

CLAIMS

WHAT IS CLAIMED IS:

1. A recombinant polypeptide comprising:
 - an amino acid sequence having one or more 70%, 80%, 90%, 95%, 99%, or 100% sequence identity to an interleukin 12 subunit p40 (IL-12p40) polypeptide having the amino acid sequence of SEQ ID NO: 1;
 - and further comprising one or more amino acid substitution at a position corresponding to an amino acid residue selected from the group consisting of X37, X39, X40, X41, X80, X81, X82, X106, X108, X115, X216, X217, X218, and X219 of SEQ ID NO: 1.
- ~~2. The recombinant polypeptide of claim 1, wherein the one or more amino acid substitution is at a position corresponding to an amino acid residue selected from the group consisting of X37, X39, X40, X81, X82, X106, X217, and X219 of SEQ ID NO: 1.~~
- ~~3. The recombinant polypeptide of any one of claims 1 to 2, wherein the one or more amino acid substitution is independently selected from the group consisting of an alanine (A) substitution, an arginine (R) substitution, an asparagine (N) substitution, an aspartic acid (D) substitution, a leucine (L) substitution, a lysine (K) substitution, a phenylalanine (F) substitution, a lysine substitution, a glutamine (Q) substitution, a glutamic acid (E) substitution, a serine (S) substitution, and a threonine (T) substitution.~~
42. The recombinant polypeptide of ~~any one of claims 1 to 3~~, wherein the one or more amino acid substitution is at a position corresponding to an amino acid residue selected from the group consisting of W37, P39, D40, A41, K80, E81, F82, K106, E108, D115, H216, K217, L218, and K219 of SEQ ID NO: 1.
- ~~5. The recombinant polypeptide of any one of claims 1 to 4, wherein the one or more amino acid substitution is at a position corresponding to an amino acid residue selected from the group consisting of W37, P39, D40, E81, F82, K106, K217, and K219 of SEQ ID NO: 1.~~
63. The recombinant polypeptide of any one of claims 1 to ~~25~~, comprising an amino acid sequence having at least 70%, 80%, 90%, 95%, 99%, or 100% sequence identity to SEQ ID

NO: 1, and further comprising the amino acid substitutions corresponding to the following amino acid substitutions:

- a) W37A;
- b) P39A;
- c) D40A;
- d) E81A;
- e) F82A;
- f) K106;
- g) D109A;
- h) K217A;
- i) K219A;
- j) E81A/F82A;
- k) W37A/E81A/F82A;
- l) E81A/F82A/K106A;
- m) E81A/F82A/K106A/K219A;
- n) E81A/F82A/K106A/K217A;
- o) 81A/F82A/K106A/E108A/D115A;
- p) E81F/F82A;
- q) E81K/F82A;
- r) E81L/F82A;
- s) E81H/F82A;
- t) E81S/F82A;
- u) E81A/F82A/K106N;
- v) E81A/F82A/K106Q;
- w) E81A/F82A/K106T;
- x) E81A/F82A/K106R; or
- (y) P39A/D40A/E81A/F82A.

74. The recombinant polypeptide of any one of claims 1 to 36, comprising an amino acid sequence selected from the group consisting of SEQ ID NOS: 3-8 and 13-16.

85. A recombinant polypeptide comprising:

an amino acid sequence having one or more 70%, 80%, 90%, 95%, 99%, or 100%

sequence identity to an interleukin 12 subunit p40 (IL-12p40) polypeptide having the amino acid sequence of SEQ ID NO: 2;

and further comprising one or more amino acid substitution at a position corresponding to an amino acid residue selected from the group consisting of X37, X39, X40, X41, X80, X81, X82, X106, X108, X115, X216, X217, X218, and X219 of SEQ ID NO: 2.

