CLAIMS

The invention claimed is:

1. A single-chain IL-12 polypeptide comprising, from N- to C-terminus:

   i. a first IL-12 p40 domain (p40N),

   ii. an optional first peptide linker,

   iii. an IL-12 p35 domain,

   iv. an optional second peptide linker,

   v. a second IL-12 p40 domain (p40C);

wherein the first IL-12 p40 domain (p40N) is an N-terminal fragment of a p40 subunit; the IL-12 p35 domain is a mature p35 subunit or fragment thereof; and the second IL-12 p40 domain (p40C) is a C-terminal fragment of a p40 subunit, wherein the single-chain IL-12 polypeptide retains at least one IL-12 biological activity, wherein the p35 insert does not occur between amino acid residues 259 and 260 of the p40 subunit corresponding to SEQ ID NO:2.

2. The single chain IL-12 polypeptide of claim 1, which comprises an N-terminal signal peptide domain.

3. The single chain IL-12 polypeptide of claim 1, wherein the polypeptide does not comprise a first peptide linker, does not comprise a second peptide linker, or does not comprise a first peptide linker and does not comprise a second peptide linker.

4. The single chain IL-12 polypeptide of claim 2, wherein the polypeptide does not comprise a first peptide linker, does not comprise a second peptide linker, or does not comprise a first peptide linker and does not comprise a second peptide linker.

5. The single chain IL-12 polypeptide of claim 1, wherein said polypeptide comprises an amino acid sequence at least 85% identical, at least 90% identical, at least 95% identical, at least 97% identical, at least 98% identical, or at least 99% identical to the polypeptide sequence of amino acids 23 to 533 of SEQ ID NO: 10.

6. The single chain IL-12 polypeptide of claim 5, comprising amino acids 23 to 533 of SEQ ID NO: 10.

7. The single chain IL-12 polypeptide of claim 2, wherein said polypeptide comprises an amino acid sequence at least 85% identical, at least 90% identical, at least 95% identical, at least 97% identical, at least 98% identical, or at least 99% identical to the polypeptide sequence of SEQ ID NO: 10.

8. The single chain IL-12 polypeptide of claim 7, comprising the amino acid sequence of SEQ ID NO: 10.

9. The single chain IL-12 polypeptide of claim 1, wherein the first and second peptide linkers each comprise a number of amino acid residues selected from the group consisting of:

a) 0 amino acids;
b) 1 amino acid;
c) 2 amino acids;
d) 3 amino acids;
e) 4 amino acids;
f) 5 amino acids;
g) 6 amino acids;
h) 7 amino acids;
i) 8 amino acids;
j) 9 amino acids; and,
k) 10 amino acids.

10. The single chain IL-12 polypeptide of claim 9, wherein the amino acid residues in either the first or second peptide linker, or in both peptide linkers, comprise any combination of one or more amino acids selected from the group consisting of:

a) Glycine (Gly);
b) Serine (Ser);
c) Alanine (Ala);

d) Threonine (Thr); and,

e) Proline (Pro).

11. The single chain IL-12 polypeptide of claim 1, wherein the first and second peptide linkers are selected from Thr-Pro-Ser (SEQ ID NO: 41) and Ser-Gly-Pro-Ala-Pro (SEQ ID NO: 42).

12. The single chain IL-12 polypeptide of claim 1, wherein p40N comprises a polypeptide sequence at least 85% identical, at least 90% identical, at least 95% identical, at least 97% identical, at least 98% identical, or at least 99% identical to amino acids 18 to 288, amino acids 18 to 298, amino acids 28 to 288, or amino acids 28 to 298 of SEQ ID NO: 2.

13. The single chain IL-12 polypeptide of claim 1, wherein p40N comprises a fragment of the polypeptide sequence of SEQ ID NO: 2.

