IL-12 HETERODIMERIC FC-FUSION PROTEINS

CROSS-REFERENCE TO RELATED APPLICATIONS

[0001] This application claims the benefit of U.S. Provisional Application No. 62/740,813, filed October 3, 2018, U.S. Provisional Application No. 62/810,038 filed February 25, 2019, and U.S. Provisional Application No. 62/848,512 filed May 15, 2019 all of which are incorporated herein by reference in their entirety for all purposes.

BACKGROUND OF THE INVENTION

[0002] In order for T cells to mount an effective anti-tumor response, three things must occur. T cells must first engage antigenic tumor peptides presented by MHC in the tumor environment. Second, costimulatory molecules must bind to the T cells. And third, the T cells must be induced by cytokines such as IL-12 and IL-2 to produce costimulatory cytokines such as IFNγ which allows differentiation and expansion. Recognition of tumor peptides alone in the absence of cytokine induction leads to T cells becoming anergic, thereby leading to tolerance. Accordingly, a very promising approach in cancer immunotherapy is cytokine-based treatments. In fact, IL-2 has been approved for use in patients with metastatic renal-cell carcinoma and malignant melanoma. However, there are currently no approved uses of recombinant IL-12 in humans.

[0003] Recombinant IL-12 is a promising cytokine-based treatment due to its broad effect in activating the immune system. However, IL-12 has thus far faced hurdles in human clinical trials due to toxicity. As with other cytokines, the short half-life of IL-12 requires frequent bolus injections.

[0004] Additionally, IL-12 is composed of an α-chain (the p35 subunit; IL-12p35) and a ß-chain (the p40 subunit; IL-12p40) covalently linked to form the biologically active IL-12 heterodimer. IL-12 exerts its cell signaling function through binding by binding to a dimeric IL-12 receptor complex composed of IL-12 receptor ß1 (IL-12Rß1) and IL-12 receptor ß2 (IL-12Rß2) on T cells

and inducing IFNγ secretion. However, the IL-12p40 subunit can also exist as a homodimer which has been reported to antagonize IL-12 activity by competing for binding to IL-12 receptor.

[0005] The present invention addresses the short half-life of IL-12 by providing novel IL-12-Fc fusion proteins, as well as novel IL-12 variants with decreased potency.

BRIEF SUMMARY OF THE INVENTION

[0006] In one aspect, the present invention provides a heterodimeric Fc fusion protein comprising: a) a first fusion protein comprising a variant IL-12p40 subunit domain and a first Fc domain, wherein said IL-12p40 subunit domain is covalently attached to the N-terminus of said first Fc domain; and b) a second fusion protein comprising an IL-12p35 subunit domain and a second Fc domain, wherein said IL-12p35 subunit domain is covalently attached to the N-terminus of said Fc domain; wherein said first and said second Fc domains comprise modifications promoting heterodimerization of said first and said second Fc domains. In some embodiments, the present invention provides a heterodimeric Fc fusion protein, wherein said IL-12p40 subunit has one or more amino acid substitutions selected from the group consisting of: E59K, E59Q, D18N, D18K, E32Q, E33Q, D34N, D34K, Q42E, S43E, S43K, E45Q, Q56E, D62N, E73Q, D87N, K99E, K99Y, E100Q, N103D, N103Q, N113D, N113Q, Q144E, D161N, R159E, K163E, E187Q, N200D, N200Q, N218Q, Q229E, E235Q, C252S, Q256N, K258E, K260E, E262Q, K264E, N281D, N281Q, and E299Q.

[0007] In some embodiments, the present invention provides a heterodimeric Fc fusion protein, wherein said IL-12p40 subunit has amino acid substitutions selected from the group consisting of N103D/N113D/N200D/N281D, Q42E/E45Q, E45Q/Q56E, Q42E/E59Q, Q56E/E59Q, Q42E/E45Q/Q56E, E45Q/Q56E/E59Q, E32Q/E59Q, D34N/E59K, D34N/E59K/K99E, D34K/E59K/K99E, E32Q/D34N/E59K/K99E, E32K/D34N/E59K/K99E, D34N/E59Q, E59Q/E187Q, S43E/E59Q, S43K/E49Q, E59Q/K163E, E59Q/K99E, E59Q/K258E, E59Q/K260E, E59K/K99E, D18K/E59K/K99E, E59K/K99E/K264E, E59K/K99Y, E59Y/K99Y, E59Y/K99E, E45K/E59K/K99E, E59K/K99E/Q144E, E59K/K99E/Q144K, E59K/K99E/R159E, E59K/K99E/K264E,

D18K/E59K/K99E/K264E, DI8K/E59K/K99E/C252S, D18K/E59K/K99E/C252S/K264E, E59K/K99Y/C252S, E59K/K99E/C252S/K264E, E59K/K99E/C252S, N103D/N113D, N103D/N200D, N103D/N281D, N113D/N200D, N113D/N281D, N200D/N281D, N103D/N113D/N200D, N103D/N113D/N281D, N103D/N200D/N281D, N113D/N200D/N281D, N103Q/N113Q, N103Q/N200Q, N103Q/N281Q, N113Q/N200Q, N113Q/N281Q, N200Q/N281Q, N103Q/N113Q/N200Q, N103Q/N113Q/N281Q, N103Q/N200Q/N281Q, N113Q/N200Q/N281Q, N103Q/N113Q/N200Q/N281Q, E59K/K99E/N103Q/C252S/K264E, E59K/K99E/N113Q/C252S/K264E, E59K/K99E/N200Q/C252S/K264E, E59K/K99E/N281Q/C252S/K264E, E59K/K99E/N103Q/N113Q/C252S/K264E, E59K/K99E/N103Q/N200Q/C252S/K264E, E59K/K99E/N103Q/N281Q/C252S/K264E, E59K/K99E/N113Q/N200Q/C252S/K264E, E59K/K99E/N113Q/N281Q/C252S/K264E, E59K/K99E/N200Q/N281Q/C252S/K264E, E59K/K99E/N103Q/N113Q/N200Q/C252S/K264E, E59K/K99E/N103Q/N200Q/N281Q/C252S/K264E, E59K/K99E/N113Q/N200Q/N281Q/C252S/K264E, and E59K/K99E/N103Q/N113Q/N200Q/N281Q/C252S/K264E.

[0008] In some embodiments, the present invention provides a heterodimeric Fc fusion protein, wherein said IL-12p40 subunit has a polypeptide sequence selected from the group consisting of: i) SEQ ID NO:57 (IL-12p40(N103D)), ii) SEQ ID NO:58 (IL-12p40(N113D)), iii) SEQ ID NO:59 (IL-12p40(N200D)), iv) SEQ ID NO:60 (IL-12p40(N281D)), v) SEQ ID NO:61 (IL-12p40(N103D/N113D/N200D/N281D)), vi) SEQ ID NO:62 (IL-12p40(Q42E)), vii) SEQ ID NO:63 (IL-12p40(E45Q)), viii) SEQ ID NO:64 (IL-12p40(Q56E)), ix) SEQ ID NO:65 (IL-12p40(E59Q)), x) SEQ ID NO:66 (IL-12p40(D62N)), xi) SEQ ID NO:67 (IL-12p40(Q42E/E45Q)), xii) SEQ ID NO:68 (IL-12p40(E45Q/Q56E)), xiii) SEQ ID NO:69 (IL-12p40(Q42E/E59Q)), xiv) SEQ ID NO:70 (IL-12p40(Q56E/E59Q)), xv) SEQ ID NO:71 (IL-12p40(Q42E/E45Q/Q56E)), xvi) SEQ ID NO:72 (IL-12p40(E45Q/Q56E/E59Q)), xvii) SEQ ID NO:73 (IL-12p40(D161N)), xviii) SEQ ID NO:74 (IL-12p40(E73Q)), xix) SEQ ID NO:75 (IL-12p40(Q144E)), xx) SEQ ID NO:76 (IL-12p40(E262Q)), xxi) SEQ ID NO:77 (IL-12p40(E100Q)), xxii) SEQ ID NO:78 (IL-12p40(D18N)), xxiii) SEQ ID NO:79 (IL-12p40(E33Q)), xxiv) SEQ ID NO:80 (IL-12p40(Q229E)), xxv) SEQ ID NO:81 (IL-

12p40(E235Q)), xxvi) SEQ ID NO:82 (IL-12p40(Q256N)), xxvii) SEQ ID NO:83 (IL-12p40(E299Q)), xxviii) SEQ ID NO:84 (IL-12p40(D87N)), xxix) IL-12p40(E32Q), xxx) IL-12p40(D34N), xxxi) IL-12p40(S43E), xxxii) IL-12p40(S43K), xxxiii) SEQ ID NO:XXX (IL-12p40 (E59K/K99E/N103Q/N113Q/N200Q/N281Q/C252S/K264E)), xxxiv) SEQ ID NO:205 (IL-12p40(E59K)), xxxv) IL-12p40(K99E), xxxvi) IL-12p40(K163E), xxxvii) IL-12p40(E187Q), xxxviii) IL-12p40(K258E), xxxix) IL-12p40(K260E), xl) SEQ ID NO:206 (IL-12p40(E32Q/E59Q)), xli) SEQ ID NO:207 (IL-12p40(D34N/E59Q)), xlii) SEQ ID NO:208 (IL-12p40(E59Q/E187Q)), xliii) SEQ ID NO:209 (IL-12p40(S43E/E59Q)), xliv) SEQ ID NO:210 (IL-12p40(S43K/E49Q)), xlv) SEQ ID NO:211 (IL-12p40(E59Q/K163E)), xlvi) SEQ ID NO:212 (IL-12p40(E59Q/K99E)), xlvii) SEQ ID NO:213 (IL-12p40(E59Q/K258E)), xlviii) SEQ ID NO:214 (IL-12p40(E59Q/K260E)), xlix) SEQ ID NO: XXX (IL-12p40 (D34N/E59K)), l) SEQ ID NO: XXX (IL-12p40(E59K/K99E)), li) SEQ ID NO: XXX (IL-12p40(D18K/E59K/K99E)), lii) SEQ ID NO: XXX (IL-12p40 (E59K/K99E/K264E)), liii) SEQ ID NO: XXX (IL-12p40 (E59K/K99Y)), liv) SEQ ID NO: XXX (IL-12p40 (E59Y/K99E)), lv) SEQ ID NO: XXX (IL-12p40 (E45K/E59K/K99E)), lvi) SEQ ID NO: XXX (IL-12p40 (E59K/K99E/Q144E)), lvii) SEQ ID NO: XXX (IL-12p40 (E59K/K99E/Q144K)), lviii) SEQ ID NO: XXX (IL-12p40 (E59K/K99E/R159E)), lix) SEQ ID NO: XXX (IL-12p40 (E59K/K99E/K264E)), lx) SEQ ID NO: XXX (IL-12p40 (D18K/E59K/K99E/K264E)), lxi) SEQ ID NO: XXX (IL-12p40 (C252S)), lxii) SEQ ID NO: XXX (IL-12p40 (DI8K/E59K/K99E/C252S)), lxiii) SEQ ID NO: XXX (IL-12p40 (D18K/E59K/K99E/C252S/K264E)), lxiv) SEQ ID NO: XXX (IL-12p40 (E59K/K99Y/C252S)), lxv) SEQ ID NO: XXX (IL-12p40 (E59K/K99E/C252S/K264E)), lxvi) SEQ ID NO: XXX (IL-12p40 (E59K/K99E/C252S)), lxvii) SEQ ID NO: XXX (IL-12p40 (N103D/N113D)), lxviii) SEQ ID NO: XXX (IL-12p40 (N103D/N200D)), lxix) SEQ ID NO: XXX (IL-12p40 (N103D/N281D)), lxx) SEQ ID NO: XXX (IL-12p40 (N113D/N200D)), lxxi) SEQ ID NO: XXX (IL-12p40 (N113D/N281D)), lxxii) SEQ ID NO: XXX (IL-12p40 (N200D/N281D)), lxxiii) SEQ ID NO: XXX (IL-12p50 (N103D/N113D/N200D)), lxxiv) SEQ ID NO: XXX (IL-12p40 (N103D/N113D/N281D)), lxxv) SEQ ID NO: XXX (IL-12p40 (N103D/N200D/N281D)), lxxvi) SEQ ID NO: XXX (IL-12p40 (N113D/N200D/N281D)), lxxvii) SEQ ID NO: XXX (IL-12p40 (N103Q)), lxxviii) SEQ ID NO: XXX (IL-12p40 (N113Q)), lxxix) SEQ ID NO: XXX (IL-12p40 (N200Q)), lxxx) SEQ ID NO: XXX (IL-12p40 (N281Q)), lxxxi) SEQ ID NO: XXX (IL-12p40

(N103Q/N113Q)), lxxxii) SEQ ID NO: XXX (IL-12p40 (N103Q/N200Q)), lxxxiii) SEQ ID NO: XXX (IL-12p40 (N103Q/N281Q)), lxxxiv) SEQ ID NO: XXX (IL-12p40 (N113Q/N200Q)), lxxxv) SEQ ID NO: XXX (IL-12p40 (N113Q/N281Q)), lxxxvi) SEQ ID NO: XXX (IL-12p40 (N200Q/N281Q)), lxxxvii) SEQ ID NO: XXX (IL-12p40 (N103Q/N113Q/N200Q)), lxxxviii) SEQ ID NO: XXX (IL-12p40 (N103Q/N113Q/N281Q)), lxxxix) SEQ ID NO: XXX (IL-12p40 (N103Q/N200Q/N281Q)), xc) SEQ ID NO: XXX (IL-12p40 (N113Q/N200Q/N281Q)), xci) SEQ ID NO:XXX (IL-12p40 (N103Q/N113Q/N200Q/N281Q)), xcii) SEQ ID NO:XXX (IL-12p40 (D34N/E59K/K99E)), xciii) SEQ ID NO:XXX (IL-12p40 (D34K/E59K/K99E)), xciv) SEQ ID NO:XXX (IL-12p40 (E32Q/D34N/E59K/K99E)), xcv) SEQ ID NO:XXX (IL-12p40 (E32K/D34N/E59K/K99E)), xcvi) SEQ ID NO: XXX (IL-12p40 (E59Y/K99Y)), xcvii) SEQ ID NO:XXX (IL-12p40 (E59K/K99E/N103Q/C252S/K264E)), xcviii) SEQ ID NO:XXX (IL-12p40 (E59K/K99E/N113Q/C252S/K264E)), xcix) SEQ ID NO:XXX (IL-12p40 (E59K/K99E/N200Q/C252S/K264E)), c) SEQ ID NO:XXX (IL-12p40 (E59K/K99E/N281Q/C252S/K264E)), ci) SEQ ID NO:XXX (IL-12p40 (E59K/K99E/N103Q/N113Q/C252S/K264E)), cii) SEQ ID NO:XXX (IL-12p40 (E59K/K99E/N103Q/N200Q/C252S/K264E)), ciii) SEQ ID NO:XXX (IL-12p40 (E59K/K99E/N103Q/N281Q/C252S/K264E)), civ) SEQ ID NO:XXX (IL-12p40 (E59K/K99E/N113Q/N200Q/C252S/K264E)), cv) SEQ ID NO:XXX (IL-12p40 (E59K/K99E/N113Q/N281Q/C252S/K264E)), cvi) SEQ ID NO:XXX (IL-12p40 (E59K/K99E/N200Q/N281Q/C252S/K264E)), cvii) SEQ ID NO:XXX (IL-12p40 (E59K/K99E/N103Q/N113Q/N200Q/C252S/K264E)), cviii) SEQ ID NO:XXX (IL-12p40 (E59K/K99E/N103Q/N200Q/N281Q/C252S/K264E)), and cix) SEQ ID NO:XXX (IL-12p40 (E59K/K99E/N113Q/N200Q/N281Q/C252S/K264E)).

[0009] In some embodiments, the present invention provides a heterodimeric Fc fusion protein, wherein said variant IL-12p40 subunit has at least 90% identity to a polypeptide sequence selected from the group consisting of SEQ ID NO:3 (human IL-12 subunit beta (IL-12p40) precursor sequence) and SEQ ID NO:4 (human IL-12 subunit beta (IL-12p40) mature form sequence), and/or said IL-12p35 subunit has a polypeptide sequence selected from the group consisting of SEQ ID

NO:1 (human IL-12 subunit alpha (IL-12p35) precursor sequence) and SEQ ID NO:2 (human IL-12 subunit alpha (IL-12p35) mature form sequence).

[0010] In some embodiments, the present invention provides a heterodimeric Fc fusion protein, wherein said IL-12p35 subunit is a variant IL-12p35 subunit.

[0011] In some embodiments, the present invention provides a heterodimeric Fc fusion protein, wherein said IL-12p40 subunit is a variant IL-12p40 subunit having altered affinity for IL-12 receptor subunit beta-1 (IL-12Rß1), IL-12 receptor subunit beta-2 (IL-12Rß2), and/or IL-12 receptor complex; and/or said IL-12p35 subunit is a variant IL-12p35 subunit having altered affinity for IL-12 receptor subunit beta-1 (IL-12Rß1), IL-12 receptor subunit beta-2 (IL-12Rß2), and/or IL-12 receptor complex.

[0012] In some embodiments, the present invention provides a heterodimeric Fc fusion protein, wherein said variant IL-12p35 subunit has one or more amino acid substitutions selected from the group consisting of N21D, Q35D, E38Q, D55Q, D55K, N71D, N71Q, L75A, N76D, E79Q, N85D, N85Q, L89A, F96A, M97A, L124A, M125A, Q130E, Q135E, N136D, E143Q, Q146E, N151D, N151K, E153K, E153Q, K158E, E162Q, E163Q, D165N, I171A, N195D, and N195Q.

[0013] In some embodiments, the present invention provides a heterodimeric Fc fusion protein, wherein said variant IL-12p35 subunit has amino acid substitutions selected from the group consisting of: N71D/N85D/N195D, N151D/E153Q, N151D/D165N, Q130E/N151D, N151D/K158E, E79Q/N151D, D55Q/N151D, N136D/N151D, N21D/N151D, E143Q/N151D, N71Q/N85Q, N71Q/N195Q, N85Q/N195Q, N71Q/N85Q/N195Q, N71D/N85D, N71D/N195D, and N85D/N195D.

[0014] In some embodiments, the present invention provides a heterodimeric Fc fusion protein, wherein said variant IL-12p35 subunit has a polypeptide sequence selected from the group consisting of: i) SEQ ID NO:113 (IL-12p35(N71D)), ii) SEQ ID NO:114 (IL-12p35(N85D)), iii) SEQ ID NO:115 (IL-12p35(N195D)), iv) SEQ ID NO:116 (IL-12p35(N71D/N85D/N195D)), v) SEQ ID NO:117 (IL-12p35(E153Q)), vi) SEQ ID NO:118 (IL-12p35(E38Q)), vii) SEQ ID NO:119 (IL-

12p35(N151D)), viii) SEQ ID NO:120 (IL-12p35(Q135E)), ix) SEQ ID NO:121 (IL-12p35(Q35D)), x) SEQ ID NO:122 (IL-12p35(Q146E)), xi) SEQ ID NO:123 (IL-12p35(N76D)), xii) SEQ ID NO:124 (IL-12p35(E162Q)), xiii) SEQ ID NO:125 (IL-12p35(E163Q)), xiv) IL-12p35(N21D), xv) SEQ ID NO:XXX (IL-12p35(D55Q)), xvi) IL-12p35(E79Q), xvii) IL-12p35(Q130E), xviii) IL-12p35(N136D), xix) IL-12p35(E143Q), xx) SEQ ID NO:227 (IL-12p35(N151K)), xxi) SEQ ID NO:226 (IL-12p35(E153K)), xxii) IL-12p35(K158E), xxiii) IL-12p35(D165N), xxiv) SEQ ID NO:225 (IL-12p35(N151D/E153Q)), xxv) SEQ ID NO:228 (IL-12p35(N151D/D165N)), xxvi) SEQ ID NO:229 (IL-12p35(Q130E/N151D)), xxvii) SEQ ID NO:230 (IL-12p35(N151D/K158E)), xxviii) SEQ ID NO:231 (IL-12p35(E79Q/N151D)), xxix) SEQ ID NO:232 (IL-12p35(D55Q/N151D)), xxx) SEQ ID NO:233 (IL-12p35(N136D/N151D)), xxxi) SEQ ID NO:234 (IL-12p35(N21D/N151D)), xxxii) SEQ ID NO:235 (IL-12p35(E143Q/N151D)), xxxiii) SEQ ID NO: XXX (IL-12p35(F96A)), xxxiv) SEQ ID NO: XXX (IL-12p35(M97A)), xxxv) SEQ ID NO: XXX (IL-12p35(L89A)), xxxvi) SEQ ID NO: XXX (IL-12p35(L124A)), xxxvii) SEQ ID NO: XXX (IL-12p35(M125A)), xxxviii) SEQ ID NO: XXX (IL-12p35(L75A)), xxxiv) SEQ ID NO: XXX (IL-12p35(I171A)), xxxv) SEQ ID NO: XXX (IL-12p35 (N71Q)), xxxvi) SEQ ID NO: XXX (IL-12p35 (N85Q)), xxxvii) SEQ ID NO: XXX (IL-12p35 (N195Q)), xxxviii) SEQ ID NO: XXX (IL-12p35 (N71Q/N85Q)), xxxix) SEQ ID NO: XXX (IL-12p35 (N71Q/N195Q)), xl) SEQ ID NO: XXX (IL-12p35 (N85Q/N195Q), xli) SEQ ID NO: XXX (IL-12p35 (N71Q/N85Q/N195Q)), xlii) SEQ ID NO: XXX (IL-12p35 (N71D/N85D)), xliii) SEQ ID NO: XXX (IL-12p35 (N71D/N195D), xliv) SEQ ID NO: XXX (IL-12p35 (N85D/N195D)), xlv) SEQ ID NO: XXX (IL-12p35 (D55Q)), and xlvi) SEQ ID NO: XXX (IL-12p35 (D55K)).

[0015] In some embodiments, the present invention provides a heterodimeric Fc fusion protein, wherein said modifications promoting heterodimerization of said first and said second Fc domains are a set of amino acid substitutions selected from the group consisting of L368D/K370S and S364K; L368D/K370S and S364K/E357L; L368D/K370S and S364K/E357Q; T411E/K360E/Q362E and D401K; L368E/K370S and S364K; K370S and S364K/E357Q; T366S/L368A/Y407V and T366W; T366S/L368A/Y407V/Y349C and T366W/S354C, according to EU numbering.

[0016] In some embodiments, the present invention provides a heterodimeric Fc fusion protein, wherein said variant IL-12p40 subunit domain is attached to said first Fc domain using a first domain linker and/or said IL-12p35 subunit domain is attached to said second Fc domain using a second domain linker.

[0017] In some embodiments, the present invention provides a heterodimeric Fc fusion protein, wherein said first and/or said second Fc domains have an additional set of amino acid substitutions comprising Q295E/N384D/Q418E/N421D, according to EU numbering.

[0018] In some embodiments, the present invention provides a heterodimeric Fc fusion protein, wherein said first and/or said second Fc domains have an additional set of amino acid substitutions selected from the group consisting of G236R/L328R, E233P/L234V/L235A/G236_/S239K, E233P/L234V/L235A/G236_/S239K/A327G, E233P/L234V/L235A/G236_/S267K/A327G, E233P/L234V/L235A/G236_, and E233P/L234V/L235A/G236_/S267K, according to EU numbering.

[0019] In some embodiments, the present invention provides a heterodimeric Fc fusion protein, wherein said first and second Fc domains further comprise amino acid substitutions M428L/N424S.

[0020] In some embodiments, the present invention provides a heterodimeric Fc fusion protein, wherein said heterodimeric Fc fusion protein comprises:

[0021] a) said first fusion protein having a polypeptide sequence selected from the group consisting of: i) SEQ ID NO:47 (XENP27201 Chain 1), ii) SEQ ID NO:85 (XenD24752), iii) SEQ ID NO:86 (XenD24753), iv) SEQ ID NO:87 (XenD24754), v) SEQ ID NO:88 (XenD24755), vi) SEQ ID NO:89 (XenD24756), vii) SEQ ID NO:90 (XenD24757), viii) SEQ ID NO:91 (XenD24758), ix) SEQ ID NO:92 (XenD24759), x) SEQ ID NO:93 (XenD24760), xi) SEQ ID NO:94 (XenD24761), xii) SEQ ID NO:95 (XenD24762), xiii) SEQ ID NO:96 (XenD24763), xiv) SEQ ID NO:97 (XenD24764), xv) SEQ ID NO:98 (XenD24765), xvi) SEQ ID NO:99 (XenD24766), xvii) SEQ ID NO:100 (XenD24767), xviii) SEQ ID NO:101 (XenD24768), xix) SEQ ID NO:102 (XenD24769), xx) SEQ ID NO:103

(XenD24770), xxi) SEQ ID NO:104 (XenD24771), xxii) SEQ ID NO:105 (XenD24772), xxiii) SEQ ID NO:106 (XenD24773), xxiv) SEQ ID NO:107 (XenD24774), xxv) SEQ ID NO:108 (XenD24775), xxvi) SEQ ID NO:109 (XenD24776), xxvii) SEQ ID NO:110 (XenD24777), xxviii) SEQ ID NO:111 (XenD24778), xxix) SEQ ID NO:112 (XenD24792), xxx) SEQ ID NO:215 (XenD25922), xxxi) SEQ ID NO:216 (XenD25923), xxxii) SEQ ID NO:217 (XenD25924), xxxiii) SEQ ID NO:218 (XenD25925), xxxiv) SEQ ID NO:219 (XenD25926), xxxv) SEQ ID NO:220(XenD25927), xxxvi) SEQ ID NO:221 (XenD25928), xxxvii) SEQ ID NO:222 (XenD25929), xxxviii) SEQ ID NO:223 (XenD25930), xxxix) SEQ ID NO:224 (XenD25931), xl) SEQ ID NO:XXX (XenD26411), xli) SEQ ID NO:XXX (XenD26412), xlii) SEQ ID NO:XXX (XenD26413), xliii) SEQ ID NO:XXX (XenD26414), xliv) SEQ ID NO:XXX (XenD26415), xlv) SEQ ID NO:XXX (XenD26416), xlvi) SEQ ID NO:XXX (XenD26417), xlvii) SEQ ID NO:XXX (XenD26418), xlviii) SEQ ID NO:XXX (XenD27070), xlix) SEQ ID NO:XXX (XenD27071), l) SEQ ID NO:XXX (XenD27072), li) SEQ ID NO:XXX (XenD27073), lii) SEQ ID NO:XXX (XenD27074), liii) SEQ ID NO:XXX (XenD27075), liv) SEQ ID NO:XXX (XenD27076), lv) SEQ ID NO:XXX (XenD27077), lvi) SEQ ID NO:XXX (XenD27078), lvii) SEQ ID NO:XXX (XenD28173), lviii) SEQ ID NO:XXX (XenD24876), lix) SEQ ID NO:XXX (XenD27162), lx) SEQ ID NO:XXX (XenD27163), lxi) SEQ ID NO:XXX (XenD27164), lxii) SEQ ID NO:XXX (XenD27165) lxiii) SEQ ID NO:XXX (XENP31582 Chain 1), lxiv) SEQ ID NO:XXX (XENP31583 Chain 1), lxv) SEQ ID NO:XXX (XENP31584 Chain 1), lxvi) SEQ ID NO:XXX (XENP32187 Chain 1), lxvii) SEQ ID NO:XXX (XENP32188 Chain 1), lxviii) SEQ ID NO:XXX (XENP32189, Chain 1), lxix) SEQ ID NO:XXX (XENP32190 Chain 1), lxx) SEQ ID NO:XXX (XENP32191 Chain 1), lxxi) SEQ ID NO:XXX (XENP32991 Chain 1), lxxii) SEQ ID NO:XXX (XENP32992 Chain 1), lxxiii) SEQ ID NO:XXX (XENP32993 Chain 1), lxxiv) SEQ ID NO:XXX (XENP32994 Chain 1), lxxv) SEQ ID NO:XXX (XENP32995 Chain 1), lxxvi) SEQ ID NO:XXX (XENP32996 Chain 1), lxxvi) SEQ ID NO:XXX (XENP32997 Chain 1), lxxvii) SEQ ID NO:XXX (XENP32998 Chain 1), lxxvii) SEQ ID NO:XXX (XENP32999 Chain 1), lxxviii) SEQ ID NO:XXX (XENP33000 Chain 1), lxxix) SEQ ID NO:XXX (XENP33001 Chain 1), lxxx) SEQ ID NO:XXX (XENP33002 Chain 1), lxxxi) SEQ ID NO:XXX (XENP33003 Chain 1), lxxxii) SEQ ID NO:XXX (XENP33004 Chain 1), lxxxiii) SEQ ID NO:XXX (XENP33005 Chain 1), lxxxiv) SEQ ID NO:XXX (XENP33006 Chain 1), lxxxv) SEQ ID NO:XXX (XENP33007 Chain 1), lxxxvi) SEQ ID

NO:XXX (XENP33008 Chain 1), lxxxvii) SEQ ID NO:XXX (XENP33008 Chain 1), lxxxviii) SEQ ID NO:XXX (XENP33009 Chain 1), lxxxix) SEQ ID NO:XXX (XENP33010 Chain 1), and xc) SEQ ID NO:XXX (XENP33011 Chain 1); and

[0022] b) said second fusion protein having a polypeptide sequence selected from the group consisting of: i) SEQ ID NO:48 (XENP27201 Chain 2), ii) SEQ ID NO:126 (XenD24779), iii) SEQ ID NO:127 (XenD24780), iv) SEQ ID NO:128 (XenD24781), v) SEQ ID NO:129 (XenD24782), vi) SEQ ID NO:130 (XenD24783), vii) SEQ ID NO:131 (XenD24784), viii) SEQ ID NO:132 (XenD24785), ix) SEQ ID NO:133 (XenD24786), x) SEQ ID NO:134 (XenD24787), xi) SEQ ID NO:135 (XenD24788), xii) SEQ ID NO:136 (XenD24789), xiii) SEQ ID NO:137 (XenD24790), xiv) SEQ ID NO:138 (XenD24791), xv) SEQ ID NO:236 (XenD25911), xvi) SEQ ID NO:237 (XenD25912), xvii) SEQ ID NO:238 (XenD25913), xviii) SEQ ID NO:239 (XenD25914), xix) SEQ ID NO:240 (XenD25915), xx) SEQ ID NO:241 (XenD25916), xxi) SEQ ID NO:242 (XenD25917), xxii) SEQ ID NO:243 (XenD25918), xxiii) SEQ ID NO:244 (XenD25919), xxiv) SEQ ID NO:245 (XenD25920), xxv) SEQ ID NO:246 (XenD25921), xxvi) SEQ ID NO:XXX (XenD26427), xxvii) SEQ ID NO:XXX (XenD26428), xxviii) SEQ ID NO:XXX (XenD27089), xxix) SEQ ID NO:XXX (XenD27090), xxx) SEQ ID NO:XXX (XenD27091), xxxi) SEQ ID NO:XXX (XenD27092), xxxii) SEQ ID NO:XXX (XenD27093), xxxiii) SEQ ID NO:XXX (XenD27094), xxxix) SEQ ID NO:XXX (XenD24877), xl) SEQ ID NO:XXX (XenD27166), xli) SEQ ID NO:XXX (XENP31582 Chain 2), xlii) SEQ ID NO:XXX (XENP31583 Chain 2), xliii) SEQ ID NO:XXX (XENP31584 Chain 2), xliv) SEQ ID NO:XXX (XENP32187 Chain 2), xlv) SEQ ID NO:XXX (XENP32188 Chain 2), xlvi) SEQ ID NO:XXX (XENP32189, Chain 2), xlvii) SEQ ID NO:XXX (XENP32190 Chain 2), xlviii) SEQ ID NO:XXX (XENP32191 Chain 2), xlix) SEQ ID NO:XXX (XENP32991 Chain 2), l) SEQ ID NO:XXX (XENP32992 Chain 2), li) SEQ ID NO:XXX (XENP32993 Chain 2), lii) SEQ ID NO:XXX (XENP32994 Chain 2), liii) SEQ ID NO:XXX (XENP32995 Chain 2), liv) SEQ ID NO:XXX (XENP32996 Chain 2), lv) SEQ ID NO:XXX (XENP32997 Chain 2), lvi) SEQ ID NO:XXX (XENP32998 Chain 2), lvii) SEQ ID NO:XXX (XENP32999 Chain 2), lviii) SEQ ID NO:XXX (XENP33000 Chain 2), lix) SEQ ID NO:XXX (XENP33001 Chain 2), lx) SEQ ID NO:XXX (XENP33002 Chain 2), lxi) SEQ ID NO:XXX (XENP33003 Chain 2), lxii) SEQ ID NO:XXX

(XENP33004 Chain 2), lxiii) SEQ ID NO:XXX (XENP33005 Chain 2), lxiv) SEQ ID NO:XXX (XENP33006 Chain 2), lxv) SEQ ID NO:XXX (XENP33007 Chain 2), lxvi) SEQ ID NO:XXX (XENP33008 Chain 2), lxvii) SEQ ID NO:XXX (XENP33008 Chain 2), lxviii) SEQ ID NO:XXX (XENP33009 Chain 2), lxix) SEQ ID NO:XXX (XENP33010 Chain 2), and lxx) SEQ ID NO:XXX (XENP33011 Chain 2).

[0023] In some embodiments, the present invention provides a heterodimeric Fc fusion protein, wherein said heterodimeric Fc fusion protein is selected from the group consisting of XENP27201, XENP28820, XENP28821, XENP28822, XENP28823, XENP28824, XENP28825, XENP28826, XENP28827, XENP28828, XENP28829, XENP28830, XENP28831, XENP28832, XENP28833, XENP28834, XENP28835, XENP28836, XENP28837, XENP28838, XENP28839, XENP28840, XENP28841, XENP28842, XENP28843, XENP28844, XENP28845, XENP28846, XENP28847, XENP28848, XENP28849, XENP28850, XENP28851, XENP28852, XENP29949, XENP29950, XENP29951, XENP29952, XENP30597, XENP30598, XENP30599, XENP30600, XENP30601, XENP30602, XENP30603, XENP30604, XENP30605, XENP30606. XENP30307, XENP30308, XENP30609, XENP31250, XENP31251, XENP31252, XENP31253, XENP31254, XENP31255, XENP31256, XENP31257, XENP31258, XENP31259, XENP31260, XENP31261, XENP31262, XENP31263, XENP31264, XENP31265, XENP31286, XENP31142, XENP31143, XENP31144, XENP31145, XENP31146, XENP31582, XENP31583, XENP31584, XENP32186, XENP32187, XENP32188, XENP32189, XENP32190, XENP32191, XENP32991, XENP32992, XENP32993, XENP32994, XENP32995, XENP32996, XENP32997, XENP32998, XENP32999, XENP33000, XENP33001, XENP33002, XENP33003, XENP33004, XENP33005, XENP33006, XENP33007, XENP33008, XENP33008, XENP33009, XENP33010, and XENP33011.

[0024] In some embodiments, the present invention provides a heterodimeric Fc fusion protein, wherein said heterodimeric Fc fusion protein is selected from the group consisting of: XENP31251, XENP31254, XENP31258, XENP32186, XENP32187, XENP32188, XENP32189, XENP32190, and XENP32191.

[0025] In some embodiments, the present invention provides a heterodimeric Fc fusion protein, further comprising one or more modifications to the IL-12p40 subunit selected from the group consisting of: N103Q, N113Q, N200Q, and N281Q.

[0026] In some embodiments, the present invention provides a composition comprising a heterodimeric Fc fusion protein for use in treating cancer in a subject.

[0027] In some embodiments, the present invention provides one or more nucleic acids encoding a heterodimeric Fc fusion protein.

[0028] In some embodiments, the present invention provides a host cell comprising said one or more nucleic acids encoding a heterodimeric Fc fusion protein.

[0029] In some embodiments, the present invention provides a method of making a heterodimeric Fc fusion protein, said method comprising culturing a host cell under conditions whereby said heterodimeric Fc fusion protein is produced.

[0030] In some embodiments, the present invention provides a method of purifying a heterodimeric Fc fusion protein, said method comprising: a) providing a composition comprising the heterodimeric Fc fusion protein; b) loading said composition onto an ion exchange column; and c) collecting a fraction containing said heterodimeric Fc fusion protein.

[0031] In some embodiments, the present invention provides a heterodimeric Fc fusion protein wherein said IL-12p40 subunit has one or more amino acid modifications at amino acid residues selected from the group consisting of E3, D7, E12, D14, W15, P17, D18, A19, P20, G21, E22, M23, D29, E32, E33, D34, L40, D41, Q42, S43, E45, L47, T54, I55, Q56, K58, E59, F60, G61, D62, Q65, Y66, E73, K84, E86, D87, G88, I89, W90, D93, D97, K99, E100, K102, N103, K104, F106, E110, N113, Y114, D129, D142, Q144, E156, R159, D161, N162, K163, D166, D170, Q172, D174, A176, C177, P178, A179, A180, E181, S183, P185, E187, N200, S204, F206, R208, D209, D214, N218, Q220, N226, Q229, E231, E235, T242, P243, S245, Y246, F247, S248, C252, Q256, K258, K260, E262, K264, D265, D270, N281, Q289, D290, R291, Y292, Y293, and E299.

[0032] In some embodiments, the present invention provides a heterodimeric Fc fusion protein wherein said IL-12p35 subunit has one or more amino acid modifications as amino acid residues selected from the group consisting of Q20, N21, Q35, E38, S44, E45, E46, H49, K54, D55, T59, V60, E61, C63, L64, P65, E67, L68, N71, S73, C74, L75, N76, E79, N85, L89, F96, M97, L124, M125, Q130, Q135, N136, E143, Q146, N151, E153, K158, E162, E163, D165, I171, R181, I182, R183, V185, T186, D188, R189, V190, S192, Y193, N195, and A196.

[0033] In another aspect, the present invention provides a heterodimeric Fc fusion protein comprising: a) a first fusion protein comprising a first protein domain and a first Fc domain, wherein said first protein domain is covalently attached to the C-terminus of said first Fc domain; andb) a second fusion protein comprising a second protein domain and a second Fc domain, wherein said second protein domain is covalently attached to the C-terminus of said Fc domain; wherein said first and said second Fc domains comprise modifications promoting heterodimerization of said first and said second Fc domains and wherein said first protein domain comprises an IL-12p40 subunit and said second protein domain comprises an IL-12p35 subunit.

[0034] In some embodiments, the present invention provides a heterodimeric Fc fusion protein wherein said modifications promoting heterodimerization of said first and said second Fc domains are a set of amino acid substitutions selected from the group consisting of L368D/K370S and S364K; L368D/K370S and S364K/E357L; L368D/K370S and S364K/E357Q; T411E/K360E/Q362E and D401K; L368E/K370S and S364K; K370S and S364K/E357Q and T366S/L368A/Y407V : T366W (optionally including a bridging disulfide, T366S/L368A/Y407V/Y349C : T366W/S354C), according to EU numbering.

[0035] In some embodiments, the present invention provides a heterodimeric Fc fusion protein wherein said first protein domain is attached to said first Fc domain using a first domain linker and/or said second protein domain is attached to said second Fc domain using a second domain linker.

[0036] In some embodiments, the present invention provides a heterodimeric Fc fusion protein wherein said first and/or said second Fc domains have an additional set of amino acid substitutions comprising Q295E/N384D/Q418E/N421D, according to EU numbering.

[0037] In some embodiments, the present invention provides a heterodimeric Fc fusion protein wherein said first and/or said second Fc domains have an additional set of amino acid substitutions selected from the group consisting of G236R/L328R, E233P/L234V/L235A/G236_/S239K, E233P/L234V/L235A/G236_/S239K/A327G, E233P/L234V/L235A/G236_/S267K/A327G, E233P/L234V/L235A/G236_, and E233P/L234V/L235A/G236_/S267K, according to EU numbering.

[0038] In some embodiments, the present invention provides a heterodimeric Fc fusion protein wherein said IL-12p40 subunit has a polypeptide sequence selected form the group consisting of SEQ ID NO:3 (human IL-12 subunit beta (IL-12p40) precursor sequence) and SEQ ID NO:4 (human IL-12 subunit beta (IL-12p40) mature form sequence), and/or said IL-12p35 subunit has a polypeptide sequence selected from the group consisting of SEQ ID NO:1 (human IL-12 subunit alpha (IL-12p35) precursor sequence) and SEQ ID NO:2 (human IL-12 subunit alpha (IL-12p35) mature form sequence).

[0039] In some embodiments, the present invention provides a heterodimeric Fc fusion protein wherein said first and second Fc domains further comprise amino acid substitutions M428L/N424S.

[0040] In some embodiments, the present invention provides a heterodimeric Fc fusion protein wherein said IL-12p40 subunit is a variant IL-12p40 subunit and/or said IL-12p35 subunit is a variant IL-12p35 subunit.

[0041] In some embodiments, the present invention provides a heterodimeric Fc fusion protein wherein said IL-12p40 subunit is a variant IL-12p40 subunit having altered affinity for IL-12 receptor subunit beta-1 (IL-12Rß1), IL-12 receptor subunit beta-2 (IL-12Rß2), and/or IL-12 receptor complex; and/or said IL-12p35 subunit is a variant IL-12p35 subunit having altered

affinity for IL-12 receptor subunit beta-1 (IL-12Rß1), IL-12 receptor subunit beta-2 (IL-12Rß2), and/or IL-12 receptor complex.

[0042] In some embodiments, the present invention provides a heterodimeric Fc fusion protein wherein said IL-12p40 subunit has one or more amino acid modifications at amino acid residues selected from the group consisting of E3, D7, E12, D14, W15, P17, D18, A19, P20, G21, E22, M23, D29, E32, E33, D34, L40, D41, Q42, S43, E45, L47, T54, I55, Q56, K58, E59, F60, G61, D62, Q65, Y66, E73, K84, E86, D87, G88, I89, W90, D93, D97, K99, E100, K102, N103, K104, F106, E110, N113, Y114, D129, D142, Q144, E156, R159, D161, N162, K163, D166, D170, Q172, D174, A176, C177, P178, A179, A180, E181, S183, P185, E187, N200, S204, F206, R208, D209, D214, N218, Q220, N226, Q229, E231, E235, T242, P243, S245, Y246, F247, S248, C252, Q256, K258, K260, E262, K264, D265, D270, N281, Q289, D290, R291, Y292, Y293, and E299.

[0043] In some embodiments, the present invention provides a heterodimeric Fc fusion protein wherein said IL-12p40 subunit has one or more amino acid substitutions selected from the group consisting of D18N, D18K, E32Q, E33Q, D34N, D34K, Q42E, S43E, S43K, E45Q, Q56E, E59Q, E59K, D62N, E73Q, D87N, K99E, K99Y, E100Q, N103D, N103Q, N113D, N113Q, Q144E, D161N, R159E, K163E, E187Q, N200D, N200Q, N218Q, Q229E, E235Q, C252S, Q256N, K258E, K260E, E262Q, K264E, N281D, N281Q, and E299Q.

[0044] In some embodiments, the present invention provides a heterodimeric Fc fusion protein wherein said IL-12p40 subunit has amino acid substitutions selected from the group consisting of N103D/N113D/N200D/N281D, Q42E/E45Q, E45Q/Q56E, Q42E/E59Q, Q56E/E59Q, Q42E/E45Q/Q56E, E45Q/Q56E/E59Q, E32Q/E59Q, D34N/E59K, D34N/E59K/K99E, D34K/E59K/K99E, E32Q/D34N/E59K/K99E, E32K/D34N/E59K/K99E, D34N/E59Q, E59Q/E187Q, S43E/E59Q, S43K/E49Q, E59Q/K163E, E59Q/K99E, E59Q/K258E, E59Q/K260E, E59K/K99E, D18K/E59K/K99E, E59K/K99E/K264E, E59K/K99Y, E59Y/K99Y, E59Y/K99E, E45K/E59K/K99E, E59K/K99E/Q144E, E59K/K99E/Q144K, E59K/K99E/R159E, E59K/K99E/K264E, D18K/E59K/K99E/K264E, DI8K/E59K/K99E/C252S, D18K/E59K/K99E/C252S/K264E, E59K/K99Y/C252S, E59K/K99E/C252S/K264E, E59K/K99E/C252S, N103D/N113D,

N103D/N200D, N103D/N281D, N113D/N200D, N113D/N281D, N200D/N281D, N103D/N113D/N200D, N103D/N113D/N281D, N103D/N200D/N281D, N113D/N200D/N281D, N103Q/N113Q, N103Q/N200Q, N103Q/N281Q, N113Q/N200Q, N113Q/N281Q, N200Q/N281Q, N103Q/N113Q/N200Q, N103Q/N113Q/N281Q, N103Q/N200Q/N281Q, N113Q/N200Q/N281Q, N103Q/N113Q/N200Q/N281Q, E59K/K99E/N103Q/C252S/K264E, E59K/K99E/N113Q/C252S/K264E, E59K/K99E/N200Q/C252S/K264E, E59K/K99E/N281Q/C252S/K264E, E59K/K99E/N103Q/N113Q/C252S/K264E, E59K/K99E/N103Q/N200Q/C252S/K264E, E59K/K99E/N103Q/N281Q/C252S/K264E, E59K/K99E/N113Q/N200Q/C252S/K264E, E59K/K99E/N113Q/N281Q/C252S/K264E, E59K/K99E/N200Q/N281Q/C252S/K264E, E59K/K99E/N103Q/N113Q/N200Q/C252S/K264E, E59K/K99E/N103Q/N200Q/N281Q/C252S/K264E, E59K/K99E/N113Q/N200Q/N281Q/C252S/K264E, and E59K/K99E/N103Q/N113Q/N200Q/N281Q/C252S/K264E.

[0045] In some embodiments, the present invention provides a heterodimeric Fc fusion protein wherein said IL-12p40 subunit has amino acid substitutions selected from the group consisting of N103D/N113D/N200D/N281D, Q42E/E45Q, E45Q/Q56E, Q42E/E59Q, Q56E/E59Q, Q42E/E45Q/Q56E, E45Q/Q56E/E59Q, E32Q/E59Q, D34N/E59K, D34N/E59K/K99E, D34K/E59K/K99E, E32Q/D34N/E59K/K99E, E32K/D34N/E59K/K99E, D34N/E59Q, E59Q/E187Q, S43E/E59Q, S43K/E49Q, E59Q/K163E, E59Q/K99E, E59Q/K258E, E59Q/K260E, E59K/K99E, D18K/E59K/K99E, E59K/K99E/K264E, E59K/K99Y, E59Y/K99Y, E59Y/K99E, E45K/E59K/K99E, E59K/K99E/Q144E, E59K/K99E/Q144K, E59K/K99E/R159E, E59K/K99E/K264E, D18K/E59K/K99E/K264E, DI8K/E59K/K99E/C252S, D18K/E59K/K99E/C252S/K264E, E59K/K99Y/C252S, E59K/K99E/C252S/K264E, E59K/K99E/C252S, N103D/N113D, N103D/N200D, N103D/N281D, N113D/N200D, N113D/N281D, N200D/N281D, N103D/N113D/N200D, N103D/N113D/N281D, N103D/N200D/N281D, N113D/N200D/N281D, N103Q/N113Q, N103Q/N200Q, N103Q/N281Q, N113Q/N200Q, N113Q/N281Q, N200Q/N281Q, N103Q/N113Q/N200Q, N103Q/N113Q/N281Q, N103Q/N200Q/N281Q, N113Q/N200Q/N281Q, N103Q/N113Q/N200Q/N281Q, E59K/K99E/N103Q/C252S/K264E,

E59K/K99E/N113Q/C252S/K264E, E59K/K99E/N200Q/C252S/K264E,
E59K/K99E/N281Q/C252S/K264E, E59K/K99E/N103Q/N113Q/C252S/K264E,
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E59K/K99E/N103Q/N200Q/N281Q/C252S/K264E,
E59K/K99E/N113Q/N200Q/N281Q/C252S/K264E, and
E59K/K99E/N103Q/N113Q/N200Q/N281Q/C252S/K264E.

[0046] In some embodiments, the present invention provides a heterodimeric Fc fusion protein wherein said IL-12p40 subunit has a polypeptide sequence selected from the group consisting of: i) SEQ ID NO:57 (IL-12p40(N103D)), ii) SEQ ID NO:58 (IL-12p40(N113D)), iii) SEQ ID NO:59 (IL-12p40(N200D)), iv) SEQ ID NO:60 (IL-12p40(N281D)), v) SEQ ID NO:61 (IL-12p40(N103D/N113D/N200D/N281D)), vi) SEQ ID NO:62 (IL-12p40(Q42E)), vii) SEQ ID NO:63 (IL-12p40(E45Q)), viii) SEQ ID NO:64 (IL-12p40(Q56E)), ix) SEQ ID NO:65 (IL-12p40(E59Q)), x) SEQ ID NO:66 (IL-12p40(D62N)), xi) SEQ ID NO:67 (IL-12p40(Q42E/E45Q)), xii) SEQ ID NO:68 (IL-12p40(E45Q/Q56E)), xiii) SEQ ID NO:69 (IL-12p40(Q42E/E59Q)), xiv) SEQ ID NO:70 (IL-12p40(Q56E/E59Q)), xv) SEQ ID NO:71 (IL-12p40(Q42E/E45Q/Q56E)), xvi) SEQ ID NO:72 (IL-12p40(E45Q/Q56E/E59Q)), xvii) SEQ ID NO:73 (IL-12p40(D161N)), xviii) SEQ ID NO:74 (IL-12p40(E73Q)), xix) SEQ ID NO:75 (IL-12p40(Q144E)), xx) SEQ ID NO:76 (IL-12p40(E262Q)), xxi) SEQ ID NO:77 (IL-12p40(E100Q)), xxii) SEQ ID NO:78 (IL-12p40(D18N)), xxiii) SEQ ID NO:79 (IL-12p40(E33Q)), xxiv) SEQ ID NO:80 (IL-12p40(Q229E)), xxv) SEQ ID NO:81 (IL-12p40(E235Q)), xxvi) SEQ ID NO:82 (IL-12p40(Q256N)), xxvii) SEQ ID NO:83 (IL-12p40(E299Q)), xxviii) SEQ ID NO:84 (IL-12p40(D87N)), xxix) IL-12p40(E32Q), xxx) IL-12p40(D34N), xxxi) IL-12p40(S43E), xxxii) IL-12p40(S43K), xxxiii) SEQ ID NO:XXX (IL-12p40 (E59K/K99E/N103Q/N113Q/N200Q/N281Q/C252S/K264E)), xxxiv) SEQ ID NO:205 (IL-12p40(E59K)), xxxv) IL-12p40(K99E), xxxvi) IL-12p40(K163E), xxxvii) IL-12p40(E187Q), xxxviii) IL-12p40(K258E), xxxix) IL-12p40(K260E), xl) SEQ ID NO:206 (IL-12p40(E32Q/E59Q)), xli) SEQ ID NO:207 (IL-12p40(D34N/E59Q)), xlii) SEQ ID NO:208 (IL-12p40(E59Q/E187Q)),

xliii) SEQ ID NO:209 (IL-12p40(S43E/E59Q)), xliv) SEQ ID NO:210 (IL-12p40(S43K/E49Q)), xlv) SEQ ID NO:211 (IL-12p40(E59Q/K163E)), xlvi) SEQ ID NO:212 (IL-12p40(E59Q/K99E)), xlvii) SEQ ID NO:213 (IL-12p40(E59Q/K258E)), xlviii) SEQ ID NO:214 (IL-12p40(E59Q/K260E)), xlix) SEQ ID NO: XXX (IL-12p40 (D34N/E59K)), l) SEQ ID NO: XXX (IL-12p40(E59K/K99E)), li) SEQ ID NO: XXX (IL-12p40(D18K/E59K/K99E)), lii) SEQ ID NO: XXX (IL-12p40 (E59K/K99E/K264E)), liii) SEQ ID NO: XXX (IL-12p40 (E59K/K99Y)), liv) SEQ ID NO: XXX (IL-12p40 (E59Y/K99E)), lv) SEQ ID NO: XXX (IL-12p40 (E45K/E59K/K99E)), lvi) SEQ ID NO: XXX (IL-12p40 (E59K/K99E/Q144E)), lvii) SEQ ID NO: XXX (IL-12p40 (E59K/K99E/Q144K)), lviii) SEQ ID NO: XXX (IL-12p40 (E59K/K99E/R159E)), lix) SEQ ID NO: XXX (IL-12p40 (E59K/K99E/K264E)), lx) SEQ ID NO: XXX (IL-12p40 (D18K/E59K/K99E/K264E)), lxi) SEQ ID NO: XXX (IL-12p40 (C252S)), lxii) SEQ ID NO: XXX (IL-12p40 (DI8K/E59K/K99E/C252S)), lxiii) SEQ ID NO: XXX (IL-12p40 (D18K/E59K/K99E/C252S/K264E)), lxiv) SEQ ID NO: XXX (IL-12p40 (E59K/K99Y/C252S)), lxv) SEQ ID NO: XXX (IL-12p40 (E59K/K99E/C252S/K264E)), lxvi) SEQ ID NO: XXX (IL-12p40 (E59K/K99E/C252S)), lxvii) SEQ ID NO: XXX (IL-12p40 (N103D/N113D)), lxviii) SEQ ID NO: XXX (IL-12p40 (N103D/N200D)), lxix) SEQ ID NO: XXX (IL-12p40 (N103D/N281D)), lxx) SEQ ID NO: XXX (IL-12p40 (N113D/N200D)), lxxi) SEQ ID NO: XXX (IL-12p40 (N113D/N281D)), lxxii) SEQ ID NO: XXX (IL-12p40 (N200D/N281D)), lxxiii) SEQ ID NO: XXX (IL-12p50 (N103D/N113D/N200D)), lxxiv) SEQ ID NO: XXX (IL-12p40 (N103D/N113D/N281D)), lxxv) SEQ ID NO: XXX (IL-12p40 (N103D/N200D/N281D)), lxxvi) SEQ ID NO: XXX (IL-12p40 (N113D/N200D/N281D)), lxxvii) SEQ ID NO: XXX (IL-12p40 (N103Q)), lxxviii) SEQ ID NO: XXX (IL-12p40 (N113Q)), lxxix) SEQ ID NO: XXX (IL-12p40 (N200Q)), lxxx) SEQ ID NO: XXX (IL-12p40 (N281Q)), lxxxi) SEQ ID NO: XXX (IL-12p40 (N103Q/N113Q)), lxxxii) SEQ ID NO: XXX (IL-12p40 (N103Q/N200Q)), lxxxiii) SEQ ID NO: XXX (IL-12p40 (N103Q/N281Q)), lxxxiv) SEQ ID NO: XXX (IL-12p40 (N113Q/N200Q)), lxxxv) SEQ ID NO: XXX (IL-12p40 (N113Q/N281Q)), lxxxvi) SEQ ID NO: XXX (IL-12p40 (N200Q/N281Q)), lxxxvii) SEQ ID NO: XXX (IL-12p40 (N103Q/N113Q/N200Q)), lxxxviii) SEQ ID NO: XXX (IL-12p40 (N103Q/N113Q/N281Q)), lxxxix) SEQ ID NO: XXX (IL-12p40 (N103Q/N200Q/N281Q)), xc) SEQ ID NO: XXX (IL-12p40 (N113Q/N200Q/N281Q)), xci) SEQ ID NO:XXX (IL-12p40 (N103Q/N113Q/N200Q/N281Q)), xcii) SEQ ID NO:XXX (IL-12p40 (D34N/E59K/K99E)), xciii)

SEQ ID NO:XXX (IL-12p40 (D34K/E59K/K99E)), xciv) SEQ ID NO:XXX (IL-12p40 (E32Q/D34N/E59K/K99E)), xcv) SEQ ID NO:XXX (IL-12p40 (E32K/D34N/E59K/K99E)), xcvi) SEQ ID NO: XXX (IL-12p40 (E59Y/K99Y)), xcvii) SEQ ID NO:XXX (IL-12p40 (E59K/K99E/N103Q/C252S/K264E)), xcviii) SEQ ID NO:XXX (IL-12p40 (E59K/K99E/N113Q/C252S/K264E)), xcix) SEQ ID NO:XXX (IL-12p40 (E59K/K99E/N200Q/C252S/K264E)), c) SEQ ID NO:XXX (IL-12p40 (E59K/K99E/N281Q/C252S/K264E)), ci) SEQ ID NO:XXX (IL-12p40 (E59K/K99E/N103Q/N113Q/C252S/K264E)), cii) SEQ ID NO:XXX (IL-12p40 (E59K/K99E/N103Q/N200Q/C252S/K264E)), ciii) SEQ ID NO:XXX (IL-12p40 (E59K/K99E/N103Q/N281Q/C252S/K264E)), civ) SEQ ID NO:XXX (IL-12p40 (E59K/K99E/N113Q/N200Q/C252S/K264E)), cv) SEQ ID NO:XXX (IL-12p40 (E59K/K99E/N113Q/N281Q/C252S/K264E)), cvi) SEQ ID NO:XXX (IL-12p40 (E59K/K99E/N200Q/N281Q/C252S/K264E)), cvii) SEQ ID NO:XXX (IL-12p40 (E59K/K99E/N103Q/N113Q/N200Q/C252S/K264E)), cviii) SEQ ID NO:XXX (IL-12p40 (E59K/K99E/N103Q/N200Q/N281Q/C252S/K264E)), and cix) SEQ ID NO:XXX (IL-12p40 (E59K/K99E/N113Q/N200Q/N281Q/C252S/K264E)).In some embodiments, the present invention provides a heterodimeric Fc fusion protein wherein said IL-12p35 subunit has one or more amino acid modifications as amino acid residues selected from the group consisting of Q20, N21, Q35, E38, S44, E45, E46, H49, K54, D55, T59, V60, E61, C63, L64, P65, E67, L68, N71, S73, C74, L75, N76, E79, N85, L89, F96, M97, L124, M125, Q130, Q135, N136, E143, Q146, N151, E153, K158, E162, E163, D165, I171, R181, I182, R183, V185, T186, D188, R189, V190, S192, Y193, N195, and A196.

[0047] In some embodiments, the present invention provides a heterodimeric Fc fusion protein wherein said IL-12p35 subunit has one or more amino acid substitutions selected from the group consisting of N21D, Q35D, E38Q, D55Q, D55K, N71D, N71Q, L75A, N76D, E79Q, N85D, N85Q, L89A, F96A, M97A, L124A, M125A, Q130E, Q135E, N136D, E143Q, Q146E, N151D, N151K, E153K, E153Q, K158E, E162Q, E163Q, D165N, I171A, N195D, and N195Q.

[0048] In some embodiments, the present invention provides a heterodimeric Fc fusion protein wherein said IL-12p35 subunit has amino acid substitutions selected from the group consisting of N71D/N85D/N195D, N151D/E153Q, N151D/D165N, Q130E/N151D, N151D/K158E, E79Q/N151D, D55Q/N151D, N136D/N151D, N21D/N151D, E143Q/N151D, N71Q/N85Q, N71Q/N195Q, N85Q/N195Q, N71Q/N85Q/N195Q, N71D/N85D, N71D/N195D, and N85D/N195D.

[0049] In some embodiments, the present invention provides a heterodimeric Fc fusion protein wherein said IL-12p35 subunit has a polypeptide sequence selected from the group consisting of: i) SEQ ID NO:113 (IL-12p35(N71D)), ii) SEQ ID NO:114 (IL-12p35(N85D)), iii) SEQ ID NO:115 (IL-12p35(N195D)), iv) SEQ ID NO:116 (IL-12p35(N71D/N85D/N195D)), v) SEQ ID NO:117 (IL-12p35(E153Q)), vi) SEQ ID NO:118 (IL-12p35(E38Q)), vii) SEQ ID NO:119 (IL-12p35(N151D)), viii) SEQ ID NO:120 (IL-12p35(Q135E)), ix) SEQ ID NO:121 (IL-12p35(Q35D)), x) SEQ ID NO:122 (IL-12p35(Q146E)), xi) SEQ ID NO:123 (IL-12p35(N76D)), xii) SEQ ID NO:124 (IL-12p35(E162Q)), xiii) SEQ ID NO:125 (IL-12p35(E163Q)), xiv) IL-12p35(N21D), xv) SEQ ID NO:XXX (IL-12p35(D55Q)), xvi) IL-12p35(E79Q), xvii) IL-12p35(Q130E), xviii) IL-12p35(N136D), xix) IL-12p35(E143Q), xx) SEQ ID NO:227 (IL-12p35(N151K)), xxi) SEQ ID NO:226 (IL-12p35(E153K)), xxii) IL-12p35(K158E), xxiii) IL-12p35(D165N), xxiv) SEQ ID NO:225 (IL-12p35(N151D/E153Q)), xxv) SEQ ID NO:228 (IL-12p35(N151D/D165N)), xxvi) SEQ ID NO:229 (IL-12p35(Q130E/N151D)), xxvii) SEQ ID NO:230 (IL-12p35(N151D/K158E)), xxviii) SEQ ID NO:231 (IL-12p35(E79Q/N151D)), xxix) SEQ ID NO:232 (IL-12p35(D55Q/N151D)), xxx) SEQ ID NO:233 (IL-12p35(N136D/N151D)), xxxi) SEQ ID NO:234 (IL-12p35(N21D/N151D)), xxxii) SEQ ID NO:235 (IL-12p35(E143Q/N151D)), xxxiii) SEQ ID NO: XXX (IL-12p35(F96A)), xxxiv) SEQ ID NO: XXX (IL-12p35(M97A)), xxxv) SEQ ID NO: XXX (IL-12p35(L89A)), xxxvi) SEQ ID NO: XXX (IL-12p35(L124A)), xxxvii) SEQ ID NO: XXX (IL-12p35(M125A)), xxxviii) SEQ ID NO: XXX (IL-12p35(L75A)), xxxiv) SEQ ID NO: XXX (IL-12p35(I171A)), xxxv) SEQ ID NO: XXX (IL-12p35 (N71Q)), xxxvi) SEQ ID NO: XXX (IL-12p35 (N85Q)), xxxvii) SEQ ID NO: XXX (IL-12p35 (N195Q)), xxxviii) SEQ ID NO: XXX (IL-12p35 (N71Q/N85Q)), xxxix) SEQ ID NO: XXX (IL-12p35 (N71Q/N195Q)), xl) SEQ ID NO: XXX (IL-12p35 (N85Q/N195Q), xli) SEQ ID NO: XXX (IL-12p35 (N71Q/N85Q/N195Q)), xlii) SEQ ID NO:

XXX (IL-12p35 (N71D/N85D)), xliii) SEQ ID NO: XXX (IL-12p35 (N71D/N195D), xliv) SEQ ID NO: XXX (IL-12p35 (N85D/N195D)), xlv) SEQ ID NO: XXX (IL-12p35 (D55Q)), and xlvi) SEQ ID NO: XXX (IL-12p35 (D55K)).

[0050] In some embodiments, the present invention provides a heterodimeric Fc fusion protein wherein said heterodimeric Fc fusion protein comprises: a) said first fusion protein having a polypeptide sequence of SEQ ID NO:49 (XENP27202 Chain 1), and b) said second fusion protein having a polypeptide sequence of SEQ ID NO:50 (XENP27202 Chain 2).

[0051] In some embodiments, the present invention provides a heterodimeric Fc fusion protein wherein said heterodimeric Fc fusion protein is XENP27202.

[0052] In some embodiments, the present invention provides a composition comprising a heterodimeric Fc fusion protein for use in treating cancer in a subject.

[0053] In some embodiments, the present invention provides one or more nucleic acids encoding a heterodimeric Fc fusion protein.

[0054] In some embodiments, the present invention provides a host cell comprising said one or more nucleic acids encoding a heterodimeric Fc fusion protein.

[0055] In some embodiments, the present invention provides a method of making a heterodimeric Fc fusion protein, said method comprising culturing a host cell under conditions whereby said heterodimeric Fc fusion protein is produced.

[0056] In some embodiments, the present invention provides a method of purifying a heterodimeric Fc fusion protein, said method comprising: a) providing a composition comprising the heterodimeric Fc fusion protein; b) loading said composition onto an ion exchange column; andc) collecting a fraction containing said heterodimeric Fc fusion protein.

[0057] In another aspect, the present invention provides a heterodimeric Fc fusion protein comprising: a) a fusion protein comprising a first protein domain, a second protein domain and a first Fc domain, wherein said first protein domain is covalently attached to said second protein

domain, and wherein said second protein domain is covalently attached to the N-terminus of said first Fc domain; and b) a second Fc domain; wherein said first and said second Fc domains comprise modifications promoting heterodimerization of said first and said second Fc domains and wherein said first protein domain comprises an IL-12p40 subunit and said second protein domain comprises an IL-12p35 subunit.

[0058] In some embodiments, the present invention provides a heterodimeric Fc fusion protein wherein said modifications promoting heterodimerization of said first and said second Fc domains are a set of amino acid substitutions selected from the group consisting of L368D/K370S and S364K; L368D/K370S and S364K/E357L; L368D/K370S and S364K/E357Q; T411E/K360E/Q362E and D401K; L368E/K370S and S364K; K370S and S364K/E357Q and T366S/L368A/Y407V : T366W (optionally including a bridging disulfide, T366S/L368A/Y407V/Y349C : T366W/S354C), according to EU numbering.

[0059] In some embodiments, the present invention provides a heterodimeric Fc fusion protein wherein said first protein domain is attached to said second protein domain using a first domain linker and/or said second protein domain is attached to said first Fc domain using a second domain linker.

[0060] In some embodiments, the present invention provides a heterodimeric Fc fusion protein wherein said first and/or said second Fc domains have an additional set of amino acid substitutions comprising Q295E/N384D/Q418E/N421D, according to EU numbering.

[0061] In some embodiments, the present invention provides a heterodimeric Fc fusion protein wherein said first and/or said second Fc domains have an additional set of amino acid substitutions selected from the group consisting of G236R/L328R, E233P/L234V/L235A/G236_/S239K, E233P/L234V/L235A/G236_/S239K/A327G, E233P/L234V/L235A/G236_/S267K/A327G, E233P/L234V/L235A/G236_, and E233P/L234V/L235A/G236_/S267K, according to EU numbering.

[0062] In some embodiments, the present invention provides a heterodimeric Fc fusion protein wherein said IL-12p40 subunit has a polypeptide sequence selected form the group consisting of SEQ ID NO:3 (human IL-12 subunit beta (IL-12p40) precursor sequence) and SEQ ID NO:4 (human IL-12 subunit beta (IL-12p40) mature form sequence), and/or said IL-12p35 subunit has a polypeptide sequence selected from the group consisting of SEQ ID NO:1 (human IL-12 subunit alpha (IL-12p35) precursor sequence) and SEQ ID NO:2 (human IL-12 subunit alpha (IL-12p35) mature form sequence).

[0063] In some embodiments, the present invention provides a heterodimeric Fc fusion protein wherein said first and second Fc domains further comprise amino acid substitutions M428L/N424S.

[0064] In some embodiments, the present invention provides a heterodimeric Fc fusion protein wherein said IL-12p40 subunit is a variant IL-12p40 subunit and/or said IL-12p35 subunit is a variant IL-12p35 subunit.

[0065] In some embodiments, the present invention provides a heterodimeric Fc fusion protein wherein said IL-12p40 subunit is a variant IL-12p40 subunit having altered affinity for IL-12 receptor subunit beta-1 (IL-12Rß1), IL-12 receptor subunit beta-2 (IL-12Rß2), and/or IL-12 receptor complex; and/or said IL-12p35 subunit is a variant IL-12p35 subunit having altered affinity for IL-12 receptor subunit beta-1 (IL-12Rß1), IL-12 receptor subunit beta-2 (IL-12Rß2), and/or IL-12 receptor complex.

[0066] In some embodiments, the present invention provides a heterodimeric Fc fusion protein wherein said IL-12p40 subunit has one or more amino acid modifications at amino acid residues selected from the group consisting of E3, D7, E12, D14, W15, P17, D18, A19, P20, G21, E22, M23, D29, E32, E33, D34, L40, D41, Q42, S43, E45, L47, T54, I55, Q56, K58, E59, F60, G61, D62, Q65, Y66, E73, K84, E86, D87, G88, I89, W90, D93, D97, K99, E100, K102, N103, K104, F106, E110, N113, Y114, D129, D142, Q144, E156, R159, D161, N162, K163, D166, D170, Q172, D174, A176, C177, P178, A179, A180, E181, S183, P185, E187, N200, S204, F206, R208, D209, D214, N218, Q220, N226, Q229,

E231, E235, T242, P243, S245, Y246, F247, S248, Q256, K158, C252, K260, E262, K264, D265, D270, N281, Q289, D290, R291, Y292, Y293, and E299.

[0067] In some embodiments, the present invention provides a heterodimeric Fc fusion protein wherein said IL-12p40 subunit has one or more amino acid substitutions selected from the group consisting of D18N, D18K, E32Q, E33Q, D34N, D34K, Q42E, S43E, S43K, E45Q, Q56E, E59Q, E59K, D62N, E73Q, D87N, K99E, K99Y, E100Q, N103D, N103Q, N113D, N113Q, Q144E, D161N, R159E, K163E, E187Q, N200D, N200Q, N218Q, Q229E, E235Q, C252S, Q256N, K258E, K260E, E262Q, K264E, N281D, N281Q, and E299Q.

[0068] In some embodiments, the present invention provides a heterodimeric Fc fusion protein wherein said IL-12p40 subunit has amino acid substitutions selected from the group consisting of N103D/N113D/N200D/N281D, Q42E/E45Q, E45Q/Q56E, Q42E/E59Q, Q56E/E59Q, Q42E/E45Q/Q56E, E45Q/Q56E/E59Q, E32Q/E59Q, D34N/E59K, D34N/E59K/K99E, D34K/E59K/K99E, E32Q/D34N/E59K/K99E, E32K/D34N/E59K/K99E, D34N/E59Q, E59Q/E187Q, S43E/E59Q, S43K/E49Q, E59Q/K163E, E59Q/K99E, E59Q/K258E, E59Q/K260E, E59K/K99E, D18K/E59K/K99E, E59K/K99E/K264E, E59K/K99Y, E59Y/K99Y, E59Y/K99E, E45K/E59K/K99E, E59K/K99E/Q144E, E59K/K99E/Q144K, E59K/K99E/R159E, E59K/K99E/K264E, D18K/E59K/K99E/K264E, DI8K/E59K/K99E/C252S, D18K/E59K/K99E/C252S/K264E, E59K/K99Y/C252S, E59K/K99E/C252S/K264E, E59K/K99E/C252S, N103D/N113D, N103D/N200D, N103D/N281D, N113D/N200D, N113D/N281D, N200D/N281D, N103D/N113D/N200D, N103D/N113D/N281D, N103D/N200D/N281D, N113D/N200D/N281D, N103Q/N113Q, N103Q/N200Q, N103Q/N281Q, N113Q/N200Q, N113Q/N281Q, N200Q/N281Q, N103Q/N113Q/N200Q, N103Q/N113Q/N281Q, N103Q/N200Q/N281Q, N113Q/N200Q/N281Q, N103Q/N113Q/N200Q/N281Q, E59K/K99E/N103Q/C252S/K264E, E59K/K99E/N113Q/C252S/K264E, E59K/K99E/N200Q/C252S/K264E, E59K/K99E/N281Q/C252S/K264E, E59K/K99E/N103Q/N113Q/C252S/K264E, E59K/K99E/N103Q/N200Q/C252S/K264E, E59K/K99E/N103Q/N281Q/C252S/K264E, E59K/K99E/N113Q/N200Q/C252S/K264E, E59K/K99E/N113Q/N281Q/C252S/K264E, E59K/K99E/N200Q/N281Q/C252S/K264E, E59K/K99E/N103Q/N113Q/N200Q/C252S/K264E,

E59K/K99E/N103Q/N200Q/N281Q/C252S/K264E,
E59K/K99E/N113Q/N200Q/N281Q/C252S/K264E, and
E59K/K99E/N103Q/N113Q/N200Q/N281Q/C252S/K264E.

[0069] In some embodiments, the present invention provides a heterodimeric Fc fusion protein wherein said IL-12p40 subunit has a polypeptide sequence selected from the group consisting of: i) SEQ ID NO:57 (IL-12p40(N103D)), ii) SEQ ID NO:58 (IL-12p40(N113D)), iii) SEQ ID NO:59 (IL-12p40(N200D)), iv) SEQ ID NO:60 (IL-12p40(N281D)), v) SEQ ID NO:61 (IL-12p40(N103D/N113D/N200D/N281D)), vi) SEQ ID NO:62 (IL-12p40(Q42E)), vii) SEQ ID NO:63 (IL-12p40(E45Q)), viii) SEQ ID NO:64 (IL-12p40(Q56E)), ix) SEQ ID NO:65 (IL-12p40(E59Q)), x) SEQ ID NO:66 (IL-12p40(D62N)), xi) SEQ ID NO:67 (IL-12p40(Q42E/E45Q)), xii) SEQ ID NO:68 (IL-12p40(E45Q/Q56E)), xiii) SEQ ID NO:69 (IL-12p40(Q42E/E59Q)), xiv) SEQ ID NO:70 (IL-12p40(Q56E/E59Q)), xv) SEQ ID NO:71 (IL-12p40(Q42E/E45Q/Q56E)), xvi) SEQ ID NO:72 (IL-12p40(E45Q/Q56E/E59Q)), xvii) SEQ ID NO:73 (IL-12p40(D161N)), xviii) SEQ ID NO:74 (IL-12p40(E73Q)), xix) SEQ ID NO:75 (IL-12p40(Q144E)), xx) SEQ ID NO:76 (IL-12p40(E262Q)), xxi) SEQ ID NO:77 (IL-12p40(E100Q)), xxii) SEQ ID NO:78 (IL-12p40(D18N)), xxiii) SEQ ID NO:79 (IL-12p40(E33Q)), xxiv) SEQ ID NO:80 (IL-12p40(Q229E)), xxv) SEQ ID NO:81 (IL-12p40(E235Q)), xxvi) SEQ ID NO:82 (IL-12p40(Q256N)), xxvii) SEQ ID NO:83 (IL-12p40(E299Q)), xxviii) SEQ ID NO:84 (IL-12p40(D87N)), xxix) IL-12p40(E32Q), xxx) IL-12p40(D34N), xxxi) IL-12p40(S43E), xxxii) IL-12p40(S43K), xxxiii) SEQ ID NO:XXX (IL-12p40 (E59K/K99E/N103Q/N113Q/N200Q/N281Q/C252S/K264E)), xxxiv) SEQ ID NO:205 (IL-12p40(E59K)), xxxv) IL-12p40(K99E), xxxvi) IL-12p40(K163E), xxxvii) IL-12p40(E187Q), xxxviii) IL-12p40(K258E), xxxix) IL-12p40(K260E), xl) SEQ ID NO:206 (IL-12p40(E32Q/E59Q)), xli) SEQ ID NO:207 (IL-12p40(D34N/E59Q)), xlii) SEQ ID NO:208 (IL-12p40(E59Q/E187Q)), xliii) SEQ ID NO:209 (IL-12p40(S43E/E59Q)), xliv) SEQ ID NO:210 (IL-12p40(S43K/E49Q)), xlv) SEQ ID NO:211 (IL-12p40(E59Q/K163E)), xlvi) SEQ ID NO:212 (IL-12p40(E59Q/K99E)), xlvii) SEQ ID NO:213 (IL-12p40(E59Q/K258E)), xlviii) SEQ ID NO:214 (IL-12p40(E59Q/K260E)), xlix) SEQ ID NO: XXX (IL-12p40 (D34N/E59K)), l) SEQ ID NO: XXX (IL-12p40(E59K/K99E)), li) SEQ ID NO: XXX (IL-12p40(D18K/E59K/K99E)), lii) SEQ ID NO: XXX (IL-12p40 (E59K/K99E/K264E)),

liii) SEQ ID NO: XXX (IL-12p40 (E59K/K99Y)), liv) SEQ ID NO: XXX (IL-12p40 (E59Y/K99E)), lv) SEQ ID NO: XXX (IL-12p40 (E45K/E59K/K99E)), lvi) SEQ ID NO: XXX (IL-12p40 (E59K/K99E/Q144E)), lvii) SEQ ID NO: XXX (IL-12p40 (E59K/K99E/Q144K)), lviii) SEQ ID NO: XXX (IL-12p40 (E59K/K99E/R159E)), lix) SEQ ID NO: XXX (IL-12p40 (E59K/K99E/K264E)), lx) SEQ ID NO: XXX (IL-12p40 (D18K/E59K/K99E/K264E)), lxi) SEQ ID NO: XXX (IL-12p40 (C252S)), lxii) SEQ ID NO: XXX (IL-12p40 (DI8K/E59K/K99E/C252S)), lxiii) SEQ ID NO: XXX (IL-12p40 (D18K/E59K/K99E/C252S/K264E)), lxiv) SEQ ID NO: XXX (IL-12p40 (E59K/K99Y/C252S)), lxv) SEQ ID NO: XXX (IL-12p40 (E59K/K99E/C252S/K264E)), lxvi) SEQ ID NO: XXX (IL-12p40 (E59K/K99E/C252S)), lxvii) SEQ ID NO: XXX (IL-12p40 (N103D/N113D)), lxviii) SEQ ID NO: XXX (IL-12p40 (N103D/N200D)), lxix) SEQ ID NO: XXX (IL-12p40 (N103D/N281D)), lxx) SEQ ID NO: XXX (IL-12p40 (N113D/N200D)), lxxi) SEQ ID NO: XXX (IL-12p40 (N113D/N281D)), lxxii) SEQ ID NO: XXX (IL-12p40 (N200D/N281D)), lxxiii) SEQ ID NO: XXX (IL-12p50 (N103D/N113D/N200D)), lxxiv) SEQ ID NO: XXX (IL-12p40 (N103D/N113D/N281D)), lxxv) SEQ ID NO: XXX (IL-12p40 (N103D/N200D/N281D)), lxxvi) SEQ ID NO: XXX (IL-12p40 (N113D/N200D/N281D)), lxxvii) SEQ ID NO: XXX (IL-12p40 (N103Q)), lxxviii) SEQ ID NO: XXX (IL-12p40 (N113Q)), lxxix) SEQ ID NO: XXX (IL-12p40 (N200Q)), lxxx) SEQ ID NO: XXX (IL-12p40 (N281Q)), lxxxi) SEQ ID NO: XXX (IL-12p40 (N103Q/N113Q)), lxxxii) SEQ ID NO: XXX (IL-12p40 (N103Q/N200Q)), lxxxiii) SEQ ID NO: XXX (IL-12p40 (N103Q/N281Q)), lxxxiv) SEQ ID NO: XXX (IL-12p40 (N113Q/N200Q)), lxxxv) SEQ ID NO: XXX (IL-12p40 (N113Q/N281Q)), lxxxvi) SEQ ID NO: XXX (IL-12p40 (N200Q/N281Q)), lxxxvii) SEQ ID NO: XXX (IL-12p40 (N103Q/N113Q/N200Q)), lxxxviii) SEQ ID NO: XXX (IL-12p40 (N103Q/N113Q/N281Q)), lxxxix) SEQ ID NO: XXX (IL-12p40 (N103Q/N200Q/N281Q)), xc) SEQ ID NO: XXX (IL-12p40 (N113Q/N200Q/N281Q)), xci) SEQ ID NO:XXX (IL-12p40 (N103Q/N113Q/N200Q/N281Q)), xcii) SEQ ID NO:XXX (IL-12p40 (D34N/E59K/K99E)), xciii) SEQ ID NO:XXX (IL-12p40 (D34K/E59K/K99E)), xciv) SEQ ID NO:XXX (IL-12p40 (E32Q/D34N/E59K/K99E)), xcv) SEQ ID NO:XXX (IL-12p40 (E32K/D34N/E59K/K99E)), xcvi) SEQ ID NO: XXX (IL-12p40 (E59Y/K99Y)), xcvii) SEQ ID NO:XXX (IL-12p40 (E59K/K99E/N103Q/C252S/K264E)), xcviii) SEQ ID NO:XXX (IL-12p40 (E59K/K99E/N113Q/C252S/K264E)), xcix) SEQ ID NO:XXX (IL-12p40

(E59K/K99E/N200Q/C252S/K264E)), c) SEQ ID NO:XXX (IL-12p40 (E59K/K99E/N281Q/C252S/K264E)), ci) SEQ ID NO:XXX (IL-12p40 (E59K/K99E/N103Q/N113Q/C252S/K264E)), cii) SEQ ID NO:XXX (IL-12p40 (E59K/K99E/N103Q/N200Q/C252S/K264E)), ciii) SEQ ID NO:XXX (IL-12p40 (E59K/K99E/N103Q/N281Q/C252S/K264E)), civ) SEQ ID NO:XXX (IL-12p40 (E59K/K99E/N113Q/N200Q/C252S/K264E)), cv) SEQ ID NO:XXX (IL-12p40 (E59K/K99E/N113Q/N281Q/C252S/K264E)), cvi) SEQ ID NO:XXX (IL-12p40 (E59K/K99E/N200Q/N281Q/C252S/K264E)), cvii) SEQ ID NO:XXX (IL-12p40 (E59K/K99E/N103Q/N113Q/N200Q/C252S/K264E)), cviii) SEQ ID NO:XXX (IL-12p40 (E59K/K99E/N103Q/N200Q/N281Q/C252S/K264E)), and cix) SEQ ID NO:XXX (IL-12p40 (E59K/K99E/N113Q/N200Q/N281Q/C252S/K264E)).

[0070] In some embodiments, the present invention provides a heterodimeric Fc fusion protein wherein said IL-12p35 subunit has one or more amino acid modifications as amino acid residues selected from the group consisting of Q20, N21, Q35, E38, S44, E45, E46, H49, K54, D55, T59, V60, E61, C63, L64, P65, E67, L68, N71, S73, C74, L75, N76, E79, N85, L89, F96, M97, L124, M125, Q130, Q135, N136, E143, Q146, N151, E153, K158, E162, E163, D165, I171, R181, I182, R183, V185, T186, D188, R189, V190, S192, Y193, N195, and A196.

