CLAIMS

WHAT IS CLAIMED IS:

1. A modified p40 domain comprising one or more amino acid substitutions at one or more positions of E45, D62 and D161 relative to the wild-type human mature IL12 p40 domain sequence set forth in SEQ ID NO: 10, wherein the one or more amino acid substitutions comprise one or more of D62H, D62I, D161R and D161S.

2. The modified p40 domain of claim 1, wherein the one or more amino acid substitutions further comprise one or more of E45K and D62N.

3. The modified p40 domain of claim 1 or claim 2, further comprising W15H, W15K, W15R, D18G, K58H, K58W, E59D, E59G, E59R, F60D, F60E, F60K, F60R, F60V, K84E, K84I, K84L, K84V, K84W, K84Y, E86L, E86R, E86S, E86W, D93E, D93H, D93R, D93W, K197D, K197E, K197Q, K197T, or K197W, or a combination thereof.

4. A modified p40 domain comprising one or more amino acid substitutions relative to the wild-type human mature IL12 p40 domain sequence set forth in SEQ ID NO: 10, wherein the one or more amino acid substitutions comprise W15H, W15K, W15R, D18G, K58H, K58W, E59D, E59G, E59R, F60D, F60E, F60K, F60R, F60V, D62H, D62I, K84E, K84I, K84L, K84V, K84W, K84Y, E86L, E86R, E86S, E86W, D93E, D93H, D93R, D93W, D161R, D161S, K197D, K197E, K197Q, K197T, or K197W, or a combination thereof.

5. The modified p40 domain of claim 4, wherein the modified p40 domain comprises one or more, two or more, or three or more amino acid substitutions.

6. The modified p40 domain of claim 5, wherein the one or more amino acid substitutions comprise W15H, W15K, D18G, E45K, K58H, K58W, E59G, E59R, F60V, F60R, D62H, D62I, D62N, K84E, K84W, K84Y, E86L, E86S, E86W, D93H, D93W, D161R, D161S, K197E, K197Q, K197T, or K197W, or a combination thereof.

7. The modified p40 domain of claim 5 or claim 6, wherein the one or more amino acid substitutions comprise W15K, F60R, or K84Y, or a combination thereof.

8. The modified p40 domain of claim 5, wherein the one or more amino acid substitutions comprise W15H_K84L, K58H_K84I, E59D_K84W, E59G_K84W, E59R_K84E, E59R_K84W, E59R_E86W, E59D_D93H, E59R_D93R, E59R_K197E, E59R_K197W, F60E_K84W, F60R_K84Y, F60K_K197W, F60R_K197W, K84I_E86R, K84E_D93H, K84I_D93H, K84V_D93H, K84W_D93W, K84I_D161R, K84W_D161R, K84W_K197E, K84W_K197Q, K84W_K197W, E86W_D93E, E86R_K197D, E86W_K197W, W15H_K84L_K197Q, K58H_E86R_K197D, E59D_K84W_K197W,

F60R_K84E_K197W, K84I_E86R_D93H, or W15R_E59D_F60D_K197W, or a combination thereof.

9. The modified p40 domain of claim 5, wherein the one or more amino acid substitutions comprise W15H_K84L, E59D_D93H, E59R_D93R, F60K_K197W, F60R_K84Y, K84I_E86R, K84W_D161R, K84W_K197W, E86R_K197D, E86W_D93E, W15H_K84L_K197Q, K58H_E86R_K197D, E59D_K84W_K197W, F60R_K84E_K197W, K84I_E86R_D93H, or W15R_E59D_F60D_K197W, or a combination thereof.

10. The modified p40 domain of claim 5, wherein the one or more amino acid substitutions comprise K58H_K84I, E59D_K84W, E59G_K84W, E59R_E86W, E59R_K197E, E59R_K197W, E59R_K84E, E59R_K84W, F60E_K84W, F60R_K197W, K84E_D93H, K84I_D161R, K84I_D93H, K84V_D93H, K84W_D93W, K84W_K197E, K84W_K197Q, or E86W_K197W, or a combination thereof.

11. The modified p40 domain of claim 5, wherein the one or more amino acid substitutions comprise W15H_K84L, K58H_K84I, E59D_K84W, E59G_K84W, E59R_K84E, E59R_K84W, E59R_E86W, E59D_D93H, E59R_D93R, E59R_K197E, E59R_K197W, F60E_K84W, F60R_K84Y, F60K_K197W, F60R_K197W, K84I_E86R, K84E_D93H, K84I_D93H, K84V_D93H, K84W_D93W, K84I_D161R, K84W_D161R, K84W_K197E, K84W_K197Q, K84W_K197W, E86W_D93E, E86R_K197D, or E86W_K197W, or a combination thereof.

