

BISPECIFIC FC FUSION PROTEINS WITH sPD-1 AND IL-15

0. SEQUENCE LISTING

[0000] This instant application contains sequence listing which has been submitted in ASCII format and is hereby incorporated by reference in its entirety. Said ASCII copy, created on July 15, 2022, is named 121076-5005-WO_SL.txt and is 373,978 bytes in size.

I. FIELD

[0001] This invention relates to the field of biomolecules (e.g., cytokine and/or immune checkpoint receptors) induced or stimulated biological responses, more particularly to the field of IL-15 and/or PD-1 modulated biological responses. Specifically, this invention relates to bispecific Fc fusion proteins comprising an IL-15 domain, an Interleukin-15 receptor alpha (IL-15R α) sushi domain, an Fc domain and a soluble PD-1 (sPD-1) variant domain, polynucleotides encoding the bispecific Fc fusion proteins, methods of making the bispecific Fc fusion proteins, and methods of using the bispecific Fc fusion proteins, for example, in preventing and/or treating diseases (e.g., cancers, infections, etc.), modulating immune functions, promoting T cell and/or Natural Killer (NK) cell cytotoxicity, etc.

II. BACKGROUND

[0002] The cytokine, interleukin-15 (IL-15), is a member of the four alpha-helix bundle family of lymphokines. IL-15 plays an important role in modulating the activity of both the innate and adaptive immune system, e.g., maintenance of the memory T-cell response to invading pathogens, inhibition of apoptosis, activation of dendritic cells, and induction of NK cell proliferation and cytotoxic activity (US10899821B2, hereby entirely incorporated by reference).

[0003] The IL-15 receptor consists of the IL-2/IL-15R β and γ c subunits in association with a unique ligand-specific subunit, IL-15R α , which is homologous to IL-2R α . These receptor proteins contain protein-binding motifs termed “sushi domains.” In both humans and mice, these receptors and their cognate ligands are physically linked in the genome (Clinical Immunology (Fifth Edition) Principles and Practice 2019, Chapter 9: J.O’Shea, Massimo Gadina, Richard M. Siegel. Cytokines and Cytokine Receptors. John p. 127-155, hereby entirely incorporated by reference).

[0004] IL-15 binds to the IL-15R α , forming cell-surface complexes. IL-15 specifically binds to the IL-15R α with high affinity via the “sushi domain” in exon 2 of the extracellular domain of the receptor. After trans-endosomal recycling and migration back to the cell surface, these IL-15 complexes acquire the property to activate bystander cells expressing the IL-15R $\beta\gamma$ low-affinity receptor complex and induce IL-15-mediated signaling pathway (US10,265,382B2, hereby entirely incorporated by reference). IL-15 signaling is essential for normal immune system functions. It stimulates T cell proliferation and inhibits IL-2-mediated activation-induced cell death.

[0009] The present disclosure relates to bispecific Fc fusion proteins comprising sPD-1 variant, IL-15 and IL-15R α sushi domain having improved properties (e.g., increased binding affinity for PD-L1 and/or PD-L2, enhanced agonist activity of IL-15, extended half-life, and synergistic efficacy for treating cancer and/or infections) as well as methods of making and using such bispecific Fc fusion proteins in treating patients with cancers, infections and immune-related diseases.

III. BRIEF SUMMARY

[0010] The present disclosure provides, inter alia, bispecific Fc fusion proteins comprising an IL-15 domain, an IL-15R α sushi domain, an Fc domain and sPD-1 variant domain, polynucleotides encoding the bispecific Fc fusion proteins, methods of making the bispecific Fc fusion proteins, and methods of using the bispecific Fc fusion proteins, for example, in treating diseases in which the adaptive immune system is suppressed or an increase in the magnitude or level of immune response is needed (e.g., cancers, infections, etc.), modulating immune functions, promoting T cell and/or NK cell cytotoxicity, etc.

[0011] In one aspect, the present disclosure provides a bispecific Fc fusion protein comprising:

- a) an IL-15R α sushi domain;
- b) an IL-15 domain;
- c) an Fc domain; and
- d) a soluble PD-1 (sPD-1) variant domain.

[0012] In an additional example, the present disclosure provides the Fc fusion protein as disclosed herein, where the Fc fusion protein further comprises a first domain linker, a second domain linker, and a third domain linker.

[0013] In a further example, the present disclosure provides the Fc fusion protein as disclosed herein, wherein the first domain linker, the second domain linker, and the third domain linker are selected from the group consisting of SEQ ID NO:12, SEQ ID NO:13, SEQ ID NO:14, SEQ ID NO:15, SEQ ID NO:16, SEQ ID NO:17, SEQ ID NO:18, SEQ ID NO:19, SEQ ID NO:20, SEQ ID NO:21, (GS) $_n$, (SEQ ID NO: 98) (GSGGS) $_n$, (SEQ ID NO: 99) (GGGGS) $_n$ (SEQ ID NO: 100), and (GGGS) $_n$ (SEQ ID NO: 101), wherein n is selected from the group consisting of 1, 2, 3, 4 and 5.

[0014] In an additional example, the present disclosure provides the Fc fusion protein as disclosed herein, wherein the first domain linker is selected from the group consisting of SEQ ID NO:12, SEQ

ID NO:13, SEQ ID NO:14, SEQ ID NO:15, SEQ ID NO:16, SEQ ID NO:17, SEQ ID NO:18, SEQ ID NO:19, SEQ ID NO:20, and SEQ ID NO:21.

[0015] In a further example, the present disclosure provides the Fc fusion protein as disclosed herein, wherein the second domain linker is selected from the group consisting of SEQ ID NO:12, SEQ ID NO:13, SEQ ID NO:14, SEQ ID NO:15, SEQ ID NO:16, SEQ ID NO:17, SEQ ID NO:18, SEQ ID NO:19, SEQ ID NO:20, and SEQ ID NO:21.

[0016] In an additional example, the present disclosure provides the Fc fusion protein as disclosed herein, wherein the third domain linker is selected from the group consisting of SEQ ID NO:12, SEQ ID NO:13, SEQ ID NO:14, SEQ ID NO:15, SEQ ID NO:16, SEQ ID NO:17, SEQ ID NO:18, SEQ ID NO:19, SEQ ID NO:20, and SEQ ID NO:21.

[0017] In a further example, the present disclosure provides the Fc fusion protein as disclosed herein, wherein the Fc fusion protein comprises, from N- to C-terminus:

- a) the IL-15R α sushi domain;
- b) the first domain linker;
- c) the IL-15 domain;
- d) the second domain linker;
- e) the Fc domain;
- f) the third domain linker; and
- g) the SPD-1 variant domain

[0018] In an additional example, the present disclosure provides the Fc fusion protein as disclosed herein, wherein the Fc fusion protein comprises, from N- to C-terminus:

- a) the IL-15 domain;
- b) the first domain linker;
- c) the IL-15R α sushi domain;
- d) the second domain linker;
- e) the Fc domain;
- f) the third domain linker; and
- g) the SPD-1 variant domain.

[0019] In a further example, the present disclosure provides the Fc fusion protein as disclosed herein, wherein the Fc fusion protein comprises, from N- to C-terminus:

- a) the sPD-1 variant domain;
- b) the first domain linker;
- c) the Fc domain;
- d) the second domain linker;
- e) the IL-15 domain;
- f) the third domain linker; and
- g) the IL-15R α sushi domain.

[0020] In an additional example, the present disclosure provides the Fc fusion protein as disclosed herein, wherein the Fc fusion protein comprises, from N- to C-terminus:

- a) the sPD-1 variant domain;
- b) the first domain linker;
- c) the Fc domain;
- d) the second domain linker;
- e) the IL-15R α sushi domain;
- f) the third domain linker; and
- g) the IL-15 domain.

[0021] In a further example, the present disclosure provides the Fc fusion protein as disclosed herein, wherein the Fc fusion protein comprises, from N- to C-terminus:

- a) the IL-15R α sushi domain;
- b) the first domain linker;
- c) the IL-15 domain;
- d) the second domain linker;
- e) the sPD-1 variant domain;
- f) the third domain linker; and
- g) the Fc domain.

