15/Rα-Fc fusions (and controls) as indicated A) percentage proliferating cells (determined based on CFSE dilution) and B) by cell counts. The data show that TIM-3-targeted IL-15/Rα-Fc fusions are much more potent in inducing proliferation of NK cells in comparison to untargeted IL-15(D30N/E64Q/N65D)/Rα-Fc fusion (as well as control RSV-targeted IL-15/Rα-Fc fusion).

[0095] Figure 36 depicts activation of CD8+ T cells as indicated by A) percentage CD8 memory T cells expressing CD25, B) percentage CD8 naive T cells expressing CD25, C) percentage CD4 memory T cells expressing CD25, and D) percentage CD4 naive T cells expressing CD25 following incubation with TIM-3-targeted IL-15/Rα-Fc fusions (and controls). The data show that TIM-3-targeted IL-15/Rα-Fc fusions appear to upregulate CD25 in CD8 memory and naive T cells more potently in comparison to untargeted IL-15(D30N/E64Q/N65D)/Rα-Fc fusion (as well as control RSV-targeted IL-15/Rα-Fc fusion).

[0096] Figure 37 depicts activation of CD8+ T cells as indicated by A) HLA-DR MFI on CD8 memory T cells, B) percentage CD8 memory T cells expressing HLA-DR, C) HLA-DR MFI on CD8 naive T cells, and D) percentage CD8 naive T cells expressing HLA-DR following incubation with TIM-3-targeted IL-15/Rα-Fc fusions (and controls).

[0097] Figure 38 depicts activation of CD4+ T cells as indicated by A) HLA-DR MFI on CD4 memory T cells, B) percentage CD4 memory T cells expressing HLA-DR, C) HLA-DR MFI on CD4 naive T cells, and D) percentage CD4 naive T cells expressing HLA-DR following incubation with TIM-3-targeted IL-15/Rα-Fc fusions (and controls).

[0098] Figure 39 depicts the sequences of XENP22853, an IL-15/Rα-heteroFc fusion comprising a wild-type IL-15 and Xtend Fc (M428L/N434S) variant. IL-15 and IL-15Rα(sushi) are underlined, linkers are double underlined (although as will be appreciated by those in the art, the linkers can be replaced by other linkers, some of which are depicted in the Figures, and slashes (/) indicate the border(s) between IL-15, IL-15Rα, linkers, and constant/Fc regions.

[0099] Figure 40 depicts the sequences of XENP24113, an IL-15/Rα-heteroFc fusion comprising a IL-15(N4D/N65D) variant and Xtend Fc (M428L/N434S) variant. IL-15 and IL-15Rα(sushi) are underlined, linkers are double underlined (although as will be appreciated by those in the art, the linkers can be replaced by other linkers, some of which are depicted in the Figures, and slashes (/) indicate the border(s) between IL-15, IL-15Rα, linkers, and constant/Fc regions.

[00100] Figure 41 depicts the sequences of XENP24294, an scIL-15/Rα-Fc fusion comprising a IL-15(N4D/N65D) variant and Xtend Fc (M428L/N434S) substitution. IL-15

and IL-15Rα(sushi) are underlined, linkers are double underlined (although as will be appreciated by those in the art, the linkers can be replaced by other linkers, some of which are depicted in the Figures, and slashes (/) indicate the border(s) between IL-15, IL-15Rα, linkers, and constant/Fc regions.

[00101] Figure 42 depicts the sequences of XENP24306, an IL-15/Rα-heteroFc fusion comprising a IL-15(D30N/E64Q/N65D) variant and Xtend Fc (M428L/N434S) substitution. IL-15 and IL-15Rα(sushi) are underlined, linkers are double underlined (although as will be appreciated by those in the art, the linkers can be replaced by other linkers, some of which are depicted in the Figures, and slashes (/) indicate the border(s) between IL-15, IL-15Rα, linkers, and constant/Fc regions.

[00102] Figure 43 depicts the serum concentration of the indicated test articles over time in cynomolgus monkeys following a first dose at the indicated relative concentrations.

[00103] Figure 44A-Figure 44C depict sequences of illustrative scIL-15/Rα-Fc fusions comprising additional IL-15 potency variants. IL-15 and IL-15Rα(sushi) are underlined, linkers are double underlined (although as will be appreciated by those in the art, the linkers can be replaced by other linkers, some of which are depicted in Figures some of which are depicted in Figures 9 and 10), and slashes (/) indicate the border(s) between IL-15, IL-15Rα, linkers, variable regions, and constant/Fc regions.

[00104] Figure 45 depicts percentage of A) CD4+CD45RA-, B) CD4+CD45RA+, C) CD8+CD45RA-, D) CD8+CD45RA+, E) CD16+ NK cells, F) CD56+ NK cells, and G) γδ cells expression Ki67 following incubation of PBMCs with the indicated test articles for 3 days.

[00105] Figure 46 depicts sequences of illustrative TIM-3-targeted IL-15/Rα-Fc fusions comprising IL-15(D30N/N65D) variant. The CDRs are in bold. As noted herein and is true for every sequence herein containing CDRs, the exact identification of the CDR locations may be slightly different depending on the numbering used as is shown in Table 2, and thus included herein are not only the CDRs that are underlined but also CDRs included within the VH and VL domains using other numbering systems. IL-15 and IL-15Rα(sushi) are underlined, linkers are double underlined (although as will be appreciated by those in the

art, the linkers can be replaced by other linkers, some of which are depicted in Figures 9 and 10), and slashes (/) indicate the border(s) between IL-15, IL-15Rα, linkers, variable regions, and constant/Fc regions.

[00106] Figure 47 depicts sequences of illustrative TIM-3-targeted IL-15/Rα-Fc fusions comprising IL-15(D30N/E64Q/N65D) variant. The CDRs are in bold. As noted herein and is true for every sequence herein containing CDRs, the exact identification of the CDR locations may be slightly different depending on the numbering used as is shown in Table 2, and thus included herein are not only the CDRs that are underlined but also CDRs included within the VH and VL domains using other numbering systems. IL-15 and IL-15Rα(sushi) are underlined, linkers are double underlined (although as will be appreciated by those in the art, the linkers can be replaced by other linkers, some of which are depicted in Figures 9 and 10), and slashes (/) indicate the border(s) between IL-15, IL-15Rα, linkers, variable regions, and constant/Fc regions.

[00107] Figure 48A and Figure 48B depict sequences of illustrative TIM-3-targeted IL-15/Rα-Fc fusions comprising Xtend (M428L/N434S) substitutions for enhancing serum half-life. The CDRs are in bold. As noted herein and is true for every sequence herein containing CDRs, the exact identification of the CDR locations may be slightly different depending on the numbering used as is shown in Table 2, and thus included herein are not only the CDRs that are underlined but also CDRs included within the VH and VL domains using other numbering systems. IL-15 and IL-15Rα(sushi) are underlined, linkers are double underlined (although as will be appreciated by those in the art, the linkers can be replaced by other linkers, some of which are depicted in Figures 9 and 10), and slashes (/) indicate the border(s) between IL-15, IL-15Rα, linkers, variable regions, and constant/Fc regions. It should be noted that any of the sequences depicted herein may include or exclude the M428L/N434S substitutions.

[00108] Figure 49A-49C depicts the sequences of XENP26007, XENP29481, and XENP30432, control RSV-targeted IL-15/Rα-Fc fusions. The CDRs are underlined. As noted herein and is true for every sequence herein containing CDRs, the exact identification of the CDR locations may be slightly different depending on the numbering used as is shown in Table 2, and thus included herein are not only the CDRs that are underlined but also CDRs

included within the VH and VL domains using other numbering systems. IL-15 and IL-15Rα(sushi) are italicized, linkers are double underlined (although as will be appreciated by those in the art, the linkers can be replaced by other linkers, some of which are depicted in Figures some of which are depicted in Figures 9 and 10), and slashes (/) indicate the border(s) between IL-15, IL-15Rα, linkers, variable regions, and constant/Fc regions.

DETAILED DESCRIPTION OF THE INVENTION

I. Definitions

[00109] In order that the application may be more completely understood, several definitions are set forth below. Such definitions are meant to encompass grammatical equivalents.

[00110] By "ablation" herein is meant a decrease or removal of activity. Thus for example, "ablating FcγR binding" means the Fc region amino acid variant has less than 50% starting binding as compared to an Fc region not containing the specific variant, with less than 70-80-90-95-98% loss of activity being preferred, and in general, with the activity being below the level of detectable binding in a Biacore assay. Of particular use in the ablation of FcγR binding are those shown in Figure 6. However, unless otherwise noted, the Fc monomers of the invention retain binding to the FcRn receptor.

[00111] By "ADCC" or "antibody dependent cell-mediated cytotoxicity" as used herein is meant the cell-mediated reaction wherein nonspecific cytotoxic cells that express FcγRs recognize bound antibody on a target cell and subsequently cause lysis of the target cell. ADCC is correlated with binding to FcγRIIIa; increased binding to FcγRIIIa leads to an increase in ADCC activity. As is discussed herein, many embodiments of the invention ablate ADCC activity entirely.

[00112] By "ADCP" or antibody dependent cell-mediated phagocytosis as used herein is meant the cell-mediated reaction wherein nonspecific cytotoxic cells that express FcγRs recognize bound antibody on a target cell and subsequently cause phagocytosis of the target cell.

[00113] By "antigen binding domain" or "ABD" herein is meant a set of six **C**omplementary **D**etermining **R**egions (CDRs) that, when present as part of a polypeptide sequence, specifically binds a target antigen as discussed herein. Thus, a "TIM-3 antigen binding domain" binds a human TIM-3 antigen as outlined herein. As is known in the art, these CDRs are generally present as a first set of variable heavy CDRs (vhCDRs or V_HCDRs) and a second set of variable light CDRs (vlCDRs or V_LCDRs), each comprising three CDRs: vhCDR1, vhCDR2, vhCDR3 for the heavy chain and vlCDR1, vlCDR2 and vlCDR3 for the light. The CDRs are present in the variable heavy and variable light domains, respectively, and together form an Fv region. Thus, in some cases, the six CDRs of the antigen binding domain are contributed by a variable heavy and variable light chain. In a "Fab" format, the set of 6 CDRs are contributed by two different polypeptide sequences, the variable heavy domain (VH or vh or V_H; containing the vhCDR1, vhCDR2 and vhCDR3) and the variable light domain (VL or vl or V_L; containing the vlCDR1, vlCDR2 and vlCDR3), with the C-terminus of the VH domain being attached to the N-terminus of the CH1 domain of the heavy chain and the C-terminus of the VL domain being attached to the N-terminus of the constant light domain (and thus forming the light chain). In a scFv format, theVH and VL domains are covalently attached, generally through the use of a linker as outlined herein, into a single polypeptide sequence, which can be either (starting from the N-terminus)VH-linker-VL or VL-linker-vh, with the former being generally preferred (including optional domain linkers on each side, depending on the format used (e.g., from Figure 1 of US 62/353,511).

[00114] By "modification" herein is meant an amino acid substitution, insertion, and/or deletion in a polypeptide sequence or an alteration to a moiety chemically linked to a protein. For example, a modification may be an altered carbohydrate or PEG structure attached to a protein. By "amino acid modification" herein is meant an amino acid substitution, insertion, and/or deletion in a polypeptide sequence. For clarity, unless otherwise noted, the amino acid modification is always to an amino acid coded for by DNA, e.g., the 20 amino acids that have codons in DNA and RNA.

[00115] By "amino acid substitution" or "substitution" herein is meant the replacement of an amino acid at a particular position in a parent polypeptide sequence with a different

amino acid. In particular, in some embodiments, the substitution is to an amino acid that is not naturally occurring at the particular position, either not naturally occurring within the organism or in any organism. For example, the substitution E272Y refers to a variant polypeptide, in this case an Fc variant, in which the glutamic acid at position 272 is replaced with tyrosine. For clarity, a protein which has been engineered to change the nucleic acid coding sequence but not change the starting amino acid (for example exchanging CGG (encoding arginine) to CGA (still encoding arginine) to increase host organism expression levels) is not an "amino acid substitution"; that is, despite the creation of a new gene encoding the same protein, if the protein has the same amino acid at the particular position that it started with, it is not an amino acid substitution.

[00116] By "amino acid insertion" or "insertion" as used herein is meant the addition of an amino acid sequence at a particular position in a parent polypeptide sequence. For example, -233E or 233E designates an insertion of glutamic acid after position 233 and before position 234. Additionally, -233ADE or A233ADE designates an insertion of AlaAspGlu after position 233 and before position 234.

[00117] By "amino acid deletion" or "deletion" as used herein is meant the removal of an amino acid sequence at a particular position in a parent polypeptide sequence. For example, E233- or E233#, E233() or E233del designates a deletion of glutamic acid at position 233. Additionally, EDA233- or EDA233# designates a deletion of the sequence GluAspAla that begins at position 233.

[00118] By "variant protein" or "protein variant", or "variant" as used herein is meant a protein that differs from that of a parent protein by virtue of at least one amino acid modification. Protein variant may refer to the protein itself, a composition comprising the protein, or the amino sequence that encodes it. Preferably, the protein variant has at least one amino acid modification compared to the parent protein, e.g., from about one to about seventy amino acid modifications, and preferably from about one to about five amino acid modifications compared to the parent. As described below, in some embodiments the parent polypeptide, for example an Fc parent polypeptide, is a human wild type sequence, such as the Fc region from IgG1, IgG2, IgG3 or IgG4. The protein variant sequence herein will preferably possess at least about 80% identity with a parent protein sequence, and most

preferably at least about 90% identity, more preferably at least about 95-98-99% identity. Variant protein can refer to the variant protein itself, compositions comprising the protein variant, or the DNA sequence that encodes it.

[00119] Accordingly, by "Fc variant" or "variant Fc" as used herein is meant a protein comprising an amino acid modification in an Fc domain. The Fc variants of the present invention are defined according to the amino acid modifications that compose them. Thus, for example, N434S or 434S is an Fc variant with the substitution serine at position 434 relative to the parent Fc polypeptide, wherein the numbering is according to the EU index. Likewise, M428L/N434S defines an Fc variant with the substitutions M428L and N434S relative to the parent Fc polypeptide. The identity of the WT amino acid may be unspecified, in which case the aforementioned variant is referred to as 428L/434S. It is noted that the order in which substitutions are provided is arbitrary, that is to say that, for example, 428L/434S is the same Fc variant as M428L/N434S, and so on. For all positions discussed in the present invention that relate to antibodies, unless otherwise noted, amino acid position numbering is according to the EU index. The EU index or EU index as in Kabat or EU numbering scheme refers to the numbering of the EU antibody (Edelman et al., 1969, Proc Natl Acad Sci USA 63:78-85, hereby entirely incorporated by reference). The modification can be an addition, deletion, or substitution. Substitutions can include naturally occurring amino acids and, in some cases, synthetic amino acids. Examples include U.S. Pat. No. 6,586,207; WO 98/48032; WO 03/073238; US2004-0214988A1; WO 05/35727A2; WO 05/74524A2; J. W. Chin et al., (2002), Journal of the American Chemical Society 124:9026-9027; J. W. Chin, & P. G. Schultz, (2002), ChemBioChem 11:1135-1137; J. W. Chin, et al., (2002), PICAS United States of America 99:11020-11024; and, L. Wang, & P. G. Schultz, (2002), Chem. 1-10, all entirely incorporated by reference.

[00120] As used herein, "protein" herein is meant at least two covalently attached amino acids, which includes proteins, polypeptides, oligopeptides and peptides.

[00121] By "residue" as used herein is meant a position in a protein and its associated amino acid identity. For example, Asparagine 297 (also referred to as Asn297 or N297) is a residue at position 297 in the human antibody IgG1.

[00122] By "Fab" or "Fab region" as used herein is meant the polypeptide that comprises the VH, CH1, VL, and CL immunoglobulin domains. Fab may refer to this region in isolation, or this region in the context of a full length antibody, antibody fragment or Fab fusion protein.

[00123] By "Fv" or "Fv fragment" or "Fv region" as used herein is meant a polypeptide that comprises the VL and VH domains of a single antibody. As will be appreciated by those in the art, these generally are made up of two chains, or can be combined (generally with a linker as discussed herein) to form an scFv.

[00124] By "single chain Fv" or "scFv" herein is meant a variable heavy domain covalently attached to a variable light domain, generally using a scFv linker as discussed herein, to form a scFv or scFv domain. A scFv domain can be in either orientation from N- to C-terminus (VH-linker-VL or VL-linker-VH).

[00125] By "IgG subclass modification" or "isotype modification" as used herein is meant an amino acid modification that converts one amino acid of one IgG isotype to the corresponding amino acid in a different, aligned IgG isotype. For example, because IgG1 comprises a tyrosine and IgG2 a phenylalanine at EU position 296, a F296Y substitution in IgG2 is considered an IgG subclass modification.

[00126] By "non-naturally occurring modification" as used herein is meant an amino acid modification that is not isotypic. For example, because none of the IgGs comprise a serine at position 434, the substitution 434S in IgG1, IgG2, IgG3, or IgG4 (or hybrids thereof) is considered a non-naturally occurring modification.

[00127] By "amino acid" and "amino acid identity" as used herein is meant one of the 20 naturally occurring amino acids that are coded for by DNA and RNA.

[00128] By "effector function" as used herein is meant a biochemical event that results from the interaction of an antibody Fc region with an Fc receptor or ligand. Effector functions include but are not limited to ADCC, ADCP, and CDC.

[00129] By "Fc gamma receptor", "FcγR" or "FcgammaR" as used herein is meant any member of the family of proteins that bind the IgG antibody Fc region and is encoded by an FcγR gene. In humans this family includes but is not limited to FcγRI (CD64), including

isoforms FcγRIa, FcγRIb, and FcγRIc; FcγRII (CD32), including isoforms FcγRIIa (including allotypes H131 and R131), FcγRIIb (including FcγRIIb-1 and FcγRIIb-2), and FcγRIIc; and FcγRIII (CD16), including isoforms FcγRIIIa (including allotypes V158 and F158) and FcγRIIIb (including allotypes FcγRIIb-NA1 and FcγRIIb-NA2) (Jefferis et al., 2002, Immunol Lett 82:57-65, entirely incorporated by reference), as well as any undiscovered human FcγRs or FcγR isoforms or allotypes.

[00130] By "FcRn" or "neonatal Fc Receptor" as used herein is meant a protein that binds the IgG antibody Fc region and is encoded at least in part by an FcRn gene. As is known in the art, the functional FcRn protein comprises two polypeptides, often referred to as the heavy chain and light chain. The light chain is beta-2-microglobulin and the heavy chain is encoded by the FcRn gene. Unless otherwise noted herein, FcRn or an FcRn protein refers to the complex of FcRn heavy chain with beta-2-microglobulin. A variety of FcRn variants can be used to increase binding to the FcRn receptor, and in some cases, to increase serum half-life. In general, unless otherwise noted, the Fc monomers of the invention retain binding to the FcRn receptor (and, as noted below, can include amino acid variants to increase binding to the FcRn receptor).

[00131] By "parent polypeptide" as used herein is meant a starting polypeptide that is subsequently modified to generate a variant. The parent polypeptide may be a naturally occurring polypeptide, or a variant or engineered version of a naturally occurring polypeptide. Parent polypeptide may refer to the polypeptide itself, compositions that comprise the parent polypeptide, or the amino acid sequence that encodes it.

[00132] By "Fc" or "Fc region" or "Fc domain" as used herein is meant the polypeptide comprising the constant region of an antibody excluding the first constant region immunoglobulin domain (e.g., CH1) and in some cases, part of the hinge. For IgG, the Fc domain comprises immunoglobulin domains CH2 and CH3 (Cγ2 and Cγ3) and the lower hinge region between CH1 (Cγ1) and CH2 (Cγ2). Thus, in some cases, the Fc domain includes, from N- to C-terminal, CH2-CH3 and hinge-CH2-CH3. In some embodiments, the Fc domain is that from IgG1, IgG2, IgG3 or IgG4, with IgG1 hinge-CH2-CH3 and IgG4 hinge-CH2-CH3 finding particular use in many embodiments. Additionally, in certain embodiments, wherein the Fc domain is a human IgG1 Fc domain, the hinge includes a

C220S amino acid substitution. Furthermore, in some meboidments where the Fc domain is a human IgG4 Fc domain, the hinge includes a S228P amino acid substitution. Although the boundaries of the Fc region may vary, the human IgG heavy chain Fc region is usually defined to include residues C226 or P230 to its carboxyl-terminus, wherein the numbering is according to the EU index as in Kabat. Accordingly, "CH" domains in the context of IgG are as follows: "CH1" refers to positions 118-215 according to the EU index as in Kabat. "Hinge" refers to positions 216-230 according to the EU index as in Kabat. "CH2" refers to positions 231-340 according to the EU index as in Kabat, and "CH3" refers to positions 341-447 according to the EU index as in Kabat. Thus, the "Fc domain" includes the -CH2-CH3 domain, and optionally a hinge domain (hinge-CH2-CH3).

[00133] As will be appreciated by those in the art, the exact numbering and placement of the heavy constant region domains can be different among different numbering systems. A useful comparison of heavy constant region numbering according to EU and Kabat is as below, see Edelman et al., 1969, Proc Natl Acad Sci USA 63:78-85 and Kabat et al., 1991, Sequences of Proteins of Immunological Interest, 5th Ed., United States Public Health Service, National Institutes of Health, Bethesda, entirely incorporated by reference.

Table 1

	EU Numbering	Kabat Numbering
CH1	118-215	114-223
Hinge	216-230	226-243
CH2	231-340	244-360
CH3	341-447	361-478

[00134] In the embodiments herein, when a scFv or IL-15 complex is attached to an Fc domain, it is the C-terminus of the scFv, IL-15 or IL-15Rα construct that is attached to the Fc domain via a domain linker; for example, a hinge domain as depicted in Figure 8. In some embodiments, as is more fully described below, amino acid modifications are made to the Fc

region, for example to alter binding to one or more FcγR receptors or to the FcRn receptor, and to enable heterodimer formation and purification, as outlined herein.

[00135] By "heavy constant region" herein is meant the CH1-hinge-CH2-CH3 portion of an antibody.

[00136] By "Fc fusion protein" or "immunoadhesin" herein is meant a protein comprising an Fc region, generally linked (optionally through a linker moiety, as described herein) to a different protein, such as to IL-15 and/or IL-15R, as described herein. In some instances, two Fc fusion proteins can form a homodimeric Fc fusion protein or a heterodimeric fusion protein with the latter being preferred. In some cases, one monomer of the heterodimeric fusion protein comprises an Fc domain alone (e.g., an empty Fc domain) and the other monomer is a Fc fusion, comprising a variant Fc domain and a protein domain, such as a receptor, ligand or other binding partner.

[00137] By "position" as used herein is meant a location in the sequence of a protein. Positions may be numbered sequentially, or according to an established format, for example the EU index for antibody numbering.

[00138] By "strandedness" in the context of the monomers of the heterodimeric antibodies of the invention herein is meant that, similar to the two strands of DNA that "match", heterodimerization variants are incorporated into each monomer so as to preserve the ability to "match" to form heterodimers. For example, if some pI variants are engineered into monomer A (e.g., making the pI higher) then steric variants that are "charge pairs" that can be utilized as well do not interfere with the pI variants, e.g., the charge variants that make a pI higher are put on the same "strand" or "monomer" to preserve both functionalities. Similarly, for "skew" variants that come in pairs of a set as more fully outlined below, the skilled artisan will consider pI in deciding into which strand or monomer that incorporates one set of the pair will go, such that pI separation is maximized using the pI of the skews as well.

[00139] By "target cell" as used herein is meant a cell that expresses the target antigen, in this case, TIM-3.

[00140] By "variable region" as used herein is meant the region of an immunoglobulin that comprises one or more Ig domains substantially encoded by any of the Vκ, Vλ, and/or VH genes that make up the kappa, lambda, and heavy chain immunoglobulin genetic loci respectively.

[00141] By "wild type or WT" herein is meant an amino acid sequence or a nucleotide sequence that is found in nature, including allelic variations. A WT protein has an amino acid sequence or a nucleotide sequence that has not been intentionally modified.

[00142] The TIM-3 targeted heterodimeric proteins of the present invention are generally isolated or recombinant. "Isolated," when used to describe the various polypeptides disclosed herein, means a polypeptide that has been identified and separated and/or recovered from a cell or cell culture from which it was expressed. Ordinarily, an isolated polypeptide will be prepared by at least one purification step. An "isolated protein," refers to a protein which is substantially free of other proteins having different binding specificities. "Recombinant" means the proteins are generated using recombinant nucleic acid techniques in exogeneous host cells.

[00143] "Percent (%) amino acid sequence identity" with respect to a protein sequence is defined as the percentage of amino acid residues in a candidate sequence that are identical with the amino acid residues in the specific (parental) sequence, after aligning the sequences and introducing gaps, if necessary, to achieve the maximum percent sequence identity, and not considering any conservative substitutions as part of the sequence identity. Alignment for purposes of determining percent amino acid sequence identity can be achieved in various ways that are within the skill in the art, for instance, using publicly available computer software such as BLAST, BLAST-2, ALIGN or Megalign (DNASTAR) software. Those skilled in the art can determine appropriate parameters for measuring alignment, including any algorithms needed to achieve maximal alignment over the full length of the sequences being compared. One particular program is the ALIGN-2 program outlined at paragraphs [0279] to [0280] of US Pub. No. 20160244525, hereby incorporated by reference.

[00144] The degree of identity between an amino acid sequence of the present invention ("invention sequence") and the parental amino acid sequence is calculated as the

number of exact matches in an alignment of the two sequences, divided by the length of the "invention sequence," or the length of the parental sequence, whichever is the shortest. The result is expressed in percent identity.

[00145] In some embodiments, two or more amino acid sequences are at least 50%, 60%, 70%, 80%, or 90% identical. In some embodiments, two or more amino acid sequences are at least 95%, 97%, 98%, 99%, or even 100% identical.

[00146] "Specific binding" or "specifically binds to" or is "specific for" a particular antigen or an epitope (in this case, human TIM-3) means binding that is measurably different from a non-specific interaction. Specific binding can be measured, for example, by determining binding of a molecule compared to binding of a control molecule, which generally is a molecule of similar structure that does not have binding activity. For example, specific binding can be determined by competition with a control molecule that is similar to the target.

[00147] Specific binding for a particular antigen or an epitope can be exhibited, for example, by an antibody having a KD for an antigen or epitope of at least about 10^{-4} M, at least about 10^{-5} M, at least about 10^{-6} M, at least about 10^{-7} M, at least about 10^{-8} M, at least about 10^{-9} M, alternatively at least about 10^{-10} M, at least about 10^{-11} M, at least about 10^{-12} M, or greater, where KD refers to a dissociation rate of a particular antibody-antigen interaction. Typically, an antibody that specifically binds an antigen will have a KD that is 20-, 50-, 100-, 500-, 1000-, 5,000-, 10,000- or more times greater for a control molecule relative to the antigen or epitope.

[00148] Also, specific binding for a particular antigen or an epitope can be exhibited, for example, by an antibody having a KA or Ka for an antigen or epitope of at least 20-, 50-, 100-, 500-, 1000-, 5,000-, 10,000- or more times greater for the epitope relative to a control, where KA or Ka refers to an association rate of a particular antibody-antigen interaction. Binding affinity is generally measured using a Biacore assay.

II. Introduction

[00149] The invention provides heterodimeric fusion proteins that contain an IL-15 complex on one side and an anti-human TIM-3 antigen binding domain on the other. Thus,

the heterodimeric fusion proteins of the invention can bind to the checkpoint TIM-3 antigen and can complex with the common gamma chain (γc; CD132) and/or the IL-2 receptor β-chain (IL-2Rβ; CD122). In general, the heterodimeric fusion proteins of the invention have three functional components: an IL-15/IL-15Rα(sushi) component, generally referred to herein as an "IL-15 complex", an anti-TIM-3 ABD component which serves as a "targeting" moiety by bringing the fusion protein to a cell expressing TIM-3, and an Fc component, each of which can take different forms and each of which can be combined with the other components in any configuration.

[00150] In general, as is more fully described herein, the fusion proteins of the invention are heterodimeric proteins that are based on the association of antibody Fc domains. That is, by using two different variant Fc domains that have been engineered to favor the formation of heterodimers over homodimers, the heterodimeric proteins are formed. In this case, one of the variant Fc domains is fused to an IL-15/RA complex and the other has a TIM-3 ABD as more fully outlined herein. By including optional pI variants, the heterodimers can be more easily purified away from the homodimers. Additionally, the inclusion of ablation variants eliminates the effector functions of the Fc domains.

A. IL-15/IL-15Rα(sushi) domains

[00151] As shown in the figures, the IL-15 complex can take several forms. As stated above, the IL-15 protein on its own is less stable than when complexed with the IL-15Rα protein. As is known in the art, the IL-15Rα protein contains a "sushi domain", which is the shortest region of the receptor that retains IL-15 binding activity. Thus, while heterodimeric fusion proteins comprising the entire IL-15Rα protein can be made, preferred embodiments herein include complexes that just use the sushi domain, the sequence of which is shown in the figures.

[00152] Accordingly, the IL-15 complex generally comprises the IL-15 protein and the sushi domain of IL IL-15Rα (unless otherwise noted that the full length sequence is used, "IL-15Rα", "IL-15Rα(sushi)", "IL-15RA" and "sushi" are used interchangeably throughout).

[00153] Importantly, the IL-15 component is generally engineered to reduce its potency. In many embodiments, the wild-type IL-15 is too potent and can cause undesirable

toxicity. Accordingly, the IL-15 component of the IL-15 complex can have one or more amino acid substitutions that result in decreased activity. Various amino acid substitutions were made (see Figure 19) and tested (see Figure 20). Of particular interest in some embodiments are a double variant, N4D/N65D or D30N/N65D, or a triple variant, D30N/E64Q/N65D.

[00154] The targeted IL-15/IL-15Rα heterodimeric fusion proteins of the present invention include an IL-15/IL-15 receptor alpha (IL-15Rα)-Fc fusion monomer; reference is made to US2018/0118828, filed 16, October 2017, U.S. Ser. No. 62/408,655, filed on October 14, 2016, U.S. Ser. No. 62/416,087, filed on October November 1, 2016, U.S. Ser. No. 62/443,465, filed on January 6, 2017, U.S. Ser. No. 62/477,926, filed on March 28, 2017, and U.S. Ser. No. 62/659,571, filed on April 18, 2018, hereby incorporated by reference in their entirety and in particular for the sequences outlined therein. In some cases, the IL-15 and IL-15 receptor alpha (IL-15Rα) protein domains are in different orientations. Exemplary embodiments of IL-15/IL-15Rα-Fc fusion monomers are provided in XENP21480 (chain 1; Figure 64A), XENP22022 (chain 1, Figure 64D), XENP22112, (chains 1 and 3; Figure 64E), XENP22641 (chains 2 and 4; Figure 64F), XENP22642, (chains 1 and 4; Figure 64H) and XENP22644 (chains 1 and 4; Figure 64I) as described, for example, in US2018/0118828.

1. IL-15 Variants

[00155] In some embodiments, the human IL-15 protein has the amino acid sequence set forth in NCBI Ref. Seq. No. NP_000576.1 as shown in Figures 2. In some cases, the coding sequence of human IL-15 is set forth in NCBI Ref. Seq. No. NM_000585. An exemplary IL-15 protein of the Fc fusion heterodimeric protein outlined herein can have the amino acid sequence of SEQ ID NO:2 or amino acids 49-162 of SEQ ID NO:1. In some embodiments, the IL-15 protein has at least 90%, e.g., 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or more sequence identity to SEQ ID NO:2. In some embodiments, the IL-15 protein has the amino acid sequence set forth in SEQ ID NO:2 except with the amino acid substitution N72D. In other embodiments, the IL-15 protein has the amino acid sequence of SEQ ID NO:2 except with one or more amino acid substitutions selected from the group consisting of C42S, L45C, Q48C, V49C, L52C, E53C, E87C, and E89C. In some aspects, the IL-15 protein has one or more amino acid substitutions selected from the group consisting of

N1D, N4D, D8N, D30N, D61N, E64Q, N65D, and Q108E. In other embodiments, the amino acid substitutions are N4D/N65D or D30N/N65D. In some embodiments, the amino acid substitution is Q108E. In certain embodiments, the amino acid substitution is N65D. In other embodiments, the amino acid substitutions are D30N/E64Q/N65D. In certain embodiments, the amino acid substitution is N65D. In some instances, the amino acid substitutions are N1D/N65D. In some instances, the amino acid substitutions are D30N/N65D. Optionally, the IL-15 protein also has an N72D substitution. The IL-15 protein of the Fc fusion protein can have 1, 2, 3, 4, 5, 6, 7, 8 or 9 amino acid substitutions. In some embodiments, the IL-15 protein of the Fc fusion protein comprises a D30N substitution. In some embodiments, the IL-15 protein of the Fc fusion protein comprises a N65D substitution. In some embodiments, the IL-15 protein of the Fc fusion contains one or more amino acid substitutions at the IL-15:CD132 interface. In certain embodiments, the Fc fusion protein described herein induces proliferation of NK cells and CD8+ T cells.

[00156] In some embodiments, the human IL-15 receptor alpha (IL-15Rα) protein has the amino acid sequence set forth in NCBI Ref. Seq. No. NP_002180.1 or SEQ ID NO:3. In some cases, the coding sequence of human IL-15Rα is set forth in NCBI Ref. Seq. No. NM_002189.3. An exemplary the IL-15Rα protein of the Fc fusion heterodimeric protein outlined herein can comprise or consist of the sushi domain of SEQ ID NO:3 (e.g., amino acids 31-95 of SEQ ID NO:3), or in other words, the amino acid sequence of SEQ ID NO:4. In some embodiments, the IL-15Rα protein has the amino acid sequence of SEQ ID NO:4 and an amino acid insertion selected from the group consisting of D96, P97, A98, D96/P97, D96/C97, D96/P97/A98, D96/P97/C98, and D96/C97/A98, wherein the amino acid position is relative to full-length human IL-15Rα protein or SEQ ID NO:3. For instance, amino acid(s) such as D (e.g., Asp), P (e.g., Pro), A (e.g., Ala), DP (e.g., Asp-Pro), DC (e.g., Asp-Cys), DPA (e.g., Asp-Pro-Ala), DPC (e.g., Asp-Pro-Cys), or DCA (e.g., Asp-Cys-Ala) can be added to the C-terminus of the IL-15Rα protein of SEQ ID NO:4. In some embodiments, the IL-15Rα protein has the amino acid sequence of SEQ ID NO:4 and one or more amino acid substitutions selected from the group consisting of K34C, A37C, G38C, S40C, and L42C, wherein the amino acid position is relative to SEQ ID NO:4. The IL-15Rα protein can have 1,

2, 3, 4, 5, 6, 7, 8 or more amino acid mutations (e.g., substitutions, insertions and/or deletions).

2. IL-15/RA Complexes

[00157] As outlined herein, the IL-15 variants and the sushi domain can be complexed in at least three different ways.

[00158] In some embodiments, as shown in Figures 21B, for example, the IL-15 protein and the IL-15Rα(sushi) are not covalently attached, but rather are self-assembled through regular ligand-ligand interactions. As is more fully described herein, it can be either the IL-15 domain or the sushi domain that is covalently linked to the Fc domain (generally using an optional domain linker). Again, of particular use in this embodiment are a double variant, N4D/N65D or D30N/N65D, or a triple variant, D30N/E64Q/N65D, used with a wild type sushi domain.

[00159] In alternative embodiments, the variant IL-15 can be complexed to the sushi domain using a domain linker, such that they are covalently attached as generally shown in Figures 21D; this figure depicts the sushi domain as the N-terminal domain, although this can be reversed. Again, of particular use in this embodiment are a double variant, N4D/N65D or D30N/N65D, or a triple variant, D30N/E64Q/N65D, used with a wild type sushi domain.

[00160] Alternatively, each of the IL-15 and sushi domains can be engineered to contain a cysteine amino acid, that forms a disulfide bond to form the complex as is generally shown in Figures 21C, again, with either the IL-15 domain or the sushi domain being covalently attached (using an optional domain linker) to the Fc domain. Again, of particular use in this embodiment are a double variant, N4D/N65D or D30N/N65D (additionally including an amino acid substitution to cysteine), or a triple variant, D30N/E64Q/N65D (additionally including an amino acid substitution to cysteine), used with a sushi domain also comprising an amino acid substitution to provide a cysteine.

[00161] Additional particular embodiments are outlined below.

B. Anti-TIM-3 components

[00162] In some embodiments, the heterodimeric fusion proteins provided herein include some antibody components.

[00163] Traditional antibody structural units typically comprise a tetramer. Each tetramer is typically composed of two identical pairs of polypeptide chains, each pair having one "light" (typically having a molecular weight of about 25 kDa) and one "heavy" chain (typically having a molecular weight of about 50-70 kDa). Human light chains are classified as kappa and lambda light chains. The present invention is directed to antibodies or antibody fragments (antibody monomers) that generally are based on the IgG class, which has several subclasses, including, but not limited to IgG1, IgG2, IgG3, and IgG4. In general, IgG1, IgG2 and IgG4 are used more frequently than IgG3. It should be noted that IgG1 has different allotypes with polymorphisms at 356 (D or E) and 358 (L or M). The sequences depicted herein use the 356D/358M allotype, however the other allotype is included herein. That is, any sequence inclusive of an IgG1 Fc domain included herein can have 356E/358L replacing the 356D/358M allotype.

[00164] In addition, many of the monomer sequences herein have at least one the cysteines at position 220 replaced by a serine, to reduce disulfide formation. Specifically included within the sequences herein are one or both of these cysteines replaced (C220S).

[00165] Thus, "isotype" as used herein is meant any of the subclasses of immunoglobulins defined by the chemical and antigenic characteristics of their constant regions.

[00166] The amino-terminal portion of each chain includes a variable region of about 100 to 110 or more amino acids primarily responsible for antigen recognition, generally referred to in the art and herein as the "Fv domain" or "Fv region". In the variable region, three loops are gathered for each of the V domains of the heavy chain and light chain to form an antigen-binding site. Each of the loops is referred to as a complementarity-determining region (hereinafter referred to as a "CDR"), in which the variation in the amino acid sequence is most significant. "Variable" refers to the fact that certain segments of the variable region differ extensively in sequence among antibodies. Variability within the variable region is not evenly distributed. Instead, the V regions consist of relatively

invariant stretches called framework regions (FRs) of 15-30 amino acids separated by shorter regions of extreme variability called "hypervariable regions" that are each 9-15 amino acids long or longer.

[00167] Each VH and VL is composed of three hypervariable regions ("complementary determining regions," "CDRs") and four FRs, arranged from amino-terminus to carboxy-terminus in the following order: FR1-CDR1-FR2-CDR2-FR3-CDR3-FR4.

[00168] The hypervariable region generally encompasses amino acid residues from about amino acid residues 24-34 (LCDR1; "L" denotes light chain), 50-56 (LCDR2) and 89-97 (LCDR3) in the light chain variable region and around about 31-35B (HCDR1; "H" denotes heavy chain), 50-65 (HCDR2), and 95-102 (HCDR3) in the heavy chain variable region; Kabat et al., SEQUENCES OF PROTEINS OF IMMUNOLOGICAL INTEREST, 5th Ed. Public Health Service, National Institutes of Health, Bethesda, Md. (1991) and/or those residues forming a hypervariable loop (e.g. residues 26-32 (LCDR1), 50-52 (LCDR2) and 91-96 (LCDR3) in the light chain variable region and 26-32 (HCDR1), 53-55 (HCDR2) and 96-101 (HCDR3) in the heavy chain variable region; Chothia and Lesk (1987) J. Mol. Biol. 196:901-917. Specific CDRs of the invention are described below.

[00169] As will be appreciated by those in the art, the exact numbering and placement of the CDRs can be different among different numbering systems. However, it should be understood that the disclosure of a variable heavy and/or variable light sequence includes the disclosure of the associated (inherent) CDRs. Accordingly, the disclosure of each variable heavy region is a disclosure of the vhCDRs (e.g. vhCDR1, vhCDR2 and vhCDR3) and the disclosure of each variable light region is a disclosure of the vlCDRs (e.g. vlCDR1, vlCDR2 and vlCDR3).

[00170] A useful comparison of CDR numbering is as below, see Lafranc et al., Dev. Comp. Immunol. 27(1):55-77 (2003):

[00171] TABLE 2

	Kabat+ Chothia	IMGT	Kabat	AbM	Chothia	Contact	Xencor
vhCDR1	26-35	27-38	31-35	26-35	26-32	30-35	27-35
vhCDR2	50-65	56-65	50-65	50-58	52-56	47-58	54-61
vhCDR3	95-102	105-117	95-102	95-102	95-102	93-101	103-116
vlCDR1	24-34	27-38	24-34	24-34	24-34	30-36	27-38
vlCDR2	50-56	56-65	50-56	50-56	50-56	46-55	56-62
vlCDR3	89-97	105-117	89-97	89-97	89-97	89-96	97-105

[00172] Throughout the present specification, the Kabat numbering system is generally used when referring to a residue in the variable domain (approximately, residues 1-107 of the light chain variable region and residues 1-113 of the heavy chain variable region) and the EU numbering system for Fc regions (e.g, Kabat et al., supra (1991)).

[00173] The present invention provides a large number of different CDR sets. In this case, a "full CDR set" comprises the three variable light and three variable heavy CDRs, e.g. a vlCDR1, vlCDR2, vlCDR3, vhCDR1, vhCDR2 and vhCDR3. These can be part of a larger variable light or variable heavy domain, respectfully. In addition, as more fully outlined herein, the variable heavy and variable light domains can be on separate polypeptide chains, when a heavy and light chain is used (for example when Fabs are used), or on a single polypeptide chain in the case of scFv sequences.

[00174] The CDRs contribute to the formation of the antigen-binding, or more specifically, epitope binding site of antibodies. "Epitope" refers to a determinant that interacts with a specific antigen binding site in the variable region of an antibody molecule known as a paratope. Epitopes are groupings of molecules such as amino acids or sugar side chains and usually have specific structural characteristics, as well as specific charge characteristics. A single antigen may have more than one epitope.

[00175] The epitope may comprise amino acid residues directly involved in the binding (also called immunodominant component of the epitope) and other amino acid residues, which are not directly involved in the binding, such as amino acid residues which are effectively blocked by the specifically antigen binding peptide; in other words, the amino acid residue is within the footprint of the specifically antigen binding peptide.

[00176] Epitopes may be either conformational or linear. A conformational epitope is produced by spatially juxtaposed amino acids from different segments of the linear polypeptide chain. A linear epitope is one produced by adjacent amino acid residues in a polypeptide chain. Conformational and nonconformational epitopes may be distinguished in that the binding to the former but not the latter is lost in the presence of denaturing solvents.

[00177] An epitope typically includes at least 3, and more usually, at least 5 or 8-10 amino acids in a unique spatial conformation. Antibodies that recognize the same epitope can be verified in a simple immunoassay showing the ability of one antibody to block the binding of another antibody to a target antigen, for example "binning." As outlined below, the invention not only includes the enumerated antigen binding domains and antibodies herein, but those that compete for binding with the epitopes bound by the enumerated antigen binding domains.

[00178] The carboxy-terminal portion of each chain defines a constant region primarily responsible for effector function. Kabat et al. collected numerous primary sequences of the variable regions of heavy chains and light chains. Based on the degree of conservation of the sequences, they classified individual primary sequences into the CDR and the framework and made a list thereof (see SEQUENCES OF IMMUNOLOGICAL INTEREST, 5th edition, NIH publication, No. 91-3242, E.A. Kabat et al., entirely incorporated by reference).

[00179] In the IgG subclass of immunoglobulins, there are several immunoglobulin domains in the heavy chain. By "immunoglobulin (Ig) domain" herein is meant a region of an immunoglobulin having a distinct tertiary structure. Of interest in the present invention are the heavy chain domains, including, the constant heavy (CH) domains and the hinge

domains. In the context of IgG antibodies, the IgG isotypes each have three CH regions. Accordingly, "CH" domains in the context of IgG are as follows: "CH1" refers to positions 118-220 according to the EU index as in Kabat. "CH2" refers to positions 237-340 according to the EU index as in Kabat, and "CH3" refers to positions 341-447 according to the EU index as in Kabat. As shown herein and described below, the pI variants can be in one or more of the CH regions, as well as the hinge region, discussed below.

[00180] Another type of Ig domain of the heavy chain is the hinge region. By "hinge" or "hinge region" or "antibody hinge region" or "immunoglobulin hinge region" herein is meant the flexible polypeptide comprising the amino acids between the first and second constant domains of an antibody. Structurally, the IgG CH1 domain ends at EU position 220, and the IgG CH2 domain begins at residue EU position 237. Thus for IgG the antibody hinge is herein defined to include positions 221 (D221 in IgG1) to 236 (G236 in IgG1), wherein the numbering is according to the EU index as in Kabat. In some embodiments, for example in the context of an Fc region, the lower hinge is included, with the "lower hinge" generally referring to positions 226 or 230. As noted herein, pI variants can be made in the hinge region as well.

[00181] The light chain generally comprises two domains, the variable light domain (containing the light chain CDRs and together with the variable heavy domains forming the Fv region), and a constant light chain region (often referred to as CL or Cκ).

[00182] Another region of interest for additional substitutions, outlined herein, is the Fc region.

[00183] Thus, the present heterodimeric fusion proteins provided herein include one or more antibody domains. As described herein and known in the art, the heterodimeric antibodies provided herein comprise different domains within the heavy and light chains, which can be overlapping as well. These domains include, but are not limited to, the Fc domain, the CH1 domain, the CH2 domain, the CH3 domain, the hinge domain, the heavy constant domain (CH1-hinge-Fc domain or CH1-hinge-CH2-CH3), the variable heavy domain, the variable light domain, the light constant domain, Fab domains and scFv domains.

[00184] As generally outlined herein, the heterodimeric proteins of the invention include one or more Fvs that bind human TIM-3. "Hepatitis A virus cellular receptor 2," "HAVCR2," "T-cell immunoglobulin and mucin-domain containing-3," "TIM-3," "TIM3," "CD366" (e.g., Genebank Accession Numbers NM_032782 and NP_116171 (human)) refers to an immune checkpoint that belongs to TIM family cell surface receptor proteins. Together with PD-1 and LAG-3, TIM-3 mediates the CD8+ T cell exhaustion. TIM-3 expression is upregulated in tumor-infiltrating lymphocytes in lung, gastric, head and neck cancer, schwannoma, melanoma and follicular B-cell non-Hodgkin lymphoma and may interact with the PD-1 pathway in the dysfunction al CD8+ and Tregs in cancer. Exemplary sequences for TIM-3 are depicted in Figure 3.

[00185] This Fv, or anti-TIM-3 component (the anti-TIM-3 antigen binding domain or TIM-3 ABD) of the subject heterodimer fusion proteins is generally a set of 6 CDRs and/or a variable heavy domain and a variable light domain that form an Fv domain that can bind human TIM-3. As described herein, there are a number of different formats that can be used, generally either by using a scFv or a Fab as outlined herein.

[00186] In certain embodiments, the ABDs of the invention comprise a heavy chain variable region with frameworks from a particular germline heavy chain immunoglobulin gene and/or a light chain variable region from a particular germline light chain immunoglobulin gene. For example, such ABDs may comprise or consist of a human ABD comprising heavy or light chain variable regions that are "the product of" or "derived from" a particular germline sequence. An ABD that is "the product of" or "derived from" a human germline immunoglobulin sequence can be identified as such by comparing the amino acid sequence of the ABD to the amino acid sequences of human germline immunoglobulins and selecting the human germline immunoglobulin sequence that is closest in sequence (i.e., greatest % identity) to the sequence of the ABD. An ABD that is "the product of" or "derived from" a particular human germline immunoglobulin sequence may contain amino acid differences as compared to the germline sequence, due to, for example, CDRs, naturally-occurring somatic mutations or intentional introduction of site-directed mutation. However, a humanized ABD typically is at least 90% identical in amino acids sequence to an amino acid sequence encoded by a human germline immunoglobulin gene and contains amino acid

residues that identify the ABD as being derived from human sequences when compared to the germline immunoglobulin amino acid sequences of other species (e.g., murine germline sequences). In certain cases, a humanized ABD may be at least 95, 96, 97, 98 or 99%, or even at least 96%, 97%, 98%, or 99% identical in amino acid sequence to the amino acid sequence encoded by the germline immunoglobulin gene. Typically, a humanized ABD derived from a particular human germline sequence will display no more than 10-20 amino acid differences from the amino acid sequence encoded by the human germline immunoglobulin gene (prior to the introduction of any skew, pI and ablation variants herein; that is, the number of variants is generally low, prior to the introduction of the variants of the invention). In certain cases, the humanized ABD may display no more than 5, or even no more than 4, 3, 2, or 1 amino acid difference from the amino acid sequence encoded by the germline immunoglobulin gene (again, prior to the introduction of any skew, pI and ablation variants herein; that is, the number of variants is generally low, prior to the introduction of the variants of the invention). In one embodiment, the parent ABD has been affinity matured, as is known in the art. Structure-based methods may be employed for humanization and affinity maturation, for example as described in USSN 11/004,590. Selection based methods may be employed to humanize and/or affinity mature antibody variable regions, including but not limited to methods described in Wu et al., 1999, J. Mol. Biol. 294:151-162; Baca et al., 1997, J. Biol. Chem. 272(16):10678-10684; Rosok et al., 1996, J. Biol. Chem. 271(37): 22611-22618; Rader et al., 1998, Proc. Natl. Acad. Sci. USA 95: 8910-8915; Krauss et al., 2003, Protein Engineering 16(10):753-759, all entirely incorporated by reference. Other humanization methods may involve the grafting of only parts of the CDRs, including but not limited to methods described in USSN 09/810,510; Tan et al., 2002, J. Immunol. 169:1119-1125; De Pascalis et al., 2002, J. Immunol. 169:3076-3084, all entirely incorporated by reference.

[00187] As shown herein, the anti-TIM-3 ABD can be in the form of either a Fab or an scFv.

[00188] In some embodiments, for example as depicted in Figures 21B and C, the anti-TIM-3 ABD is a scFv, wherein the VH and VL domains are joined using an scFv linker, which can be optionally a charged scFv linker. As will be appreciated by those in the art, the

scFv can be assembled from N- to C-terminus, as N-VH-scFv linker-VL-C or as N-VL-scFv linker-VH-C, with the C terminus of the scFv domain generally being linked to the hinge-CH2-CH3 Fc domain, wherein the hinge in this case serving as a domain linker. Suitable Fvs (including CDR sets and variable heavy/variable light domains) can be used in scFv formats or Fab formats are shown in the Figures as well as disclosed in WO2017/218707, the contents are hereby incorporated in its entirety for all purposes, and in particular for the TIM-3 ABDs in Figure 13, the data in Figure 21 and Figure 22 and SEQ ID NO:s 20765-20884, SEQ ID NO:s 37587-37698 and SEQ ID NO:s 36347-36706 sequences in the sequence listing.

[00189] As will further be appreciated by those in the art, all or part of the hinge (which can also be a wild type hinge from IgG1, IgG2 or IgG4 or a variant thereof, such as the IgG4 S241P or S228P hinge variant with the substitution proline at position 228 relative to the parent IgG4 hinge polypeptide (wherein the numbering S228P is according to the EU index and the S241P is the Kabat numbering)) can be used as the domain linker between the scFv and the CH2-CH3 domain, or a different domain linker such as depicted in the Figures can be used.

[00190] Alternatively, the TIM-3 ABD can be in the form of a Fab fragment. In this embodiment, the ABD is made up of a variable heavy domain, contributed by a heavy chain, and a variable light domain, contributed by a light chain. Suitable Fvs (including CDR sets and variable heavy/variable light domains) can be used in scFv formats or Fab formats are shown in the Figures as well as disclosed in WO2017/218707, the contents are hereby incorporated in its entirety for all purposes, and in particular for the TIM-3 ABDs in Figure 13, the data in Figure 21 and Figure 22 and SEQ ID NO:s 20765-20884, SEQ ID NO:s 37587-37698 and SEQ ID NO:s 36347-36706 sequences in the sequence listing.

[00191] As will be appreciated by those in the art, suitable TIM-3 binding domains can comprise a set of 6 CDRs as depicted in the sequence listing and figures (e.g., Figures 12 and 13), either as they are underlined/bolded or, in the case where a different numbering scheme is used as described herein and as shown in Table 2, as the CDRs that are identified using other alignments within the variable heavy (VH) domain and variable light domain (VL) sequences of those depicted in the figures (e.g., Figures 12 and 13A-C)and the sequence listing. Suitable TIM-3 ABDs that find use in the subject targeted IL-15/IL-15Rα

heterodimeric fusion proteins can also include the entire VH and VL sequences as depicted in these sequences and figures, used as scFvs or as Fabs.

[00192] In one embodiment, the TIM-3 antigen binding domain includes the 6 CDRs (i.e., vhCDR1-3 and vlCDR1-3) of any of the TIM-3 binding domains described in Figures 12 and 13A-C or the sequence listing.

[00193] In addition to the parental CDR sets disclosed in the figures and sequence listing that form an ABD to TIM-3, provided herein are variant TIM-3 ABDS having CDRs that include at least one modification of the TIM-3 ABD CDRs disclosed herein (e.g., Figures 12 and 13A-C). In one embodiment, the heterodimeric fusion protein includes a TIM-3 ABD that includes a set of 6 CDRs with 1, 2, 3, 4, 5, 6, 7, 8, 9, 10 amino acid modifications as compared to the 6 CDRs of a TIM-3 ABD as depicted in Figures 112 and 3A-C or the sequence listing. In certain embodiments, the TIM-3 ABD is capable of binding TIM-3 antigen, as measured by at least one of a Biacore, surface plasmon resonance (SPR) and/or BLI (biolayer interferometry, e.g., Octet assay) assay, with the latter finding particular use in many embodiments.

[00194] In one embodiment, the TIM-3 ABD of the subject targeted IL-15/IL-15Rα heterodimeric fusion protein includes 6 CDRs that are at least 90, 95, 97, 98 or 99% identical to the 6 CDRs of a TIM-3 ABD as depicted in Figures 12 and 13A-C or the sequence listing. In certain embodiments, the TIM-3 ABD is capable of binding to the TIM-3, as measured by at least one of a Biacore, surface plasmon resonance (SPR) and/or BLI (biolayer interferometry, e.g., Octet assay) assay, with the latter finding particular use in many embodiments.

[00195] In one embodiment of the subject targeted IL-15/IL-15Rα heterodimeric fusion protein, the TIM-3 antigen binding domain includes the 6 CDRs (i.e., vhCDR1-3 and vlCDR1-3) of one of the following TIM-3 ABDs: 3H3[TIM-3]_H0_L0, 3H3[TIM-3]_H1_L2, 3H3[TIM-3]_H1_L2.1, APE137[TIM-3], APE5121[TIM-3], ABTIM3-hum03[TIM-3], ABTIM3-hum11[TIM-3], ABTIM3-hum21[TIM-3], 4177[TIM-3], 4545[Tim-3], 8213[TIM-3], mAb15[TIM-3], mAb58[TIM-3], TIM3-0433[TIM-3], TIM3-0434[TIM-3], TIM3-0438[TIM3],

and TIM3-0443[TIM3] (see, e.g., Figures 12 and 13A-C). In an exemplary embodiments, the TIM-3ABD is 3H3[TIM-3]_H1_L2.1.

[00196] In one embodiment, the TIM-3 antigen binding domain is a variant TIM-3 antigen binding domain that includes 6 CDRs (i.e., vhCDR1-3 and vlCDR1-3), where the 6 CDRs include 1, 2, 3, 4, 5, 6, 7, 8, 9, or 10 modifications as compared to the 6 CDRs of one of the following TIM-3 ABDs: 3H3[TIM-3]_H0_L0, 3H3[TIM-3]_H1_L2, 3H3[TIM-3]_H1_L2.1, APE137[TIM-3], APE5121[TIM-3], ABTIM3-hum03[TIM-3], ABTIM3-hum11[TIM-3], ABTIM3-hum21[TIM-3], 4177[TIM-3], 4545[Tim-3], 8213[TIM-3], mAb15[TIM-3], mAb58[TIM-3], TIM3-0433[TIM-3], TIM3-0434[TIM-3], TIM3-0438[TIM3], and TIM3-0443[TIM3] (see, e.g., Figures 12 and 13A-C). In an exemplary embodiments, the TIM-3ABD is 3H3[TIM-3]_H1_L2.1.

[00197] In one embodiment, the TIM-3 antigen binding domain of the IL-15/IL-15Rα heterodimeric fusion protein is a variant TIM-3 antigen binding domain that includes 6 CDRs (i.e., vhCDR1-3 and vlCDR1-3), where the 6 CDRs are at least 90, 95, 97, 98 or 99% identical as compared to the 6 CDRs of one of the following TIM-3 ABDs: 3H3[TIM-3]_H0_L0, 3H3[TIM-3]_H1_L2, 3H3[TIM-3]_H1_L2.1, APE137[TIM-3], APE5121[TIM-3], ABTIM3-hum03[TIM-3], ABTIM3-hum11[TIM-3], ABTIM3-hum21[TIM-3], 4177[TIM-3], 4545[Tim-3], 8213[TIM-3], mAb15[TIM-3], mAb58[TIM-3], TIM3-0433[TIM-3], TIM3-0434[TIM-3], TIM3-0438[TIM3], and TIM3-0443[TIM3] (see, e.g., Figures 12 and 13A-C). In an exemplary embodiments, the TIM-3ABD is 3H3[TIM-3]_H1_L2.1.

[00198] In some embodiments, the TIM-3 ABD of the IL-15/IL-15Rα heterodimeric fusion protein includes the variable heavy domain (VH) and variable light domain (VL) of any of the LAG-ABDs disclosed herein, including, but not limited to those disclosed in Figures 12 and 13A-C. In addition to the parental TIM-3 variable heavy and variable light domains disclosed herein, provided herein are subject targeted IL-15/IL-15Rα heterodimeric fusion proteins having one or more TIM-3 ABDs that include a variable heavy domain and/or a variable light domain that are variants of a TIM-3 ABD VH and VL domain disclosed herein. In one embodiment, the variant VH domain and/or VL domain has from 1, 2, 3, 4, 5, 6, 7, 8, 9 or 10 amino acid changes from a VH and/or VL domain of a TIM-3 ABD depicted in Figures 12, 13A-C or the sequence listing. In certain embodiments, the TIM-3

ABD is capable of binding to TIM-3, as measured at least one of a Biacore, surface plasmon resonance (SPR) and/or BLI (biolayer interferometry, e.g., Octet assay) assay, with the latter finding particular use in many embodiments.

[00199] In one embodiment, the variant VH and/or VL domain of the IL-15/IL-15Rα heterodimeric fusion protein is at least 90, 95, 97, 98 or 99% identical to the VH and/or VL of a TIM-3 ABD as depicted in Figures 12 and 13A-C or the sequence listing. In certain embodiments, the TIM-3 ABD is capable of binding to TIM-3, as measured by at least one of a Biacore, surface plasmon resonance (SPR) and/or BLI (biolayer interferometry, e.g., Octet assay) assay, with the latter finding particular use in many embodiments.

[00200] In some embodiments, the TIM-3 ABD includes the VH and VL of a one of the following TIM-3 ABDs: 3H3[TIM-3]_H0_L0, 3H3[TIM-3]_H1_L2, 3H3[TIM-3]_H1_L2.1, APE137[TIM-3], APE5121[TIM-3], ABTIM3-hum03[TIM-3], ABTIM3-hum11[TIM-3], ABTIM3-hum21[TIM-3], 4177[TIM-3], 4545[Tim-3], 8213[TIM-3], mAb15[TIM-3], mAb58[TIM-3], TIM3-0433[TIM-3], TIM3-0434[TIM-3], TIM3-0438[TIM3], and TIM3-0443[TIM3] (see, e.g., Figures 12 and 13A-C). In an exemplary embodiments, the TIM-3ABD is 3H3[TIM-3]_H1_L2.1.

[00201] In some embodiments, the TIM-3 ABD includes a VH and VL, where the VH and/or VL includes 1, 2, 3, 4, 5, 6, 7, 8, 9 or 10 amino acid modifications as compared to a VH and/or VL of one of the following TIM-3 ABDs: 3H3[TIM-3]_H0_L0, 3H3[TIM-3]_H1_L2, 3H3[TIM-3]_H1_L2.1, APE137[TIM-3], APE5121[TIM-3], ABTIM3-hum03[TIM-3], ABTIM3-hum11[TIM-3], ABTIM3-hum21[TIM-3], 4177[TIM-3], 4545[Tim-3], 8213[TIM-3], mAb15[TIM-3], mAb58[TIM-3], TIM3-0433[TIM-3], TIM3-0434[TIM-3], TIM3-0438[TIM3], and TIM3-0443[TIM3] (see, e.g., Figures 12 and 13A-C). In an exemplary embodiments, the TIM-3ABD is 3H3[TIM-3]_H1_L2.1.

[00202] In certain embodiments, the TIM-3 ABD includes a VH and VL, where the VH and VL are at least 90, 95, 97, 98 or 99% identical as compared to a VH and VL of one of the following TIM-3 ABDs: 3H3[TIM-3]_H0_L0, 3H3[TIM-3]_H1_L2, 3H3[TIM-3]_H1_L2.1, APE137[TIM-3], APE5121[TIM-3], ABTIM3-hum03[TIM-3], ABTIM3-hum11[TIM-3], ABTIM3-hum21[TIM-3], 4177[TIM-3], 4545[Tim-3], 8213[TIM-3], mAb15[TIM-3],

mAb58[TIM-3], TIM3-0433[TIM-3], TIM3-0434[TIM-3], TIM3-0438[TIM3], and TIM3-0443[TIM3] (see, e.g., Figures 12 and 13A-C). In an exemplary embodiments, the TIM-3ABD is 3H3[TIM-3]_H1_L2.1.

C. Fc domains

[00203] The Fc domain component of the invention is as described herein, which generally contains skew variants and/or optional pI variants and/or ablation variants are outlined herein. See for example the disclosure of WO2017/218707 under the heading "IV Heterodimeric Antibodies", including sections IV.A, IV.B, IV.C, IV.D, IV.E, IV.F, IV.G, IV.H and IV.I, all of which are expressly incorporated by reference in their entirety. Of particular use in the heterodimeric proteins of the present invention are Fc domains containing "skew variants", "pI variants", "ablation variants" and FcRn variants as outlined therein. Particularly useful combinations of such variants are depicted, for example, Figures 7A-F.

[00204] The Fc domains can be derived from IgG Fc domains, e.g., IgG1, IgG2, IgG3 or IgG4 Fc domains. In an exemplary embodiment, the subject heterodimeric fusion protein provided herein includes an IgG1 Fc domain. The following describes Fc domains that are useful for IL-15/IL-15Rα Fc fusion monomers and anti-TIM-3 antibody fragments of the targeted IL-15/IL-15Rα heterodimeric fusion proteins.

[00205] Thus, the "Fc domain" includes the -CH2-CH3 domain, and optionally a hinge domain, and can be from human IgG1, IgG2, IgG3 or IgG4, with Fc domains derived from IgG1. In some of the embodiments herein, when a protein fragment, e.g., IL-15 or IL-15Rα is attached to an Fc domain, it is the C-terminus of the IL-15 or IL-15Rα construct that is attached to all or part of the hinge of the Fc domain. In other embodiments, when a protein fragment, e.g., IL-15 or IL-15Rα, is attached to an Fc domain, it is the C-terminus of the IL-15 or IL-15Rα construct that is attached to the CH1 domain of the Fc domain.

[00206] In some of the constructs and sequences outlined herein of an Fc domain protein, the C-terminus of the IL-15 or IL-15Rα protein fragment is attached to the N-terminus of a domain linker, the C-terminus of which is attached to the N-terminus of a constant Fc domain (N-IL-15 or IL-15Rα protein fragment-linker-Fc domain-C) although that can be switched (N- Fc domain-linker- IL-15 or IL-15Rα protein fragment -C). In other

constructs and sequence outlined herein, C-terminus of a first protein fragment is attached to the N-terminus of a second protein fragment, optionally via a domain linker, the C-terminus of the second protein fragment is attached to the N-terminus of a constant Fc domain, optionally via a domain linker. In yet other constructs and sequences outlined herein, a constant Fc domain that is not attached to a first protein fragment or a second protein fragment is provided. A heterodimeric fusion protein can contain two or more of the exemplary monomeric Fc domain proteins described herein. Any domain linker can be used to attach a IL-15 or IL-15Rα protein fragment to an Fc domain of the heterodimeric fusion protein provided herein. In some embodiments, the linker is any one of the linkers in Figures 8.

[00207] In some embodiments, the linker is a "domain linker", used to link any two domains (e.g., IL-15 or IL-15Rα protein fragment to Fc domain or scFv to Fc domain) as outlined herein together, some of which are depicted in **Error! Reference source not found.**. While any suitable linker can be used, many embodiments utilize a glycine-serine polymer, including for example (GS)n, (GSGGS)n, (GGGGS)n, and (GGGS)n, where n is an integer of at least one (and generally from 1 to 2 to 3 to 4 to 5) as well as any peptide sequence that allows for recombinant attachment of the two domains with sufficient length and flexibility to allow each domain to retain its biological function. In some cases, and with attention being paid to "strandedness", as outlined below, charged domain linkers.

[00208] In one embodiment, the heterodimeric fusion proteins contain at least two constant domains which can be engineered to produce heterodimers, such as pI engineering. Other Fc domains that can be used include fragments that contain one or more of the CH1, CH2, CH3, and hinge domains of the invention that have been pI engineered. In particular, the formats depicted in Figures 21 are heterodimeric fusion proteins, meaning that the protein has two associated Fc sequences self-assembled into a heterodimeric Fc domain and at least one fusion protein (e.g., 1, 2 or more fusion proteins) as more fully described below. In some cases, a first fusion protein is linked to a first Fc and a second fusion protein is linked to a second Fc. In other cases, a first fusion protein is linked to a first Fc, and the first fusion protein is non-covalently attached to a second fusion protein that is not linked to an Fc. In some cases, the heterodimeric fusion protein contains a first fusion protein linked to a

second fusion protein which is linked a first Fc sequence, and a second Fc sequence that is not linked to either the first or second fusion proteins.

[00209] Accordingly, in some embodiments the present invention provides heterodimeric fusion proteins that rely on the use of two different heavy chain variant Fc sequences, that will self-assemble to form a heterodimeric Fc domain fusion polypeptide.

[00210] The present invention is directed to novel constructs to provide heterodimeric fusion proteins that allow binding to one or more binding partners, ligands or receptors. The heterodimeric fusion constructs are based on the self-assembling nature of the two Fc domains of the heavy chains of antibodies, e.g., two "monomers" that assemble into a "dimer". Heterodimeric Fc fusions are made by altering the amino acid sequence of each monomer as more fully discussed below. Thus, the present invention is generally directed to the creation of heterodimeric fusion proteins which can co-engage binding partner(s) or ligand(s) or receptor(s) in several ways, relying on amino acid variants in the constant regions that are different on each chain to promote heterodimeric formation and/or allow for ease of purification of heterodimers over the homodimers. Specific variants that are included in the Fc domains of specific embodiments of the subject heterodimeric fusion protein are described in greater detail below.

1. Heterodimerization Variants

[00211] The present invention provides heterodimeric proteins, including heterodimeric fusion proteins in a variety of formats. Such heterodimeric proteins include two different Fc domains (one on each of the first and second monomers) that include modifications that facilitate the heterodimerization of the first and second monomers and/or allow for ease of purification of heterodimers over homodimers, collectively referred to herein as "heterodimerization variants." As discussed below, heterodimerization variants can include skew variants (e.g., the "knobs and holes" and "charge pairs" variants described below) as well as "pI variants" that facilitates the separation of homodimers away from heterodimers. As is generally described in US Patent No. US 9,605,084, hereby incorporated by reference in its entirety and specifically as below for the discussion of heterodimerization variants, useful mechanisms for heterodimerization include "knobs and holes" ("KIH") as described in US Patent No. US 9,605,084, "electrostatic steering" or "charge pairs" as

described in US Patent No. US 9,605,084, pI variants as described in US Patent No. US 9,605,084, and general additional Fc variants as outlined in US Patent No. US 9,605,084 and below.

a. Skew Variants

[00212] In some embodiments, the subject heterodimeric protein includes skew variants, which are one or more amino acid modifications in a first Fc domain (A) and/or a second Fc domain (B) that favor the formation of Fc heterodimers (Fc dimers that include the first and the second Fc domain; A-B) over Fc homodimers (Fc dimers that include two of the first Fc domain or two of the second Fc domain; A-A or B-B). Suitable skew variants are included in the Figure 29 of US Publ. App. No. 2016/0355608, hereby incorporated by reference in its entirety and specifically for its disclosure of skew variants, as well as in Figure 4.

[00213] One mechanism for skew variants is generally referred to in the art as "knobs and holes," referring to amino acid engineering that creates steric influences to favor heterodimeric formation and disfavor homodimeric formation, as described in USSN 61/596,846, Ridgway et al., Protein Engineering 9(7):617 (1996); Atwell et al., J. Mol. Biol. 1997 270:26; US Patent No. 8,216,805, all of which are hereby incorporated by reference in their entirety and specifically for the disclosure of "knobs and holes" mutations. This is sometime referred to herein as "steric variants." The figures identify a number of "monomer A – monomer B" pairs that rely on "knobs and holes". In addition, as described in Merchant et al., Nature Biotech. 16:677 (1998), these "knobs and holes" mutations can be combined with disulfide bonds to further favor formation of Fc heterodimers.

[00214] An additional mechanism for skew variants that finds use in the generation of heterodimers is sometimes referred to as "electrostatic steering" as described in Gunasekaran et al., J. Biol. Chem. 285(25):19637 (2010), hereby incorporated by reference in its entirety. This is sometimes referred to herein as "charge pairs." In this embodiment, electrostatics are used to skew the formation towards heterodimerization. As those in the art will appreciate, these may also have an effect on pI, and thus on purification, and thus could in some cases also be considered pI variants. However, as these were generated to force heterodimerization and were not used as purification tools, they are classified as "skew

variants." These include, but are not limited to, D221E/P228E/L368E paired with D221R/P228R/K409R (e.g., these are "monomer" corresponding sets) and C220E/P228E/368E paired with C220R/E224R/P228R/K409R.

[00215] In some embodiments, the skew variants advantageously and simultaneously favor heterodimerization based on both the "knobs and holes" mechanism as well as the "electrostatic steering" mechanisms described above. In some embodiments, the heterodimeric protein includes one or more sets of such heterodimerization skew variants. These variants come in "pairs" of "sets." That is, one set of the pair is incorporated into the first monomer and the other set of the pair is incorporated into the second monomer. Exemplary "skew variants' in this category include S364K/E357Q : L368D/K370S; L368D/K370S : S364K; L368E/K370S : S364K; T411T/E360E/Q362E : D401K; L368D/K370S : S364K/E357L; K370S : S364K/E357Q; or a T366S/L368A/Y407V : T366W (optionally including a bridging disulfide, T366S/L368A/Y407V/Y349C : T366W/S354C) "skew" variant amino acid substitution sets. In terms of nomenclature, the pair "S364K/E357Q : L368D/K370S" means that one of the monomers includes an Fc domain that includes the amino acid substitutions S364K and E357Q and the other monomer includes an Fc domain that includes the amino acid substitutions L368D and K370S; as above, the "strandedness" of these pairs depends on the starting pI. It should be noted that these sets do not necessarily behave as "knobs in holes" variants, with a one-to-one correspondence between a residue on one monomer and a residue on the other. That is, these pairs of sets may instead form an interface between the two monomers that encourages heterodimer formation and discourages homodimer formation, allowing the percentage of heterodimers that spontaneously form under biological conditions to be over 90%, rather than the expected 50% (25 % homodimer A/A:50% heterodimer A/B:25% homodimer B/B).

[00216] In exemplary embodiments, the heterodimeric fusion protein includes a S364K/E357Q : L368D/K370S; L368D/K370S : S364K; L368E/K370S : S364K; T411T/E360E/Q362E : D401K; L368D/K370S : S364K/E357L; K370S : S364K/E357Q; or a T366S/L368A/Y407V : T366W (optionally including a bridging disulfide, T366S/L368A/Y407V/Y349C : T366W/S354C) "skew" variant amino acid substitution set. In

an exemplary embodiment, the heterodimeric fusion protein includes a "S364K/E357Q : L368D/K370S" amino acid substitution set.

[00217] In some embodiments, the skew variants provided herein are independently incorporated with other modifications, including, but not limited to, other skew variants (see, e.g., in Figure 37 of US Publ. App. No. 2012/0149876, herein incorporated by reference, particularly for its disclosure of skew variants), pI variants, isotpypic variants, FcRn variants, ablation variants, etc. into one or both of the first and second Fc domains of the heterodimeric fusion protein. Further, individual modifications can also independently and optionally be included or excluded from the subject heterodimeric fusion proteins.

b. pI (Isoelectric point) Variants for Heterodimers

[00218] In some embodiments, the heterodimeric fusion protein includes purification variants that advantageously allow for the separation of heterodimeric fusion proteins from homodimeric proteins ("pI variants").

[00219] In general, as will be appreciated by those in the art, there are two general categories of pI variants: those that increase the pI of the protein (basic changes) and those that decrease the pI of the protein (acidic changes). As described herein, all combinations of these variants can be done: one monomer may be wild type, or a variant that does not display a significantly different pI from wild-type, and the other can be either more basic or more acidic. Alternatively, each monomer is changed, one to more basic and one to more acidic.

