SUMMARY

This disclosure relates to engineered IL-21 variants, protein constructs (e.g., fusion proteins or protein complexes), and methods of use thereof. In some examples, the variants and protein constructs include a non-native disulfide bond formed by mutating 1, 2, 3, 4, 5, 6, or more than 6 residues (e.g., a pair of residues) of wild-type IL-21 to cysteines, without interfering the overall structure or residues critical for IL-21 receptor interaction. As a result, the variants or protein constructs can be more stable than wild-type IL-21. In some examples, the protein constructs showed enhanced thermostability (e.g., increased T_{agg} and T_{onset}) and higher potency to relieve immune suppression (e.g., Treg-mediated T cell suppression) than wild-type IL-21. Therefore, the engineered IL-21 variants and protein constructs thereof described herein can be used for cancer treatment with minimal toxicity.

Also provided herein are methods for screening a cytokine (e.g., IL-21) with increased stability and/or anti-tumor efficacy.

In one aspect, the disclosure provides an engineered IL-21 polypeptide, wherein the engineered IL-21 polypeptide comprises a non-native disulfide bond and an amino acid sequence that is at least 80% identical to SEQ ID NO: 2, wherein two or more amino acid residues corresponding to positions selected from the group consisting of positions 8, 19, 29, 31, 33, 36, 39, 56, 61,62, 63, 80, 86, 105, 106, 107, 110, 112 and 117 of SEQ ID NO: 2 are substituted with cysteine (C).

In another aspect, the disclosure provides a fusion protein comprising, optionally from N-terminus to C-terminus: (a) optionally a His-tag; (b) a human serum albumin (HSA); (c)a linker peptide, and (d) an engineered IL-21 polypeptide.

In another aspect, the disclosure provides a protein complex comprising: (a) a first polypeptide comprising from N-terminus to C-terminus: an optional first hinge region, a first Fc region, an optional linker peptide, and the engineered IL-21 polypeptide of the disclosure; and (b) a second polypeptide comprising from N-terminus to C-terminus, an optional second hinge region, and a second Fc region.

In another aspect, the disclosure provides a use of the engineered IL-21 polypeptide of the disclosure, the fusion protein of the disclosure, the protein complex of the disclosure in the manufacture of a medicament for inducing STAT-3 phosphorylation, and/or relieving Treg-mediated T cell suppression, and/or inducing proliferation of immune cells (e.g., T cells or NK cells), and/or inducing primary NK cell cytotoxicity.

In another aspect, the disclosure provides a pharmaceutical composition comprising the engineered IL-21 polypeptide of the disclosure, the fusion protein of the disclosure, or the protein complex of the disclosure; and a pharmaceutically acceptable carrier.

In another aspect, the disclosure provides a nucleic acid encoding the engineered IL-21 polypeptide of the disclosure, the fusion protein of the disclosure, or the protein complex of the disclosure.

In another aspect, the disclosure provides a vector comprising the nucleic acid of the disclosure.

In another aspect, the disclosure provides a cell comprising the nucleic acid of the disclosure or the vector of the disclosure.

In another aspect, the disclosure provides a use of a therapeutically effective amount of a composition comprising the engineered IL-21 polypeptide of the disclosure, the fusion protein of the disclosure, or the protein complex of the disclosure in the manufacture of a medicament for treating a subject having cancer.

In another aspect, the disclosure provides method of improving the stability of a protein (e.g., cytokine), comprising (a) providing a 3D structure of the protein (e.g., cytokine), (b) measuring distance of the Calpha atoms of one or more of amino acid residues in the 3D structure; and (c) selecting two amino acid residues from the one or more amino acid residues, wherein the Calpha atoms of the two selected amino acid residues are within 3-7 angstroms.

In one example, the disclosure is related to an engineered IL-21 polypeptide, in some examples, the engineered IL-21 polypeptide comprises a non-native disulfide bond. In some examples, the engineered IL-21 polypeptide comprises an amino acid sequence that is at least 80% identical to SEQ ID NO: 2. In some examples, the engineered IL-21 polypeptide can bind to a complex formed by human IL-21 receptor (IL-21R) and common cytokine γ chain (γc).