[0071] In some embodiments, the present invention provides a heterodimeric Fc fusion protein wherein said IL-12p35 subunit has one or more amino acid substitutions selected from the group consisting of N21D, Q35D, E38Q, D55Q, D55K, N71D, N71Q, L75A, N76D, E79Q, N85D, N85Q, L89A, F96A, M97A, L124A, M125A, Q130E, Q135E, N136D, E143Q, Q146E, N151D, N151K, E153K, E153Q, K158E, E162Q, E163Q, D165N, I171A, N195D, and N195Q.

[0072] In some embodiments, the present invention provides a heterodimeric Fc fusion protein wherein said IL-12p35 subunit has amino acid substitutions selected from the group consisting of N71D/N85D/N195D, N151D/E153Q, N151D/D165N, Q130E/N151D, N151D/K158E, E79Q/N151D, D55Q/N151D, N136D/N151D, N21D/N151D, E143Q/N151D, N71Q/N85Q,

N71Q/N195Q, N85Q/N195Q, N71Q/N85Q/N195Q, N71D/N85D, N71D/N195D, and N85D/N195D.

[0073] In some embodiments, the present invention provides a heterodimeric Fc fusion protein wherein said IL-12p35 subunit has a polypeptide sequence selected from the group consisting of: i) SEQ ID NO:113 (IL-12p35(N71D)), ii) SEQ ID NO:114 (IL-12p35(N85D)), iii) SEQ ID NO:115 (IL-12p35(N195D)), iv) SEQ ID NO:116 (IL-12p35(N71D/N85D/N195D)), v) SEQ ID NO:117 (IL-12p35(E153Q)), vi) SEQ ID NO:118 (IL-12p35(E38Q)), vii) SEQ ID NO:119 (IL-12p35(N151D)), viii) SEQ ID NO:120 (IL-12p35(Q135E)), ix) SEQ ID NO:121 (IL-12p35(Q35D)), x) SEQ ID NO:122 (IL-12p35(Q146E)), xi) SEQ ID NO:123 (IL-12p35(N76D)), xii) SEQ ID NO:124 (IL-12p35(E162Q)), xiii) SEQ ID NO:125 (IL-12p35(E163Q)), xiv) IL-12p35(N21D), xv) SEQ ID NO:XXX (IL-12p35(D55Q)), xvi) IL-12p35(E79Q), xvii) IL-12p35(Q130E), xviii) IL-12p35(N136D), xix) IL-12p35(E143Q), xx) SEQ ID NO:227 (IL-12p35(N151K)), xxi) SEQ ID NO:226 (IL-12p35(E153K)), xxii) IL-12p35(K158E), xxiii) IL-12p35(D165N), xxiv) SEQ ID NO:225 (IL-12p35(N151D/E153Q)), xxv) SEQ ID NO:228 (IL-12p35(N151D/D165N)), xxvi) SEQ ID NO:229 (IL-12p35(Q130E/N151D)), xxvii) SEQ ID NO:230 (IL-12p35(N151D/K158E)), xxviii) SEQ ID NO:231 (IL-12p35(E79Q/N151D)), xxix) SEQ ID NO:232 (IL-12p35(D55Q/N151D)), xxx) SEQ ID NO:233 (IL-12p35(N136D/N151D)), xxxi) SEQ ID NO:234 (IL-12p35(N21D/N151D)), xxxii) SEQ ID NO:235 (IL-12p35(E143Q/N151D)), xxxiii) SEQ ID NO: XXX (IL-12p35(F96A)), xxxiv) SEQ ID NO: XXX (IL-12p35(M97A)), xxxv) SEQ ID NO: XXX (IL-12p35(L89A)), xxxvi) SEQ ID NO: XXX (IL-12p35(L124A)), xxxvii) SEQ ID NO: XXX (IL-12p35(M125A)), xxxviii) SEQ ID NO: XXX (IL-12p35(L75A)), xxxiv) SEQ ID NO: XXX (IL-12p35(I171A)), xxxv) SEQ ID NO: XXX (IL-12p35 (N71Q)), xxxvi) SEQ ID NO: XXX (IL-12p35 (N85Q)), xxxvii) SEQ ID NO: XXX (IL-12p35 (N195Q)), xxxviii) SEQ ID NO: XXX (IL-12p35 (N71Q/N85Q)), xxxix) SEQ ID NO: XXX (IL-12p35 (N71Q/N195Q)), xl) SEQ ID NO: XXX (IL-12p35 (N85Q/N195Q), xli) SEQ ID NO: XXX (IL-12p35 (N71Q/N85Q/N195Q)), xlii) SEQ ID NO: XXX (IL-12p35 (N71D/N85D)), xliii) SEQ ID NO: XXX (IL-12p35 (N71D/N195D), xliv) SEQ ID NO: XXX (IL-12p35 (N85D/N195D)), xlv) SEQ ID NO: XXX (IL-12p35 (D55Q)), and xlvi) SEQ ID NO: XXX (IL-12p35 (D55K)).

[0074] In some embodiments, the present invention provides a heterodimeric Fc fusion protein wherein said heterodimeric Fc fusion protein comprises: a) said fusion protein having a polypeptide sequence of SEQ ID NO:51 (XENP27203 Chain 1), and b) said second Fc domain having a polypeptide sequence of SEQ ID NO:52 (XENP27203 Chain 2).

[0075] In some embodiments, the present invention provides a heterodimeric Fc fusion protein wherein said heterodimeric Fc fusion protein is XENP27203.

[0076] In some embodiments, the present invention provides a heterodimeric Fc fusion protein wherein said heterodimeric Fc fusion protein is XENP31290.

[0077] In some embodiments, the present invention provides a composition comprising a heterodimeric Fc fusion protein for use in treating cancer in a subject.

[0078] In some embodiments, the present invention provides one or more nucleic acids encoding a heterodimeric Fc fusion protein.

[0079] In some embodiments, the present invention provides a host cell comprising said one or more nucleic acids encoding a heterodimeric Fc fusion protein.

[0080] In some embodiments, the present invention provides a method of making a heterodimeric Fc fusion protein, said method comprising culturing a host cell under conditions whereby said heterodimeric Fc fusion protein is produced.

[0081] In some embodiments, the present invention provides a method of purifying a heterodimeric Fc fusion protein, said method comprising: a) providing a composition comprising the heterodimeric Fc fusion protein; b) loading said composition onto an ion exchange column; andc) collecting a fraction containing said heterodimeric Fc fusion protein.

[0082] In another aspect, the present invention provides a heterodimeric Fc fusion protein comprising:a) a fusion protein comprising a first protein domain, a second protein domain and a first Fc domain, wherein said first protein domain is covalently attached to said second protein domain, and wherein said second protein domain is covalently attached to the N-terminus of said

first Fc domain; and b) a second Fc domain; wherein said first and said second Fc domains comprise modifications promoting heterodimerization of said first and said second Fc domains and wherein said first protein domain comprises an IL-12p35 subunit and said second protein domain comprises an IL-12p40 subunit.

[0083] In some embodiments, the present invention provides a heterodimeric Fc fusion protein wherein said modifications promoting heterodimerization of said first and said second Fc domains are a set of amino acid substitutions selected from the group consisting of L368D/K370S and S364K; L368D/K370S and S364K/E357L; L368D/K370S and S364K/E357Q; T411E/K360E/Q362E and D401K; L368E/K370S and S364K; K370S and S364K/E357Q and T366S/L368A/Y407V : T366W (optionally including a bridging disulfide, T366S/L368A/Y407V/Y349C : T366W/S354C), according to EU numbering.

[0084] In some embodiments, the present invention provides a heterodimeric Fc fusion protein wherein said first protein domain is attached to said second protein domain using a first domain linker and/or said second protein domain is attached to said first Fc domain using a second domain linker.

[0085] In some embodiments, the present invention provides a heterodimeric Fc fusion protein wherein said first and/or said second Fc domains have an additional set of amino acid substitutions comprising Q295E/N384D/Q418E/N421D, according to EU numbering.

[0086] In some embodiments, the present invention provides a heterodimeric Fc fusion protein wherein said first and/or said second Fc domains have an additional set of amino acid substitutions selected from the group consisting of G236R/L328R, E233P/L234V/L235A/G236_/S239K, E233P/L234V/L235A/G236_/S239K/A327G, E233P/L234V/L235A/G236_/S267K/A327G, E233P/L234V/L235A/G236_, and E233P/L234V/L235A/G236_/S267K, according to EU numbering.

[0087] In some embodiments, the present invention provides a heterodimeric Fc fusion protein wherein said IL-12p40 subunit has a polypeptide sequence selected form the group consisting of

SEQ ID NO:3 (human IL-12 subunit beta (IL-12p40) precursor sequence) and/or SEQ ID NO:4 (human IL-12 subunit beta (IL-12p40) mature form sequence), and said IL-12p35 subunit has a polypeptide sequence selected from the group consisting of SEQ ID NO:1 (human IL-12 subunit alpha (IL-12p35) precursor sequence) and SEQ ID NO:2 (human IL-12 subunit alpha (IL-12p35) mature form sequence).

[0088] In some embodiments, the present invention provides a heterodimeric Fc fusion protein wherein said first and second Fc domains further comprise amino acid substitutions M428L/N424S.

[0089] In some embodiments, the present invention provides a heterodimeric Fc fusion protein wherein said IL-12p40 subunit is a variant IL-12p40 subunit and/or said IL-12p35 subunit is a variant IL-12p35 subunit.

[0090] In some embodiments, the present invention provides a heterodimeric Fc fusion protein wherein said IL-12p40 subunit is a variant IL-12p40 subunit having altered affinity for IL-12 receptor subunit beta-1 (IL-12Rß1), IL-12 receptor subunit beta-2 (IL-12Rß2), and/or IL-12 receptor complex; and/or said IL-12p35 subunit is a variant IL-12p35 subunit having altered affinity for IL-12 receptor subunit beta-1 (IL-12Rß1), IL-12 receptor subunit beta-2 (IL-12Rß2), and/or IL-12 receptor complex.

[0091] In some embodiments, the present invention provides a heterodimeric Fc fusion protein wherein said IL-12p40 subunit has one or more amino acid modifications at amino acid residues selected from the group consisting of E3, D7, E12, D14, W15, P17, D18, A19, P20, G21, E22, M23, D29, E32, E33, D34, L40, D41, Q42, S43, E45, L47, T54, I55, Q56, K58, E59, F60, G61, D62, Q65, Y66, E73, K84, E86, D87, G88, I89, W90, D93, D97, K99, E100, K102, N103, K104, F106, E110, N113, Y114, D129, D142, Q144, E156, R159, D161, N162, K163, D166, D170, Q172, D174, A176, C177, P178, A179, A180, E181, S183, P185, E187, N200, S204, F206, R208, D209, D214, N218, Q220, N226, Q229, E231, E235, T242, P243, S245, Y246, F247, S248, C252, Q256, K258, K260, E262, K264, D265, D270, N281, Q289, D290, R291, Y292, Y293, and E299.

[0092] In some embodiments, the present invention provides a heterodimeric Fc fusion protein wherein said IL-12p40 subunit has one or more amino acid substitutions selected from the group consisting of D18N, D18K, E32Q, E33Q, D34N, D34K, Q42E, S43E, S43K, E45Q, Q56E, E59Q, E59K, D62N, E73Q, D87N, K99E, K99Y, E100Q, N103D, N103Q, N113D, N113Q, Q144E, D161N, R159E, K163E, E187Q, N200D, N200Q, N218Q, Q229E, E235Q, C252S, Q256N, K258E, K260E, E262Q, K264E, N281D, N281Q, and E299Q.

[0093] In some embodiments, the present invention provides a heterodimeric Fc fusion protein wherein said IL-12p40 subunit has amino acid substitutions selected from the group consisting of N103D/N113D/N200D/N281D, Q42E/E45Q, E45Q/Q56E, Q42E/E59Q, Q56E/E59Q, Q42E/E45Q/Q56E, E45Q/Q56E/E59Q, E32Q/E59Q, D34N/E59K, D34N/E59K/K99E, D34K/E59K/K99E, E32Q/D34N/E59K/K99E, E32K/D34N/E59K/K99E, D34N/E59Q, E59Q/E187Q, S43E/E59Q, S43K/E49Q, E59Q/K163E, E59Q/K99E, E59Q/K258E, E59Q/K260E, E59K/K99E, D18K/E59K/K99E, E59K/K99E/K264E, E59K/K99Y, E59Y/K99Y, E59Y/K99E, E45K/E59K/K99E, E59K/K99E/Q144E, E59K/K99E/Q144K, E59K/K99E/R159E, E59K/K99E/K264E, D18K/E59K/K99E/K264E, DI8K/E59K/K99E/C252S, D18K/E59K/K99E/C252S/K264E, E59K/K99Y/C252S, E59K/K99E/C252S/K264E, E59K/K99E/C252S, N103D/N113D, N103D/N200D, N103D/N281D, N113D/N200D, N113D/N281D, N200D/N281D, N103D/N113D/N200D, N103D/N113D/N281D, N103D/N200D/N281D, N113D/N200D/N281D, N103Q/N113Q, N103Q/N200Q, N103Q/N281Q, N113Q/N200Q, N113Q/N281Q, N200Q/N281Q, N103Q/N113Q/N200Q, N103Q/N113Q/N281Q, N103Q/N200Q/N281Q, N113Q/N200Q/N281Q, N103Q/N113Q/N200Q/N281Q, E59K/K99E/N103Q/C252S/K264E, E59K/K99E/N113Q/C252S/K264E, E59K/K99E/N200Q/C252S/K264E, E59K/K99E/N281Q/C252S/K264E, E59K/K99E/N103Q/N113Q/C252S/K264E, E59K/K99E/N103Q/N200Q/C252S/K264E, E59K/K99E/N103Q/N281Q/C252S/K264E, E59K/K99E/N113Q/N200Q/C252S/K264E, E59K/K99E/N113Q/N281Q/C252S/K264E, E59K/K99E/N200Q/N281Q/C252S/K264E, E59K/K99E/N103Q/N113Q/N200Q/C252S/K264E, E59K/K99E/N103Q/N200Q/N281Q/C252S/K264E,

E59K/K99E/N113Q/N200Q/N281Q/C252S/K264E, and
E59K/K99E/N103Q/N113Q/N200Q/N281Q/C252S/K264E.

[0094] In some embodiments, the present invention provides a heterodimeric Fc fusion protein wherein said IL-12p40 subunit has a polypeptide sequence selected from the group consisting of: i) SEQ ID NO:57 (IL-12p40(N103D)), ii) SEQ ID NO:58 (IL-12p40(N113D)), iii) SEQ ID NO:59 (IL-12p40(N200D)), iv) SEQ ID NO:60 (IL-12p40(N281D)), v) SEQ ID NO:61 (IL-12p40(N103D/N113D/N200D/N281D)), vi) SEQ ID NO:62 (IL-12p40(Q42E)), vii) SEQ ID NO:63 (IL-12p40(E45Q)), viii) SEQ ID NO:64 (IL-12p40(Q56E)), ix) SEQ ID NO:65 (IL-12p40(E59Q)), x) SEQ ID NO:66 (IL-12p40(D62N)), xi) SEQ ID NO:67 (IL-12p40(Q42E/E45Q)), xii) SEQ ID NO:68 (IL-12p40(E45Q/Q56E)), xiii) SEQ ID NO:69 (IL-12p40(Q42E/E59Q)), xiv) SEQ ID NO:70 (IL-12p40(Q56E/E59Q)), xv) SEQ ID NO:71 (IL-12p40(Q42E/E45Q/Q56E)), xvi) SEQ ID NO:72 (IL-12p40(E45Q/Q56E/E59Q)), xvii) SEQ ID NO:73 (IL-12p40(D161N)), xviii) SEQ ID NO:74 (IL-12p40(E73Q)), xix) SEQ ID NO:75 (IL-12p40(Q144E)), xx) SEQ ID NO:76 (IL-12p40(E262Q)), xxi) SEQ ID NO:77 (IL-12p40(E100Q)), xxii) SEQ ID NO:78 (IL-12p40(D18N)), xxiii) SEQ ID NO:79 (IL-12p40(E33Q)), xxiv) SEQ ID NO:80 (IL-12p40(Q229E)), xxv) SEQ ID NO:81 (IL-12p40(E235Q)), xxvi) SEQ ID NO:82 (IL-12p40(Q256N)), xxvii) SEQ ID NO:83 (IL-12p40(E299Q)), xxviii) SEQ ID NO:84 (IL-12p40(D87N)), xxix) IL-12p40(E32Q), xxx) IL-12p40(D34N), xxxi) IL-12p40(S43E), xxxii) IL-12p40(S43K), xxxiii) SEQ ID NO:XXX (IL-12p40 (E59K/K99E/N103Q/N113Q/N200Q/N281Q/C252S/K264E)), xxxiv) SEQ ID NO:205 (IL-12p40(E59K)), xxxv) IL-12p40(K99E), xxxvi) IL-12p40(K163E), xxxvii) IL-12p40(E187Q), xxxviii) IL-12p40(K258E), xxxix) IL-12p40(K260E), xl) SEQ ID NO:206 (IL-12p40(E32Q/E59Q)), xli) SEQ ID NO:207 (IL-12p40(D34N/E59Q)), xlii) SEQ ID NO:208 (IL-12p40(E59Q/E187Q)), xliii) SEQ ID NO:209 (IL-12p40(S43E/E59Q)), xliv) SEQ ID NO:210 (IL-12p40(S43K/E49Q)), xlv) SEQ ID NO:211 (IL-12p40(E59Q/K163E)), xlvi) SEQ ID NO:212 (IL-12p40(E59Q/K99E)), xlvii) SEQ ID NO:213 (IL-12p40(E59Q/K258E)), xlviii) SEQ ID NO:214 (IL-12p40(E59Q/K260E)), xlix) SEQ ID NO: XXX (IL-12p40 (D34N/E59K)), l) SEQ ID NO: XXX (IL-12p40(E59K/K99E)), li) SEQ ID NO: XXX (IL-12p40(D18K/E59K/K99E)), lii) SEQ ID NO: XXX (IL-12p40 (E59K/K99E/K264E)), liii) SEQ ID NO: XXX (IL-12p40 (E59K/K99Y)), liv) SEQ ID NO: XXX (IL-12p40 (E59Y/K99E)), lv)

SEQ ID NO: XXX (IL-12p40 (E45K/E59K/K99E)), lvi) SEQ ID NO: XXX (IL-12p40 (E59K/K99E/Q144E)), lvii) SEQ ID NO: XXX (IL-12p40 (E59K/K99E/Q144K)), lviii) SEQ ID NO: XXX (IL-12p40 (E59K/K99E/R159E)), lix) SEQ ID NO: XXX (IL-12p40 (E59K/K99E/K264E)), lx) SEQ ID NO: XXX (IL-12p40 (D18K/E59K/K99E/K264E)), lxi) SEQ ID NO: XXX (IL-12p40 (C252S)), lxii) SEQ ID NO: XXX (IL-12p40 (DI8K/E59K/K99E/C252S)), lxiii) SEQ ID NO: XXX (IL-12p40 (D18K/E59K/K99E/C252S/K264E)), lxiv) SEQ ID NO: XXX (IL-12p40 (E59K/K99Y/C252S)), lxv) SEQ ID NO: XXX (IL-12p40 (E59K/K99E/C252S/K264E)), lxvi) SEQ ID NO: XXX (IL-12p40 (E59K/K99E/C252S)), lxvii) SEQ ID NO: XXX (IL-12p40 (N103D/N113D)), lxviii) SEQ ID NO: XXX (IL-12p40 (N103D/N200D)), lxix) SEQ ID NO: XXX (IL-12p40 (N103D/N281D)), lxx) SEQ ID NO: XXX (IL-12p40 (N113D/N200D)), lxxi) SEQ ID NO: XXX (IL-12p40 (N113D/N281D)), lxxii) SEQ ID NO: XXX (IL-12p40 (N200D/N281D)), lxxiii) SEQ ID NO: XXX (IL-12p50 (N103D/N113D/N200D)), lxxiv) SEQ ID NO: XXX (IL-12p40 (N103D/N113D/N281D)), lxxv) SEQ ID NO: XXX (IL-12p40 (N103D/N200D/N281D)), lxxvi) SEQ ID NO: XXX (IL-12p40 (N113D/N200D/N281D)), lxxvii) SEQ ID NO: XXX (IL-12p40 (N103Q)), lxxviii) SEQ ID NO: XXX (IL-12p40 (N113Q)), lxxix) SEQ ID NO: XXX (IL-12p40 (N200Q)), lxxx) SEQ ID NO: XXX (IL-12p40 (N281Q)), lxxxi) SEQ ID NO: XXX (IL-12p40 (N103Q/N113Q)), lxxxii) SEQ ID NO: XXX (IL-12p40 (N103Q/N200Q)), lxxxiii) SEQ ID NO: XXX (IL-12p40 (N103Q/N281Q)), lxxxiv) SEQ ID NO: XXX (IL-12p40 (N113Q/N200Q)), lxxxv) SEQ ID NO: XXX (IL-12p40 (N113Q/N281Q)), lxxxvi) SEQ ID NO: XXX (IL-12p40 (N200Q/N281Q)), lxxxvii) SEQ ID NO: XXX (IL-12p40 (N103Q/N113Q/N200Q)), lxxxviii) SEQ ID NO: XXX (IL-12p40 (N103Q/N113Q/N281Q)), lxxxix) SEQ ID NO: XXX (IL-12p40 (N103Q/N200Q/N281Q)), xc) SEQ ID NO: XXX (IL-12p40 (N113Q/N200Q/N281Q)), xci) SEQ ID NO:XXX (IL-12p40 (N103Q/N113Q/N200Q/N281Q)), xcii) SEQ ID NO:XXX (IL-12p40 (D34N/E59K/K99E)), xciii) SEQ ID NO:XXX (IL-12p40 (D34K/E59K/K99E)), xciv) SEQ ID NO:XXX (IL-12p40 (E32Q/D34N/E59K/K99E)), xcv) SEQ ID NO:XXX (IL-12p40 (E32K/D34N/E59K/K99E)), xcvi) SEQ ID NO: XXX (IL-12p40 (E59Y/K99Y)), xcvii) SEQ ID NO:XXX (IL-12p40 (E59K/K99E/N103Q/C252S/K264E)), xcviii) SEQ ID NO:XXX (IL-12p40 (E59K/K99E/N113Q/C252S/K264E)), xcix) SEQ ID NO:XXX (IL-12p40 (E59K/K99E/N200Q/C252S/K264E)), c) SEQ ID NO:XXX (IL-12p40

(E59K/K99E/N281Q/C252S/K264E)), ci) SEQ ID NO:XXX (IL-12p40 (E59K/K99E/N103Q/N113Q/C252S/K264E)), cii) SEQ ID NO:XXX (IL-12p40 (E59K/K99E/N103Q/N200Q/C252S/K264E)), ciii) SEQ ID NO:XXX (IL-12p40 (E59K/K99E/N103Q/N281Q/C252S/K264E)), civ) SEQ ID NO:XXX (IL-12p40 (E59K/K99E/N113Q/N200Q/C252S/K264E)), cv) SEQ ID NO:XXX (IL-12p40 (E59K/K99E/N113Q/N281Q/C252S/K264E)), cvi) SEQ ID NO:XXX (IL-12p40 (E59K/K99E/N200Q/N281Q/C252S/K264E)), cvii) SEQ ID NO:XXX (IL-12p40 (E59K/K99E/N103Q/N113Q/N200Q/C252S/K264E)), cviii) SEQ ID NO:XXX (IL-12p40 (E59K/K99E/N103Q/N200Q/N281Q/C252S/K264E)), and cix) SEQ ID NO:XXX (IL-12p40 (E59K/K99E/N113Q/N200Q/N281Q/C252S/K264E)).

[0095] In some embodiments, the present invention provides a heterodimeric Fc fusion protein wherein said IL-12p35 subunit has one or more amino acid modifications as amino acid residues selected from the group consisting of Q20, N21, Q35, E38, S44, E45, E46, H49, K54, D55, T59, V60, E61, C63, L64, P65, E67, L68, N71, S73, C74, L75, N76, E79, N85, L89, F96, M97, L124, M125, Q130, Q135, N136, E143, Q146, N151, E153, K158, E162, E163, D165, I171, R181, I182, R183, V185, T186, D188, R189, V190, S192, Y193, N195, and A196.

[0096] In some embodiments, the present invention provides a heterodimeric Fc fusion protein wherein said IL-12p35 subunit has one or more amino acid substitutions selected from the group consisting of N21D, Q35D, E38Q, D55Q, D55K, N71D, N71Q, L75A, N76D, E79Q, N85D, N85Q, L89A, F96A, M97A, L124A, M125A, Q130E, Q135E, N136D, E143Q, Q146E, N151D, N151K, E153K, E153Q, K158E, E162Q, E163Q, D165N, I171A, N195D, and N195Q.

[0097] In some embodiments, the present invention provides a heterodimeric Fc fusion protein wherein said IL-12p35 subunit has amino acid substitutions selected from the group consisting of N71D/N85D/N195D, N151D/E153Q, N151D/D165N, Q130E/N151D, N151D/K158E, E79Q/N151D, D55Q/N151D, N136D/N151D, N21D/N151D, E143Q/N151D, N71Q/N85Q, N71Q/N195Q, N85Q/N195Q, N71Q/N85Q/N195Q, N71D/N85D, N71D/N195D, and N85D/N195D.

[0098] In some embodiments, the present invention provides a heterodimeric Fc fusion protein wherein said IL-12p35 subunit has a polypeptide sequence selected from the group consisting of: i) SEQ ID NO:113 (IL-12p35(N71D)), ii) SEQ ID NO:114 (IL-12p35(N85D)), iii) SEQ ID NO:115 (IL-12p35(N195D)), iv) SEQ ID NO:116 (IL-12p35(N71D/N85D/N195D)), v) SEQ ID NO:117 (IL-12p35(E153Q)), vi) SEQ ID NO:118 (IL-12p35(E38Q)), vii) SEQ ID NO:119 (IL-12p35(N151D)), viii) SEQ ID NO:120 (IL-12p35(Q135E)), ix) SEQ ID NO:121 (IL-12p35(Q35D)), x) SEQ ID NO:122 (IL-12p35(Q146E)), xi) SEQ ID NO:123 (IL-12p35(N76D)), xii) SEQ ID NO:124 (IL-12p35(E162Q)), xiii) SEQ ID NO:125 (IL-12p35(E163Q)), xiv) IL-12p35(N21D), xv) SEQ ID NO:XXX (IL-12p35(D55Q)), xvi) IL-12p35(E79Q), xvii) IL-12p35(Q130E), xviii) IL-12p35(N136D), xix) IL-12p35(E143Q), xx) SEQ ID NO:227 (IL-12p35(N151K)), xxi) SEQ ID NO:226 (IL-12p35(E153K)), xxii) IL-12p35(K158E), xxiii) IL-12p35(D165N), xxiv) SEQ ID NO:225 (IL-12p35(N151D/E153Q)), xxv) SEQ ID NO:228 (IL-12p35(N151D/D165N)), xxvi) SEQ ID NO:229 (IL-12p35(Q130E/N151D)), xxvii) SEQ ID NO:230 (IL-12p35(N151D/K158E)), xxviii) SEQ ID NO:231 (IL-12p35(E79Q/N151D)), xxix) SEQ ID NO:232 (IL-12p35(D55Q/N151D)), xxx) SEQ ID NO:233 (IL-12p35(N136D/N151D)), xxxi) SEQ ID NO:234 (IL-12p35(N21D/N151D)), xxxii) SEQ ID NO:235 (IL-12p35(E143Q/N151D)), xxxiii) SEQ ID NO: XXX (IL-12p35(F96A)), xxxiv) SEQ ID NO: XXX (IL-12p35(M97A)), xxxv) SEQ ID NO: XXX (IL-12p35(L89A)), xxxvi) SEQ ID NO: XXX (IL-12p35(L124A)), xxxvii) SEQ ID NO: XXX (IL-12p35(M125A)), xxxviii) SEQ ID NO: XXX (IL-12p35(L75A)), xxxiv) SEQ ID NO: XXX (IL-12p35(I171A)), xxxv) SEQ ID NO: XXX (IL-12p35 (N71Q)), xxxvi) SEQ ID NO: XXX (IL-12p35 (N85Q)), xxxvii) SEQ ID NO: XXX (IL-12p35 (N195Q)), xxxviii) SEQ ID NO: XXX (IL-12p35 (N71Q/N85Q)), xxxix) SEQ ID NO: XXX (IL-12p35 (N71Q/N195Q)), xl) SEQ ID NO: XXX (IL-12p35 (N85Q/N195Q), xli) SEQ ID NO: XXX (IL-12p35 (N71Q/N85Q/N195Q)), xlii) SEQ ID NO: XXX (IL-12p35 (N71D/N85D)), xliii) SEQ ID NO: XXX (IL-12p35 (N71D/N195D), xliv) SEQ ID NO: XXX (IL-12p35 (N85D/N195D)), xlv) SEQ ID NO: XXX (IL-12p35 (D55Q)), and xlvi) SEQ ID NO: XXX (IL-12p35 (D55K)).

[0099] In some embodiments, the present invention provides a heterodimeric Fc fusion protein wherein said heterodimeric Fc fusion protein comprises: a) said fusion protein having a

polypeptide sequence of SEQ ID NO:53 (XENP27204 Chain 1), and b) said second Fc domain having a polypeptide sequence of SEQ ID NO:54 (XENP27204 Chain 2).

[00100] In some embodiments, the present invention provides a heterodimeric Fc fusion protein wherein said heterodimeric Fc fusion protein is XENP27204.

[00101] In some embodiments, the present invention provides a composition comprising a heterodimeric Fc fusion protein for use in treating cancer in a subject.

[00102] In some embodiments, the present invention provides one or more nucleic acids encoding a heterodimeric Fc fusion protein.

[00103] In some embodiments, the present invention provides a host cell comprising said one or more nucleic acids encoding a heterodimeric Fc fusion protein.

[00104] In some embodiments, the present invention provides a method of making a heterodimeric Fc fusion protein, said method comprising culturing a host cell under conditions whereby said heterodimeric Fc fusion protein is produced.

[00105] In some embodiments, the present invention provides a method of purifying a heterodimeric Fc fusion protein, said method comprising: a) providing a composition comprising the heterodimeric Fc fusion protein; b) loading said composition onto an ion exchange column; andc) collecting a fraction containing said heterodimeric Fc fusion protein.

[00106] In another aspect, the present invention provides a heterodimeric Fc fusion protein comprising: a) a fusion protein comprising a first protein domain, a second protein domain and a first Fc domain, wherein said first protein domain is attached to the C-terminus of said first Fc domain, and wherein said second protein domain is covalently attached to said first protein domain; and b) a second Fc domain; wherein said first and said second Fc domains comprise modifications promoting heterodimerization of said first and said second Fc domains and wherein said first protein domain comprises an IL-12p40 subunit and said second protein domain comprises an IL-12p35 subunit.

[00107] In some embodiments, the present invention provides a heterodimeric Fc fusion protein wherein said modifications promoting heterodimerization of said first and said second Fc domains are a set of amino acid substitutions selected from the group consisting of L368D/K370S and S364K; L368D/K370S and S364K/E357L; L368D/K370S and S364K/E357Q; T411E/K360E/Q362E and D401K; L368E/K370S and S364K; K370S and S364K/E357Q and T366S/L368A/Y407V : T366W (optionally including a bridging disulfide, T366S/L368A/Y407V/Y349C : T366W/S354C), according to EU numbering.

[00108] In some embodiments, the present invention provides a heterodimeric Fc fusion protein wherein said first protein domain is attached to said second protein domain using a first domain linker and/or said second protein domain is attached to said first Fc domain using a second domain linker.

[00109] In some embodiments, the present invention provides a heterodimeric Fc fusion protein wherein said first and/or said second Fc domains have an additional set of amino acid substitutions comprising Q295E/N384D/Q418E/N421D, according to EU numbering.

[00110] In some embodiments, the present invention provides a heterodimeric Fc fusion protein wherein said first and/or said second Fc domains have an additional set of amino acid substitutions selected from the group consisting of G236R/L328R, E233P/L234V/L235A/G236_/S239K, E233P/L234V/L235A/G236_/S239K/A327G, E233P/L234V/L235A/G236_/S267K/A327G, E233P/L234V/L235A/G236_, and E233P/L234V/L235A/G236_/S267K, according to EU numbering.

[00111] In some embodiments, the present invention provides a heterodimeric Fc fusion protein wherein said IL-12p40 subunit has a polypeptide sequence selected form the group consisting of SEQ ID NO:3 (human IL-12 subunit beta (IL-12p40) precursor sequence) and SEQ ID NO:4 (human IL-12 subunit beta (IL-12p40) mature form sequence), and/or said IL-12p35 subunit has a polypeptide sequence selected from the group consisting of SEQ ID NO:1 (human IL-12 subunit alpha (IL-12p35) precursor sequence) and SEQ ID NO:2 (human IL-12 subunit alpha (IL-12p35) mature form sequence).

[00112] In some embodiments, the present invention provides a heterodimeric Fc fusion protein wherein said first and second Fc domains further comprise amino acid substitutions M428L/N424S.

[00113] In some embodiments, the present invention provides a heterodimeric Fc fusion protein wherein said IL-12p40 subunit is a variant IL-12p40 subunit and/or said IL-12p35 subunit is a variant IL-12p35 subunit.

[00114] In some embodiments, the present invention provides a heterodimeric Fc fusion protein wherein said IL-12p40 subunit is a variant IL-12p40 subunit having altered affinity for IL-12 receptor subunit beta-1 (IL-12Rß1), IL-12 receptor subunit beta-2 (IL-12Rß2), and/or IL-12 receptor complex; and/or said IL-12p35 subunit is a variant IL-12p35 subunit having altered affinity for IL-12 receptor subunit beta-1 (IL-12Rß1), IL-12 receptor subunit beta-2 (IL-12Rß2), and/or IL-12 receptor complex.

[00115] In some embodiments, the present invention provides a heterodimeric Fc fusion protein wherein said IL-12p40 subunit has one or more amino acid modifications at amino acid residues selected from the group consisting of E3, D7, E12, D14, W15, P17, D18, A19, P20, G21, E22, M23, D29, E32, E33, D34, L40, D41, Q42, S43, E45, L47, T54, I55, Q56, K58, E59, F60, G61, D62, Q65, Y66, E73, K84, E86, D87, G88, I89, W90, D93, D97, K99, E100, K102, N103, K104, F106, E110, N113, Y114, D129, D142, Q144, E156, R159, D161, N162, K163, D166, D170, Q172, D174, A176, C177, P178, A179, A180, E181, S183, P185, E187, N200, S204, F206, R208, D209, D214, N218, Q220, N226, Q229, E231, E235, T242, P243, S245, Y246, F247, S248, C252, Q256, K258, K260, E262, K264, D265, D270, N281, Q289, D290, R291, Y292, Y293, and E299.

[00116] In some embodiments, the present invention provides a heterodimeric Fc fusion protein wherein said IL-12p40 subunit has one or more amino acid substitutions selected from the group consisting of D18N, D18K, E32Q, E33Q, D34N, D34K, Q42E, S43E, S43K, E45Q, Q56E, E59Q, E59K, D62N, E73Q, D87N, K99E, K99Y, E100Q, N103D, N103Q, N113D, N113Q, Q144E, D161N, R159E, K163E, E187Q, N200D, N200Q, N218Q, Q229E, E235Q, C252S, Q256N, K258E, K260E, E262Q, K264E, N281D, N281Q, and E299Q.

[00117] In some embodiments, the present invention provides a heterodimeric Fc fusion protein wherein said IL-12p40 subunit has amino acid substitutions selected from the group consisting of N103D/N113D/N200D/N281D, Q42E/E45Q, E45Q/Q56E, Q42E/E59Q, Q56E/E59Q, Q42E/E45Q/Q56E, E45Q/Q56E/E59Q, E32Q/E59Q, D34N/E59K, D34N/E59K/K99E, D34K/E59K/K99E, E32Q/D34N/E59K/K99E, E32K/D34N/E59K/K99E, D34N/E59Q, E59Q/E187Q, S43E/E59Q, S43K/E49Q, E59Q/K163E, E59Q/K99E, E59Q/K258E, E59Q/K260E, E59K/K99E, D18K/E59K/K99E, E59K/K99E/K264E, E59K/K99Y, E59Y/K99Y, E59Y/K99E, E45K/E59K/K99E, E59K/K99E/Q144E, E59K/K99E/Q144K, E59K/K99E/R159E, E59K/K99E/K264E, D18K/E59K/K99E/K264E, DI8K/E59K/K99E/C252S, D18K/E59K/K99E/C252S/K264E, E59K/K99Y/C252S, E59K/K99E/C252S/K264E, E59K/K99E/C252S, N103D/N113D, N103D/N200D, N103D/N281D, N113D/N200D, N113D/N281D, N200D/N281D, N103D/N113D/N200D, N103D/N113D/N281D, N103D/N200D/N281D, N113D/N200D/N281D, N103Q/N113Q, N103Q/N200Q, N103Q/N281Q, N113Q/N200Q, N113Q/N281Q, N200Q/N281Q, N103Q/N113Q/N200Q, N103Q/N113Q/N281Q, N103Q/N200Q/N281Q, N113Q/N200Q/N281Q, N103Q/N113Q/N200Q/N281Q, E59K/K99E/N103Q/C252S/K264E, E59K/K99E/N113Q/C252S/K264E, E59K/K99E/N200Q/C252S/K264E, E59K/K99E/N281Q/C252S/K264E, E59K/K99E/N103Q/N113Q/C252S/K264E, E59K/K99E/N103Q/N200Q/C252S/K264E, E59K/K99E/N103Q/N281Q/C252S/K264E, E59K/K99E/N113Q/N200Q/C252S/K264E, E59K/K99E/N113Q/N281Q/C252S/K264E, E59K/K99E/N200Q/N281Q/C252S/K264E, E59K/K99E/N103Q/N113Q/N200Q/C252S/K264E, E59K/K99E/N103Q/N200Q/N281Q/C252S/K264E, E59K/K99E/N113Q/N200Q/N281Q/C252S/K264E, and E59K/K99E/N103Q/N113Q/N200Q/N281Q/C252S/K264E.

[00118] In some embodiments, the present invention provides a heterodimeric Fc fusion protein wherein said IL-12p40 subunit has a polypeptide sequence selected from the group consisting of: i) SEQ ID NO:57 (IL-12p40(N103D)), ii) SEQ ID NO:58 (IL-12p40(N113D)), iii) SEQ ID NO:59 (IL-12p40(N200D)), iv) SEQ ID NO:60 (IL-12p40(N281D)), v) SEQ ID NO:61 (IL-12p40(N103D/N113D/N200D/N281D)), vi) SEQ ID NO:62 (IL-12p40(Q42E)), vii) SEQ ID NO:63

(IL-12p40(E45Q)), viii) SEQ ID NO:64 (IL-12p40(Q56E)), ix) SEQ ID NO:65 (IL-12p40(E59Q)), x) SEQ ID NO:66 (IL-12p40(D62N)), xi) SEQ ID NO:67 (IL-12p40(Q42E/E45Q)), xii) SEQ ID NO:68 (IL-12p40(E45Q/Q56E)), xiii) SEQ ID NO:69 (IL-12p40(Q42E/E59Q)), xiv) SEQ ID NO:70 (IL-12p40(Q56E/E59Q)), xv) SEQ ID NO:71 (IL-12p40(Q42E/E45Q/Q56E)), xvi) SEQ ID NO:72 (IL-12p40(E45Q/Q56E/E59Q)), xvii) SEQ ID NO:73 (IL-12p40(D161N)), xviii) SEQ ID NO:74 (IL-12p40(E73Q)), xix) SEQ ID NO:75 (IL-12p40(Q144E)), xx) SEQ ID NO:76 (IL-12p40(E262Q)), xxi) SEQ ID NO:77 (IL-12p40(E100Q)), xxii) SEQ ID NO:78 (IL-12p40(D18N)), xxiii) SEQ ID NO:79 (IL-12p40(E33Q)), xxiv) SEQ ID NO:80 (IL-12p40(Q229E)), xxv) SEQ ID NO:81 (IL-12p40(E235Q)), xxvi) SEQ ID NO:82 (IL-12p40(Q256N)), xxvii) SEQ ID NO:83 (IL-12p40(E299Q)), xxviii) SEQ ID NO:84 (IL-12p40(D87N)), xxix) IL-12p40(E32Q), xxx) IL-12p40(D34N), xxxi) IL-12p40(S43E), xxxii) IL-12p40(S43K), xxxiii) SEQ ID NO:XXX (IL-12p40 (E59K/K99E/N103Q/N113Q/N200Q/N281Q/C252S/K264E)), xxxiv) SEQ ID NO:205 (IL-12p40(E59K)), xxxv) IL-12p40(K99E), xxxvi) IL-12p40(K163E), xxxvii) IL-12p40(E187Q), xxxviii) IL-12p40(K258E), xxxix) IL-12p40(K260E), xl) SEQ ID NO:206 (IL-12p40(E32Q/E59Q)), xli) SEQ ID NO:207 (IL-12p40(D34N/E59Q)), xlii) SEQ ID NO:208 (IL-12p40(E59Q/E187Q)), xliii) SEQ ID NO:209 (IL-12p40(S43E/E59Q)), xliv) SEQ ID NO:210 (IL-12p40(S43K/E49Q)), xlv) SEQ ID NO:211 (IL-12p40(E59Q/K163E)), xlvi) SEQ ID NO:212 (IL-12p40(E59Q/K99E)), xlvii) SEQ ID NO:213 (IL-12p40(E59Q/K258E)), xlviii) SEQ ID NO:214 (IL-12p40(E59Q/K260E)), xlix) SEQ ID NO: XXX (IL-12p40 (D34N/E59K)), l) SEQ ID NO: XXX (IL-12p40(E59K/K99E)), li) SEQ ID NO: XXX (IL-12p40(D18K/E59K/K99E)), lii) SEQ ID NO: XXX (IL-12p40 (E59K/K99E/K264E)), liii) SEQ ID NO: XXX (IL-12p40 (E59K/K99Y)), liv) SEQ ID NO: XXX (IL-12p40 (E59Y/K99E)), lv) SEQ ID NO: XXX (IL-12p40 (E45K/E59K/K99E)), lvi) SEQ ID NO: XXX (IL-12p40 (E59K/K99E/Q144E)), lvii) SEQ ID NO: XXX (IL-12p40 (E59K/K99E/Q144K)), lviii) SEQ ID NO: XXX (IL-12p40 (E59K/K99E/R159E)), lix) SEQ ID NO: XXX (IL-12p40 (E59K/K99E/K264E)), lx) SEQ ID NO: XXX (IL-12p40 (D18K/E59K/K99E/K264E)), lxi) SEQ ID NO: XXX (IL-12p40 (C252S)), lxii) SEQ ID NO: XXX (IL-12p40 (DI8K/E59K/K99E/C252S)), lxiii) SEQ ID NO: XXX (IL-12p40 (D18K/E59K/K99E/C252S/K264E)), lxiv) SEQ ID NO: XXX (IL-12p40 (E59K/K99Y/C252S)), lxv) SEQ ID NO: XXX (IL-12p40 (E59K/K99E/C252S/K264E)), lxvi) SEQ ID NO: XXX (IL-12p40 (E59K/K99E/C252S)), lxvii) SEQ ID NO: XXX (IL-12p40 (N103D/N113D)),

lxviii) SEQ ID NO: XXX (IL-12p40 (N103D/N200D)), lxix) SEQ ID NO: XXX (IL-12p40 (N103D/N281D)), lxx) SEQ ID NO: XXX (IL-12p40 (N113D/N200D)), lxxi) SEQ ID NO: XXX (IL-12p40 (N113D/N281D)), lxxii) SEQ ID NO: XXX (IL-12p40 (N200D/N281D)), lxxiii) SEQ ID NO: XXX (IL-12p50 (N103D/N113D/N200D)), lxxiv) SEQ ID NO: XXX (IL-12p40 (N103D/N113D/N281D)), lxxv) SEQ ID NO: XXX (IL-12p40 (N103D/N200D/N281D)), lxxvi) SEQ ID NO: XXX (IL-12p40 (N113D/N200D/N281D)), lxxvii) SEQ ID NO: XXX (IL-12p40 (N103Q)), lxxviii) SEQ ID NO: XXX (IL-12p40 (N113Q)), lxxix) SEQ ID NO: XXX (IL-12p40 (N200Q)), lxxx) SEQ ID NO: XXX (IL-12p40 (N281Q)), lxxxi) SEQ ID NO: XXX (IL-12p40 (N103Q/N113Q)), lxxxii) SEQ ID NO: XXX (IL-12p40 (N103Q/N200Q)), lxxxiii) SEQ ID NO: XXX (IL-12p40 (N103Q/N281Q)), lxxxiv) SEQ ID NO: XXX (IL-12p40 (N113Q/N200Q)), lxxxv) SEQ ID NO: XXX (IL-12p40 (N113Q/N281Q)), lxxxvi) SEQ ID NO: XXX (IL-12p40 (N200Q/N281Q)), lxxxvii) SEQ ID NO: XXX (IL-12p40 (N103Q/N113Q/N200Q)), lxxxviii) SEQ ID NO: XXX (IL-12p40 (N103Q/N113Q/N281Q)), lxxxix) SEQ ID NO: XXX (IL-12p40 (N103Q/N200Q/N281Q)), xc) SEQ ID NO: XXX (IL-12p40 (N113Q/N200Q/N281Q)), xci) SEQ ID NO:XXX (IL-12p40 (N103Q/N113Q/N200Q/N281Q)), xcii) SEQ ID NO:XXX (IL-12p40 (D34N/E59K/K99E)), xciii) SEQ ID NO:XXX (IL-12p40 (D34K/E59K/K99E)), xciv) SEQ ID NO:XXX (IL-12p40 (E32Q/D34N/E59K/K99E)), xcv) SEQ ID NO:XXX (IL-12p40 (E32K/D34N/E59K/K99E)), xcvi) SEQ ID NO: XXX (IL-12p40 (E59Y/K99Y)), xcvii) SEQ ID NO:XXX (IL-12p40 (E59K/K99E/N103Q/C252S/K264E)), xcviii) SEQ ID NO:XXX (IL-12p40 (E59K/K99E/N113Q/C252S/K264E)), xcix) SEQ ID NO:XXX (IL-12p40 (E59K/K99E/N200Q/C252S/K264E)), c) SEQ ID NO:XXX (IL-12p40 (E59K/K99E/N281Q/C252S/K264E)), ci) SEQ ID NO:XXX (IL-12p40 (E59K/K99E/N103Q/N113Q/C252S/K264E)), cii) SEQ ID NO:XXX (IL-12p40 (E59K/K99E/N103Q/N200Q/C252S/K264E)), ciii) SEQ ID NO:XXX (IL-12p40 (E59K/K99E/N103Q/N281Q/C252S/K264E)), civ) SEQ ID NO:XXX (IL-12p40 (E59K/K99E/N113Q/N200Q/C252S/K264E)), cv) SEQ ID NO:XXX (IL-12p40 (E59K/K99E/N113Q/N281Q/C252S/K264E)), cvi) SEQ ID NO:XXX (IL-12p40 (E59K/K99E/N200Q/N281Q/C252S/K264E)), cvii) SEQ ID NO:XXX (IL-12p40 (E59K/K99E/N103Q/N113Q/N200Q/C252S/K264E)), cviii) SEQ ID NO:XXX (IL-12p40

(E59K/K99E/N103Q/N200Q/N281Q/C252S/K264E)), and cix) SEQ ID NO:XXX (IL-12p40 (E59K/K99E/N113Q/N200Q/N281Q/C252S/K264E)).

[00119] In some embodiments, the present invention provides a heterodimeric Fc fusion protein wherein said IL-12p35 subunit has one or more amino acid modifications as amino acid residues selected from the group consisting of Q20, N21, Q35, E38, S44, E45, E46, H49, K54, D55, T59, V60, E61, C63, L64, P65, E67, L68, N71, S73, C74, L75, N76, E79, N85, L89, F96, M97, L124, M125, Q130, Q135, N136, E143, Q146, N151, E153, K158, E162, E163, D165, I171, R181, I182, R183, V185, T186, D188, R189, V190, S192, Y193, N195, and A196.

[00120] In some embodiments, the present invention provides a heterodimeric Fc fusion protein wherein said IL-12p35 subunit has one or more amino acid substitutions selected from the group consisting of N21D, Q35D, E38Q, D55Q, D55K, N71D, N71Q, L75A, N76D, E79Q, N85D, N85Q, L89A, F96A, M97A, L124A, M125A, Q130E, Q135E, N136D, E143Q, Q146E, N151D, N151K, E153K, E153Q, K158E, E162Q, E163Q, D165N, I171A, N195D, and N195Q.

[00121] In some embodiments, the present invention provides a heterodimeric Fc fusion protein wherein said IL-12p35 subunit has amino acid substitutions selected from the group consisting of N71D/N85D/N195D, N151D/E153Q, N151D/D165N, Q130E/N151D, N151D/K158E, E79Q/N151D, D55Q/N151D, N136D/N151D, N21D/N151D, E143Q/N151D, N71Q/N85Q, N71Q/N195Q, N85Q/N195Q, N71Q/N85Q/N195Q, N71D/N85D, N71D/N195D, and N85D/N195D.

[00122] In some embodiments, the present invention provides a heterodimeric Fc fusion protein wherein said IL-12p35 subunit has a polypeptide sequence selected from the group consisting of: i) SEQ ID NO:113 (IL-12p35(N71D)), ii) SEQ ID NO:114 (IL-12p35(N85D)), iii) SEQ ID NO:115 (IL-12p35(N195D)), iv) SEQ ID NO:116 (IL-12p35(N71D/N85D/N195D)), v) SEQ ID NO:117 (IL-12p35(E153Q)), vi) SEQ ID NO:118 (IL-12p35(E38Q)), vii) SEQ ID NO:119 (IL-12p35(N151D)), viii) SEQ ID NO:120 (IL-12p35(Q135E)), ix) SEQ ID NO:121 (IL-12p35(Q35D)), x) SEQ ID NO:122 (IL-12p35(Q146E)), xi) SEQ ID NO:123 (IL-12p35(N76D)), xii) SEQ ID NO:124 (IL-12p35(E162Q)), xiii) SEQ ID NO:125 (IL-12p35(E163Q)), xiv) IL-12p35(N21D), xv) SEQ ID

NO:XXX (IL-12p35(D55Q)), xvi) IL-12p35(E79Q), xvii) IL-12p35(Q130E), xviii) IL-12p35(N136D), xix) IL-12p35(E143Q), xx) SEQ ID NO:227 (IL-12p35(N151K)), xxi) SEQ ID NO:226 (IL-12p35(E153K)), xxii) IL-12p35(K158E), xxiii) IL-12p35(D165N), xxiv) SEQ ID NO:225 (IL-12p35(N151D/E153Q)), xxv) SEQ ID NO:228 (IL-12p35(N151D/D165N)), xxvi) SEQ ID NO:229 (IL-12p35(Q130E/N151D)), xxvii) SEQ ID NO:230 (IL-12p35(N151D/K158E)), xxviii) SEQ ID NO:231 (IL-12p35(E79Q/N151D)), xxix) SEQ ID NO:232 (IL-12p35(D55Q/N151D)), xxx) SEQ ID NO:233 (IL-12p35(N136D/N151D)), xxxi) SEQ ID NO:234 (IL-12p35(N21D/N151D)), xxxii) SEQ ID NO:235 (IL-12p35(E143Q/N151D)), xxxiii) SEQ ID NO: XXX (IL-12p35(F96A)), xxxiv) SEQ ID NO: XXX (IL-12p35(M97A)), xxxv) SEQ ID NO: XXX (IL-12p35(L89A)), xxxvi) SEQ ID NO: XXX (IL-12p35(L124A)), xxxvii) SEQ ID NO: XXX (IL-12p35(M125A)), xxxviii) SEQ ID NO: XXX (IL-12p35(L75A)), xxxiv) SEQ ID NO: XXX (IL-12p35(I171A)), xxxv) SEQ ID NO: XXX (IL-12p35 (N71Q)), xxxvi) SEQ ID NO: XXX (IL-12p35 (N85Q)), xxxvii) SEQ ID NO: XXX (IL-12p35 (N195Q)), xxxviii) SEQ ID NO: XXX (IL-12p35 (N71Q/N85Q)), xxxix) SEQ ID NO: XXX (IL-12p35 (N71Q/N195Q)), xl) SEQ ID NO: XXX (IL-12p35 (N85Q/N195Q), xli) SEQ ID NO: XXX (IL-12p35 (N71Q/N85Q/N195Q)), xlii) SEQ ID NO: XXX (IL-12p35 (N71D/N85D)), xliii) SEQ ID NO: XXX (IL-12p35 (N71D/N195D), xliv) SEQ ID NO: XXX (IL-12p35 (N85D/N195D)), xlv) SEQ ID NO: XXX (IL-12p35 (D55Q)), and xlvi) SEQ ID NO: XXX (IL-12p35 (D55K)).

[00123] In some embodiments, the present invention provides a composition comprising a heterodimeric Fc fusion protein for use in treating cancer in a subject.

[00124] In some embodiments, the present invention provides one or more nucleic acids encoding a heterodimeric Fc fusion protein.

[00125] In some embodiments, the present invention provides a host cell comprising said one or more nucleic acids encoding a heterodimeric Fc fusion protein.

[00126] In some embodiments, the present invention provides a method of making a heterodimeric Fc fusion protein, said method comprising culturing a host cell under conditions whereby said heterodimeric Fc fusion protein is produced.

[00127] In some embodiments, the present invention provides a method of purifying a heterodimeric Fc fusion protein, said method comprising: a) providing a composition comprising the heterodimeric Fc fusion protein; b) loading said composition onto an ion exchange column; andc) collecting a fraction containing said heterodimeric Fc fusion protein.

[00128] In another aspect, the present invention provides a heterodimeric Fc fusion protein comprising: a) a fusion protein comprising a first protein domain, a second protein domain and a first Fc domain, wherein said first protein domain is attached to the C-terminus of said first Fc domain, and wherein said second protein domain is covalently attached to said first protein domain; and b) a second Fc domain; wherein said first and said second Fc domains comprise modifications promoting heterodimerization of said first and said second Fc domains and wherein said first protein domain comprises an IL-12p35 subunit and said second protein domain comprises an IL-12p40 subunit.

[00129] In some embodiments, the present invention provides a heterodimeric Fc fusion protein, wherein said modifications promoting heterodimerization of said first and said second Fc domains are a set of amino acid substitutions selected from the group consisting of L368D/K370S and S364K; L368D/K370S and S364K/E357L; L368D/K370S and S364K/E357Q; T411E/K360E/Q362E and D401K; L368E/K370S and S364K; K370S and S364K/E357Q and T366S/L368A/Y407V : T366W (optionally including a bridging disulfide, T366S/L368A/Y407V/Y349C : T366W/S354C), according to EU numbering.

[00130] In some embodiments, the present invention provides a heterodimeric Fc fusion protein wherein said first protein domain is attached to said second protein domain using a first domain linker and/or said second protein domain is attached to said first Fc domain using a second domain linker.

[00131] In some embodiments, the present invention provides a heterodimeric Fc fusion protein wherein said first and/or said second Fc domains have an additional set of amino acid substitutions comprising Q295E/N384D/Q418E/N421D, according to EU numbering.

[00132] In some embodiments, the present invention provides a heterodimeric Fc fusion protein wherein said first and/or said second Fc domains have an additional set of amino acid substitutions selected from the group consisting of G236R/L328R, E233P/L234V/L235A/G236_/S239K, E233P/L234V/L235A/G236_/S239K/A327G, E233P/L234V/L235A/G236_/S267K/A327G, E233P/L234V/L235A/G236_, and E233P/L234V/L235A/G236_/S267K, according to EU numbering.

[00133] In some embodiments, the present invention provides a heterodimeric Fc fusion protein wherein said IL-12p40 subunit has a polypeptide sequence selected form the group consisting of SEQ ID NO:3 (human IL-12 subunit beta (IL-12p40) precursor sequence) and SEQ ID NO:4 (human IL-12 subunit beta (IL-12p40) mature form sequence), and/or said IL-12p35 subunit has a polypeptide sequence selected from the group consisting of SEQ ID NO:1 (human IL-12 subunit alpha (IL-12p35) precursor sequence) and SEQ ID NO:2 (human IL-12 subunit alpha (IL-12p35) mature form sequence).

[00134] In some embodiments, the present invention provides a heterodimeric Fc fusion protein wherein said first and second Fc domains further comprise amino acid substitutions M428L/N424S.

[00135] In some embodiments, the present invention provides a heterodimeric Fc fusion protein wherein said IL-12p40 subunit is a variant IL-12p40 subunit and/or said IL-12p35 subunit is a variant IL-12p35 subunit.

[00136] In some embodiments, the present invention provides a heterodimeric Fc fusion protein wherein said IL-12p40 subunit is a variant IL-12p40 subunit having altered affinity for IL-12 receptor subunit beta-1 (IL-12Rß1), IL-12 receptor subunit beta-2 (IL-12Rß2), and/or IL-12 receptor complex; and/or said IL-12p35 subunit is a variant IL-12p35 subunit having altered

affinity for IL-12 receptor subunit beta-1 (IL-12Rß1), IL-12 receptor subunit beta-2 (IL-12Rß2), and/or IL-12 receptor complex.

[00137] In some embodiments, the present invention provides a heterodimeric Fc fusion protein wherein said IL-12p40 subunit has one or more amino acid modifications at amino acid residues selected from the group consisting of E3, D7, E12, D14, W15, P17, D18, A19, P20, G21, E22, M23, D29, E32, E33, D34, L40, D41, Q42, S43, E45, L47, T54, I55, Q56, K58, E59, F60, G61, D62, Q65, Y66, E73, K84, E86, D87, G88, I89, W90, D93, D97, K99, E100, K102, N103, K104, F106, E110, N113, Y114, D129, D142, Q144, E156, R159, D161, N162, K163, D166, D170, Q172, D174, A176, C177, P178, A179, A180, E181, S183, P185, E187, N200, S204, F206, R208, D209, D214, N218, Q220, N226, Q229, E231, E235, T242, P243, S245, Y246, F247, S248, C252, Q256, K258, K260, E262, K264, D265, D270, N281, Q289, D290, R291, Y292, Y293, and E299.

[00138] In some embodiments, the present invention provides a heterodimeric Fc fusion protein wherein said IL-12p40 subunit has one or more amino acid substitutions selected from the group consisting of D18N, D18K, E32Q, E33Q, D34N, D34K, Q42E, S43E, S43K, E45Q, Q56E, E59Q, E59K, D62N, E73Q, D87N, K99E, K99Y, E100Q, N103D, N103Q, N113D, N113Q, Q144E, D161N, R159E, K163E, E187Q, N200D, N200Q, N218Q, Q229E, E235Q, C252S, Q256N, K258E, K260E, E262Q, K264E, N281D, N281Q, and E299Q.

[00139]

[00140] In some embodiments, the present invention provides a heterodimeric Fc fusion protein wherein said IL-12p40 subunit has amino acid substitutions selected from the group consisting of N103D/N113D/N200D/N281D, Q42E/E45Q, E45Q/Q56E, Q42E/E59Q, Q56E/E59Q, Q42E/E45Q/Q56E, E45Q/Q56E/E59Q, E32Q/E59Q, D34N/E59K, D34N/E59K/K99E, D34K/E59K/K99E, E32Q/D34N/E59K/K99E, E32K/D34N/E59K/K99E, D34N/E59Q, E59Q/E187Q, S43E/E59Q, S43K/E49Q, E59Q/K163E, E59Q/K99E, E59Q/K258E, E59Q/K260E, E59K/K99E, D18K/E59K/K99E, E59K/K99E/K264E, E59K/K99Y, E59Y/K99Y, E59Y/K99E, E45K/E59K/K99E, E59K/K99E/Q144E, E59K/K99E/Q144K, E59K/K99E/R159E, E59K/K99E/K264E, D18K/E59K/K99E/K264E, DI8K/E59K/K99E/C252S, D18K/E59K/K99E/C252S/K264E,

E59K/K99Y/C252S, E59K/K99E/C252S/K264E, E59K/K99E/C252S, N103D/N113D, N103D/N200D, N103D/N281D, N113D/N200D, N113D/N281D, N200D/N281D, N103D/N113D/N200D, N103D/N113D/N281D, N103D/N200D/N281D, N113D/N200D/N281D, N103Q/N113Q, N103Q/N200Q, N103Q/N281Q, N113Q/N200Q, N113Q/N281Q, N200Q/N281Q, N103Q/N113Q/N200Q, N103Q/N113Q/N281Q, N103Q/N200Q/N281Q, N113Q/N200Q/N281Q, N103Q/N113Q/N200Q/N281Q, E59K/K99E/N103Q/C252S/K264E, E59K/K99E/N113Q/C252S/K264E, E59K/K99E/N200Q/C252S/K264E, E59K/K99E/N281Q/C252S/K264E, E59K/K99E/N103Q/N113Q/C252S/K264E, E59K/K99E/N103Q/N200Q/C252S/K264E, E59K/K99E/N103Q/N281Q/C252S/K264E, E59K/K99E/N113Q/N200Q/C252S/K264E, E59K/K99E/N113Q/N281Q/C252S/K264E, E59K/K99E/N200Q/N281Q/C252S/K264E, E59K/K99E/N103Q/N113Q/N200Q/C252S/K264E, E59K/K99E/N103Q/N200Q/N281Q/C252S/K264E, E59K/K99E/N113Q/N200Q/N281Q/C252S/K264E, and E59K/K99E/N103Q/N113Q/N200Q/N281Q/C252S/K264E.

[00141] In some embodiments, the present invention provides a heterodimeric Fc fusion protein wherein said IL-12p40 subunit has a polypeptide sequence selected from the group consisting of: i) SEQ ID NO:57 (IL-12p40(N103D)), ii) SEQ ID NO:58 (IL-12p40(N113D)), iii) SEQ ID NO:59 (IL-12p40(N200D)), iv) SEQ ID NO:60 (IL-12p40(N281D)), v) SEQ ID NO:61 (IL-12p40(N103D/N113D/N200D/N281D)), vi) SEQ ID NO:62 (IL-12p40(Q42E)), vii) SEQ ID NO:63 (IL-12p40(E45Q)), viii) SEQ ID NO:64 (IL-12p40(Q56E)), ix) SEQ ID NO:65 (IL-12p40(E59Q)), x) SEQ ID NO:66 (IL-12p40(D62N)), xi) SEQ ID NO:67 (IL-12p40(Q42E/E45Q)), xii) SEQ ID NO:68 (IL-12p40(E45Q/Q56E)), xiii) SEQ ID NO:69 (IL-12p40(Q42E/E59Q)), xiv) SEQ ID NO:70 (IL-12p40(Q56E/E59Q)), xv) SEQ ID NO:71 (IL-12p40(Q42E/E45Q/Q56E)), xvi) SEQ ID NO:72 (IL-12p40(E45Q/Q56E/E59Q)), xvii) SEQ ID NO:73 (IL-12p40(D161N)), xviii) SEQ ID NO:74 (IL-12p40(E73Q)), xix) SEQ ID NO:75 (IL-12p40(Q144E)), xx) SEQ ID NO:76 (IL-12p40(E262Q)), xxi) SEQ ID NO:77 (IL-12p40(E100Q)), xxii) SEQ ID NO:78 (IL-12p40(D18N)), xxiii) SEQ ID NO:79 (IL-12p40(E33Q)), xxiv) SEQ ID NO:80 (IL-12p40(Q229E)), xxv) SEQ ID NO:81 (IL-12p40(E235Q)), xxvi) SEQ ID NO:82 (IL-12p40(Q256N)), xxvii) SEQ ID NO:83 (IL-

12p40(E299Q)), xxviii) SEQ ID NO:84 (IL-12p40(D87N)), xxix) IL-12p40(E32Q), xxx) IL-12p40(D34N), xxxi) IL-12p40(S43E), xxxii) IL-12p40(S43K), xxxiii) SEQ ID NO:XXX (IL-12p40 (E59K/K99E/N103Q/N113Q/N200Q/N281Q/C252S/K264E)), xxxiv) SEQ ID NO:205 (IL-12p40(E59K)), xxxv) IL-12p40(K99E), xxxvi) IL-12p40(K163E), xxxvii) IL-12p40(E187Q), xxxviii) IL-12p40(K258E), xxxix) IL-12p40(K260E), xl) SEQ ID NO:206 (IL-12p40(E32Q/E59Q)), xli) SEQ ID NO:207 (IL-12p40(D34N/E59Q)), xlii) SEQ ID NO:208 (IL-12p40(E59Q/E187Q)), xliii) SEQ ID NO:209 (IL-12p40(S43E/E59Q)), xliv) SEQ ID NO:210 (IL-12p40(S43K/E49Q)), xlv) SEQ ID NO:211 (IL-12p40(E59Q/K163E)), xlvi) SEQ ID NO:212 (IL-12p40(E59Q/K99E)), xlvii) SEQ ID NO:213 (IL-12p40(E59Q/K258E)), xlviii) SEQ ID NO:214 (IL-12p40(E59Q/K260E)), xlix) SEQ ID NO: XXX (IL-12p40 (D34N/E59K)), l) SEQ ID NO: XXX (IL-12p40(E59K/K99E)), li) SEQ ID NO: XXX (IL-12p40(D18K/E59K/K99E)), lii) SEQ ID NO: XXX (IL-12p40 (E59K/K99E/K264E)), liii) SEQ ID NO: XXX (IL-12p40 (E59K/K99Y)), liv) SEQ ID NO: XXX (IL-12p40 (E59Y/K99E)), lv) SEQ ID NO: XXX (IL-12p40 (E45K/E59K/K99E)), lvi) SEQ ID NO: XXX (IL-12p40 (E59K/K99E/Q144E)), lvii) SEQ ID NO: XXX (IL-12p40 (E59K/K99E/Q144K)), lviii) SEQ ID NO: XXX (IL-12p40 (E59K/K99E/R159E)), lix) SEQ ID NO: XXX (IL-12p40 (E59K/K99E/K264E)), lx) SEQ ID NO: XXX (IL-12p40 (D18K/E59K/K99E/K264E)), lxi) SEQ ID NO: XXX (IL-12p40 (C252S)), lxii) SEQ ID NO: XXX (IL-12p40 (DI8K/E59K/K99E/C252S)), lxiii) SEQ ID NO: XXX (IL-12p40 (D18K/E59K/K99E/C252S/K264E)), lxiv) SEQ ID NO: XXX (IL-12p40 (E59K/K99Y/C252S)), lxv) SEQ ID NO: XXX (IL-12p40 (E59K/K99E/C252S/K264E)), lxvi) SEQ ID NO: XXX (IL-12p40 (E59K/K99E/C252S)), lxvii) SEQ ID NO: XXX (IL-12p40 (N103D/N113D)), lxviii) SEQ ID NO: XXX (IL-12p40 (N103D/N200D)), lxix) SEQ ID NO: XXX (IL-12p40 (N103D/N281D)), lxx) SEQ ID NO: XXX (IL-12p40 (N113D/N200D)), lxxi) SEQ ID NO: XXX (IL-12p40 (N113D/N281D)), lxxii) SEQ ID NO: XXX (IL-12p40 (N200D/N281D)), lxxiii) SEQ ID NO: XXX (IL-12p50 (N103D/N113D/N200D)), lxxiv) SEQ ID NO: XXX (IL-12p40 (N103D/N113D/N281D)), lxxv) SEQ ID NO: XXX (IL-12p40 (N103D/N200D/N281D)), lxxvi) SEQ ID NO: XXX (IL-12p40 (N113D/N200D/N281D)), lxxvii) SEQ ID NO: XXX (IL-12p40 (N103Q)), lxxviii) SEQ ID NO: XXX (IL-12p40 (N113Q)), lxxix) SEQ ID NO: XXX (IL-12p40 (N200Q)), lxxx) SEQ ID NO: XXX (IL-12p40 (N281Q)), lxxxi) SEQ ID NO: XXX (IL-12p40 (N103Q/N113Q)), lxxxii) SEQ ID NO: XXX (IL-12p40 (N103Q/N200Q)), lxxxiii) SEQ ID NO: XXX

(IL-12p40 (N103Q/N281Q)), lxxxiv) SEQ ID NO: XXX (IL-12p40 (N113Q/N200Q)), lxxxv) SEQ ID NO: XXX (IL-12p40 (N113Q/N281Q)), lxxxvi) SEQ ID NO: XXX (IL-12p40 (N200Q/N281Q)), lxxxvii) SEQ ID NO: XXX (IL-12p40 (N103Q/N113Q/N200Q)), lxxxviii) SEQ ID NO: XXX (IL-12p40 (N103Q/N113Q/N281Q)), lxxxix) SEQ ID NO: XXX (IL-12p40 (N103Q/N200Q/N281Q)), xc) SEQ ID NO: XXX (IL-12p40 (N113Q/N200Q/N281Q)), xci) SEQ ID NO:XXX (IL-12p40 (N103Q/N113Q/N200Q/N281Q)), xcii) SEQ ID NO:XXX (IL-12p40 (D34N/E59K/K99E)), xciii) SEQ ID NO:XXX (IL-12p40 (D34K/E59K/K99E)), xciv) SEQ ID NO:XXX (IL-12p40 (E32Q/D34N/E59K/K99E)), xcv) SEQ ID NO:XXX (IL-12p40 (E32K/D34N/E59K/K99E)), xcvi) SEQ ID NO: XXX (IL-12p40 (E59Y/K99Y)), xcvii) SEQ ID NO:XXX (IL-12p40 (E59K/K99E/N103Q/C252S/K264E)), xcviii) SEQ ID NO:XXX (IL-12p40 (E59K/K99E/N113Q/C252S/K264E)), xcix) SEQ ID NO:XXX (IL-12p40 (E59K/K99E/N200Q/C252S/K264E)), c) SEQ ID NO:XXX (IL-12p40 (E59K/K99E/N281Q/C252S/K264E)), ci) SEQ ID NO:XXX (IL-12p40 (E59K/K99E/N103Q/N113Q/C252S/K264E)), cii) SEQ ID NO:XXX (IL-12p40 (E59K/K99E/N103Q/N200Q/C252S/K264E)), ciii) SEQ ID NO:XXX (IL-12p40 (E59K/K99E/N103Q/N281Q/C252S/K264E)), civ) SEQ ID NO:XXX (IL-12p40 (E59K/K99E/N113Q/N200Q/C252S/K264E)), cv) SEQ ID NO:XXX (IL-12p40 (E59K/K99E/N113Q/N281Q/C252S/K264E)), cvi) SEQ ID NO:XXX (IL-12p40 (E59K/K99E/N200Q/N281Q/C252S/K264E)), cvii) SEQ ID NO:XXX (IL-12p40 (E59K/K99E/N103Q/N113Q/N200Q/C252S/K264E)), cviii) SEQ ID NO:XXX (IL-12p40 (E59K/K99E/N103Q/N200Q/N281Q/C252S/K264E)), and cix) SEQ ID NO:XXX (IL-12p40 (E59K/K99E/N113Q/N200Q/N281Q/C252S/K264E)).

[00142] In some embodiments, the present invention provides a heterodimeric Fc fusion protein wherein said IL-12p35 subunit has one or more amino acid modifications as amino acid residues selected from the group consisting of Q20, N21, Q35, E38, S44, E45, E46, H49, K54, D55, T59, V60, E61, C63, L64, P65, E67, L68, N71, S73, C74, L75, N76, E79, N85, L89, F96, M97, L124, M125, Q130, Q135, N136, E143, Q146, N151, E153, K158, E162, E163, D165, I171, R181, I182, R183, V185, T186, D188, R189, V190, S192, Y193, N195, and A196.

[00143] In some embodiments, the present invention provides a heterodimeric Fc fusion protein wherein said IL-12p35 subunit has one or more amino acid substitutions selected from the group consisting of N21D, Q35D, E38Q, D55Q, D55K, N71D, N71Q, L75A, N76D, E79Q, N85D, N85Q, L89A, F96A, M97A, L124A, M125A, Q130E, Q135E, N136D, E143Q, Q146E, N151D, N151K, E153K, E153Q, K158E, E162Q, E163Q, D165N, I171A, N195D, and N195Q.

[00144] In some embodiments, the present invention provides a heterodimeric Fc fusion protein wherein said IL-12p35 subunit has amino acid substitutions selected from the group consisting of N71D/N85D/N195D, N151D/E153Q, N151D/D165N, Q130E/N151D, N151D/K158E, E79Q/N151D, D55Q/N151D, N136D/N151D, N21D/N151D, E143Q/N151D, N71Q/N85Q, N71Q/N195Q, N85Q/N195Q, N71Q/N85Q/N195Q, N71D/N85D, N71D/N195D, and N85D/N195D.

[00145] In some embodiments, the present invention provides a heterodimeric Fc fusion protein wherein said IL-12p35 subunit has a polypeptide sequence selected from the group consisting of: i) SEQ ID NO:113 (IL-12p35(N71D)), ii) SEQ ID NO:114 (IL-12p35(N85D)), iii) SEQ ID NO:115 (IL-12p35(N195D)), iv) SEQ ID NO:116 (IL-12p35(N71D/N85D/N195D)), v) SEQ ID NO:117 (IL-12p35(E153Q)), vi) SEQ ID NO:118 (IL-12p35(E38Q)), vii) SEQ ID NO:119 (IL-12p35(N151D)), viii) SEQ ID NO:120 (IL-12p35(Q135E)), ix) SEQ ID NO:121 (IL-12p35(Q35D)), x) SEQ ID NO:122 (IL-12p35(Q146E)), xi) SEQ ID NO:123 (IL-12p35(N76D)), xii) SEQ ID NO:124 (IL-12p35(E162Q)), xiii) SEQ ID NO:125 (IL-12p35(E163Q)), xiv) IL-12p35(N21D), xv) SEQ ID NO:XXX (IL-12p35(D55Q)), xvi) IL-12p35(E79Q), xvii) IL-12p35(Q130E), xviii) IL-12p35(N136D), xix) IL-12p35(E143Q), xx) SEQ ID NO:227 (IL-12p35(N151K)), xxi) SEQ ID NO:226 (IL-12p35(E153K)), xxii) IL-12p35(K158E), xxiii) IL-12p35(D165N), xxiv) SEQ ID NO:225 (IL-12p35(N151D/E153Q)), xxv) SEQ ID NO:228 (IL-12p35(N151D/D165N)), xxvi) SEQ ID NO:229 (IL-12p35(Q130E/N151D)), xxvii) SEQ ID NO:230 (IL-12p35(N151D/K158E)), xxviii) SEQ ID NO:231 (IL-12p35(E79Q/N151D)), xxix) SEQ ID NO:232 (IL-12p35(D55Q/N151D)), xxx) SEQ ID NO:233 (IL-12p35(N136D/N151D)), xxxi) SEQ ID NO:234 (IL-12p35(N21D/N151D)), xxxii) SEQ ID NO:235 (IL-12p35(E143Q/N151D)), xxxiii) SEQ ID NO: XXX (IL-12p35(F96A)), xxxiv) SEQ ID NO: XXX (IL-12p35(M97A)), xxxv) SEQ ID NO: XXX (IL-12p35(L89A)), xxxvi) SEQ ID NO: XXX (IL-12p35(L124A)), xxxvii) SEQ ID NO:

XXX (IL-12p35(M125A)), xxxviii) SEQ ID NO: XXX (IL-12p35(L75A)), xxxiv) SEQ ID NO: XXX (IL-12p35(I171A)), xxxv) SEQ ID NO: XXX (IL-12p35 (N71Q)), xxxvi) SEQ ID NO: XXX (IL-12p35 (N85Q)), xxxvii) SEQ ID NO: XXX (IL-12p35 (N195Q)), xxxviii) SEQ ID NO: XXX (IL-12p35 (N71Q/N85Q)), xxxix) SEQ ID NO: XXX (IL-12p35 (N71Q/N195Q)), xl) SEQ ID NO: XXX (IL-12p35 (N85Q/N195Q), xli) SEQ ID NO: XXX (IL-12p35 (N71Q/N85Q/N195Q)), xlii) SEQ ID NO: XXX (IL-12p35 (N71D/N85D)), xliii) SEQ ID NO: XXX (IL-12p35 (N71D/N195D), xliv) SEQ ID NO: XXX (IL-12p35 (N85D/N195D)), xlv) SEQ ID NO: XXX (IL-12p35 (D55Q)), and xlvi) SEQ ID NO: XXX (IL-12p35 (D55K)).

[00146] In some embodiments, the present invention provides a composition comprising a heterodimeric Fc fusion protein for use in treating cancer in a subject.

[00147] In some embodiments, the present invention provides one or more nucleic acids encoding a heterodimeric Fc fusion protein.

[00148] In some embodiments, the present invention provides a host cell comprising said one or more nucleic acids encoding a heterodimeric Fc fusion protein.

[00149] In some embodiments, the present invention provides a method of making a heterodimeric Fc fusion protein, said method comprising culturing a host cell under conditions whereby said heterodimeric Fc fusion protein is produced.

[00150] In some embodiments, the present invention provides a method of purifying a heterodimeric Fc fusion protein, said method comprising: a) providing a composition comprising the heterodimeric Fc fusion protein; b) loading said composition onto an ion exchange column; andc) collecting a fraction containing said heterodimeric Fc fusion protein.

[00151] In another aspect, the present invention provides a heterodimeric complex comprising: a) an IL-12p40 subunit, and b) an IL-12p35 subunit.

[00152] In some embodiments, the present invention provides a heterodimeric complex wherein said IL-12p40 subunit is covalently attached to said IL-12p35 subunit using domain linker.

[00153] In some embodiments, the present invention provides a heterodimeric complex wherein said IL-12p40 subunit has a polypeptide sequence selected form the group consisting of SEQ ID NO:3 (human IL-12 subunit beta (IL-12p40) precursor sequence) and SEQ ID NO:4 (human IL-12 subunit beta (IL-12p40) mature form sequence), and/or said IL-12p35 subunit has a polypeptide sequence selected from the group consisting of SEQ ID NO:1 (human IL-12 subunit alpha (IL-12p35) precursor sequence) and SEQ ID NO:2 (human IL-12 subunit alpha (IL-12p35) mature form sequence).

[00154] In some embodiments, the present invention provides a heterodimeric complex wherein said first and second Fc domains further comprise amino acid substitutions M428L/N424S.

[00155] In some embodiments, the present invention provides a heterodimeric complex wherein said IL-12p40 subunit is a variant IL-12p40 subunit and/or said IL-12p35 subunit is a variant IL-12p35 subunit.

[00156] In some embodiments, the present invention provides a heterodimeric complex wherein said IL-12p40 subunit is a variant IL-12p40 subunit having altered affinity for IL-12 receptor subunit beta-1 (IL-12Rß1), IL-12 receptor subunit beta-2 (IL-12Rß2), and/or IL-12 receptor complex; and/or said IL-12p35 subunit is a variant IL-12p35 subunit having altered affinity for IL-12 receptor subunit beta-1 (IL-12Rß1), IL-12 receptor subunit beta-2 (IL-12Rß2), and/or IL-12 receptor complex.

[00157] In some embodiments, the present invention provides a heterodimeric complex wherein said IL-12p40 subunit has one or more amino acid modifications at amino acid residues selected from the group consisting of E3, D7, E12, D14, W15, P17, D18, A19, P20, G21, E22, M23, D29, E32, E33, D34, L40, D41, Q42, S43, E45, L47, T54, I55, Q56, K58, E59, F60, G61, D62, Q65, Y66, E73, K84, E86, D87, G88, I89, W90, D93, D97, K99, E100, K102, N103, K104, F106, E110, N113, Y114, D129, D142, Q144, E156, R159, D161, N162, K163, D166, D170, Q172, D174, A176, C177, P178, A179, A180, E181, S183, P185, E187, N200, S204, F206, R208, D209, D214, N218, Q220, N226, Q229, E231, E235, T242, P243, S245, Y246, F247, S248, C252, Q256, K258, K260, E262, K264, D265, D270, N281, Q289, D290, R291, Y292, Y293, and E299.

[00158] In some embodiments, the present invention provides a heterodimeric complex wherein said IL-12p40 subunit has one or more amino acid substitutions selected from the group consisting of D18N, D18K, E32Q, E33Q, D34N, D34K, Q42E, S43E, S43K, E45Q, Q56E, E59Q, E59K, D62N, E73Q, D87N, K99E, K99Y, E100Q, N103D, N103Q, N113D, N113Q, Q144E, D161N, R159E, K163E, E187Q, N200D, N200Q, N218Q, Q229E, E235Q, C252S, Q256N, K258E, K260E, E262Q, K264E, N281D, N281Q, and E299Q.

[00159]

[00160] In some embodiments, the present invention provides a heterodimeric complex wherein said IL-12p40 subunit has amino acid substitutions selected from the group consisting of N103D/N113D/N200D/N281D, Q42E/E45Q, E45Q/Q56E, Q42E/E59Q, Q56E/E59Q, Q42E/E45Q/Q56E, E45Q/Q56E/E59Q, E32Q/E59Q, D34N/E59K, D34N/E59K/K99E, D34K/E59K/K99E, E32Q/D34N/E59K/K99E, E32K/D34N/E59K/K99E, D34N/E59Q, E59Q/E187Q, S43E/E59Q, S43K/E49Q, E59Q/K163E, E59Q/K99E, E59Q/K258E, E59Q/K260E, E59K/K99E, D18K/E59K/K99E, E59K/K99E/K264E, E59K/K99Y, E59Y/K99Y, E59Y/K99E, E45K/E59K/K99E, E59K/K99E/Q144E, E59K/K99E/Q144K, E59K/K99E/R159E, E59K/K99E/K264E, D18K/E59K/K99E/K264E, DI8K/E59K/K99E/C252S, D18K/E59K/K99E/C252S/K264E, E59K/K99Y/C252S, E59K/K99E/C252S/K264E, E59K/K99E/C252S, N103D/N113D, N103D/N200D, N103D/N281D, N113D/N200D, N113D/N281D, N200D/N281D, N103D/N113D/N200D, N103D/N113D/N281D, N103D/N200D/N281D, N113D/N200D/N281D, N103Q/N113Q, N103Q/N200Q, N103Q/N281Q, N113Q/N200Q, N113Q/N281Q, N200Q/N281Q, N103Q/N113Q/N200Q, N103Q/N113Q/N281Q, N103Q/N200Q/N281Q, N113Q/N200Q/N281Q, N103Q/N113Q/N200Q/N281Q, E59K/K99E/N103Q/C252S/K264E, E59K/K99E/N113Q/C252S/K264E, E59K/K99E/N200Q/C252S/K264E, E59K/K99E/N281Q/C252S/K264E, E59K/K99E/N103Q/N113Q/C252S/K264E, E59K/K99E/N103Q/N200Q/C252S/K264E, E59K/K99E/N103Q/N281Q/C252S/K264E, E59K/K99E/N113Q/N200Q/C252S/K264E, E59K/K99E/N113Q/N281Q/C252S/K264E, E59K/K99E/N200Q/N281Q/C252S/K264E, E59K/K99E/N103Q/N113Q/N200Q/C252S/K264E, E59K/K99E/N103Q/N200Q/N281Q/C252S/K264E,

E59K/K99E/N113Q/N200Q/N281Q/C252S/K264E, and
E59K/K99E/N103Q/N113Q/N200Q/N281Q/C252S/K264E.