[0022] In an additional example, the present disclosure provides the Fc fusion protein as disclosed herein, wherein the Fc fusion protein comprises, from N- to C-terminus:

- a) the IL-15 domain;
- b) the first domain linker;
- c) the IL-15R α sushi domain;
- d) the second domain linker;
- e) the sPD-1 variant domain;
- f) the third domain linker; and
- g) the Fc domain.

[0023] In a further example, the present disclosure provides the Fc fusion protein as disclosed herein, wherein the Fc fusion protein comprises, from N- to C-terminus:

- a) the sPD-1 variant domain;
- b) the first domain linker;
- c) the IL-15 domain;
- d) the second domain linker;
- e) the IL-15R α sushi domain;
- f) the third domain linker; and
- g) the Fc domain.

[0024] In an additional example, the present disclosure provides the Fc fusion protein as disclosed herein, wherein the Fc fusion protein comprises, from N- to C-terminus:

- a) the sPD-1 variant domain;
- b) the first domain linker;
- c) the IL-15R α sushi domain;
- d) the second domain linker;
- e) the IL-15 domain;
- f) the third domain linker; and
- g) the Fc domain.

[0025] In a further example, the present disclosure provides the Fc fusion protein as disclosed herein, wherein the Fc fusion protein comprises, from N- to C-terminus:

- a) the Fc domain;
- b) the first domain linker;
- c) the IL-15 domain;
- d) the second domain linker;
- e) the IL-15R α sushi domain;
- f) the third domain linker; and
- g) the SPD-1 variant domain

[0026] In an additional example, the present disclosure provides the Fc fusion protein as disclosed herein, wherein the Fc fusion protein comprises, from N- to C-terminus:

- a) the Fc domain;
- b) the first domain linker;
- c) the IL-15R α sushi domain;
- d) the second domain linker;
- e) the IL-15 domain;
- f) the third domain linker; and
- g) the SPD-1 variant domain.

[0027] In a further example, the present disclosure provides the Fc fusion protein as disclosed herein, wherein the Fc fusion protein comprises, from N- to C-terminus:

- a) the Fc domain;
- b) the first domain linker;
- c) the SPD-1 variant domain;
- d) the second domain linker;
- e) the IL-15 domain;
- f) the third domain linker; and
- g) the IL-15R α sushi domain.

[0028] In an additional example, the present disclosure provides the Fc fusion protein as disclosed herein, wherein the Fc fusion protein comprises, from N- to C-terminus:

- a) the Fc domain;
- b) the first domain linker;
- c) the SPD-1 variant domain;
- d) the second domain linker;
- e) the IL-15R α sushi domain;
- f) the third domain linker; and
- g) the IL-15 domain.

[0029] In a further example, the present disclosure provides the Fc fusion protein as disclosed herein,

wherein the domain linker linking the IL-15 domain and the IL-15R α sushi domain is selected from the group consisting of SEQ ID NO:13, SEQ ID NO:14, SEQ ID NO:15, SEQ ID NO:16, SEQ ID NO:17, and SEQ ID NO:18; and

wherein the other two domain linkers are selected from the group consisting of SEQ ID NO:19, SEQ ID NO:20, and SEQ ID NO:21.

[0030] In an additional example, the present disclosure provides the Fc fusion protein as disclosed herein,

wherein the domain linker linking the IL-15 domain and the IL-15R α sushi domain has the amino acid sequence of SEQ ID NO:15; and

wherein the other two domain linkers are selected from the group consisting of SEQ ID NO:19, SEQ ID NO:20, and SEQ ID NO:21.

[0031] In a further example, the present disclosure provides the Fc fusion protein as disclosed herein,

wherein the domain linker linking the IL-15 domain and the IL-15R α sushi domain has the amino acid sequence of SEQ ID NO:18; and

wherein the other two domain linkers are selected from the group consisting of SEQ ID NO:19, SEQ ID NO:20, and SEQ ID NO:21.

[0032] In an additional example, the present disclosure provides the Fc fusion protein as disclosed herein, wherein the SPD-1 variant domain comprises one or more amino acid substitutions at positions

corresponding to positions selected from the group consisting of positions 38, 63, 65, 92, 100, 103, 108 and 116 of SEQ ID NO: 1.

[0033] In a further example, the present disclosure provides the Fc fusion protein as disclosed herein, wherein the sPD-1 variant domain comprises an amino acid substitution at a position corresponding to position 38 of SEQ ID NO:1.

[0034] In an additional example, the present disclosure provides the Fc fusion protein as disclosed herein, wherein the sPD-1 variant domain comprises an amino acid substitution at a position corresponding to position 63 of SEQ ID NO: 1.

[0035] In a further example, the present disclosure provides the Fc fusion protein as disclosed herein, wherein the sPD-1 variant domain comprises an amino acid substitution at a position corresponding to position 65 of SEQ ID NO: 1.

[0036] In an additional example, the present disclosure provides the Fc fusion protein as disclosed herein, wherein the sPD-1 variant domain comprises an amino acid substitution at a position corresponding to position 92 of SEQ ID NO: 1.

[0037] In a further example, the present disclosure provides the Fc fusion protein as disclosed herein, wherein the sPD-1 variant domain comprises an amino acid substitution at a position corresponding to position 100 of SEQ ID NO: 1.

[0038] In an additional example, the present disclosure provides the Fc fusion protein as disclosed herein, wherein the sPD-1 variant domain comprises an amino acid substitution at a position corresponding to position 103 of SEQ ID NO: 1.

[0039] In a further example, the present disclosure provides the Fc fusion protein as disclosed herein, wherein the sPD-1 variant domain comprises an amino acid substitution at a position corresponding to position 108 of SEQ ID NO:1.

[0040] In an additional example, the present disclosure provides the Fc fusion protein as disclosed herein, wherein the sPD-1 variant domain comprises an amino acid substitution at a position corresponding to position 116 of SEQ ID NO:1.

[0041] In a further example, the present disclosure provides the Fc fusion protein as disclosed herein, wherein said one or more amino acid substitutions occur at two of said positions, three of said positions, four of said positions, five of said positions, six of said positions, seven of said positions or eight of said positions.

[0042] In an additional example, the present disclosure provides the Fc fusion protein as disclosed herein, wherein the sPD-1 variant domain comprises an amino acid sequence having at least 96% sequence identity to SEQ ID NO:1.

[0043] In a further example, the present disclosure provides the Fc fusion protein as disclosed herein, wherein the sPD-1 variant domain comprises one or more amino acid substitutions selected from the group consisting of S38G, S63G, P65L, N92S, G100S, S103V, A108I, and A116V of SEQ ID NO:1.

[0044] In an additional example, the present disclosure provides the Fc fusion protein as disclosed herein, wherein the sPD-1 variant domain comprises a set of amino acid substitutions N92S/G100S/S103V/A108I/A116V of SEQ ID NO:1.

[0045] In a further example, the present disclosure provides the Fc fusion protein as disclosed herein, wherein the sPD-1 variant domain comprises a set of amino acid substitutions S38G/S63G/P65L/N92S/G100S/S103V/A108I/A116V of SEQ ID NO:1.

[0046] In an additional example, the present disclosure provides the Fc fusion protein as disclosed herein, wherein the sPD-1 variant domain comprises a set of amino acid substitutions S38G/S63G/P65L/G100S/S103V/A108I/A116V of SEQ ID NO:1.

[0047] In a further example, the present disclosure provides the Fc fusion protein as disclosed herein, wherein the sPD-1 variant domain comprises a set of amino acid substitutions P65L/G100S/S103V/A108I/A116V of SEQ ID NO:1.

[0048] In an additional example, the present disclosure provides the Fc fusion protein as disclosed herein, wherein the sPD-1 variant domain comprises a set of amino acid substitutions S63G/G100S/S103V/A108I/A116V of SEQ ID NO:1.

[0049] In a further example, the present disclosure provides the Fc fusion protein as disclosed herein, wherein the sPD-1 variant domain comprises a set of amino acid substitutions S63G/P65L/G100S/S103V/A108I/A116V of SEQ ID NO:1.