12. The modified p40 domain of claim 5, wherein the one or more amino acid substitutions comprise W15H_K84L, E59D_D93H, E59R_D93R, F60K_K197W, F60R_K84Y, K84I_E86R, K84W_D161R, K84W_K197W, E86R_K197D, or E86W_D93E, or a combination thereof.

13. The modified p40 domain of claim 5, wherein the one or more amino acid substitutions comprise W15H_K84L_K197Q, K58H_E86R_K197D, E59D_K84W_K197W, F60R_K84E_K197W, K84I_E86R_D93H or W15R_E59D_F60D_K197W, or a combination thereof.

14. The modified p40 domain of claim 5, wherein the modified p40 domain comprises an amino acid sequence having at least about 90%, 95%, 97%, 99%, or 100% sequence identity to the amino acid sequence set forth in any one of SEQ ID NOS: 12-14, 16, 18, 19, 21-34 and 36-56.

15. The modified p40 domain of any one of claims 10-11, wherein the modified p40 domain comprises an amino acid substitution or a set of amino acid substitutions selected from: W15R_E59D_F60D_K197W, W15H_K84L_K197Q, E59D_K84W_K197W,

K58H_E86R_K197D, W15H_K84L, F60R_K84E_K197W, K84W_K197W, F60K_K197W, E86R_K197D, K84I_E86R_D93H, E59R_D93R, K84I_E86R, W15K, K84W_D161R, E45R_K58S_E59S_K195D, E59D_D93H, F60R_K84Y, E86W_D93E, K58S_E59S_K195D, K84E, K197T, E59R, W15H, K84W, K197W, E59S, D161R or E86W, or a combination thereof.

16. The modified p40 domain of claim 15, wherein the modified p40 domain comprises an amino acid substitution or a set of amino acid substitutions selected from: W15H_K84L_K197Q, E59D_K84W_K197W, K84W_K197W, F60K_K197W, E59R_D93R, W15K, or F60R_K84Y, or a combination thereof.

17. The modified p40 domain of any one of claims 1-16, wherein the modified p40 domain has a binding affinity to at least one of its cognate receptors that is reduced from about 5-fold to about 1000-fold, from about 5-fold to about 800-fold, from about 5-fold to about 600-fold, from about 10-fold to about 500-fold, from about 10-fold to about 300-fold, or from about 20-fold to about 200-fold, relative to the binding affinity of the unmodified wildtype p40 domain having the sequence set forth in SEQ ID NO: 10, and as determined in a reporter gene assay (RGA).

18. The modified p40 domain of claim 17, wherein the modified p40 domain has a binding affinity to at least one of its cognate receptors that is reduced from about 20-fold to about 200-fold.

19. The modified p40 domain of claim 18, wherein the at least one cognate receptor comprises IL12Rβ1.

20. The modified p40 domain of any one of claims 1-19, wherein the modified p40 domain has a thermostability as measured by a melting temperature that is within ±5 ℃, ±4 ℃, ±3 ℃, ±2 ℃ or ±1 ℃ of that of the unmodified wildtype p40 domain that has the sequence set forth in SEQ ID NO: 10, and wherein the melting temperature is determined by Differential Scanning Fluorimetry (DSF) or Differential Scanning Calorimetry (DSC).

21. An IL12 fusion protein comprising an IL12 polypeptide comprising the modified p40 domain of any one of claims 1-20, and a p35 domain.

22. The IL12 fusion protein of claim 21, wherein the p35 domain comprises or consists of an amino acid sequence having at least about 90%, 95%, 97%, 99% or 100% sequence identity to the amino acid sequence set forth in SEQ ID NO: 11.

23. The IL12 fusion protein of claim 21, wherein the N-terminus of the p35 domain is coupled to the C-terminus of the modified p40 domain either directly or via a first linker.

24. The IL12 fusion protein of claim 23, wherein the first linker has the sequence of $(G_4S)_x$, and wherein x is 1, 2, 3 or 4.

25. The IL12 fusion protein of any one of claims 21-24, further comprising a heterodimeric Fc (HetFc) domain comprising a first Fc polypeptide and a second Fc polypeptide, thereby forming an IL12 HetFc fusion protein.

26. The IL12 HetFc fusion protein of claim 25, wherein the IL12 polypeptide is coupled to the first Fc polypeptide either directly or via a second linker.

27. The IL12 HetFc fusion protein of claim 26, wherein the IL12 polypeptide is coupled to the C-terminus of the first Fc polypeptide.

28. The IL12 HetFc fusion protein of any one of claims 26-27, wherein the IL12 polypeptide is coupled to the C-terminus of the first Fc polypeptide via the N-terminus of the modified p40 domain.