[00161] In some embodiments, the present invention provides a heterodimeric complex wherein said IL-12p40 subunit has a polypeptide sequence selected from the group consisting of: i) SEQ ID NO:57 (IL-12p40(N103D)), ii) SEQ ID NO:58 (IL-12p40(N113D)), iii) SEQ ID NO:59 (IL-12p40(N200D)), iv) SEQ ID NO:60 (IL-12p40(N281D)), v) SEQ ID NO:61 (IL-12p40(N103D/N113D/N200D/N281D)), vi) SEQ ID NO:62 (IL-12p40(Q42E)), vii) SEQ ID NO:63 (IL-12p40(E45Q)), viii) SEQ ID NO:64 (IL-12p40(Q56E)), ix) SEQ ID NO:65 (IL-12p40(E59Q)), x) SEQ ID NO:66 (IL-12p40(D62N)), xi) SEQ ID NO:67 (IL-12p40(Q42E/E45Q)), xii) SEQ ID NO:68 (IL-12p40(E45Q/Q56E)), xiii) SEQ ID NO:69 (IL-12p40(Q42E/E59Q)), xiv) SEQ ID NO:70 (IL-12p40(Q56E/E59Q)), xv) SEQ ID NO:71 (IL-12p40(Q42E/E45Q/Q56E)), xvi) SEQ ID NO:72 (IL-12p40(E45Q/Q56E/E59Q)), xvii) SEQ ID NO:73 (IL-12p40(D161N)), xviii) SEQ ID NO:74 (IL-12p40(E73Q)), xix) SEQ ID NO:75 (IL-12p40(Q144E)), xx) SEQ ID NO:76 (IL-12p40(E262Q)), xxi) SEQ ID NO:77 (IL-12p40(E100Q)), xxii) SEQ ID NO:78 (IL-12p40(D18N)), xxiii) SEQ ID NO:79 (IL-12p40(E33Q)), xxiv) SEQ ID NO:80 (IL-12p40(Q229E)), xxv) SEQ ID NO:81 (IL-12p40(E235Q)), xxvi) SEQ ID NO:82 (IL-12p40(Q256N)), xxvii) SEQ ID NO:83 (IL-12p40(E299Q)), xxviii) SEQ ID NO:84 (IL-12p40(D87N)), xxix) IL-12p40(E32Q), xxx) IL-12p40(D34N), xxxi) IL-12p40(S43E), xxxii) IL-12p40(S43K), xxxiii) SEQ ID NO:XXX (IL-12p40 (E59K/K99E/N103Q/N113Q/N200Q/N281Q/C252S/K264E)), xxxiv) SEQ ID NO:205 (IL-12p40(E59K)), xxxv) IL-12p40(K99E), xxxvi) IL-12p40(K163E), xxxvii) IL-12p40(E187Q), xxxviii) IL-12p40(K258E), xxxix) IL-12p40(K260E), xl) SEQ ID NO:206 (IL-12p40(E32Q/E59Q)), xli) SEQ ID NO:207 (IL-12p40(D34N/E59Q)), xlii) SEQ ID NO:208 (IL-12p40(E59Q/E187Q)), xliii) SEQ ID NO:209 (IL-12p40(S43E/E59Q)), xliv) SEQ ID NO:210 (IL-12p40(S43K/E49Q)), xlv) SEQ ID NO:211 (IL-12p40(E59Q/K163E)), xlvi) SEQ ID NO:212 (IL-12p40(E59Q/K99E)), xlvii) SEQ ID NO:213 (IL-12p40(E59Q/K258E)), xlviii) SEQ ID NO:214 (IL-12p40(E59Q/K260E)), xlix) SEQ ID NO: XXX (IL-12p40 (D34N/E59K)), l) SEQ ID NO: XXX (IL-12p40(E59K/K99E)), li) SEQ ID NO: XXX (IL-12p40(D18K/E59K/K99E)), lii) SEQ ID NO: XXX (IL-12p40 (E59K/K99E/K264E)), liii) SEQ ID NO: XXX (IL-12p40 (E59K/K99Y)), liv) SEQ ID NO: XXX (IL-12p40 (E59Y/K99E)), lv)

SEQ ID NO: XXX (IL-12p40 (E45K/E59K/K99E)), lvi) SEQ ID NO: XXX (IL-12p40 (E59K/K99E/Q144E)), lvii) SEQ ID NO: XXX (IL-12p40 (E59K/K99E/Q144K)), lviii) SEQ ID NO: XXX (IL-12p40 (E59K/K99E/R159E)), lix) SEQ ID NO: XXX (IL-12p40 (E59K/K99E/K264E)), lx) SEQ ID NO: XXX (IL-12p40 (D18K/E59K/K99E/K264E)), lxi) SEQ ID NO: XXX (IL-12p40 (C252S)), lxii) SEQ ID NO: XXX (IL-12p40 (DI8K/E59K/K99E/C252S)), lxiii) SEQ ID NO: XXX (IL-12p40 (D18K/E59K/K99E/C252S/K264E)), lxiv) SEQ ID NO: XXX (IL-12p40 (E59K/K99Y/C252S)), lxv) SEQ ID NO: XXX (IL-12p40 (E59K/K99E/C252S/K264E)), lxvi) SEQ ID NO: XXX (IL-12p40 (E59K/K99E/C252S)), lxvii) SEQ ID NO: XXX (IL-12p40 (N103D/N113D)), lxviii) SEQ ID NO: XXX (IL-12p40 (N103D/N200D)), lxix) SEQ ID NO: XXX (IL-12p40 (N103D/N281D)), lxx) SEQ ID NO: XXX (IL-12p40 (N113D/N200D)), lxxi) SEQ ID NO: XXX (IL-12p40 (N113D/N281D)), lxxii) SEQ ID NO: XXX (IL-12p40 (N200D/N281D)), lxxiii) SEQ ID NO: XXX (IL-12p50 (N103D/N113D/N200D)), lxxiv) SEQ ID NO: XXX (IL-12p40 (N103D/N113D/N281D)), lxxv) SEQ ID NO: XXX (IL-12p40 (N103D/N200D/N281D)), lxxvi) SEQ ID NO: XXX (IL-12p40 (N113D/N200D/N281D)), lxxvii) SEQ ID NO: XXX (IL-12p40 (N103Q)), lxxviii) SEQ ID NO: XXX (IL-12p40 (N113Q)), lxxix) SEQ ID NO: XXX (IL-12p40 (N200Q)), lxxx) SEQ ID NO: XXX (IL-12p40 (N281Q)), lxxxi) SEQ ID NO: XXX (IL-12p40 (N103Q/N113Q)), lxxxii) SEQ ID NO: XXX (IL-12p40 (N103Q/N200Q)), lxxxiii) SEQ ID NO: XXX (IL-12p40 (N103Q/N281Q)), lxxxiv) SEQ ID NO: XXX (IL-12p40 (N113Q/N200Q)), lxxxv) SEQ ID NO: XXX (IL-12p40 (N113Q/N281Q)), lxxxvi) SEQ ID NO: XXX (IL-12p40 (N200Q/N281Q)), lxxxvii) SEQ ID NO: XXX (IL-12p40 (N103Q/N113Q/N200Q)), lxxxviii) SEQ ID NO: XXX (IL-12p40 (N103Q/N113Q/N281Q)), lxxxix) SEQ ID NO: XXX (IL-12p40 (N103Q/N200Q/N281Q)), xc) SEQ ID NO: XXX (IL-12p40 (N113Q/N200Q/N281Q)), xci) SEQ ID NO:XXX (IL-12p40 (N103Q/N113Q/N200Q/N281Q)), xcii) SEQ ID NO:XXX (IL-12p40 (D34N/E59K/K99E)), xciii) SEQ ID NO:XXX (IL-12p40 (D34K/E59K/K99E)), xciv) SEQ ID NO:XXX (IL-12p40 (E32Q/D34N/E59K/K99E)), xcv) SEQ ID NO:XXX (IL-12p40 (E32K/D34N/E59K/K99E)), xcvi) SEQ ID NO: XXX (IL-12p40 (E59Y/K99Y)), xcvii) SEQ ID NO:XXX (IL-12p40 (E59K/K99E/N103Q/C252S/K264E)), xcviii) SEQ ID NO:XXX (IL-12p40 (E59K/K99E/N113Q/C252S/K264E)), xcix) SEQ ID NO:XXX (IL-12p40 (E59K/K99E/N200Q/C252S/K264E)), c) SEQ ID NO:XXX (IL-12p40

(E59K/K99E/N281Q/C252S/K264E)), ci) SEQ ID NO:XXX (IL-12p40 (E59K/K99E/N103Q/N113Q/C252S/K264E)), cii) SEQ ID NO:XXX (IL-12p40 (E59K/K99E/N103Q/N200Q/C252S/K264E)), ciii) SEQ ID NO:XXX (IL-12p40 (E59K/K99E/N103Q/N281Q/C252S/K264E)), civ) SEQ ID NO:XXX (IL-12p40 (E59K/K99E/N113Q/N200Q/C252S/K264E)), cv) SEQ ID NO:XXX (IL-12p40 (E59K/K99E/N113Q/N281Q/C252S/K264E)), cvi) SEQ ID NO:XXX (IL-12p40 (E59K/K99E/N200Q/N281Q/C252S/K264E)), cvii) SEQ ID NO:XXX (IL-12p40 (E59K/K99E/N103Q/N113Q/N200Q/C252S/K264E)), cviii) SEQ ID NO:XXX (IL-12p40 (E59K/K99E/N103Q/N200Q/N281Q/C252S/K264E)), and cix) SEQ ID NO:XXX (IL-12p40 (E59K/K99E/N113Q/N200Q/N281Q/C252S/K264E)).

[00162] In some embodiments, the present invention provides a heterodimeric complex wherein said IL-12p35 subunit has one or more amino acid modifications as amino acid residues selected from the group consisting of Q20, N21, Q35, E38, S44, E45, E46, H49, K54, D55, T59, V60, E61, C63, L64, P65, E67, L68, N71, S73, C74, L75, N76, E79, N85, L89, F96, M97, L124, M125, Q130, Q135, N136, E143, Q146, N151, E153, K158, E162, E163, D165, I171, R181, I182, R183, V185, T186, D188, R189, V190, S192, Y193, N195, and A196.

[00163] In some embodiments, the present invention provides a heterodimeric complex wherein said IL-12p35 subunit has one or more amino acid substitutions selected from the group consisting of N21D, Q35D, E38Q, D55Q, D55K, N71D, N71Q, L75A, N76D, E79Q, N85D, N85Q, L89A, F96A, M97A, L124A, M125A, Q130E, Q135E, N136D, E143Q, Q146E, N151D, N151K, E153K, E153Q, K158E, E162Q, E163Q, D165N, I171A, N195D, and N195Q.

[00164] In some embodiments, the present invention provides a heterodimeric complex wherein said IL-12p35 subunit has amino acid substitutions selected from the group consisting of N71D/N85D/N195D, N151D/E153Q, N151D/D165N, Q130E/N151D, N151D/K158E, E79Q/N151D, D55Q/N151D, N136D/N151D, N21D/N151D, E143Q/N151D, N71Q/N85Q, N71Q/N195Q, N85Q/N195Q, N71Q/N85Q/N195Q, N71D/N85D, N71D/N195D, and N85D/N195D.

[00165] In some embodiments, the present invention provides a heterodimeric complex wherein said IL-12p35 subunit has a polypeptide sequence selected from the group consisting of: i) SEQ ID NO:113 (IL-12p35(N71D)), ii) SEQ ID NO:114 (IL-12p35(N85D)), iii) SEQ ID NO:115 (IL-12p35(N195D)), iv) SEQ ID NO:116 (IL-12p35(N71D/N85D/N195D)), v) SEQ ID NO:117 (IL-12p35(E153Q)), vi) SEQ ID NO:118 (IL-12p35(E38Q)), vii) SEQ ID NO:119 (IL-12p35(N151D)), viii) SEQ ID NO:120 (IL-12p35(Q135E)), ix) SEQ ID NO:121 (IL-12p35(Q35D)), x) SEQ ID NO:122 (IL-12p35(Q146E)), xi) SEQ ID NO:123 (IL-12p35(N76D)), xii) SEQ ID NO:124 (IL-12p35(E162Q)), xiii) SEQ ID NO:125 (IL-12p35(E163Q)), xiv) IL-12p35(N21D), xv) SEQ ID NO:XXX (IL-12p35(D55Q)), xvi) IL-12p35(E79Q), xvii) IL-12p35(Q130E), xviii) IL-12p35(N136D), xix) IL-12p35(E143Q), xx) SEQ ID NO:227 (IL-12p35(N151K)), xxi) SEQ ID NO:226 (IL-12p35(E153K)), xxii) IL-12p35(K158E), xxiii) IL-12p35(D165N), xxiv) SEQ ID NO:225 (IL-12p35(N151D/E153Q)), xxv) SEQ ID NO:228 (IL-12p35(N151D/D165N)), xxvi) SEQ ID NO:229 (IL-12p35(Q130E/N151D)), xxvii) SEQ ID NO:230 (IL-12p35(N151D/K158E)), xxviii) SEQ ID NO:231 (IL-12p35(E79Q/N151D)), xxix) SEQ ID NO:232 (IL-12p35(D55Q/N151D)), xxx) SEQ ID NO:233 (IL-12p35(N136D/N151D)), xxxi) SEQ ID NO:234 (IL-12p35(N21D/N151D)), xxxii) SEQ ID NO:235 (IL-12p35(E143Q/N151D)), xxxiii) SEQ ID NO: XXX (IL-12p35(F96A)), xxxiv) SEQ ID NO: XXX (IL-12p35(M97A)), xxxv) SEQ ID NO: XXX (IL-12p35(L89A)), xxxvi) SEQ ID NO: XXX (IL-12p35(L124A)), xxxvii) SEQ ID NO: XXX (IL-12p35(M125A)), xxxviii) SEQ ID NO: XXX (IL-12p35(L75A)), xxxiv) SEQ ID NO: XXX (IL-12p35(I171A)), xxxv) SEQ ID NO: XXX (IL-12p35 (N71Q)), xxxvi) SEQ ID NO: XXX (IL-12p35 (N85Q)), xxxvii) SEQ ID NO: XXX (IL-12p35 (N195Q)), xxxviii) SEQ ID NO: XXX (IL-12p35 (N71Q/N85Q)), xxxix) SEQ ID NO: XXX (IL-12p35 (N71Q/N195Q)), xl) SEQ ID NO: XXX (IL-12p35 (N85Q/N195Q), xli) SEQ ID NO: XXX (IL-12p35 (N71Q/N85Q/N195Q)), xlii) SEQ ID NO: XXX (IL-12p35 (N71D/N85D)), xliii) SEQ ID NO: XXX (IL-12p35 (N71D/N195D), xliv) SEQ ID NO: XXX (IL-12p35 (N85D/N195D)), xlv) SEQ ID NO: XXX (IL-12p35 (D55Q)), and xlvi) SEQ ID NO: XXX (IL-12p35 (D55K)).

[00166] In some embodiments, the present invention provides a composition comprising a heterodimeric complex for use in treating cancer in a subject.

[00167] In some embodiments, the present invention provides one or more nucleic acids encoding a heterodimeric complex.

[00168] In some embodiments, the present invention provides a host cell comprising said one or more nucleic acids encoding a heterodimeric complex.

[00169] In some embodiments, the present invention provides a method of making a heterodimeric complex, said method comprising culturing a host cell under conditions whereby said heterodimeric complex is produced.

[00170] In some embodiments, the present invention provides a method of purifying a heterodimeric complex, said method comprising: a) providing a composition comprising the heterodimeric complex; b) loading said composition onto an ion exchange column; and c) collecting a fraction containing said heterodimeric complex.

[00171] In another aspect, the present invention provides a variant IL-12p40 subunit having altered affinity for IL-12 receptor subunit beta-1 (IL-12Rß1), IL-12 receptor subunit beta-2 (IL-12Rß2), and/or IL-12 receptor complex.

[00172] In some embodiments, the present invention provides a variant IL-12p40 subunit wherein said variant IL-12p40 subunit has one or more amino acid modifications at amino acid residues selected from the group consisting of E3, D7, E12, D14, W15, P17, D18, A19, P20, G21, E22, M23, D29, E32, E33, D34, L40, D41, Q42, S43, E45, L47, T54, I55, Q56, K58, E59, F60, G61, D62, Q65, Y66, E73, K84, E86, D87, G88, I89, W90, D93, D97, K99, E100, K102, N103, K104, F106, E110, N113, Y114, D129, D142, Q144, E156, R159, D161, N162, K163, D166, D170, Q172, D174, A176, C177, P178, A179, A180, E181, S183, P185, E187, N200, S204, F206, R208, D209, D214, N218, Q220, N226, Q229, E231, E235, T242, P243, S245, Y246, F247, S248, Q256, C252, K258, K260, E262, K264, D265, D270, N281, Q289, D290, R291, Y292, Y293, and E299.

In some embodiments, the present invention provides a variant IL-12p40 subunit wherein said variant IL-12p40 subunit has one or more amino acid substitutions selected from the group consisting of D18N, D18K, E32Q, E33Q, D34N, D34K, Q42E, S43E, S43K, E45Q, Q56E, E59Q, E59K,

D62N, E73Q, D87N, K99E, K99Y, E100Q, N103D, N103Q, N113D, N113Q, Q144E, D161N, R159E, K163E, E187Q, N200D, N200Q, N218Q, Q229E, E235Q, C252S, Q256N, K258E, K260E, E262Q, K264E, N281D, N281Q, and E299Q.

[00173] In some embodiments, the present invention provides a variant IL-12p40 subunit wherein said variant IL-12p40 subunit has amino acid substitutions selected from the group consisting of N103D/N113D/N200D/N281D, Q42E/E45Q, E45Q/Q56E, Q42E/E59Q, Q56E/E59Q, Q42E/E45Q/Q56E, E45Q/Q56E/E59Q, E32Q/E59Q, D34N/E59K, D34N/E59K/K99E, D34K/E59K/K99E, E32Q/D34N/E59K/K99E, E32K/D34N/E59K/K99E, D34N/E59Q, E59Q/E187Q, S43E/E59Q, S43K/E49Q, E59Q/K163E, E59Q/K99E, E59Q/K258E, E59Q/K260E, E59K/K99E, D18K/E59K/K99E, E59K/K99E/K264E, E59K/K99Y, E59Y/K99Y, E59Y/K99E, E45K/E59K/K99E, E59K/K99E/Q144E, E59K/K99E/Q144K, E59K/K99E/R159E, E59K/K99E/K264E, D18K/E59K/K99E/K264E, DI8K/E59K/K99E/C252S, D18K/E59K/K99E/C252S/K264E, E59K/K99Y/C252S, E59K/K99E/C252S/K264E, E59K/K99E/C252S, N103D/N113D, N103D/N200D, N103D/N281D, N113D/N200D, N113D/N281D, N200D/N281D, N103D/N113D/N200D, N103D/N113D/N281D, N103D/N200D/N281D, N113D/N200D/N281D, N103Q/N113Q, N103Q/N200Q, N103Q/N281Q, N113Q/N200Q, N113Q/N281Q, N200Q/N281Q, N103Q/N113Q/N200Q, N103Q/N113Q/N281Q, N103Q/N200Q/N281Q, N113Q/N200Q/N281Q, N103Q/N113Q/N200Q/N281Q, E59K/K99E/N103Q/C252S/K264E, E59K/K99E/N113Q/C252S/K264E, E59K/K99E/N200Q/C252S/K264E, E59K/K99E/N281Q/C252S/K264E, E59K/K99E/N103Q/N113Q/C252S/K264E, E59K/K99E/N103Q/N200Q/C252S/K264E, E59K/K99E/N103Q/N281Q/C252S/K264E, E59K/K99E/N113Q/N200Q/C252S/K264E, E59K/K99E/N113Q/N281Q/C252S/K264E, E59K/K99E/N200Q/N281Q/C252S/K264E, E59K/K99E/N103Q/N113Q/N200Q/C252S/K264E, E59K/K99E/N103Q/N200Q/N281Q/C252S/K264E, E59K/K99E/N113Q/N200Q/N281Q/C252S/K264E, and E59K/K99E/N103Q/N113Q/N200Q/N281Q/C252S/K264E.

[00174] In some embodiments, the present invention provides a variant IL-12p40 subunit wherein said variant IL-12p40 subunit has a polypeptide sequence selected from the group

consisting of: i) SEQ ID NO:57 (IL-12p40(N103D)), ii) SEQ ID NO:58 (IL-12p40(N113D)), iii) SEQ ID NO:59 (IL-12p40(N200D)), iv) SEQ ID NO:60 (IL-12p40(N281D)), v) SEQ ID NO:61 (IL-12p40(N103D/N113D/N200D/N281D)), vi) SEQ ID NO:62 (IL-12p40(Q42E)), vii) SEQ ID NO:63 (IL-12p40(E45Q)), viii) SEQ ID NO:64 (IL-12p40(Q56E)), ix) SEQ ID NO:65 (IL-12p40(E59Q)), x) SEQ ID NO:66 (IL-12p40(D62N)), xi) SEQ ID NO:67 (IL-12p40(Q42E/E45Q)), xii) SEQ ID NO:68 (IL-12p40(E45Q/Q56E)), xiii) SEQ ID NO:69 (IL-12p40(Q42E/E59Q)), xiv) SEQ ID NO:70 (IL-12p40(Q56E/E59Q)), xv) SEQ ID NO:71 (IL-12p40(Q42E/E45Q/Q56E)), xvi) SEQ ID NO:72 (IL-12p40(E45Q/Q56E/E59Q)), xvii) SEQ ID NO:73 (IL-12p40(D161N)), xviii) SEQ ID NO:74 (IL-12p40(E73Q)), xix) SEQ ID NO:75 (IL-12p40(Q144E)), xx) SEQ ID NO:76 (IL-12p40(E262Q)), xxi) SEQ ID NO:77 (IL-12p40(E100Q)), xxii) SEQ ID NO:78 (IL-12p40(D18N)), xxiii) SEQ ID NO:79 (IL-12p40(E33Q)), xxiv) SEQ ID NO:80 (IL-12p40(Q229E)), xxv) SEQ ID NO:81 (IL-12p40(E235Q)), xxvi) SEQ ID NO:82 (IL-12p40(Q256N)), xxvii) SEQ ID NO:83 (IL-12p40(E299Q)), xxviii) SEQ ID NO:84 (IL-12p40(D87N)), xxix) IL-12p40(E32Q), xxx) IL-12p40(D34N), xxxi) IL-12p40(S43E), xxxii) IL-12p40(S43K), xxxiii) SEQ ID NO:XXX (IL-12p40 (E59K/K99E/N103Q/N113Q/N200Q/N281Q/C252S/K264E)), xxxiv) SEQ ID NO:205 (IL-12p40(E59K)), xxxv) IL-12p40(K99E), xxxvi) IL-12p40(K163E), xxxvii) IL-12p40(E187Q), xxxviii) IL-12p40(K258E), xxxix) IL-12p40(K260E), xl) SEQ ID NO:206 (IL-12p40(E32Q/E59Q)), xli) SEQ ID NO:207 (IL-12p40(D34N/E59Q)), xlii) SEQ ID NO:208 (IL-12p40(E59Q/E187Q)), xliii) SEQ ID NO:209 (IL-12p40(S43E/E59Q)), xliv) SEQ ID NO:210 (IL-12p40(S43K/E49Q)), xlv) SEQ ID NO:211 (IL-12p40(E59Q/K163E)), xlvi) SEQ ID NO:212 (IL-12p40(E59Q/K99E)), xlvii) SEQ ID NO:213 (IL-12p40(E59Q/K258E)), xlviii) SEQ ID NO:214 (IL-12p40(E59Q/K260E)), xlix) SEQ ID NO: XXX (IL-12p40 (D34N/E59K)), l) SEQ ID NO: XXX (IL-12p40(E59K/K99E)), li) SEQ ID NO: XXX (IL-12p40(D18K/E59K/K99E)), lii) SEQ ID NO: XXX (IL-12p40 (E59K/K99E/K264E)), liii) SEQ ID NO: XXX (IL-12p40 (E59K/K99Y)), liv) SEQ ID NO: XXX (IL-12p40 (E59Y/K99E)), lv) SEQ ID NO: XXX (IL-12p40 (E45K/E59K/K99E)), lvi) SEQ ID NO: XXX (IL-12p40 (E59K/K99E/Q144E)), lvii) SEQ ID NO: XXX (IL-12p40 (E59K/K99E/Q144K)), lviii) SEQ ID NO: XXX (IL-12p40 (E59K/K99E/R159E)), lix) SEQ ID NO: XXX (IL-12p40 (E59K/K99E/K264E)), lx) SEQ ID NO: XXX (IL-12p40 (D18K/E59K/K99E/K264E)), lxi) SEQ ID NO: XXX (IL-12p40 (C252S)), lxii) SEQ ID NO: XXX (IL-12p40 (DI8K/E59K/K99E/C252S)), lxiii) SEQ ID NO: XXX

(IL-12p40 (D18K/E59K/K99E/C252S/K264E)), lxiv) SEQ ID NO: XXX (IL-12p40 (E59K/K99Y/C252S)), lxv) SEQ ID NO: XXX (IL-12p40 (E59K/K99E/C252S/K264E)), lxvi) SEQ ID NO: XXX (IL-12p40 (E59K/K99E/C252S)), lxvii) SEQ ID NO: XXX (IL-12p40 (N103D/N113D)), lxviii) SEQ ID NO: XXX (IL-12p40 (N103D/N200D)), lxix) SEQ ID NO: XXX (IL-12p40 (N103D/N281D)), lxx) SEQ ID NO: XXX (IL-12p40 (N113D/N200D)), lxxi) SEQ ID NO: XXX (IL-12p40 (N113D/N281D)), lxxii) SEQ ID NO: XXX (IL-12p40 (N200D/N281D)), lxxiii) SEQ ID NO: XXX (IL-12p50 (N103D/N113D/N200D)), lxxiv) SEQ ID NO: XXX (IL-12p40 (N103D/N113D/N281D)), lxxv) SEQ ID NO: XXX (IL-12p40 (N103D/N200D/N281D)), lxxvi) SEQ ID NO: XXX (IL-12p40 (N113D/N200D/N281D)), lxxvii) SEQ ID NO: XXX (IL-12p40 (N103Q)), lxxviii) SEQ ID NO: XXX (IL-12p40 (N113Q)), lxxix) SEQ ID NO: XXX (IL-12p40 (N200Q)), lxxx) SEQ ID NO: XXX (IL-12p40 (N281Q)), lxxxi) SEQ ID NO: XXX (IL-12p40 (N103Q/N113Q)), lxxxii) SEQ ID NO: XXX (IL-12p40 (N103Q/N200Q)), lxxxiii) SEQ ID NO: XXX (IL-12p40 (N103Q/N281Q)), lxxxiv) SEQ ID NO: XXX (IL-12p40 (N113Q/N200Q)), lxxxv) SEQ ID NO: XXX (IL-12p40 (N113Q/N281Q)), lxxxvi) SEQ ID NO: XXX (IL-12p40 (N200Q/N281Q)), lxxxvii) SEQ ID NO: XXX (IL-12p40 (N103Q/N113Q/N200Q)), lxxxviii) SEQ ID NO: XXX (IL-12p40 (N103Q/N113Q/N281Q)), lxxxix) SEQ ID NO: XXX (IL-12p40 (N103Q/N200Q/N281Q)), xc) SEQ ID NO: XXX (IL-12p40 (N113Q/N200Q/N281Q)), xci) SEQ ID NO:XXX (IL-12p40 (N103Q/N113Q/N200Q/N281Q)), xcii) SEQ ID NO:XXX (IL-12p40 (D34N/E59K/K99E)), xciii) SEQ ID NO:XXX (IL-12p40 (D34K/E59K/K99E)), xciv) SEQ ID NO:XXX (IL-12p40 (E32Q/D34N/E59K/K99E)), xcv) SEQ ID NO:XXX (IL-12p40 (E32K/D34N/E59K/K99E)), xcvi) SEQ ID NO: XXX (IL-12p40 (E59Y/K99Y)), xcvii) SEQ ID NO:XXX (IL-12p40 (E59K/K99E/N103Q/C252S/K264E)), xcviii) SEQ ID NO:XXX (IL-12p40 (E59K/K99E/N113Q/C252S/K264E)), xcix) SEQ ID NO:XXX (IL-12p40 (E59K/K99E/N200Q/C252S/K264E)), c) SEQ ID NO:XXX (IL-12p40 (E59K/K99E/N281Q/C252S/K264E)), ci) SEQ ID NO:XXX (IL-12p40 (E59K/K99E/N103Q/N113Q/C252S/K264E)), cii) SEQ ID NO:XXX (IL-12p40 (E59K/K99E/N103Q/N200Q/C252S/K264E)), ciii) SEQ ID NO:XXX (IL-12p40 (E59K/K99E/N103Q/N281Q/C252S/K264E)), civ) SEQ ID NO:XXX (IL-12p40 (E59K/K99E/N113Q/N200Q/C252S/K264E)), cv) SEQ ID NO:XXX (IL-12p40

(E59K/K99E/N113Q/N281Q/C252S/K264E)), cvi) SEQ ID NO:XXX (IL-12p40 (E59K/K99E/N200Q/N281Q/C252S/K264E)), cvii) SEQ ID NO:XXX (IL-12p40 (E59K/K99E/N103Q/N113Q/N200Q/C252S/K264E)), cviii) SEQ ID NO:XXX (IL-12p40 (E59K/K99E/N103Q/N200Q/N281Q/C252S/K264E)), and cix) SEQ ID NO:XXX (IL-12p40 (E59K/K99E/N113Q/N200Q/N281Q/C252S/K264E)).

[00175] In some embodiments, the present invention provides a composition comprising a variant IL-12p40 subunit.

[00176] In some embodiments, the present invention provides a nucleic acid encoding a variant IL-12p40 subunit.

[00177] In some embodiments, the present invention provides a host cell comprising said nucleic acid encoding a variant IL-12p40 subunit.

[00178] In some embodiments, the present invention provides a method of making a variant IL-12p40 subunit, said method comprising culturing a host cell according to claim H8 under conditions whereby said variant IL-12p40 subunit is produced.

[00179] In another aspect, the present invention provides a variant IL-12p35 subunit having altered affinity for IL-12 receptor subunit beta-1 (IL-12Rß1), IL-12 receptor subunit beta-2 (IL-12Rß2), and/or IL-12 receptor complex.

[00180] In some embodiments, the present invention provides a variant IL-12p35 subunit, wherein said variant IL-12p35 subunit has one or more amino acid modifications as amino acid residues selected from the group consisting of Q20, N21, Q35, E38, S44, E45, E46, H49, K54, D55, T59, V60, E61, C63, L64, P65, E67, L68, N71, S73, C74, L75, N76, E79, N85, L89, F96, M97, L124, M125, Q130, Q135, N136, E143, Q146, N151, E153, K158, E162, E163, D165, I171, R181, I182, R183, V185, T186, D188, R189, V190, S192, Y193, N195, and A196.

[00181] In some embodiments, the present invention provides a variant IL-12p35 subunit, wherein said variant IL-12p35 subunit has one or more amino acid substitutions selected from the group

consisting of N21D, Q35D, E38Q, D55Q, D55K, N71D, N71Q, L75A, N76D, E79Q, N85D, N85Q, L89A, F96A, M97A, L124A, M125A, Q130E, Q135E, N136D, E143Q, Q146E, N151D, N151K, E153K, E153Q, K158E, E162Q, E163Q, D165N, I171A, N195D, and N195Q.

[00182] In some embodiments, the present invention provides a variant IL-12p35 subunit, wherein said variant IL-12p35 subunit amino acid substitutions selected from the group consisting of N71D/N85D/N195D, N151D/E153Q, N151D/D165N, Q130E/N151D, N151D/K158E, E79Q/N151D, D55Q/N151D, N136D/N151D, N21D/N151D, E143Q/N151D, N71Q/N85Q, N71Q/N195Q, N85Q/N195Q, N71Q/N85Q/N195Q, N71D/N85D, N71D/N195D, and N85D/N195D.

[00183] In some embodiments, the present invention provides a variant IL-12p35 subunit, wherein said variant IL-12p35 subunit has amino acid substitutions N71D/N85D/N195D.

[00184] In some embodiments, the present invention provides a variant IL-12p35 subunit wherein said variant IL-12p35 subunit has a polypeptide sequence selected from the group consisting of: i) SEQ ID NO:113 (IL-12p35(N71D)), ii) SEQ ID NO:114 (IL-12p35(N85D)), iii) SEQ ID NO:115 (IL-12p35(N195D)), iv) SEQ ID NO:116 (IL-12p35(N71D/N85D/N195D)), v) SEQ ID NO:117 (IL-12p35(E153Q)), vi) SEQ ID NO:118 (IL-12p35(E38Q)), vii) SEQ ID NO:119 (IL-12p35(N151D)), viii) SEQ ID NO:120 (IL-12p35(Q135E)), ix) SEQ ID NO:121 (IL-12p35(Q35D)), x) SEQ ID NO:122 (IL-12p35(Q146E)), xi) SEQ ID NO:123 (IL-12p35(N76D)), xii) SEQ ID NO:124 (IL-12p35(E162Q)), xiii) SEQ ID NO:125 (IL-12p35(E163Q)), xiv) IL-12p35(N21D), xv) SEQ ID NO:XXX (IL-12p35(D55Q)), xvi) IL-12p35(E79Q), xvii) IL-12p35(Q130E), xviii) IL-12p35(N136D), xix) IL-12p35(E143Q), xx) SEQ ID NO:227 (IL-12p35(N151K)), xxi) SEQ ID NO:226 (IL-12p35(E153K)), xxii) IL-12p35(K158E), xxiii) IL-12p35(D165N), xxiv) SEQ ID NO:225 (IL-12p35(N151D/E153Q)), xxv) SEQ ID NO:228 (IL-12p35(N151D/D165N)), xxvi) SEQ ID NO:229 (IL-12p35(Q130E/N151D)), xxvii) SEQ ID NO:230 (IL-12p35(N151D/K158E)), xxviii) SEQ ID NO:231 (IL-12p35(E79Q/N151D)), xxix) SEQ ID NO:232 (IL-12p35(D55Q/N151D)), xxx) SEQ ID NO:233 (IL-12p35(N136D/N151D)), xxxi) SEQ ID NO:234 (IL-12p35(N21D/N151D)), xxxii) SEQ ID NO:235 (IL-12p35(E143Q/N151D)), xxxiii) SEQ ID NO: XXX (IL-12p35(F96A)), xxxiv) SEQ ID NO: XXX (IL-12p35(M97A)), xxxv) SEQ

ID NO: XXX (IL-12p35(L89A)), xxxvi) SEQ ID NO: XXX (IL-12p35(L124A)), xxxvii) SEQ ID NO: XXX (IL-12p35(M125A)), xxxviii) SEQ ID NO: XXX (IL-12p35(L75A)), xxxiv) SEQ ID NO: XXX (IL-12p35(I171A)), xxxv) SEQ ID NO: XXX (IL-12p35 (N71Q)), xxxvi) SEQ ID NO: XXX (IL-12p35 (N85Q)), xxxvii) SEQ ID NO: XXX (IL-12p35 (N195Q)), xxxviii) SEQ ID NO: XXX (IL-12p35 (N71Q/N85Q)), xxxix) SEQ ID NO: XXX (IL-12p35 (N71Q/N195Q)), xl) SEQ ID NO: XXX (IL-12p35 (N85Q/N195Q), xli) SEQ ID NO: XXX (IL-12p35 (N71Q/N85Q/N195Q)), xlii) SEQ ID NO: XXX (IL-12p35 (N71D/N85D)), xliii) SEQ ID NO: XXX (IL-12p35 (N71D/N195D), xliv) SEQ ID NO: XXX (IL-12p35 (N85D/N195D)), xlv) SEQ ID NO: XXX (IL-12p35 (D55Q)), and xlvi) SEQ ID NO: XXX (IL-12p35 (D55K)).

[00185] In some embodiments, the present invention provides a composition comprising a variant IL-12p35 subunit.

[00186] In some embodiments, the present invention provides a nucleic acid encoding a variant IL-12p35 subunit.

[00187] In some embodiments, the present invention provides a host cell comprising said nucleic acid encoding a variant IL-12p35 subunit.

[00188] In some embodiments, the present invention provides a variant IL-12p35 subunit, said method comprising culturing a host cell under conditions whereby said variant IL-12p35 subunit is produced.

[00189] In another aspect, the present invention provides a heterodimeric protein comprising: a) a first fusion protein comprising an IL-12p40 subunit domain covalently attached to a first Fc domain; and b) a second fusion protein comprising an IL-12p35 subunit domain covalent attached to a second Fc domain; wherein said first and second Fc domains a set of amino acid substitutions selected from the group consisting of L368D/K370S and S364K; L368D/K370S and S364K/E357L; L368D/K370S and S364K/E357Q; T411E/K360E/Q362E and D401K; L368E/K370S and S364K; K370S and S364K/E357Q; T366S/L368A/Y407V and T366W; and T366S/L368A/Y407V/Y349C and T366W/S354C; according to EU numbering.

[00190] In some embodiments, the present invention provides a heterodimeric protein, wherein said IL-12p40 subunit domain is attached N-terminal to said first Fc domain and said IL-12p35 subunit domain is attached N-terminal to said second Fc domain.

[00191] In some embodiments, the present invention provides a heterodimeric protein, wherein said IL-12p40 subunit domain is attached C-terminal to said first Fc domain and said IL-12p35 subunit domain is attached C-terminal to said second Fc domain.

[00192] In some embodiments, the present invention provides a heterodimeric protein, wherein said Il-12p40 subunit domain is attached to said first Fc domain using a domain linker and said IL-12p35 subunit domain is attached to said second Fc domain using a domain linker.

[00193] In some embodiments, the present invention provides a heterodimeric protein wherein said first and second Fc domain comprises the IgG1 hinge-CH2-CH3.

[00194] In some embodiments, the present invention provides a heterodimeric protein, wherein said first fusion protein comprises, from N- to C-terminal, an IL-12p40 subunit domain-domain linker-hinge-CH2-CH3 and said second fusion protein comprises, from N- to C-terminal, an IL-12p35 subunit domain-domain linker-hinge-CH2-CH3.

[00195] In some embodiments, the present invention provides a heterodimeric protein, wherein said first fusion protein comprises, from N- to C-terminal, hinge-CH2-CH3-domain linker-IL-12p40 subunit domain and said second fusion protein comprises, from N- to C-terminal, hinge-CH2-CH3-domain linker-IL-12p35 subunit domain.

[00196] In some embodiments, the present invention provides a heterodimeric protein wherein said IL-12p40 subunit has a sequence selected from the group consisting of: i) SEQ ID NO:57 (IL-12p40(N103D)), ii) SEQ ID NO:58 (IL-12p40(N113D)), iii) SEQ ID NO:59 (IL-12p40(N200D)), iv) SEQ ID NO:60 (IL-12p40(N281D)), v) SEQ ID NO:61 (IL-12p40(N103D/N113D/N200D/N281D)), vi) SEQ ID NO:62 (IL-12p40(Q42E)), vii) SEQ ID NO:63 (IL-12p40(E45Q)), viii) SEQ ID NO:64 (IL-12p40(Q56E)), ix) SEQ ID NO:65 (IL-12p40(E59Q)), x) SEQ ID NO:66 (IL-12p40(D62N)), xi) SEQ ID NO:67 (IL-12p40(Q42E/E45Q)), xii) SEQ ID

NO:68 (IL-12p40(E45Q/Q56E)), xiii) SEQ ID NO:69 (IL-12p40(Q42E/E59Q)), xiv) SEQ ID NO:70 (IL-12p40(Q56E/E59Q)), xv) SEQ ID NO:71 (IL-12p40(Q42E/E45Q/Q56E)), xvi) SEQ ID NO:72 (IL-12p40(E45Q/Q56E/E59Q)), xvii) SEQ ID NO:73 (IL-12p40(D161N)), xviii) SEQ ID NO:74 (IL-12p40(E73Q)), xix) SEQ ID NO:75 (IL-12p40(Q144E)), xx) SEQ ID NO:76 (IL-12p40(E262Q)), xxi) SEQ ID NO:77 (IL-12p40(E100Q)), xxii) SEQ ID NO:78 (IL-12p40(D18N)), xxiii) SEQ ID NO:79 (IL-12p40(E33Q)), xxiv) SEQ ID NO:80 (IL-12p40(Q229E)), xxv) SEQ ID NO:81 (IL-12p40(E235Q)), xxvi) SEQ ID NO:82 (IL-12p40(Q256N)), xxvii) SEQ ID NO:83 (IL-12p40(E299Q)), xxviii) SEQ ID NO:84 (IL-12p40(D87N)), xxix) IL-12p40(E32Q), xxx) IL-12p40(D34N), xxxi) IL-12p40(S43E), xxxii) IL-12p40(S43K), xxxiii) SEQ ID NO:XXX (IL-12p40 (E59K/K99E/N103Q/N113Q/N200Q/N281Q/C252S/K264E)), xxxiv) SEQ ID NO:205 (IL-12p40(E59K)), xxxv) IL-12p40(K99E), xxxvi) IL-12p40(K163E), xxxvii) IL-12p40(E187Q), xxxviii) IL-12p40(K258E), xxxix) IL-12p40(K260E), xl) SEQ ID NO:206 (IL-12p40(E32Q/E59Q)), xli) SEQ ID NO:207 (IL-12p40(D34N/E59Q)), xlii) SEQ ID NO:208 (IL-12p40(E59Q/E187Q)), xliii) SEQ ID NO:209 (IL-12p40(S43E/E59Q)), xliv) SEQ ID NO:210 (IL-12p40(S43K/E49Q)), xlv) SEQ ID NO:211 (IL-12p40(E59Q/K163E)), xlvi) SEQ ID NO:212 (IL-12p40(E59Q/K99E)), xlvii) SEQ ID NO:213 (IL-12p40(E59Q/K258E)), xlviii) SEQ ID NO:214 (IL-12p40(E59Q/K260E)), xlix) SEQ ID NO: XXX (IL-12p40 (D34N/E59K)), l) SEQ ID NO: XXX (IL-12p40(E59K/K99E)), li) SEQ ID NO: XXX (IL-12p40(D18K/E59K/K99E)), lii) SEQ ID NO: XXX (IL-12p40 (E59K/K99E/K264E)), liii) SEQ ID NO: XXX (IL-12p40 (E59K/K99Y)), liv) SEQ ID NO: XXX (IL-12p40 (E59Y/K99E)), lv) SEQ ID NO: XXX (IL-12p40 (E45K/E59K/K99E)), lvi) SEQ ID NO: XXX (IL-12p40 (E59K/K99E/Q144E)), lvii) SEQ ID NO: XXX (IL-12p40 (E59K/K99E/Q144K)), lviii) SEQ ID NO: XXX (IL-12p40 (E59K/K99E/R159E)), lix) SEQ ID NO: XXX (IL-12p40 (E59K/K99E/K264E)), lx) SEQ ID NO: XXX (IL-12p40 (D18K/E59K/K99E/K264E)), lxi) SEQ ID NO: XXX (IL-12p40 (C252S)), lxii) SEQ ID NO: XXX (IL-12p40 (DI8K/E59K/K99E/C252S)), lxiii) SEQ ID NO: XXX (IL-12p40 (D18K/E59K/K99E/C252S/K264E)), lxiv) SEQ ID NO: XXX (IL-12p40 (E59K/K99Y/C252S)), lxv) SEQ ID NO: XXX (IL-12p40 (E59K/K99E/C252S/K264E)), lxvi) SEQ ID NO: XXX (IL-12p40 (E59K/K99E/C252S)), lxvii) SEQ ID NO: XXX (IL-12p40 (N103D/N113D)), lxviii) SEQ ID NO: XXX (IL-12p40 (N103D/N200D)), lxix) SEQ ID NO: XXX (IL-12p40 (N103D/N281D)), lxx) SEQ ID NO: XXX (IL-12p40 (N113D/N200D)), lxxi) SEQ ID NO: XXX (IL-

12p40 (N113D/N281D)), lxxii) SEQ ID NO: XXX (IL-12p40 (N200D/N281D)), lxxiii) SEQ ID NO: XXX (IL-12p50 (N103D/N113D/N200D)), lxxiv) SEQ ID NO: XXX (IL-12p40 (N103D/N113D/N281D)), lxxv) SEQ ID NO: XXX (IL-12p40 (N103D/N200D/N281D)), lxxvi) SEQ ID NO: XXX (IL-12p40 (N113D/N200D/N281D)), lxxvii) SEQ ID NO: XXX (IL-12p40 (N103Q)), lxxviii) SEQ ID NO: XXX (IL-12p40 (N113Q)), lxxix) SEQ ID NO: XXX (IL-12p40 (N200Q)), lxxx) SEQ ID NO: XXX (IL-12p40 (N281Q)), lxxxi) SEQ ID NO: XXX (IL-12p40 (N103Q/N113Q)), lxxxii) SEQ ID NO: XXX (IL-12p40 (N103Q/N200Q)), lxxxiii) SEQ ID NO: XXX (IL-12p40 (N103Q/N281Q)), lxxxiv) SEQ ID NO: XXX (IL-12p40 (N113Q/N200Q)), lxxxv) SEQ ID NO: XXX (IL-12p40 (N113Q/N281Q)), lxxxvi) SEQ ID NO: XXX (IL-12p40 (N200Q/N281Q)), lxxxvii) SEQ ID NO: XXX (IL-12p40 (N103Q/N113Q/N200Q)), lxxxviii) SEQ ID NO: XXX (IL-12p40 (N103Q/N113Q/N281Q)), lxxxix) SEQ ID NO: XXX (IL-12p40 (N103Q/N200Q/N281Q)), xc) SEQ ID NO: XXX (IL-12p40 (N113Q/N200Q/N281Q)), xci) SEQ ID NO:XXX (IL-12p40 (N103Q/N113Q/N200Q/N281Q)), xcii) SEQ ID NO:XXX (IL-12p40 (D34N/E59K/K99E)), xciii) SEQ ID NO:XXX (IL-12p40 (D34K/E59K/K99E)), xciv) SEQ ID NO:XXX (IL-12p40 (E32Q/D34N/E59K/K99E)), xcv) SEQ ID NO:XXX (IL-12p40 (E32K/D34N/E59K/K99E)), xcvi) SEQ ID NO: XXX (IL-12p40 (E59Y/K99Y)), xcvii) SEQ ID NO:XXX (IL-12p40 (E59K/K99E/N103Q/C252S/K264E)), xcviii) SEQ ID NO:XXX (IL-12p40 (E59K/K99E/N113Q/C252S/K264E)), xcix) SEQ ID NO:XXX (IL-12p40 (E59K/K99E/N200Q/C252S/K264E)), c) SEQ ID NO:XXX (IL-12p40 (E59K/K99E/N281Q/C252S/K264E)), ci) SEQ ID NO:XXX (IL-12p40 (E59K/K99E/N103Q/N113Q/C252S/K264E)), cii) SEQ ID NO:XXX (IL-12p40 (E59K/K99E/N103Q/N200Q/C252S/K264E)), ciii) SEQ ID NO:XXX (IL-12p40 (E59K/K99E/N103Q/N281Q/C252S/K264E)), civ) SEQ ID NO:XXX (IL-12p40 (E59K/K99E/N113Q/N200Q/C252S/K264E)), cv) SEQ ID NO:XXX (IL-12p40 (E59K/K99E/N113Q/N281Q/C252S/K264E)), cvi) SEQ ID NO:XXX (IL-12p40 (E59K/K99E/N200Q/N281Q/C252S/K264E)), cvii) SEQ ID NO:XXX (IL-12p40 (E59K/K99E/N103Q/N113Q/N200Q/C252S/K264E)), cviii) SEQ ID NO:XXX (IL-12p40 (E59K/K99E/N103Q/N200Q/N281Q/C252S/K264E)), and cix) SEQ ID NO:XXX (IL-12p40 (E59K/K99E/N113Q/N200Q/N281Q/C252S/K264E)).

[00197] In some embodiments, the present invention provides a heterodimeric protein wherein said IL-12p35 subunit has a sequence selected from the group consisting of: i) SEQ ID NO:113 (IL-12p35(N71D)), ii) SEQ ID NO:114 (IL-12p35(N85D)), iii) SEQ ID NO:115 (IL-12p35(N195D)), iv) SEQ ID NO:116 (IL-12p35(N71D/N85D/N195D)), v) SEQ ID NO:117 (IL-12p35(E153Q)), vi) SEQ ID NO:118 (IL-12p35(E38Q)), vii) SEQ ID NO:119 (IL-12p35(N151D)), viii) SEQ ID NO:120 (IL-12p35(Q135E)), ix) SEQ ID NO:121 (IL-12p35(Q35D)), x) SEQ ID NO:122 (IL-12p35(Q146E)), xi) SEQ ID NO:123 (IL-12p35(N76D)), xii) SEQ ID NO:124 (IL-12p35(E162Q)), xiii) SEQ ID NO:125 (IL-12p35(E163Q)), xiv) IL-12p35(N21D), xv) SEQ ID NO:XXX (IL-12p35(D55Q)), xvi) IL-12p35(E79Q), xvii) IL-12p35(Q130E), xviii) IL-12p35(N136D), xix) IL-12p35(E143Q), xx) SEQ ID NO:227 (IL-12p35(N151K)), xxi) SEQ ID NO:226 (IL-12p35(E153K)), xxii) (IL-12p35(K158E)), xxiii) IL-12p35(D165N), xxiv) SEQ ID NO:225 (IL-12p35(N151D/E153Q)), xxv) SEQ ID NO:228 (IL-12p35(N151D/D165N)), xxvi) SEQ ID NO:229 (IL-12p35(Q130E/N151D)), xxvii) SEQ ID NO:230 (IL-12p35(N151D/K158E)), xxviii) SEQ ID NO:231 (IL-12p35(E79Q/N151D)), xxix) SEQ ID NO:232 (IL-12p35(D55Q/N151D)), xxx) SEQ ID NO:233 (IL-12p35(N136D/N151D)), xxxi) SEQ ID NO:234 (IL-12p35(N21D/N151D)), xxxii) SEQ ID NO:235 (IL-12p35(E143Q/N151D)),xxxiii) SEQ ID NO: XXX (IL-12p35(F96A)),xxxiv) SEQ ID NO: XXX (IL-12p35(M97A)), xxxv) SEQ ID NO: XXX (IL-12p35(L89A)),xxxvi) SEQ ID NO: XXX (IL-12p35(L124A)),xxxvii) SEQ ID NO: XXX (IL-12p35(M125A)),xxxviii) SEQ ID NO: XXX (IL-12p35(L75A)), xxxiv) SEQ ID NO: XXX (IL-12p35(I171A)),xxxv) SEQ ID NO: XXX (IL-12p35 (N71Q)), xxxvi) SEQ ID NO: XXX (IL-12p35 (N85Q)), xxxvii) SEQ ID NO: XXX (IL-12p35 (N195Q)), xxxviii) SEQ ID NO: XXX (IL-12p35 (N71Q/N85Q)),xxxix) SEQ ID NO: XXX (IL-12p35 (N71Q/N195Q)),lx) SEQ ID NO: XXX (IL-12p35 (N85Q/N195Q),lxi) SEQ ID NO: XXX (IL-12p35 (N71Q/N85Q/N195Q)),lxii) SEQ ID NO: XXX (IL-12p35 (N71D/N85D)), lxiii) SEQ ID NO: XXX (IL-12p35 (N71D/N195D),lxiv) SEQ ID NO: XXX (IL-12p35 (N85D/N195D)), lxv) SEQ ID NO: XXX (IL-12p35 (D55Q)), and lxvi) SEQ ID NO: XXX (IL-12p35 (D55K)).

[00198] In another aspect, the present invention comprises a heterodimeric protein comprising: a) a first Fc domain; and b) a fusion protein comprising: i) a second Fc domain; ii) an IL-12p40

subunit domain; and iii) an IL-12p35 subunit domain; wherein said first and second Fc domains a set of amino acid substitutions selected from the group consisting of L368D/K370S and S364K; L368D/K370S and S364K/E357L; L368D/K370S and S364K/E357Q; T411E/K360E/Q362E and D401K; L368E/K370S and S364K; K370S and S364K/E357Q; T366S/L368A/Y407V and T366W; and T366S/L368A/Y407V/Y349C and T366W/S354C; according to EU numbering.

[00199] In some embodiments, the present invention provides a heterodimeric protein wherein said fusion protein comprises, from N- to C-terminal: IL-12p40 subunit domain-domain linker-IL-12p35 subunit domain-domain linker-hinge-CH2-CH3.

[00200] In some embodiments, the present invention provides a heterodimeric protein wherein said fusion protein comprises, from N- to C-terminal: IL-12p35 subunit domain-domain linker-IL-12p40 subunit domain-domain linker-hinge-CH2-CH3.

[00201] In some embodiments, the present invention provides a heterodimeric protein wherein said fusion protein comprises, from N- to C-terminal: hinge-CH2-CH3-domain linker-IL-12p35 subunit domain-domain linker-IL-12p40 subunit domain.

[00202] In some embodiments, the present invention provides a heterodimeric protein wherein said fusion protein comprises, from N- to C-terminal: hinge-CH2-CH3-domain linker-IL-12p40 subunit domain-domain linker-IL-12p35 subunit domain.

[00203] In some embodiments, the present invention provides a heterodimeric protein wherein said IL-12p40 subunit has a sequence selected from the group consisting of: i) SEQ ID NO:57 (IL-12p40(N103D)), ii) SEQ ID NO:58 (IL-12p40(N113D)), iii) SEQ ID NO:59 (IL-12p40(N200D)), iv) SEQ ID NO:60 (IL-12p40(N281D)), v) SEQ ID NO:61 (IL-12p40(N103D/N113D/N200D/N281D)), vi) SEQ ID NO:62 (IL-12p40(Q42E)), vii) SEQ ID NO:63 (IL-12p40(E45Q)), viii) SEQ ID NO:64 (IL-12p40(Q56E)), ix) SEQ ID NO:65 (IL-12p40(E59Q)), x) SEQ ID NO:66 (IL-12p40(D62N)), xi) SEQ ID NO:67 (IL-12p40(Q42E/E45Q)), xii) SEQ ID NO:68 (IL-12p40(E45Q/Q56E)), xiii) SEQ ID NO:69 (IL-12p40(Q42E/E59Q)), xiv) SEQ ID NO:70 (IL-12p40(Q56E/E59Q)), xv) SEQ ID NO:71 (IL-12p40(Q42E/E45Q/Q56E)), xvi) SEQ ID NO:72

(IL-12p40(E45Q/Q56E/E59Q)), xvii) SEQ ID NO:73 (IL-12p40(D161N)), xviii) SEQ ID NO:74 (IL-12p40(E73Q)), xix) SEQ ID NO:75 (IL-12p40(Q144E)), xx) SEQ ID NO:76 (IL-12p40(E262Q)), xxi) SEQ ID NO:77 (IL-12p40(E100Q)), xxii) SEQ ID NO:78 (IL-12p40(D18N)), xxiii) SEQ ID NO:79 (IL-12p40(E33Q)), xxiv) SEQ ID NO:80 (IL-12p40(Q229E)), xxv) SEQ ID NO:81 (IL-12p40(E235Q)), xxvi) SEQ ID NO:82 (IL-12p40(Q256N)), xxvii) SEQ ID NO:83 (IL-12p40(E299Q)), xxviii) SEQ ID NO:84 (IL-12p40(D87N)), xxix) IL-12p40(E32Q), xxx) IL-12p40(D34N), xxxi) IL-12p40(S43E), xxxii) IL-12p40(S43K), xxxiii) SEQ ID NO:XXX (IL-12p40 (E59K/K99E/N103Q/N113Q/N200Q/N281Q/C252S/K264E)), xxxiv) SEQ ID NO:205 (IL-12p40(E59K)), xxxv) IL-12p40(K99E), xxxvi) IL-12p40(K163E), xxxvii) IL-12p40(E187Q), xxxviii) IL-12p40(K258E), xxxix) IL-12p40(K260E), xl) SEQ ID NO:206 (IL-12p40(E32Q/E59Q)), xli) SEQ ID NO:207 (IL-12p40(D34N/E59Q)), xlii) SEQ ID NO:208 (IL-12p40(E59Q/E187Q)), xliii) SEQ ID NO:209 (IL-12p40(S43E/E59Q)), xliv) SEQ ID NO:210 (IL-12p40(S43K/E49Q)), xlv) SEQ ID NO:211 (IL-12p40(E59Q/K163E)), xlvi) SEQ ID NO:212 (IL-12p40(E59Q/K99E)), xlvii) SEQ ID NO:213 (IL-12p40(E59Q/K258E)), xlviii) SEQ ID NO:214 (IL-12p40(E59Q/K260E)), xlix) SEQ ID NO: XXX (IL-12p40 (D34N/E59K)), l) SEQ ID NO: XXX (IL-12p40(E59K/K99E)), li) SEQ ID NO: XXX (IL-12p40(D18K/E59K/K99E)), lii) SEQ ID NO: XXX (IL-12p40 (E59K/K99E/K264E)), liii) SEQ ID NO: XXX (IL-12p40 (E59K/K99Y)), liv) SEQ ID NO: XXX (IL-12p40 (E59Y/K99E)), lv) SEQ ID NO: XXX (IL-12p40 (E45K/E59K/K99E)), lvi) SEQ ID NO: XXX (IL-12p40 (E59K/K99E/Q144E)), lvii) SEQ ID NO: XXX (IL-12p40 (E59K/K99E/Q144K)), lviii) SEQ ID NO: XXX (IL-12p40 (E59K/K99E/R159E)), lix) SEQ ID NO: XXX (IL-12p40 (E59K/K99E/K264E)), lx) SEQ ID NO: XXX (IL-12p40 (D18K/E59K/K99E/K264E)), lxi) SEQ ID NO: XXX (IL-12p40 (C252S)), lxii) SEQ ID NO: XXX (IL-12p40 (DI8K/E59K/K99E/C252S)), lxiii) SEQ ID NO: XXX (IL-12p40 (D18K/E59K/K99E/C252S/K264E)), lxiv) SEQ ID NO: XXX (IL-12p40 (E59K/K99Y/C252S)), lxv) SEQ ID NO: XXX (IL-12p40 (E59K/K99E/C252S/K264E)), lxvi) SEQ ID NO: XXX (IL-12p40 (E59K/K99E/C252S)), lxvii) SEQ ID NO: XXX (IL-12p40 (N103D/N113D)), lxviii) SEQ ID NO: XXX (IL-12p40 (N103D/N200D)), lxix) SEQ ID NO: XXX (IL-12p40 (N103D/N281D)), lxx) SEQ ID NO: XXX (IL-12p40 (N113D/N200D)), lxxi) SEQ ID NO: XXX (IL-12p40 (N113D/N281D)), lxxii) SEQ ID NO: XXX (IL-12p40 (N200D/N281D)), lxxiii) SEQ ID NO: XXX (IL-12p50 (N103D/N113D/N200D)), lxxiv) SEQ ID NO: XXX (IL-12p40

(N103D/N113D/N281D)), lxxv) SEQ ID NO: XXX (IL-12p40 (N103D/N200D/N281D)), lxxvi) SEQ ID NO: XXX (IL-12p40 (N113D/N200D/N281D)), lxxvii) SEQ ID NO: XXX (IL-12p40 (N103Q)), lxxviii) SEQ ID NO: XXX (IL-12p40 (N113Q)), lxxix) SEQ ID NO: XXX (IL-12p40 (N200Q)), lxxx) SEQ ID NO: XXX (IL-12p40 (N281Q)), lxxxi) SEQ ID NO: XXX (IL-12p40 (N103Q/N113Q)), lxxxii) SEQ ID NO: XXX (IL-12p40 (N103Q/N200Q)), lxxxiii) SEQ ID NO: XXX (IL-12p40 (N103Q/N281Q)), lxxxiv) SEQ ID NO: XXX (IL-12p40 (N113Q/N200Q)), lxxxv) SEQ ID NO: XXX (IL-12p40 (N113Q/N281Q)), lxxxvi) SEQ ID NO: XXX (IL-12p40 (N200Q/N281Q)), lxxxvii) SEQ ID NO: XXX (IL-12p40 (N103Q/N113Q/N200Q)), lxxxviii) SEQ ID NO: XXX (IL-12p40 (N103Q/N113Q/N281Q)), lxxxix) SEQ ID NO: XXX (IL-12p40 (N103Q/N200Q/N281Q)), xc) SEQ ID NO: XXX (IL-12p40 (N113Q/N200Q/N281Q)), xci) SEQ ID NO:XXX (IL-12p40 (N103Q/N113Q/N200Q/N281Q)), xcii) SEQ ID NO:XXX (IL-12p40 (D34N/E59K/K99E)), xciii) SEQ ID NO:XXX (IL-12p40 (D34K/E59K/K99E)), xciv) SEQ ID NO:XXX (IL-12p40 (E32Q/D34N/E59K/K99E)), xcv) SEQ ID NO:XXX (IL-12p40 (E32K/D34N/E59K/K99E)), xcvi) SEQ ID NO: XXX (IL-12p40 (E59Y/K99Y)), xcvii) SEQ ID NO:XXX (IL-12p40 (E59K/K99E/N103Q/C252S/K264E)), xcviii) SEQ ID NO:XXX (IL-12p40 (E59K/K99E/N113Q/C252S/K264E)), xcix) SEQ ID NO:XXX (IL-12p40 (E59K/K99E/N200Q/C252S/K264E)), c) SEQ ID NO:XXX (IL-12p40 (E59K/K99E/N281Q/C252S/K264E)), ci) SEQ ID NO:XXX (IL-12p40 (E59K/K99E/N103Q/N113Q/C252S/K264E)), cii) SEQ ID NO:XXX (IL-12p40 (E59K/K99E/N103Q/N200Q/C252S/K264E)), ciii) SEQ ID NO:XXX (IL-12p40 (E59K/K99E/N103Q/N281Q/C252S/K264E)), civ) SEQ ID NO:XXX (IL-12p40 (E59K/K99E/N113Q/N200Q/C252S/K264E)), cv) SEQ ID NO:XXX (IL-12p40 (E59K/K99E/N113Q/N281Q/C252S/K264E)), cvi) SEQ ID NO:XXX (IL-12p40 (E59K/K99E/N200Q/N281Q/C252S/K264E)), cvii) SEQ ID NO:XXX (IL-12p40 (E59K/K99E/N103Q/N113Q/N200Q/C252S/K264E)), cviii) SEQ ID NO:XXX (IL-12p40 (E59K/K99E/N103Q/N200Q/N281Q/C252S/K264E)), and cix) SEQ ID NO:XXX (IL-12p40 (E59K/K99E/N113Q/N200Q/N281Q/C252S/K264E)).

[00204] In some embodiments, the present invention provides a heterodimeric protein wherein said IL-12p35 subunit has a sequence selected from the group consisting of: i) SEQ ID NO:113 (IL-12p35(N71D)), ii) SEQ ID NO:114 (IL-12p35(N85D)), iii) SEQ ID NO:115 (IL-12p35(N195D)), iv) SEQ ID NO:116 (IL-12p35(N71D/N85D/N195D)), v) SEQ ID NO:117 (IL-12p35(E153Q)), vi) SEQ ID NO:118 (IL-12p35(E38Q)), vii) SEQ ID NO:119 (IL-12p35(N151D)), viii) SEQ ID NO:120 (IL-12p35(Q135E)), ix) SEQ ID NO:121 (IL-12p35(Q35D)), x) SEQ ID NO:122 (IL-12p35(Q146E)), xi) SEQ ID NO:123 (IL-12p35(N76D)), xii) SEQ ID NO:124 (IL-12p35(E162Q)), xiii) SEQ ID NO:125 (IL-12p35(E163Q)), xiv) IL-12p35(N21D), xv) SEQ ID NO:XXX (IL-12p35(D55Q)), xvi) IL-12p35(E79Q), xvii) IL-12p35(Q130E), xviii) IL-12p35(N136D), xix) IL-12p35(E143Q), xx) SEQ ID NO:227 (IL-12p35(N151K)), xxi) SEQ ID NO:226 (IL-12p35(E153K)), xxii) IL-12p35(K158E), xxiii) IL-12p35(D165N), xxiv) SEQ ID NO:225 (IL-12p35(N151D/E153Q)), xxv) SEQ ID NO:228 (IL-12p35(N151D/D165N)), xxvi) SEQ ID NO:229 (IL-12p35(Q130E/N151D)), xxvii) SEQ ID NO:230 (IL-12p35(N151D/K158E)), xxviii) SEQ ID NO:231 (IL-12p35(E79Q/N151D)), xxix) SEQ ID NO:232 (IL-12p35(D55Q/N151D)), xxx) SEQ ID NO:233 (IL-12p35(N136D/N151D)), xxxi) SEQ ID NO:234 (IL-12p35(N21D/N151D)), xxxii) SEQ ID NO:235 (IL-12p35(E143Q/N151D)),xxxiii) SEQ ID NO: XXX (IL-12p35(F96A)),xxxiv) SEQ ID NO: XXX (IL-12p35(M97A)), xxxv) SEQ ID NO: XXX (IL-12p35(L89A)),xxxvi) SEQ ID NO: XXX (IL-12p35(L124A)),xxxvii) SEQ ID NO: XXX (IL-12p35(M125A)),xxxviii) SEQ ID NO: XXX (IL-12p35(L75A)), xxxiv) SEQ ID NO: XXX (IL-12p35(I171A)),xxxv) SEQ ID NO: XXX (IL-12p35 (N71Q)), xxxvi) SEQ ID NO: XXX (IL-12p35 (N85Q)), xxxvii) SEQ ID NO: XXX (IL-12p35 (N195Q)), xxxviii) SEQ ID NO: XXX (IL-12p35 (N71Q/N85Q)),xxxix) SEQ ID NO: XXX (IL-12p35 (N71Q/N195Q)),lx) SEQ ID NO: XXX (IL-12p35 (N85Q/N195Q),lxi) SEQ ID NO: XXX (IL-12p35 (N71Q/N85Q/N195Q)),lxii) SEQ ID NO: XXX (IL-12p35 (N71D/N85D)), lxiii) SEQ ID NO: XXX (IL-12p35 (N71D/N195D),lxiv) SEQ ID NO: XXX (IL-12p35 (N85D/N195D)), lxv) SEQ ID NO: XXX (IL-12p35 (D55Q)), and lxvi) SEQ ID NO: XXX (IL-12p35 (D55K)).

[00205] In another aspect, the present invention provides a homodimeric Fc fusion protein comprising a first monomer and a second monomer each comprising, from N- to C-terminal, an

IL-12p40 subunit domain- an optional first domain linker-an IL-12p35 subunit domain-an optional second domain linker- an Fc domain.

[00206] In some embodiments, the present invention provides a homodimeric Fc fusion protein, wherein said modifications promoting homodimerization of said Fc domain are a set of amino acid substitutions selected from the group consisting of L368D/K370S; S364K; S364K/E357L; S364K/E357Q; T411E/K360E/Q362E; D401K; T366S/L368A/Y407V; T366W; T366S/L368A/Y407V/Y349C; and T366W/S354C, according to EU numbering.

[00207] In some embodiments, the present invention provides a homodimeric Fc fusion protein wherein said first domain linker and said second domain linker have the same amino acid sequence.

[00208] In some embodiments, the present invention provides a homodimeric Fc fusion protein wherein said Fc domain has an additional set of amino acid substitutions comprising Q295E/N384D/Q418E/N421D, according to EU numbering.

[00209] In some embodiments, the present invention provides a homodimeric Fc fusion protein, wherein said Fc domain has an additional set of amino acid substitutions selected from the group consisting of G236R/L328R, E233P/L234V/L235A/G236_/S239K, E233P/L234V/L235A/G236_/S239K/A327G, E233P/L234V/L235A/G236_/S267K/A327G, E233P/L234V/L235A/G236_, and E233P/L234V/L235A/G236_/S267K, according to EU numbering.

[00210] In some embodiments, the present invention provides a homodimeric Fc fusion protein wherein said IL-12p40 subunit has a polypeptide sequence selected form the group consisting of SEQ ID NO:XX (human IL-12 subunit beta (IL-12p40) precursor sequence) and SEQ ID NO:XX (human IL-12 subunit beta (IL-12p40) mature form sequence), and said IL-12p35 subunit has a polypeptide sequence selected from the group consisting of SEQ ID NO:XX (human IL-12 subunit alpha (IL-12p35) precursor sequence) and SEQ ID NO:XX (human IL-12 subunit alpha (IL-12p35) mature form sequence).

[00211] In some embodiments, the present invention provides a homodimeric Fc fusion protein wherein each of said Fc domain further comprises amino acid substitutions M428L/N424S.

[00212] In some embodiments, the present invention provides a homodimeric Fc fusion protein wherein said IL-12p40 subunit is a variant IL-12p40 subunit and/or said IL-12p35 subunit is a variant IL-12p35 subunit.

[00213] In some embodiments, the present invention provides a homodimeric Fc fusion protein wherein said IL-12p40 subunit is a variant IL-12p40 subunit having altered affinity for IL-12 receptor subunit beta-1 (IL-12Rß1), IL-12 receptor subunit beta-2 (IL-12Rß2), and/or IL-12 receptor complex; and/or said IL-12p35 subunit is a variant IL-12p35 subunit having altered affinity for IL-12 receptor subunit beta-1 (IL-12Rß1), IL-12 receptor subunit beta-2 (IL-12Rß2), and/or IL-12 receptor complex.

[00214] In some embodiments, the present invention provides a homodimeric Fc fusion protein wherein said IL-12p40 subunit has one or more amino acid modifications at amino acid residues selected from the group consisting of E3, D7, E12, D14, W15, P17, D18, A19, P20, G21, E22, M23, D29, E32, E33, D34, L40, D41, Q42, S43, E45, L47, T54, I55, Q56, K58, E59, F60, G61, D62, Q65, Y66, E73, K84, E86, D87, G88, I89, W90, D93, D97, K99, E100, K102, N103, K104, F106, E110, N113, Y114, D129, D142, Q144, E156, R159, D161, N162, K163, D166, D170, Q172, D174, A176, C177, P178, A179, A180, E181, S183, P185, E187, N200, S204, F206, R208, D209, D214, N218, Q220, N226, Q229, E231, E235, T242, P243, S245, Y246, F247, S248, C252S, Q256, K158, K260, E262, K264, D265, D270, N281, Q289, D290, R291, Y292, Y293, and E299.

[00215] In some embodiments, the present invention provides a homodimeric Fc fusion protein wherein said IL-12p40 subunit has one or more amino acid substitutions selected from the group consisting of D18N, D18K, E32Q, E33Q, D34N, D34K, Q42E, S43E, S43K, E45Q, Q56E, E59Q, E59K, D62N, E73Q, D87N, K99E, K99Y, E100Q, N103D, N103Q, N113D, N113Q, Q144E, D161N, R159E, K163E, E187Q, N200D, N200Q, N218Q, Q229E, E235Q, C252S, Q256N, K258E, K260E, E262Q, K264E, N281D, N281Q, and E299Q.

[00216] In some embodiments, the present invention provides a homodimeric Fc fusion protein wherein said IL-12p40 subunit has amino acid substitutions selected from the group consisting of N103D/N113D/N200D/N281D, Q42E/E45Q, E45Q/Q56E, Q42E/E59Q, Q56E/E59Q, Q42E/E45Q/Q56E, E45Q/Q56E/E59Q, E32Q/E59Q, D34N/E59K, D34N/E59K/K99E, D34K/E59K/K99E, E32Q/D34N/E59K/K99E, E32K/D34N/E59K/K99E, D34N/E59Q, E59Q/E187Q, S43E/E59Q, S43K/E49Q, E59Q/K163E, E59Q/K99E, E59Q/K258E, E59Q/K260E, E59K/K99E, D18K/E59K/K99E, E59K/K99E/K264E, E59K/K99Y, E59Y/K99Y, E59Y/K99E, E45K/E59K/K99E, E59K/K99E/Q144E, E59K/K99E/Q144K, E59K/K99E/R159E, E59K/K99E/K264E, D18K/E59K/K99E/K264E, DI8K/E59K/K99E/C252S, D18K/E59K/K99E/C252S/K264E, E59K/K99Y/C252S, E59K/K99E/C252S/K264E, E59K/K99E/C252S, N103D/N113D, N103D/N200D, N103D/N281D, N113D/N200D, N113D/N281D, N200D/N281D, N103D/N113D/N200D, N103D/N113D/N281D, N103D/N200D/N281D, N113D/N200D/N281D, N103Q/N113Q, N103Q/N200Q, N103Q/N281Q, N113Q/N200Q, N113Q/N281Q, N200Q/N281Q, N103Q/N113Q/N200Q, N103Q/N113Q/N281Q, N103Q/N200Q/N281Q, N113Q/N200Q/N281Q, N103Q/N113Q/N200Q/N281Q, E59K/K99E/N103Q/C252S/K264E, E59K/K99E/N113Q/C252S/K264E, E59K/K99E/N200Q/C252S/K264E, E59K/K99E/N281Q/C252S/K264E, E59K/K99E/N103Q/N113Q/C252S/K264E, E59K/K99E/N103Q/N200Q/C252S/K264E, E59K/K99E/N103Q/N281Q/C252S/K264E, E59K/K99E/N113Q/N200Q/C252S/K264E, E59K/K99E/N113Q/N281Q/C252S/K264E, E59K/K99E/N200Q/N281Q/C252S/K264E, E59K/K99E/N103Q/N113Q/N200Q/C252S/K264E, E59K/K99E/N103Q/N200Q/N281Q/C252S/K264E, E59K/K99E/N113Q/N200Q/N281Q/C252S/K264E, and E59K/K99E/N103Q/N113Q/N200Q/N281Q/C252S/K264E.

[00217] In some embodiments, the present invention provides a homodimeric Fc fusion protein wherein said IL-12p40 subunit has a polypeptide sequence selected from the group consisting of: i) SEQ ID NO:57 (IL-12p40(N103D)), ii) SEQ ID NO:58 (IL-12p40(N113D)), iii) SEQ ID NO:59 (IL-12p40(N200D)), iv) SEQ ID NO:60 (IL-12p40(N281D)), v) SEQ ID NO:61 (IL-12p40(N103D/N113D/N200D/N281D)), vi) SEQ ID NO:62 (IL-12p40(Q42E)), vii) SEQ ID NO:63

(IL-12p40(E45Q)), viii) SEQ ID NO:64 (IL-12p40(Q56E)), ix) SEQ ID NO:65 (IL-12p40(E59Q)), x) SEQ ID NO:66 (IL-12p40(D62N)), xi) SEQ ID NO:67 (IL-12p40(Q42E/E45Q)), xii) SEQ ID NO:68 (IL-12p40(E45Q/Q56E)), xiii) SEQ ID NO:69 (IL-12p40(Q42E/E59Q)), xiv) SEQ ID NO:70 (IL-12p40(Q56E/E59Q)), xv) SEQ ID NO:71 (IL-12p40(Q42E/E45Q/Q56E)), xvi) SEQ ID NO:72 (IL-12p40(E45Q/Q56E/E59Q)), xvii) SEQ ID NO:73 (IL-12p40(D161N)), xviii) SEQ ID NO:74 (IL-12p40(E73Q)), xix) SEQ ID NO:75 (IL-12p40(Q144E)), xx) SEQ ID NO:76 (IL-12p40(E262Q)), xxi) SEQ ID NO:77 (IL-12p40(E100Q)), xxii) SEQ ID NO:78 (IL-12p40(D18N)), xxiii) SEQ ID NO:79 (IL-12p40(E33Q)), xxiv) SEQ ID NO:80 (IL-12p40(Q229E)), xxv) SEQ ID NO:81 (IL-12p40(E235Q)), xxvi) SEQ ID NO:82 (IL-12p40(Q256N)), xxvii) SEQ ID NO:83 (IL-12p40(E299Q)), xxviii) SEQ ID NO:84 (IL-12p40(D87N)), xxix) IL-12p40(E32Q), xxx) IL-12p40(D34N), xxxi) IL-12p40(S43E), xxxii) IL-12p40(S43K), xxxiii) SEQ ID NO:XXX (IL-12p40 (E59K/K99E/N103Q/N113Q/N200Q/N281Q/C252S/K264E)), xxxiv) SEQ ID NO:205 (IL-12p40(E59K)), xxxv) IL-12p40(K99E), xxxvi) IL-12p40(K163E), xxxvii) IL-12p40(E187Q), xxxviii) IL-12p40(K258E), xxxix) IL-12p40(K260E), xl) SEQ ID NO:206 (IL-12p40(E32Q/E59Q)), xli) SEQ ID NO:207 (IL-12p40(D34N/E59Q)), xlii) SEQ ID NO:208 (IL-12p40(E59Q/E187Q)), xliii) SEQ ID NO:209 (IL-12p40(S43E/E59Q)), xliv) SEQ ID NO:210 (IL-12p40(S43K/E49Q)), xlv) SEQ ID NO:211 (IL-12p40(E59Q/K163E)), xlvi) SEQ ID NO:212 (IL-12p40(E59Q/K99E)), xlvii) SEQ ID NO:213 (IL-12p40(E59Q/K258E)), xlviii) SEQ ID NO:214 (IL-12p40(E59Q/K260E)), xlix) SEQ ID NO: XXX (IL-12p40 (D34N/E59K)), l) SEQ ID NO: XXX (IL-12p40(E59K/K99E)), li) SEQ ID NO: XXX (IL-12p40(D18K/E59K/K99E)), lii) SEQ ID NO: XXX (IL-12p40 (E59K/K99E/K264E)), liii) SEQ ID NO: XXX (IL-12p40 (E59K/K99Y)), liv) SEQ ID NO: XXX (IL-12p40 (E59Y/K99E)), lv) SEQ ID NO: XXX (IL-12p40 (E45K/E59K/K99E)), lvi) SEQ ID NO: XXX (IL-12p40 (E59K/K99E/Q144E)), lvii) SEQ ID NO: XXX (IL-12p40 (E59K/K99E/Q144K)), lviii) SEQ ID NO: XXX (IL-12p40 (E59K/K99E/R159E)), lix) SEQ ID NO: XXX (IL-12p40 (E59K/K99E/K264E)), lx) SEQ ID NO: XXX (IL-12p40 (D18K/E59K/K99E/K264E)), lxi) SEQ ID NO: XXX (IL-12p40 (C252S)), lxii) SEQ ID NO: XXX (IL-12p40 (DI8K/E59K/K99E/C252S)), lxiii) SEQ ID NO: XXX (IL-12p40 (D18K/E59K/K99E/C252S/K264E)), lxiv) SEQ ID NO: XXX (IL-12p40 (E59K/K99Y/C252S)), lxv) SEQ ID NO: XXX (IL-12p40 (E59K/K99E/C252S/K264E)), lxvi) SEQ ID NO: XXX (IL-12p40 (E59K/K99E/C252S)), lxvii) SEQ ID NO: XXX (IL-12p40 (N103D/N113D)),

lxviii) SEQ ID NO: XXX (IL-12p40 (N103D/N200D)), lxix) SEQ ID NO: XXX (IL-12p40 (N103D/N281D)), lxx) SEQ ID NO: XXX (IL-12p40 (N113D/N200D)), lxxi) SEQ ID NO: XXX (IL-12p40 (N113D/N281D)), lxxii) SEQ ID NO: XXX (IL-12p40 (N200D/N281D)), lxxiii) SEQ ID NO: XXX (IL-12p50 (N103D/N113D/N200D)), lxxiv) SEQ ID NO: XXX (IL-12p40 (N103D/N113D/N281D)), lxxv) SEQ ID NO: XXX (IL-12p40 (N103D/N200D/N281D)), lxxvi) SEQ ID NO: XXX (IL-12p40 (N113D/N200D/N281D)), lxxvii) SEQ ID NO: XXX (IL-12p40 (N103Q)), lxxviii) SEQ ID NO: XXX (IL-12p40 (N113Q)), lxxix) SEQ ID NO: XXX (IL-12p40 (N200Q)), lxxx) SEQ ID NO: XXX (IL-12p40 (N281Q)), lxxxi) SEQ ID NO: XXX (IL-12p40 (N103Q/N113Q)), lxxxii) SEQ ID NO: XXX (IL-12p40 (N103Q/N200Q)), lxxxiii) SEQ ID NO: XXX (IL-12p40 (N103Q/N281Q)), lxxxiv) SEQ ID NO: XXX (IL-12p40 (N113Q/N200Q)), lxxxv) SEQ ID NO: XXX (IL-12p40 (N113Q/N281Q)), lxxxvi) SEQ ID NO: XXX (IL-12p40 (N200Q/N281Q)), lxxxvii) SEQ ID NO: XXX (IL-12p40 (N103Q/N113Q/N200Q)), lxxxviii) SEQ ID NO: XXX (IL-12p40 (N103Q/N113Q/N281Q)), lxxxix) SEQ ID NO: XXX (IL-12p40 (N103Q/N200Q/N281Q)), xc) SEQ ID NO: XXX (IL-12p40 (N113Q/N200Q/N281Q)), xci) SEQ ID NO:XXX (IL-12p40 (N103Q/N113Q/N200Q/N281Q)), xcii) SEQ ID NO:XXX (IL-12p40 (D34N/E59K/K99E)), xciii) SEQ ID NO:XXX (IL-12p40 (D34K/E59K/K99E)), xciv) SEQ ID NO:XXX (IL-12p40 (E32Q/D34N/E59K/K99E)), xcv) SEQ ID NO:XXX (IL-12p40 (E32K/D34N/E59K/K99E)), xcvi) SEQ ID NO: XXX (IL-12p40 (E59Y/K99Y)), xcvii) SEQ ID NO:XXX (IL-12p40 (E59K/K99E/N103Q/C252S/K264E)), xcviii) SEQ ID NO:XXX (IL-12p40 (E59K/K99E/N113Q/C252S/K264E)), xcix) SEQ ID NO:XXX (IL-12p40 (E59K/K99E/N200Q/C252S/K264E)), c) SEQ ID NO:XXX (IL-12p40 (E59K/K99E/N281Q/C252S/K264E)), ci) SEQ ID NO:XXX (IL-12p40 (E59K/K99E/N103Q/N113Q/C252S/K264E)), cii) SEQ ID NO:XXX (IL-12p40 (E59K/K99E/N103Q/N200Q/C252S/K264E)), ciii) SEQ ID NO:XXX (IL-12p40 (E59K/K99E/N103Q/N281Q/C252S/K264E)), civ) SEQ ID NO:XXX (IL-12p40 (E59K/K99E/N113Q/N200Q/C252S/K264E)), cv) SEQ ID NO:XXX (IL-12p40 (E59K/K99E/N113Q/N281Q/C252S/K264E)), cvi) SEQ ID NO:XXX (IL-12p40 (E59K/K99E/N200Q/N281Q/C252S/K264E)), cvii) SEQ ID NO:XXX (IL-12p40 (E59K/K99E/N103Q/N113Q/N200Q/C252S/K264E)), cviii) SEQ ID NO:XXX (IL-12p40

(E59K/K99E/N103Q/N200Q/N281Q/C252S/K264E)), and cix) SEQ ID NO:XXX (IL-12p40 (E59K/K99E/N113Q/N200Q/N281Q/C252S/K264E)).