In some examples, the engineered IL-21 polypeptide comprises one or more of the following: (a) the amino acid that corresponds to position 8 of SEQ ID NO: 2 is cysteine (C); (b) the amino acid that corresponds to position 19 of SEQ ID NO: 2 is C; (c) the amino acid that corresponds to position 29 of SEQ ID NO: 2 is C; (d) the amino acid that corresponds to position 31 of SEQ ID NO: 2 is C; (e) the amino acid that corresponds to position 33 of SEQ ID NO: 2 is C; (f) the amino acid that corresponds to position 36 of SEQ ID NO: 2 is C; (g) the amino acid that corresponds to position 39 of SEQ ID NO: 2 is C; and (h) the amino acid that corresponds to position 56 of SEQ ID NO: 2 is C.

In some examples, the engineered IL-21 polypeptide comprises one or more of the following: (a) the amino acid that corresponds to position 61 of SEQ ID NO: 2 is C; (b) the amino acid that corresponds to position 62 of SEQ ID NO: 2 is C; (c) the amino acid that corresponds to position 63 of SEQ ID NO: 2 is C; (d) the amino acid that corresponds to position 80 of SEQ ID NO: 2 is C; (e) the amino acid that corresponds to position 86 of SEQ ID NO: 2 is C; (f) the amino acid that corresponds to position 105 of SEQ ID NO: 2 is

C; (g) the amino acid that corresponds to position 106 of SEQ ID NO: 2 is C; (h) the amino acid that corresponds to position 107 of SEQ ID NO: 2 is C; (i) the amino acid that corresponds to position 110 of SEQ ID NO: 2 is C; (j) the amino acid that corresponds to position 112 of SEQ ID NO: 2 is C; and (k) the amino acid that corresponds to position 117 of SEQ ID NO: 2 is C. In some examples, the engineered IL-21 polypeptide comprises one or more of the following: (a) the amino acid that corresponds to position 8 of SEQ ID NO: 2 is C, and the amino acid that corresponds to position 86 of SEQ ID NO: 2 is C; (b) the amino acid that corresponds to position 19 of SEQ ID NO: 2 is C, and the amino acid that corresponds to position 117 of SEQ ID NO: 2 is C; (c) the amino acid that corresponds to position 29 of SEQ ID NO: 2 is C, and the amino acid that corresponds to position 110 of SEQ ID NO: 2 is C; (d) the amino acid that corresponds to position 31 of SEQ ID NO: 2 is C, and the amino acid that corresponds to position 62 of SEQ ID NO: 2 is C; (e) the amino acid that corresponds to position 31 of SEQ ID NO: 2 is C, and the amino acid that corresponds to position 63 of SEQ ID NO: 2 is C; (f) the amino acid that corresponds to position 33 of SEQ ID NO: 2 is C, and the amino acid that corresponds to position 61 of SEQ ID NO: 2 is C; (g) the amino acid that corresponds to position 33 of SEQ ID NO: 2 is C, and the amino acid that corresponds to position 63 of SEQ ID NO: 2 is C; (h) the amino acid that corresponds to position 36 of SEQ ID NO: 2 is C, and the amino acid that corresponds to position 106 of SEQ ID NO: 2 is C; (i) the amino acid that corresponds to position 36 of SEQ ID NO: 2 is C, and the amino acid that corresponds to position 107 of SEQ ID NO: 2 is C; (j) the amino acid that corresponds to position 39 of SEQ ID NO: 2 is C, and the amino acid that corresponds to position 105 of SEQ ID NO: 2 is C; (k) the amino acid that corresponds to position 39 of SEQ ID NO: 2 is C, and the amino acid that corresponds to position 107 of SEQ ID NO: 2 is C; (l) the amino acid that corresponds to position 39 of SEQ ID NO: 2 is C, and the amino acid that corresponds to position 112 of SEQ ID NO: 2 is C; and (m) the amino acid that corresponds to position 56 of SEQ ID NO: 2 is C, and the amino acid that corresponds to position 80 of SEQ ID NO: 2 is C.