[0050] In an additional example, the present disclosure provides the Fc fusion protein as disclosed herein, wherein the sPD-1 variant domain comprises a set of amino acid substitutions G100S/S103V/A108I/A116V of SEQ ID NO:1.

[0051] In a further example, the present disclosure provides the Fc fusion protein as disclosed herein, wherein the sPD-1 variant domain comprises a set of amino acid substitutions G100S/S103V/A108I of SEQ ID NO:1.

[0052] In an additional example, the present disclosure provides the Fc fusion protein as disclosed herein, wherein the sPD-1 variant domain comprises an amino acid sequence selected from the group consisting of SEQ ID NO:2, SEQ ID NO:3, SEQ ID NO:4, SEQ ID NO:5, SEQ ID NO:6, SEQ ID NO:7, SEQ ID NO:8 and SEQ ID NO:9.

[0053] In a further example, the present disclosure provides the Fc fusion protein as disclosed herein, wherein the sPD-1 variant domain comprises the amino acid sequence of SEQ ID NO:7.

[0054] In an additional example, the present disclosure provides the Fc fusion protein as disclosed herein, wherein the IL-15 domain comprises the amino acid sequence of SEQ ID NO:10.

[0055] In a further example, the present disclosure provides the Fc fusion protein as disclosed herein, wherein the IL-15R α sushi domain comprises the amino acid sequence of SEQ ID NO:11.

[0056] In an additional example, the present disclosure provides the Fc fusion protein as disclosed herein, wherein the Fc domain is a human IgG Fc domain or a variant human IgG Fc domain.

[0057] In a further example, the present disclosure provides the Fc fusion protein as disclosed herein, wherein the human IgG Fc domain comprises hinge-CH2-CH3 of human IgG4.

[0058] In an additional example, the present disclosure provides the Fc fusion protein as disclosed herein, wherein the Fc domain is a variant human IgG Fc domain.

[0059] In a further example, the present disclosure provides the Fc fusion protein as disclosed herein, wherein said variant human IgG Fc domain comprises hinge-CH2-CH3 of human IgG4 with a substitution corresponding to S228P as set forth in SEQ ID NO:25.

[0060] In an additional example, the present disclosure provides the Fc fusion protein comprising the amino acid sequence of SEQ ID NO:26.

[0061] In a further example, the present disclosure provides the Fc fusion protein comprising the amino acid sequence of SEQ ID NO:27.

[0062] In an additional example, the present disclosure provides the Fc fusion protein comprising the amino acid sequence of SEQ ID NO:28.

[0063] In a further example, the present disclosure provides the Fc fusion protein comprising the amino acid sequence of SEQ ID NO:29.

[0064] In an additional example, the present disclosure provides the Fc fusion protein comprising the amino acid sequence of SEQ ID NO:30.

[0065] In a further example, the present disclosure provides the Fc fusion protein comprising the amino acid sequence of SEQ ID NO:31.

[0066] In an additional example, the present disclosure provides the Fc fusion protein comprising the amino acid sequence of SEQ ID NO:32.

[0067] In a further example, the present disclosure provides the Fc fusion protein comprising the amino acid sequence of SEQ ID NO:33.

[0068] In an additional example, the present disclosure provides the Fc fusion protein comprising the amino acid sequence of SEQ ID NO:34.

[0069] In a further example, the present disclosure provides the Fc fusion protein comprising the amino acid sequence of SEQ ID NO:35.

[0070] In an additional example, the present disclosure provides the Fc fusion protein comprising the amino acid sequence of SEQ ID NO:36.

[0071] In a further example, the present disclosure provides the Fc fusion protein comprising the amino acid sequence of SEQ ID NO:37.

[0072] In an additional example, the present disclosure provides the Fc fusion protein comprising the amino acid sequence of SEQ ID NO:62.

[0073] In a further example, the present disclosure provides the Fc fusion protein comprising the amino acid sequence of SEQ ID NO:63.

[0074] In an additional example, the present disclosure provides the Fc fusion protein comprising the amino acid sequence of SEQ ID NO:64.

[0075] In a further example, the present disclosure provides the Fc fusion protein comprising the amino acid sequence of SEQ ID NO:65.

[0076] In an additional example, the present disclosure provides the Fc fusion protein comprising the amino acid sequence of SEQ ID NO:66.

[0077] In a further example, the present disclosure provides the Fc fusion protein comprising the amino acid sequence of SEQ ID NO:68.

[0078] In an additional example, the present disclosure provides the Fc fusion protein comprising the amino acid sequence of SEQ ID NO:69.

[0079] In a further example, the present disclosure provides the Fc fusion protein comprising the amino acid sequence of SEQ ID NO:70.

[0080] In an additional example, the present disclosure provides the Fc fusion protein comprising the amino acid sequence of SEQ ID NO:71.

[0081] In a further example, the present disclosure provides the Fc fusion protein comprising the amino acid sequence of SEQ ID NO:72.

[0082] In an additional aspect, the present disclosure provides a pharmaceutical composition comprising the Fc fusion protein as disclosed herein and a pharmaceutically acceptable carrier, excipient and/or stabilizer.

[0083] In a further aspect, the present disclosure provides a nucleic acid encoding the Fc fusion protein as disclosed herein.

[0084] In an additional example, the present disclosure provides the nucleic acid encoding the Fc fusion protein as disclosed herein, wherein the nucleic acid is codon optimized for a host organism for expression of the Fc fusion protein in said organism.

[0085] In a further aspect, the present disclosure provides an expression vector comprising the nucleic acid as disclosed herein.

[0086] In an additional aspect, the present disclosure provides a method of making the bispecific Fc fusion protein as disclosed herein comprising: a) culturing the host cell as disclosed herein under conditions wherein said Fc fusion protein is expressed; and b) recovering said Fc fusion protein.

[0087] In a further aspect, the present disclosure provides a nucleic acid encoding a preprotein comprising a signal peptide and the Fc fusion protein as disclosed herein.

[0088] In an additional example, the present disclosure provides the nucleic acid encoding a preprotein comprising a signal peptide and the Fc fusion protein as disclosed herein, wherein the signal peptide comprises an amino acid sequence having at least 85% sequence identity to SEQ ID NO:22 or SEQ ID NO:23.

[0089] In a further example, the present disclosure provides the nucleic acid encoding a preprotein comprising a signal peptide and the Fc fusion protein as disclosed herein, wherein the signal peptide comprises the amino acid sequence of SEQ ID NO:22.

[0090] In an additional example, the present disclosure provides the nucleic acid encoding a preprotein comprising a signal peptide and the Fc fusion protein as disclosed herein, wherein the signal peptide comprises the amino acid sequence of SEQ ID NO:23.

[0091] In a further example, the present disclosure provides the nucleic acid encoding the preprotein as disclosed herein, wherein the preprotein comprises an amino acid sequence selected from the group

consisting of SEQ ID NO:38, SEQ ID NO:39, SEQ ID NO:40, SEQ ID NO:41, SEQ ID NO:42, SEQ ID NO:43, SEQ ID NO:44, SEQ ID NO:45, SEQ ID NO:46, SEQ ID NO:47, SEQ ID NO:48, SEQ ID NO:49, SEQ ID NO:50, SEQ ID NO:51, SEQ ID NO:52, SEQ ID NO:53, SEQ ID NO:54, SEQ ID NO:55, SEQ ID NO:56, SEQ ID NO:57, SEQ ID NO:58, SEQ ID NO:59, SEQ ID NO:60, SEQ ID NO:61, SEQ ID NO:73, SEQ ID NO:74, SEQ ID NO:75, SEQ ID NO:76, SEQ ID NO:77, SEQ ID NO:78, SEQ ID NO:79, SEQ ID NO:80, SEQ ID NO:81, SEQ ID NO:82 and SEQ ID NO:83.

[0092] In an additional aspect, the present disclosure provides an expression vector comprising the nucleic acid as disclosed herein.

[0093] In a further aspect, the present disclosure provides a host cell comprising the nucleic acid as disclosed herein, or the expression vector as disclosed herein.

[0094] In an additional aspect, the present disclosure provides a method of making a bispecific Fc fusion protein comprising: a) culturing the host cell as disclosed herein under conditions wherein said Fc fusion protein is expressed; and b) recovering said Fc fusion protein.