~~9. The recombinant polypeptide of claim 8, wherein the one or more amino acid substitution is at a position corresponding to an amino acid residue selected from the group consisting of X37, X39, X40, X81, X82, X106, X217, and X219 of SEQ ID NO: 2.~~

~~10. The recombinant polypeptide of any one of claims 8 to 9, wherein the one or more amino acid substitution is independently selected from the group consisting of an alanine (A) substitution, an arginine (R) substitution, an asparagine (N) substitution, an aspartic acid (D) substitution, a leucine (L) substitution, a lysine (K) substitution, a phenylalanine (F) substitution, a lysine substitution, a glutamine (Q) substitution, a glutamic acid (E) substitution, a serine (S) substitution, and a threonine (T) substitution.~~

~~116.~~ The recombinant polypeptide of ~~any one of claims 58 to 10~~, wherein the one or more amino acid substitution is at a position corresponding to an amino acid residue selected from the group consisting of W37, P39, D40, A41, K80, E81, F82, K106, E108, D115, H216, K217, L218, and E219 of SEQ ID NO: 2.

~~12. The recombinant polypeptide of any one of claims 8 to 11, wherein the one or more amino acid substitution is at a position corresponding to an amino acid residue selected from the group consisting of P39, D40, E81, F82, K106, K217, and E219 of SEQ ID NO: 2.~~

~~137.~~ The recombinant polypeptide of any one of claims ~~58 to 612~~, comprising an amino acid sequence having one or more 70%, 80%, 90%, 95%, 99%, or 100% sequence identity to SEQ ID NO: 2, and further comprising the amino acid substitutions corresponding to the following amino acid substitutions:

- a) W37A;
- b) P39A;
- c) D40A;
- d) E81A;

- e) F82A;
- f) K106;
- g) D109A;
- h) K217A;
- i) E219A;
- j) E81A/F82A;
- k) W37A/E81A/F82A;
- l) E81A/F82A/K106A;
- m) E81A/F82A/K106A/K217A;
- n) E81F/F82A;
- o) E81K/F82A;
- p) E81L/F82A;
- q) E81H/F82A;
- r) E81S/F82A;
- s) E81A/F82A/K106N;
- t) E81A/F82A/K106Q;
- u) E81A/F82A/K106T;
- v) E81A/F82A/K106R; or
- w) P39A/D40A/E81A/F82A.

148. The recombinant polypeptide of ~~any one of claims 58 to 713~~, comprising an amino acid sequence selected from the group consisting of SEQ ID NOS: 9-11 and 17-25.

159. The recombinant polypeptide of any one of claims 1 to 814, wherein:

(a) the recombinant polypeptide has an altered binding affinity for interleukin-12 receptor, subunit beta 1 (IL-12R β 1) compared to binding affinity of a reference polypeptide lacking the one or more amino acid substitution; and/or

(b) the recombinant polypeptide has a reduced binding affinity for IL-12R β 1 compared to binding affinity of a reference polypeptide lacking the one or more amino acid substitution; and/or

(c) the recombinant polypeptide has binding affinity for IL-12R β 1 reduced by about 10% to about 100% compared to binding affinity of a reference polypeptide lacking the one or more amino acid substitution, as determined by surface plasmon resonance (SPR).

~~16. The recombinant polypeptide of claim 15, wherein the recombinant polypeptide has a reduced binding affinity for IL-12R β 1 compared to binding affinity of a reference polypeptide lacking the one or more amino acid substitution.~~

~~17. The recombinant polypeptide of any one of claim 15 to 15, wherein the recombinant polypeptide has binding affinity for IL-12R β 1 reduced by about 10% to about 100% compared to binding affinity of a reference polypeptide lacking the one or more amino acid substitution, as determined by surface plasmon resonance (SPR).~~

~~18~~10. The recombinant polypeptide of ~~any one of claim 9~~15 to 17, wherein the recombinant polypeptide, when combined with an interleukin 12 subunit p35 (IL-12p35) polypeptide, has a reduced capability to stimulate STAT4 signaling compared to a reference polypeptide lacking the one or more amino acid substitution.