In some examples, the engineered IL-21 polypeptide comprises one or more of the following: (a) the amino acid that corresponds to position 12 of SEQ ID NO: 2 is M; (b) the amino acid that corresponds to position 16 of SEQ ID NO: 2 is R; (c) the amino acid that corresponds to position 19 of SEQ ID NO: 2 is I; (d) the amino acid that corresponds to position 23 of SEQ ID NO: 2 is D; (e) the amino acid that corresponds to position 105 of SEQ ID NO: 2 is E; (f) the amino acid that corresponds to position 114 of SEQ ID NO: 2 is E; (g) the amino acid that corresponds to position 118 of SEQ ID NO:

2 is S; (h) the amino acid that corresponds to position 121 of SEQ ID NO: 2 is Q; (i) the amino acid that corresponds to position 122 of SEQ ID NO: 2 is K; (j) the amino acid that corresponds to position 124 of SEQ ID NO: 2 is I; (k) the amino acid that corresponds to position 125 of SEQ ID NO: 2 is H; and (l) the amino acid that corresponds to position 128 of SEQ ID NO: 2 is L.

In some examples, the engineered IL-21 polypeptide described herein comprises an amino acid sequence that is at least 85%, 90%, 95%, 96%, 97%, 98%, 99%, or 100% identical to SEQ ID NO: 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, or 15. In some examples, the engineered IL-21 polypeptide comprises an amino acid sequence that is at least 90% identical to SEQ ID NO: 3. In some examples, the engineered IL-21 polypeptide comprises an amino acid sequence that is at least 90% identical to SEQ ID NO: 5. In some examples, the engineered IL-21 polypeptide comprises an amino acid sequence that is at least 90% identical to SEQ ID NO: 9. In some examples, the engineered IL-21 polypeptide comprises an amino acid sequence that is at least 90% identical to SEQ ID NO: 10.

In some examples, the engineered IL-21 polypeptide can induce proliferation of immune cells (e.g., T cells or NK cells). In some examples, the engineered IL-21 polypeptide can induce STAT-3 phosphorylation.

In one example, the disclosure is related to a fusion protein comprising the engineered IL-21 polypeptide described herein. In some examples, the fusion protein described herein further comprises a human serum albumin (HSA). In some examples, the HSA comprises an amino acid sequence that is at least 85%, 90%, 95%, 96%, 97%, 98%, 99%, or 100% identical to SEQ ID NO: 26. In some examples, the engineered IL-21 polypeptide is linked to the C-terminus to the HSA via a linker peptide. In some examples, the linker peptide comprises an amino acid sequence that is at least 85%, 90%, 95%, 96%, 97%, 98%, 99%, or 100% identical to SEQ ID NO: 25. In some examples, the fusion protein further comprises a His-tag, optionally at the N-terminus. In some examples, the fusion protein comprises an amino acid sequence that is at least 85%, 90%, 95%, 96%, 97%, 98%, 99%, or 100% identical to SEQ ID NO: 17, 18, 19, 20, or 21.

In one example, the disclosure is related to a fusion protein comprising, optionally from N-terminus to C-terminus: (a) optionally a His-tag; (b) a HSA; (c) a linker peptide, and (d) an engineered IL-21 polypeptide. In some examples, the His-tag comprises an amino acid sequence that is at least 85%, 90%, 95%, 96%, 97%, 98%, 99%, or 100% identical to SEQ ID NO: 22. In some examples, the HSA comprises an amino acid sequence that is at

least 85%, 90%, 95%, 96%, 97%, 98%, 99%, or 100% identical to SEQ ID NO: 26. In some examples, the linker peptide comprises an amino acid sequence that is at least 85%, 90%, 95%, 96%, 97%, 98%, 99%, or 100% identical to SEQ ID NO: 25. In some examples, the engineered IL-21 polypeptide comprises an amino acid sequence that is at least 85%, 90%, 95%, 96%, 97%, 98%, 99%, or 100% identical to SEQ ID NO: 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, or 15.

In some examples, the fusion protein described herein further comprises an Fc region.

In some examples, the fusion protein can relieve Treg-mediated T cell suppression. In some examples, the fusion protein can induce primary NK cell cytotoxicity.