[0095] In a further aspect, the present disclosure provides use of one or more said Fc fusion proteins as disclosed herein, said pharmaceutical composition as disclosed herein, or said preprotein encoded by the nucleic acid as disclosed herein in the manufacture of a medicament for treating, reducing or preventing metastasis or invasion of a tumor in a subject with cancer. In one example, the present disclosure provides a method of treating, reducing or preventing metastasis or invasion of a tumor in a subject with cancer, the method comprising administering to the subject a therapeutically effective dose of one or more said Fc fusion proteins as disclosed herein or the pharmaceutical composition as disclosed herein.

[0096] In an additional example, the present disclosure provides the method of treating, reducing or preventing metastasis or invasion of a tumor in a subject with cancer as disclosed herein, wherein the tumor is a solid tumor.

[0097] In a further example, the present disclosure provides the method of treating, reducing or preventing metastasis or invasion of a tumor in a subject with cancer as disclosed herein, wherein the cancer is a colorectal cancer.

[0098] In an additional aspect, the present disclosure provides the method of treating, reducing or preventing metastasis or invasion of a tumor in a subject with cancer as disclosed herein, wherein the effective dose of the one or more Fc fusion proteins or the pharmaceutical composition inhibits, reduces, or modulates signal transduction mediated by the wild-type PD-1 in the subject.

[0099] In a further example, the present disclosure provides the method of treating, reducing or preventing metastasis or invasion of a tumor in a subject with cancer as disclosed herein, wherein the effective dose of the one or more Fc fusion proteins or the pharmaceutical composition increases a T cell response in the subject.

[00100] In an additional aspect, the present disclosure provides use of one or more said Fc fusion proteins as disclosed herein, said pharmaceutical composition as disclosed herein or said preprotein encoded by the nucleic acid as disclosed herein in the manufacture of a medicament for preventing or treating an infection in a subject. In one example, the present disclosure provides a method of preventing or treating an infection in a subject, the method comprising administering to the subject a therapeutically effective dose of one or more said Fc fusion proteins as disclosed herein or the pharmaceutical composition as disclosed herein.

[00101] In a further example, the present disclosure provides the method of preventing or treating an infection in a subject as disclosed herein, wherein the infection is selected from the group consisting of a fungal infection, bacterial infection and viral infection.

[00102] In an additional example, the present disclosure provides the method of preventing or treating an infection in a subject as disclosed herein, wherein the effective dose of the one or more Fc fusion proteins or the pharmaceutical composition inhibits, reduces, or modulates signal transduction mediated by the wild-type PD-1 in the subject.

[00103] In a further example, the method of preventing or treating an infection in a subject as disclosed herein, wherein the effective dose of the one or more Fc fusion proteins or the pharmaceutical composition increases a T cell response in the subject.

[00104] In an additional aspect, the present disclosure provides use of one or more said Fc fusion proteins as disclosed herein, said pharmaceutical composition as disclosed herein or said preprotein encoded by the nucleic acid as disclosed herein in the manufacture of a medicament for preventing or treating an IL-15 mediated disease or disorder in a subject, wherein the IL-15 mediated disease or disorder is a cancer or an infectious disease. In another example, the present disclosure provides a method of preventing or treating an IL-15 mediated disease or disorder in a subject, the method comprising administering to the subject a therapeutically effective dose of one or more said Fc fusion proteins as disclosed herein or the pharmaceutical composition as disclosed herein, wherein the IL-15 mediated disease or disorder is a cancer or an infectious disease.

[00105] In an additional example, the present disclosure provides the method of preventing or treating an IL-15 mediated disease or disorder in a subject as disclosed herein, wherein the cancer is colorectal cancer.

[00106] In a further example, the present disclosure provides the method of preventing or treating an IL-15 mediated disease or disorder in a subject as disclosed herein, wherein the infectious disease is a viral infection.

[00107] In an additional example, the present disclosure provides a method of preventing or treating an immunodeficiency or lymphopenia in a subject, comprising administering to the subject a therapeutically effective dose of one or more said Fc fusion proteins as disclosed herein or said pharmaceutical composition as disclosed herein.

[00108] In a further aspect, the present disclosure provides use of one or more said Fc fusion proteins as disclosed herein, said pharmaceutical composition as disclosed herein or said preprotein encoded by the nucleic acid as disclosed herein in the manufacture of a medicament for preventing or treating an immunodeficiency or lymphopenia in a subject. In one example, the present disclosure provided a method of enhancing IL-15-mediated immune function in a subject in need thereof, comprising administering to the subject a

therapeutically effective dose of one or more said Fc fusion proteins as disclosed herein or said pharmaceutical composition as disclosed herein.

[00109] In an additional example, the present disclosure provides use of one or more said Fc fusion proteins as disclosed herein, said pharmaceutical composition as disclosed herein or said preprotein encoded by the nucleic acid as disclosed herein in the manufacture of a medicament for enhancing IL-15-mediated immune function in a subject in need thereof. In one example, the present disclosure provided the method of enhancing IL-15-mediated immune function in a subject in need thereof as disclosed herein, wherein the enhanced IL-15-mediated immune function comprises proliferation of lymphocytes, inhibition of apoptosis of lymphocytes, antibody production, activation of antigen presenting cells and/or antigen presentation.

[00110] In a further example, the present disclosure provides the method of enhancing IL-15-mediated immune function in a subject in need thereof as disclosed herein, wherein the enhanced IL-15-mediated immune function comprises activation or proliferation of CD4⁺ T cells, CD8⁺ T cells, B cells, memory T cells, memory B cells, dendritic cells, other antigen presenting cells, macrophages, mast cells, natural killer T cells (NKT cells), tumor-resident T cells, CD122⁺ T cells, and/or natural killer cells (NK cells).

[00111] In an additional aspect, the present disclosure provides use of one or more said Fc fusion proteins as disclosed herein, said pharmaceutical composition as disclosed herein or said preprotein encoded by the nucleic acid as disclosed herein in the manufacture of a medicament for promoting T cell cytotoxicity or NK cell cytotoxicity in a subject in need thereof. In another example, the present disclosure provides a method of promoting T cell cytotoxicity or NK cell cytotoxicity in a subject in need thereof, comprising administering to the subject a therapeutically effective dose of one or more said Fc fusion proteins as disclosed herein or said pharmaceutical composition as disclosed herein.

IV. BRIEF DESCRIPTION OF THE DRAWINGS

[00112] **Figure 1** provides flowchart illustrations of molecule cell line development including culture medium and selection drug used.

[00113] **Figure 2** provides a Table of pooled clones corresponding to each sequence including culture medium and selection drug used.

[00114] **Figure 3A** shows results of cell viability recovery of Sequence 1 clone G1 to G4. **Figure 3B** shows results of cell viability recovery of Sequence 2 clone G5 to G8. **Figure 3C** shows results of cell viability recovery of Sequence 1-1 clone G9 to G12. **Figure 3D** shows results of cell viability recovery of Sequence 2-1 clone G13 to G16.

[00115] **Figure 4** shows tabulated productivity and cell growth profile of recovered clone pools by 11 days fed-batch culture.

[00116] **Figure 5A** shows results for SDS-PAGE of recovered pools by 11 days fed-batch culture using Harvested Cell Culture Fluid (HCCF), clone G1 to G8. **Figure 5B** shows results for

NA1 and FcγRIIb-NA2) (Jefferis et al., 2002, Immunol Lett 82:57-65, hereby entirely incorporated by reference), as well as any undiscovered human FcγRs or FcγR isoforms or allotypes. An FcγR may be from any organism, including, but not limited to, humans, mice, rats, rabbits, and monkeys. Mouse FcγRs include but are not limited to FcγRI (CD64), FcγRII (CD32), FcγRIII (CD16), and FcγRIII-2 (CD16-2), as well as any undiscovered mouse FcγRs or FcγR isoforms or allotypes.

[00187] 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.