29. The IL12 HetFc fusion protein of any one of claims 25-28, further comprising a masking moiety, thereby forming a masked IL12 HetFc fusion protein, wherein the masking moiety is capable of non-covalently interacting with the modified p40 domain, thereby masking the modified p40 domain and reducing the binding affinity (K_D) of the modified p40 domain for binding to at least one of its cognate receptors when compared to an unmasked modified p40 domain.

30. The masked IL12 HetFc fusion protein of claim 29, wherein the masking moiety is coupled to the C-terminus of the second Fc polypeptide either directly or via a third linker.

31. The masked IL12 HetFc fusion protein of claim 30, wherein the third linker is a protease-cleavable linker.

32. The masked IL12 HetFc fusion protein of any one of claims 26-31, wherein the second linker and the third linker each comprise or consist of an amino acid sequence spanning from 5 to about 50 amino acids.

33. The masked IL12 HetFc fusion protein of claim 32, wherein the second linker comprises or consists of the amino acid sequence set forth in SEQ ID NO: 132, and the third linker comprises or consists of the amino acid sequence set forth in SEQ ID NO: 134.

34. The masked IL12 HetFc fusion protein of any one of claims 29-33, wherein the masking moiety comprises or consists of an scFv domain comprising a V_H domain coupled either directly or via a fourth linker to a V_L domain.

35. The masked IL12 HetFc fusion protein of claim 34, wherein the fourth linker comprises or consists of the amino acid sequence set forth in SEQ ID NO: 135.

36. The masked IL12 HetFc fusion protein of any one of claims 34-35, wherein the V_H domain comprises or consists of an amino acid sequence having about 95%, 97%, 99%, or 100% sequence identity to the sequence set forth in SEQ ID NO: 2, and the V_L domain comprises or consists of an amino acid sequence having about 95%, 97%, 99%, or 100% sequence identity to the sequence set forth in SEQ ID NO: 3.

37. The masked IL12 HetFc fusion protein of any one of claims 29-36, wherein the masking moiety is capable of reducing the binding affinity of the modified p40 domain to the at least one cognate receptor by at least about 10-fold, 15-fold, 20-fold, 30-fold, 40-fold, 50-fold, 100-fold, 200-fold, or at least about 300-fold, compared to a corresponding polypeptide construct that does not comprise the masking moiety.

38. The masked IL12 HetFc fusion protein of claim 37, wherein the at least one cognate receptor comprises IL12Rβ1.

39. A masked IL12 HetFc fusion protein comprising:

(i) an IL-12 polypeptide comprising the modified p40 domain according to any one of claims 1-20 coupled via the linker $(G_4S)_4$ to a p35 domain comprising or consisting of the amino acid sequence set forth in SEQ ID NO: 11;

(ii) a heterodimeric Fc domain comprising a first Fc polypeptide and a second Fc polypeptide; and

(iii) a masking moiety (MM) comprising an anti-IL12 scFv domain,

wherein:

the IL12 polypeptide is coupled either directly or via a second linker to the C-terminus of the first Fc polypeptide, and

the masking moiety is coupled either directly or via a third linker to the C-terminus of the second Fc polypeptide and is capable of non-covalently interacting with the IL12 polypeptide, thereby reducing the binding affinity of the IL12 polypeptide to at least one of its cognate receptors.

40. The masked IL12 HetFc fusion protein of claim 39, comprising or consisting of two polypeptide chains, from N- to C-terminus, (i) an Fc-IL12 polypeptide chain and (ii) an Fc-MM polypeptide chain.

41. The masked IL12 HetFc fusion protein of claim 40, comprising a polypeptide chain comprising an amino acid sequence having at least about 90%, 95%, 97%, 99%, or 100% sequence identity to any of the amino acid sequences set forth in SEQ ID NOs: 61-89.

42. The masked IL12 HetFc fusion protein of any one of claims 40-41, wherein the Fc-IL12 polypeptide chain comprises or consists of the amino acid sequence set forth in any one of SEQ ID NOs: 61-89.

43. The masked IL12 HetFc fusion protein of any one of claims 40-42, comprising a polypeptide chain comprising an amino acid sequence having at least about 90%, 95%, 97%, 99%, or 100% sequence identity to the amino acid sequence set forth in SEQ ID NO: 60.

44. The masked IL12 HetFc fusion protein of any one of claims 40-43, wherein the Fc-MM polypeptide chain comprises or consists of the amino acid sequence set forth in SEQ ID NO: 60.