14. The single chain IL-12 polypeptide of claim 13, wherein p40N comprises a fragment of the polypeptide sequence of SEQ ID NO: 2, wherein the first residue of said fragment begins at a position selected from the group consisting of:

a) amino acid residue 18 of SEQ ID NO: 2;

b) amino acid residue 19 of SEQ ID NO: 2;

c) amino acid residue 20 of SEQ ID NO: 2;

d) amino acid residue 21 of SEQ ID NO: 2;

e) amino acid residue 22 of SEQ ID NO: 2;

f) amino acid residue 23 of SEQ ID NO: 2;

g) amino acid residue 24 of SEQ ID NO: 2;

h) amino acid residue 25 of SEQ ID NO: 2;

i) amino acid residue 26 of SEQ ID NO: 2;

j) amino acid residue 27 of SEQ ID NO: 2; and,

k) amino acid residue 28 of SEQ ID NO: 2,

and, wherein the last residue of said fragment ends at a position selected from the group consisting of:

l) amino acid residue 288 of SEQ ID NO: 2;

m) amino acid residue 289 of SEQ ID NO: 2;

n) amino acid residue 290 of SEQ ID NO: 2;

o) amino acid residue 291 of SEQ ID NO: 2;

p) amino acid residue 292 of SEQ ID NO: 2;

q) amino acid residue 293 of SEQ ID NO: 2;

r) amino acid residue 294 of SEQ ID NO: 2;

s) amino acid residue 295 of SEQ ID NO: 2;

t) amino acid residue 296 of SEQ ID NO: 2;

u) amino acid residue 297 of SEQ ID NO: 2; and,

v) amino acid residue 298 of SEQ ID NO: 2.

15. The single chain IL-12 polypeptide of claim 1, wherein p35 comprises a polypeptide sequence at least 85% identical, at least 90% identical, at least 95% identical, at least 97% identical, at least 98% identical, or at least 99% identical to amino acids 52 to 247, amino acids 52 to 253, amino acids 62 to 247, or amino acids 62 to 253 of SEQ ID NO: 4.

16. The single chain IL-12 polypeptide of claim 1, wherein p35 comprises a fragment of the polypeptide sequence of SEQ ID NO: 4.

17. The single chain IL-12 polypeptide of claim 16, wherein p35 comprises a fragment of the polypeptide sequence of SEQ ID NO:4, wherein the first residue of said fragment begins at a position selected from the group consisting of:

a) amino acid residue 52 of SEQ ID NO: 4;

b) amino acid residue 53 of SEQ ID NO: 4;

c) amino acid residue 54 of SEQ ID NO: 4;

d) amino acid residue 55 of SEQ ID NO: 4;

e) amino acid residue 56 of SEQ ID NO: 4;

f) amino acid residue 57 of SEQ ID NO: 4;

g) amino acid residue 58 of SEQ ID NO: 4;

h) amino acid residue 59 of SEQ ID NO: 4;

i) amino acid residue 60 of SEQ ID NO: 4;

j) amino acid residue 61 of SEQ ID NO: 4; and,

k) amino acid residue 62 of SEQ ID NO: 4,

and, wherein the last residue of said fragment ends at a position selected from the group consisting of:

l) amino acid residue 247 of SEQ ID NO: 4;

m) amino acid residue 248 of SEQ ID NO: 4;

n) amino acid residue 249 of SEQ ID NO: 4;

o) amino acid residue 250 of SEQ ID NO: 4;

p) amino acid residue 251 of SEQ ID NO: 4;

q) amino acid residue 252 of SEQ ID NO: 4; and,

r) amino acid residue 253 of SEQ ID NO: 4.

18. The single chain IL-12 polypeptide of claim 1, wherein p40C comprises a polypeptide sequence at least 85% identical, at least 90% identical, at least 95% identical, at least 97% identical, at least 98% identical, or at least 99% identical to amino acids 289 to 322, amino acids 289 to 328, amino acids 299 to 322, or amino acids 299 to 328 of SEQ ID NO: 2.

19. The single chain IL-12 polypeptide of claim 1, wherein p40C comprises a fragment of the polypeptide sequence of SEQ ID NO: 2.

20. The single chain IL-12 polypeptide of claim 19, wherein p40C comprises a fragment of the polypeptide sequence of SEQ ID NO: 2, wherein the first residue of said p40C fragment begins at a position selected from the group consisting of:

a) amino acid residue 289 of SEQ ID NO: 2;

b) amino acid residue 290 of SEQ ID NO: 2;

c) amino acid residue 291 of SEQ ID NO: 2;

d) amino acid residue 292 of SEQ ID NO: 2;

e) amino acid residue 293 of SEQ ID NO: 2;

f) amino acid residue 294 of SEQ ID NO: 2;

g) amino acid residue 295 of SEQ ID NO: 2;

h) amino acid residue 296 of SEQ ID NO: 2;

i) amino acid residue 297 of SEQ ID NO: 2;

j) amino acid residue 298 of SEQ ID NO: 2; and,

k) amino acid residue 299 of SEQ ID NO: 2,

and, wherein the last residue of said fragment ends at a position selected from the group consisting of:

l) amino acid residue 322 of SEQ ID NO: 2;

m) amino acid residue 323 of SEQ ID NO: 2;

n) amino acid residue 324 of SEQ ID NO: 2;

o) amino acid residue 325 of SEQ ID NO: 2;

p) amino acid residue 326 of SEQ ID NO: 2;

q) amino acid residue 327 of SEQ ID NO: 2; and,

r) amino acid residue 328 of SEQ ID NO: 2.