[00188] By "linker", "domain linker", "linker domain" or "linker peptide" as used herein have a length that is adequate to link two molecules in such a way that they assume the correct conformation relative to one another so that they retain the desired activity. The linker or linker peptide may predominantly include the following amino acid residues: Gly, Ser, Leu, or Gln. In one embodiment, the linker is from about 1 to 50 amino acids in length, preferably about 1 to 20 amino acids in length. In one embodiment, linkers of 1 to 20 amino acids in length may be used, with from about 8 to about 20 amino acids finding use in some embodiments. Useful linkers include glycine-serine polymers, including for example (GS)_n, (GSGGS)_n (SEQ ID NO: 102), (GGGGS)_n (SEQ ID NO: 103), and (GGGS)_n (SEQ ID NO: 104), where n is an integer of at least one (and generally from 3 to 4), glycine-alanine polymers, alanine-serine polymers, and other flexible linkers. Alternatively, a variety of nonproteinaceous polymers, including, but not limited to, polyethylene glycol (PEG), polypropylene glycol, polyoxyalkylenes, or copolymers of polyethylene glycol and polypropylene glycol, may find use as linkers.

[00189] In some embodiments, the linker is a "domain linker", used to link any two domains as outlined herein together, such as to link the sPD-1 variant domain with (variant) Fc domain, or link the IL-15 domain or the IL-15Rα sushi domain with (variant) Fc domain. While any suitable linker can be used, many embodiments utilize a glycine-serine polymer, including for example (GS)_n, (GSGGS)_n (SEQ ID NO: 102), (GGGGS)_n (SEQ ID NO: 103), and (GGGS)_n (SEQ ID NO: 104), where n is an integer of at least one (and generally from 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 embodiments, the linker has the sequence selected from the group consisting of SEQ ID NO:12, SEQ ID NO:13, SEQ ID NO:14, SEQ ID NO:15, SEQ ID NO:16, SEQ ID NO:17, SEQ ID NO:18, SEQ ID NO:19, SEQ ID NO:20 and SEQ ID NO:21.

[00190] By "target cell" as used herein is meant a cell that expresses a target polypeptide or protein.

[00191] By "host cell" in the context of producing the bispecific Fc fusion proteins comprising sPD-1 variant domain, IL-15 domain and IL-15Rα domain according to the disclosure

[00280] In some embodiments, the IL-15R α sushi domain comprises the amino acid sequence of the sushi domain of human IL-15R α . In some embodiments, the IL-15R α sushi domain comprises the amino acid sequence of the ECD of human IL-15R α sushi domain as set forth in SEQ ID NO:11.

[00281] In some embodiments, the IL-15R α sushi domain is a biologically active fragment or variant of the sushi domain of human IL-15R α , and has at least about 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98% or 99% but less than 100% sequence identity to the amino acid sequence of the sushi domain of human IL-15R α . In some embodiments, the IL-15 domain is a biologically active fragment or variant of the ECD of human IL-15R α sushi domain as set forth in SEQ ID NO:11, and has at least about 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98% or 99% but less than 100% sequence identity to the amino acid sequence of SEQ ID NO:11.

5. Domain Linkers

[00282] The four domains described above (i.e., an sPD-1 variant domain, an IL-15 domain, an IL-15R α sushi domain, and an Fc domain) are generally linked using domain linkers as described herein. In the context of the present disclosure, what is important is that sPD-1 variant domain and IL-15 domain/IL-15R α sushi domain are attached to the Fc domain using a flexible linker in such a way that the three domains -sPD-1 variant, IL-15 (with IL-15R α sushi domain) and Fc domain can act independently. This can be accomplished in a variety of ways, using traditional linkers and/or the hinge linker.

[00283] While any suitable linker can be used, the domain linker may predominantly include the following amino acid residues: Gly, Ser, Leu, or Gln. Useful linkers include glycine-serine polymers, including for example (GS) $_n$, (GSGGS) $_n$ (SEQ ID NO: 102), (GGGGS) $_n$ (SEQ ID NO: 103), and (GGGS) $_n$ (SEQ ID NO: 104), where n is an integer of at least one (and generally from 3 to 5). In some embodiments, the linkers include glycine-alanine polymers, alanine-serine polymers, and other flexible linkers that allow for recombinant attachment of the two domains with sufficient length and flexibility to allow each domain to retain its biological function. In some embodiments, a variety of nonproteinaceous polymers, including, but not limited to, polyethylene glycol (PEG), polypropylene glycol, polyoxyalkylenes, or copolymers of polyethylene glycol and polypropylene glycol, may find use as linkers.

[00284] In some embodiments, the hinge domain of a human IgG antibody (e.g., IgG1, IgG2, IgG3 and IgG4) is used. In some cases, the hinge domain can contain amino acid substitutions as well. For example, as shown in SEQ ID NO:25 of Figure 21, a hinge domain from IgG4 comprising a S228P variant is used.

[00285] In some embodiments, the domain linker is a combination of a hinge domain and a flexible linker, such as an IgG4 hinge with a S228P with a GGSGGGGS linker (SEQ ID NO: 19) as well.

[00286] In one embodiment, the linker is from about 1 to 50 amino acids in length, preferably about 5 to 20 amino acids in length.

[00287] In some embodiments, a domain linker is used to link the SPD-1 variant domain with the (variant) Fc domain. In some embodiments, said domain linker comprises the amino acid sequence selected from the group consisting of SEQ ID NO:12, SEQ ID NO:13, SEQ ID NO:14, SEQ ID NO:15, SEQ ID NO:16, SEQ ID NO:17, SEQ ID NO:18, SEQ ID NO:19, SEQ ID NO:20, and SEQ ID NO:21. In some embodiments, said domain linker comprises the amino acid sequence of SEQ ID NO:12. In some embodiments, said domain linker comprises the amino acid sequence of SEQ ID NO:13. In some embodiments, said domain linker comprises the amino acid sequence of SEQ ID NO:14. In some embodiments, said domain linker comprises the amino acid sequence of SEQ ID NO:15. In some embodiments, said domain linker comprises the amino acid sequence of SEQ ID NO:16. In some embodiments, said domain linker comprises the amino acid sequence of SEQ ID NO:17. In some embodiments, said domain linker comprises the amino acid sequence of SEQ ID NO:18. In some embodiments, said domain linker comprises the amino acid sequence of SEQ ID NO:19. In some embodiments, said domain linker comprises the amino acid sequence of SEQ ID NO:20. In some embodiments, said domain linker comprises the amino acid sequence of SEQ ID NO:21.

[00288] In some embodiments, a domain linker is used to link the IL-15 domain with the (variant) Fc domain. In some embodiments, said domain linker comprises the amino acid sequence selected from the group consisting of SEQ ID NO:12, SEQ ID NO:13, SEQ ID NO:14, SEQ ID NO:15, SEQ ID NO:16, SEQ ID NO:17, SEQ ID NO:18, SEQ ID NO:19, SEQ ID NO:20, and SEQ ID NO:21. In some embodiments, said domain linker comprises the amino acid sequence of SEQ ID NO:12. In some embodiments, said domain linker comprises the amino acid sequence of SEQ ID NO:13. In some embodiments, said domain linker comprises the amino acid sequence of SEQ ID NO:14. In some embodiments, said domain linker comprises the amino acid sequence of SEQ ID NO:15. In some embodiments, said domain linker comprises the amino acid sequence of SEQ ID NO:16. In some embodiments, said domain linker comprises the amino acid sequence of SEQ ID NO:17. In some embodiments, said domain linker comprises the amino acid sequence of SEQ ID NO:18. In some embodiments, said domain linker comprises the amino acid sequence of SEQ ID NO:19. In some embodiments, said domain linker comprises the amino acid sequence of SEQ ID NO:20. In some embodiments, said domain linker comprises the amino acid sequence of SEQ ID NO:21.

NO:16, SEQ ID NO:17, SEQ ID NO:18, SEQ ID NO:19, SEQ ID NO:20, SEQ ID NO:21, (GS)_n (SEQ ID NO: 98), (GSGGS)_n (SEQ ID NO: 99), (GGGGS)_n (SEQ ID NO: 100), and (GGGS)_n (SEQ ID NO: 101), wherein n is selected from the group consisting of 1, 2, 3, 4 and 5.

