CLAIMS

1. An Interleukin-12 (IL-12) Fc fusion protein comprising a first polypeptide chain and a second polypeptide chain, wherein

a) the first polypeptide chain comprises a first Fc domain and an IL-12p35 subunit and an IL-12p40 subunit of IL-12, and

b) the second polypeptide chain comprises a second Fc domain and a masking moiety that binds to the IL-12p35 and/or IL-12p40 subunit in the first polypeptide chain;

wherein the first and second polypeptide chain are linked via the first Fc domain and the second Fc domain,

wherein the IL-12p35 subunit or the IL-12p40 subunit is linked to the C-terminus of the first Fc domain via a first peptide linker, which first peptide linker is protease-cleavable,

wherein the masking moiety is linked to the C-terminus of the second Fc domain via a second linker, preferably a peptide linker, and

wherein the first or the second polypeptide chain further comprises a binding moiety selected from the group consisting of: a collagen binding moiety, a heparin binding moiety, and a fibronectin binding moiety.

2. The IL-12 Fc fusion protein according to claim 1, wherein the binding moiety is linked to the C-terminus of the IL-12p35 subunit or to the C-terminus of the IL-12p40 subunit, or the binding moiety is linked to the C-terminus of the masking moiety, and in each case optionally via a third polypeptide linker.

3. The IL-12 Fc fusion protein according to claim 1 or 2, wherein the binding moiety is located between the IL-12p35 subunit and the IL-12p40 subunit, or the binding moiety is located between the C-terminus of the first Fc domain and the N-terminus of the IL-12p35 subunit or the N-terminus of the IL-12p40 subunit, and in either case the binding moiety may be optionally flanked on one or both sides by a linker or linkers, preferably a peptide linker.

4. The IL-12 Fc fusion protein according to any one of the preceding claims, wherein the binding moiety is a collagen binding moiety.

5. The IL-12 Fc fusion protein according to claim 4, wherein the collagen binding moiety binds to collagen I.

6. The IL-12 Fc fusion protein according to claim 5, wherein the collagen binding moiety binds to collagen I and has the sequence LxxLxLxxN (SEQ ID NO:41), wherein L is Leucine and N is Asparagine and x is any amino acid.

7. The IL-12 Fc fusion protein according to claim 6, wherein the collagen binding moiety has a length of 20 amino acids (aa), 19aa, 18aa, 17aa, 16aa, 15aa, 14aa, 13aa, 12aa, 11aa, 10a, or 9aa.

8. The IL-12 Fc fusion protein according to any one of the preceding claims, wherein the collagen binding moiety comprises or consists of any one of the amino acid sequences of SEQ ID NOs:40-47.

9. The IL-12 Fc fusion protein according to any one of claims 1 to 3, wherein the binding moiety is a heparin binding moiety.

10. The IL-12 Fc fusion protein according to claim 9, wherein the heparin binding moiety has the sequence VRIQRKKEKMKET (SEQ ID NO:50).

11. The IL-12 Fc fusion protein according to claim 4, wherein the collagen binding moiety binds to collagen IV.

12. The IL-12 Fc fusion protein according to claim 11, wherein the collagen binding moiety has the sequence KLWVLPK (SEQ ID NO:40).

13. The IL-12 Fc fusion protein according to any one of claims 1 to 3, wherein the binding moiety is a fibronectin binding moiety.

14. The IL-12 Fc fusion protein according to claim 13, wherein the fibronectin binding moiety has the sequence GGWSHW (SEQ ID NO:49).

15. The IL-12 Fc fusion protein according to any one of the preceding claims, wherein the IL-12p35 subunit and the IL-12p40 subunit are human.

16. The IL-12 Fc fusion protein according to any one of the preceding claims, wherein the IL-12p35 subunit comprises a polypeptide having at least 95% identity to SEQ ID NO:1 and the IL-12p40 subunit comprises a polypeptide having at least 95% identity to SEQ ID NO:2, preferably the IL-12p35 subunit comprises the polypeptide of SEQ ID NO:1 and the IL-12p40 subunit comprises the polypeptide of SEQ ID NO:2.

17. The IL-12 Fc fusion protein according to any one of the preceding claims, wherein the IL-12p40 subunit and the IL-12p35 subunit are linked in a single-chain having the configuration IL-12p40—IL-12p35 or IL-12p35—IL-12p40.

18. The IL-12 Fc fusion protein according to claim 17, wherein the single-chain IL-12p40—IL-12p35 is linked via its IL-12p40 subunit to the C-terminus of the first Fc domain, or the single-chain IL-12p35—IL-12p40 is linked via its IL-12p35 subunit to the first Fc domain, and in both cases via the first peptide linker, which first peptide linker is protease-cleavable.