[00220] There are several basic mechanisms that can lead to ease of purifying heterodimeric proteins. One such mechanism relies on the use of pI variants which include one or more modifications that affect the isoelectric point of one or both of the monomers of the fusion protein, such that each monomer, and subsequently each dimeric species, has a different pI, thus allowing the isoelectric purification of A-A, A-B and B-B dimeric proteins. Alternatively, some formats also allow separation on the basis of size. As is further outlined above, it is also possible to "skew" the formation of heterodimers over homodimers using

skew variants. Thus, a combination of heterodimerization skew variants and pI variants find particular use in the subject heterodimeric fusion proteins provided herein.

[00221] Additionally, as more fully outlined below, depending on the format of the heterodimeric fusion protein, pI variants can be either contained within the constant region and/or Fc domains of a monomer, and/or domain linkers can be used. In some embodiments, the heterodimeric fusion protein includes additional modifications for alternative functionalities can also create pI changes, such as Fc, FcRn and KO variants.

[00222] In the embodiments that utilizes pI as a separation mechanism to allow the purification of heterodimeric fusion proteins, amino acid modifications can be introduced into one or both of the monomers of the heterodimeric fusion protein. That is, the pI of one of the monomers (referred to herein for simplicity as "monomer A") can be engineered away from monomer B, or both monomer A and B can be changed, with the pI of monomer A increasing and the pI of monomer B decreasing. As discussed, the pI changes of either or both monomers can be done by removing or adding a charged residue (e.g., a neutral amino acid is replaced by a positively or negatively charged amino acid residue, e.g., glutamine to glutamic acid), changing a charged residue from positive or negative to the opposite charge (e.g. aspartic acid to lysine) or changing a charged residue to a neutral residue (e.g., loss of a charge; lysine to serine.). A number of these variants are shown in the figures, including, Figures 4 and 5.

[00223] Creating a sufficient change in pI in at least one of the monomers such that heterodimers can be separated from homodimers can be done by using a "wild type" heavy chain constant region and a variant region that has been engineered to either increase or decrease its pI (wt A : B+ or wt A : B-), or by increasing one region and decreasing the other region (A+ : B- or A- : B+).

[00224] Thus, in general, a component of some embodiments of the present subject fusion proteins are amino acid variants in the Fc domains or constant domain regions that are directed to altering the isoelectric point (pI) of at least one, if not both, of the monomers of a dimeric protein by incorporating amino acid substitutions ("pI variants" or "pI substitutions") into one or both of the monomers. The separation of the heterodimers from

the two homodimers can be accomplished if the pIs of the two monomers differ by as little as 0.1 pH unit, with 0.2, 0.3, 0.4 and 0.5 or greater all finding use in the present invention.

[00225] As will be appreciated by those in the art, the number of pI variants to be included on each or both monomer(s) of a heterodimeric fusion protein to achieve good separation will depend in part on the starting pI of the components. That is, to determine which monomer to engineer or in which "direction" (e.g., more positive or more negative), the sequences of the Fc domains and any IL-15, IL-15Rα or linker included in each monomer are calculated and a decision is made from there based on the pIs of the monomers. As is known in the art, different Fc domains, linkers IL-15, and IL-15Rα will have different starting pIs. In general, as outlined herein, the pIs are engineered to result in a total pI difference of each monomer of at least about 0.1 logs, with 0.2 to 0.5 being preferred as outlined herein.

[00226] In general, as will be appreciated by those in the art, there are two general categories of amino acid modifications that affect pI: those that increase the pI of the protein (basic changes) and those that decrease the pI of the protein (acidic changes). As described herein, all combinations of these variants can be used: one monomer may include a wild type Fc domain, or a variant Fc domain that does not display a significantly different pI from wild-type, and the other monomer includes a Fc domain that is either more basic or more acidic. Alternatively, each monomer may be changed, one to more basic and one to more acidic.

[00227] In the case where pI variants are used to achieve heterodimerization, a more modular approach to designing and purifying heterodimeric fusion proteins is provided. Thus, in some embodiments, heterodimerization variants (including skew and pI variants) must be engineered. In addition, in some embodiments, the possibility of immunogenicity resulting from the pI variants is significantly reduced by importing pI variants from different IgG isotypes such that pI is changed without introducing significant immunogenicity (see isotypic variants below). Thus, an additional problem to be solved is the elucidation of low pI constant domains with high human sequence content, e.g. the minimization or avoidance of non-human residues at any particular position. Alternatively or in addition to isotypic substitutions, the possibility of immunogenicity resulting from the

pI variants is significantly reduced by utilizing isosteric substitutions (e.g., Asn to Asp; and Gln to Glu).

[00228] A side benefit that can occur with this pI engineering is also the extension of serum half-life and increased FcRn binding. That is, as described in US Publ. App. No. US 2012/0028304 (incorporated by reference in its entirety and specifically for the disclosure of pI variants that provide additional function), lowering the pI of antibody constant domains (including those found in Fc fusions) can lead to longer serum retention in vivo. These pI variants for increased serum half-life also facilitate pI changes for purification.

[00229] In addition, it should be noted that the pI variants of the heterodimerization variants give an additional benefit for the analytics and quality control process of Fc fusion proteins, as the ability to either eliminate, minimize and distinguish when homodimers are present is significant. Similarly, the ability to reliably test the reproducibility of the heterodimeric fusion protein production is important.

[00230] Exemplary combinations of pI variants are shown in Figures 4 and 5, and Figure 30 of US Publ. App. No. 2016/0355608, all of which are herein incorporated by reference in its entirety and specifically for the disclosure of pI variants. As outlined herein and shown in the figures, these changes are shown relative to IgG1, but all isotypes can be altered this way, as well as isotype hybrids. In the case where the heavy chain constant domain is from IgG2-4, R133E and R133Q can also be used.

[00231] In some embodiments, modifications are made in the hinge of the Fc domain, including positions 208, 216, 217, 218, 219, 220, 221, 222, 223, 224, 225, 226, 227, 228, 229, and 230 based on EU numbering. Thus, pI mutations and particularly substitutions can be made in one or more of positions 216-230, with 1, 2, 3, 4 or 5 mutations finding use. Again, all possible combinations are contemplated, alone or with other pI variants in other domains.

[00232] Specific substitutions that find use in lowering the pI of hinge domains include, but are not limited to, a deletion at position 221, a non-native valine or threonine at position 222, a deletion at position 223, a non-native glutamic acid at position 224, a deletion at position 225, a deletion at position 235 and a deletion or a non-native alanine at position

236. In some cases, only pI substitutions are done in the hinge domain, and in others, these substitution(s) are added to other pI variants in other domains in any combination.

[00233] In some embodiments, mutations can be made in the CH2 region, including positions 233, 234, 235, 236, 274, 296, 300, 309, 320, 322, 326, 327, 334 and 339, based on EU numbering. It should be noted that changes in 233-236 can be made to increase effector function (along with 327A) in the IgG2 backbone. Again, all possible combinations of these 14 positions can be made; e.g., a heterodimeric fusion protein may include a variant Fc domain with 1, 2, 3, 4, 5, 6, 7, 8, 9 or 10 CH2 pI substitutions.

[00234] Specific substitutions that find use in lowering the pI of CH2 domains include, but are not limited to, a non-native glutamine or glutamic acid at position 274, a non-native phenylalanine at position 296, a non-native phenylalanine at position 300, a non-native valine at position 309, a non-native glutamic acid at position 320, a non-native glutamic acid at position 322, a non-native glutamic acid at position 326, a non-native glycine at position 327, a non-native glutamic acid at position 334, a non-native threonine at position 339, and all possible combinations within CH2 and with other domains.

[00235] In this embodiment, the modifications can be independently and optionally selected from position 355, 359, 362, 384, 389,392, 397, 418, 419, 444 and 447 (EU numbering) of the CH3 region. Specific substitutions that find use in lowering the pI of CH3 domains include, but are not limited to, a non-native glutamine or glutamic acid at position 355, a non-native serine at position 384, a non-native asparagine or glutamic acid at position 392, a non-native methionine at position 397, a non-native glutamic acid at position 419, a non-native glutamic acid at position 359, a non-native glutamic acid at position 362, a non-native glutamic acid at position 389, a non-native glutamic acid at position 418, a non-native glutamic acid at position 444, and a deletion or non-native aspartic acid at position 447. Exemplary embodiments of pI variants are provided in Figure 5.

[00236] In one embodiment, the heterodimeric fusion protein includes a monomer with a variant Fc domain having pI variant modifications 295E/384D/418E/421D (Q295E/N384D/Q418E/N421D when relative to human IgG1). In one embodiment, the heterodimeric fusion protein includes a monomer with a variant Fc domain having pI

variant modifications 208D/295E/384D/418E/421D (N208D/Q295E/N384D/Q418E/N421D when relative to human IgG1). In some embodiments, the heterodimeric fusion protein includes a monomer with a variant Fc domain having pI variant modifications 295E/384D/418E/421D (Q295E/N384D/Q418E/N421D when relative to human IgG1). In one embodiment, the heterodimeric fusion protein includes a monomer with a variant Fc domain having pI variant modifications 196K/199T/217R/228R/276K (Q196K/I199T/P217R/P228R/N276K) when relative to human IgG1).

[00237] In one embodiment, the heterodimeric fusion protein includes a monomer with a variant Fc domain having pI variant modifications 217R/228R/276K (P217R/P228R/N276K when relative to human IgG1). Additional exemplary pI variant modification that can be incorporated into the Fc domain of a subject are depicted in Figure 5.

2. Additional Fc Variants for Additional Functionality

[00238] In addition to pI amino acid variants, there are a number of useful Fc amino acid modification that can be made for a variety of reasons, including, but not limited to, altering binding to one or more FcγR receptors, altered binding to FcRn receptors, etc.

[00239] Accordingly, the proteins of the invention can include amino acid modifications, including the heterodimerization variants outlined herein, which includes the pI variants and steric variants. Each set of variants can be independently and optionally included or excluded from any particular heterodimeric protein.

a. FcγR Variants

[00240] Accordingly, there are a number of useful Fc substitutions that can be made to alter binding to one or more of the FcγR receptors. Substitutions that result in increased binding as well as decreased binding can be useful. For example, it is known that increased binding to FcγRIIIa results in increased ADCC (antibody dependent cell-mediated cytotoxicity; the cell-mediated reaction wherein nonspecific cytotoxic cells that express FcγRs recognize bound antibody on a target cell and subsequently cause lysis of the target cell). Similarly, decreased binding to FcγRIIb (an inhibitory receptor) can be beneficial as well in some circumstances. Amino acid substitutions that find use in the present invention include those listed in USSNs 11/124,620 (particularly Figure 41), 11/174,287, 11/396,495,

11/538,406, all of which are expressly incorporated herein by reference in their entirety and specifically for the variants disclosed therein. Particular variants that find use include, but are not limited to, 236A, 239D, 239E, 332E, 332D, 239D/332E, 267D, 267E, 328F, 267E/328F, 236A/332E, 239D/332E/330Y, 239D, 332E/330L, 243A, 243L, 264A, 264V and 299T.

[00241] In addition, amino acid substitutions that increase affinity for FcγRIIc can also be included in the Fc domain variants outlined herein. The substitutions described in, for example, USSNs 11/124,620 and 14/578,305 are useful.

[00242] In addition, there are additional Fc substitutions that find use in increased binding to the FcRn receptor and increased serum half-life, as specifically disclosed in USSN 12/341,769, hereby incorporated by reference in its entirety, including, but not limited to, 434S, 434A, 428L, 308F, 259I, 428L/434S, 259I/308F, 436I/428L, 436I or V/434S, 436V/428L and 259I/308F/428L.

b. Ablation Variants

[00243] Similarly, another category of functional variants are "FcγR ablation variants" or "Fc knock out (FcKO or KO)" variants. In these embodiments, for some therapeutic applications, it is desirable to reduce or remove the normal binding of the Fc domain to one or more or all of the Fcγ receptors (e.g., FcγR1, FcγRIIa, FcγRIIb, FcγRIIIa, etc.) to avoid additional mechanisms of action. That is, for example, in many embodiments, particularly in the use of bispecific immunomodulatory antibodies desirable to ablate FcγRIIIa binding to eliminate or significantly reduce ADCC activity such that one of the Fc domains comprises one or more Fcγ receptor ablation variants. These ablation variants are depicted in Figure 31 of USSN 15/141,350, all of which are herein incorporated by reference in its entirety, and each can be independently and optionally included or excluded, with preferred aspects utilizing ablation variants selected from the group consisting of G236R/L328R, E233P/L234V/L235A/G236del/S239K, E233P/L234V/L235A/G236del/S267K, E233P/L234V/L235A/G236del/S239K/A327G, E233P/L234V/L235A/G236del/S267K/A327G and E233P/L234V/L235A/G236del, according to the EU index. It should be noted that the ablation variants referenced herein ablate FcγR binding but generally not FcRn binding.

[00244] Exemplary ablation variants are provided in Figure 5.

c. Combination of Heterodimeric and Fc Variants

[00245] As will be appreciated by those in the art, all of the recited heterodimerization variants (including skew and/or pI variants) can be optionally and independently combined in any way, as long as they retain their "strandedness" or "monomer partition". In addition, all of these variants can be combined into any of the heterodimerization formats.

[00246] In the case of pI variants, while embodiments finding particular use are shown in the Figures, other combinations can be generated, following the basic rule of altering the pI difference between two monomers to facilitate purification.

[00247] In addition, any of the heterodimerization variants, skew and pI, are also independently and optionally combined with Fc ablation variants, Fc variants, FcRn variants, as generally outlined herein.

[00248] In addition, a monomeric Fc domain can comprise a set of amino acid substitutions that includes C220S/S267K/L368D/K370S or C220S/S267K/S364K/E357Q.

[00249] In addition, the heterodimeric fusion proteins can comprise skew variants (e.g., a set of amino acid substitutions as shown in Figures 1A-1C of USSN 15/141,350, all of which are herein incorporated by reference in its entirety), with particularly useful skew variants being selected from the group consisting of S364K/E357Q : L368D/K370S; L368D/K370S : S364K; L368E/K370S : S364K; T411E/K360E/Q362E : D401K; L368D/K370S : S364K/E357L, K370S : S364K/E357Q, T366S/L368A/Y407V : T366W and T366S/L368A/Y407V/Y349C : T366W/S354C, optionally ablation variants, optionally charged domain linkers and the heavy chain comprises pI variants.

[00250] In some embodiments, the Fc domain comprising an amino acid substitution selected from the group consisting of: 236R, 239D, 239E, 243L, M252Y, V259I, 267D, 267E, 298A, V308F, 328F, 328R, 330L, 332D, 332E, M428L, N434A, N434S, 236R/328R, 239D/332E, M428L, 236R/328F, V259I/V308F, 267E/328F, M428L/N434S, Y436I/M428L, Y436V/M428L, Y436I/N434S, Y436V/N434S, 239D/332E/330L, M252Y/S254T/T256E, V259I/V308F/M428L, E233P/L234V/L235A/G236del/S267K, G236R/L328R and PVA/S267K. In some cases, the Fc domain comprises the amino acid substitution 239D/332E. In other cases, the Fc domain comprises the amino acid substitution G236R/L328R or PVA/S267K.

[00251] In one embodiment, a particular combination of skew and pI variants that finds use in the present invention is T366S/L368A/Y407V : T366W (optionally including a bridging disulfide, T366S/L368A/Y407V/Y349C : T366W/S354C) with one monomer comprises Q295E/N384D/Q418E/N481D and the other a positively charged domain linker. As will be appreciated in the art, the "knobs in holes" variants do not change pI, and thus can be used on either monomer. Useful combination of variants that can be used in particular formats of the invention are included in Figures 7A-7F.

III. Targeted IL-15/IL-15Rα Fc Fusion x TIM-3 ABD Heterodimeric Proteins

[00252] Provided herein are heterodimeric fusion proteins that can bind to the checkpoint inhibitor TIM-3 antigen and can complex with the common gamma chain (γc; CD132) and/or the Il-2 receptor β-chain (IL-2Rβ; CD122). The heterodimeric fusion proteins can contain an IL-15/IL-15Rα-Fc fusion protein and an antibody fusion protein. The IL-15/IL-15Rα-Fc fusion protein can include as IL-15 protein (generally including amino acid substitutions) covalently attached to an IL-15Rα, and an Fc domain. Optionally, the IL-15 protein and IL-15Rα protein are noncovalently attached.

IV. Useful Formats of the Invention

[00253] As shown in Figures 21, there are a number of useful formats of the targeted IL-15/IL-15Rα-Fc heterodimeric fusion proteins of the invention. In general, the heterodimeric fusion proteins of the invention have three functional components: an IL-15/IL-15Rα(sushi) component, an anti-TIM-3 component, and an Fc component, each of which can take different forms as outlined herein and each of which can be combined with the other components in any configuration.

[00254] The first and the second variant Fc domains can have a set of amino acid substitutions selected from the group consisting of a) S267K/L368D/K370S : S267K/S364K/E357Q; b) S364K/E357Q : L368D/K370S; c) L368D/K370S : S364K; d) L368E/K370S : S364K; e) T411E/K360E/Q362E : D401K; f) L368D/K370S : S364K/E357L and g) K370S : S364K/E357Q, according to EU numbering. In an exemplary embodiment, the skew variants are S364K/E357Q : L368D/K370S.

[00255] In some embodiments, the first and/or the second Fc domains have an additional set of pI amino acid substitutions selected from the following pI variants: Q295E/N384D/Q418E/N421D, N208/Q295E/N384D/Q418E/N421D or Q196K/I199T/P217R/P228R/N276K, according to EU numbering.

[00256] Optionally, the first and/or the second Fc domains have an additional set of ablation ("FcKO") variants selected from the following FcKO variants: G236R/L328R, E233P/L234V/L235A/G236del/S239K, E233P/L234V/L235A/G236del/S267K, E233P/L234V/L235A/G236del/S239K/A327G, E233P/L234V/L235A/G236del/S267K/A327G and E233P/L234V/L235A/G236del, according to EU numbering.

[00257] Optionally, the first and/or second Fc domains have 428L/434S variants for half-life extension.

[00258] In embodiments wherein a hinge or partial hinge is used to link an Fc domain to a scFv, IL-15 or IL-15Rα domain, the hinge may optional include a C220S substitution to prevent the hinge from forming undesirable disulfide bonds with any light chains.

[00259] Exemplary formats of the subject heterodimeric fusion proteins are provided below.

A. scIL-15/Rα X scFv

[00260] One embodiment is shown in Figures 21A, and comprises two monomers. The first monomer comprises, from N- to C-terminus, the IL-15Rα(sushi) domain-(domain linker)-IL-15 variant-(domain linker)-CH2-CH3 (with the second domain linker frequently being a hinge domain), and the second monomer comprises VH-scFv linker-VL-hinge-CH2-CH3 or VL-scFv linker-VH-hinge-CH2-CH3, although in either orientation a domain linker can be substituted for the hinge. This is generally referred to as "scIL-15/Rα X scFv", with the "sc" standing for "single chain" referring to the attachment of the IL-15 variant and IL-15Rα(sushi) domain using a covalent linker. Preferred combinations of variants for this embodiment are found in Figures 21A and B.

[00261] In one embodiment, the targeted IL-15/IL-15Rα heterodimeric protein is an "scIL-15/Rα X scFv" format heterodimeric protein that includes: a) a first monomer that includes, from N- to C-terminus, an IL-15Rα(sushi) domain-(domain linker)-IL-15 variant-

(domain linker)-CH2-CH3, where CH2-CH3 is a first Fc domain; and b) a second monomer that includes, from N- to C-terminus, an anti-TIM-3 scFv-(domain linker)-CH2-CH3, where CH2-CH3 is a second Fc domain. Any useful domain linker can be used to attach the various components of the heterodimeric protein including, but not limited to those in Figures 8 and 9A-C. In an exemplary embodiment, the domain linkers that attach the IL-15 variant to the first Fc domain and the anti-TIM-3 scFv to the second Fc domain are each antibody hinge domains.

[00262] In some embodiments, the anti-TIM-3 scFv includes a variable heavy domain (VH) covalently attached to a variable light domain (VL) by an scFv linker (e.g., Figures 9A-C). In one embodiment, the anti-TIM-3 scFv is from N- to C-terminus VH-scFv linker-VL. In another embodiment, the anti-TIM-3 scFv is from N- to C-terminus VL-scFv linker-VH. The C-terminus of the anti-TIM-3 scFv is attached to the N terminus of the first Fc domain by a domain linker (e.g., an antibody hinge domain).

[00263] In the scIL-15/Rα X scFv format, one preferred embodiment utilizes an anti-TIM-3 ABD having any of the variable heavy and light domain pairs as shown in Figure 12 and 13A-C.

[00264] In the scIL-15/Rα X scFv format, one preferred embodiment utilizes an anti-TIM-3 ABD having the variable heavy and light domain pair of 3H3[TIM-3]_H1_L2.1 as shown in Figure 12. In one embodiment, the "scIL-15/Rα X scFv" format heterodimeric protein includes: a) a first monomer that includes, from N- to C-terminus, an IL-15Rα(sushi) domain-(domain linker)-IL-15 variant-(hinge)-CH2-CH3, where CH2-CH3 is a first Fc domain; and b) a second monomer that includes, from N- to C-terminus, an anti –TIM-3 scFv-(hinge)-CH2-CH3, where CH2-CH3 is a second Fc domain, and where the anti-TIM-3 scFv includes the variable heavy domain and variable light domain of 3H3[TIM-3]_H1_L2.1.

[00265] In the scIL-15/Rα X scFv format, one preferred embodiment utilizes an IL-15 variant that includes amino acid substitutions N4D/N65D, D30N/N65D, or D30N/E64Q/N65D. In one embodiment, the "scIL-15/Rα X scFv" format heterodimeric protein includes: a) a first monomer that includes, from N- to C-terminus, an IL-15Rα(sushi) domain-(domain linker)-IL-15 variant-(hinge)-CH2-CH3, where CH2-CH3 is a first Fc

domain; and b) a second monomer that includes, from N- to C-terminus, anti-TIM-3 scFv-(hinge)-CH2-CH3, where CH2-CH3 is a second Fc domain, and where the IL-15 variant includes amino acid substitutions N4D/N65D, D30N/N65D, or D30N/E64Q/N65D. In an exemplary embodiment, the TIM-3 scFv includes the VH and VL of any of the TIM-3 ABDs in Figures 12 and 13A-C and the IL-15 variant includes amino acid substitutions N4D/N65D. In another exemplary embodiment, the TIM-3 scFv includes the VH and VL of any of the TIM-3 ABDs in Figures 12 and 13A-C and the IL-15 variant includes amino acid substitutions D30N/N65D. In yet another exemplary embodiment, the TIM-3 scFv includes the VH and VL of any of the TIM-3 ABDs in Figures 12 and 13A-C and the IL-15 variant includes amino acid substitutions D30N/E64Q/N65D.

[00266] In the scIL-15/Rα X scFv format, one preferred embodiment utilizes an anti-TIM-3 ABD having the variable heavy and light domain pair of 3H3[TIM-3]_H1_L2.1 as shown in Figure 12, with either the IL-15 N4D/N65D variant or the IL-15 D30N/N65D variant or the IL-15 D30N/E64Q/N65D variant. In one embodiment, the "scIL-15/Rα X scFv" format heterodimeric protein includes: a) a first monomer that includes, from N- to C-terminus, an IL-15Rα(sushi) domain-(domain linker)-IL-15 variant-(hinge)-CH2-CH3, where CH2-CH3 is a first Fc domain; and b) a second monomer that includes, from N- to C-terminus, anti-TIM-3 scFv-(hinge)-CH2-CH3, where CH2-CH3 is a second Fc domain, where the anti-TIM-3 scFv includes the variable heavy domain and variable light domain of 3H3[TIM-3]_H1_L2.1, and where the IL-15 variant includes amino acid substitutions N4D/N65D, D30N/N65D, or D30N/E64Q/N65D.

[00267] In the scIL-15/Rα X scFv format, one preferred embodiment utilizes the skew variant pair S364K/E357Q : L368D/K370S. In one embodiment, the targeted IL-15/IL-15Rα heterodimeric protein is an "scIL-15/Rα X scFv" format heterodimeric protein that includes: a) a first monomer that includes, from N- to C-terminus, an IL-15Rα(sushi) domain-(domain linker)-IL-15 variant-(domain linker)-CH2-CH3, where CH2-CH3 is a first variant Fc domain; and b) a second monomer that includes, from N- to C-terminus, anti-TIM-3 scFv-(domain linker)-CH2-CH3, where CH2-CH3 is a second variant Fc domain, and where the first and second variant Fc domains include the skew variant pair S364K/E357Q : L368D/K370S. In an exemplary embodiment, the first variant Fc domain includes skew

variants L368D/K370S, and the second variant Fc domain includes skew variants L368D/K370S.

[00268] In one embodiment, the targeted IL-15/IL-15Rα heterodimeric protein is an "scIL-15/Rα X scFv" format heterodimeric protein that includes: a) a first monomer that includes, from N- to C-terminus, an IL-15Rα(sushi) domain-(domain linker)-IL-15 variant-(domain linker)-CH2-CH3, where CH2-CH3 is a first variant Fc domain; and b) a second monomer that includes, from N- to C-terminus, anti-TIM-3 scFv-(domain linker)-CH2-CH3, where CH2-CH3 is a second variant Fc domain, where the IL-15 variant includes amino acid substitutions N4D/N65D, D30N/N65D, or D30N/E64Q/N65D, and where the first and second variant Fc domains include the skew variant pair S364K/E357Q : L368D/K370S. In an exemplary embodiment, the first variant Fc domain includes skew variants L368D/K370S, and the second variant Fc domain includes skew variants L368D/K370S. In an exemplary embodiment, the TIM-3 scFv includes the VH and VL of any of the TIM-3 ABDs in Figures 12 and 13A-C and the IL-15 variant includes amino acid substitutions N4D/N65D. In another exemplary embodiment, the TIM-3 scFv includes the VH and VL of any of the TIM-3 ABDs in Figures 12 and 13A-C and the IL-15 variant includes amino acid substitutions D30N/N65D. In yet another exemplary embodiment, the TIM-3 scFv includes the VH and VL of any of the TIM-3 ABDs in Figures 12 and 13A-C and the IL-15 variant includes amino acid substitutions D30N/E64Q/N65D.

[00269] In the scIL-15/Rα X scFv format, one preferred embodiment utilizes an anti-TIM-3 ABD having the variable heavy and light domain pair of 3H3[TIM-3]_H1_L2.1 as shown in Figure 12 and the skew variant pair S364K/E357Q : L368D/K370S. In one embodiment, the "scIL-15/Rα X scFv" format heterodimeric protein includes: a) a first monomer that includes, from N- to C-terminus, an IL-15Rα(sushi) domain-(domain linker)-IL-15 variant-(hinge)-CH2-CH3, where CH2-CH3 is a first variant Fc domain; and b) a second monomer that includes, from N- to C-terminus, anti-TIM-3 scFv-(hinge)-CH2-CH3, where CH2-CH3 is a second variant Fc domain, where the anti-TIM-3 scFv includes the variable heavy domain and variable light domain of 3H3[TIM-3]_H1_L2.1, and where the first and second variant Fc domains include the skew variant pair S364K/E357Q : L368D/K370S. In an exemplary embodiment, the first variant Fc domain includes skew

variants L368D/K370S, and the second variant Fc domain includes skew variants L368D/K370S.

[00270] In the scIL-15/Rα X scFv format, one preferred embodiment utilizes an anti-TIM-3 ABD having the variable heavy and light domain pair of 3H3[TIM-3]_H1_L2.1 as shown in Figure 12 and the skew variant pair S364K/E357Q : L368D/K370S with either the IL-15 N4D/N65D variant or the IL-15 D30N/N65D variant or the IL-15 D30N/E64Q/N65D variant. In one embodiment, the "scIL-15/Rα X scFv" format heterodimeric protein includes: a) a first monomer that includes, from N- to C-terminus, an IL-15Rα(sushi) domain-(domain linker)-IL-15 variant-(hinge)-CH2-CH3, where CH2-CH3 is a first variant Fc domain; and b) a second monomer that includes, from N- to C-terminus, anti-TIM-3 scFv-(hinge)-CH2-CH3, where CH2-CH3 is a second variant Fc domain, where the anti-TIM-3 scFv includes the variable heavy domain and variable light domain of 3H3[TIM-3]_H1_L2.1, where the IL-15 variant includes amino acid substitutions N4D/N65D, D30N/N65D, or D30N/E64Q/N65D, and where the first and second variant Fc domains include the skew variant pair S364K/E357Q : L368D/K370S. In an exemplary embodiment, the first variant Fc domain includes skew variants L368D/K370S, and the second variant Fc domain includes skew variants L368D/K370S. In a particular embodiment, the IL-15 variant includes amino acid substitutions N4D/N65D and the scFv includes the variable heavy and light domain pair of 3H3[TIM-3]_H1_L2.1. In one embodiment, the IL-15 variant includes amino acid substitutions D30N/N65D and the scFv includes the variable heavy and light domain pair of 3H3[TIM-3]_H1_L2.1. In yet another embodiment, the IL-15 variant is the IL-15 D30N/E64Q/N65D variant and the scFv includes the variable heavy and light domain pair of 3H3[TIM-3]_H1_L2.1.

[00271] In the scIL-15/Rα X scFv format, one preferred embodiment utilizes the skew variant set S364K/E357Q : L368D/K370S, the pI variants Q295E/N384D/Q418E/N421D, the ablation variants E233P/L234V/L235A/G236_/S267K on both first and second monomers, and optionally the 428L/434S variants on both first and second monomers.

[00272] In one embodiment, the "scIL-15/Rα X scFv" format heterodimeric protein includes: a) a first monomer that includes, from N- to C-terminus, an IL-15Rα(sushi) domain-(domain linker)-IL-15 variant-(domain linker)-CH2-CH3, where CH2-CH3 is a first

variant Fc domain; and b) a second monomer that includes, from N- to C-terminus, anti-TIM-3 scFv-(hinge)-CH2-CH3, where CH2-CH3 is a second variant Fc domain; where the first variant Fc domain includes skew variants L368D/K370S and the second variant Fc domain includes skew variants S364K/E357Q, where the first and second variant Fc domains each include FcKO variants E233P/L234V/L235A/G236del/S267K, where the first variant Fc domain includes pI variants Q295E/N384D/Q418E/N421D, and where numbering is according to EU numbering. In some embodiments, the hinge of the first and second monomers also each include amino acid substitution C220S. In certain embodiments, the first and second variant Fc domains each further include half-life extension variants M428L/N434S. In an exemplary embodiment, the IL-15 variant includes amino acid substitutions N4D/N65D, D30N/N65D, or D30N/E64Q/N65D. In an exemplary embodiment, the TIM-3 scFv includes the VH and VL of any of the TIM-3 ABDs in Figures 12 and 13A-C and the IL-15 variant includes amino acid substitutions N4D/N65D. In another exemplary embodiment, the TIM-3 scFv includes the VH and VL of any of the TIM-3 ABDs in Figures 12 and 13A-C and the IL-15 variant includes amino acid substitutions D30N/N65D. In yet another exemplary embodiment, the TIM-3 scFv includes the VH and VL of any of the TIM-3 ABDs in Figures 12 and 13A-C and the IL-15 variant includes amino acid substitutions D30N/E64Q/N65D.

[00273] In the scIL-15/Rα X scFv format, one preferred embodiment utilizes an anti-TIM-3 ABD having the variable heavy and light domain pair 3H3[TIM-3]_H1_L2.1 as shown in Figure 12 with the Figures 21A format, the skew variant set S364K/E357Q : L368D/K370S, the pI variants Q295E/N384D/Q418E/N421D, the ablation variants E233P/L234V/L235A/G236_/S267K on both first and second monomers, and optionally the 428L/434S variants on both first and second monomers.

[00274] In one embodiment, the "scIL-15/Rα X scFv" format heterodimeric protein includes: a) a first monomer that includes, from N- to C-terminus, an IL-15Rα(sushi) domain-(domain linker)-IL-15 variant-(domain linker)-CH2-CH3, where CH2-CH3 is a first variant Fc domain; and b) a second monomer that includes, from N- to C-terminus, anti-TIM-3 scFv-(hinge)-CH2-CH3, where CH2-CH3 is a second variant Fc domain; where the anti-TIM-3 scFv includes the variable heavy domain and variable light domain of 3H3[TIM-

3]_H1_L2.1, where the first variant Fc domain includes skew variants L368D/K370S and the second variant Fc domain includes skew variants S364K/E357Q, where the first and second variant Fc domains each include FcKO variants E233P/L234V/L235A/G236del/S267K, where the first variant Fc domain includes pI variants Q295E/N384D/Q418E/N421D, and where numbering is according to EU numbering. In some embodiments, the hinge of the first and second monomers also each include amino acid substitution C220S. In certain embodiments, the first and second variant Fc domains each further include half-life extension variants M428L/N434S. In a particular embodiment, the IL-15 variant includes amino acid substitutions N4D/N65D and the scFv includes the variable heavy and light domain pair of 3H3[TIM-3]_H1_L2.1. In one embodiment, the IL-15 variant includes amino acid substitutions D30N/N65D and the scFv includes the variable heavy and light domain pair of 3H3[TIM-3]_H1_L2.1. In yet another embodiment, the IL-15 variant is the IL-15 D30N/E64Q/N65D variant and the scFv includes the variable heavy and light domain pair of 3H3[TIM-3]_H1_L2.1.

B. scFv X ncIL-15/Rα

[00275] This embodiment is shown in Figures 21B, and comprises three monomers. The first monomer comprises, from N- to C-terminus, the IL-15Rα(sushi) domain-domain linker-CH2-CH3, and the second monomer comprisesVH-scFv linker-VL-hinge-CH2-CH3 or VL-scFv linker-vh-hinge-CH2-CH3, although in either orientation a domain linker can be substituted for the hinge. The third monomer is the variant IL-15 domain. This is generally referred to as "ncIL-15/Rα X scFv" or "scFv X ncIL-15/Rα" with the "nc" standing for "non-covalent" referring to the self-assembing non-covalent attachment of the IL-15 variant and IL-15Rα(sushi) domain.

[00276] In one embodiment, the targeted IL-15/IL-15Rα heterodimeric protein is an "scFv X ncIL-15/Rα" format heterodimeric protein that includes: a) a first monomer that includes, from N- to C-terminus, an anti-TIM-3 scFv-(domain linker)-CH2-CH3, where CH2-CH3 is a first Fc domain; b) a second monomer that includes, from N- to C-terminus, an IL-15Rα(sushi) domain-(domain linker)-CH2-CH3, where CH2-CH3 is a second Fc domain; and c) an IL-15 variant, where the IL-15 variant and the IL-15Rα(sushi) domain form an IL-15 complex. Any useful domain linker can be used to attach the various components of the

heterodimeric protein including, but not limited to those in Figures 8 and 9A-C. In an exemplary embodiment, the domain linkers that attach the anti-TIM-3 scFv to the first Fc domain and the IL-15Rα(sushi) domain to the second Fc domain are each antibody hinge domains.

[00277] In some embodiments, the anti-TIM-3 scFv includes a variable heavy domain (VH) covalently attached to a variable light domain (VL) by an scFv linker (e.g., Figures 9A-C). In one embodiment, the anti-TIM-3 scFv is, from N- to C-terminus, VH-scFv linker-VL. In another embodiment, the anti-TIM-3 scFv is, from N- to C-terminus, VL-scFv linker-VH. The C-terminus of the anti-TIM-3 scFv is attached to the N terminus of the first Fc domain by a domain linker (e.g., an antibody hinge domain).

[00278] In the ncIL-15/Rα X scFv format, one preferred embodiment utilizes an anti-TIM-3 ABD having any of the variable heavy and light domain pairs as shown in Figure 12.

[00279] In the ncIL-15/Rα X scFv format, one preferred embodiment utilizes an anti-TIM-3 ABD having the variable heavy and light domain pair of 3H3[TIM-3]_H1_L2.1 as shown in Figure 12.

[00280] In one embodiment, the targeted IL-15/IL-15Rα heterodimeric protein is an "scFv X ncIL-15/Rα" format heterodimeric protein that includes: a) a first monomer that includes, from N- to C-terminus, an anti-TIM-3 scFv-(domain linker)-CH2-CH3, where CH2-CH3 is a first Fc domain; b) a second monomer that includes, from N- to C-terminus, an IL-15Rα(sushi) domain-(domain linker)-CH2-CH3, where CH2-CH3 is a second Fc domain; and c) an IL-15 variant, where the IL-15 variant and the IL-15Rα(sushi) domain form an IL-15 complex, and where the anti-TIM-3 scFv includes the variable heavy domain and variable light domain of 3H3[TIM-3]_H1_L2.1.

[00281] In the ncIL-15/Rα X scFv format, one preferred embodiment utilizes an IL-15 variant that includes amino acid substitutions N4D/N65D, D30N/N65D, or D30N/E64Q/N65D. In one embodiment, the targeted IL-15/IL-15Rα heterodimeric protein is an "scFv X ncIL-15/Rα" format heterodimeric protein that includes: a) a first monomer that includes, from N- to C-terminus, an anti-TIM-3 scFv-(domain linker)-CH2-CH3, where CH2-CH3 is a first Fc domain; b) a second monomer that includes, from N- to C-terminus, an IL-

15Rα(sushi) domain-(domain linker)-CH2-CH3, where CH2-CH3 is a second Fc domain; and c) an IL-15 variant, where the IL-15 variant and the IL-15Rα(sushi) domain form an IL-15 complex, and where the IL-15 variant includes amino acid substitutions N4D/N65D, D30N/N65D, or D30N/E64Q/N65D. In an exemplary embodiment, the TIM-3 scFv includes the VH and VL of any of the TIM-3 ABDs in Figures 12 and 13A-C and the IL-15 variant includes amino acid substitutions N4D/N65D. In another exemplary embodiment, the TIM-3 scFv includes the VH and VL of any of the TIM-3 ABDs in Figures 12 and 13A-C and the IL-15 variant includes amino acid substitutions D30N/N65D. In yet another exemplary embodiment, the TIM-3 scFv includes the VH and VL of any of the TIM-3 ABDs in Figures 12 and 13A-C and the IL-15 variant includes amino acid substitutions D30N/E64Q/N65D.

[00282] In the ncIL-15/Rα X scFv format, one preferred embodiment utilizes an anti-TIM-3 ABD having the variable heavy and light domain pair of 3H3[TIM-3]_H1_L2.1 as shown in Figure 12, with either the IL-15 N4D/N65D variant or the IL-15 D30N/N65D variant or the IL-15 D30N/E64Q/N65D variant. In one embodiment, the targeted IL-15/IL-15Rα heterodimeric protein is an "scFv X ncIL-15/Rα" format heterodimeric protein that includes: a) a first monomer that includes, from N- to C-terminus, an anti-TIM-3 scFv-(domain linker)-CH2-CH3, where CH2-CH3 is a first Fc domain; b) a second monomer that includes, from N- to C-terminus, an IL-15Rα(sushi) domain-(domain linker)-CH2-CH3, where CH2-CH3 is a second Fc domain; and c) an IL-15 variant, where the IL-15 variant and the IL-15Rα(sushi) domain form an IL-15 complex, where the anti-TIM-3 scFv includes the variable heavy domain and variable light domain of 3H3[TIM-3]_H1_L2.1, and where the IL-15 variant includes amino acid substitutions N4D/N65D, D30N/N65D, or D30N/E64Q/N65D. In a particular embodiment, the IL-15 variant includes amino acid substitutions N4D/N65D and the scFv includes the variable heavy and light domain pair of 3H3[TIM-3]_H1_L2.1. In one embodiment, the IL-15 variant includes amino acid substitutions D30N/N65D and the scFv includes the variable heavy and light domain pair of 3H3[TIM-3]_H1_L2.1. In yet another embodiment, the IL-15 variant includes amino acid substitutions D30N/E64Q/N65D and the scFv includes the variable heavy and light domain pair of 3H3[TIM-3]_H1_L2.1.

[00283] In the ncIL-15/Rα X scFv format, one preferred embodiment utilizes the skew variant pair S364K/E357Q : L368D/K370S. In one embodiment, the targeted IL-15/IL-15Rα heterodimeric protein is an "scFv X ncIL-15/Rα" format heterodimeric protein that includes: a) a first monomer that includes, from N- to C-terminus, an anti-TIM-3 scFv-(domain linker)-CH2-CH3, where CH2-CH3 is a first variant Fc domain; b) a second monomer that includes, from N- to C-terminus, an IL-15Rα(sushi) domain-(domain linker)-CH2-CH3, where CH2-CH3 is a second variant Fc domain; and c) an IL-15 variant, where the IL-15 variant and the IL-15Rα(sushi) domain form an IL-15 complex, and where the first and second variant Fc domains include the skew variant pair S364K/E357Q : L368D/K370S. In an exemplary embodiment, the first variant Fc domain includes skew variants L368D/K370S, and the second variant Fc domain includes skew variants S364K/E357Q.

[00284] In one embodiment, the targeted IL-15/IL-15Rα heterodimeric protein is an "scFv X ncIL-15/Rα" format heterodimeric protein that includes: a) a first monomer that includes, from N- to C-terminus, an anti-TIM-3 scFv-(domain linker)-CH2-CH3, where CH2-CH3 is a first variant Fc domain; b) a second monomer that includes, from N- to C-terminus, an IL-15Rα(sushi) domain-(domain linker)-CH2-CH3, where CH2-CH3 is a second variant Fc domain; and c) an IL-15 variant, where the IL-15 variant and the IL-15Rα(sushi) domain form an IL-15 complex, where the IL-15 variant includes amino acid substitutions N4D/N65D, D30N/N65D, or D30N/E64Q/N65D, and where the first and second variant Fc domains include the skew variant pair S364K/E357Q : L368D/K370S. In an exemplary embodiment, the first variant Fc domain includes skew variants L368D/K370S, and the second variant Fc domain includes skew variants S364K/E357Q. In an exemplary embodiment, the TIM-3 scFv includes the VH and VL of any of the TIM-3 ABDs in Figures 12 and 13A-C and the IL-15 variant includes amino acid substitutions N4D/N65D. In another exemplary embodiment, the TIM-3 scFv includes the VH and VL of any of the TIM-3 ABDs in Figures 12 and 13A-C and the IL-15 variant includes amino acid substitutions D30N/N65D. In yet another exemplary embodiment, the TIM-3 scFv includes the VH and VL of any of the TIM-3 ABDs in Figures 12 and 13A-C and the IL-15 variant includes amino acid substitutions D30N/E64Q/N65D.

[00285] In the ncIL-15/Rα X scFv format, one preferred embodiment utilizes an anti-TIM-3 ABD having the variable heavy and light domain pair of 3H3[TIM-3]_H1_L2.1 as shown in Figure 12 and the skew variant pair S364K/E357Q : L368D/K370S. In one embodiment, the targeted IL-15/IL-15Rα heterodimeric protein is an "scFv X ncIL-15/Rα" format heterodimeric protein that includes: a) a first monomer that includes, from N- to C-terminus, an anti-TIM-3 scFv-(domain linker)-CH2-CH3, where CH2-CH3 is a first variant Fc domain; b) a second monomer that includes, from N- to C-terminus, an IL-15Rα(sushi) domain-(domain linker)-CH2-CH3, where CH2-CH3 is a second variant Fc domain; and c) an IL-15 variant, where the IL-15 variant and the IL-15Rα(sushi) domain form an IL-15 complex, where the anti-TIM-3 scFv includes the variable heavy domain and variable light domain of 3H3[TIM-3]_H1_L2.1, and where the first and second variant Fc domains include the skew variant pair S364K/E357Q : L368D/K370S.

[00286] In the ncIL-15/Rα X scFv format, one preferred embodiment utilizes an anti-TIM-3 ABD having the variable heavy and light domain pair of 3H3[TIM-3]_H1_L2.1 as shown in Figure 12 and the skew variant pair S364K/E357Q : L368D/K370S with either the IL-15 N4D/N65D variant or the IL-15 D30N/N65D variant or the IL-15 D30N/E64Q/N65D variant. In an exemplary embodiment, the first variant Fc domain includes skew variants L368D/K370S, and the second variant Fc domain includes skew variants L368D/K370S. In one embodiment, the targeted IL-15/IL-15Rα heterodimeric protein is an "scFv X ncIL-15/Rα" format heterodimeric protein that includes: a) a first monomer that includes, from N- to C-terminus, an anti-TIM-3 scFv-(domain linker)-CH2-CH3, where CH2-CH3 is a first variant Fc domain; b) a second monomer that includes, from N- to C-terminus, an IL-15Rα(sushi) domain-(domain linker)-CH2-CH3, where CH2-CH3 is a second variant Fc domain; and c) an IL-15 variant, where the IL-15 variant and the IL-15Rα(sushi) domain form an IL-15 complex, where the anti-TIM-3 scFv includes the variable heavy domain and variable light domain of 3H3[TIM-3]_H1_L2.1, where the IL-15 variant includes amino acid substitutions N4D/N65D, D30N/N65D, or D30N/E64Q/N65D, and where the first and second variant Fc domains include the skew variant pair S364K/E357Q : L368D/K370S. In a particular embodiment, the IL-15 variant includes amino acid substitutions N4D/N65D and the scFv includes the variable heavy and light domain pair of 3H3[TIM-3]_H1_L2.1. In one

embodiment, the IL-15 variant includes amino acid substitutions D30N/N65D and the scFv includes the variable heavy and light domain pair of 3H3[TIM-3]_H1_L2.1. In yet another embodiment, the IL-15 variant includes amino acid substitutions D30N/E64Q/N65D and the scFv includes the variable heavy and light domain pair of 3H3[TIM-3]_H1_L2.1.

[00287] In the ncIL-15/Rα X scFv format, one preferred embodiment utilizes the skew variant set S364K/E357Q : L368D/K370S, the pI variants Q295E/N384D/Q418E/N421D, the ablation variants E233P/L234V/L235A/G236_/S267K on both first and second monomers, and optionally the 428L/434S variants on both first and second monomers.

[00288] In one embodiment, the targeted IL-15/IL-15Rα heterodimeric protein is an "scFv X ncIL-15/Rα" format heterodimeric protein that includes: a) a first monomer that includes, from N- to C-terminus, an anti-TIM-3 scFv-(domain linker)-CH2-CH3, where CH2-CH3 is a first variant Fc domain; b) a second monomer that includes, from N- to C-terminus, an IL-15Rα(sushi) domain-(domain linker)-CH2-CH3, where CH2-CH3 is a second variant Fc domain; and c) an IL-15 variant, where the IL-15 variant and the IL-15Rα(sushi) domain form an IL-15 complex, where the first variant Fc domain includes skew variants L368D/K370S and the second variant Fc domain includes skew variants S364K/E357Q, where the first and second variant Fc domains each include FcKO variants E233P/L234V/L235A/G236del/S267K, where the first variant Fc domain includes pI variants Q295E/N384D/Q418E/N421D, and where numbering is according to EU numbering. In some embodiments, the hinge of the first and second monomers also each include amino acid substitution C220S. In certain embodiments, the first and second variant Fc domains each further include half-life extension variants M428L/N434S. In an exemplary embodiment, the IL-15 variant includes amino acid substitutions N4D/N65D, D30N/N65D, or D30N/E64Q/N65D. In an exemplary embodiment, the TIM-3 scFv includes the VH and VL of any of the TIM-3 ABDs in Figures 12 and 13A-C and the IL-15 variant includes amino acid substitutions N4D/N65D. In another exemplary embodiment, the TIM-3 scFv includes the VH and VL of any of the TIM-3 ABDs in Figures 12 and 13A-C and the IL-15 variant includes amino acid substitutions D30N/N65D. In yet another exemplary embodiment, the TIM-3 scFv includes the VH and VL of any of the TIM-3 ABDs in Figures 12 and 13A-C and the IL-15 variant includes amino acid substitutions D30N/E64Q/N65D.

[00289] In the ncIL-15/Rα X scFv format, one preferred embodiment utilizes an anti-TIM-3 ABD the variable heavy and light domain pair of 3H3[TIM-3]_H1_L2.1 as shown in Figure 12 with the Figures 21B format, the skew variant set S364K/E357Q : L368D/K370S, the pI variants Q295E/N384D/Q418E/N421D, the ablation variants E233P/L234V/L235A/G236_/S267K on both first and second monomers, and optionally the 428L/434S variants on both first and second monomers.

[00290] In one embodiment, the targeted IL-15/IL-15Rα heterodimeric protein is an "scFv X ncIL-15/Rα" format heterodimeric protein that includes: a) a first monomer that includes, from N- to C-terminus, an anti-TIM-3 scFv-(domain linker)-CH2-CH3, where CH2-CH3 is a first variant Fc domain; b) a second monomer that includes, from N- to C-terminus, an IL-15Rα(sushi) domain-(domain linker)-CH2-CH3, where CH2-CH3 is a second variant Fc domain; and c) an IL-15 variant, where the IL-15 variant and the IL-15Rα(sushi) domain form an IL-15 complex, where the anti-TIM-3 scFv includes the variable heavy domain and variable light domain of 3H3[TIM-3]_H1_L2.1, where the first variant Fc domain includes skew variants L368D/K370S and the second variant Fc domain includes skew variants S364K/E357Q, where the first and second variant Fc domains each include FcKO variants E233P/L234V/L235A/G236del/S267K, where the first variant Fc domain includes pI variants Q295E/N384D/Q418E/N421D, and where numbering is according to EU numbering. In some embodiments, the hinge of the first and second monomers also each include amino acid substitution C220S. In certain embodiments, the first and second variant Fc domains each further include half-life extension variants M428L/N434S. In a particular embodiment, the IL-15 variant includes amino acid substitutions N4D/N65D and the scFv includes the variable heavy and light domain pair of 3H3[TIM-3]_H1_L2.1. In one embodiment, the IL-15 variant includes amino acid substitutions D30N/N65D and the scFv includes the variable heavy and light domain pair of 3H3[TIM-3]_H1_L2.1. In another embodiment, the IL-15 variant includes amino acid substitutions D30N/E64Q/N65D and the scFv includes the variable heavy and light domain pair of 3H3[TIM-3]_H1_L2.1.

C. scFv X dsIL-15/Rα

[00291] This embodiment is shown in Figures 21C, and comprises three monomers. The first monomer comprises, from N- to C-terminus, the IL-15Rα(sushi) domain-domain

linker-CH2-CH3, wherein the IL-15Rα(sushi) domain has an engineered cysteine residue and the second monomer comprises VH-scFv linker-VL-hinge-CH2-CH3 or VL-scFv linker-vh-hinge-CH2-CH3, although in either orientation a domain linker can be substituted for the hinge. The third monomer is the variant IL-15 domain, also engineered to have a cysteine variant amino acid, thus allowing a disulfide bridge to form between the IL-15Rα(sushi) domain and the variant IL-15 domain. This is generally referred to as "scFv X dsIL-15/Rα" or "dsIL-15/Rα X scFv", with the "ds" standing for "disulfide".

[00292] In one embodiment, the targeted IL-15/IL-15Rα heterodimeric protein is an "scFv X dsIL-15/Rα" format heterodimeric protein that includes: a) a first monomer that includes, from N- to C-terminus, an anti-TIM-3 scFv-(domain linker)-CH2-CH3, where CH2-CH3 is a first Fc domain; b) a second monomer that includes, from N- to C-terminus, an IL-15Rα(sushi) domain-(domain linker)-CH2-CH3, where CH2-CH3 is a second Fc domain and the IL-15Rα(sushi) domain includes an amino acid substitution for a cysteine residue; and c) an IL-15 variant that includes an amino acid substitution for a cysteine residue, and where the cysteine residue on the IL-15 variant and the cysteine residue on the IL-15Rα(sushi) domain form a disulfide bond. Any useful domain linker can be used to attach the various components of the heterodimeric protein including, but not limited to those in Figures 8 and 9A-C. In an exemplary embodiment, the domain linkers that attach the anti-TIM-3 scFv to the first Fc domain and the IL-15Rα(sushi) domain to the second Fc domain and are each antibody hinge domains.

[00293] Any useful domain linker can be used to attach the various components of the heterodimeric protein including, but not limited to those in Figures 8 and 9A-C. In an exemplary embodiment, the domain linkers that attach the anti-TIM-3 scFv to the first Fc domain and the IL-15Rα(sushi) domain to the second Fc domain and are each antibody hinge domains (e.g., an antibody hinge domain).

[00294] In some embodiments, the anti-TIM-3 scFv includes a variable heavy domain (VH) covalently attached to a variable light domain (VL) by an scFv linker (e.g., Figures 9A-C). In one embodiment, the anti-TIM-3 scFv is from N- to C-terminus VH-scFv linker-VL. In another embodiment, the anti-TIM-3 scFv is from N- to C-terminus VL-scFv linker-VH.

The C-terminus of the anti-TIM-3 scFv is attached to the N terminus of the first Fc domain by a domain linker (e.g., an antibody hinge domain).

[00295] In the dsIL-15/Rα X scFv format, one preferred embodiment utilizes an anti-TIM-3 ABD having any of the variable heavy and light domain pairs as shown in Figure 12 and 13A-C.

[00296] In the dsIL-15/Rα X scFv format, one preferred embodiment utilizes an anti-TIM-3 ABD having the variable heavy and light domain pair of 3H3[TIM-3]_H1_L2.1 as shown in Figure 12.

[00297] In one embodiment, the targeted IL-15/IL-15Rα heterodimeric protein is an "scFv X dsIL-15/Rα" format heterodimeric protein that includes: a) a first monomer that includes, from N- to C-terminus, an anti-TIM-3 scFv-(domain linker)-CH2-CH3, where CH2-CH3 is a first Fc domain; b) a second monomer that includes, from N- to C-terminus, an IL-15Rα(sushi) domain-(domain linker)-CH2-CH3, where CH2-CH3 is a second Fc domain and the IL-15Rα(sushi) domain includes an amino acid substitution for a cysteine residue; and c) an IL-15 variant that includes an amino acid substitution for a cysteine residue, where the cysteine residue on the IL-15 variant and the cysteine residue on the IL-15Rα(sushi) domain form a disulfide bond, and where the anti-TIM-3 scFv includes the variable heavy domain and variable light domain of 3H3[TIM-3]_H1_L2.1.

[00298] In the dsIL-15/Rα X scFv format, one preferred embodiment utilizes the IL-15 N4D/N65D variant or the IL-15 D30N/N65D variant or the IL-15 D30N/E64Q/N65D variant, as well as appropriate cysteine substitutions. In on embodiment, the targeted IL-15/IL-15Rα heterodimeric protein is an "scFv X dsIL-15/Rα" format heterodimeric protein that includes: a) a first monomer that includes, from N- to C-terminus, an anti-TIM-3 scFv-(domain linker)-CH2-CH3, where CH2-CH3 is a first Fc domain; b) a second monomer that includes, from N- to C-terminus, an IL-15Rα(sushi) domain-(domain linker)-CH2-CH3, where CH2-CH3 is a second Fc domain and the IL-15Rα(sushi) domain includes an amino acid substitution for a cysteine residue; and c) an IL-15 variant that includes an amino acid substitution for a cysteine residue, where the cysteine residue on the IL-15 variant and the cysteine residue on the IL-15Rα(sushi) domain form a disulfide bond, and where the IL-15 variant includes

amino acid substitutions N4D/N65D, D30N/N65D, or D30N/E64Q/N65D. In an exemplary embodiment, the TIM-3 scFv includes the VH and VL of any of the TIM-3 ABDs in Figures 12 and 13A-C and the IL-15 variant includes amino acid substitutions N4D/N65D. In another exemplary embodiment, the TIM-3 scFv includes the VH and VL of any of the TIM-3 ABDs in Figures 12 and 13A-C and the IL-15 variant includes amino acid substitutions D30N/N65D. In yet another exemplary embodiment, the TIM-3 scFv includes the VH and VL of any of the TIM-3 ABDs in Figures 12 and 13A-C and the IL-15 variant includes amino acid substitutions D30N/E64Q/N65D.

[00299] In the dsIL-15/Rα X scFv format, one preferred embodiment utilizes an anti-TIM-3 ABD having the variable heavy and light domain pair of 3H3[TIM-3]_H1_L2.1 as shown in Figure 12, with either the IL-15 N4D/N65D variant or the IL-15 D30N/N65D variant or the IL-15 D30N/E64Q/N65D variant, as well as appropriate cysteine substitutions. In one embodiment, the targeted IL-15/IL-15Rα heterodimeric protein is an "scFv X dsIL-15/Rα" format heterodimeric protein that includes: a) a first monomer that includes, from N- to C-terminus, an anti-TIM-3 scFv-(domain linker)-CH2-CH3, where CH2-CH3 is a first Fc domain; b) a second monomer that includes, from N- to C-terminus, an IL-15Rα(sushi) domain-(domain linker)-CH2-CH3, where CH2-CH3 is a second Fc domain and the IL-15Rα(sushi) domain includes an amino acid substitution for a cysteine residue; and c) an IL-15 variant that includes an amino acid substitution for a cysteine residue, where the cysteine residue on the IL-15 variant and the cysteine residue on the IL-15Rα(sushi) domain form a disulfide bond, where the anti-TIM-3 scFv includes the variable heavy domain and variable light domain of 3H3[TIM-3]_H1_L2.1, and where the IL-15 variant includes amino acid substitutions N4D/N65D, D30N/N65D, or D30N/E64Q/N65D. In a particular embodiment, the IL-15 variant includes amino acid substitutions N4D/N65D and the scFv includes the variable heavy and light domain pair of 3H3[TIM-3]_H1_L2.1. In one embodiment, the IL-15 variant includes amino acid substitutions D30N/N65D and the scFv includes the variable heavy and light domain pair of 3H3[TIM-3]_H1_L2.1. In another embodiment, the IL-15 variant includes amino acid substitutions D30N/E64Q/N65D and the scFv includes the variable heavy and light domain pair of 3H3[TIM-3]_H1_L2.1.

[00300] In the dsIL-15/Rα X scFv format, one preferred embodiment utilizes the skew variant pair S364K/E357Q : L368D/K370S. In one embodiment, the targeted IL-15/IL-15Rα heterodimeric protein is an "scFv X dsIL-15/Rα" format heterodimeric protein that includes: a) a first monomer that includes, from N- to C-terminus, an anti-TIM-3 scFv-(domain linker)-CH2-CH3, where CH2-CH3 is a first variant Fc domain; b) a second monomer that includes, from N- to C-terminus, an IL-15Rα(sushi) domain-(domain linker)-CH2-CH3, where CH2-CH3 is a second variant Fc domain and the IL-15Rα(sushi) domain includes an amino acid substitution for a cysteine residue; and c) an IL-15 variant that includes an amino acid substitution for a cysteine residue, where the cysteine residue on the IL-15 variant and the cysteine residue on the IL-15Rα(sushi) domain form a disulfide bond, and where the first and second variant Fc domains include the skew variant pair S364K/E357Q : L368D/K370S. In an exemplary embodiment, the first variant Fc domain includes skew variants L368D/K370S, and the second variant Fc domain includes skew variants S364K/E357Q.

[00301] In one embodiment, the targeted IL-15/IL-15Rα heterodimeric protein is an "scFv X dsIL-15/Rα" format heterodimeric protein that includes: a) a first monomer that includes, from N- to C-terminus, an anti-TIM-3 scFv-(domain linker)-CH2-CH3, where CH2-CH3 is a first variant Fc domain; b) a second monomer that includes, from N- to C-terminus, an IL-15Rα(sushi) domain-(domain linker)-CH2-CH3, where CH2-CH3 is a second variant Fc domain and the IL-15Rα(sushi) domain includes an amino acid substitution for a cysteine residue; and c) an IL-15 variant that includes an amino acid substitution for a cysteine residue, where the cysteine residue on the IL-15 variant and the cysteine residue on the IL-15Rα(sushi) domain form a disulfide bond, where the IL-15 variant includes amino acid substitutions N4D/N65D, D30N/N65D, or D30N/E64Q/N65D, and where the first and second variant Fc domains include the skew variant pair S364K/E357Q : L368D/K370S. In an exemplary embodiment, the first variant Fc domain includes skew variants L368D/K370S, and the second variant Fc domain includes skew variants S364K/E357Q. In an exemplary embodiment, the TIM-3 scFv includes the VH and VL of any of the TIM-3 ABDs in Figures 12 and 13A-C and the IL-15 variant includes amino acid substitutions N4D/N65D. In another exemplary embodiment, the TIM-3 scFv includes the VH and VL of any of the TIM-3 ABDs in Figures 12 and 13A-C and the IL-15 variant includes amino acid substitutions

D30N/N65D. In yet another exemplary embodiment, the TIM-3 scFv includes the VH and VL of any of the TIM-3 ABDs in Figures 12 and 13A-C and the IL-15 variant includes amino acid substitutions D30N/E64Q/N65D.

[00302] In the dsIL-15/Rα X scFv format, one preferred embodiment utilizes an anti-TIM-3 ABD having the variable heavy and light domain pair of 3H3[TIM-3]_H1_L2.1 as shown in Figure 12 and the skew variant pair S364K/E357Q : L368D/K370S. In one embodiment, the targeted IL-15/IL-15Rα heterodimeric protein is an "scFv X dsIL-15/Rα" format heterodimeric protein that includes: a) a first monomer that includes, from N- to C-terminus, an anti-TIM-3 scFv-(domain linker)-CH2-CH3, where CH2-CH3 is a first variant Fc domain; b) a second monomer that includes, from N- to C-terminus, an IL-15Rα(sushi) domain-(domain linker)-CH2-CH3, where CH2-CH3 is a second variant Fc domain and the IL-15Rα(sushi) domain includes an amino acid substitution for a cysteine residue; and c) an IL-15 variant that includes an amino acid substitution for a cysteine residue, where the cysteine residue on the IL-15 variant and the cysteine residue on the IL-15Rα(sushi) domain form a disulfide bond, where the anti-TIM-3 scFv includes the variable heavy domain and variable light domain of 3H3[TIM-3]_H1_L2.1, and where the first and second variant Fc domains include the skew variant pair S364K/E357Q : L368D/K370S.

[00303] In the dsIL-15/Rα X scFv format, one preferred embodiment utilizes an anti-TIM-3 ABD having the variable heavy and light domain pair of 3H3[TIM-3]_H1_L2.1 as shown in Figure 12 and the skew variant pair S364K/E357Q : L368D/K370S with either the IL-15 N4D/N65D variant or the IL-15 D30N/N65D variant or the IL-15 D30N/E64Q/N65D variant with the appropriate cysteine substitutions.

[00304] In one embodiment, the targeted IL-15/IL-15Rα heterodimeric protein is an "scFv X dsIL-15/Rα" format heterodimeric protein that includes: a) a first monomer that includes, from N- to C-terminus, an anti-TIM-3 scFv-(domain linker)-CH2-CH3, where CH2-CH3 is a first variant Fc domain; b) a second monomer that includes, from N- to C-terminus, an IL-15Rα(sushi) domain-(domain linker)-CH2-CH3, where CH2-CH3 is a second variant Fc domain and the IL-15Rα(sushi) domain includes an amino acid substitution for a cysteine residue; and c) an IL-15 variant that includes an amino acid substitution for a cysteine residue, where the cysteine residue on the IL-15 variant and the cysteine residue on the IL-

15Rα(sushi) domain form a disulfide bond, where the anti-TIM-3 scFv includes the variable heavy domain and variable light domain of 3H3[TIM-3]_H1_L2.1, where the IL-15 variant includes amino acid substitutions N4D/N65D, D30N/N65D, or D30N/E64Q/N65D, and where the first and second variant Fc domains include the skew variant pair S364K/E357Q : L368D/K370S. In an exemplary embodiment, the first variant Fc domain includes skew variants L368D/K370S, and the second variant Fc domain includes skew variants S364K/E357Q. In a particular embodiment, the IL-15 variant includes amino acid substitutions N4D/N65D and the scFv includes the variable heavy and light domain pair of 3H3[TIM-3]_H1_L2.1. In one embodiment, the IL-15 variant includes amino acid substitutions D30N/N65D and the scFv includes the variable heavy and light domain pair of 3H3[TIM-3]_H1_L2.1. In another embodiment, the IL-15 variant includes amino acid substitutions D30N/E64Q/N65D and the scFv includes the variable heavy and light domain pair of 3H3[TIM-3]_H1_L2.1.

[00305] In the dsIL-15/Rα X scFv format, one preferred embodiment utilizes the skew variant set S364K/E357Q : L368D/K370S, the pI variants Q295E/N384D/Q418E/N421D, the ablation variants E233P/L234V/L235A/G236_/S267K on both first and second monomers, and optionally the 428L/434S variants on both first and second monomers.

[00306] In one embodiment, the targeted IL-15/IL-15Rα heterodimeric protein is an "scFv X dsIL-15/Rα" format heterodimeric protein that includes: a) a first monomer that includes, from N- to C-terminus, an anti-TIM-3 scFv-(hinge)-CH2-CH3, where CH2-CH3 is a first variant Fc domain; b) a second monomer that includes, from N- to C-terminus, an IL-15Rα(sushi) domain-(domain linker)-CH2-CH3, where CH2-CH3 is a second variant Fc domain and the IL-15Rα(sushi) domain includes an amino acid substitution for a cysteine residue; and c) an IL-15 variant that includes an amino acid substitution for a cysteine residue, where the cysteine residue on the IL-15 variant and the cysteine residue on the IL-15Rα(sushi) domain form a disulfide bond, where the first variant Fc domain includes skew variants L368D/K370S and the second variant Fc domain includes skew variants S364K/E357Q, where the first and second variant Fc domains each include FcKO variants E233P/L234V/L235A/G236del/S267K, where the first variant Fc domain includes pI variants Q295E/N384D/Q418E/N421D, and where numbering is according to EU numbering. In

some embodiments, the hinge of the first monomer and second monomer also each include amino acid substitution C220S. In certain embodiments, the first and second variant Fc domains each further include half-life extension variants M428L/N434S. In an exemplary embodiment, the IL-15 variant includes amino acid substitutions N4D/N65D, D30N/N65D, or D30N/E64Q/N65D. In an exemplary embodiment, the TIM-3 scFv includes the VH and VL of any of the TIM-3 ABDs in Figures 12 and 13A-C and the IL-15 variant includes amino acid substitutions N4D/N65D. In another exemplary embodiment, the TIM-3 scFv includes the VH and VL of any of the TIM-3 ABDs in Figures 12 and 13A-C and the IL-15 variant includes amino acid substitutions D30N/N65D. In yet another exemplary embodiment, the TIM-3 scFv includes the VH and VL of any of the TIM-3 ABDs in Figures 12 and 13A-C and the IL-15 variant includes amino acid substitutions D30N/E64Q/N65D.

[00307] In the dsIL-15/Rα X scFv format, one preferred embodiment utilizes an anti-TIM-3 ABD the variable heavy and light domain pair of 3H3[TIM-3]_H1_L2.1 as shown in Figure 12 with the Figures 21C format, the skew variant set S364K/E357Q : L368D/K370S, the pI variants Q295E/N384D/Q418E/N421D, the ablation variants E233P/L234V/L235A/G236_/S267K on both first and second monomers, and optionally the 428L/434S variants on both first and second monomers.

[00308] In one embodiment, the targeted IL-15/IL-15Rα heterodimeric protein is an "scFv X dsIL-15/Rα" format heterodimeric protein that includes: a) a first monomer that includes, from N- to C-terminus, an anti-TIM-3 scFv-(hinge)-CH2-CH3, where CH2-CH3 is a first variant Fc domain; b) a second monomer that includes, from N- to C-terminus, an IL-15Rα(sushi) domain-(domain linker)-CH2-CH3, where CH2-CH3 is a second variant Fc domain and the IL-15Rα(sushi) domain includes an amino acid substitution for a cysteine residue; and c) an IL-15 variant that includes an amino acid substitution for a cysteine residue, where the cysteine residue on the IL-15 variant and the cysteine residue on the IL-15Rα(sushi) domain form a disulfide bond, where the anti-TIM-3 scFv includes the variable heavy domain and variable light domain of 3H3[TIM-3]_H1_L2.1, where the first variant Fc domain includes skew variants L368D/K370S and the second variant Fc domain includes skew variants S364K/E357Q, where the first and second variant Fc domains each include FcKO variants E233P/L234V/L235A/G236del/S267K, where the first variant Fc domain

includes pI variants Q295E/N384D/Q418E/N421D, and where numbering is according to EU numbering. In some embodiments, the hinge of the first monomer and second monomer also each include amino acid substitution C220S. In certain embodiments, the first and second variant Fc domains each further include half-life extension variants M428L/N434S. In a particular embodiment, the IL-15 variant includes amino acid substitutions N4D/N65D and the scFv includes the variable heavy and light domain pair of 3H3[TIM-3]_H1_L2.1. In one embodiment, the IL-15 variant includes amino acid substitutions D30N/N65D and the scFv includes the variable heavy and light domain pair of 3H3[TIM-3]_H1_L2.1. In yet another embodiment, the IL-15 variant includes amino acid substitutions D30N/E64Q/N65D and the scFv includes the variable heavy and light domain pair of 3H3[TIM-3]_H1_L2.1.

D. scIL-15/Rα X Fab

[00309] This embodiment is shown in Figures 21D, and comprises three monomers. The first monomer comprises, from N- to C-terminus, the IL-15Rα(sushi) domain-(domain linker)-variant IL-15-domain linker-CH2-CH3 and the second monomer comprises a heavy chain, VH-CH1-hinge-CH2-CH3. The third monomer is a light chain, VL-CL. This is generally referred to as "scIL-15/Rα X Fab", with the "sc" standing for "single chain". The scIL-15/Rα x Fab format (see Figures 21D) comprises IL-15Rα(sushi) fused to a variant IL-15 by a variable length linker (termed "scIL-15/Rα") which is then fused to the N-terminus of a heterodimeric Fc-region (inclusive of the hinge). The second monomer is a heavy chain, VH-CH1-hinge-CH2-CH3, while a corresponding light chain (the third monomer) is transfected separately so as to form a Fab with the VH.

[00310] In one embodiment, the targeted IL-15/IL-15Rα heterodimeric protein is an "scIL-15/Rα X Fab" format heterodimeric protein that includes: a) a first monomer that includes, from N- to C-terminus, an IL-15Rα(sushi) domain-(domain linker)-IL-15 variant-(domain linker)-CH2-CH3, where CH2-CH3 is a first Fc domain; b) a second monomer that includes, from N- to C-terminus, a VH-CH1-hinge-CH2-CH3, where VH is a variable heavy domain and CH2-CH3 is a second Fc domain, and c) a light chain that includes from, N- to C-terminus, VL-VC, where VL is a variable light domain, where VH and VL form a TIM-3 binding domain. Any useful domain linker can be used to attach the various components of the heterodimeric protein including, but not limited to those in Figures 8 and 9A-C. In an

exemplary embodiment, the domain linkers that attach the IL-15 variant to the first Fc domain is an antibody hinge domain (e.g., an antibody hinge domain).

[00311] In the scIL-15/Rα X Fab format, one preferred embodiment utilizes an anti-TIM-3 ABD having any of the variable heavy and light domain pairs as shown in Figure 12 and 13A-C.

[00312] In the scIL-15/Rα X Fab format, one preferred embodiment utilizes an anti-TIM-3 ABD having the variable heavy and light domain pair of 3H3[TIM-3]_H1_L2.1 as shown in Figure 12.

[00313] In one embodiment, 2A11_H1.144_L2.142 the targeted IL-15/IL-15Rα heterodimeric protein is an "scIL-15/Rα X Fab" format heterodimeric protein that includes: a) a first monomer that includes, from N- to C-terminus, an IL-15Rα(sushi) domain-(domain linker)-IL-15 variant-(domain linker)-CH2-CH3, where CH2-CH3 is a first Fc domain; b) a second monomer that includes, from N- to C-terminus, a VH-CH1-hinge-CH2-CH3, where VH is a variable heavy domain and CH2-CH3 is a second Fc domain, and c) a light chain that includes from, N- to C-terminus, VL-VC, where VL is a variable light domain, and where VH and VL are the variable heavy domain and variable light domain of 3H3[TIM-3]_H1_L2.1, respectively.

[00314] In the scIL-15/Rα X Fab format, one preferred embodiment utilizes an IL-15 variant that includes amino acid substitutions N4D/N65D, D30N/N65D, or D30N/E64Q/N65D. In one embodiment, the targeted IL-15/IL-15Rα heterodimeric protein is an "scIL-15/Rα X Fab" format heterodimeric protein that includes: a) a first monomer that includes, from N- to C-terminus, an IL-15Rα(sushi) domain-(domain linker)-IL-15 variant-(domain linker)-CH2-CH3, where CH2-CH3 is a first Fc domain; b) a second monomer that includes, from N- to C-terminus, a VH-CH1-hinge-CH2-CH3, where VH is a variable heavy domain and CH2-CH3 is a second Fc domain, and c) a light chain that includes from, N- to C-terminus, VL-VC, where VL is a variable light domain, where VH and VL form a TIM-3 binding domain, and where the IL-15 variant includes amino acid substitutions N4D/N65D, D30N/N65D, or D30N/E64Q/N65D. In an exemplary embodiment, the VH and VL are the VH and VL of any of the TIM-3 ABDs in Figures 12 and 13A-C and the IL-15 variant

includes amino acid substitutions N4D/N65D. In another exemplary embodiment, the VH and VL are the VH and VL of any of the TIM-3 ABDs in Figures 12 and 13A-C and the IL-15 variant includes amino acid substitutions D30N/N65D. In yet another exemplary embodiment, the VH and VL are the VH and VL of any of the TIM-3 ABDs in Figures 12 and 13A-C and the IL-15 variant includes amino acid substitutions D30N/E64Q/N65D.