[00299] In some embodiments, the second domain linker as described herein is selected from the group consisting of SEQ ID NO:12, SEQ ID NO:13, SEQ ID NO:14, SEQ ID NO:15, SEQ ID NO:16, SEQ ID NO:17, SEQ ID NO:18, SEQ ID NO:19, SEQ ID NO:20, SEQ ID NO:21, (GS)_n (SEQ ID NO: 98), (GSGGS)_n (SEQ ID NO: 99), (GGGGS)_n (SEQ ID NO: 100), and (GGGS)_n (SEQ ID NO: 101), wherein n is selected from the group consisting of 1, 2, 3, 4 and 5.

[00300] In some embodiments, the third domain linker as described herein is selected from the group consisting of SEQ ID NO:12, SEQ ID NO:13, SEQ ID NO:14, SEQ ID NO:15, SEQ ID NO:16, SEQ ID NO:17, SEQ ID NO:18, SEQ ID NO:19, SEQ ID NO:20, SEQ ID NO:21, (GS)_n (SEQ ID NO: 98), (GSGGS)_n (SEQ ID NO: 99), (GGGGS)_n (SEQ ID NO: 100), and (GGGS)_n (SEQ ID NO: 101), wherein n is selected from the group consisting of 1, 2, 3, 4 and 5.

[00301] In some embodiments, a domain linker is used to link the sPD-1 variant domain with the (variant) Fc domain, wherein said domain linker comprises the amino acid sequence selected from the group consisting of SEQ ID NO:19, SEQ ID NO:20, and SEQ ID NO:21. In some embodiments, the domain linker used to link the sPD-1 variant domain with the (variant) Fc domain comprises the amino acid sequence of SEQ ID NO:19. In some embodiments, the domain linker used to link the sPD-1 variant domain with the (variant) Fc domain comprises the amino acid sequence of SEQ ID NO:20. In some embodiments, the domain linker used to link the sPD-1 variant domain with the (variant) Fc domain comprises the amino acid sequence of SEQ ID NO:21.

[00302] In some embodiments, a domain linker is used to link the IL-15 domain with the (variant) Fc domain, wherein said domain linker comprises the amino acid sequence selected from the group consisting of SEQ ID NO:19, SEQ ID NO:20, and SEQ ID NO:21. In some embodiments, the domain linker used to link the IL-15 variant domain with the (variant) Fc domain comprises the amino acid sequence of SEQ ID NO:19. In some embodiments, the domain linker used to link the IL-15 variant domain with the (variant) Fc domain comprises the amino acid sequence of SEQ ID NO:20. In some embodiments, the domain linker used to link the IL-15 variant domain with the (variant) Fc domain comprises the amino acid sequence of SEQ ID NO:21.

[00303] In some embodiments, a domain linker is used to link the IL-15R α sushi domain with the (variant) Fc domain, wherein said domain linker comprises the amino acid sequence selected from the group consisting of SEQ ID NO:19, SEQ ID NO:20, and SEQ ID NO:21. In some embodiments, the domain linker used to link the IL-15R α sushi domain with the (variant) Fc domain comprises the

WHAT IS CLAIMED IS:

1. A bispecific Fc fusion protein comprising:
 - a) an IL-15R α sushi domain;
 - b) an IL-15 domain;
 - c) an Fc domain; and
 - d) a soluble PD-1 (sPD-1) variant domain.
2. The Fc fusion protein according to claim 1, further comprising:
a first domain linker, a second domain linker, and a third domain linker.
3. The Fc fusion protein according to claim 2, wherein the first domain linker, the second domain linker, and the third domain linker are selected from the group consisting of SEQ ID NO:12, SEQ ID NO:13, SEQ ID NO:14, SEQ ID NO:15, SEQ ID NO:16, SEQ ID NO:17, SEQ ID NO:18, SEQ ID NO:19, SEQ ID NO:20, SEQ ID NO:21, (GS)_n, (GSGGS)_n, (GGGGS)_n, and (GGGS)_n, wherein n is selected from the group consisting of 1, 2, 3, 4 and 5.
4. The Fc fusion protein according to claim 2 or 3, wherein the first domain linker is selected from the group consisting of SEQ ID NO:12, SEQ ID NO:13, SEQ ID NO:14, SEQ ID NO:15, SEQ ID NO:16, SEQ ID NO:17, SEQ ID NO:18, SEQ ID NO:19, SEQ ID NO:20, and SEQ ID NO:21.
5. The Fc fusion protein according to any one of claims 2-4, wherein the second domain linker is selected from the group consisting of SEQ ID NO:12, SEQ ID NO:13, SEQ ID NO:14, SEQ ID NO:15, SEQ ID NO:16, SEQ ID NO:17, SEQ ID NO:18, SEQ ID NO:19, SEQ ID NO:20, and SEQ ID NO:21.
6. The Fc fusion protein according to any one of claims 2-5, wherein the third domain linker is selected from the group consisting of SEQ ID NO:12, SEQ ID NO:13, SEQ ID NO:14, SEQ ID NO:15, SEQ ID NO:16, SEQ ID NO:17, SEQ ID NO:18, SEQ ID NO:19, SEQ ID NO:20, and SEQ ID NO:21.

7. The Fc fusion protein according to any preceding claim comprising, from N- to C-terminus:
 - a) the IL-15R α sushi domain;
 - b) the first domain linker;
 - c) the IL-15 domain;
 - d) the second domain linker;
 - e) the Fc domain;
 - f) the third domain linker; and
 - g) the SPD-1 variant domain.

8. The Fc fusion protein according to any one of claims 2-6 comprising, from N- to C-terminus:
 - a) the IL-15 domain;
 - b) the first domain linker;
 - c) the IL-15R α sushi domain;
 - d) the second domain linker;
 - e) the Fc domain;
 - f) the third domain linker; and
 - g) the SPD-1 variant domain.

9. The Fc fusion protein according to any one of claims 2-6 comprising, from N- to C-terminus:
 - a) the SPD-1 variant domain;
 - b) the first domain linker;
 - c) the Fc domain;
 - d) the second domain linker;
 - e) the IL-15 domain;
 - f) the third domain linker; and

- g) the IL-15R α sushi domain.
10. The Fc fusion protein according to any one of claims 2-6 comprising, from N- to C-terminus:
- a) the SPD-1 variant domain;
 - b) the first domain linker;
 - c) the Fc domain;
 - d) the second domain linker;
 - e) the IL-15R α sushi domain;
 - f) the third domain linker; and
 - g) the IL-15 domain.
11. The Fc fusion protein according to any one of claims 2-6 comprising, from N- to C-terminus:
- a) the IL-15R α sushi domain;
 - b) the first domain linker;
 - c) the IL-15 domain;
 - d) the second domain linker;
 - e) the SPD-1 variant domain;
 - f) the third domain linker; and
 - g) the Fc domain.
12. The Fc fusion protein according to any one of claims 2-6 comprising, from N- to C-terminus:
- a) the IL-15 domain;
 - b) the first domain linker;
 - c) the IL-15R α sushi domain;
 - d) the second domain linker;
 - e) the SPD-1 variant domain;

- f) the third domain linker; and
 - g) the Fc domain.
13. The Fc fusion protein according to any one of claims 2-6 comprising, from N- to C-terminus:
- a) the SPD-1 variant domain;
 - b) the first domain linker;
 - c) the IL-15 domain;
 - d) the second domain linker;
 - e) the IL-15R α sushi domain;
 - f) the third domain linker; and
 - g) the Fc domain.
14. The Fc fusion protein according to any one of claims 2-6 comprising, from N- to C-terminus:
- a) the SPD-1 variant domain;
 - b) the first domain linker;
 - c) the IL-15R α sushi domain;
 - d) the second domain linker;
 - e) the IL-15 domain;
 - f) the third domain linker; and
 - g) the Fc domain.
15. The Fc fusion protein according to any one of claims 2-6 comprising, from N- to C-terminus:
- a) the Fc domain;
 - b) the first domain linker;
 - c) the IL-15 domain;
 - d) the second domain linker;

- e) the IL-15R α sushi domain;
 - f) the third domain linker; and
 - g) the SPD-1 variant domain.
16. The Fc fusion protein according to any one of claims 2-6 comprising, from N- to C-terminus:
- a) the Fc domain;
 - b) the first domain linker;
 - c) the IL-15R α sushi domain;
 - d) the second domain linker;
 - e) the IL-15 domain;
 - f) the third domain linker; and
 - g) the SPD-1 variant domain.
17. The Fc fusion protein according to any one of claims 2-6 comprising, from N- to C-terminus:
- a) the Fc domain;
 - b) the first domain linker;
 - c) the SPD-1 variant domain;
 - d) the second domain linker;
 - e) the IL-15 domain;
 - f) the third domain linker; and
 - g) the IL-15R α sushi domain.
18. The Fc fusion protein according to any one of claims 2-6 comprising, from N- to C-terminus:
- a) the Fc domain;
 - b) the first domain linker;
 - c) the SPD-1 variant domain;

- d) the second domain linker;
- e) the IL-15R α sushi domain;
- f) the third domain linker; and
- g) the IL-15 domain.