19. The IL-12 Fc fusion protein according to claim 17, wherein the IL-12p40 subunit and the IL-12p35 subunit are linked to each other via a linker that is rich in amino acid residues glycine and serine, preferably having a length of 5 to 20 amino acids and only including the amino acids glycine and serine, more preferably a glycine and serine linker having the amino acid sequence of SEQ ID NO:22.

20. The IL-12 Fc fusion protein according to claim 17, wherein the single-chain IL-12p40—IL-12p35 comprises a polypeptide having at least 95% identity to SEQ ID NO:8, or the single-chain IL-12p35—IL-12p40 comprises a polypeptide having at least 95% identity to SEQ ID NO:9.

21. The IL-12 Fc fusion protein according to any one of the preceding claims, wherein the second peptide linker is not protease-cleavable.

22. The IL-12 Fc fusion protein according to any one of the preceding claims, wherein the masking moiety binds to the IL-12p40 subunit and is selected from the group consisting of: an IL-12 receptor or an IL-12p40 binding fragment thereof, an scFv, or an immunoglobulin single variable domain, preferably a VHH.

23. The IL-12 Fc fusion protein according to any one of the preceding claims, wherein the first and the second Fc domain each comprise one or more mutations that promote heterodimerization of the Fc domains.

24. The IL-12 Fc fusion protein according to claim 23, wherein (a) the first Fc domain is a human IgG1 Fc domain comprising the mutation T366W and the second Fc domain is a human IgG1 Fc domain comprising the mutations T366S, L368A and Y407V, or (b) the first Fc domain is a human IgG1 Fc domain comprising the mutations T366S, L368A and Y407V and the second Fc domain is a human IgG1 Fc domain comprising the mutation T366W.

25. The IL-12 Fc fusion protein according to any one of the preceding claims, wherein the first and the second Fc domain are human IgG1 Fc domains and one of the first or the second Fc domain comprises the mutations H435R and Y436F.

26. The IL-12 Fc fusion protein according to any one of the preceding claims, wherein the first and the second Fc domain are human IgG1 Fc domains and either the first Fc domain, or the second Fc domain, or both Fc domains comprise the mutations L234A and L235A.

27. The IL-12 Fc fusion protein according to any one of the preceding claims, wherein the first Fc domain comprises the amino acid sequence of SEQ ID NO:15 and the second Fc domain comprises the amino acid sequence of SEQ ID NO:16, OR the first Fc domain comprises the amino acid sequence of SEQ ID NO:17 and the second Fc domain comprises the amino acid sequence of SEQ ID NO:18, OR the first Fc domain comprises the amino acid sequence of SEQ ID NO:16 and the

second Fc domain comprises the amino acid sequence of SEQ ID NO:15, OR the first Fc domain comprises the amino acid sequence of SEQ ID NO:18 and the second Fc domain comprises the amino acid sequence of SEQ ID NO:17.

28. The IL-12 Fc fusion protein according to any one of the preceding claims, wherein the protease-cleavable linker is cleavable by a matrix metalloproteinase (MMP), preferably an MMP-2, MMP-9, or MMP-13.

29. The IL-12 Fc fusion protein according to claim 28, wherein the protease-cleavable linker comprises or consists of any one of the amino acid sequences of SEQ ID NOs:232-241.

30. An IL-12 Fc fusion protein comprising a first polypeptide chain and a second polypeptide chain, wherein

a) the first polypeptide chain comprises or consists of the amino acid sequence of SEQ ID NO:208 and the second polypeptide chain comprises or consists of the amino acid sequence of SEQ ID NO:209,

b) the first polypeptide chain comprises or consists of the amino acid sequence of SEQ ID NO:210 and the second polypeptide chain comprises or consists of the amino acid sequence of SEQ ID NO:211,

c) the first polypeptide chain comprises or consists of the amino acid sequence of SEQ ID NO:212 and the second polypeptide chain comprises or consists of the amino acid sequence of SEQ ID NO:213,

d) the first polypeptide chain comprises or consists of the amino acid sequence of SEQ ID NO:214 and the second polypeptide chain comprises or consists of the amino acid sequence of SEQ ID NO:215,

e) the first polypeptide chain comprises or consists of the amino acid sequence of SEQ ID NO:216 and the second polypeptide chain comprises or consists of the amino acid sequence of SEQ ID NO:217,

f) the first polypeptide chain comprises or consists of the amino acid sequence of SEQ ID NO:218 and the second polypeptide chain comprises or consists of the amino acid sequence of SEQ ID NO:219,