[00218] In some embodiments, the present invention provides a homodimeric Fc fusion protein wherein said IL-12p35 subunit has one or more amino acid modifications as amino acid residues selected from the group consisting of Q20, N21, Q35, E38, S44, E45, E46, H49, K54, D55, T59, V60, E61, C63, L64, P65, E67, L68, N71, S73, C74, L75, N76, E79, N85, L89, F96, M97, L124, M125, Q130, Q135, N136, E143, Q146, N151, E153, K158, E162, E163, D165, I171, R181, I182, R183, V185, T186, D188, R189, V190, S192, Y193, N195, and A196.

[00219] In some embodiments, the present invention provides a homodimeric Fc fusion protein wherein said IL-12p35 subunit has one or more amino acid substitutions selected from the group consisting of N21D, Q35D, E38Q, D55Q, D55K, N71D, N71Q, L75A, N76D, E79Q, N85D, N85Q, L89A, F96A, M97A, L124A, M125A, Q130E, Q135E, N136D, E143Q, Q146E, N151D, N151K, E153K, E153Q, K158E, E162Q, E163Q, D165N, I171A, N195D, and N195Q.

[00220] In some embodiments, the present invention provides a homodimeric Fc fusion protein wherein said IL-12p35 subunit has amino acid substitutions selected from the group consisting of N71D/N85D/N195D, N151D/E153Q, N151D/D165N, Q130E/N151D, N151D/K158E, E79Q/N151D, D55Q/N151D, N136D/N151D, N21D/N151D, E143Q/N151D, N71Q/N85Q, N71Q/N195Q, N85Q/N195Q, N71Q/N85Q/N195Q, N71D/N85D, N71D/N195D, and N85D/N195D.

[00221] In some embodiments, the present invention provides a homodimeric Fc fusion protein wherein said IL-12p35 subunit has a polypeptide sequence selected from the group consisting of: i) SEQ ID NO:113 (IL-12p35(N71D)), ii) SEQ ID NO:114 (IL-12p35(N85D)), iii) SEQ ID NO:115 (IL-12p35(N195D)), iv) SEQ ID NO:116 (IL-12p35(N71D/N85D/N195D)), v) SEQ ID NO:117 (IL-12p35(E153Q)), vi) SEQ ID NO:118 (IL-12p35(E38Q)), vii) SEQ ID NO:119 (IL-12p35(N151D)), viii) SEQ ID NO:120 (IL-12p35(Q135E)), ix) SEQ ID NO:121 (IL-12p35(Q35D)), x) SEQ ID NO:122 (IL-12p35(Q146E)), xi) SEQ ID NO:123 (IL-12p35(N76D)), xii) SEQ ID NO:124 (IL-12p35(E162Q)), xiii) SEQ ID NO:125 (IL-12p35(E163Q)), xiv) IL-12p35(N21D), xv) SEQ ID NO:XXX (IL-12p35(D55Q)),

xvi) IL-12p35(E79Q), xvii) IL-12p35(Q130E), xviii) IL-12p35(N136D), xix) IL-12p35(E143Q), xx) SEQ ID NO:227 (IL-12p35(N151K)), xxi) SEQ ID NO:226 (IL-12p35(E153K)), xxii) IL-12p35(K158E), xxiii) IL-12p35(D165N), xxiv) SEQ ID NO:225 (IL-12p35(N151D/E153Q)), xxv) SEQ ID NO:228 (IL-12p35(N151D/D165N)), xxvi) SEQ ID NO:229 (IL-12p35(Q130E/N151D)), xxvii) SEQ ID NO:230 (IL-12p35(N151D/K158E)), xxviii) SEQ ID NO:231 (IL-12p35(E79Q/N151D)), xxix) SEQ ID NO:232 (IL-12p35(D55Q/N151D)), xxx) SEQ ID NO:233 (IL-12p35(N136D/N151D)), xxxi) SEQ ID NO:234 (IL-12p35(N21D/N151D)), xxxii) SEQ ID NO:235 (IL-12p35(E143Q/N151D)), xxxiii) SEQ ID NO: XXX (IL-12p35(F96A)), xxxiv) SEQ ID NO: XXX (IL-12p35(M97A)), xxxv) SEQ ID NO: XXX (IL-12p35(L89A)), xxxvi) SEQ ID NO: XXX (IL-12p35(L124A)), xxxvii) SEQ ID NO: XXX (IL-12p35(M125A)), xxxviii) SEQ ID NO: XXX (IL-12p35(L75A)), xxxiv) SEQ ID NO: XXX (IL-12p35(I171A)), xxxv) SEQ ID NO: XXX (IL-12p35 (N71Q)), xxxvi) SEQ ID NO: XXX (IL-12p35 (N85Q)), xxxvii) SEQ ID NO: XXX (IL-12p35 (N195Q)), xxxviii) SEQ ID NO: XXX (IL-12p35 (N71Q/N85Q)), xxxix) SEQ ID NO: XXX (IL-12p35 (N71Q/N195Q)), xl) SEQ ID NO: XXX (IL-12p35 (N85Q/N195Q), xli) SEQ ID NO: XXX (IL-12p35 (N71Q/N85Q/N195Q)), xlii) SEQ ID NO: XXX (IL-12p35 (N71D/N85D)), xliii) SEQ ID NO: XXX (IL-12p35 (N71D/N195D), xliv) SEQ ID NO: XXX (IL-12p35 (N85D/N195D)), xlv) SEQ ID NO: XXX (IL-12p35 (D55Q)), and xlvi) SEQ ID NO: XXX (IL-12p35 (D55K)).

[00222] In some embodiments, the present invention provides a homodimeric Fc fusion protein wherein said first and second monomers are XENP31289.

[00223] In some embodiments, the present invention provides a homodimeric Fc fusion protein wherein said first and second monomers are XENP31291.

[00224] In some embodiments, the present invention provides a homodimeric Fc fusion protein composition comprising a homodimeric Fc fusion protein for use in treating cancer in a subject.

[00225] In some embodiments, the present invention provides a one or more nucleic acids encoding a homodimeric Fc fusion protein.

[00226] In some embodiments, the present invention provides a host cell comprising said one or more nucleic acids encoding a homodimeric Fc fusion protein.

[00227] In some embodiments, the present invention provides a method of making a homodimeric Fc fusion protein, said method comprising culturing a host cell under conditions whereby said homodimeric Fc fusion protein is produced.

[00228] In some embodiments, the present invention provides a method of purifying a homodimeric Fc fusion protein, said method comprising: a) providing a composition comprising the homodimeric Fc fusion protein; b) loading said composition onto an ion exchange column; and c) collecting a fraction containing said homodimeric Fc fusion protein.

[00229] In another aspect, the present invention provides a homodimeric Fc fusion protein comprising a first monomer and a second monomer each comprising, from N- to C-terminal, an IL-12p35 subunit domain-an optional first domain linker-an IL-12p40 subunit domain-an optional second domain linker-an Fc domain.

[00230] In some embodiments, the present invention provides a homodimeric Fc fusion protein, wherein said modifications promoting homodimerization of said Fc domain are a set of amino acid substitutions selected from the group consisting of L368D/K370S; S364K; S364K/E357L; S364K/E357Q; T411E/K360E/Q362E; D401K; T366S/L368A/Y407V; T366W; T366S/L368A/Y407V/Y349C; and T366W/S354C, according to EU numbering.

[00231] In some embodiments, the present invention provides a homodimeric Fc fusion protein wherein said first domain linker and said second domain linker have the same amino acid sequence.

[00232] In some embodiments, the present invention provides a homodimeric Fc fusion protein wherein said Fc domain has an additional set of amino acid substitutions comprising Q295E/N384D/Q418E/N421D, according to EU numbering.

[00233] In some embodiments, the present invention provides a homodimeric Fc fusion protein, wherein said Fc domain has an additional set of amino acid substitutions selected from the group consisting of G236R/L328R, E233P/L234V/L235A/G236_/S239K, E233P/L234V/L235A/G236_/S239K/A327G, E233P/L234V/L235A/G236_/S267K/A327G, E233P/L234V/L235A/G236_, and E233P/L234V/L235A/G236_/S267K, according to EU numbering.

[00234] In some embodiments, the present invention provides a homodimeric Fc fusion protein wherein said IL-12p40 subunit has a polypeptide sequence selected form the group consisting of SEQ ID NO:3 (human IL-12 subunit beta (IL-12p40) precursor sequence) and SEQ ID NO:4 (human IL-12 subunit beta (IL-12p40) mature form sequence), and said IL-12p35 subunit has a polypeptide sequence selected from the group consisting of SEQ ID NO:1 (human IL-12 subunit alpha (IL-12p35) precursor sequence) and SEQ ID NO:2 (human IL-12 subunit alpha (IL-12p35) mature form sequence).

[00235] In some embodiments, the present invention provides a homodimeric Fc fusion protein wherein each of said Fc domain further comprises amino acid substitutions M428L/N424S.

[00236] In some embodiments, the present invention provides a homodimeric Fc fusion protein wherein said IL-12p40 subunit is a variant IL-12p40 subunit and/or said IL-12p35 subunit is a variant IL-12p35 subunit.

[00237] In some embodiments, the present invention provides a homodimeric Fc fusion protein wherein said IL-12p40 subunit is a variant IL-12p40 subunit having altered affinity for IL-12 receptor subunit beta-1 (IL-12Rß1), IL-12 receptor subunit beta-2 (IL-12Rß2), and/or IL-12 receptor complex; and/or said IL-12p35 subunit is a variant IL-12p35 subunit having altered affinity for IL-12 receptor subunit beta-1 (IL-12Rß1), IL-12 receptor subunit beta-2 (IL-12Rß2), and/or IL-12 receptor complex.

[00238] In some embodiments, the present invention provides a homodimeric Fc fusion protein wherein said IL-12p40 subunit has one or more amino acid modifications at amino acid residues

selected from the group consisting of E3, D7, E12, D14, W15, P17, D18, A19, P20, G21, E22, M23, D29, E32, E33, D34, L40, D41, Q42, S43, E45, L47, T54, I55, Q56, K58, E59, F60, G61, D62, Q65, Y66, E73, K84, E86, D87, G88, I89, W90, D93, D97, K99, E100, K102, N103, K104, F106, E110, N113, Y114, D129, D142, Q144, E156, R159, D161, N162, K163, D166, D170, Q172, D174, A176, C177, P178, A179, A180, E181, S183, P185, E187, N200, S204, F206, R208, D209, D214, N218, Q220, N226, Q229, E231, E235, T242, P243, S245, Y246, F247, S248, C252S, Q256, K158, K260, E262, K264, D265, D270, N281, Q289, D290, R291, Y292, Y293, and E299.

[00239] In some embodiments, the present invention provides a homodimeric Fc fusion protein wherein said IL-12p40 subunit has one or more amino acid substitutions selected from the group consisting of D18N, D18K, E32Q, E33Q, D34N, D34K, Q42E, S43E, S43K, E45Q, Q56E, E59Q, E59K, D62N, E73Q, D87N, K99E, K99Y, E100Q, N103D, N103Q, N113D, N113Q, Q144E, D161N, R159E, K163E, E187Q, N200D, N200Q, N218Q, Q229E, E235Q, C252S, Q256N, K258E, K260E, E262Q, K264E, N281D, N281Q, and E299Q.

[00240] In some embodiments, the present invention provides a homodimeric Fc fusion protein wherein said IL-12p40 subunit has amino acid substitutions selected from the group consisting of N103D/N113D/N200D/N281D, Q42E/E45Q, E45Q/Q56E, Q42E/E59Q, Q56E/E59Q, Q42E/E45Q/Q56E, E45Q/Q56E/E59Q, E32Q/E59Q, D34N/E59K, D34N/E59K/K99E, D34K/E59K/K99E, E32Q/D34N/E59K/K99E, E32K/D34N/E59K/K99E, D34N/E59Q, E59Q/E187Q, S43E/E59Q, S43K/E49Q, E59Q/K163E, E59Q/K99E, E59Q/K258E, E59Q/K260E, E59K/K99E, D18K/E59K/K99E, E59K/K99E/K264E, E59K/K99Y, E59Y/K99Y, E59Y/K99E, E45K/E59K/K99E, E59K/K99E/Q144E, E59K/K99E/Q144K, E59K/K99E/R159E, E59K/K99E/K264E, D18K/E59K/K99E/K264E, DI8K/E59K/K99E/C252S, D18K/E59K/K99E/C252S/K264E, E59K/K99Y/C252S, E59K/K99E/C252S/K264E, E59K/K99E/C252S, N103D/N113D, N103D/N200D, N103D/N281D, N113D/N200D, N113D/N281D, N200D/N281D, N103D/N113D/N200D, N103D/N113D/N281D, N103D/N200D/N281D, N113D/N200D/N281D, N103Q/N113Q, N103Q/N200Q, N103Q/N281Q, N113Q/N200Q, N113Q/N281Q, N200Q/N281Q, N103Q/N113Q/N200Q, N103Q/N113Q/N281Q, N103Q/N200Q/N281Q, N113Q/N200Q/N281Q, N103Q/N113Q/N200Q/N281Q, E59K/K99E/N103Q/C252S/K264E,

E59K/K99E/N113Q/C252S/K264E, E59K/K99E/N200Q/C252S/K264E,
E59K/K99E/N281Q/C252S/K264E, E59K/K99E/N103Q/N113Q/C252S/K264E,
E59K/K99E/N103Q/N200Q/C252S/K264E, E59K/K99E/N103Q/N281Q/C252S/K264E,
E59K/K99E/N113Q/N200Q/C252S/K264E, E59K/K99E/N113Q/N281Q/C252S/K264E,
E59K/K99E/N200Q/N281Q/C252S/K264E, E59K/K99E/N103Q/N113Q/N200Q/C252S/K264E,
E59K/K99E/N103Q/N200Q/N281Q/C252S/K264E,
E59K/K99E/N113Q/N200Q/N281Q/C252S/K264E, and
E59K/K99E/N103Q/N113Q/N200Q/N281Q/C252S/K264E.

In some embodiments, the present invention provides a homodimeric Fc fusion protein wherein said IL-12p40 subunit has a polypeptide sequence selected from the group consisting of:

i) SEQ ID NO:57 (IL-12p40(N103D)), ii) SEQ ID NO:58 (IL-12p40(N113D)), iii) SEQ ID NO:59 (IL-12p40(N200D)), iv) SEQ ID NO:60 (IL-12p40(N281D)), v) SEQ ID NO:61 (IL-12p40(N103D/N113D/N200D/N281D)), vi) SEQ ID NO:62 (IL-12p40(Q42E)), vii) SEQ ID NO:63 (IL-12p40(E45Q)), viii) SEQ ID NO:64 (IL-12p40(Q56E)), ix) SEQ ID NO:65 (IL-12p40(E59Q)), x) SEQ ID NO:66 (IL-12p40(D62N)), xi) SEQ ID NO:67 (IL-12p40(Q42E/E45Q)), xii) SEQ ID NO:68 (IL-12p40(E45Q/Q56E)), xiii) SEQ ID NO:69 (IL-12p40(Q42E/E59Q)), xiv) SEQ ID NO:70 (IL-12p40(Q56E/E59Q)), xv) SEQ ID NO:71 (IL-12p40(Q42E/E45Q/Q56E)), xvi) SEQ ID NO:72 (IL-12p40(E45Q/Q56E/E59Q)), xvii) SEQ ID NO:73 (IL-12p40(D161N)), xviii) SEQ ID NO:74 (IL-12p40(E73Q)), xix) SEQ ID NO:75 (IL-12p40(Q144E)), xx) SEQ ID NO:76 (IL-12p40(E262Q)), xxi) SEQ ID NO:77 (IL-12p40(E100Q)), xxii) SEQ ID NO:78 (IL-12p40(D18N)), xxiii) SEQ ID NO:79 (IL-12p40(E33Q)), xxiv) SEQ ID NO:80 (IL-12p40(Q229E)), xxv) SEQ ID NO:81 (IL-12p40(E235Q)), xxvi) SEQ ID NO:82 (IL-12p40(Q256N)), xxvii) SEQ ID NO:83 (IL-12p40(E299Q)), xxviii) SEQ ID NO:84 (IL-12p40(D87N)), xxix) IL-12p40(E32Q), xxx) IL-12p40(D34N), xxxi) IL-12p40(S43E), xxxii) IL-12p40(S43K), xxxiii) SEQ ID NO:XXX (IL-12p40 (E59K/K99E/N103Q/N113Q/N200Q/N281Q/C252S/K264E)), xxxiv) SEQ ID NO:205 (IL-12p40(E59K)), xxxv) IL-12p40(K99E), xxxvi) IL-12p40(K163E), xxxvii) IL-12p40(E187Q), xxxviii) IL-12p40(K258E), xxxix) IL-12p40(K260E), xl) SEQ ID NO:206 (IL-12p40(E32Q/E59Q)), xli) SEQ ID NO:207 (IL-12p40(D34N/E59Q)), xlii) SEQ ID NO:208 (IL-12p40(E59Q/E187Q)),

xliii) SEQ ID NO:209 (IL-12p40(S43E/E59Q)), xliv) SEQ ID NO:210 (IL-12p40(S43K/E49Q)), xlv) SEQ ID NO:211 (IL-12p40(E59Q/K163E)), xlvi) SEQ ID NO:212 (IL-12p40(E59Q/K99E)), xlvii) SEQ ID NO:213 (IL-12p40(E59Q/K258E)), xlviii) SEQ ID NO:214 (IL-12p40(E59Q/K260E)), xlix) SEQ ID NO: XXX (IL-12p40 (D34N/E59K)), l) SEQ ID NO: XXX (IL-12p40(E59K/K99E)), li) SEQ ID NO: XXX (IL-12p40(D18K/E59K/K99E)), lii) SEQ ID NO: XXX (IL-12p40 (E59K/K99E/K264E)), liii) SEQ ID NO: XXX (IL-12p40 (E59K/K99Y)), liv) SEQ ID NO: XXX (IL-12p40 (E59Y/K99E)), lv) SEQ ID NO: XXX (IL-12p40 (E45K/E59K/K99E)), lvi) SEQ ID NO: XXX (IL-12p40 (E59K/K99E/Q144E)), lvii) SEQ ID NO: XXX (IL-12p40 (E59K/K99E/Q144K)), lviii) SEQ ID NO: XXX (IL-12p40 (E59K/K99E/R159E)), lix) SEQ ID NO: XXX (IL-12p40 (E59K/K99E/K264E)), lx) SEQ ID NO: XXX (IL-12p40 (D18K/E59K/K99E/K264E)), lxi) SEQ ID NO: XXX (IL-12p40 (C252S)), lxii) SEQ ID NO: XXX (IL-12p40 (DI8K/E59K/K99E/C252S)), lxiii) SEQ ID NO: XXX (IL-12p40 (D18K/E59K/K99E/C252S/K264E)), lxiv) SEQ ID NO: XXX (IL-12p40 (E59K/K99Y/C252S)), lxv) SEQ ID NO: XXX (IL-12p40 (E59K/K99E/C252S/K264E)), lxvi) SEQ ID NO: XXX (IL-12p40 (E59K/K99E/C252S)), lxvii) SEQ ID NO: XXX (IL-12p40 (N103D/N113D)), lxviii) SEQ ID NO: XXX (IL-12p40 (N103D/N200D)), lxix) SEQ ID NO: XXX (IL-12p40 (N103D/N281D)), lxx) SEQ ID NO: XXX (IL-12p40 (N113D/N200D)), lxxi) SEQ ID NO: XXX (IL-12p40 (N113D/N281D)), lxxii) SEQ ID NO: XXX (IL-12p40 (N200D/N281D)), lxxiii) SEQ ID NO: XXX (IL-12p50 (N103D/N113D/N200D)), lxxiv) SEQ ID NO: XXX (IL-12p40 (N103D/N113D/N281D)), lxxv) SEQ ID NO: XXX (IL-12p40 (N103D/N200D/N281D)), lxxvi) SEQ ID NO: XXX (IL-12p40 (N113D/N200D/N281D)), lxxvii) SEQ ID NO: XXX (IL-12p40 (N103Q)), lxxviii) SEQ ID NO: XXX (IL-12p40 (N113Q)), lxxix) SEQ ID NO: XXX (IL-12p40 (N200Q)), lxxx) SEQ ID NO: XXX (IL-12p40 (N281Q)), lxxxi) SEQ ID NO: XXX (IL-12p40 (N103Q/N113Q)), lxxxii) SEQ ID NO: XXX (IL-12p40 (N103Q/N200Q)), lxxxiii) SEQ ID NO: XXX (IL-12p40 (N103Q/N281Q)), lxxxiv) SEQ ID NO: XXX (IL-12p40 (N113Q/N200Q)), lxxxv) SEQ ID NO: XXX (IL-12p40 (N113Q/N281Q)), lxxxvi) SEQ ID NO: XXX (IL-12p40 (N200Q/N281Q)), lxxxvii) SEQ ID NO: XXX (IL-12p40 (N103Q/N113Q/N200Q)), lxxxviii) SEQ ID NO: XXX (IL-12p40 (N103Q/N113Q/N281Q)), lxxxix) SEQ ID NO: XXX (IL-12p40 (N103Q/N200Q/N281Q)), xc) SEQ ID NO: XXX (IL-12p40 (N113Q/N200Q/N281Q)), xci) SEQ ID NO:XXX (IL-12p40 (N103Q/N113Q/N200Q/N281Q)), xcii) SEQ ID NO:XXX (IL-12p40 (D34N/E59K/K99E)), xciii)

SEQ ID NO:XXX (IL-12p40 (D34K/E59K/K99E)), xciv) SEQ ID NO:XXX (IL-12p40 (E32Q/D34N/E59K/K99E)), xcv) SEQ ID NO:XXX (IL-12p40 (E32K/D34N/E59K/K99E)), xcvi) SEQ ID NO: XXX (IL-12p40 (E59Y/K99Y)), xcvii) SEQ ID NO:XXX (IL-12p40 (E59K/K99E/N103Q/C252S/K264E)), xcviii) SEQ ID NO:XXX (IL-12p40 (E59K/K99E/N113Q/C252S/K264E)), xcix) SEQ ID NO:XXX (IL-12p40 (E59K/K99E/N200Q/C252S/K264E)), c) SEQ ID NO:XXX (IL-12p40 (E59K/K99E/N281Q/C252S/K264E)), ci) SEQ ID NO:XXX (IL-12p40 (E59K/K99E/N103Q/N113Q/C252S/K264E)), cii) SEQ ID NO:XXX (IL-12p40 (E59K/K99E/N103Q/N200Q/C252S/K264E)), ciii) SEQ ID NO:XXX (IL-12p40 (E59K/K99E/N103Q/N281Q/C252S/K264E)), civ) SEQ ID NO:XXX (IL-12p40 (E59K/K99E/N113Q/N200Q/C252S/K264E)), cv) SEQ ID NO:XXX (IL-12p40 (E59K/K99E/N113Q/N281Q/C252S/K264E)), cvi) SEQ ID NO:XXX (IL-12p40 (E59K/K99E/N200Q/N281Q/C252S/K264E)), cvii) SEQ ID NO:XXX (IL-12p40 (E59K/K99E/N103Q/N113Q/N200Q/C252S/K264E)), cviii) SEQ ID NO:XXX (IL-12p40 (E59K/K99E/N103Q/N200Q/N281Q/C252S/K264E)), and cix) SEQ ID NO:XXX (IL-12p40 (E59K/K99E/N113Q/N200Q/N281Q/C252S/K264E)).

[00241] In some embodiments, the present invention provides a homodimeric Fc fusion protein wherein said IL-12p35 subunit has one or more amino acid modifications as amino acid residues selected from the group consisting of Q20, N21, Q35, E38, S44, E45, E46, H49, K54, D55, T59, V60, E61, C63, L64, P65, E67, L68, N71, S73, C74, L75, N76, E79, N85, L89, F96, M97, L124, M125, Q130, Q135, N136, E143, Q146, N151, E153, K158, E162, E163, D165, I171, R181, I182, R183, V185, T186, D188, R189, V190, S192, Y193, N195, and A196.

[00242] In some embodiments, the present invention provides a homodimeric Fc fusion protein wherein said IL-12p35 subunit has one or more amino acid substitutions selected from the group consisting of N21D, Q35D, E38Q, D55Q, D55K, N71D, N71Q, L75A, N76D, E79Q, N85D, N85Q, L89A, F96A, M97A, L124A, M125A, Q130E, Q135E, N136D, E143Q, Q146E, N151D, N151K, E153K, E153Q, K158E, E162Q, E163Q, D165N, I171A, N195D, and N195Q.

[00243] In some embodiments, the present invention provides a homodimeric Fc fusion protein wherein said IL-12p35 subunit has amino acid substitutions selected from the group consisting of N71D/N85D/N195D, N151D/E153Q, N151D/D165N, Q130E/N151D, N151D/K158E, E79Q/N151D, D55Q/N151D, N136D/N151D, N21D/N151D, E143Q/N151D, N71Q/N85Q, N71Q/N195Q, N85Q/N195Q, N71Q/N85Q/N195Q, N71D/N85D, N71D/N195D, and N85D/N195D.

[00244] In some embodiments, the present invention provides a homodimeric Fc fusion protein wherein said IL-12p35 subunit has a polypeptide sequence selected from the group consisting of: i) SEQ ID NO:113 (IL-12p35(N71D)), ii) SEQ ID NO:114 (IL-12p35(N85D)), iii) SEQ ID NO:115 (IL-12p35(N195D)), iv) SEQ ID NO:116 (IL-12p35(N71D/N85D/N195D)), v) SEQ ID NO:117 (IL-12p35(E153Q)), vi) SEQ ID NO:118 (IL-12p35(E38Q)), vii) SEQ ID NO:119 (IL-12p35(N151D)), viii) SEQ ID NO:120 (IL-12p35(Q135E)), ix) SEQ ID NO:121 (IL-12p35(Q35D)), x) SEQ ID NO:122 (IL-12p35(Q146E)), xi) SEQ ID NO:123 (IL-12p35(N76D)), xii) SEQ ID NO:124 (IL-12p35(E162Q)), xiii) SEQ ID NO:125 (IL-12p35(E163Q)), xiv) IL-12p35(N21D), xv) SEQ ID NO:XXX (IL-12p35(D55Q)), xvi) IL-12p35(E79Q), xvii) IL-12p35(Q130E), xviii) IL-12p35(N136D), xix) IL-12p35(E143Q), xx) SEQ ID NO:227 (IL-12p35(N151K)), xxi) SEQ ID NO:226 (IL-12p35(E153K)), xxii) IL-12p35(K158E), xxiii) IL-12p35(D165N), xxiv) SEQ ID NO:225 (IL-12p35(N151D/E153Q)), xxv) SEQ ID NO:228 (IL-12p35(N151D/D165N)), xxvi) SEQ ID NO:229 (IL-12p35(Q130E/N151D)), xxvii) SEQ ID NO:230 (IL-12p35(N151D/K158E)), xxviii) SEQ ID NO:231 (IL-12p35(E79Q/N151D)), xxix) SEQ ID NO:232 (IL-12p35(D55Q/N151D)), xxx) SEQ ID NO:233 (IL-12p35(N136D/N151D)), xxxi) SEQ ID NO:234 (IL-12p35(N21D/N151D)), xxxii) SEQ ID NO:235 (IL-12p35(E143Q/N151D)), xxxiii) SEQ ID NO: XXX (IL-12p35(F96A)), xxxiv) SEQ ID NO: XXX (IL-12p35(M97A)), xxxv) SEQ ID NO: XXX (IL-12p35(L89A)), xxxvi) SEQ ID NO: XXX (IL-12p35(L124A)), xxxvii) SEQ ID NO: XXX (IL-12p35(M125A)), xxxviii) SEQ ID NO: XXX (IL-12p35(L75A)), xxxiv) SEQ ID NO: XXX (IL-12p35(I171A)), xxxv) SEQ ID NO: XXX (IL-12p35 (N71Q)), xxxvi) SEQ ID NO: XXX (IL-12p35 (N85Q)), xxxvii) SEQ ID NO: XXX (IL-12p35 (N195Q)), xxxviii) SEQ ID NO: XXX (IL-12p35 (N71Q/N85Q)), xxxix) SEQ ID NO: XXX (IL-12p35 (N71Q/N195Q)), xl) SEQ ID NO: XXX (IL-12p35 (N85Q/N195Q), xli) SEQ ID NO: XXX (IL-12p35 (N71Q/N85Q/N195Q)), xlii) SEQ ID NO:

XXX (IL-12p35 (N71D/N85D)), xliii) SEQ ID NO: XXX (IL-12p35 (N71D/N195D), xliv) SEQ ID NO: XXX (IL-12p35 (N85D/N195D)), xlv) SEQ ID NO: XXX (IL-12p35 (D55Q)), and xlvi) SEQ ID NO: XXX (IL-12p35 (D55K)).

[00245] In some embodiments, the present invention provides a homodimeric Fc fusion protein wherein said first and second monomers are XENP31289.

[00246] In some embodiments, the present invention provides a homodimeric Fc fusion protein wherein said first and second monomers are XENP31291.

[00247] In some embodiments, the present invention provides a homodimeric Fc fusion protein composition comprising a homodimeric Fc fusion protein for use in treating cancer in a subject.

[00248] In some embodiments, the present invention provides a one or more nucleic acids encoding a homodimeric Fc fusion protein.

[00249] In some embodiments, the present invention provides a host cell comprising said one or more nucleic acids encoding a homodimeric Fc fusion protein.

[00250] In some embodiments, the present invention provides a method of making a homodimeric Fc fusion protein, said method comprising culturing a host cell under conditions whereby said homodimeric Fc fusion protein is produced.

[00251] In some embodiments, the present invention provides a method of purifying a homodimeric Fc fusion protein, said method comprising: a) providing a composition comprising the homodimeric Fc fusion protein; b) loading said composition onto an ion exchange column; and c) collecting a fraction containing said homodimeric Fc fusion protein.

[00252] In another aspect, the present invention provides a homodimeric Fc fusion protein comprising a first monomer and a second monomer each comprising, from N- to C-terminal, an Fc domain-an optional first domain linker-an IL-12p40 subunit domain-an optional second domain linker-an IL-12p35 subunit domain .

[00253] In some embodiments, the present invention provides a homodimeric Fc fusion protein, wherein said modifications promoting homodimerization of said Fc domain are a set of amino acid substitutions selected from the group consisting of L368D/K370S; S364K; S364K/E357L; S364K/E357Q; T411E/K360E/Q362E; D401K; T366S/L368A/Y407V; T366W; T366S/L368A/Y407V/Y349C; and T366W/S354C, according to EU numbering.

[00254] In some embodiments, the present invention provides a homodimeric Fc fusion protein wherein said first domain linker and said second domain linker have the same amino acid sequence.

[00255] In some embodiments, the present invention provides a homodimeric Fc fusion protein wherein said Fc domain has an additional set of amino acid substitutions comprising Q295E/N384D/Q418E/N421D, according to EU numbering.

[00256] In some embodiments, the present invention provides a homodimeric Fc fusion protein, wherein said Fc domain has an additional set of amino acid substitutions selected from the group consisting of G236R/L328R, E233P/L234V/L235A/G236_/S239K, E233P/L234V/L235A/G236_/S239K/A327G, E233P/L234V/L235A/G236_/S267K/A327G, E233P/L234V/L235A/G236_, and E233P/L234V/L235A/G236_/S267K, according to EU numbering.

[00257] In some embodiments, the present invention provides a homodimeric Fc fusion protein wherein said IL-12p40 subunit has a polypeptide sequence selected form the group consisting of SEQ ID NO:3 (human IL-12 subunit beta (IL-12p40) precursor sequence) and SEQ ID NO:4 (human IL-12 subunit beta (IL-12p40) mature form sequence), and said IL-12p35 subunit has a polypeptide sequence selected from the group consisting of SEQ ID NO:1 (human IL-12 subunit alpha (IL-12p35) precursor sequence) and SEQ ID NO:2 (human IL-12 subunit alpha (IL-12p35) mature form sequence).

[00258] In some embodiments, the present invention provides a homodimeric Fc fusion protein wherein each of said Fc domain further comprises amino acid substitutions M428L/N424S.

[00259] In some embodiments, the present invention provides a homodimeric Fc fusion protein wherein said IL-12p40 subunit is a variant IL-12p40 subunit and/or said IL-12p35 subunit is a variant IL-12p35 subunit.

[00260] In some embodiments, the present invention provides a homodimeric Fc fusion protein wherein said IL-12p40 subunit is a variant IL-12p40 subunit having altered affinity for IL-12 receptor subunit beta-1 (IL-12Rß1), IL-12 receptor subunit beta-2 (IL-12Rß2), and/or IL-12 receptor complex; and/or said IL-12p35 subunit is a variant IL-12p35 subunit having altered affinity for IL-12 receptor subunit beta-1 (IL-12Rß1), IL-12 receptor subunit beta-2 (IL-12Rß2), and/or IL-12 receptor complex.

[00261] In some embodiments, the present invention provides a homodimeric Fc fusion protein wherein said IL-12p40 subunit has one or more amino acid modifications at amino acid residues selected from the group consisting of E3, D7, E12, D14, W15, P17, D18, A19, P20, G21, E22, M23, D29, E32, E33, D34, L40, D41, Q42, S43, E45, L47, T54, I55, Q56, K58, E59, F60, G61, D62, Q65, Y66, E73, K84, E86, D87, G88, I89, W90, D93, D97, K99, E100, K102, N103, K104, F106, E110, N113, Y114, D129, D142, Q144, E156, R159, D161, N162, K163, D166, D170, Q172, D174, A176, C177, P178, A179, A180, E181, S183, P185, E187, N200, S204, F206, R208, D209, D214, N218, Q220, N226, Q229, E231, E235, T242, P243, S245, Y246, F247, S248, C252S, Q256, K158, K260, E262, K264, D265, D270, N281, Q289, D290, R291, Y292, Y293, and E299.

[00262] In some embodiments, the present invention provides a homodimeric Fc fusion protein wherein said IL-12p40 subunit has one or more amino acid substitutions selected from the group consisting of D18N, D18K, E32Q, E33Q, D34N, D34K, Q42E, S43E, S43K, E45Q, Q56E, E59Q, E59K, D62N, E73Q, D87N, K99E, K99Y, E100Q, N103D, N103Q, N113D, N113Q, Q144E, D161N, R159E, K163E, E187Q, N200D, N200Q, N218Q, Q229E, E235Q, C252S, Q256N, K258E, K260E, E262Q, K264E, N281D, N281Q, and E299Q.

[00263] In some embodiments, the present invention provides a homodimeric Fc fusion protein wherein said IL-12p40 subunit has amino acid substitutions selected from the group consisting of N103D/N113D/N200D/N281D, Q42E/E45Q, E45Q/Q56E, Q42E/E59Q, Q56E/E59Q,

Q42E/E45Q/Q56E, E45Q/Q56E/E59Q, E32Q/E59Q, D34N/E59K, D34N/E59K/K99E, D34K/E59K/K99E, E32Q/D34N/E59K/K99E, E32K/D34N/E59K/K99E, D34N/E59Q, E59Q/E187Q, S43E/E59Q, S43K/E49Q, E59Q/K163E, E59Q/K99E, E59Q/K258E, E59Q/K260E, E59K/K99E, D18K/E59K/K99E, E59K/K99E/K264E, E59K/K99Y, E59Y/K99Y, E59Y/K99E, E45K/E59K/K99E, E59K/K99E/Q144E, E59K/K99E/Q144K, E59K/K99E/R159E, E59K/K99E/K264E, D18K/E59K/K99E/K264E, DI8K/E59K/K99E/C252S, D18K/E59K/K99E/C252S/K264E, E59K/K99Y/C252S, E59K/K99E/C252S/K264E, E59K/K99E/C252S, N103D/N113D, N103D/N200D, N103D/N281D, N113D/N200D, N113D/N281D, N200D/N281D, N103D/N113D/N200D, N103D/N113D/N281D, N103D/N200D/N281D, N113D/N200D/N281D, N103Q/N113Q, N103Q/N200Q, N103Q/N281Q, N113Q/N200Q, N113Q/N281Q, N200Q/N281Q, N103Q/N113Q/N200Q, N103Q/N113Q/N281Q, N103Q/N200Q/N281Q, N113Q/N200Q/N281Q, N103Q/N113Q/N200Q/N281Q, E59K/K99E/N103Q/C252S/K264E, E59K/K99E/N113Q/C252S/K264E, E59K/K99E/N200Q/C252S/K264E, E59K/K99E/N281Q/C252S/K264E, E59K/K99E/N103Q/N113Q/C252S/K264E, E59K/K99E/N103Q/N200Q/C252S/K264E, E59K/K99E/N103Q/N281Q/C252S/K264E, E59K/K99E/N113Q/N200Q/C252S/K264E, E59K/K99E/N113Q/N281Q/C252S/K264E, E59K/K99E/N200Q/N281Q/C252S/K264E, E59K/K99E/N103Q/N113Q/N200Q/C252S/K264E, E59K/K99E/N103Q/N200Q/N281Q/C252S/K264E, E59K/K99E/N113Q/N200Q/N281Q/C252S/K264E, and E59K/K99E/N103Q/N113Q/N200Q/N281Q/C252S/K264E.

[00264] In some embodiments, the present invention provides a homodimeric Fc fusion protein wherein said IL-12p40 subunit has a polypeptide sequence selected from the group consisting of: i) SEQ ID NO:57 (IL-12p40(N103D)), ii) SEQ ID NO:58 (IL-12p40(N113D)), iii) SEQ ID NO:59 (IL-12p40(N200D)), iv) SEQ ID NO:60 (IL-12p40(N281D)), v) SEQ ID NO:61 (IL-12p40(N103D/N113D/N200D/N281D)), vi) SEQ ID NO:62 (IL-12p40(Q42E)), vii) SEQ ID NO:63 (IL-12p40(E45Q)), viii) SEQ ID NO:64 (IL-12p40(Q56E)), ix) SEQ ID NO:65 (IL-12p40(E59Q)), x) SEQ ID NO:66 (IL-12p40(D62N)), xi) SEQ ID NO:67 (IL-12p40(Q42E/E45Q)), xii) SEQ ID NO:68 (IL-12p40(E45Q/Q56E)), xiii) SEQ ID NO:69 (IL-12p40(Q42E/E59Q)), xiv) SEQ ID NO:70 (IL-

12p40(Q56E/E59Q)), xv) SEQ ID NO:71 (IL-12p40(Q42E/E45Q/Q56E)), xvi) SEQ ID NO:72 (IL-12p40(E45Q/Q56E/E59Q)), xvii) SEQ ID NO:73 (IL-12p40(D161N)), xviii) SEQ ID NO:74 (IL-12p40(E73Q)), xix) SEQ ID NO:75 (IL-12p40(Q144E)), xx) SEQ ID NO:76 (IL-12p40(E262Q)), xxi) SEQ ID NO:77 (IL-12p40(E100Q)), xxii) SEQ ID NO:78 (IL-12p40(D18N)), xxiii) SEQ ID NO:79 (IL-12p40(E33Q)), xxiv) SEQ ID NO:80 (IL-12p40(Q229E)), xxv) SEQ ID NO:81 (IL-12p40(E235Q)), xxvi) SEQ ID NO:82 (IL-12p40(Q256N)), xxvii) SEQ ID NO:83 (IL-12p40(E299Q)), xxviii) SEQ ID NO:84 (IL-12p40(D87N)), xxix) IL-12p40(E32Q), xxx) IL-12p40(D34N), xxxi) IL-12p40(S43E), xxxii) IL-12p40(S43K), xxxiii) SEQ ID NO:XXX (IL-12p40 (E59K/K99E/N103Q/N113Q/N200Q/N281Q/C252S/K264E)), xxxiv) SEQ ID NO:205 (IL-12p40(E59K)), xxxv) IL-12p40(K99E), xxxvi) IL-12p40(K163E), xxxvii) IL-12p40(E187Q), xxxviii) IL-12p40(K258E), xxxix) IL-12p40(K260E), xl) SEQ ID NO:206 (IL-12p40(E32Q/E59Q)), xli) SEQ ID NO:207 (IL-12p40(D34N/E59Q)), xlii) SEQ ID NO:208 (IL-12p40(E59Q/E187Q)), xliii) SEQ ID NO:209 (IL-12p40(S43E/E59Q)), xliv) SEQ ID NO:210 (IL-12p40(S43K/E49Q)), xlv) SEQ ID NO:211 (IL-12p40(E59Q/K163E)), xlvi) SEQ ID NO:212 (IL-12p40(E59Q/K99E)), xlvii) SEQ ID NO:213 (IL-12p40(E59Q/K258E)), xlviii) SEQ ID NO:214 (IL-12p40(E59Q/K260E)), xlix) SEQ ID NO: XXX (IL-12p40 (D34N/E59K)), l) SEQ ID NO: XXX (IL-12p40(E59K/K99E)), li) SEQ ID NO: XXX (IL-12p40(D18K/E59K/K99E)), lii) SEQ ID NO: XXX (IL-12p40 (E59K/K99E/K264E)), liii) SEQ ID NO: XXX (IL-12p40 (E59K/K99Y)), liv) SEQ ID NO: XXX (IL-12p40 (E59Y/K99E)), lv) SEQ ID NO: XXX (IL-12p40 (E45K/E59K/K99E)), lvi) SEQ ID NO: XXX (IL-12p40 (E59K/K99E/Q144E)), lvii) SEQ ID NO: XXX (IL-12p40 (E59K/K99E/Q144K)), lviii) SEQ ID NO: XXX (IL-12p40 (E59K/K99E/R159E)), lix) SEQ ID NO: XXX (IL-12p40 (E59K/K99E/K264E)), lx) SEQ ID NO: XXX (IL-12p40 (D18K/E59K/K99E/K264E)), lxi) SEQ ID NO: XXX (IL-12p40 (C252S)), lxii) SEQ ID NO: XXX (IL-12p40 (DI8K/E59K/K99E/C252S)), lxiii) SEQ ID NO: XXX (IL-12p40 (D18K/E59K/K99E/C252S/K264E)), lxiv) SEQ ID NO: XXX (IL-12p40 (E59K/K99Y/C252S)), lxv) SEQ ID NO: XXX (IL-12p40 (E59K/K99E/C252S/K264E)), lxvi) SEQ ID NO: XXX (IL-12p40 (E59K/K99E/C252S)), lxvii) SEQ ID NO: XXX (IL-12p40 (N103D/N113D)), lxviii) SEQ ID NO: XXX (IL-12p40 (N103D/N200D)), lxix) SEQ ID NO: XXX (IL-12p40 (N103D/N281D)), lxx) SEQ ID NO: XXX (IL-12p40 (N113D/N200D)), lxxi) SEQ ID NO: XXX (IL-12p40 (N113D/N281D)), lxxii) SEQ ID NO: XXX (IL-12p40 (N200D/N281D)), lxxiii) SEQ ID NO: XXX (IL-12p50

(N103D/N113D/N200D)), lxxiv) SEQ ID NO: XXX (IL-12p40 (N103D/N113D/N281D)), lxxv) SEQ ID NO: XXX (IL-12p40 (N103D/N200D/N281D)), lxxvi) SEQ ID NO: XXX (IL-12p40 (N113D/N200D/N281D)), lxxvii) SEQ ID NO: XXX (IL-12p40 (N103Q)), lxxviii) SEQ ID NO: XXX (IL-12p40 (N113Q)), lxxix) SEQ ID NO: XXX (IL-12p40 (N200Q)), lxxx) SEQ ID NO: XXX (IL-12p40 (N281Q)), lxxxi) SEQ ID NO: XXX (IL-12p40 (N103Q/N113Q)), lxxxii) SEQ ID NO: XXX (IL-12p40 (N103Q/N200Q)), lxxxiii) SEQ ID NO: XXX (IL-12p40 (N103Q/N281Q)), lxxxiv) SEQ ID NO: XXX (IL-12p40 (N113Q/N200Q)), lxxxv) SEQ ID NO: XXX (IL-12p40 (N113Q/N281Q)), lxxxvi) SEQ ID NO: XXX (IL-12p40 (N200Q/N281Q)), lxxxvii) SEQ ID NO: XXX (IL-12p40 (N103Q/N113Q/N200Q)), lxxxviii) SEQ ID NO: XXX (IL-12p40 (N103Q/N113Q/N281Q)), lxxxix) SEQ ID NO: XXX (IL-12p40 (N103Q/N200Q/N281Q)), xc) SEQ ID NO: XXX (IL-12p40 (N113Q/N200Q/N281Q)), xci) SEQ ID NO:XXX (IL-12p40 (N103Q/N113Q/N200Q/N281Q)), xcii) SEQ ID NO:XXX (IL-12p40 (D34N/E59K/K99E)), xciii) SEQ ID NO:XXX (IL-12p40 (D34K/E59K/K99E)), xciv) SEQ ID NO:XXX (IL-12p40 (E32Q/D34N/E59K/K99E)), xcv) SEQ ID NO:XXX (IL-12p40 (E32K/D34N/E59K/K99E)), xcvi) SEQ ID NO: XXX (IL-12p40 (E59Y/K99Y)), xcvii) SEQ ID NO:XXX (IL-12p40 (E59K/K99E/N103Q/C252S/K264E)), xcviii) SEQ ID NO:XXX (IL-12p40 (E59K/K99E/N113Q/C252S/K264E)), xcix) SEQ ID NO:XXX (IL-12p40 (E59K/K99E/N200Q/C252S/K264E)), c) SEQ ID NO:XXX (IL-12p40 (E59K/K99E/N281Q/C252S/K264E)), ci) SEQ ID NO:XXX (IL-12p40 (E59K/K99E/N103Q/N113Q/C252S/K264E)), cii) SEQ ID NO:XXX (IL-12p40 (E59K/K99E/N103Q/N200Q/C252S/K264E)), ciii) SEQ ID NO:XXX (IL-12p40 (E59K/K99E/N103Q/N281Q/C252S/K264E)), civ) SEQ ID NO:XXX (IL-12p40 (E59K/K99E/N113Q/N200Q/C252S/K264E)), cv) SEQ ID NO:XXX (IL-12p40 (E59K/K99E/N113Q/N281Q/C252S/K264E)), cvi) SEQ ID NO:XXX (IL-12p40 (E59K/K99E/N200Q/N281Q/C252S/K264E)), cvii) SEQ ID NO:XXX (IL-12p40 (E59K/K99E/N103Q/N113Q/N200Q/C252S/K264E)), cviii) SEQ ID NO:XXX (IL-12p40 (E59K/K99E/N103Q/N200Q/N281Q/C252S/K264E)), and cix) SEQ ID NO:XXX (IL-12p40 (E59K/K99E/N113Q/N200Q/N281Q/C252S/K264E)).

[00265] In some embodiments, the present invention provides a homodimeric Fc fusion protein wherein said IL-12p35 subunit has one or more amino acid modifications as amino acid residues selected from the group consisting of Q20, N21, Q35, E38, S44, E45, E46, H49, K54, D55, T59, V60, E61, C63, L64, P65, E67, L68, N71, S73, C74, L75, N76, E79, N85, L89, F96, M97, L124, M125, Q130, Q135, N136, E143, Q146, N151, E153, K158, E162, E163, D165, I171, R181, I182, R183, V185, T186, D188, R189, V190, S192, Y193, N195, and A196.

[00266] In some embodiments, the present invention provides a homodimeric Fc fusion protein wherein said IL-12p35 subunit has one or more amino acid substitutions selected from the group consisting of N21D, Q35D, E38Q, D55Q, D55K, N71D, N71Q, L75A, N76D, E79Q, N85D, N85Q, L89A, F96A, M97A, L124A, M125A, Q130E, Q135E, N136D, E143Q, Q146E, N151D, N151K, E153K, E153Q, K158E, E162Q, E163Q, D165N, I171A, N195D, and N195Q.

[00267] In some embodiments, the present invention provides a homodimeric Fc fusion protein wherein said IL-12p35 subunit has amino acid substitutions selected from the group consisting of N71D/N85D/N195D, N151D/E153Q, N151D/D165N, Q130E/N151D, N151D/K158E, E79Q/N151D, D55Q/N151D, N136D/N151D, N21D/N151D, E143Q/N151D, N71Q/N85Q, N71Q/N195Q, N85Q/N195Q, N71Q/N85Q/N195Q, N71D/N85D, N71D/N195D, and N85D/N195D.

[00268] In some embodiments, the present invention provides a homodimeric Fc fusion protein wherein said IL-12p35 subunit has a polypeptide sequence selected from the group consisting of: i) SEQ ID NO:113 (IL-12p35(N71D)), ii) SEQ ID NO:114 (IL-12p35(N85D)), iii) SEQ ID NO:115 (IL-12p35(N195D)), iv) SEQ ID NO:116 (IL-12p35(N71D/N85D/N195D)), v) SEQ ID NO:117 (IL-12p35(E153Q)), vi) SEQ ID NO:118 (IL-12p35(E38Q)), vii) SEQ ID NO:119 (IL-12p35(N151D)), viii) SEQ ID NO:120 (IL-12p35(Q135E)), ix) SEQ ID NO:121 (IL-12p35(Q35D)), x) SEQ ID NO:122 (IL-12p35(Q146E)), xi) SEQ ID NO:123 (IL-12p35(N76D)), xii) SEQ ID NO:124 (IL-12p35(E162Q)), xiii) SEQ ID NO:125 (IL-12p35(E163Q)), xiv) IL-12p35(N21D), xv) SEQ ID NO:XXX (IL-12p35(D55Q)), xvi) IL-12p35(E79Q), xvii) IL-12p35(Q130E), xviii) IL-12p35(N136D), xix) IL-12p35(E143Q), xx) SEQ ID NO:227 (IL-12p35(N151K)), xxi) SEQ ID NO:226 (IL-12p35(E153K)), xxii) IL-

12p35(K158E), xxiii) IL-12p35(D165N), xxiv) SEQ ID NO:225 (IL-12p35(N151D/E153Q)), xxv) SEQ ID NO:228 (IL-12p35(N151D/D165N)), xxvi) SEQ ID NO:229 (IL-12p35(Q130E/N151D)), xxvii) SEQ ID NO:230 (IL-12p35(N151D/K158E)), xxviii) SEQ ID NO:231 (IL-12p35(E79Q/N151D)), xxix) SEQ ID NO:232 (IL-12p35(D55Q/N151D)), xxx) SEQ ID NO:233 (IL-12p35(N136D/N151D)), xxxi) SEQ ID NO:234 (IL-12p35(N21D/N151D)), xxxii) SEQ ID NO:235 (IL-12p35(E143Q/N151D)), xxxiii) SEQ ID NO: XXX (IL-12p35(F96A)), xxxiv) SEQ ID NO: XXX (IL-12p35(M97A)), xxxv) SEQ ID NO: XXX (IL-12p35(L89A)), xxxvi) SEQ ID NO: XXX (IL-12p35(L124A)), xxxvii) SEQ ID NO: XXX (IL-12p35(M125A)), xxxviii) SEQ ID NO: XXX (IL-12p35(L75A)), xxxiv) SEQ ID NO: XXX (IL-12p35(I171A)), xxxv) SEQ ID NO: XXX (IL-12p35 (N71Q)), xxxvi) SEQ ID NO: XXX (IL-12p35 (N85Q)), xxxvii) SEQ ID NO: XXX (IL-12p35 (N195Q)), xxxviii) SEQ ID NO: XXX (IL-12p35 (N71Q/N85Q)), xxxix) SEQ ID NO: XXX (IL-12p35 (N71Q/N195Q)), xl) SEQ ID NO: XXX (IL-12p35 (N85Q/N195Q), xli) SEQ ID NO: XXX (IL-12p35 (N71Q/N85Q/N195Q)), xlii) SEQ ID NO: XXX (IL-12p35 (N71D/N85D)), xliii) SEQ ID NO: XXX (IL-12p35 (N71D/N195D), xliv) SEQ ID NO: XXX (IL-12p35 (N85D/N195D)), xlv) SEQ ID NO: XXX (IL-12p35 (D55Q)), and xlvi) SEQ ID NO: XXX (IL-12p35 (D55K)).

[00269] In some embodiments, the present invention provides a homodimeric Fc fusion protein wherein said first and second monomers are XENP31289.

[00270] In some embodiments, the present invention provides a homodimeric Fc fusion protein wherein said first and second monomers are XENP31291.

[00271] In some embodiments, the present invention provides a homodimeric Fc fusion protein composition comprising a homodimeric Fc fusion protein for use in treating cancer in a subject.

[00272] In some embodiments, the present invention provides a one or more nucleic acids encoding a homodimeric Fc fusion protein.

[00273] In some embodiments, the present invention provides a host cell comprising said one or more nucleic acids encoding a homodimeric Fc fusion protein.

[00274] In some embodiments, the present invention provides a method of making a homodimeric Fc fusion protein, said method comprising culturing a host cell under conditions whereby said homodimeric Fc fusion protein is produced.

[00275] In some embodiments, the present invention provides a method of purifying a homodimeric Fc fusion protein, said method comprising: a) providing a composition comprising the homodimeric Fc fusion protein; b) loading said composition onto an ion exchange column; and c) collecting a fraction containing said homodimeric Fc fusion protein.

[00276] In another aspect, the present invention provides a homodimeric Fc fusion protein comprising a first monomer and a second monomer each comprising, from N- to C-terminal, an Fc domain-an optional first domain linker-an IL-12p35 subunit domain –an optional second domain linker-an IL-12p40 subunit domain.

[00277] In some embodiments, the present invention provides a homodimeric Fc fusion protein, wherein said modifications promoting homodimerization of said Fc domain are a set of amino acid substitutions selected from the group consisting of L368D/K370S; S364K; S364K/E357L; S364K/E357Q; T411E/K360E/Q362E; D401K; T366S/L368A/Y407V; T366W; T366S/L368A/Y407V/Y349C; and T366W/S354C, according to EU numbering.

[00278] In some embodiments, the present invention provides a homodimeric Fc fusion protein wherein said first domain linker and said second domain linker have the same amino acid sequence.

[00279] In some embodiments, the present invention provides a homodimeric Fc fusion protein wherein said Fc domain has an additional set of amino acid substitutions comprising Q295E/N384D/Q418E/N421D, according to EU numbering.

[00280] In some embodiments, the present invention provides a homodimeric Fc fusion protein, wherein said Fc domain has an additional set of amino acid substitutions selected from the group consisting of G236R/L328R, E233P/L234V/L235A/G236_/S239K, E233P/L234V/L235A/G236_/S239K/A327G, E233P/L234V/L235A/G236_/S267K/A327G,

E233P/L234V/L235A/G236_, and E233P/L234V/L235A/G236_/S267K, according to EU numbering.

[00281] In some embodiments, the present invention provides a homodimeric Fc fusion protein wherein said IL-12p40 subunit has a polypeptide sequence selected form the group consisting of SEQ ID NO:3 (human IL-12 subunit beta (IL-12p40) precursor sequence) and SEQ ID NO:4 (human IL-12 subunit beta (IL-12p40) mature form sequence), and said IL-12p35 subunit has a polypeptide sequence selected from the group consisting of SEQ ID NO:1 (human IL-12 subunit alpha (IL-12p35) precursor sequence) and SEQ ID NO:2 (human IL-12 subunit alpha (IL-12p35) mature form sequence).

[00282] In some embodiments, the present invention provides a homodimeric Fc fusion protein wherein each of said Fc domain further comprises amino acid substitutions M428L/N424S.

[00283] In some embodiments, the present invention provides a homodimeric Fc fusion protein wherein said IL-12p40 subunit is a variant IL-12p40 subunit and/or said IL-12p35 subunit is a variant IL-12p35 subunit.

[00284] In some embodiments, the present invention provides a homodimeric Fc fusion protein wherein said IL-12p40 subunit is a variant IL-12p40 subunit having altered affinity for IL-12 receptor subunit beta-1 (IL-12Rß1), IL-12 receptor subunit beta-2 (IL-12Rß2), and/or IL-12 receptor complex; and/or said IL-12p35 subunit is a variant IL-12p35 subunit having altered affinity for IL-12 receptor subunit beta-1 (IL-12Rß1), IL-12 receptor subunit beta-2 (IL-12Rß2), and/or IL-12 receptor complex.

[00285] In some embodiments, the present invention provides a homodimeric Fc fusion protein wherein said IL-12p40 subunit has one or more amino acid modifications at amino acid residues selected from the group consisting of E3, D7, E12, D14, W15, P17, D18, A19, P20, G21, E22, M23, D29, E32, E33, D34, L40, D41, Q42, S43, E45, L47, T54, I55, Q56, K58, E59, F60, G61, D62, Q65, Y66, E73, K84, E86, D87, G88, I89, W90, D93, D97, K99, E100, K102, N103, K104, F106, E110, N113, Y114, D129, D142, Q144, E156, R159, D161, N162, K163, D166, D170, Q172, D174, A176, C177, P178,

A179, A180, E181, S183, P185, E187, N200, S204, F206, R208, D209, D214, N218, Q220, N226, Q229, E231, E235, T242, P243, S245, Y246, F247, S248, C252S, Q256, K158, K260, E262, K264, D265, D270, N281, Q289, D290, R291, Y292, Y293, and E299.

[00286] In some embodiments, the present invention provides a homodimeric Fc fusion protein wherein said IL-12p40 subunit has one or more amino acid substitutions selected from the group consisting of D18N, D18K, E32Q, E33Q, D34N, D34K, Q42E, S43E, S43K, E45Q, Q56E, E59Q, E59K, D62N, E73Q, D87N, K99E, K99Y, E100Q, N103D, N103Q, N113D, N113Q, Q144E, D161N, R159E, K163E, E187Q, N200D, N200Q, N218Q, Q229E, E235Q, C252S, Q256N, K258E, K260E, E262Q, K264E, N281D, N281Q, and E299Q.

[00287] In some embodiments, the present invention provides a homodimeric Fc fusion protein wherein said IL-12p40 subunit has amino acid substitutions selected from the group consisting of N103D/N113D/N200D/N281D, Q42E/E45Q, E45Q/Q56E, Q42E/E59Q, Q56E/E59Q, Q42E/E45Q/Q56E, E45Q/Q56E/E59Q, E32Q/E59Q, D34N/E59K, D34N/E59K/K99E, D34K/E59K/K99E, E32Q/D34N/E59K/K99E, E32K/D34N/E59K/K99E, D34N/E59Q, E59Q/E187Q, S43E/E59Q, S43K/E49Q, E59Q/K163E, E59Q/K99E, E59Q/K258E, E59Q/K260E, E59K/K99E, D18K/E59K/K99E, E59K/K99E/K264E, E59K/K99Y, E59Y/K99Y, E59Y/K99E, E45K/E59K/K99E, E59K/K99E/Q144E, E59K/K99E/Q144K, E59K/K99E/R159E, E59K/K99E/K264E, D18K/E59K/K99E/K264E, DI8K/E59K/K99E/C252S, D18K/E59K/K99E/C252S/K264E, E59K/K99Y/C252S, E59K/K99E/C252S/K264E, E59K/K99E/C252S, N103D/N113D, N103D/N200D, N103D/N281D, N113D/N200D, N113D/N281D, N200D/N281D, N103D/N113D/N200D, N103D/N113D/N281D, N103D/N200D/N281D, N113D/N200D/N281D, N103Q/N113Q, N103Q/N200Q, N103Q/N281Q, N113Q/N200Q, N113Q/N281Q, N200Q/N281Q, N103Q/N113Q/N200Q, N103Q/N113Q/N281Q, N103Q/N200Q/N281Q, N113Q/N200Q/N281Q, N103Q/N113Q/N200Q/N281Q, E59K/K99E/N103Q/C252S/K264E, E59K/K99E/N113Q/C252S/K264E, E59K/K99E/N200Q/C252S/K264E, E59K/K99E/N281Q/C252S/K264E, E59K/K99E/N103Q/N113Q/C252S/K264E, E59K/K99E/N103Q/N200Q/C252S/K264E, E59K/K99E/N103Q/N281Q/C252S/K264E, E59K/K99E/N113Q/N200Q/C252S/K264E, E59K/K99E/N113Q/N281Q/C252S/K264E,

E59K/K99E/N200Q/N281Q/C252S/K264E, E59K/K99E/N103Q/N113Q/N200Q/C252S/K264E, E59K/K99E/N103Q/N200Q/N281Q/C252S/K264E, E59K/K99E/N113Q/N200Q/N281Q/C252S/K264E, and E59K/K99E/N103Q/N113Q/N200Q/N281Q/C252S/K264E.

[00288] In some embodiments, the present invention provides a homodimeric Fc fusion protein wherein said IL-12p40 subunit has a polypeptide sequence selected from the group consisting of: i) SEQ ID NO:57 (IL-12p40(N103D)), ii) SEQ ID NO:58 (IL-12p40(N113D)), iii) SEQ ID NO:59 (IL-12p40(N200D)), iv) SEQ ID NO:60 (IL-12p40(N281D)), v) SEQ ID NO:61 (IL-12p40(N103D/N113D/N200D/N281D)), vi) SEQ ID NO:62 (IL-12p40(Q42E)), vii) SEQ ID NO:63 (IL-12p40(E45Q)), viii) SEQ ID NO:64 (IL-12p40(Q56E)), ix) SEQ ID NO:65 (IL-12p40(E59Q)), x) SEQ ID NO:66 (IL-12p40(D62N)), xi) SEQ ID NO:67 (IL-12p40(Q42E/E45Q)), xii) SEQ ID NO:68 (IL-12p40(E45Q/Q56E)), xiii) SEQ ID NO:69 (IL-12p40(Q42E/E59Q)), xiv) SEQ ID NO:70 (IL-12p40(Q56E/E59Q)), xv) SEQ ID NO:71 (IL-12p40(Q42E/E45Q/Q56E)), xvi) SEQ ID NO:72 (IL-12p40(E45Q/Q56E/E59Q)), xvii) SEQ ID NO:73 (IL-12p40(D161N)), xviii) SEQ ID NO:74 (IL-12p40(E73Q)), xix) SEQ ID NO:75 (IL-12p40(Q144E)), xx) SEQ ID NO:76 (IL-12p40(E262Q)), xxi) SEQ ID NO:77 (IL-12p40(E100Q)), xxii) SEQ ID NO:78 (IL-12p40(D18N)), xxiii) SEQ ID NO:79 (IL-12p40(E33Q)), xxiv) SEQ ID NO:80 (IL-12p40(Q229E)), xxv) SEQ ID NO:81 (IL-12p40(E235Q)), xxvi) SEQ ID NO:82 (IL-12p40(Q256N)), xxvii) SEQ ID NO:83 (IL-12p40(E299Q)), xxviii) SEQ ID NO:84 (IL-12p40(D87N)), xxix) IL-12p40(E32Q), xxx) IL-12p40(D34N), xxxi) IL-12p40(S43E), xxxii) IL-12p40(S43K), xxxiii) SEQ ID NO:XXX (IL-12p40 (E59K/K99E/N103Q/N113Q/N200Q/N281Q/C252S/K264E)), xxxiv) SEQ ID NO:205 (IL-12p40(E59K)), xxxv) IL-12p40(K99E), xxxvi) IL-12p40(K163E), xxxvii) IL-12p40(E187Q), xxxviii) IL-12p40(K258E), xxxix) IL-12p40(K260E), xl) SEQ ID NO:206 (IL-12p40(E32Q/E59Q)), xli) SEQ ID NO:207 (IL-12p40(D34N/E59Q)), xlii) SEQ ID NO:208 (IL-12p40(E59Q/E187Q)), xliii) SEQ ID NO:209 (IL-12p40(S43E/E59Q)), xliv) SEQ ID NO:210 (IL-12p40(S43K/E49Q)), xlv) SEQ ID NO:211 (IL-12p40(E59Q/K163E)), xlvi) SEQ ID NO:212 (IL-12p40(E59Q/K99E)), xlvii) SEQ ID NO:213 (IL-12p40(E59Q/K258E)), xlviii) SEQ ID NO:214 (IL-12p40(E59Q/K260E)), xlix) SEQ ID NO: XXX (IL-12p40 (D34N/E59K)), l) SEQ ID NO: XXX (IL-12p40(E59K/K99E)), li) SEQ ID NO:

XXX (IL-12p40(D18K/E59K/K99E)), lii) SEQ ID NO: XXX (IL-12p40 (E59K/K99E/K264E)), liii) SEQ ID NO: XXX (IL-12p40 (E59K/K99Y)), liv) SEQ ID NO: XXX (IL-12p40 (E59Y/K99E)), lv) SEQ ID NO: XXX (IL-12p40 (E45K/E59K/K99E)), lvi) SEQ ID NO: XXX (IL-12p40 (E59K/K99E/Q144E)), lvii) SEQ ID NO: XXX (IL-12p40 (E59K/K99E/Q144K)), lviii) SEQ ID NO: XXX (IL-12p40 (E59K/K99E/R159E)), lix) SEQ ID NO: XXX (IL-12p40 (E59K/K99E/K264E)), lx) SEQ ID NO: XXX (IL-12p40 (D18K/E59K/K99E/K264E)), lxi) SEQ ID NO: XXX (IL-12p40 (C252S)), lxii) SEQ ID NO: XXX (IL-12p40 (DI8K/E59K/K99E/C252S)), lxiii) SEQ ID NO: XXX (IL-12p40 (D18K/E59K/K99E/C252S/K264E)), lxiv) SEQ ID NO: XXX (IL-12p40 (E59K/K99Y/C252S)), lxv) SEQ ID NO: XXX (IL-12p40 (E59K/K99E/C252S/K264E)), lxvi) SEQ ID NO: XXX (IL-12p40 (E59K/K99E/C252S)), lxvii) SEQ ID NO: XXX (IL-12p40 (N103D/N113D)), lxviii) SEQ ID NO: XXX (IL-12p40 (N103D/N200D)), lxix) SEQ ID NO: XXX (IL-12p40 (N103D/N281D)), lxx) SEQ ID NO: XXX (IL-12p40 (N113D/N200D)), lxxi) SEQ ID NO: XXX (IL-12p40 (N113D/N281D)), lxxii) SEQ ID NO: XXX (IL-12p40 (N200D/N281D)), lxxiii) SEQ ID NO: XXX (IL-12p50 (N103D/N113D/N200D)), lxxiv) SEQ ID NO: XXX (IL-12p40 (N103D/N113D/N281D)), lxxv) SEQ ID NO: XXX (IL-12p40 (N103D/N200D/N281D)), lxxvi) SEQ ID NO: XXX (IL-12p40 (N113D/N200D/N281D)), lxxvii) SEQ ID NO: XXX (IL-12p40 (N103Q)), lxxviii) SEQ ID NO: XXX (IL-12p40 (N113Q)), lxxix) SEQ ID NO: XXX (IL-12p40 (N200Q)), lxxx) SEQ ID NO: XXX (IL-12p40 (N281Q)), lxxxi) SEQ ID NO: XXX (IL-12p40 (N103Q/N113Q)), lxxxii) SEQ ID NO: XXX (IL-12p40 (N103Q/N200Q)), lxxxiii) SEQ ID NO: XXX (IL-12p40 (N103Q/N281Q)), lxxxiv) SEQ ID NO: XXX (IL-12p40 (N113Q/N200Q)), lxxxv) SEQ ID NO: XXX (IL-12p40 (N113Q/N281Q)), lxxxvi) SEQ ID NO: XXX (IL-12p40 (N200Q/N281Q)), lxxxvii) SEQ ID NO: XXX (IL-12p40 (N103Q/N113Q/N200Q)), lxxxviii) SEQ ID NO: XXX (IL-12p40 (N103Q/N113Q/N281Q)), lxxxix) SEQ ID NO: XXX (IL-12p40 (N103Q/N200Q/N281Q)), xc) SEQ ID NO: XXX (IL-12p40 (N113Q/N200Q/N281Q)), xci) SEQ ID NO:XXX (IL-12p40 (N103Q/N113Q/N200Q/N281Q)), xcii) SEQ ID NO:XXX (IL-12p40 (D34N/E59K/K99E)), xciii) SEQ ID NO:XXX (IL-12p40 (D34K/E59K/K99E)), xciv) SEQ ID NO:XXX (IL-12p40 (E32Q/D34N/E59K/K99E)), xcv) SEQ ID NO:XXX (IL-12p40 (E32K/D34N/E59K/K99E)), xcvi) SEQ ID NO: XXX (IL-12p40 (E59Y/K99Y)), xcvii) SEQ ID NO:XXX (IL-12p40 (E59K/K99E/N103Q/C252S/K264E)), xcviii) SEQ ID NO:XXX (IL-12p40 (E59K/K99E/N113Q/C252S/K264E)), xcix) SEQ ID NO:XXX (IL-12p40

(E59K/K99E/N200Q/C252S/K264E)), c) SEQ ID NO:XXX (IL-12p40 (E59K/K99E/N281Q/C252S/K264E)), ci) SEQ ID NO:XXX (IL-12p40 (E59K/K99E/N103Q/N113Q/C252S/K264E)), cii) SEQ ID NO:XXX (IL-12p40 (E59K/K99E/N103Q/N200Q/C252S/K264E)), ciii) SEQ ID NO:XXX (IL-12p40 (E59K/K99E/N103Q/N281Q/C252S/K264E)), civ) SEQ ID NO:XXX (IL-12p40 (E59K/K99E/N113Q/N200Q/C252S/K264E)), cv) SEQ ID NO:XXX (IL-12p40 (E59K/K99E/N113Q/N281Q/C252S/K264E)), cvi) SEQ ID NO:XXX (IL-12p40 (E59K/K99E/N200Q/N281Q/C252S/K264E)), cvii) SEQ ID NO:XXX (IL-12p40 (E59K/K99E/N103Q/N113Q/N200Q/C252S/K264E)), cviii) SEQ ID NO:XXX (IL-12p40 (E59K/K99E/N103Q/N200Q/N281Q/C252S/K264E)), and cix) SEQ ID NO:XXX (IL-12p40 (E59K/K99E/N113Q/N200Q/N281Q/C252S/K264E)).

[00289] In some embodiments, the present invention provides a homodimeric Fc fusion protein wherein said IL-12p35 subunit has one or more amino acid modifications as amino acid residues selected from the group consisting of Q20, N21, Q35, E38, S44, E45, E46, H49, K54, D55, T59, V60, E61, C63, L64, P65, E67, L68, N71, S73, C74, L75, N76, E79, N85, L89, F96, M97, L124, M125, Q130, Q135, N136, E143, Q146, N151, E153, K158, E162, E163, D165, I171, R181, I182, R183, V185, T186, D188, R189, V190, S192, Y193, N195, and A196.

[00290] In some embodiments, the present invention provides a homodimeric Fc fusion protein wherein said IL-12p35 subunit has one or more amino acid substitutions selected from the group consisting of N21D, Q35D, E38Q, D55Q, D55K, N71D, N71Q, L75A, N76D, E79Q, N85D, N85Q, L89A, F96A, M97A, L124A, M125A, Q130E, Q135E, N136D, E143Q, Q146E, N151D, N151K, E153K, E153Q, K158E, E162Q, E163Q, D165N, I171A, N195D, and N195Q.

[00291] In some embodiments, the present invention provides a homodimeric Fc fusion protein wherein said IL-12p35 subunit has amino acid substitutions selected from the group consisting of N71D/N85D/N195D, N151D/E153Q, N151D/D165N, Q130E/N151D, N151D/K158E, E79Q/N151D, D55Q/N151D, N136D/N151D, N21D/N151D, E143Q/N151D, N71Q/N85Q,

N71Q/N195Q, N85Q/N195Q, N71Q/N85Q/N195Q, N71D/N85D, N71D/N195D, and N85D/N195D.

[00292] In some embodiments, the present invention provides a homodimeric Fc fusion protein wherein said IL-12p35 subunit has a polypeptide sequence selected from the group consisting of: i) SEQ ID NO:113 (IL-12p35(N71D)), ii) SEQ ID NO:114 (IL-12p35(N85D)), iii) SEQ ID NO:115 (IL-12p35(N195D)), iv) SEQ ID NO:116 (IL-12p35(N71D/N85D/N195D)), v) SEQ ID NO:117 (IL-12p35(E153Q)), vi) SEQ ID NO:118 (IL-12p35(E38Q)), vii) SEQ ID NO:119 (IL-12p35(N151D)), viii) SEQ ID NO:120 (IL-12p35(Q135E)), ix) SEQ ID NO:121 (IL-12p35(Q35D)), x) SEQ ID NO:122 (IL-12p35(Q146E)), xi) SEQ ID NO:123 (IL-12p35(N76D)), xii) SEQ ID NO:124 (IL-12p35(E162Q)), xiii) SEQ ID NO:125 (IL-12p35(E163Q)), xiv) IL-12p35(N21D), xv) SEQ ID NO:XXX (IL-12p35(D55Q)), xvi) IL-12p35(E79Q), xvii) IL-12p35(Q130E), xviii) IL-12p35(N136D), xix) IL-12p35(E143Q), xx) SEQ ID NO:227 (IL-12p35(N151K)), xxi) SEQ ID NO:226 (IL-12p35(E153K)), xxii) IL-12p35(K158E), xxiii) IL-12p35(D165N), xxiv) SEQ ID NO:225 (IL-12p35(N151D/E153Q)), xxv) SEQ ID NO:228 (IL-12p35(N151D/D165N)), xxvi) SEQ ID NO:229 (IL-12p35(Q130E/N151D)), xxvii) SEQ ID NO:230 (IL-12p35(N151D/K158E)), xxviii) SEQ ID NO:231 (IL-12p35(E79Q/N151D)), xxix) SEQ ID NO:232 (IL-12p35(D55Q/N151D)), xxx) SEQ ID NO:233 (IL-12p35(N136D/N151D)), xxxi) SEQ ID NO:234 (IL-12p35(N21D/N151D)), xxxii) SEQ ID NO:235 (IL-12p35(E143Q/N151D)), xxxiii) SEQ ID NO: XXX (IL-12p35(F96A)), xxxiv) SEQ ID NO: XXX (IL-12p35(M97A)), xxxv) SEQ ID NO: XXX (IL-12p35(L89A)), xxxvi) SEQ ID NO: XXX (IL-12p35(L124A)), xxxvii) SEQ ID NO: XXX (IL-12p35(M125A)), xxxviii) SEQ ID NO: XXX (IL-12p35(L75A)), xxxiv) SEQ ID NO: XXX (IL-12p35(I171A)), xxxv) SEQ ID NO: XXX (IL-12p35 (N71Q)), xxxvi) SEQ ID NO: XXX (IL-12p35 (N85Q)), xxxvii) SEQ ID NO: XXX (IL-12p35 (N195Q)), xxxviii) SEQ ID NO: XXX (IL-12p35 (N71Q/N85Q)), xxxix) SEQ ID NO: XXX (IL-12p35 (N71Q/N195Q)), xl) SEQ ID NO: XXX (IL-12p35 (N85Q/N195Q), xli) SEQ ID NO: XXX (IL-12p35 (N71Q/N85Q/N195Q)), xlii) SEQ ID NO: XXX (IL-12p35 (N71D/N85D)), xliii) SEQ ID NO: XXX (IL-12p35 (N71D/N195D), xliv) SEQ ID NO: XXX (IL-12p35 (N85D/N195D)), xlv) SEQ ID NO: XXX (IL-12p35 (D55Q)), and xlvi) SEQ ID NO: XXX (IL-12p35 (D55K)).

[00293] In some embodiments, the present invention provides a homodimeric Fc fusion protein wherein said first and second monomers are XENP31289.

[00294] In some embodiments, the present invention provides a homodimeric Fc fusion protein wherein said first and second monomers are XENP31291.

[00295] In some embodiments, the present invention provides a homodimeric Fc fusion protein composition comprising a homodimeric Fc fusion protein for use in treating cancer in a subject.

[00296] In some embodiments, the present invention provides a one or more nucleic acids encoding a homodimeric Fc fusion protein.

[00297] In some embodiments, the present invention provides a host cell comprising said one or more nucleic acids encoding a homodimeric Fc fusion protein.

[00298] In some embodiments, the present invention provides a method of making a homodimeric Fc fusion protein, said method comprising culturing a host cell under conditions whereby said homodimeric Fc fusion protein is produced.

[00299] In some embodiments, the present invention provides a method of purifying a homodimeric Fc fusion protein, said method comprising: a) providing a composition comprising the homodimeric Fc fusion protein; b) loading said composition onto an ion exchange column; and c) collecting a fraction containing said homodimeric Fc fusion protein.

[00300] In another aspect, the present invention provides a homodimeric Fc fusion protein comprising a first monomer and a second monomer each comprising, from N- to C-terminal, an IL-12p40 subunit domain- a first domain linker-an IL-12p35 subunit domain – a second domain linker-Fc domain.

[00301] In some embodiments, the present invention provides a homodimeric Fc fusion protein, wherein said modifications promoting homodimerization of said Fc domain are a set of amino acid substitutions selected from the group consisting of L368D/K370S; S364K; S364K/E357L;

S364K/E357Q; T411E/K360E/Q362E; D401K; T366S/L368A/Y407V; T366W; T366S/L368A/Y407V/Y349C; and T366W/S354C, according to EU numbering.

[00302] In some embodiments, the present invention provides a homodimeric Fc fusion protein wherein said first domain linker and said second domain linker have the same amino acid sequence.

[00303] In some embodiments, the present invention provides a homodimeric Fc fusion protein wherein said Fc domain has an additional set of amino acid substitutions comprising Q295E/N384D/Q418E/N421D, according to EU numbering.

[00304] In some embodiments, the present invention provides a homodimeric Fc fusion protein, wherein said Fc domain has an additional set of amino acid substitutions selected from the group consisting of G236R/L328R, E233P/L234V/L235A/G236_/S239K, E233P/L234V/L235A/G236_/S239K/A327G, E233P/L234V/L235A/G236_/S267K/A327G, E233P/L234V/L235A/G236_, and E233P/L234V/L235A/G236_/S267K, according to EU numbering.

[00305] In some embodiments, the present invention provides a homodimeric Fc fusion protein wherein said IL-12p40 subunit has a polypeptide sequence selected form the group consisting of SEQ ID NO:3 (human IL-12 subunit beta (IL-12p40) precursor sequence) and SEQ ID NO:4 (human IL-12 subunit beta (IL-12p40) mature form sequence), and said IL-12p35 subunit has a polypeptide sequence selected from the group consisting of SEQ ID NO:1 (human IL-12 subunit alpha (IL-12p35) precursor sequence) and SEQ ID NO:2 (human IL-12 subunit alpha (IL-12p35) mature form sequence).

[00306] In some embodiments, the present invention provides a homodimeric Fc fusion protein wherein each of said Fc domain further comprises amino acid substitutions M428L/N424S.

[00307] In some embodiments, the present invention provides a homodimeric Fc fusion protein wherein said IL-12p40 subunit is a variant IL-12p40 subunit and/or said IL-12p35 subunit is a variant IL-12p35 subunit.

[00308] In some embodiments, the present invention provides a homodimeric Fc fusion protein wherein said IL-12p40 subunit is a variant IL-12p40 subunit having altered affinity for IL-12 receptor subunit beta-1 (IL-12Rß1), IL-12 receptor subunit beta-2 (IL-12Rß2), and/or IL-12 receptor complex; and/or said IL-12p35 subunit is a variant IL-12p35 subunit having altered affinity for IL-12 receptor subunit beta-1 (IL-12Rß1), IL-12 receptor subunit beta-2 (IL-12Rß2), and/or IL-12 receptor complex.

[00309] In some embodiments, the present invention provides a homodimeric Fc fusion protein wherein said IL-12p40 subunit has one or more amino acid modifications at amino acid residues selected from the group consisting of E3, D7, E12, D14, W15, P17, D18, A19, P20, G21, E22, M23, D29, E32, E33, D34, L40, D41, Q42, S43, E45, L47, T54, I55, Q56, K58, E59, F60, G61, D62, Q65, Y66, E73, K84, E86, D87, G88, I89, W90, D93, D97, K99, E100, K102, N103, K104, F106, E110, N113, Y114, D129, D142, Q144, E156, R159, D161, N162, K163, D166, D170, Q172, D174, A176, C177, P178, A179, A180, E181, S183, P185, E187, N200, S204, F206, R208, D209, D214, N218, Q220, N226, Q229, E231, E235, T242, P243, S245, Y246, F247, S248, C252S, Q256, K158, K260, E262, K264, D265, D270, N281, Q289, D290, R291, Y292, Y293, and E299.