[00315] In the scIL-15/Rα X Fab format, one preferred embodiment utilizes an anti-TIM-3 ABD having the variable heavy and light domain pair of 3H3[TIM-3]_H1_L2.1 as shown in Figure 12, with either the IL-15 N4D/N65D variant or the IL-15 D30N/N65D variant or the IL-15 D30N/E64Q/N65D variant. In one embodiment, the targeted IL-15/IL-15Rα heterodimeric protein is an "scIL-15/Rα X Fab" format heterodimeric protein that includes: a) a first monomer that includes, from N- to C-terminus, an IL-15Rα(sushi) domain-(domain linker)-IL-15 variant-(domain linker)-CH2-CH3, where CH2-CH3 is a first Fc domain; b) a second monomer that includes, from N- to C-terminus, a VH-CH1-hinge-CH2-CH3, where VH is a variable heavy domain and CH2-CH3 is a second Fc domain, and c) a light chain that includes from, N- to C-terminus, VL-VC, where VL is a variable light domain, where VH and VL are the variable heavy domain and variable light domain of 3H3[TIM-3]_H1_L2.1, and where the IL-15 variant includes amino acid substitutions N4D/N65D, D30N/N65D, or D30N/E64Q/N65D. In a particular embodiment, the IL-15 variant includes amino acid substitutions N4D/N65D and VH and VL are the variable heavy domain and variable light domain of 3H3[TIM-3]_H1_L2.1. In one embodiment, the IL-15 variant includes amino acid substitutions D30N/N65D and VH and VL are the variable heavy domain and variable light domain of 3H3[TIM-3]_H1_L2.1. In yet another embodiment, the IL-15 variant includes amino acid substitutions D30N/E64Q/N65D and VH and VL are the variable heavy domain and variable light domain of 3H3[TIM-3]_H1_L2.1.

[00316] In the scIL-15/Rα X Fab format, one preferred embodiment utilizes the skew variant pair S364K/E357Q : L368D/K370S. In one embodiment, the targeted IL-15/IL-15Rα heterodimeric protein is an "scIL-15/Rα X Fab" format heterodimeric protein that includes: a) a first monomer that includes, from N- to C-terminus, an IL-15Rα(sushi) domain-(domain linker)-IL-15 variant-(domain linker)-CH2-CH3, where CH2-CH3 is a first variant Fc domain; b) a second monomer that includes, from N- to C-terminus, a VH-CH1-hinge-CH2-

CH3, where VH is a variable heavy domain and CH2-CH3 is a second variant Fc domain, and c) a light chain that includes from, N- to C-terminus, VL-VC, where VL is a variable light domain, where VH and VL form a TIM-3 binding domain, and where the first and second variant Fc domains include the skew variant pair S364K/E357Q : L368D/K370S. In an exemplary embodiment, the first variant Fc domain includes skew variants L368D/K370S, and the second variant Fc domain includes skew variants S364K/E357Q. In an exemplary embodiment, the VH and VL are the VH and VL of any of the TIM-3 ABDs in Figures 12 and 13A-C and the IL-15 variant includes amino acid substitutions N4D/N65D. In another exemplary embodiment, the VH and VL are the VH and VL of any of the TIM-3 ABDs in Figures 12 and 13A-C and the IL-15 variant includes amino acid substitutions D30N/N65D. In yet another exemplary embodiment, the VH and VL are the VH and VL of any of the TIM-3 ABDs in Figures 12 and 13A-C and the IL-15 variant includes amino acid substitutions D30N/E64Q/N65D.

[00317] In one embodiment, the targeted IL-15/IL-15Rα heterodimeric protein is an "scIL-15/Rα X Fab" format heterodimeric protein that includes: a) a first monomer that includes, from N- to C-terminus, an IL-15Rα(sushi) domain-(domain linker)-IL-15 variant-(domain linker)-CH2-CH3, where CH2-CH3 is a first variant Fc domain; b) a second monomer that includes, from N- to C-terminus, a VH-CH1-hinge-CH2-CH3, where VH is a variable heavy domain and CH2-CH3 is a second variant Fc domain, and c) a light chain that includes from, N- to C-terminus, VL-VC, where VL is a variable light domain, where VH and VL form a TIM-3 binding domain, where the IL-15 variant includes amino acid substitutions N4D/N65D, D30N/N65D, or D30N/E64Q/N65D, and where the first and second variant Fc domains include the skew variant pair S364K/E357Q : L368D/K370S. In an exemplary embodiment, the first variant Fc domain includes skew variants L368D/K370S, and the second variant Fc domain includes skew variants S364K/E357Q.

[00318] In the scIL-15/Rα X Fab format, one preferred embodiment utilizes an anti-TIM-3 ABD having the variable heavy and light domain pair of 3H3[TIM-3]_H1_L2.1 as shown in Figure 12 and the skew variant pair S364K/E357Q : L368D/K370S. In one embodiment, the targeted IL-15/IL-15Rα heterodimeric protein is an "scIL-15/Rα X Fab" format heterodimeric protein that includes: a) a first monomer that includes, from N- to C-

terminus, an IL-15Rα(sushi) domain-(domain linker)-IL-15 variant-(domain linker)-CH2-CH3, where CH2-CH3 is a first variant Fc domain; b) a second monomer that includes, from N- to C-terminus, a VH-CH1-hinge-CH2-CH3, where VH is a variable heavy domain and CH2-CH3 is a second variant Fc domain, and c) a light chain that includes from, N- to C-terminus, VL-VC, where VL is a variable light domain, where VH and VL are the variable heavy domain and variable light domain of 3H3[TIM-3]_H1_L2.1, respectively, and where the first and second variant Fc domains include the skew variant pair S364K/E357Q : L368D/K370S.

[00319] In the scIL-15/Rα X Fab format, one preferred embodiment utilizes an anti-TIM-3 ABD having the variable heavy and light domain pair of 3H3[TIM-3]_H1_L2.1 as shown in Figure 12 and the skew variant pair S364K/E357Q : L368D/K370S with either the IL-15 N4D/N65D variant or the IL-15 D30N/N65D variant or the IL-15 D30N/E64Q/N65D variant. In one embodiment, the targeted IL-15/IL-15Rα heterodimeric protein is an "scIL-15/Rα X Fab" format heterodimeric protein that includes: a) a first monomer that includes, from N- to C-terminus, an IL-15Rα(sushi) domain-(domain linker)-IL-15 variant-(domain linker)-CH2-CH3, where CH2-CH3 is a first variant Fc domain; b) a second monomer that includes, from N- to C-terminus, a VH-CH1-hinge-CH2-CH3, where VH is a variable heavy domain and CH2-CH3 is a second variant Fc domain, and c) a light chain that includes from, N- to C-terminus, VL-VC, where VL is a variable light domain, where VH and VL are the variable heavy domain and variable light domain of 3H3[TIM-3]_H1_L2.1, where the IL-15 variant includes amino acid substitutions N4D/N65D, D30N/N65D, or D30N/E64Q/N65D, and where the first and second variant Fc domains include the skew variant pair S364K/E357Q : L368D/K370S. In an exemplary embodiment, the first variant Fc domain includes skew variants L368D/K370S, and the second variant Fc domain includes skew variants S364K/E357Q. In a particular embodiment, the IL-15 variant includes amino acid substitutions N4D/N65D and VH and VL are the variable heavy domain and variable light domain of 3H3[TIM-3]_H1_L2.1. In one embodiment, the IL-15 variant includes amino acid substitutions D30N/N65D and VH and VL are the variable heavy domain and variable light domain of 3H3[TIM-3]_H1_L2.1. In another embodiment, the IL-15 variant includes amino

acid substitutions D30N/E64Q/N65D and VH and VL are the variable heavy domain and variable light domain of 3H3[TIM-3]_H1_L2.1.

[00320] In the scIL-15/Rα X Fab format, one preferred embodiment utilizes the skew variant set S364K/E357Q : L368D/K370S, the pI variants Q295E/N384D/Q418E/N421D, the ablation variants E233P/L234V/L235A/G236_/S267K on both first and second monomers, and optionally the 428L/434S variants on both first and second monomers.

[00321] In one embodiment, the targeted IL-15/IL-15Rα heterodimeric protein is an "scIL-15/Rα X Fab" format heterodimeric protein that includes: a) a first monomer that includes, from N- to C-terminus, an IL-15Rα(sushi) domain-(domain linker)-IL-15 variant-(domain linker)-CH2-CH3, where CH2-CH3 is a first variant Fc domain; b) a second monomer that includes, from N- to C-terminus, a VH-CH1-hinge-CH2-CH3, where VH is a variable heavy domain and CH2-CH3 is a second variant Fc domain, and c) a light chain that includes from, N- to C-terminus, VL-VC, where VL is a variable light domain, where VH and VL form a TIM-3 binding domain, where the first variant Fc domain includes skew variants L368D/K370S and the second variant Fc domain includes skew variants S364K/E357Q, where the first and second variant Fc domains each include FcKO variants E233P/L234V/L235A/G236del/S267K, where the first variant Fc domain includes pI variants Q295E/N384D/Q418E/N421D, and where numbering is according to EU numbering. In some embodiments, the hinge of the first monomer also includes amino acid substitution C220S. In certain embodiments, the first and second variant Fc domains each further include half-life extension variants M428L/N434S. In an exemplary embodiment, the IL-15 variant includes amino acid substitutions N4D/N65D, D30N/N65D, or D30N/E64Q/N65D. In an exemplary embodiment, the VH and VL are the VH and VL of any of the TIM-3 ABDs in Figures 12 and 13A-C and the IL-15 variant includes amino acid substitutions N4D/N65D. In another exemplary embodiment, the VH and VL are the VH and VL of any of the TIM-3 ABDs in Figures 12 and 13A-C and the IL-15 variant includes amino acid substitutions D30N/N65D. In yet another exemplary embodiment, the VH and VL are the VH and VL of any of the TIM-3 ABDs in Figures 12 and 13A-C and the IL-15 variant includes amino acid substitutions D30N/E64Q/N65D.

[00322] In the scIL-15/Rα X Fab format, one preferred embodiment utilizes an anti-TIM-3 ABD the variable heavy and light domain pair of 3H3[TIM-3]_H1_L2.1 as shown in Figure 12 with the Figures 21D format, the skew variant set S364K/E357Q : L368D/K370S, the pI variants Q295E/N384D/Q418E/N421D, the ablation variants E233P/L234V/L235A/G236_/S267K on both first and second monomers, and optionally the 428L/434S variants on both first and second monomers.

[00323] In one embodiment, the targeted IL-15/IL-15Rα heterodimeric protein is an "scIL-15/Rα X Fab" format heterodimeric protein that includes: a) a first monomer that includes, from N- to C-terminus, an IL-15Rα(sushi) domain-(domain linker)-IL-15 variant-(domain linker)-CH2-CH3, where CH2-CH3 is a first variant Fc domain; b) a second monomer that includes, from N- to C-terminus, a VH-CH1-hinge-CH2-CH3, where VH is a variable heavy domain and CH2-CH3 is a second variant Fc domain, and c) a light chain that includes from, N- to C-terminus, VL-VC, where VL is a variable light domain, where VH and VL are the variable heavy domain and variable light domain of 3H3[TIM-3]_H1_L2.1, where the first variant Fc domain includes skew variants L368D/K370S and the second variant Fc domain includes skew variants S364K/E357Q, where the first and second variant Fc domains each include FcKO variants E233P/L234V/L235A/G236del/S267K, where the first variant Fc domain includes pI variants Q295E/N384D/Q418E/N421D, and where numbering is according to EU numbering. In some embodiments, the hinge of the first monomer also includes amino acid substitution C220S. In certain embodiments, the first and second variant Fc domains each further include half-life extension variants M428L/N434S. In a particular embodiment, the IL-15 variant includes amino acid substitutions N4D/N65D and the scFv includes the variable heavy and light domain pair of 3H3[TIM-3]_H1_L2.1. In one embodiment, the IL-15 variant includes amino acid substitutions D30N/N65D and the scFv includes the variable heavy and light domain pair of 3H3[TIM-3]_H1_L2.1. In yet another embodiment, the IL-15 variant includes amino acid substitutions D30N/E64Q/N65D and the scFv includes the variable heavy and light domain pair of 3H3[TIM-3]_H1_L2.1.

E. Fab X ncIL-15/Rα

[00324] This embodiment is shown in Figures 21E, and comprises four monomers. The first monomer comprises, from N- to C-terminus, the IL-15Rα(sushi)domain-(domain linker)-CH2-CH3, and the second monomer comprises a heavy chain, VH-CH1-hinge-CH2-CH3. The third monomer is the light chain that includes, from N-to C-terminus, a variable light domain (VL) and a light constant domain(CL). The fourth monomer is a variant IL-15 domain. This is generally referred to as "Fab X ncIL-15/Rα", with the "nc" standing for "non-covalent" referring to the self-assembling non-covalent attachment of the IL-15 variant and IL-15Rα(sushi)domain.

[00325] In one embodiment, the targeted IL-15/IL-15Rα heterodimeric protein is an "Fab X ncIL-15/Rα"format heterodimeric protein that includes: a) a first monomer that includes, from N- to C-terminus, a VH-CH1-hinge-CH2-CH3, where VH is a variable heavy domain and CH2-CH3 is a first Fc domain; b) a second monomer that includes, from N- to C-terminus, an IL-15Rα(sushi) domain-(domain linker)-CH2-CH3, where CH2-CH3 is a second Fc domain; c) a third monomer that includes from, N- to C-terminus, VL-VC, where VL is a variable light domain, and d) a fourth monomer comprising an IL-15 variant, where the VH and the VL form a TIM-3 binding domain, and where the IL-15 and IL-15Rα(sushi) domain form an IL-15 complex. Any useful domain linker can be used to attach the various components of the heterodimeric protein including, but not limited to those in Figures 8 and 9A-C. In an exemplary embodiment, the domain linkers that attach the IL-15Rα(sushi) domain to the second Fc domain is an antibody hinge domain (e.g., an antibody hinge domain).

[00326] In the Fab X ncIL-15/Rα format, one preferred embodiment utilizes an anti-TIM-3 ABD having any of the variable heavy and light domain pairs as shown in Figure 12.

[00327] In the Fab X ncIL-15/Rα format, one preferred embodiment utilizes an anti-TIM-3 ABD having the variable heavy and light domain pair of 3H3[TIM-3]_H1_L2.1 as shown in Figure 12.

[00328] In one embodiment, the targeted IL-15/IL-15Rα heterodimeric protein is a "Fab X ncIL-15/Rα"format heterodimeric protein that includes: a) a first monomer that includes, from N- to C-terminus, a VH-CH1-hinge-CH2-CH3, where VH is a variable heavy

domain and CH2-CH3 is a first Fc domain; b) a second monomer that includes, from N- to C-terminus, an IL-15Rα(sushi) domain-(domain linker)-CH2-CH3, where CH2-CH3 is a second Fc domain; c) a third monomer that includes from, N- to C-terminus, VL-VC, where VL is a variable light domain, and d) a fourth monomer comprising an IL-15 variant, where the VH and the VL are the variable heavy domain and variable light domain of 3H3[TIM-3]_H1_L2.1, respectively, and where the IL-15 and IL-15Rα(sushi) domain form an IL-15 complex.

[00329] In the Fab X ncIL-15/Rα format, one preferred embodiment utilizes an IL-15 variant that includes amino acid substitutions N4D/N65D, D30N/N65D, or D30N/E64Q/N65D. In one embodiment, the targeted IL-15/IL-15Rα heterodimeric protein is a "Fab X ncIL-15/Rα"format heterodimeric protein that includes: a) a first monomer that includes, from N- to C-terminus, a VH-CH1-hinge-CH2-CH3, where VH is a variable heavy domain and CH2-CH3 is a first Fc domain; b) a second monomer that includes, from N- to C-terminus, an IL-15Rα(sushi) domain-(domain linker)-CH2-CH3, where CH2-CH3 is a second Fc domain; c) a third monomer that includes from, N- to C-terminus, VL-VC, where VL is a variable light domain, and d) a fourth monomer comprising an IL-15 variant, where the VH and the VL form a TIM-3 binding domain, where the IL-15 and IL-15Rα(sushi) domain form an IL-15 complex, and where the IL-15 variant includes amino acid substitutions N4D/N65D, D30N/N65D, or D30N/E64Q/N65D. In an exemplary embodiment, the VH and VL are the VH and VL of any of the TIM-3 ABDs in Figures 12 and 13A-C and the IL-15 variant includes amino acid substitutions N4D/N65D. In another exemplary embodiment, the VH and VL are the VH and VL of any of the TIM-3 ABDs in Figures 12 and 13A-C and the IL-15 variant includes amino acid substitutions D30N/N65D. In yet another exemplary embodiment, the VH and VL are the VH and VL of any of the TIM-3 ABDs in Figures 12 and 13A-C and the IL-15 variant includes amino acid substitutions D30N/E64Q/N65D.

[00330] In the Fab X ncIL-15/Rα format, one preferred embodiment utilizes an anti-TIM-3 ABD having the variable heavy and light domain pair of 3H3[TIM-3]_H1_L2.1 as shown in Figure 12, with either the IL-15 N4D/N65D variant or the IL-15 D30N/N65D variant or the IL-15 D30N/E64Q/N65D variant. In one embodiment, the targeted IL-15/IL-

15Rα heterodimeric protein is a "Fab X ncIL-15/Rα"format heterodimeric protein that includes: a) a first monomer that includes, from N- to C-terminus, a VH-CH1-hinge-CH2-CH3, where VH is a variable heavy domain and CH2-CH3 is a first Fc domain; b) a second monomer that includes, from N- to C-terminus, an IL-15Rα(sushi) domain-(domain linker)-CH2-CH3, where CH2-CH3 is a second Fc domain; c) a third monomer that includes from, N- to C-terminus, VL-VC, where VL is a variable light domain, and d) a fourth monomer comprising an IL-15 variant, where the VH and the VL form a TIM-3 binding domain, where the IL-15 and IL-15Rα(sushi) domain form an IL-15 complex, where VH and VL are the variable heavy domain and variable light domain of 3H3[TIM-3]_H1_L2.1, and where the IL-15 variant includes amino acid substitutions N4D/N65D, D30N/N65D, or D30N/E64Q/N65D. In a particular embodiment, the IL-15 variant includes amino acid substitutions N4D/N65D and VH and VL are the variable heavy domain and variable light domain of 3H3[TIM-3]_H1_L2.1. In one embodiment, the IL-15 variant includes amino acid substitutions D30N/N65D and VH and VL are the variable heavy domain and variable light domain of 3H3[TIM-3]_H1_L2.1. In yet another embodiment, the IL-15 variant includes amino acid substitutions D30N/E64Q/N65D and VH and VL are the variable heavy domain and variable light domain of 3H3[TIM-3]_H1_L2.1.

[00331] In the Fab X ncIL-15/Rα format, one preferred embodiment utilizes the skew variant pair S364K/E357Q : L368D/K370S. In one embodiment, the targeted IL-15/IL-15Rα heterodimeric protein is an "Fab X ncIL-15/Rα"format heterodimeric protein that includes: a) a first monomer that includes, from N- to C-terminus, a VH-CH1-hinge-CH2-CH3, where VH is a variable heavy domain and CH2-CH3 is a first variant Fc domain; b) a second monomer that includes, from N- to C-terminus, an IL-15Rα(sushi) domain-(domain linker)-CH2-CH3, where CH2-CH3 is a second variant Fc domain; c) a third monomer that includes from, N- to C-terminus, VL-VC, where VL is a variable light domain, and d) a fourth monomer comprising an IL-15 variant, where the VH and the VL form a TIM-3 binding domain, where the IL-15 and IL-15Rα(sushi) domain form an IL-15 complex, and where the first and second variant Fc domains include the skew variant pair S364K/E357Q : L368D/K370S. In an exemplary embodiment, the first variant Fc domain includes skew

variants L368D/K370S, and the second variant Fc domain includes skew variants S364K/E357Q.

[00332] In one embodiment, the targeted IL-15/IL-15Rα heterodimeric protein is an "Fab X ncIL-15/Rα"format heterodimeric protein that includes: a) a first monomer that includes, from N- to C-terminus, a VH-CH1-hinge-CH2-CH3, where VH is a variable heavy domain and CH2-CH3 is a first variant Fc domain; b) a second monomer that includes, from N- to C-terminus, an IL-15Rα(sushi) domain-(domain linker)-CH2-CH3, where CH2-CH3 is a second variant Fc domain; c) a third monomer that includes from, N- to C-terminus, VL-VC, where VL is a variable light domain, and d) a fourth monomer comprising an IL-15 variant, where the VH and the VL form a TIM-3 binding domain, where the IL-15 and IL-15Rα(sushi) domain form an IL-15 complex, where the IL-15 variant includes amino acid substitutions N4D/N65D, D30N/N65D, or D30N/E64Q/N65D, and where the first and second variant Fc domains include the skew variant pair S364K/E357Q : L368D/K370S. In an exemplary embodiment, the first variant Fc domain includes skew variants L368D/K370S, and the second variant Fc domain includes skew variants S364K/E357Q. In an exemplary embodiment, the VH and VL are the VH and VL of any of the TIM-3 ABDs in Figures 12 and 13A-C and the IL-15 variant includes amino acid substitutions N4D/N65D. In another exemplary embodiment, the VH and VL are the VH and VL of any of the TIM-3 ABDs in Figures 12 and 13A-C and the IL-15 variant includes amino acid substitutions D30N/N65D. In yet another exemplary embodiment, the VH and VL are the VH and VL of any of the TIM-3 ABDs in Figures 12 and 13A-C and the IL-15 variant includes amino acid substitutions D30N/E64Q/N65D.

[00333] In the Fab X ncIL-15/Rα format, one preferred embodiment utilizes an anti-TIM-3 ABD having the variable heavy and light domain pair of 3H3[TIM-3]_H1_L2.1 as shown in Figure 12 and the skew variant pair S364K/E357Q : L368D/K370S. In one embodiment, the targeted IL-15/IL-15Rα heterodimeric protein is a "Fab X ncIL-15/Rα"format heterodimeric protein that includes: a) a first monomer that includes, from N- to C-terminus, a VH-CH1-hinge-CH2-CH3, where VH is a variable heavy domain and CH2-CH3 is a first variant Fc domain; b) a second monomer that includes, from N- to C-terminus, an IL-15Rα(sushi) domain-(domain linker)-CH2-CH3, where CH2-CH3 is a second variant

Fc domain; c) a third monomer that includes from, N- to C-terminus, VL-VC, where VL is a variable light domain, and d) a fourth monomer comprising an IL-15 variant, where the VH and the VL are the variable heavy domain and variable light domain of 3H3[TIM-3]_H1_L2.1, respectively, where the IL-15 and IL-15Rα(sushi) domain form an IL-15 complex and where the first and second variant Fc domains include the skew variant pair S364K/E357Q : L368D/K370S.

[00334] In the Fab X ncIL-15/Rα format, one preferred embodiment utilizes an anti-TIM-3 ABD having the variable heavy and light domain pair of 3H3[TIM-3]_H1_L2.1 as shown in Figure 12 and the skew variant pair S364K/E357Q : L368D/K370S with either the IL-15 N4D/N65D variant or the IL-15 D30N/N65D variant or the IL-15 D30N/E64Q/N65D variant.

[00335] In one embodiment, the targeted IL-15/IL-15Rα heterodimeric protein is a "Fab X ncIL-15/Rα"format heterodimeric protein that includes: a) a first monomer that includes, from N- to C-terminus, a VH-CH1-hinge-CH2-CH3, where VH is a variable heavy domain and CH2-CH3 is a first variant Fc domain; b) a second monomer that includes, from N- to C-terminus, an IL-15Rα(sushi) domain-(domain linker)-CH2-CH3, where CH2-CH3 is a second variant Fc domain; c) a third monomer that includes from, N- to C-terminus, VL-VC, where VL is a variable light domain, and d) a fourth monomer comprising an IL-15 variant, where the VH and the VL form a TIM-3 binding domain, where the IL-15 and IL-15Rα(sushi) domain form an IL-15 complex, where VH and VL are the variable heavy domain and variable light domain of 3H3[TIM-3]_H1_L2.1, where the IL-15 variant includes amino acid substitutions N4D/N65D, D30N/N65D, or D30N/E64Q/N65D, and where the first and second variant Fc domains include the skew variant pair S364K/E357Q : L368D/K370S. In an exemplary embodiment, the first variant Fc domain includes skew variants L368D/K370S, and the second variant Fc domain includes skew variants S364K/E357Q. In a particular embodiment, the IL-15 variant includes amino acid substitutions N4D/N65D and VH and VL are the variable heavy domain and variable light domain of 3H3[TIM-3]_H1_L2.1. In one embodiment, the IL-15 variant includes amino acid substitutions D30N/N65D and VH and VL are the variable heavy domain and variable light domain of 3H3[TIM-3]_H1_L2.1. In another embodiment, the IL-15 variant includes amino acid

substitutions D30N/E64Q/N65D and VH and VL are the variable heavy domain and variable light domain of 3H3[TIM-3]_H1_L2.1.

[00336] In the Fab X ncIL-15/Rα format, one preferred embodiment utilizes the skew variant set S364K/E357Q : L368D/K370S, the pI variants Q295E/N384D/Q418E/N421D, the ablation variants E233P/L234V/L235A/G236_/S267K on both first and second monomers, and optionally the 428L/434S variants on both first and second monomers.

[00337] In one embodiment, the targeted IL-15/IL-15Rα heterodimeric protein is a "Fab X ncIL-15/Rα"format heterodimeric protein that includes: a) a first monomer that includes, from N- to C-terminus, a VH-CH1-hinge-CH2-CH3, where VH is a variable heavy domain and CH2-CH3 is a first variant Fc domain; b) a second monomer that includes, from N- to C-terminus, an IL-15Rα(sushi) domain-(domain linker)-CH2-CH3, where CH2-CH3 is a second variant Fc domain; c) a third monomer that includes from, N- to C-terminus, VL-VC, where VL is a variable light domain, and d) a fourth monomer comprising an IL-15 variant, where the VH and the VL form a TIM-3 binding domain, where the IL-15 and IL-15Rα(sushi) domain form an IL-15 complex, where the first variant Fc domain includes skew variants L368D/K370S and the second variant Fc domain includes skew variants S364K/E357Q, where the first and second variant Fc domains each include FcKO variants E233P/L234V/L235A/G236del/S267K, where the hinge-first variant Fc domain of the first monomer includes pI variants N208D/Q295E/N384D/Q418E/N421D, and where numbering is according to EU numbering. In some embodiments, the hinge of the second monomer also includes amino acid substitution C220S. In certain embodiments, the first and second variant Fc domains each further include half-life extension variants M428L/N434S. In an exemplary embodiment, the IL-15 variant includes amino acid substitutions N4D/N65D, D30N/N65D, or D30N/E64Q/N65D. In an exemplary embodiment, the VH and VL are the VH and VL of any of the TIM-3 ABDs in Figures 12 and 13A-C and the IL-15 variant includes amino acid substitutions N4D/N65D. In another exemplary embodiment, the VH and VL are the VH and VL of any of the TIM-3 ABDs in Figures 12 and 13A-C and the IL-15 variant includes amino acid substitutions D30N/N65D. In yet another exemplary embodiment, the VH and VL are the VH and VL of any of the TIM-3 ABDs in Figures 12 and 13A-C and the IL-15 variant includes amino acid substitutions D30N/E64Q/N65D.

[00338] In the Fab X ncIL-15/Rα format, one preferred embodiment utilizes an anti-TIM-3 ABD the variable heavy and light domain pair of 3H3[TIM-3]_H1_L2.1 as shown in Figure 12 with the Figures 21E format, the skew variant set S364K/E357Q : L368D/K370S, the pI variants Q295E/N384D/Q418E/N421D, the ablation variants E233P/L234V/L235A/G236_/S267K on both first and second monomers, and optionally the 428L/434S variants on both first and second monomers.

[00339] In one embodiment, the targeted IL-15/IL-15Rα heterodimeric protein is a "Fab X ncIL-15/Rα"format heterodimeric protein that includes: a) a first monomer that includes, from N- to C-terminus, a VH-CH1-hinge-CH2-CH3, where VH is a variable heavy domain and CH2-CH3 is a first variant Fc domain; b) a second monomer that includes, from N- to C-terminus, an IL-15Rα(sushi) domain-(domain linker)-CH2-CH3, where CH2-CH3 is a second variant Fc domain; c) a third monomer that includes from, N- to C-terminus, VL-VC, where VL is a variable light domain, and d) a fourth monomer comprising an IL-15 variant, where the VH and the VL form a TIM-3 binding domain, where the IL-15 and IL-15Rα(sushi) domain form an IL-15 complex, where VH and VL are the variable heavy domain and variable light domain of 3H3[TIM-3]_H1_L2.1, where the first variant Fc domain includes skew variants L368D/K370S and the second variant Fc domain includes skew variants S364K/E357Q, where the first and second variant Fc domains each include FcKO variants E233P/L234V/L235A/G236del/S267K, where the hinge-first variant Fc domain of the first monomer includes pI variants N208D/Q295E/N384D/Q418E/N421D, and where numbering is according to EU numbering. In some embodiments, the hinge of the second monomer also includes amino acid substitution C220S. In certain embodiments, the first and second variant Fc domains each further include half-life extension variants M428L/N434S. In a particular embodiment, the IL-15 variant includes amino acid substitutions N4D/N65D and VH and VL are the variable heavy domain and variable light domain of 3H3[TIM-3]_H1_L2.1. In one embodiment, the IL-15 variant includes amino acid substitutions D30N/N65D and VH and VL are the variable heavy domain and variable light domain of 3H3[TIM-3]_H1_L2.1. In another embodiment, the IL-15 variant includes amino acid substitutions D30N/E64Q/N65D and VH and VL are the variable heavy domain and variable light domain of 3H3[TIM-3]_H1_L2.1.

F. Fab X dsIL-15/Rα

[00340] This embodiment is shown in Figures 21F, and comprises four monomers. The first monomer comprises, from N- to C-terminus, the IL-15Rα(sushi)domain-domain linker-CH2-CH3, wherein the IL-15Rα(sushi)domain has been engineered to contain a cysteine residue, and the second monomer comprises a heavy chain, VH-CH1-hinge-CH2-CH3. The third monomer is a light chain that includes, from N-to C-terminus, a variable light domain (VL) and a constant light domain (CL). The fourth monomer is the variant IL-15 domain, also engineered to have a cysteine residue, such that a disulfide bridge is formed under native cellular conditions. This is generally referred to as "Fab X dsIL-15/Rα", with the "ds" standing for "disulfide".

[00341] In one embodiment, the targeted IL-15/IL-15Rα heterodimeric protein is an "Fab X dsIL-15/Rα" format heterodimeric protein that includes: a) a first monomer that includes, from N- to C-terminus, a VH-hinge-CH2-CH3, where VH is a variable heavy domain and CH2-CH3 is a first Fc domain; b) a second monomer that includes, from N- to C-terminus, an IL-15Rα(sushi) domain-(domain linker)-CH2-CH3, where CH2-CH3 is a second Fc domain and the IL-15Rα(sushi) domain includes an amino acid substitution for a cysteine residue; c) a third monomer that includes, from N- to C-terminus, a VL-CL, where VL is a variable light domain; and d) an IL-15 variant that includes an amino acid substitution for a cysteine residue, where the VH and VL form a TIM-3 binding domain, and where the cysteine residue on the IL-15 variant and the cysteine residue on the IL-15Rα(sushi) domain form a disulfide bond. Any useful domain linker can be used to attach the various components of the heterodimeric protein including, but not limited to those in Figures 8 and 9A-C. In an exemplary embodiment, the domain linkers that attach the IL-15Rα(sushi) domain to the second Fc domain is an antibody hinge domain (e.g., an antibody hinge domain).

[00342] In the Fab X dsIL-15/Rα format, one preferred embodiment utilizes an anti-TIM-3 ABD having any of the variable heavy and light domain pairs as shown in Figure 12.

[00343] In the Fab X dsIL-15/Rα format, one preferred embodiment utilizes an anti-TIM-3 ABD having the variable heavy and light domain pair of 3H3[TIM-3]_H1_L2.1 as shown in Figure 12.

[00344] In one embodiment, the targeted IL-15/IL-15Rα heterodimeric protein is an "Fab X dsIL-15/Rα" format heterodimeric protein that includes: a) a first monomer that includes, from N- to C-terminus, a VH-hinge-CH2-CH3, where VH is a variable heavy domain and CH2-CH3 is a first Fc domain; b) a second monomer that includes, from N- to C-terminus, an IL-15Rα(sushi) domain-(domain linker)-CH2-CH3, where CH2-CH3 is a second Fc domain and the IL-15Rα(sushi) domain includes an amino acid substitution for a cysteine residue; c) a third monomer that includes, from N- to C-terminus, a VL-CL, where VL is a variable light domain; and d) an IL-15 variant that includes an amino acid substitution for a cysteine residue, where the VH and VL are the variable heavy domain and variable light domain of 3H3[TIM-3]_H1_L2.1, respectively, and where the cysteine residue on the IL-15 variant and the cysteine residue on the IL-15Rα(sushi) domain form a disulfide bond.

[00345] In the Fab X dsIL-15/Rα format, one preferred embodiment utilizes an IL-15 variant that includes amino acid substitutions N4D/N65D, D30N/N65D, or D30N/E64Q/N65D, with the appropriate cysteine amino acid substitutions. In one embodiment, the targeted IL-15/IL-15Rα heterodimeric protein is an "Fab X dsIL-15/Rα" format heterodimeric protein that includes: a) a first monomer that includes, from N- to C-terminus, a VH-hinge-CH2-CH3, where VH is a variable heavy domain and CH2-CH3 is a first Fc domain; b) a second monomer that includes, from N- to C-terminus, an IL-15Rα(sushi) domain-(domain linker)-CH2-CH3, where CH2-CH3 is a second Fc domain and the IL-15Rα(sushi) domain includes an amino acid substitution for a cysteine residue; c) a third monomer that includes, from N- to C-terminus, a VL-CL, where VL is a variable light domain; and d) an IL-15 variant that includes an amino acid substitution for a cysteine residue, where the VH and VL form a TIM-3 binding domain, where the cysteine residue on the IL-15 variant and the cysteine residue on the IL-15Rα(sushi) domain form a disulfide bond, and where the IL-15 variant includes amino acid substitutions N4D/N65D, D30N/N65D, or D30N/E64Q/N65D. In an exemplary embodiment, the VH and VL are the

VH and VL of any of the TIM-3 ABDs in Figures 12 and 13A-C and the IL-15 variant includes amino acid substitutions N4D/N65D. In another exemplary embodiment, the VH and VL are the VH and VL of any of the TIM-3 ABDs in Figures 12 and 13A-C and the IL-15 variant includes amino acid substitutions D30N/N65D. In yet another exemplary embodiment, the VH and VL are the VH and VL of any of the TIM-3 ABDs in Figures 12 and 13A-C and the IL-15 variant includes amino acid substitutions D30N/E64Q/N65D.

[00346] In the Fab X dsIL-15/Rα format, one preferred embodiment utilizes an anti-TIM-3 ABD having the variable heavy and light domain pair of 3H3[TIM-3]_H1_L2.1 as shown in Figure 12, with either the IL-15 N4D/N65D variant or the IL-15 D30N/N65D variant or the IL-15 D30N/E64Q/N65D variant, with the appropriate cysteine amino acid substitutions. In one embodiment, the targeted IL-15/IL-15Rα heterodimeric protein is an "Fab X dsIL-15/Rα" format heterodimeric protein that includes: a) a first monomer that includes, from N- to C-terminus, a VH-hinge-CH2-CH3, where VH is a variable heavy domain and CH2-CH3 is a first Fc domain; b) a second monomer that includes, from N- to C-terminus, an IL-15Rα(sushi) domain-(domain linker)-CH2-CH3, where CH2-CH3 is a second Fc domain and the IL-15Rα(sushi) domain includes an amino acid substitution for a cysteine residue; c) a third monomer that includes, from N- to C-terminus, a VL-CL, where VL is a variable light domain; and d) an IL-15 variant that includes an amino acid substitution for a cysteine residue, where the VH and VL form a TIM-3 binding domain, where the cysteine residue on the IL-15 variant and the cysteine residue on the IL-15Rα(sushi) domain form a disulfide bond, where VH and VL are the variable heavy domain and variable light domain of 3H3[TIM-3]_H1_L2.1, and where the IL-15 variant includes amino acid substitutions N4D/N65D, D30N/N65D, or D30N/E64Q/N65D. In a particular embodiment, the IL-15 variant includes amino acid substitutions N4D/N65D and VH and VL are the variable heavy domain and variable light domain of 3H3[TIM-3]_H1_L2.1. In one embodiment, the IL-15 variant includes amino acid substitutions D30N/N65D and VH and VL are the variable heavy domain and variable light domain of 3H3[TIM-3]_H1_L2.1. In another embodiment, the IL-15 variant includes amino acid substitutions D30N/E64Q/N65D and VH and VL are the variable heavy domain and variable light domain of 3H3[TIM-3]_H1_L2.1.

[00347] In the Fab X dsIL-15/Rα format, one preferred embodiment utilizes the skew variant pair S364K/E357Q : L368D/K370S. In one embodiment, the targeted IL-15/IL-15Rα heterodimeric protein is an "Fab X dsIL-15/Rα" format heterodimeric protein that includes: a) a first monomer that includes, from N- to C-terminus, a VH-hinge-CH2-CH3, where VH is a variable heavy domain and CH2-CH3 is a first variant Fc domain; b) a second monomer that includes, from N- to C-terminus, an IL-15Rα(sushi) domain-(domain linker)-CH2-CH3, where CH2-CH3 is a second variant Fc domain and the IL-15Rα(sushi) domain includes an amino acid substitution for a cysteine residue; c) a third monomer that includes, from N- to C-terminus, a VL-CL, where VL is a variable light domain; and d) an IL-15 variant that includes an amino acid substitution for a cysteine residue, where the VH and VL form a TIM-3 binding domain, where the cysteine residue on the IL-15 variant and the cysteine residue on the IL-15Rα(sushi) domain form a disulfide bond, and where the first and second variant Fc domains include the skew variant pair S364K/E357Q : L368D/K370S. In an exemplary embodiment, the first variant Fc domain includes skew variants L368D/K370S, and the second variant Fc domain includes skew variants S364K/E357Q.

[00348] In one embodiment, the targeted IL-15/IL-15Rα heterodimeric protein is an "Fab X dsIL-15/Rα" format heterodimeric protein that includes: a) a first monomer that includes, from N- to C-terminus, a VH-hinge-CH2-CH3, where VH is a variable heavy domain and CH2-CH3 is a first variant Fc domain; b) a second monomer that includes, from N- to C-terminus, an IL-15Rα(sushi) domain-(domain linker)-CH2-CH3, where CH2-CH3 is a second variant Fc domain and the IL-15Rα(sushi) domain includes an amino acid substitution for a cysteine residue; c) a third monomer that includes, from N- to C-terminus, a VL-CL, where VL is a variable light domain; and d) an IL-15 variant that includes an amino acid substitution for a cysteine residue, where the VH and VL form a TIM-3 binding domain, where the cysteine residue on the IL-15 variant and the cysteine residue on the IL-15Rα(sushi) domain form a disulfide bond, where the IL-15 variant includes amino acid substitutions N4D/N65D, D30N/N65D, or D30N/E64Q/N65D, and where the first and second variant Fc domains include the skew variant pair S364K/E357Q : L368D/K370S. In an exemplary embodiment, the first variant Fc domain includes skew variants L368D/K370S, and the second variant Fc domain includes skew variants S364K/E357Q. In an exemplary

embodiment, the VH and VL are the VH and VL of any of the TIM-3 ABDs in Figures 12 and 13A-C and the IL-15 variant includes amino acid substitutions N4D/N65D. In another exemplary embodiment, the VH and VL are the VH and VL of any of the TIM-3 ABDs in Figures 12 and 13A-C and the IL-15 variant includes amino acid substitutions D30N/N65D. In yet another exemplary embodiment, the VH and VL are the VH and VL of any of the TIM-3 ABDs in Figures 12 and 13A-C and the IL-15 variant includes amino acid substitutions D30N/E64Q/N65D.

[00349] In the Fab X dsIL-15/Rα format, one preferred embodiment utilizes an anti-TIM-3 ABD having the variable heavy and light domain pair of 3H3[TIM-3]_H1_L2.1 as shown in Figure 12 and the skew variant pair S364K/E357Q : L368D/K370S. In one embodiment, the targeted IL-15/IL-15Rα heterodimeric protein is an "Fab X dsIL-15/Rα" format heterodimeric protein that includes: a) a first monomer that includes, from N- to C-terminus, a VH-hinge-CH2-CH3, where VH is a variable heavy domain and CH2-CH3 is a first variant Fc domain; b) a second monomer that includes, from N- to C-terminus, an IL-15Rα(sushi) domain-(domain linker)-CH2-CH3, where CH2-CH3 is a variant second Fc domain and the IL-15Rα(sushi) domain includes an amino acid substitution for a cysteine residue; c) a third monomer that includes, from N- to C-terminus, a VL-CL, where VL is a variable light domain; and d) an IL-15 variant that includes an amino acid substitution for a cysteine residue, where the VH and VL are the variable heavy domain and variable light domain of 3H3[TIM-3]_H1_L2.1, respectively, where the cysteine residue on the IL-15 variant and the cysteine residue on the IL-15Rα(sushi) domain form a disulfide bond, and where the first and second variant Fc domains include the skew variant pair S364K/E357Q : L368D/K370S.

[00350] In the Fab X dsIL-15/Rα format, one preferred embodiment utilizes an anti-TIM-3 ABD having the variable heavy and light domain pair of 3H3[TIM-3]_H1_L2.1 as shown in Figure 12 and the skew variant pair S364K/E357Q : L368D/K370S with either the IL-15 N4D/N65D variant or the IL-15 D30N/N65D variant or the IL-15 D30N/E64Q/N65D variant with appropriate cysteine substitutions. In one embodiment, the targeted IL-15/IL-15Rα heterodimeric protein is an "Fab X dsIL-15/Rα" format heterodimeric protein that includes: a) a first monomer that includes, from N- to C-terminus, a VH-hinge-CH2-CH3,

where VH is a variable heavy domain and CH2-CH3 is a first variant Fc domain; b) a second monomer that includes, from N- to C-terminus, an IL-15Rα(sushi) domain-(domain linker)-CH2-CH3, where CH2-CH3 is a second variant Fc domain and the IL-15Rα(sushi) domain includes an amino acid substitution for a cysteine residue; c) a third monomer that includes, from N- to C-terminus, a VL-CL, where VL is a variable light domain; and d) an IL-15 variant that includes an amino acid substitution for a cysteine residue, where the VH and VL form a TIM-3 binding domain, where the cysteine residue on the IL-15 variant and the cysteine residue on the IL-15Rα(sushi) domain form a disulfide bond, where VH and VL are the variable heavy domain and variable light domain of 3H3[TIM-3]_H1_L2.1, where the IL-15 variant includes amino acid substitutions N4D/N65D, D30N/N65D, or D30N/E64Q/N65D, and where the first and second variant Fc domains include the skew variant pair S364K/E357Q : L368D/K370S. In a particular embodiment, the IL-15 variant includes amino acid substitutions N4D/N65D and VH and VL are the variable heavy domain and variable light domain of 3H3[TIM-3]_H1_L2.1. In one embodiment, the IL-15 variant includes amino acid substitutions D30N/N65D and VH and VL are the variable heavy domain and variable light domain of 3H3[TIM-3]_H1_L2.1. In another embodiment, the IL-15 variant includes amino acid substitutions D30N/E64Q/N65D and VH and VL are the variable heavy domain and variable light domain of 3H3[TIM-3]_H1_L2.1.

[00351] In the Fab X dsIL-15/Rα format, one preferred embodiment utilizes the skew variant set S364K/E357Q : L368D/K370S, the pI variants Q295E/N384D/Q418E/N421D, the ablation variants E233P/L234V/L235A/G236_/S267K on both first and second monomers, and optionally the 428L/434S variants on both first and second monomers.

[00352] In one embodiment, the targeted IL-15/IL-15Rα heterodimeric protein is an "Fab X dsIL-15/Rα" format heterodimeric protein that includes: a) a first monomer that includes, from N- to C-terminus, a VH-hinge-CH2-CH3, where VH is a variable heavy domain and CH2-CH3 is a first variant Fc domain; b) a second monomer that includes, from N- to C-terminus, an IL-15Rα(sushi) domain-(domain linker)-CH2-CH3, where CH2-CH3 is a second variant Fc domain and the IL-15Rα(sushi) domain includes an amino acid substitution for a cysteine residue; c) a third monomer that includes, from N- to C-terminus, a VL-CL, where VL is a variable light domain; and d) an IL-15 variant that includes an

amino acid substitution for a cysteine residue, where the VH and VL form a TIM-3 binding domain, where the cysteine residue on the IL-15 variant and the cysteine residue on the IL-15Rα(sushi) domain form a disulfide bond, where the first variant Fc domain includes skew variants L368D/K370S and the second variant Fc domain includes skew variants S364K/E357Q, where the first and second variant Fc domains each include FcKO variants E233P/L234V/L235A/G236del/S267K, where the hinge-first variant Fc domain of the first monomer includes pI variants N208D/Q295E/N384D/Q418E/N421D, and where numbering is according to EU numbering. In some embodiments, the hinge of the second monomer also includes amino acid substitution C220S. In certain embodiments, the first and second variant Fc domains each further include half-life extension variants M428L/N434S. In an exemplary embodiment, the IL-15 variant includes amino acid substitutions N4D/N65D, D30N/N65D, or D30N/E64Q/N65D. In an exemplary embodiment, the VH and VL are the VH and VL of any of the TIM-3 ABDs in Figures 12 and 13A-C and the IL-15 variant includes amino acid substitutions N4D/N65D. In another exemplary embodiment, the VH and VL are the VH and VL of any of the TIM-3 ABDs in Figures 12 and 13A-C and the IL-15 variant includes amino acid substitutions D30N/N65D. In yet another exemplary embodiment, the VH and VL are the VH and VL of any of the TIM-3 ABDs in Figures 12 and 13A-C and the IL-15 variant includes amino acid substitutions D30N/E64Q/N65D.

[00353] In the Fab X dsIL-15/Rα format, one preferred embodiment utilizes an anti-TIM-3 ABD having the variable heavy and light domain pair of 3H3[TIM-3]_H1_L2.1 as shown in Figure 12 with the Figures 21F format, the skew variant set S364K/E357Q : L368D/K370S, the pI variants Q295E/N384D/Q418E/N421D, the ablation variants E233P/L234V/L235A/G236_/S267K on both first and second monomers, and optionally the 428L/434S variants on both first and second monomers.

[00354] In one embodiment, the targeted IL-15/IL-15Rα heterodimeric protein is an "Fab X dsIL-15/Rα" format heterodimeric protein that includes: a) a first monomer that includes, from N- to C-terminus, a VH-hinge-CH2-CH3, where VH is a variable heavy domain and CH2-CH3 is a first variant Fc domain; b) a second monomer that includes, from N- to C-terminus, an IL-15Rα(sushi) domain-(domain linker)-CH2-CH3, where CH2-CH3 is a second variant Fc domain and the IL-15Rα(sushi) domain includes an amino acid

substitution for a cysteine residue; c) a third monomer that includes, from N- to C-terminus, a VL-CL, where VL is a variable light domain; and d) an IL-15 variant that includes an amino acid substitution for a cysteine residue, where the VH and VL form a TIM-3 binding domain, where the cysteine residue on the IL-15 variant and the cysteine residue on the IL-15Rα(sushi) domain form a disulfide bond, where VH and VL are the variable heavy domain and variable light domain of 3H3[TIM-3]_H1_L2.1, where the first variant Fc domain includes skew variants L368D/K370S and the second variant Fc domain includes skew variants S364K/E357Q, where the first and second variant Fc domains each include FcKO variants E233P/L234V/L235A/G236del/S267K, where the hinge-first variant Fc domain of the first monomer includes pI variants N208D/Q295E/N384D/Q418E/N421D, and where numbering is according to EU numbering. In some embodiments, the hinge of the second monomer also includes amino acid substitution C220S. In a particular embodiment, the IL-15 variant includes amino acid substitutions N4D/N65D and VH and VL are the variable heavy domain and variable light domain of 3H3[TIM-3]_H1_L2.1. In one embodiment, the IL-15 variant includes amino acid substitutions D30N/N65D and VH and VL are the variable heavy domain and variable light domain of 3H3[TIM-3]_H1_L2.1. In another embodiment, the IL-15 variant includes amino acid substitutions D30N/E64Q/N65D and VH and VL are the variable heavy domain and variable light domain of 3H3[TIM-3]_H1_L2.1.

G. mAb-scIL-15/Rα

[00355] This embodiment is shown in Figures 21G, and comprises three monomers (although the fusion protein is a tetramer). The first monomer comprises a heavy chain, VH-CH1-hinge-CH2-CH3. The second monomer comprises a heavy chain with a scIL-15 complex, VH-CH1-hinge-CH2-CH3-domain linker- IL-15Rα(sushi)domain-domain linker-IL-15 variant. The third (and fourth) monomer are light chains, VL-CL. This is generally referred to as "mAb-scIL-15/Rα", with the "sc" standing for "single chain". This binds the TIM-3 molecule bivalently.

[00356] In one embodiment, the targeted IL-15/IL-15Rα heterodimeric protein is an "mAb-scIL-15/Rα" format heterodimeric protein that includes: a) a first monomer that includes, from N- to C-terminus, a VH-hinge-CH2-CH3, where CH2-CH3 is a first Fc domain; b) a second monomer that includes, from N- to C-terminus, a VH-hinge-CH2-CH3-

(domain linker)-IL-15Rα(sushi) domain-(domain linker)-IL-15 variant, where CH2-CH3 is a second Fc domain; and c) a third and fourth monomer that each include, from N- to C-terminus, a VL-CL, where VL is a variable light domain, where the VH of the first monomer and the VL of the third monomer form a first TIM-3 binding domain, where the VH of the second monomer and the VL of the fourth monomer form a second TIM-3 binding domain, and where the IL-15 variant and the IL-15Rα(sushi) domain form an IL-15 complex. Any useful domain linker can be used to attach the various components of the heterodimeric protein including, but not limited to those in Figures 8 and 9A-C.

[00357] In the mAb-scIL-15/Rα format, one preferred embodiment utilizes an anti-TIM-3 ABD having any of the variable heavy and light domain pairs as shown in Figure 12.

[00358] In the mAb-scIL-15/Rα format, one preferred embodiment utilizes an anti-TIM-3 ABD having the variable heavy and light domain pair of 3H3[TIM-3]_H1_L2.1 as shown in Figure 12.

[00359] In one embodiment, the targeted IL-15/IL-15Rα heterodimeric protein is an "mAb-scIL-15/Rα" format heterodimeric protein that includes: a) a first monomer that includes, from N- to C-terminus, a VH-hinge-CH2-CH3, where CH2-CH3 is a first Fc domain; b) a second monomer that includes, from N- to C-terminus, a VH-hinge-CH2-CH3-(domain linker)-IL-15Rα(sushi) domain-(domain linker)-IL-15 variant, where CH2-CH3 is a second Fc domain; and c) a third and fourth monomer that each include, from N- to C-terminus, a VL-CL, where VL is a variable light domain, where the VH of the first monomer and the VL of the third monomer are the variable heavy domain and variable light domain of 3H3[TIM-3]_H1_L2.1, respectively, where the VH of the second monomer and the VL of the fourth monomer are the variable heavy domain and variable light domain of 3H3[TIM-3]_H1_L2.1, respectively, and where the IL-15 variant and the IL-15Rα(sushi) domain form an IL-15 complex.

[00360] In the mAb-scIL-15/Rα format, one preferred embodiment utilizes an IL-15 variant that includes amino acid substitutions N4D/N65D, D30N/N65D, or D30N/E64Q/N65D. In one embodiment, the targeted IL-15/IL-15Rα heterodimeric protein is an "mAb-scIL-15/Rα" format heterodimeric protein that includes: a) a first monomer that

includes, from N- to C-terminus, a VH-hinge-CH2-CH3, where CH2-CH3 is a first Fc domain; b) a second monomer that includes, from N- to C-terminus, a VH-hinge-CH2-CH3-(domain linker)-IL-15Rα(sushi) domain-(domain linker)-IL-15 variant, where CH2-CH3 is a second Fc domain; and c) a third and fourth monomer that each include, from N- to C-terminus, a VL-CL, where VL is a variable light domain, where the VH of the first monomer and the VL of the third monomer form a first TIM-3 binding domain, where the VH of the second monomer and the VL of the fourth monomer form a second TIM-3 binding domain, where the IL-15 variant and the IL-15Rα(sushi) domain form an IL-15 complex, and where the IL-15 variant includes amino acid substitutions N4D/N65D, D30N/N65D, or D30N/E64Q/N65D. In an exemplary embodiment, the VH and VL are the VH and VL of any of the TIM-3 ABDs in Figures 12 and 13A-C and the IL-15 variant includes amino acid substitutions N4D/N65D. In another exemplary embodiment, the VH and VL are the VH and VL of any of the TIM-3 ABDs in Figures 12 and 13A-C and the IL-15 variant includes amino acid substitutions D30N/N65D. In yet another exemplary embodiment, the VH and VL are the VH and VL of any of the TIM-3 ABDs in Figures 12 and 13A-C and the IL-15 variant includes amino acid substitutions D30N/E64Q/N65D.

[00361] In the mAb-scIL-15/Rα format, one preferred embodiment utilizes an anti-TIM-3 ABD having the variable heavy and light domain pair of 3H3[TIM-3]_H1_L2.1 as shown in Figure 12, with either the IL-15 N4D/N65D variant or the IL-15 D30N/N65D variant or the IL-15 D30N/E64Q/N65D variant. In one embodiment, the targeted IL-15/IL-15Rα heterodimeric protein is an "mAb-scIL-15/Rα" format heterodimeric protein that includes: a) a first monomer that includes, from N- to C-terminus, a VH-hinge-CH2-CH3, where CH2-CH3 is a first Fc domain; b) a second monomer that includes, from N- to C-terminus, a VH-hinge-CH2-CH3-(domain linker)-IL-15Rα(sushi) domain-(domain linker)-IL-15 variant, where CH2-CH3 is a second Fc domain; and c) a third and fourth monomer that each include, from N- to C-terminus, a VL-CL, where VL is a variable light domain, where the VH of the first monomer and the VL of the third monomer form a first TIM-3 binding domain, where the VH of the second monomer and the VL of the fourth monomer form a second TIM-3 binding domain, where the IL-15 variant and the IL-15Rα(sushi) domain form an IL-15 complex, where VH and VL are the variable heavy domain and

variable light domain of 3H3[TIM-3]_H1_L2.1, and where the IL-15 variant includes amino acid substitutions N4D/N65D, D30N/N65D, or D30N/E64Q/N65D. In a particular embodiment, the IL-15 variant includes amino acid substitutions N4D/N65D and VH and VL are the variable heavy domain and variable light domain of 3H3[TIM-3]_H1_L2.1. In one embodiment, the IL-15 variant includes amino acid substitutions D30N/N65D and VH and VL are the variable heavy domain and variable light domain of 3H3[TIM-3]_H1_L2.1. In another embodiment, the IL-15 variant includes amino acid substitutions D30N/E64Q/N65D and VH and VL are the variable heavy domain and variable light domain of 3H3[TIM-3]_H1_L2.1.

[00362] In the mAb-scIL-15/Rα format, one preferred embodiment utilizes the skew variant pair S364K/E357Q : L368D/K370S. In one embodiment, the targeted IL-15/IL-15Rα heterodimeric protein is an "mAb-scIL-15/Rα" format heterodimeric protein that includes: a) a first monomer that includes, from N- to C-terminus, a VH-hinge-CH2-CH3, where CH2-CH3 is a first variant Fc domain; b) a second monomer that includes, from N- to C-terminus, a VH-hinge-CH2-CH3-(domain linker)-IL-15Rα(sushi) domain-(domain linker)-IL-15 variant, where CH2-CH3 is a second variant Fc domain; and c) a third and fourth monomer that each include, from N- to C-terminus, a VL-CL, where VL is a variable light domain, where the VH of the first monomer and the VL of the third monomer form a first TIM-3 binding domain, where the VH of the second monomer and the VL of the fourth monomer form a second TIM-3 binding domain, where the IL-15 variant and the IL-15Rα(sushi) domain form an IL-15 complex, and where the first and second variant Fc domains include the skew variant pair S364K/E357Q : L368D/K370S. In an exemplary embodiment, the first variant Fc domain includes skew variants L368D and K370S, and the second variant Fc domain includes skew variants S364K and E357Q. In an exemplary embodiment, the first variant Fc domain includes skew variants S364K and E357Q, the second variant Fc domain includes skew variants E357Q L368D and K370S.

[00363] In one embodiment, the targeted IL-15/IL-15Rα heterodimeric protein is an "mAb-scIL-15/Rα" format heterodimeric protein that includes: a) a first monomer that includes, from N- to C-terminus, a VH-hinge-CH2-CH3, where CH2-CH3 is a first variant Fc domain; b) a second monomer that includes, from N- to C-terminus, a VH-hinge-CH2-CH3-

(domain linker)-IL-15Rα(sushi) domain-(domain linker)-IL-15 variant, where CH2-CH3 is a second variant Fc domain; and c) a third and fourth monomer that each include, from N- to C-terminus, a VL-CL, where VL is a variable light domain, where the VH of the first monomer and the VL of the third monomer form a first TIM-3 binding domain, where the VH of the second monomer and the VL of the fourth monomer form a second TIM-3 binding domain, where the IL-15 variant and the IL-15Rα(sushi) domain form an IL-15 complex, where the IL-15 variant includes amino acid substitutions N4D/N65D, D30N/N65D, or D30N/E64Q/N65D, and where the first and second variant Fc domains include the skew variant pair S364K/E357Q : L368D/K370S. In an exemplary embodiment, the first variant Fc domain includes skew variants L368D and K370S, and the second variant Fc domain includes skew variants S364K and E357Q. In an exemplary embodiment, the first variant Fc domain includes skew variants S364K and E357Q, and the second variant Fc domain includes skew variants L368D and K370S. In an exemplary embodiment, the VH and VL are the VH and VL of any of the TIM-3 ABDs in Figures 12 and 13A-C and the IL-15 variant includes amino acid substitutions N4D/N65D. In another exemplary embodiment, the VH and VL are the VH and VL of any of the TIM-3 ABDs in Figures 12 and 13A-C and the IL-15 variant includes amino acid substitutions D30N/N65D. In yet another exemplary embodiment, the VH and VL are the VH and VL of any of the TIM-3 ABDs in Figures 12 and 13A-C and the IL-15 variant includes amino acid substitutions D30N/E64Q/N65D.

[00364] In the mAb-scIL-15/Rα format, one preferred embodiment utilizes an anti-TIM-3 ABD having the variable heavy and light domain pair of 3H3[TIM-3]_H1_L2.1 as shown in Figure 12 and the skew variant pair S364K/E357Q : L368D/K370S. In one embodiment, the targeted IL-15/IL-15Rα heterodimeric protein is an "mAb-scIL-15/Rα" format heterodimeric protein that includes: a) a first monomer that includes, from N- to C-terminus, a VH-hinge-CH2-CH3, where CH2-CH3 is a first variant Fc domain; b) a second monomer that includes, from N- to C-terminus, a VH-hinge-CH2-CH3-(domain linker)-IL-15Rα(sushi) domain-(domain linker)-IL-15 variant, where CH2-CH3 is a second variant Fc domain; and c) a third and fourth monomer that each include, from N- to C-terminus, a VL-CL, where VL is a variable light domain, where the VH of the first monomer and the VL of the third monomer are the variable heavy domain and variable light domain of 3H3[TIM-

3]_H1_L2.1, respectively, where the VH of the second monomer and the VL of the fourth monomer are the variable heavy domain and variable light domain of 3H3[TIM-3]_H1_L2.1, respectively, where the IL-15 variant and the IL-15Rα(sushi) domain form an IL-15 complex, where the IL-15 variant and the IL-15Rα(sushi) domain form an IL-15 complex, and where the first and second variant Fc domains include the skew variant pair S364K/E357Q : L368D/K370S. In an exemplary embodiment, the first variant Fc domain includes skew variants L368D and K370S, and the second variant Fc domain includes skew variants S364K and E357Q. In an exemplary embodiment, the first variant Fc domain includes skew variants S364K and E357Q, and the second variant Fc domain includes skew variants L368D and K370S.

[00365] In the mAb-scIL-15/Rα format, one preferred embodiment utilizes an anti-TIM-3 ABD having the variable heavy and light domain pair of 3H3[TIM-3]_H1_L2.1 as shown in Figure 12 and the skew variant pair S364K/E357Q : L368D/K370S with either the IL-15 N4D/N65D variant or the IL-15 D30N/N65D variant or the IL-15 D30N/E64Q/N65D variant. In one embodiment, the targeted IL-15/IL-15Rα heterodimeric protein is an "mAb-scIL-15/Rα" format heterodimeric protein that includes: a) a first monomer that includes, from N- to C-terminus, a VH-hinge-CH2-CH3, where CH2-CH3 is a first variant Fc domain; b) a second monomer that includes, from N- to C-terminus, a VH-hinge-CH2-CH3-(domain linker)-IL-15Rα(sushi) domain-(domain linker)-IL-15 variant, where CH2-CH3 is a second variant Fc domain; and c) a third and fourth monomer that each include, from N- to C-terminus, a VL-CL, where VL is a variable light domain, where the VH of the first monomer and the VL of the third monomer form a first TIM-3 binding domain, where the VH of the second monomer and the VL of the fourth monomer form a second TIM-3 binding domain, where the IL-15 variant and the IL-15Rα(sushi) domain form an IL-15 complex, where VH and VL are the variable heavy domain and variable light domain of 3H3[TIM-3]_H1_L2.1, where the IL-15 variant includes amino acid substitutions N4D/N65D, D30N/N65D, or D30N/E64Q/N65D, and where the first and second variant Fc domains include the skew variant pair S364K/E357Q : L368D/K370S. In an exemplary embodiment, the first variant Fc domain includes skew variants L368D and K370S, and the second variant Fc domain includes skew variants S364K and E357Q. In a particular embodiment, the IL-15 variant

includes amino acid substitutions N4D/N65D and VH and VL are the variable heavy domain and variable light domain of 3H3[TIM-3]_H1_L2.1. In one embodiment, the IL-15 variant includes amino acid substitutions D30N/N65D and VH and VL are the variable heavy domain and variable light domain of 3H3[TIM-3]_H1_L2.1. In another embodiment, the IL-15 variant includes amino acid substitutions D30N/E64Q/N65D and VH and VL are the variable heavy domain and variable light domain of 3H3[TIM-3]_H1_L2.1. In an exemplary embodiment, the first variant Fc domain includes skew variants L368D and K370S, and the second variant Fc domain includes skew variants S364K and E357Q. In an exemplary embodiment, the first variant Fc domain includes skew variants S364K and E357Q, and the second variant Fc domain includes skew variants L368D and K370S.

[00366] In the mAb-scIL-15/Rα format, one preferred embodiment utilizes the skew variant set S364K/E357Q : L368D/K370S, the pI variants N208D/Q295E/N384D/Q418D/N421D and/or Q196K/I199T/P271R/P228R/N276K, the ablation variants E233P/L234V/L235A/G236_/S267K on both first and second monomers, and optionally the 428L/434S variants on both first and second monomers.

[00367] In one embodiment, the targeted IL-15/IL-15Rα heterodimeric protein is an "mAb-scIL-15/Rα" format heterodimeric protein that includes: a) a first monomer that includes, from N- to C-terminus, a VH-hinge-CH2-CH3, where CH2-CH3 is a first variant Fc domain; b) a second monomer that includes, from N- to C-terminus, a VH-hinge-CH2-CH3-(domain linker)-IL-15Rα(sushi) domain-(domain linker)-IL-15 variant, where CH2-CH3 is a second variant Fc domain; and c) a third and fourth monomer that each include, from N- to C-terminus, a VL-CL, where VL is a variable light domain, where the VH of the first monomer and the VL of the third monomer form a first TIM-3 binding domain, where the VH of the second monomer and the VL of the fourth monomer form a second TIM-3 binding domain, where the IL-15 variant and the IL-15Rα(sushi) domain form an IL-15 complex, where the first variant Fc domain includes skew variants L368D/K370S and the second variant Fc domain include the skew variant pair S364K/E357Q, where the first and second variant Fc domains each include FcKO variants E233P/L234V/L235A/G236del/S267K, where the hinge-first variant Fc domain of the first monomer includes pI substitutions N208D/Q295E/N384D/Q418D/N421D and the hinge-second variant Fc domain of the second

monomer includes pI variants Q196K/I199T/P271R/P228R/N276K, and where numbering is according to EU numbering. In one embodiment, the targeted IL-15/IL-15Rα heterodimeric protein is an "mAb-scIL-15/Rα" format heterodimeric protein that includes: a) a first monomer that includes, from N- to C-terminus, a VH-hinge-CH2-CH3, where CH2-CH3 is a first variant Fc domain; b) a second monomer that includes, from N- to C-terminus, a VH-hinge-CH2-CH3-(domain linker)-IL-15Rα(sushi) domain-(domain linker)-IL-15 variant, where CH2-CH3 is a second variant Fc domain; and c) a third and fourth monomer that each include, from N- to C-terminus, a VL-CL, where VL is a variable light domain, where the VH of the first monomer and the VL of the third monomer form a first TIM-3 binding domain, where the VH of the second monomer and the VL of the fourth monomer form a second TIM-3 binding domain, where the IL-15 variant and the IL-15Rα(sushi) domain form an IL-15 complex, where the first variant Fc domain includes skew variants L368D/K370S and the second variant Fc domain include the skew variant pair S364K/E357Q, where the first and second variant Fc domains each include FcKO variants E233P/L234V/L235A/G236del/S267K, where the hinge-first variant Fc domain of the first monomer includes pI substitutions N208D/Q295E/N384D/Q418D/N421D, and where numbering is according to EU numbering. In one embodiment, the targeted IL-15/IL-15Rα heterodimeric protein is an "mAb-scIL-15/Rα" format heterodimeric protein that includes: a) a first monomer that includes, from N- to C-terminus, a VH-hinge-CH2-CH3, where CH2-CH3 is a first variant Fc domain; b) a second monomer that includes, from N- to C-terminus, a VH-hinge-CH2-CH3-(domain linker)-IL-15Rα(sushi) domain-(domain linker)-IL-15 variant, where CH2-CH3 is a second variant Fc domain; and c) a third and fourth monomer that each include, from N- to C-terminus, a VL-CL, where VL is a variable light domain, where the VH of the first monomer and the VL of the third monomer form a first TIM-3 binding domain, where the VH of the second monomer and the VL of the fourth monomer form a second TIM-3 binding domain, where the IL-15 variant and the IL-15Rα(sushi) domain form an IL-15 complex, where the first variant Fc domain includes skew variants L368D/K370S and the second variant Fc domain include the skew variant pair S364K/E357Q, where the first and second variant Fc domains each include FcKO variants E233P/L234V/L235A/G236del/S267K, where the hinge-second variant Fc domain of the

second monomer includes pI variants Q196K/I199T/P271R/P228R/N276K, and where numbering is according to EU numbering. In one embodiment, the targeted IL-15/IL-15Rα heterodimeric protein is an "mAb-scIL-15/Rα" format heterodimeric protein that includes: a) a first monomer that includes, from N- to C-terminus, a VH-hinge-CH2-CH3, where CH2-CH3 is a first variant Fc domain; b) a second monomer that includes, from N- to C-terminus, a VH-hinge-CH2-CH3-(domain linker)-IL-15Rα(sushi) domain-(domain linker)-IL-15 variant, where CH2-CH3 is a second variant Fc domain; and c) a third and fourth monomer that each include, from N- to C-terminus, a VL-CL, where VL is a variable light domain, where the VH of the first monomer and the VL of the third monomer form a first TIM-3 binding domain, where the VH of the second monomer and the VL of the fourth monomer form a second TIM-3 binding domain, where the IL-15 variant and the IL-15Rα(sushi) domain form an IL-15 complex, where the first variant Fc domain includes skew variants S364K/E357Q and the second variant Fc domain include the skew variant pair L368D/K370S, where the first and second variant Fc domains each include FcKO variants E233P/L234V/L235A/G236del/S267K, where the hinge-first variant Fc domain of the first monomer includes pI substitutions Q196K/I199T/P271R/P228R/N276K and the hinge-second variant Fc domain of the second monomer includes pI variants N208D/Q295E/N384D/Q418D/N421D, and where numbering is according to EU numbering. In one embodiment, the targeted IL-15/IL-15Rα heterodimeric protein is an "mAb-scIL-15/Rα" format heterodimeric protein that includes: a) a first monomer that includes, from N- to C-terminus, a VH-hinge-CH2-CH3, where CH2-CH3 is a first variant Fc domain; b) a second monomer that includes, from N- to C-terminus, a VH-hinge-CH2-CH3-(domain linker)-IL-15Rα(sushi) domain-(domain linker)-IL-15 variant, where CH2-CH3 is a second variant Fc domain; and c) a third and fourth monomer that each include, from N- to C-terminus, a VL-CL, where VL is a variable light domain, where the VH of the first monomer and the VL of the third monomer form a first TIM-3 binding domain, where the VH of the second monomer and the VL of the fourth monomer form a second TIM-3 binding domain, where the IL-15 variant and the IL-15Rα(sushi) domain form an IL-15 complex, where the first variant Fc domain includes skew variants S364K/E357Q and the second variant Fc domain include the skew variant pair L368D/K370S, where the first and second variant Fc

domains each include FcKO variants E233P/L234V/L235A/G236del/S267K, where the hinge-first variant Fc domain of the first monomer includes pI substitutions Q196K/I199T/P271R/P228R/N276K, and where numbering is according to EU numbering. In one embodiment, the targeted IL-15/IL-15Rα heterodimeric protein is an "mAb-scIL-15/Rα" format heterodimeric protein that includes: a) a first monomer that includes, from N- to C-terminus, a VH-hinge-CH2-CH3, where CH2-CH3 is a first variant Fc domain; b) a second monomer that includes, from N- to C-terminus, a VH-hinge-CH2-CH3-(domain linker)-IL-15Rα(sushi) domain-(domain linker)-IL-15 variant, where CH2-CH3 is a second variant Fc domain; and c) a third and fourth monomer that each include, from N- to C-terminus, a VL-CL, where VL is a variable light domain, where the VH of the first monomer and the VL of the third monomer form a first TIM-3 binding domain, where the VH of the second monomer and the VL of the fourth monomer form a second TIM-3 binding domain, where the IL-15 variant and the IL-15Rα(sushi) domain form an IL-15 complex, where the first variant Fc domain includes skew variants S364K/E357Q and the second variant Fc domain include the skew variant pair L368D/K370S, where the first and second variant Fc domains each include FcKO variants E233P/L234V/L235A/G236del/S267K, where the hinge-first variant Fc domain of the hinge-second variant Fc domain of the second monomer includes pI variants N208D/Q295E/N384D/Q418D/N421D, and where numbering is according to EU numbering. In certain embodiments, the first and second variant Fc domains each further include half-life extension variants M428L/N434S. In an exemplary embodiment, the IL-15 variant includes amino acid substitutions N4D/N65D, D30N/N65D, or D30N/E64Q/N65D. In an exemplary embodiment, the VH and VL are the VH and VL of any of the TIM-3 ABDs in Figures 12 and 13A-C and the IL-15 variant includes amino acid substitutions N4D/N65D. In another exemplary embodiment, the VH and VL are the VH and VL of any of the TIM-3 ABDs in Figures 12 and 13A-C and the IL-15 variant includes amino acid substitutions D30N/N65D. In yet another exemplary embodiment, the VH and VL are the VH and VL of any of the TIM-3 ABDs in Figures 12 and 13A-C and the IL-15 variant includes amino acid substitutions D30N/E64Q/N65D.

[00368] In the mAb-scIL-15/Rα format, one preferred embodiment utilizes an anti-TIM-3 ABD the variable heavy and light domain pair of 3H3[TIM-3]_H1_L2.1 as shown in

Figure 12 with the Figures 21G format, the skew variant set S364K/E357Q : L368D/K370S, the pI variants N208D/Q295E/N384D/Q418D/N421D and/or Q196K/I199T/P271R/P228R/N276K, the ablation variants E233P/L234V/L235A/G236_/S267K on both first and second monomers, and optionally the 428L/434S variants on both first and second monomers.