In one example, the disclosure is related to a protein complex comprising: (a) a first polypeptide comprising from N-terminus to C-terminus: an optional first hinge region, a first Fc region, an optional linker peptide, and the engineered IL-21 polypeptide described herein; and (b) a second polypeptide comprising from N-terminus to C-terminus, an optional second hinge region, a second Fc region. In some examples, the first hinge region, the first Fc region, the second hinge region, and/or the second Fc region are derived from human IgG4. In some examples, the first Fc region and/or the second Fc region comprise one or more knob-into-hole (KIH) mutations. In some examples, the first Fc region comprises a sequence that is at least 80%, 90%, 95%, or 100% to SEQ ID NO: 33, and the second Fc region comprises a sequence that is at least 80%, 90%, 95%, or 100% to SEQ ID NO: 32. In some examples, the first hinge region and/or the second hinge region comprise a sequence that is at least 80%, 90%, 95%, or 100% to SEQ ID NO: 31. In some examples, the linker peptide comprises a sequence that is at least 80%, 90%, 95%, or 100% to SEQ ID NO: 34. In some examples, protein complex described herein comprises one of the followings: (1) the first polypeptide comprises a sequence that is at least 80%, 90%, 95%, or 100% to SEQ ID NO: 35, and the first polypeptide comprises a sequence that is at least 80%, 90%, 95%, or 100% to SEQ ID NO: 29; (2) the first polypeptide comprises a sequence that is at least 80%, 90%, 95%, or 100% to SEQ ID NO: 36, and the first polypeptide comprises a sequence that is at least 80%, 90%, 95%, or 100% to SEQ ID NO: 29; (3) the first polypeptide comprises a sequence that is at least 80%, 90%, 95%, or 100% to SEQ ID NO: 37, and the first polypeptide comprises a sequence that is at least 80%, 90%, 95%, or 100% to SEQ ID NO: 29; (4) the first polypeptide comprises a sequence that is at least 80%, 90%, 95%, or 100% to SEQ ID NO: 38, and the first polypeptide comprises a sequence that is at

least 80%, 90%, 95%, or 100% to SEQ ID NO: 29; or (5) the first polypeptide comprises a sequence that is at least 80%, 90%, 95%, or 100% to SEQ ID NO: 39, and the first polypeptide comprises a sequence that is at least 80%, 90%, 95%, or 100% to SEQ ID NO: 29.

In some examples, the protein complex can induce STAT-3 phosphorylation and/or relieve Treg-mediated T cell suppression. In some examples, the protein complex can induce proliferation of immune cells (e.g., T cells or NK cells), and/or induce primary NK cell cytotoxicity.

In one example, the disclosure is related to a pharmaceutical composition comprising the engineered IL-21 polypeptide, the fusion protein, or the protein complex described herein; and a pharmaceutically acceptable carrier.

In one example, the disclosure is related to a nucleic acid encoding the engineered IL-21 polypeptide, the fusion protein, or the protein complex described herein. In one example, the disclosure is related to a vector comprising the nucleic acid described herein.

In one example, the disclosure is related to a cell comprising the nucleic acid or the vector described herein. In some examples, the cell is a Expi293 cell or CHO-S cell.

In one example, the disclosure is related to a method of producing an engineered IL-21 polypeptide or a fusion protein comprising the engineered IL-21 polypeptide, the method comprising (a) culturing the cell described herein under conditions sufficient for the cell to produce the engineered IL-21 polypeptide or the fusion protein; and (b) collecting the engineered IL-21 polypeptide, the fusion protein, or the protein complex produced by the cell.

In one example, the disclosure is related to a method of treating a subject having cancer, the method comprising administering a therapeutically effective amount of a composition comprising the engineered IL-21 polypeptide, the fusion protein, or the protein complex described herein, to the subject. In some examples, the subject has a solid tumor or a hematologic cancer. In some examples, the cancer is melanoma, renal cell carcinoma (RCC), lymphoma, esophageal adenocarcinoma, lung cancer, hepatocellular carcinoma, cholangiocarcinoma, gallbladder cancer, gastric cancer, pancreatic cancer, colorectal cancer, endometrial carcinoma, ovarian cancer, bladder cancer, or prostate cancer.

In one example, the disclosure is related to a method of decreasing the rate of tumor growth, the method comprising contacting a tumor cell with an effective amount of a composition comprising the engineered IL-21 polypeptide, the fusion protein, or the protein complex described herein.

In one example, the disclosure is related to a method of killing a tumor cell, the method comprising contacting a tumor cell with an effective amount of a composition comprising the engineered IL-21 polypeptide, the fusion protein, or the protein complex described herein.