~~19~~11. The recombinant polypeptide of any one of claims ~~9~~15 to ~~10~~18, wherein the recombinant polypeptide, when combined with an interleukin 23 subunit p19 (IL-23p19) polypeptide, has a reduced capability to stimulate STAT3 signaling compared to a reference polypeptide lacking the one or more amino acid substitution.

~~20. The recombinant polypeptide of any one of claims 18 to 19, wherein the STAT3 signaling and/or STAT4 signaling is determined by an assay selected from the group consisting of by a gene expression assay, a phospho-flow signaling assay, and an enzyme-linked immunosorbent assay (ELISA).~~

~~21~~12. The recombinant polypeptide of any one of claims ~~9~~15 to ~~11~~20, wherein the one or more amino acid substitution results in a cell-type biased signaling of the downstream signal transduction mediated through interleukin-12 (IL-12) and/or interleukin-23 (IL-23) compared to a reference polypeptide lacking the one or more amino acid substitution.

~~22~~13. The recombinant polypeptide of claim ~~12~~24, wherein:

(a) the cell-type biased signaling comprises a reduced capability of the recombinant polypeptide to stimulate IL-12-mediated signaling in natural killer (NK) cells; and/or

- (b) the cell-type biased signaling comprises a substantially unaltered capability of the recombinant polypeptide to stimulate IL-12 signaling in CD8+ T cells; and/or
- (c) the one or more amino acid substitution results in a reduced capability of the recombinant polypeptide to stimulate IL-12 signaling in NK cells while substantially retains its capability to stimulate IL-12 signaling in CD8+ T cells.

~~23. The recombinant polypeptide of any one of claim 21 to 22, wherein the cell-type biased signaling comprises a substantially unaltered capability of the recombinant polypeptide to stimulate IL-12 signaling in CD8+ T cells.~~

~~24. The recombinant polypeptide of any one of claim 21 to 23, wherein the one or more amino acid substitution results in a reduced capability of the recombinant polypeptide to stimulate IL-12 signaling in NK cells while substantially retains its capability to stimulate IL-12 signaling in CD8+ T cells.~~

2514. A recombinant nucleic acid molecule comprising a nucleic acid sequence encoding a polypeptide that comprises an amino acid sequence having at least 90% sequence identity to the amino acid sequence of the polypeptide of any one of claims 1 to 1324.

~~26. The nucleic acid molecule of claim 25, wherein the nucleic acid sequence is operably linked to a heterologous nucleic acid sequence.~~

~~27. The nucleic acid molecule of any one of claims 25 to 26, wherein the nucleic acid molecule is further defined as an expression cassette or an expression vector.~~

2815. A recombinant cell comprising:

- a) a recombinant polypeptide according to any one of claims 1 to 1324; and/or
- b) a recombinant nucleic acid according to ~~any one of claims 1425 to 27.~~

~~2916.~~ The recombinant cell of claim 1528, wherein the recombinant cell is a eukaryotic cell.

~~3017.~~ The recombinant cell of claim 1629, wherein the eukaryotic cell is a mammalian cell

~~3118.~~ A cell culture comprising at least one recombinant cell of any one of claims 1528 to 1730 and a culture medium.

~~3219.~~ A method for producing a recombinant polypeptide, comprising:

- a) providing one or more recombinant cells of any one of claims 1528 to 1730; and

- b) culturing the one or more recombinant cells in a culture medium such that the cells produce the polypeptide encoded by the recombinant nucleic acid molecule.

~~33. The method of claim 32, further comprising isolating and/or purifying the produced polypeptide.~~

~~34~~20. The method of ~~any one of claims 1932 to 33~~, further comprising structurally modifying the produced polypeptide to increase half-life.

~~35~~21. The method of claim ~~20~~34, wherein said modification comprises one or more alterations selected from the group consisting of fusion to a human Fc antibody fragment, fusion to albumin, and PEGylation.