[00369] In one embodiment, the targeted IL-15/IL-15Rα heterodimeric protein is an "mAb-scIL-15/Rα" format heterodimeric protein that includes: a) a first monomer that includes, from N- to C-terminus, a VH-hinge-CH2-CH3, where CH2-CH3 is a first variant Fc domain; b) a second monomer that includes, from N- to C-terminus, a VH-hinge-CH2-CH3-(domain linker)-IL-15Rα(sushi) domain-(domain linker)-IL-15 variant, where CH2-CH3 is a second variant Fc domain; and c) a third and fourth monomer that each include, from N- to C-terminus, a VL-CL, where VL is a variable light domain, where the VH of the first monomer and the VL of the third monomer form a first TIM-3 binding domain, where the VH of the second monomer and the VL of the fourth monomer form a second TIM-3 binding domain, where the IL-15 variant and the IL-15Rα(sushi) domain form an IL-15 complex, where VH and VL are the variable heavy domain and variable light domain of 3H3[TIM-3]_H1_L2.1, where the first variant Fc domain includes skew variants L368D/K370S and the second variant Fc domain include the skew variant pair S364K/E357Q, where the first and second variant Fc domains each include FcKO variants E233P/L234V/L235A/G236del/S267K, where the hinge-first variant Fc domain of the first monomer includes pI substitutions N208D/Q295E/N384D/Q418D/N421D and the hinge-second variant Fc domain of the second monomer includes pI variants Q196K/I199T/P271R/P228R/N276K, and where numbering is according to EU numbering. In one embodiment, the targeted IL-15/IL-15Rα heterodimeric protein is an "mAb-scIL-15/Rα" format heterodimeric protein that includes: a) a first monomer that includes, from N- to C-terminus, a VH-hinge-CH2-CH3, where CH2-CH3 is a first variant Fc domain; b) a second monomer that includes, from N- to C-terminus, a VH-hinge-CH2-CH3-(domain linker)-IL-15Rα(sushi) domain-(domain linker)-IL-15 variant, where CH2-CH3 is a second variant Fc domain; and c) a third and fourth monomer that each include, from N- to C-terminus, a VL-CL, where VL is a variable light domain, where the VH of the first monomer and the VL of the third monomer form a first TIM-3 binding

domain, where the VH of the second monomer and the VL of the fourth monomer form a second TIM-3 binding domain, where the IL-15 variant and the IL-15Rα(sushi) domain form an IL-15 complex, where VH and VL are the variable heavy domain and variable light domain of 3H3[TIM-3]_H1_L2.1, where the first variant Fc domain includes skew variants L368D/K370S and the second variant Fc domain include the skew variant pair S364K/E357Q, where the first and second variant Fc domains each include FcKO variants E233P/L234V/L235A/G236del/S267K, where the hinge-first variant Fc domain of the first monomer includes pI substitutions N208D/Q295E/N384D/Q418D/N421D, and where numbering is according to EU numbering. In one embodiment, the targeted IL-15/IL-15Rα heterodimeric protein is an "mAb-scIL-15/Rα" format heterodimeric protein that includes: a) a first monomer that includes, from N- to C-terminus, a VH-hinge-CH2-CH3, where CH2-CH3 is a first variant Fc domain; b) a second monomer that includes, from N- to C-terminus, a VH-hinge-CH2-CH3-(domain linker)-IL-15Rα(sushi) domain-(domain linker)-IL-15 variant, where CH2-CH3 is a second variant Fc domain; and c) a third and fourth monomer that each include, from N- to C-terminus, a VL-CL, where VL is a variable light domain, where the VH of the first monomer and the VL of the third monomer form a first TIM-3 binding domain, where the VH of the second monomer and the VL of the fourth monomer form a second TIM-3 binding domain, where the IL-15 variant and the IL-15Rα(sushi) domain form an IL-15 complex, where VH and VL are the variable heavy domain and variable light domain of 3H3[TIM-3]_H1_L2.1, where the first variant Fc domain includes skew variants L368D/K370S and the second variant Fc domain include the skew variant pair S364K/E357Q, where the first and second variant Fc domains each include FcKO variants E233P/L234V/L235A/G236del/S267K, where the hinge-second variant Fc domain of the second monomer includes pI variants Q196K/I199T/P271R/P228R/N276K, and where numbering is according to EU numbering. In one embodiment, the targeted IL-15/IL-15Rα heterodimeric protein is an "mAb-scIL-15/Rα" format heterodimeric protein that includes: a) a first monomer that includes, from N- to C-terminus, a VH-hinge-CH2-CH3, where CH2-CH3 is a first variant Fc domain; b) a second monomer that includes, from N- to C-terminus, a VH-hinge-CH2-CH3-(domain linker)-IL-15Rα(sushi) domain-(domain linker)-IL-15 variant, where CH2-CH3 is a second variant Fc domain; and c) a third and fourth monomer

that each include, from N- to C-terminus, a VL-CL, where VL is a variable light domain, where the VH of the first monomer and the VL of the third monomer form a first TIM-3 binding domain, where the VH of the second monomer and the VL of the fourth monomer form a second TIM-3 binding domain, where the IL-15 variant and the IL-15Rα(sushi) domain form an IL-15 complex, where VH and VL are the variable heavy domain and variable light domain of 3H3[TIM-3]_H1_L2.1, where the first variant Fc domain includes skew variants S364K/E357Q and the second variant Fc domain include the skew variant pair L368D/K370S, where the first and second variant Fc domains each include FcKO variants E233P/L234V/L235A/G236del/S267K, where the hinge-first variant Fc domain of the first monomer includes pI substitutions Q196K/I199T/P271R/P228R/N276K and the hinge-second variant Fc domain of the second monomer includes pI variants N208D/Q295E/N384D/Q418D/N421D, and where numbering is according to EU numbering. In one embodiment, the targeted IL-15/IL-15Rα heterodimeric protein is an "mAb-scIL-15/Rα" format heterodimeric protein that includes: a) a first monomer that includes, from N- to C-terminus, a VH-hinge-CH2-CH3, where CH2-CH3 is a first variant Fc domain; b) a second monomer that includes, from N- to C-terminus, a VH-hinge-CH2-CH3-(domain linker)-IL-15Rα(sushi) domain-(domain linker)-IL-15 variant, where CH2-CH3 is a second variant Fc domain; and c) a third and fourth monomer that each include, from N- to C-terminus, a VL-CL, where VL is a variable light domain, where the VH of the first monomer and the VL of the third monomer form a first TIM-3 binding domain, where the VH of the second monomer and the VL of the fourth monomer form a second TIM-3 binding domain, where the IL-15 variant and the IL-15Rα(sushi) domain form an IL-15 complex, where VH and VL are the variable heavy domain and variable light domain of 3H3[TIM-3]_H1_L2.1, where the first variant Fc domain includes skew variants S364K/E357Q and the second variant Fc domain include the skew variant pair L368D/K370S, where the first and second variant Fc domains each include FcKO variants E233P/L234V/L235A/G236del/S267K, where the hinge-first variant Fc domain of the first monomer includes pI substitutions Q196K/I199T/P271R/P228R/N276K, and where numbering is according to EU numbering. In one embodiment, the targeted IL-15/IL-15Rα heterodimeric protein is an "mAb-scIL-15/Rα" format heterodimeric protein that includes: a) a first monomer that includes, from N- to C-

terminus, a VH-hinge-CH2-CH3, where CH2-CH3 is a first variant Fc domain; b) a second monomer that includes, from N- to C-terminus, a VH-hinge-CH2-CH3-(domain linker)-IL-15Rα(sushi) domain-(domain linker)-IL-15 variant, where CH2-CH3 is a second variant Fc domain; and c) a third and fourth monomer that each include, from N- to C-terminus, a VL-CL, where VL is a variable light domain, where the VH of the first monomer and the VL of the third monomer form a first TIM-3 binding domain, where the VH of the second monomer and the VL of the fourth monomer form a second TIM-3 binding domain, where the IL-15 variant and the IL-15Rα(sushi) domain form an IL-15 complex, where VH and VL are the variable heavy domain and variable light domain of 3H3[TIM-3]_H1_L2.1, where the first variant Fc domain includes skew variants S364K/E357Q and the second variant Fc domain include the skew variant pair L368D/K370S, where the first and second variant Fc domains each include FcKO variants E233P/L234V/L235A/G236del/S267K, where the hinge-first variant Fc domain of the hinge-second variant Fc domain of the second monomer includes pI variants N208D/Q295E/N384D/Q418D/N421D, and where numbering is according to EU numbering. In certain embodiments, the first and second variant Fc domains each further include half-life extension variants M428L/N434S. In a particular embodiment, the IL-15 variant includes amino acid substitutions N4D/N65D and VH and VL are the variable heavy domain and variable light domain of 3H3[TIM-3]_H1_L2.1. In one embodiment, the IL-15 variant includes amino acid substitutions D30N/N65D and VH and VL are the variable heavy domain and variable light domain of 3H3[TIM-3]_H1_L2.1. In another embodiment, the IL-15 variant includes amino acid substitutions D30N/E64Q/N65D and VH and VL are the variable heavy domain and variable light domain of 3H3[TIM-3]_H1_L2.1.

H. mAb-ncIL-15/Rα

[00370] This embodiment is shown in Figures 21H, and comprises four monomers (although the heterodimeric fusion protein is a pentamer). The first monomer comprises a heavy chain, VH-CH1-hinge-CH2-CH3. The second monomer comprises a heavy chain with an IL-15Rα(sushi) domain: e.g., VH-CH1-hinge-CH2-CH3-domain linker-IL-15Rα(sushi) domain. The third monomer is a variant IL-15 domain. The fourth (and fifth) monomer are

light chains, VL-CL. This is generally referred to as "mAb-ncIL-15/Rα", with the "nc" standing for "non-covalent". This also binds the TIM-3 bivalently.

[00371] In one embodiment, the targeted IL-15/IL-15Rα heterodimeric protein is an "mAb-ncIL-15/Rα" format heterodimeric protein that includes: a) a first monomer that includes, from N- to C-terminus, a VH-hinge-CH2-CH3, where CH2-CH3 is a first Fc domain; b) a second monomer that includes, from N- to C-terminus, a VH-hinge-CH2-CH3-(domain linker)-IL-15Rα(sushi) domain-(domain linker), where CH2-CH3 is a second Fc domain; c) a third monomer that includes an IL-15 variant; and d) a fourth and fifth monomer that each include, from N- to C-terminus, a VL-CL, where VL is a variable light domain, where the VH of the first monomer and the VL of the fourth monomer form a first TIM-3 binding domain, where the VH of the second monomer and the VL of the fifth monomer form a second TIM-3 binding domain, and where the IL-15 variant and the IL-15Rα(sushi) domain form an IL-15 complex. Any useful domain linker can be used to attach the various components of the heterodimeric protein including, but not limited to those in Figures 8 and 9A-C.

[00372] In the mAb-ncIL-15/Rα format, one preferred embodiment utilizes an anti-TIM-3 ABD having any of the variable heavy and light domain pairs as shown in Figure 12.

[00373] In the mAb-ncIL-15/Rα format, one preferred embodiment utilizes an anti-TIM-3 ABD having the variable heavy and light domain pair of 3H3[TIM-3]_H1_L2.1 as shown in Figure 12.

[00374] In one embodiment, the targeted IL-15/IL-15Rα heterodimeric protein is an "mAb-ncIL-15/Rα" format heterodimeric protein that includes: a) a first monomer that includes, from N- to C-terminus, a VH-hinge-CH2-CH3, where CH2-CH3 is a first Fc domain; b) a second monomer that includes, from N- to C-terminus, a VH-hinge-CH2-CH3-(domain linker)-IL-15Rα(sushi) domain-(domain linker), where CH2-CH3 is a second Fc domain; c) a third monomer that includes an IL-15 variant; and d) a fourth and fifth monomer that each include, from N- to C-terminus, a VL-CL, where VL is a variable light domain, where the VH of the first monomer and the VL of the fourth monomer are the variable heavy domain and variable light domain of 3H3[TIM-3]_H1_L2.1, respectively,

where the VH of the second monomer and the VL of the fifth monomer are the variable heavy domain and variable light domain of 3H3[TIM-3]_H1_L2.1, respectively, and where the IL-15 variant and the IL-15Rα(sushi) domain form an IL-15 complex.

[00375] In the mAb-ncIL-15/Rα format, one preferred embodiment utilizes an IL-15 variant that includes amino acid substitutions N4D/N65D, D30N/N65D, or D30N/E64Q/N65D. In one embodiment, the targeted IL-15/IL-15Rα heterodimeric protein is an "mAb-ncIL-15/Rα" format heterodimeric protein that includes: a) a first monomer that includes, from N- to C-terminus, a VH-hinge-CH2-CH3, where CH2-CH3 is a first Fc domain; b) a second monomer that includes, from N- to C-terminus, a VH-hinge-CH2-CH3-(domain linker)-IL-15Rα(sushi) domain-(domain linker), where CH2-CH3 is a second Fc domain; c) a third monomer that includes an IL-15 variant; and d) a fourth and fifth monomer that each include, from N- to C-terminus, a VL-CL, where VL is a variable light domain, where the VH of the first monomer and the VL of the fourth monomer form a first TIM-3 binding domain, where the VH of the second monomer and the VL of the fifth monomer form a second TIM-3 binding domain, where the IL-15 variant and the IL-15Rα(sushi) domain form an IL-15 complex, where the IL-15 variant and the IL-15Rα(sushi) domain form an IL-15 complex, and where the IL-15 variant includes amino acid substitutions N4D/N65D, D30N/N65D, or D30N/E64Q/N65D. In an exemplary embodiment, the VH and VL are the VH and VL of any of the TIM-3 ABDs in Figures 12 and 13A-C and the IL-15 variant includes amino acid substitutions N4D/N65D. In another exemplary embodiment, the VH and VL are the VH and VL of any of the TIM-3 ABDs in Figures 12 and 13A-C and the IL-15 variant includes amino acid substitutions D30N/N65D. In yet another exemplary embodiment, the VH and VL are the VH and VL of any of the TIM-3 ABDs in Figures 12 and 13A-C and the IL-15 variant includes amino acid substitutions D30N/E64Q/N65D.

[00376] In the mAb-ncIL-15/Rα format, one preferred embodiment utilizes an anti-TIM-3 ABD having the variable heavy and light domain pair of 3H3[TIM-3]_H1_L2.1 as shown in Figure 12, with either the IL-15 N4D/N65D variant or the IL-15 D30N/N65D variant or the IL-15 D30N/E64Q/N65D variant. In one embodiment, the targeted IL-15/IL-15Rα heterodimeric protein is an "mAb-ncIL-15/Rα" format heterodimeric protein that

includes: a) a first monomer that includes, from N- to C-terminus, a VH-hinge-CH2-CH3, where CH2-CH3 is a first Fc domain; b) a second monomer that includes, from N- to C-terminus, a VH-hinge-CH2-CH3-(domain linker)-IL-15Rα(sushi) domain-(domain linker), where CH2-CH3 is a second Fc domain; c) a third monomer that includes an IL-15 variant; and d) a fourth and fifth monomer that each include, from N- to C-terminus, a VL-CL, where VL is a variable light domain, where the VH of the first monomer and the VL of the fourth monomer form a first TIM-3 binding domain, where the VH of the second monomer and the VL of the fifth monomer form a second TIM-3 binding domain, where the IL-15 variant and the IL-15Rα(sushi) domain form an IL-15 complex, where the IL-15 variant and the IL-15Rα(sushi) domain form an IL-15 complex, where VH and VL are the variable heavy domain and variable light domain of 3H3[TIM-3]_H1_L2.1, and where the IL-15 variant includes amino acid substitutions N4D/N65D, D30N/N65D, or D30N/E64Q/N65D. In a particular embodiment, the IL-15 variant includes amino acid substitutions N4D/N65D and VH and VL are the variable heavy domain and variable light domain of 3H3[TIM-3]_H1_L2.1. In one embodiment, the IL-15 variant includes amino acid substitutions D30N/N65D and VH and VL are the variable heavy domain and variable light domain of 3H3[TIM-3]_H1_L2.1. In another embodiment, the IL-15 variant includes amino acid substitutions D30N/E64Q/N65D and VH and VL are the variable heavy domain and variable light domain of 3H3[TIM-3]_H1_L2.1.

[00377] In the mAb-ncIL-15/Rα format, one preferred embodiment utilizes the skew variant pair S364K/E357Q : L368D/K370S. In one embodiment, the targeted IL-15/IL-15Rα heterodimeric protein is an "mAb-ncIL-15/Rα" format heterodimeric protein that includes: a) a first monomer that includes, from N- to C-terminus, a VH-hinge-CH2-CH3, where CH2-CH3 is a first variant Fc domain; b) a second monomer that includes, from N- to C-terminus, a VH-hinge-CH2-CH3-(domain linker)-IL-15Rα(sushi) domain-(domain linker), where CH2-CH3 is a second variant Fc domain; c) a third monomer that includes an IL-15 variant; and d) a fourth and fifth monomer that each include, from N- to C-terminus, a VL-CL, where VL is a variable light domain, where the VH of the first monomer and the VL of the fourth monomer form a first TIM-3 binding domain, where the VH of the second monomer and the VL of the fifth monomer form a second TIM-3 binding domain, where the IL-15 variant and

the IL-15Rα(sushi) domain form an IL-15 complex, and where the first and second variant Fc domains include the skew variant pair S364K/E357Q : L368D/K370S. In an exemplary embodiment, the first variant Fc domain includes skew variants L368D/K370S, and the second variant Fc domain includes skew variants S364K/E357Q. In another exemplary embodiment, the first variant Fc domain includes skew variants S364K/E357Q, and the second variant Fc domain includes skew variants L368D/K370S.

[00378] In one embodiment, the targeted IL-15/IL-15Rα heterodimeric protein is an "mAb-ncIL-15/Rα" format heterodimeric protein that includes: a) a first monomer that includes, from N- to C-terminus, a VH-hinge-CH2-CH3, where CH2-CH3 is a first variant Fc domain; b) a second monomer that includes, from N- to C-terminus, a VH-hinge-CH2-CH3-(domain linker)-IL-15Rα(sushi) domain-(domain linker), where CH2-CH3 is a second variant Fc domain; c) a third monomer that includes an IL-15 variant; and d) a fourth and fifth monomer that each include, from N- to C-terminus, a VL-CL, where VL is a variable light domain, where the VH of the first monomer and the VL of the fourth monomer form a first TIM-3 binding domain, where the VH of the second monomer and the VL of the fifth monomer form a second TIM-3 binding domain, where the IL-15 variant and the IL-15Rα(sushi) domain form an IL-15 complex, where the IL-15 variant includes amino acid substitutions N4D/N65D, D30N/N65D, or D30N/E64Q/N65D, and where the first and second variant Fc domains include the skew variant pair S364K/E357Q : L368D/K370S. In an exemplary embodiment, the first variant Fc domain includes skew variants L368D/K370S, and the second variant Fc domain includes skew variants S364K/E357Q. In another exemplary embodiment, the first variant Fc domain includes skew variants S364K/E357Q, and the second variant Fc domain includes skew variants L368D/K370S. In an exemplary embodiment, the VH and VL are the VH and VL of any of the TIM-3 ABDs in Figures 12 and 13A-C and the IL-15 variant includes amino acid substitutions N4D/N65D. In another exemplary embodiment, the VH and VL are the VH and VL of any of the TIM-3 ABDs in Figures 12 and 13A-C and the IL-15 variant includes amino acid substitutions D30N/N65D. In yet another exemplary embodiment, the VH and VL are the VH and VL of any of the TIM-3 ABDs in Figures 12 and 13A-C and the IL-15 variant includes amino acid substitutions D30N/E64Q/N65D.

[00379] In the mAb-ncIL-15/Rα format, one preferred embodiment utilizes an anti-TIM-3 ABD having the variable heavy and light domain pair of 3H3[TIM-3]_H1_L2.1 as shown in Figure 12 and the skew variant pair S364K/E357Q : L368D/K370S. In one embodiment, the targeted IL-15/IL-15Rα heterodimeric protein is an "mAb-ncIL-15/Rα" format heterodimeric protein that includes: a) a first monomer that includes, from N- to C-terminus, a VH-hinge-CH2-CH3, where CH2-CH3 is a first variant Fc domain; b) a second monomer that includes, from N- to C-terminus, a VH-hinge-CH2-CH3-(domain linker)-IL-15Rα(sushi) domain-(domain linker), where CH2-CH3 is a second variant Fc domain; c) a third monomer that includes an IL-15 variant; and d) a fourth and fifth monomer that each include, from N- to C-terminus, a VL-CL, where VL is a variable light domain, where the VH of the first monomer and the VL of the fourth monomer are the variable heavy domain and variable light domain of 3H3[TIM-3]_H1_L2.1, respectively, where the VH of the second monomer and the VL of the fifth monomer are the variable heavy domain and variable light domain of 3H3[TIM-3]_H1_L2.1, respectively, where the IL-15 variant and the IL-15Rα(sushi) domain form an IL-15 complex, and where the first and second variant Fc domains include the skew variant pair S364K/E357Q : L368D/K370S.

[00380] In the mAb-ncIL-15/Rα format, one preferred embodiment utilizes an anti-TIM-3 ABD having the variable heavy and light domain pair of 3H3[TIM-3]_H1_L2.1 as shown in Figure 12 and the skew variant pair S364K/E357Q : L368D/K370S with either the IL-15 N4D/N65D variant or the IL-15 D30N/N65D variant or the IL-15 D30N/E64Q/N65D variant. In one embodiment, the targeted IL-15/IL-15Rα heterodimeric protein is an "mAb-ncIL-15/Rα" format heterodimeric protein that includes: a) a first monomer that includes, from N- to C-terminus, a VH-hinge-CH2-CH3, where CH2-CH3 is a first variant Fc domain; b) a second monomer that includes, from N- to C-terminus, a VH-hinge-CH2-CH3-(domain linker)-IL-15Rα(sushi) domain-(domain linker), where CH2-CH3 is a second variant Fc domain; c) a third monomer that includes an IL-15 variant; and d) a fourth and fifth monomer that each include, from N- to C-terminus, a VL-CL, where VL is a variable light domain, where the VH of the first monomer and the VL of the fourth monomer form a first TIM-3 binding domain, where the VH of the second monomer and the VL of the fifth monomer form a second TIM-3 binding domain, where the IL-15 variant and the IL-

15Rα(sushi) domain form an IL-15 complex, where the IL-15 variant and the IL-15Rα(sushi) domain form an IL-15 complex, where VH and VL are the variable heavy domain and variable light domain of 3H3[TIM-3]_H1_L2.1, where the IL-15 variant includes amino acid substitutions N4D/N65D, D30N/N65D, or D30N/E64Q/N65D, and where the first and second variant Fc domains include the skew variant pair S364K/E357Q : L368D/K370S. In an exemplary embodiment, the first variant Fc domain includes skew variants L368D/K370S, and the second variant Fc domain includes skew variants S364K/E357Q. In an exemplary embodiment, the first variant Fc domain includes skew variants S364K/E357Q, and the second variant Fc domain includes skew variants L368D/K370S. In a particular embodiment, the IL-15 variant includes amino acid substitutions N4D/N65D and VH and VL are the variable heavy domain and variable light domain of 3H3[TIM-3]_H1_L2.1. In one embodiment, the IL-15 variant includes amino acid substitutions D30N/N65D and VH and VL are the variable heavy domain and variable light domain of 3H3[TIM-3]_H1_L2.1. In another embodiment, the IL-15 variant includes amino acid substitutions D30N/E64Q/N65D and VH and VL are the variable heavy domain and variable light domain of 3H3[TIM-3]_H1_L2.1.

[00381] In the mAb-ncIL-15/Rα format, one preferred embodiment utilizes the skew variant set S364K/E357Q : L368D/K370S, the pI variants N208D/Q295E/N384D/Q418D/N421D and/or Q196K/I199T/P271R/P228R/N276K, the ablation variants E233P/L234V/L235A/G236_/S267K on both first and second monomers, and optionally the 428L/434S variants on both first and second monomers.

[00382] In one embodiment, the targeted IL-15/IL-15Rα heterodimeric protein is an "mAb-ncIL-15/Rα" format heterodimeric protein that includes: a) a first monomer that includes, from N- to C-terminus, a VH-hinge-CH2-CH3, where CH2-CH3 is a first variant Fc domain; b) a second monomer that includes, from N- to C-terminus, a VH-hinge-CH2-CH3-(domain linker)-IL-15Rα(sushi) domain-(domain linker), where CH2-CH3 is a second variant Fc domain; c) a third monomer that includes an IL-15 variant; and d) a fourth and fifth monomer that each include, from N- to C-terminus, a VL-CL, where VL is a variable light domain, where the VH of the first monomer and the VL of the fourth monomer form a first TIM-3 binding domain, where the VH of the second monomer and the VL of the fifth

monomer form a second TIM-3 binding domain, where the IL-15 variant and the IL-15Rα(sushi) domain form an IL-15 complex, where the IL-15 variant and the IL-15Rα(sushi) domain form an IL-15 complex, where the first variant Fc domain includes skew variants L368D/K370S and the second variant Fc domain include the skew variant pair S364K/E357Q, where the first and second variant Fc domains each include FcKO variants E233P/L234V/L235A/G236del/S267K, where the hinge-first variant Fc domain of the first monomer includes pI substitutions N208D/Q295E/N384D/Q418D/N421D and the hinge-second variant Fc domain of the second monomer includes pI variants Q196K/I199T/P271R/P228R/N276K, and where numbering is according to EU numbering. In one embodiment, the targeted IL-15/IL-15Rα heterodimeric protein is an "mAb-ncIL-15/Rα" format heterodimeric protein that includes: a) a first monomer that includes, from N- to C-terminus, a VH-hinge-CH2-CH3, where CH2-CH3 is a first variant Fc domain; b) a second monomer that includes, from N- to C-terminus, a VH-hinge-CH2-CH3-(domain linker)-IL-15Rα(sushi) domain-(domain linker), where CH2-CH3 is a second variant Fc domain; c) a third monomer that includes an IL-15 variant; and d) a fourth and fifth monomer that each include, from N- to C-terminus, a VL-CL, where VL is a variable light domain, where the VH of the first monomer and the VL of the fourth monomer form a first TIM-3 binding domain, where the VH of the second monomer and the VL of the fifth monomer form a second TIM-3 binding domain, where the IL-15 variant and the IL-15Rα(sushi) domain form an IL-15 complex, where the IL-15 variant and the IL-15Rα(sushi) domain form an IL-15 complex, where the first variant Fc domain includes skew variants L368D/K370S and the second variant Fc domain include the skew variant pair S364K/E357Q, where the first and second variant Fc domains each include FcKO variants E233P/L234V/L235A/G236del/S267K, where the hinge-first variant Fc domain of the first monomer includes pI substitutions N208D/Q295E/N384D/Q418D/N421D, and where numbering is according to EU numbering. In one embodiment, the targeted IL-15/IL-15Rα heterodimeric protein is an "mAb-ncIL-15/Rα" format heterodimeric protein that includes: a) a first monomer that includes, from N- to C-terminus, a VH-hinge-CH2-CH3, where CH2-CH3 is a first variant Fc domain; b) a second monomer that includes, from N- to C-terminus, a VH-hinge-CH2-CH3-(domain linker)-IL-15Rα(sushi) domain-(domain linker), where CH2-CH3 is a second variant Fc

domain; c) a third monomer that includes an IL-15 variant; and d) a fourth and fifth monomer that each include, from N- to C-terminus, a VL-CL, where VL is a variable light domain, where the VH of the first monomer and the VL of the fourth monomer form a first TIM-3 binding domain, where the VH of the second monomer and the VL of the fifth monomer form a second TIM-3 binding domain, where the IL-15 variant and the IL-15Rα(sushi) domain form an IL-15 complex, where the IL-15 variant and the IL-15Rα(sushi) domain form an IL-15 complex, where the first variant Fc domain includes skew variants L368D/K370S and the second variant Fc domain include the skew variant pair S364K/E357Q, where the first and second variant Fc domains each include FcKO variants E233P/L234V/L235A/G236del/S267K, where the hinge-second variant Fc domain of the second monomer includes pI variants Q196K/I199T/P271R/P228R/N276K, and where numbering is according to EU numbering. In one embodiment, the targeted IL-15/IL-15Rα heterodimeric protein is an "mAb-ncIL-15/Rα" format heterodimeric protein that includes: a) a first monomer that includes, from N- to C-terminus, a VH-hinge-CH2-CH3, where CH2-CH3 is a first variant Fc domain; b) a second monomer that includes, from N- to C-terminus, a VH-hinge-CH2-CH3-(domain linker)-IL-15Rα(sushi) domain-(domain linker), where CH2-CH3 is a second variant Fc domain; c) a third monomer that includes an IL-15 variant; and d) a fourth and fifth monomer that each include, from N- to C-terminus, a VL-CL, where VL is a variable light domain, where the VH of the first monomer and the VL of the fourth monomer form a first TIM-3 binding domain, where the VH of the second monomer and the VL of the fifth monomer form a second TIM-3 binding domain, where the IL-15 variant and the IL-15Rα(sushi) domain form an IL-15 complex, where the IL-15 variant and the IL-15Rα(sushi) domain form an IL-15 complex, where the first variant Fc domain includes skew variants S364K/E357Q and the second variant Fc domain include the skew variant pair L368D/K370S, where the first and second variant Fc domains each include FcKO variants E233P/L234V/L235A/G236del/S267K, where the hinge-first variant Fc domain of the first monomer includes pI substitutions Q196K/I199T/P271R/P228R/N276K and the hinge-second variant Fc domain of the second monomer includes pI variants N208D/Q295E/N384D/Q418D/N421D, and where numbering is according to EU numbering. In one embodiment, the targeted IL-15/IL-15Rα heterodimeric protein is an "mAb-ncIL-

15/Rα" format heterodimeric protein that includes: a) a first monomer that includes, from N- to C-terminus, a VH-hinge-CH2-CH3, where CH2-CH3 is a first variant Fc domain; b) a second monomer that includes, from N- to C-terminus, a VH-hinge-CH2-CH3-(domain linker)-IL-15Rα(sushi) domain-(domain linker), where CH2-CH3 is a second variant Fc domain; c) a third monomer that includes an IL-15 variant; and d) a fourth and fifth monomer that each include, from N- to C-terminus, a VL-CL, where VL is a variable light domain, where the VH of the first monomer and the VL of the fourth monomer form a first TIM-3 binding domain, where the VH of the second monomer and the VL of the fifth monomer form a second TIM-3 binding domain, where the IL-15 variant and the IL-15Rα(sushi) domain form an IL-15 complex, where the IL-15 variant and the IL-15Rα(sushi) domain form an IL-15 complex, where the first variant Fc domain includes skew variants S364K/E357Q and the second variant Fc domain include the skew variant pair L368D/K370S, where the first and second variant Fc domains each include FcKO variants E233P/L234V/L235A/G236del/S267K, where the hinge-first variant Fc domain of the first monomer includes pI substitutions Q196K/I199T/P271R/P228R/N276K, and where numbering is according to EU numbering. In one embodiment, the targeted IL-15/IL-15Rα heterodimeric protein is an "mAb-ncIL-15/Rα" format heterodimeric protein that includes: a) a first monomer that includes, from N- to C-terminus, a VH-hinge-CH2-CH3, where CH2-CH3 is a first variant Fc domain; b) a second monomer that includes, from N- to C-terminus, a VH-hinge-CH2-CH3-(domain linker)-IL-15Rα(sushi) domain-(domain linker), where CH2-CH3 is a second variant Fc domain; c) a third monomer that includes an IL-15 variant; and d) a fourth and fifth monomer that each include, from N- to C-terminus, a VL-CL, where VL is a variable light domain, where the VH of the first monomer and the VL of the fourth monomer form a first TIM-3 binding domain, where the VH of the second monomer and the VL of the fifth monomer form a second TIM-3 binding domain, where the IL-15 variant and the IL-15Rα(sushi) domain form an IL-15 complex, where the IL-15 variant and the IL-15Rα(sushi) domain form an IL-15 complex, where the first variant Fc domain includes skew variants S364K/E357Q and the second variant Fc domain include the skew variant pair L368D/K370S, where the first and second variant Fc domains each include FcKO variants E233P/L234V/L235A/G236del/S267K, where the hinge-second variant Fc domain of the

second monomer includes pI variants N208D/Q295E/N384D/Q418D/N421D, and where numbering is according to EU numbering. In certain embodiments, the first and second variant Fc domains each further include half-life extension variants M428L/N434S. In an exemplary embodiment, the IL-15 variant includes amino acid substitutions N4D/N65D, D30N/N65D, or D30N/E64Q/N65D. In an exemplary embodiment, the VH and VL are the VH and VL of any of the TIM-3 ABDs in Figures 12 and 13A-C and the IL-15 variant includes amino acid substitutions N4D/N65D. In another exemplary embodiment, the VH and VL are the VH and VL of any of the TIM-3 ABDs in Figures 12 and 13A-C and the IL-15 variant includes amino acid substitutions D30N/N65D. In yet another exemplary embodiment, the VH and VL are the VH and VL of any of the TIM-3 ABDs in Figures 12 and 13A-C and the IL-15 variant includes amino acid substitutions D30N/E64Q/N65D.

[00383] In the mAb-ncIL-15/Rα format, one preferred embodiment utilizes an anti-TIM-3 ABD variable heavy and light domain pair of 3H3[TIM-3]_H1_L2.1 as shown in Figure 12 with the Figures 21H format, the skew variant set S364K/E357Q : L368D/K370S, the pI variants N208D/Q295E/N384D/Q418D/N421D and/or Q196K/I199T/P271R/P228R/N276K, the ablation variants E233P/L234V/L235A/G236_/S267K on both first and second monomers, and optionally the 428L/434S variants on both first and second monomers. In an exemplary embodiment, the IL-15 variant includes amino acid substitutions N4D/N65D, D30N/N65D, or D30N/E64Q/N65D.

[00384] In one embodiment, the targeted IL-15/IL-15Rα heterodimeric protein is an "mAb-ncIL-15/Rα" format heterodimeric protein that includes: a) a first monomer that includes, from N- to C-terminus, a VH-hinge-CH2-CH3, where CH2-CH3 is a first variant Fc domain; b) a second monomer that includes, from N- to C-terminus, a VH-hinge-CH2-CH3-(domain linker)-IL-15Rα(sushi) domain-(domain linker), where CH2-CH3 is a second variant Fc domain; c) a third monomer that includes an IL-15 variant; and d) a fourth and fifth monomer that each include, from N- to C-terminus, a VL-CL, where VL is a variable light domain, where the VH of the first monomer and the VL of the fourth monomer form a first TIM-3 binding domain, where the VH of the second monomer and the VL of the fifth monomer form a second TIM-3 binding domain, where the IL-15 variant and the IL-15Rα(sushi) domain form an IL-15 complex, where the IL-15 variant and the IL-15Rα(sushi)

domain form an IL-15 complex, where VH and VL are the variable heavy domain and variable light domain of 3H3[TIM-3]_H1_L2.1, where the first variant Fc domain includes skew variants L368D/K370S and the second variant Fc domain include the skew variant pair S364K/E357Q, where the first and second variant Fc domains each include FcKO variants E233P/L234V/L235A/G236del/S267K, where the hinge-first variant Fc domain of the first monomer includes pI substitutions N208D/Q295E/N384D/Q418D/N421D and the hinge-second variant Fc domain of the second monomer includes pI variants Q196K/I199T/P271R/P228R/N276K, and where numbering is according to EU numbering. In one embodiment, the targeted IL-15/IL-15Rα heterodimeric protein is an "mAb-ncIL-15/Rα" format heterodimeric protein that includes: a) a first monomer that includes, from N- to C-terminus, a VH-hinge-CH2-CH3, where CH2-CH3 is a first variant Fc domain; b) a second monomer that includes, from N- to C-terminus, a VH-hinge-CH2-CH3-(domain linker)-IL-15Rα(sushi) domain-(domain linker), where CH2-CH3 is a second variant Fc domain; c) a third monomer that includes an IL-15 variant; and d) a fourth and fifth monomer that each include, from N- to C-terminus, a VL-CL, where VL is a variable light domain, where the VH of the first monomer and the VL of the fourth monomer form a first TIM-3 binding domain, where the VH of the second monomer and the VL of the fifth monomer form a second TIM-3 binding domain, where the IL-15 variant and the IL-15Rα(sushi) domain form an IL-15 complex, where the IL-15 variant and the IL-15Rα(sushi) domain form an IL-15 complex, where VH and VL are the variable heavy domain and variable light domain of 3H3[TIM-3]_H1_L2.1, where the first variant Fc domain includes skew variants L368D/K370S and the second variant Fc domain include the skew variant pair S364K/E357Q, where the first and second variant Fc domains each include FcKO variants E233P/L234V/L235A/G236del/S267K, where the hinge-first variant Fc domain of the first monomer includes pI substitutions N208D/Q295E/N384D/Q418D/N421D, and where numbering is according to EU numbering. In one embodiment, the targeted IL-15/IL-15Rα heterodimeric protein is an "mAb-ncIL-15/Rα" format heterodimeric protein that includes: a) a first monomer that includes, from N- to C-terminus, a VH-hinge-CH2-CH3, where CH2-CH3 is a first variant Fc domain; b) a second monomer that includes, from N- to C-terminus, a VH-hinge-CH2-CH3-(domain linker)-IL-15Rα(sushi) domain-(domain linker), where CH2-

CH3 is a second variant Fc domain; c) a third monomer that includes an IL-15 variant; and d) a fourth and fifth monomer that each include, from N- to C-terminus, a VL-CL, where VL is a variable light domain, where the VH of the first monomer and the VL of the fourth monomer form a first TIM-3 binding domain, where the VH of the second monomer and the VL of the fifth monomer form a second TIM-3 binding domain, where the IL-15 variant and the IL-15Rα(sushi) domain form an IL-15 complex, where the IL-15 variant and the IL-15Rα(sushi) domain form an IL-15 complex, where VH and VL are the variable heavy domain and variable light domain of 3H3[TIM-3]_H1_L2.1, where the first variant Fc domain includes skew variants L368D/K370S and the second variant Fc domain include the skew variant pair S364K/E357Q, where the first and second variant Fc domains each include FcKO variants E233P/L234V/L235A/G236del/S267K, where the hinge-second variant Fc domain of the second monomer includes pI variants Q196K/I199T/P271R/P228R/N276K, and where numbering is according to EU numbering. In one embodiment, the targeted IL-15/IL-15Rα heterodimeric protein is an "mAb-ncIL-15/Rα" format heterodimeric protein that includes: a) a first monomer that includes, from N- to C-terminus, a VH-hinge-CH2-CH3, where CH2-CH3 is a first variant Fc domain; b) a second monomer that includes, from N- to C-terminus, a VH-hinge-CH2-CH3-(domain linker)-IL-15Rα(sushi) domain-(domain linker), where CH2-CH3 is a second variant Fc domain; c) a third monomer that includes an IL-15 variant; and d) a fourth and fifth monomer that each include, from N- to C-terminus, a VL-CL, where VL is a variable light domain, where the VH of the first monomer and the VL of the fourth monomer form a first TIM-3 binding domain, where the VH of the second monomer and the VL of the fifth monomer form a second TIM-3 binding domain, where the IL-15 variant and the IL-15Rα(sushi) domain form an IL-15 complex, where the IL-15 variant and the IL-15Rα(sushi) domain form an IL-15 complex, where VH and VL are the variable heavy domain and variable light domain of 3H3[TIM-3]_H1_L2.1, where the first variant Fc domain includes skew variants S364K/E357Q and the second variant Fc domain include the skew variant pair L368D/K370S, where the first and second variant Fc domains each include FcKO variants E233P/L234V/L235A/G236del/S267K, where the hinge-first variant Fc domain of the first monomer includes pI substitutions Q196K/I199T/P271R/P228R/N276K and the hinge-second variant Fc domain of the second monomer includes pI variants

N208D/Q295E/N384D/Q418D/N421D, and where numbering is according to EU numbering. In one embodiment, the targeted IL-15/IL-15Rα heterodimeric protein is an "mAb-ncIL-15/Rα" format heterodimeric protein that includes: a) a first monomer that includes, from N- to C-terminus, a VH-hinge-CH2-CH3, where CH2-CH3 is a first variant Fc domain; b) a second monomer that includes, from N- to C-terminus, a VH-hinge-CH2-CH3-(domain linker)-IL-15Rα(sushi) domain-(domain linker), where CH2-CH3 is a second variant Fc domain; c) a third monomer that includes an IL-15 variant; and d) a fourth and fifth monomer that each include, from N- to C-terminus, a VL-CL, where VL is a variable light domain, where the VH of the first monomer and the VL of the fourth monomer form a first TIM-3 binding domain, where the VH of the second monomer and the VL of the fifth monomer form a second TIM-3 binding domain, where the IL-15 variant and the IL-15Rα(sushi) domain form an IL-15 complex, where the IL-15 variant and the IL-15Rα(sushi) domain form an IL-15 complex, where VH and VL are the variable heavy domain and variable light domain of 3H3[TIM-3]_H1_L2.1, where the first variant Fc domain includes skew variants S364K/E357Q and the second variant Fc domain include the skew variant pair L368D/K370S, where the first and second variant Fc domains each include FcKO variants E233P/L234V/L235A/G236del/S267K, where the hinge-first variant Fc domain of the first monomer includes pI substitutions Q196K/I199T/P271R/P228R/N276K, and where numbering is according to EU numbering. In one embodiment, the targeted IL-15/IL-15Rα heterodimeric protein is an "mAb-ncIL-15/Rα" format heterodimeric protein that includes: a) a first monomer that includes, from N- to C-terminus, a VH-hinge-CH2-CH3, where CH2-CH3 is a first variant Fc domain; b) a second monomer that includes, from N- to C-terminus, a VH-hinge-CH2-CH3-(domain linker)-IL-15Rα(sushi) domain-(domain linker), where CH2-CH3 is a second variant Fc domain; c) a third monomer that includes an IL-15 variant; and d) a fourth and fifth monomer that each include, from N- to C-terminus, a VL-CL, where VL is a variable light domain, where the VH of the first monomer and the VL of the fourth monomer form a first TIM-3 binding domain, where the VH of the second monomer and the VL of the fifth monomer form a second TIM-3 binding domain, where the IL-15 variant and the IL-15Rα(sushi) domain form an IL-15 complex, where the IL-15 variant and the IL-15Rα(sushi) domain form an IL-15 complex, where VH and VL are the variable heavy

domain and variable light domain of 3H3[TIM-3]_H1_L2.1, where the first variant Fc domain includes skew variants S364K/E357Q and the second variant Fc domain include the skew variant pair L368D/K370S, where the first and second variant Fc domains each include FcKO variants E233P/L234V/L235A/G236del/S267K, where the hinge-second variant Fc domain of the second monomer includes pI variants N208D/Q295E/N384D/Q418D/N421D, and where numbering is according to EU numbering. In certain embodiments, the first and second variant Fc domains each further include half-life extension variants M428L/N434S. In a particular embodiment, the IL-15 variant includes amino acid substitutions N4D/N65D and VH and VL are the variable heavy domain and variable light domain of 3H3[TIM-3]_H1_L2.1. In one embodiment, the IL-15 variant includes amino acid substitutions D30N/N65D and VH and VL are the variable heavy domain and variable light domain of 3H3[TIM-3]_H1_L2.1. In another embodiment, the IL-15 variant includes amino acid substitutions D30N/E64Q/N65D and VH and VL are the variable heavy domain and variable light domain of 3H3[TIM-3]_H1_L2.1.

I. mAb-dsIL-15/Rα

[00385] This embodiment is shown in Figures 21I, and comprises four monomers (although the heterodimeric fusion protein is a pentamer). The first monomer comprises a heavy chain, VH-CH1-hinge-CH2-CH3. The second monomer comprises a heavy chain with an IL-15Rα(sushi) domain: e.g., VH-CH1-hinge-CH2-CH3-domain linker- IL-15Rα(sushi) domain, where the IL-15Rα(sushi) domain has been engineered to contain a cysteine residue. The third monomer is a variant IL-15 domain, which has been engineered to contain a cysteine residue, such that the IL-15 complex is formed under physiological conditions. The fourth (and fifth) monomer are light chains, VL-CL. This is generally referred to as "mAb-dsIL-15/Rα", with the "ds" standing for "disulfide", and it binds TIM-3 bivalently.

[00386] In one embodiment, the targeted IL-15/IL-15Rα heterodimeric protein is an "mAb-dsIL-15/Rα" format heterodimeric protein that includes: a) a first monomer that includes, from N- to C-terminus, a VH-hinge-CH2-CH3, where CH2-CH3 is a first Fc domain; b) a second monomer that includes, from N- to C-terminus, a VH-hinge-CH2-CH3-(domain linker)-IL-15Rα(sushi) domain-(domain linker), where the IL-15Rα(sushi) domain

includes an amino acid substitution for a cysteine residue and CH2-CH3 is a second Fc domain; c) a third monomer that includes an IL-15 variant that includes an amino acid substitution for a cysteine residue; and d) a fourth and fifth monomer that each include, from N- to C-terminus, a VL-CL, where VL is a variable light domain, where the VH of the first monomer and the VL of the fourth monomer form a first TIM-3 binding domain, where the VH of the second monomer and the VL of the fifth monomer form a second TIM-3 binding domain, and where the cysteine residue on the IL-15 variant and the cysteine residue on the IL-15Rα(sushi) domain form a disulfide bond. Any useful domain linker can be used to attach the various components of the heterodimeric protein including, but not limited to those in Figures 8 and 9A-C.

[00387] In the mAb-dsIL-15/Rα format, one preferred embodiment utilizes an anti-TIM-3 ABD having any of the variable heavy and light domain pairs as shown in Figure 12.

[00388] In the mAb-dsIL-15/Rα format, one preferred embodiment utilizes an anti-TIM-3 ABD having the variable heavy and light domain pair of 3H3[TIM-3]_H1_L2.1 as shown in Figure 12.

[00389] In one embodiment, the targeted IL-15/IL-15Rα heterodimeric protein is an "mAb-dsIL-15/Rα" format heterodimeric protein that includes: a) a first monomer that includes, from N- to C-terminus, a VH-hinge-CH2-CH3, where CH2-CH3 is a first Fc domain; b) a second monomer that includes, from N- to C-terminus, a VH-hinge-CH2-CH3-(domain linker)-IL-15Rα(sushi) domain-(domain linker), where the IL-15Rα(sushi) domain includes an amino acid substitution for a cysteine residue and CH2-CH3 is a second Fc domain; c) a third monomer that includes an IL-15 variant that includes an amino acid substitution for a cysteine residue; and d) a fourth and fifth monomer that each include, from N- to C-terminus, a VL-CL, where VL is a variable light domain, where the VH of the first monomer and the VL of the fourth monomer are the variable heavy domain and variable light domain 3H3[TIM-3]_H1_L2.1, respectively, where the VH of the second monomer and the VL of the fifth monomer are the variable heavy domain and variable light domain of 3H3[TIM-3]_H1_L2.1, respectively, and where the cysteine residue on the IL-15 variant and the cysteine residue on the IL-15Rα(sushi) domain form a disulfide bond.

[00390] In the mAb-dsIL-15/Rα format, one preferred embodiment utilizes an IL-15 variant that includes amino acid substitutions N4D/N65D, D30N/N65D, or D30N/E64Q/N65D, with the appropriate cysteine amino acid substitutions. In one embodiment, the targeted IL-15/IL-15Rα heterodimeric protein is an "mAb-dsIL-15/Rα" format heterodimeric protein that includes: a) a first monomer that includes, from N- to C-terminus, a VH-hinge-CH2-CH3, where CH2-CH3 is a first Fc domain; b) a second monomer that includes, from N- to C-terminus, a VH-hinge-CH2-CH3-(domain linker)-IL-15Rα(sushi) domain-(domain linker), where the IL-15Rα(sushi) domain includes an amino acid substitution for a cysteine residue and CH2-CH3 is a second Fc domain; c) a third monomer that includes an IL-15 variant that includes an amino acid substitution for a cysteine residue; and d) a fourth and fifth monomer that each include, from N- to C-terminus, a VL-CL, where VL is a variable light domain, where the VH of the first monomer and the VL of the fourth monomer form a first TIM-3 binding domain, where the VH of the second monomer and the VL of the fifth monomer form a second TIM-3 binding domain, where the cysteine residue on the IL-15 variant and the cysteine residue on the IL-15Rα(sushi) domain form a disulfide bond, and where the IL-15 variant includes amino acid substitutions N4D/N65D, D30N/N65D, or D30N/E64Q/N65D. In an exemplary embodiment, the VH and VL are the VH and VL of any of the TIM-3 ABDs in Figures 12 and 13A-C and the IL-15 variant includes amino acid substitutions N4D/N65D. In another exemplary embodiment, the VH and VL are the VH and VL of any of the TIM-3 ABDs in Figures 12 and 13A-C and the IL-15 variant includes amino acid substitutions D30N/N65D. In yet another exemplary embodiment, the VH and VL are the VH and VL of any of the TIM-3 ABDs in Figures 12 and 13A-C and the IL-15 variant includes amino acid substitutions D30N/E64Q/N65D.

[00391] In the mAb-dsIL-15/Rα format, one preferred embodiment utilizes an anti-TIM-3 ABD having the variable heavy and light domain pair of 3H3[TIM-3]_H1_L2.1 as shown in Figure 12, with either the IL-15 N4D/N65D variant or the IL-15 D30N/N65D variant or the IL-15 D30N/E64Q/N65D variant, with the appropriate cysteine amino acid substitutions. In one embodiment, the targeted IL-15/IL-15Rα heterodimeric protein is an "mAb-dsIL-15/Rα" format heterodimeric protein that includes: a) a first monomer that includes, from N- to C-terminus, a VH-hinge-CH2-CH3, where CH2-CH3 is a first Fc

domain; b) a second monomer that includes, from N- to C-terminus, a VH-hinge-CH2-CH3-(domain linker)-IL-15Rα(sushi) domain-(domain linker), where the IL-15Rα(sushi) domain includes an amino acid substitution for a cysteine residue and CH2-CH3 is a second Fc domain; c) a third monomer that includes an IL-15 variant that includes an amino acid substitution for a cysteine residue; and d) a fourth and fifth monomer that each include, from N- to C-terminus, a VL-CL, where VL is a variable light domain, where the VH of the first monomer and the VL of the fourth monomer form a first TIM-3 binding domain, where the VH of the second monomer and the VL of the fifth monomer form a second TIM-3 binding domain, where the cysteine residue on the IL-15 variant and the cysteine residue on the IL-15Rα(sushi) domain form a disulfide bond, where VH and VL are the variable heavy domain and variable light domain of 3H3[TIM-3]_H1_L2.1, and where the IL-15 variant includes amino acid substitutions N4D/N65D, D30N/N65D, or D30N/E64Q/N65D. In a particular embodiment, the IL-15 variant includes amino acid substitutions N4D/N65D and VH and VL are the variable heavy domain and variable light domain of 3H3[TIM-3]_H1_L2.1. In one embodiment, the IL-15 variant includes amino acid substitutions D30N/N65D and VH and VL are the variable heavy domain and variable light domain of 3H3[TIM-3]_H1_L2.1. In another embodiment, the IL-15 variant includes amino acid substitutions D30N/E64Q/N65D and VH and VL are the variable heavy domain and variable light domain of 3H3[TIM-3]_H1_L2.1.

[00392] In the mAb-dsIL-15/Rα format, one preferred embodiment utilizes the skew variant pair S364K/E357Q : L368D/K370S. In one embodiment, the targeted IL-15/IL-15Rα heterodimeric protein is an "mAb-dsIL-15/Rα" format heterodimeric protein that includes: a) a first monomer that includes, from N- to C-terminus, a VH-hinge-CH2-CH3, where CH2-CH3 is a first variant Fc domain; b) a second monomer that includes, from N- to C-terminus, a VH-hinge-CH2-CH3-(domain linker)-IL-15Rα(sushi) domain-(domain linker), where the IL-15Rα(sushi) domain includes an amino acid substitution for a cysteine residue and CH2-CH3 is a second variant Fc domain; c) a third monomer that includes an IL-15 variant that includes an amino acid substitution for a cysteine residue; and d) a fourth and fifth monomer that each include, from N- to C-terminus, a VL-CL, where VL is a variable light domain, where the VH of the first monomer and the VL of the fourth monomer form a first

TIM-3 binding domain, where the VH of the second monomer and the VL of the fifth monomer form a second TIM-3 binding domain, where the cysteine residue on the IL-15 variant and the cysteine residue on the IL-15Rα(sushi) domain form a disulfide bond, and where the first and second variant Fc domains include the skew variant pair S364K/E357Q : L368D/K370S. In an exemplary embodiment, the first variant Fc domain includes skew variants L368D/K370S, and the second variant Fc domain includes skew variants S364K/E357Q. In another exemplary embodiment, the first variant Fc domain includes skew variants S364K/E357Q, and the second variant Fc domain includes skew variants L368D/K370S.

[00393] In one embodiment, the targeted IL-15/IL-15Rα heterodimeric protein is an "mAb-dsIL-15/Rα" format heterodimeric protein that includes: a) a first monomer that includes, from N- to C-terminus, a VH-hinge-CH2-CH3, where CH2-CH3 is a first variant Fc domain; b) a second monomer that includes, from N- to C-terminus, a VH-hinge-CH2-CH3-(domain linker)-IL-15Rα(sushi) domain-(domain linker), where the IL-15Rα(sushi) domain includes an amino acid substitution for a cysteine residue and CH2-CH3 is a second variant Fc domain; c) a third monomer that includes an IL-15 variant that includes an amino acid substitution for a cysteine residue; and d) a fourth and fifth monomer that each include, from N- to C-terminus, a VL-CL, where VL is a variable light domain, where the VH of the first monomer and the VL of the fourth monomer form a first TIM-3 binding domain, where the VH of the second monomer and the VL of the fifth monomer form a second TIM-3 binding domain, where the cysteine residue on the IL-15 variant and the cysteine residue on the IL-15Rα(sushi) domain form a disulfide bond, where the IL-15 variant includes amino acid substitutions N4D/N65D, D30N/N65D, or D30N/E64Q/N65D, and where the first and second variant Fc domains include the skew variant pair S364K/E357Q : L368D/K370S. In an exemplary embodiment, the first variant Fc domain includes skew variants L368D/K370S, and the second variant Fc domain includes skew variants S364K/E357Q. In another exemplary embodiment, the first variant Fc domain includes skew variants S364K/E357Q, and the second variant Fc domain includes skew variants L368D/K370S. In an exemplary embodiment, the VH and VL are the VH and VL of any of the TIM-3 ABDs in Figures 12 and 13A-C and the IL-15 variant includes amino acid substitutions N4D/N65D. In another

exemplary embodiment, the VH and VL are the VH and VL of any of the TIM-3 ABDs in Figures 12 and 13A-C and the IL-15 variant includes amino acid substitutions D30N/N65D. In yet another exemplary embodiment, the VH and VL are the VH and VL of any of the TIM-3 ABDs in Figures 12 and 13A-C and the IL-15 variant includes amino acid substitutions D30N/E64Q/N65D.

[00394] In the mAb-dsIL-15/Rα format, one preferred embodiment utilizes an anti-TIM-3 ABD having the variable heavy and light domain pair of 3H3[TIM-3]_H1_L2.1 as shown in Figure 12 and the skew variant pair S364K/E357Q : L368D/K370S. In one embodiment, the targeted IL-15/IL-15Rα heterodimeric protein is an "mAb-dsIL-15/Rα" format heterodimeric protein that includes: a) a first monomer that includes, from N- to C-terminus, a VH-hinge-CH2-CH3, where CH2-CH3 is a first variant Fc domain; b) a second monomer that includes, from N- to C-terminus, a VH-hinge-CH2-CH3-(domain linker)-IL-15Rα(sushi) domain-(domain linker), where the IL-15Rα(sushi) domain includes an amino acid substitution for a cysteine residue and CH2-CH3 is a second variant Fc domain; c) a third monomer that includes an IL-15 variant that includes an amino acid substitution for a cysteine residue; and d) a fourth and fifth monomer that each include, from N- to C-terminus, a VL-CL, where VL is a variable light domain, where the VH of the first monomer and the VL of the fourth monomer are the variable heavy domain and variable light domain of 3H3[TIM-3]_H1_L2.1, respectively, where the VH of the second monomer and the VL of the fifth monomer are the variable heavy domain and variable light domain of 3H3[TIM-3]_H1_L2.1, respectively, where the cysteine residue on the IL-15 variant and the cysteine residue on the IL-15Rα(sushi) domain form a disulfide bond, and where the first and second variant Fc domains include the skew variant pair S364K/E357Q : L368D/K370S.

[00395] In the mAb-dsIL-15/Rα format, one preferred embodiment utilizes an anti-TIM-3 ABD having the variable heavy and light domain pair of 3H3[TIM-3]_H1_L2.1 as shown in Figure 12 and the skew variant pair S364K/E357Q : L368D/K370S with either the IL-15 N4D/N65D variant or the IL-15 D30N/N65D variant or the IL-15 D30N/E64Q/N65D variant with appropriate cysteine substitutions. In one embodiment, the targeted IL-15/IL-15Rα heterodimeric protein is an "mAb-dsIL-15/Rα" format heterodimeric protein that includes: a) a first monomer that includes, from N- to C-terminus, a VH-hinge-CH2-CH3,

where CH2-CH3 is a first Fc domain; b) a second monomer that includes, from N- to C-terminus, a VH-hinge-CH2-CH3-(domain linker)-IL-15Rα(sushi) domain-(domain linker), where the IL-15Rα(sushi) domain includes an amino acid substitution for a cysteine residue and CH2-CH3 is a second Fc domain; c) a third monomer that includes an IL-15 variant that includes an amino acid substitution for a cysteine residue; and d) a fourth and fifth monomer that each include, from N- to C-terminus, a VL-CL, where VL is a variable light domain, where the VH of the first monomer and the VL of the fourth monomer form a first TIM-3 binding domain, where the VH of the second monomer and the VL of the fifth monomer form a second TIM-3 binding domain where the cysteine residue on the IL-15 variant and the cysteine residue on the IL-15Rα(sushi) domain form a disulfide bond, where VH and VL are the variable heavy domain and variable light domain of 3H3[TIM-3]_H1_L2.1, where the IL-15 variant includes amino acid substitutions N4D/N65D, D30N/N65D, or D30N/E64Q/N65D, and where the first and second variant Fc domains include the skew variant pair S364K/E357Q : L368D/K370S. In an exemplary embodiment, the first variant Fc domain includes skew variants L368D/K370S, and the second variant Fc domain includes skew variants S364K/E357Q. In an exemplary embodiment, the first variant Fc domain includes skew variants S364K/E357Q, and the second variant Fc domain includes skew variants L368D/K370S. In a particular embodiment, the IL-15 variant includes amino acid substitutions N4D/N65D and VH and VL are the variable heavy domain and variable light domain of 3H3[TIM-3]_H1_L2.1. In one embodiment, the IL-15 variant includes amino acid substitutions D30N/N65D and VH and VL are the variable heavy domain and variable light domain of 3H3[TIM-3]_H1_L2.1. In another embodiment, the IL-15 variant includes amino acid substitutions D30N/E64Q/N65D and VH and VL are the variable heavy domain and variable light domain of 3H3[TIM-3]_H1_L2.1.

[00396] In the mAb-dsIL-15/Rα format, one preferred embodiment utilizes the skew variant set S364K/E357Q : L368D/K370S, the pI variants N208D/Q295E/N384D/Q418D/N421D and/or Q196K/I199T/P271R/P228R/N276K, the ablation variants E233P/L234V/L235A/G236_/S267K on both first and second monomers, and optionally the 428L/434S variants on both first and second monomers.

[00397] In one embodiment, the targeted IL-15/IL-15Rα heterodimeric protein is an "mAb-dsIL-15/Rα" format heterodimeric protein that includes: a) a first monomer that includes, from N- to C-terminus, a VH-hinge-CH2-CH3, where CH2-CH3 is a first Fc domain; b) a second monomer that includes, from N- to C-terminus, a VH-hinge-CH2-CH3-(domain linker)-IL-15Rα(sushi) domain-(domain linker), where the IL-15Rα(sushi) domain includes an amino acid substitution for a cysteine residue and CH2-CH3 is a second Fc domain; c) a third monomer that includes an IL-15 variant that includes an amino acid substitution for a cysteine residue; and d) a fourth and fifth monomer that each include, from N- to C-terminus, a VL-CL, where VL is a variable light domain, where the VH of the first monomer and the VL of the fourth monomer form a first TIM-3 binding domain, where the VH of the second monomer and the VL of the fifth monomer form a second TIM-3 binding domain, where the cysteine residue on the IL-15 variant and the cysteine residue on the IL-15Rα(sushi) domain form a disulfide bond, where the first variant Fc domain includes skew variants L368D/K370S and the second variant Fc domain include the skew variant pair S364K/E357Q, where the first and second variant Fc domains each include FcKO variants E233P/L234V/L235A/G236del/S267K, where the hinge-first variant Fc domain of the first monomer includes pI substitutions N208D/Q295E/N384D/Q418D/N421D and the hinge-second variant Fc domain of the second monomer includes pI variants Q196K/I199T/P271R/P228R/N276K, and where numbering is according to EU numbering. In one embodiment, the targeted IL-15/IL-15Rα heterodimeric protein is an "mAb-dsIL-15/Rα" format heterodimeric protein that includes: a) a first monomer that includes, from N- to C-terminus, a VH-hinge-CH2-CH3, where CH2-CH3 is a first Fc domain; b) a second monomer that includes, from N- to C-terminus, a VH-hinge-CH2-CH3-(domain linker)-IL-15Rα(sushi) domain-(domain linker), where the IL-15Rα(sushi) domain includes an amino acid substitution for a cysteine residue and CH2-CH3 is a second Fc domain; c) a third monomer that includes an IL-15 variant that includes an amino acid substitution for a cysteine residue; and d) a fourth and fifth monomer that each include, from N- to C-terminus, a VL-CL, where VL is a variable light domain, where the VH of the first monomer and the VL of the fourth monomer form a first TIM-3 binding domain, where the VH of the second monomer and the VL of the fifth

monomer form a second TIM-3 binding domain, where the cysteine residue on the IL-15 variant and the cysteine residue on the IL-15Rα(sushi) domain form a disulfide bond, where the first variant Fc domain includes skew variants L368D/K370S and the second variant Fc domain include the skew variant pair S364K/E357Q, where the first and second variant Fc domains each include FcKO variants E233P/L234V/L235A/G236del/S267K, where the hinge-first variant Fc domain of the first monomer includes pI substitutions N208D/Q295E/N384D/Q418D/N421D, and where numbering is according to EU numbering. In one embodiment, the targeted IL-15/IL-15Rα heterodimeric protein is an "mAb-dsIL-15/Rα" format heterodimeric protein that includes: a) a first monomer that includes, from N- to C-terminus, a VH-hinge-CH2-CH3, where CH2-CH3 is a first Fc domain; b) a second monomer that includes, from N- to C-terminus, a VH-hinge-CH2-CH3-(domain linker)-IL-15Rα(sushi) domain-(domain linker), where the IL-15Rα(sushi) domain includes an amino acid substitution for a cysteine residue and CH2-CH3 is a second Fc domain; c) a third monomer that includes an IL-15 variant that includes an amino acid substitution for a cysteine residue; and d) a fourth and fifth monomer that each include, from N- to C-terminus, a VL-CL, where VL is a variable light domain, where the VH of the first monomer and the VL of the fourth monomer form a first TIM-3 binding domain, where the VH of the second monomer and the VL of the fifth monomer form a second TIM-3 binding domain, where the cysteine residue on the IL-15 variant and the cysteine residue on the IL-15Rα(sushi) domain form a disulfide bond, where the first variant Fc domain includes skew variants L368D/K370S and the second variant Fc domain include the skew variant pair S364K/E357Q, where the first and second variant Fc domains each include FcKO variants E233P/L234V/L235A/G236del/S267K, where the hinge-second variant Fc domain of the second monomer includes pI variants Q196K/I199T/P271R/P228R/N276K, and where numbering is according to EU numbering. In one embodiment, the targeted IL-15/IL-15Rα heterodimeric protein is an "mAb-dsIL-15/Rα" format heterodimeric protein that includes: a) a first monomer that includes, from N- to C-terminus, a VH-hinge-CH2-CH3, where CH2-CH3 is a first Fc domain; b) a second monomer that includes, from N- to

C-terminus, a VH-hinge-CH2-CH3-(domain linker)-IL-15Rα(sushi) domain-(domain linker), where the IL-15Rα(sushi) domain includes an amino acid substitution for a cysteine residue and CH2-CH3 is a second Fc domain; c) a third monomer that includes an IL-15 variant that includes an amino acid substitution for a cysteine residue; and d) a fourth and fifth monomer that each include, from N- to C-terminus, a VL-CL, where VL is a variable light domain, where the VH of the first monomer and the VL of the fourth monomer form a first TIM-3 binding domain, where the VH of the second monomer and the VL of the fifth monomer form a second TIM-3 binding domain, where the cysteine residue on the IL-15 variant and the cysteine residue on the IL-15Rα(sushi) domain form a disulfide bond, where the first variant Fc domain includes skew variants S364K/E357Q and the second variant Fc domain include the skew variant pair L368D/K370S, where the first and second variant Fc domains each include FcKO variants E233P/L234V/L235A/G236del/S267K, where the hinge-first variant Fc domain of the first monomer includes pI substitutions Q196K/I199T/P271R/P228R/N276K and the hinge-second variant Fc domain of the second monomer includes pI variants N208D/Q295E/N384D/Q418D/N421D, and where numbering is according to EU numbering. In one embodiment, the targeted IL-15/IL-15Rα heterodimeric protein is an "mAb-dsIL-15/Rα" format heterodimeric protein that includes: a) a first monomer that includes, from N- to C-terminus, a VH-hinge-CH2-CH3, where CH2-CH3 is a first Fc domain; b) a second monomer that includes, from N- to C-terminus, a VH-hinge-CH2-CH3-(domain linker)-IL-15Rα(sushi) domain-(domain linker), where the IL-15Rα(sushi) domain includes an amino acid substitution for a cysteine residue and CH2-CH3 is a second Fc domain; c) a third monomer that includes an IL-15 variant that includes an amino acid substitution for a cysteine residue; and d) a fourth and fifth monomer that each include, from N- to C-terminus, a VL-CL, where VL is a variable light domain, where the VH of the first monomer and the VL of the fourth monomer form a first TIM-3 binding domain, where the VH of the second monomer and the VL of the fifth monomer form a second TIM-3 binding domain, where the cysteine residue on the IL-15 variant and the cysteine residue on the IL-15Rα(sushi) domain form a disulfide bond, where the first variant Fc domain

includes skew variants S364K/E357Q and the second variant Fc domain include the skew variant pair L368D/K370S, where the first and second variant Fc domains each include FcKO variants E233P/L234V/L235A/G236del/S267K, where the hinge-first variant Fc domain of the first monomer includes pI substitutions Q196K/I199T/P271R/P228R/N276K, and where numbering is according to EU numbering. In one embodiment, the targeted IL-15/IL-15Rα heterodimeric protein is an "mAb-dsIL-15/Rα" format heterodimeric protein that includes: a) a first monomer that includes, from N- to C-terminus, a VH-hinge-CH2-CH3, where CH2-CH3 is a first Fc domain; b) a second monomer that includes, from N- to C-terminus, a VH-hinge-CH2-CH3-(domain linker)-IL-15Rα(sushi) domain-(domain linker), where the IL-15Rα(sushi) domain includes an amino acid substitution for a cysteine residue and CH2-CH3 is a second Fc domain; c) a third monomer that includes an IL-15 variant that includes an amino acid substitution for a cysteine residue; and d) a fourth and fifth monomer that each include, from N- to C-terminus, a VL-CL, where VL is a variable light domain, where the VH of the first monomer and the VL of the fourth monomer form a first TIM-3 binding domain, where the VH of the second monomer and the VL of the fifth monomer form a second TIM-3 binding domain, where the cysteine residue on the IL-15 variant and the cysteine residue on the IL-15Rα(sushi) domain form a disulfide bond, where the first variant Fc domain includes skew variants S364K/E357Q and the second variant Fc domain include the skew variant pair L368D/K370S, where the first and second variant Fc domains each include FcKO variants E233P/L234V/L235A/G236del/S267K, where the hinge-second variant Fc domain of the second monomer includes pI variants N208D/Q295E/N384D/Q418D/N421D, and where numbering is according to EU numbering. In certain embodiments, the first and second variant Fc domains each further include half-life extension variants M428L/N434S. In an exemplary embodiment, the IL-15 variant includes amino acid substitutions N4D/N65D, D30N/N65D, or D30N/E64Q/N65D. In an exemplary embodiment, the IL-15 variant includes amino acid substitutions N4D/N65D, D30N/N65D, or D30N/E64Q/N65D. In an exemplary embodiment, the VH and VL are the VH and VL of any of the TIM-3 ABDs in Figures 12 and 13A-C and the IL-15

variant includes amino acid substitutions N4D/N65D. In another exemplary embodiment, the VH and VL are the VH and VL of any of the TIM-3 ABDs in Figures 12 and 13A-C and the IL-15 variant includes amino acid substitutions D30N/N65D. In yet another exemplary embodiment, the VH and VL are the VH and VL of any of the TIM-3 ABDs in Figures 12 and 13A-C and the IL-15 variant includes amino acid substitutions D30N/E64Q/N65D.

[00398] In the mAb-dsIL-15/Rα format, one preferred embodiment utilizes an anti-TIM-3 ABD variable heavy and light domain pair of 3H3[TIM-3]_H1_L2.1 as shown in Figure 12 with the Figures 21I format, the skew variant set S364K/E357Q : L368D/K370S, the pI variants N208D/Q295E/N384D/Q418D/N421D and/or Q196K/I199T/P271R/P228R/N276K, the ablation variants E233P/L234V/L235A/G236_/S267K on both first and second monomers, and optionally the 428L/434S variants on both first and second monomers.