19. The Fc fusion protein according to any one of claims 7, 8, 11 and 12,

wherein the first domain linker is selected from the group consisting of SEQ ID NO:13, SEQ ID NO:14, SEQ ID NO:15, SEQ ID NO:16, SEQ ID NO:17, and SEQ ID NO:18;

wherein the second domain linker is selected from the group consisting of SEQ ID NO:19, SEQ ID NO:20, and SEQ ID NO:21; and

wherein the third domain linker is selected from the group consisting of SEQ ID NO:19, SEQ ID NO:20, and SEQ ID NO:21.

20. The Fc fusion protein according to any one of claims 9, 10, 17 and 18,

wherein the first domain linker is selected from the group consisting of SEQ ID NO:19, SEQ ID NO:20, and SEQ ID NO:21;

wherein the second domain linker is selected from the group consisting of SEQ ID NO:19, SEQ ID NO:20, and SEQ ID NO:21; and

wherein the third domain linker is selected from the group consisting of SEQ ID NO:13, SEQ ID NO:14, SEQ ID NO:15, SEQ ID NO:16, SEQ ID NO:17, and SEQ ID NO:18.

21. The Fc fusion protein according to any one of claims 13-16,

wherein the first domain linker is selected from the group consisting of SEQ ID NO:19, SEQ ID NO:20, and SEQ ID NO:21;

wherein the second domain linker is selected from the group consisting of SEQ ID NO:13, SEQ ID NO:14, SEQ ID NO:15, SEQ ID NO:16, SEQ ID NO:17, and SEQ ID NO:18; and

wherein the third domain linker is selected from the group consisting of SEQ ID NO:19, SEQ ID NO:20, and SEQ ID NO:21.

22. The Fc fusion protein according to any preceding claim, wherein the sPD-1 variant domain comprises one or more amino acid substitutions at positions corresponding to positions selected from the group consisting of positions 38, 63, 65, 92, 100, 103, 108 and 116 of SEQ ID NO: 1.
23. The Fc fusion protein according to claim 22, wherein the sPD-1 variant domain comprises an amino acid substitution at a position corresponding to position 38 of SEQ ID NO: 1.
24. The Fc fusion protein according to claim 22 or claim 23, wherein the sPD-1 variant domain comprises an amino acid substitution at a position corresponding to position 63 of SEQ ID NO: 1.
25. The Fc fusion protein according to any one of claims 22-24, wherein the sPD-1 variant domain comprises an amino acid substitution at a position corresponding to position 65 of SEQ ID NO: 1.
26. The Fc fusion protein according to any one of claims 22-25, wherein the sPD-1 variant domain comprises an amino acid substitution at a position corresponding to position 92 of SEQ ID NO: 1.
27. The Fc fusion protein according to any one of claims 22-26, wherein the sPD-1 variant domain comprises an amino acid substitution at a position corresponding to position 100 of SEQ ID NO: 1.
28. The Fc fusion protein according to any one of claims 22-27, wherein the sPD-1 variant domain comprises an amino acid substitution at a position corresponding to position 103 of SEQ ID NO: 1.
29. The Fc fusion protein according to any one of claims 22-28, wherein the sPD-1 variant domain comprises an amino acid substitution at a position corresponding to position 108 of SEQ ID NO: 1.

30. The Fc fusion protein according to any one of claims 22-29, wherein the sPD-1 variant domain comprises an amino acid substitution at a position corresponding to position 116 of SEQ ID NO:1.
31. The Fc fusion protein according to any one of claims 22-30, wherein said one or more amino acid substitutions occur at two of said positions, three of said positions, four of said positions, five of said positions, six of said positions, seven of said positions or eight of said positions.
32. The Fc fusion protein according to any one of claims 22-31, wherein the sPD-1 variant domain comprises an amino acid sequence having at least 96% sequence identity to SEQ ID NO:1.
33. The Fc fusion protein according to any one of claims 22-31, wherein said one or more amino acid substitutions are selected from the group consisting of S38G, S63G, P65L, N92S, G100S, S103V, A108I, and A116V of SEQ ID NO:1.
34. The Fc fusion protein according to claim 33, wherein the sPD-1 variant domain comprises a set of amino acid substitutions N92S/G100S/S103V/A108I/A116V of SEQ ID NO:1.
35. The Fc fusion protein according to claim 33, wherein the sPD-1 variant domain comprises a set of amino acid substitutions S38G/S63G/P65L/N92S/G100S/S103V/A108I/A116V of SEQ ID NO:1.
36. The Fc fusion protein according to claim 33, wherein the sPD-1 variant domain comprises a set of amino acid substitutions S38G/S63G/P65L/G100S/S103V/A108I/A116V of SEQ ID NO:1.
37. The Fc fusion protein according to claim 33, wherein the sPD-1 variant domain comprises a set of amino acid substitutions P65L/G100S/S103V/A108I/A116V of SEQ ID NO:1.
38. The Fc fusion protein according to claim 33, wherein the sPD-1 variant domain comprises a set of amino acid substitutions S63G/G100S/S103V/A108I/A116V of SEQ ID NO:1.

39. The Fc fusion protein according to claim 33, wherein the sPD-1 variant domain comprises a set of amino acid substitutions S63G/P65L/G100S/S103V/A108I/A116V of SEQ ID NO:1.
40. The Fc fusion protein according to claim 33, wherein the sPD-1 variant domain comprises a set of amino acid substitutions G100S/S103V/A108I/A116V of SEQ ID NO:1.
41. The Fc fusion protein according to claim 33, wherein the sPD-1 variant domain comprises a set of amino acid substitutions G100S/S103V/A108I of SEQ ID NO:1.
42. The Fc fusion protein according to claim 33, wherein the sPD-1 variant domain comprises an amino acid sequence selected from the group consisting of SEQ ID NO:2, SEQ ID NO:3, SEQ ID NO:4, SEQ ID NO:5, SEQ ID NO:6, SEQ ID NO:7, SEQ ID NO:8 and SEQ ID NO:9.
43. The Fc fusion protein according to claim 42, wherein the sPD-1 variant domain comprises the amino acid sequence of SEQ ID NO:7.
44. The Fc fusion protein according to any preceding claim, wherein the IL-15 domain comprises the amino acid sequence of SEQ ID NO:10.
45. The Fc fusion protein according to any preceding claim, wherein the IL-15R α sushi domain comprises the amino acid sequence of SEQ ID NO:11.
46. The Fc fusion protein according to any preceding claim, wherein the Fc domain is a human IgG Fc domain or a variant human IgG Fc domain.
47. The Fc fusion protein according to claim 46, wherein the human IgG Fc domain comprises hinge-CH2-CH3 of human IgG4.