g) the first polypeptide chain comprises or consists of the amino acid sequence of SEQ ID NO:220 and the second polypeptide chain comprises or consists of the amino acid sequence of SEQ ID NO:221,

h) the first polypeptide chain comprises or consists of the amino acid sequence of SEQ ID NO:222 and the second polypeptide chain comprises or consists of the amino acid sequence of SEQ ID NO:223,

i) the first polypeptide chain comprises or consists of the amino acid sequence of SEQ ID NO:224 and the second polypeptide chain comprises or consists of the amino acid sequence of SEQ ID NO:225,

j) the first polypeptide chain comprises or consists of the amino acid sequence of SEQ ID NO:226 and the second polypeptide chain comprises or consists of the amino acid sequence of SEQ ID NO:227,

k) the first polypeptide chain comprises or consists of the amino acid sequence of SEQ ID NO:228 and the second polypeptide chain comprises or consists of the amino acid sequence of SEQ ID NO:229,

l) the first polypeptide chain comprises or consists of the amino acid sequence of SEQ ID NO:230 and the second polypeptide chain comprises or consists of the amino acid sequence of SEQ ID NO:231, OR

m) the first polypeptide chain comprises or consists of the amino acid sequence of SEQ ID NO:242 and the second polypeptide chain comprises or consists of the amino acid sequence of SEQ ID NO:243.

31. The IL-12 Fc fusion protein according to any one of claims 1 to 29, wherein the masking moiety comprises an IL-12 binding immunoglobulin single variable domain comprising the three CDRs contained within any one of the sequences of SEQ ID NOs:61-109.

32. The IL-12 Fc fusion protein according to claim 31, wherein said immunoglobulin single variable domain comprises any one of the amino acid sequences of SEQ ID NOs:61-109.

33. A cleavage product capable of binding to a human IL-12 receptor comprising the IL-12 cytokine after proteolytic cleavage of the cleavable linker as defined in any one of the IL-12 Fc fusion proteins of claims 1 to 32.

34. The cleavage product according to claim 33 comprising the IL-12 cytokine and the binding moiety.

35. The cleavage product according to claim 34 comprising or consisting of the amino acid sequence of any one of SEQ ID NOs:208, 210, 212, 214, 216, 218, 220, 222, 224, 226, 228, 230 or 242 after proteolytic cleavage of the cleavable linker.

36. An IL-12 binding immunoglobulin single variable domain comprising the three CDRs contained within any one of the sequences of SEQ ID NOs:61-109.

37. The IL-12 binding immunoglobulin single variable domain of claim 36, wherein said immunoglobulin single variable domain is a VHH.

38. The IL-12 binding immunoglobulin single variable domain of claim 36, wherein said immunoglobulin single variable domain comprises the amino acid sequence of any one of SEQ ID NOs:61-109.

39. A nucleic acid encoding at least one polypeptide of the IL-12 Fc fusion protein of any one of claims 1 to 32, or a nucleic acid encoding one of the polypeptide chains of an IL-12 Fc fusion protein of any one of claims 1 to 32, or a nucleic acid encoding an IL-12 binding immunoglobulin single variable domain of any one of claims 36 to 38.

40. A vector comprising the nucleic acid of claim 39, optionally wherein the vector comprises nucleic acids encoding both chains of the IL-12 Fc fusion protein.

41. A host cell comprising the nucleic acid of claim 39 or the vector of claim 40, optionally wherein the cell comprises one or more nucleic acids encoding both chains of the IL-12 Fc fusion protein.

42. A method of producing an IL-12 Fc fusion protein comprising culturing the host cell of claim 41 under a condition that produces the fusion protein and optionally purifying said IL-12 Fc fusion protein.

43. A composition comprising the IL-12 Fc fusion protein of any one of claims 1 to 32.

44. A pharmaceutical composition comprising the IL-12 Fc fusion protein of any one of claims 1 to 32 and a pharmaceutically acceptable carrier.

45. A kit comprising the IL-12 Fc fusion protein of any one of claims 1 to 32, or the composition of claim 43, or the pharmaceutical composition of claim 44.

46. An IL-12 Fc fusion protein as defined in any one of claims 1 to 32 for use in medicine.

47. A cleavage product as defined in any one of claims 33 to 35 for use in medicine.

48. An IL-12 Fc fusion protein according to any one of claims 1 to 32 for use in treating or preventing cancer.

49. Use of an IL-12 Fc fusion protein according to any one of claims 1 to 32 in the manufacture of a medicament.

50. Use of an IL-12 Fc fusion protein according to any one of claims 1 to 32 in the manufacture of a medicament for reduction of the incidence of or treatment of cancer.