[00399] In one embodiment, the targeted IL-15/IL-15Rα heterodimeric protein is an "mAb-dsIL-15/Rα" format heterodimeric protein that includes: a) a first monomer that includes, from N- to C-terminus, a VH-hinge-CH2-CH3, where CH2-CH3 is a first Fc domain; b) a second monomer that includes, from N- to C-terminus, a VH-hinge-CH2-CH3-(domain linker)-IL-15Rα(sushi) domain-(domain linker), where the IL-15Rα(sushi) domain includes an amino acid substitution for a cysteine residue and CH2-CH3 is a second Fc domain; c) a third monomer that includes an IL-15 variant that includes an amino acid substitution for a cysteine residue; and d) a fourth and fifth monomer that each include, from N- to C-terminus, a VL-CL, where VL is a variable light domain, where the VH of the first monomer and the VL of the fourth monomer form a first TIM-3 binding domain, where the VH of the second monomer and the VL of the fifth monomer form a second TIM-3 binding domain, where the cysteine residue on the IL-15 variant and the cysteine residue on the IL-15Rα(sushi) domain form a disulfide bond, where VH and VL are the variable heavy domain and variable light domain of 3H3[TIM-3]_H1_L2.1, where the first variant Fc domain includes skew variants L368D/K370S and the second variant Fc domain include the skew variant pair S364K/E357Q, where the first and second variant Fc domains each include FcKO variants E233P/L234V/L235A/G236del/S267K, where the hinge-first variant Fc domain of the first monomer includes pI substitutions

N208D/Q295E/N384D/Q418D/N421D and the hinge-second variant Fc domain of the second monomer includes pI variants Q196K/I199T/P271R/P228R/N276K, and where numbering is according to EU numbering. In one embodiment, the targeted IL-15/IL-15Rα heterodimeric protein is an "mAb-dsIL-15/Rα" format heterodimeric protein that includes: a) a first monomer that includes, from N- to C-terminus, a VH-hinge-CH2-CH3, where CH2-CH3 is a first Fc domain; b) a second monomer that includes, from N- to C-terminus, a VH-hinge-CH2-CH3-(domain linker)-IL-15Rα(sushi) domain-(domain linker), where the IL-15Rα(sushi) domain includes an amino acid substitution for a cysteine residue and CH2-CH3 is a second Fc domain; c) a third monomer that includes an IL-15 variant that includes an amino acid substitution for a cysteine residue; and d) a fourth and fifth monomer that each include, from N- to C-terminus, a VL-CL, where VL is a variable light domain, where the VH of the first monomer and the VL of the fourth monomer form a first TIM-3 binding domain, where the VH of the second monomer and the VL of the fifth monomer form a second TIM-3 binding domain, where the cysteine residue on the IL-15 variant and the cysteine residue on the IL-15Rα(sushi) domain form a disulfide bond, where VH and VL are the variable heavy domain and variable light domain of 3H3[TIM-3]_H1_L2.1, where the first variant Fc domain includes skew variants L368D/K370S and the second variant Fc domain include the skew variant pair S364K/E357Q, where the first and second variant Fc domains each include FcKO variants E233P/L234V/L235A/G236del/S267K, where the hinge-first variant Fc domain of the first monomer includes pI substitutions N208D/Q295E/N384D/Q418D/N421D, and where numbering is according to EU numbering. In one embodiment, the targeted IL-15/IL-15Rα heterodimeric protein is an "mAb-dsIL-15/Rα" format heterodimeric protein that includes: a) a first monomer that includes, from N- to C-terminus, a VH-hinge-CH2-CH3, where CH2-CH3 is a first Fc domain; b) a second monomer that includes, from N- to C-terminus, a VH-hinge-CH2-CH3-(domain linker)-IL-15Rα(sushi) domain-(domain linker), where the IL-15Rα(sushi) domain includes an amino acid substitution for a cysteine residue and CH2-CH3 is a second Fc domain; c) a third monomer that includes an IL-15 variant that includes an amino acid substitution for a cysteine residue; and d) a fourth and fifth

monomer that each include, from N- to C-terminus, a VL-CL, where VL is a variable light domain, where the VH of the first monomer and the VL of the fourth monomer form a first TIM-3 binding domain, where the VH of the second monomer and the VL of the fifth monomer form a second TIM-3 binding domain, where the cysteine residue on the IL-15 variant and the cysteine residue on the IL-15Rα(sushi) domain form a disulfide bond, where VH and VL are the variable heavy domain and variable light domain of 3H3[TIM-3]_H1_L2.1, where the first variant Fc domain includes skew variants L368D/K370S and the second variant Fc domain include the skew variant pair S364K/E357Q, where the first and second variant Fc domains each include FcKO variants E233P/L234V/L235A/G236del/S267K, where the hinge-second variant Fc domain of the second monomer includes pI variants Q196K/I199T/P271R/P228R/N276K, and where numbering is according to EU numbering. In one embodiment, the targeted IL-15/IL-15Rα heterodimeric protein is an "mAb-dsIL-15/Rα" format heterodimeric protein that includes: a) a first monomer that includes, from N- to C-terminus, a VH-hinge-CH2-CH3, where CH2-CH3 is a first Fc domain; b) a second monomer that includes, from N- to C-terminus, a VH-hinge-CH2-CH3-(domain linker)-IL-15Rα(sushi) domain-(domain linker), where the IL-15Rα(sushi) domain includes an amino acid substitution for a cysteine residue and CH2-CH3 is a second Fc domain; c) a third monomer that includes an IL-15 variant that includes an amino acid substitution for a cysteine residue; and d) a fourth and fifth monomer that each include, from N- to C-terminus, a VL-CL, where VL is a variable light domain, where the VH of the first monomer and the VL of the fourth monomer form a first TIM-3 binding domain, where the VH of the second monomer and the VL of the fifth monomer form a second TIM-3 binding domain, where the cysteine residue on the IL-15 variant and the cysteine residue on the IL-15Rα(sushi) domain form a disulfide bond, where VH and VL are the variable heavy domain and variable light domain of 3H3[TIM-3]_H1_L2.1, where the first variant Fc domain includes skew variants S364K/E357Q and the second variant Fc domain include the skew variant pair L368D/K370S, where the first and second variant Fc domains each include FcKO variants E233P/L234V/L235A/G236del/S267K, where the hinge-first variant Fc domain of the

first monomer includes pI substitutions Q196K/I199T/P271R/P228R/N276K and the hinge-second variant Fc domain of the second monomer includes pI variants N208D/Q295E/N384D/Q418D/N421D, and where numbering is according to EU numbering. In one embodiment, the targeted IL-15/IL-15Rα heterodimeric protein is an "mAb-dsIL-15/Rα" format heterodimeric protein that includes: a) a first monomer that includes, from N- to C-terminus, a VH-hinge-CH2-CH3, where CH2-CH3 is a first Fc domain; b) a second monomer that includes, from N- to C-terminus, a VH-hinge-CH2-CH3-(domain linker)-IL-15Rα(sushi) domain-(domain linker), where the IL-15Rα(sushi) domain includes an amino acid substitution for a cysteine residue and CH2-CH3 is a second Fc domain; c) a third monomer that includes an IL-15 variant that includes an amino acid substitution for a cysteine residue; and d) a fourth and fifth monomer that each include, from N- to C-terminus, a VL-CL, where VL is a variable light domain, where the VH of the first monomer and the VL of the fourth monomer form a first TIM-3 binding domain, where the VH of the second monomer and the VL of the fifth monomer form a second TIM-3 binding domain, where the cysteine residue on the IL-15 variant and the cysteine residue on the IL-15Rα(sushi) domain form a disulfide bond, where VH and VL are the variable heavy domain and variable light domain of 3H3[TIM-3]_H1_L2.1, where the first variant Fc domain includes skew variants S364K/E357Q and the second variant Fc domain include the skew variant pair L368D/K370S, where the first and second variant Fc domains each include FcKO variants E233P/L234V/L235A/G236del/S267K, where the hinge-first variant Fc domain of the first monomer includes pI substitutions Q196K/I199T/P271R/P228R/N276K, and where numbering is according to EU numbering. In one embodiment, the targeted IL-15/IL-15Rα heterodimeric protein is an "mAb-dsIL-15/Rα" format heterodimeric protein that includes: a) a first monomer that includes, from N- to C-terminus, a VH-hinge-CH2-CH3, where CH2-CH3 is a first Fc domain; b) a second monomer that includes, from N- to C-terminus, a VH-hinge-CH2-CH3-(domain linker)-IL-15Rα(sushi) domain-(domain linker), where the IL-15Rα(sushi) domain includes an amino acid substitution for a cysteine residue and CH2-CH3 is a second Fc domain; c) a third monomer that includes an IL-15 variant that includes an amino acid

substitution for a cysteine residue; and d) a fourth and fifth monomer that each include, from N- to C-terminus, a VL-CL, where VL is a variable light domain, where the VH of the first monomer and the VL of the fourth monomer form a first TIM-3 binding domain, where the VH of the second monomer and the VL of the fifth monomer form a second TIM-3 binding domain, where the cysteine residue on the IL-15 variant and the cysteine residue on the IL-15Rα(sushi) domain form a disulfide bond, where VH and VL are the variable heavy domain and variable light domain of 3H3[TIM-3]_H1_L2.1, where the first variant Fc domain includes skew variants S364K/E357Q and the second variant Fc domain include the skew variant pair L368D/K370S, where the first and second variant Fc domains each include FcKO variants E233P/L234V/L235A/G236del/S267K, where the hinge-second variant Fc domain of the second monomer includes pI variants N208D/Q295E/N384D/Q418D/N421D, and where numbering is according to EU numbering. In certain embodiments, the first and second variant Fc domains each further include half-life extension variants M428L/N434S. In a particular embodiment, the IL-15 variant includes amino acid substitutions N4D/N65D and VH and VL are the variable heavy domain and variable light domain of 3H3[TIM-3]_H1_L2.1. In one embodiment, the IL-15 variant includes amino acid substitutions D30N/N65D and VH and VL are the variable heavy domain and variable light domain of 3H3[TIM-3]_H1_L2.1. In another embodiment, the IL-15 variant includes amino acid substitutions D30N/E64Q/N65D and VH and VL are the variable heavy domain and variable light domain of 3H3[TIM-3]_H1_L2.1.

J. Central-IL-15/Rα

[00400] This embodiment is shown in Figures 21J, and comprises four monomers forming a tetramer. The first monomer comprises a VH-CH1-[optional domain linker]-IL-15 variant-[optional domain linker]-CH2-CH3, with the second optional domain linker sometimes being the hinge domain. The second monomer comprises a VH-CH1-[optional domain linker]- IL-15Rα(sushi) domain-[optional domain linker]-CH2-CH3, with the second optional domain linker sometimes being the hinge domain. The third (and fourth) monomers are light chains, VL-CL. This is generally referred to as "central-IL-15/Rα".

[00401] In one embodiment, the targeted IL-15/IL-15Rα heterodimeric protein is an "central-IL-15/Rα"format heterodimeric protein that includes: a) a first monomer that includes, from N- to C-terminus, a VH-(domain linker)-IL-15 variant-(domain linker)-CH2-CH3, where CH2-CH3 is a first Fc domain; b) a second monomer that includes, from N- to C-terminus, a VH-(domain linker)- IL-15Rα(sushi) domain-(domain linker)-CH2-CH3, where CH2-CH3 is a second Fc domain; and d) a third and fourth monomer that each include from N-to C-terminus, a VL-CL, where the VH of the first monomer and the VL of the third monomer form a first TIM-3 binding domain, where the VH of the second monomer and the VL of the fourth monomer form a second TIM-3 binding domain, and where the IL-15 variant and the IL-15Rα(sushi) domain form an IL-15 complex. Any useful domain linker can be used to attach the various components of the heterodimeric protein including, but not limited to those in Figures 8 and 9A-C. In an exemplary embodiment, the domain linkers that attach the IL-15 variant to the first Fc domain and the IL-15Rα(sushi) domain to the second Fc domain are each antibody hinge domains.

[00402] In the central-IL-15/Rα format, one preferred embodiment utilizes an anti-TIM-3 ABD having any of the variable heavy and light domain pairs as shown in Figure 12.

[00403] In the central-IL-15/Rα format, one preferred embodiment utilizes an anti-TIM-3 ABD having the variable heavy and light domain pair of 3H3[TIM-3]_H1_L2.1 as shown in Figure 12.

[00404] In one embodiment, the targeted IL-15/IL-15Rα heterodimeric protein is an "central-IL-15/Rα"format heterodimeric protein that includes: a) a first monomer that includes, from N- to C-terminus, a VH-(domain linker)-IL-15 variant-(domain linker)-CH2-CH3, where CH2-CH3 is a first Fc domain; b) a second monomer that includes, from N- to C-terminus, a VH-(domain linker)- IL-15Rα(sushi) domain-(domain linker)-CH2-CH3, where CH2-CH3 is a second Fc domain; and d) a third and fourth monomer that each include from N-to C-terminus, a VL-CL, where the VH of the first monomer and the VL of the third monomer are the variable heavy domain and variable light domain of 3H3[TIM-3]_H1_L2.1, respectively, where the VH of the second monomer and the VL of the fourth monomer are the variable heavy domain and variable light domain of 3H3[TIM-3]_H1_L2.1,

respectively, and where the IL-15 variant and the IL-15Rα(sushi) domain form an IL-15 complex.

[00405] In the "central-IL-15/Rα"format, one preferred embodiment utilizes an IL-15 variant that includes amino acid substitutions N4D/N65D, D30N/N65D, or D30N/E64Q/N65D. In one embodiment, the targeted IL-15/IL-15Rα heterodimeric protein is an "central-IL-15/Rα"format heterodimeric protein that includes: a) a first monomer that includes, from N- to C-terminus, a VH-(domain linker)-IL-15 variant-(domain linker)-CH2-CH3, where CH2-CH3 is a first Fc domain; b) a second monomer that includes, from N- to C-terminus, a VH-(domain linker)- IL-15Rα(sushi) domain-(domain linker)-CH2-CH3, where CH2-CH3 is a second Fc domain; and d) a third and fourth monomer that each include from N-to C-terminus, a VL-CL, where the VH of the first monomer and the VL of the third monomer form a first TIM-3 binding domain, where the VH of the second monomer and the VL of the fourth monomer form a second TIM-3 binding domain, where the IL-15 variant and the IL-15Rα(sushi) domain form an IL-15 complex, and where the IL-15 variant includes amino acid substitutions N4D/N65D, D30N/N65D, or D30N/E64Q/N65D. In an exemplary embodiment, the VH and VL are the VH and VL of any of the TIM-3 ABDs in Figures 12 and 13A-C and the IL-15 variant includes amino acid substitutions N4D/N65D. In another exemplary embodiment, the VH and VL are the VH and VL of any of the TIM-3 ABDs in Figures 12 and 13A-C and the IL-15 variant includes amino acid substitutions D30N/N65D. In yet another exemplary embodiment, the VH and VL are the VH and VL of any of the TIM-3 ABDs in Figures 12 and 13A-C and the IL-15 variant includes amino acid substitutions D30N/E64Q/N65D.

[00406] In the central-IL-15/Rαformat, one preferred embodiment utilizes an anti-TIM-3 ABD having the variable heavy and light domain pair of 3H3[TIM-3]_H1_L2.1 as shown in Figure 12, with either the IL-15 N4D/N65D variant or the IL-15 D30N/N65D variant or the IL-15 D30N/E64Q/N65D variant. In one embodiment, the targeted IL-15/IL-15Rα heterodimeric protein is an "central-IL-15/Rα"format heterodimeric protein that includes: a) a first monomer that includes, from N- to C-terminus, a VH-(domain linker)-IL-15 variant-(domain linker)-CH2-CH3, where CH2-CH3 is a first Fc domain; b) a second monomer that includes, from N- to C-terminus, a VH-(domain linker)- IL-15Rα(sushi)

domain-(domain linker)-CH2-CH3, where CH2-CH3 is a second Fc domain; and d) a third and fourth monomer that each include from N-to C-terminus, a VL-CL, where the VH of the first monomer and the VL of the third monomer form a first TIM-3 binding domain, where the VH of the second monomer and the VL of the fourth monomer form a second TIM-3 binding domain, where the IL-15 variant and the IL-15Rα(sushi) domain form an IL-15 complex, where VH and VL are the variable heavy domain and variable light domain of 3H3[TIM-3]_H1_L2.1, and where the IL-15 variant includes amino acid substitutions N4D/N65D, D30N/N65D, or D30N/E64Q/N65D. In certain embodiments, the first and second variant Fc domains each further include half-life extension variants M428L/N434S. In a particular embodiment, the IL-15 variant includes amino acid substitutions N4D/N65D and VH and VL are the variable heavy domain and variable light domain of 3H3[TIM-3]_H1_L2.1. In one embodiment, the IL-15 variant includes amino acid substitutions D30N/N65D and VH and VL are the variable heavy domain and variable light domain of 3H3[TIM-3]_H1_L2.1. In another embodiment, the IL-15 variant includes amino acid substitutions D30N/E64Q/N65D and VH and VL are the variable heavy domain and variable light domain of 3H3[TIM-3]_H1_L2.1.

[00407] In the central-IL-15/Rα format, one preferred embodiment utilizes the skew variant pair S364K/E357Q : L368D/K370S. In one embodiment, the targeted IL-15/IL-15Rα heterodimeric protein is an "central-IL-15/Rα"format heterodimeric protein that includes: a) a first monomer that includes, from N- to C-terminus, a VH-(domain linker)-IL-15 variant-(domain linker)-CH2-CH3, where CH2-CH3 is a first Fc domain; b) a second monomer that includes, from N- to C-terminus, a VH-(domain linker)- IL-15Rα(sushi) domain-(domain linker)-CH2-CH3, where CH2-CH3 is a second Fc domain; and d) a third and fourth monomer that each include from N-to C-terminus, a VL-CL, where the VH of the first monomer and the VL of the third monomer form a first TIM-3 binding domain, where the VH of the second monomer and the VL of the fourth monomer form a second TIM-3 binding domain, where the IL-15 variant and the IL-15Rα(sushi) domain form an IL-15 complex, and where the first and second variant Fc domains include the skew variant pair S364K/E357Q : L368D/K370S. In an exemplary embodiment, the first variant Fc domain includes skew variants S364K and E357Q, and the second variant Fc domain includes skew variants L368D

and K370S. In another exemplary embodiment, the first variant Fc domain includes skew variants L368D and K370S, and the second variant Fc domain includes skew variants S364K and E357Q.

[00408] In one embodiment, the targeted IL-15/IL-15Rα heterodimeric protein is an "central-IL-15/Rα"format heterodimeric protein that includes: a) a first monomer that includes, from N- to C-terminus, a VH-(domain linker)-IL-15 variant-(domain linker)-CH2-CH3, where CH2-CH3 is a first Fc domain; b) a second monomer that includes, from N- to C-terminus, a VH-(domain linker)- IL-15Rα(sushi) domain-(domain linker)-CH2-CH3, where CH2-CH3 is a second Fc domain; and d) a third and fourth monomer that each include from N-to C-terminus, a VL-CL, where the VH of the first monomer and the VL of the third monomer form a first TIM-3 binding domain, where the VH of the second monomer and the VL of the fourth monomer form a second TIM-3 binding domain, where the IL-15 variant and the IL-15Rα(sushi) domain form an IL-15 complex, where the IL-15 variant includes amino acid substitutions N4D/N65D, D30N/N65D, or D30N/E64Q/N65D, and where the first and second variant Fc domains include the skew variant pair S364K/E357Q : L368D/K370S. In an exemplary embodiment, the first variant Fc domain includes skew variants S364K and E357Q, and the second variant Fc domain includes skew variants L368D and K370S. In another exemplary embodiment, the first variant Fc domain includes skew variants L368D and K370S, and the second variant Fc domain includes skew variants S364K and E357Q. In an exemplary embodiment, the VH and VL are the VH and VL of any of the TIM-3 ABDs in Figures 12 and 13A-C and the IL-15 variant includes amino acid substitutions N4D/N65D. In another exemplary embodiment, the VH and VL are the VH and VL of any of the TIM-3 ABDs in Figures 12 and 13A-C and the IL-15 variant includes amino acid substitutions D30N/N65D. In yet another exemplary embodiment, the VH and VL are the VH and VL of any of the TIM-3 ABDs in Figures 12 and 13A-C and the IL-15 variant includes amino acid substitutions D30N/E64Q/N65D.

[00409] In the central-IL-15/Rα format, one preferred embodiment utilizes an anti-TIM-3 ABD having the variable heavy and light domain pair of 3H3[TIM-3]_H1_L2.1 as shown in Figure 12 and the skew variant pair S364K/E357Q : L368D/K370S. In one embodiment, the targeted IL-15/IL-15Rα heterodimeric protein is an "central-IL-

15/Rα"format heterodimeric protein that includes: a) a first monomer that includes, from N- to C-terminus, a VH-(domain linker)-IL-15 variant-(domain linker)-CH2-CH3, where CH2-CH3 is a first variant Fc domain; b) a second monomer that includes, from N- to C-terminus, a VH-(domain linker)- IL-15Rα(sushi) domain-(domain linker)-CH2-CH3, where CH2-CH3 is a second variant Fc domain; and d) a third and fourth monomer that each include from N- to C-terminus, a VL-CL, where the VH of the first monomer and the VL of the third monomer are the variable heavy domain and variable light domain of 3H3[TIM-3]_H1_L2.1, respectively, where the VH of the second monomer and the VL of the fourth monomer are the variable heavy domain and variable light domain of 3H3[TIM-3]_H1_L2.1, respectively, where the IL-15 variant and the IL-15Rα(sushi) domain form an IL-15 complex, and where the first and second variant Fc domains include the skew variant pair S364K/E357Q : L368D/K370S.

[00410] In the central-IL-15/Rα format, one preferred embodiment utilizes an anti-TIM-3 ABD having the variable heavy and light domain pair of 3H3[TIM-3]_H1_L2.1 as shown in Figure 12 and the skew variant pair S364K/E357Q : L368D/K370S with either the IL-15 N4D/N65D variant or the IL-15 D30N/E64Q/N65D variant with appropriate cysteine substitutions. In one embodiment, the targeted IL-15/IL-15Rα heterodimeric protein is an "central-IL-15/Rα"format heterodimeric protein that includes: a) a first monomer that includes, from N- to C-terminus, a VH-(domain linker)-IL-15 variant-(domain linker)-CH2-CH3, where CH2-CH3 is a first variant Fc domain; b) a second monomer that includes, from N- to C-terminus, a VH-(domain linker)- IL-15Rα(sushi) domain-(domain linker)-CH2-CH3, where CH2-CH3 is a second variant Fc domain; and d) a third and fourth monomer that each include from N-to C-terminus, a VL-CL, where the VH of the first monomer and the VL of the third monomer form a first TIM-3 binding domain, where the VH of the second monomer and the VL of the fourth monomer form a second TIM-3 binding domain, where the IL-15 variant and the IL-15Rα(sushi) domain form an IL-15 complex, where VH and VL are the variable heavy domain and variable light domain of 3H3[TIM-3]_H1_L2.1, where the IL-15 variant includes amino acid substitutions N4D/N65D, D30N/N65D, or D30N/E64Q/N65D, and where the first and second variant Fc domains include the skew variant pair S364K/E357Q : L368D/K370S. In an exemplary embodiment, the first variant Fc

domain includes skew variants S364K and E357Q, and the second variant Fc domain includes skew variants L368D and K370S. In another exemplary embodiment, the first variant Fc domain includes skew variants L368D and K370S, and the second variant Fc domain includes skew variants S364K and E357Q. In certain embodiments, the first and second variant Fc domains each further include half-life extension variants M428L/N434S. In a particular embodiment, the IL-15 variant includes amino acid substitutions N4D/N65D and VH and VL are the variable heavy domain and variable light domain of 3H3[TIM-3]_H1_L2.1. In one embodiment, the IL-15 variant includes amino acid substitutions D30N/N65D and VH and VL are the variable heavy domain and variable light domain of 3H3[TIM-3]_H1_L2.1. In another embodiment, the IL-15 variant includes amino acid substitutions D30N/E64Q/N65D and VH and VL are the variable heavy domain and variable light domain of 3H3[TIM-3]_H1_L2.1.

[00411] In the central-IL-15/Rα format, one preferred embodiment utilizes the skew variant set S364K/E357Q : L368D/K370S, the pI variants Q295E/N384D/Q418E/N421D, the ablation variants E233P/L234V/L235A/G236_/S267K on both first and second monomers, and optionally the 428L/434S variants on both first and second monomers.

[00412] In one embodiment, the targeted IL-15/IL-15Rα heterodimeric protein is an "central-IL-15/Rα"format heterodimeric protein that includes: a) a first monomer that includes, from N- to C-terminus, a VH-(domain linker)-IL-15 variant-(hinge)-CH2-CH3, where CH2-CH3 is a first variant Fc domain; b) a second monomer that includes, from N- to C-terminus, a VH-(domain linker)- IL-15Rα(sushi) domain-(hinge)-CH2-CH3, where CH2-CH3 is a second variant Fc domain; and d) a third and fourth monomer that each include from N-to C-terminus, a VL-CL, where the VH of the first monomer and the VL of the third monomer form a first TIM-3 binding domain, where the VH of the second monomer and the VL of the fourth monomer form a second TIM-3 binding domain, where the IL-15 variant and the IL-15Rα(sushi) domain form an IL-15 complex, where the first variant Fc domain includes skew variants L368D/K370S and the second variant Fc domain include the skew variant pair S364K/E357Q, where the first and second variant Fc domains each include FcKO variants E233P/L234V/L235A/G236del/S267K, where the first variant Fc domain includes pI substitutions Q295E/N384D/Q418D/N421D, and where

numbering is according to EU numbering. In one embodiment, the targeted IL-15/IL-15Rα heterodimeric protein is an "central-IL-15/Rα"format heterodimeric protein that includes: a) a first monomer that includes, from N- to C-terminus, a VH-(domain linker)-IL-15 variant-(hinge)-CH2-CH3, where CH2-CH3 is a first variant Fc domain; b) a second monomer that includes, from N- to C-terminus, a VH-(domain linker)- IL-15Rα(sushi) domain-(hinge)-CH2-CH3, where CH2-CH3 is a second variant Fc domain; and d) a third and fourth monomer that each include from N-to C-terminus, a VL-CL, where the VH of the first monomer and the VL of the third monomer form a first TIM-3 binding domain, where the VH of the second monomer and the VL of the fourth monomer form a second TIM-3 binding domain, where the IL-15 variant and the IL-15Rα(sushi) domain form an IL-15 complex, where the first variant Fc domain includes skew variants S364K/E357Q and the second variant Fc domain include the skew variant pair L368D/K370S, where the first and second variant Fc domains each include FcKO variants E233P/L234V/L235A/G236del/S267K, where the second variant Fc domain of the second monomer includes pI substitutions Q295E/N384D/Q418D/N421D, and where numbering is according to EU numbering. In certain embodiments, the first and second variant Fc domains each further include half-life extension variants M428L/N434S. In an exemplary embodiment, the IL-15 variant includes amino acid substitutions N4D/N65D, D30N/N65D, or D30N/E64Q/N65D. In an exemplary embodiment, the IL-15 variant includes amino acid substitutions N4D/N65D, D30N/N65D, or D30N/E64Q/N65D. In an exemplary embodiment, the VH and VL are the VH and VL of any of the TIM-3 ABDs in Figures 12 and 13A-C and the IL-15 variant includes amino acid substitutions N4D/N65D. In another exemplary embodiment, the VH and VL are the VH and VL of any of the TIM-3 ABDs in Figures 12 and 13A-C and the IL-15 variant includes amino acid substitutions D30N/N65D. In yet another exemplary embodiment, the VH and VL are the VH and VL of any of the TIM-3 ABDs in Figures 12 and 13A-C and the IL-15 variant includes amino acid substitutions D30N/E64Q/N65D.

[00413] In the central-IL-15/Rα format, one preferred embodiment utilizes an anti-TIM-3 ABD having the variable heavy and light domain pair of 3H3[TIM-3]_H1_L2.1 as shown in Figure 12 with the Figures 21K, the skew variant set S364K/E357Q : L368D/K370S,

the pI variants Q295E/N384D/Q418E/N421D, the ablation variants E233P/L234V/L235A/G236_/S267K on both first and second monomers, and optionally the 428L/434S variants on both first and second monomers. In one embodiment, the targeted IL-15/IL-15Rα heterodimeric protein is an "central-IL-15/Rα"format heterodimeric protein that includes: a) a first monomer that includes, from N- to C-terminus, a VH-(domain linker)-IL-15 variant-(hinge)-CH2-CH3, where CH2-CH3 is a first variant Fc domain; b) a second monomer that includes, from N- to C-terminus, a VH-(domain linker)- IL-15Rα(sushi) domain-(hinge)-CH2-CH3, where CH2-CH3 is a second variant Fc domain; and d) a third and fourth monomer that each include from N-to C-terminus, a VL-CL, where the VH of the first monomer and the VL of the third monomer form a first TIM-3 binding domain, where the VH of the second monomer and the VL of the fourth monomer form a second TIM-3 binding domain, where the IL-15 variant and the IL-15Rα(sushi) domain form an IL-15 complex, where VH and VL are the variable heavy domain and variable light domain of 3H3[TIM-3]_H1_L2.1, where the first variant Fc domain includes skew variants L368D/K370S and the second variant Fc domain include the skew variant pair S364K/E357Q, where the first and second variant Fc domains each include FcKO variants E233P/L234V/L235A/G236del/S267K, where the first variant Fc domain includes pI substitutions Q295E/N384D/Q418D/N421D, and where numbering is according to EU numbering. In one embodiment, the targeted IL-15/IL-15Rα heterodimeric protein is an "central-IL-15/Rα"format heterodimeric protein that includes: a) a first monomer that includes, from N- to C-terminus, a VH-(domain linker)-IL-15 variant-(hinge)-CH2-CH3, where CH2-CH3 is a first variant Fc domain; b) a second monomer that includes, from N- to C-terminus, a VH-(domain linker)- IL-15Rα(sushi) domain-(hinge)-CH2-CH3, where CH2-CH3 is a second variant Fc domain; and d) a third and fourth monomer that each include from N-to C-terminus, a VL-CL, where the VH of the first monomer and the VL of the third monomer form a first TIM-3 binding domain, where the VH of the second monomer and the VL of the fourth monomer form a second TIM-3 binding domain, where the IL-15 variant and the IL-15Rα(sushi) domain form an IL-15 complex, where VH and VL are the variable heavy domain and variable light domain of 3H3[TIM-3]_H1_L2.1, where the first variant Fc domain includes skew variants S364K/E357Q

and the second variant Fc domain include the skew variant pair L368D/K370S, where the first and second variant Fc domains each include FcKO variants E233P/L234V/L235A/G236del/S267K, where the second variant Fc domain of the second monomer includes pI substitutions Q295E/N384D/Q418D/N421D, and where numbering is according to EU numbering. In certain embodiments, the first and second variant Fc domains each further include half-life extension variants M428L/N434S. In certain embodiments, the first and second variant Fc domains each further include half-life extension variants M428L/N434S. In a particular embodiment, the IL-15 variant includes amino acid substitutions N4D/N65D and VH and VL are the variable heavy domain and variable light domain of 3H3[TIM-3]_H1_L2.1. In one embodiment, the IL-15 variant includes amino acid substitutions D30N/N65D and VH and VL are the variable heavy domain and variable light domain of 3H3[TIM-3]_H1_L2.1. In another embodiment, the IL-15 variant includes amino acid substitutions D30N/E64Q/N65D and VH and VL are the variable heavy domain and variable light domain of 3H3[TIM-3]_H1_L2.1.

K. Central-scIL-15/Rα

[00414] This embodiment is shown in Figures 21K, and comprises four monomers forming a tetramer. The first monomer comprises a VH-CH1-[optional domain linker]- IL-15Rα(sushi) domain-domain linker-IL-15 variant-[optional domain linker]-CH2-CH3, with the second optional domain linker sometimes being the hinge domain. The second monomer comprises a VH-CH1-hinge-CH2-CH3. The third (and fourth) monomers are light chains, VL-CL. This is generally referred to as "central-scIL-15/Rα", with the "sc" standing for "single chain".

[00415] In one embodiment, the targeted IL-15/IL-15Rα heterodimeric protein is an "central-scIL-15/Rα"format heterodimeric protein that includes: a) a first monomer that includes, from N- to C-terminus, a VH-(domain linker)-IL-15Rα(sushi) domain-(domain linker)-IL-15 variant -(domain linker)-CH2-CH3, where CH2-CH3 is a first Fc domain; b) a second monomer that includes, from N- to C-terminus, a VH-hinge-CH2-CH3, where CH2-CH3 is a second Fc domain; and d) a third and fourth monomer that each include from N-to C-terminus, a VL-CL, where the VH of the first monomer and the VL of the third monomer form a first TIM-3 binding domain, where the VH of the second monomer and the VL of the

fourth monomer form a second TIM-3 binding domain, and where the IL-15 variant and the IL-15Rα(sushi) domain form an IL-15 complex. Any useful domain linker can be used to attach the various components of the heterodimeric protein including, but not limited to those in Figures 8 and 9A-C. In an exemplary embodiment, the domain linker that attaches the IL-15 variant to the first Fc domain is an antibody hinge domain.

[00416] In the central-scIL-15/Rα format, one preferred embodiment utilizes an anti-TIM-3 ABD having any of the variable heavy and light domain pairs as shown in Figure 12.

[00417] In the central-scIL-15/Rα format, one preferred embodiment utilizes an anti-TIM-3 ABD having the variable heavy and light domain pair of 3H3[TIM-3]_H1_L2.1 as shown in as shown in Figure 12.

[00418] In one embodiment, the targeted IL-15/IL-15Rα heterodimeric protein is an "central-scIL-15/Rα"format heterodimeric protein that includes: a) a first monomer that includes, from N- to C-terminus, a VH-(domain linker)- IL-15Rα(sushi) domain -(domain linker)- IL-15 variant -(domain linker)-CH2-CH3, where CH2-CH3 is a first Fc domain; b) a second monomer that includes, from N- to C-terminus, a VH-hinge-CH2-CH3, where CH2-CH3 is a second Fc domain; and d) a third and fourth monomer that each include from N-to C-terminus, a VL-CL, where the VH of the first monomer and the VL of the third monomer are the variable heavy domain and variable light domain of 7G8_H3.30_L1.34, respectively, where the VH of the second monomer and the VL of the fourth monomer are the variable heavy domain and variable light domain of 3H3[TIM-3]_H1_L2.1, respectively, and where the IL-15 variant and the IL-15Rα(sushi) domain form an IL-15 complex.

[00419] In the central-scIL-15/Rα format, one preferred embodiment utilizes an IL-15 variant that includes amino acid substitutions N4D/N65D, D30N/N65D, or D30N/E64Q/N65D. In one embodiment, the targeted IL-15/IL-15Rα heterodimeric protein is an "central-scIL-15/Rα"format heterodimeric protein that includes: a) a first monomer that includes, from N- to C-terminus, a VH-(domain linker)- IL-15Rα(sushi) domain -(domain linker)-IL-15 variant-(domain linker)-CH2-CH3, where CH2-CH3 is a first Fc domain; b) a second monomer that includes, from N- to C-terminus, a VH-hinge-CH2-CH3, where CH2-CH3 is a second Fc domain; and d) a third and fourth monomer that each include from N-to

C-terminus, a VL-CL, where the VH of the first monomer and the VL of the third monomer form a first TIM-3 binding domain, where the VH of the second monomer and the VL of the fourth monomer form a second TIM-3 binding domain, where the IL-15 variant and the IL-15Rα(sushi) domain form an IL-15 complex, and where the IL-15 variant includes amino acid substitutions N4D/N65D, D30N/N65D, or D30N/E64Q/N65D. In an exemplary embodiment, the VH and VL are the VH and VL of any of the TIM-3 ABDs in Figures 12 and 13A-C and the IL-15 variant includes amino acid substitutions N4D/N65D. In another exemplary embodiment, the VH and VL are the VH and VL of any of the TIM-3 ABDs in Figures 12 and 13A-C and the IL-15 variant includes amino acid substitutions D30N/N65D. In yet another exemplary embodiment, the VH and VL are the VH and VL of any of the TIM-3 ABDs in Figures 12 and 13A-C and the IL-15 variant includes amino acid substitutions D30N/E64Q/N65D.

[00420] In the central-scIL-15/Rα format, one preferred embodiment utilizes an anti-TIM-3 ABD having the variable heavy and light domain pair of 3H3[TIM-3]_H1_L2.1 as shown in Figure 12, with either the IL-15 N4D/N65D variant or the IL-15 D30N/N65D variant or the IL-15 D30N/E64Q/N65D variant. In one embodiment, the targeted IL-15/IL-15Rα heterodimeric protein is an "central-scIL-15/Rα"format heterodimeric protein that includes: a) a first monomer that includes, from N- to C-terminus, a VH-(domain linker)- IL-15Rα(sushi) domain -(domain linker)-IL-15 variant-(domain linker)-CH2-CH3, where CH2-CH3 is a first Fc domain; b) a second monomer that includes, from N- to C-terminus, a VH-hinge-CH2-CH3, where CH2-CH3 is a second Fc domain; and d) a third and fourth monomer that each include from N-to C-terminus, a VL-CL, where the VH of the first monomer and the VL of the third monomer form a first TIM-3 binding domain, where the VH of the second monomer and the VL of the fourth monomer form a second TIM-3 binding domain, where the IL-15 variant and the IL-15Rα(sushi) domain form an IL-15 complex, where VH and VL are the variable heavy domain and variable light domain of 3H3[TIM-3]_H1_L2.1, and where the IL-15 variant includes amino acid substitutions N4D/N65D, D30N/N65D, or D30N/E64Q/N65D. In a particular embodiment, the IL-15 variant includes amino acid substitutions N4D/N65D and VH and VL are the variable heavy domain and variable light domain of 3H3[TIM-3]_H1_L2.1. In one embodiment, the IL-15 variant

includes amino acid substitutions D30N/N65D and VH and VL are the variable heavy domain and variable light domain of 3H3[TIM-3]_H1_L2.1. In another embodiment, the IL-15 variant includes amino acid substitutions D30N/E64Q/N65D and VH and VL are the variable heavy domain and variable light domain of 3H3[TIM-3]_H1_L2.1.

[00421] In the central-scIL-15/Rα format, one preferred embodiment utilizes the skew variant pair S364K/E357Q : L368D/K370S. In one embodiment, the targeted IL-15/IL-15Rα heterodimeric protein is an "central-scIL-15/Rα"format heterodimeric protein that includes: a) a first monomer that includes, from N- to C-terminus, a VH-(domain linker)- IL-15Rα(sushi) domain -(domain linker)- IL-15 variant -(domain linker)-CH2-CH3, where CH2-CH3 is a first variant Fc domain; b) a second monomer that includes, from N- to C-terminus, a VH-hinge-CH2-CH3, where CH2-CH3 is a second variant Fc domain; and d) a third and fourth monomer that each include from N-to C-terminus, a VL-CL, where the VH of the first monomer and the VL of the third monomer form a first TIM-3 binding domain, where the VH of the second monomer and the VL of the fourth monomer form a second TIM-3 binding domain, where the IL-15 variant and the IL-15Rα(sushi) domain form an IL-15 complex, and where the first and second variant Fc domains include the skew variant pair S364K/E357Q : L368D/K370S. In an exemplary embodiment, the first variant Fc domain includes skew variants L368D and K370S, and the second variant Fc domain includes skew variants S364K and E357Q.

[00422] In one embodiment, the targeted IL-15/IL-15Rα heterodimeric protein is an "central-scIL-15/Rα"format heterodimeric protein that includes: a) a first monomer that includes, from N- to C-terminus, a VH-(domain linker)- IL-15Rα(sushi) domain -(domain linker)- IL-15 variant -(domain linker)-CH2-CH3, where CH2-CH3 is a first variant Fc domain; b) a second monomer that includes, from N- to C-terminus, a VH-hinge-CH2-CH3, where CH2-CH3 is a second variant Fc domain; and d) a third and fourth monomer that each include from N-to C-terminus, a VL-CL, where the VH of the first monomer and the VL of the third monomer form a first TIM-3 binding domain, where the VH of the second monomer and the VL of the fourth monomer form a second TIM-3 binding domain, where the IL-15 variant and the IL-15Rα(sushi) domain form an IL-15 complex, where the IL-15 variant includes amino acid substitutions N4D/N65D, D30N/N65D, or D30N/E64Q/N65D,

and where the first and second variant Fc domains include the skew variant pair S364K/E357Q : L368D/K370S. In an exemplary embodiment, the first variant Fc domain includes skew variants L368D and K370S, and the second variant Fc domain includes skew variants S364K and E357Q. In an exemplary embodiment, the VH and VL are the VH and VL of any of the TIM-3 ABDs in Figures 12 and 13A-C and the IL-15 variant includes amino acid substitutions N4D/N65D. In another exemplary embodiment, the VH and VL are the VH and VL of any of the TIM-3 ABDs in Figures 12 and 13A-C and the IL-15 variant includes amino acid substitutions D30N/N65D. In yet another exemplary embodiment, the VH and VL are the VH and VL of any of the TIM-3 ABDs in Figures 12 and 13A-C and the IL-15 variant includes amino acid substitutions D30N/E64Q/N65D.

[00423] In the central-scIL-15/Rα format, one preferred embodiment utilizes an anti-TIM-3 ABD having the variable heavy and light domain pair of 3H3[TIM-3]_H1_L2.1 as shown in Figure 12 and the skew variant pair S364K/E357Q : L368D/K370S. In one embodiment, the targeted IL-15/IL-15Rα heterodimeric protein is an "central-scIL-15/Rα"format heterodimeric protein that includes: a) a first monomer that includes, from N- to C-terminus, a VH-(domain linker)- IL-15Rα(sushi) domain -(domain linker)- IL-15 variant -(domain linker)-CH2-CH3, where CH2-CH3 is a first variant Fc domain; b) a second monomer that includes, from N- to C-terminus, a VH-hinge-CH2-CH3, where CH2-CH3 is a second variant Fc domain; and d) a third and fourth monomer that each include from N-to C-terminus, a VL-CL, where the VH of the first monomer and the VL of the third monomer are the variable heavy domain and variable light domain 3H3[TIM-3]_H1_L2.1, respectively, where the VH of the second monomer and the VL of the fourth monomer are the variable heavy domain and variable light domain of 3H3[TIM-3]_H1_L2.1, respectively, where the IL-15 variant and the IL-15Rα(sushi) domain form an IL-15 complex, and where the first and second variant Fc domains include the skew variant pair S364K/E357Q : L368D/K370S. In an exemplary embodiment, the first variant Fc domain includes skew variants L368D and K370S, and the second variant Fc domain includes skew variants S364K and E357Q.

[00424] In the central-scIL-15/Rα format, one preferred embodiment utilizes an anti-TIM-3 ABD having the variable heavy and light domain pair of 3H3[TIM-3]_H1_L2.1 as shown in Figure 12 and the skew variant pair S364K/E357Q : L368D/K370S with either the

IL-15 N4D/N65D variant or the IL-15 D30N/N65D variant or the IL-15 D30N/E64Q/N65D variant. In one embodiment, the targeted IL-15/IL-15Rα heterodimeric protein is an "central-scIL-15/Rα"format heterodimeric protein that includes: a) a first monomer that includes, from N- to C-terminus, a VH-(domain linker)- IL-15Rα(sushi) domain -(domain linker)- IL-15 variant-(domain linker)-CH2-CH3, where CH2-CH3 is a first variant Fc domain; b) a second monomer that includes, from N- to C-terminus, a VH-hinge-CH2-CH3, where CH2-CH3 is a second variant Fc domain; and d) a third and fourth monomer that each include from N-to C-terminus, a VL-CL, where the VH of the first monomer and the VL of the third monomer form a first TIM-3 binding domain, where the VH of the second monomer and the VL of the fourth monomer form a second TIM-3 binding domain, where the IL-15 variant and the IL-15Rα(sushi) domain form an IL-15 complex, where VH and VL are the variable heavy domain and variable light domain of 3H3[TIM-3]_H1_L2.1, where the IL-15 variant includes amino acid substitutions N4D/N65D, D30N/N65D, or D30N/E64Q/N65D, and where the first and second variant Fc domains include the skew variant pair L368D/K370S : S364K/E357Q. In an exemplary embodiment, the first variant Fc domain includes skew variants L368D/K370S, and the second variant Fc domain includes skew variants S364K/E357Q. In a particular embodiment, the IL-15 variant includes amino acid substitutions N4D/N65D and VH and VL are the variable heavy domain and variable light domain of 3H3[TIM-3]_H1_L2.1. In one embodiment, the IL-15 variant includes amino acid substitutions D30N/N65D and VH and VL are the variable heavy domain and variable light domain of 3H3[TIM-3]_H1_L2.1. In another embodiment, the IL-15 variant includes amino acid substitutions D30N/E64Q/N65D and VH and VL are the variable heavy domain and variable light domain of 3H3[TIM-3]_H1_L2.1.

[00425] In the central-scIL-15/Rα format, one preferred embodiment utilizes the skew variant set S364K/E357Q : L368D/K370S, the pI variants Q295E/N384D/Q418E/N421D, the ablation variants E233P/L234V/L235A/G236_/S267K on both first and second monomers, and optionally the 428L/434S variants on both first and second monomers.

[00426] In one embodiment, the targeted IL-15/IL-15Rα heterodimeric protein is an "central-scIL-15/Rα"format heterodimeric protein that includes: a) a first monomer that includes, from N- to C-terminus, a VH-(domain linker)- IL-15Rα(sushi) domain -(domain

linker)- IL-15 variant -(hinge)-CH2-CH3, where CH2-CH3 is a first variant Fc domain; b) a second monomer that includes, from N- to C-terminus, a VH-hinge-CH2-CH3, where CH2-CH3 is a second variant Fc domain; and d) a third and fourth monomer that each include from N-to C-terminus, a VL-CL, where the VH of the first monomer and the VL of the third monomer form a first TIM-3 binding domain, where the VH of the second monomer and the VL of the fourth monomer form a second TIM-3 binding domain, where the IL-15 variant and the IL-15Rα(sushi) domain form an IL-15 complex, where the first variant Fc domain includes skew variants L368D/K370S and the second variant Fc domain includes skew variants S364K/E357Q, where the first and second variant Fc domains each include FcKO variants E233P/L234V/L235A/G236del/S267K, where the first variant Fc domain includes pI variants Q295E/N384D/Q418E/N421D, and where numbering is according to EU numbering. In an exemplary embodiment, the IL-15 variant includes amino acid substitutions N4D/N65D, D30N/N65D, or D30N/E64Q/N65D. In an exemplary embodiment, the IL-15 variant includes amino acid substitutions N4D/N65D, D30N/N65D, or D30N/E64Q/N65D. In an exemplary embodiment, the VH and VL are the VH and VL of any of the TIM-3 ABDs in Figures 12 and 13A-C and the IL-15 variant includes amino acid substitutions N4D/N65D. In another exemplary embodiment, the VH and VL are the VH and VL of any of the TIM-3 ABDs in Figures 12 and 13A-C and the IL-15 variant includes amino acid substitutions D30N/N65D. In yet another exemplary embodiment, the VH and VL are the VH and VL of any of the TIM-3 ABDs in Figures 12 and 13A-C and the IL-15 variant includes amino acid substitutions D30N/E64Q/N65D.

[00427] In the central-scIL-15/Rα format, one preferred embodiment utilizes an anti-TIM-3 ABD having the variable heavy and light domain pair of 3H3[TIM-3]_H1_L2.1 as shown in Figure 12 with the Figures 21K format, the skew variant set S364K/E357Q : L368D/K370S, the pI variants Q295E/N384D/Q418E/N421D, the ablation variants E233P/L234V/L235A/G236_/S267K on both first and second monomers, and optionally the 428L/434S variants on both first and second monomers.

[00428] In one embodiment, the targeted IL-15/IL-15Rα heterodimeric protein is an "central-scIL-15/Rα"format heterodimeric protein that includes: a) a first monomer that includes, from N- to C-terminus, a VH-(domain linker)- IL-15Rα(sushi) domain -(domain

linker)- IL-15 variant -(hinge)-CH2-CH3, where CH2-CH3 is a first variant Fc domain; b) a second monomer that includes, from N- to C-terminus, a VH-hinge-CH2-CH3, where CH2-CH3 is a second variant Fc domain; and d) a third and fourth monomer that each include from N-to C-terminus, a VL-CL, where the VH of the first monomer and the VL of the third monomer form a first TIM-3 binding domain, where the VH of the second monomer and the VL of the fourth monomer form a second TIM-3 binding domain, where the IL-15 variant and the IL-15Rα(sushi) domain form an IL-15 complex, where VH and VL are the variable heavy domain and variable light domain of 3H3[TIM-3]_H1_L2.1, where the first variant Fc domain includes skew variants L368D/K370S and the second variant Fc domain includes skew variants S364K/E357Q, where the first and second variant Fc domains each include FcKO variants E233P/L234V/L235A/G236del/S267K, where the first variant Fc domain includes pI variants Q295E/N384D/Q418E/N421D, and where numbering is according to EU numbering. In certain embodiments, the hinge of the first monomer further includes variant C220S. In certain embodiments, the first and second variant Fc domains each further include half-life extension variants M428L/N434S. In a particular embodiment, the IL-15 variant includes amino acid substitutions N4D/N65D and VH and VL are the variable heavy domain and variable light domain of 3H3[TIM-3]_H1_L2.1. In one embodiment, the IL-15 variant includes amino acid substitutions D30N/N65D and VH and VL are the variable heavy domain and variable light domain of 3H3[TIM-3]_H1_L2.1. In another embodiment, the IL-15 variant includes amino acid substitutions D30N/E64Q/N65D and VH and VL are the variable heavy domain and variable light domain of 3H3[TIM-3]_H1_L2.1.

V. Particularly Useful Embodiments of the Invention

[00429] The present invention provides a targeted IL-15/IL-15Rα-Fc heterodimeric protein comprising at least two monomers, one of which contains an anti-TIM-3 ABD and the other that contains an IL-15/RA complex, joined using heterodimeric Fc domains.

[00430] In some embodiments, the first and the second variant Fc domains have a set of amino acid substitutions selected from the group consisting of S267K/L368D/K370S : S267K/S364K/E357Q; S364K/E357Q : L368D/K370S; L368D/K370S : S364K; L368E/K370S :

S364K; T411E/K360E/Q362E : D401K; L368D/K370S : S364K/E357L and K370S : S364K/E357Q, according to EU numbering.

[00431] In some instances, the first and/or the second variant Fc domains have an additional set of amino acid substitutions comprising Q295E/N384D/Q418E/N421D, according to EU numbering. In some cases, the first and/or the second Fc domains have an additional set of amino acid substitutions consisting of G236R/L328R, E233P/L234V/L235A/G236del/S239K, E233P/L234V/L235A/G236del/S267K, E233P/L234V/L235A/G236del/S239K/A327G, E233P/L234V/L235A/G236del/S267K/A327G and E233P/L234V/L235A/G236del, according to EU numbering.

[00432] In some embodiments, the IL-15 protein has a polypeptide sequence selected from the group consisting of SEQ ID NO:1 (full-length human IL-15) and SEQ ID NO:2 (truncated human IL-15), and the IL-15Rα protein has a polypeptide sequence selected from the group consisting of SEQ ID NO:3 (full-length human IL-15Rα) and SEQ ID NO:4 (sushi domain of human IL-15Rα).

[00433] In embodiments the IL-15 protein and the IL-15Rα protein can have a set of amino acid substitutions selected from the group consisting of E87C : D96/P97/C98; E87C : D96/C97/A98; V49C : S40C; L52C : S40C; E89C : K34C; Q48C : G38C; E53C : L42C; C42S : A37C; and L45C : A37C, respectively.

[00434] In some embodiments, the IL-15 protein is a variant protein that has a sequence selected from Figure 19 and Figure 20 to reduce potency. In some embodiments, the IL-15 protein is a variant protein having one or more amino acid substitutions at the IL-15:CD132 interface.

[00435] In some embodiments, the TIM-3 antigen binding domain comprises an anti-TIM-3 scFv or an anti-TIM-3 Fab. In an exemplary embodiment, the TIM-3 ABD includes the VH and VL of any of the TIM-3 ABDs depicted in Figures 12 and 13A-C.

[00436] In an exemplary embodiment, the targeted IL-15/IL-15Rα heterodimeric protein is an "scIL-15/Rα X Fab" format heterodimeric protein that includes: a) a first monomer that includes, from N- to C-terminus, an IL-15Rα(sushi) domain-(domain linker)-IL-15 variant-(domain linker)-CH2-CH3, where CH2-CH3 is a first variant Fc domain; b) a

second monomer that includes, from N- to C-terminus, a VH-CH1-hinge-CH2-CH3, where VH is a variable heavy domain and CH2-CH3 is a second variant Fc domain, and c) a light chain that includes from, N- to C-terminus, VL-VC, where VL is a variable light domain, where VH and VL form a TIM-3 binding domain, where the IL-15 variant is an IL-15 N4D/N65D variant, where the first variant Fc domain includes skew variants L368D/K370S and the second variant Fc domain includes skew variants S364K/E357Q, where the first and second variant Fc domains each include FcKO variants E233P/L234V/L235A/G236del/S267K, where the first variant Fc domain includes pI variants Q295E/N384D/Q418E/N421D, and where numbering is according to EU numbering. In certain embodiments, the first and second variant Fc domains each further include half-life extension variants M428L/N434S. In certain embodiments, the hinge of the first monomer includes also includes amino acid substitution C220S and the first and second variant Fc domains each further include half-life extension variants M428L/N434S. In some embodiments, the VH and VL are the variable heavy domain and variable light domain of any of the TIM-3 ABDs in Figures 12 or 13A-C. In some embodiments, the VH and VL are the variable heavy domain and variable light domain of 3H3[TIM-3]_H1_L2.1 (Figure 12).

[00437] In an exemplary embodiment, the targeted IL-15/IL-15Rα heterodimeric protein is an "scIL-15/Rα X Fab" format heterodimeric protein that includes: a) a first monomer that includes, from N- to C-terminus, an IL-15Rα(sushi) domain-(domain linker)-IL-15 variant-(domain linker)-CH2-CH3, where CH2-CH3 is a first variant Fc domain; b) a second monomer that includes, from N- to C-terminus, a VH-CH1-hinge-CH2-CH3, where VH is a variable heavy domain and CH2-CH3 is a second variant Fc domain, and c) a light chain that includes from, N- to C-terminus, VL-VC, where VL is a variable light domain, where VH and VL form a TIM-3 binding domain, where the IL-15 variant is an IL-15 D30N/N65D variant, where the first variant Fc domain includes skew variants L368D/K370S and the second variant Fc domain includes skew variants S364K/E357Q, where the first and second variant Fc domains each include FcKO variants E233P/L234V/L235A/G236del/S267K, where the first variant Fc domain includes pI variants Q295E/N384D/Q418E/N421D, and where numbering is according to EU numbering. In some embodiments, the hinge of the first monomer also includes amino acid substitution C220S. In certain embodiments, the

first and second variant Fc domains each further include half-life extension variants M428L/N434S. In certain embodiments, the hinge of the first monomer includes also includes amino acid substitution C220S and the first and second variant Fc domains each further include half-life extension variants M428L/N434S. In some embodiments, the VH and VL are the variable heavy domain and variable light domain of any of the TIM-3 ABDs in Figures 12 or 13A-C. In some embodiments, the VH and VL are the variable heavy domain and variable light domain of 3H3[TIM-3]_H1_L2.1 (Figure 12).

[00438] In an exemplary embodiment, the targeted IL-15/IL-15Rα heterodimeric protein is an "scIL-15/Rα X Fab" format heterodimeric protein that includes: a) a first monomer that includes, from N- to C-terminus, an IL-15Rα(sushi) domain-(domain linker)-IL-15 variant-(domain linker)-CH2-CH3, where CH2-CH3 is a first variant Fc domain; b) a second monomer that includes, from N- to C-terminus, a VH-CH1-hinge-CH2-CH3, where VH is a variable heavy domain and CH2-CH3 is a second variant Fc domain, and c) a light chain that includes from, N- to C-terminus, VL-VC, where VL is a variable light domain, where VH and VL form a TIM-3 binding domain, where the IL-15 variant is an IL-15 D30N/E64Q/N65D variant, where the first variant Fc domain includes skew variants L368D/K370S and the second variant Fc domain includes skew variants S364K/E357Q, where the first and second variant Fc domains each include FcKO variants E233P/L234V/L235A/G236del/S267K, where the first variant Fc domain includes pI variants Q295E/N384D/Q418E/N421D, and where numbering is according to EU numbering. In some embodiments, the hinge of the first monomer also includes amino acid substitution C220S. In certain embodiments, the first and second variant Fc domains each further include half-life extension variants M428L/N434S. In certain embodiments, the hinge of the first monomer includes also includes amino acid substitution C220S and the first and second variant Fc domains each further include half-life extension variants M428L/N434S. In some embodiments, the VH and VL are the variable heavy domain and variable light domain of any of the TIM-3 ABDs in Figures 12 or 13A-C. In some embodiments, the VH and VL are the variable heavy domain and variable light domain of 3H3[TIM-3]_H1_L2.1 (Figure 12).

[00439] Useful "backbone" sequences that can be included in the "scIL-15/Rα X Fab" format heterodimeric protein are depicted in Figure 10. In some embodiments, the "scIL-

15/Rα X Fab" format heterodimeric protein that includes: a) a first monomer that includes, from N- to C-terminus, an IL-15Rα(sushi) domain-(domain linker)-IL-15 variant-(hinge)-CH2-CH3, where hinge-CH2-CH3 has the amino acid sequence of Chain 2 of "Backbone 1" in Figure 10 (SEQ ID NO: XXX); b) a second monomer that includes, from N- to C-terminus, a VH-CH1-hinge-CH2-CH3, where VH is a variable heavy domain and CH1-hinge-CH2-CH3 has the amino acid sequence of Chain 1 of "Backbone 1" in Figure 10 (SEQ ID NO: XXX), and c) a light chain that includes from, N- to C-terminus, VL-VC, where VL is a variable light domain and VC has the sequence of "Constant Light Chain – Kappa" in Figure 11 (SEQ ID NO: XXX). In certain embodiments, the "scIL-15/Rα X Fab" format heterodimeric protein that includes: a) a first monomer that includes, from N- to C-terminus, an IL-15Rα(sushi) domain-(domain linker)-IL-15 variant-(hinge)-CH2-CH3, where hinge-CH2-CH3 has the amino acid sequence of Chain 2 of "Backbone 2" in Figure 10 (SEQ ID NO: XXX); b) a second monomer that includes, from N- to C-terminus, a VH-CH1-hinge-CH2-CH3, where VH is a variable heavy domain and CH1-hinge-CH2-CH3 has the amino acid sequence of Chain 1 of "Backbone 2" in Figure 10 (SEQ ID NO: XXX), and c) a light chain that includes from, N- to C-terminus, VL-VC, where VL is a variable light domain and VC has the sequence of "Constant Light Chain – Kappa" in Figure 11 (SEQ ID NO: XXX). In some embodiments, the "scIL-15/Rα X Fab" format heterodimeric protein that includes: a) a first monomer that includes, from N- to C-terminus, an IL-15Rα(sushi) domain-(domain linker)-IL-15 variant-(hinge)-CH2-CH3, where hinge-CH2-CH3 has the amino acid sequence of Chain 2 of "Backbone 3" in Figure 10 (SEQ ID NO: XXX); b) a second monomer that includes, from N- to C-terminus, a VH-CH1-hinge-CH2-CH3, where VH is a variable heavy domain and CH1-hinge-CH2-CH3 has the amino acid sequence of Chain 1 of "Backbone 3" in Figure 10 (SEQ ID NO: XXX), and c) a light chain that includes from, N- to C-terminus, VL-VC, where VL is a variable light domain and VC has the sequence of "Constant Light Chain – Kappa" in Figure 11 (SEQ ID NO: XXX). In an exemplary embodiment, the IL-15 variant includes amino acid substitutions N4D/N65D, D30N/N65D, or D30N/E64Q/N65D. In an exemplary embodiment, the IL-15 variant includes amino acid substitutions N4D/N65D, D30N/N65D, or D30N/E64Q/N65D. In an exemplary embodiment, the VH and VL are the VH and VL of any of the TIM-3 ABDs in Figures 12 and 13A-C and the IL-15

variant includes amino acid substitutions N4D/N65D. In another exemplary embodiment, the VH and VL are the VH and VL of any of the TIM-3 ABDs in Figures 12 and 13A-C and the IL-15 variant includes amino acid substitutions D30N/N65D. In yet another exemplary embodiment, the VH and VL are the VH and VL of any of the TIM-3 ABDs in Figures 12 and 13A-C and the IL-15 variant includes amino acid substitutions D30N/E64Q/N65D.

[00440] Particularly preferred TIM-3 targeted IL-15/IL-15Rα-Fc heterodimeric fusion proteins include XENP27974, XENP27979, XENC1000, XENC1001, XENC1002, and XENC1003 "scIL-15/Rα X Fab" format heterodimeric protein. Exemplary embodiments of the TIM-3 targeted IL-15/IL-15Rα-Fc heterodimeric fusion proteins are shown in as described in Figure 22 and Figure 29, Figure 46, Figure 47 and Figures 48A and B. respectively.

VI. Nucleic Acids of the Invention

[00441] The invention further provides nucleic acid compositions encoding the targeted IL-15/IL-15Rα-Fc heterodimeric fusion proteins of the invention (or, in the case of a monomer Fc domain protein, nucleic acids encoding those as well).

[00442] As will be appreciated by those in the art, the nucleic acid compositions will depend on the format of the targeted IL-15/IL-15Rα-Fc heterodimeric fusion protein. Thus, for example, when the format requires three amino acid sequences, three nucleic acid sequences can be incorporated into one or more expression vectors for expression. Similarly, some formats only two nucleic acids are needed; again, they can be put into one or two expression vectors, or four or 5. As noted herein, some constructs have two copies of a light chain, for example.

[00443] As is known in the art, the nucleic acids encoding the components of the invention can be incorporated into expression vectors as is known in the art, and depending on the host cells used to produce the targeted IL-15/IL-15Rα-Fc heterodimeric fusion proteins of the invention. Generally the nucleic acids are operably linked to any number of regulatory elements (promoters, origin of replication, selectable markers, ribosomal binding sites, inducers, etc.). The expression vectors can be extra-chromosomal or integrating vectors.

[00444] The nucleic acids and/or expression vectors of the invention are then transformed into any number of different types of host cells as is well known in the art, including mammalian, bacterial, yeast, insect and/or fungal cells, with mammalian cells (e.g., CHO cells), finding use in many embodiments.

[00445] In some embodiments, nucleic acids encoding each monomer, as applicable depending on the format, are each contained within a single expression vector, generally under different or the same promoter controls. In embodiments of particular use in the present invention, each of these two or three nucleic acids are contained on a different expression vector.

[00446] The targeted IL-15/IL-15Rα-Fc heterodimeric fusion protein of the invention are made by culturing host cells comprising the expression vector(s) as is well known in the art. Once produced, traditional fusion protein or antibody purification steps are done, including an ion exchange chromatography step. As discussed herein, having the pIs of the two monomers differ by at least 0.5 can allow separation by ion exchange chromatography or isoelectric focusing, or other methods sensitive to isoelectric point. That is, the inclusion of pI substitutions that alter the isoelectric point (pI) of each monomer so that such that each monomer has a different pI and the heterodimer also has a distinct pI, thus facilitating isoelectric purification of the heterodimer (e.g., anionic exchange columns, cationic exchange columns). These substitutions also aid in the determination and monitoring of any contaminating homodimers post-purification (e.g., IEF gels, cIEF, and analytical IEX columns).

VII. Biological and Biochemical Functionality of TIM-3 Antibody x IL-15/IL-15Rα Heterodimeric Immunomodulatory Fusion Proteins

[00447] Generally the targeted IL-15/IL-15Rα-Fc heterodimeric fusion proteins of the invention are administered to patients with cancer, and efficacy is assessed, in a number of ways as described herein. Thus, while standard assays of efficacy can be run, such as cancer load, size of tumor, evaluation of presence or extent of metastasis, etc., immuno-oncology treatments can be assessed on the basis of immune status evaluations as well. This can be done in a number of ways, including both in vitro and in vivo assays. For example, evaluation of changes in immune status along with "old fashioned" measurements such as

tumor burden, size, invasiveness, LN involvement, metastasis, etc. can be done. Thus, any or all of the following can be evaluated: the inhibitory effects of the heterodimeric proteins on $CD4^+$ T cell activation or proliferation, $CD8^+$ T (CTL) cell activation or proliferation, $CD8^+$ T cell-mediated cytotoxic activity and/or CTL mediated cell depletion, NK cell activity and NK mediated cell depletion, the potentiating effects of the heterodimeric protein on Treg cell differentiation and proliferation and Treg- or myeloid derived suppressor cell (MDSC)-mediated immunosuppression or immune tolerance, and/or the effects of heterodimeric protein on proinflammatory cytokine production by immune cells, e.g., IL-2, IFN-ɣ or TNF-α production by T or other immune cells.

[00448] In some embodiments, assessment of treatment is done by evaluating immune cell proliferation, using for example, CFSE dilution method, Ki67 intracellular staining of immune effector cells, and ^{3}H-thymidine incorporation method.

[00449] In some embodiments, assessment of treatment is done by evaluating the increase in gene expression or increased protein levels of activation-associated markers, including one or more of: CD25, CD69, CD137, ICOS, PD1, GITR, OX40, and cell degranulation measured by surface expression of CD107A.

[00450] In general, gene expression assays are done as is known in the art.

[00451] In general, protein expression measurements are also similarly done as is known in the art.

[00452] In some embodiments, assessment of treatment is done by assessing cytotoxic activity measured by target cell viability detection via estimating numerous cell parameters such as enzyme activity (including protease activity), cell membrane permeability, cell adherence, ATP production, co-enzyme production, and nucleotide uptake activity. Specific examples of these assays include, but are not limited to, Trypan Blue or PI staining, ^{51}Cr or ^{35}S release method, LDH activity, MTT and/or WST assays, Calcein-AM assay, Luminescent based assay, and others.

[00453] In some embodiments, assessment of treatment is done by assessing T cell activity measured by cytokine production, measure either intracellularly in culture

supernatant using cytokines including, but not limited to, IFNγ, TNFα, GM-CSF, IL2, IL6, IL4, IL5, IL10, IL13 using well known techniques.

[00454] Accordingly, assessment of treatment can be done using assays that evaluate one or more of the following: (i) increases in immune response, (ii) increases in activation of αβ and/or γδ T cells, (iii) increases in cytotoxic T cell activity, (iv) increases in NK and/or NKT cell activity, (v) alleviation of αβ and/or γδ T-cell suppression, (vi) increases in pro-inflammatory cytokine secretion, (vii) increases in IL-2 secretion; (viii) increases in interferon-ɣ production, (ix) increases in Th1 response, (x) decreases in Th2 response, (xi) decreases or eliminates cell number and/or activity of at least one of regulatory T cells (Tregs).

A. Assays to Measure Efficacy

[00455] In some embodiments, T cell activation is assessed using a Mixed Lymphocyte Reaction (MLR) assay as is known in the art. An increase in activity indicates immunostimulatory activity. Appropriate increases in activity are outlined below.

[00456] In one embodiment, the signaling pathway assay measures increases or decreases in immune response as measured for an example by phosphorylation or de-phosphorylation of different factors, or by measuring other post translational modifications. An increase in activity indicates immunostimulatory activity. Appropriate increases in activity are outlined below.

[00457] In one embodiment, the signaling pathway assay measures increases or decreases in activation of αβ and/or γδ T cells as measured for an example by cytokine secretion or by proliferation or by changes in expression of activation markers like for an example CD137, CD107a, PD1, etc. An increase in activity indicates immunostimulatory activity. Appropriate increases in activity are outlined below.

[00458] In one embodiment, the signaling pathway assay measures increases or decreases in cytotoxic T cell activity as measured for an example by direct killing of target cells like for an example cancer cells or by cytokine secretion or by proliferation or by changes in expression of activation markers like for an example CD137, CD107a, PD1, etc.

An increase in activity indicates immunostimulatory activity. Appropriate increases in activity are outlined below.

[00459] In one embodiment, the signaling pathway assay measures increases or decreases in NK and/or NKT cell activity as measured for an example by direct killing of target cells like for an example cancer cells or by cytokine secretion or by changes in expression of activation markers like for an example CD107a, etc. An increase in activity indicates immunostimulatory activity. Appropriate increases in activity are outlined below.

[00460] In one embodiment, the signaling pathway assay measures increases or decreases in $\alpha\beta$ and/or $\gamma\delta$ T-cell suppression, as measured for an example by cytokine secretion or by proliferation or by changes in expression of activation markers like for an example CD137, CD107a, PD1, etc. An increase in activity indicates immunostimulatory activity. Appropriate increases in activity are outlined below.

[00461] In one embodiment, the signaling pathway assay measures increases or decreases in pro-inflammatory cytokine secretion as measured for example by ELISA or by Luminex or by Multiplex bead based methods or by intracellular staining and FACS analysis or by Alispot etc. An increase in activity indicates immunostimulatory activity. Appropriate increases in activity are outlined below.

[00462] In one embodiment, the signaling pathway assay measures increases or decreases in IL-2 secretion as measured for example by ELISA or by Luminex or by Multiplex bead based methods or by intracellular staining and FACS analysis or by Alispot etc. An increase in activity indicates immunostimulatory activity. Appropriate increases in activity are outlined below.

[00463] In one embodiment, the signaling pathway assay measures increases or decreases in interferon-γ production as measured for example by ELISA or by Luminex or by Multiplex bead based methods or by intracellular staining and FACS analysis or by Alispot etc. An increase in activity indicates immunostimulatory activity. Appropriate increases in activity are outlined below.

[00464] In one embodiment, the signaling pathway assay measures increases or decreases in Th1 response as measured for an example by cytokine secretion or by changes

in expression of activation markers. An increase in activity indicates immunostimulatory activity. Appropriate increases in activity are outlined below.

[00465] In one embodiment, the signaling pathway assay measures increases or decreases in Th2 response as measured for an example by cytokine secretion or by changes in expression of activation markers. An increase in activity indicates immunostimulatory activity. Appropriate increases in activity are outlined below.

[00466] In one embodiment, the signaling pathway assay measures increases or decreases cell number and/or activity of at least one of regulatory T cells (Tregs), as measured for example by flow cytometry or by IHC. A decrease in response indicates immunostimulatory activity. Appropriate decreases are the same as for increases, outlined below.

[00467] In one embodiment, the signaling pathway assay measures increases or decreases in M2 macrophages cell numbers, as measured for example by flow cytometry or by IHC. A decrease in response indicates immunostimulatory activity. Appropriate decreases are the same as for increases, outlined below.

[00468] In one embodiment, the signaling pathway assay measures increases or decreases in M2 macrophage pro-tumorigenic activity, as measured for an example by cytokine secretion or by changes in expression of activation markers. A decrease in response indicates immunostimulatory activity. Appropriate decreases are the same as for increases, outlined below.

[00469] In one embodiment, the signaling pathway assay measures increases or decreases in N2 neutrophils increase, as measured for example by flow cytometry or by IHC. A decrease in response indicates immunostimulatory activity. Appropriate decreases are the same as for increases, outlined below.

[00470] In one embodiment, the signaling pathway assay measures increases or decreases in N2 neutrophils pro-tumorigenic activity, as measured for an example by cytokine secretion or by changes in expression of activation markers. A decrease in response indicates immunostimulatory activity. Appropriate decreases are the same as for increases, outlined below.

[00471] In one embodiment, the signaling pathway assay measures increases or decreases in inhibition of T cell activation, as measured for an example by cytokine secretion or by proliferation or by changes in expression of activation markers like for an example CD137, CD107a, PD1, etc. An increase in activity indicates immunostimulatory activity. Appropriate increases in activity are outlined below.

[00472] In one embodiment, the signaling pathway assay measures increases or decreases in inhibition of CTL activation as measured for an example by direct killing of target cells like for an example cancer cells or by cytokine secretion or by proliferation or by changes in expression of activation markers like for an example CD137, CD107a, PD1, etc. An increase in activity indicates immunostimulatory activity. Appropriate increases in activity are outlined below.

[00473] In one embodiment, the signaling pathway assay measures increases or decreases in $\alpha\beta$ and/or $\gamma\delta$ T cell exhaustion as measured for an example by changes in expression of activation markers. A decrease in response indicates immunostimulatory activity. Appropriate decreases are the same as for increases, outlined below.

[00474] In one embodiment, the signaling pathway assay measures increases or decreases $\alpha\beta$ and/or $\gamma\delta$ T cell response as measured for an example by cytokine secretion or by proliferation or by changes in expression of activation markers like for an example CD137, CD107a, PD1, etc. An increase in activity indicates immunostimulatory activity. Appropriate increases in activity are outlined below.

[00475] In one embodiment, the signaling pathway assay measures increases or decreases in stimulation of antigen-specific memory responses as measured for an example by cytokine secretion or by proliferation or by changes in expression of activation markers like for an example CD45RA, CCR7 etc. An increase in activity indicates immunostimulatory activity. Appropriate increases in activity are outlined below. .

[00476] In one embodiment, the signaling pathway assay measures increases or decreases in apoptosis or lysis of cancer cells as measured for an example by cytotoxicity assays such as for an example MTT, Cr release, Calcine AM, or by flow cytometry based assays like for an example CFSE dilution or propidium iodide staining etc. An increase in

activity indicates immunostimulatory activity. Appropriate increases in activity are outlined below.

[00477] In one embodiment, the signaling pathway assay measures increases or decreases in stimulation of cytotoxic or cytostatic effect on cancer cells. as measured for an example by cytotoxicity assays such as for an example MTT, Cr release, Calcine AM, or by flow cytometry based assays like for an example CFSE dilution or propidium iodide staining etc. An increase in activity indicates immunostimulatory activity. Appropriate increases in activity are outlined below.

[00478] In one embodiment, the signaling pathway assay measures increases or decreases direct killing of cancer cells as measured for an example by cytotoxicity assays such as for an example MTT, Cr release, Calcine AM, or by flow cytometry based assays like for an example CFSE dilution or propidium iodide staining etc. An increase in activity indicates immunostimulatory activity. Appropriate increases in activity are outlined below.

[00479] In one embodiment, the signaling pathway assay measures increases or decreases Th17 activity as measured for an example by cytokine secretion or by proliferation or by changes in expression of activation markers. An increase in activity indicates immunostimulatory activity. Appropriate increases in activity are outlined below.

[00480] In one embodiment, the signaling pathway assay measures increases or decreases in induction of complement dependent cytotoxicity and/or antibody dependent cell-mediated cytotoxicity, as measured for an example by cytotoxicity assays such as for an example MTT, Cr release, Calcine AM, or by flow cytometry based assays like for an example CFSE dilution or propidium iodide staining etc. An increase in activity indicates immunostimulatory activity. Appropriate increases in activity are outlined below.

[00481] In one embodiment, T cell activation is measured for an example by direct killing of target cells like for an example cancer cells or by cytokine secretion or by proliferation or by changes in expression of activation markers like for an example CD137, CD107a, PD1, etc. For T-cells, increases in proliferation, cell surface markers of activation (e.g., CD25, CD69, CD137, PD1), cytotoxicity (ability to kill target cells), and cytokine

production (e.g., IL-2, IL-4, IL-6, IFNγ, TNF-a, IL-10, IL-17A) would be indicative of immune modulation that would be consistent with enhanced killing of cancer cells.

[00482] In one embodiment, NK cell activation is measured for example by direct killing of target cells like for an example cancer cells or by cytokine secretion or by changes in expression of activation markers like for an example CD107a, etc. For NK cells, increases in proliferation, cytotoxicity (ability to kill target cells and increases CD107a, granzyme, and perforin expression), cytokine production (e.g., IFNγ and TNF), and cell surface receptor expression (e.g., CD25) would be indicative of immune modulation that would be consistent with enhanced killing of cancer cells.

[00483] In one embodiment, γδ T cell activation is measured for example by cytokine secretion or by proliferation or by changes in expression of activation markers.

[00484] In one embodiment, Th1 cell activation is measured for example by cytokine secretion or by changes in expression of activation markers.

[00485] Appropriate increases in activity or response (or decreases, as appropriate as outlined above), are increases of 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90%, 95% or 98 to 99% percent over the signal in either a reference sample or in control samples, for example test samples that do not contain a heterodimeric protein of the invention. Similarly, increases of at least one-, two-, three-, four- or five-fold as compared to reference or control samples show efficacy.

VIII. Treatments

[00486] Once made, the compositions of the invention find use in a number of oncology applications, by treating cancer, generally by promoting T cell activation (e.g., T cells are no longer suppressed) with the binding of the heterodimeric fusion proteins of the invention.

[00487] Accordingly, the targeted IL-15/IL-15Rα-Fc heterodimeric compositions of the invention find use in the treatment of these cancers.

A. Targeted IL-15/IL-15Rα-Fc Heterodimeric Protein Compositions for *In Vivo* Administration

[00488] Formulations of the antibodies used in accordance with the present invention are prepared for storage by mixing an antibody having the desired degree of purity with optional pharmaceutically acceptable carriers, excipients or stabilizers (as generally outlined in Remington's Pharmaceutical Sciences 16th edition, Osol, A. Ed. [1980]), in the form of lyophilized formulations or aqueous solutions. Acceptable carriers, buffers, excipients, or stabilizers are nontoxic 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 glucose, mannose, or dextrins; 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).

B. Combination Therapies

[00489] In some embodiments, the heterodimeric proteins of the invention can be used in combination therapies with antibodies that bind to different checkpoint proteins, e.g., not TIM-3 antibodies. In this way, the targeted IL-15/IL-15Rα-Fc binding domains of the additional antibody do not compete for binding with the targeted IL-15/IL-15Rα-Fc heterodimeric protein. In this way, a sort of "triple combination" therapy is achieved, as three receptors are engaged (two from the targeted IL-15/IL-15Rα-Fc heterodimeric protein and one from the additional antibody). As discussed herein, the heterodimeric protein can have different valencies and specifities as outlined herein.