~~36~~22. A recombinant polypeptide produced by the method of any one of claims ~~1932 to 21~~35.

~~37~~23. A pharmaceutical composition comprising:

- a) a recombinant polypeptide according to any one of claims 1-~~24~~13 and ~~22~~36;
- b) a recombinant nucleic acid according to ~~any one of claims 1425 to 27~~;
- c) a recombinant cell according to any one of claims to ~~1528 to 1730~~; and/or
- c) a pharmaceutically acceptable carrier.

~~38. The pharmaceutical composition of claim 37, wherein the composition comprises a recombinant polypeptide according to any one of claims 1-24 and 36, and a pharmaceutically acceptable carrier.~~

~~39. The pharmaceutical composition of claim 37, wherein the composition comprises a recombinant nucleic acid according to any one of claims 25 to 27, and a pharmaceutically acceptable carrier.~~

~~40~~24. A method for modulating IL-12 mediated signal transduction in a subject, the method comprising administering to the subject Use of a composition comprising:

- a) a recombinant IL-12p40 polypeptide according to any one of claims 1-~~13~~24 and ~~22~~36;
- b) a recombinant nucleic acid according to ~~any one of claims 1425 to 27~~;
- c) a recombinant cell according to any one of claims to ~~1528 to 1730~~; and/or
- d) a pharmaceutically composition according to claims ~~2337 to 39~~,

in the preparation of a medicament for modulating IL-12-mediated signal transduction in a subject.

4125. The ~~method-use~~ of claim 2440, further comprising ~~administering to the subject~~ an IL-12p35 polypeptide, or nucleic acid encoding the IL-12p35 polypeptide, to be administered to the subject.
4226. ~~A method for modulating IL-23 mediated signal transduction in a subject, the method comprising administering to the subject~~ Use of a composition comprising:
- a) a recombinant IL-12p40 polypeptide according to any one of claims 1-1324 and 2236;
 - b) a recombinant nucleic acid according to ~~any one of claims~~ 1425 to 27;
 - c) a recombinant cell according to any one of claims to 1528 to 1730; and/or
 - d) a pharmaceutically composition according to claims 2337 to 39,
- in the preparation of a medicament for modulating IL-23-mediated signal transduction in a subject..
4327. The ~~method-use~~ of claim 2642, further comprising ~~administering to the subject~~ an IL-12p35 polypeptide, or nucleic acid encoding the IL-23p19 polypeptide, to be administered to the subject.
4428. ~~A method for the treatment of a condition in a subject in need thereof, the method comprising administering to the subject~~ Use of a composition comprising:
- a) a recombinant IL-12p40 polypeptide according to any one of claims 1-1324 and 2236;
 - b) a recombinant nucleic acid according to ~~any one of claims~~ 1425 to 27;
 - c) a recombinant cell according to any one of claims to 1528 to 1730; and/or
 - d) a pharmaceutically composition according to claims 2337 to 39,
- in the preparation of a medicament for the treatment of a condition in a subject.
4529. The ~~method-use~~ of claim 2844, further comprising ~~administering to the subject~~:
- a) an IL-12p35 polypeptide;
 - b) an IL-23p19 polypeptide; and/or
 - c) nucleic acid encoding (a) or (b) above,
- to be administered to the subject.
4630. The ~~method-use~~ of any one of claims 2840 to 2945, wherein:

(a) the recombinant polypeptide has an altered binding affinity for interleukin-12 receptor, beta 1 (IL-12R β 1) compared to binding affinity of a reference polypeptide lacking the one or more amino acid substitution; and/or

(b) the recombinant polypeptide has a reduced binding affinity for IL-12R β 1 compared to binding affinity of a reference polypeptide lacking the one or more amino acid substitution; and/or

(c) the recombinant polypeptide has binding affinity for IL-12R β 1 reduced by about 10% to about 100% compared to binding affinity of a reference polypeptide lacking the one or more amino acid substitution, as determined by surface plasmon resonance (SPR).