In one example, the disclosure is related to a method of improving the stability of a protein (e.g., cytokine), comprising (a) providing a 3D structure of the protein (e.g., cytokine), (b) measuring distance of the C_{alpha} atoms of one or more of amino acid residues in the 3D structure; and (c) selecting two amino acid residues from the one or more amino acid residues, in some examples, the C_{alpha} atoms of the two selected amino acid residues are within 3-7 angstroms (e.g., 4.5-6.5 angstroms). In some examples, the method described herein further comprises expressing a protein variant (e.g., a cytokine variant), in some examples, the protein variant comprises a non-native disulfide bond formed by mutating the two selected amino acid residues to cysteines. In some examples, the mutating the two selected amino acid residues to cysteines does not substantially change the 3D structure of the protein (e.g., cytokine).

In one example, the disclosure is related to a method of screening a cytokine variant with an improved anti-tumor efficacy, comprising (a) providing a 3D structure of the cytokine, (b) measuring distance of the C_{alpha} atoms of one or more of amino acid residues in the 3D structure; and (c) selecting two amino acid residues from the one or more amino acid residues, in some examples, the C_{alpha} atoms of the two selected amino acid residues are within 3-7 angstroms (e.g., 4.5-6.5 angstroms). In some examples, the method described herein further comprises (d) expressing a cytokine variant, in some examples, the variant comprises a non-native disulfide bond formed by mutating the two selected amino acid residues to cysteines; (e) administering the cytokine variant to a tumor-bearing animal; and (f) determining tumor growth (e.g., by measuring tumor volume) in the tumor-bearing animal.

In some examples, the protein or cytokine described herein is IL-21 (e.g., human IL-21).

In some examples, the protein or cytokine has no more than 90, 100, 110, 120, 130, 140, 150, 160, 170, 180, 190, or 200 amino acid residues.

As used herein, the term “non-native disulfide bond” refers to a disulfide bond that does not naturally exist in a wild-type protein. In some examples, the non-native disulfide bond is formed by two cysteine residues, wherein at least one of them is a mutation. In some examples, two of them are mutations. In some examples, at least one or two cysteine residues are introduced by insertion. In some examples, a deletion changes the distance

between two existing cysteine residues, which then forms a disulfide bond that does not exist in a wild-type protein.

As used herein, the term "engineered IL-21 polypeptide" refers to a polypeptide derived from a wild-type IL-21 polypeptide or a portion thereof, optionally with one or more mutations (e.g., insertions, deletions, or substitutions). In some examples, the engineered IL-21 polypeptide comprises or consists of an amino acid sequence corresponding to amino acids 25-162 of human IL-21 (SEQ ID NO: 1). In some examples, the engineered IL-21 polypeptide has one or more (e.g., 1, 2, 3, 4, 5, 6, 7, 8, 9, or 10) mutations (e.g., amino acids are substituted by cysteine).

As used herein, the term "protein construct" refers to a complex having one or more polypeptides. In some examples, the protein construct is a fusion protein, e.g., a fusion protein including a HSA and an engineered IL-21 polypeptide (e.g., any of the engineered IL-21 polypeptides described herein). In some examples, the protein construct has two or more polypeptides, wherein the polypeptides can associate with each other, forming a dimer or a multimer (e.g., a trimer). In some examples, the protein construct is a heterodimeric Fc-fused IL-21 (e.g., any of the heterodimeric Fc-fused IL-21 or its variants described herein).

As used herein, the term "cancer" refers to cells having the capacity for uncontrolled autonomous growth. Examples of such cells include cells having an abnormal state or condition characterized by rapidly proliferating cell growth. The term is meant to include cancerous growths, e.g., tumors; oncogenic processes, metastatic tissues, and malignantly transformed cells, tissues, or organs, irrespective of histopathologic type or stage of invasiveness. Also included are malignancies of the various organ systems, such as respiratory, cardiovascular, renal, reproductive, hematological, neurological, hepatic, gastrointestinal, and endocrine systems; as well as adenocarcinomas which include malignancies such as most colon cancers, renal-cell carcinoma, prostate cancer and/or testicular tumors, non-small cell carcinoma of the lung, and cancer of the small intestine. Cancer that is "naturally arising" includes any cancer that is not experimentally induced by implantation of cancer cells into a subject, and includes, for example, spontaneously arising cancer, cancer caused by exposure of a patient to a carcinogen(s), cancer resulting from insertion of a transgenic oncogene or knockout of a tumor suppressor gene, and cancer caused by infections, e.g., viral infections. The term "carcinoma" is art recognized and refers to malignancies of epithelial or endocrine tissues. The term also includes carcinosarcomas, which include malignant tumors composed of carcinomatous and sarcomatous tissues. An