[00490] Surprisingly, as shown herein, these combinations can result in synergistic effects when co-administered. In this context, "co-administration" means that the two moieties can be administered simultaneously or sequentially. That is, in some cases, the

drugs may be administered simultaneously, although generally this is through the use of two separate IV infusions; that is, the drugs are generally not combined into a single dosage unit. Alternatively, co-administration includes the sequential administration of the two separate drugs, either in a single day or separate days (including separate days over time).

1. Anti-PD-1 Antibodies for use in Co-Administration Therapies

[00491] As is known in the art, there are two currently approved anti-PD-1 antibodies and many more in clinical testing. Thus, suitable anti-PD-1 antibodies for use in combination therapies as outlined herein include, but are not limited to, the two currently FDA approved antibodies, pembrolizumab and nivolizumab, as well as those in clinical testing currently, including, but not limited to, tislelizumab, Sym021, REGN2810 (developed by Rengeneron), JNJ-63723283 (developed by J and J), SHR-1210, pidilizumab, AMP-224, MEDIo680, PDR001 and CT-001, as well as others outlined in Liu et al., J. Hemat. & Oncol. (2017)10:136, the antibodies therein expressly incorporated by reference. As above, anti-PD-1 antibodies are used in combination when the targeted IL-15/IL-15Rα-Fc fusion proteins of the invention do not have an antigen binding domain that binds PD-1.

2. Anti-PD-L1 Antibodies for Use in Co-Administration Therapies

[00492] In some embodiments, anti-PD-L1 antibodies are used in combination. As is known in the art, there are three currently approved anti-PD-L1 antibodies and many more in clinical testing. Thus, suitable anti-PD-L1 antibodies for use in combination therapies as outlined herein include, but are not limited to, the three currently FDA approved antibodies, atezolizumab, avelumab, durvalumab, as well as those in clinical testing currently, including, but not limited to, LY33000054 and CS1001, as well as others outlined in Liu et al., J. Hemat. & Oncol. (2017)10:136, the antibodies therein expressly incorporated by reference. As above, anti-PD-L1 antibodies are used in combination when the targeted IL-15/IL-15Rα-Fc fusion proteins of the invention do not have an antigen binding domain that binds PD-L1.

3. Anti-TIGIT Antibodies for Use in Co-Administration Therapies

[00493] In some embodiments, anti-TIGIT antibodies can be used in combination with the targeted IL-15/IL-15Rα-Fc fusion proteins of the invention. There are several TIGIT antibodies in clinical development, BMS-986207, OMP-313M32 and MTIG7192A. As above,

anti-TIGIT antibodies are used in combination when the targeted IL-15/IL-15Rα-Fc fusion protein of the invention do not have an antigen binding domain that binds TIGIT.

4. Anti-CTLA-4 Antibodies for Use in Co-Administration Therapies

[00494] In some embodiments, anti-CTLA-4 antibodies can be used in combination with the targeted IL-15/IL-15Rα-Fc fusion protein of the invention. Ipilimumab has been approved, and there are several more in development, including CP-675,206 and AGEN-1884. As above, anti-CTLA-4 antibodies are used in combination when the targeted IL-15/IL-15Rα-Fc fusion proteins of the invention do not have an antigen binding domain that binds CTLA-4.

5. Anti-TIM-3 Antibodies for Use in Co-Administration Therapies

In some embodiments, anti-TIM-3 antibodies can be used in combination with the targeted IL-15/IL-15Rα-Fc fusion protein of the invention. There are several TIM-3 antibodies in clinical development including BMS-986016, LAG525 and REGN3767. As above, anti-TIM-3 antibodies are used in combination when the targeted IL-15/IL-15Rα-Fc fusion proteins of the invention do not have an antigen binding domain that binds TIM-3.

C. Administrative Modalities

[00495] The targeted IL-15/IL-15Rα-Fc fusion proteins and chemotherapeutic agents of the invention are administered to a subject, in accord with known methods, such as intravenous administration as a bolus or by continuous infusion over a period of time.

D. Treatment Modalities

[00496] In the methods of the invention, therapy is used to provide a positive therapeutic response with respect to a disease or condition. By "positive therapeutic response" is intended an improvement in the disease or condition, and/or an improvement in the symptoms associated with the disease or condition. For example, a positive therapeutic response would refer to one or more of the following improvements in the disease: (1) a reduction in the number of neoplastic cells; (2) an increase in neoplastic cell death; (3) inhibition of neoplastic cell survival; (5) inhibition (i.e., slowing to some extent, preferably halting) of tumor growth; (6) an increased patient survival rate; and (7) some relief from one or more symptoms associated with the disease or condition.

[00497] Positive therapeutic responses in any given disease or condition can be determined by standardized response criteria specific to that disease or condition. Tumor response can be assessed for changes in tumor morphology (i.e., overall tumor burden, tumor size, and the like) using screening techniques such as magnetic resonance imaging (MRI) scan, x-radiographic imaging, computed tomographic (CT) scan, bone scan imaging, endoscopy, and tumor biopsy sampling including bone marrow aspiration (BMA) and counting of tumor cells in the circulation.

[00498] In addition to these positive therapeutic responses, the subject undergoing therapy may experience the beneficial effect of an improvement in the symptoms associated with the disease.

[00499] Treatment according to the present invention includes a "therapeutically effective amount" of the medicaments used. A "therapeutically effective amount" refers to an amount effective, at dosages and for periods of time necessary, to achieve a desired therapeutic result.

[00500] A therapeutically effective amount may vary according to factors such as the disease state, age, sex, and weight of the individual, and the ability of the medicaments to elicit a desired response in the individual. A therapeutically effective amount is also one in which any toxic or detrimental effects of the antibody or antibody portion are outweighed by the therapeutically beneficial effects.

[00501] A "therapeutically effective amount" for tumor therapy may also be measured by its ability to stabilize the progression of disease. The ability of a compound to inhibit cancer may be evaluated in an animal model system predictive of efficacy in human tumors.

[00502] Alternatively, this property of a composition may be evaluated by examining the ability of the compound to inhibit cell growth or to induce apoptosis by in vitro assays known to the skilled practitioner. A therapeutically effective amount of a therapeutic compound may decrease tumor size, or otherwise ameliorate symptoms in a subject. One of ordinary skill in the art would be able to determine such amounts based on such factors as

the subject's size, the severity of the subject's symptoms, and the particular composition or route of administration selected.

[00503] Dosage regimens are adjusted to provide the optimum desired response (e.g., a therapeutic response). For example, a single bolus may be administered, several divided doses may be administered over time or the dose may be proportionally reduced or increased as indicated by the exigencies of the therapeutic situation. Parenteral compositions may be formulated in dosage unit form for ease of administration and uniformity of dosage. Dosage unit form as used herein refers to physically discrete units suited as unitary dosages for the subjects to be treated; each unit contains a predetermined quantity of active compound calculated to produce the desired therapeutic effect in association with the required pharmaceutical carrier.

[00504] The specification for the dosage unit forms of the present invention are dictated by and directly dependent on (a) the unique characteristics of the active compound and the particular therapeutic effect to be achieved, and (b) the limitations inherent in the art of compounding such an active compound for the treatment of sensitivity in individuals.

[00505] The efficient dosages and the dosage regimens for the targeted IL-15/IL-15Rα-Fc fusion protein used in the present invention depend on the disease or condition to be treated and may be determined by the persons skilled in the art.

[00506] An exemplary, non-limiting range for a therapeutically effective amount of the targeted IL-15/IL-15Rα-Fc fusion protein used in the present invention is about 0.1-100 mg/kg.

[00507] All cited references are herein expressly incorporated by reference in their entirety.

[00508] Whereas particular embodiments of the invention have been described above for purposes of illustration, it will be appreciated by those skilled in the art that numerous variations of the details may be made without departing from the invention as described in the appended claims.

IX. Examples

[00509] Examples are provided below to illustrate the present invention. These examples are not meant to constrain the present invention to any particular application or theory of operation. For all constant region positions discussed in the present invention, numbering is according to the EU index as in Kabat (Kabat et al., 1991, Sequences of Proteins of Immunological Interest, 5th Ed., United States Public Health Service, National Institutes of Health, Bethesda, entirely incorporated by reference). Those skilled in the art of antibodies will appreciate that this convention consists of nonsequential numbering in specific regions of an immunoglobulin sequence, enabling a normalized reference to conserved positions in immunoglobulin families. Accordingly, the positions of any given immunoglobulin as defined by the EU index will not necessarily correspond to its sequential sequence.

[00510] General and specific scientific techniques are outlined in US Publications 2015/0307629, 2014/0288275 and WO2014/145806, all of which are expressly incorporated by reference in their entirety and particularly for the techniques outlined therein. Examples 1 and 2 from USSN 62,416, 087, filed on November 1, 2016 are expressly incorporated by reference in their entirety, including the corresponding figures. Additionally, USSNs 62/408,655, 62/443,465, 62/477,926, 15/785,401, 62/416,087 and 15/785,393 are expressly incorporated by reference in their entirety, and specifically for all the sequences, Figures and Legends therein.

A. Example 1: Anti-TIM-3 ABDs

[00511] Examples of antigen-binding domains which bind TIM-3 are described in WO2017/218707, herein incorporated by reference, the contents are hereby incorporated in its entirety for all purposes, and in particular for the TIM-3 ABDs in Figure 13, the data in Figure 21 and Figure 22 and SEQ ID NO:s 20765-20884, SEQ ID NO:s 37587-37698 and SEQ ID NO:s 36347-36706 sequences in the sequence listing (which can be formatted either as scFvs or as Fabs as discussed therein and herein). Additional illustrative sequences of anti-TIM-3 Fvs are depicted in Figure 12 and Figures 13. Additional non-limiting examples of TIM-3 ABDs which may find use in the TIM-3-targeted IL-15/Rα-Fc fusion proteins of the invention are depicted in Figure 16 and in the sequence listing.

B. Example 2: TIM-3-targeted IL-15/Rα-Fc fusions

[00512] Reference is made to WO2018/071919 which describes IL-15/RA –Fc fusions that do not contain ABDs as are generally depicted in Figure 9 and Figure 39. WO2018/071919 is expressly incorporated by reference herein, and specifically for all of the sequences, formats, Figures and Legends therein.

2A: Generation of TIM-3-targeted IL-15/Rα-Fc fusions

[00513] Plasmids coding for IL-15, IL-15Rα sushi domain, or the anti-TIM-3 variable regions were constructed by standard gene synthesis, followed by subcloning into a pTT5 expression vector containing Fc fusion partners (e.g., constant regions as depicted in Figure 13). Cartoon schematics of illustrative TIM-3-targeted IL-15/Rα-Fc fusions are depicted in Figures 21.

[00514] The "scIL-15/Rα x scFv" format (Figures 21A) comprises IL-15Rα(sushi) fused to IL-15 by a variable length linker (termed "scIL-15/Rα") which is then fused to the N-terminus of a heterodimeric Fc-region, with an scFv fused to the other side of the heterodimeric Fc.

[00515] The "scFv x ncIL-15/Rα" format (Figures 21B) comprises an scFv fused to the N-terminus of a heterodimeric Fc-region, with IL-15Rα(sushi) fused to the other side of the heterodimeric Fc, while IL-15 is transfected separately so that a non-covalent IL-15/Rα complex is formed.

[00516] The "scFv x dsIL-15/Rα" format (Figures 21C) is the same as the "scFv x ncIL-15/Rα" format, but wherein IL-15Rα(sushi) and IL-15 are covalently linked as a result of engineered cysteines.

[00517] The "scIL-15/Rα x Fab" format (Figures 21D) comprises IL-15Rα(sushi) fused to IL-15 by a variable length linker (termed "scIL-15/Rα") which is then fused to the N-terminus of a heterodimeric Fc-region, with a variable heavy chain (VH) fused to the other side of the heterodimeric Fc, while a corresponding light chain is transfected separately so as to form a Fab with the VH. Sequences for illustrative TIM-3-targeted IL-15/Rα-Fc fusion proteins of this format are depicted in Figure 61.

[00518] The "ncIL-15/Rα x Fab" format (Figures 21E) comprises a VH fused to the N-terminus of a heterodimeric Fc-region, with IL-15Rα(sushi) fused to the other side of the heterodimeric Fc, while a corresponding light chain is transfected separately so as to form a Fab with the VH, and while IL-15 is transfected separately so that a non-covalent IL-15/Rα complex is formed.

[00519] The "dsIL-15/Rα x Fab" format (Figures 21F) is the same as the "ncIL-15/Rα x Fab" format, but wherein IL-15Rα(sushi) and IL-15 are covalently linked as a result of engineered cysteines.

[00520] The "mAb-scIL-15/Rα" format (Figures 21G) comprises VH fused to the N-terminus of a first and a second heterodimeric Fc, with IL-15 is fused to IL-15Rα(sushi) which is then further fused to the C-terminus of one of the heterodimeric Fc-region, while corresponding light chains are transfected separately so as to form a Fabs with the VHs.

[00521] The "mAb-ncIL-15/Rα" format (Figures 21H) comprises VH fused to the N-terminus of a first and a second heterodimeric Fc, with IL-15Rα(sushi) fused to the C-terminus of one of the heterodimeric Fc-region, while corresponding light chains are transfected separately so as to form a Fabs with the VHs, and while and while IL-15 is transfected separately so that a non-covalent IL-15/Rα complex is formed.

[00522] The "mAb-dsIL-15/Rα" format (Figures 21I) is the same as the "mAb-ncIL-15/Rα" format, but wherein IL-15Rα(sushi) and IL-15 are covalently linked as a result of engineered cysteines.

[00523] The "central-IL-15/Rα" format (Figures 21J) comprises a VH recombinantly fused to the N-terminus of IL-15 which is then further fused to one side of a heterodimeric Fc and a VH recombinantly fused to the N-terminus of IL-15Rα(sushi) which is then further fused to the other side of the heterodimeric Fc, while corresponding light chains are transfected separately so as to form a Fabs with the VHs.

[00524] The "central-scIL-15/Rα" format (Figures 21K) comprises a VH fused to the N-terminus of IL-15Rα(sushi) which is fused to IL-15 which is then further fused to one side of a heterodimeric Fc and a VH fused to the other side of the heterodimeric Fc, while corresponding light chains are transfected separately so as to form a Fabs with the VHs.

2B: TIM-3-targeted IL-15/Rα-Fc fusions enhance GVHD, and combines synergistically with anti-PD-1 antibody

[00525] Illustrative TIM-3-targeted IL-15/Rα-Fc fusion protein, XENP27974 alone or in combination with (a bivalent anti-PD-1 mAb based on nivolumab with ablated effector function; sequences for which is depicted in Figure 23), was evaluated in a Graft-versus-Host Disease (GVHD) model conducted in NSG (NOD-SCID-gamma) immunodeficient mice. When the NSG mice are injected with human PBMCs, the human PBMCs develop an autoimmune response against mouse cells. Dosing of NSG mice injected with human PBMCs followed with TIM-3-targeted IL-15/Rα-Fc fusion proteins proliferate the engrafted T cells and enhances engraftment.

[00526] 10 million human PBMCs were engrafted into NSG mice via IV-OSP on Day -1 followed by dosing with the indicated test articles at the indicated concentrations on Days 0, 7, 14, and 21. Counts of various lymphocyte populations were performed on Days 6 and 10, data for which are depicted in Figures 24 to Figures 27. Body weights of mice were measured over time and depicted in Figure 28 as percentage of initial body weight. The data show that dosing XENP27974 following engraftment with human PBMCs enhanced GVHD as indicated by increased T cell (CD8+ and CD4+), NK cell, and CD45+ cell counts as well as decreased body weight in comparison to engraftment with PBMC alone. Notably, XENP27974 enhanced GVHD to a greater extent than dosing with XENP16432 alone. Additionally, the data show that XENP27974 combined synergistically with XENP16432 in enhancing GVHD as indicated by the death of all mice by Day 19 following dosing with a combination of XENP27974 and XENP16432. This suggests that, in an immuno-oncology setting, treatment with TIM-3-targeted IL-15/Rα-Fc fusion proteins alone or in combination with checkpoint blockade antibodies will proliferate tumor-infiltrating lymphocytes and enhance anti-tumor activity.

2C: In vitro characterization of TIM-3-targeted IL-15/Rα-Fc fusions

[00527] The TIM-3-targeted IL-15/Rα-Fc fusions were further characterized in a cell proliferation assay. Human PBMCs were stimulated for 48 hours with 500 ng/ml plate-bound anti-CD3 (OKT3) and then labeled with CFSE and incubated with the following test articles for 4 days at 37°C: XENP27974 (TIM-3-targeted IL-15/Rα-Fc fusion based on 2A5B4

and having N4D/N65D IL-15 variant); XENP24306 (control untargeted IL-15(D30N/E64Q/N65D)/Rα-Fc fusion having D30N/E64Q/N65D IL-15 variant); and XENP26007 (control RSV-targeted IL-15/Rα-Fc fusion having N4D/N65D IL-15 variant). Cells were stained with the following antibodies: anti-CD8-PerCP-Cy5.5 (SK1), anti-CD3-PE-Cy7 (OKT3), anti-CD45RO-APC-Fire750 (UCHL1), anti-HLA-DR-Alexa700 (L243), anti-CD16-BV605 (3G6), anti-CD56-BV605 (HCD56), anti-CD25-BV711 (M-A251), anti-CD45RA-BV785 (HI100), anti-CD4-BUV395 (SK3), and Zombie Aqua-BV510 and analyzed by flow for various cell populations.

[00528] The proliferation of various T cell populations based on CFSE dilution (Zombie Aqua to exclude dead cells) was investigated, data for which are depicted in Figures 30-35. The data show that the TIM-3-targeted IL-15/Rα-Fc fusion is much more potent in inducing proliferation of both $CD8^+$ and $CD4^+$ T cells in comparison to untargeted IL-15(D30N/E64Q/N65D)/Rα-Fc fusion (as well as control RSV-targeted IL-15/Rα-Fc fusion). Notably, the TIM-3-targeted IL-15/Rα-Fc fusions preferentially targets memory T cells over naive T cells, suggesting that in a clinical setting, the TIM-3-targeted IL-15/Rα-Fc fusions will be selective for activated tumor-infiltrating lymphocytes in the tumor environment. Additionally, as shown in Figure 35, TIM-3-targeted IL-15/Rα-Fc fusions are much more potent in inducing proliferation of NK cells.

[00529] The activation of various T cell populations based on expression of CD25 (a late stage T cell activation marker) and HLA-DR (another activation marker) was also investigated, data for which are depicted in Figures 36-38. The data depicted in Figure 36 show that TIM-3-targeted IL-15/Rα-Fc fusions appear more potent in inducing activation of CD8 T cell populations in comparison to untargeted IL-15(D30N/E64Q/N65D)/Rα-Fc fusion (as well as control RSV-targeted IL-15/Rα-Fc fusion).

C. Example 3: TIM-3-targeted IL-15/Rα-Fc fusions with tuned IL-15 Potency

3A: IL-15(D30N/N65D) variant

[00530] In a study investigating the pharmacokinetics of IL-15-Fc potency variants with Xtend, cynomolgus monkeys were administered a first single intravenous (i.v.) dose of XENP22853 (WT IL-15/Rα-heteroFc with Xtend; sequences depicted in Figure 39), XENP24306 (IL-15(D30N/E64Q/N65D)/Rα-heteroFc with Xtend; sequences depicted in

Figure 42), XENP24113 (IL-15(N4D/N65D)/Rα-heteroFc with Xtend; sequences depicted in Figure 40), and XENP24294 (scIL-15(N4D/N65D)/Rα-Fc with Xtend; sequences depicted in Figure 41) at varying concentrations.

[00531] Figure 43 depicts the serum concentration of the test articles over time following the first dose. As expected, incorporating potency variants in addition to Xtend substitution (as in XENP24306 and XENP24113) greatly improves the pharmacokinetics of IL-15-Fc fusions (in comparison to XENP22583). Unexpectedly, however, IL-15/Rα-heteroFc fusion XENP24113 and scIL-15/Rα-Fc fusion XENP24294 (which have the same IL-15(N4D/N65D) potency variant) demonstrated reduced pharmacokinetics in comparison to XENP24306. This suggests that the reduced pharmacokinetics was due to the particular IL-15 potency variant rather than the format of the IL-15-Fc fusion. While a decrease in pharmacokinetics for XENP24113 and XENP24294 was expected on the basis of previous findings which demonstrated that the IL-15-Fc fusions having IL-15(N4D/N65D) variant had greater in vitro potency than IL-15-Fc fusions having the IL-15(D30N/E64Q/N65D) variant, the decrease in pharmacokinetics was unexpectedly disproportionate to the increase in potency. Accordingly, identification of alternative IL-15 potency variants for use in the TIM-3-targeted IL-15-Fc fusions of the invention was carried out.

[00532] It is noted that IL-15(N4D/N65D) has both its substitutions at the IL-15 interface responsible for binding to CD122, while IL-15(D30N/E64Q/N65D) has two substitutions (E64Q and N65D) at IL-15:CD122 interface; and one substitution (D30N) at the IL-15 interface responsible for binding to CD132. Accordingly, it is believed that the modification at the IL-15:CD132 interface may contribute to the superior pharmacokinetics observed for XENP24306. Notably, it was observed that scIL-15/Rα-Fc fusions comprising IL-15(N4D/N65D) variant and IL-15(D30N/N65D) variant demonstrated very similar potency in vitro, as depicted in Figure 45. In view of the above, illustrative TIM-3-targeted IL-15-Fc fusion comprising the IL-15(D30N/N65D) variants were conceived, sequences for which are depicted in Figure 46. A control RSV-targeted IL-15/Rα-Fc fusion protein XENP29481 with IL-15(D30N/N65D) variant was also generated, sequences for which are depicted in Figure 49.

3B: IL-15(D30N/E64Q/N65D) variant

[00533] Although the TIM-3-targeted IL-15/Rα-Fc fusions were designed with the aim to be targeted to the tumor environment via the TIM-3-targeting arm, the cytokine moiety is still capable of signaling before reaching the tumor site and may contribute to systemic toxicity. Accordingly, further reduce the IL-15 potency TIM-3-targeted IL-15/Rα-Fc fusions with IL-15(D30N/E64Q/N65D) variant were constructed to further reduce the IL-15 potency, which as illustrated in Example 2C, has drastically reduced activity and in Figure 45. Sequences for illustrative TIM-3-targeted IL-15/Rα-Fc fusions comprising IL-15(D30N/E64Q/N65D) variant are depicted in Figure 47. Additionally, XENP30432, a RSV-targeted IL-15/Rα-Fc fusion comprising IL-15(D30N/E64Q/N65D) variant (sequences for which are depicted in Figure 49), was constructed to act as a surrogate for investigating the behavior of TIM-3-targeted IL-15/Rα-Fc fusions comprising IL-15(D30N/E64Q/N65D) variant outside of the tumor environment.

WHAT IS CLAIMED IS:

1. A heterodimeric fusion protein comprising:

a) a first monomer comprising, from N-to C-terminal:

i) an IL-15Rα(sushi) domain;

ii) a first domain linker;

iii) an IL-15 variant;

iv) a hinge; and

v) a first variant Fc domain comprising CH2-CH3; and

b) a second monomer comprising, from N-to C-terminal, VH-CH1-hinge-CH2-CH3, wherein the CH2-CH3 is a second variant Fc domain; and

c) a third monomer comprising a VL-CL,

wherein the VH and VL are a variable heavy domain and a variable light domain, respectively, that form a humanTIM-3 antigen binding domain,

wherein the first variant Fc domain comprises skew variants L368D/K370S and the second variant Fc domain comprises skew variants S364K/E357Q,

wherein the first and second variant Fc domains each comprise FcKO variants E233P/L234V/L235A/G236del/S267K,

wherein the first variant Fc domain comprises pI variants Q295E/N384D/Q418E/N421D, and wherein numbering is according to EU numbering.

2. A heterodimeric fusion protein according to claim 1, wherein the hinge of the first monomer comprises amino acid substitution C220S, and wherein numbering is according to EU numbering.

3. A heterodimeric fusion protein according to claim 1 or 2, wherein the first and second variant Fc domains each further comprise half-life extension variants M428:/N434S.

4. A heterodimeric fusion protein according to any one of claims 1 to 3, the wherein the IL-15 variant comprises an amino acid substitution(s) selected from the group consisting of N1D, N4D, D8N, D30N, D61N, E64Q, N65D, Q108E, N4D/N65D, D30N/N65D, and D30N/E64Q/N65D.

5. A heterodimeric fusion protein according to claim 4, wherein the IL-15 variant comprises amino acid substitutions N4D/N65D, D30N/N65D, or D30N/E64Q/N65D

6. A heterodimeric fusion protein according to any one of claims 1 to 5, wherein the VH and VL are the variable heavy domain and variable domain of any of the TIM-3 antigen binding domains in Figures 12 and 13.

7. A heterodimeric fusion protein according to claim 6, wherein the TIM-3 antigen binding domain is 3H3_H1_L2.1.

8. A heterodimeric fusion protein according to claim 1, wherein the heterodimeric fusion protein is selected from the group consisting of: XENP27974, XENP27979, XENC1000, XENC1001, XENC1002, and XENC1003.

9. A nucleic acid composition comprising:

a) a first nucleic acid encoding said first monomer of any of claims 1 to 8;

b) a second nucleic acid encoding said second monomer of any of claims 1 to 8; and

c) a third nucleic acid encoding said third monomer of any of claims 1 to 8; respectively.

10. An expression vector composition comprising:

a) a first expression vector comprising said first nucleic acid of claim 9;

b) a second expression vector comprising said second nucleic acid of claim 9;

c) a third expression vector comprising said third nucleic acid of claim 9.

11. A host cell comprising the expression vector composition according to claim 10.

12. A method of making a heterodimeric fusion protein comprising culturing the host cell of claim 11 and recovering the heterodimeric fusion protein from the cell culture.

13. A method of treating a patient in need thereof comprising administering to the patient the heterodimeric fusion protein according to any one of claims 1 to 8.

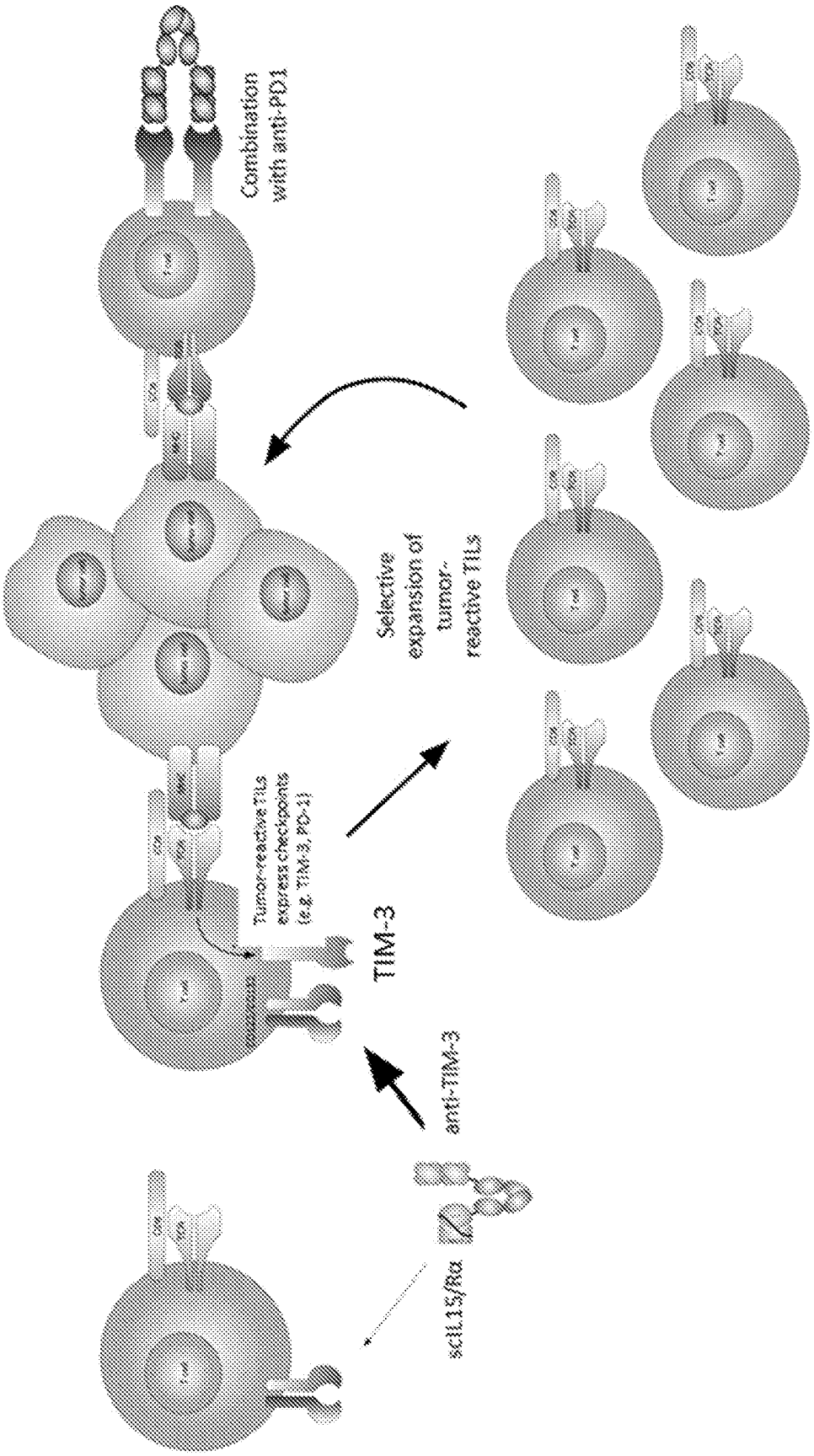

Combination with anti-PD1
Selective expansion of tumor-reactive TILs
Tumor-reactive TILs express checkpoints (e.g. TIM-3, PD-1)
TIM-3
anti-TIM-3
scIL15/Rα

Figure 1

Figure 2A

Human IL-15 precursor sequence SEQ ID NO:1

>sp|P40933
MRISKPHLRSISIQCYLCLLLNSHFLTEAGIHVFILGCFSAGLPKTEANWVNVISDLKKIEDLIQSMHIDATLYTES
DVHPSCKVTAMKCFLLELQVISLESGDASIHDTVENLIILANNSLSSNGNVTESGCKECEELEEKNIKEFLQSFVHI
VQMFINTS

Human IL-15 mature form sequence SEQ ID NO:2

>sp|P40933|49-162
NWVNVISDLKKIEDLIQSMHIDATLYTESDVHPSCKVTAMKCFLLELQVISLESGDASIHDTVENLIILANNSLSSN
GNVTESGCKECEELEEKNIKEFLQSFVHIVQMFINTS

Human IL-15Rα sequence SEQ ID NO:3

>sp|Q13261
MAPRRARGCRTLGLPALLLLLLLRPPATRGITCPPPMSVEHADIWVKSYSLYSRERYICNSGFKRKAGTSSLTECVL
NKATNVAHWTTPSLKCIRDPALVHQRPAPPSTVTTAGVTPQPESLSPSGKEPAASSPSSNNTAATTAAIVPGSQLMP
SKSPSTGTTEISSHESSHGTPSQTTAKNWELTASASHQPPGVYPQGHSDTTVAISTSTVLLCGLSAVSLLACYLKSR
QTPPLASVEMEAMEALPVTWGTSSRDEDLENCSHHL

Human IL-15Rα, extracellular domain SEQ ID NO:5

>sp|Q13261|31-205
ITCPPPMSVEHADIWVKSYSLYSRERYICNSGFKRKAGTSSLTECVLNKATNVAHWTTPSLKCIRDPALVHQRPAPP
STVTTAGVTPQPESLSPSGKEPAASSPSSNNTAATTAAIVPGSQLMPSKSPSTGTTEISSHESSHGTPSQTTAKNWE
LTASASHQPPGVYPQGHSDTT

Human IL-15Rα, sushi domain SEQ ID NO:4

>sp|Q13261|31-95
ITCPPPMSVEHADIWVKSYSLYSRERYICNSGFKRKAGTSSLTECVLNKATNVAHWTTPSLKCIR

Human IL-15Rß sequence (SEQ ID NO:XXX)

>sp|P14784
MAAPALSWRLPLLILLLPLATSWASAAVNGTSQFTCFYNSRANISCVWSQDGALQDTSCQVHAWPDRRRWNQTCELL
PVSQASWACNLILGAPDSQKLTTVDIVTLRVLCREGVRWRVMAIQDFKPFENLRLMAPISLQVVHVETHRCNISWEI
SQASHYFERHLEFEARTLSPGHTWEEAPLLTLKQKQEWICLETLTPDTQYEFQVRVKPLQGEFTTWSPWSQPLAFRT
KPAALGKDTIPWLGHLLVGLSGAFGFIILVYLLINCRNTGPWLKKVLKCNTPDPSKFFSQLSSEHGGDVQKWLSSPF
PSSSFSPGGLAPEISPLEVLERDKVTQLLLQQDKVPEPASLSSNHSLTSCFTNQGYFFFHLPDALEIEACQVYFTYD
PYSEEDPDEGVAGAPTGSSPQPLQPLSGEDDAYCTFPSRDDLLLFSPSLLGGPSPPSTAPGGSGAGEERMPPSLQER
VPRDWDPQPLGPPTPGVPDLVDFQPPPELVLREAGEEVPDAGPREGVSFPWSRPPGQGEFRALNARLPLNTDAYLSL
QELQGQDPTHLV

Human IL-15Rß, extracellular domain (SEQ ID NO:XXX)

>sp|P14784|27-240
AVNGTSQFTCFYNSRANISCVWSQDGALQDTSCQVHAWPDRRRWNQTCELLPVSQASWACNLILGAPDSQKLTTVDI
VTLRVLCREGVRWRVMAIQDFKPFENLRLMAPISLQVVHVETHRCNISWEISQASHYFERHLEFEARTLSPGHTWEE
APLLTLKQKQEWICLETLTPDTQYEFQVRVKPLQGEFTTWSPWSQPLAFRTKPAALGKDT

Figure 2B

Human common gamma chain sequence (SEQ ID NO:XXX)

>sp|P31785
MLKPSLPFTSLLFLQLPLLGVGLNTTILTPNGNEDTTADFFLTTMPTDSLSVSTLPLPEVQCFVFNVEYMNCTWNSS
SEPQPTNLTLHYWYKNSDNDKVQKCSHYLFSEEITSGCQLQKKEIHLYQTFVVQLQDPREPRRQATQMLKLQNLVIP
WAPENLTLHKLSESQLELNWNNRFLNHCLEHLVQYRTDWDHSWTEQSVDYRHKFSLPSVDGQKRYTFRVRSRFNPLC
GSAQHWSEWSHPIHWGSNTSKENPFLFALEAVVISVGSMGLIISLLCVYFWLERTMPRIPTLKNLEDLVTEYHGNFS
AWSGVSKGLAESLQPDYSERLCLVSEIPPKGGALGEGPGASPCNQHSPYWAPPCYTLKPET

Human common gamma chain, extracellular domain (SEQ ID NO:XXX)

>sp|P31785|23-262
LNTTILTPNGNEDTTADFFLTTMPTDSLSVSTLPLPEVQCFVFNVEYMNCTWNSSSEPQPTNLTLHYWYKNSDNDKV
QKCSHYLFSEEITSGCQLQKKEIHLYQTFVVQLQDPREPRRQATQMLKLQNLVIPWAPENLTLHKLSESQLELNWNN
RFLNHCLEHLVQYRTDWDHSWTEQSVDYRHKFSLPSVDGQKRYTFRVRSRFNPLCGSAQHWSEWSHPIHWGSNTSKE
NPFLFALEA

Figure 3

Human TIM-3 sequence (SEQ ID NO:XXX)

>sp|Q8TDQ0

MFSHLPFDCVLLLLLLLLTRSSEVEYRAEVGQNAYLPCFYTPAAPGNLVPVCWGKGACPVFECGNVVLRTDERDVNY
WTSRYWLNGDFRKGDVSLTIENVTLADSGIYCCRIQIPGIMNDEKFNLKLVIKPAKVTPAPTRQRDFTAAFPRMLTT
RGHGPAETQTLGSLPDINLTQISTLANELRDSRLANDLRDSGATIRIGIYIGAGICAGLALALIFGALIFKWYSHSK
EKIQNLSLISLANLPPSGLANAVAEGIRSEENIYTIEENVYEVEEPNEYYCYVSSRQQPSQPLGCRFAM

Human TIM-3 sequence, extracellular domain (SEQ ID NO:XXX)

>sp|Q8TDQ0|22-202

SEVEYRAEVGQNAYLPCFYTPAAPGNLVPVCWGKGACPVFECGNVVLRTDERDVNYWTSRYWLNGDFRKGDVSLTIE
NVTLADSGIYCCRIQIPGIMNDEKFNLKLVIKPAKVTPAPTRQRDFTAAFPRMLTTRGHGPAETQTLGSLPDINLTQ
ISTLANELRDSRLANDLRDSGATIRIG

Macaca fascicularis TIM-3 sequence (predicted) (SEQ ID NO:XXX)

>gi|355750365|gb|EHH54703.1

MFSHLPFDCVLLLLLLLLTRSSEVEYIAEVGQNAYLPCSYTPAPPGNLVPVCWGKGACPVFDCSNVVLRTDNRDVND
RTSGRYWLKGDFHKGDVSLTIENVTLADSGVYCCRIQIPGIMNDEKHNVKLVVIKPAKVTPAPTLQRDLTSAFPRML
TTGEHGPAETQTPGSLPDVNLTVSNFFCELQIFTLTNELRDSGATIRTAIYIAAGISAGLALALIFGALIFKWYSHS
KEKTQNLSLISLANIPPSGLANAVAEGIRSEENIYTIEEDVYEVEEPNEYYCYVSSGQQPSQPLGCRVAMP

Macaca fascicularis TIM-3 sequence, extracellular domain (predicted) (SEQ ID NO:XXX)

>gi|355750365|gb|EHH54703.1|22-203

SEVEYIAEVGQNAYLPCSYTPAPPGNLVPVCWGKGACPVFDCSNVVLRTDNRDVNDRTSGRYWLKGDFHKGDVSLTI
ENVTLADSGVYCCRIQIPGIMNDEKHNVKLVVIKPAKVTPAPTLQRDLTSAFPRMLTTGEHGPAETQTPGSLPDVNL
TVSNFFCELQIFTLTNELRDSGATIRTA

Figure 4A

Monomer 1	Monomer 2
F405A	T394F
S364D	Y349K
S364E	L368K
S364E	Y349K
S364F	K370G
S364H	Y349K
S364H	Y349T
S364Y	K370G
T411K	K370E
V397S/F405A	T394F
K370R/T411K	K370E/T411E
L351E/S364D	Y349K/L351K
L351E/S364E	Y349K/L351K
L351E/T366D	L351K/T366K
P395T/V397S/F405A	T394F
S364D/K370G	S364Y/K370R
S364D/T394F	Y349K/F405A
S364E/F405A	Y349K/T394F
S364E/F405S	Y349K/T394Y
S364E/T411E	Y349K/D401K
S364H/D401K	Y349T/T411E
S364H/F405A	Y349T/T394F
S364H/T394F	Y349T/F405A
Y349C/S364E	Y349K/S354C
L351E/S364D/F405A	Y349K/L351K/T394F
L351K/S364H/D401K	Y349T/L351E/T411E
S364E/T411E/F405A	Y349K/T394F/D401K
S364H/D401K/F405A	Y349T/T394F/T411E
S364H/F405A/T411E	Y349T/T394F/D401K

Figure 4B

Monomer 1	Monomer 2
K370E/T411D	T411K
L368E/K409E	L368K
Y349T/T394F/S354C	S364H/F405A/Y349C
T411E	D401K
T411E	D401R/T411R
Q347E/K360E	Q347R
L368E	S364K
L368E/K370S	S364K
L368E/K370T	S364K
L368E/D401R	S364K
L368E/D401N	S364K
L368E	E357S/S364K
L368E	S364K/K409E
L368E	S364K/K409V
L368D	S364K
L368D/K370S	S364K
L368D/K370S	S364K/E357L
L368D/K370S	S364K/E357Q
T411E/K360E/Q362E	D401K
K370S	S364K
L368E/K370S	S364K/E357Q
K370S	S364K/E357Q
T411E/K360D	D401K
T411E/K360E	D401K
T411E/Q362E	D401K
T411E/N390D	D401K
T411E	D401K/Q347K
T411E	D401K/Q347R
T411E/K360D/Q362E	D401K

Figure 4C

Monomer 1	Monomer 2
T411E/K360E/N390D	D401K
T411E/Q362E/N390D	D401K
T411E/Q347R	D401K/K360D
T411E/Q347R	D401K/K360E
T411E/K360	D401K/Q347K
T411E/K360D	D401K/Q347R
T411E/K360E	D401K/Q347K
T411E/K360E	D401K/Q347R
T411E/S364K	D401K/K370S
T411E/K370S	D401K/S364K
Q347E	E357Q
Q347E	E357Q/Q362K
K360D/Q362E	Q347R
K360D/Q362E	D401K
K360D/Q362E	Q347R/D401K
K360E/Q362E	Q347R
K360E/Q362E	D401K
K360E/Q362E	Q347R/D401K
Q362E/N390D	D401K
Q347E/K360D	D401N
K360D	Q347R/N390K
K360D	N390K/D401N
K360E	Y349H
K370S/Q347E	S364K
K370S/E357L	S364K
K370S/E357Q	S364K
K370S/Q347E/E357L	S364K
K370S/Q347E/E357Q	S364K

Figure 4D

Monomer 1	Monomer 2
L368D/K370S/Q347E	S364K
L368D/K370S/E357L	S364K
L368D/K370S/E357Q	S364K
L368D/K370S/Q347E/E357L	S364K
L368D/K370S/Q347E/E357Q	S364K
L368E/K370S/Q347E	S364K
L368E/K370S/E357L	S364K
L368E/K370S/E357Q	S364K
L368E/K370S/Q347E/E357L	S364K
L368E/K370S/Q347E/E357Q	S364K
L368D/K370T/Q347E	S364K
L368D/K370T/E357L	S364K
L368D/K370T/E357Q	S364K
L368D/K370T/Q347E/E357L	S364K
L368D/K370T/Q347E/E357Q	S364K
L368E/K370T/Q347E	S364K
L368E/K370T/E357L	S364K
L368E/K370T/E357Q	S364K
L368E/K370T/Q347E/E357L	S364K
L368E/K370T/Q347E/E357Q	S364K
T411E/Q362E	D401K/T411K
T411E/N390D	D401K/T411K
T411E/Q362E	D401R/T411R
T411E/N390D	D401R/T411R
Y407T	T366Y
F405A	T394W
T366Y/F405A	T394W/Y407T
Y407A	T366W
T366S/L368A/Y407V	T366W
T366S/L368A/Y407V/Y349C	T366W/S354C

Figure 4E

Monomer 1	Monomer 2
K392D/K409D	E356K/D399K
K370D/K392D/K409D	E356K/E357K/D399K
I199T/N203D/K247Q/R355Q/N384S/K392N/V397M/Q419E/K447_	Q196K/I199T/P217R/P228R/N276K
I199T/N203D/K247Q/R355Q/N384S/K392N/V397M/Q419E/K447_	Q196K/I199T/N276K
K247Q/R355Q/N384S/K392N/V397M/Q419E/K447_	P217R/P228R/N276K
K247Q/R355Q/N384S/K392N/V397M/Q419E/K447_	N276K
N384S/K392N/V397M/Q419E	N276K
D221E/P228E/L368E	D221R/P228R/K409R
C220E/P228E/L368E	C220R/E224R/P228R/K409R
F405L	K409R
T366I/K392M/T394W	F405A/Y407V
T366V/K409F	L351Y/Y407A
T366A/K392E/K409F/T411E	D399R/S400R/Y407A
L351K	L351E
I199T/N203D/K247Q/R355Q/Q419E/K447_	Q196K/I199T/P217R/P228R/N276K
I199T/N203D/K247Q/R355Q/Q419E/K447_	Q196K/I199T/N276K
K247Q/R355Q/Q419E/K447_	P217R/P228R/N276K
K247Q/R355Q/Q419E/K447_	N276K
I199T/N203D/K274Q/R355Q/N384S/K392N/V397M/Q419E/K447_	
N208D/Q295E/N384D/Q418E/N421D	
N208D/Q295E/Q418E/N421D	
Q196K/I199T/P217R/P228R/N276K	
Q196K/I199T/N276K	
K274Q/R355Q/N384S/K392N/V397M/Q419E/K447_	
Q295E/N384D/Q418E/N421D	
Q295E/Q418E/N421D	
P217R/P228R/N276K	
N276K	
E269Q/E272Q/E283Q/E357Q	
E269Q/E272Q/E283Q	
E269Q/E272Q	
E269Q/E283Q	
E272Q/E283Q	
E269Q	

Figure 5

Variant constant region	Substitutions
pI_ISO(-)	I199T/N203D/K274Q/R355Q/N384S/K392N/V397M/Q419E/K447_
pI_ISO(-)-Fc only	K274Q/R355Q/N384S/K392N/V397M/Q419E/K447_
pI_(-)_isosteric A	N208D/Q295E/N384D/Q418E/N421D
pI_(-)_isosteric A-Fc only	Q295E/N384D/Q418E/N421D
pI_(-)_isosteric_B	N208D/Q295E/Q418E/N421D
pI_(-)_isosteric_B-Fc only	Q295E/Q418E/N421D
pI_ISO(+RR)	Q196K/I199T/P217R/P228R/N276K
pI_ISO(+RR)-Fc only	P217R/P228R/N276K
pI_ISO(+)	Q196K/I199T/N276K
pI_ISO(+)-Fc only	N276K
pI_(+)_isosteric_A	E269Q/E272Q/E283Q/E357Q
pI_(+)_isosteric_B	E269Q/E272Q/E283Q
pI_(+)_isosteric_E269Q/E272Q	E269Q/E272Q
pI_(+)_isosteric_E269Q/E283Q	E269Q/E283Q
pI_(+)_isosteric_E272Q/E283Q	E272Q/E283Q
pI_(+)_isosteric_E269Q	E269Q

Figure 6

Ablation Variants

G236R
S239G
S239K
S239Q
S239R
V266D
S267K
S267R
H268K
E269R
299R
299K
K322A
A327G
A327L
A327N
A327Q
L328E
P329K
A330L
A330S/P331S
I332K
I332R
V266D/A327Q
V266D/P329K
S267R/A327Q
S267R/P329K
G236R/L328R
E233P/L234V/L235A/G236_/S239K
E233P/L234V/L235A/G236_/S267K
E233P/L234V/L235A/G236_/S239K/A327G
E233P/L234V/L235A/G236_/S267K/A327G
E233P/L234V/L235A/G236_
S239K/S267K
267K/P329K

Figure 7A

scIL-15/Rα-Fc monomer (-)	**scFv-Fc monomer (+)**
C220S	C220S
Heterodimer skew variants L368D/K370S	Heterodimer skew variants S364K/E357Q
Isosteric pI substitutions Q295E/N384D/Q418E/N421D	
FcKO E233P/L234V/L235A/G236_/S267K	FcKO E233P/L234V/L235A/G236_/S267K
±M428L/N434S	±M428L/N434S

Figure 7B

scFv-Fc monomer (-)	**IL-15Rα(sushi)-Fc monomer (+)**
C220S	C220S
Heterodimer skew variants L368D/K370S	Heterodimer skew variants S364K/E357Q
Isosteric pI substitutions Q295E/N384D/Q418E/N421D	
FcKO E233P/L234V/L235A/G236_/S267K	FcKO E233P/L234V/L235A/G236_/S267K
±M428L/N434S	±M428L/N434S

Figure 7C

scIL-15/Rα-Fc monomer (-)	**Heavy Chain (+)**
C220S	
Heterodimer skew variants L368D/K370S	Heterodimer skew variants S364K/E357Q
Isosteric pI substitutions Q295E/N384D/Q418E/N421D	
FcKO E233P/L234V/L235A/G236_/S267K	FcKO E233P/L234V/L235A/G236_/S267K
±M428L/N434S	±M428L/N434S

Figure 7D

Heavy Chain (-)	**IL-15Rα(sushi)-Fc monomer (+)**
	C220S
Heterodimer skew variants L368D/K370S	Heterodimer skew variants S364K/E357Q
Isosteric pI substitutions N208D/Q295E/N384D/Q418E/N421D	
FcKO E233P/L234V/L235A/G236_/S267K	FcKO E233P/L234V/L235A/G236_/S267K
±M428L/N434S	±M428L/N434S

Figure 7E

Heavy Chain-IL-15Rα(sushi) (-)	**Heavy Chain (+)**
Heterodimer skew variants L368D/K370S	Heterodimer skew variants S364K/E357Q
Isosteric pI substitutions N208D/Q295E/N384D/Q418E/N421D	Isosteric pI subsitutions Q196K/I199T/P217R/P228R/N276K
FcKO E233P/L234V/L235A/G236_/S267K	FcKO E233P/L234V/L235A/G236_/S267K
±M428L/N434S	±M428L/N434S

Figure 7F

Heavy Chain (-)	**Heavy Chain-IL-15Rα(sushi) (+)**
Heterodimer skew variants L368D/K370S	Heterodimer skew variants S364K/E357Q
Isosteric pI substitutions N208D/Q295E/N384D/Q418E/N421D	Isosteric pI substitutions Q196K/I199T/P217R/P228R/N276K
FcKO E233P/L234V/L235A/G236_/S267K	FcKO E233P/L234V/L235A/G236_/S267K
±M428L/N434S	±M428L/N434S

Figure 8

Name	Sequence	SEQ ID NO:
$(GGGGS)_1$ or GGGGS	GGGGS	SEQ ID NO: XXX
$(GGGGS)_2$	GGGGSGGGGS	SEQ ID NO: XXX
$(GGGGS)_3$	GGGGSGGGGSGGGGS	SEQ ID NO: XXX
$(GGGGS)_4$	GGGGSGGGGSGGGGSGGGGS	SEQ ID NO: XXX
$(GGGGS)_5$	GGGGSGGGGSGGGGSGGGGSGGGGS	SEQ ID NO: XXX
$(GGGGS)_6$	GGGGSGGGGSGGGGSGGGGSGGGGSGGGGS	SEQ ID NO: XXX
$(GGGGS)_7$	GGGGSGGGGSGGGGSGGGGSGGGGSGGGGSGGGGS	SEQ ID NO: XXX
30AA-linker	DPALVHQRPAPPGGGGSGGGGSGGGGSGGG	SEQ ID NO: XXX
$(GKPGS)_1$ or GKPGS	GKPGS	SEQ ID NO: XXX
$(GKPGS)_5$	GKPGSGKPGSGKPGSGKPGSGKPGS	SEQ ID NO: XXX
$(GKPGS)_6$	GKPGSGKPGSGKPGSGKPGSGKPGSGKPGS	SEQ ID NO: XXX
$(GGGES)_1$ or GGGES	GGGES	SEQ ID NO: XXX

Additional useful domain linkers

KTHTCPPCP (“half hinge”)	SEQ ID NO:XXX
EPKSSDKTHTCPPCP (“full hinge C220S variant”)	SEQ ID NO:XXX
GGGGSGGGGSKTHTCPPCP (“flex half hinge”)	SEQ ID NO:XXX
GKPGSGKPGSKTHTCPPCP (“charged half hinge1”)	SEQ ID NO:XXX
GKPGSKTHTCPPCP (“charged half hinge2”)	SEQ ID NO:XXX

Figure 9A

Positive Charged scFv Linkers

Name	Sequence	Length	Charge	SEQ ID NO:
Gly-Ser 15	GGGGSGGGGSGGGGS	15	0	SEQ ID NO: XXX
Whitlow linker	GSTSGSGKPGSGEGSTKG	18	+1	SEQ ID NO: XXX
6paxA_1 (+A)	IRPRAIGGSKPRVA	14	+4	SEQ ID NO: XXX
+B	GKGGSGKGGSGKGGS	15	+3	SEQ ID NO: XXX
+C	GGKGSGGKGSGGKGS	15	+3	SEQ ID NO: XXX
+D	GGGKSGGGKSGGGKS	15	+3	SEQ ID NO: XXX
+E	GKGKSGKGKSGKGKS	15	+6	SEQ ID NO: XXX
+F	GGGKSGGKGSGKGGS	15	+3	SEQ ID NO: XXX
+G	GKPGSGKPGSGKPGS	15	+3	SEQ ID NO: XXX
+H	GKPGSGKPGSGKPGSGKPGS	20	+4	SEQ ID NO: XXX
+I	GKGKSGKGKSGKGKSGKGKS	20	+8	SEQ ID NO: XXX

Figure 9B

Negative Charged scFv Linkers

Name	Sequence	Length	Charge	SEQ ID NO:
Gly-Ser 20	GGGGSGGGGSGGGGSGGGGS	20	0	SEQ ID NO: XXX
3hsc_2 (-A)	STAGDTHLGGEDFD	14	-4	SEQ ID NO: XXX
-B	GEGGSGEGGSGEGGS	15	-3	SEQ ID NO: XXX
-C	GGEGSGGEGSGGEGS	15	-3	SEQ ID NO: XXX
-D	GGGESGGGESGGGES	15	-3	SEQ ID NO: XXX
-E	GEGESGEGESGEGES	15	-6	SEQ ID NO: XXX
-F	GGGESGGEGSGEGGS	15	-3	SEQ ID NO: XXX
-G	GEGESGEGESGEGESGEGES	20	-8	SEQ ID NO: XXX

Figure 10

IL-15/Rα x anti-TIM-3 Backbone 1

>Chain 1 (SEQ ID NO:XXX)
/ASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSSLGTQTYICNVNHKPSNT
KVDKKVEPKSCDKTHTCPPCPAPPVAGPSVFLFPPKPKDTLMISRTPEVTCVVVDVKHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVV
SVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSREQMTKNQVKLTCLVKGFYPSDIAVEWESNGQPENNYKTTPP
VLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGK

>Chain 2 (SEQ ID NO:XXX)
/EPKSSDKTHTCPPCPAPPVAGPSVFLFPPKPKDTLMISRTPEVTCVVVDVKHEDPEVKFNWYVDGVEVHNAKTKPREEEYNSTYRVVSVLTV
LHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSREEMTKNQVSLTCDVSGFYPSDIAVEWESDGQPENNYKTTPPVLDSD
GSFFLYSKLTVDKSRWEQGDVFSCSVMHEALHNHYTQKSLSLSPGK

IL-15/Rα x anti-TIM-3 Backbone 2

>Chain 1 (SEQ ID NO:XXX)
/ASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSSLGTQTYICNVNHKPSDT
KVDKKVEPKSCDKTHTCPPCPAPPVAGPSVFLFPPKPKDTLMISRTPEVTCVVVDVKHEDPEVKFNWYVDGVEVHNAKTKPREEEYNSTYRVV
SVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSREEMTKNQVSLTCDVSGFYPSDIAVEWESDGQPENNYKTTPP
VLDSDGSFFLYSKLTVDKSRWEQGDVFSCSVMHEALHNHYTQKSLSLSPGK

>Chain 2 (SEQ ID NO:XXX)
/EPKSSDKTHTCPPCPAPPVAGPSVFLFPPKPKDTLMISRTPEVTCVVVDVKHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTV
LHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSREQMTKNQVKLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSD
GSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGK

IL-15/Rα x anti-TIM-3 Backbone 3

>Chain 1 (SEQ ID NO:XXX)
/ASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSSLGTQTYICNVNHKPSDT
KVDKKVEPKSCDKTHTCPPCPAPPVAGPSVFLFPPKPKDTLMISRTPEVTCVVVDVKHEDPEVKFNWYVDGVEVHNAKTKPREEEYNSTYRVV
SVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSREEMTKNQVSLTCDVSGFYPSDIAVEWESDGQPENNYKTTPP
VLDSDGSFFLYSKLTVDKSRWEQGDVFSCSVMHEALHNHYTQKSLSLSPGK

>Chain 2 (SEQ ID NO:XXX)
/ASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSSLGTKTYTCNVNHKPSNT
KVDKKVERKSCDKTHTCPRCPAPPVAGPSVFLFPPKPKDTLMISRTPEVTCVVVDVKHEDPEVKFKWYVDGVEVHNAKTKPREEQYNSTYRVV
SVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSREQMTKNQVKLTCLVKGFYPSDIAVEWESNGQPENNYKTTPP
VLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGK

Figure 11

Constant Light Chain – Kappa (SEQ ID NO:XXX)
/RTVAAPSVFIFPPSDEQLKSGTASVVCLLNNFYPREAKVQWKVDNALQSGNSQESVTEQDSKDSTYSLSSTLTLSKADYEKHKVYACEVTHQ
GLSSPVTKSFNRGEC

Constant Light Chain – Lambda (SEQ ID NO:XXX)
/GQPKAAPSVTLFPPSSEELQANKATLVCLISDFYPGAVTVAWKADSSPVKAGVETTTPSKQSNNKYAASSYLSLTP
EQWKSHRSYSCQVTHEGSTVEKTVAPTECS

Figure 12

>3H3[TIM-3]_H0L0 Variable Heavy (SEQ ID NO:XXX-XXX)
QVQLKESGPGLVAPSQSLSITCTVSGFSLNGYGVNWVRQPPGKGLEWLGMIWGDGSTDYNSALKSRLSISKDNSKSQ
VFLKMNSLQTDDTARYYCARSYYTSDEDYWGQGTLVTVSA

>3H3[TIM-3]_H0L0 Variable Light (SEQ ID NO:XXX-XXX)
DIVMSQSPSSLAVSAGEKVTMSCKSSQSLLNSRTRKNYLAWYQQKPGQSPKLLIYWASTRESGVPDRFTGSGSGTDF
TLTISSVLAEDLAVYYCKQSYSLRTFGGGTKLEIK

>3H3[TIM-3]_H1L2 Variable Heavy (SEQ ID NO:XXX-XXX)
QVTLKESGPVLVKPTETLTLTCTVSGFSLNGYGVNWVRQPPGKGLEWLAMIWGDGSTDYNSALKSRLTISKDNSKSQ
VVLTMTNMDPVDTATYYCARSYYTSDEDYWGQGTLVTVSS

>3H3[TIM-3]_H1L2 Variable Light (SEQ ID NO:XXX-XXX)
DIVLTQSPDSLAVSLGERATINCKSSQSLLNSRTRKNYLAWYQQKPGQSPKLLIYWASTRESGVPDRFTGSGSGTDF
TLTISSLQAEDVAVYYCKQSYSLRTFGGGTKVEIK

>3H3[TIM-3]_H1L2.1 Variable Heavy (SEQ ID NO:XXX-XXX)
QVTLKESGPVLVKPTETLTLTCTVSGFSLNGYGVNWVRQPPGKGLEWLAMIWGDGSTDYNSALKSRLTISKDNSKSQ
VVLTMTNMDPVDTATYYCARSYYTSDEDYWGQGTLVTVSS

>3H3[TIM-3]_H1L2.1 Variable Light (SEQ ID NO:XXX-XXX)
DIVMTQSPDSLAVSLGERATINCKSSQSLLNSRTRKNYLAWYQQKPGQSPKLLIYWASTRESGVPDRFTGSGSGTDF
TLTISSLQAEDVAVYYCKQSYSLRTFGGGTKVEIK

Figure 13A

>APE5137[TIM-3] Variable Heavy (SEQ ID NO:XXX-XXX)
EVQLLESGGGLVQPGGSLRLSCAAASGFTFSSYDMSWVRQAPGKGLDWVSTISGGGTYTYYQDSVKGRFTISRDNSK
NTLYLQMNSLRAEDTAVYYCASMDYWGQGTTVTVSSA

>APE5137[TIM-3] Variable Light (SEQ ID NO:XXX-XXX)
DIQMTQSPSSLSASVGDRVTITCRASQSIRRYLNWYHQKPGKAPKLLIYGASTLQSGVPSRFSGSGSGTDFTLTISS
LQPEDFAVYYCQQSHSAPLTFGGGTKVEIKR

>APE5121[TIM-3] Variable Heavy (SEQ ID NO:XXX-XXX)
EVQVLESGGGLVQPGGSLRLYCVASGFTFSGSYAMSWVRQAPGKGLEWVSAISGSGGSTYYADSVKGRFTISRDNSK
NTLYLQMNSLRAEDTAVYYCAKKYYVGPADYWGQGTLVTVSSG

>APE5121[TIM-3] Variable Light (SEQ ID NO:XXX-XXX)
DIVMTQSPDSLAVSLGERATINCKSSQSVLYSSNNKNYLAWYQHKPGQPPKLLIYWASTRESGVPDRFSGSGSGTDF
TLTISSLQAEDVAVYYCQQYYSSPLTFGGGTKIEVK

>ABTIM3-hum03[TIM-3] Variable Heavy ((SEQ ID NO:XXX-XXX)
QVQLVQSGAEVKKPGASVKVSCKASGYTFTSYNMHWVRQAPGQGLEWIGDIYPGQGDTSYNQKFKGRATMTADKSTS
TVYMELSSLRSEDTAVYYCARVGGAFPMDYWGQGTLVTVSS

>ABTIM3-hum03[TIM-3] Variable Light (SEQ ID NO:XXX-XXX)
DIVLTQSPDSLAVSLGERATINCRASESVEYYGTSLMQWYQQKPGQPPKLLIYAASNVESGVPDRFSGSGSGTDFTL
TISSLQAEDVAVYYCQQSRKDPSTFGGGTKVEIK

>ABTIM3-hum11[TIM-3] Variable Heavy (SEQ ID NO:XXX-XXX)
QVQLVQSGAEVKKPGSSVKVSCKASGYTFTSYNMHWVRQAPGQGLEWMGDIYPGNGDTSYNQKFKGRVTITADKSTS
TVYMELSSLRSEDTAVYYCARVGGAFPMDYWGQGTTVTVSS

>ABTIM3-hum11[TIM-3] Variable Light (SEQ ID NO:XXX-XXX)
AIQLTQSPSSLSASVGDRVTITCRASESVEYYGTSLMQWYQQKPGKAPKLLIYAASNVESGVPSRFSGSGSGTDFTL
TISSLQPEDFATYFCQQSRKDPSTFGGGTKVEIK

>ABTIM3-hum21[TIM-3] Variable Heavy (SEQ ID NO:XXX-XXX))
QVQLVQSGAEVKKPGASVKVSCKASGYTFTSYNMHWVRQAPGQGLEWIGDIYPGQGDTSYNQKFKGRATMTADKSTS
TVYMELSSLRSEDTAVYYCARVGGAFPMDYWGQGTLVTVSS

>ABTIM3-hum21[TIM-3] Variable Light (SEQ ID NO:XXX-XXX))
DIVLTQSPDSLAVSLGERATINCRASESVEYYGTSLMQWYQQKPGQPPKLLIYAASNVESGVPDRFSGSGSGTDFTL
TISSLQAEDVAVYYCQQSRKDPSTFGGGTKVEIK

>4177[TIM-3] Variable Heavy (SEQ ID NO:XXX)
QVQLQESGPGLVKPSETLSLTCTVSGGSISSYYWSWIRQPPGKGLEWIGYIFHSGSTNYNPSLKSRVTISVDTSKNQ
FSLKLSSVTAADTAVYYCARDGEYFDMLTGFDYWGQGTLVTVSS

Figure 13B

>4177[TIM-3] Variable Light (SEQ ID NO:XXX-XXX)
RCDIQMTQSPSSLSASVGDRVTITCRASQGISSWLAWYQQKPEKAPKSLIYAASSLQSGVPSRFSGSGSGTDFTLTI
SSLQPEDFATYYCQQYNSYPRTFGQGTKVEIK

>4545[TIM-3] Variable Heavy (SEQ ID NO:XXX-XXX)
QVQLQESGPGLVKPSETLSLTCTVSGGSFSRGGYYWNWIRQPPGKGLEWIGYIYYSGSTNYNPSLKSRVTISLDTSK
NQFSLKLSSVTAADTAVYYCARDHYSSSWTFDYWGQGTLVTVSS

>4545[TIM-3] Variable Light (SEQ ID NO:XXX-XXX)
EIVLTQSPATLSLSPGERATLSCRASQSVSSYLAWYQQKPGQAPRLLIYDASNRATGIPARFSGSGSGTDFTLTISS
LEPEDFAVYYCQQRSNWPPTFGQGTKLEIK

>8213[TIM-3] Variable Heavy (SEQ ID NO:XXX-XXX)
QVQLVQSGAEVKKPGASVKVSCKASGYTFTSYWMHWVRQAPGQGLEWMGEINPSNGRTNYNEKFKTRVTITADTSTS
TAYMELSSLRSEDTAVYYCARGYYLYFDYWGQGTLVTVSS

>8213[TIM-3] Variable Light (SEQ ID NO:XXX-XXX)
DIQMTQSPSSLSASVGDRVTITCHASQGIRINIGWYQQKPGKAPKLLIYHGTNLEDGVPSRFSGSGSGTDFTLTISS
LQPEDFATYYCVQYGQFPWTFGQGTKLEIK

>mAb15[TIM-3] Variable Heavy (SEQ ID NO:XXX-XXX)
QVQLKESGPGLVAPSQSLSITCTVSGFSLTGYGVTWVRQPPGKGLEWLGMIWGDGNTDYNSGLKSRLNISKDNSKSQ
VFLKMNSLQTDDTARYYCARSYYYGPPDYWGQGTTLTVSS

>mAb15[TIM-3] Variable Light (SEQ ID NO:XXX-XXX)
DIVMTQSPSSLAMSVGQKVTMSCKSSQSLLNSRSQKNYLAWYQRKPGQSPKLLLYFASTRESGVPDRFIGSGSGTDF
TLTISSVQAEDLADYFCHQHYNTPYTFGGGTKLEIK

>mAb58[TIM-3] Variable Heavy (SEQ ID NO:XXX-XXX)
QIQLVQSGPELKKPGETVKISCKASGYTFTTYGMSWVKQAPGKGLKLMGWINTYSGAPTYADDFKGRFAFSLETSAS
AAYLQINNLKNEDTATYFCARKPPHYYVNSFDYWGQGTTLTVSS

>mAb58[TIM-3] Variable Light (SEQ ID NO:XXX-XXX)
DIVMTQSPATLSVTPGDRVSLSCRASQSISDYLHWYQQKSHESPRLLIKYASQSISGIPSRFSGSGSGSDFTLSINS
VEPEDVGVYYCQNGHSFPYTFGGGTKLEIK

>TIM3-0433[TIM-3] Variable Heavy (SEQ ID NO:XXX-XXX)
QITLKESGPTLVKPTQTLTLTCTFSGFSLSTSGMSVGWIRQPPGKGLEWLAHIWLNDDVFFNPALKSRLTITKDTSK
NQVVLTMTNMDPVDTATYYCVRANGYLYALDYWGQGTLVTVSS

>TIM3-0433[TIM-3] Variable Light (SEQ ID NO:XXX)
ETTLTQSPAFMSATPGDKVNIACSASSSVSYTQWYQQKPGEAPKLWIYDAFKLAPGIPPRFSGSGYGTDFTLTINNI
ESEDAAYYFCHQWSSYPWTFGQGTKLEIK

Figure 13C

>TIM3-0434[TIM-3] Variable Heavy (SEQ ID NO:XXX-XXX)
QITLKESGPTLVKPTQTLTLTCTFSGFSLSTSGMSVGWIRQPPGKGLEWLAHIWLNDDVFFNPALKSRLTITKDTSK
NQVVLTMTNMDPVDTATYYCVRANGYLYALDYWGQGTLVTVSS

>TIM3-0434[TIM-3] Variable Light (SEQ ID NO:XXX-XXX)
DIQLTQSPSFLSASVGDRVTITCSASSSVSYTQWYQQKPGKAPKLWIYDAFKLAPGVPSRFSGSGSGTEFTLTISSL
QPEDFATYFCHQWSSYPWTFGQGTKLEIK

>TIM3-0438[TIM-3] Variable Heavy (SEQ ID NO:XXX-XXX)
EVQLVESGGGLVQPGGSLRLSCAASGFNIKTTYMHWVRQAPGKGLEWVGRIDPADDNTKYAPKFQGKATISADTSKN
TAYLQMNSLRAEDTAVYYCVRDFGYVAWFAYWGQGTLVTVSS

>TIM3-0438[TIM-3] Variable Light (SEQ ID NO:XXX-XXX)
DIVMTQSPLSLPVTPGEPASISCRASQSVDNYVAWYLQKPGQSPQLLIYYASNRYIGVPDRFSGSGSGTDFTLKISR
VEAEDVGVYYCQQHYSSPYTFGQGTKVEIK

>TIM3-0443[TIM-3] Variable Heavy (SEQ ID NO:XXX-XXX)
EVQLVESGGGLVQPGGSLRLSCAASGFNIKTTYMHWVRQAPGKGLEWVGRIDPADDNTKYAPKFQGKATISADTSKN
TAYLQMNSLRAEDTAVYYCVRDFGYVAWFAYWGQGTLVTFSS

>TIM3-0443[TIM-3] Variable Light (SEQ ID NO:XXX-XXX)
DIVMTQSPLSLPVTPGEPASISCRASQSVDNYVAWYLQKPGQSPQLLIYYASNRYIGVPDRFSGSGSGTDFTLKISR
VEAEDVGVYYCQQHYSSPYTFGQGTKVEIK

Figure 14

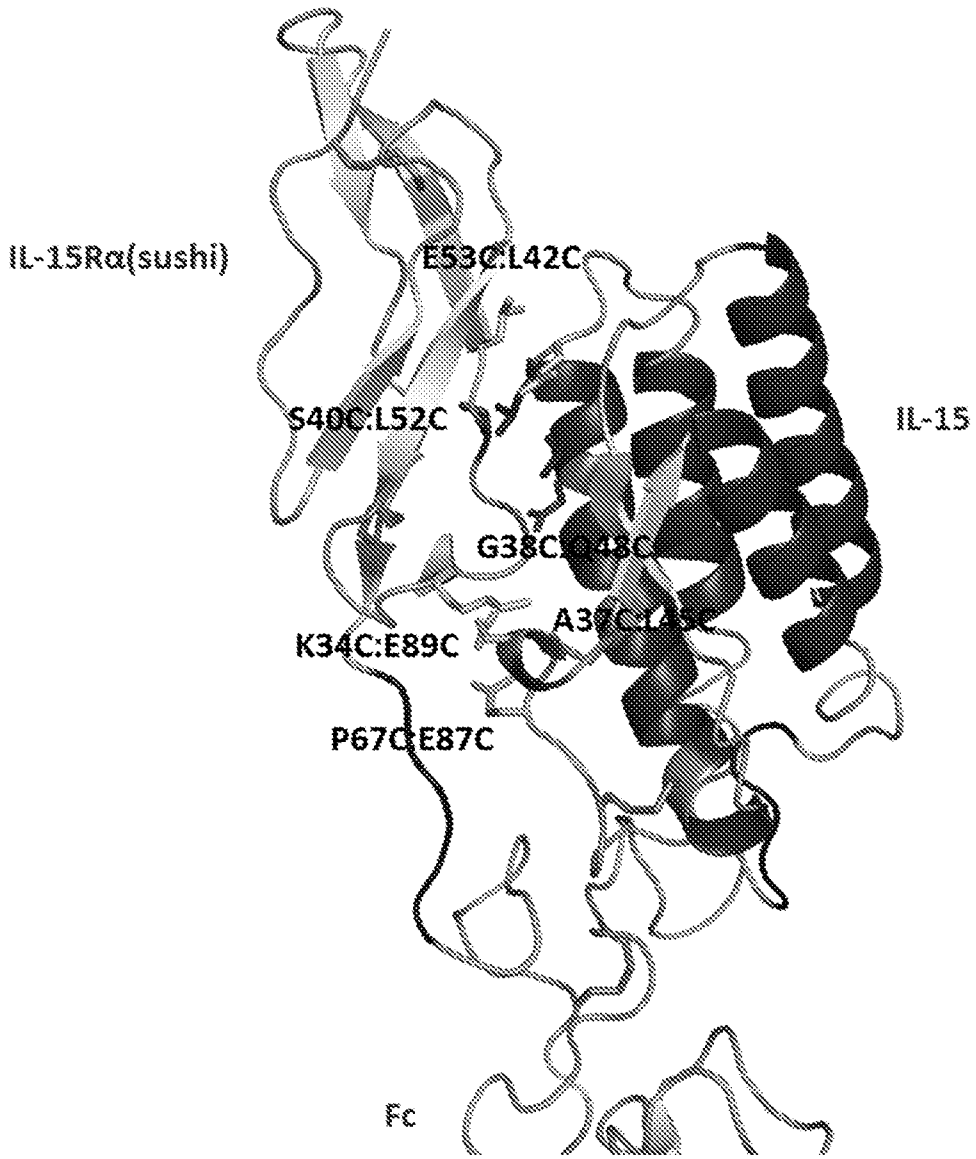

IL-15Rα(sushi)
E53C:L42C
S40C:L52C
IL-15
G38C:Q48C
A37C:L45C
K34C:E89C
P67C:E87C
Fc

Figure 15

IL-15Rα(sushi-D96) (SEQ ID NO:XXX)
ITCPPPMSVEHADIWVKSYSLYSRERYICNSGFKRKAGTSSLTECVLNKATNVAHWTTPSLKCIRD

IL-15Rα(sushi-D96/P97) (SEQ ID NO:XXX)
ITCPPPMSVEHADIWVKSYSLYSRERYICNSGFKRKAGTSSLTECVLNKATNVAHWTTPSLKCIRDP

IL-15Rα(sushi-D96/P97/A98) (SEQ ID NO:XXX)
ITCPPPMSVEHADIWVKSYSLYSRERYICNSGFKRKAGTSSLTECVLNKATNVAHWTTPSLKCIRDPA

Figure 16

IL-15(E87C) (SEQ ID NO:XXX)
NWVNVISDLKKIEDLIQSMHIDATLYTESDVHPSCKVTAMKCFLLELQVISLESGDASIHDTVENLIILANNSLSSN
GNVTESGCKCCEELEEKNIKEFLQSFVHIVQMFINTS