~~47. The method use of any one of claims 40 to 46, wherein the recombinant polypeptide has a reduced binding affinity for IL-12R β 1 compared to binding affinity of a reference polypeptide lacking the one or more amino acid substitution.~~

~~48. The method use of any one of claims 40 to 47, wherein the recombinant polypeptide has binding affinity for IL-12R β 1 reduced by about 10% to about 100% compared to binding affinity of a reference polypeptide lacking the one or more amino acid substitution, as determined by surface plasmon resonance (SPR).~~

~~49~~31. The ~~method use of any one of claims 30~~40 to 48, wherein the reduced binding affinity of the recombinant polypeptide to IL-12R β 1 receptor results in a reduction in STAT4-mediated signaling compared to a reference polypeptide lacking the one or more amino acid substitution.

~~50~~32. The ~~method use of any one of claims 30~~40 to ~~31~~49, wherein the reduced binding affinity of the recombinant polypeptide to IL-12R β 1 receptor results in a reduction in STAT3-mediated signaling compared to a reference polypeptide lacking the one or more amino acid substitution.

~~51. The method of any one of claims 49 to 50, wherein the STAT3 signaling and/or STAT4 signaling is determined by an assay selected from the group consisting of by a gene expression assay, a phospho-flow signaling assay, and an enzyme-linked immunosorbent assay (ELISA).~~

~~5233.~~ The ~~method-use~~ of any one of claims ~~2840~~ to ~~3251~~, wherein the administered composition to be administered results in a cell-type biased signaling of the downstream signal transduction mediated by interleukin-12 (IL-12) and/or by interleukin-23 (IL-23) compared to a reference polypeptide lacking the one or more amino acid substitution.

~~5334.~~ The ~~method-use~~ of claim ~~52 33~~, wherein:

(a) the cell-type biased signaling comprises a reduced capability of the recombinant polypeptide to stimulate IL-12-mediated signaling in NK cells; and/or

(b) the cell-type biased signaling comprises a substantially unaltered capability of the recombinant polypeptide to stimulate IL-12 signaling in CD8+ T cells; and/or

(c) the composition to be administered results in a reduced capability of the recombinant polypeptide to stimulate IL-12 signaling in NK cells while substantially retains its capability to stimulate IL-12 signaling in CD8+ T cells; and/or

(d) the composition to be administered substantially retains the recombinant polypeptide's capability to stimulate expression of interferon gamma (INF γ) in CD8+ T cells; and/or

(e) the composition to be administered enhances antitumor immunity in a tumor microenvironment.

~~54. The method of any one of claims 52 to 53, wherein the cell-type biased signaling comprises a substantially unaltered capability of the recombinant polypeptide to stimulate IL-12 signaling in CD8+ T cells.~~

~~55. The method of any one of claims 40 to 54, wherein the administered composition results in a reduced capability of the recombinant polypeptide to stimulate IL-12 signaling in NK cells while substantially retains its capability to stimulate IL-12 signaling in CD8+ T cells.~~

~~56. The methods of claim 55, wherein the administered composition substantially retains the recombinant polypeptide's capability to stimulate expression of interferon gamma (INF γ) in CD8+ T cells.~~

~~57. The method of any one of claims 40 to 56, wherein the administered composition enhances antitumor immunity in a tumor microenvironment.~~

~~5835.~~ The ~~method-use~~ of any one of claims ~~2440~~ to ~~3457~~, wherein the subject is a mammal.

~~59. The method of claim 58, wherein the mammal is a human.~~

~~6036.~~ The method-use of any one of claims 2440 to 3559, wherein the subject has or is suspected of having a condition associated with IL-12p40 mediated signaling.