48. The Fc fusion protein according to claim 46, wherein the Fc domain is a variant human IgG Fc domain.
49. The Fc fusion protein according to claim 48, wherein said variant human IgG Fc domain comprises hinge-CH2-CH3 of human IgG4 with a substitution corresponding to S228P as set forth in SEQ ID NO: 25.
50. The Fc fusion protein according to claim 7 comprising the amino acid sequence of SEQ ID NO:26.
51. The Fc fusion protein according to claim 7 comprising the amino acid sequence of SEQ ID NO:27.
52. The Fc fusion protein according to claim 7 comprising the amino acid sequence of SEQ ID NO:28.
53. The Fc fusion protein according to claim 7 comprising the amino acid sequence of SEQ ID NO:29.
54. The Fc fusion protein according to claim 7 comprising the amino acid sequence of SEQ ID NO:30.
55. The Fc fusion protein according to claim 7 comprising the amino acid sequence of SEQ ID NO:31.
56. The Fc fusion protein according to claim 7 comprising the amino acid sequence of SEQ ID NO:32.

57. The Fc fusion protein according to claim 7 comprising the amino acid sequence of SEQ ID NO:33.

58. The Fc fusion protein according to claim 7 comprising the amino acid sequence of SEQ ID NO:34.

59. The Fc fusion protein according to claim 7 comprising the amino acid sequence of SEQ ID NO:35.

60. The Fc fusion protein according to claim 7 comprising the amino acid sequence of SEQ ID NO:36.

61. The Fc fusion protein according to claim 7 comprising the amino acid sequence of SEQ ID NO:37.

62. The Fc fusion protein according to claim 8 comprising the amino acid sequence of SEQ ID NO:62.

63. The Fc fusion protein according to claim 9 comprising the amino acid sequence of SEQ ID NO:63.

64. The Fc fusion protein according to claim 10 comprising the amino acid sequence of SEQ ID NO:64.

65. The Fc fusion protein according to claim 11 comprising the amino acid sequence of SEQ ID NO:65.

66. The Fc fusion protein according to claim 12 comprising the amino acid sequence of SEQ ID NO:66.

67. The Fc fusion protein according to claim 13 comprising the amino acid sequence of SEQ ID NO:67.
68. The Fc fusion protein according to claim 14 comprising the amino acid sequence of SEQ ID NO:68.
69. The Fc fusion protein according to claim 15 comprising the amino acid sequence of SEQ ID NO:69.
70. The Fc fusion protein according to claim 16 comprising the amino acid sequence of SEQ ID NO:70.
71. The Fc fusion protein according to claim 17 comprising the amino acid sequence of SEQ ID NO:71.
72. The Fc fusion protein according to claim 18 comprising the amino acid sequence of SEQ ID NO:72.
73. A pharmaceutical composition comprising the Fc fusion protein according to any preceding claim and a pharmaceutically acceptable carrier, excipient and/or stabilizer.
74. A nucleic acid encoding the Fc fusion protein according to any one of claims 1-72.
75. The nucleic acid according to claim 74, wherein the nucleic acid is codon optimized for a host organism for expression of the Fc fusion protein in said organism.
76. An expression vector comprising the nucleic acid of claim 74 or 75.

77. A host cell comprising the nucleic acid of claim 74 or 75, or the expression vector of claim 76.

78. A method of making a bispecific Fc fusion protein comprising: a) culturing the host cell of claim 77 under conditions wherein said Fc fusion protein is expressed; and b) recovering said Fc fusion protein.

79. A nucleic acid encoding a preprotein comprising a signal peptide and the Fc fusion protein of any one of claims 1-72.

80. The nucleic acid according to claim 79, wherein the signal peptide comprises an amino acid sequence having at least 85% sequence identity to SEQ ID NO:22 or SEQ ID NO:23.

81. The nucleic acid according to claim 80, wherein the signal peptide comprises the amino acid sequence of SEQ ID NO:22.

82. The nucleic acid according to claim 80, wherein the signal peptide comprises the amino acid sequence of SEQ ID NO:23.

83. The nucleic acid according to claim 80, wherein the preprotein comprises an amino acid sequence selected from the group consisting of SEQ ID NO:38, SEQ ID NO:39, SEQ ID NO:40, SEQ ID NO:41, SEQ ID NO:42, SEQ ID NO:43, SEQ ID NO:44, SEQ ID NO:45, SEQ ID NO:46, SEQ ID NO:47, SEQ ID NO:48, SEQ ID NO:49, SEQ ID NO:50, SEQ ID NO:51, SEQ ID NO:52, SEQ ID NO:53, SEQ ID NO:54, SEQ ID NO:55, SEQ ID NO:56, SEQ ID NO:57, SEQ ID NO:58, SEQ ID NO:59, SEQ ID NO:60, SEQ ID NO:61, SEQ ID NO:73, SEQ ID NO:74, SEQ ID NO:75, SEQ ID NO:76, SEQ ID NO:77, SEQ ID NO:78, SEQ ID NO:79, SEQ ID NO:80, SEQ ID NO:81, SEQ ID NO:82 and SEQ ID NO:83.

84. An expression vector comprising the nucleic acid of any one of claims 79-83.

85. A host cell comprising the nucleic acid of any one of claims 79-83, or the expression vector of claim 84.
86. A method of making a bispecific Fc fusion protein comprising: a) culturing the host cell of claim 85 under conditions wherein said Fc fusion protein is expressed; and b) recovering said Fc fusion protein.
87. Use of one or more said Fc fusion proteins of any one of claims 1-72, said pharmaceutical composition of claim 73, or said preprotein encoded by the nucleic acid of any one of claims 79-83 in the manufacture of a medicament for treating, reducing or preventing metastasis or invasion of a tumor in a subject with cancer.
88. The use according to claim 87, wherein the tumor is a solid tumor.
89. The use according to claim 87 or claim 88, wherein the cancer is a colorectal cancer.
90. Use of one or more said Fc fusion proteins of any one of claims 1-72, said pharmaceutical composition of claim 73 or said preprotein encoded by the nucleic acid of any one of claims 79-83 in the manufacture of a medicament for preventing or treating an infection in a subject.
91. The use according to claim 90, wherein the infection is selected from the group consisting of a fungal infection, bacterial infection and viral infection.
92. The use according to any one of claims 87 to 91, wherein the effective dose of the one or more Fc fusion proteins or the pharmaceutical composition inhibits, reduces, or modulates signal transduction mediated by the wild-type PD-1 in the subject.

93. The use according to any one of claims 87 to 91, wherein the effective dose of the one or more Fc fusion proteins or the pharmaceutical composition increases a T cell response in the subject.
94. Use of one or more said Fc fusion proteins of any one of claims 1-72, said pharmaceutical composition of claim 73 or said preprotein encoded by the nucleic acid of any one of claims 79-83 in the manufacture of a medicament for preventing or treating an IL-15 mediated disease or disorder in a subject, wherein the IL-15 mediated disease or disorder is a cancer or an infectious disease.
95. The use according to claim 94, wherein the cancer is colorectal cancer.
96. The use according to claim 94, wherein the infectious disease is a viral infection.
97. Use of one or more said Fc fusion proteins of any one of claims 1-72, said pharmaceutical composition of claim 73 or said preprotein encoded by the nucleic acid of any one of claims 79-83 in the manufacture of a medicament for preventing or treating an immunodeficiency or lymphopenia in a subject.
98. Use of one or more said Fc fusion proteins of any one of claims 1-72, said pharmaceutical composition of claim 73 or said preprotein encoded by the nucleic acid of any one of claims 79-83 in the manufacture of a medicament for enhancing IL-15-mediated immune function in a subject in need thereof.
99. The use according to claim 98, wherein the enhanced IL-15-mediated immune function comprises proliferation of lymphocytes, inhibition of apoptosis of lymphocytes, antibody production, activation of antigen presenting cells and/or antigen presentation.
100. The use according to claim 98 or 99, wherein the enhanced IL-15-mediated immune function comprises activation or proliferation of CD4⁺ T cells, CD8⁺ T cells, B cells, memory T cells, memory B cells, dendritic cells, other antigen presenting cells, macrophages, mast cells, natural killer T cells (NKT cells), tumor-resident T cells, CD122⁺ T cells, and/or natural killer cells (NK cells).

101. Use of one or more said Fc fusion proteins of any one of claims 1-72, said pharmaceutical composition of claim 73 or said preprotein encoded by the nucleic acid of any one of claims 79-83 in the manufacture of a medicament for promoting T cell cytotoxicity or NK cell cytotoxicity in a subject in need thereof.