[00310] In some embodiments, the present invention provides a homodimeric Fc fusion protein wherein said IL-12p40 subunit has one or more amino acid substitutions selected from the group consisting of D18N, D18K, E32Q, E33Q, D34N, D34K, Q42E, S43E, S43K, E45Q, Q56E, E59Q, E59K, D62N, E73Q, D87N, K99E, K99Y, E100Q, N103D, N103Q, N113D, N113Q, Q144E, D161N, R159E, K163E, E187Q, N200D, N200Q, N218Q, Q229E, E235Q, C252S, Q256N, K258E, K260E, E262Q, K264E, N281D, N281Q, and E299Q.

[00311] In some embodiments, the present invention provides a homodimeric Fc fusion protein wherein said IL-12p40 subunit has amino acid substitutions selected from the group consisting of N103D/N113D/N200D/N281D, Q42E/E45Q, E45Q/Q56E, Q42E/E59Q, Q56E/E59Q, Q42E/E45Q/Q56E, E45Q/Q56E/E59Q, E32Q/E59Q, D34N/E59K, D34N/E59K/K99E, D34K/E59K/K99E, E32Q/D34N/E59K/K99E, E32K/D34N/E59K/K99E, D34N/E59Q, E59Q/E187Q, S43E/E59Q, S43K/E49Q, E59Q/K163E, E59Q/K99E, E59Q/K258E, E59Q/K260E, E59K/K99E,

D18K/E59K/K99E, E59K/K99E/K264E, E59K/K99Y, E59Y/K99Y, E59Y/K99E, E45K/E59K/K99E, E59K/K99E/Q144E, E59K/K99E/Q144K, E59K/K99E/R159E, E59K/K99E/K264E, D18K/E59K/K99E/K264E, DI8K/E59K/K99E/C252S, D18K/E59K/K99E/C252S/K264E, E59K/K99Y/C252S, E59K/K99E/C252S/K264E, E59K/K99E/C252S, N103D/N113D, N103D/N200D, N103D/N281D, N113D/N200D, N113D/N281D, N200D/N281D, N103D/N113D/N200D, N103D/N113D/N281D, N103D/N200D/N281D, N113D/N200D/N281D, N103Q/N113Q, N103Q/N200Q, N103Q/N281Q, N113Q/N200Q, N113Q/N281Q, N200Q/N281Q, N103Q/N113Q/N200Q, N103Q/N113Q/N281Q, N103Q/N200Q/N281Q, N113Q/N200Q/N281Q, N103Q/N113Q/N200Q/N281Q, E59K/K99E/N103Q/C252S/K264E, E59K/K99E/N113Q/C252S/K264E, E59K/K99E/N200Q/C252S/K264E, E59K/K99E/N281Q/C252S/K264E, E59K/K99E/N103Q/N113Q/C252S/K264E, E59K/K99E/N103Q/N200Q/C252S/K264E, E59K/K99E/N103Q/N281Q/C252S/K264E, E59K/K99E/N113Q/N200Q/C252S/K264E, E59K/K99E/N113Q/N281Q/C252S/K264E, E59K/K99E/N200Q/N281Q/C252S/K264E, E59K/K99E/N103Q/N113Q/N200Q/C252S/K264E, E59K/K99E/N103Q/N200Q/N281Q/C252S/K264E, E59K/K99E/N113Q/N200Q/N281Q/C252S/K264E, and E59K/K99E/N103Q/N113Q/N200Q/N281Q/C252S/K264E.

In some embodiments, the present invention provides a homodimeric Fc fusion protein wherein said IL-12p40 subunit has a polypeptide sequence selected from the group consisting of:

i) SEQ ID NO:57 (IL-12p40(N103D)), ii) SEQ ID NO:58 (IL-12p40(N113D)), iii) SEQ ID NO:59 (IL-12p40(N200D)), iv) SEQ ID NO:60 (IL-12p40(N281D)), v) SEQ ID NO:61 (IL-12p40(N103D/N113D/N200D/N281D)), vi) SEQ ID NO:62 (IL-12p40(Q42E)), vii) SEQ ID NO:63 (IL-12p40(E45Q)), viii) SEQ ID NO:64 (IL-12p40(Q56E)), ix) SEQ ID NO:65 (IL-12p40(E59Q)), x) SEQ ID NO:66 (IL-12p40(D62N)), xi) SEQ ID NO:67 (IL-12p40(Q42E/E45Q)), xii) SEQ ID NO:68 (IL-12p40(E45Q/Q56E)), xiii) SEQ ID NO:69 (IL-12p40(Q42E/E59Q)), xiv) SEQ ID NO:70 (IL-12p40(Q56E/E59Q)), xv) SEQ ID NO:71 (IL-12p40(Q42E/E45Q/Q56E)), xvi) SEQ ID NO:72 (IL-12p40(E45Q/Q56E/E59Q)), xvii) SEQ ID NO:73 (IL-12p40(D161N)), xviii) SEQ ID NO:74 (IL-12p40(E73Q)), xix) SEQ ID NO:75 (IL-12p40(Q144E)), xx) SEQ ID NO:76 (IL-12p40(E262Q)),

xxi) SEQ ID NO:77 (IL-12p40(E100Q)), xxii) SEQ ID NO:78 (IL-12p40(D18N)), xxiii) SEQ ID NO:79 (IL-12p40(E33Q)), xxiv) SEQ ID NO:80 (IL-12p40(Q229E)), xxv) SEQ ID NO:81 (IL-12p40(E235Q)), xxvi) SEQ ID NO:82 (IL-12p40(Q256N)), xxvii) SEQ ID NO:83 (IL-12p40(E299Q)), xxviii) SEQ ID NO:84 (IL-12p40(D87N)), xxix) IL-12p40(E32Q), xxx) IL-12p40(D34N), xxxi) IL-12p40(S43E), xxxii) IL-12p40(S43K), xxxiii) SEQ ID NO:XXX (IL-12p40 (E59K/K99E/N103Q/N113Q/N200Q/N281Q/C252S/K264E)), xxxiv) SEQ ID NO:205 (IL-12p40(E59K)), xxxv) IL-12p40(K99E), xxxvi) IL-12p40(K163E), xxxvii) IL-12p40(E187Q), xxxviii) IL-12p40(K258E), xxxix) IL-12p40(K260E), xl) SEQ ID NO:206 (IL-12p40(E32Q/E59Q)), xli) SEQ ID NO:207 (IL-12p40(D34N/E59Q)), xlii) SEQ ID NO:208 (IL-12p40(E59Q/E187Q)), xliii) SEQ ID NO:209 (IL-12p40(S43E/E59Q)), xliv) SEQ ID NO:210 (IL-12p40(S43K/E49Q)), xlv) SEQ ID NO:211 (IL-12p40(E59Q/K163E)), xlvi) SEQ ID NO:212 (IL-12p40(E59Q/K99E)), xlvii) SEQ ID NO:213 (IL-12p40(E59Q/K258E)), xlviii) SEQ ID NO:214 (IL-12p40(E59Q/K260E)), xlix) SEQ ID NO: XXX (IL-12p40 (D34N/E59K)), l) SEQ ID NO: XXX (IL-12p40(E59K/K99E)), li) SEQ ID NO: XXX (IL-12p40(D18K/E59K/K99E)), lii) SEQ ID NO: XXX (IL-12p40 (E59K/K99E/K264E)), liii) SEQ ID NO: XXX (IL-12p40 (E59K/K99Y)), liv) SEQ ID NO: XXX (IL-12p40 (E59Y/K99E)), lv) SEQ ID NO: XXX (IL-12p40 (E45K/E59K/K99E)), lvi) SEQ ID NO: XXX (IL-12p40 (E59K/K99E/Q144E)), lvii) SEQ ID NO: XXX (IL-12p40 (E59K/K99E/Q144K)), lviii) SEQ ID NO: XXX (IL-12p40 (E59K/K99E/R159E)), lix) SEQ ID NO: XXX (IL-12p40 (E59K/K99E/K264E)), lx) SEQ ID NO: XXX (IL-12p40 (D18K/E59K/K99E/K264E)), lxi) SEQ ID NO: XXX (IL-12p40 (C252S)), lxii) SEQ ID NO: XXX (IL-12p40 (DI8K/E59K/K99E/C252S)), lxiii) SEQ ID NO: XXX (IL-12p40 (D18K/E59K/K99E/C252S/K264E)), lxiv) SEQ ID NO: XXX (IL-12p40 (E59K/K99Y/C252S)), lxv) SEQ ID NO: XXX (IL-12p40 (E59K/K99E/C252S/K264E)), lxvi) SEQ ID NO: XXX (IL-12p40 (E59K/K99E/C252S)), lxvii) SEQ ID NO: XXX (IL-12p40 (N103D/N113D)), lxviii) SEQ ID NO: XXX (IL-12p40 (N103D/N200D)), lxix) SEQ ID NO: XXX (IL-12p40 (N103D/N281D)), lxx) SEQ ID NO: XXX (IL-12p40 (N113D/N200D)), lxxi) SEQ ID NO: XXX (IL-12p40 (N113D/N281D)), lxxii) SEQ ID NO: XXX (IL-12p40 (N200D/N281D)), lxxiii) SEQ ID NO: XXX (IL-12p50 (N103D/N113D/N200D)), lxxiv) SEQ ID NO: XXX (IL-12p40 (N103D/N113D/N281D)), lxxv) SEQ ID NO: XXX (IL-12p40 (N103D/N200D/N281D)), lxxvi) SEQ ID NO: XXX (IL-12p40 (N113D/N200D/N281D)), lxxvii) SEQ ID NO: XXX (IL-12p40

(N103Q)), lxxviii) SEQ ID NO: XXX (IL-12p40 (N113Q)), lxxix) SEQ ID NO: XXX (IL-12p40 (N200Q)), lxxx) SEQ ID NO: XXX (IL-12p40 (N281Q)), lxxxi) SEQ ID NO: XXX (IL-12p40 (N103Q/N113Q)), lxxxii) SEQ ID NO: XXX (IL-12p40 (N103Q/N200Q)), lxxxiii) SEQ ID NO: XXX (IL-12p40 (N103Q/N281Q)), lxxxiv) SEQ ID NO: XXX (IL-12p40 (N113Q/N200Q)), lxxxv) SEQ ID NO: XXX (IL-12p40 (N113Q/N281Q)), lxxxvi) SEQ ID NO: XXX (IL-12p40 (N200Q/N281Q)), lxxxvii) SEQ ID NO: XXX (IL-12p40 (N103Q/N113Q/N200Q)), lxxxviii) SEQ ID NO: XXX (IL-12p40 (N103Q/N113Q/N281Q)), lxxxix) SEQ ID NO: XXX (IL-12p40 (N103Q/N200Q/N281Q)), xc) SEQ ID NO: XXX (IL-12p40 (N113Q/N200Q/N281Q)), xci) SEQ ID NO:XXX (IL-12p40 (N103Q/N113Q/N200Q/N281Q)), xcii) SEQ ID NO:XXX (IL-12p40 (D34N/E59K/K99E)), xciii) SEQ ID NO:XXX (IL-12p40 (D34K/E59K/K99E)), xciv) SEQ ID NO:XXX (IL-12p40 (E32Q/D34N/E59K/K99E)), xcv) SEQ ID NO:XXX (IL-12p40 (E32K/D34N/E59K/K99E)), xcvi) SEQ ID NO: XXX (IL-12p40 (E59Y/K99Y)), xcvii) SEQ ID NO:XXX (IL-12p40 (E59K/K99E/N103Q/C252S/K264E)), xcviii) SEQ ID NO:XXX (IL-12p40 (E59K/K99E/N113Q/C252S/K264E)), xcix) SEQ ID NO:XXX (IL-12p40 (E59K/K99E/N200Q/C252S/K264E)), c) SEQ ID NO:XXX (IL-12p40 (E59K/K99E/N281Q/C252S/K264E)), ci) SEQ ID NO:XXX (IL-12p40 (E59K/K99E/N103Q/N113Q/C252S/K264E)), cii) SEQ ID NO:XXX (IL-12p40 (E59K/K99E/N103Q/N200Q/C252S/K264E)), ciii) SEQ ID NO:XXX (IL-12p40 (E59K/K99E/N103Q/N281Q/C252S/K264E)), civ) SEQ ID NO:XXX (IL-12p40 (E59K/K99E/N113Q/N200Q/C252S/K264E)), cv) SEQ ID NO:XXX (IL-12p40 (E59K/K99E/N113Q/N281Q/C252S/K264E)), cvi) SEQ ID NO:XXX (IL-12p40 (E59K/K99E/N200Q/N281Q/C252S/K264E)), cvii) SEQ ID NO:XXX (IL-12p40 (E59K/K99E/N103Q/N113Q/N200Q/C252S/K264E)), cviii) SEQ ID NO:XXX (IL-12p40 (E59K/K99E/N103Q/N200Q/N281Q/C252S/K264E)), and cix) SEQ ID NO:XXX (IL-12p40 (E59K/K99E/N113Q/N200Q/N281Q/C252S/K264E)).

[00312] In some embodiments, the present invention provides a homodimeric Fc fusion protein wherein said IL-12p35 subunit has one or more amino acid modifications as amino acid residues selected from the group consisting of Q20, N21, Q35, E38, S44, E45, E46, H49, K54, D55, T59, V60,

E61, C63, L64, P65, E67, L68, N71, S73, C74, L75, N76, E79, N85, L89, F96, M97, L124, M125, Q130, Q135, N136, E143, Q146, N151, E153, K158, E162, E163, D165, I171, R181, I182, R183, V185, T186, D188, R189, V190, S192, Y193, N195, and A196.

[00313] In some embodiments, the present invention provides a homodimeric Fc fusion protein wherein said IL-12p35 subunit has one or more amino acid substitutions selected from the group consisting of N21D, Q35D, E38Q, D55Q, D55K, N71D, N71Q, L75A, N76D, E79Q, N85D, N85Q, L89A, F96A, M97A, L124A, M125A, Q130E, Q135E, N136D, E143Q, Q146E, N151D, N151K, E153K, E153Q, K158E, E162Q, E163Q, D165N, I171A, N195D, and N195Q.

[00314] In some embodiments, the present invention provides a homodimeric Fc fusion protein wherein said IL-12p35 subunit has amino acid substitutions selected from the group consisting of N71D/N85D/N195D, N151D/E153Q, N151D/D165N, Q130E/N151D, N151D/K158E, E79Q/N151D, D55Q/N151D, N136D/N151D, N21D/N151D, E143Q/N151D, N71Q/N85Q, N71Q/N195Q, N85Q/N195Q, N71Q/N85Q/N195Q, N71D/N85D, N71D/N195D, and N85D/N195D.

[00315] In some embodiments, the present invention provides a homodimeric Fc fusion protein wherein said IL-12p35 subunit has a polypeptide sequence selected from the group consisting of: i) SEQ ID NO:113 (IL-12p35(N71D)), ii) SEQ ID NO:114 (IL-12p35(N85D)), iii) SEQ ID NO:115 (IL-12p35(N195D)), iv) SEQ ID NO:116 (IL-12p35(N71D/N85D/N195D)), v) SEQ ID NO:117 (IL-12p35(E153Q)), vi) SEQ ID NO:118 (IL-12p35(E38Q)), vii) SEQ ID NO:119 (IL-12p35(N151D)), viii) SEQ ID NO:120 (IL-12p35(Q135E)), ix) SEQ ID NO:121 (IL-12p35(Q35D)), x) SEQ ID NO:122 (IL-12p35(Q146E)), xi) SEQ ID NO:123 (IL-12p35(N76D)), xii) SEQ ID NO:124 (IL-12p35(E162Q)), xiii) SEQ ID NO:125 (IL-12p35(E163Q)), xiv) IL-12p35(N21D), xv) SEQ ID NO:XXX (IL-12p35(D55Q)), xvi) IL-12p35(E79Q), xvii) IL-12p35(Q130E), xviii) IL-12p35(N136D), xix) IL-12p35(E143Q), xx) SEQ ID NO:227 (IL-12p35(N151K)), xxi) SEQ ID NO:226 (IL-12p35(E153K)), xxii) IL-12p35(K158E), xxiii) IL-12p35(D165N), xxiv) SEQ ID NO:225 (IL-12p35(N151D/E153Q)), xxv) SEQ ID NO:228 (IL-12p35(N151D/D165N)), xxvi) SEQ ID NO:229 (IL-12p35(Q130E/N151D)), xxvii) SEQ ID NO:230 (IL-12p35(N151D/K158E)), xxviii) SEQ ID NO:231 (IL-12p35(E79Q/N151D)), xxix)

SEQ ID NO:232 (IL-12p35(D55Q/N151D)), xxx) SEQ ID NO:233 (IL-12p35(N136D/N151D)), xxxi) SEQ ID NO:234 (IL-12p35(N21D/N151D)), xxxii) SEQ ID NO:235 (IL-12p35(E143Q/N151D)), xxxiii) SEQ ID NO: XXX (IL-12p35(F96A)), xxxiv) SEQ ID NO: XXX (IL-12p35(M97A)), xxxv) SEQ ID NO: XXX (IL-12p35(L89A)), xxxvi) SEQ ID NO: XXX (IL-12p35(L124A)), xxxvii) SEQ ID NO: XXX (IL-12p35(M125A)), xxxviii) SEQ ID NO: XXX (IL-12p35(L75A)), xxxiv) SEQ ID NO: XXX (IL-12p35(I171A)), xxxv) SEQ ID NO: XXX (IL-12p35 (N71Q)), xxxvi) SEQ ID NO: XXX (IL-12p35 (N85Q)), xxxvii) SEQ ID NO: XXX (IL-12p35 (N195Q)), xxxviii) SEQ ID NO: XXX (IL-12p35 (N71Q/N85Q)), xxxix) SEQ ID NO: XXX (IL-12p35 (N71Q/N195Q)), xl) SEQ ID NO: XXX (IL-12p35 (N85Q/N195Q), xli) SEQ ID NO: XXX (IL-12p35 (N71Q/N85Q/N195Q)), xlii) SEQ ID NO: XXX (IL-12p35 (N71D/N85D)), xliii) SEQ ID NO: XXX (IL-12p35 (N71D/N195D), xliv) SEQ ID NO: XXX (IL-12p35 (N85D/N195D)), xlv) SEQ ID NO: XXX (IL-12p35 (D55Q)), and xlvi) SEQ ID NO: XXX (IL-12p35 (D55K)).

[00316] In some embodiments, the present invention provides a homodimeric Fc fusion protein wherein said first and second monomers are XENP31289.

[00317] In some embodiments, the present invention provides a homodimeric Fc fusion protein wherein said first and second monomers are XENP31291.

[00318] In some embodiments, the present invention provides a homodimeric Fc fusion protein composition comprising a homodimeric Fc fusion protein for use in treating cancer in a subject.

[00319] In some embodiments, the present invention provides a one or more nucleic acids encoding a homodimeric Fc fusion protein.

[00320] In some embodiments, the present invention provides a host cell comprising said one or more nucleic acids encoding a homodimeric Fc fusion protein.

[00321] In some embodiments, the present invention provides a method of making a homodimeric Fc fusion protein, said method comprising culturing a host cell under conditions whereby said homodimeric Fc fusion protein is produced.

[00322] In some embodiments, the present invention provides a method of purifying a homodimeric Fc fusion protein, said method comprising: a) providing a composition comprising the homodimeric Fc fusion protein; b) loading said composition onto an ion exchange column; and c) collecting a fraction containing said homodimeric Fc fusion protein.

[00323] In another aspect, the present invention provides a homodimeric Fc fusion protein comprising a first monomer and a second monomer each comprising, from N- to C-terminal, an IL-12p35 subunit domain – a first domain linker-an IL-12p40 subunit domain-a second domain linker-Fc domain.

[00324] In some embodiments, the present invention provides a homodimeric Fc fusion protein, wherein said modifications promoting homodimerization of said Fc domain are a set of amino acid substitutions selected from the group consisting of L368D/K370S; S364K; S364K/E357L; S364K/E357Q; T411E/K360E/Q362E; D401K; T366S/L368A/Y407V; T366W; T366S/L368A/Y407V/Y349C; and T366W/S354C, according to EU numbering.

[00325] In some embodiments, the present invention provides a homodimeric Fc fusion protein wherein said first domain linker and said second domain linker have the same amino acid sequence.

[00326] In some embodiments, the present invention provides a homodimeric Fc fusion protein wherein said Fc domain has an additional set of amino acid substitutions comprising Q295E/N384D/Q418E/N421D, according to EU numbering.

[00327] In some embodiments, the present invention provides a homodimeric Fc fusion protein, wherein said Fc domain has an additional set of amino acid substitutions selected from the group consisting of G236R/L328R, E233P/L234V/L235A/G236_/S239K, E233P/L234V/L235A/G236_/S239K/A327G, E233P/L234V/L235A/G236_/S267K/A327G, E233P/L234V/L235A/G236_, and E233P/L234V/L235A/G236_/S267K, according to EU numbering.

[00328] In some embodiments, the present invention provides a homodimeric Fc fusion protein wherein said IL-12p40 subunit has a polypeptide sequence selected form the group consisting of SEQ ID NO:3 (human IL-12 subunit beta (IL-12p40) precursor sequence) and SEQ ID NO:4 (human IL-12 subunit beta (IL-12p40) mature form sequence), and said IL-12p35 subunit has a polypeptide sequence selected from the group consisting of SEQ ID NO:1 (human IL-12 subunit alpha (IL-12p35) precursor sequence) and SEQ ID NO:2 (human IL-12 subunit alpha (IL-12p35) mature form sequence).

[00329] In some embodiments, the present invention provides a homodimeric Fc fusion protein wherein each of said Fc domain further comprises amino acid substitutions M428L/N424S.

[00330] In some embodiments, the present invention provides a homodimeric Fc fusion protein wherein said IL-12p40 subunit is a variant IL-12p40 subunit and/or said IL-12p35 subunit is a variant IL-12p35 subunit.

[00331] In some embodiments, the present invention provides a homodimeric Fc fusion protein wherein said IL-12p40 subunit is a variant IL-12p40 subunit having altered affinity for IL-12 receptor subunit beta-1 (IL-12Rß1), IL-12 receptor subunit beta-2 (IL-12Rß2), and/or IL-12 receptor complex; and/or said IL-12p35 subunit is a variant IL-12p35 subunit having altered affinity for IL-12 receptor subunit beta-1 (IL-12Rß1), IL-12 receptor subunit beta-2 (IL-12Rß2), and/or IL-12 receptor complex.

[00332] In some embodiments, the present invention provides a homodimeric Fc fusion protein wherein said IL-12p40 subunit has one or more amino acid modifications at amino acid residues selected from the group consisting of E3, D7, E12, D14, W15, P17, D18, A19, P20, G21, E22, M23, D29, E32, E33, D34, L40, D41, Q42, S43, E45, L47, T54, I55, Q56, K58, E59, F60, G61, D62, Q65, Y66, E73, K84, E86, D87, G88, I89, W90, D93, D97, K99, E100, K102, N103, K104, F106, E110, N113, Y114, D129, D142, Q144, E156, R159, D161, N162, K163, D166, D170, Q172, D174, A176, C177, P178, A179, A180, E181, S183, P185, E187, N200, S204, F206, R208, D209, D214, N218, Q220, N226, Q229, E231, E235, T242, P243, S245, Y246, F247, S248, C252S, Q256, K158, K260, E262, K264, D265, D270, N281, Q289, D290, R291, Y292, Y293, and E299.

[00333] In some embodiments, the present invention provides a homodimeric Fc fusion protein wherein said IL-12p40 subunit has one or more amino acid substitutions selected from the group consisting of D18N, D18K, E32Q, E33Q, D34N, D34K, Q42E, S43E, S43K, E45Q, Q56E, E59Q, E59K, D62N, E73Q, D87N, K99E, K99Y, E100Q, N103D, N103Q, N113D, N113Q, Q144E, D161N, R159E, K163E, E187Q, N200D, N200Q, N218Q, Q229E, E235Q, C252S, Q256N, K258E, K260E, E262Q, K264E, N281D, N281Q, and E299Q.

[00334] In some embodiments, the present invention provides a homodimeric Fc fusion protein wherein said IL-12p40 subunit has amino acid substitutions selected from the group consisting of N103D/N113D/N200D/N281D, Q42E/E45Q, E45Q/Q56E, Q42E/E59Q, Q56E/E59Q, Q42E/E45Q/Q56E, E45Q/Q56E/E59Q, E32Q/E59Q, D34N/E59K, D34N/E59K/K99E, D34K/E59K/K99E, E32Q/D34N/E59K/K99E, E32K/D34N/E59K/K99E, D34N/E59Q, E59Q/E187Q, S43E/E59Q, S43K/E49Q, E59Q/K163E, E59Q/K99E, E59Q/K258E, E59Q/K260E, E59K/K99E, D18K/E59K/K99E, E59K/K99E/K264E, E59K/K99Y, E59Y/K99Y, E59Y/K99E, E45K/E59K/K99E, E59K/K99E/Q144E, E59K/K99E/Q144K, E59K/K99E/R159E, E59K/K99E/K264E, D18K/E59K/K99E/K264E, DI8K/E59K/K99E/C252S, D18K/E59K/K99E/C252S/K264E, E59K/K99Y/C252S, E59K/K99E/C252S/K264E, E59K/K99E/C252S, N103D/N113D, N103D/N200D, N103D/N281D, N113D/N200D, N113D/N281D, N200D/N281D, N103D/N113D/N200D, N103D/N113D/N281D, N103D/N200D/N281D, N113D/N200D/N281D, N103Q/N113Q, N103Q/N200Q, N103Q/N281Q, N113Q/N200Q, N113Q/N281Q, N200Q/N281Q, N103Q/N113Q/N200Q, N103Q/N113Q/N281Q, N103Q/N200Q/N281Q, N113Q/N200Q/N281Q, N103Q/N113Q/N200Q/N281Q, E59K/K99E/N103Q/C252S/K264E, E59K/K99E/N113Q/C252S/K264E, E59K/K99E/N200Q/C252S/K264E, E59K/K99E/N281Q/C252S/K264E, E59K/K99E/N103Q/N113Q/C252S/K264E, E59K/K99E/N103Q/N200Q/C252S/K264E, E59K/K99E/N103Q/N281Q/C252S/K264E, E59K/K99E/N113Q/N200Q/C252S/K264E, E59K/K99E/N113Q/N281Q/C252S/K264E, E59K/K99E/N200Q/N281Q/C252S/K264E, E59K/K99E/N103Q/N113Q/N200Q/C252S/K264E, E59K/K99E/N103Q/N200Q/N281Q/C252S/K264E,

E59K/K99E/N113Q/N200Q/N281Q/C252S/K264E, and E59K/K99E/N103Q/N113Q/N200Q/N281Q/C252S/K264E.

In some embodiments, the present invention provides a homodimeric Fc fusion protein wherein said IL-12p40 subunit has a polypeptide sequence selected from the group consisting of:

i) SEQ ID NO:57 (IL-12p40(N103D)), ii) SEQ ID NO:58 (IL-12p40(N113D)), iii) SEQ ID NO:59 (IL-12p40(N200D)), iv) SEQ ID NO:60 (IL-12p40(N281D)), v) SEQ ID NO:61 (IL-12p40(N103D/N113D/N200D/N281D)), vi) SEQ ID NO:62 (IL-12p40(Q42E)), vii) SEQ ID NO:63 (IL-12p40(E45Q)), viii) SEQ ID NO:64 (IL-12p40(Q56E)), ix) SEQ ID NO:65 (IL-12p40(E59Q)), x) SEQ ID NO:66 (IL-12p40(D62N)), xi) SEQ ID NO:67 (IL-12p40(Q42E/E45Q)), xii) SEQ ID NO:68 (IL-12p40(E45Q/Q56E)), xiii) SEQ ID NO:69 (IL-12p40(Q42E/E59Q)), xiv) SEQ ID NO:70 (IL-12p40(Q56E/E59Q)), xv) SEQ ID NO:71 (IL-12p40(Q42E/E45Q/Q56E)), xvi) SEQ ID NO:72 (IL-12p40(E45Q/Q56E/E59Q)), xvii) SEQ ID NO:73 (IL-12p40(D161N)), xviii) SEQ ID NO:74 (IL-12p40(E73Q)), xix) SEQ ID NO:75 (IL-12p40(Q144E)), xx) SEQ ID NO:76 (IL-12p40(E262Q)), xxi) SEQ ID NO:77 (IL-12p40(E100Q)), xxii) SEQ ID NO:78 (IL-12p40(D18N)), xxiii) SEQ ID NO:79 (IL-12p40(E33Q)), xxiv) SEQ ID NO:80 (IL-12p40(Q229E)), xxv) SEQ ID NO:81 (IL-12p40(E235Q)), xxvi) SEQ ID NO:82 (IL-12p40(Q256N)), xxvii) SEQ ID NO:83 (IL-12p40(E299Q)), xxviii) SEQ ID NO:84 (IL-12p40(D87N)), xxix) IL-12p40(E32Q), xxx) IL-12p40(D34N), xxxi) IL-12p40(S43E), xxxii) IL-12p40(S43K), xxxiii) SEQ ID NO:XXX (IL-12p40 (E59K/K99E/N103Q/N113Q/N200Q/N281Q/C252S/K264E)), xxxiv) SEQ ID NO:205 (IL-12p40(E59K)), xxxv) IL-12p40(K99E), xxxvi) IL-12p40(K163E), xxxvii) IL-12p40(E187Q), xxxviii) IL-12p40(K258E), xxxix) IL-12p40(K260E), xl) SEQ ID NO:206 (IL-12p40(E32Q/E59Q)), xli) SEQ ID NO:207 (IL-12p40(D34N/E59Q)), xlii) SEQ ID NO:208 (IL-12p40(E59Q/E187Q)), xliii) SEQ ID NO:209 (IL-12p40(S43E/E59Q)), xliv) SEQ ID NO:210 (IL-12p40(S43K/E49Q)), xlv) SEQ ID NO:211 (IL-12p40(E59Q/K163E)), xlvi) SEQ ID NO:212 (IL-12p40(E59Q/K99E)), xlvii) SEQ ID NO:213 (IL-12p40(E59Q/K258E)), xlviii) SEQ ID NO:214 (IL-12p40(E59Q/K260E)), xlix) SEQ ID NO: XXX (IL-12p40 (D34N/E59K)), l) SEQ ID NO: XXX (IL-12p40(E59K/K99E)), li) SEQ ID NO: XXX (IL-12p40(D18K/E59K/K99E)), lii) SEQ ID NO: XXX (IL-12p40 (E59K/K99E/K264E)), liii) SEQ ID NO: XXX (IL-12p40 (E59K/K99Y)), liv) SEQ ID NO: XXX (IL-12p40 (E59Y/K99E)), lv)

SEQ ID NO: XXX (IL-12p40 (E45K/E59K/K99E)), lvi) SEQ ID NO: XXX (IL-12p40 (E59K/K99E/Q144E)), lvii) SEQ ID NO: XXX (IL-12p40 (E59K/K99E/Q144K)), lviii) SEQ ID NO: XXX (IL-12p40 (E59K/K99E/R159E)), lix) SEQ ID NO: XXX (IL-12p40 (E59K/K99E/K264E)), lx) SEQ ID NO: XXX (IL-12p40 (D18K/E59K/K99E/K264E)), lxi) SEQ ID NO: XXX (IL-12p40 (C252S)), lxii) SEQ ID NO: XXX (IL-12p40 (DI8K/E59K/K99E/C252S)), lxiii) SEQ ID NO: XXX (IL-12p40 (D18K/E59K/K99E/C252S/K264E)), lxiv) SEQ ID NO: XXX (IL-12p40 (E59K/K99Y/C252S)), lxv) SEQ ID NO: XXX (IL-12p40 (E59K/K99E/C252S/K264E)), lxvi) SEQ ID NO: XXX (IL-12p40 (E59K/K99E/C252S)), lxvii) SEQ ID NO: XXX (IL-12p40 (N103D/N113D)), lxviii) SEQ ID NO: XXX (IL-12p40 (N103D/N200D)), lxix) SEQ ID NO: XXX (IL-12p40 (N103D/N281D)), lxx) SEQ ID NO: XXX (IL-12p40 (N113D/N200D)), lxxi) SEQ ID NO: XXX (IL-12p40 (N113D/N281D)), lxxii) SEQ ID NO: XXX (IL-12p40 (N200D/N281D)), lxxiii) SEQ ID NO: XXX (IL-12p50 (N103D/N113D/N200D)), lxxiv) SEQ ID NO: XXX (IL-12p40 (N103D/N113D/N281D)), lxxv) SEQ ID NO: XXX (IL-12p40 (N103D/N200D/N281D)), lxxvi) SEQ ID NO: XXX (IL-12p40 (N113D/N200D/N281D)), lxxvii) SEQ ID NO: XXX (IL-12p40 (N103Q)), lxxviii) SEQ ID NO: XXX (IL-12p40 (N113Q)), lxxix) SEQ ID NO: XXX (IL-12p40 (N200Q)), lxxx) SEQ ID NO: XXX (IL-12p40 (N281Q)), lxxxi) SEQ ID NO: XXX (IL-12p40 (N103Q/N113Q)), lxxxii) SEQ ID NO: XXX (IL-12p40 (N103Q/N200Q)), lxxxiii) SEQ ID NO: XXX (IL-12p40 (N103Q/N281Q)), lxxxiv) SEQ ID NO: XXX (IL-12p40 (N113Q/N200Q)), lxxxv) SEQ ID NO: XXX (IL-12p40 (N113Q/N281Q)), lxxxvi) SEQ ID NO: XXX (IL-12p40 (N200Q/N281Q)), lxxxvii) SEQ ID NO: XXX (IL-12p40 (N103Q/N113Q/N200Q)), lxxxviii) SEQ ID NO: XXX (IL-12p40 (N103Q/N113Q/N281Q)), lxxxix) SEQ ID NO: XXX (IL-12p40 (N103Q/N200Q/N281Q)), xc) SEQ ID NO: XXX (IL-12p40 (N113Q/N200Q/N281Q)), xci) SEQ ID NO:XXX (IL-12p40 (N103Q/N113Q/N200Q/N281Q)), xcii) SEQ ID NO:XXX (IL-12p40 (D34N/E59K/K99E)), xciii) SEQ ID NO:XXX (IL-12p40 (D34K/E59K/K99E)), xciv) SEQ ID NO:XXX (IL-12p40 (E32Q/D34N/E59K/K99E)), xcv) SEQ ID NO:XXX (IL-12p40 (E32K/D34N/E59K/K99E)), xcvi) SEQ ID NO: XXX (IL-12p40 (E59Y/K99Y)), xcvii) SEQ ID NO:XXX (IL-12p40 (E59K/K99E/N103Q/C252S/K264E)), xcviii) SEQ ID NO:XXX (IL-12p40 (E59K/K99E/N113Q/C252S/K264E)), xcix) SEQ ID NO:XXX (IL-12p40 (E59K/K99E/N200Q/C252S/K264E)), c) SEQ ID NO:XXX (IL-12p40

(E59K/K99E/N281Q/C252S/K264E)), ci) SEQ ID NO:XXX (IL-12p40 (E59K/K99E/N103Q/N113Q/C252S/K264E)), cii) SEQ ID NO:XXX (IL-12p40 (E59K/K99E/N103Q/N200Q/C252S/K264E)), ciii) SEQ ID NO:XXX (IL-12p40 (E59K/K99E/N103Q/N281Q/C252S/K264E)), civ) SEQ ID NO:XXX (IL-12p40 (E59K/K99E/N113Q/N200Q/C252S/K264E)), cv) SEQ ID NO:XXX (IL-12p40 (E59K/K99E/N113Q/N281Q/C252S/K264E)), cvi) SEQ ID NO:XXX (IL-12p40 (E59K/K99E/N200Q/N281Q/C252S/K264E)), cvii) SEQ ID NO:XXX (IL-12p40 (E59K/K99E/N103Q/N113Q/N200Q/C252S/K264E)), cviii) SEQ ID NO:XXX (IL-12p40 (E59K/K99E/N103Q/N200Q/N281Q/C252S/K264E)), and cix) SEQ ID NO:XXX (IL-12p40 (E59K/K99E/N113Q/N200Q/N281Q/C252S/K264E)).

[00335] In some embodiments, the present invention provides a homodimeric Fc fusion protein wherein said IL-12p35 subunit has one or more amino acid modifications as amino acid residues selected from the group consisting of Q20, N21, Q35, E38, S44, E45, E46, H49, K54, D55, T59, V60, E61, C63, L64, P65, E67, L68, N71, S73, C74, L75, N76, E79, N85, L89, F96, M97, L124, M125, Q130, Q135, N136, E143, Q146, N151, E153, K158, E162, E163, D165, I171, R181, I182, R183, V185, T186, D188, R189, V190, S192, Y193, N195, and A196.

[00336] In some embodiments, the present invention provides a homodimeric Fc fusion protein wherein said IL-12p35 subunit has one or more amino acid substitutions selected from the group consisting of N21D, Q35D, E38Q, D55Q, D55K, N71D, N71Q, L75A, N76D, E79Q, N85D, N85Q, L89A, F96A, M97A, L124A, M125A, Q130E, Q135E, N136D, E143Q, Q146E, N151D, N151K, E153K, E153Q, K158E, E162Q, E163Q, D165N, I171A, N195D, and N195Q.

[00337] In some embodiments, the present invention provides a homodimeric Fc fusion protein wherein said IL-12p35 subunit has amino acid substitutions selected from the group consisting of N71D/N85D/N195D, N151D/E153Q, N151D/D165N, Q130E/N151D, N151D/K158E, E79Q/N151D, D55Q/N151D, N136D/N151D, N21D/N151D, E143Q/N151D, N71Q/N85Q, N71Q/N195Q, N85Q/N195Q, N71Q/N85Q/N195Q, N71D/N85D, N71D/N195D, and N85D/N195D.

[00338] In some embodiments, the present invention provides a homodimeric Fc fusion protein wherein said IL-12p35 subunit has a polypeptide sequence selected from the group consisting of: i) SEQ ID NO:113 (IL-12p35(N71D)), ii) SEQ ID NO:114 (IL-12p35(N85D)), iii) SEQ ID NO:115 (IL-12p35(N195D)), iv) SEQ ID NO:116 (IL-12p35(N71D/N85D/N195D)), v) SEQ ID NO:117 (IL-12p35(E153Q)), vi) SEQ ID NO:118 (IL-12p35(E38Q)), vii) SEQ ID NO:119 (IL-12p35(N151D)), viii) SEQ ID NO:120 (IL-12p35(Q135E)), ix) SEQ ID NO:121 (IL-12p35(Q35D)), x) SEQ ID NO:122 (IL-12p35(Q146E)), xi) SEQ ID NO:123 (IL-12p35(N76D)), xii) SEQ ID NO:124 (IL-12p35(E162Q)), xiii) SEQ ID NO:125 (IL-12p35(E163Q)), xiv) IL-12p35(N21D), xv) SEQ ID NO:XXX (IL-12p35(D55Q)), xvi) IL-12p35(E79Q), xvii) IL-12p35(Q130E), xviii) IL-12p35(N136D), xix) (IL-12p35(E143Q)), xx) SEQ ID NO:227 (IL-12p35(N151K)), xxi) SEQ ID NO:226 (IL-12p35(E153K)), xxii) IL-12p35(K158E), xxiii) IL-12p35(D165N), xxiv) SEQ ID NO:225 (IL-12p35(N151D/E153Q)), xxv) SEQ ID NO:228 (IL-12p35(N151D/D165N)), xxvi) SEQ ID NO:229 (IL-12p35(Q130E/N151D)), xxvii) SEQ ID NO:230 (IL-12p35(N151D/K158E)), xxviii) SEQ ID NO:231 (IL-12p35(E79Q/N151D)), xxix) SEQ ID NO:232 (IL-12p35(D55Q/N151D)), xxx) SEQ ID NO:233 (IL-12p35(N136D/N151D)), xxxi) SEQ ID NO:234 (IL-12p35(N21D/N151D)), xxxii) SEQ ID NO:235 (IL-12p35(E143Q/N151D)), xxxiii) SEQ ID NO: XXX (IL-12p35(F96A)), xxxiv) SEQ ID NO: XXX (IL-12p35(M97A)), xxxv) SEQ ID NO: XXX (IL-12p35(L89A)), xxxvi) SEQ ID NO: XXX (IL-12p35(L124A)), xxxvii) SEQ ID NO: XXX (IL-12p35(M125A)), xxxviii) SEQ ID NO: XXX (IL-12p35(L75A)), xxxiv) SEQ ID NO: XXX (IL-12p35(I171A)), xxxv) SEQ ID NO: XXX (IL-12p35 (N71Q)), xxxvi) SEQ ID NO: XXX (IL-12p35 (N85Q)), xxxvii) SEQ ID NO: XXX (IL-12p35 (N195Q)), xxxviii) SEQ ID NO: XXX (IL-12p35 (N71Q/N85Q)), xxxix) SEQ ID NO: XXX (IL-12p35 (N71Q/N195Q)), xl) SEQ ID NO: XXX (IL-12p35 (N85Q/N195Q), xli) SEQ ID NO: XXX (IL-12p35 (N71Q/N85Q/N195Q)), xlii) SEQ ID NO: XXX (IL-12p35 (N71D/N85D)), xliii) SEQ ID NO: XXX (IL-12p35 (N71D/N195D), xliv) SEQ ID NO: XXX (IL-12p35 (N85D/N195D)), xlv) SEQ ID NO: XXX (IL-12p35 (D55Q)), and xlvi) SEQ ID NO: XXX (IL-12p35 (D55K)).

[00339] In some embodiments, the present invention provides a homodimeric Fc fusion protein wherein said first and second monomers are XENP31289.

[00340] In some embodiments, the present invention provides a homodimeric Fc fusion protein wherein said first and second monomers are XENP31291.

[00341] In some embodiments, the present invention provides a homodimeric Fc fusion protein composition comprising a homodimeric Fc fusion protein for use in treating cancer in a subject.

[00342] In some embodiments, the present invention provides a one or more nucleic acids encoding a homodimeric Fc fusion protein.

[00343] In some embodiments, the present invention provides a host cell comprising said one or more nucleic acids encoding a homodimeric Fc fusion protein.

[00344] In some embodiments, the present invention provides a method of making a homodimeric Fc fusion protein, said method comprising culturing a host cell under conditions whereby said homodimeric Fc fusion protein is produced.

[00345] In some embodiments, the present invention provides a method of purifying a homodimeric Fc fusion protein, said method comprising: a) providing a composition comprising the homodimeric Fc fusion protein; b) loading said composition onto an ion exchange column; and c) collecting a fraction containing said homodimeric Fc fusion protein.

[00346] In another aspect, the present invention provides a homodimeric Fc fusion protein comprising a first monomer and a second monomer each comprising, from N- to C-terminal, a Fc domain-a first domain linker-an IL-12p40 subunit domain-a second domain linker- an IL-12p35 subunit domain.

[00347] In some embodiments, the present invention provides a homodimeric Fc fusion protein, wherein said modifications promoting homodimerization of said Fc domain are a set of amino acid substitutions selected from the group consisting of L368D/K370S; S364K; S364K/E357L; S364K/E357Q; T411E/K360E/Q362E; D401K; T366S/L368A/Y407V; T366W; T366S/L368A/Y407V/Y349C; and T366W/S354C, according to EU numbering.

[00348] In some embodiments, the present invention provides a homodimeric Fc fusion protein wherein said first domain linker and said second domain linker have the same amino acid sequence.

[00349] In some embodiments, the present invention provides a homodimeric Fc fusion protein wherein said Fc domain has an additional set of amino acid substitutions comprising Q295E/N384D/Q418E/N421D, according to EU numbering.

[00350] In some embodiments, the present invention provides a homodimeric Fc fusion protein, wherein said Fc domain has an additional set of amino acid substitutions selected from the group consisting of G236R/L328R, E233P/L234V/L235A/G236_/S239K, E233P/L234V/L235A/G236_/S239K/A327G, E233P/L234V/L235A/G236_/S267K/A327G, E233P/L234V/L235A/G236_, and E233P/L234V/L235A/G236_/S267K, according to EU numbering.

[00351] In some embodiments, the present invention provides a homodimeric Fc fusion protein wherein said IL-12p40 subunit has a polypeptide sequence selected form the group consisting of SEQ ID NO:3 (human IL-12 subunit beta (IL-12p40) precursor sequence) and SEQ ID NO:4 (human IL-12 subunit beta (IL-12p40) mature form sequence), and said IL-12p35 subunit has a polypeptide sequence selected from the group consisting of SEQ ID NO:1 (human IL-12 subunit alpha (IL-12p35) precursor sequence) and SEQ ID NO:2 (human IL-12 subunit alpha (IL-12p35) mature form sequence).

[00352] In some embodiments, the present invention provides a homodimeric Fc fusion protein wherein each of said Fc domain further comprises amino acid substitutions M428L/N424S.

[00353] In some embodiments, the present invention provides a homodimeric Fc fusion protein wherein said IL-12p40 subunit is a variant IL-12p40 subunit and/or said IL-12p35 subunit is a variant IL-12p35 subunit.

[00354] In some embodiments, the present invention provides a homodimeric Fc fusion protein wherein said IL-12p40 subunit is a variant IL-12p40 subunit having altered affinity for IL-12

receptor subunit beta-1 (IL-12Rß1), IL-12 receptor subunit beta-2 (IL-12Rß2), and/or IL-12 receptor complex; and/or said IL-12p35 subunit is a variant IL-12p35 subunit having altered affinity for IL-12 receptor subunit beta-1 (IL-12Rß1), IL-12 receptor subunit beta-2 (IL-12Rß2), and/or IL-12 receptor complex.

[00355] In some embodiments, the present invention provides a homodimeric Fc fusion protein wherein said IL-12p40 subunit has one or more amino acid modifications at amino acid residues selected from the group consisting of E3, D7, E12, D14, W15, P17, D18, A19, P20, G21, E22, M23, D29, E32, E33, D34, L40, D41, Q42, S43, E45, L47, T54, I55, Q56, K58, E59, F60, G61, D62, Q65, Y66, E73, K84, E86, D87, G88, I89, W90, D93, D97, K99, E100, K102, N103, K104, F106, E110, N113, Y114, D129, D142, Q144, E156, R159, D161, N162, K163, D166, D170, Q172, D174, A176, C177, P178, A179, A180, E181, S183, P185, E187, N200, S204, F206, R208, D209, D214, N218, Q220, N226, Q229, E231, E235, T242, P243, S245, Y246, F247, S248, C252S, Q256, K158, K260, E262, K264, D265, D270, N281, Q289, D290, R291, Y292, Y293, and E299.

[00356] In some embodiments, the present invention provides a homodimeric Fc fusion protein wherein said IL-12p40 subunit has one or more amino acid substitutions selected from the group consisting of D18N, D18K, E32Q, E33Q, D34N, D34K, Q42E, S43E, S43K, E45Q, Q56E, E59Q, E59K, D62N, E73Q, D87N, K99E, K99Y, E100Q, N103D, N103Q, N113D, N113Q, Q144E, D161N, R159E, K163E, E187Q, N200D, N200Q, N218Q, Q229E, E235Q, C252S, Q256N, K258E, K260E, E262Q, K264E, N281D, N281Q, and E299Q.

[00357] In some embodiments, the present invention provides a homodimeric Fc fusion protein wherein said IL-12p40 subunit has amino acid substitutions selected from the group consisting of N103D/N113D/N200D/N281D, Q42E/E45Q, E45Q/Q56E, Q42E/E59Q, Q56E/E59Q, Q42E/E45Q/Q56E, E45Q/Q56E/E59Q, E32Q/E59Q, D34N/E59K, D34N/E59K/K99E, D34K/E59K/K99E, E32Q/D34N/E59K/K99E, E32K/D34N/E59K/K99E, D34N/E59Q, E59Q/E187Q, S43E/E59Q, S43K/E49Q, E59Q/K163E, E59Q/K99E, E59Q/K258E, E59Q/K260E, E59K/K99E, D18K/E59K/K99E, E59K/K99E/K264E, E59K/K99Y, E59Y/K99Y, E59Y/K99E, E45K/E59K/K99E, E59K/K99E/Q144E, E59K/K99E/Q144K, E59K/K99E/R159E, E59K/K99E/K264E,

D18K/E59K/K99E/K264E, DI8K/E59K/K99E/C252S, D18K/E59K/K99E/C252S/K264E, E59K/K99Y/C252S, E59K/K99E/C252S/K264E, E59K/K99E/C252S, N103D/N113D, N103D/N200D, N103D/N281D, N113D/N200D, N113D/N281D, N200D/N281D, N103D/N113D/N200D, N103D/N113D/N281D, N103D/N200D/N281D, N113D/N200D/N281D, N103Q/N113Q, N103Q/N200Q, N103Q/N281Q, N113Q/N200Q, N113Q/N281Q, N200Q/N281Q, N103Q/N113Q/N200Q, N103Q/N113Q/N281Q, N103Q/N200Q/N281Q, N113Q/N200Q/N281Q, N103Q/N113Q/N200Q/N281Q, E59K/K99E/N103Q/C252S/K264E, E59K/K99E/N113Q/C252S/K264E, E59K/K99E/N200Q/C252S/K264E, E59K/K99E/N281Q/C252S/K264E, E59K/K99E/N103Q/N113Q/C252S/K264E, E59K/K99E/N103Q/N200Q/C252S/K264E, E59K/K99E/N103Q/N281Q/C252S/K264E, E59K/K99E/N113Q/N200Q/C252S/K264E, E59K/K99E/N113Q/N281Q/C252S/K264E, E59K/K99E/N200Q/N281Q/C252S/K264E, E59K/K99E/N103Q/N113Q/N200Q/C252S/K264E, E59K/K99E/N103Q/N200Q/N281Q/C252S/K264E, E59K/K99E/N113Q/N200Q/N281Q/C252S/K264E, and E59K/K99E/N103Q/N113Q/N200Q/N281Q/C252S/K264E.

In some embodiments, the present invention provides a homodimeric Fc fusion protein wherein said IL-12p40 subunit has a polypeptide sequence selected from the group consisting of:

i) SEQ ID NO:57 (IL-12p40(N103D)), ii) SEQ ID NO:58 (IL-12p40(N113D)), iii) SEQ ID NO:59 (IL-12p40(N200D)), iv) SEQ ID NO:60 (IL-12p40(N281D)), v) SEQ ID NO:61 (IL-12p40(N103D/N113D/N200D/N281D)), vi) SEQ ID NO:62 (IL-12p40(Q42E)), vii) SEQ ID NO:63 (IL-12p40(E45Q)), viii) SEQ ID NO:64 (IL-12p40(Q56E)), ix) SEQ ID NO:65 (IL-12p40(E59Q)), x) SEQ ID NO:66 (IL-12p40(D62N)), xi) SEQ ID NO:67 (IL-12p40(Q42E/E45Q)), xii) SEQ ID NO:68 (IL-12p40(E45Q/Q56E)), xiii) SEQ ID NO:69 (IL-12p40(Q42E/E59Q)), xiv) SEQ ID NO:70 (IL-12p40(Q56E/E59Q)), xv) SEQ ID NO:71 (IL-12p40(Q42E/E45Q/Q56E)), xvi) SEQ ID NO:72 (IL-12p40(E45Q/Q56E/E59Q)), xvii) SEQ ID NO:73 (IL-12p40(D161N)), xviii) SEQ ID NO:74 (IL-12p40(E73Q)), xix) SEQ ID NO:75 (IL-12p40(Q144E)), xx) SEQ ID NO:76 (IL-12p40(E262Q)), xxi) SEQ ID NO:77 (IL-12p40(E100Q)), xxii) SEQ ID NO:78 (IL-12p40(D18N)), xxiii) SEQ ID NO:79 (IL-12p40(E33Q)), xxiv) SEQ ID NO:80 (IL-12p40(Q229E)), xxv) SEQ ID NO:81 (IL-

12p40(E235Q)), xxvi) SEQ ID NO:82 (IL-12p40(Q256N)), xxvii) SEQ ID NO:83 (IL-12p40(E299Q)), xxviii) SEQ ID NO:84 (IL-12p40(D87N)), xxix) IL-12p40(E32Q), xxx) IL-12p40(D34N), xxxi) IL-12p40(S43E), xxxii) IL-12p40(S43K), xxxiii) SEQ ID NO:XXX (IL-12p40 (E59K/K99E/N103Q/N113Q/N200Q/N281Q/C252S/K264E)), xxxiv) SEQ ID NO:205 (IL-12p40(E59K)), xxxv) IL-12p40(K99E), xxxvi) IL-12p40(K163E), xxxvii) IL-12p40(E187Q), xxxviii) IL-12p40(K258E), xxxix) IL-12p40(K260E), xl) SEQ ID NO:206 (IL-12p40(E32Q/E59Q)), xli) SEQ ID NO:207 (IL-12p40(D34N/E59Q)), xlii) SEQ ID NO:208 (IL-12p40(E59Q/E187Q)), xliii) SEQ ID NO:209 (IL-12p40(S43E/E59Q)), xliv) SEQ ID NO:210 (IL-12p40(S43K/E49Q)), xlv) SEQ ID NO:211 (IL-12p40(E59Q/K163E)), xlvi) SEQ ID NO:212 (IL-12p40(E59Q/K99E)), xlvii) SEQ ID NO:213 (IL-12p40(E59Q/K258E)), xlviii) SEQ ID NO:214 (IL-12p40(E59Q/K260E)), xlix) SEQ ID NO: XXX (IL-12p40 (D34N/E59K)), l) SEQ ID NO: XXX (IL-12p40(E59K/K99E)), li) SEQ ID NO: XXX (IL-12p40(D18K/E59K/K99E)), lii) SEQ ID NO: XXX (IL-12p40 (E59K/K99E/K264E)), liii) SEQ ID NO: XXX (IL-12p40 (E59K/K99Y)), liv) SEQ ID NO: XXX (IL-12p40 (E59Y/K99E)), lv) SEQ ID NO: XXX (IL-12p40 (E45K/E59K/K99E)), lvi) SEQ ID NO: XXX (IL-12p40 (E59K/K99E/Q144E)), lvii) SEQ ID NO: XXX (IL-12p40 (E59K/K99E/Q144K)), lviii) SEQ ID NO: XXX (IL-12p40 (E59K/K99E/R159E)), lix) SEQ ID NO: XXX (IL-12p40 (E59K/K99E/K264E)), lx) SEQ ID NO: XXX (IL-12p40 (D18K/E59K/K99E/K264E)), lxi) SEQ ID NO: XXX (IL-12p40 (C252S)), lxii) SEQ ID NO: XXX (IL-12p40 (DI8K/E59K/K99E/C252S)), lxiii) SEQ ID NO: XXX (IL-12p40 (D18K/E59K/K99E/C252S/K264E)), lxiv) SEQ ID NO: XXX (IL-12p40 (E59K/K99Y/C252S)), lxv) SEQ ID NO: XXX (IL-12p40 (E59K/K99E/C252S/K264E)), lxvi) SEQ ID NO: XXX (IL-12p40 (E59K/K99E/C252S)), lxvii) SEQ ID NO: XXX (IL-12p40 (N103D/N113D)), lxviii) SEQ ID NO: XXX (IL-12p40 (N103D/N200D)), lxix) SEQ ID NO: XXX (IL-12p40 (N103D/N281D)), lxx) SEQ ID NO: XXX (IL-12p40 (N113D/N200D)), lxxi) SEQ ID NO: XXX (IL-12p40 (N113D/N281D)), lxxii) SEQ ID NO: XXX (IL-12p40 (N200D/N281D)), lxxiii) SEQ ID NO: XXX (IL-12p50 (N103D/N113D/N200D)), lxxiv) SEQ ID NO: XXX (IL-12p40 (N103D/N113D/N281D)), lxxv) SEQ ID NO: XXX (IL-12p40 (N103D/N200D/N281D)), lxxvi) SEQ ID NO: XXX (IL-12p40 (N113D/N200D/N281D)), lxxvii) SEQ ID NO: XXX (IL-12p40 (N103Q)), lxxviii) SEQ ID NO: XXX (IL-12p40 (N113Q)), lxxix) SEQ ID NO: XXX (IL-12p40 (N200Q)), lxxx) SEQ ID NO: XXX (IL-12p40 (N281Q)), lxxxi) SEQ ID NO: XXX (IL-12p40

(N103Q/N113Q)), lxxxii) SEQ ID NO: XXX (IL-12p40 (N103Q/N200Q)), lxxxiii) SEQ ID NO: XXX (IL-12p40 (N103Q/N281Q)), lxxxiv) SEQ ID NO: XXX (IL-12p40 (N113Q/N200Q)), lxxxv) SEQ ID NO: XXX (IL-12p40 (N113Q/N281Q)), lxxxvi) SEQ ID NO: XXX (IL-12p40 (N200Q/N281Q)), lxxxvii) SEQ ID NO: XXX (IL-12p40 (N103Q/N113Q/N200Q)), lxxxviii) SEQ ID NO: XXX (IL-12p40 (N103Q/N113Q/N281Q)), lxxxix) SEQ ID NO: XXX (IL-12p40 (N103Q/N200Q/N281Q)), xc) SEQ ID NO: XXX (IL-12p40 (N113Q/N200Q/N281Q)), xci) SEQ ID NO:XXX (IL-12p40 (N103Q/N113Q/N200Q/N281Q)), xcii) SEQ ID NO:XXX (IL-12p40 (D34N/E59K/K99E)), xciii) SEQ ID NO:XXX (IL-12p40 (D34K/E59K/K99E)), xciv) SEQ ID NO:XXX (IL-12p40 (E32Q/D34N/E59K/K99E)), xcv) SEQ ID NO:XXX (IL-12p40 (E32K/D34N/E59K/K99E)), xcvi) SEQ ID NO: XXX (IL-12p40 (E59Y/K99Y)), xcvii) SEQ ID NO:XXX (IL-12p40 (E59K/K99E/N103Q/C252S/K264E)), xcviii) SEQ ID NO:XXX (IL-12p40 (E59K/K99E/N113Q/C252S/K264E)), xcix) SEQ ID NO:XXX (IL-12p40 (E59K/K99E/N200Q/C252S/K264E)), c) SEQ ID NO:XXX (IL-12p40 (E59K/K99E/N281Q/C252S/K264E)), ci) SEQ ID NO:XXX (IL-12p40 (E59K/K99E/N103Q/N113Q/C252S/K264E)), cii) SEQ ID NO:XXX (IL-12p40 (E59K/K99E/N103Q/N200Q/C252S/K264E)), ciii) SEQ ID NO:XXX (IL-12p40 (E59K/K99E/N103Q/N281Q/C252S/K264E)), civ) SEQ ID NO:XXX (IL-12p40 (E59K/K99E/N113Q/N200Q/C252S/K264E)), cv) SEQ ID NO:XXX (IL-12p40 (E59K/K99E/N113Q/N281Q/C252S/K264E)), cvi) SEQ ID NO:XXX (IL-12p40 (E59K/K99E/N200Q/N281Q/C252S/K264E)), cvii) SEQ ID NO:XXX (IL-12p40 (E59K/K99E/N103Q/N113Q/N200Q/C252S/K264E)), cviii) SEQ ID NO:XXX (IL-12p40 (E59K/K99E/N103Q/N200Q/N281Q/C252S/K264E)), and cix) SEQ ID NO:XXX (IL-12p40 (E59K/K99E/N113Q/N200Q/N281Q/C252S/K264E)).

[00358] In some embodiments, the present invention provides a homodimeric Fc fusion protein wherein said IL-12p35 subunit has one or more amino acid modifications as amino acid residues selected from the group consisting of Q20, N21, Q35, E38, S44, E45, E46, H49, K54, D55, T59, V60, E61, C63, L64, P65, E67, L68, N71, S73, C74, L75, N76, E79, N85, L89, F96, M97, L124, M125, Q130,

Q135, N136, E143, Q146, N151, E153, K158, E162, E163, D165, I171, R181, I182, R183, V185, T186, D188, R189, V190, S192, Y193, N195, and A196.

[00359] In some embodiments, the present invention provides a homodimeric Fc fusion protein wherein said IL-12p35 subunit has one or more amino acid substitutions selected from the group consisting of N21D, Q35D, E38Q, D55Q, D55K, N71D, N71Q, L75A, N76D, E79Q, N85D, N85Q, L89A, F96A, M97A, L124A, M125A, Q130E, Q135E, N136D, E143Q, Q146E, N151D, N151K, E153K, E153Q, K158E, E162Q, E163Q, D165N, I171A, N195D, and N195Q.

[00360] In some embodiments, the present invention provides a homodimeric Fc fusion protein wherein said IL-12p35 subunit has amino acid substitutions selected from the group consisting of N71D/N85D/N195D, N151D/E153Q, N151D/D165N, Q130E/N151D, N151D/K158E, E79Q/N151D, D55Q/N151D, N136D/N151D, N21D/N151D, E143Q/N151D, N71Q/N85Q, N71Q/N195Q, N85Q/N195Q, N71Q/N85Q/N195Q, N71D/N85D, N71D/N195D, and N85D/N195D.

[00361] In some embodiments, the present invention provides a homodimeric Fc fusion protein wherein said IL-12p35 subunit has a polypeptide sequence selected from the group consisting of: i) SEQ ID NO:113 (IL-12p35(N71D)), ii) SEQ ID NO:114 (IL-12p35(N85D)), iii) SEQ ID NO:115 (IL-12p35(N195D)), iv) SEQ ID NO:116 (IL-12p35(N71D/N85D/N195D)), v) SEQ ID NO:117 (IL-12p35(E153Q)), vi) SEQ ID NO:118 (IL-12p35(E38Q)), vii) SEQ ID NO:119 (IL-12p35(N151D)), viii) SEQ ID NO:120 (IL-12p35(Q135E)), ix) SEQ ID NO:121 (IL-12p35(Q35D)), x) SEQ ID NO:122 (IL-12p35(Q146E)), xi) SEQ ID NO:123 (IL-12p35(N76D)), xii) SEQ ID NO:124 (IL-12p35(E162Q)), xiii) SEQ ID NO:125 (IL-12p35(E163Q)), xiv) IL-12p35(N21D), xv) SEQ ID NO:XXX (IL-12p35(D55Q)), xvi) IL-12p35(E79Q), xvii) IL-12p35(Q130E), xviii) IL-12p35(N136D), xix) IL-12p35(E143Q), xx) SEQ ID NO:227 (IL-12p35(N151K)), xxi) SEQ ID NO:226 (IL-12p35(E153K)), xxii) IL-12p35(K158E), xxiii) IL-12p35(D165N), xxiv) SEQ ID NO:225 (IL-12p35(N151D/E153Q)), xxv) SEQ ID NO:228 (IL-12p35(N151D/D165N)), xxvi) SEQ ID NO:229 (IL-12p35(Q130E/N151D)), xxvii) SEQ ID NO:230 (IL-12p35(N151D/K158E)), xxviii) SEQ ID NO:231 (IL-12p35(E79Q/N151D)), xxix) SEQ ID NO:232 (IL-12p35(D55Q/N151D)), xxx) SEQ ID NO:233 (IL-12p35(N136D/N151D)), xxxi)

SEQ ID NO:234 (IL-12p35(N21D/N151D)), xxxii) SEQ ID NO:235 (IL-12p35(E143Q/N151D)), xxxiii) SEQ ID NO: XXX (IL-12p35(F96A)), xxxiv) SEQ ID NO: XXX (IL-12p35(M97A)), xxxv) SEQ ID NO: XXX (IL-12p35(L89A)), xxxvi) SEQ ID NO: XXX (IL-12p35(L124A)), xxxvii) SEQ ID NO: XXX (IL-12p35(M125A)), xxxviii) SEQ ID NO: XXX (IL-12p35(L75A)), xxxiv) SEQ ID NO: XXX (IL-12p35(I171A)), xxxv) SEQ ID NO: XXX (IL-12p35 (N71Q)), xxxvi) SEQ ID NO: XXX (IL-12p35 (N85Q)), xxxvii) SEQ ID NO: XXX (IL-12p35 (N195Q)), xxxviii) SEQ ID NO: XXX (IL-12p35 (N71Q/N85Q)), xxxix) SEQ ID NO: XXX (IL-12p35 (N71Q/N195Q)), xl) SEQ ID NO: XXX (IL-12p35 (N85Q/N195Q), xli) SEQ ID NO: XXX (IL-12p35 (N71Q/N85Q/N195Q)), xlii) SEQ ID NO: XXX (IL-12p35 (N71D/N85D)), xliii) SEQ ID NO: XXX (IL-12p35 (N71D/N195D), xliv) SEQ ID NO: XXX (IL-12p35 (N85D/N195D)), xlv) SEQ ID NO: XXX (IL-12p35 (D55Q)), and xlvi) SEQ ID NO: XXX (IL-12p35 (D55K)).

[00362] In some embodiments, the present invention provides a homodimeric Fc fusion protein wherein said first and second monomers are XENP31289.

[00363] In some embodiments, the present invention provides a homodimeric Fc fusion protein wherein said first and second monomers are XENP31291.

[00364] In some embodiments, the present invention provides a homodimeric Fc fusion protein composition comprising a homodimeric Fc fusion protein for use in treating cancer in a subject.

[00365] In some embodiments, the present invention provides a one or more nucleic acids encoding a homodimeric Fc fusion protein.

[00366] In some embodiments, the present invention provides a host cell comprising said one or more nucleic acids encoding a homodimeric Fc fusion protein.

[00367] In some embodiments, the present invention provides a method of making a homodimeric Fc fusion protein, said method comprising culturing a host cell under conditions whereby said homodimeric Fc fusion protein is produced.

[00368] In some embodiments, the present invention provides a method of purifying a homodimeric Fc fusion protein, said method comprising: a) providing a composition comprising the homodimeric Fc fusion protein; b) loading said composition onto an ion exchange column; and c) collecting a fraction containing said homodimeric Fc fusion protein.

[00369] In another aspect, the present invention provides a homodimeric Fc fusion protein comprising a first monomer and a second monomer each comprising, from N- to C-terminal, a Fc domain-a first domain linker-an IL-12p35 subunit domain- a second domain linker-an IL-12p40 subunit domain.

[00370] In some embodiments, the present invention provides a homodimeric Fc fusion protein, wherein said modifications promoting homodimerization of said Fc domain are a set of amino acid substitutions selected from the group consisting of L368D/K370S; S364K; S364K/E357L; S364K/E357Q; T411E/K360E/Q362E; D401K; T366S/L368A/Y407V; T366W; T366S/L368A/Y407V/Y349C; and T366W/S354C, according to EU numbering.

[00371] In some embodiments, the present invention provides a homodimeric Fc fusion protein wherein said first domain linker and said second domain linker have the same amino acid sequence.

[00372] In some embodiments, the present invention provides a homodimeric Fc fusion protein wherein said Fc domain has an additional set of amino acid substitutions comprising Q295E/N384D/Q418E/N421D, according to EU numbering.

[00373] In some embodiments, the present invention provides a homodimeric Fc fusion protein, wherein said Fc domain has an additional set of amino acid substitutions selected from the group consisting of G236R/L328R, E233P/L234V/L235A/G236_/S239K, E233P/L234V/L235A/G236_/S239K/A327G, E233P/L234V/L235A/G236_/S267K/A327G, E233P/L234V/L235A/G236_, and E233P/L234V/L235A/G236_/S267K, according to EU numbering.

[00374] In some embodiments, the present invention provides a homodimeric Fc fusion protein wherein said IL-12p40 subunit has a polypeptide sequence selected form the group consisting of SEQ ID NO:3 (human IL-12 subunit beta (IL-12p40) precursor sequence) and SEQ ID NO:4 (human IL-12 subunit beta (IL-12p40) mature form sequence), and said IL-12p35 subunit has a polypeptide sequence selected from the group consisting of SEQ ID NO:1 (human IL-12 subunit alpha (IL-12p35) precursor sequence) and SEQ ID NO:2 (human IL-12 subunit alpha (IL-12p35) mature form sequence).

[00375] In some embodiments, the present invention provides a homodimeric Fc fusion protein wherein each of said Fc domain further comprises amino acid substitutions M428L/N424S.

[00376] In some embodiments, the present invention provides a homodimeric Fc fusion protein wherein said IL-12p40 subunit is a variant IL-12p40 subunit and/or said IL-12p35 subunit is a variant IL-12p35 subunit.

[00377] In some embodiments, the present invention provides a homodimeric Fc fusion protein wherein said IL-12p40 subunit is a variant IL-12p40 subunit having altered affinity for IL-12 receptor subunit beta-1 (IL-12Rß1), IL-12 receptor subunit beta-2 (IL-12Rß2), and/or IL-12 receptor complex; and/or said IL-12p35 subunit is a variant IL-12p35 subunit having altered affinity for IL-12 receptor subunit beta-1 (IL-12Rß1), IL-12 receptor subunit beta-2 (IL-12Rß2), and/or IL-12 receptor complex.

[00378] In some embodiments, the present invention provides a homodimeric Fc fusion protein wherein said IL-12p40 subunit has one or more amino acid modifications at amino acid residues selected from the group consisting of E3, D7, E12, D14, W15, P17, D18, A19, P20, G21, E22, M23, D29, E32, E33, D34, L40, D41, Q42, S43, E45, L47, T54, I55, Q56, K58, E59, F60, G61, D62, Q65, Y66, E73, K84, E86, D87, G88, I89, W90, D93, D97, K99, E100, K102, N103, K104, F106, E110, N113, Y114, D129, D142, Q144, E156, R159, D161, N162, K163, D166, D170, Q172, D174, A176, C177, P178, A179, A180, E181, S183, P185, E187, N200, S204, F206, R208, D209, D214, N218, Q220, N226, Q229, E231, E235, T242, P243, S245, Y246, F247, S248, C252S, Q256, K158, K260, E262, K264, D265, D270, N281, Q289, D290, R291, Y292, Y293, and E299.

[00379] In some embodiments, the present invention provides a homodimeric Fc fusion protein wherein said IL-12p40 subunit has one or more amino acid substitutions selected from the group consisting of D18N, D18K, E32Q, E33Q, D34N, D34K, Q42E, S43E, S43K, E45Q, Q56E, E59Q, E59K, D62N, E73Q, D87N, K99E, K99Y, E100Q, N103D, N103Q, N113D, N113Q, Q144E, D161N, R159E, K163E, E187Q, N200D, N200Q, N218Q, Q229E, E235Q, C252S, Q256N, K258E, K260E, E262Q, K264E, N281D, N281Q, and E299Q.

[00380] In some embodiments, the present invention provides a homodimeric Fc fusion protein wherein said IL-12p40 subunit has amino acid substitutions selected from the group consisting of N103D/N113D/N200D/N281D, Q42E/E45Q, E45Q/Q56E, Q42E/E59Q, Q56E/E59Q, Q42E/E45Q/Q56E, E45Q/Q56E/E59Q, E32Q/E59Q, D34N/E59K, D34N/E59K/K99E, D34K/E59K/K99E, E32Q/D34N/E59K/K99E, E32K/D34N/E59K/K99E, D34N/E59Q, E59Q/E187Q, S43E/E59Q, S43K/E49Q, E59Q/K163E, E59Q/K99E, E59Q/K258E, E59Q/K260E, E59K/K99E, D18K/E59K/K99E, E59K/K99E/K264E, E59K/K99Y, E59Y/K99Y, E59Y/K99E, E45K/E59K/K99E, E59K/K99E/Q144E, E59K/K99E/Q144K, E59K/K99E/R159E, E59K/K99E/K264E, D18K/E59K/K99E/K264E, DI8K/E59K/K99E/C252S, D18K/E59K/K99E/C252S/K264E, E59K/K99Y/C252S, E59K/K99E/C252S/K264E, E59K/K99E/C252S, N103D/N113D, N103D/N200D, N103D/N281D, N113D/N200D, N113D/N281D, N200D/N281D, N103D/N113D/N200D, N103D/N113D/N281D, N103D/N200D/N281D, N113D/N200D/N281D, N103Q/N113Q, N103Q/N200Q, N103Q/N281Q, N113Q/N200Q, N113Q/N281Q, N200Q/N281Q, N103Q/N113Q/N200Q, N103Q/N113Q/N281Q, N103Q/N200Q/N281Q, N113Q/N200Q/N281Q, N103Q/N113Q/N200Q/N281Q, E59K/K99E/N103Q/C252S/K264E, E59K/K99E/N113Q/C252S/K264E, E59K/K99E/N200Q/C252S/K264E, E59K/K99E/N281Q/C252S/K264E, E59K/K99E/N103Q/N113Q/C252S/K264E, E59K/K99E/N103Q/N200Q/C252S/K264E, E59K/K99E/N103Q/N281Q/C252S/K264E, E59K/K99E/N113Q/N200Q/C252S/K264E, E59K/K99E/N113Q/N281Q/C252S/K264E, E59K/K99E/N200Q/N281Q/C252S/K264E, E59K/K99E/N103Q/N113Q/N200Q/C252S/K264E, E59K/K99E/N103Q/N200Q/N281Q/C252S/K264E,

E59K/K99E/N113Q/N200Q/N281Q/C252S/K264E, and E59K/K99E/N103Q/N113Q/N200Q/N281Q/C252S/K264E.

In some embodiments, the present invention provides a homodimeric Fc fusion protein wherein said IL-12p40 subunit has a polypeptide sequence selected from the group consisting of:

i) SEQ ID NO:57 (IL-12p40(N103D)), ii) SEQ ID NO:58 (IL-12p40(N113D)), iii) SEQ ID NO:59 (IL-12p40(N200D)), iv) SEQ ID NO:60 (IL-12p40(N281D)), v) SEQ ID NO:61 (IL-12p40(N103D/N113D/N200D/N281D)), vi) SEQ ID NO:62 (IL-12p40(Q42E)), vii) SEQ ID NO:63 (IL-12p40(E45Q)), viii) SEQ ID NO:64 (IL-12p40(Q56E)), ix) SEQ ID NO:65 (IL-12p40(E59Q)), x) SEQ ID NO:66 (IL-12p40(D62N)), xi) SEQ ID NO:67 (IL-12p40(Q42E/E45Q)), xii) SEQ ID NO:68 (IL-12p40(E45Q/Q56E)), xiii) SEQ ID NO:69 (IL-12p40(Q42E/E59Q)), xiv) SEQ ID NO:70 (IL-12p40(Q56E/E59Q)), xv) SEQ ID NO:71 (IL-12p40(Q42E/E45Q/Q56E)), xvi) SEQ ID NO:72 (IL-12p40(E45Q/Q56E/E59Q)), xvii) SEQ ID NO:73 (IL-12p40(D161N)), xviii) SEQ ID NO:74 (IL-12p40(E73Q)), xix) SEQ ID NO:75 (IL-12p40(Q144E)), xx) SEQ ID NO:76 (IL-12p40(E262Q)), xxi) SEQ ID NO:77 (IL-12p40(E100Q)), xxii) SEQ ID NO:78 (IL-12p40(D18N)), xxiii) SEQ ID NO:79 (IL-12p40(E33Q)), xxiv) SEQ ID NO:80 (IL-12p40(Q229E)), xxv) SEQ ID NO:81 (IL-12p40(E235Q)), xxvi) SEQ ID NO:82 (IL-12p40(Q256N)), xxvii) SEQ ID NO:83 (IL-12p40(E299Q)), xxviii) SEQ ID NO:84 (IL-12p40(D87N)), xxix) IL-12p40(E32Q), xxx) IL-12p40(D34N), xxxi) IL-12p40(S43E), xxxii) IL-12p40(S43K), xxxiii) SEQ ID NO:XXX (IL-12p40 (E59K/K99E/N103Q/N113Q/N200Q/N281Q/C252S/K264E)), xxxiv) SEQ ID NO:205 (IL-12p40(E59K)), xxxv) IL-12p40(K99E), xxxvi) IL-12p40(K163E), xxxvii) IL-12p40(E187Q), xxxviii) IL-12p40(K258E), xxxix) IL-12p40(K260E), xl) SEQ ID NO:206 (IL-12p40(E32Q/E59Q)), xli) SEQ ID NO:207 (IL-12p40(D34N/E59Q)), xlii) SEQ ID NO:208 (IL-12p40(E59Q/E187Q)), xliii) SEQ ID NO:209 (IL-12p40(S43E/E59Q)), xliv) SEQ ID NO:210 (IL-12p40(S43K/E49Q)), xlv) SEQ ID NO:211 (IL-12p40(E59Q/K163E)), xlvi) SEQ ID NO:212 (IL-12p40(E59Q/K99E)), xlvii) SEQ ID NO:213 (IL-12p40(E59Q/K258E)), xlviii) SEQ ID NO:214 (IL-12p40(E59Q/K260E)), xlix) SEQ ID NO: XXX (IL-12p40 (D34N/E59K)), l) SEQ ID NO: XXX (IL-12p40(E59K/K99E)), li) SEQ ID NO: XXX (IL-12p40(D18K/E59K/K99E)), lii) SEQ ID NO: XXX (IL-12p40 (E59K/K99E/K264E)), liii) SEQ ID NO: XXX (IL-12p40 (E59K/K99Y)), liv) SEQ ID NO: XXX (IL-12p40 (E59Y/K99E)), lv)

SEQ ID NO: XXX (IL-12p40 (E45K/E59K/K99E)), lvi) SEQ ID NO: XXX (IL-12p40 (E59K/K99E/Q144E)), lvii) SEQ ID NO: XXX (IL-12p40 (E59K/K99E/Q144K)), lviii) SEQ ID NO: XXX (IL-12p40 (E59K/K99E/R159E)), lix) SEQ ID NO: XXX (IL-12p40 (E59K/K99E/K264E)), lx) SEQ ID NO: XXX (IL-12p40 (D18K/E59K/K99E/K264E)), lxi) SEQ ID NO: XXX (IL-12p40 (C252S)), lxii) SEQ ID NO: XXX (IL-12p40 (DI8K/E59K/K99E/C252S)), lxiii) SEQ ID NO: XXX (IL-12p40 (D18K/E59K/K99E/C252S/K264E)), lxiv) SEQ ID NO: XXX (IL-12p40 (E59K/K99Y/C252S)), lxv) SEQ ID NO: XXX (IL-12p40 (E59K/K99E/C252S/K264E)), lxvi) SEQ ID NO: XXX (IL-12p40 (E59K/K99E/C252S)), lxvii) SEQ ID NO: XXX (IL-12p40 (N103D/N113D)), lxviii) SEQ ID NO: XXX (IL-12p40 (N103D/N200D)), lxix) SEQ ID NO: XXX (IL-12p40 (N103D/N281D)), lxx) SEQ ID NO: XXX (IL-12p40 (N113D/N200D)), lxxi) SEQ ID NO: XXX (IL-12p40 (N113D/N281D)), lxxii) SEQ ID NO: XXX (IL-12p40 (N200D/N281D)), lxxiii) SEQ ID NO: XXX (IL-12p50 (N103D/N113D/N200D)), lxxiv) SEQ ID NO: XXX (IL-12p40 (N103D/N113D/N281D)), lxxv) SEQ ID NO: XXX (IL-12p40 (N103D/N200D/N281D)), lxxvi) SEQ ID NO: XXX (IL-12p40 (N113D/N200D/N281D)), lxxvii) SEQ ID NO: XXX (IL-12p40 (N103Q)), lxxviii) SEQ ID NO: XXX (IL-12p40 (N113Q)), lxxix) SEQ ID NO: XXX (IL-12p40 (N200Q)), lxxx) SEQ ID NO: XXX (IL-12p40 (N281Q)), lxxxi) SEQ ID NO: XXX (IL-12p40 (N103Q/N113Q)), lxxxii) SEQ ID NO: XXX (IL-12p40 (N103Q/N200Q)), lxxxiii) SEQ ID NO: XXX (IL-12p40 (N103Q/N281Q)), lxxxiv) SEQ ID NO: XXX (IL-12p40 (N113Q/N200Q)), lxxxv) SEQ ID NO: XXX (IL-12p40 (N113Q/N281Q)), lxxxvi) SEQ ID NO: XXX (IL-12p40 (N200Q/N281Q)), lxxxvii) SEQ ID NO: XXX (IL-12p40 (N103Q/N113Q/N200Q)), lxxxviii) SEQ ID NO: XXX (IL-12p40 (N103Q/N113Q/N281Q)), lxxxix) SEQ ID NO: XXX (IL-12p40 (N103Q/N200Q/N281Q)), xc) SEQ ID NO: XXX (IL-12p40 (N113Q/N200Q/N281Q)), xci) SEQ ID NO:XXX (IL-12p40 (N103Q/N113Q/N200Q/N281Q)), xcii) SEQ ID NO:XXX (IL-12p40 (D34N/E59K/K99E)), xciii) SEQ ID NO:XXX (IL-12p40 (D34K/E59K/K99E)), xciv) SEQ ID NO:XXX (IL-12p40 (E32Q/D34N/E59K/K99E)), xcv) SEQ ID NO:XXX (IL-12p40 (E32K/D34N/E59K/K99E)), xcvi) SEQ ID NO: XXX (IL-12p40 (E59Y/K99Y)), xcvii) SEQ ID NO:XXX (IL-12p40 (E59K/K99E/N103Q/C252S/K264E)), xcviii) SEQ ID NO:XXX (IL-12p40 (E59K/K99E/N113Q/C252S/K264E)), xcix) SEQ ID NO:XXX (IL-12p40 (E59K/K99E/N200Q/C252S/K264E)), c) SEQ ID NO:XXX (IL-12p40

(E59K/K99E/N281Q/C252S/K264E)), ci) SEQ ID NO:XXX (IL-12p40 (E59K/K99E/N103Q/N113Q/C252S/K264E)), cii) SEQ ID NO:XXX (IL-12p40 (E59K/K99E/N103Q/N200Q/C252S/K264E)), ciii) SEQ ID NO:XXX (IL-12p40 (E59K/K99E/N103Q/N281Q/C252S/K264E)), civ) SEQ ID NO:XXX (IL-12p40 (E59K/K99E/N113Q/N200Q/C252S/K264E)), cv) SEQ ID NO:XXX (IL-12p40 (E59K/K99E/N113Q/N281Q/C252S/K264E)), cvi) SEQ ID NO:XXX (IL-12p40 (E59K/K99E/N200Q/N281Q/C252S/K264E)), cvii) SEQ ID NO:XXX (IL-12p40 (E59K/K99E/N103Q/N113Q/N200Q/C252S/K264E)), cviii) SEQ ID NO:XXX (IL-12p40 (E59K/K99E/N103Q/N200Q/N281Q/C252S/K264E)), and cix) SEQ ID NO:XXX (IL-12p40 (E59K/K99E/N113Q/N200Q/N281Q/C252S/K264E)).