~~6137.~~ The method-use of claim 3660, wherein the IL-12p40 mediated signaling is IL-12 mediated signaling or IL-23 mediated signaling.

~~62. The method of claim 60, wherein the condition is a cancer, an immune disease, or a chronic infection.~~

~~63. The method of claim 62, wherein the immune disease is an autoimmune disease.~~

~~64. The method of claim 63, wherein the autoimmune disease is selected from the group consisting of rheumatoid arthritis, insulin-dependent diabetes mellitus, hemolytic anemias, rheumatic fever, thyroiditis, Crohn's disease, myasthenia gravis, glomerulonephritis, autoimmune hepatitis, multiple sclerosis, alopecia areata, psoriasis, vitiligo, dystrophic epidermolysis bullosa, systemic lupus erythematosus, moderate to severe plaque psoriasis, psoriatic arthritis, Crohn's disease, ulcerative colitis, and graft vs. host disease.~~

~~65. The method of claim 62, wherein the condition is a cancer selected from the group consisting of an acute myeloma leukemia, an anaplastic lymphoma, an astrocytoma, a B-cell cancer, a breast cancer, a colon cancer, an ependymoma, an esophageal cancer, a glioblastoma, a glioma, a leiomyosarcoma, a liposarcoma, a liver cancer, a lung cancer, a mantle cell lymphoma, a melanoma, a neuroblastoma, a non-small cell lung cancer, an oligodendroglioma, an ovarian cancer, a pancreatic cancer, a peripheral T-cell lymphoma, a renal cancer, a sarcoma, a stomach cancer, a carcinoma, a mesothelioma, and a sarcoma.~~

~~6638.~~ The method-use of any one of claims 2440 to 3765, wherein the composition is to be administered to the subject individually as a first therapy or in combination with a second therapy.

~~67. The method of claim 66, wherein the second therapy is selected from the group consisting of chemotherapy, radiotherapy, immunotherapy, hormonal therapy, toxin therapy, or surgery.~~

~~68. The method of any one of claims 66 to 67, wherein the first therapy and the second therapy are administered concomitantly.~~

- ~~69. The method of any one of claims 66 to 68, wherein the first therapy is administered at the same time as the second therapy.~~
- ~~70. The method of any one of claims 66 to 68, wherein the first therapy and the second therapy are administered sequentially.~~
- ~~71. The method of claim 70, wherein the first therapy is administered before the second therapy.~~
- ~~72. The method of claim 70, wherein the first therapy is administered after the second therapy.~~
- ~~73. The method of claim 70, wherein the first therapy is administered before and/or after the second therapy.~~
- ~~74. The method of any one of claims 66 to 73, wherein the first therapy and the second therapy are administered in rotation.~~
- ~~75. The method of any one of claims 66 to 67, wherein the first therapy and the second therapy are administered together in a single formulation.~~
7639. A kit for modulating IL-12p40 mediated signal transduction, modulating IL-12p40-mediated signal transduction, or treating a condition in a subject in need thereof, the system comprising:
- a) a recombinant polypeptide according to any one of claims 1-~~13~~24 and 2236;
 - b) a recombinant nucleic acid according to ~~any one of claims 1425 to 27~~;
 - c) a recombinant cell according to any one of claims 1528 to 1731; and/or
 - d) a pharmaceutical composition according to ~~any one of claims 2337 to 39~~; and instructions for performing the method of any one of claims 40 to 75.
- ~~7740.~~ Use of the following for the manufacture of a medicament, for the treatment and/or prevention of a condition associated with a health condition associated with a perturbation in IL-12-p40 mediated signal transduction:
- a) a recombinant polypeptide according to any one of claims 1-~~13~~24 and 2236;
 - b) a recombinant nucleic acid according to ~~any one of claims 1425 to 27~~;
 - c) a recombinant cell according to any one of claims 1528 to 1731; and/or
 - d) a pharmaceutical composition according to ~~any one of claims 2337 to 39~~.