21. A polynucleotide comprising a nucleic acid sequence encoding the single chain IL-12 polypeptide of any one of claims 1 to 20.

22. The polynucleotide of claim 21, wherein the polynucleotide comprises a nucleic acid sequence at least 85% identical, at least 90% identical, at least 95% identical, at least 97% identical, at least 98% identical, at least 99% identical to nucleotides 67 to 1599 of SEQ ID NO: 9.

23. The polynucleotide of claim 22, wherein the polynucleotide comprises nucleic acids 67 to 1599 of SEQ ID NO: 9.

24. A vector comprising the polynucleotide of any one of claims 21 to 23.

25. The vector of claim 24, wherein the vector further comprises a gene switch capable of regulating expression of the single-chain IL-12 polypeptide.

26. The vector of claim 25, wherein the gene switch is an EcR-based gene switch.

27. The vector of any one of claims 24 to 26, wherein the vector is an adenovirus, an adeno-associated virus, a retrovirus, or a lentivirus.

28. An isolated host cell or a non-human organism transformed or transfected with the vector of any one of claims 24 to 27.

29. The isolated host cell of claim 28, wherein the host cell is an immune cell or a stem cell.

30. The isolated host cell of claim 29, wherein the immune cell is a dendritic cell, a macrophage cell, a neutrophil cell, a mast cell, an eosinophil cell, a basophil cell, a natural killer cell, or a lymphocyte cell.

31. The isolated host cell of claim 30, wherein the lymphocyte cell is a T cell.

32. The isolated host cell of claim 29, wherein the stem cell is a mesenchymal stem cell, an endometrial stem cell, or an embryonic stem cell.

33. The isolated host cell of claim 32, wherein the endometrial stem cell is an endometrial regenerative cell (ERC).

34. A medicament comprising the polypeptide of any one claims 1 to 20, the polynucleotide of any one of claims 21 to 23, the vector of any one of claims 24 to 27, or the isolated host cell of any one of claims 28 to 33.

35. Use of the medicament of claim 34, for the treatment of a condition selected from the group consisting of: cancer, an infectious disease, and an immune system disorder.

36. The use of the medicament of claim 35, for the treatment of cancer wherein the cancer is selected from the group consisting of: breast cancer, prostate cancer, lymphoma, skin cancer,

pancreatic cancer, colon cancer, melanoma, malignant melanoma, ovarian cancer, brain cancer, primary brain carcinoma, head-neck cancer, glioma, glioblastoma, liver cancer, bladder cancer, non-small cell lung cancer, head or neck carcinoma, breast carcinoma, ovarian carcinoma, lung carcinoma, small-cell lung carcinoma, Wilms' tumor, cervical carcinoma, testicular carcinoma, bladder carcinoma, pancreatic carcinoma, stomach carcinoma, colon carcinoma, prostatic carcinoma, genitourinary carcinoma, thyroid carcinoma, esophageal carcinoma, myeloma, multiple myeloma, adrenal carcinoma, renal cell carcinoma, endometrial carcinoma, adrenal cortex carcinoma, malignant pancreatic insulinoma, malignant carcinoid carcinoma, choriocarcinoma, mycosis fungoides, malignant hypercalcemia, cervical hyperplasia, leukemia, acute lymphocytic leukemia, chronic lymphocytic leukemia, acute myelogenous leukemia, chronic myelogenous leukemia, chronic granulocytic leukemia, acute granulocytic leukemia, hairy cell leukemia, neuroblastoma, rhabdomyosarcoma, Kaposi's sarcoma, polycythemia vera, essential thrombocytosis, Hodgkin's disease, non-Hodgkin's lymphoma, soft-tissue sarcoma, mesothelioma, osteogenic sarcoma, primary macroglobulinemia, and retinoblastoma.