IL-15(V49C) (SEQ ID NO:XXX)
NWVNVISDLKKIEDLIQSMHIDATLYTESDVHPSCKVTAMKCFLLELQCISLESGDASIHDTVENLIILANNSLSSN
GNVTESGCKECEELEEKNIKEFLQSFVHIVQMFINTS

IL-15(L52C) (SEQ ID NO:XXX)
NWVNVISDLKKIEDLIQSMHIDATLYTESDVHPSCKVTAMKCFLLELQVISCESGDASIHDTVENLIILANNSLSSN
GNVTESGCKECEELEEKNIKEFLQSFVHIVQMFINTS

IL-15(E89C) (SEQ ID NO:XXX)
NWVNVISDLKKIEDLIQSMHIDATLYTESDVHPSCKVTAMKCFLLELQVISLESGDASIHDTVENLIILANNSLSSN
GNVTESGCKECCELEEKNIKEFLQSFVHIVQMFINTS

IL-15(Q48C) (SEQ ID NO:XXX)
NWVNVISDLKKIEDLIQSMHIDATLYTESDVHPSCKVTAMKCFLLELCVISLESGDASIHDTVENLIILANNSLSSN
GNVTESGCKECEELEEKNIKEFLQSFVHIVQMFINTS

IL-15(E53C) (SEQ ID NO:XXX)
NWVNVISDLKKIEDLIQSMHIDATLYTESDVHPSCKVTAMKCFLLELQVISLCSGDASIHDTVENLIILANNSLSSN
GNVTESGCKECEELEEKNIKEFLQSFVHIVQMFINTS

IL-15(C42S) (SEQ ID NO:XXX)
NWVNVISDLKKIEDLIQSMHIDATLYTESDVHPSCKVTAMKSFLLELQVISLESGDASIHDTVENLIILANNSLSSN
GNVTESGCKECEELEEKNIKEFLQSFVHIVQMFINTS

IL-15(L45C) (SEQ ID NO:XXX)
NWVNVISDLKKIEDLIQSMHIDATLYTESDVHPSCKVTAMKCFLCELQVISLESGDASIHDTVENLIILANNSLSSN
GNVTESGCKECEELEEKNIKEFLQSFVHIVQMFINTS

Figure 17

IL-15Rα(sushi-D96/C97) (SEQ ID NO:XXX)
ITCPPPMSVEHADIWVKSYSLYSRERYICNSGFKRKAGTSSLTECVLNKATNVAHWTTPSLKCIRDC

IL-15Rα(sushi-D96/P97/C98) (SEQ ID NO:XXX)
ITCPPPMSVEHADIWVKSYSLYSRERYICNSGFKRKAGTSSLTECVLNKATNVAHWTTPSLKCIRDPC

IL-15Rα(sushi-D96/C97/A98) (SEQ ID NO:XXX)
ITCPPPMSVEHADIWVKSYSLYSRERYICNSGFKRKAGTSSLTECVLNKATNVAHWTTPSLKCIRDCA

IL-15Rα(sushi-S40C) (SEQ ID NO:XXX)
ITCPPPMSVEHADIWVKSYSLYSRERYICNSGFKRKAGTCSLTECVLNKATNVAHWTTPSLKCIR

IL-15Rα(sushi-K34C) (SEQ ID NO:XXX)
ITCPPPMSVEHADIWVKSYSLYSRERYICNSGFCRKAGTSSLTECVLNKATNVAHWTTPSLKCIR

IL-15Rα(sushi-G38C) (SEQ ID NO:XXX)
ITCPPPMSVEHADIWVKSYSLYSRERYICNSGFKRKACTSSLTECVLNKATNVAHWTTPSLKCIR

IL-15Rα(sushi-L42C) (SEQ ID NO:XXX)
ITCPPPMSVEHADIWVKSYSLYSRERYICNSGFKRKAGTSSCTECVLNKATNVAHWTTPSLKCIR

IL-15Rα(sushi-A37C) (SEQ ID NO:XXX)
ITCPPPMSVEHADIWVKSYSLYSRERYICNSGFKRKCGTSSLTECVLNKATNVAHWTTPSLKCIR

Figure 18

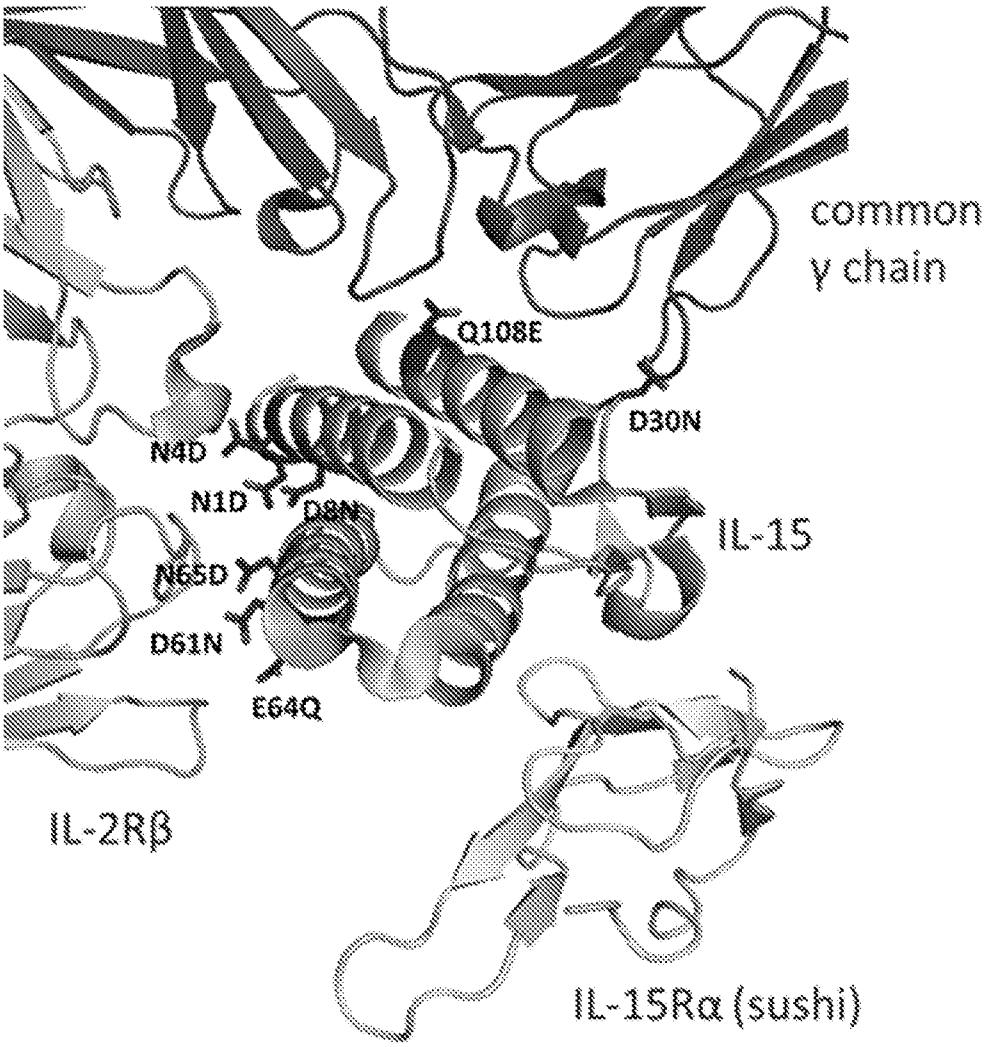

common
γ chain
Q108E
D30N
N4D
N1D
D8N
N65D
D61N
E64Q
IL-15
IL-2Rβ
IL-15Rα (sushi)

Figure 19A

N1D (SEQ ID NO:XXX)
DWVNVISDLKKIEDLIQSMHIDATLYTESDVHPSCKVTAMKCFLLELQVISLESGDASIHDTVENLIILANNSLSSNGNVTESGCKECEELEE
KNIKEFLQSFVHIVQMFINTS

N4D (SEQ ID NO:XXX)
NWVDVISDLKKIEDLIQSMHIDATLYTESDVHPSCKVTAMKCFLLELQVISLESGDASIHDTVENLIILANNSLSSNGNVTESGCKECEELEE
KNIKEFLQSFVHIVQMFINTS

D8N (SEQ ID NO:XXX)
NWVNVISNLKKIEDLIQSMHIDATLYTESDVHPSCKVTAMKCFLLELQVISLESGDASIHDTVENLIILANNSLSSNGNVTESGCKECEELEE
KNIKEFLQSFVHIVQMFINTS

D30N (SEQ ID NO:XXX)
NWVNVISDLKKIEDLIQSMHIDATLYTESNVHPSCKVTAMKCFLLELQVISLESGDASIHDTVENLIILANNSLSSNGNVTESGCKECEELEE
KNIKEFLQSFVHIVQMFINTS

D61N (SEQ ID NO:XXX)
NWVNVISDLKKIEDLIQSMHIDATLYTESDVHPSCKVTAMKCFLLELQVISLESGDASIHNTVENLIILANNSLSSNGNVTESGCKECEELEE
KNIKEFLQSFVHIVQMFINTS

E64Q (SEQ ID NO:XXX)
NWVNVISDLKKIEDLIQSMHIDATLYTESDVHPSCKVTAMKCFLLELQVISLESGDASIHDTVQNLIILANNSLSSNGNVTESGCKECEELEE
KNIKEFLQSFVHIVQMFINTS

N65D (SEQ ID NO:XXX)
NWVNVISDLKKIEDLIQSMHIDATLYTESDVHPSCKVTAMKCFLLELQVISLESGDASIHDTVEDLIILANNSLSSNGNVTESGCKECEELEE
KNIKEFLQSFVHIVQMFINTS

Q108E (SEQ ID NO:XXX)
NWVNVISDLKKIEDLIQSMHIDATLYTESDVHPSCKVTAMKCFLLELQVISLESGDASIHDTVENLIILANNSLSSNGNVTESGCKECEELEE
KNIKEFLQSFVHIVEMFINTS

N1D/D61N (SEQ ID NO:XXX)
DWVNVISDLKKIEDLIQSMHIDATLYTESDVHPSCKVTAMKCFLLELQVISLESGDASIHNTVENLIILANNSLSSNGNVTESGCKECEELEE
KNIKEFLQSFVHIVQMFINTS

N1D/E64Q (SEQ ID NO:XXX)
DWVNVISDLKKIEDLIQSMHIDATLYTESDVHPSCKVTAMKCFLLELQVISLESGDASIHDTVQNLIILANNSLSSNGNVTESGCKECEELEE
KNIKEFLQSFVHIVQMFINTS

N4D/D61N (SEQ ID NO:XXX)
NWVDVISDLKKIEDLIQSMHIDATLYTESDVHPSCKVTAMKCFLLELQVISLESGDASIHNTVENLIILANNSLSSNGNVTESGCKECEELEE
KNIKEFLQSFVHIVQMFINTS

N4D/E64Q (SEQ ID NO:XXX)
NWVDVISDLKKIEDLIQSMHIDATLYTESDVHPSCKVTAMKCFLLELQVISLESGDASIHDTVQNLIILANNSLSSNGNVTESGCKECEELEE
KNIKEFLQSFVHIVQMFINTS

D8N/D61N (SEQ ID NO:XXX)
NWVNVISNLKKIEDLIQSMHIDATLYTESDVHPSCKVTAMKCFLLELQVISLESGDASIHNTVENLIILANNSLSSNGNVTESGCKECEELEE
KNIKEFLQSFVHIVQMFINTS

Figure 19B

D8N/E64Q (SEQ ID NO:XXX)
NWVNVISNLKKIEDLIQSMHIDATLYTESDVHPSCKVTAMKCFLLELQVISLESGDASIHDTVQNLIILANNSLSSNGNVTESGCKECEELEE
KNIKEFLQSFVHIVQMFINTS

D61N/E64Q (SEQ ID NO:XXX)
NWVNVISDLKKIEDLIQSMHIDATLYTESDVHPSCKVTAMKCFLLELQVISLESGDASIHNTVQNLIILANNSLSSNGNVTESGCKECEELEE
KNIKEFLQSFVHIVQMFINTS

E64Q/Q108E (SEQ ID NO:XXX)
NWVNVISDLKKIEDLIQSMHIDATLYTESDVHPSCKVTAMKCFLLELQVISLESGDASIHDTVQNLIILANNSLSSNGNVTESGCKECEELEE
KNIKEFLQSFVHIVEMFINTS

N1D/N4D/D8N (SEQ ID NO:XXX)
DWVDVISNLKKIEDLIQSMHIDATLYTESDVHPSCKVTAMKCFLLELQVISLESGDASIHDTVENLIILANNSLSSNGNVTESGCKECEELEE
KNIKEFLQSFVHIVQMFINTS

D61N/E64Q/N65D (SEQ ID NO:XXX)
NWVNVISDLKKIEDLIQSMHIDATLYTESDVHPSCKVTAMKCFLLELQVISLESGDASIHNTVQDLIILANNSLSSNGNVTESGCKECEELEE
KNIKEFLQSFVHIVQMFINTS

N1D/D61N/E64Q/Q108E (SEQ ID NO:XXX)
DWVNVISDLKKIEDLIQSMHIDATLYTESDVHPSCKVTAMKCFLLELQVISLESGDASIHNTVQNLIILANNSLSSNGNVTESGCKECEELEE
KNIKEFLQSFVHIVEMFINTS

N4D/D61N/E64Q/Q108E (SEQ ID NO:XXX)
NWVDVISDLKKIEDLIQSMHIDATLYTESDVHPSCKVTAMKCFLLELQVISLESGDASIHNTVQNLIILANNSLSSNGNVTESGCKECEELEE
KNIKEFLQSFVHIVEMFINTS

N1D/N65D (SEQ ID NO:XXX)
DWVNVISDLKKIEDLIQSMHIDATLYTESDVHPSCKVTAMKCFLLELQVISLESGDASIHDTVEDLIILANNSLSSNGNVTESGCKECEELEE
KNIKEFLQSFVHIVQMFINTS

N1D/Q108E (SEQ ID NO:XXX)
DWVNVISDLKKIEDLIQSMHIDATLYTESDVHPSCKVTAMKCFLLELQVISLESGDASIHDTVENLIILANNSLSSNGNVTESGCKECEELEE
KNIKEFLQSFVHIVEMFINTS

N4D/N65D (SEQ ID NO:XXX)
NWVDVISDLKKIEDLIQSMHIDATLYTESDVHPSCKVTAMKCFLLELQVISLESGDASIHDTVEDLIILANNSLSSNGNVTESGCKECEELEE
KNIKEFLQSFVHIVQMFINTS

D30N/N65D (SEQ ID NO:XXX)
NWVNVISDLKKIEDLIQSMHIDATLYTESNVHPSCKVTAMKCFLLELQVISLESGDASIHDTVEDLIILANNSLSSNGNVTESGCKECEELEE
KNIKEFLQSFVHIVQMFINTS

D30N/Q108E (SEQ ID NO:XXX)
NWVNVISDLKKIEDLIQSMHIDATLYTESNVHPSCKVTAMKCFLLELQVISLESGDASIHDTVENLIILANNSLSSNGNVTESGCKECEELEE
KNIKEFLQSFVHIVEMFINTS

N65D/Q108E (SEQ ID NO:XXX)
NWVNVISDLKKIEDLIQSMHIDATLYTESDVHPSCKVTAMKCFLLELQVISLESGDASIHDTVEDLIILANNSLSSNGNVTESGCKECEELEE
KNIKEFLQSFVHIVEMFINTS

Figure 19C

E64Q/N65D (SEQ ID NO:XXX)
NWVNVISDLKKIEDLIQSMHIDATLYTESDVHPSCKVTAMKCFLLELQVISLESGDASIHDTVQDLIILANNSLSSNGNVTESGCKECEELEE
KNIKEFLQSFVHIVQMFINTS

N1D/N4D/N65D (SEQ ID NO:XXX)
DWVDVISDLKKIEDLIQSMHIDATLYTESDVHPSCKVTAMKCFLLELQVISLESGDASIHDTVEDLIILANNSLSSNGNVTESGCKECEELEE
KNIKEFLQSFVHIVQMFINTS

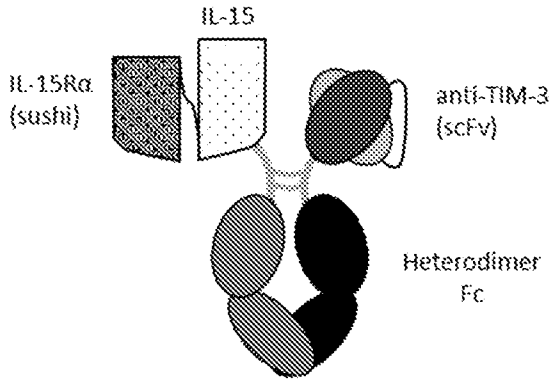
D30N/E64Q/N65D (SEQ ID NO:XXX)
NWVNVISDLKKIEDLIQSMHIDATLYTESNVHPSCKVTAMKCFLLELQVISLESGDASIHDTVQDLIILANNSLSSNGNVTESGCKECEELEE
KNIKEFLQSFVHIVQMFINTS

N4D/D61N/N65D (SEQ ID NO:XXX)
NWVDVISDLKKIEDLIQSMHIDATLYTESDVHPSCKVTAMKCFLLELQVISLESGDASIHNTVEDLIILANNSLSSNGNVTESGCKECEELEE
KNIKEFLQSFVHIVQMFINTS

Figure 20

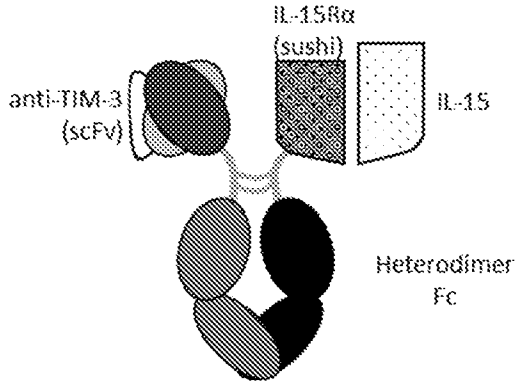
XENP	Variant	EC50 pM (NK cells)	Fold reduced (NK cells)	EC50 pM (CD8 T cells)	Fold reduced (CD8 T cells)
20818	WT	200.6		637.1	
21478	single-chain	848.5	4.2	4982.0	7.8
22815	N1D	281.3	1.4	1051.0	1.6
22816	N4D	321.9	1.6	1190.0	1.9
22817	D8N	very weak	very weak	very weak	very weak
22818	D30N	376.3	1.9	1366.0	2.1
22819	D61N	5934.0	29.6	161937.0	>100
22820	E64Q	877.0	4.4	2858.0	4.5
22821	N65D	2883.0	14.4	6928.0	10.9
22822	Q108E	9777.0	48.7	very weak	>100
22823	N1D/D61N	918.0	4.6	4225.0	6.6
22824	N1D/E64Q	1091.0	5.4	4228.0	6.6
22825	N4D/D61N	309.0	1.5	1070.0	1.7
22826	N4D/E64Q	very weak	very weak	very weak	very weak
22827	D8N/D61N	ND	ND	ND	ND
22828	D8N/E64Q	597.7	3.0	1658.0	2.6
22829	D61N/E64Q	458.2	2.3	2115.0	3.3
22830	E64Q/Q108E	436.6	2.2	1815.0	2.8
22831	N1D/N4D/D8N	very weak	very weak	very weak	very weak
22832	D61N/E64Q/N65D	ND	ND	ND	ND
22833	N1D/D61N/E64Q/Q108E	ND	ND	ND	ND
22834	N4D/D61N/E64Q/Q108E	very weak	very weak	very weak	very weak

Figure 21A



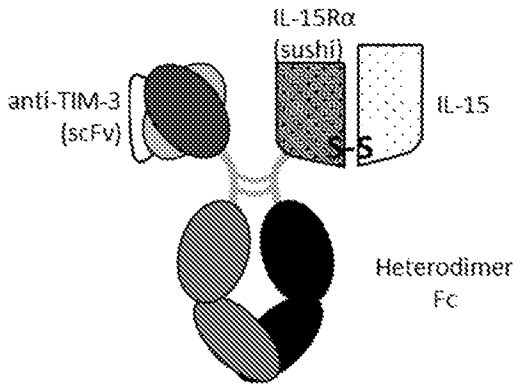
scIL-15/Rα x scFv

Figure 21B



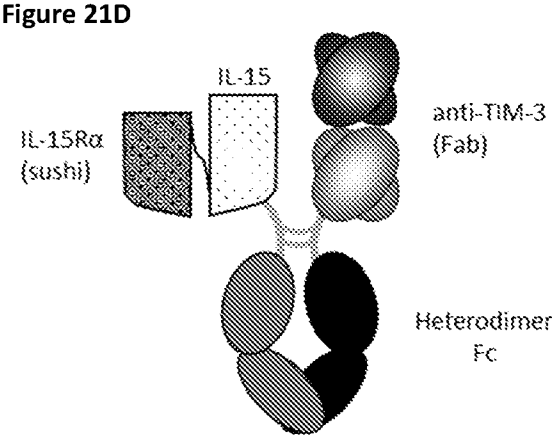
scFv x ncIL-15/Rα

Figure 21C



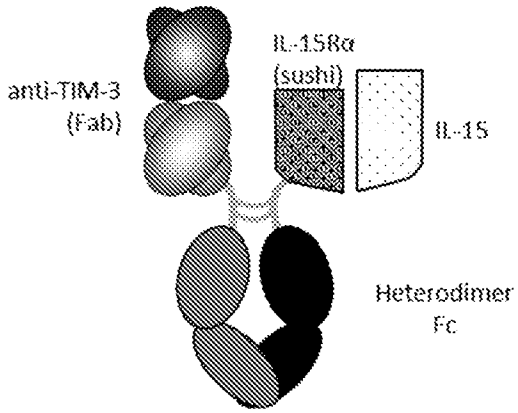
scFv x dsIL-15/Rα

Figure 21D



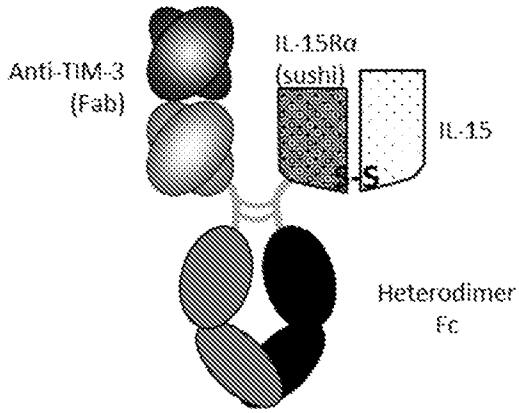
scIL-15/Rα x Fab
Example: XENP27974

Figure 21E



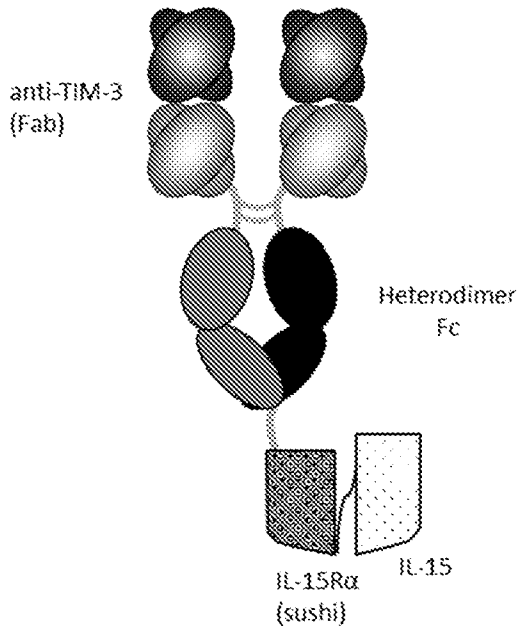
Fab x ncIL-15/Rα

Figure 21F



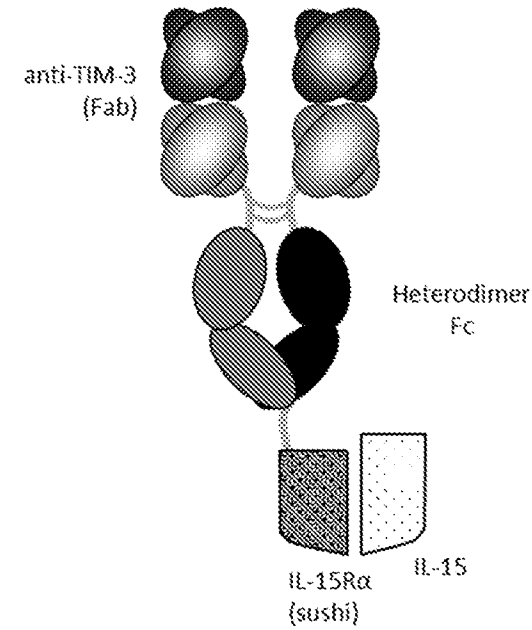
Fab x dsIL-15/Rα

Figure 21G



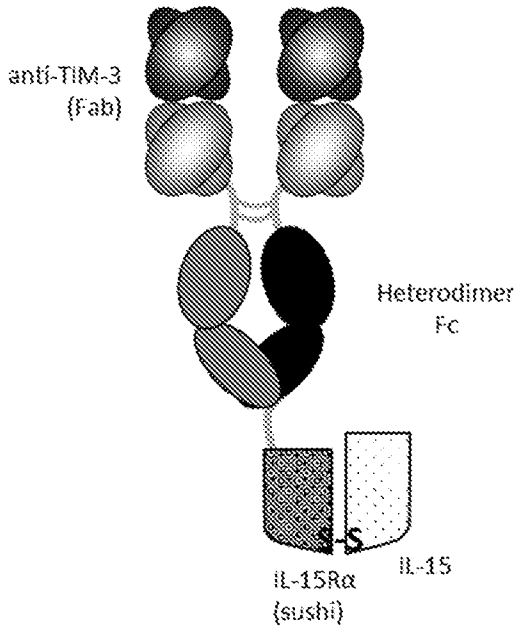
mAb-scIL-15/Rα

Figure 21H



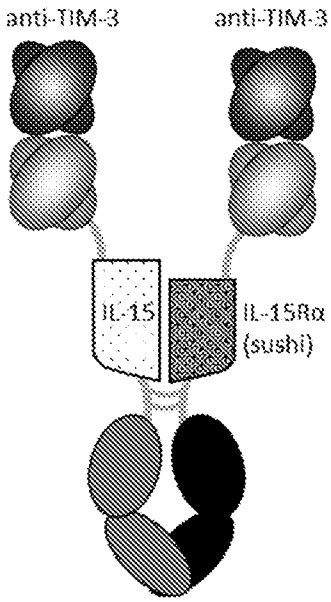
mAb-ncIL-15/Rα

Figure 21I



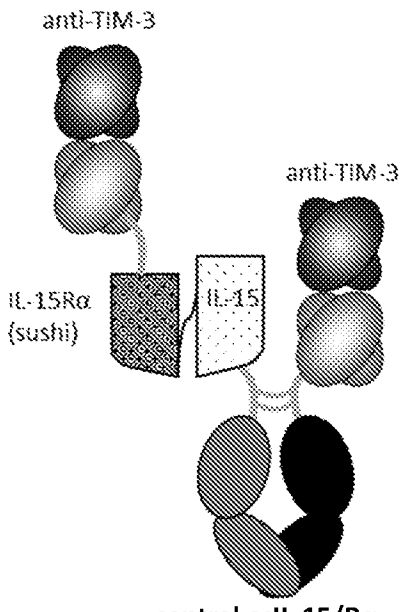
mAb-dsIL-15/Rα

Figure 21J



central-IL-15/Rα

Figure 21K



central-scIL-15/Rα

Figure 22

>XENP027974 human IL15Ra(sushi) (GGGGS)5-human IL15(N4D/N65D;single-Chain)-3H3[TIM-3] H1 L2.1 Fab IgG1 Fc(216) IgG1 pI(-) Isosteric A C220S/PVA /S267K/L368D/K370S-IgG1 PVA /S267K/S364K/E357Q

Chain 1 human_IL15Ra(sushi)_(GGGGS)5-human_IL15(N4D/N65D;single-Chain)_Fc(216)_IgG1_pI(-)_Isosteric_A_C220S/PVA_/S267K/L368D/K370S scIL-15/Ra-Fc Chain **(SEQ ID NO:XXX)**

ITCPPPMSVEHADIWVKSYSLYSRERYICNSGFKRKAGTSSLTECVLNKATNVAHWTTPSLKCIR/GGGGSGGGGSG
GGGSGGGGSGGGGS/NWVDVISDLKKIEDLIQSMHIDATLYTESDVHPSCKVTAMKCFLLELQVISLESGDASIHDT
VEDLIILANNSLSSNGNVTESGCKECEELEEKNIKEFLQSFVHIVQMFINTS/EPKSSDKTHTCPPCPAPPVAGPSV
FLFPPKPKDTLMISRTPEVTCVVVDVKHEDPEVKFNWYVDGVEVHNAKTKPREEEYNSTYRVVSVLTVLHQDWLNGK
EYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSREEMTKNQVSLTCDVSGFYPSDIAVEWESDGQPENNYKTT
PPVLDSDGSFFLYSKLTVDKSRWEQGDVFSCSVMHEALHNHYTQKSLSLSPGK

Chain 2 3H3[TIM-3]_H1_L2.1_Fab_IgG1_PVA_/S267K/S364K/E357Q Fab-Fc Heavy Chain **(SEQ ID NO:XXX)**

QVTLKESGPVLVKPTETLTLTCTVSGFSLN**GYGVN**WVRQPPGKGLEWLA**MIWGDGSTDYNSALKS**RLTISKDNSKSQ
VVLTMTNMDPVDTATYYCAR**SYYTSDEDY**WGQGTLVTVSS/ASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEP
VTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSSLGTQTYICNVNHKPSNTKVDKKVEPKSCDKTHTCPPCP
APPVAGPSVFLFPPKPKDTLMISRTPEVTCVVVDVKHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTV
LHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSREQMTKNQVKLTCLVKGFYPSDIAVEWESNG
QPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGK

Chain 3 3H3[TIM-3]_H1_L2.1_Fab Light Chain **(SEQ ID NO:XXX)**

DIVMTQSPDSLAVSLGERATINC**KSSQSLLNSRTRKNYLA**WYQQKPGQSPKLLIY**WASTRES**GVPDRFTGSGSGTDF
TLTISSLQAEDVAVYYC**KQSYSLRT**FGGGTKVEIK/RTVAAPSVFIFPPSDEQLKSGTASVVCLLNNFYPREAKVQW
KVDNALQSGNSQESVTEQDSKDSTYSLSSTLTLSKADYEKHKVYACEVTHQGLSSPVTKSFNRGEC

Figure 23

XENP016432 Nivolumab_H0L0_IgG1_PVA_/S267K Heavy Chain **(SEQ ID NO:XXX)**

QVQLVESGGGVVQPGRSLRLDCKASGITFSNSGMHWVRQAPGKGLEWVAVIWYDGSKRYYADSVKGRFTISRDNSKN
TLFLQMNSLRAEDTAVYYCATNDDYWGQGTLVTVSS/ASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVS
WNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSSLGTQTYICNVNHKPSNTKVDKKVEPKSCDKTHTCPPCPAPPV
AGPSVFLFPPKPKDTLMISRTPEVTCVVVDVKHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQD
WLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSREEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPEN
NYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGK

XENP016432 Nivolumab_H0L0_IgG1_PVA_/S267K Light Chain **(SEQ ID NO:XXX)**

EIVLTQSPATLSLSPGERATLSCRASQSVSSYLAWYQQKPGQAPRLLIYDASNRATGIPARFSGSGSGTDFTLTISS
LEPEDFAVYYCQQSSNWPRTFGQGTKVEIK/RTVAAPSVFIFPPSDEQLKSGTASVVCLLNNFYPREAKVQWKVDNA
LQSGNSQESVTEQDSKDSTYSLSSTLTLSKADYEKHKVYACEVTHQGLSSPVTKSFNRGEC

Figure 24A

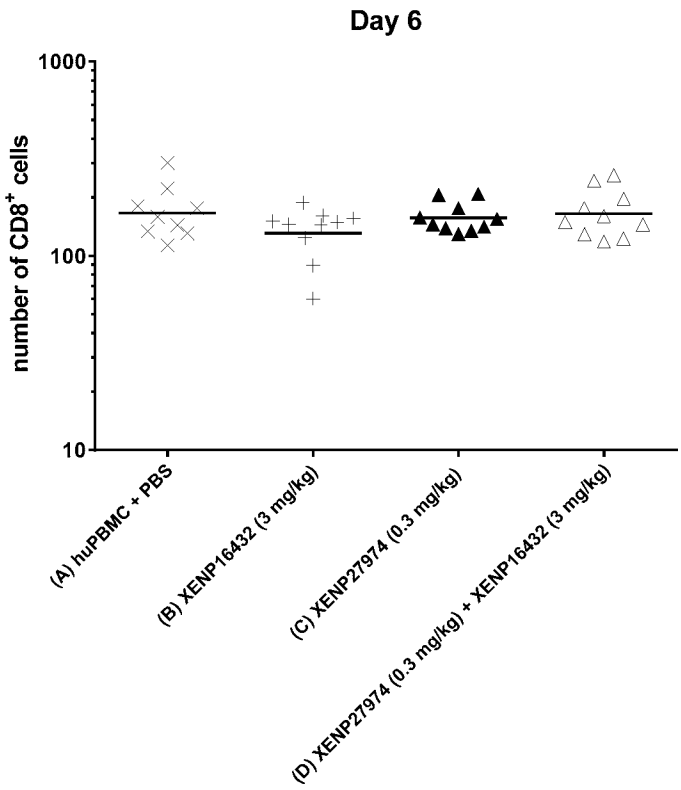


Figure 24B

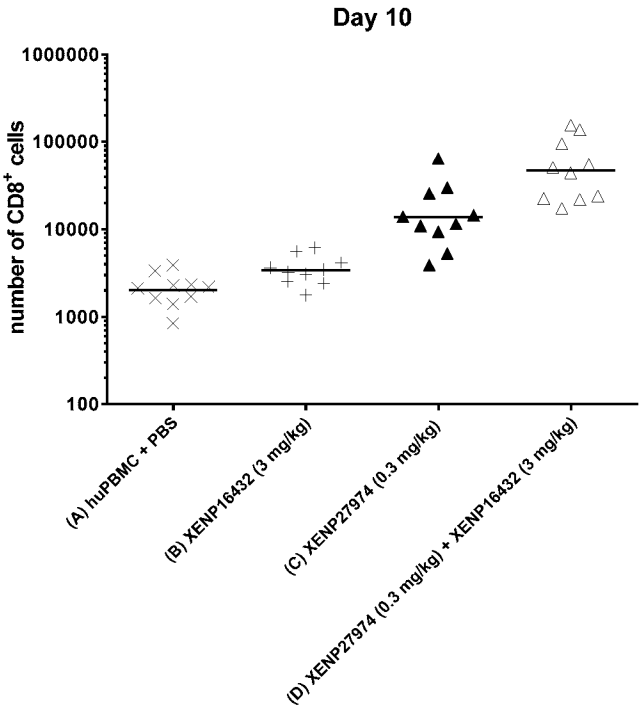


Figure 25A

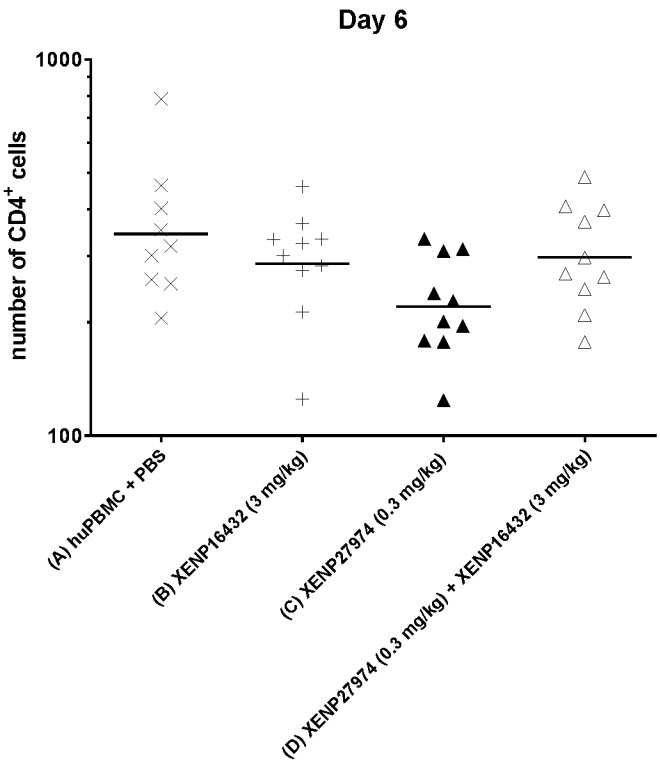


Figure 25B

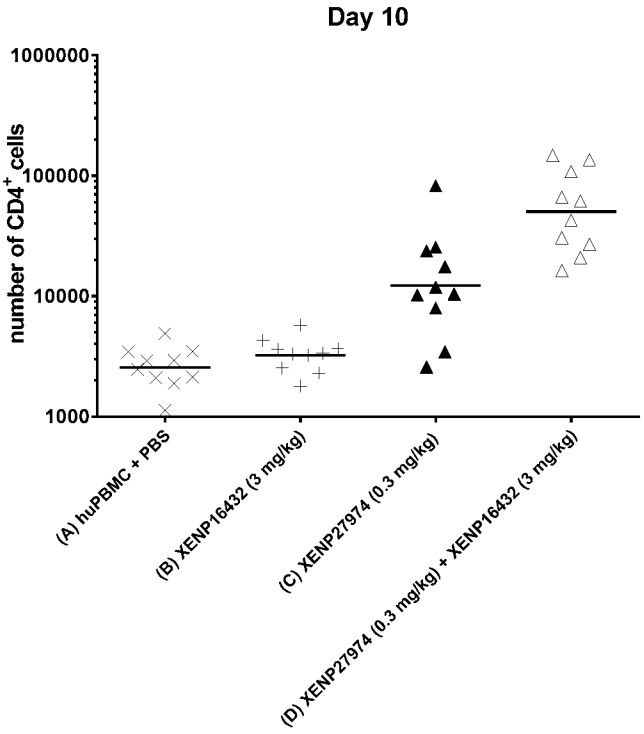


Figure 26A

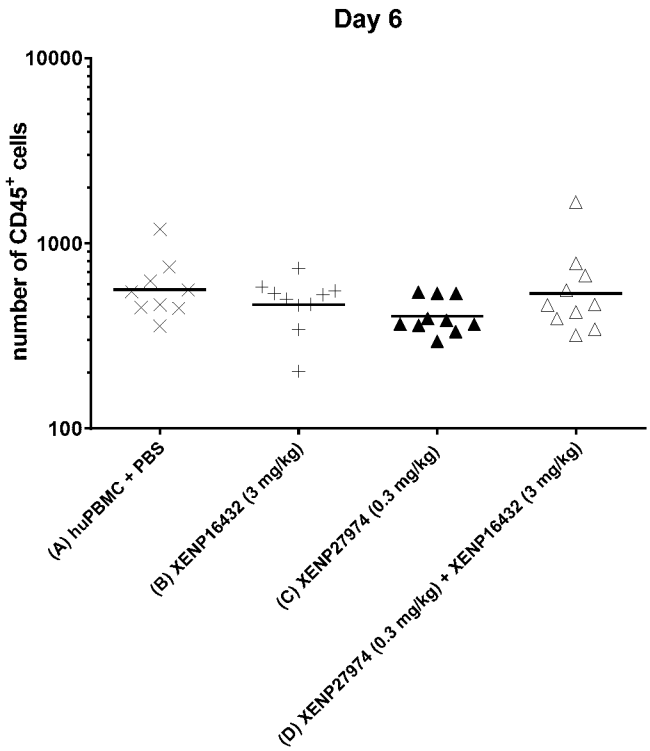


Figure 26B

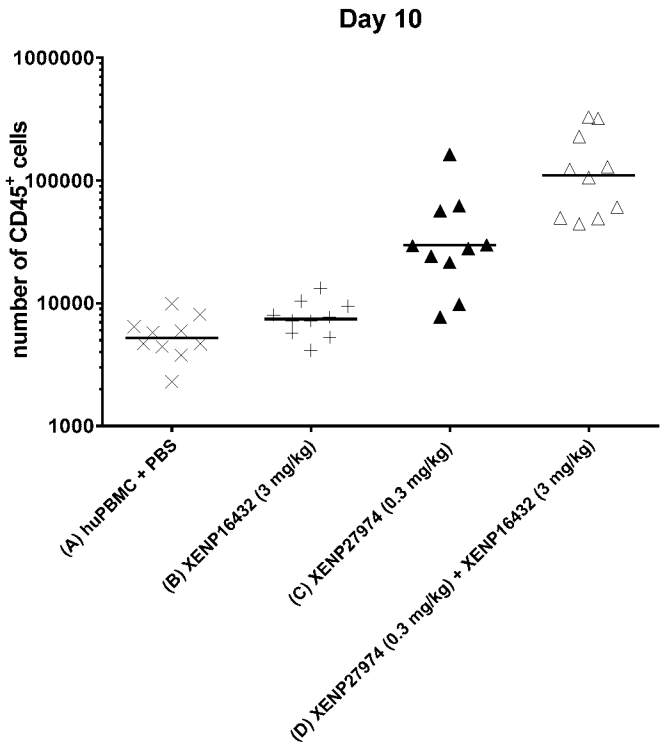


Figure 27A

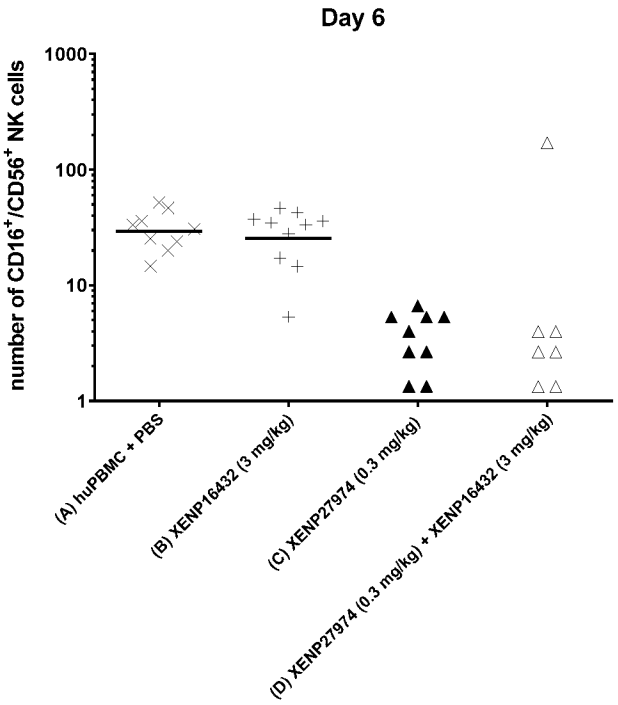


Figure 27B

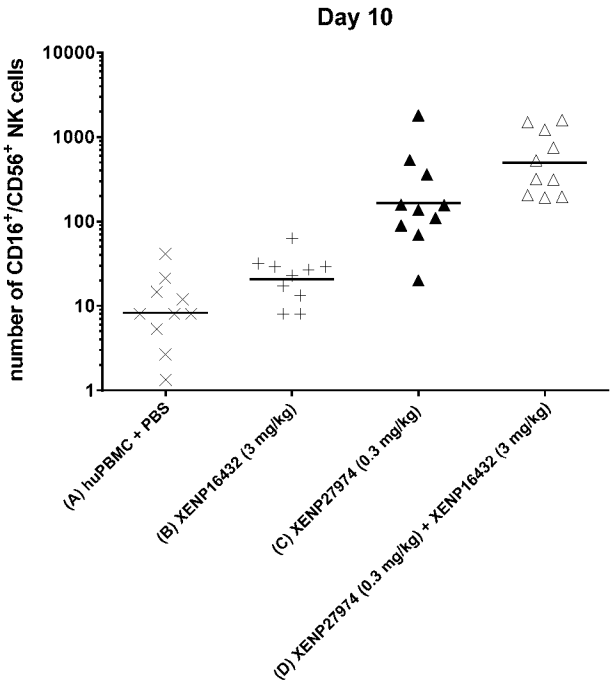


Figure 28

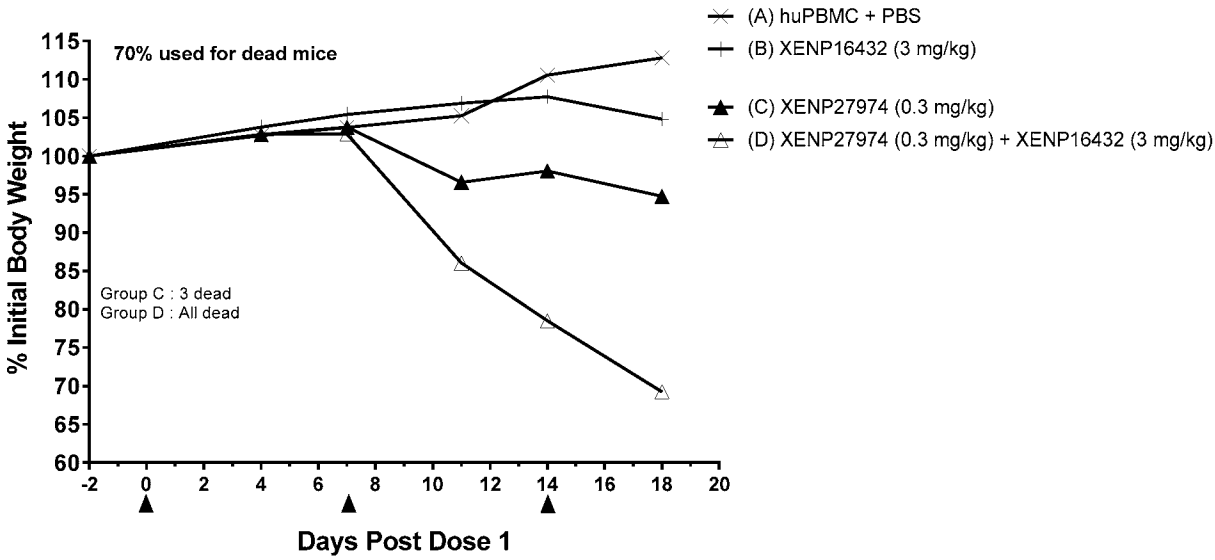

70% used for dead mice
Group C : 3 dead
Group D : All dead
% Initial Body Weight
Days Post Dose 1
(A) huPBMC + PBS
(B) XENP16432 (3 mg/kg)
(C) XENP27974 (0.3 mg/kg)
(D) XENP27974 (0.3 mg/kg) + XENP16432 (3 mg/kg)

Figure 29

>XENP027979 human_IL15Ra(sushi)_(GGGGS)5-human_IL15(N4D/N65D;single-Chain)-3H3[TIM-3]_H1_L2.1_Fab_IgG1_Fc(216)_IgG1_pI(-) _Isosteric_A_C220S/PVA_/S267K/L368D/K370S/M428L/N434S-IgG1_PVA_/S267K/S364K/E357Q/M428L/N434S

Chain 1 human_IL15Ra(sushi)_(GGGGS)5-human_IL15(N4D/N65D;single-Chain)_Fc(216)_IgG1_pI(-)_Isosteric_A_C220S/PVA_/S267K/L368D/K370S/M428L/N434S scIL-15/Ra-Fc Chain **(SEQ ID NO:XXX)**

ITCPPPMSVEHADIWVKSYSLYSRERYICNSGFKRKAGTSSLTECVLNKATNVAHWTTPSLKCIR/GGGGSGGGGSGGG GSGGGGSGGGGS/NWVDVISDLKKIEDLIQSMHIDATLYTESDVHPSCKVTAMKCFLLELQVISLESGDASIHDTVEDLII LANNSLSSNGNVTESGCKECEELEEKNIKEFLQSFVHIVQMFINTS/EPKSSDKTHTCPPCPAPPVAGPSVFLFPPKPKDTL MISRTPEVTCVVVDVKHEDPEVKFNWYVDGVEVHNAKTKPREEEYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKAL PAPIEKTISKAKGQPREPQVYTLPPSREEMTKNQVSLTCDVSGFYPSDIAVEWESDGQPENNYKTTPPVLDSDGSFFLYS KLTVDKSRWEQGDVFSCSVLHEALHSHYTQKSLSLSPGK

Chain 2 3H3[TIM-3]_H1_L2.1_Fab_IgG1_PVA_/S267K/S364K/E357Q/M428L/N434S Fab-Fc Heavy Chain **(SEQ ID NO:XXX)**

QVTLKESGPVLVKPTETLTLTCTVSGFSLN**GYGVN**WVRQPPGKGLEWLA**MIWGDGSTDYNSALKS**RLTISKDNSKSQV VLTMTNMDPVDTATYYCAR**SYYTSDEDY**WGQGTLVTVSS/ASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTV SWNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSSLGTQTYICNVNHKPSNTKVDKKVEPKSCDKTHTCPPCPAPPVAG PSVFLFPPKPKDTLMISRTPEVTCVVVDVKHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLN GKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSREQMTKNQVKLTCLVKGFYPSDIAVEWESNGQPENNYKTT PPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVLHEALHSHYTQKSLSLSPGK

Chain 3 3H3[TIM-3]_H1_L2.1_Fab Light Chain **(SEQ ID NO:XXX)**

DIVMTQSPDSLAVSLGERATINC**KSSQSLLNSRTRKNYLA**WYQQKPGQSPKLLIY**WASTRES**GVPDRFTGSGSGTDFTL TISSLQAEDVAVYYC**KQSYSLRT**FGGGTKVEIK/RTVAAPSVFIFPPSDEQLKSGTASVVCLLNNFYPREAKVQWKVDNA LQSGNSQESVTEQDSKDSTYSLSSTLTLSKADYEKHKVYACEVTHQGLSSPVTKSFNRGEC

Figure 30

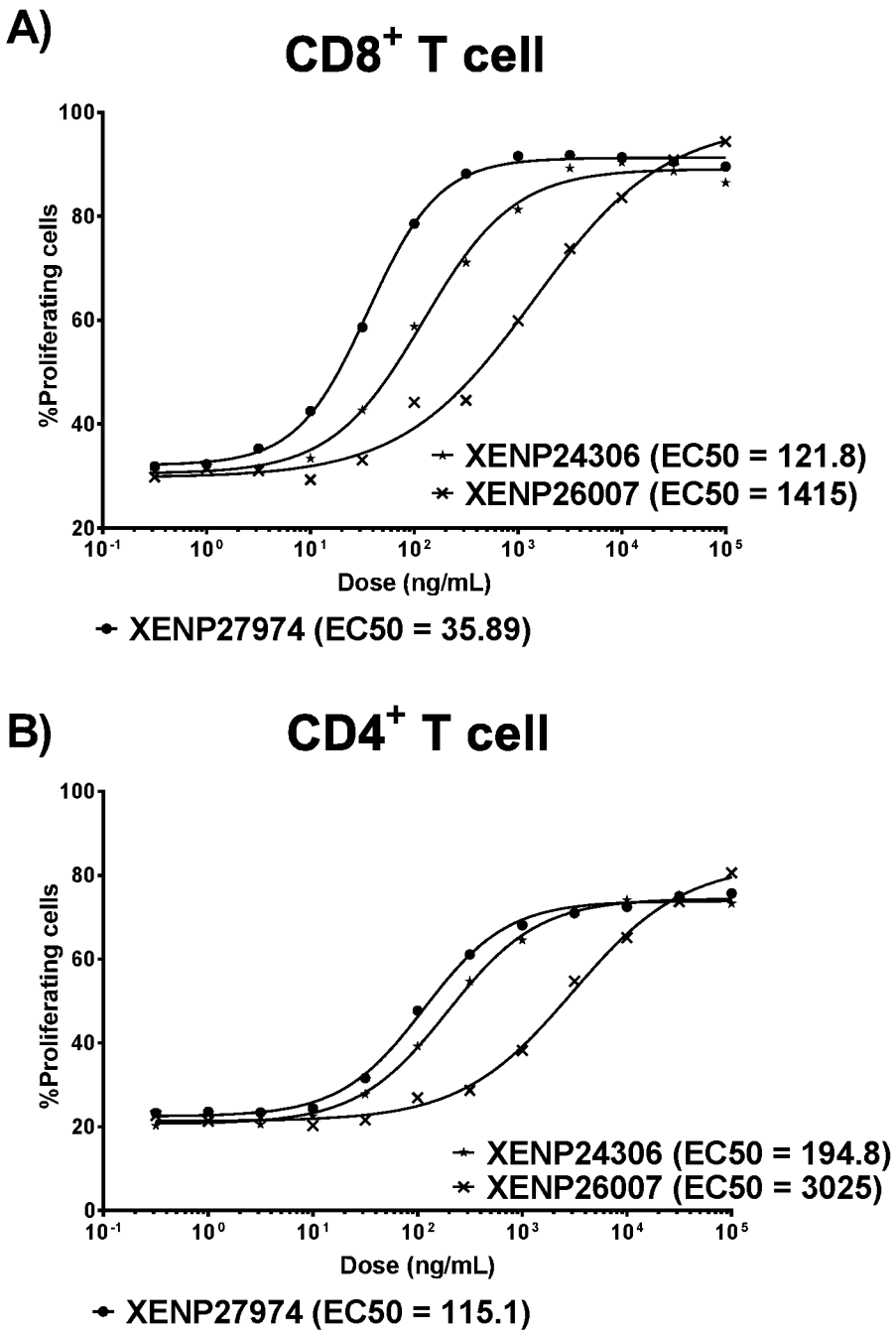

A)
CD8+ T cell
%Proliferating cells
Dose (ng/mL)
XENP24306 (EC50 = 121.8)
XENP26007 (EC50 = 1415)
XENP27974 (EC50 = 35.89)
B)
CD4+ T cell
%Proliferating cells
Dose (ng/mL)
XENP24306 (EC50 = 194.8)
XENP26007 (EC50 = 3025)
XENP27974 (EC50 = 115.1)

Figure 31

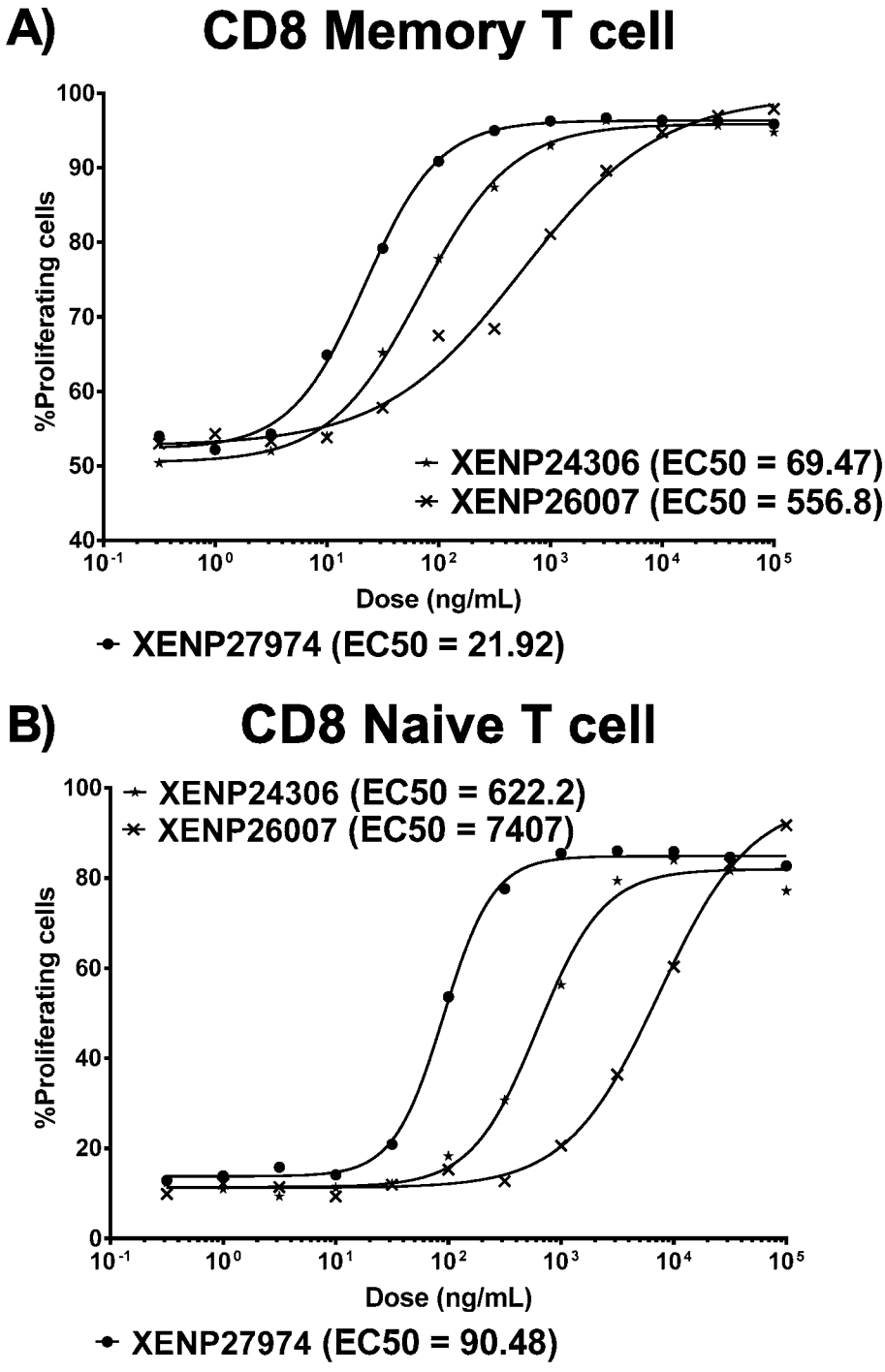

A)
CD8 Memory T cell
%Proliferating cells
Dose (ng/mL)
XENP24306 (EC50 = 69.47)
XENP26007 (EC50 = 556.8)
XENP27974 (EC50 = 21.92)
B)
CD8 Naive T cell
%Proliferating cells
Dose (ng/mL)
XENP24306 (EC50 = 622.2)
XENP26007 (EC50 = 7407)
XENP27974 (EC50 = 90.48)

Figure 32

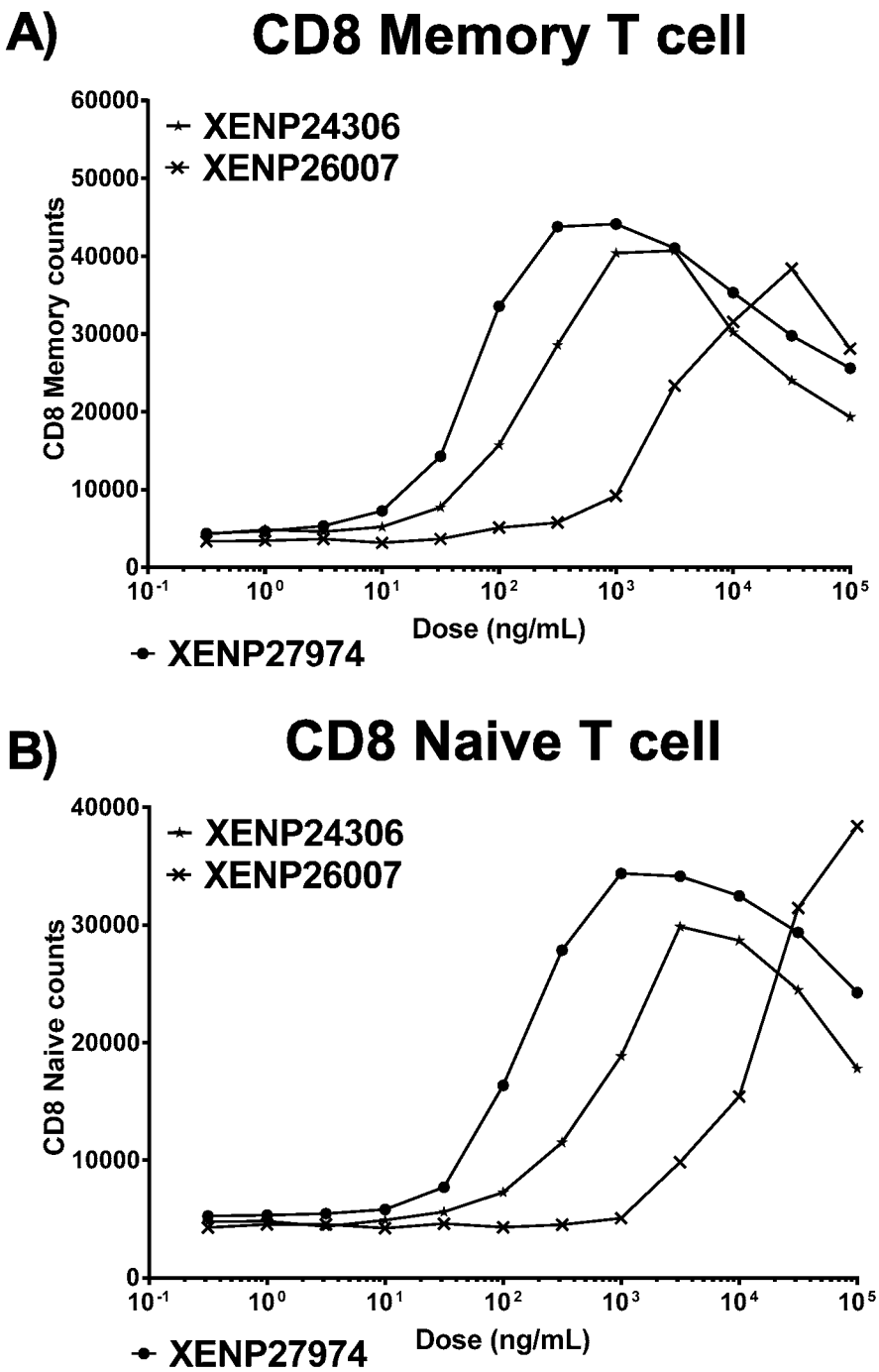
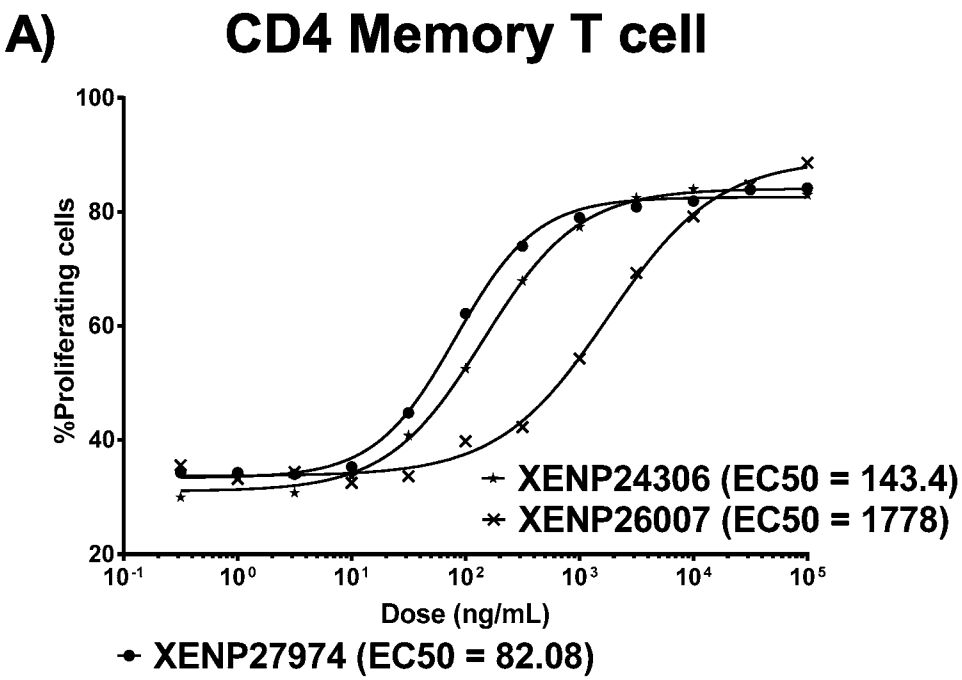

A)
CD8 Memory T cell
CD8 Memory counts
60000
50000
40000
30000
20000
10000
0
XENP24306
XENP26007
XENP27974
10-1
100
101
102
103
104
105
Dose (ng/mL)
B)
CD8 Naive T cell
CD8 Naive counts
40000
30000
20000
10000
0
XENP24306
XENP26007
XENP27974
10-1
100
101
102
103
104
105
Dose (ng/mL)

Figure 33


A)
CD4 Memory T cell
%Proliferating cells
Dose (ng/mL)
XENP24306 (EC50 = 143.4)
XENP26007 (EC50 = 1778)
XENP27974 (EC50 = 82.08)

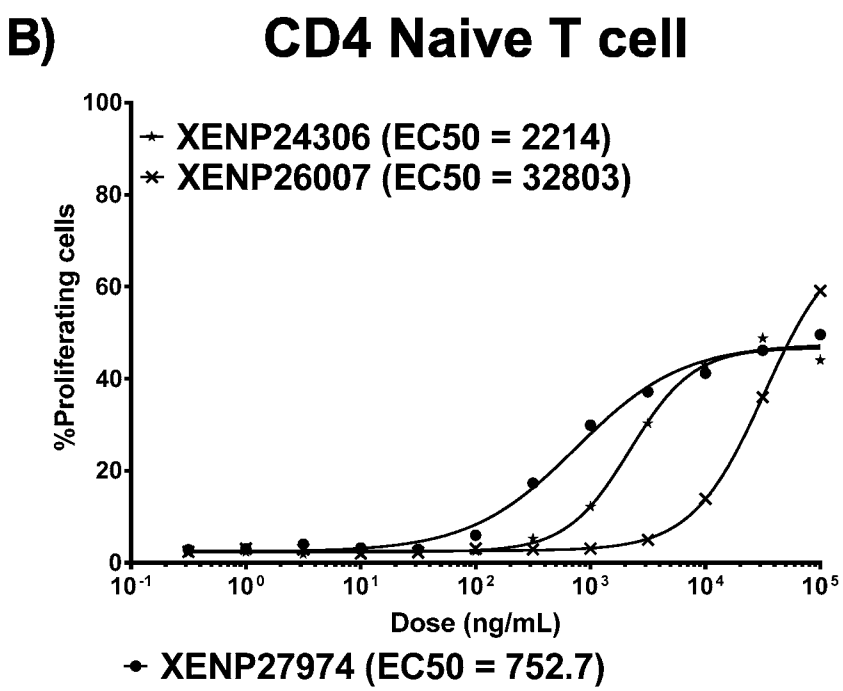

B)
CD4 Naive T cell
%Proliferating cells
Dose (ng/mL)
XENP24306 (EC50 = 2214)
XENP26007 (EC50 = 32803)
XENP27974 (EC50 = 752.7)

Figure 34

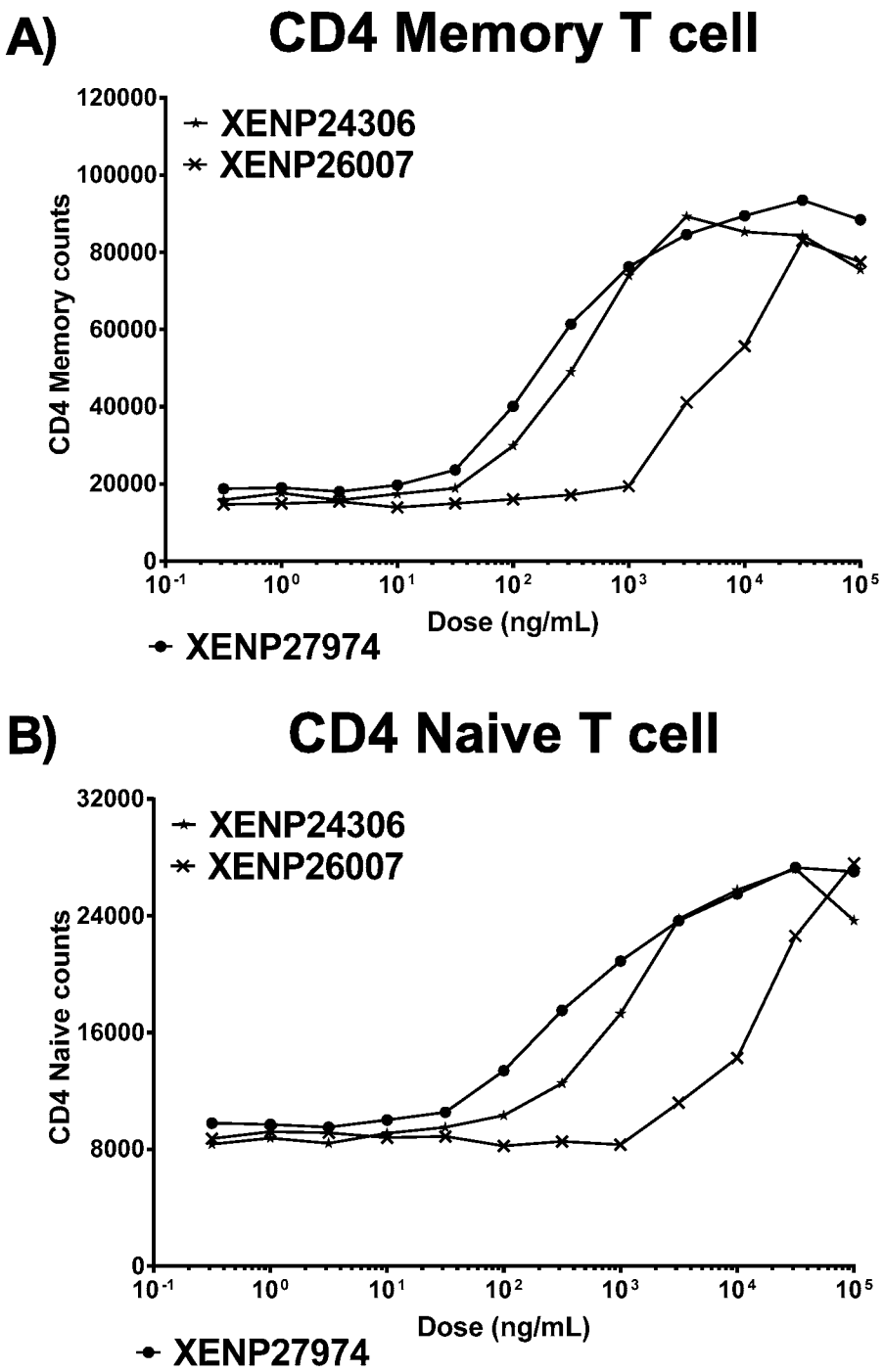
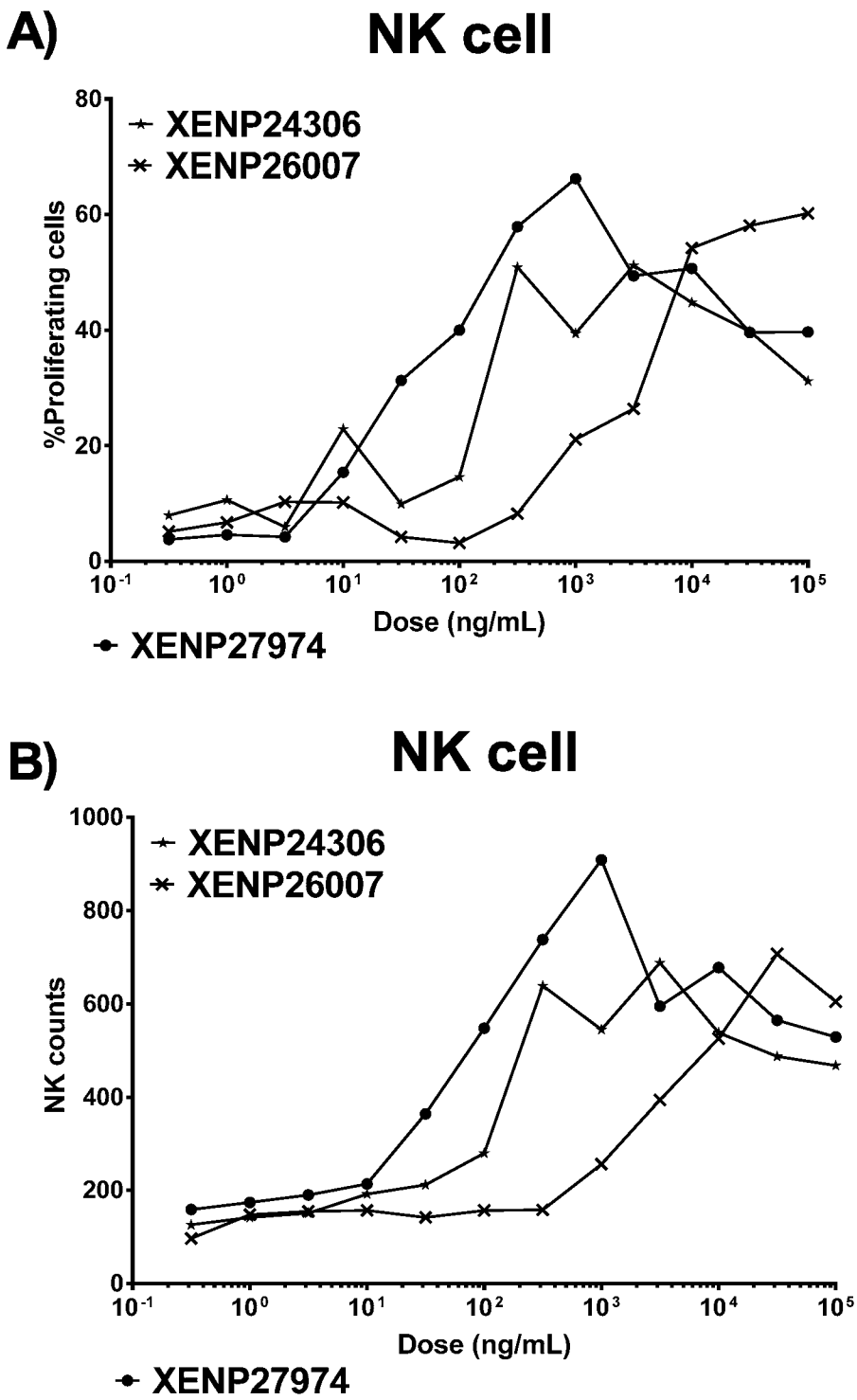