"adenocarcinoma" refers to a carcinoma derived from glandular tissue or in which the tumor cells form recognizable glandular structures. The term "sarcoma" is art recognized and refers to malignant tumors of mesenchymal derivation. The term "hematopoietic neoplastic disorders" includes diseases involving hyperplastic/neoplastic cells of hematopoietic origin. A hematopoietic neoplastic disorder can arise from myeloid, lymphoid or erythroid lineages, or precursor cells thereof. A hematologic cancer is a cancer that begins in blood-forming tissue, such as the bone marrow, or in the cells of the immune system. Examples of hematologic cancer include e.g., leukemia, lymphoma, and multiple myeloma etc.

As used herein, the terms "subject" and "patient" are used interchangeably throughout the specification and describe an animal, human or non-human, to whom treatment according to the methods of the present disclosure is provided. Veterinary and non-veterinary applications are contemplated in the present disclosure. Human patients can be adult humans or juvenile humans (e.g., humans below the age of 18 years old). In addition to humans, patients include but are not limited to mice, rats, hamsters, guinea-pigs, rabbits, ferrets, cats, dogs, and primates. Included are, for example, non-human primates (e.g., monkey, chimpanzee, gorilla, and the like), rodents (e.g., rats, mice, gerbils, hamsters, ferrets, rabbits), lagomorphs, swine (e.g., pig, miniature pig), equine, canine, feline, bovine, and other domestic, farm, and zoo animals.

As used herein, the terms "polypeptide," "peptide," and "protein" are used interchangeably to refer to polymers of amino acids of any length of at least two amino acids.

As used herein, the terms "polynucleotide," "nucleic acid molecule," and "nucleic acid sequence" are used interchangeably herein to refer to polymers of nucleotides of any length of at least two nucleotides, and include, without limitation, DNA, RNA, DNA/RNA hybrids, and modifications thereof.

Unless otherwise defined, all technical and scientific terms used herein have the same meaning as commonly understood by one of ordinary skill in the art to which this disclosure belongs. Methods and materials are described herein for use in the present disclosure; other, suitable methods and materials known in the art can also be used. The materials, methods, and examples are illustrative only and not intended to be limiting. All publications, patent applications, patents, sequences, database entries, and other references mentioned herein are incorporated by reference in their entirety. In case of conflict, the present specification, including definitions, will control.

Other features and advantages of the disclosure will be apparent from the following detailed description and figures, and from the claims.

WHAT IS CLAIMED IS:

1. An engineered IL-21 polypeptide, wherein the engineered IL-21 polypeptide comprises a non-native disulfide bond and an amino acid sequence that is at least 80% identical to SEQ ID NO: 2, wherein two or more amino acid residues corresponding to positions selected from the group consisting of positions 8, 19, 29, 31, 33, 36, 39, 56, 61,62, 63, 80, 86, 105, 106, 107, 110, 112 and 117 of SEQ ID NO: 2 are substituted with cysteine (C).

2. The engineered IL-21 polypeptide of claim 1, wherein the engineered IL-21 polypeptide can bind to a complex formed by human IL-21 receptor (IL-21R) and common cytokine γ chain (γc).