[00381]

[00382] In some embodiments, the present invention provides a homodimeric Fc fusion protein wherein said IL-12p35 subunit has one or more amino acid modifications as amino acid residues selected from the group consisting of Q20, N21, Q35, E38, S44, E45, E46, H49, K54, D55, T59, V60, E61, C63, L64, P65, E67, L68, N71, S73, C74, L75, N76, E79, N85, L89, F96, M97, L124, M125, Q130, Q135, N136, E143, Q146, N151, E153, K158, E162, E163, D165, I171, R181, I182, R183, V185, T186, D188, R189, V190, S192, Y193, N195, and A196.

[00383] In some embodiments, the present invention provides a homodimeric Fc fusion protein wherein said IL-12p35 subunit has one or more amino acid substitutions selected from the group consisting of N21D, Q35D, E38Q, D55Q, D55K, N71D, N71Q, L75A, N76D, E79Q, N85D, N85Q, L89A, F96A, M97A, L124A, M125A, Q130E, Q135E, N136D, E143Q, Q146E, N151D, N151K, E153K, E153Q, K158E, E162Q, E163Q, D165N, I171A, N195D, and N195Q.

[00384] In some embodiments, the present invention provides a homodimeric Fc fusion protein wherein said IL-12p35 subunit has amino acid substitutions selected from the group consisting of N71D/N85D/N195D, N151D/E153Q, N151D/D165N, Q130E/N151D, N151D/K158E, E79Q/N151D, D55Q/N151D, N136D/N151D, N21D/N151D, E143Q/N151D, N71Q/N85Q,

N71Q/N195Q, N85Q/N195Q, N71Q/N85Q/N195Q, N71D/N85D, N71D/N195D, and N85D/N195D.

[00385] In some embodiments, the present invention provides a homodimeric Fc fusion protein wherein said IL-12p35 subunit has a polypeptide sequence selected from the group consisting of: i) SEQ ID NO:113 (IL-12p35(N71D)), ii) SEQ ID NO:114 (IL-12p35(N85D)), iii) SEQ ID NO:115 (IL-12p35(N195D)), iv) SEQ ID NO:116 (IL-12p35(N71D/N85D/N195D)), v) SEQ ID NO:117 (IL-12p35(E153Q)), vi) SEQ ID NO:118 (IL-12p35(E38Q)), vii) SEQ ID NO:119 (IL-12p35(N151D)), viii) SEQ ID NO:120 (IL-12p35(Q135E)), ix) SEQ ID NO:121 (IL-12p35(Q35D)), x) SEQ ID NO:122 (IL-12p35(Q146E)), xi) SEQ ID NO:123 (IL-12p35(N76D)), xii) SEQ ID NO:124 (IL-12p35(E162Q)), xiii) SEQ ID NO:125 (IL-12p35(E163Q)), xiv) IL-12p35(N21D), xv) SEQ ID NO:XXX (IL-12p35(D55Q)), xvi) IL-12p35(E79Q), xvii) IL-12p35(Q130E), xviii) IL-12p35(N136D), xix) IL-12p35(E143Q), xx) SEQ ID NO:227 (IL-12p35(N151K)), xxi) SEQ ID NO:226 (IL-12p35(E153K)), xxii) IL-12p35(K158E), xxiii) IL-12p35(D165N), xxiv) SEQ ID NO:225 (IL-12p35(N151D/E153Q)), xxv) SEQ ID NO:228 (IL-12p35(N151D/D165N)), xxvi) SEQ ID NO:229 (IL-12p35(Q130E/N151D)), xxvii) SEQ ID NO:230 (IL-12p35(N151D/K158E)), xxviii) SEQ ID NO:231 (IL-12p35(E79Q/N151D)), xxix) SEQ ID NO:232 (IL-12p35(D55Q/N151D)), xxx) SEQ ID NO:233 (IL-12p35(N136D/N151D)), xxxi) SEQ ID NO:234 (IL-12p35(N21D/N151D)), xxxii) SEQ ID NO:235 (IL-12p35(E143Q/N151D)), xxxiii) SEQ ID NO: XXX (IL-12p35(F96A)), xxxiv) SEQ ID NO: XXX (IL-12p35(M97A)), xxxv) SEQ ID NO: XXX (IL-12p35(L89A)), xxxvi) SEQ ID NO: XXX (IL-12p35(L124A)), xxxvii) SEQ ID NO: XXX (IL-12p35(M125A)), xxxviii) SEQ ID NO: XXX (IL-12p35(L75A)), xxxiv) SEQ ID NO: XXX (IL-12p35(I171A)), xxxv) SEQ ID NO: XXX (IL-12p35 (N71Q)), xxxvi) SEQ ID NO: XXX (IL-12p35 (N85Q)), xxxvii) SEQ ID NO: XXX (IL-12p35 (N195Q)), xxxviii) SEQ ID NO: XXX (IL-12p35 (N71Q/N85Q)), xxxix) SEQ ID NO: XXX (IL-12p35 (N71Q/N195Q)), xl) SEQ ID NO: XXX (IL-12p35 (N85Q/N195Q), xli) SEQ ID NO: XXX (IL-12p35 (N71Q/N85Q/N195Q)), xlii) SEQ ID NO: XXX (IL-12p35 (N71D/N85D)), xliii) SEQ ID NO: XXX (IL-12p35 (N71D/N195D), xliv) SEQ ID NO: XXX (IL-12p35 (N85D/N195D)), xlv) SEQ ID NO: XXX (IL-12p35 (D55Q)), and xlvi) SEQ ID NO: XXX (IL-12p35 (D55K)).

[00386] In some embodiments, the present invention provides a homodimeric Fc fusion protein wherein said first and second monomers are XENP31289.

[00387] In some embodiments, the present invention provides a homodimeric Fc fusion protein wherein said first and second monomers are XENP31291.

[00388] In some embodiments, the present invention provides a homodimeric Fc fusion protein composition comprising a homodimeric Fc fusion protein for use in treating cancer in a subject.

[00389] In some embodiments, the present invention provides a one or more nucleic acids encoding a homodimeric Fc fusion protein.

[00390] In some embodiments, the present invention provides a host cell comprising said one or more nucleic acids encoding a homodimeric Fc fusion protein.

[00391] In some embodiments, the present invention provides a method of making a homodimeric Fc fusion protein, said method comprising culturing a host cell under conditions whereby said homodimeric Fc fusion protein is produced.

[00392] In some embodiments, the present invention provides a method of purifying a homodimeric Fc fusion protein, said method comprising: a) providing a composition comprising the homodimeric Fc fusion protein; b) loading said composition onto an ion exchange column; and c) collecting a fraction containing said homodimeric Fc fusion protein.

[00393] In another aspect, the present invention provides a method of treating cancer in a patient in need thereof, the method comprising administering a therapeutically effective amount of a heterodimeric or homodimeric Fc fusion protein according to any of the previous claims to said patient.

[00394] In some embodiments, the present invention provides a method of treating cancer in a patient in need thereof, further comprising administering a therapeutically effective amount of a checkpoint blockade antibody.

[00395] In some further embodiments, the present invention provides a method of treating cancer in a patient in need thereof, wherein said checkpoint blockade antibody is selected from an anti-PD-1 antibody, an anti-PD-L1 antibody, an anti-TIM3 antibody, an anti-TIGIT antibody, an anti-LAG3 antibody, and an anti-CTLA-4 antibody.

[00396] In some further embodiments, the present invention provides a method of treating cancer in a patient in need thereof, wherein said anti-PD-1 antibody is nivolumab, pembrolizumab, or pidilizumab.

[00397] In some further embodiments, the present invention provides a method of treating cancer in a patient in need thereof, wherein said anti-PD-L1 antibody is atezolizumab, avelumab, or durbalumab.

[00398] In some further embodiments, the present invention provides a method of treating cancer in a patient in need thereof, wherein the patient exhibits an increase in lymphocytes following administration.

[00399] In some further embodiments, the present invention provides a method of treating cancer in a patient in need thereof, wherein the patient exhibits an increase in peripheral CD8+ T cells following administration.

[00400] In some embodiments, the present invention provides method of treating cancer in a patient in need thereof wherein the IL-12 Fc fusion protein is administered before the checkpoint inhibitor to increase checkpoint expression prior to treatment.

[00401] In some embodiments, the present invention provides a method of treating cancer in a patient in need thereof wherein the IL-12 Fc fusion protein is administered before the checkpoint inhibitor to increase checkpoint expression prior to treatment.

[00402] In another aspect, the present invention provides a method of inducing T cell expansion in a patient in need thereof comprising administering a therapeutically effective amount of a

heterodimeric or homodimeric Fc fusion protein according any of the previous claims to said patient.

[00403] In some further embodiments, the present invention provides a method of inducing T cell expansion in a patient in need thereof, further comprising administering a therapeutically effective amount of a checkpoint blockade antibody.

[00404] In some further embodiments, the present invention provides a method of inducing T cell expansion in a patient in need thereof, wherein said checkpoint blockade antibody is selected from an anti-PD-1 antibody, an anti-PD-L1 antibody, an anti-TIM3 antibody, an anti-TIGIT antibody, an anti-LAG3 antibody, and an anti-CTLA-4 antibody.

[00405] In some further embodiments, the present invention provides a method of inducing T cell expansion in a patient in need thereof, wherein said anti-PD-1 antibody is nivolumab, pembrolizumab, or pidilizumab.

[00406] In some other further embodiments, the present invention provides a method of inducing T cell expansion in a patient in need thereof, wherein said anti-PD-L1 antibody is atezolizumab, avelumab, or durbalumab.

[00407] In some further embodiments, the present invention provides a method of inducing T cell expansion in a patient in need thereof, wherein the T cell expansion is at least a 2-fold increase in T cells.

[00408] In some embodiments, the present invention provides a method of inducing T cell expansion in a patient in need thereof wherein the IL-12 Fc fusion protein is administered before the checkpoint inhibitor to increase checkpoint expression prior to treatment.

[00409] In some embodiments, the present invention provides a method of inducing T cell expansion in a patient in need thereof wherein the IL-12 Fc fusion protein is administered before the checkpoint inhibitor to increase checkpoint expression prior to treatment.

[00410] In one aspect the present invention provides an IL-12p40 subunit. In another aspect the present invention provides an IL-12p35 subunit. In a further aspect the present invention provides a heterodimeric complex comprising a) an IL-12p40 subunit and b) a IL-12p35 subunit.

BRIEF DESCRIPTION OF THE DRAWINGS

[00411] Figure 1A and 1B depicts the sequences for IL-12 and its receptors.

[00412] Figure 2A-2E depicts useful pairs of Fc heterodimerization variant sets (including skew and pI variants). Variants without a corresponding "monomer 2" are pI variants which can be used alone on either monomer.

[00413] Figure 3 depicts a list of isosteric variant antibody constant regions and their respective substitutions. pI_(-) indicates lower pI variants, while pI_(+) indicates higher pI variants. These can be optionally and independently combined with other heterodimerization variants of the inventions (and other variant types as well, as outlined herein.)

[00414] Figure 4 depicts useful ablation variants that ablate FcγR binding (sometimes referred to as "knock outs" or "KO" variants). Generally, ablation variants are found on both monomers, although in some cases they may be on only one monomer.

[00415] Figure 5 shows particularly useful embodiments of "non-cytokine" components of the invention.

[00416] Figure 6 depicts a number of exemplary domain linkers. In some embodiments, these linkers find use linking the IL-12p35 subunit, the IL-12p40 subunit, or the single-chain IL-12 complex to the N-terminus of the Fc region. In some embodiments, these linkers find use fusing IL-12p35 subunit to the IL-12p40 subunit in the single-chain IL-12 complex. It is important to note that the scIL-12 complex can comprise either IL-12p35 N-terminally linked to IL-12p40 or IL-12p40 N-terminally linked to IL-12p35. Also, in some cases as described herein, the hinge portion of an Fc domain serves as a domain linker, which can be combined with any of these linkers as well.

[00417] Figure 7A-7E shows the sequences of several useful IL-12-Fc fusion backbones based on human IgG1, without the cytokine sequences (e.g. the IL-12p35 subunit, the IL-12p40 subunit, or the scIL-12 complex). Backbone 1 is based on human IgG1 (356E/358M allotype), and includes C220S on both chains, the S364K/E357Q : L368D/K370S skew variants, the Q295E/N384D/Q418E/N421D pI variants on the chain with L368D/K370S skew variants and the E233P/L234V/L235A/G236del/S267K ablation variants on both chains. Backbone 2 is based on human IgG1 (356E/358M allotype), and includes C220S on both chains, the S364K : L368D/K370S skew variants, the Q295E/N384D/Q418E/N421D pI variants on the chain with L368D/K370S skew variants and the E233P/L234V/L235A/G236del/S267K ablation variants on both chains. Backbone 3 is based on human IgG1 (356E/358M allotype), and includes C220S on both chains, the S364K : L368E/K370S skew variants, the Q295E/N384D/Q418E/N421D pI variants on the chain with L368E/K370S skew variants and the E233P/L234V/L235A/G236del/S267K ablation variants on both chains. Backbone 4 is based on human IgG1 (356E/358M allotype), and includes C220S on both chains, the D401K : K360E/Q362E/T411E skew variants, the Q295E/N384D/Q418E/N421D pI variants on the chain with K360E/Q362E/T411E skew variants and the E233P/L234V/L235A/G236del/S267K ablation variants on both chains. Backbone 5 is based on human IgG1 (356D/358L allotype), and includes C220S on both chains, the S364K/E357Q : L368D/K370S skew variants, the Q295E/N384D/Q418E/N421D pI variants on the chain with L368D/K370S skew variants and the E233P/L234V/L235A/G236del/S267K ablation variants on both chains. Backbone 6 is based on human IgG1 (356E/358M allotype), and includes C220S on both chains, the S364K/E357Q : L368D/K370S skew variants, Q295E/N384D/Q418E/N421D pI variants on the chain with L368D/K370S skew variants and the E233P/L234V/L235A/G236del/S267K ablation variants on both chains, as well as an N297A variant on both chains. Backbone 7 is identical to 6 except the mutation is N297S. Alternative formats for backbones 6 and 7 can exclude the ablation variants E233P/L234V/L235A/G236del/S267K in both chains. Backbone 8 is based on human IgG4, and includes the S364K/E357Q : L368D/K370S skew variants, the Q295E/N384D/Q418E/N421D pI variants on the chain with L368D/K370S skew variants, as well as a S228P (EU numbering, this is S241P in Kabat) variant on both chains that ablates Fab arm exchange as is known in the art.

Backbone 9 is based on human IgG2, and includes the S364K/E357Q : L368D/K370S skew variants, the Q295E/N384D/Q418E/N421D pI variants on the chain with L368D/K370S skew variants. Backbone 10 is based on human IgG2, and includes the S364K/E357Q : L368D/K370S skew variants, the Q295E/N384D/Q418E/N421D pI variants on the chain with L368D/K370S skew variants as well as a S267K variant on both chains. Backbone 11 is identical to backbone 1, except it includes M428L/N434S Xtend mutations. Backbone 12 is based on human IgG1 (356E/358M allotype), and includes C220S on both identical chain, the the E233P/L234V/L235A/G236del/S267K ablation variants on both identical chains. Backbone 13 is based on human IgG1 (356E/358M allotype), and includes C220S on both chain, the S364K/E357Q : L368D/K370S skew variants, the P217R/P229R/N276K pI variants on the chain with S364K/E357Q skew variants and the E233P/L234V/L235A/G236del/S267K ablation variants on both chains.

[00418] As will be appreciated by those in the art and outlined below, these sequences can be used with any IL-12-Fc fusion formats outlined herein, including but not limited to IL-12-heteroFc, heteroFc-IL-12, and scIL-12-Fc formats as schematically depicted in Figure 8. It should be noted that for heteroFc-IL-12 fusions, the backbones may further comprise deletion of K447. Additionally, any IL-12p35 and/or IL-12p40 variants can be incorporated into these Figure 7 backbones in any combination.

[00419] Included within each of these backbones are sequences that are 90, 95, 98 and 99% identical (as defined herein) to the recited sequences, and/or contain from 1, 2, 3, 4, 5, 6, 7, 8, 9 or 10 additional amino acid substitutions (as compared to the "parent" of the Figure, which, as will be appreciated by those in the art, already contain a number of amino acid modifications as compared to the parental human IgG1 (or IgG2 or IgG4, depending on the backbone). That is, the recited backbones may contain additional amino acid modifications (generally amino acid substitutions) in addition to the skew, pI and ablation variants contained within the backbones of this figure.

[00420] Figures 8A-8F depict illustrative formats for the IL-12-Fc fusion proteins of the present invention. The N-terminal IL-12 heterodimeric Fc fusion or "IL-12-heteroFc" (Figure 8A) format comprises the IL-12p40 subunit recombinantly fused to the N-terminus of one side of a heterodimeric Fc and the IL-12p35 subunit recombinantly fused to N-terminus of the other side of the heterodimeric Fc. The IL-12p35 and IL-12p40 subunits may be linked to their respective Fc chains by a domain linker. The C-terminal IL-12 heterodimeric Fc fusion or "heteroFc-IL-12" (Figure 8B) format comprises the IL-12p40 subunit recombinantly fused to the C-terminus of one side of a heterodimeric Fc and the IL-12p35 subunit recombinantly fused to the C-terminus of the other side of the heterodimeric Fc. The IL-12p35 and IL-12p40 subunits may be linked to their respective Fc chains by a domain linker. The N-terminal single-chain IL-12-Fc fusion or "scIL-12-Fc" (Figures 8C-D) format comprises a single-chain IL-12 complex (or "scIL-12 complex") recombinantly fused to the N-terminus of one side of a heterodimeric Fc (optionally via a domain linker), with the other side of the molecule being a "Fc-only" or "empty-Fc" heterodimeric Fc. The C-terminal single-chain IL-12-Fc fusion or "Fc-scIL-12" (Figures 8E-F) format comprises a scIL-12 complex recombinantly fused to the C-terminus of one side of a heterodimeric Fc (optionally via a domain linker), with the other side of the molecule being a "Fc-only" or "empty-Fc" heterodimeric Fc. The scIL-12 complex can comprise either IL-12p35 N-terminally linked to IL-12p40 or IL-12p40 N-terminally linked to IL-12p35, optionally but generally with a domain linker. The order of the two subunits in the scIL-12 complex may be designated as follows: "scIL-12(p40/p35)", wherein the IL-12p40 subunit is N-terminally linked to the IL-12p35 subunit, or "scIL-12(p35/p40)", wherein the IL-12p35 is N-terminally linked to the IL-12p40 subunit.

[00421] Figure 9 depicts the sequences of XENP27201, an illustrative IL-12-Fc fusion protein of the "IL-12-heteroFc" format, that contains the wild-type IL-12p40 and wild-type IL-12p35 sequences. Linkers are double underlined (although as will be appreciated by those in the art, the linkers can be replaced by other linkers, some of which are depicted in Figure 6), and slashes (/) indicate the border(s) between IL-12p35, IL-12p40, linkers, and Fc regions.

[00422] Figure 10 depicts the sequences of XENP27202, an illustrative IL-12-Fc fusion protein of the "heteroFc-IL-12" format, that contains the wild-type IL-12p40 and wild-type IL-12p35 sequences. Linkers are double underlined (although as will be appreciated by those in the art, the linkers can be replaced by other linkers, some of which are depicted in Figure 6), and slashes (/) indicate the border(s) between IL-12p35, IL-12p40, linkers, and Fc regions.

[00423] Figure 11 depicts the sequences of XENP27203 and XENP27204, illustrative IL-12-Fc fusion proteins of the "scIL-12-Fc" format, that contains the wild-type IL-12p40 and wild-type IL-12p35 sequences. Linkers are double underlined (although as will be appreciated by those in the art, the linkers can be replaced by other linkers, some of which are depicted in Figure 6), and slashes (/) indicate the border(s) between IL-12p35, IL-12p40, linkers, and Fc regions.

[00424] Figure 12A-12B depict A) chromatogram illustrating purification part 2 of XENP27201 (anion exchange chromatography following protein A chromatography), and the purity and homogeneity of peak B isolated from anion exchange separation as depicted in Figure 12A in comparison to peak A as determined by B) analytical size-exclusion chromatography with multi-angle light scattering (aSEC-MALS) and C) analytical anion exchange chromatography (analytical AIEX). Figure 12B also depicts the molecular weight of protein species in peaks as determined by multi-angle light scattering.

[00425] Figure 13A-13C depicts A) chromatogram illustrating purification part 2 of XENP27203 (anion exchange chromatography following protein A chromatography), and the purity and homogeneity of peak B isolated from anion exchange separation as depicted in Figure 13A in comparison to peak A as determined by B) analytical size-exclusion chromatography with multi-angle light scattering (SEC-MALS) and C) analytical anion exchange chromatography. Figure 13B also depicts the molecular weight of protein species in peaks as determined by multi-angle light scattering.

[00426] Figure 14A-14B depicts cartoon schematics for A) bivalent IL-12p40-Fc fusion and B) bivalent IL-12p35-Fc fusion. Each fusion comprises either IL-12p40 or IL-12p35 subunits

recombinant fused to the N-terminus of a homodimeric Fc. The subunits may have a domain linker between their respective C-terminus and the N-terminus of the Fc region.

[00427] Figure 15 depicts the sequences of XENP27560, a bivalent IL-12p40-Fc fusion, and XENP27561, a bivalent IL-12p35-Fc fusion (cartoon schematics depicted in Figure 14), that contains the wild-type IL-12p40 and wild-type IL-12p35 sequences. Linkers are double underlined (although as will be appreciated by those in the art, the linkers can be replaced by other linkers, some of which are depicted in Figure 6), and slashes (/) indicate the border(s) between IL-12p35, IL-12p40, linkers, and Fc regions.

[00428] Figure 16A-16D depicts STAT4 phosphorylation on A) $CD4^{+}CD45RA^{-}CD25^{hi}$ T cells, B) $CD8^{+}CD45RA^{-}CD25^{hi}$ T cells, C) γδ T cells, and D) NK cells following incubation of activated PBMCs with the indicated test articles.

[00429] Figure 17 depicts residues on IL-12p40 (based on IL-12p40 mature form sequence) predicted to contribute to the binding of IL-12p40 with IL-12 receptors.

[00430] Figure 18 depicts aspartic acid, glutamic acid, asparagine, and glutamine residues on IL-12p40 (based on IL-12p40 mature form sequence) identified using the QuaSAR package in MOE to have an ASA score (water accessible surface area calculated using a radius of 1.4 Å for the water molecule and a polyhedral representation for each atom) of at least 19.

[00431] Figure 19 depicts residues on IL-12p40 (based on IL-12p40 mature form sequence) predicted to be in contact with IL-23 receptors (based on crystal structure deposited in the PDB with accession number 5MZV) as well as the predicted contact type(s). "D" indicates contact predicted based on proximity. "H" indicates contact predicted based on potential hydrogen bond. "I" indicates contact predicted based on potential salt bridge. "A" indicates contact predicted based potential arene binding.

[00432] Figure 20A-20I depicts sequences for illustrative IL-12p40 variants designed with the view to reduce the affinity of the IL-12 heterodimeric complex for the IL-12 receptors and/or remove putative glycosylation sites. Modified amino acids are underlined and in bold.

[00433] Figure 21A-21J depicts the amino acid sequences for illustrative IL-12p40 variants with Fc fusion partners. Domain linkers are double-underlined, and IL-12p40 variants are italicized.

[00434] Figure 22 depicts aspartic acid, glutamic acid, asparagine, and glutamine residues on IL-12p35 (based on IL-12p35 mature form sequence) identified using the QuaSAR package in MOE to have an ASA score (water accessible surface area calculated using a radius of 1.4 Å for the water molecule and a polyhedral representation for each atom) of at least 103.

[00435] Figure 23A-23C depicts sequences for illustrative IL-12p35 variants designed with the view to reduce the affinity of the IL-12 heterodimeric complex for the IL-12 receptors and/or remove putative glycosylation sites. Modified amino acids are underlined and in bold.

[00436] Figure 24A-24D depicts the amino acid sequences for illustrative IL-12p35 variants with Fc fusion partners. Domain linkers are double-underlined, and IL-12p35 variants are italicized.

[00437] Figure 25A-25Q depicts sequences for illustrative variant IL-12-Fc fusions designed with the view to reduce the affinity of the IL-12-Fc fusions for IL-12 receptors or to remove putative glycosylation sites. Linkers are double underlined (although as will be appreciated by those in the art, the linkers can be replaced by other linkers, some of which are depicted in Figure 6), and slashes (/) indicate the border(s) between IL-12p35, IL-12p40, linkers, and Fc regions.

[00438] Figure 26A-26B depicts STAT4 phosphorylation on A) $CD4^+CD45RA^+CD25^+$ T cells and B) $CD8^+CD45RA^+CD25^+$ T cells following incubation of activated PBMCs with IL-12-Fc fusions comprising IL-12p40 variants engineered with an aim to reduce affinity and potency

[00439] Figure 27A-27B depicts STAT4 phosphorylation on A) $CD4^+CD45RA^+CD25^+$ T cells and B) $CD8^+CD45RA^+CD25^+$ T cells following incubation of activated PBMCs with IL-12-Fc fusions comprising IL-12p35 variants engineered with an aim to reduce affinity and potency

[00440] Figure 28 depicts the EC50 (for STAT4 phosphorylation) as IL-12-Fc fusions comprising IL-12p40 or IL-12p35 variants and the fold decrease in EC50 relative to WT IL-12-Fc XENP27201. 27201-1 and 27201-2 represent two separately produced batches of XENP27201.

[00441] Figure 29A-29B depicts sequences for illustrative IL-12p40 variants designed with the view to reduce the affinity of the IL-12 heterodimeric complex for the IL-12 receptors. Modified amino acids are underlined and in bold.

[00442] Figure 30A-30D depicts the amino acid sequences for illustrative IL-12p40 variants with Fc fusion partner. Domain linkers are double-underlined, and IL-12p40 variants are italicized.

[00443] Figure 31A-31B depicts sequences for illustrative IL-12p35 variants designed with the view to reduce the affinity of the IL-12 heterodimeric complex for the IL-12 receptors. Modified amino acids are underlined and in bold.

[00444] Figure 32A-32C depicts the amino acid sequences for illustrative IL-12p35 variants with Fc fusion partners. Domain linkers are double-underlined, and IL-12p35 variants are italicized.

[00445] Figure 33A-33P depicts sequences for illustrative variant IL-12-Fc fusions designed with the view to reduce the affinity of the IL-12-Fc fusions for IL-12 receptors. Linkers are double underlined (although as will be appreciated by those in the art, the linkers can be replaced by other linkers, some of which are depicted in Figure 6), and slashes (/) indicate the border(s) between IL-12p35, IL-12p40, linkers, and Fc regions.

[00446] Figure 34A and 34B depicts STAT4 phosphorylation on A) $CD4^+CD45RA^+CD25^+$ T cells and B) $CD8^+CD45RA^+CD25^+$ T cells following incubation of activated PBMCs with IL-12-Fc fusions comprising IL-12p40 variants engineered with an aim to reduce affinity and potency.

[00447] Figure 35A and 35B depicts STAT4 phosphorylation on A) $CD4^+CD45RA^+CD25^+$ T cells and B) $CD8^+CD45RA^+CD25^+$ T cells following incubation of activated PBMCs with IL-12-Fc fusions comprising IL-12p40 and/or IL-12p35 variants engineered with an aim to reduce affinity and potency.

[00448] Figure 36 depicts the EC50 (for STAT4 phosphorylation) of IL-12-Fc fusions comprising IL-12p40 and/or IL-12p35 variants and the fold decrease in EC50 relative to WT IL-12-Fc

XENP27201. The data show that potency was reduced by up to 12-fold by an IL-12-Fc fusion comprising only a E59K substitution in the IL-12p40 subunit.

[00449] Figure 37A-37B depicts sequences for illustrative IL-12p40 variants designed with the view to reduce the affinity of the IL-12 heterodimeric complex for the IL-12 receptors. Modified amino acids are underlined and in bold.

[00450] Figure 38 depicts sequences for illustrative IL-12p35 variants designed with the view to reduce the affinity of the IL-12 heterodimeric complex for the IL-12 receptors. Modified amino acids are underlined and in bold.

[00451] Figure 39A-39I depicts sequences for illustrative variant IL-12-Fc fusions designed with the view to reduce the affinity of the IL-12-Fc fusions for IL-12 receptors. Linkers are double underlined (although as will be appreciated by those in the art, the linkers can be replaced by other linkers, some of which are depicted in Figure 6), and slashes (/) indicate the border(s) between IL-12p35, IL-12p40, linkers, and Fc regions.

[00452] Figure 40A-40B depicts STAT4 phosphorylation on A) $CD4^+CD45RA^+CD25^+$ T cells and B) $CD8^+CD45RA^+CD25^+$ T cells following incubation of activated PBMCs with IL-12-Fc fusions comprising Il-12p40 and/or IL-12p35 variants engineered with an aim to reduce affinity and potency.

[00453] Figure 41 depicts the EC50 (for STAT4 phosphorylation) of IL-12-Fc fusions comprising IL-12p40 and/or IL-12p35 variants and the fold decrease in EC50 relative to WT IL-12-Fc XENP27201. The data show that potency was reduced by up to 100-fold.

[00454] Figure 42A-42B depicts sequences for illustrative IL-12p40 variants designed with the view to reduce the affinity of the IL-12 heterodimeric complex for the IL-12 receptors. Modified amino acids are underlined and in bold.

[00455] Figure 43 depicts sequences for illustrative IL-12p35 variants designed with the view to reduce the affinity of the IL-12 heterodimeric complex for the IL-12 receptors. Modified amino acids are underlined and in bold.

[00456] Figure 44A-44K depicts sequences for illustrative variant IL-12-Fc fusions designed with the view to reduce the affinity of the IL-12-Fc fusions for IL-12 receptors. Linkers are double underlined (although as will be appreciated by those in the art, the linkers can be replaced by other linkers, some of which are depicted in Figure 6), and slashes (/) indicate the border(s) between IL-12p35, IL-12p40, linkers, and Fc regions.

[00457] Figure 45A-45C depicts sequences for illustrative variant IL-12-Fc fusions designed with the view to reduce the affinity of the IL-12-Fc fusions for IL-12 receptors, further engineered with Xtend Fc (M428L/N434S) for extending half-life. Linkers are double underlined (although as will be appreciated by those in the art, the linkers can be replaced by other linkers, some of which are depicted in Figure 6), and slashes (/) indicate the border(s) between IL-12p35, IL-12p40, linkers, and Fc regions. It should be noted that these sequences are provided for illustrative purposes, and that any of the sequences depicted in the other Figures may also include Xtend Fc (M428L/N434S) for extending half-life.

[00458] Figure 46A-46D depict STAT4 phosphorylation on A) $CD4^{+}CD45RA^{+}CD25^{+}$ T cells, B) $CD4^{+}CD45RA^{-}CD25^{+}$ T cells, C) $CD8^{+}CD45RA^{+}CD25^{+}$ T cells, and D) $CD8^{+}CD45RA^{-}CD25^{+}$ T cells following incubation of activated PBMCs with IL-12-Fc fusions comprising IL-12p40 and/or IL-12p35 variants engineered with an aim to reduce affinity and potency.

[00459] Figure 47 depicts the EC50 (for STAT4 phosphorylation) of IL-12-Fc fusions comprising IL-12p40 and/or IL-12p35 variants and the fold decrease in EC50 relative to WT IL-12-Fc XENP27201.

[00460] Figures 48A-48D depict illustrative formats for bivalent IL-12-Fc fusion proteins of the present invention. The bivalent N-terminal single-chain (Figures 48A-B) format comprises two identical monomers each comprising a scIL-12 complex recombinant fused to the N-terminus of

a homodimeric Fc chain (optionally via a domain linker). The bivalent C-terminal single-chain (Figures 48C-D) format comprises two identical monomers each comprising a scIL-12 complex recombinant fused to the C-terminus of a homodimeric Fc chain (optionally via a domain linker). The scIL-12 complex can comprise either IL-12p35 N-terminally linked to IL-12p40 or IL-12p40 N-terminally linked to IL-12p35, optionally with a domain linker. The order of the two subunits in the scIL-12 complex may be designated as follows: "scIL-12(p40/p35)", wherein the IL-12p40 subunit is N-terminally linked to the IL-12p35 subunit, or "scIL-12(p35/p40)", wherein the IL-12p35 is N-terminally linked to the IL-12p40 subunit.

[00461] Figure 49 depicts the sequences for XENP31289 and XENP31291, illustrative IL-12-Fc fusion proteins of the (scIL-12(p40/p35))$_2$-Fc format. XENP31289 contains the wild-type IL-12p40 and wild-type IL-12p35 subunits. XENP31291 contains the IL-12p40(E59K/K99E) variant and wild-type IL-12p35 subunits. Linkers are double underlined (although as will be appreciated by those in the art, the linkers can be replaced by other linkers, some of which are depicted in Figure 6), and slashes (/) indicate the border(s) between IL-12p35, IL-12p40, linkers, and Fc regions.

[00462] Figure 50 depicts the sequences for XENP31290, an illustrative IL-12-Fc fusion protein of the scIL-12(p40/p35)-Fc format, that contains the IL-12p40(E59K/K99E) variant and wild-type IL-12p35 subunits. Linkers are double underlined (although as will be appreciated by those in the art, the linkers can be replaced by other linkers, some of which are depicted in Figure 6), and slashes (/) indicate the border(s) between IL-12p35, IL-12p40, linkers, and Fc regions.

[00463] Figures 51A-51D depict STAT4 phosphorylation on A) $CD4^+CD45RA^+CD25^+$ T cells, B) $CD4^+CD45RA^-CD25^+$ T cells, C) $CD8^+CD45RA^+CD25^+$ T cells, and D) $CD8^+CD45RA^-CD25^+$ T cells following incubation of activated PBMCs with IL-12-Fc fusions in the scIL-12(p40/p35)-Fc and (scIL-12(p40/p35))$_2$-Fc formats with either WT IL-12p40 subunits or variant IL-12p40(E59K/K99E) subunits. The data show that the IL-12-Fc fusions in scIL-12(p40/p35)-Fc and (scIL-12(p40/p35))$_2$-Fc fusions comprising variant IL-12p40(E59K/K99E) subunits demonstrated reduced potency relative to IL-12-Fc fusions comprising WT IL-12p40 subunits.

[00464] Figure 52 depicts the EC50 (for STAT4 phosphorylation) of IL-12-Fc fusions in the scIL-12(p40/p35)-Fc and (scIL-12(p40/p35))$_2$-Fc formats with either WT IL-12p40 subunits or variant IL-12p40(E59K/K99E) subunits.

[00465] Figure 53 depicts the sequences for XENP16432, anti-PD-1 mAb based on nivolumab and IgG1 backbone with E233**P**/L234**V**/L235**A**/G236**del**/S267**K** ablation variant.

[00466] Figures 54A-54D depict the change in tumor volume (as determined by caliper measurements) on A) Day 11, B) Day 13, and C) Day 15 as well as D) over time in pp65-MCF7 and huPBMC-engrafted NSG mice dosed with PBS, XENP16432 (a bivalent anti-PD-1 mAb), or XENP29952 (a reduced potency IL-12-Fc fusion in the IL-12-heteroFc format comprising variant IL-12p40(E59K)). XENP29952 significantly enhanced anti-tumor activity by Day 11 as indicated by change in tumor volume (statistics performed on baseline corrected data using unpaired t-test).

[00467] Figures 55A-55F depict A) CD45 cell, B) CD3$^+$ T cell, C) CD4$^+$ T cell, D) CD8$^+$ T cell, E) NK cell counts as well as F) CD4$^+$ T cell to CD8$^+$ T cell ratio in pp65-MCF7 and huPBMC-engrafted NSG mice on Day 14 following PBMC-engraftment and first dose of PBS, XENP16432 (a bivalent anti-PD-1 mAb), or XENP29952 (a reduced potency IL-12-Fc fusion in the IL-12-heteroFc format comprising variant IL-12p40(E59K)). XENP29952 had significantly enhanced expansion of CD45$^+$, CD3$^+$ T cells, CD4$^+$ T cells, CD8$^+$ T cells, and NK cells by Day 14 in comparison to both PBS control and checkpoint blockade by XENP16432 (statistics performed on log-transformed data using unpaired t-test).

[00468] Figures 56A-56D depict serum IFNγ concentrations on A) Day 7 and B) Day 14, and serum CD25 concentrations on C) Day 7 and D) Day 14 in pp65-MCF7 and huPBMC-engrafted NSG mice following PBMC-engraftment and first dose of PBS, XENP16432 (a bivalent anti-PD-1 mAb), or XENP29952 (a reduced potency IL-12-Fc fusion in the IL-12-heteroFc format comprising variant IL-12p40(E59K)). XENP29952 significantly enhanced secretion of IFNγ and CD25 by Day 7 in comparison to checkpoint blockade by XENP16432 (statistics performed on log-transformed date using unpaired t-test).

[00469] Figures 57A-57I depict change in body weight (as an indicator of GVHD) by A) Day 3, B) Day 6, C) Day 10, D) Day 13, E) Day 17, F) Day 20, G) Day 24, and H) Day 27, as well as I) over time in huPBMC-engrafted NSG mice dosed with PBS or XENP29952 (a reduced potency IL-12-Fc fusion in the IL-12-heteroFc format comprising variant IL-12p40(E59K)), XENP30597 (a reduced potency IL-12-Fc fusion in the IL-12-heteroFc format comprising variant IL-12p40(E59K/K99E)), XENP31254 (a reduced potency IL-12-Fc fusion in the IL-12-heteroFc format comprising variant IL-12p40(D18K/E59K/K99E)), XENP31251 (a reduced potency IL-12-Fc fusion in the IL-12-heteroFc format comprising variant IL-12p40(E59K/K99Y)), or XENP31258 (a reduced potency IL-12-Fc fusion in the IL-12-heteroFc format comprising variant IL-12p40(E59K/K99E/K264E)) at 0.3 or 0.03 mg/kg. Notably, the IL-12-Fc fusion test articles induced varying degrees of GVHD which correlated with their in vitro potency. Additionally, the data show a dose response for the test articles (i.e. enhanced GVHD by 0.3 mg/kg vs. 0.03 mg/kg).

[00470] Figures 58A-58C depict PD-1 expression on $CD8^+$ T cells (as an indicator of activation) in huPBMC-engrafted NSG mice on A) Day 7, B) Day 10, and C) Day 14 following PBMC-engraftment and first dose of PBS or XENP29952 (a reduced potency IL-12-Fc fusion in the IL-12-heteroFc format comprising variant IL-12p40(E59K)), XENP30597 (a reduced potency IL-12-Fc fusion in the IL-12-heteroFc format comprising variant IL-12p40(E59K/K99E)), XENP31254 (a reduced potency IL-12-Fc fusion in the IL-12-heteroFc format comprising variant IL-12p40(D18K/E59K/K99E)), XENP31251 (a reduced potency IL-12-Fc fusion in the IL-12-heteroFc format comprising variant IL-12p40(E59K/K99Y)), or XENP31258 (a reduced potency IL-12-Fc fusion in the IL-12-heteroFc format comprising variant IL-12p40(E59K/K99E/K264E)) at 0.3 or 0.03 mg/kg. Notably, the IL-12-Fc fusion test articles induced varying degrees of $CD8^+$ T cell activation which correlated with their in vitro potency. Additionally, the data show a dose response for the test articles (i.e. enhanced $CD8^+$ T cell activation by 0.3 mg/kg vs. 0.03 mg/kg).

[00471] Figures 59A-59C depict PD-1 expression on $CD4^+$ T cells (as an indicator of activation) in huPBMC-engrafted NSG mice on A) Day 7, B) Day 10, and C) Day 14 following PBMC-engraftment and first dose of PBS or XENP29952 (a reduced potency IL-12-Fc fusion in the IL-12-heteroFc format comprising variant IL-12p40(E59K)), XENP30597 (a reduced potency IL-12-Fc

fusion in the IL-12-heteroFc format comprising variant IL-12p40(E59K/K99E)), XENP31254 (a reduced potency IL-12-Fc fusion in the IL-12-heteroFc format comprising variant IL-12p40(D18K/E59K/K99E)), XENP31251 (a reduced potency IL-12-Fc fusion in the IL-12-heteroFc format comprising variant IL-12p40(E59K/K99Y)), or XENP31258 (a reduced potency IL-12-Fc fusion in the IL-12-heteroFc format comprising variant IL-12p40(E59K/K99E/K264E)) at 0.3 or 0.03 mg/kg. Notably, the IL-12-Fc fusion test articles induced varying degrees of $CD4^+$ T cell activation which correlated with their in vitro potency. Additionally, the data show a dose response for the test articles (i.e. enhanced $CD4^+$ T cell activation by 0.3 mg/kg vs. 0.03 mg/kg).

[00472] Figures 60A-60D depict serum concentration of IFNγ in huPBMC-engrafted NSG mice on Days A) 7, B) 10, C) 14 and D) 31 following PBMC-engraftment and first dose of PBS or XENP29952 (a reduced potency IL-12-Fc fusion in the IL-12-heteroFc format comprising variant IL-12p40(E59K)), XENP30597 (a reduced potency IL-12-Fc fusion in the IL-12-heteroFc format comprising variant IL-12p40(E59K/K99E)), XENP31254 (a reduced potency IL-12-Fc fusion in the IL-12-heteroFc format comprising variant IL-12p40(D18K/E59K/K99E)), XENP31251 (a reduced potency IL-12-Fc fusion in the IL-12-heteroFc format comprising variant IL-12p40(E59K/K99Y)), or XENP31258 (a reduced potency IL-12-Fc fusion in the IL-12-heteroFc format comprising variant IL-12p40(E59K/K99E/K264E)) at 0.3 or 0.03 mg/kg. Notably, the IL-12-Fc fusion test articles induced varying levels of IFNγ secretion which correlated with their in vitro potency. Additionally, the data show a dose response for the test articles (i.e. enhanced IFNγ secretion induced by 0.3 mg/kg vs. 0.03 mg/kg).

[00473] Figures 61A-61K depict STAT4 phosphorylation on A) $CD4^+CD45RA^-CD25^+$ T cells, B) $CD4^+CD45RA^+CD25^+$ T cells, C) $CD4^+CD45RA^+CD25$- T cells, D) $CD4^+CD45RA^-CD25^-$ T cells, E) $CD8^+CD45RA^-CD25^+$ T cells, F) $CD8^+CD45RA^+CD25^+$ T cells, G) $CD8^+CD45RA^+CD25^-$ T cells, H) $CD8^+CD45RA^-CD25^-$ T cells, I) Tregs, J) γδ T cells, and K) $CD56^+$ NK cells following incubation of activated PBMCs (from a first donor; Donor 1) with recombinant human IL-12, XENP29952 (a reduced potency IL-12-Fc fusion in the IL-12-heteroFc format comprising variant IL-12p40(E59K)), XENP30597 (a reduced potency IL-12-Fc fusion in the IL-12-heteroFc format comprising variant IL-12p40(E59K/K99E)), XENP31254 (a reduced potency IL-12-Fc fusion in the

IL-12-heteroFc format comprising variant IL-12p40(D18K/E59K/K99E)), XENP31251 (a reduced potency IL-12-Fc fusion in the IL-12-heteroFc format comprising variant IL-12p40(E59K/K99Y)), or XENP31258 (a reduced potency IL-12-Fc fusion in the IL-12-heteroFc format comprising variant IL-12p40(E59K/K99E/K264E)). The data show a potency ladder with XENP29952 as the most potent variant, XENP31254 and XENP31258 as the least potent variants, and XENP30597 and XENP31251 falling in between. Notably, the degree of GVHD and T cell activation as induced by the reduced potency IL-12-Fc fusion variants *in vivo* correlated with the *in vitro* potency.

[00474] Figures 62A-62D depict the change in tumor volume (as determined by caliper measurements) on A) Day 14, B) Day 16, and C) Day 21 as well as D) over time in pp65-MCF7 and huPBMC-engrafted NSG mice dosed with PBS, XENP16432 (a bivalent anti-PD-1 mAb), XENP31258, XENP31251, or a combination of XENP31251 and XENP16432. XENP31258 significantly enhanced anti-tumor activity by Day 14, XENP31251 (alone or in combination with XENP16432) significantly enhanced anti-tumor activity by Day 16 in comparison to treatment with PBS; and XENP31251 in combination with XENP16432 significantly enhanced anti-tumor activity by Day 21 in comparison to treatment with XENP16432 alone (statistics performed on baseline corrected data using Mann-Whitney test).

[00475] Figures 63A-63F depict A) CD45 cell, B) $CD3^+$ T cell, C) $CD4^+$ T cell, D) $CD8^+$ T cell, E) NK cell counts as well as F) $CD8^+$ T cell to $CD4^+$ T cell ratio in pp65-MCF7 and huPBMC-engrafted NSG mice on Day 14 following PBMC-engraftment and first dose of PBS, XENP16432 (a bivalent anti-PD-1 mAb), XENP31258, XENP31251, or a combination of XENP31251 and XENP16432. Notably, the data show that treatment with XENP31251 in combination with XENP16432 significantly enhanced lymphocyte expansion in comparison to either XENP31251 or XENP16432 alone, indicating that IL-12-Fc fusions combine productively with checkpoint blockade.

[00476] Figures 64A-64C depict sequences for illustrative variant IL-12-Fc fusions designed with the view to reduce the affinity of the IL-12-Fc fusions for IL-12 receptors, further engineered with Xtend Fc (M428L/N434S) for extending half-life. Linkers are double underlined (although as will be appreciated by those in the art, the linkers can be replaced by other linkers, some of which are

depicted in Figure 6), and slashes (/) indicate the border(s) between Il-12p35, IL-12p40, linkers, and Fc regions. It should be noted that these sequences are provided for illustrative purposes, and that any of the sequences depicted in the other Figures may also include Xtend Fc (M428L/N434S) for extending half-life.

[00477] Figures 65A-C depict sequences for illustrative IL-12p40 variants engineered with C252S with the view to remove the free cysteine (in addition to expression and affinity/potency variants).

[00478] Figures 66A-66Q depict sequences for illustrative variant IL-12-Fc fusions engineered with C252S in the IL-12p40 subunit with the view to remove the free cysteine(in addition to expression and affinity/potency variants). Linkers are double underlined (although as will be appreciated by those in the art, the linkers can be replaced by other linkers, some of which are depicted in Figure 6), and slashes (/) indicate the border(s) between IL-12p35, IL-12p40, linkers, and Fc regions.

[00479] Figures 67A-67D depicts STAT4 phosphorylation on A) $CD8^+CD45RA^-CD25^+$ T cells, B) $CD8^+CD45RA^+CD25^+$ T, C) $CD4^+CD45RA^-CD25^+$ T, D) $CD4^+CD45RA^+CD25^+$ T, and E) NK cells following incubation of activated PBMCs with IL-12-Fc comprising IL-12p40 variants with or without additional engineering to remove free cysteine. The data show that most of the variants comprising C252S in the IL-12p40 subunit demonstrated similar, albeit slightly improved, potency in comparison to the variants without the C252S modification

DETAILED DESCRIPTION OF THE INVENTION

I. Overview

[00480] The present invention is directed to novel heterodimeric fusion protein constructs containing IL-12 subunits and Fc domains. As noted above, IL-12 is composed of an α-chain (the p35 subunit; IL-12p35) and a ß-chain (the p40 subunit; IL-12p40) covalently linked to form the biologically active IL-12 heterodimer. IL-12 exerts its cell signaling function through binding by binding to a dimeric IL-12 receptor complex composed of IL-12 receptor ß1 (IL-12Rß1) and

IL-12 receptor ß2 (IL-12Rß2) on T cells and inducing IFNγ secretion. However, the IL-12p40 subunit can also exist as a homodimer which has been reported to antagonize IL-12 activity by competing for binding to IL-12 receptor. Accordingly, the present invention addresses the short half-life of IL-12 and the potential formation of antagonistic IL12p40 homodimers by providing IL-12-Fc fusion proteins, as well as novel IL-12 variants with decreased potency. As generally shown in Figure 8, the heterodimeric fusion proteins of the invention can take on a variety of conformations.

[00481] This application incorporates by reference 62/740,813 filed October 3, 2018 and 62/828,512 filed May 15, 2019, more specifically for Figure 17 in 62/740,813 and Figures 62A-62K and Figures 63A-63D in 62/828,512 as well as the Figure Legends and the mention of these figures in the corresponding specification.

II. Definitions

[00482] In order that the application may be more completely understood, several definitions are set forth below. Such definitions are meant to encompass grammatical equivalents.

[00483] By “ablation” herein is meant a decrease or removal of binding and/or activity. Thus for example, “ablating FcγR binding” means the Fc region amino acid variant has less than 50% starting binding as compared to an Fc region not containing the specific variant, with less than 70-80-90-95-98% loss of binding being preferred, and in general, with the binding being below the level of detectable binding in a Biacore assay. Of particular use in the ablation of FcγR binding are those shown in Figure 4. However, unless otherwise noted, the Fc monomers of the invention retain binding to the FcRn.

[00484] By "ADCC" or "antibody dependent cell-mediated cytotoxicity" as used herein is meant the cell-mediated reaction wherein nonspecific cytotoxic cells that express FcγRs recognize bound antibody on a target cell and subsequently cause lysis of the target cell. ADCC is correlated with binding to FcγRIIIa; increased binding to FcγRIIIa leads to an increase in ADCC

activity. As is discussed herein, many embodiments of the invention ablate ADCC activity entirely.

[00485] By "modification" herein is meant an amino acid substitution, insertion, and/or deletion in a polypeptide sequence or an alteration to a moiety chemically linked to a protein. For example, a modification may be an altered carbohydrate or PEG structure attached to a protein. By "amino acid modification" herein is meant an amino acid substitution, insertion, and/or deletion in a polypeptide sequence. For clarity, unless otherwise noted, the amino acid modification is always to an amino acid coded for by DNA, e.g., the 20 amino acids that have codons in DNA and RNA.

[00486] By "amino acid substitution" or "substitution" herein is meant the replacement of an amino acid at a particular position in a parent polypeptide sequence with a different amino acid. In particular, in some embodiments, the substitution is to an amino acid that is not naturally occurring at the particular position, either not naturally occurring within the organism or in any organism. For example, the substitution E272Y or 272Y refers to a variant polypeptide, in this case an Fc variant, in which the glutamic acid at position 272 is replaced with tyrosine. For clarity, a protein which has been engineered to change the nucleic acid coding sequence but not to change the starting amino acid (for example exchanging CGG (encoding arginine) to CGA (still encoding arginine) to increase host organism expression levels) is not an “amino acid substitution”; that is, despite the creation of a new gene encoding the same protein, if the protein has the same amino acid at the particular position that it started with, it is not an amino acid substitution.

[00487] By "amino acid insertion" or "insertion" as used herein is meant the addition of an amino acid residue or sequence at a particular position in a parent polypeptide sequence. For example, -233E designates an insertion of glutamic acid after position 233 and before position 234. Additionally, -233ADE or A233ADE designates an insertion of AlaAspGlu after position 233 and before position 234.

[00488] By "amino acid deletion" or "deletion" as used herein is meant the removal of an amino acid residue or sequence at a particular position in a parent polypeptide sequence. For example, E233-, E233#, E233() or E233del designates a deletion of glutamic acid at position 233. Additionally, EDA233- or EDA233# designates a deletion of the sequence GluAspAla that begins at position 233.

[00489] By "variant protein", "protein variant", or "variant" as used herein is meant a protein that differs from that of a parent protein by virtue of at least one modification. Protein variant may refer to the protein itself, a composition comprising the protein, the amino acid sequence that encodes it, or the DNA sequence that encodes it. Preferably, the protein variant has at least one amino acid modification compared to the parent protein, e.g. from about one to about seventy amino acid modifications, and preferably from about one to about five amino acid modifications compared to the parent. The modification can be an addition, deletion, or substitution. As described below, in some embodiments the parent polypeptide, for example an Fc parent polypeptide, is a human wild type sequence, such as the Fc region from IgG1, IgG2, IgG3 or IgG4. The protein variant sequence herein will preferably possess at least about 80% identity with a parent protein sequence, and most preferably at least about 90% identity, more preferably at least about 95-98-99% identity.

[00490] As used herein, by "protein" is meant at least two covalently attached amino acids, which includes proteins, polypeptides, oligopeptides and peptides. When a biologically functional unit comprises two or more proteins, each protein may be referred to as a "monomer" or as a "subunit" or as a "domain"; and the biologically functional molecule may be referred to as a "complex".

[00491] As used herein, by "protein" is meant at least two covalently attached amino acids, which includes proteins, polypeptides, oligopeptides, and peptides. When a biologically functional molecule or complex comprises two or more proteins, each protein may be referred to as a "monomer" or as a "subunit" or as a "domain"; and the biologically functional molecule may be referred to as a "complex" In some embodiments, the two or more proteins of a

functional complex are non-covalently attached. In some embodiments, the term “monomer” refers to a polypeptide or protein comprising one or more components, fragments, or subunits of a protein(s), and the components, fragments, or subunits are covalently attached.

[00492] By "residue" as used herein is meant a position in a protein and its associated amino acid identity. For example, Asparagine 297 (also referred to as Asn297 or N297) is a residue at position 297 in the human antibody IgG1.

[00493] By "IgG subclass modification" or "isotype modification" as used herein is meant an amino acid modification that converts one amino acid of one IgG isotype to the corresponding amino acid in a different, aligned IgG isotype. For example, because IgG1 comprises a tyrosine and IgG2 a phenylalanine at EU position 296, a F296Y substitution in IgG2 is considered an IgG subclass modification.

[00494] The carboxy-terminal portion of each IgG chain defines a constant region primarily responsible for effector function. Kabat et al. collected numerous primary sequences of the variable regions of heavy chains and light chains. Based on the degree of conservation of the sequences, they classified individual primary sequences into the CDRs and the framework and made a list thereof (see SEQUENCES OF IMMUNOLOGICAL INTEREST, 5th edition, NIH publication, No. 91-3242, E.A. Kabat et al., entirely incorporated by reference). Throughout the present specification, the Kabat numbering system is generally used when referring to a residue in the variable domain (approximately, residues 1-107 of the light chain variable region and residues 1-113 of the heavy chain variable region) and the EU numbering system for Fc regions (e.g., Kabat et al., supra (1991)).

[00495] In the IgG subclass of immunoglobulins, there are several immunoglobulin domains in the heavy chain. By “immunoglobulin (Ig) domain” herein is meant a region of an immunoglobulin having a distinct tertiary structure. Of interest in the present invention are the heavy chain domains, including, the constant heavy (CH) domains and the hinge domains. In the context of IgG antibodies, the IgG isotypes each have three CH regions. Accordingly, “CH” domains in the context of IgG are as follows: “CH1” refers to positions 118-215 according to the

EU index as in Kabat. “Hinge” refers to positions 216-230 according to the EU index as in Kabat. “CH2” refers to positions 231-340 according to the EU index as in Kabat, and “CH3” refers to positions 341-447 according to the EU index as in Kabat. As shown in Table 1, the exact numbering and placement of the heavy chain domains can be different among different numbering systems. As shown herein and described below, the pI variants can be in one or more of the CH regions, as well as the hinge region, discussed below.

[00496] Another type of Ig domain of the heavy chain is the hinge region. By “hinge” or “hinge region” or “antibody hinge region” or “immunoglobulin hinge region” herein is meant the flexible polypeptide comprising the amino acids between the first and second heavy chain constant domains of an antibody. Structurally, the IgG CH1 domain ends at EU position 215, and the IgG CH2 domain begins at residue EU position 231. Thus for IgG the antibody hinge is herein defined to include positions 216 (E216 in IgG1) to 230 (P230 in IgG1), wherein the numbering is according to the EU index as in Kabat. In some embodiments, for example in the context of an Fc region, the hinge is included, generally referring to positions 216-230. As noted herein, pI variants can be made in the hinge region as well.

[00497] By "non-naturally occurring modification" as used herein is meant an amino acid modification that is not isotypic. For example, because none of the IgGs comprise a serine at position 434, the substitution 434S in IgG1, IgG2, IgG3, or IgG4 (or hybrids thereof) is considered a non-naturally occurring modification.

[00498] By "amino acid" and "amino acid identity" as used herein is meant one of the 20 naturally occurring amino acids that are coded for by DNA and RNA.

[00499] By "effector function" as used herein is meant a biochemical event that results from the interaction of an antibody Fc region with an Fc receptor or ligand. Effector functions include but are not limited to ADCC, ADCP, and CDC.

[00500] By "IgG Fc ligand" or “Fc ligand” as used herein is meant a molecule, preferably a polypeptide, from any organism that binds to the Fc region of an IgG antibody to form an Fc/Fc

ligand complex. Fc ligands include but are not limited to FcγRIs, FcγRIIs, FcγRIIIs, FcRn, C1q, C3, mannan binding lectin, mannose receptor, staphylococcal protein A, streptococcal protein G, and viral FcγR. Fc ligands also include Fc receptor homologs (FcRH), which are a family of Fc receptors that are homologous to the FcγRs (Davis et al., 2002, Immunological Reviews 190:123-136, entirely incorporated by reference). Fc ligands may include undiscovered molecules that bind Fc. Particular IgG Fc ligands are FcRn and Fc gamma receptors.

[00501] By "Fc gamma receptor", "FcγR" or "FcgammaR" as used herein is meant any member of the family of proteins that bind the IgG antibody Fc region and is encoded by an FcγR gene. In humans this family includes but is not limited to FcγRI (CD64), including isoforms FcγRIa, FcγRIb, and FcγRIc; FcγRII (CD32), including isoforms FcγRIIa (including allotypes H131 and R131), FcγRIIb (including FcγRIIb-1 and FcγRIIb-2), and FcγRIIc; and FcγRIII (CD16), including isoforms FcγRIIIa (including allotypes V158 and F158) and FcγRIIIb (including allotypes FcγRIIb-NA1 and FcγRIIb-NA2) (Jefferis et al., 2002, Immunol Lett 82:57-65, entirely incorporated by reference), as well as any undiscovered human FcγRs or FcγR isoforms or allotypes. An FcγR may be from any organism, including but not limited to humans, mice, rats, rabbits, and monkeys. Mouse FcγRs include but are not limited to FcγRI (CD64), FcγRII (CD32), FcγRIII (CD16), and FcγRIII-2 (CD16-2), as well as any undiscovered mouse FcγRs or FcγR isoforms or allotypes.

[00502] By "FcRn" or "neonatal Fc receptor" as used herein is meant a protein that binds the IgG antibody Fc region and is encoded at least in part by an FcRn gene. The FcRn may be from any organism, including but not limited to humans, mice, rats, rabbits, and monkeys. As is known in the art, the functional FcRn protein comprises two polypeptides, often referred to as the heavy chain and light chain. The light chain is beta-2-microglobulin (ß2-microglobulin) and the heavy chain is encoded by the FcRn gene. Unless otherwise noted herein, FcRn or an FcRn protein refers to the complex of FcRn heavy chain with ß2-microglobulin. A variety of Fc variants can be used to increase binding to the FcRn, and in some cases, to increase serum half-life. In general, unless otherwise noted, the Fc monomers of the invention retain binding to the FcRn (and, as noted below, can include amino acid variants to increase binding to the FcRn).

[00503] By "parent polypeptide" as used herein is meant a starting polypeptide that is subsequently modified to generate a variant. The parent polypeptide may be a naturally occurring polypeptide, or a variant or engineered version of a naturally occurring polypeptide. Parent polypeptide may refer to the polypeptide itself, compositions that comprise the parent polypeptide, or the amino acid sequence that encodes it.

[00504] By "Fc" or "Fc region" or "Fc domain" as used herein is meant the polypeptide comprising the constant region of an antibody, in some instances, excluding all of the first constant region immunoglobulin domain (e.g., CH1) or a portion thereof, and in some cases, optionally including all or part of the hinge. For IgG, the Fc domain comprises immunoglobulin domains CH2 and CH3 (Cγ2 and Cγ3), and optionally all or a portion of the hinge region between CH1 (Cγ1) and CH2 (Cγ2). Thus, in some cases, the Fc domain includes, from N- to C-terminal, CH2-CH3 and hinge-CH2-CH3. In some embodiments, the Fc domain is that from IgG1, IgG2, IgG3 or IgG4, with IgG1 hinge-CH2-CH3 and IgG4 hinge-CH2-CH3 finding particular use in many embodiments. Additionally, in the case of human IgG1 Fc domains, frequently the hinge includes a C220S amino acid substitution. Furthermore, in the case of human IgG4 Fc domains, frequently the hinge includes a S228P amino acid substitution. Although the boundaries of the Fc region may vary, the human IgG heavy chain Fc region is usually defined to include residues E216, C226, or A231 to its carboxyl-terminus, wherein the numbering is according to the EU index as in Kabat. In some embodiments, as is more fully described below, amino acid modifications are made to the Fc region, for example to alter binding to one or more FcγR or to the FcRn.

[00505] As will be appreciated by those in the art, the exact numbering and placement of the heavy constant region domains can be different among different numbering systems. A useful comparison of heavy constant region numbering according to EU and Kabat is as below, see Edelman et al., 1969, Proc Natl Acad Sci USA 63:78-85 and Kabat et al., 1991, Sequences of Proteins of Immunological Interest, 5th Ed., United States Public Health Service, National Institutes of Health, Bethesda, entirely incorporated by reference.

Table 1

	EU Numbering	Kabat Numbering
CH1	118-215	114-223
Hinge	216-230	226-243
CH2	231-340	244-360
CH3	341-447	361-478

[00506] "Fc variant" or "variant Fc" as used herein is meant a protein comprising an amino acid modification in an Fc domain. The modification can be an addition, deletion, or substitution. The Fc variants of the present invention are defined according to the amino acid modifications that compose them. Thus, for example, N434S or 434S is an Fc variant with the substitution for serine at position 434 relative to the parent Fc polypeptide, wherein the numbering is according to the EU index. Likewise, M428L/N434S defines an Fc variant with the substitutions M428L and N434S relative to the parent Fc polypeptide. The identity of the WT amino acid may be unspecified, in which case the aforementioned variant is referred to as 428L/434S. It is noted that the order in which substitutions are provided is arbitrary, that is to say that, for example, 428L/434S is the same Fc variant as 434S/428L, and so on. For all positions discussed in the present invention that relate to antibodies or derivatives and fragments thereof, unless otherwise noted, amino acid position numbering is according to the EU index. The EU index or EU index as in Kabat or EU numbering scheme refers to the numbering of the EU antibody (Edelman et al., 1969, Proc Natl Acad Sci USA 63:78-85, hereby entirely incorporated by reference). The modification can be an addition, deletion, or substitution.

[00507] By “fusion protein” as used herein is meant covalent joining of at least two proteins or protein domains. Fusion proteins may comprise artificial sequences, e.g. a domain linker, variant Fc domains, a variant IL-12p40 subunit domain, a variant IL-12p35 subunit domain, etc. as described herein. By “Fc fusion protein” or “immunoadhesin” herein is meant a protein

comprising an Fc region, generally linked (optionally through a domain linker, as described herein) to one or more different protein domains. Accordingly, an "IL-12 Fc fusion" comprises an Fc region linked (optionally but usually through a domain linker) to an IL-12p40 subunit, an IL12p35 subunit and/or single-chain IL-12 complex (scIL-12), as described herein. In some instances, two Fc fusion proteins can form a homodimeric Fc fusion protein or a heterodimeric Fc fusion protein with the latter being preferred in some instances. In some cases, one monomer of the heterodimeric Fc fusion protein comprises an Fc domain alone (e.g., an "empty Fc domain") and the other monomer is an Fc fusion, comprising a variant Fc domain and one or two IL-12 subunit domains, as outlined herein.

[00508] By "position" as used herein is meant a location in the sequence of a protein. Positions may be numbered sequentially, or according to an established format, for example the EU index for antibody numbering.

[00509] By "strandedness" in the context of the monomers of the heterodimeric proteins of the invention herein is meant that, similar to the two strands of DNA that "match", heterodimerization variants are incorporated into each monomer so as to preserve, create, and/or enhance the ability to "match" to form heterodimers. For example, if some pI variants are engineered into monomer A (e.g. making the pI higher), then steric variants that are "charge pairs" that can be utilized as well do not interfere with the pI variants, e.g. the charge variants that make a pI higher are put on the same "strand" or "monomer" to preserve both functionalities. Similarly, for "skew" variants that come in pairs of a set as more fully outlined below, the skilled artisan will consider pI in deciding into which strand or monomer that incorporates one set of the pair will go, such that pI separation is maximized using the pI of the skews as well.

[00510] By "wild type or WT" herein is meant an amino acid sequence or a nucleotide sequence that is found in nature, including allelic variations. A WT protein has an amino acid sequence or a nucleotide sequence that has not been intentionally modified.

[00511] The heterodimeric proteins of the present invention are generally isolated or recombinant. “Isolated,” when used to describe the various polypeptides disclosed herein, means a polypeptide that has been identified and separated and/or recovered from a cell or cell culture from which it was expressed. Ordinarily, an isolated polypeptide will be prepared by at least one purification step. An “isolated protein,” refers to a protein which is substantially free of other proteins from a cell culture such as host cell proteins. “Recombinant” means the proteins are generated using recombinant nucleic acid techniques in exogeneous host cells.

[00512] "Percent (%) amino acid sequence identity" with respect to a protein sequence is defined as the percentage of amino acid residues in a candidate sequence that are identical with the amino acid residues in the specific (parental) sequence, after aligning the sequences and introducing gaps, if necessary, to achieve the maximum percent sequence identity, and not considering any conservative substitutions as part of the sequence identity. Alignment for purposes of determining percent amino acid sequence identity can be achieved in various ways that are within the skill in the art, for instance, using publicly available computer software such as BLAST, BLAST-2, ALIGN or Megalign (DNASTAR) software. Those skilled in the art can determine appropriate parameters for measuring alignment, including any algorithms needed to achieve maximal alignment over the full length of the sequences being compared. One particular program is the ALIGN-2 program outlined at paragraphs [0279] to [0280] of US Pub. No. 20160244525, hereby incorporated by reference.

[00513] The degree of identity between an amino acid sequence of the present invention ("invention sequence") and the parental amino acid sequence is calculated as the number of exact matches in an alignment of the two sequences, divided by the length of the "invention sequence," or the length of the parental sequence, whichever is the shortest. The result is expressed in percent identity.

[00514] In some embodiments, two or more amino acid sequences are at least 50%, 60%, 70%, 80%, or 90% identical. In some embodiments, two or more amino acid sequences are at least 95%, 97%, 98%, 99%, or even 100% identical.

[00515] By "IL-12p40 subunit domain" herein is meant the ß-chain (the p40 subunit; IL-12p40). As discussed herein, the IL-12p40 subunit domain can be a wild-type human sequence (e.g. SEQ ID NO:XX from Figure 1) or a variant thereof, as more fully discussed below (e.g. see Figures 20, 23 and 29, for example).

[00516] By "IL-12p35 subunit domain" herein is meant α-chain (the p35 subunit; IL-12p35). As discussed herein, the IL-12p35 subunit domain can be a wild-type human sequence (e.g. SEQ ID NO:XX from Figure 1) or a variant thereof, as more fully discussed below (e.g. see Figures 20, 23 and 29, for example).

[00517] The IL-12 subunit domains of the invention, when associated together, specifically bind to a dimeric IL-12 receptor complex comprising IL-12 receptor β1 and IL-12 receptor β2. The strength, or affinity, of specific binding can be expressed in terms of dissociation constant (K_D) of the interaction, wherein a smaller K_D represents greater affinity and a larger K_D represents lower affinity. Binding properties can be determined by methods well known in the art such as bio-layer interferometry and surface plasmon resonance based methods, including Biacore and Octet methodologies. One such method entails measuring the rates of antigen-binding site/antigen or receptor/ligand complex association and dissociation, wherein rates depend on the concentration of the complex partners, the affinity of the interaction, and geometric parameters that equally influence the rate in both directions. Thus, both the association rate (k_a) and the dissociation rate (k_d) can be determined, and the ratio of k_d/k_a is equal to the dissociation constant K_D (See Nature 361:186-187 (1993) and Davies et al. (1990) Annual Rev Biochem 59:439-473), both of which are incorporated by reference in their entirety for the methods therein.

[00518] Specific binding for a particular molecule can be exhibited, for example, by a molecule having a K_D for a ligand (generally a receptor, in this case) of at least about 10^{-4} M, at least about 10^{-5} M, at least about 10^{-6} M, at least about 10^{-7} M, at least about 10^{-8} M, at least about 10^{-9} M, alternatively at least about 10^{-10} M, at least about 10^{-11} M, at least about 10^{-12} M, or greater.

Typically, a molecule that specifically binds its receptor will have a K_D that is 20-, 50-, 100-, 500-, 1000-, 5,000-, 10,000- or more times greater for a control molecule relative to the receptor.

[00519] Also, specific binding for a particular molecule can be exhibited, for example, by a molecule having a k_a or association rate for a ligand or receptor of at least 20-, 50-, 100-, 500-, 1000-, 5,000-, 10,000- or more times greater for the receptor relative to a control.

[00520] By "fused" or "covalently linked" is herein meant that the components (e.g., an IL-12 subunit and an Fc domain) are linked by peptide bonds, either directly or indirectly via domain linkers, outlined herein.

[00521] As used herein, the term "single-chain" refers to a molecule comprising amino acid domains linearly linked by peptide bonds. In certain embodiments, the biologically functional IL-12 is a single chain IL-12 complex or "scIL-12", i.e. the IL-12p35 subunit and the IL-12p40 subunit are fused to form a single peptide chain. In a particular such embodiment, the C-terminus of the IL-12p35 subunit is connected to the N-terminus of the IL-12p40 subunit, designated "scIL-12(p35/p40)". In another particular such embodiment, the C-terminus of the IL-12p40 subunit is connected to the N-terminus of the IL-12p35 subunit, designated "scIL-12(p40/p35)". Additionally, when these complexes are further fused to an Fc domain, they are still a "single chain". It should be noted that these single chain constructs, where the p35 and p40 subunits are on the same amino acid chain, still are part of a heterodimeric complex containing two amino acid chains (e.g. as shown in Figure 8C and 8D, the scIL-12(p35/p40) component and the "empty arm" Fc component). That is, there are two single chains that make up the heterodimeric complex.

[00522] The fusion proteins of the invention can take on a variety of formats, including heterodimeric formats such as those depicted in Figure 8, as well as homodimeric formats such as those depicted in Figure 14 and Figure 48.

III. Heterodimeric Fc Fusion Proteins

In some aspects, the present invention relates to heterodimeric Fc fusion proteins that include an Fc region, generally linked (optionally through a domain linker) to one or more different IL-12 protein domains. These heterodimeric fusion proteins can take on a large number of different formats, as are generally depicted in Figure 8. In one aspect, the heterodimeric Fc fusion protein is an IL-12 heterodimeric Fc fusion protein that includes IL-12p40 and IL-12p35 subunits in different orientations, such that they present together to bind to the IL-12 receptor complex of IL-12Rβ1/β2. The Fc domains can be derived from IgG Fc domains, e.g., IgG1, IgG2, IgG3 or IgG4 Fc domains, with IgG1 and IgG4 Fc domains finding particular use in the invention. As described herein, IgG1 Fc domains may be used, often, but not always in conjunction with ablation variants to ablate effector function. Similarly, when low effector function is desired, IgG4 Fc domains may be used.

[00523] As described herein and known in the art, the heterodimeric proteins of the invention comprise different domains, which can be overlapping as well. These domains include, but are not limited to, the Fc domain, the CH2 domain, the CH3 domain and the hinge domain, an IL-12p40 subunit domain and an Il-12p35 subunit domain. As described herein, these domains are linked together in different formats, as generally outlined in Figure 8.

[00524] In some of the embodiments herein, when a protein fragment, e.g., IL-12p40 or IL-12p35 is attached to an Fc domain, it is the C-terminus of the protein fragment that is attached to all or part of the hinge of the Fc domain; for example, it is generally attached to the sequence EPKS which is the beginning of the IgG1 hinge. In other of the embodiments herein, when a protein fragment, e.g., IL-12p40 or IL-12p35 is attached to an Fc domain, it is the N-terminus of the protein fragment that is attached to the C-terminus of the CH3 domain.

[00525] In some of the constructs and sequences outlined herein of an Fc domain protein, the C-terminus of the IL-12p40 or IL-12p35 protein fragment is attached to the N-terminus of a domain linker, the C-terminus of which is attached to the N-terminus of a constant Fc domain (N- IL-12p40 or IL-12p35 protein fragment-linker-Fc domain-C) although that can be switched

(N-Fc domain-linker- IL-12p40 or IL-12p35 protein fragment-C). In other constructs and sequences outlined herein, the C-terminus of a first protein fragment is attached to the N-terminus of a second protein fragment, optionally via a domain linker, the C-terminus of the second protein fragment is attached to the N-terminus of a constant Fc domain, optionally via a domain linker. In yet another construct, the N-terminus of a first protein fragment is attached to the C-terminus of a second protein fragment, optionally via a domain linker, the N-terminus of the second protein fragment is attached to the C-terminus of a constant Fc domain, optionally via a domain linker. In yet other constructs and sequences outlined herein, a constant Fc domain that is not attached to a first protein fragment or a second protein fragment is provided. A heterodimer Fc fusion protein can contain two or more of the exemplary monomeric Fc domain proteins described herein.

[00526] Accordingly, in some embodiments the present invention provides heterodimeric Fc fusion proteins that rely on the use of two different heavy chain variant Fc sequences, that will self-assemble to form a heterodimeric Fc domain fusion polypeptide. In one embodiment, heterodimeric Fc fusion proteins contain at least two constant domains which can be engineered to produce heterodimers, such as pI engineering. Other Fc domains that can be used include fragments that contain one or more of the CH1, CH2, CH3, and hinge domains of the invention that have been pI engineered. In particular, the formats depicted in Figures 8A-F are heterodimeric Fc fusion proteins, meaning that the protein has two associated Fc sequences self-assembled into a heterodimeric Fc domain and at least one protein fragment (e.g., 1, 2 or more protein fragments). In some cases, a first protein fragment is linked to a first Fc sequence and a second protein fragment is linked to a second Fc sequence. In some cases, the heterodimeric Fc fusion protein contains a first protein fragment linked to a second protein fragment which is linked to a first Fc sequence, and a second Fc sequence that is not linked to either the first or second protein fragments.

[00527] The present invention is directed to novel constructs to provide heterodimeric Fc fusion proteins that allow binding to one or more binding partners, ligands or receptors. The heterodimeric Fc fusion constructs are based on the self-assembling nature of the two Fc

domains of the heavy chains of antibodies, e.g., two "monomers" that assemble into a "dimer". Heterodimeric Fc fusions are made by altering the amino acid sequence of each monomer as more fully discussed below. Thus, the present invention is generally directed to the creation of heterodimeric Fc fusion proteins which can co-engage binding partner(s) or ligand(s) or receptor(s) in several ways, relying on amino acid variants in the constant regions that are different on each chain to promote heterodimeric formation and/or allow for ease of purification of heterodimers over the homodimers. There are a number of mechanisms that can be used to generate the heterodimers of the present invention. In addition, as will be appreciated by those in the art, these mechanisms can be combined to ensure high heterodimerization. Thus, amino acid variants that lead to the production of heterodimers are referred to as "heterodimerization variants". As discussed below, heterodimerization variants can include steric variants (e.g. the "knobs and holes" or "skew" variants described below and the "charge pairs" variants described below) as well as "pI variants", which allows purification of homodimers away from heterodimers. As is generally described in WO2014/145806, hereby incorporated by reference in its entirety and specifically as below for the discussion of "heterodimerization variants", useful mechanisms for heterodimerization include "knobs and holes" ("KIH"; sometimes described herein as "skew" variants (see discussion in WO2014/145806)), "electrostatic steering" or "charge pairs" as described in WO2014/145806, pI variants as described in WO2014/145806, and general additional Fc variants as outlined in WO2014/145806 and below.

[00528] In the present invention, there are several basic mechanisms that can lead to ease of purifying heterodimeric proteins and antibodies; one relies on the use of pI variants, such that each monomer, and subsequently each dimeric species, has a different pI, thus allowing the isoelectric purification of A-A, A-B and B-B dimeric proteins. Alternatively, some formats also allow separation on the basis of size. As is further outlined below, it is also possible to "skew" the formation of heterodimers over homodimers. Thus, a combination of steric heterodimerization variants and pI or charge pair variants find particular use in the invention.

[00529] In general, embodiments of particular use in the present invention rely on sets of variants that include skew variants, that encourage heterodimerization formation over

homodimerization formation, coupled with pI variants, which increase the pI difference between the two monomers and each dimeric species.

[00530] Additionally, as more fully outlined below, depending on the format of the heterodimer Fc fusion protein, pI variants can be either contained within the constant and/or Fc domains of a monomer, or domain linkers can be used. That is, the invention provides pI variants that are on one or both of the monomers, and/or charged domain linkers as well. In addition, additional amino acid engineering for alternative functionalities may also confer pI changes, such as Fc, FcRn and KO variants.

[00531] In the present invention that utilizes pI as a separation mechanism to allow the purification of heterodimeric proteins, amino acid variants can be introduced into one or both of the monomer polypeptides; that is, the pI of one of the monomers (referred to herein for simplicity as "monomer A") can be engineered away from monomer B, or both monomer A and B can be changed, with the pI of monomer A increasing and the pI of monomer B decreasing. As discussed, the pI changes of either or both monomers can be done by removing or adding a charged residue (e.g., a neutral amino acid is replaced by a positively or negatively charged amino acid residue, e.g., glutamine to glutamic acid), changing a charged residue from positive or negative to the opposite charge (e.g. aspartic acid to lysine) or changing a charged residue to a neutral residue (e.g., loss of a charge; lysine to serine.). A number of these variants are shown in the Figures.

[00532] Accordingly, this embodiment of the present invention provides for creating a sufficient change in pI in at least one of the monomers such that heterodimers can be separated from homodimers. As will be appreciated by those in the art, and as discussed further below, this can be done by using a "wild type" heavy chain constant region and a variant region that has been engineered to either increase or decrease its pI (wt A : B+ or wt A : B-), or by increasing one region and decreasing the other region (A+ : B- or A- : B+).

[00533] Thus, in general, a component of some embodiments of the present invention are amino acid variants in the constant regions that are directed to altering the isoelectric point (pI) of at

least one, if not both, of the monomers of a dimeric protein by incorporating amino acid substitutions ("pI variants" or "pI substitutions") into one or both of the monomers. The separation of the heterodimers from the two homodimers can be accomplished if the pIs of the two monomers differ by as little as 0.1 pH unit, with 0.2, 0.3, 0.4 and 0.5 or greater all finding use in the present invention.

[00534] As will be appreciated by those in the art, the number of pI variants to be included on each or both monomer(s) to get good separation will depend in part on the starting pI of the components. That is, to determine which monomer to engineer or in which "direction" (e.g., more positive or more negative), the sequences of the Fc domains, and in some cases, the protein domain(s) linked to the Fc domain are calculated and a decision is made from there. As is known in the art, different Fc domains and/or protein domains will have different starting pIs which are exploited in the present invention. In general, as outlined herein, the pIs are engineered to result in a total pI difference of each monomer of at least about 0.1 logs, with 0.2 to 0.5 being preferred as outlined herein.

[00535] Furthermore, as will be appreciated by those in the art and outlined herein, in some embodiments, heterodimers can be separated from homodimers on the basis of size. As shown in the Figures, for example, several of the formats allow separation of heterodimers and homodimers on the basis of size.

[00536] In the case where pI variants are used to achieve heterodimerization, by using the constant region(s) of Fc domains(s), a more modular approach to designing and purifying heterodimeric Fc fusion proteins is provided. Thus, in some embodiments, heterodimerization variants (including skew and purification heterodimerization variants) must be engineered. In addition, in some embodiments, the possibility of immunogenicity resulting from the pI variants is significantly reduced by importing pI variants from different IgG isotypes such that pI is changed without introducing significant immunogenicity. Thus, an additional problem to be solved is the elucidation of low pI constant domains with high human sequence content, e.g. the minimization or avoidance of non-human residues at any particular position.

[00537] A side benefit that can occur with this pI engineering is also the extension of serum half-life and increased FcRn binding. That is, as described in USSN 13/194,904 (incorporated by reference in its entirety), lowering the pI of antibody constant domains (including those found in antibodies and Fc fusions) can lead to longer serum retention in vivo. These pI variants for increased serum half life also facilitate pI changes for purification.

[00538] In addition, it should be noted that the pI variants of the heterodimerization variants give an additional benefit for the analytics and quality control process of Fc fusion proteins, as the ability to either eliminate, minimize and distinguish when homodimers are present is significant. Similarly, the ability to reliably test the reproducibility of the heterodimeric Fc fusion protein production is important.

A. Heterodimerization Variants

[00539] The present invention provides heterodimeric proteins, including heterodimeric Fc fusion proteins in a variety of formats, which utilize heterodimeric variants to allow for heterodimer formation and/or purification away from homodimers. The heterodimeric fusion constructs are based on the self-assembling nature of the two Fc domains, e.g., two "monomers" that assemble into a "dimer".

[00540] There are a number of suitable pairs of sets of heterodimerization skew variants. These variants come in "pairs" of "sets". That is, one set of the pair is incorporated into the first monomer and the other set of the pair is incorporated into the second monomer. It should be noted that these sets do not necessarily behave as "knobs in holes" variants, with a one-to-one correspondence between a residue on one monomer and a residue on the other; that is, these pairs of sets form an interface between the two monomers that encourages heterodimer formation and discourages homodimer formation, allowing the percentage of heterodimers that spontaneously form under biological conditions to be over 90%, rather than the expected 50% (25 % homodimer A/A:50% heterodimer A/B:25% homodimer B/B).