A)
CD4 Memory T cell
120000
100000
80000
60000
40000
20000
0
CD4 Memory counts
XENP24306
XENP26007
XENP27974
10-1
100
101
102
103
104
105
Dose (ng/mL)
B)
CD4 Naive T cell
32000
24000
16000
8000
0
CD4 Naive counts
XENP24306
XENP26007
XENP27974
Dose (ng/mL)

Figure 35


A)
NK cell
XENP24306
XENP26007
%Proliferating cells
Dose (ng/mL)
XENP27974
B)
NK cell
XENP24306
XENP26007
NK counts
Dose (ng/mL)
XENP27974

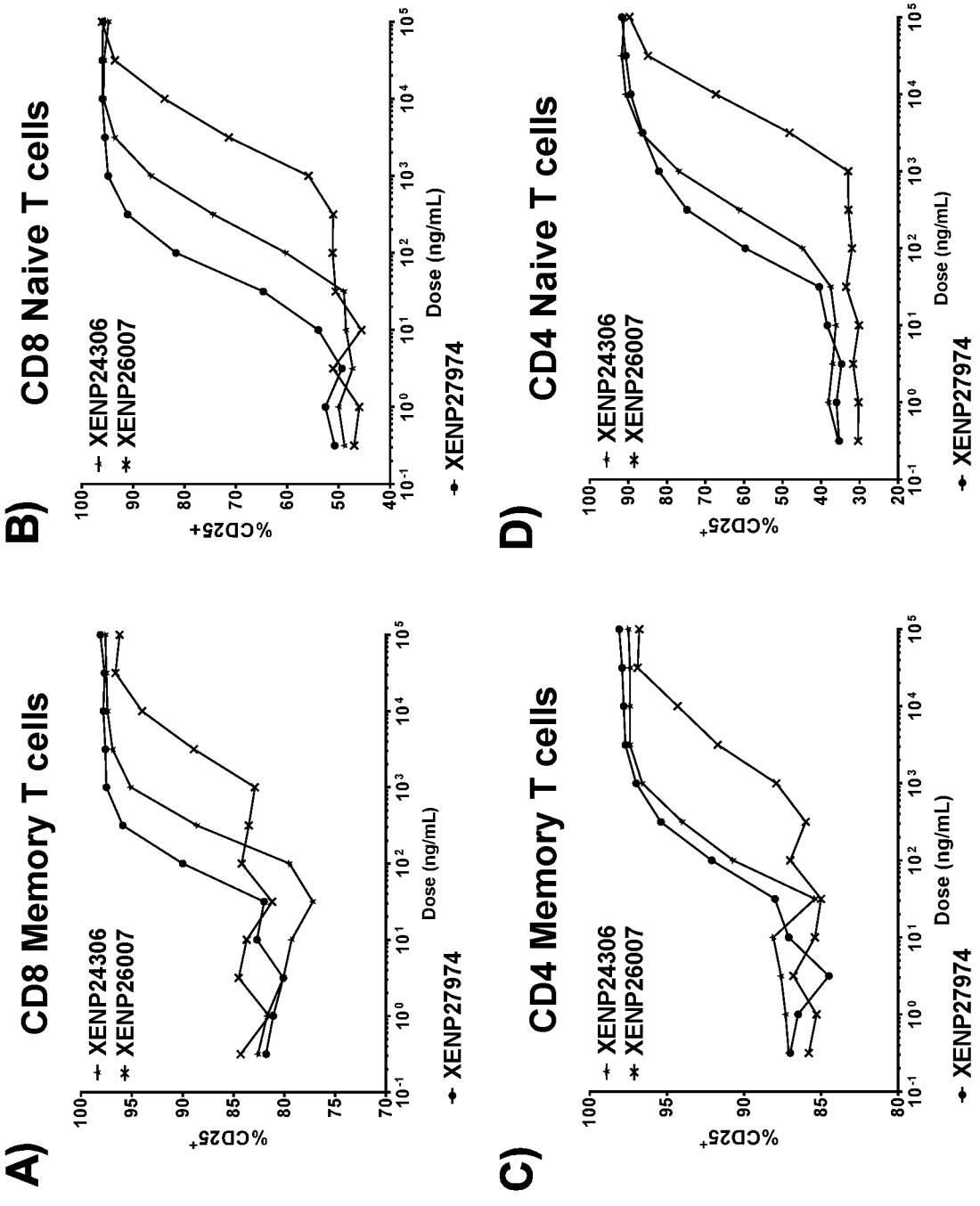

A)
CD8 Memory T cells
B)
CD8 Naive T cells
C)
CD4 Memory T cells
D)
CD4 Naive T cells
XENP24306
XENP26007
XENP27974
%CD25+
Dose (ng/mL)

Figure 36

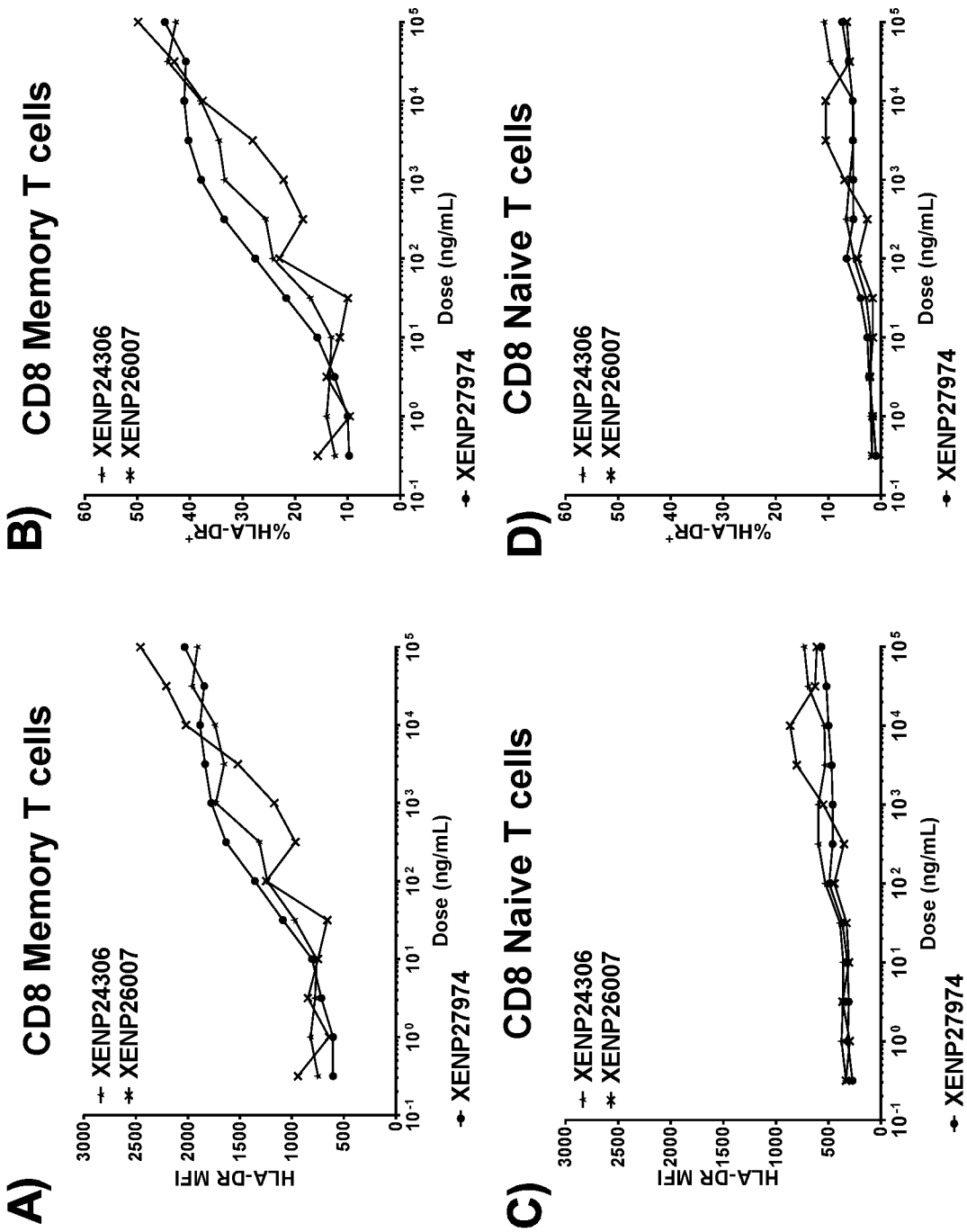

A)
CD8 Memory T cells
HLA-DR MFI
Dose (ng/mL)
XENP24306
XENP26007
XENP27974
B)
CD8 Memory T cells
%HLA-DR+
Dose (ng/mL)
XENP24306
XENP26007
XENP27974
C)
CD8 Naive T cells
HLA-DR MFI
Dose (ng/mL)
XENP24306
XENP26007
XENP27974
D)
CD8 Naive T cells
%HLA-DR+
Dose (ng/mL)
XENP24306
XENP26007
XENP27974

Figure 37

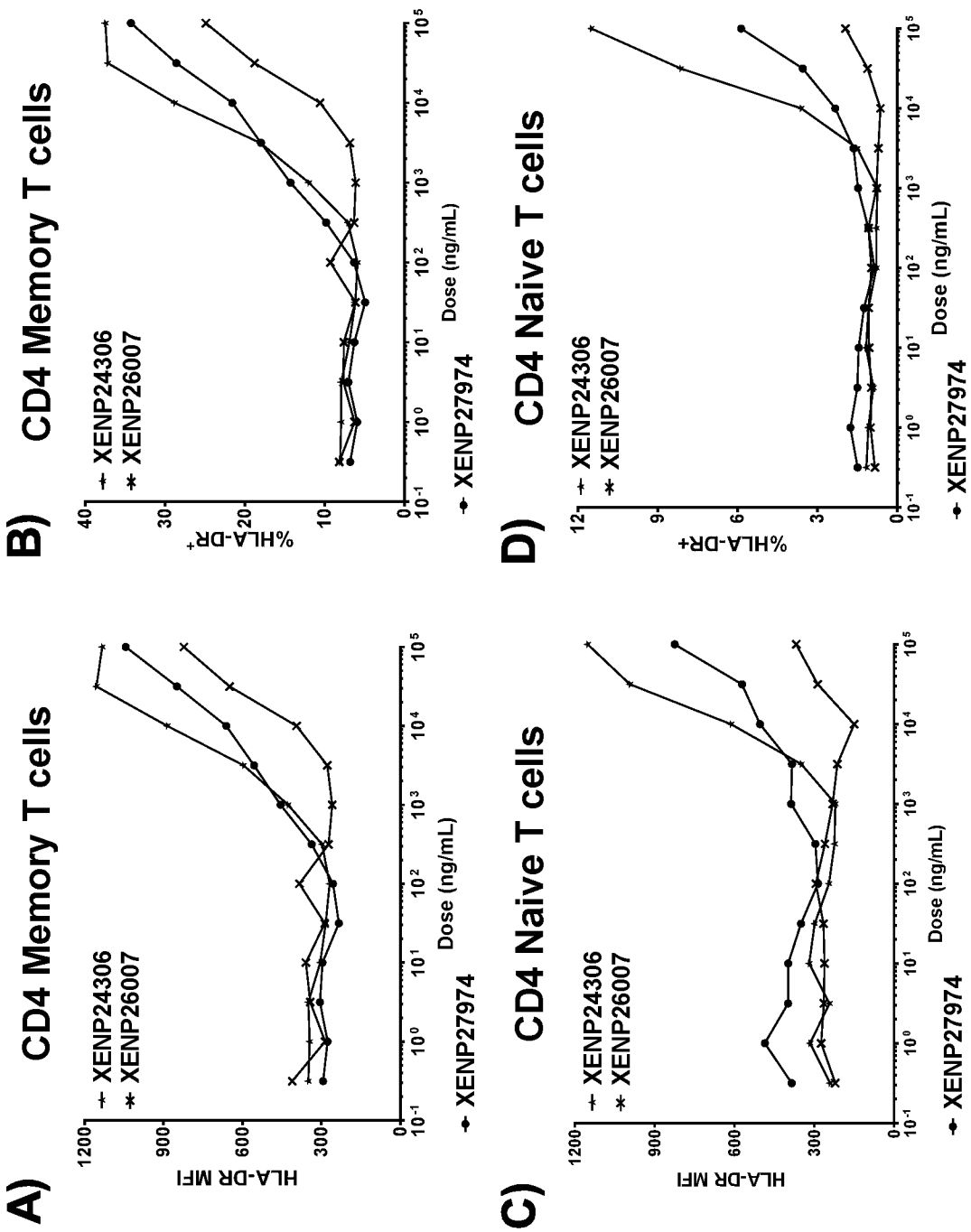

A)
CD4 Memory T cells
HLA-DR MFI
Dose (ng/mL)
XENP24306
XENP26007
XENP27974
B)
CD4 Memory T cells
%HLA-DR+
Dose (ng/mL)
XENP24306
XENP26007
XENP27974
C)
CD4 Naive T cells
HLA-DR MFI
Dose (ng/mL)
XENP24306
XENP26007
XENP27974
D)
CD4 Naive T cells
%HLA-DR+
Dose (ng/mL)
XENP24306
XENP26007
XENP27974

Figure 38

Figure 39

>XENP22853 human_IL15_(GGGGS)1-human_IL15Ra(Sushi)_(GGGGS)1_Fc(216)_IgG1_pI(-)_Isosteric_A_C220S/PVA_/S267K/L368D/K370S/M428L/N434S-Fc(216)_IgG1_C220S/PVA_/S267K/S364K/E357Q/M428L/N434S

XENP22853 Chain 1 - human_IL15_(GGGGS)1-Fc(216)_IgG1_pI(-)_Isosteric_A_C220S/PVA_/S267K/L368D/K370S/M428L/N434S (SEQ ID NO:XXX)

NWVNVISDLKKIEDLIQSMHIDATLYTESDVHPSCKVTAMKCFLLELQVISLESGDASIHDTVENLIILANNSLSSNGNVTESGCKECEELEEKNIKEFLQSFVHIVQMFINTS/GGGGS/EPKSSDKTHTCPPCPAPPVAGPSVFLFPPKPKDTLMISRTPEVTCVVVDVKHEDPEVKFNWYVDGVEVHNAKTKPREEEYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSREEMTKNQVSLTCDVSGFYPSDIAVEWESDGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWEQGDVFSCSVLHEALHSHYTQKSLSLSPGK

XENP22853 Chain 2 - human_IL15Ra(Sushi)_(GGGGS)1-Fc(216)_IgG1_C220S/PVA_/S267K/S364K/E357Q/M428L/N434S **(SEQ ID NO:XXX)**

ITCPPPMSVEHADIWVKSYSLYSRERYICNSGFKRKAGTSSLTECVLNKATNVAHWTTPSLKCIR/GGGGS/EPKSSDKTHTCPPCPAPPVAGPSVFLFPPKPKDTLMISRTPEVTCVVVDVKHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSREQMTKNQVKLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVLHEALHSHYTQKSLSLSPGK

Figure 40

>XENP24113 human_IL15_N4D/N65D_(GGGGS)1-human_IL15Ra(Sushi)_(GGGGS)1_Fc(216)_IgG1_pI(-)_Isosteric_A_C220S/PVA_/S267K/L368D/K370S/M428L/N434S-Fc(216)_IgG1_C220S/PVA_/S267K/S364K/E357Q/M428L/N434S

XENP24113 Chain 1 - human_IL15_N4D/N65D_(GGGGS)1-Fc(216)_IgG1_pI(-)_Isosteric_A_C220S/PVA_/S267K/L368D/K370S/M428L/N434S (SEQ ID NO:XXX)

NWVDVISDLKKIEDLIQSMHIDATLYTESDVHPSCKVTAMKCFLLELQVISLESGDASIHDTVEDLIILANNSLSSNGNVTESGCKECEELEEKNIKEFLQSFVHIVQMFINTS/GGGGS/EPKSSDKTHTCPPCPAPPVAGPSVFLFPPKPKDTLMISRTPEVTCVVVDVKHEDPEVKFNWYVDGVEVHNAKTKPREEEYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSREEMTKNQVSLTCDVSGFYPSDIAVEWESDGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWEQGDVFSCSVLHEALHSHYTQKSLSLSPGK

XENP24113 Chain 2 - human_IL15Ra(Sushi)_(GGGGS)1-Fc(216)_IgG1_C220S/PVA_/S267K/S364K/E357Q/M428L/N434S (SEQ ID NO:XXX)

ITCPPPMSVEHADIWVKSYSLYSRERYICNSGFKRKAGTSSLTECVLNKATNVAHWTTPSLKCIR/GGGGS/EPKSSDKTHTCPPCPAPPVAGPSVFLFPPKPKDTLMISRTPEVTCVVVDVKHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSREQMTKNQVKLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVLHEALHSHYTQKSLSLSPGK

Figure 41

>XENP24294 human_IL15Ra(sushi)_(GGGGS)5-human_IL15_N4D/N65D_(single-Chain)-empty-Fc_Fc(216)_IgG1_pI(-)_Isosteric_A_C220S/PVA_/S267K/L368D/K370S/M428L/N434S-Fc(216)_IgG1_C220S/PVA_/S267K/S364K/E357Q/M428L/N434S

XENP24294 Chain 1 - human_IL15Ra(sushi)_(GGGGS)5-human_IL15_N4D/N65D_(single-Chain)-Fc(216)_IgG1_pI(-)_Isosteric_A_C220S/PVA_/S267K/L368D/K370S/M428L/N434S (SEQ ID NO:XXX)

ITCPPPMSVEHADIWVKSYSLYSRERYICNSGFKRKAGTSSLTECVLNKATNVAHWTTPSLKCIR/GGGGSGGGGSGGGGSGGGGSGGGGS/NWVDVISDLKKIEDLIQSMHIDATLYTESDVHPSCKVTAMKCFLLELQVISLESGDASIHDTVEDLIILANNSLSSNGNVTESGCKECEELEEKNIKEFLQSFVHIVQMFINTS/EPKSSDKTHTCPPCPAPPVAGPSVFLFPPKPKDTLMISRTPEVTCVVVDVKHEDPEVKFNWYVDGVEVHNAKTKPREEEYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSREEMTKNQVSLTCDVSGFYPSDIAVEWESDGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWEQGDVFSCSVLHEALHSHYTQKSLSLSPGK

XENP24294 Chain 2 - empty-Fc(216)_IgG1_C220S/PVA_/S267K/S364K/E357Q/M428L/N434S (SEQ ID NO:XXX)

EPKSSDKTHTCPPCPAPPVAGPSVFLFPPKPKDTLMISRTPEVTCVVVDVKHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSREQMTKNQVKLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVLHEALHSHYTQKSLSLSPGK

Figure 42

>XENP24306 human_IL15_D30N/E64Q/N65D_(GGGGS)1-human_IL15Ra(Sushi)_(GGGGS)1_Fc(216)_IgG1_pI(-)_Isosteric_A_C220S/PVA_/S267K/L368D/K370S/M428L/N434S-Fc(216)_IgG1_C220S/PVA_/S267K/S364K/E357Q/M428L/N434S

XENP24306 Chain 1 - human_IL15_D30N/E64Q/N65D_(GGGGS)1-Fc(216)_IgG1_pI(-)_Isosteric_A_C220S/PVA_/S267K/L368D/K370S/M428L/N434S (SEQ ID NO:XXX)

NWVNVISDLKKIEDLIQSMHIDATLYTESNVHPSCKVTAMKCFLLELQVISLESGDASIHDTVQDLIILANNSLSSNGNVTESGCKECEELEEKNIKEFLQSFVHIVQMFINTS/GGGGS/EPKSSDKTHTCPPCPAPPVAGPSVFLFPPKPKDTLMISRTPEVTCVVVDVKHEDPEVKFNWYVDGVEVHNAKTKPREEEYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSREEMTKNQVSLTCDVSGFYPSDIAVEWESDGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWEQGDVFSCSVLHEALHSHYTQKSLSLSPGK

XENP24306 Chain 2 - human_IL15Ra(Sushi)_(GGGGS)1-Fc(216)_IgG1_C220S/PVA_/S267K/S364K/E357Q/M428L/N434S (SEQ ID NO:XXX)

ITCPPPMSVEHADIWVKSYSLYSRERYICNSGFKRKAGTSSLTECVLNKATNVAHWTTPSLKCIR/GGGGS/EPKSSDKTHTCPPCPAPPVAGPSVFLFPPKPKDTLMISRTPEVTCVVVDVKHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSREQMTKNQVKLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVLHEALHSHYTQKSLSLSPGK

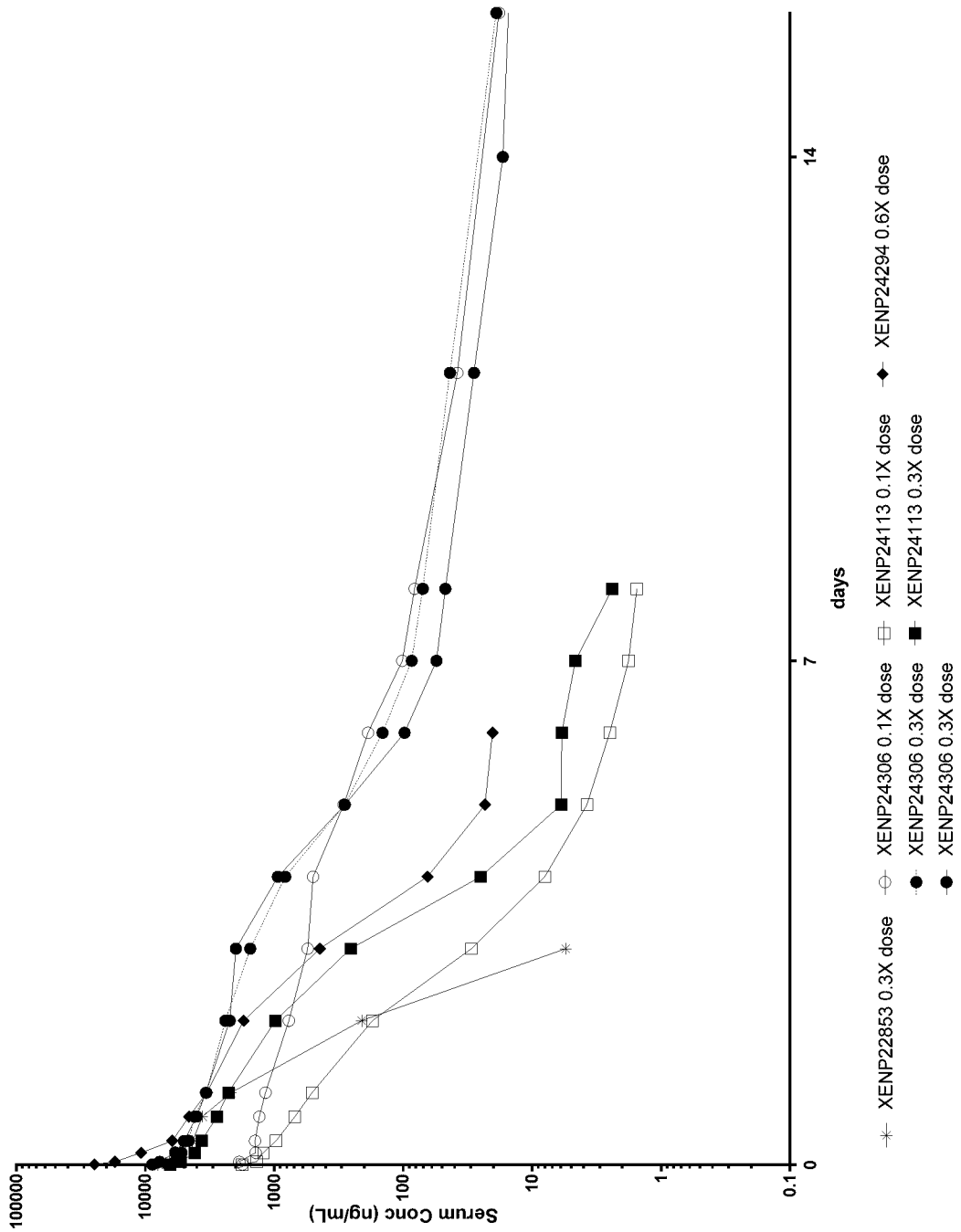

Serum Conc (ng/mL)
100000
10000
1000
100
10
1
0.1
0
7
14
days
XENP22853 0.3X dose
XENP24306 0.1X dose
XENP24306 0.3X dose
XENP24306 0.3X dose
XENP24113 0.1X dose
XENP24113 0.3X dose
XENP24294 0.6X dose

Figure 43

Figure 44A

>XENP21993 human_IL15Ra(sushi)_(GGGGS)5-human_IL15(single-chain)-empty-Fc_Fc(216)_IgG1_pI(-)_Isosteric_A_C220S/PVA_/S267K/L368D/K370S-Fc(216)_IgG1_C220S/PVA_/S267K/S364K/E357Q

Chain 1 - human_IL15Ra(sushi)_(GGGGS)5-human_IL15(single-chain)_Fc(216)_IgG1_pI(-)_Isosteric_A_C220S/PVA_/S267K/L368D/K370S (SEQ ID NO:XXX)
ITCPPPMSVEHADIWVKSYSLYSRERYICNSGFKRKAGTSSLTECVLNKATNVAHWTTPSLKCIR/GGGGSGGGGSGGGGSGGGGSGGGGS/NWVNVISDLKKIEDLIQSMHIDATLYTESDVHPSCKVTAMKCFLLELQVISLESGDASIHDTVENLIILANNSLSSNGNVTESGCKECEELEEKNIKEFLQSFVHIVQMFINTS/EPKSSDKTHTCPPCPAPPVAGPSVFLFPPKPKDTLMISRTPEVTCVVVDVKHEDPEVKFNWYVDGVEVHNAKTKPREEEYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSREEMTKNQVSLTCDVSGFYPSDIAVEWESDGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWEQGDVFSCSVMHEALHNHYTQKSLSLSPGK

Chain 2 - empty_Fc(216)_IgG1_C220S/PVA_/S267K/S364K/E357Q (SEQ ID NO:XXX)
EPKSSDKTHTCPPCPAPPVAGPSVFLFPPKPKDTLMISRTPEVTCVVVDVKHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSREQMTKNQVKLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGK

>XENP24050 human_IL15Ra(sushi)_(GGGGS)5-human_IL15_N4D/N65D_(single-chain)-empty-Fc_Fc(216)_IgG1_pI(-)_Isosteric_A_C220S/PVA_/S267K/L368D/K370S-Fc(216)_IgG1_C220S/PVA_/S267K/S364K/E357Q

Chain 1 - human_IL15Ra(sushi)_(GGGGS)5-human_IL15_N4D/N65D_(single-chain)_Fc(216)_IgG1_pI(-)_Isosteric_A_C220S/PVA_/S267K/L368D/K370S (SEQ ID NO:XXX)
ITCPPPMSVEHADIWVKSYSLYSRERYICNSGFKRKAGTSSLTECVLNKATNVAHWTTPSLKCIR/GGGGSGGGGSGGGGSGGGGSGGGGS/NWVDVISDLKKIEDLIQSMHIDATLYTESDVHPSCKVTAMKCFLLELQVISLESGDASIHDTVEDLIILANNSLSSNGNVTESGCKECEELEEKNIKEFLQSFVHIVQMFINTS/EPKSSDKTHTCPPCPAPPVAGPSVFLFPPKPKDTLMISRTPEVTCVVVDVKHEDPEVKFNWYVDGVEVHNAKTKPREEEYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSREEMTKNQVSLTCDVSGFYPSDIAVEWESDGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWEQGDVFSCSVMHEALHNHYTQKSLSLSPGK

Chain 2 - empty_Fc(216)_IgG1_C220S/PVA_/S267K/S364K/E357Q (SEQ ID NO:XXX)
EPKSSDKTHTCPPCPAPPVAGPSVFLFPPKPKDTLMISRTPEVTCVVVDVKHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSREQMTKNQVKLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGK

Figure 44B

>XENP29281 human_IL15Ra(sushi)_(GGGGS)5-human_IL15_D30N_(single-chain)-empty-Fc_Fc(216)_IgG1_pI(-)_Isosteric_A_C220S/PVA_/S267K/L368D/K370S-Fc(216)_IgG1_C220S/PVA_/S267K/S364K/E357Q

Chain 1 - human_IL15Ra(sushi)_(GGGGS)5-human_IL15_D30N_(single-chain)_Fc(216)_IgG1_pI(-)_Isosteric_A_C220S/PVA_/S267K/L368D/K370S (SEQ ID NO:XXX)
ITCPPPMSVEHADIWVKSYSLYSRERYICNSGFKRKAGTSSLTECVLNKATNVAHWTTPSLKCIR/GGGGSGGGGSGGGGSGGGGSGGGGS/NWVNVISDLKKIEDLIQSMHIDATLYTESNVHPSCKVTAMKCFLLELQVISLESGDASIHDTVENLIILANNSLSSNGNVTESGCKECEELEEKNIKEFLQSFVHIVQMFINTS/EPKSSDKTHTCPPCPAPPVAGPSVFLFPPKPKDTLMISRTPEVTCVVVDVKHEDPEVKFNWYVDGVEVHNAKTKPREEEYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSREEMTKNQVSLTCDVSGFYPSDIAVEWESDGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWEQGDVFSCSVMHEALHNHYTQKSLSLSPGK

Chain 2 - empty_Fc(216)_IgG1_C220S/PVA_/S267K/S364K/E357Q (SEQ ID NO:XXX)
EPKSSDKTHTCPPCPAPPVAGPSVFLFPPKPKDTLMISRTPEVTCVVVDVKHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSREQMTKNQVKLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGK

>XENP29285 human_IL15Ra(sushi)_(GGGGS)5-human_IL15_D30N/N65D_(single-chain)-empty-Fc_Fc(216)_IgG1_pI(-)_Isosteric_A_C220S/PVA_/S267K/L368D/K370S-Fc(216)_IgG1_C220S/PVA_/S267K/S364K/E357Q

Chain 1 - human_IL15Ra(sushi)_(GGGGS)5-human_IL15_D30N/N65D_(single-chain)_Fc(216)_IgG1_pI(-)_Isosteric_A_C220S/PVA_/S267K/L368D/K370S (SEQ ID NO:XXX)
ITCPPPMSVEHADIWVKSYSLYSRERYICNSGFKRKAGTSSLTECVLNKATNVAHWTTPSLKCIR/GGGGSGGGGSGGGGSGGGGSGGGGS/NWVNVISDLKKIEDLIQSMHIDATLYTESNVHPSCKVTAMKCFLLELQVISLESGDASIHDTVEDLIILANNSLSSNGNVTESGCKECEELEEKNIKEFLQSFVHIVQMFINTS/EPKSSDKTHTCPPCPAPPVAGPSVFLFPPKPKDTLMISRTPEVTCVVVDVKHEDPEVKFNWYVDGVEVHNAKTKPREEEYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSREEMTKNQVSLTCDVSGFYPSDIAVEWESDGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWEQGDVFSCSVMHEALHNHYTQKSLSLSPGK

Chain 2 - empty_Fc(216)_IgG1_C220S/PVA_/S267K/S364K/E357Q (SEQ ID NO:XXX)
EPKSSDKTHTCPPCPAPPVAGPSVFLFPPKPKDTLMISRTPEVTCVVVDVKHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSREQMTKNQVKLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGK

Figure 44C

>XENP29286 human_IL15Ra(sushi)_(GGGGS)5-human_IL15_D30N/E64Q/N65D_(single-chain)-empty-Fc_Fc(216)_IgG1_pI(-)_Isosteric_A_C220S/PVA_/S267K/L368D/K370S-Fc(216)_IgG1_C220S/PVA_/S267K/S364K/E357Q

Chain 1 - human_IL15Ra(sushi)_(GGGGS)5-human_IL15_D30N/E64Q/N65D_(single-chain)_Fc(216)_IgG1_pI(-)_Isosteric_A_C220S/PVA_/S267K/L368D/K370S (SEQ ID NO:XXX)
ITCPPPMSVEHADIWVKSYSLYSRERYICNSGFKRKAGTSSLTECVLNKATNVAHWTTPSLKCIR/GGGGSGGGGSGGGGSGGGGSGGGGS/NWVNVISDLKKIEDLIQSMHIDATLYTESNVHPSCKVTAMKCFLLELQVISLESGDASIHDTVQDLIILANNSLSSNGNVTESGCKECEELEEKNIKEFLQSFVHIVQMFINTS/EPKSSDKTHTCPPCPAPPVAGPSVFLFPPKPKDTLMISRTPEVTCVVVDVKHEDPEVKFNWYVDGVEVHNAKTKPREEEYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSREEMTKNQVSLTCDVSGFYPSDIAVEWESDGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWEQGDVFSCSVMHEALHNHYTQKSLSLSPGK

Chain 2 - empty_Fc(216)_IgG1_C220S/PVA_/S267K/S364K/E357Q (SEQ ID NO:XXX)
EPKSSDKTHTCPPCPAPPVAGPSVFLFPPKPKDTLMISRTPEVTCVVVDVKHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSREQMTKNQVKLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGK

Figure 45A

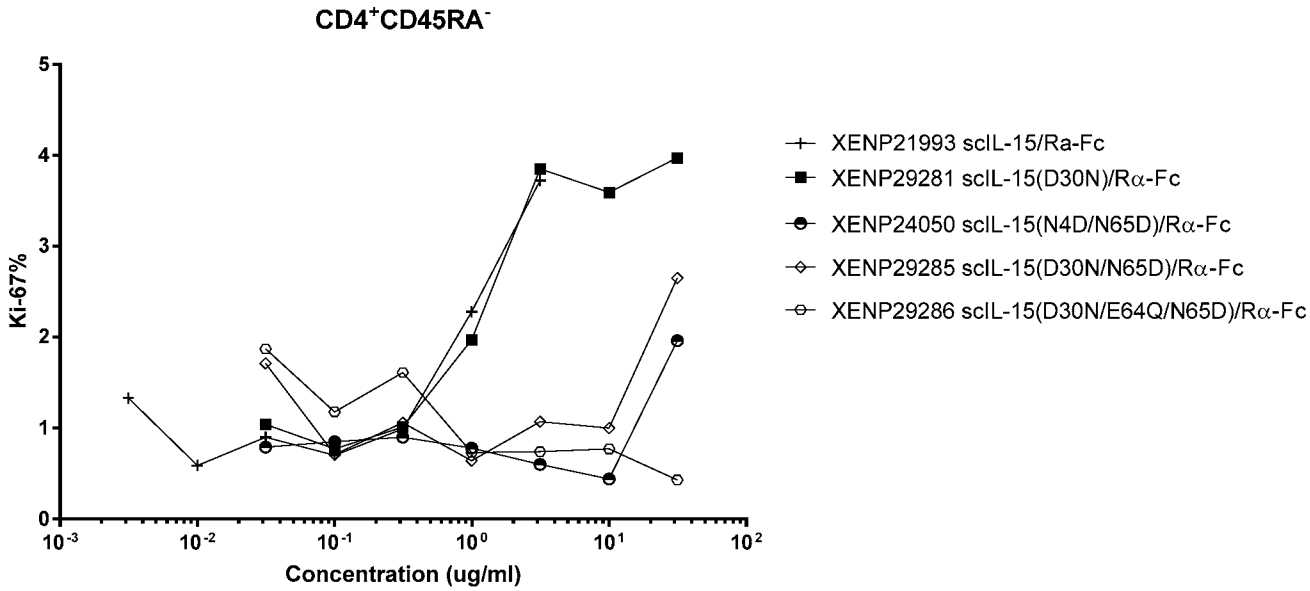


Figure 45B

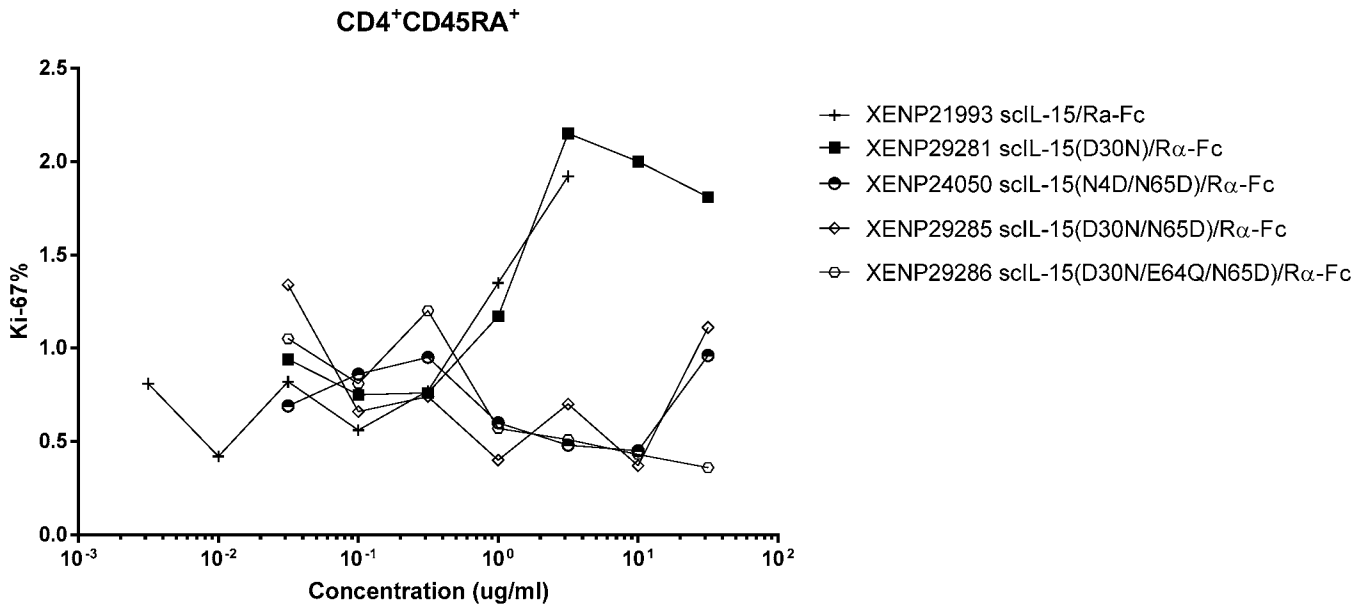


Figure 45C

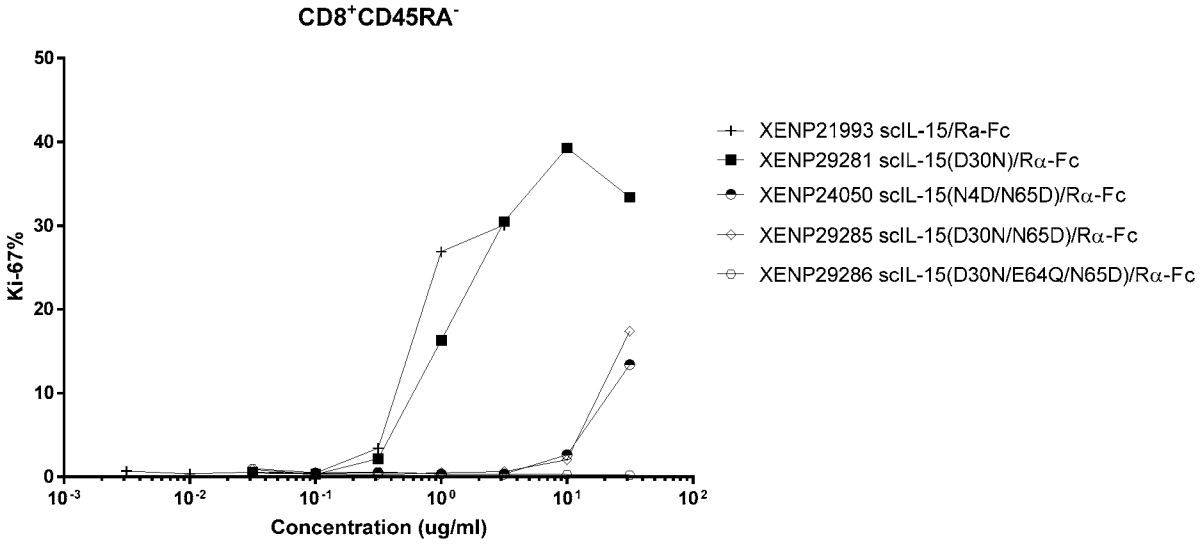


Figure 45D

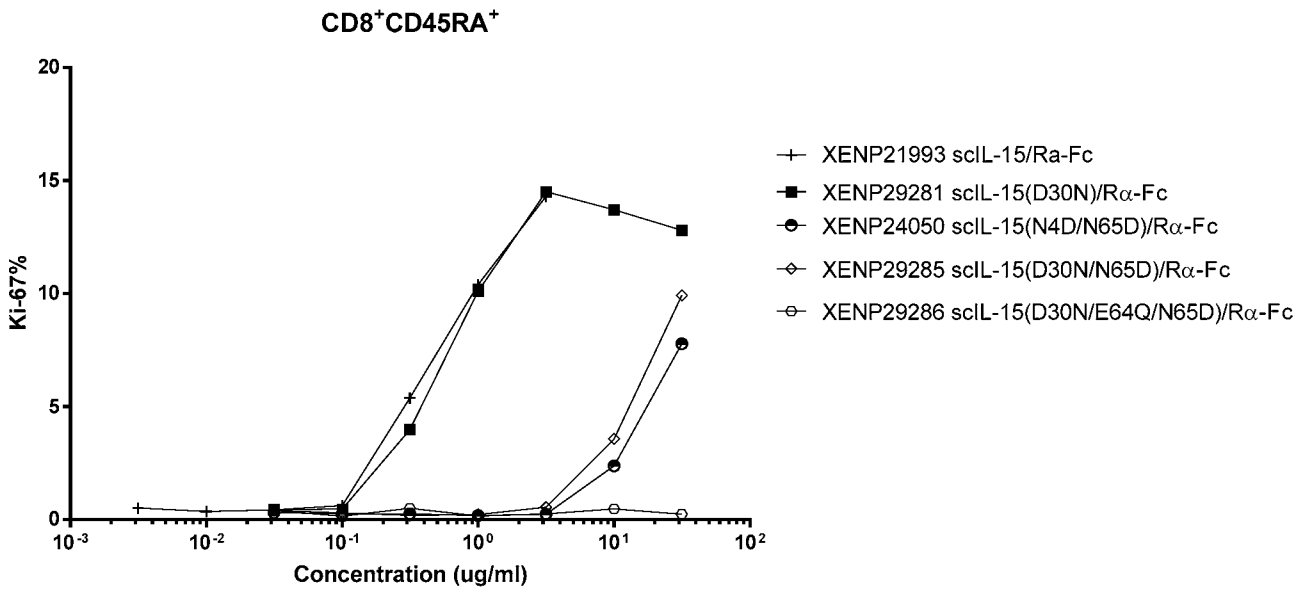


Figure 45E

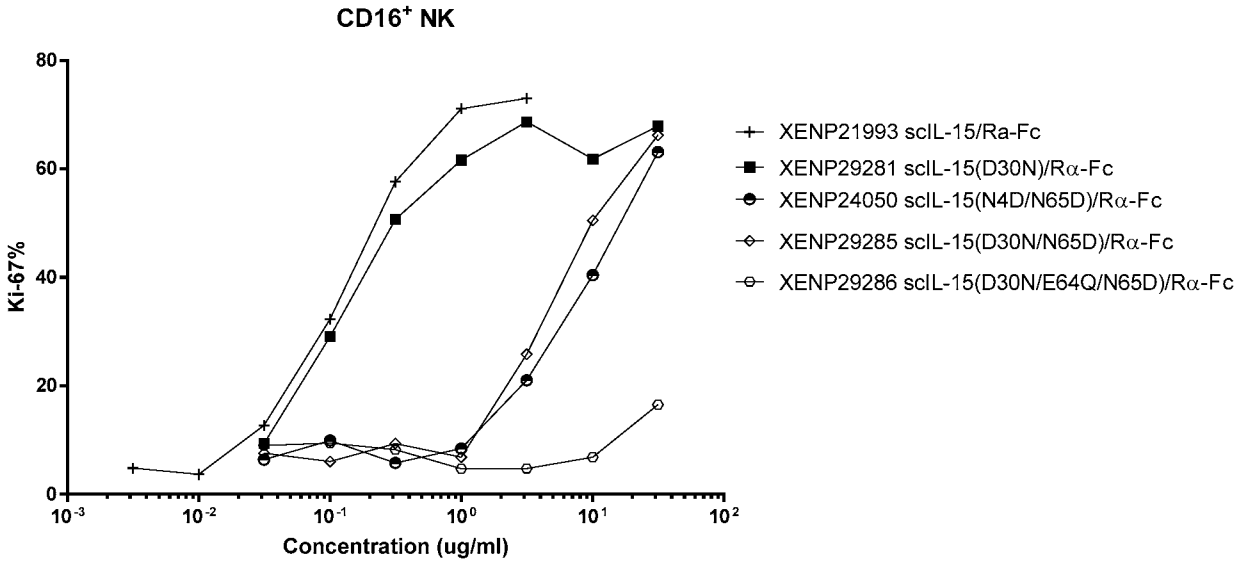
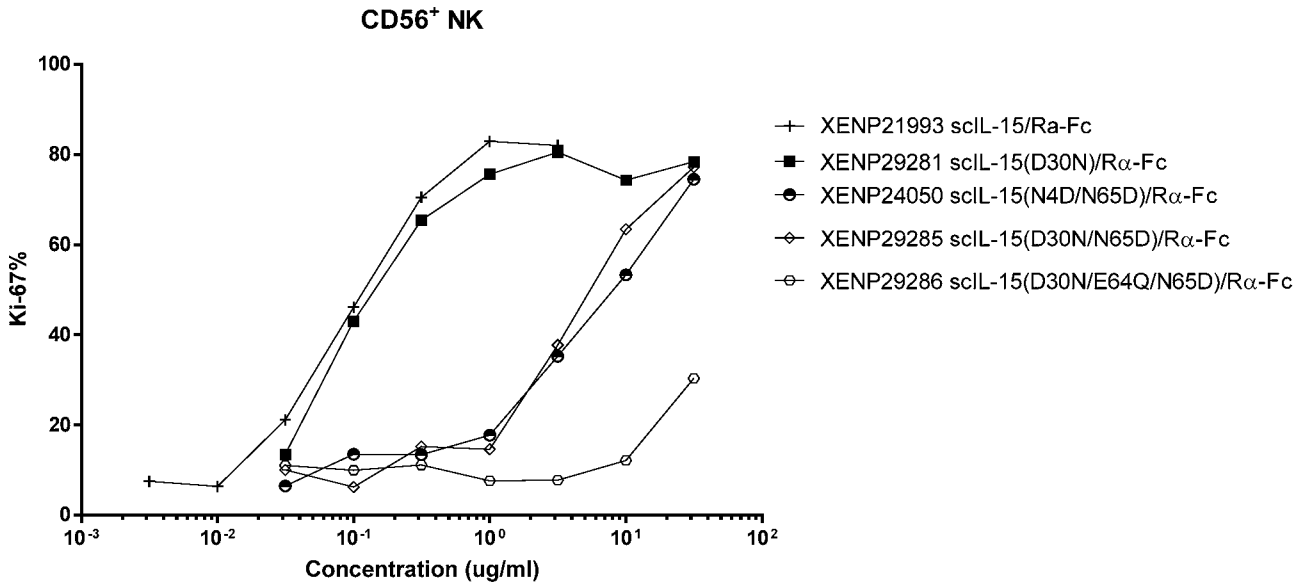


Figure 45F



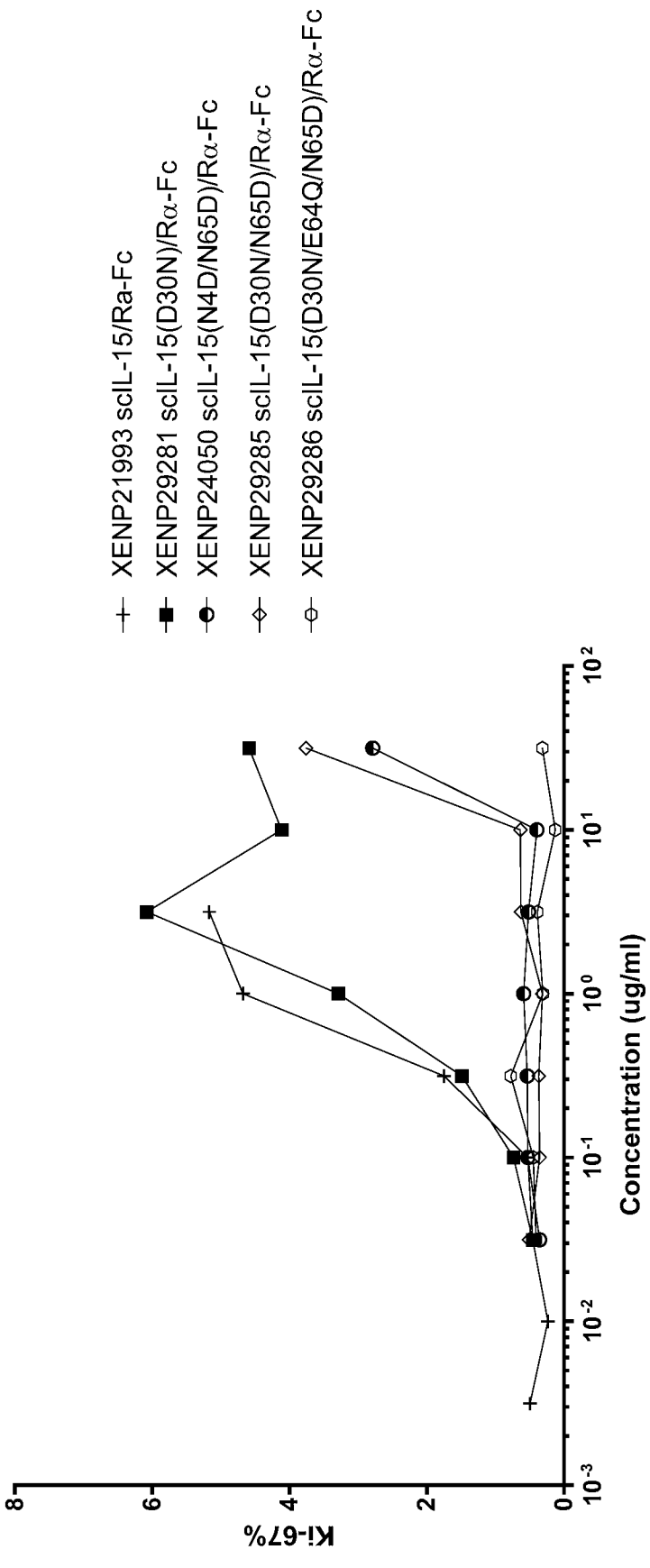

γδ
XENP21993 sclL-15/Ra-Fc
XENP29281 sclL-15(D30N)/Rα-Fc
XENP24050 sclL-15(N4D/N65D)/Rα-Fc
XENP29285 sclL-15(D30N/N65D)/Rα-Fc
XENP29286 sclL-15(D30N/E64Q/N65D)/Rα-Fc
Ki-67%
0
2
4
6
8
10-3
10-2
10-1
100
101
102
Concentration (ug/ml)

Figure 45G

Figure 46

> XENC1000 human_IL15Ra(sushi)_(GGGGS)5-human_IL15(D30N/N65D;single-Chain)-3H3[TIM-3]_H1_L2.1_Fab_IgG1_Fc(216)_IgG1_pI(-)_Isosteric_A_C220S/PVA_/S267K/L368D/K370S-IgG1_PVA_/S267K/S364K/E357Q

Chain 1 human_IL15Ra(sushi)_(GGGGS)5-human_IL15(D30N/N65D;single-Chain)_Fc(216)_IgG1_pI(-)_Isosteric_A_C220S/PVA_/S267K/L368D/K370S scIL-15/Ra-Fc Chain **(SEQ ID NO:XXX)**
ITCPPPMSVEHADIWVKSYSLYSRERYICNSGFKRKAGTSSLTECVLNKATNVAHWTTPSLKCIR/GGGGSGGGGSGGGGSGGGGSGGGGS/NWVNVISDLKKIEDLIQSMHIDATLYTESNVHPSCKVTAMKCFLLELQVISLESGDASIHDTVEDLII LANNSLSSNGNVTESGCKECEELEEKNIKEFLQSFVHIVQMFINTS/EPKSSDKTHTCPPCPAPPVAGPSVFLFPPKPKDTL MISRTPEVTCVVVDVKHEDPEVKFNWYVDGVEVHNAKTKPREEEYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKAL PAPIEKTISKAKGQPREPQVYTLPPSREEMTKNQVSLTCDVSGFYPSDIAVEWESDGQPENNYKTTPPVLDSDGSFFLYS KLTVDKSRWEQGDVFSCSVMHEALHNHYTQKSLSLSPGK

Chain 2 3H3[TIM-3]_H1_L2.1_Fab_IgG1_PVA_/S267K/S364K/E357Q Fab-Fc Heavy Chain **(SEQ ID NO:XXX)**
QVTLKESGPVLVKPTETLTLTCTVSGFSLN**GYGVN**WVRQPPGKGLEWLA**MIWGDGSTDYNSALKS**RLTISKDNSKSQV VLTMTNMDPVDTATYYCAR**SYYTSDEDY**WGQGTLVTVSS/ASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTV SWNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSSLGTQTYICNVNHKPSNTKVDKKVEPKSCDKTHTCPPCPAPPVAG PSVFLFPPKPKDTLMISRTPEVTCVVVDVKHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLN GKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSREQMTKNQVKLTCLVKGFYPSDIAVEWESNGQPENNYKTT PPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGK

Chain 3 3H3[TIM-3]_H1_L2.1_Fab Light Chain **(SEQ ID NO:XXX)**
DIVMTQSPDSLAVSLGERATINC**KSSQSLLNSRTRKNYLA**WYQQKPGQSPKLLIY**WASTRES**GVPDRFTGSGSGTDFTL TISSLQAEDVAVYYC**KQSYSLRT**FGGGTKVEIK/RTVAAPSVFIFPPSDEQLKSGTASVVCLLNNFYPREAKVQWKVDNA LQSGNSQESVTEQDSKDSTYSLSSTLTLSKADYEKHKVYACEVTHQGLSSPVTKSFNRGEC

Figure 47

> XENC1001 human_IL15Ra(sushi)_(GGGGS)5-human_IL15(D30N/E64Q/N65D;single-Chain)-3H3[TIM-3]_H1_L2.1_Fab_IgG1_Fc(216)_IgG1_pI(-)_Isosteric_A_C220S/PVA_/S267K/L368D/K370S-IgG1_PVA_/S267K/S364K/E357Q

Chain 1 human_IL15Ra(sushi)_(GGGGS)5-human_IL15(D30N/E64Q/N65D;single-Chain)_Fc(216)_IgG1_pI(-)_Isosteric_A_C220S/PVA_/S267K/L368D/K370S scIL-15/Ra-Fc Chain **(SEQ ID NO:XXX)**
ITCPPPMSVEHADIWVKSYSLYSRERYICNSGFKRKAGTSSLTECVLNKATNVAHWTTPSLKCIR/GGGGSGGGGSGGGGSGGGGSGGGGS/NWVNVISDLKKIEDLIQSMHIDATLYTESNVHPSCKVTAMKCFLLELQVISLESGDASIHDTVQDLII LANNSLSSNGNVTESGCKECEELEEKNIKEFLQSFVHIVQMFINTS/EPKSSDKTHTCPPCPAPPVAGPSVFLFPPKPKDTL MISRTPEVTCVVVDVKHEDPEVKFNWYVDGVEVHNAKTKPREEEYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKAL PAPIEKTISKAKGQPREPQVYTLPPSREEMTKNQVSLTCDVSGFYPSDIAVEWESDGQPENNYKTTPPVLDSDGSFFLYS KLTVDKSRWEQGDVFSCSVMHEALHNHYTQKSLSLSPGK

Chain 2 3H3[TIM-3]_H1_L2.1_Fab_IgG1_PVA_/S267K/S364K/E357Q Fab-Fc Heavy Chain **(SEQ ID NO:XXX)**
QVTLKESGPVLVKPTETLTLTCTVSGFSLN**GYGVN**WVRQPPGKGLEWLA**MIWGDGSTDYNSALKS**RLTISKDNSKSQV VLTMTNMDPVDTATYYCAR**SYYTSDEDY**WGQGTLVTVSS/ASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTV SWNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSSLGTQTYICNVNHKPSNTKVDKKVEPKSCDKTHTCPPCPAPPVAG PSVFLFPPKPKDTLMISRTPEVTCVVVDVKHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLN GKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSREQMTKNQVKLTCLVKGFYPSDIAVEWESNGQPENNYKTT PPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGK

Chain 3 3H3[TIM-3]_H1_L2.1_Fab Light Chain **(SEQ ID NO:XXX)**
DIVMTQSPDSLAVSLGERATINC**KSSQSLLNSRTRKNYLA**WYQQKPGQSPKLLIY**WASTRES**GVPDRFTGSGSGTDFTL TISSLQAEDVAVYYC**KQSYSLRT**FGGGTKVEIK/RTVAAPSVFIFPPSDEQLKSGTASVVCLLNNFYPREAKVQWKVDNA LQSGNSQESVTEQDSKDSTYSLSSTLTLSKADYEKHKVYACEVTHQGLSSPVTKSFNRGEC

Figure 48A

> XENC1002 human_IL15Ra(sushi)_(GGGGS)5-human_IL15(D30N/N65D;single-Chain)-3H3[TIM-3]_H1_L2.1_Fab_IgG1_Fc(216)_IgG1_pI(-)_Isosteric_A_C220S/PVA_/S267K/L368D/K370S/M428L/N434S-IgG1_PVA_/S267K/S364K/E357Q/M428L/N434S

Chain 1 human_IL15Ra(sushi)_(GGGGS)5-human_IL15(D30N/N65D;single-Chain **(SEQ ID NO:XXX)** *)_Fc(216)_IgG1_pI(-)_Isosteric_A_C220S/PVA_/S267K/L368D/K370S/M428L/N434S scIL-15/Ra-Fc Chain*
ITCPPPMSVEHADIWVKSYSLYSRERYICNSGFKRKAGTSSLTECVLNKATNVAHWTTPSLKCIR/GGGGSGGGGSGGGGSGGGGSGGGGS/NWVNVISDLKKIEDLIQSMHIDATLYTESNVHPSCKVTAMKCFLLELQVISLESGDASIHDTVEDLILANNSLSSNGNVTESGCKECEELEEKNIKEFLQSFVHIVQMFINTS/EPKSSDKTHTCPPCPAPPVAGPSVFLFPPKPKDTLMISRTPEVTCVVVDVKHEDPEVKFNWYVDGVEVHNAKTKPREEEYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSREEMTKNQVSLTCDVSGFYPSDIAVEWESDGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWEQGDVFSCSVLHEALHSHYTQKSLSLSPGK

Chain 2 3H3[TIM-3]_H1_L2.1_Fab_IgG1_PVA_/S267K/S364K/E357Q/M428L/N434S Fab-Fc Heavy Chain

(SEQ ID NO:XXX)
QVTLKESGPVLVKPTETLTLTCTVSGFSLN**GYGVN**WVRQPPGKGLEWLA**MIWGDGSTDYNSALKS**RLTISKDNSKSQVVLTMTNMDPVDTATYYCAR**SYYTSDEDY**WGQGTLVTVSS/ASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSSLGTQTYICNVNHKPSNTKVDKKVEPKSCDKTHTCPPCPAPPVAGPSVFLFPPKPKDTLMISRTPEVTCVVVDVKHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSREQMTKNQVKLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVLHEALHSHYTQKSLSLSPGK

Chain 3 3H3[TIM-3]_H1_L2.1_Fab Light Chain **(SEQ ID NO:XXX)**
DIVMTQSPDSLAVSLGERATINC**KSSQSLLNSRTRKNYLA**WYQQKPGQSPKLLIY**WASTRES**GVPDRFTGSGSGTDFTLTISSLQAEDVAVYYC**KQSYSLRT**FGGGTKVEIK/RTVAAPSVFIFPPSDEQLKSGTASVVCLLNNFYPREAKVQWKVDNALQSGNSQESVTEQDSKDSTYSLSSTLTLSKADYEKHKVYACEVTHQGLSSPVTKSFNRGEC

Figure 48B

> XENC1003 human_IL15Ra(sushi)_(GGGGS)5-human_IL15(D30N/E64Q/N65D;single-Chain)-3H3[TIM-3]_H1_L2.1_Fab_IgG1_Fc(216)_IgG1_pI(-)_Isosteric_A_C220S/PVA_/S267K/L368D/K370S/M428L/N434S-IgG1_PVA_/S267K/S364K/E357Q/M428L/N434S

Chain 1 human_IL15Ra(sushi)_(GGGGS)5-human_IL15(D30N/E64Q/N65D;single-Chain)_Fc(216)_IgG1_pI(-)_Isosteric_A_C220S/PVA_/S267K/L368D/K370S/M428L/N434S scIL-15/Ra-Fc Chain **(SEQ ID NO:XXX)**

ITCPPPMSVEHADIWVKSYSLYSRERYICNSGFKRKAGTSSLTECVLNKATNVAHWTTPSLKCIR/GGGGSGGGGSGGGGSGGGGSGGGGS/NWVNVISDLKKIEDLIQSMHIDATLYTESNVHPSCKVTAMKCFLLELQVISLESGDASIHDTVQDLIILANNSLSSNGNVTESGCKECEELEEKNIKEFLQSFVHIVQMFINTS/EPKSSDKTHTCPPCPAPPVAGPSVFLFPPKPKDTLMISRTPEVTCVVVDVKHEDPEVKFNWYVDGVEVHNAKTKPREEEYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSREEMTKNQVSLTCDVSGFYPSDIAVEWESDGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWEQGDVFSCSVLHEALHSHYTQKSLSLSPGK

Chain 2 3H3[TIM-3]_H1_L2.1_Fab_IgG1_PVA_/S267K/S364K/E357Q/M428L/N434S Fab-Fc Heavy Chain

(SEQ ID NO:XXX)

QVTLKESGPVLVKPTETLTLTCTVSGFSLN**GYGVN**WVRQPPGKGLEWLA**MIWGDGSTDYNSALKS**RLTISKDNSKSQVVLTMTNMDPVDTATYYCAR**SYYTSDEDY**WGQGTLVTVSS/ASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSSLGTQTYICNVNHKPSNTKVDKKVEPKSCDKTHTCPPCPAPPVAGPSVFLFPPKPKDTLMISRTPEVTCVVVDVKHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSREQMTKNQVKLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVLHEALHSHYTQKSLSLSPGK

Chain 3 3H3[TIM-3]_H1_L2.1_Fab Light Chain **(SEQ ID NO:XXX)**

DIVMTQSPDSLAVSLGERATINC**KSSQSLLNSRTRKNYLA**WYQQKPGQSPKLLIY**WASTRES**GVPDRFTGSGSGTDFTLTISSLQAEDVAVYYC**KQSYSLRT**FGGGTKVEIK/RTVAAPSVFIFPPSDEQLKSGTASVVCLLNNFYPREAKVQWKVDNALQSGNSQESVTEQDSKDSTYSLSSTLTLSKADYEKHKVYACEVTHQGLSSPVTKSFNRGEC

Figure 49A

>XENP26007 human_IL15Ra(sushi)_(GGGGS)5-human_IL15(N4D/N65D;single-Chain)-Numax_IgG1_Fc(216)_IgG1_pI(-)_Isosteric_A_C220S/PVA_/S267K/L368D/K370S-IgG1_PVA_/S267K/S364K/E357Q

Chain 1 - human_IL15Ra(sushi)_(GGGGS)5-human_IL15(N4D/N65D;single-Chain)_Fc(216)_IgG1_pI(-)_Isosteric_A_C220S/PVA_/S267K/L368D/K370S scIL-15/Rα-Fc Chain (SEQ ID NO:XXX)

ITCPPPMSVEHADIWVKSYSLYSRERYICNSGFKRKAGTSSLTECVLNKATNVAHWTTPSLKCIR/GGGGSGGGGSGGGGSGGGGSGGGGS/NWVDVISDLKKIEDLIQSMHIDATLYTESDVHPSCKVTAMKCFLLELQVISLESGDASIHDTVEDLII LANNSLSSNGNVTESGCKECEELEEKNIKEFLQSFVHIVQMFINTS/EPKSSDKTHTCPPCPAPPVAGPSVFLFPPKPKDTL MISRTPEVTCVVVDVKHEDPEVKFNWYVDGVEVHNAKTKPREEEYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKAL PAPIEKTISKAKGQPREPQVYTLPPSREEMTKNQVSLTCDVSGFYPSDIAVEWESDGQPENNYKTTPPVLDSDGSFFLYS KLTVDKSRWEQGDVFSCSVMHEALHNHYTQKSLSLSPGK

Chain 2 - Numax_IgG1_PVA_/S267K/S364K/E357Q Heavy Chain (SEQ ID NO:XXX)

QVTLRESGPALVKPTQTLTLTCTFSGFSLSTAGMSVGWIRQPPGKALEWLADIWWDDKKHYNPSLKDRLTISKDTSKN QVVLKVTNMDPADTATYYCARDMIFNFYFDVWGQGTTVTVSS/ASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPE PVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSSLGTQTYICNVNHKPSNTKVDKKVEPKSCDKTHTCPPCPAP PVAGPSVFLFPPKPKDTLMISRTPEVTCVVVDVKHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQ DWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSREQMTKNQVKLTCLVKGFYPSDIAVEWESNGQPEN NYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGK

Chain 3 - Numax Light Chain (SEQ ID NO:XXX)

DIQMTQSPSTLSASVGDRVTITCSASSRVGYMHWYQQKPGKAPKLLIYDTSKLASGVPSRFSGSGSGTEFTLTISSLQPD DFATYYCFQGSGYPFTFGGGTKVEIK/RTVAAPSVFIFPPSDEQLKSGTASVVCLLNNFYPREAKVQWKVDNALQSGNS QESVTEQDSKDSTYSLSSTLTLSKADYEKHKVYACEVTHQGLSSPVTKSFNRGEC

Figure 49B

>XENP29481 human_IL15Ra(sushi)_(GGGGS)5-human_IL15(D30N/N65D;single-chain)-Numax_IgG1_Fc(216)_IgG1_pI(-)_Isosteric_A_C220S/PVA_/S267K/L368D/K370S-IgG1_PVA_/S267K/S364K/E357Q

Chain 1 - human_IL15Ra(sushi)_(GGGGS)5-human_IL15(D30N/N65D;single-chain)_Fc(216)_IgG1_pI(-)_Isosteric_A_C220S/PVA_/S267K/L368D/K370S (SEQ ID NO:XXX)

ITCPPPMSVEHADIWVKSYSLYSRERYICNSGFKRKAGTSSLTECVLNKATNVAHWTTPSLKCIR/GGGGSGGGGSGGGGSGGGGSGGGGS/NWVNVISDLKKIEDLIQSMHIDATLYTESNVHPSCKVTAMKCFLLELQVISLESGDASIHDTVEDLII LANNSLSSNGNVTESGCKECEELEEKNIKEFLQSFVHIVQMFINTS/EPKSSDKTHTCPPCPAPPVAGPSVFLFPPKPKDTL MISRTPEVTCVVVDVKHEDPEVKFNWYVDGVEVHNAKTKPREEEYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKAL PAPIEKTISKAKGQPREPQVYTLPPSREEMTKNQVSLTCDVSGFYPSDIAVEWESDGQPENNYKTTPPVLDSDGSFFLYS KLTVDKSRWEQGDVFSCSVMHEALHNHYTQKSLSLSPGK

Chain 2 - Numax_IgG1_PVA_/S267K/S364K/E357Q (SEQ ID NO:XXX)

QVTLRESGPALVKPTQTLTLTCTFSGFSLSTAGMSVGWIRQPPGKALEWLADIWWDDKKHYNPSLKDRLTISKDTSKN QVVLKVTNMDPADTATYYCARDMIFNFYFDVWGQGTTVTVSS/ASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPE PVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSSLGTQTYICNVNHKPSNTKVDKKVEPKSCDKTHTCPPCPAP PVAGPSVFLFPPKPKDTLMISRTPEVTCVVVDVKHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQ DWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSREQMTKNQVKLTCLVKGFYPSDIAVEWESNGQPEN NYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGK

Chain 3 - Numax LC (SEQ ID NO:XXX)

DIQMTQSPSTLSASVGDRVTITCSASSRVGYMHWYQQKPGKAPKLLIYDTSKLASGVPSRFSGSGSGTEFTLTISSLQPD DFATYYCFQGSGYPFTFGGGTKVEIK/RTVAAPSVFIFPPSDEQLKSGTASVVCLLNNFYPREAKVQWKVDNALQSGNS QESVTEQDSKDSTYSLSSTLTLSKADYEKHKVYACEVTHQGLSSPVTKSFNRGEC

Figure 49C

>XENP30432 human_IL15Ra(sushi)_(GGGGS)5-human_IL15(D30N/E64Q/N65D;single-chain)-Numax_IgG1_Fc(216)_IgG1_pI(-)_Isosteric_A_C220S/PVA_/S267K/L368D/K370S-IgG1_PVA_/S267K/S364K/E357Q

Chain 1 - human_IL15Ra(sushi)_(GGGGS)5-human_IL15(D30N/E64Q/N65D;single-chain)_Fc(216)_IgG1_pI(-)_Isosteric_A_C220S/PVA_/S267K/L368D/K370S (SEQ ID NO:XXX)

ITCPPPMSVEHADIWVKSYSLYSRERYICNSGFKRKAGTSSLTECVLNKATNVAHWTTPSLKCIR/GGGGSGGGGSGGGGSGGGGSGGGGS/NWVNVISDLKKIEDLIQSMHIDATLYTESNVHPSCKVTAMKCFLLELQVISLESGDASIHDTVQDLII LANNSLSSNGNVTESGCKECEELEEKNIKEFLQSFVHIVQMFINTS/EPKSSDKTHTCPPCPAPPVAGPSVFLFPPKPKDTL MISRTPEVTCVVVDVKHEDPEVKFNWYVDGVEVHNAKTKPREEEYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKAL PAPIEKTISKAKGQPREPQVYTLPPSREEMTKNQVSLTCDVSGFYPSDIAVEWESDGQPENNYKTTPPVLDSDGSFFLYS KLTVDKSRWEQGDVFSCSVMHEALHNHYTQKSLSLSPGK

Chain 2 - Numax_VH_IgG1_IgG1_PVA_/S267K/S364K/E357Q (SEQ ID NO:XXX)

QVTLRESGPALVKPTQTLTLTCTFSGFSLSTAGMSVGWIRQPPGKALEWLADIWWDDKKHYNPSLKDRLTISKDTSKN QVVLKVTNMDPADTATYYCARDMIFNFYFDVWGQGTTVTVSS/ASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPE PVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSSLGTQTYICNVNHKPSNTKVDKKVEPKSCDKTHTCPPCPAP PVAGPSVFLFPPKPKDTLMISRTPEVTCVVVDVKHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQ DWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSREQMTKNQVKLTCLVKGFYPSDIAVEWESNGQPEN NYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGK

Chain 3 - Numax LC (SEQ ID NO:XXX)

DIQMTQSPSTLSASVGDRVTITCSASSRVGYMHWYQQKPGKAPKLLIYDTSKLASGVPSRFSGSGSGTEFTLTISSLQPD DFATYYCFQGSGYPFTFGGGTKVEIK/RTVAAPSVFIFPPSDEQLKSGTASVVCLLNNFYPREAKVQWKVDNALQSGNS QESVTEQDSKDSTYSLSSTLTLSKADYEKHKVYACEVTHQGLSSPVTKSFNRGEC

INTERNATIONAL SEARCH REPORT

International application No
PCT/US2019/028192

A. CLASSIFICATION OF SUBJECT MATTER
INV. C07K16/28 A61K38/17 A61K38/20 C07K14/54 C07K14/715 A61P35/00
ADD.
According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
C07K A61K A61P

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)
EPO-Internal, EMBASE, BIOSIS, Sequence Search, WPI Data

C. DOCUMENTS CONSIDERED TO BE RELEVANT

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Y	WO 2015/018528 A1 (CYTUNE PHARMA [FR]; UNIV PARIS DESCARTES [FR] ET AL.) 12 February 2015 (2015-02-12) claims 1,3	1-13
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[X] Further documents are listed in the continuation of Box C.

[X] See patent family annex.

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Date of the actual completion of the international search	Date of mailing of the international search report
16 July 2019	05/08/2019

Name and mailing address of the ISA/	Authorized officer
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C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

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