3. The engineered IL-21 polypeptide of claim 1 or 2, wherein the engineered IL-21 polypeptide comprises one or more of the following:
   (a) the amino acid that corresponds to position 8 of SEQ ID NO: 2 is C, and the amino acid that corresponds to position 86 of SEQ ID NO: 2 is C;
   (b) the amino acid that corresponds to position 19 of SEQ ID NO: 2 is C, and the amino acid that corresponds to position 117 of SEQ ID NO: 2 is C;
   (c) the amino acid that corresponds to position 29 of SEQ ID NO: 2 is C, and the amino acid that corresponds to position 110 of SEQ ID NO: 2 is C;
   (d) the amino acid that corresponds to position 31 of SEQ ID NO: 2 is C, and the amino acid that corresponds to position 62 of SEQ ID NO: 2 is C;
   (e) the amino acid that corresponds to position 31 of SEQ ID NO: 2 is C, and the amino acid that corresponds to position 63 of SEQ ID NO: 2 is C;
   (f) the amino acid that corresponds to position 33 of SEQ ID NO: 2 is C, and the amino acid that corresponds to position 61 of SEQ ID NO: 2 is C;
   (g) the amino acid that corresponds to position 33 of SEQ ID NO: 2 is C, and the amino acid that corresponds to position 63 of SEQ ID NO: 2 is C;
   (h) the amino acid that corresponds to position 36 of SEQ ID NO: 2 is C, and the amino acid that corresponds to position 106 of SEQ ID NO: 2 is C;
   (i) the amino acid that corresponds to position 36 of SEQ ID NO: 2 is C, and the amino acid that corresponds to position 107 of SEQ ID NO: 2 is C;
   (j) the amino acid that corresponds to position 39 of SEQ ID NO: 2 is C, and the amino acid that corresponds to position 105 of SEQ ID NO: 2 is C;

(k) the amino acid that corresponds to position 39 of SEQ ID NO: 2 is C, and the amino acid that corresponds to position 107 of SEQ ID NO: 2 is C;
(l) the amino acid that corresponds to position 39 of SEQ ID NO: 2 is C, and the amino acid that corresponds to position 112 of SEQ ID NO: 2 is C; and
(m) the amino acid that corresponds to position 56 of SEQ ID NO: 2 is C, and the amino acid that corresponds to position 80 of SEQ ID NO: 2 is C.

4. The engineered IL-21 polypeptide of any one of claims 1-3, comprising an amino acid sequence that is at least 85%, 90%, 95%, 96%, 97%, 98%, 99%, or 100% identical to SEQ ID NO: 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, or 15.

5. The engineered IL-21 polypeptide of any one of claims 1-4, wherein the engineered IL-21 polypeptide comprises one or more of the following: (a) an amino acid sequence that is at least 90% identical to SEQ ID NO: 3;
(b) an amino acid sequence that is at least 90% identical to SEQ ID NO: 5;
(c) an amino acid sequence that is at least 90% identical to SEQ ID NO: 9; and
(d) an amino acid sequence that is at least 90% identical to SEQ ID NO: 10.

6. A fusion protein comprising, optionally from N-terminus to C-terminus:
(a) optionally a His-tag;
(b) a human serum albumin (HSA);
(c) a linker peptide; and
(d) an engineered IL-21 polypeptide.

7. The fusion protein of claim 6, wherein the engineered IL-21 polypeptide is linked to the C-terminus to the HSA via a linker peptide.

8. The fusion protein of claim 6 or 7, wherein the His-tag comprises an amino acid sequence that is at least 85%, 90%, 95%, 96%, 97%, 98%, 99%, or 100% identical to SEQ ID NO: 22; wherein the HSA comprises an amino acid sequence that is at least 85%, 90%, 95%, 96%, 97%, 98%, 99%, or 100% identical to SEQ ID NO: 26; wherein the linker peptide comprises an amino acid sequence that is at least 85%, 90%, 95%, 96%, 97%, 98%, 99%, or 100% identical to SEQ ID NO: 25; and/or wherein the engineered IL-21 polypeptide comprises an amino acid sequence of any one of claims 1-5.

9. The fusion protein of any one of claims 6-8, wherein the fusion protein comprises an amino acid sequence that is at least 85%, 90%, 95%, 96%, 97%, 98%, 99%, or 100% identical to SEQ ID NO: 17, 18, 19, 20, or 21.

10. The fusion protein of any one of claims 6-9, further comprising an Fc region.

11. A protein complex comprising:
(a) a first polypeptide comprising from N-terminus to C-terminus: an optional first hinge region, a first Fc region, an optional linker peptide, and the engineered IL-21 polypeptide of any one of claims 1-5; and
(b) a second polypeptide comprising from N-terminus to C-terminus, an optional second hinge region, and a second Fc region.

12. The protein complex of claim 11, wherein the first hinge region, the first Fc region, the second hinge region, and/or the second Fc region are derived from human IgG with reduced or no effector function.