B. Steric Variants

[00541] In some embodiments, the formation of heterodimers can be facilitated by the addition of steric variants. That is, by changing amino acids in each heavy chain, different heavy chains are more likely to associate to form the heterodimeric structure than to form homodimers with the same Fc amino acid sequences. Suitable steric variants are included in the Figure 29 of USSN 15/141,350, all of which is hereby incorporated by reference in its entirety, as well as in Figure 2.

[00542] One mechanism is generally referred to in the art as "knobs and holes", referring to amino acid engineering that creates steric influences to favor heterodimeric formation and disfavor homodimeric formation, as described in USSN 61/596,846, Ridgway et al., Protein Engineering 9(7):617 (1996); Atwell et al., J. Mol. Biol. 1997 270:26; US Patent No. 8,216,805, all of which are hereby incorporated by reference in their entirety. The Figures identify a number of "monomer A – monomer B" pairs that rely on "knobs and holes". In addition, as described in Merchant et al., Nature Biotech. 16:677 (1998), these "knobs and hole" mutations can be combined with disulfide bonds to skew formation to heterodimerization.

[00543] An additional mechanism that finds use in the generation of heterodimers is sometimes referred to as "electrostatic steering" as described in Gunasekaran et al., J. Biol. Chem. 285(25):19637 (2010), hereby incorporated by reference in its entirety. This is sometimes referred to herein as "charge pairs". In this embodiment, electrostatics are used to skew the formation towards heterodimerization. As those in the art will appreciate, these may also have an effect on pI, and thus on purification, and thus could in some cases also be considered pI variants. However, as these were generated to force heterodimerization and were not used as purification tools, they are classified as "steric variants". These include, but are not limited to, D221E/P228E/L368E paired with D221R/P228R/K409R (e.g., these are "monomer" corresponding sets) and C220E/P228E/368E paired with C220R/E224R/P228R/K409R.

[00544] Additional monomer A and monomer B variants can be combined with other variants, optionally and independently in any amount, such as pI variants outlined herein or other steric

variants that are shown in Figure 37 of US 2012/0149876, all of which are incorporated expressly by reference herein.

[00545] In some embodiments, the steric variants outlined herein can be optionally and independently incorporated with any pI variant (or other variants such as Fc variants, FcRn variants, etc.) into one or both monomers, and can be independently and optionally included or excluded from the proteins of the invention.

[00546] A list of suitable skew variants is found in Figure 2. Of particular use in many embodiments are the pairs of sets including, but not limited to, S364K/E357Q : L368D/K370S; L368D/K370S : S364K; L368E/K370S : S364K; T411T/E360E/Q362E : D401K; L368D/K370S : S364K/E357L; K370S : S364K/E357Q; and T366S/L368A/Y407V : T366W (optionally including a bridging disulfide, T366S/L368A/Y407V/Y349C : T366W/S354C). In terms of nomenclature, the pair "S364K/E357Q : L368D/K370S" means that one of the monomers has the double variant set S364K/E357Q and the other has the double variant set L368D/K370S; as above, the "strandedness" of these pairs depends on the starting pI.

C. pI (Isoelectric point) Variants for Heterodimers

[00547] In general, as will be appreciated by those in the art, there are two general categories of pI variants: those that increase the pI of the protein (basic changes) and those that decrease the pI of the protein (acidic changes). As described herein, all combinations of these variants can be used: one monomer may be wild type, or a variant that does not display a significantly different pI from wild-type, and the other can be either more basic or more acidic. Alternatively, each monomer may be changed, one to more basic and one to more acidic.

[00548] Preferred combinations of pI variants are shown in Figure 30 of USSN 15/141,350, all of which are herein incorporated by reference in its entirety. As outlined herein and shown in the figures, these changes are shown relative to IgG1, but all isotypes can be altered this way, as well as isotype hybrids. In the case where the heavy chain constant domain is from IgG2-4, R133E and R133Q can also be used.

[00549] In one embodiment, a preferred combination of pI variants has one monomer comprising 208D/295E/384D/418E/421D variants (N208D/Q295E/N384D/Q418E/N421D when relative to human IgG1) if one of the Fc monomers includes a CH1 domain. In some instances, the second monomer comprising a positively charged domain linker, including $(GKPGS)_4$. In some cases, the first monomer includes a CH1 domain, including position 208. Accordingly, in constructs that do not include a CH1 domain (for example for heterodimeric Fc fusion proteins that do not utilize a CH1 domain on one of the domains), a preferred negative pI variant Fc set includes 295E/384D/418E/421D variants (Q295E/N384D/Q418E/N421D when relative to human IgG1).

[00550] In some embodiments, mutations are made in the hinge of the Fc domain, including positions 216, 217, 218, 219, 220, 221, 222, 223, 224, 225, 226, 227, 228, 229, and 230. Thus, pI mutations and particularly substitutions can be made in one or more of positions 216-230, with 1, 2, 3, 4 or 5 mutations finding use in the present invention. Again, all possible combinations are contemplated, alone or with other pI variants in other domains.

[00551] Specific substitutions that find use in lowering the pI of hinge domains include, but are not limited to, a deletion at position 221, a non-native valine or threonine at position 222, a deletion at position 223, a non-native glutamic acid at position 224, a deletion at position 225, a deletion at position 235 and a deletion or a non-native alanine at position 236. In some cases, only pI substitutions are done in the hinge domain, and in others, these substitution(s) are added to other pI variants in other domains in any combination.

[00552] In some embodiments, mutations can be made in the CH2 region, including positions 233, 234, 235, 236, 274, 296, 300, 309, 320, 322, 326, 327, 334 and 339. It should be noted that changes in 233-236 can be made to increase effector function (along with 327A) in the IgG2 backbone. Again, all possible combinations of these 14 positions can be made; e.g., a pI antibody may have 1, 2, 3, 4, 5, 6, 7, 8, 9 or 10 CH2 pI substitutions.

[00553] Specific substitutions that find use in lowering the pI of CH2 domains include, but are not limited to, a non-native glutamine or glutamic acid at position 274, a non-native

phenylalanine at position 296, a non-native phenylalanine at position 300, a non-native valine at position 309, a non-native glutamic acid at position 320, a non-native glutamic acid at position 322, a non-native glutamic acid at position 326, a non-native glycine at position 327, a non-native glutamic acid at position 334, a non-native threonine at position 339, and all possible combinations within CH2 and with other domains.

[00554] In this embodiment, the mutations can be independently and optionally selected from position 355, 359, 362, 384, 389,392, 397, 418, 419, 444 and 447. Specific substitutions that find use in lowering the pI of CH3 domains include, but are not limited to, a non-native glutamine or glutamic acid at position 355, a non-native serine at position 384, a non-native asparagine or glutamic acid at position 392, a non-native methionine at position 397, a non-native glutamic acid at position 419, a non-native glutamic acid at position 359, a non-native glutamic acid at position 362, a non-native glutamic acid at position 389, a non-native glutamic acid at position 418, a non-native glutamic acid at position 444, and a deletion or non-native aspartic acid at position 447.

D. Isotypic Variants

[00555] In addition, many embodiments of the invention rely on the "importation" of pI amino acids at particular positions from one IgG isotype into another, thus reducing or eliminating the possibility of unwanted immunogenicity being introduced into the variants. A number of these are shown in Figure 21 of US Publ. App. No. 2014/0370013, hereby incorporated by reference. That is, IgG1 is a common isotype for therapeutic antibodies for a variety of reasons, including high effector function. However, the heavy constant region of IgG1 has a higher pI than that of IgG2 (8.10 versus 7.31). By introducing IgG2 residues at particular positions into the IgG1 backbone, the pI of the resulting monomer is lowered (or increased) and additionally exhibits longer serum half-life. For example, IgG1 has a glycine (pI 5.97) at position 137, and IgG2 has a glutamic acid (pI 3.22); importing the glutamic acid will affect the pI of the resulting protein. As is described below, a number of amino acid substitutions are generally required to significantly

affect the pI of the variant Fc fusion protein. However, it should be noted as discussed below that even changes in IgG2 molecules allow for increased serum half-life.

[00556] In other embodiments, non-isotypic amino acid changes are made, either to reduce the overall charge state of the resulting protein (e.g., by changing a higher pI amino acid to a lower pI amino acid), or to allow accommodations in structure for stability, etc. as is more further described below.

[00557] In addition, by pI engineering both the heavy and light constant domains, significant changes in each monomer of the heterodimer can be seen. As discussed herein, having the pIs of the two monomers differ by at least 0.5 can allow separation by ion exchange chromatography or isoelectric focusing, or other methods sensitive to isoelectric point.

E. Calculating pI

[00558] The pI of each monomer can depend on the pI of the variant heavy chain constant domain and the pI of the total monomer, including the variant heavy chain constant domain and the fusion partner. Thus, in some embodiments, the change in pI is calculated on the basis of the variant heavy chain constant domain, using the chart in the Figure 19 of US Publ. App. No. 2014/0370013. As discussed herein, which monomer to engineer is generally decided by the inherent pI of each monomer.

F. pI Variants that also confer better FcRn in vivo binding

[00559] In the case where the pI variant decreases the pI of the monomer, they can have the added benefit of improving serum retention in vivo.

[00560] Although still under examination, Fc regions are believed to have longer half-lives in vivo, because binding to FcRn at pH 6 in an endosome sequesters the Fc (Ghetie and Ward, 1997 Immunol Today. 18(12): 592-598, entirely incorporated by reference). The endosomal compartment then recycles the Fc to the cell surface. Once the compartment opens to the extracellular space, the higher pH, ~7.4, induces the release of Fc back into the blood. In mice,

Dall' Acqua et al. showed that Fc mutants with increased FcRn binding at pH 6 and pH 7.4 actually had reduced serum concentrations and the same half-life as wild-type Fc (Dall' Acqua et al. 2002, J. Immunol. 169:5171-5180, entirely incorporated by reference). The increased affinity of Fc for FcRn at pH 7.4 is thought to forbid the release of the Fc back into the blood. Therefore, the Fc mutations that will increase Fc's half-life in vivo will ideally increase FcRn binding at the lower pH while still allowing release of Fc at higher pH. The amino acid histidine changes its charge state in the pH range of 6.0 to 7.4. Therefore, it is not surprising to find His residues at important positions in the Fc/FcRn complex.

[00561] There are a number of Fc substitutions that find use in increased binding to the FcRn and increased serum half-life, as specifically disclosed in USSN 12/341,769, hereby incorporated by reference in its entirety, including, but not limited to, 434A, 428L, 308F, 259I, 428L/434S, 259I/308F, 436I/428L, 436I, 434S, 252Y/428L, 252Y/434S, 428L/434S, 436V/428L and 259I/308F/428L.

G. Additional Fc Variants for Additional Functionality

[00562] In addition to pI amino acid variants, there are a number of useful Fc amino acid modification that can be made for a variety of reasons, including, but not limited to, altering binding to one or more FcγR, altered binding to FcRn, etc.

[00563] Accordingly, the proteins of the invention can include amino acid modifications, including the heterodimerization variants outlined herein, which includes the pI variants and steric variants. Each set of variants can be independently and optionally included or excluded from any particular heterodimeric protein.

H. FcγR Variants

[00564] Accordingly, there are a number of useful Fc substitutions that can be made to alter binding to one or more of the Fcγ receptors. Substitutions that result in increased binding as well as decreased binding can be useful. For example, it is known that increased binding to FcγRIIIa results in increased ADCC (antibody dependent cell-mediated cytotoxicity; the cell-

mediated reaction wherein nonspecific cytotoxic cells that express FcγRs recognize bound antibody on a target cell and subsequently cause lysis of the target cell). Similarly, decreased binding to FcγRIIb (an inhibitory receptor) can be beneficial as well in some circumstances. Amino acid substitutions that find use in the present invention include those listed in USSNs 11/124,620 (particularly Figure 41), 11/174,287, 11/396,495, 11/538,406, all of which are expressly incorporated herein by reference in their entirety and specifically for the variants disclosed therein. Particular variants that find use include, but are not limited to, 236A, 239D, 239E, 332E, 332D, 239D/332E, 267D, 267E, 328F, 267E/328F, 236A/332E, 239D/332E/330Y, 239D, 332E/330L, 243A, 243L, 264A, 264V and 299T.

[00565] In addition, amino acid substitutions that increase affinity for FcγRIIc can also be included in the Fc domain variants outlined herein. The substitutions described in, for example, USSNs 11/124,620 and 14/578,305 are useful.

I. Ablation Variants

[00566] Similarly, another category of functional variants are "FcγR ablation variants" or "Fc knock out (FcKO or KO)" variants. In these embodiments, for some therapeutic applications, it is desirable to reduce or remove the normal binding of the Fc domain to one or more or all of the Fcγ receptors (e.g., FcγR1, FcγRIIa, FcγRIIb, FcγRIIIa, etc.) to avoid additional mechanisms of action. That is, for example, in many embodiments, particularly in the use of immunomodulatory proteins, it is desirable to ablate FcγRIIIa binding to eliminate or significantly reduce ADCC activity such that one of the Fc domains comprises one or more Fcγ receptor ablation variants. These ablation variants are depicted in Figure 31 of USSN 15/141,350, all of which are herein incorporated by reference in its entirety, and each can be independently and optionally included or excluded, with preferred aspects utilizing ablation variants selected from the group consisting of G236R/L328R, E233P/L234V/L235A/G236del/S239K, E233P/L234V/L235A/G236del/S267K, E233P/L234V/L235A/G236del/S239K/A327G, E233P/L234V/L235A/G236del/S267K/A327G and E233P/L234V/L235A/G236del, according to the

EU index. It should be noted that the ablation variants referenced herein ablate FcγR binding but generally not FcRn binding.

J. Combination of Heterodimeric and Fc Variants

[00567] As will be appreciated by those in the art, all of the recited heterodimerization variants (including skew and/or pI variants) can be optionally and independently combined in any way, as long as they retain their "strandedness" or "monomer partition". In addition, all of these variants can be combined into any of the heterodimerization formats.

[00568] In the case of pI variants, while embodiments finding particular use are shown in the Figures, other combinations can be generated, following the basic rule of altering the pI difference between two monomers to facilitate purification.

[00569] In addition, any of the heterodimerization variants, skew and pI, may also be independently and optionally combined with Fc ablation variants, Fc variants, FcRn variants, as generally outlined herein.

[00570] In addition, a monomeric Fc domain can comprise a set of amino acid substitutions that includes C220S/S267K/L368D/K370S or C220S/S267K/S364K/E357Q.

[00571] In addition, the heterodimeric Fc fusion proteins can comprise skew variants (e.g., a set of amino acid substitutions as shown in Figures 1A-1C of USSN 15/141,350, all of which are herein incorporated by reference in its entirety), with particularly useful skew variants being selected from the group consisting of S364K/E357Q : L368D/K370S; L368D/K370S : S364K; L368E/K370S : S364K; T411T/E360E/Q362E : D401K; L368D/K370S : S364K/E357L; K370S : S364K/E357Q; T366S/L368A/Y407V : T366W; and T366S/L368A/Y407V : T366W (optionally including a bridging disulfide, T366S/L368A/Y407V/Y349C : T366W/S354C), optionally ablation variants, optionally charged domain linkers; and optionally pI variants.

[00572] In some embodiments, the Fc domain comprises one or more amino acid substitutions selected from the group consisting of: 236R, S239D, S239E, F243L, M252Y, V259I, S267D, S267E,

S67K, S298A, V308F, L328F, L328R, 330L, I332D, I332E, M428L, N434A, N434S, 236R/L328R, S239D/I332E, 236R/L328F, V259I/V308F, S267E/L328F, M428L/N43S, Y436I/M428L, N436V/M428L, V436I/N434S, Y436V/N434S, S239D/I332E/330L, M252Y/S54T/T256E, V259I/V308F/M428L, E233P/L234V/L235A/G236_/S239K, E233P/L234V/L235A/G236_/S239K/A327G, E233P/L234V/L235A/G236_/S267K/A327G, E233P/L234V/L235A/G236_, and E233P/L234V/L235A/G236_/S267K according to EU index.

[00573] In one embodiment, a particular combination of skew and pI variants that finds use in the present invention is T366S/L368A/Y407V : T366W (optionally including a bridging disulfide, T366S/L368A/Y407V/Y349C : T366W/S354C) with one monomer comprising Q295E/N384D/Q418E/N481D and the other a positively charged domain linker. As will be appreciated in the art, the "knobs in holes" variants do not change pI, and thus can be used on either monomer.

IV. Homodimeric Fc Fusion Proteins

[00574] In some aspects, the present invention relates to homodimeric Fc fusion proteins that include an Fc region, generally linked (optionally through a domain linker) to one or more different protein domains. These formats are generally shown in Figure 14 and Figure 48. In one aspect, the homodimeric Fc fusion protein is an IL-12 homodimeric Fc fusion protein that includes IL-12p40 and IL-12p35 subunits in different orientations, such that they present together to bind to the IL-12 receptor complex of IL-12Rβ1/β2. The Fc domains can be derived from IgG Fc domains, e.g., IgG1, IgG2, IgG3 or IgG4 Fc domains, with IgG1 and IgG4 Fc domains finding particular use in the invention. As described herein, IgG1 Fc domains may be used, often, but not always in conjunction with ablation variants to ablate effector function. Similarly, when low effector function is desired, IgG4 Fc domains may be used.

[00575] In one aspect, a homodimeric Fc fusion protein comprises first monomer and a second monomer comprising, from N- to C-terminal, IL-12p40 subunit-optional linker-IL-12p35 subunit-optional linker-Fc domain. In some embodiments, a homodimeric Fc fusion protein comprises a first monomer and a second monomer comprising, from N- to C-terminal, IL-12p40

subunit-linker-IL-12p35 subunit-linker-Fc domain. An example of an embodiment is provided in Figure 48A.

[00576] In another aspect, a homodimeric Fc fusion protein comprises a first monomer and a second monomer comprising, from N- to C-terminal, IL-12p35 subunit-optional linker-IL-12p40 subunit-optional linker-Fc domain. In some embodiments, a homodimeric Fc fusion protein comprises a first monomer and a second monomer comprising, from N- to C-terminal, IL-12p35 subunit-linker-IL-12p40 subunit-linker-Fc domain. An example of an embodiment is provided in Figure 48B.

[00577] In another aspect, a homodimeric Fc fusion protein comprises a first monomer and a second monomer comprising, from N- to C-terminal, Fc domain-optional linker-IL-12p40 subunit-optional linker-IL-12p35 subunit. In some embodiments, a homodimeric Fc fusion protein comprising a first monomer and a second monomer comprises, from N- to C-terminal, Fc domain-linker-IL-12p40 subunit-linker-IL-12p35 subunit. An example of an embodiment is provided in Figure 48C.

[00578] In another aspect, a homodimeric Fc fusion protein comprises a first monomer and a second monomer comprising, from N- to C-terminal, Fc domain-optional linker-IL-12p35 subunit-optional linker-IL-12p40 subunit. In some embodiments, a homodimeric Fc fusion protein comprising a first monomer and a second monomer comprises, from N- to C-terminal, Fc domain-linker-IL-12p35 subunit-linker-IL-12p40 subunit. An example of an embodiment is provided in Figure 48D.

[00579] In some embodiments, the bivalent IL-12p40-Fc format provides a homodimeric Fc fusion protein comprising: a) a first monomer comprising, from N- to C-terminal: i) a IL-12p40 domain protein; ii) an optional first domain linker; iii) a first variant Fc domain protein; and b) a second monomer comprising, from N- to C-terminal: i) a IL-12p40 domain protein; ii) optionally a second domain linker; iii) a second variant Fc domain protein.

[00580] In some embodiments, the bivalent IL-12p35-Fc format provides a homodimeric Fc fusion protein comprising: a) a first monomer comprising, from N- to C-terminal: i) a IL-12p35 domain protein; ii) an optional first domain linker; iii) a first variant Fc domain protein; and b) a second monomer comprising, from N- to C-terminal: i) a IL-12p35 domain protein; ii) optionally a second domain linker; iii) a second variant Fc domain protein.

[00581] In some embodiments, the (scIL-12(p40/p35))$_2$-Fc format provides a homodimeric Fc fusion protein comprising: a) a first monomer comprising, from N- to C-terminal: i) a IL-12p40 domain protein; ii) an optional first domain linker; iii) a IL-12p35 domain protein; iv) a optional second domain linker; v) a first variant Fc domain protein; and b) a second monomer comprising, from N- to C-terminal: i) a IL-12p40 domain protein; ii) optionally a third domain linker; iii) a IL-12p35 domain protein; iv) optionally a fourth domain linker; v) a second variant Fc domain protein.

[00582] In some embodiments, the (scIL-12(p35/p40))$_2$-Fc format provides a homodimeric Fc fusion protein comprising: a) a first monomer comprising, from N- to C-terminal: i) a IL-12p35 domain protein; ii) an optional first domain linker; iii) a IL-12p40 domain protein; iv) a optional second domain linker; v) a first variant Fc domain protein; and b) a second monomer comprising, from N- to C-terminal: i) a IL-12p35 domain protein; ii) optionally a third domain linker; iii) a IL-12p40 domain protein; iv) optionally a fourth domain linker; v) a second variant Fc domain protein.

[00583] In some embodiments, the Fc-(scIL-12(p40/p35))$_2$ format provides a homodimeric Fc fusion protein comprising: a) a first monomer comprising, from N- to C-terminal: i) a first variant Fc domain protein; ii) an optional first domain linker; iii) a IL-12p40 domain protein; iv) an optional second domain linker; v) a IL-12p35 domain protein; and b) a second monomer comprising, from N- to C-terminal: i) a second variant Fc domain protein; ii) an optional first domain linker; iii) a IL-12p40 domain protein; iv) an optional second domain linker; v) a IL-12p35 domain protein.

[00584] In some embodiments, the Fc-(scIL-12(p35/p40))$_2$ format provides a homodimeric Fc fusion protein comprising: a) a first monomer comprising, from N- to C-terminal: i) a first variant Fc domain protein; ii) an optional first domain linker; iii) a IL-12p35 domain protein; iv) an optional second domain linker; v) a IL-12p40 domain protein; and b) a second monomer comprising, from N- to C-terminal: i) a second variant Fc domain protein; ii) an optional first domain linker; iii) a IL-12p35 domain protein; iv) an optional second domain linker; v) a IL-12p40 domain protein.

[00585] In some embodiments, the first domain linker and said second domain linker have the same amino acid sequence. In some embodiments, the first domain linker and second domain linker have different amino acid sequences.

[00586] In some embodiments, the Fc variants comprise one or more skew, pI, and ablation variants as provided herein. In one embodiment, Fc variants comprise particular skew, pI, and ablation variants. In some embodiments, modifications promoting homodimerization of the first and the second Fc domains are a set of amino acid substitutions selected from the group consisting of L368D/K370S; S364K; S364K/E357L; S364K/E357Q; T411E/K360E/Q362E; D401K; T366S/L368A/Y407V; T366W; T366S/L368A/Y407V/Y349C; and T366W/S354C, according to EU numbering. In some embodiments, the first and/or the second Fc domains have an additional set of amino acid substitutions comprising Q295E/N384D/Q418E/N421D, according to EU numbering. In some embodiments, the first and/or the second Fc domains have an additional set of amino acid substitutions selected from the group consisting of G236R/L328R, E233P/L234V/L235A/G236_/S239K, E233P/L234V/L235A/G236_/S239K/A327G, E233P/L234V/L235A/G236_/S267K/A327G, E233P/L234V/L235A/G236_, and E233P/L234V/L235A/G236_/S267K, according to EU numbering. In some embodiments the first and second Fc domains further comprise amino acid substitutions M428L/N424S.

[00587] In this format, useful IL-12p40 protein domains include, but are not limited to, SEQ ID NO: 4 (human IL-12 subunit beta (IL-12p40) mature form sequence). In this format, useful IL-12p40 variants include, but are not limited to, E59K, E59Q, D18N, D18K, E32Q, E33Q, D34N,

D34K, Q42E, S43E, S43K, E45Q, Q56E, D62N, E73Q, D87N, K99E, K99Y, E100Q, N103D, N103Q, N113D, N113Q, Q144E, D161N, R159E, K163E, E187Q, N200D, N200Q, N218Q, Q229E, E235Q, C252S, Q256N, K258E, K260E, E262Q, K264E, N281D, N281Q, and E299Q. In this format, useful IL-12p40 variants include, but are not limited to, N103D/N113D/N200D/N281D, Q42E/E45Q, E45Q/Q56E, Q42E/E59Q, Q56E/E59Q, Q42E/E45Q/Q56E, E45Q/Q56E/E59Q, E32Q/E59Q, D34N/E59K, D34N/E59K/K99E, D34K/E59K/K99E, E32Q/D34N/E59K/K99E, E32K/D34N/E59K/K99E, D34N/E59Q, E59Q/E187Q, S43E/E59Q, S43K/E49Q, E59Q/K163E, E59Q/K99E, E59Q/K258E, E59Q/K260E, E59K/K99E, D18K/E59K/K99E, E59K/K99E/K264E, E59K/K99Y, E59Y/K99Y, E59Y/K99E, E45K/E59K/K99E, E59K/K99E/Q144E, E59K/K99E/Q144K, E59K/K99E/R159E, E59K/K99E/K264E, D18K/E59K/K99E/K264E, DI8K/E59K/K99E/C252S, D18K/E59K/K99E/C252S/K264E, E59K/K99Y/C252S, E59K/K99E/C252S/K264E, E59K/K99E/C252S, N103D/N113D, N103D/N200D, N103D/N281D, N113D/N200D, N113D/N281D, N200D/N281D, N103D/N113D/N200D, N103D/N113D/N281D, N103D/N200D/N281D, N113D/N200D/N281D, N103Q/N113Q, N103Q/N200Q, N103Q/N281Q, N113Q/N200Q, N113Q/N281Q, N200Q/N281Q, N103Q/N113Q/N200Q, N103Q/N113Q/N281Q, N103Q/N200Q/N281Q, N113Q/N200Q/N281Q, N103Q/N113Q/N200Q/N281Q, E59K/K99E/N103Q/C252S/K264E, E59K/K99E/N113Q/C252S/K264E, E59K/K99E/N200Q/C252S/K264E, E59K/K99E/N281Q/C252S/K264E, E59K/K99E/N103Q/N113Q/C252S/K264E, E59K/K99E/N103Q/N200Q/C252S/K264E, E59K/K99E/N103Q/N281Q/C252S/K264E, E59K/K99E/N113Q/N200Q/C252S/K264E, E59K/K99E/N113Q/N281Q/C252S/K264E, E59K/K99E/N200Q/N281Q/C252S/K264E, E59K/K99E/N103Q/N113Q/N200Q/C252S/K264E, E59K/K99E/N103Q/N200Q/N281Q/C252S/K264E, E59K/K99E/N113Q/N200Q/N281Q/C252S/K264E, and E59K/K99E/N103Q/N113Q/N200Q/N281Q/C252S/K264E.

[00588] In this format, IL-12p35 protein domains include SEQ ID NO: 2 (human IL-12 subunit alpha (IL-12p35) mature form sequence). In this format, useful IL-12p35 variants, include, but are not limited to, N21D, Q35D, E38Q, D55Q, D55K, N71D, N71Q, L75A, N76D, E79Q, N85D,

N85Q, L89A, F96A, M97A, L124A, M125A, Q130E, Q135E, N136D, E143Q, Q146E, N151D, N151K, E153K, E153Q, K158E, E162Q, E163Q, D165N, I171A, N195D, and N195Q. In this format, useful IL-12p35 variants, include, but are not limited to, N71D/N85D/N195D, N151D/E153Q, N151D/D165N, Q130E/N151D, N151D/K158E, E79Q/N151D, D55Q/N151D, N136D/N151D, N21D/N151D, E143Q/N151D, N71Q/N85Q, N71Q/N195Q, N85Q/N195Q, N71Q/N85Q/N195Q, N71D/N85D, N71D/N195D, and N85D/N195D.

[00589] In this format, useful embodiments include, but are not limited to, those found in Figures 14A-14B and Figure 15.

Potential embodiments of homodimeric Fc fusion proteins may use methods and compositions described in the section above entitled "Heterodimeric Fc Fusion Proteins," as appropriate with the goal of producing a homodimer rather than heterodimer. Potential embodiments of homodimeric Fc fusion proteins may use any combination of methods and compositions described herein.

V. Interleukin 12

[00590] The present invention relates to the biologically functional form of interleukin 12. IL-12 heterodimeric Fc proteins comprising a IL-12p40 variant and/or a IL-12p35 variants have reduced or decreased binding affinity to IL-12 receptors compared to a wild-type IL-12, including the p40/p35 heterodimer and the p40 homodimer. IL-12 heterodimeric Fc proteins of the present invention have reduced or decreased potency compared to wild-type IL-12. IL-12 heterodimeric Fc proteins have prolonged or increased serum half-life. IL-12 heterodimeric Fc proteins have a reduced or decreased potential for toxicity. IL-12 heterodimeric Fc proteins of the present invention have reduced or decreased potency in inducing STAT4 phosphorylation in immune cells such as, but not limited to, T cells and NK cells. IL-12p40 variants comprise amino acid modifications (e.g., substitutions, additions, and deletions) that remove potential N-glycosylation sites. IL-12p35 variants comprise amino acid modifications (e.g., substitutions, additions, and deletions) that remove potential N-glycosylation sites. In some embodiments, the IL-12 heterodimeric Fc proteins of the present invention has reduced/decreased glycosylation

compared to wild-type IL-12. In some embodiments, the glycosylation status of the IL-12 heterodimeric Fc proteins of the present invention is different than a wild-type IL-12 protein. IL-12 heterodimeric Fc proteins of the present invention provide improved pharmacokinetics compared to wild-type IL-12. Also, such IL-12 heterodimeric Fc proteins are therapeutically effective and have an improved therapeutic index such as compared to wild-type IL-12. IL-12 heterodimeric Fc proteins of the present invention can promote/induce T cell activation. In some embodiments, administration of any one of the IL-12 heterodimeric Fc proteins to a patient induces T cell activation in the patient. In some instances, administration induces IFN-gamma secretion in the patient. In some cases, administration of the IL-12 heterodimeric Fc proteins induces expansion of lymphocytes in the patient. IL-12 heterodimeric Fc proteins are useful for treating cancer such as solid tumor cancers or liquid tumor cancers. n some embodiments, administration of any one of the IL-12 heterodimeric Fc proteins to a patient, and in some cases, in combination with a checkpoint blockage agent reduces tumor size.

[00591] As stated above, the biologically functional form of interleukin 12 or "IL-12" is a heterodimer, composed of the IL-12p35 subunit (IL-12 subunit alpha) and the IL-12p40 subunit (IL-12 subunit beta), hereon designated as an "IL-12 heterodimeric complex". This complex can be used in two different formats. As shown in Figures 8A-B, the IL-12p40 subunit and the IL-12p35 subunits are not covalently attached to each other, but rather are covalently attached respectively to a first and a second Fc domain which are assembled as a heterodimer. Alternatively, the IL-12p35 and IL-12p40 subunits can be covalently attached, optionally using a domain linker (as described herein), as generally shown in Figures 8C-F, hereon designated a single-chain IL-12 complex or "scIL-12". The order of the two subunits in the scIL-12 may be designated as follows: "scIL-12(p40/p35)", wherein the IL-12p40 subunit is N-terminally linked (with or without a domain linker) to the IL-12p35 subunit, or "scIL-12(p35/p40)", wherein the IL-12p35 is N-terminally linked (with or without a domain linker) to the IL-12p40subunit.

[00592] In some embodiments, the human IL-12p35 protein has the amino acid sequence set forth in NCBI Ref. Seq. No. NP_000873.2 or SEQ ID NO:1 (Human IL-12 subunit alpha (IL-12p35) precursor sequence as depicted in Figure 1). In some cases, the coding sequence of

human IL-12p35 is set forth in NCBI Ref. Seq. No. NM_000882.3. An exemplary IL-12p35 protein of the Fc fusion heterodimeric protein outlined herein can have the amino acid sequence of SEQ ID NO:2 (Human IL-12 subunit alpha (IL-12p35) mature form sequence as depicted in Figure 1) or amino acids 23-219 of SEQ ID NO:1. In some embodiments, the IL-12p35 protein has at least 90%, e.g., 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or more sequence identity to SEQ ID NO:2. The IL-12p35 protein of the Fc fusion protein can have 1, 2, 3, 4, 5, 6, 7, 8 or 9 amino acid mutations.

[00593] In some embodiments, the human IL-12p40 protein has the amino acid sequence set forth in NCBI Ref. Seq. No. NP_002178.3 or SEQ ID NO:3 (Human IL-12 subunit beta (IL-12p40) precursor sequence as depicted in Figure 1). In some cases, the coding sequence of human IL-12p40 is set forth in NCBI Ref. Seq. No. NM_002187.3. An exemplary IL-12p40 protein of the Fc fusion protein outlined herein can have the amino acid sequence of SEQ ID NO:4 (Human IL-12 subunit beta (IL-12p40) mature form sequence as depicted in Figure 1) or amino acids 23-328 of SEQ ID NO:3. In some embodiments, the IL-12p40 protein has at least 90%, e.g., 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or more sequence identity to SEQ ID NO:4. The IL-12p40 protein of the Fc fusion protein can have 1, 2, 3, 4, 5, 6, 7, 8, or 9 amino acid mutations.

[00594] The present invention also provides variant IL-12p40 subunits and variant IL-12p35 subunits. These variants find use as part of the biologically functional IL-12 complex as well as any of the IL-12-Fc fusions described herein.

A. Expression Variants

[00595] As a preliminary matter, the IL-12p40 and IL-12p35 subunits of the invention also include variants to remove potential N-glycosylation sites designed to reduce heterogeneity.

[00596] Such potential N-glycosylation sites on IL-12p40 at which amino acid modifications can be introduced include N103, N113, N200, and N281 (numbered according to the Human IL-12 subunit beta (IL-12p40 mature form sequence as depicted in Figure 1). Illustrative modifications

at one or more of these sites may be selected from the group consisting of: N103D, N103Q, N113D, N113Q, N200D, N200Q, N281D, and N281Q. IL-12p40 expression variants can include one or more modifications at these sites. Accordingly, in one embodiment, the IL-12p40 variant comprises N103D/N113D/N200D/N281D. In one embodiment, the IL-12p40 variant comprises N103D/N113D. In one embodiment, the IL-12p40 variant comprises N103D/N200D. In one embodiment, the IL-12p40 variant comprises N103D/N281D. In one embodiment, the IL-12p40 variant comprises N113D/N200D. In one embodiment, the IL-12p40 variant comprises N113D/N281D. In one embodiment, the IL-12p40 variant comprises N200D/N281D. In one embodiment, the IL-12p40 variant comprises N103D/N113D/N200D. In one embodiment, the IL-12p40 variant comprises N103D/N113D/N281D. In one embodiment, the IL-12p40 variant comprises N/103D/N200D/N281D. In one embodiment, the IL-12p40 variant comprises N113D/N200D/N281D. In one embodiment, the IL-12p40 variant comprises N103Q/N113Q. In one embodiment, the IL-12p40 variant comprises N103Q/N200Q. In one embodiment, the IL-12p40 variant comprises N103Q/N281Q. In one embodiment, the IL-12p40 variant comprises N113Q/N200Q. In one embodiment, the IL-12p40 variant comprises N113Q/N281Q. In one embodiment, the IL-12p40 variant comprises N103Q/N113Q/N200Q. In one embodiment, the IL-12p40 variant comprises N103Q/N113Q/N281Q. In one embodiment, the IL-12p40 variant comprises N103Q/N200Q/N281Q. In one embodiment, the IL-12p40 variant comprises N113Q/N200Q/N281Q. These modifications can be used alone or in combination with any other IL-12p40 variants, such as affinity variants.

[00597]Such potential N-glycosylation sites on IL-12p35 at which amino acid modifications can be introduced at one or more of the sites selected from the group consisting of: N71, N85, and N195 (numbered according to the Human IL-12 subunit alpha (IL-12p35) mature form sequence as depicted in Figure 1). Illustrative modifications at these sites include N71D, N71Q, N85D, N85Q, N195D, and N195Q. IL-12p35 variants can include one or more modifications at these sites. Accordingly, in one embodiment, the IL-12p35 variant comprises N71D/N85D/N195D. These modifications can be used alone or in combination with any other IL-12p35 variants, such as affinity variants.

[00598] The IL-12p40 subunit has a free cysteine at position 252 (numbered according to the Human IL-12 subunit beta (IL-12p40) mature form sequence as depicted in Figure 1) which may bond with other free cysteines leading at least to heterogeneity and at worse to immunogenicity. Accordingly, IL-12p40 variants were engineered to remove the free cysteine, for example, by introducing C252S modification (although other substitutions may also be used). Modification of C252 (e.g. C252S) can be used alone or in combination with any other IL-12p40 variants, such as affinity or expression variants. Illustrative IL-12p40 variants comprising a modification at C252 to remove the free cysteine are depicted in Figure 65. Illustrative IL-12-Fc fusions proteins were generated with the additional variant IL-12p40 subunits, sequences for which are depicted in Figure 66, and produced as generally described in Example 1B. These modifications can be used alone or in combination with any other IL-12p40 variants, such as affinity variants.

B. Affinity and Potency Variants

[00599] The invention provides IL-12p40 variants and IL-12p35 variants which form biologically functional IL-12 with altered, that is either reduced or increased, binding affinity for IL-12 receptors. In some cases, the variant IL-12p40 subunit has altered, that is either reduced or increased, binding affinity for IL-12 receptor subunit beta-1 (IL-12Rß1), IL-12 receptor subunit beta-2 (IL-12Rß2), and/or the IL-12 receptor complex. In some cases, the variant IL-12p35 has altered, that is either reduced or increased, binding affinity for IL-12 receptor subunit beta-1 (IL-12Rß1), IL-12 receptor subunit beta-2 (IL-12Rß2), and/or the IL-12 receptor complex. The invention also provides IL-12p40 variants and IL-12p35 variants which form biologically functional IL-12 with altered, that is either reduced or increased, potency compared to wild-type IL-12p40 and IL-12p35.

Suitable sites on IL-12p40 at which amino acid modifications can be be introduced are selected from the group consisting of: E3, D7, E12, D14, W15, P17, D18, A19, P20, G21, E22, M23, D29, E32, E33, D34, L40, D41, Q42, S43, E45, L47, T54, I55, Q56, K58, E59, F60, G61, D62, Q65, Y66, E73, K84, E86, D87, G88, I89, W90, D93, D97, K99, E100, K102, N103, K104, F106, E110, N113,

Y114, D129, D142, Q144, E156, R159, D161, N162, K163, D166, D170, Q172, D174, A176, C177, P178, A179, A180, E181, S183, P185, E187, N200, S204, F206, R208, D209, D214, N218, Q220, N226, Q229, E231, E235, T242, P243, S245, Y246, F247, S248, C252, Q256, K258, K260, E262, K264, D265, D270, N281, Q289, D290, R291, Y292, Y293, and E299 (numbered according to the Human IL-12 subunit beta (IL-12p40 mature form sequence as depicted in Figure 1). Illustrative modifications at these sites can be selected from the group consisting of: D18N, D18K, E32Q, E33Q, D34N, D34K, Q42E, S43E, S43K, E45Q, Q56E, E59Q, E59K, D62N, E73Q, D87N, K99E, K99Y, E100Q, N103D, N103Q, N113D, N113Q, Q144E, D161N, R159E, K163E, E187Q, N200D, N200Q, N218Q, Q229E, E235Q, C252S, Q256N, K258E, K260E, E262Q, K264E, N281D, N281Q, and E299Q. IL-12p40 affinity variants can include modifications at one or more of these sites. Accordingly, in one embodiment, the IL-12p40 variant comprises N103D/N113D/N200D/N281D. In another embodiment, the IL-12p40 variant comprises Q42E/E45Q. In another embodiment, the IL-12p40 variant comprises E45Q/Q56E. In another embodiment, the IL-12p40 variant comprises Q42E/E59Q. In another embodiment, the IL-12p40 variant comprises Q56E/E59Q. In another embodiment, the IL-12p40 variant comprises Q42E/E45Q/Q56E. In another embodiment, the IL-12p40 variant comprises E45Q/Q56E/E59Q. In another embodiment, the IL-12p40 variant comprises E32Q/E59Q. In another embodiment, the IL-12p40 variant comprises D34N/E59K. In another embodiment, the IL-12p40 variant comprises D34N/E59K/K99E. In another embodiment, the IL-12p40 variant comprises D34K/E59K/K99E. In another embodiment, the IL-12p40 variant comprises E32Q/D34N/E59K/K99E. In another embodiment, the IL-12p40 variant comprises E32K/D34N/E59K/K99E. In another embodiment, the IL-12p40 variant comprises D34N/E59Q. In another embodiment, the IL-12p40 variant comprises E59Q/E187Q. In another embodiment, the IL-12p40 variant comprises S43E/E59Q. In another embodiment, the IL-12p40 variant comprises S43K/E49Q. In another embodiment, the IL-12p40 variant comprises E59Q/K163E. In another embodiment, the IL-12p40 variant comprises E59Q/K99E. In another embodiment, the IL-12p40 variant comprises E59Q/K258E. In another embodiment, the IL-12p40 variant comprises E59Q/K260E. In another embodiment, the IL-12p40 variant comprises E59K/K99E. In another embodiment, the IL-12p40 variant comprises D18K/E59K/K99E. In another

embodiment, the IL-12p40 variant comprises E59K/K99E/K264E. In another embodiment, the IL-12p40 variant comprises E59K/K99Y. In another embodiment, the IL-12p40 variant comprises E59Y/K99Y. In another embodiment, the IL-12p40 variant comprises E59Y/K99E. In another embodiment, the IL-12p40 variant comprises E45K/E59K/K99E. In another embodiment, the IL-12p40 variant comprises E59K/K99E/Q144E. In another embodiment, the IL-12p40 variant comprises E59K/K99E/Q144K. In another embodiment, the IL-12p40 variant comprises E59K/K99E/R159E. In another embodiment, the IL-12p40 variant comprises E59K/K99E/K264E. In another embodiment, the IL-12p40 variant comprises D18K/E59K/K99E/K264E. In another embodiment, the IL-12p40 variant comprises DI8K/E59K/K99E/C252S. In another embodiment, the IL-12p40 variant comprises D18K/E59K/K99E/C252S/K264E. In another embodiment, the IL-12p40 variant comprises E59K/K99Y/C252S. In another embodiment, the IL-12p40 variant comprises E59K/K99E/C252S/K264E. In another embodiment, the IL-12p40 variant comprises E59K/K99E/C252S. In another embodiment, the IL-12p40 variant comprises N103D/N113D. In another embodiment, the IL-12p40 variant comprises N103D/N200D. In another embodiment, the IL-12p40 variant comprises N103D/N281D. In another embodiment, the IL-12p40 variant comprises N113D/N200D. In another embodiment, the IL-12p40 variant comprises N113D/N281D. In another embodiment, the IL-12p40 variant comprises N200D/N281D. In another embodiment, the IL-12p40 variant comprises N103D/N113D/N200D. In another embodiment, the IL-12p40 variant comprises N103D/N113D/N281D. In another embodiment, the IL-12p40 variant comprises N103D/N200D/N281D. In another embodiment, the IL-12p40 variant comprises N113D/N200D/N281D. In another embodiment, the IL-12p40 variant comprises N103Q/N113Q. In another embodiment, the IL-12p40 variant comprises N103Q/N200Q. In another embodiment, the IL-12p40 variant comprises N103Q/N281Q. In another embodiment, the IL-12p40 variant comprises N113Q/N200Q. In another embodiment, the IL-12p40 variant comprises N113Q/N281Q. In another embodiment, the IL-12p40 variant comprises N200Q/N281Q. In another embodiment, the IL-12p40 variant comprises N103Q/N113Q/N200Q. In another embodiment, the IL-12p40 variant comprises N103Q/N113Q/N281Q,. In another embodiment, the IL-12p40 variant comprises N103Q/N200Q/N281Q. In another embodiment, the IL-12p40 variant comprises

N113Q/N200Q/N281Q. In another embodiment, the IL-12p40 variant comprises N103Q/N113Q/N200Q/N281Q. In another embodiment, the IL-12p40 variant comprises E59K/K99E/N103Q/C252S/K264E. In another embodiment, the IL-12p40 variant comprises E59K/K99E/N113Q/C252S/K264E. In another embodiment, the IL-12p40 variant comprises E59K/K99E/N200Q/C252S/K264E. In another embodiment, the IL-12p40 variant comprises E59K/K99E/N281Q/C252S/K264E. In another embodiment, the IL-12p40 variant comprises E59K/K99E/N103Q/N113Q/C252S/K264E. In another embodiment, the IL-12p40 variant comprises E59K/K99E/N103Q/N200Q/C252S/K264E. In another embodiment, the IL-12p40 variant comprises E59K/K99E/N103Q/N281Q/C252S/K264E. In another embodiment, the IL-12p40 variant comprises E59K/K99E/N113Q/N200Q/C252S/K264E. In another embodiment, the IL-12p40 variant comprises E59K/K99E/N113Q/N281Q/C252S/K264E. In another embodiment, the IL-12p40 variant comprises E59K/K99E/N200Q/N281Q/C252S/K264E. In another embodiment, the IL-12p40 variant comprises E59K/K99E/N103Q/N113Q/N200Q/C252S/K264E. In another embodiment, the IL-12p40 variant comprises E59K/K99E/N103Q/N200Q/N281Q/C252S/K264E. In another embodiment, the IL-12p40 variant comprises E59K/K99E/N113Q/N200Q/N281Q/C252S/K264E. In another embodiment, the IL-12p40 variant comprises E59K/K99E/N103Q/N113Q/N200Q/N281Q/C252S/K264E.

Additionally, these modifications can be used alone or in combination with any other IL-12p40 variants, such as expression variants.

[00600] The IL-12p40 subunit has a free cysteine at position 252 (numbered according to the Human IL-12 subunit beta (IL-12p40) mature form sequence as depicted in Figure 1) which may bond with other free cysteines leading at least to heterogeneity and at worse to immunogenicity. Accordingly, IL-12p40 variants were engineered to remove the free cysteine, for example, by introducing C252S modification (although other substitutions may also be used). Modification of C252 (e.g. C252S) can be used alone or in combination with any other IL-12p40 variants, such as affinity or expression variants. Illustrative IL-12p40 variants comprising a modification at C252 to remove the free cysteine are depicted in Figure 65. Illustrative IL-12-Fc fusions proteins were generated with the additional variant IL-12p40 subunits, sequences for

which are depicted in Figure 66, and produced as generally described in Example 1B. Additionally, these modifications can be used alone or in combination with any other IL-12p40 variants, such as expression variants.

[00601]Suitable sites on IL-12p35 at which amino acid modifications can be introduced are selected from the group consisting of: Q20, N21, Q35, E38, S44, E45, E46, H49, K54, D55, T59, V60, E61, C63, L64, P65, E67, L68, N71, S73, C74, L75, N76, E79, N85, F96, M97, L89, L124, M125, Q130, Q135, N136, E143, Q146, N151, E153, K158, E162, E163, D165, I171, R181, I182, R183, V185, T186, D188, R189, V190, S192, Y193, and A196 (numbered according to the Human IL-12 subunit alpha (IL-12p35) mature form sequence as depicted in Figure 1). Illustrative modifications are selected from the group consisting of: N21D, Q35D, E38Q, D55Q, D55K, N71D, N71Q, L75A, N76D, E79Q, N85D, N85Q, L89A, F96A, M97A, L124A, M125A, Q130E, Q135E, N136D, E143Q, Q146E, N151D, N151K, E153K, E153Q, K158E, E162Q, E163Q, D165N, I171A, N195D, and N195Q. IL-12p35 affinity variants can include modifications at one or more of these sites. Accordingly, in one embodiment, the IL-12p35 variant comprises N71D/N85D/N195D. In another embodiment, the IL-12p35 variant comprises N151D/E153Q. In another embodiment, the IL-12p35 variant comprises N151D/D165N. In another embodiment, the IL-12p35 variant comprises Q130E/N151D. In another embodiment, the IL-12p35 variant comprises N151D/K158E. In another embodiment, the IL-12p35 variant comprises E79Q/N151D. In another embodiment, the IL-12p35 variant comprises D55Q/N151D. In another embodiment, the IL-12p35 variant comprises N136D/N151D. In another embodiment, the IL-12p35 variant comprises N21D/N151D. In another embodiment, the IL-12p35 variant comprises E143Q/N151D. In another embodiment, the IL-12p35 variant comprises N71Q/N85Q. In another embodiment, the IL-12p35 variant comprises N71Q/N195Q. In another embodiment, the IL-12p35 variant comprises N85Q/N195Q. In another embodiment, the IL-12p35 variant comprises N71Q/N85Q/N195Q. In another embodiment, the IL-12p35 variant comprises N71D/N85D. In another embodiment, the IL-12p35 variant comprises N71D/N195D. In another embodiment, the IL-12p35 variant comprises N85D/N195D.

[00602] Additionally, these modifications can be used alone or in combination with any other IL-12p35 variants, such as expression variants.

[00603] A biologically functional IL-12 heterodimeric complex can comprise a wild-type IL-12p40 subunit and a wild-type IL-12p35 subunit, a variant IL-12p40 subunit and a wild-type IL-12p35 subunit, a wild-type IL-12p40 subunit and a variant IL-12p35 subunit, or a variant IL-12p40 subunit and a variant IL-12p35 subunit.

[00604] A biologically functional IL-12 bivalent homodimeric complex can comprise a wild-type IL-12p40 subunit and a wild-type IL-12p35 subunit, a variant IL-12p40 subunit and a wild-type IL-12p35 subunit, a wild-type IL-12p40 subunit and a variant IL-12p35 subunit, or a variant IL-12p40 subunit and a variant IL-12p35 subunit.

VI. Domain Linkers

[00605] In some embodiments, the IL-12p35 and IL-12p40 subunits are attached together via a linker. Optionally, the subunits are not attached via a linker. In other embodiments, the IL-12p35 and IL-12p40 subunits are noncovalently attached. In some embodiments, the IL-12p35 subunit is attached to an Fc domain via a linker. In certain embodiments, the IL-12p35 subunit is attached to an Fc domain directly, such as without a linker. In other embodiments, the IL-12p40 subunit is attached to an Fc domain via a linker. In other embodiments, the IL-12p40 subunit is attached to an Fc domain directly. In some cases, a linker is not used to attach the IL-12p35 subunit or IL-12p40 subunit to an Fc domain.

[00606] In some embodiments, the linker is a "domain linker", used to link any two domains as outlined herein together. While any suitable linker can be used, many embodiments utilize a glycine-serine polymer, including for example $(GS)_n$, $(GSGGS)_n$, $(GGGGS)_n$, and $(GGGS)_n$, where n is an integer of at least 0 (and generally from 0 to 1 to 2 to 3 to 4 to 5), as well as any peptide sequence that allows for recombinant attachment of the two domains with sufficient length and flexibility to allow each domain to retain its biological function. In certain cases, useful linkers include $(GGGGS)_0$ or $(GGGGS)_1$ or $(GGGGS)_2$. Illustrative domain linkers are depicted in Figure

6. In some cases, and with attention being paid to "strandedness", as outlined below, charged domain linkers can be used as discussed herein.

[00607] In addition, it has been previousy reported tha thte serine in Gly-Ser linkers in Fc fusions may be subject to O-glycosylation. Accordingly, a final approach to decrease heterogeneity (in the context of Fc fusions) is to replace the Gly-Ser linkers used thus far with Gly-Ala linkers.

VII. Useful formats of the Invention

[00608] As shown in Figures 8A-F there are a number of useful formats of the heterodimeric fusion proteins of the invention. In general, the heterodimeric fusion proteins of the invention have two functional components: an IL-12 heterodimer component and an Fc component, both of which can take different forms as outlined herein and both of which can be combined with the other component in any configuration.

[00609] In some embodiments, the IL-12p35 and IL-12p40 subunits are covalently linked, optionally with a domain linker, and is referred to herein as a single-chain IL-12 complex or "scIL-12". The scIL-12 can comprise either IL-12p35 N-terminally linked to IL-12p40 or IL-12p40 N-terminally linked to IL-12p35, optionally with a domain linker. The order of the two subunits in the scIL-12 may be designated as follows: "scIL-12(p40/p35)", wherein the IL-12p40 subunit is N-terminally linked (with or without a domain linker) to the IL-12p35 subunit, or "scIL-12(p35/p40)", wherein the IL-12p35 is N-terminally linked (with or without a domain linker) to the IL-12p340subunit.

[00610] In some embodiments, the IL-12p35 and IL-12p40 subunits are not covalently linked, but rather are covalently attached respectively to a first and a second Fc domain which are assembled as a heterodimer.

[00611] The first and the second Fc domains can have a set of amino acid substitutions selected from the group consisting of a) L368D/K370S and S364K; b) L368D/K370S and S364K/E357L; c) L368D/K370S and S364K/E357Q; d) S267K/L368D/K370S; e) T411E/K360E/Q362E and D401K; f) L368E/K370S and S364K; g) K370S and S364K/E357Q; and h) T366S/L368A/Y407V and T366W

(optionally including a bridging disulfide, T366S/L368A/Y407V/Y349C and T366W/S354C), according to EU numbering.

[00612] In some embodiments, the first and/or the second Fc domains have an additional set of amino acid substitutions comprising Q295E/N384D/Q418E/N421D, according to EU numbering.

[00613] Optionally, the first and/or the second Fc domains have an additional set of amino acid substitutions selected from the group consisting of G236R/L328R, E233P/L234V/L235A/G236_/S239K, E233P/L234V/L235A/G236_/S239K/A327G, E233P/L234V/L235A/G236_/S267K/A327G, E233P/L234V/L235A/G236_, and E233P/L234V/L235A/G236_/S267K, according to EU numbering.

[00614] Optionally, the first and/or second Fc domains have 428L/434S variants for half life extension.

A. IL-12-heteroFc format

[00615] In one embodiment, the present invention provides the N-terminal IL-12 heterodimeric Fc fusion or "IL-12-heteroFc" format. In this embodiment, as shown in Figure 8A, the heterodimeric fusion protein comprises two monomers. The first monomer comprises (from N- to C-terminus) IL-12p40-optional domain linker-Fc. The second monomer comprises (from N-to C-terminus) the IL-12p35-optional domain linker-Fc.

[00616] In the IL-12-heteroFc format, a preferred embodiment utilizes the skew variant pair S364K/E357Q : L368D/K370S.

[00617] In the IL-12-heteroFc format, a preferred embodiment is shown in Figure 9 (XENP27201 including chain 1 chain 2).

[00618] In some embodiments, the IL-12-heteroFc format provides a heterodimeric Fc fusion protein comprising: a) a first monomer comprising, from N- to C-terminal: i) a IL-12p40 domain protein; ii) an optional first domain linker; iii) a first variant Fc domain protein; and b)

a second monomer comprising, from N- to C-terminal: i) a IL-12p35 domain protein; ii) optionally a second domain linker; iii) a second variant Fc domain protein.

[00619] In some embodiments, the Fc variants comprise one or more skew, pI, and ablation variants as provided herein. In one embodiment, Fc variants comprise particular skew, pI, and ablation variants. In some embodiments, modifications promoting heterodimerization of the first and the second Fc domains are a set of amino acid substitutions selected from the group consisting of L368D/K370S and S364K; L368D/K370S and S364K/E357L; L368D/K370S and S364K/E357Q; T411E/K360E/Q362E and D401K; L368E/K370S and S364K; K370S and S364K/E357Q; T366S/L368A/Y407V and T366W; T366S/L368A/Y407V/Y349C and T366W/S354C, according to EU numbering. In some embodiments, the first and/or the second Fc domains have an additional set of amino acid substitutions comprising Q295E/N384D/Q418E/N421D, according to EU numbering. In some embodiments, the first and/or the second Fc domains have an additional set of amino acid substitutions selected from the group consisting of G236R/L328R, E233P/L234V/L235A/G236_/S239K, E233P/L234V/L235A/G236_/S239K/A327G, E233P/L234V/L235A/G236_/S267K/A327G, E233P/L234V/L235A/G236_, and E233P/L234V/L235A/G236_/S267K, according to EU numbering. In some embodiments the first and second Fc domains further comprise amino acid substitutions M428L/N424S.

[00620] In this format, useful IL-12p40 protein domains include, but are not limited to, SEQ ID NO: 4 (human IL-12 subunit beta (IL-12p40) mature form sequence). In this format, useful IL-12p40 variants include, but are not limited to, E59K, E59Q, D18N, D18K, E32Q, E33Q, D34N, D34K, Q42E, S43E, S43K, E45Q, Q56E, D62N, E73Q, D87N, K99E, K99Y, E100Q, N103D, N103Q, N113D, N113Q, Q144E, D161N, R159E, K163E, E187Q, N200D, N200Q, N218Q, Q229E, E235Q, C252S, Q256N, K258E, K260E, E262Q, K264E, N281D, N281Q, and E299Q. In this format, useful IL-12p40 variants include, but are not limited to, N103D/N113D/N200D/N281D, Q42E/E45Q, E45Q/Q56E, Q42E/E59Q, Q56E/E59Q, Q42E/E45Q/Q56E, E45Q/Q56E/E59Q, E32Q/E59Q, D34N/E59K, D34N/E59K/K99E, D34K/E59K/K99E, E32Q/D34N/E59K/K99E, E32K/D34N/E59K/K99E, D34N/E59Q, E59Q/E187Q, S43E/E59Q, S43K/E49Q, E59Q/K163E, E59Q/K99E, E59Q/K258E, E59Q/K260E, E59K/K99E, D18K/E59K/K99E, E59K/K99E/K264E,

E59K/K99Y, E59Y/K99Y, E59Y/K99E, E45K/E59K/K99E, E59K/K99E/Q144E, E59K/K99E/Q144K, E59K/K99E/R159E, E59K/K99E/K264E, D18K/E59K/K99E/K264E, DI8K/E59K/K99E/C252S, D18K/E59K/K99E/C252S/K264E, E59K/K99Y/C252S, E59K/K99E/C252S/K264E, E59K/K99E/C252S, N103D/N113D, N103D/N200D, N103D/N281D, N113D/N200D, N113D/N281D, N200D/N281D, N103D/N113D/N200D, N103D/N113D/N281D, N103D/N200D/N281D, N113D/N200D/N281D, N103Q/N113Q, N103Q/N200Q, N103Q/N281Q, N113Q/N200Q, N113Q/N281Q, N200Q/N281Q, N103Q/N113Q/N200Q, N103Q/N113Q/N281Q, N103Q/N200Q/N281Q, N113Q/N200Q/N281Q, N103Q/N113Q/N200Q/N281Q, E59K/K99E/N103Q/C252S/K264E, E59K/K99E/N113Q/C252S/K264E, E59K/K99E/N200Q/C252S/K264E, E59K/K99E/N281Q/C252S/K264E, E59K/K99E/N103Q/N113Q/C252S/K264E, E59K/K99E/N103Q/N200Q/C252S/K264E, E59K/K99E/N103Q/N281Q/C252S/K264E, E59K/K99E/N113Q/N200Q/C252S/K264E, E59K/K99E/N113Q/N281Q/C252S/K264E, E59K/K99E/N200Q/N281Q/C252S/K264E, E59K/K99E/N103Q/N113Q/N200Q/C252S/K264E, E59K/K99E/N103Q/N200Q/N281Q/C252S/K264E, E59K/K99E/N113Q/N200Q/N281Q/C252S/K264E, and E59K/K99E/N103Q/N113Q/N200Q/N281Q/C252S/K264E.

[00621] In this format, IL-12p35 protein domains include SEQ ID NO: 2 (human IL-12 subunit alpha (IL-12p35) mature form sequence). In this format, useful IL-12p35 variants, include, but are not limited to, N21D, Q35D, E38Q, D55Q, D55K, N71D, N71Q, L75A, N76D, E79Q, N85D, N85Q, L89A, F96A, M97A, L124A, M125A, Q130E, Q135E, N136D, E143Q, Q146E, N151D, N151K, E153K, E153Q, K158E, E162Q, E163Q, D165N, I171A, N195D, and N195Q. In this format, useful IL-12p35 variants, include, but are not limited to, N71D/N85D/N195D, N151D/E153Q, N151D/D165N, Q130E/N151D, N151D/K158E, E79Q/N151D, D55Q/N151D, N136D/N151D, N21D/N151D, E143Q/N151D, N71Q/N85Q, N71Q/N195Q, N85Q/N195Q, N71Q/N85Q/N195Q, N71D/N85D, N71D/N195D, and N85D/N195D.

[00622] In this format, useful combinations of a variant IL-12p40 subunit and IL-12p35 subunit include, but are not limited to IL-12p40(E59K/K99Y) and IL-12p35(SEQ ID NO: 2), IL-

12p40(D18K/E59K/K99E) and IL-12p35 (SEQ ID NO: 2), IL-12p40(E59K/K99E/K264E) and IL-12p35(SEQ ID NO: 2), IL-12p40(D18K/E59K/K99E/K264E) and IL-12p35(SEQ ID NO: 2), IL-12p40(D18K/E59K/K99E/C252S) and IL-12p35(SEQ ID NO: 2), IL-12p40(D18K/E59K/K99E/C252S/K264E) and IL-12p35(SEQ ID NO: 2), IL-12p40(E59K/K99Y/C252S) and IL-12p35(SEQ ID NO: 2), IL-12p40(E59K/K99E/C252S/K264E) and IL-12p35(SEQ ID NO: 2), and IL-12p40(E59K/K99E/C252S) and IL-12p35(SEQ ID NO: 2).

[00623] In this format, useful combinations of a variant IL-12p40 subunit and a IL-12p35 subunit include, but are not limited to a combination of an IL-12p35 comprising SEQ ID NO: 2 (human IL-12 subunit alpha (IL-12p35) mature form sequence) and a variantIL-12p40 comprising one or more substitutions including, but not limited, to E59K, E59Q, D18N, D18K, E32Q, E33Q, D34N, D34K, Q42E, S43E, S43K, E45Q, Q56E, D62N, E73Q, D87N, K99E, K99Y, E100Q, N103D, N103Q, N113D, N113Q, Q144E, D161N, R159E, K163E, E187Q, N200D, N200Q, N218Q, Q229E, E235Q, C252S, Q256N, K258E, K260E, E262Q, K264E, N281D, N281Q, E299Q, N103D/N113D/N200D/N281D, Q42E/E45Q, E45Q/Q56E, Q42E/E59Q, Q56E/E59Q, Q42E/E45Q/Q56E, E45Q/Q56E/E59Q, E32Q/E59Q, D34N/E59K, D34N/E59K/K99E, D34K/E59K/K99E, E32Q/D34N/E59K/K99E, E32K/D34N/E59K/K99E, D34N/E59Q, E59Q/E187Q, S43E/E59Q, S43K/E49Q, E59Q/K163E, E59Q/K99E, E59Q/K258E, E59Q/K260E, E59K/K99E, D18K/E59K/K99E, E59K/K99E/K264E, E59K/K99Y, E59Y/K99Y, E59Y/K99E, E45K/E59K/K99E, E59K/K99E/Q144E, E59K/K99E/Q144K, E59K/K99E/R159E, E59K/K99E/K264E, D18K/E59K/K99E/K264E, DI8K/E59K/K99E/C252S, D18K/E59K/K99E/C252S/K264E, E59K/K99Y/C252S, E59K/K99E/C252S/K264E, E59K/K99E/C252S, N103D/N113D, N103D/N200D, N103D/N281D, N113D/N200D, N113D/N281D, N200D/N281D, N103D/N113D/N200D, N103D/N113D/N281D, N103D/N200D/N281D, N113D/N200D/N281D, N103Q/N113Q, N103Q/N200Q, N103Q/N281Q, N113Q/N200Q, N113Q/N281Q, N200Q/N281Q, N103Q/N113Q/N200Q, N103Q/N113Q/N281Q, N103Q/N200Q/N281Q, N113Q/N200Q/N281Q, N103Q/N113Q/N200Q/N281Q, E59K/K99E/N103Q/C252S/K264E, E59K/K99E/N113Q/C252S/K264E, E59K/K99E/N200Q/C252S/K264E, E59K/K99E/N281Q/C252S/K264E, E59K/K99E/N103Q/N113Q/C252S/K264E,

E59K/K99E/N103Q/N200Q/C252S/K264E, E59K/K99E/N103Q/N281Q/C252S/K264E, E59K/K99E/N113Q/N200Q/C252S/K264E, E59K/K99E/N113Q/N281Q/C252S/K264E, E59K/K99E/N200Q/N281Q/C252S/K264E, E59K/K99E/N103Q/N113Q/N200Q/C252S/K264E, E59K/K99E/N103Q/N200Q/N281Q/C252S/K264E, E59K/K99E/N113Q/N200Q/N281Q/C252S/K264E, and E59K/K99E/N103Q/N113Q/N200Q/N281Q/C252S/K264E.

[00624] In this format, useful combinations of IL-12p40 and IL-12p35 variants include, but are not limited to a combination of an IL-12p40 comprising SEQ ID NO: 4 (human IL-12 subunit beta (IL-12p40) mature form sequence) and an IL-12p35 comprising N21D, Q35D, E38Q, D55Q, D55K, N71D, N71Q, L75A, N76D, E79Q, N85D, N85Q, L89A, F96A, M97A, L124A, M125A, Q130E, Q135E, N136D, E143Q, Q146E, N151D, N151K, E153K, E153Q, K158E, E162Q, E163Q, D165N, I171A, N195D, N195Q, N71D/N85D/N195D, N151D/E153Q, N151D/D165N, Q130E/N151D, N151D/K158E, E79Q/N151D, D55Q/N151D, N136D/N151D, N21D/N151D, E143Q/N151D, N71Q/N85Q, N71Q/N195Q, N85Q/N195Q, N71Q/N85Q/N195Q, N71D/N85D, N71D/N195D, and N85D/N195D.

[00625] In this format, useful combinations of IL-12p40 variants and IL-12p35 variants include, but are not limited to, a combination of an IL-12p40 variant comprising one or more amino acid substitution selected from the group consisting of: E59K, E59Q, D18N, D18K, E32Q, E33Q, D34N, D34K, Q42E, S43E, S43K, E45Q, Q56E, D62N, E73Q, D87N, K99E, K99Y, E100Q, N103D, N103Q, N113D, N113Q, Q144E, D161N, R159E, K163E, E187Q, N200D, N200Q, N218Q, Q229E, E235Q, C252S, Q256N, K258E, K260E, E262Q, K264E, N281D, N281Q, E299Q, N103D/N113D/N200D/N281D, Q42E/E45Q, E45Q/Q56E, Q42E/E59Q, Q56E/E59Q, Q42E/E45Q/Q56E, E45Q/Q56E/E59Q, E32Q/E59Q, D34N/E59K, D34N/E59K/K99E, D34K/E59K/K99E, E32Q/D34N/E59K/K99E, E32K/D34N/E59K/K99E, D34N/E59Q, E59Q/E187Q, S43E/E59Q, S43K/E49Q, E59Q/K163E, E59Q/K99E, E59Q/K258E, E59Q/K260E, E59K/K99E, D18K/E59K/K99E, E59K/K99E/K264E, E59K/K99Y, E59Y/K99Y, E59Y/K99E, E45K/E59K/K99E, E59K/K99E/Q144E, E59K/K99E/Q144K, E59K/K99E/R159E, E59K/K99E/K264E, D18K/E59K/K99E/K264E, DI8K/E59K/K99E/C252S, D18K/E59K/K99E/C252S/K264E,

E59K/K99Y/C252S, E59K/K99E/C252S/K264E, E59K/K99E/C252S, N103D/N113D, N103D/N200D, N103D/N281D, N113D/N200D, N113D/N281D, N200D/N281D, N103D/N113D/N200D, N103D/N113D/N281D, N103D/N200D/N281D, N113D/N200D/N281D, N103Q/N113Q, N103Q/N200Q, N103Q/N281Q, N113Q/N200Q, N113Q/N281Q, N200Q/N281Q, N103Q/N113Q/N200Q, N103Q/N113Q/N281Q, N103Q/N200Q/N281Q, N113Q/N200Q/N281Q, N103Q/N113Q/N200Q/N281Q, E59K/K99E/N103Q/C252S/K264E, E59K/K99E/N113Q/C252S/K264E, E59K/K99E/N200Q/C252S/K264E, E59K/K99E/N281Q/C252S/K264E, E59K/K99E/N103Q/N113Q/C252S/K264E, E59K/K99E/N103Q/N200Q/C252S/K264E, E59K/K99E/N103Q/N281Q/C252S/K264E, E59K/K99E/N113Q/N200Q/C252S/K264E, E59K/K99E/N113Q/N281Q/C252S/K264E, E59K/K99E/N200Q/N281Q/C252S/K264E, E59K/K99E/N103Q/N113Q/N200Q/C252S/K264E, E59K/K99E/N103Q/N200Q/N281Q/C252S/K264E, E59K/K99E/N113Q/N200Q/N281Q/C252S/K264E, and E59K/K99E/N103Q/N113Q/N200Q/N281Q/C252S/K264E; and an IL-12p35 variant comprising one or more substitutions selected from the group consisting of: N21D, Q35D, E38Q, D55Q, D55K, N71D, N71Q, L75A, N76D, E79Q, N85D, N85Q, L89A, F96A, M97A, L124A, M125A, Q130E, Q135E, N136D, E143Q, Q146E, N151D, N151K, E153K, E153Q, K158E, E162Q, E163Q, D165N, I171A, N195D, N195Q, N71D/N85D/N195D, N151D/E153Q, N151D/D165N, Q130E/N151D, N151D/K158E, E79Q/N151D, D55Q/N151D, N136D/N151D, N21D/N151D, E143Q/N151D, N71Q/N85Q, N71Q/N195Q, N85Q/N195Q, N71Q/N85Q/N195Q, N71D/N85D, N71D/N195D, and N85D/N195D.

[00626] In this format, useful embodiments include but are not limited to those in which a variant IL-12p40 subunit domain is attached to said first Fc domain using a first domain linker and/or said IL-12p35 subunit domain is attached to said second Fc domain using a second domain linker.

[00627] In this format, useful embodiments include but are not limited to XENP31251, XENP31254, XENP31258, XENP32186, XENP32187, XENP32188, XENP32189, XENP32190, and XENP32191. In further embodiments, XENP31251, XENP31254, XENP31258, XENP32186,

XENP32187, XENP32188, XENP32189, XENP32190, and XENP32191 include further glycoengineering.

[00628] In this format, useful embodiments include, but are not limited to, those found in Figures 44A-44K, and Figures 66A-66Q.

B. heteroFc-IL-12 Format

[00629] In another embodiment, the present invention provides the C-terminal IL-12 heterodimeric Fc fusion or "heteroFc-IL-12" format. In this embodiment, as shown in Figure 8B, the heterodimeric fusion protein comprises two monomers. The first monomer comprises (from N-to C-terminus) Fc-optional domain linker-IL-12p40. The second monomer comprises (from N-to C-terminus) Fc-optional domain linker-IL-12p35.

[00630] In the heteroFc-IL-12 format, a preferred embodiment utilizes the skew variant pair S364K/E357Q : L368D/K370S.

[00631] In the IL-12-heteroFc format, a preferred embodiment is shown in Figure 10 (XENP27202 including chain 1 chain 2).

[00632] In some embodiments, the heteroFc-IL-12 format provides a heterodimeric Fc fusion protein comprising: a) a first monomer comprising, from N- to C-terminal: i) a first variant Fc domain protein; ii) an optional first domain linker; iii) a IL-12p40 domain protein; and b) a second monomer comprising, from N- to C-terminal: i) a second variant Fc domain protein; ii) optionally a second domain linker; iii) a IL-12p35 domain protein.

[00633] In some embodiments, the Fc variants comprise one or more skew, pI, and ablation variants as provided herein. In one embodiment, Fc variants comprise particular skew, pI, and ablation variants. In some embodiments, modifications promoting heterodimerization of the first and the second Fc domains are a set of amino acid substitutions selected from the group consisting of L368D/K370S and S364K; L368D/K370S and S364K/E357L; L368D/K370S and S364K/E357Q; T411E/K360E/Q362E and D401K; L368E/K370S and S364K; K370S and

S364K/E357Q; T366S/L368A/Y407V and T366W; T366S/L368A/Y407V/Y349C and T366W/S354C, according to EU numbering. In some embodiments, the first and/or the second Fc domains have an additional set of amino acid substitutions comprising Q295E/N384D/Q418E/N421D, according to EU numbering. In some embodiments, the first and/or the second Fc domains have an additional set of amino acid substitutions selected from the group consisting of G236R/L328R, E233P/L234V/L235A/G236_/S239K, E233P/L234V/L235A/G236_/S239K/A327G, E233P/L234V/L235A/G236_/S267K/A327G, E233P/L234V/L235A/G236_, and E233P/L234V/L235A/G236_/S267K, according to EU numbering. In some embodiments the first and second Fc domains further comprise amino acid substitutions M428L/N424S.

[00634] In this format, useful IL-12p40 protein domains include, but are not limited to, SEQ ID NO: 4 (human IL-12 subunit beta (IL-12p40) mature form sequence). In this format, useful IL-12p40 variants include, but are not limited to, E59K, E59Q, D18N, D18K, E32Q, E33Q, D34N, D34K, Q42E, S43E, S43K, E45Q, Q56E, D62N, E73Q, D87N, K99E, K99Y, E100Q, N103D, N103Q, N113D, N113Q, Q144E, D161N, R159E, K163E, E187Q, N200D, N200Q, N218Q, Q229E, E235Q, C252S, Q256N, K258E, K260E, E262Q, K264E, N281D, N281Q, and E299Q. In this format, useful IL-12p40 variants include, but are not limited to, N103D/N113D/N200D/N281D, Q42E/E45Q, E45Q/Q56E, Q42E/E59Q, Q56E/E59Q, Q42E/E45Q/Q56E, E45Q/Q56E/E59Q, E32Q/E59Q, D34N/E59K, D34N/E59K/K99E, D34K/E59K/K99E, E32Q/D34N/E59K/K99E, E32K/D34N/E59K/K99E, D34N/E59Q, E59Q/E187Q, S43E/E59Q, S43K/E49Q, E59Q/K163E, E59Q/K99E, E59Q/K258E, E59Q/K260E, E59K/K99E, D18K/E59K/K99E, E59K/K99E/K264E, E59K/K99Y, E59Y/K99Y, E59Y/K99E, E45K/E59K/K99E, E59K/K99E/Q144E, E59K/K99E/Q144K, E59K/K99E/R159E, E59K/K99E/K264E, D18K/E59K/K99E/K264E, DI8K/E59K/K99E/C252S, D18K/E59K/K99E/C252S/K264E, E59K/K99Y/C252S, E59K/K99E/C252S/K264E, E59K/K99E/C252S, N103D/N113D, N103D/N200D, N103D/N281D, N113D/N200D, N113D/N281D, N200D/N281D, N103D/N113D/N200D, N103D/N113D/N281D, N103D/N200D/N281D, N113D/N200D/N281D, N103Q/N113Q, N103Q/N200Q, N103Q/N281Q, N113Q/N200Q, N113Q/N281Q, N200Q/N281Q, N103Q/N113Q/N200Q, N103Q/N113Q/N281Q, N103Q/N200Q/N281Q, N113Q/N200Q/N281Q, N103Q/N113Q/N200Q/N281Q,

E59K/K99E/N103Q/C252S/K264E, E59K/K99E/N113Q/C252S/K264E,
E59K/K99E/N200Q/C252S/K264E, E59K/K99E/N281Q/C252S/K264E,
E59K/K99E/N103Q/N113Q/C252S/K264E, E59K/K99E/N103Q/N200Q/C252S/K264E,
E59K/K99E/N103Q/N281Q/C252S/K264E, E59K/K99E/N113Q/N200Q/C252S/K264E,
E59K/K99E/N113Q/N281Q/C252S/K264E, E59K/K99E/N200Q/N281Q/C252S/K264E,
E59K/K99E/N103Q/N113Q/N200Q/C252S/K264E,
E59K/K99E/N103Q/N200Q/N281Q/C252S/K264E,
E59K/K99E/N113Q/N200Q/N281Q/C252S/K264E, and
E59K/K99E/N103Q/N113Q/N200Q/N281Q/C252S/K264E.

[00635] In this format, IL-12p35 protein domains include SEQ ID NO: 2 (human IL-12 subunit alpha (IL-12p35) mature form sequence). In this format, useful IL-12p35 variants, include, but are not limited to, N21D, Q35D, E38Q, D55Q, D55K, N71D, N71Q, L75A, N76D, E79Q, N85D, N85Q, L89A, F96A, M97A, L124A, M125A, Q130E, Q135E, N136D, E143Q, Q146E, N151D, N151K, E153K, E153Q, K158E, E162Q, E163Q, D165N, I171A, N195D, and N195Q. In this format, useful IL-12p35 variants, include, but are not limited to, N71D/N85D/N195D, N151D/E153Q, N151D/D165N, Q130E/N151D, N151D/K158E, E79Q/N151D, D55Q/N151D, N136D/N151D, N21D/N151D, E143Q/N151D, N71Q/N85Q, N71Q/N195Q, N85Q/N195Q, N71Q/N85Q/N195Q, N71D/N85D, N71D/N195D, and N85D/N195D.

[00636] In this format, useful combinations of a variant IL-12p40 subunit and IL-12p35 subunit include, but are not limited to IL-12p40(E59K/K99Y) and IL-12p35(SEQ ID NO: 2), IL-12p40(D18K/E59K/K99E) and IL-12p35 (SEQ ID NO: 2), IL-12p40(E59K/K99E/K264E) and IL-12p35(SEQ ID NO: 2), IL-12p40(D18K/E59K/K99E/K264E) and IL-12p35(SEQ ID NO: 2), IL-12p40(D18K/E59K/K99E/C252S) and IL-12p35(SEQ ID NO: 2), IL-12p40(D18K/E59K/K99E/C252S/K264E) and IL-12p35(SEQ ID NO: 2), IL-12p40(E59K/K99Y/C252S) and IL-12p35(SEQ ID NO: 2), IL-12p40(E59K/K99E/C252S/K264E) and IL-12p35(SEQ ID NO: 2), and IL-12p40(E59K/K99E/C252S) and IL-12p35(SEQ ID NO: 2).

[00637] In this format, useful combinations of a variant IL-12p40 subunit and a IL-12p35 subunit include, but are not limited to a combination of an IL-12p35 comprising SEQ ID NO: 2 (human IL-12 subunit alpha (IL-12p35) mature form sequence) and a variantIL-12p40 comprising one or more substitutions including, but not limited, to E59K, E59Q, D18N, D18K, E32Q, E33Q, D34N, D34K, Q42E, S43E, S43K, E45Q, Q56E, D62N, E73Q, D87N, K99E, K99Y, E100Q, N103D, N103Q, N113D, N113Q, Q144E, D161N, R159E, K163E, E187Q, N200D, N200Q, N218Q, Q229E, E235Q, C252S, Q256N, K258E, K260E, E262Q, K264E, N281D, N281Q, E299Q, N103D/N113D/N200D/N281D, Q42E/E45Q, E45Q/Q56E, Q42E/E59Q, Q56E/E59Q, Q42E/E45Q/Q56E, E45Q/Q56E/E59Q, E32Q/E59Q, D34N/E59K, D34N/E59K/K99E, D34K/E59K/K99E, E32Q/D34N/E59K/K99E, E32K/D34N/E59K/K99E, D34N/E59Q, E59Q/E187Q, S43E/E59Q, S43K/E49Q, E59Q/K163E, E59Q/K99E, E59Q/K258E, E59Q/K260E, E59K/K99E, D18K/E59K/K99E, E59K/K99E/K264E, E59K/K99Y, E59Y/K99Y, E59Y/K99E, E45K/E59K/K99E, E59K/K99E/Q144E, E59K/K99E/Q144K, E59K/K99E/R159E, E59K/K99E/K264E, D18K/E59K/K99E/K264E, DI8K/E59K/K99E/C252S, D18K/E59K/K99E/C252S/K264E, E59K/K99Y/C252S, E59K/K99E/C252S/K264E, E59K/K99E/C252S, N103D/N113D, N103D/N200D, N103D/N281D, N113D/N200D, N113D/N281D, N200D/N281D, N103D/N113D/N200D, N103D/N113D/N281D, N103D/N200D/N281D, N113D/N200D/N281D, N103Q/N113Q, N103Q/N200Q, N103Q/N281Q, N113Q/N200Q, N113Q/N281Q, N200Q/N281Q, N103Q/N113Q/N200Q, N103Q/N113Q/N281Q, N103Q/N200Q/N281Q, N113Q/N200Q/N281Q, N103Q/N113Q/N200Q/N281Q, E59K/K99E/N103Q/C252S/K264E, E59K/K99E/N113Q/C252S/K264E, E59K/K99E/N200Q/C252S/K264E, E59K/K99E/N281Q/C252S/K264E, E59K/K99E/N103Q/N113Q/C252S/K264E, E59K/K99E/N103Q/N200Q/C252S/K264E, E59K/K99E/N103Q/N281Q/C252S/K264E, E59K/K99E/N113Q/N200Q/C252S/K264E, E59K/K99E/N113Q/N281Q/C252S/K264E, E59K/K99E/N200Q/N281Q/C252S/K264E, E59K/K99E/N103Q/N113Q/N200Q/C252S/K264E, E59K/K99E/N103Q/N200Q/N281Q/C252S/K264E, E59K/K99E/N113Q/N200Q/N281Q/C252S/K264E, and E59K/K99E/N103Q/N113Q/N200Q/N281Q/C252S/K264E.

[00638] In this format, useful combinations of IL-12p40 and IL-12p35 variants include, but are not limited to a combination of an IL-12p40 comprising SEQ ID NO: 4 (human IL-12 subunit beta (IL-12p40) mature form sequence) and an IL-12p35 comprising N21D, Q35D, E38Q, D55Q, D55K, N71D, N71Q, L75A, N76D, E79Q, N85D, N85Q, L89A, F96A, M97A, L124A, M125A, Q130E, Q135E, N136D, E143Q, Q146E, N151D, N151K, E153K, E153Q, K158E, E162Q, E163Q, D165N, I171A, N195D, N195Q, N71D/N85D/N195D, N151D/E153Q, N151D/D165N, Q130E/N151D, N151D/K158E, E79Q/N151D, D55Q/N151D, N136D/N151D, N21D/N151D, E143Q/N151D, N71Q/N85Q, N71Q/N195Q, N85Q/N195Q, N71Q/N85Q/N195Q, N71D/N85D, N71D/N195D, and N85D/N195D.