45. The masked IL12 HetFc fusion protein of any one of claims 40-44, wherein (i) the Fc-IL12 polypeptide chain amino acid sequence is selected from the group consisting of the sequences of: v28046, v28047, v28048, v28049, v28050, v28051, v28053, v28054, v28055, v28056, v28057, v28058, v28059, v28060, v28061, v28062, v28063, v28064, v28065, v28066, v28067, v28068, v28069, v28070, v28071, v28072, v28074, v28075, and (ii) the Fc-MM polypeptide chain comprises or consists of the amino acid sequence of v26503.

46. An unmasked IL12 HetFc fusion protein comprising:

(i) an IL-12 polypeptide comprising the modified p40 domain according to any one of claims 1-20 coupled via the linker $(G_4S)_4$ to the p35 domain comprising or consisting of the amino acid sequence set forth in SEQ ID NO: 11; and

(ii) a heterodimeric Fc domain comprising a first Fc polypeptide and a second Fc polypeptide,

wherein the IL12 polypeptide is coupled either directly or via a second linker to the C-terminus of the first Fc polypeptide.

47. The unmasked IL12 HetFc fusion protein of claim 46, comprising or consisting of two polypeptide chains, from N- to C-terminus, (i) an Fc-IL12 polypeptide chain and (ii) an Fc polypeptide chain.

48. The unmasked IL12 HetFc fusion protein of claim 47, wherein the Fc-IL12 polypeptide chain comprises or consists of an amino acid sequence having at least about 90%, 95%, 97%, 99%, or 100% sequence identity to any of the amino acid sequences set forth in SEQ ID NOs: 61-89.

49. The unmasked IL12 HetFc fusion protein of any one of claims 47-48, wherein the Fc-IL12 polypeptide chain comprises or consists of the amino acid sequence set forth in any one of SEQ ID NOs: 61-89.

50. The unmasked IL12 HetFc fusion protein of any one of claims 47-49, comprising a polypeptide chain comprising an amino acid sequence having at least about 90%, 95%, 97%, 99%, or 100% sequence identity to the amino acid sequence set forth in SEQ ID NO: 58.

51. The unmasked IL12 HetFc fusion protein of any one of claims 47-50, wherein (i) the IL12 polypeptide chain amino acid sequence is selected from the group consisting of the sequences of: v28046, v28047, v28048, v28049, v28050, v28051, v28053, v28054, v28055, v28056, v28057, v28058, v28059, v28060, v28061, v28062, v28063, v28064, v28065, v28066, v28067, v28068, v28069, v28070, v28071, v28072, v28074, v28075, and (ii) the Fc polypeptide chain comprises or consists of the amino acid sequence of v12153.

52. A pharmaceutical composition comprising (i) the modified p40 domain of any one of claims 1-20, (ii) the masked IL12 HetFc fusion protein of any one of claims 30-45, and/or (iii) the unmasked IL12 HetFc fusion protein of claims 46-51, and a pharmaceutically acceptable carrier.

53. A nucleic acid molecule or a set of nucleic acid molecules encoding (i) the modified p40 domain of any one of claims 1-20, (ii) the masked IL12 HetFc fusion protein of any one of claims 30-45, and/or (iii) the unmasked IL12 HetFc fusion protein of claims of any one of claims 46-51.

54. A vector or a set of vectors comprising the nucleic acid molecule or the set of nucleic acid molecules of claim 53.

55. A method of identifying one or more amino acid substitutions in a p40 domain amino acid sequence to produce a modified p40 domain, the method comprising performing molecular dynamics and mutagenesis simulations, thereby identifying the one or more amino acid substitutions listed in **Table C**, wherein the one or more amino acid substitutions in the modified p40 domain amino acid sequence are relative to the sequence set forth in SEQ ID NO: 1, and wherein the one or more amino acid substitutions reduce the binding affinity (K_D) of the modified p40 domain to at least one of its cognate receptors, and relative to an unmodified p40 domain that does not comprise the one or more amino acid substitutions.

56. The method of claim 55, wherein the at least one cognate receptor comprises IL12Rβ1.

57. The method of any one of claims 55-56, wherein the binding affinity of the modified p40 domain is reduced by at least about 2-fold, 5-fold, 10-fold, 15-fold, 20-fold, 30-fold, 40-fold, 50-fold, 100-fold, 200-fold, 300-fold, 400-fold, 500-fold, or at least about 600-fold, compared to an unmodified p40 domain and as determined in a reporter gene assay.

58. A modified p40 domain of any one of claims 1-20, a masked IL12 HetFc fusion protein of any one of claims 30-45, or an unmasked IL12 HetFc fusion protein of any one of claims 46-51, for use in therapy.

59. Use of a modified p40 domain of any one of claims 1-20, a masked IL12 HetFc fusion protein of any one of claims 30-45, or an unmasked IL12 HetFc fusion protein of any one of claims 46-51, in the preparation of a medicament for the treatment of cancer.