13. The protein complex of claim 11 or 12, wherein the first Fc region and/or the second Fc region comprise one or more knob-into-hole (KIH) mutations.

14. The protein complex of any one of claims 11-13, wherein the protein complex comprises one or more of the following: (a) the first Fc region comprises a sequence that is at least 80%, 90%, 95%, or 100% to SEQ ID NO: 33, and the second Fc region comprises a sequence that is at least 80%, 90%, 95%, or 100% to SEQ ID NO: 32;
(b) the first hinge region and/or the second hinge region comprise a sequence that is at least 80%, 90%, 95%, or 100% to SEQ ID NO: 31; and
(c) the linker peptide comprises a sequence that is at least 80%, 90%, 95%, or 100% to SEQ ID NO: 34.

15. The protein complex of any one of claims 11-14, wherein:
(1) the first polypeptide comprises a sequence that is at least 80%, 90%, 95%, or 100% to SEQ ID NO: 35, and the second polypeptide comprises a sequence that is at least 80%, 90%, 95%, or 100% to SEQ ID NO: 29;

(2) the first polypeptide comprises a sequence that is at least 80%, 90%, 95%, or 100% to SEQ ID NO: 36, and the second polypeptide comprises a sequence that is at least 80%, 90%, 95%, or 100% to SEQ ID NO: 29;

(3) the first polypeptide comprises a sequence that is at least 80%, 90%, 95%, or 100% to SEQ ID NO: 37, and the second polypeptide comprises a sequence that is at least 80%, 90%, 95%, or 100% to SEQ ID NO: 29;

(4) the first polypeptide comprises a sequence that is at least 80%, 90%, 95%, or 100% to SEQ ID NO: 38, and the second polypeptide comprises a sequence that is at least 80%, 90%, 95%, or 100% to SEQ ID NO: 29; or

(5) the first polypeptide comprises a sequence that is at least 80%, 90%, 95%, or 100% to SEQ ID NO: 39, and the second polypeptide comprises a sequence that is at least 80%, 90%, 95%, or 100% to SEQ ID NO: 29.

16. Use of the engineered IL-21 polypeptide of any one of claims 1-5, the fusion protein of any one of claims 6-10, the protein complex of any one of claims 11-15 in the manufacture of a medicament for inducing STAT-3 phosphorylation, and/or relieving Treg-mediated T cell suppression, and/or inducing proliferation of immune cells (e.g., T cells or NK cells), and/or inducing primary NK cell cytotoxicity.

17. A pharmaceutical composition comprising the engineered IL-21 polypeptide of any one of claims 1-5, the fusion protein of any one of claims 6-10, or the protein complex of any one of claims 11-15; and a pharmaceutically acceptable carrier.

18. A nucleic acid encoding the engineered IL-21 polypeptide of any one of claims 1-5, the fusion protein of any one of claims 6-10, or the protein complex of any one of claims 11-15.

19. A vector comprising the nucleic acid of claim 18.

20. A cell comprising the nucleic acid of claim 18 or the vector of claim 19.

21. Use of a therapeutically effective amount of a composition comprising the engineered IL-21 polypeptide of any one of claims 1-5, the fusion protein of any one of claims 6-10, or

the protein complex of any one of claims 11-15 in the manufacture of a medicament for treating a subject having cancer .

22. A method of improving the stability of a protein (e.g., cytokine), comprising
    (a) providing a 3D structure of the protein (e.g., cytokine),
    (b) measuring distance of the C_{alpha} atoms of one or more of amino acid residues in the 3D structure; and
    (c) selecting two amino acid residues from the one or more amino acid residues, wherein the C_{alpha} atoms of the two selected amino acid residues are within 3-7 angstroms.

23. The method of claim 22, further comprising expressing a protein variant (e.g., a cytokine variant), wherein the protein variant comprises a non-native disulfide bond formed by mutating the two selected amino acid residues to cysteines.

24. The method of claim 22 or 23, wherein the mutating the two selected amino acid residues to cysteines does not substantially change the 3D structure of the protein (e.g., cytokine).

25. The method of any one of claims 22-24, wherein the protein has no more than 200 amino acid residues.

26. The method of any one of claims 22-25, wherein the protein or cytokine is IL-21 (e.g., human IL-21).