[00639] In this format, useful combinations of IL-12p40 variants and IL-12p35 variants include, but are not limited to, a combination of an IL-12p40 variant comprising one or more amino acid substitution selected from the group consisting of: E59K, E59Q, D18N, D18K, E32Q, E33Q, D34N, D34K, Q42E, S43E, S43K, E45Q, Q56E, D62N, E73Q, D87N, K99E, K99Y, E100Q, N103D, N103Q, N113D, N113Q, Q144E, D161N, R159E, K163E, E187Q, N200D, N200Q, N218Q, Q229E, E235Q, C252S, Q256N, K258E, K260E, E262Q, K264E, N281D, N281Q, E299Q, N103D/N113D/N200D/N281D, Q42E/E45Q, E45Q/Q56E, Q42E/E59Q, Q56E/E59Q, Q42E/E45Q/Q56E, E45Q/Q56E/E59Q, E32Q/E59Q, D34N/E59K, D34N/E59K/K99E, D34K/E59K/K99E, E32Q/D34N/E59K/K99E, E32K/D34N/E59K/K99E, D34N/E59Q, E59Q/E187Q, S43E/E59Q, S43K/E49Q, E59Q/K163E, E59Q/K99E, E59Q/K258E, E59Q/K260E, E59K/K99E, D18K/E59K/K99E, E59K/K99E/K264E, E59K/K99Y, E59Y/K99Y, E59Y/K99E, E45K/E59K/K99E, E59K/K99E/Q144E, E59K/K99E/Q144K, E59K/K99E/R159E, E59K/K99E/K264E, D18K/E59K/K99E/K264E, DI8K/E59K/K99E/C252S, D18K/E59K/K99E/C252S/K264E, E59K/K99Y/C252S, E59K/K99E/C252S/K264E, E59K/K99E/C252S, N103D/N113D, N103D/N200D, N103D/N281D, N113D/N200D, N113D/N281D, N200D/N281D, N103D/N113D/N200D, N103D/N113D/N281D, N103D/N200D/N281D, N113D/N200D/N281D, N103Q/N113Q, N103Q/N200Q, N103Q/N281Q, N113Q/N200Q, N113Q/N281Q, N200Q/N281Q, N103Q/N113Q/N200Q, N103Q/N113Q/N281Q, N103Q/N200Q/N281Q, N113Q/N200Q/N281Q, N103Q/N113Q/N200Q/N281Q, E59K/K99E/N103Q/C252S/K264E,

E59K/K99E/N113Q/C252S/K264E, E59K/K99E/N200Q/C252S/K264E, E59K/K99E/N281Q/C252S/K264E, E59K/K99E/N103Q/N113Q/C252S/K264E, E59K/K99E/N103Q/N200Q/C252S/K264E, E59K/K99E/N103Q/N281Q/C252S/K264E, E59K/K99E/N113Q/N200Q/C252S/K264E, E59K/K99E/N113Q/N281Q/C252S/K264E, E59K/K99E/N200Q/N281Q/C252S/K264E, E59K/K99E/N103Q/N113Q/N200Q/C252S/K264E, E59K/K99E/N103Q/N200Q/N281Q/C252S/K264E, E59K/K99E/N113Q/N200Q/N281Q/C252S/K264E, and E59K/K99E/N103Q/N113Q/N200Q/N281Q/C252S/K264E; and an IL-12p35 variant comprising one or more substitutions selected from the group consisting of: N21D, Q35D, E38Q, D55Q, D55K, N71D, N71Q, L75A, N76D, E79Q, N85D, N85Q, L89A, F96A, M97A, L124A, M125A, Q130E, Q135E, N136D, E143Q, Q146E, N151D, N151K, E153K, E153Q, K158E, E162Q, E163Q, D165N, I171A, N195D, N195Q, N71D/N85D/N195D, N151D/E153Q, N151D/D165N, Q130E/N151D, N151D/K158E, E79Q/N151D, D55Q/N151D, N136D/N151D, N21D/N151D, E143Q/N151D, N71Q/N85Q, N71Q/N195Q, N85Q/N195Q, N71Q/N85Q/N195Q, N71D/N85D, N71D/N195D, and N85D/N195D.

[00640] In this format, useful embodiments include, but are not limited to, those found in Figure 10.

C. scIL-12-Fc Format

[00641] In a further embodiment, the present invention provides the N-terminal single-chain IL-12-Fc fusion or "scIL-12-Fc" format. In this embodiment, as shown in Figures 8C-D, the heterodimeric fusion protein comprises two monomers. The first monomer comprises (from N-to-C-terminus) scIL-12-optional domain linker-Fc. The second monomer comprises an "empty" Fc, comprising all or part of the hinge-CH2-CH3. The scIL-12 may be "scIL-12(p40/p35)", wherein the IL-12p40 subunit is N-terminally linked (with or without a domain linker) to the IL-12p35 subunit, or "scIL-12(p35/p40)", wherein the IL-12p35 is N-terminally linked (with or without a domain linker) to the IL-12p40 subunit.

[00642] In the scIL-12-Fc format, a preferred embodiment utilizes the skew variant pair S364K/E357Q : L368D/K370S.

[00643] In the scIL-12-Fc format, preferred embodiments are shown in Figure 11 as XENP24203 (including chain 1 and chain 2) and XENP24204 (including chain 1 and chain 2).

[00644] In the scIL-12-Fc format, a preferred embodiment is shown in Figure 50 as XENP31290 (including chain 1 and chain 2).

[00645] In some embodiments, the scIL-12-Fc format is a bivalent IL-12-Fc fusion. In some embodiments, the bivalent IL-12-Fc fusion format (Figures 48A-B) comprises two identical monomers each comprising a scIL-12 complex recombinant fused to the N-terminus of a homodimeric Fc chain (optionally via a domain linker).

[00646] In some embodiments, the scIL-12-Fc format a heterodimeric Fc fusion protein comprising: a) a first monomer comprising, from N- to C-terminal: i) a IL-12p40 domain protein; ii) an optional first domain linker; iii) a a IL-12p35 domain protein; iv) an optional second linker; v) a first variant Fc domain protein; and b) a second monomer comprising a second variant Fc domain protein.

[00647] In some embodiments, the scIL-12-Fc format a heterodimeric Fc fusion protein comprising: a) a first monomer comprising, from N- to C-terminal: i) a IL-12p35 domain protein; ii) an optional first domain linker; iii) a a IL-12p40 domain protein; iv) an optional second linker; v) a first variant Fc domain protein; and b) a second monomer comprising a second variant Fc domain protein.

[00648] In some embodiments, the Fc variants comprise one or more skew, pI, and ablation variants as provided herein. In one embodiment, Fc variants comprise particular skew, pI, and ablation variants. In some embodiments, modifications promoting heterodimerization of the first and the second Fc domains are a set of amino acid substitutions selected from the group consisting of L368D/K370S and S364K; L368D/K370S and S364K/E357L; L368D/K370S and S364K/E357Q; T411E/K360E/Q362E and D401K; L368E/K370S and S364K; K370S and

S364K/E357Q; T366S/L368A/Y407V and T366W; T366S/L368A/Y407V/Y349C and T366W/S354C, according to EU numbering. In some embodiments, the first and/or the second Fc domains have an additional set of amino acid substitutions comprising Q295E/N384D/Q418E/N421D, according to EU numbering. In some embodiments, the first and/or the second Fc domains have an additional set of amino acid substitutions selected from the group consisting of G236R/L328R, E233P/L234V/L235A/G236_/S239K, E233P/L234V/L235A/G236_/S239K/A327G, E233P/L234V/L235A/G236_/S267K/A327G, E233P/L234V/L235A/G236_, and E233P/L234V/L235A/G236_/S267K, according to EU numbering. In some embodiments the first and second Fc domains further comprise amino acid substitutions M428L/N424S.

[00649] In this format, useful IL-12p40 protein domains include, but are not limited to, SEQ ID NO: 4 (human IL-12 subunit beta (IL-12p40) mature form sequence). In this format, useful IL-12p40 variants include, but are not limited to, E59K, E59Q, D18N, D18K, E32Q, E33Q, D34N, D34K, Q42E, S43E, S43K, E45Q, Q56E, D62N, E73Q, D87N, K99E, K99Y, E100Q, N103D, N103Q, N113D, N113Q, Q144E, D161N, R159E, K163E, E187Q, N200D, N200Q, N218Q, Q229E, E235Q, C252S, Q256N, K258E, K260E, E262Q, K264E, N281D, N281Q, and E299Q. In this format, useful IL-12p40 variants include, but are not limited to, N103D/N113D/N200D/N281D, Q42E/E45Q, E45Q/Q56E, Q42E/E59Q, Q56E/E59Q, Q42E/E45Q/Q56E, E45Q/Q56E/E59Q, E32Q/E59Q, D34N/E59K, D34N/E59K/K99E, D34K/E59K/K99E, E32Q/D34N/E59K/K99E, E32K/D34N/E59K/K99E, D34N/E59Q, E59Q/E187Q, S43E/E59Q, S43K/E49Q, E59Q/K163E, E59Q/K99E, E59Q/K258E, E59Q/K260E, E59K/K99E, D18K/E59K/K99E, E59K/K99E/K264E, E59K/K99Y, E59Y/K99Y, E59Y/K99E, E45K/E59K/K99E, E59K/K99E/Q144E, E59K/K99E/Q144K, E59K/K99E/R159E, E59K/K99E/K264E, D18K/E59K/K99E/K264E, DI8K/E59K/K99E/C252S, D18K/E59K/K99E/C252S/K264E, E59K/K99Y/C252S, E59K/K99E/C252S/K264E, E59K/K99E/C252S, N103D/N113D, N103D/N200D, N103D/N281D, N113D/N200D, N113D/N281D, N200D/N281D, N103D/N113D/N200D, N103D/N113D/N281D, N103D/N200D/N281D, N113D/N200D/N281D, N103Q/N113Q, N103Q/N200Q, N103Q/N281Q, N113Q/N200Q, N113Q/N281Q, N200Q/N281Q, N103Q/N113Q/N200Q, N103Q/N113Q/N281Q, N103Q/N200Q/N281Q, N113Q/N200Q/N281Q, N103Q/N113Q/N200Q/N281Q,

E59K/K99E/N103Q/C252S/K264E, E59K/K99E/N113Q/C252S/K264E,
E59K/K99E/N200Q/C252S/K264E, E59K/K99E/N281Q/C252S/K264E,
E59K/K99E/N103Q/N113Q/C252S/K264E, E59K/K99E/N103Q/N200Q/C252S/K264E,
E59K/K99E/N103Q/N281Q/C252S/K264E, E59K/K99E/N113Q/N200Q/C252S/K264E,
E59K/K99E/N113Q/N281Q/C252S/K264E, E59K/K99E/N200Q/N281Q/C252S/K264E,
E59K/K99E/N103Q/N113Q/N200Q/C252S/K264E,
E59K/K99E/N103Q/N200Q/N281Q/C252S/K264E,
E59K/K99E/N113Q/N200Q/N281Q/C252S/K264E, and
E59K/K99E/N103Q/N113Q/N200Q/N281Q/C252S/K264E.

[00650] In this format, IL-12p35 protein domains include SEQ ID NO: 2 (human IL-12 subunit alpha (IL-12p35) mature form sequence). In this format, useful IL-12p35 variants, include, but are not limited to, N21D, Q35D, E38Q, D55Q, D55K, N71D, N71Q, L75A, N76D, E79Q, N85D, N85Q, L89A, F96A, M97A, L124A, M125A, Q130E, Q135E, N136D, E143Q, Q146E, N151D, N151K, E153K, E153Q, K158E, E162Q, E163Q, D165N, I171A, N195D, and N195Q. In this format, useful IL-12p35 variants, include, but are not limited to, N71D/N85D/N195D, N151D/E153Q, N151D/D165N, Q130E/N151D, N151D/K158E, E79Q/N151D, D55Q/N151D, N136D/N151D, N21D/N151D, E143Q/N151D, N71Q/N85Q, N71Q/N195Q, N85Q/N195Q, N71Q/N85Q/N195Q, N71D/N85D, N71D/N195D, and N85D/N195D.

[00651] In this format, useful combinations of a variant IL-12p40 subunit and IL-12p35 subunit include, but are not limited to IL-12p40(E59K/K99Y) and IL-12p35(SEQ ID NO: 2), IL-12p40(D18K/E59K/K99E) and IL-12p35 (SEQ ID NO: 2), IL-12p40(E59K/K99E/K264E) and IL-12p35(SEQ ID NO: 2), IL-12p40(D18K/E59K/K99E/K264E) and IL-12p35(SEQ ID NO: 2), IL-12p40(D18K/E59K/K99E/C252S) and IL-12p35(SEQ ID NO: 2), IL-12p40(D18K/E59K/K99E/C252S/K264E) and IL-12p35(SEQ ID NO: 2), IL-12p40(E59K/K99Y/C252S) and IL-12p35(SEQ ID NO: 2), IL-12p40(E59K/K99E/C252S/K264E) and IL-12p35(SEQ ID NO: 2), and IL-12p40(E59K/K99E/C252S) and IL-12p35(SEQ ID NO: 2).

[00652] In this format, useful combinations of a variant IL-12p40 subunit and a IL-12p35 subunit include, but are not limited to a combination of an IL-12p35 comprising SEQ ID NO: 2 (human IL-12 subunit alpha (IL-12p35) mature form sequence) and a variantIL-12p40 comprising one or more substitutions including, but not limited, to E59K, E59Q, D18N, D18K, E32Q, E33Q, D34N, D34K, Q42E, S43E, S43K, E45Q, Q56E, D62N, E73Q, D87N, K99E, K99Y, E100Q, N103D, N103Q, N113D, N113Q, Q144E, D161N, R159E, K163E, E187Q, N200D, N200Q, N218Q, Q229E, E235Q, C252S, Q256N, K258E, K260E, E262Q, K264E, N281D, N281Q, E299Q, N103D/N113D/N200D/N281D, Q42E/E45Q, E45Q/Q56E, Q42E/E59Q, Q56E/E59Q, Q42E/E45Q/Q56E, E45Q/Q56E/E59Q, E32Q/E59Q, D34N/E59K, D34N/E59K/K99E, D34K/E59K/K99E, E32Q/D34N/E59K/K99E, E32K/D34N/E59K/K99E, D34N/E59Q, E59Q/E187Q, S43E/E59Q, S43K/E49Q, E59Q/K163E, E59Q/K99E, E59Q/K258E, E59Q/K260E, E59K/K99E, D18K/E59K/K99E, E59K/K99E/K264E, E59K/K99Y, E59Y/K99Y, E59Y/K99E, E45K/E59K/K99E, E59K/K99E/Q144E, E59K/K99E/Q144K, E59K/K99E/R159E, E59K/K99E/K264E, D18K/E59K/K99E/K264E, DI8K/E59K/K99E/C252S, D18K/E59K/K99E/C252S/K264E, E59K/K99Y/C252S, E59K/K99E/C252S/K264E, E59K/K99E/C252S, N103D/N113D, N103D/N200D, N103D/N281D, N113D/N200D, N113D/N281D, N200D/N281D, N103D/N113D/N200D, N103D/N113D/N281D, N103D/N200D/N281D, N113D/N200D/N281D, N103Q/N113Q, N103Q/N200Q, N103Q/N281Q, N113Q/N200Q, N113Q/N281Q, N200Q/N281Q, N103Q/N113Q/N200Q, N103Q/N113Q/N281Q, N103Q/N200Q/N281Q, N113Q/N200Q/N281Q, N103Q/N113Q/N200Q/N281Q, E59K/K99E/N103Q/C252S/K264E, E59K/K99E/N113Q/C252S/K264E, E59K/K99E/N200Q/C252S/K264E, E59K/K99E/N281Q/C252S/K264E, E59K/K99E/N103Q/N113Q/C252S/K264E, E59K/K99E/N103Q/N200Q/C252S/K264E, E59K/K99E/N103Q/N281Q/C252S/K264E, E59K/K99E/N113Q/N200Q/C252S/K264E, E59K/K99E/N113Q/N281Q/C252S/K264E, E59K/K99E/N200Q/N281Q/C252S/K264E, E59K/K99E/N103Q/N113Q/N200Q/C252S/K264E, E59K/K99E/N103Q/N200Q/N281Q/C252S/K264E, E59K/K99E/N113Q/N200Q/N281Q/C252S/K264E, and E59K/K99E/N103Q/N113Q/N200Q/N281Q/C252S/K264E.

[00653] In this format, useful combinations of IL-12p40 and IL-12p35 variants include, but are not limited to a combination of an IL-12p40 comprising SEQ ID NO: 4 (human IL-12 subunit beta (IL-12p40) mature form sequence) and an IL-12p35 comprising N21D, Q35D, E38Q, D55Q, D55K, N71D, N71Q, L75A, N76D, E79Q, N85D, N85Q, L89A, F96A, M97A, L124A, M125A, Q130E, Q135E, N136D, E143Q, Q146E, N151D, N151K, E153K, E153Q, K158E, E162Q, E163Q, D165N, I171A, N195D, N195Q, N71D/N85D/N195D, N151D/E153Q, N151D/D165N, Q130E/N151D, N151D/K158E, E79Q/N151D, D55Q/N151D, N136D/N151D, N21D/N151D, E143Q/N151D, N71Q/N85Q, N71Q/N195Q, N85Q/N195Q, N71Q/N85Q/N195Q, N71D/N85D, N71D/N195D, and N85D/N195D.

[00654] In this format, useful combinations of IL-12p40 variants and IL-12p35 variants include, but are not limited to, a combination of an IL-12p40 variant comprising one or more amino acid substitution selected from the group consisting of: E59K, E59Q, D18N, D18K, E32Q, E33Q, D34N, D34K, Q42E, S43E, S43K, E45Q, Q56E, D62N, E73Q, D87N, K99E, K99Y, E100Q, N103D, N103Q, N113D, N113Q, Q144E, D161N, R159E, K163E, E187Q, N200D, N200Q, N218Q, Q229E, E235Q, C252S, Q256N, K258E, K260E, E262Q, K264E, N281D, N281Q, E299Q, N103D/N113D/N200D/N281D, Q42E/E45Q, E45Q/Q56E, Q42E/E59Q, Q56E/E59Q, Q42E/E45Q/Q56E, E45Q/Q56E/E59Q, E32Q/E59Q, D34N/E59K, D34N/E59K/K99E, D34K/E59K/K99E, E32Q/D34N/E59K/K99E, E32K/D34N/E59K/K99E, D34N/E59Q, E59Q/E187Q, S43E/E59Q, S43K/E49Q, E59Q/K163E, E59Q/K99E, E59Q/K258E, E59Q/K260E, E59K/K99E, D18K/E59K/K99E, E59K/K99E/K264E, E59K/K99Y, E59Y/K99Y, E59Y/K99E, E45K/E59K/K99E, E59K/K99E/Q144E, E59K/K99E/Q144K, E59K/K99E/R159E, E59K/K99E/K264E, D18K/E59K/K99E/K264E, DI8K/E59K/K99E/C252S, D18K/E59K/K99E/C252S/K264E, E59K/K99Y/C252S, E59K/K99E/C252S/K264E, E59K/K99E/C252S, N103D/N113D, N103D/N200D, N103D/N281D, N113D/N200D, N113D/N281D, N200D/N281D, N103D/N113D/N200D, N103D/N113D/N281D, N103D/N200D/N281D, N113D/N200D/N281D, N103Q/N113Q, N103Q/N200Q, N103Q/N281Q, N113Q/N200Q, N113Q/N281Q, N200Q/N281Q, N103Q/N113Q/N200Q, N103Q/N113Q/N281Q, N103Q/N200Q/N281Q, N113Q/N200Q/N281Q, N103Q/N113Q/N200Q/N281Q, E59K/K99E/N103Q/C252S/K264E,

E59K/K99E/N113Q/C252S/K264E, E59K/K99E/N200Q/C252S/K264E, E59K/K99E/N281Q/C252S/K264E, E59K/K99E/N103Q/N113Q/C252S/K264E, E59K/K99E/N103Q/N200Q/C252S/K264E, E59K/K99E/N103Q/N281Q/C252S/K264E, E59K/K99E/N113Q/N200Q/C252S/K264E, E59K/K99E/N113Q/N281Q/C252S/K264E, E59K/K99E/N200Q/N281Q/C252S/K264E, E59K/K99E/N103Q/N113Q/N200Q/C252S/K264E, E59K/K99E/N103Q/N200Q/N281Q/C252S/K264E, E59K/K99E/N113Q/N200Q/N281Q/C252S/K264E, and E59K/K99E/N103Q/N113Q/N200Q/N281Q/C252S/K264E; and an IL-12p35 variant comprising one or more substitutions selected from the group consisting of: N21D, Q35D, E38Q, D55Q, D55K, N71D, N71Q, L75A, N76D, E79Q, N85D, N85Q, L89A, F96A, M97A, L124A, M125A, Q130E, Q135E, N136D, E143Q, Q146E, N151D, N151K, E153K, E153Q, K158E, E162Q, E163Q, D165N, I171A, N195D, N195Q, N71D/N85D/N195D, N151D/E153Q, N151D/D165N, Q130E/N151D, N151D/K158E, E79Q/N151D, D55Q/N151D, N136D/N151D, N21D/N151D, E143Q/N151D, N71Q/N85Q, N71Q/N195Q, N85Q/N195Q, N71Q/N85Q/N195Q, N71D/N85D, N71D/N195D, and N85D/N195D.

[00655] In this format, useful embodiments include, but are not limited to, those found in Figure 11.

D. Fc-scIL-12 Format

[00656] In an additional embodiment, the present invention provides the C-terminal single-chain IL-12-Fc fusion or "Fc-scIL-12" format. In this embodiment, as shown in Figures 8E-F, the heterodimeric fusion protein comprises two monomers. The first monomer comprises (from N-to-C-terminus) Fc-optional linker-scIL-12. The second monomer comprises an "empty" Fc, comprising all or part of the hinge-CH2-CH3. As above, the scIL-12 may be "scIL-12(p40/p35)", wherein the IL-12p40 subunit is N-terminally linked (with or without a domain linker) to the IL-12p35 subunit, or "scIL-12(p35/p40)", wherein the IL-12p35 is N-terminally linked (with or without a domain linker) to the IL-12p40 subunit.

[00657] In the Fc-scIL-12 format, a preferred embodiment utilizes the skew variant pair S364K/E357Q : L368D/K370S.

[00658] In some embodiments, the scIL-12-Fc format is a bivalent IL-12-Fc fusion. In some embodiments, the bivalent IL-12-Fc fusion format (Figures 48C-D) comprises two identical monomers each comprising a scIL-12 complex recombinant fused to the C-terminus of a homodimeric Fc chain (optionally via a domain linker).

[00659] In some embodiments, the scIL-12-Fc format provides a heterodimeric Fc fusion protein comprising: a) a first monomer comprising, from N- to C-terminal: i) a first variant Fc domain protein; ii) an optional first domain linker; iii) a IL-12p40 domain protein; iv) an optional second domain linker; v) a IL-12p35 domain protein; and b) a second monomer comprising a second variant Fc domain protein.

[00660] In some embodiments, the scIL-12-Fc format provides a heterodimeric Fc fusion protein comprising: a) a first monomer comprising, from N- to C-terminal: i) a first variant Fc domain protein; ii) an optional first domain linker; iii) a IL-12p35 domain protein; iv) an optional second domain linker; v) a IL-12p40 domain protein; and b) a second monomer comprising a second variant Fc domain protein.

[00661] In some embodiments, the Fc variants comprise one or more skew, pI, and ablation variants as provided herein. In one embodiment, Fc variants comprise particular skew, pI, and ablation variants. In some embodiments, modifications promoting heterodimerization of the first and the second Fc domains are a set of amino acid substitutions selected from the group consisting of L368D/K370S and S364K; L368D/K370S and S364K/E357L; L368D/K370S and S364K/E357Q; T411E/K360E/Q362E and D401K; L368E/K370S and S364K; K370S and S364K/E357Q; T366S/L368A/Y407V and T366W; T366S/L368A/Y407V/Y349C and T366W/S354C, according to EU numbering. In some embodiments, the first and/or the second Fc domains have an additional set of amino acid substitutions comprising Q295E/N384D/Q418E/N421D, according to EU numbering. In some embodiments, the first and/or the second Fc domains have an additional set of amino acid substitutions selected from the group consisting of

G236R/L328R, E233P/L234V/L235A/G236_/S239K, E233P/L234V/L235A/G236_/S239K/A327G, E233P/L234V/L235A/G236_/S267K/A327G, E233P/L234V/L235A/G236_, and E233P/L234V/L235A/G236_/S267K, according to EU numbering. In some embodiments the first and second Fc domains further comprise amino acid substitutions M428L/N424S.

[00662] In this format, useful IL-12p40 protein domains include, but are not limited to,SEQ ID NO: 4 (human IL-12 subunit beta (IL-12p40) mature form sequence). In this format, useful IL-12p40 variants include, but are not limited to, E59K, E59Q, D18N, D18K, E32Q, E33Q, D34N, D34K, Q42E, S43E, S43K, E45Q, Q56E, D62N, E73Q, D87N, K99E, K99Y, E100Q, N103D, N103Q, N113D, N113Q, Q144E, D161N, R159E, K163E, E187Q, N200D, N200Q, N218Q, Q229E, E235Q, C252S, Q256N, K258E, K260E, E262Q, K264E, N281D, N281Q, and E299Q. In this format, useful IL-12p40 variants include, but are not limited to, N103D/N113D/N200D/N281D, Q42E/E45Q, E45Q/Q56E, Q42E/E59Q, Q56E/E59Q, Q42E/E45Q/Q56E, E45Q/Q56E/E59Q, E32Q/E59Q, D34N/E59K, D34N/E59K/K99E, D34K/E59K/K99E, E32Q/D34N/E59K/K99E, E32K/D34N/E59K/K99E, D34N/E59Q, E59Q/E187Q, S43E/E59Q, S43K/E49Q, E59Q/K163E, E59Q/K99E, E59Q/K258E, E59Q/K260E, E59K/K99E, D18K/E59K/K99E, E59K/K99E/K264E, E59K/K99Y, E59Y/K99Y, E59Y/K99E, E45K/E59K/K99E, E59K/K99E/Q144E, E59K/K99E/Q144K, E59K/K99E/R159E, E59K/K99E/K264E, D18K/E59K/K99E/K264E, DI8K/E59K/K99E/C252S, D18K/E59K/K99E/C252S/K264E, E59K/K99Y/C252S, E59K/K99E/C252S/K264E, E59K/K99E/C252S, N103D/N113D, N103D/N200D, N103D/N281D, N113D/N200D, N113D/N281D, N200D/N281D, N103D/N113D/N200D, N103D/N113D/N281D, N103D/N200D/N281D, N113D/N200D/N281D, N103Q/N113Q, N103Q/N200Q, N103Q/N281Q, N113Q/N200Q, N113Q/N281Q, N200Q/N281Q, N103Q/N113Q/N200Q, N103Q/N113Q/N281Q, N103Q/N200Q/N281Q, N113Q/N200Q/N281Q, N103Q/N113Q/N200Q/N281Q, E59K/K99E/N103Q/C252S/K264E, E59K/K99E/N113Q/C252S/K264E, E59K/K99E/N200Q/C252S/K264E, E59K/K99E/N281Q/C252S/K264E, E59K/K99E/N103Q/N113Q/C252S/K264E, E59K/K99E/N103Q/N200Q/C252S/K264E, E59K/K99E/N103Q/N281Q/C252S/K264E, E59K/K99E/N113Q/N200Q/C252S/K264E, E59K/K99E/N113Q/N281Q/C252S/K264E, E59K/K99E/N200Q/N281Q/C252S/K264E,

E59K/K99E/N103Q/N113Q/N200Q/C252S/K264E,
E59K/K99E/N103Q/N200Q/N281Q/C252S/K264E,
E59K/K99E/N113Q/N200Q/N281Q/C252S/K264E, and
E59K/K99E/N103Q/N113Q/N200Q/N281Q/C252S/K264E.

[00663] In this format, IL-12p35 protein domains include SEQ ID NO: 2 (human IL-12 subunit alpha (IL-12p35) mature form sequence). In this format, useful IL-12p35 variants, include, but are not limited to, N21D, Q35D, E38Q, D55Q, D55K, N71D, N71Q, L75A, N76D, E79Q, N85D, N85Q, L89A, F96A, M97A, L124A, M125A, Q130E, Q135E, N136D, E143Q, Q146E, N151D, N151K, E153K, E153Q, K158E, E162Q, E163Q, D165N, I171A, N195D, and N195Q. In this format, useful IL-12p35 variants, include, but are not limited to, N71D/N85D/N195D, N151D/E153Q, N151D/D165N, Q130E/N151D, N151D/K158E, E79Q/N151D, D55Q/N151D, N136D/N151D, N21D/N151D, E143Q/N151D, N71Q/N85Q, N71Q/N195Q, N85Q/N195Q, N71Q/N85Q/N195Q, N71D/N85D, N71D/N195D, and N85D/N195D.

[00664] In this format, useful combinations of a variant IL-12p40 subunit and IL-12p35 subunit include, but are not limited to IL-12p40(E59K/K99Y) and IL-12p35(SEQ ID NO: 2), IL-12p40(D18K/E59K/K99E) and IL-12p35 (SEQ ID NO: 2), IL-12p40(E59K/K99E/K264E) and IL-12p35(SEQ ID NO: 2), IL-12p40(D18K/E59K/K99E/K264E) and IL-12p35(SEQ ID NO: 2), IL-12p40(D18K/E59K/K99E/C252S) and IL-12p35(SEQ ID NO: 2), IL-12p40(D18K/E59K/K99E/C252S/K264E) and IL-12p35(SEQ ID NO: 2), IL-12p40(E59K/K99Y/C252S) and IL-12p35(SEQ ID NO: 2), IL-12p40(E59K/K99E/C252S/K264E) and IL-12p35(SEQ ID NO: 2), and IL-12p40(E59K/K99E/C252S) and IL-12p35(SEQ ID NO: 2).

[00665] In this format, useful combinations of a variant IL-12p40 subunit and a IL-12p35 subunit include, but are not limited to a combination of an IL-12p35 comprising SEQ ID NO: 2 (human IL-12 subunit alpha (IL-12p35) mature form sequence) and a variantIL-12p40 comprising one or more substitutions including, but not limited, to E59K, E59Q, D18N, D18K, E32Q, E33Q, D34N, D34K, Q42E, S43E, S43K, E45Q, Q56E, D62N, E73Q, D87N, K99E, K99Y, E100Q, N103D, N103Q, N113D, N113Q, Q144E, D161N, R159E, K163E, E187Q, N200D, N200Q, N218Q, Q229E, E235Q,

C252S, Q256N, K258E, K260E, E262Q, K264E, N281D, N281Q, E299Q, N103D/N113D/N200D/N281D, Q42E/E45Q, E45Q/Q56E, Q42E/E59Q, Q56E/E59Q, Q42E/E45Q/Q56E, E45Q/Q56E/E59Q, E32Q/E59Q, D34N/E59K, D34N/E59K/K99E, D34K/E59K/K99E, E32Q/D34N/E59K/K99E, E32K/D34N/E59K/K99E, D34N/E59Q, E59Q/E187Q, S43E/E59Q, S43K/E49Q, E59Q/K163E, E59Q/K99E, E59Q/K258E, E59Q/K260E, E59K/K99E, D18K/E59K/K99E, E59K/K99E/K264E, E59K/K99Y, E59Y/K99Y, E59Y/K99E, E45K/E59K/K99E, E59K/K99E/Q144E, E59K/K99E/Q144K, E59K/K99E/R159E, E59K/K99E/K264E, D18K/E59K/K99E/K264E, DI8K/E59K/K99E/C252S, D18K/E59K/K99E/C252S/K264E, E59K/K99Y/C252S, E59K/K99E/C252S/K264E, E59K/K99E/C252S, N103D/N113D, N103D/N200D, N103D/N281D, N113D/N200D, N113D/N281D, N200D/N281D, N103D/N113D/N200D, N103D/N113D/N281D, N103D/N200D/N281D, N113D/N200D/N281D, N103Q/N113Q, N103Q/N200Q, N103Q/N281Q, N113Q/N200Q, N113Q/N281Q, N200Q/N281Q, N103Q/N113Q/N200Q, N103Q/N113Q/N281Q, N103Q/N200Q/N281Q, N113Q/N200Q/N281Q, N103Q/N113Q/N200Q/N281Q, E59K/K99E/N103Q/C252S/K264E, E59K/K99E/N113Q/C252S/K264E, E59K/K99E/N200Q/C252S/K264E, E59K/K99E/N281Q/C252S/K264E, E59K/K99E/N103Q/N113Q/C252S/K264E, E59K/K99E/N103Q/N200Q/C252S/K264E, E59K/K99E/N103Q/N281Q/C252S/K264E, E59K/K99E/N113Q/N200Q/C252S/K264E, E59K/K99E/N113Q/N281Q/C252S/K264E, E59K/K99E/N200Q/N281Q/C252S/K264E, E59K/K99E/N103Q/N113Q/N200Q/C252S/K264E, E59K/K99E/N103Q/N200Q/N281Q/C252S/K264E, E59K/K99E/N113Q/N200Q/N281Q/C252S/K264E, and E59K/K99E/N103Q/N113Q/N200Q/N281Q/C252S/K264E.

[00666] In this format, useful combinations of IL-12p40 and IL-12p35 variants include, but are not limited to a combination of an IL-12p40 comprising SEQ ID NO: 4 (human IL-12 subunit beta (IL-12p40) mature form sequence) and an IL-12p35 comprising N21D, Q35D, E38Q, D55Q, D55K, N71D, N71Q, L75A, N76D, E79Q, N85D, N85Q, L89A, F96A, M97A, L124A, M125A, Q130E, Q135E, N136D, E143Q, Q146E, N151D, N151K, E153K, E153Q, K158E, E162Q, E163Q, D165N, I171A, N195D, N195Q, N71D/N85D/N195D, N151D/E153Q, N151D/D165N,

Q130E/N151D, N151D/K158E, E79Q/N151D, D55Q/N151D, N136D/N151D, N21D/N151D, E143Q/N151D, N71Q/N85Q, N71Q/N195Q, N85Q/N195Q, N71Q/N85Q/N195Q, N71D/N85D, N71D/N195D, and N85D/N195D.

[00667] In this format, useful combinations of IL-12p40 variants and IL-12p35 variants include, but are not limited to, a combination of an IL-12p40 variant comprising one or more amino acid substitution selected from the group consisting of: E59K, E59Q, D18N, D18K, E32Q, E33Q, D34N, D34K, Q42E, S43E, S43K, E45Q, Q56E, D62N, E73Q, D87N, K99E, K99Y, E100Q, N103D, N103Q, N113D, N113Q, Q144E, D161N, R159E, K163E, E187Q, N200D, N200Q, N218Q, Q229E, E235Q, C252S, Q256N, K258E, K260E, E262Q, K264E, N281D, N281Q, E299Q, N103D/N113D/N200D/N281D, Q42E/E45Q, E45Q/Q56E, Q42E/E59Q, Q56E/E59Q, Q42E/E45Q/Q56E, E45Q/Q56E/E59Q, E32Q/E59Q, D34N/E59K, D34N/E59K/K99E, D34K/E59K/K99E, E32Q/D34N/E59K/K99E, E32K/D34N/E59K/K99E, D34N/E59Q, E59Q/E187Q, S43E/E59Q, S43K/E49Q, E59Q/K163E, E59Q/K99E, E59Q/K258E, E59Q/K260E, E59K/K99E, D18K/E59K/K99E, E59K/K99E/K264E, E59K/K99Y, E59Y/K99Y, E59Y/K99E, E45K/E59K/K99E, E59K/K99E/Q144E, E59K/K99E/Q144K, E59K/K99E/R159E, E59K/K99E/K264E, D18K/E59K/K99E/K264E, DI8K/E59K/K99E/C252S, D18K/E59K/K99E/C252S/K264E, E59K/K99Y/C252S, E59K/K99E/C252S/K264E, E59K/K99E/C252S, N103D/N113D, N103D/N200D, N103D/N281D, N113D/N200D, N113D/N281D, N200D/N281D, N103D/N113D/N200D, N103D/N113D/N281D, N103D/N200D/N281D, N113D/N200D/N281D, N103Q/N113Q, N103Q/N200Q, N103Q/N281Q, N113Q/N200Q, N113Q/N281Q, N200Q/N281Q, N103Q/N113Q/N200Q, N103Q/N113Q/N281Q, N103Q/N200Q/N281Q, N113Q/N200Q/N281Q, N103Q/N113Q/N200Q/N281Q, E59K/K99E/N103Q/C252S/K264E, E59K/K99E/N113Q/C252S/K264E, E59K/K99E/N200Q/C252S/K264E, E59K/K99E/N281Q/C252S/K264E, E59K/K99E/N103Q/N113Q/C252S/K264E, E59K/K99E/N103Q/N200Q/C252S/K264E, E59K/K99E/N103Q/N281Q/C252S/K264E, E59K/K99E/N113Q/N200Q/C252S/K264E, E59K/K99E/N113Q/N281Q/C252S/K264E, E59K/K99E/N200Q/N281Q/C252S/K264E, E59K/K99E/N103Q/N113Q/N200Q/C252S/K264E, E59K/K99E/N103Q/N200Q/N281Q/C252S/K264E,

E59K/K99E/N113Q/N200Q/N281Q/C252S/K264E, and E59K/K99E/N103Q/N113Q/N200Q/N281Q/C252S/K264E; and an IL-12p35 variant comprising one or more substitutions selected from the group consisting of: N21D, Q35D, E38Q, D55Q, D55K, N71D, N71Q, L75A, N76D, E79Q, N85D, N85Q, L89A, F96A, M97A, L124A, M125A, Q130E, Q135E, N136D, E143Q, Q146E, N151D, N151K, E153K, E153Q, K158E, E162Q, E163Q, D165N, I171A, N195D, N195Q, N71D/N85D/N195D, N151D/E153Q, N151D/D165N, Q130E/N151D, N151D/K158E, E79Q/N151D, D55Q/N151D, N136D/N151D, N21D/N151D, E143Q/N151D, N71Q/N85Q, N71Q/N195Q, N85Q/N195Q, N71Q/N85Q/N195Q, N71D/N85D, N71D/N195D, and N85D/N195D.

VIII. Useful Embodiments of the Invention

[00668] As will be appreciated by those in the art and discussed more fully below, the heterodimeric fusion proteins of the present invention can take on a wide variety of configurations, as are generally depicted in Figures 8A-F. The amino acid sequences of exemplary fusion proteins are provided in Figures 9-11.

[00669] Many of the embodiments outlined herein rely in general on the format comprising a first monomer (first fusion protein) comprising an IL-12p35 subunit domain covalently attached (optionally via a domain linker) to the N-terminus of a first Fc domain, and a second monomer (second fusion protein) comprising an IL-12p40 subunit domain covalently attached (optionally via a domain linker) to the N-terminus of a second Fc domain. Exemplary embodiments of this format include, but are not limited to XENP27201. In additional embodiments, the first monomer has a polypeptide sequence selected from the group consisting of i) SEQ ID NO:XX (XENP27201 Chain 1), ii) SEQ ID NO:XX (XenD24752), iii) SEQ ID NO:XX (XenD24753), iv) SEQ ID NO:XX (XenD24754), v) SEQ ID NO:XX (XenD24755), vi) SEQ ID NO:XX (XenD24756), vii) SEQ ID NO:XX (XenD24757), viii) SEQ ID NO:XX (XenD24758), ix) SEQ ID NO:XX (XenD24759), x) SEQ ID NO:XX (XenD24760), xi) SEQ ID NO:XX (XenD24761), xii) SEQ ID NO:XX (XenD24762), xiii) SEQ ID NO:XX (XenD24763), xiv) SEQ ID NO:XX (XenD24764), xv) SEQ ID NO:XX (XenD24765), xvi) SEQ ID NO:XX (XenD24766), xvii) SEQ ID NO:XX

(XenD24767), xviii) SEQ ID NO:XX (XenD24768), xix) SEQ ID NO:XX (XenD24769), xx) SEQ ID NO:XX (XenD24770), xxi) SEQ ID NO:XX (XenD24771), xxii) SEQ ID NO:XX (XenD24772), xxiii) SEQ ID NO:XX (XenD24773), xxiv) SEQ ID NO:XX (XenD24774), xxv) SEQ ID NO:XX (XenD24775), xxvi) SEQ ID NO:XX (XenD24776), xxvii) SEQ ID NO:XX (XenD24777), xxviii) SEQ ID NO:XX (XenD24778), xxix) SEQ ID NO:XX (XenD24792), xxx) SEQ ID NO:XX (XenD25922), xxxi) SEQ ID NO:XX (XenD25923), xxxii) SEQ ID NO:XX (XenD25924), xxxiii) SEQ ID NO:XX (XenD25925), xxxiv) SEQ ID NO:XX (XenD25926), xxxv) SEQ ID NO:XX (XenD25927), xxxvi) SEQ ID NO:XX (XenD25928), xxxvii) SEQ ID NO:XX (XenD25929), xxxviii) SEQ ID NO:XX (XenD25930), and xxxix) SEQ ID NO:XX (XenD25931); and the second monomer has a polypeptide sequence selected from the group consisting of i) SEQ ID NO:XX (XENP27201 Chain 2), ii) SEQ ID NO:XX (XenD24779), iii) SEQ ID NO:XX (XenD24780), iv) SEQ ID NO:XX (XenD24781), v) SEQ ID NO:XX (XenD24782), vi) SEQ ID NO:XX (XenD24783), vii) SEQ ID NO:XX (XenD24784), viii) SEQ ID NO:XX (XenD24785), ix) SEQ ID NO:XX (XenD24786), x) SEQ ID NO:XX (XenD24787), xi) SEQ ID NO:XX (XenD24788), xii) SEQ ID NO:XX (XenD24789), xiii) SEQ ID NO:XX (XenD24790), xiv) SEQ ID NO:XX (XenD24791), xv) SEQ ID NO:XX (XenD25911), xvi) SEQ ID NO:XX (XenD25912), xvii) SEQ ID NO:XX (XenD25913), xviii) SEQ ID NO:XX (XenD25914), xix) SEQ ID NO:XX (XenD25915), xx) SEQ ID NO:XX (XenD25916), xxi) SEQ ID NO:XX (XenD25917), xxii) SEQ ID NO:XX (XenD25918), xxiii) SEQ ID NO:XX (XenD25919), xxiv) SEQ ID NO:XX (XenD25920), and xxv) SEQ ID NO:XX (XenD25921). Particular such embodiments include, but are not limited to, XENP27201, XENP28820, XENP28821, XENP28822, XENP28823, XENP28824, XENP28825, XENP28826, XENP28827, XENP28828, XENP28829, XENP28830, XENP28831, XENP28832, XENP28833, XENP28834, XENP28835, XENP28836, XENP28837, XENP28838, XENP28839, XENP28840, XENP28841, XENP28842, XENP28843, XENP28844, XENP28845, XENP28846, XENP28847, XENP28848, XENP28849, XENP28850, XENP28851, XENP28852, XENP29949, XENP29950, XENP29951, XENP29952, XENP30597, XENP30598, XENP30599, XENP30600, XENP30601, XENP30602, XENP30603, XENP30604, XENP30605, XENP30606. XENP30307, XENP30308, XENP30609, XENP31250, XENP31251, XENP31252, XENP31253, XENP31254, XENP31255, XENP31256, XENP31257, XENP31258, XENP31259, XENP31260, XENP31261, XENP31262, XENP31263,

XENP31264, XENP31265, XENP31286, XENP31142, XENP31143, XENP31144, XENP31145, XENP31146, XENP31582, XENP31583, XENP31584, XENP32187, XENP32188, XENP32189, XENP32190, XENP32191, XENP32991, XENP32992, XENP32993, XENP32994, XENP32995, XENP32996, XENP32997, XENP32998, XENP32999, XENP33000, XENP33001, XENP33002, XENP33003, XENP33004, XENP33005, XENP33006, XENP33007, XENP33008, XENP33008, XENP33009, XENP33010, and XENP33011. A useful embodiment of a heterodimer Fc fusion protein comprises a first monomer (first fusion protein) comprising an IL-12p35 subunit domain covalently attached (optionally via a domain linker) to the C-terminus of a first Fc domain, and a second monomer (second fusion protein) comprising an IL-12p40 subunit domain covalently attached (optionally via a domain linker) to the C-terminus of a second Fc domain. Exemplary embodiments of this format include, but are not limited to XENP27202.

[00670] Another useful embodiment of a heterodimer Fc fusion protein comprises a first monomer (first fusion protein) comprising a single-chain IL-12 complex ("scIL-12") covalently attached (optionally via a domain linker) to the N-terminus of a first Fc domain, and a second Fc domain (e.g., an empty Fc domain). The scIL-12 may be "scIL-12(p40/p35)", wherein the IL-12p40 subunit is N-terminally linked (with or without a domain linker) to the IL-12p35 subunit, or "scIL-12(p35/p40)", wherein the IL-12p35 is N-terminally linked (with or without a domain linker) to the IL-12p40 subunit. Exemplary embodiments of this format includes, but is not limited to, XENP27203 and XENP27204.

[00671] A further useful embodiment of a heterodimer Fc fusion protein comprises a first monomer (first fusion protein) comprising a single-chain IL-12 complex ("scIL-12") covalently attached (optionally via a domain linker) to the C-terminus of a first Fc domain, and a second Fc domain (e.g., an empty Fc domain). The scIL-12 may be "scIL-12(p40/p35)", wherein the IL-12p40 subunit is N-terminally linked (with or without a domain linker) to the IL-12p35 subunit, or "scIL-12(p35/p40)", wherein the IL-12p35 is N-terminally linked (with or without a domain linker) to the IL-12p40 subunit.

[00672] For any of the heterodimer Fc fusion proteins outlined herein, the optional domain linkers used on the first monomer, on the second monomer, and/or in the scIL-12 can be the same or different. In addition, the first Fc domain and the second Fc domain of the heterodimeric protein can have different amino acid sequences.

[00673] The Fc domains of the present invention comprise IgG Fc domains, e.g., IgG1 Fc domains. In some embodiments, the first and second Fc domains comprising a set of amino acid substitutions selected from the group consisting of: L368D/K370S and S364K; L368D/K370S and S364K/E357L; L368D/K370S and S364K/E357Q; T411E/K360E/Q362E and D401K; L368E/K370S and S364K; K370S and S364K/E357Q and T366S/L368A/Y407V : T366W (optionally including a bridging disulfide, T366S/L368A/Y407V/Y349C : T366W/S354C), according to EU numbering. In some instances, the first and/or the second Fc domains of any of the heterodimeric Fc fusion formats outlined herein can have an additional set of amino acid substitutions comprising Q295E/N384D/Q418E/N421D, according to EU numbering. In some embodiments, the first and/or the second Fc domains have an additional set of amino acid substitutions selected from the group consisting of G236R/L328R, E233P/L234V/L235A/G236_/S239K, E233P/L234V/L235A/G236_/S239K/A327G, E233P/L234V/L235A/G236_/S267K/A327G, E233P/L234V/L235A/G236_, and E233P/L234V/L235A/G236_/S267K, according to EU numbering.

[00674] Additional heterodimerization or homodimerization variants can be independently and optionally included and selected from variants outlined in the figures. These compositions can further comprise ablation variants, pI variants, charged variants, isotypic variants, etc.

A. IL-12p40 variants

[00675] In some embodiments, the IL-12p40 subunit is a variant IL-12p40 subunit. In some particular such embodiments, the IL-12p40 subunit is a variant IL-12p40 subunit having reduced heterogeneity. In other particular such embodiments, the IL-12p40 subunit is a variant IL-12p40 subunit having altered, that is either reduced or increased, affinity for IL-12 receptor subunit beta-1 (IL-12Rß1), IL-12 receptor subunit beta-2 (IL-12Rß2), and/or IL-12 receptor

complex. In some embodiments, the variant IL-12p40 subunit has one or more amino acid modifications at amino acid residues selected from the group consisting of E3, D7, E12, D14, W15, P17, D18, A19, P20, G21, E22, M23, D29, E32, E33, D34, L40, D41, Q42, S43, E45, L47, T54, I55, Q56, K58, E59, F60, G61, D62, Q65, Y66, E73, K84, E86, D87, G88, I89, W90, D93, D97, K99, E100, K102, N103, K104, F106, E110, N113, Y114, D129, D142, Q144, E156, R159, D161, N162, K163, D166, D170, Q172, D174, A176, C177, P178, A179, A180, E181, S183, P185, E187, N200, S204, F206, R208, D209, D214, N218, Q220, N226, Q229, E231, E235, T242, P243, S245, Y246, F247, S248, C252, Q256, K258, K260, E262, K264, D265, D270, N281, Q289, D290, R291, Y292, Y293, and E299. (numbered according to the human IL-12 subunit beta (IL-12p40) mature form sequence).

[00676] In some embodiments, the variant IL-12p40 subunit has one or more amino acid substitutions selected from the group consisting of D18N, D18K, E32Q, E33Q, D34N, D34K, Q42E, S43E, S43K, E45Q, Q56E, E59Q, E59K, D62N, E73Q, D87N, K99E, K99Y, E100Q, N103D, N103Q, N113D, N113Q, Q144E, D161N, R159E, K163E, E187Q, N200D, N200Q, N218Q, Q229E, E235Q, C252S, Q256N, K258E, K260E, E262Q, K264E, N281D, N281Q, and E299Q. In some embodiments, the variant IL-12p40 subunit has amino acid substitutions selected from the group consisting of N103D/N113D/N200D/N281D, Q42E/E45Q, E45Q/Q56E, Q42E/E59Q, Q56E/E59Q, Q42E/E45Q/Q56E, E45Q/Q56E/E59Q, E32Q/E59Q, D34N/E59K, D34N/E59K/K99E, D34K/E59K/K99E, E32Q/D34N/E59K/K99E, E32K/D34N/E59K/K99E, D34N/E59Q, E59Q/E187Q, S43E/E59Q, S43K/E49Q, E59Q/K163E, E59Q/K99E, E59Q/K258E, E59Q/K260E, E59K/K99E, D18K/E59K/K99E, E59K/K99E/K264E, E59K/K99Y, E59Y/K99Y, E59Y/K99E, E45K/E59K/K99E, E59K/K99E/Q144E, E59K/K99E/Q144K, E59K/K99E/R159E, E59K/K99E/K264E, D18K/E59K/K99E/K264E, DI8K/E59K/K99E/C252S, D18K/E59K/K99E/C252S/K264E, E59K/K99Y/C252S, E59K/K99E/C252S/K264E, E59K/K99E/C252S, N103D/N113D, N103D/N200D, N103D/N281D, N113D/N200D, N113D/N281D, N200D/N281D, N103D/N113D/N200D, N103D/N113D/N281D, N103D/N200D/N281D, N113D/N200D/N281D, N103Q/N113Q, N103Q/N200Q, N103Q/N281Q, N113Q/N200Q, N113Q/N281Q, N200Q/N281Q, N103Q/N113Q/N200Q, N103Q/N113Q/N281Q, N103Q/N200Q/N281Q, N113Q/N200Q/N281Q, N103Q/N113Q/N200Q/N281Q, E59K/K99E/N103Q/C252S/K264E,

E59K/K99E/N113Q/C252S/K264E, E59K/K99E/N200Q/C252S/K264E, E59K/K99E/N281Q/C252S/K264E, E59K/K99E/N103Q/N113Q/C252S/K264E, E59K/K99E/N103Q/N200Q/C252S/K264E, E59K/K99E/N103Q/N281Q/C252S/K264E, E59K/K99E/N113Q/N200Q/C252S/K264E, E59K/K99E/N113Q/N281Q/C252S/K264E, E59K/K99E/N200Q/N281Q/C252S/K264E, E59K/K99E/N103Q/N113Q/N200Q/C252S/K264E, E59K/K99E/N103Q/N200Q/N281Q/C252S/K264E, E59K/K99E/N113Q/N200Q/N281Q/C252S/K264E, and E59K/K99E/N103Q/N113Q/N200Q/N281Q/C252S/K264E.

[00677] In some embodiments, the IL-12p40 variant has a polypeptide sequence selected from the group consisting of: i) SEQ ID NO:57 (IL-12p40(N103D)), ii) SEQ ID NO:58 (IL-12p40(N113D)), iii) SEQ ID NO:59 (IL-12p40(N200D)), iv) SEQ ID NO:60 (IL-12p40(N281D)), v) SEQ ID NO:61 (IL-12p40(N103D/N113D/N200D/N281D)), vi) SEQ ID NO:62 (IL-12p40(Q42E)), vii) SEQ ID NO:63 (IL-12p40(E45Q)), viii) SEQ ID NO:64 (IL-12p40(Q56E)), ix) SEQ ID NO:65 (IL-12p40(E59Q)), x) SEQ ID NO:66 (IL-12p40(D62N)), xi) SEQ ID NO:67 (IL-12p40(Q42E/E45Q)), xii) SEQ ID NO:68 (IL-12p40(E45Q/Q56E)), xiii) SEQ ID NO:69 (IL-12p40(Q42E/E59Q)), xiv) SEQ ID NO:70 (IL-12p40(Q56E/E59Q)), xv) SEQ ID NO:71 (IL-12p40(Q42E/E45Q/Q56E)), xvi) SEQ ID NO:72 (IL-12p40(E45Q/Q56E/E59Q)), xvii) SEQ ID NO:73 (IL-12p40(D161N)), xviii) SEQ ID NO:74 (IL-12p40(E73Q)), xix) SEQ ID NO:75 (IL-12p40(Q144E)), xx) SEQ ID NO:76 (IL-12p40(E262Q)), xxi) SEQ ID NO:77 (IL-12p40(E100Q)), xxii) SEQ ID NO:78 (IL-12p40(D18N)), xxiii) SEQ ID NO:79 (IL-12p40(E33Q)), xxiv) SEQ ID NO:80 (IL-12p40(Q229E)), xxv) SEQ ID NO:81 (IL-12p40(E235Q)), xxvi) SEQ ID NO:82 (IL-12p40(Q256N)), xxvii) SEQ ID NO:83 (IL-12p40(E299Q)), xxviii) SEQ ID NO:84 (IL-12p40(D87N)), xxix) IL-12p40(E32Q), xxx) IL-12p40(D34N), xxxi) IL-12p40(S43E), xxxii) IL-12p40(S43K), xxxiii) SEQ ID NO:XXX (IL-12p40 (E59K/K99E/N103Q/N113Q/N200Q/N281Q/C252S/K264E)), xxxiv) SEQ ID NO:205 (IL-12p40(E59K)), xxxv) IL-12p40(K99E), xxxvi) IL-12p40(K163E), xxxvii) IL-12p40(E187Q), xxxviii) IL-12p40(K258E), xxxix) IL-12p40(K260E), xl) SEQ ID NO:206 (IL-12p40(E32Q/E59Q)), xli) SEQ ID NO:207 (IL-12p40(D34N/E59Q)), xlii) SEQ ID NO:208 (IL-12p40(E59Q/E187Q)),

xliii) SEQ ID NO:209 (IL-12p40(S43E/E59Q)), xliv) SEQ ID NO:210 (IL-12p40(S43K/E49Q)), xlv) SEQ ID NO:211 (IL-12p40(E59Q/K163E)), xlvi) SEQ ID NO:212 (IL-12p40(E59Q/K99E)), xlvii) SEQ ID NO:213 (IL-12p40(E59Q/K258E)), xlviii) SEQ ID NO:214 (IL-12p40(E59Q/K260E)), xlix) SEQ ID NO: XXX (IL-12p40 (D34N/E59K)), l) SEQ ID NO: XXX (IL-12p40(E59K/K99E)), li) SEQ ID NO: XXX (IL-12p40(D18K/E59K/K99E)), lii) SEQ ID NO: XXX (IL-12p40 (E59K/K99E/K264E)), liii) SEQ ID NO: XXX (IL-12p40 (E59K/K99Y)), liv) SEQ ID NO: XXX (IL-12p40 (E59Y/K99E)), lv) SEQ ID NO: XXX (IL-12p40 (E45K/E59K/K99E)), lvi) SEQ ID NO: XXX (IL-12p40 (E59K/K99E/Q144E)), lvii) SEQ ID NO: XXX (IL-12p40 (E59K/K99E/Q144K)), lviii) SEQ ID NO: XXX (IL-12p40 (E59K/K99E/R159E)), lix) SEQ ID NO: XXX (IL-12p40 (E59K/K99E/K264E)), lx) SEQ ID NO: XXX (IL-12p40 (D18K/E59K/K99E/K264E)), lxi) SEQ ID NO: XXX (IL-12p40 (C252S)), lxii) SEQ ID NO: XXX (IL-12p40 (DI8K/E59K/K99E/C252S)), lxiii) SEQ ID NO: XXX (IL-12p40 (D18K/E59K/K99E/C252S/K264E)), lxiv) SEQ ID NO: XXX (IL-12p40 (E59K/K99Y/C252S)), lxv) SEQ ID NO: XXX (IL-12p40 (E59K/K99E/C252S/K264E)), lxvi) SEQ ID NO: XXX (IL-12p40 (E59K/K99E/C252S)), lxvii) SEQ ID NO: XXX (IL-12p40 (N103D/N113D)), lxviii) SEQ ID NO: XXX (IL-12p40 (N103D/N200D)), lxix) SEQ ID NO: XXX (IL-12p40 (N103D/N281D)), lxx) SEQ ID NO: XXX (IL-12p40 (N113D/N200D)), lxxi) SEQ ID NO: XXX (IL-12p40 (N113D/N281D)), lxxii) SEQ ID NO: XXX (IL-12p40 (N200D/N281D)), lxxiii) SEQ ID NO: XXX (IL-12p50 (N103D/N113D/N200D)), lxxiv) SEQ ID NO: XXX (IL-12p40 (N103D/N113D/N281D)), lxxv) SEQ ID NO: XXX (IL-12p40 (N103D/N200D/N281D)), lxxvi) SEQ ID NO: XXX (IL-12p40 (N113D/N200D/N281D)), lxxvii) SEQ ID NO: XXX (IL-12p40 (N103Q)), lxxviii) SEQ ID NO: XXX (IL-12p40 (N113Q)), lxxix) SEQ ID NO: XXX (IL-12p40 (N200Q)), lxxx) SEQ ID NO: XXX (IL-12p40 (N281Q)), lxxxi) SEQ ID NO: XXX (IL-12p40 (N103Q/N113Q)), lxxxii) SEQ ID NO: XXX (IL-12p40 (N103Q/N200Q)), lxxxiii) SEQ ID NO: XXX (IL-12p40 (N103Q/N281Q)), lxxxiv) SEQ ID NO: XXX (IL-12p40 (N113Q/N200Q)), lxxxv) SEQ ID NO: XXX (IL-12p40 (N113Q/N281Q)), lxxxvi) SEQ ID NO: XXX (IL-12p40 (N200Q/N281Q)), lxxxvii) SEQ ID NO: XXX (IL-12p40 (N103Q/N113Q/N200Q)), lxxxviii) SEQ ID NO: XXX (IL-12p40 (N103Q/N113Q/N281Q)), lxxxix) SEQ ID NO: XXX (IL-12p40 (N103Q/N200Q/N281Q)), xc) SEQ ID NO: XXX (IL-12p40 (N113Q/N200Q/N281Q)), xci) SEQ ID NO:XXX (IL-12p40 (N103Q/N113Q/N200Q/N281Q)), xcii) SEQ ID NO:XXX (IL-12p40 (D34N/E59K/K99E)), xciii)

SEQ ID NO:XXX (IL-12p40 (D34K/E59K/K99E)), xciv) SEQ ID NO:XXX (IL-12p40 (E32Q/D34N/E59K/K99E)), xcv) SEQ ID NO:XXX (IL-12p40 (E32K/D34N/E59K/K99E)), xcvi) SEQ ID NO: XXX (IL-12p40 (E59Y/K99Y)), xcvii) SEQ ID NO:XXX (IL-12p40 (E59K/K99E/N103Q/C252S/K264E)), xcviii) SEQ ID NO:XXX (IL-12p40 (E59K/K99E/N113Q/C252S/K264E)), xcix) SEQ ID NO:XXX (IL-12p40 (E59K/K99E/N200Q/C252S/K264E)), c) SEQ ID NO:XXX (IL-12p40 (E59K/K99E/N281Q/C252S/K264E)), ci) SEQ ID NO:XXX (IL-12p40 (E59K/K99E/N103Q/N113Q/C252S/K264E)), cii) SEQ ID NO:XXX (IL-12p40 (E59K/K99E/N103Q/N200Q/C252S/K264E)), ciii) SEQ ID NO:XXX (IL-12p40 (E59K/K99E/N103Q/N281Q/C252S/K264E)), civ) SEQ ID NO:XXX (IL-12p40 (E59K/K99E/N113Q/N200Q/C252S/K264E)), cv) SEQ ID NO:XXX (IL-12p40 (E59K/K99E/N113Q/N281Q/C252S/K264E)), cvi) SEQ ID NO:XXX (IL-12p40 (E59K/K99E/N200Q/N281Q/C252S/K264E)), cvii) SEQ ID NO:XXX (IL-12p40 (E59K/K99E/N103Q/N113Q/N200Q/C252S/K264E)), cviii) SEQ ID NO:XXX (IL-12p40 (E59K/K99E/N103Q/N200Q/N281Q/C252S/K264E)), and cix) SEQ ID NO:XXX (IL-12p40 (E59K/K99E/N113Q/N200Q/N281Q/C252S/K264E)).

B. IL-12p35 variants

[00678] In some embodiments, the IL-12p35 subunit is a variant IL-12p35 subunit. In some particular such embodiments, the IL-12p35 subunit is a variant IL-12p35 subunit having reduced heterogeneity. In other particular such embodiments, the IL-12p35 subunit is a variant IL-12p35 subunit having altered, that is either reduced or increased, affinity for IL-12 receptor subunit beta-1 (IL-12Rß1), IL-12 receptor subunit beta-2 (IL-12Rß2), and/or IL-12 receptor complex. In some embodiments, the variant IL-12p35 subunit has one or more amino acid modifications at amino acid residues selected from the group consisting of: Q20, N21, Q35, E38, S44, E45, E46, H49, K54, D55, T59, V60, E61, C63, L64, P65, E67, L68, N71, S73, C74, L75, N76, E79, N85, L89, F96, M97, L124, M125, Q130, Q135, N136, E143, Q146, N151, E153, K158, E162, E163, D165, I171, R181, I182, R183, V185, T186, D188, R189, V190, S192, Y193, N195, and A196. (numbered according to the Human IL-12 subunit alpha (IL-12p35) mature form sequence). In some embodiments, the variant

IL-12p35 subunit has one or more amino acid substitutions selected from the group consisting of: N21D, Q35D, E38Q, D55Q, D55K, N71D, N71Q, L75A, N76D, E79Q, N85D, N85Q, L89A, F96A, M97A, L124A, M125A, Q130E, Q135E, N136D, E143Q, Q146E, N151D, N151K, E153K, E153Q, K158E, E162Q, E163Q, D165N, I171A, N195D, and N195Q.In some embodiments, the variant IL-12p35 subunit has amino acid substitutions N71D/N85D/N195D, N151D/E153Q, N151D/D165N, Q130E/N151D, N151D/K158E, E79Q/N151D, D55Q/N151D, N136D/N151D, N21D/N151D, E143Q/N151D, N71Q/N85Q, N71Q/N195Q, N85Q/N195Q, N71Q/N85Q/N195Q, N71D/N85D, N71D/N195D, and N85D/N195D.. In some embodiments, the variant IL-12p35 subunit has a polypeptide sequence selected from the group consisting of: i) SEQ ID NO:113 (IL-12p35(N71D)), ii) SEQ ID NO:114 (IL-12p35(N85D)), iii) SEQ ID NO:115 (IL-12p35(N195D)), iv) SEQ ID NO:116 (IL-12p35(N71D/N85D/N195D)), v) SEQ ID NO:117 (IL-12p35(E153Q)), vi) SEQ ID NO:118 (IL-12p35(E38Q)), vii) SEQ ID NO:119 (IL-12p35(N151D)), viii) SEQ ID NO:120 (IL-12p35(Q135E)), ix) SEQ ID NO:121 (IL-12p35(Q35D)), x) SEQ ID NO:122 (IL-12p35(Q146E)), xi) SEQ ID NO:123 (IL-12p35(N76D)), xii) SEQ ID NO:124 (IL-12p35(E162Q)), xiii) SEQ ID NO:125 (IL-12p35(E163Q)), xiv) IL-12p35(N21D), xv) SEQ ID NO:XXX IL-12p35(D55Q), xvi) IL-12p35(E79Q), xvii) IL-12p35(Q130E), xviii) IL-12p35(N136D), xix) IL-12p35(E143Q), xx) SEQ ID NO:227 (IL-12p35(N151K)), xxi) SEQ ID NO:226 (IL-12p35(E153K)), xxii) IL-12p35(K158E), xxiii) IL-12p35(D165N), xxiv) SEQ ID NO:225 (IL-12p35(N151D/E153Q)), xxv) SEQ ID NO:228 (IL-12p35(N151D/D165N)), xxvi) SEQ ID NO:229 (IL-12p35(Q130E/N151D)), xxvii) SEQ ID NO:230 (IL-12p35(N151D/K158E)), xxviii) SEQ ID NO:231 (IL-12p35(E79Q/N151D)), xxix) SEQ ID NO:232 (IL-12p35(D55Q/N151D)), xxx) SEQ ID NO:233 (IL-12p35(N136D/N151D)), xxxi) SEQ ID NO:234 (IL-12p35(N21D/N151D)), xxxii) SEQ ID NO:235 (IL-12p35(E143Q/N151D)),xxxiii) SEQ ID NO: XXX (IL-12p35(F96A)),xxxiv) SEQ ID NO: XXX (IL-12p35(M97A)), xxxv) SEQ ID NO: XXX (IL-12p35(L89A)),xxxvi) SEQ ID NO: XXX (IL-12p35(L124A)),xxxvii) SEQ ID NO: XXX (IL-12p35(M125A)),xxxviii) SEQ ID NO: XXX (IL-12p35(L75A)), xxxiv) SEQ ID NO: XXX (IL-12p35(I171A)),xxxv) SEQ ID NO: XXX (IL-12p35 (N71Q)), xxxvi) SEQ ID NO: XXX (IL-12p35 (N85Q)), xxxvii) SEQ ID NO: XXX (IL-12p35 (N195Q)), xxxviii) SEQ ID NO: XXX (IL-12p35 (N71Q/N85Q)),xxxix) SEQ ID NO: XXX (IL-12p35 (N71Q/N195Q)),lx) SEQ ID NO: XXX (IL-12p35 (N85Q/N195Q),lxi) SEQ ID NO: XXX (IL-12p35 (N71Q/N85Q/N195Q)),lxii) SEQ ID NO: XXX (IL-

12p35 (N71D/N85D)), lxiii) SEQ ID NO: XXX (IL-12p35 (N71D/N195D),lxiv) SEQ ID NO: XXX (IL-12p35 (N85D/N195D)), lxv) SEQ ID NO: XXX (IL-12p35 (D55Q)), and lxvi) SEQ ID NO: XXX (IL-12p35 (D55K)).

C. Additional options

[00679] In some embodiments, the scIL-12-Fc format is a bivalent IL-12-Fc fusion. In some embodiments, the bivalent IL-12-Fc fusion format (Figures 48A-B) comprises two identical monomers each comprising a scIL-12 complex recombinant fused to the N-terminus of a homodimeric Fc chain (optionally via a domain linker). In some embodiments, the bivalent IL-12-Fc fusion format (Figures 48C-D) comprises two identical monomers each comprising a scIL-12 complex recombinant fused to the C-terminus of a homodimeric Fc chain (optionally via a domain linker). Examples include but are not limited to XENP31289 and XENP31291 (Figure 49).

[00680] In some embodiments, the IL-12 Fc fusion proteins are further engineered for extending half-life by substitutions comprising M428L and N434S. In some embodiments, the IL-12 Fc fusion proteins are further engineered for extending half-life by substitutions comprising M428L/N434S. Any of the IL-12 Fc fusions listed in herein may be engineered for extending half-life. Examples include but are not limited to XENP31582, XENP31583, and XENP31584 (Figure 65).

[00681] In some embodiments, examples of heterodimeric Fc fusion proteins include XENP27201, XENP28820, XENP28821, XENP28822, XENP28823, XENP28824, XENP28825, XENP28826, XENP28827, XENP28828, XENP28829, XENP28830, XENP28831, XENP28832, XENP28833, XENP28834, XENP28835, XENP28836, XENP28837, XENP28838, XENP28839, XENP28840, XENP28841, XENP28842, XENP28843, XENP28844, XENP28845, XENP28846, XENP28847, XENP28848, XENP28849, XENP28850, XENP28851, XENP28852, XENP29949, XENP29950, XENP29951, XENP29952, XENP30597, XENP30598, XENP30599, XENP30600, XENP30601, XENP30602, XENP30603, XENP30604, XENP30605, XENP30606. XENP30307, XENP30308, XENP30609, XENP31250, XENP31251, XENP31252, XENP31253, XENP31254,

XENP31255, XENP31256, XENP31257, XENP31258, XENP31259, XENP31260, XENP31261, XENP31262, XENP31263, XENP31264, XENP31265, XENP31286, XENP31142, XENP31143, XENP31144, XENP31145, XENP31146, XENP31582, XENP31583, XENP31584, XENP32186, XENP32187, XENP32188, XENP32189, XENP32190, XENP32191, XENP32991, XENP32992, XENP32993, XENP32994, XENP32995, XENP32996, XENP32997, XENP32998, XENP32999, XENP33000, XENP33001, XENP33002, XENP33003, XENP33004, XENP33005, XENP33006, XENP33007, XENP33008, XENP33008, XENP33009, XENP33010, and XENP33011.

[00682] In some embodiments, examples of the heterodimeric Fc fusion protein comprise a first fusion protein having a polypeptide sequence selected from the group consisting of: i) SEQ ID NO:47 (XENP27201 Chain 1), ii) SEQ ID NO:85 (XenD24752), iii) SEQ ID NO:86 (XenD24753), iv) SEQ ID NO:87 (XenD24754), v) SEQ ID NO:88 (XenD24755), vi) SEQ ID NO:89 (XenD24756), vii) SEQ ID NO:90 (XenD24757), viii) SEQ ID NO:91 (XenD24758), ix) SEQ ID NO:92 (XenD24759), x) SEQ ID NO:93 (XenD24760), xi) SEQ ID NO:94 (XenD24761), xii) SEQ ID NO:95 (XenD24762), xiii) SEQ ID NO:96 (XenD24763), xiv) SEQ ID NO:97 (XenD24764), xv) SEQ ID NO:98 (XenD24765), xvi) SEQ ID NO:99 (XenD24766), xvii) SEQ ID NO:100 (XenD24767), xviii) SEQ ID NO:101 (XenD24768), xix) SEQ ID NO:102 (XenD24769), xx) SEQ ID NO:103 (XenD24770), xxi) SEQ ID NO:104 (XenD24771), xxii) SEQ ID NO:105 (XenD24772), xxiii) SEQ ID NO:106 (XenD24773), xxiv) SEQ ID NO:107 (XenD24774), xxv) SEQ ID NO:108 (XenD24775), xxvi) SEQ ID NO:109 (XenD24776), xxvii) SEQ ID NO:110 (XenD24777), xxviii) SEQ ID NO:111 (XenD24778), xxix) SEQ ID NO:112 (XenD24792), xxx) SEQ ID NO:215 (XenD25922), xxxi) SEQ ID NO:216 (XenD25923), xxxii) SEQ ID NO:217 (XenD25924), xxxiii) SEQ ID NO:218 (XenD25925), xxxiv) SEQ ID NO:219 (XenD25926), xxxv) SEQ ID NO:220(XenD25927), xxxvi) SEQ ID NO:221 (XenD25928), xxxvii) SEQ ID NO:222 (XenD25929), xxxviii) SEQ ID NO:223 (XenD25930), xxxix) SEQ ID NO:224 (XenD25931), xl) SEQ ID NO:XXX (XenD26411),xli) SEQ ID NO:XXX (XenD26412), xlii) SEQ ID NO:XXX (XenD26413),xliii) SEQ ID NO:XXX (XenD26414), xliv) SEQ ID NO:XXX (XenD26415), xlv) SEQ ID NO:XXX (XenD26416), xlvi) SEQ ID NO:XXX (XenD26417),xlvii) SEQ ID NO:XXX (XenD26418), xlviii) SEQ ID NO:XXX (XenD27070),xlix) SEQ ID NO:XXX (XenD27071),l) SEQ ID NO:XXX (XenD27072),li) SEQ ID NO:XXX

(XenD27073), lii) SEQ ID NO:XXX (XenD27074),liii) SEQ ID NO:XXX (XenD27075),liv) SEQ ID NO:XXX (XenD27076),lv) SEQ ID NO:XXX (XenD27077)lvi) SEQ ID NO:XXX (XenD27078),lvii) SEQ ID NO:XXX (XenD28173),lviii) SEQ ID NO:XXX (XenD24876),lix) SEQ ID NO:XXX (XenD27162),lx) SEQ ID NO:XXX (XenD27163),lxi) SEQ ID NO:XXX (XenD27164),lxii) SEQ ID NO:XXX (XenD27165)lxiii) SEQ ID NO:XXX (XENP31582 Chain 1),lxiv) SEQ ID NO:XXX (XENP31583 Chain 1),lxv) SEQ ID NO:XXX (XENP31584 Chain 1),lxvi) SEQ ID NO:XXX (XENP32187 Chain 1), lxvii) SEQ ID NO:XXX (XENP32188 Chain 1),lxviii) SEQ ID NO:XXX (XENP32189, Chain 1), lxix) SEQ ID NO:XXX (XENP32190 Chain 1), lxx) SEQ ID NO:XXX (XENP32191 Chain 1),lxxi) SEQ ID NO:XXX (XENP32991 Chain 1),lxxii) SEQ ID NO:XXX (XENP32992 Chain 1),lxxiii) SEQ ID NO:XXX (XENP32993 Chain 1),lxxiv) SEQ ID NO:XXX (XENP32994 Chain 1),lxxv) SEQ ID NO:XXX (XENP32995 Chain 1),lxxvi) SEQ ID NO:XXX (XENP32996 Chain 1), lxxvi) SEQ ID NO:XXX (XENP32997 Chain 1), lxxvii) SEQ ID NO:XXX (XENP32998 Chain 1),lxxvii) SEQ ID NO:XXX (XENP32999 Chain 1),lxxviii) SEQ ID NO:XXX (XENP33000 Chain 1), lxxix) SEQ ID NO:XXX (XENP33001 Chain 1), lxxx) SEQ ID NO:XXX (XENP33002 Chain 1), lxxxi) SEQ ID NO:XXX (XENP33003 Chain 1), lxxxii) SEQ ID NO:XXX (XENP33004 Chain 1), lxxxiii) SEQ ID NO:XXX (XENP33005 Chain 1),lxxxiv) SEQ ID NO:XXX (XENP33006 Chain 1), lxxxv) SEQ ID NO:XXX (XENP33007 Chain 1), lxxxvi) SEQ ID NO:XXX (XENP33008 Chain 1), lxxxvii) SEQ ID NO:XXX (XENP33008 Chain 1),lxxxviii) SEQ ID NO:XXX (XENP33009 Chain 1),lxxxix) SEQ ID NO:XXX (XENP33010 Chain 1), and xc) SEQ ID NO:XXX (XENP33011 Chain 1); and said second fusion protein having a polypeptide sequence selected from the group consisting of: i) SEQ ID NO:48 (XENP27201 Chain 2),ii) SEQ ID NO:126 (XenD24779), iii) SEQ ID NO:127 (XenD24780), iv) SEQ ID NO:128 (XenD24781), v) SEQ ID NO:129 (XenD24782), vi) SEQ ID NO:130 (XenD24783), vii) SEQ ID NO:131 (XenD24784), viii) SEQ ID NO:132 (XenD24785), ix) SEQ ID NO:133 (XenD24786), x) SEQ ID NO:134 (XenD24787), xi) SEQ ID NO:135 (XenD24788), xii) SEQ ID NO:136 (XenD24789), xiii) SEQ ID NO:137 (XenD24790), xiv) SEQ ID NO:138 (XenD24791), xv) SEQ ID NO:236 (XenD25911), xvi) SEQ ID NO:237 (XenD25912), xvii) SEQ ID NO:238 (XenD25913), xviii) SEQ ID NO:239 (XenD25914), xix) SEQ ID NO:240 (XenD25915), xx) SEQ ID NO:241 (XenD25916), xxi) SEQ ID NO:242 (XenD25917), xxii) SEQ ID NO:243 (XenD25918), xxiii) SEQ ID NO:244 (XenD25919), xxiv) SEQ

ID NO:245 (XenD25920), xxv) SEQ ID NO:246 (XenD25921),xxvi) SEQ ID NO:XXX (XenD26427), xxvii) SEQ ID NO:XXX (XenD26428),xxviii) SEQ ID NO:XXX (XenD27089),xxix) SEQ ID NO:XXX (XenD27090),xxx) SEQ ID NO:XXX (XenD27091),xxxi) SEQ ID NO:XXX (XenD27092), xxxii) SEQ ID NO:XXX (XenD27093),xxxiii) SEQ ID NO:XXX (XenD27094),xxxix) SEQ ID NO:XXX (XenD24877),xl) SEQ ID NO:XXX (XenD27166),xli) SEQ ID NO:XXX (XENP31582 Chain 2),xlii) SEQ ID NO:XXX (XENP31583 Chain 2), xliii) SEQ ID NO:XXX (XENP31584 Chain 2), xliv) SEQ ID NO:XXX (XENP32187 Chain 2), xlv) SEQ ID NO:XXX (XENP32188 Chain 2), xlvi) SEQ ID NO:XXX (XENP32189, Chain 2), xlvii) SEQ ID NO:XXX (XENP32190 Chain 2), xlviii) SEQ ID NO:XXX (XENP32191 Chain 2), xlix) SEQ ID NO:XXX (XENP32991 Chain 2), l) SEQ ID NO:XXX (XENP32992 Chain 2), li) SEQ ID NO:XXX (XENP32993 Chain 2), lii) SEQ ID NO:XXX (XENP32994 Chain 2), liii) SEQ ID NO:XXX (XENP32995 Chain 2), liv) SEQ ID NO:XXX (XENP32996 Chain 2), lv) SEQ ID NO:XXX (XENP32997 Chain 2), lvi) SEQ ID NO:XXX (XENP32998 Chain 2), lvii) SEQ ID NO:XXX (XENP32999 Chain 2), lviii) SEQ ID NO:XXX (XENP33000 Chain 2), lix) SEQ ID NO:XXX (XENP33001 Chain 2), lx) SEQ ID NO:XXX (XENP33002 Chain 2), lxi) SEQ ID NO:XXX (XENP33003 Chain 2), lxii) SEQ ID NO:XXX (XENP33004 Chain 2), lxiii) SEQ ID NO:XXX (XENP33005 Chain 2), lxiv) SEQ ID NO:XXX (XENP33006 Chain 2), lxv) SEQ ID NO:XXX (XENP33007 Chain 2), lxvi) SEQ ID NO:XXX (XENP33008 Chain 2), lxvii) SEQ ID NO:XXX (XENP33008 Chain 2), lxviii) SEQ ID NO:XXX (XENP33009 Chain 2), lxix) SEQ ID NO:XXX (XENP33010 Chain 2), and lxx) SEQ ID NO:XXX (XENP33011 Chain 2).

[00683] Preferred embodiments include XENP31251, XENP31254, XENP31258, XENP32186, XENP32187, XENP32188, XENP32189, XENP32190, and XENP32191. In further embodiments, XENP31251, XENP31254, XENP31258, XENP32186, XENP32187, XENP32188, XENP32189, XENP32190, and XENP32191 include further glycoengineering.

IX. Nucleic Acids of the Invention

[00684] The invention further provides nucleic acid compositions encoding the heterodimeric Fc fusion protein, the IL-12 subunits, and the IL-12 heterodimeric complex of the invention (or, in the case of a monomer Fc domain protein, nucleic acids encoding those as well).

[00685] As will be appreciated by those in the art, the nucleic acid compositions will depend on the format of the heterodimeric protein. Thus, for example, when the format requires three amino acid sequences, three nucleic acid sequences can be incorporated into one or more expression vectors for expression. Similarly for some formats, only two nucleic acids are needed; again, they can be put into one or two expression vectors.

[00686] As is known in the art, the nucleic acids encoding the components of the invention can be incorporated into expression vectors as is known in the art, and depending on the host cells used to produce the heterodimeric Fc fusion proteins of the invention. Generally the nucleic acids are operably linked to any number of regulatory elements (promoters, origin of replication, selectable markers, ribosomal binding sites, inducers, etc.). The expression vectors can be extra-chromosomal or integrating vectors.

[00687] The nucleic acids and/or expression vectors of the invention are then transformed into any number of different types of host cells as is well known in the art, including mammalian, bacterial, yeast, insect and/or fungal cells, with mammalian cells (e.g. CHO cells), finding use in many embodiments.

[00688] In some embodiments, nucleic acids encoding each monomer, as applicable depending on the format, are each contained within a single expression vector, generally under different or the same promoter controls. In embodiments of particular use in the present invention, each of these two or three nucleic acids are contained on a different expression vector.

[00689] The heterodimeric Fc fusion protein of the invention are made by culturing host cells comprising the expression vector(s) as is well known in the art. Once produced, traditional fusion protein or antibody purification steps are done, including an ion exchange

chromotography step. As discussed herein, having the pIs of the two monomers differ by at least 0.5 can allow separation by ion exchange chromatography or isoelectric focusing, or other methods sensitive to isoelectric point. That is, the inclusion of pI substitutions that alter the isoelectric point (pI) of each monomer so that each monomer has a different pI and the heterodimer also has a distinct pI, thus facilitating isoelectric purification of the heterodimer (e.g., anionic exchange chromatography, cationic exchange chromatography). These substitutions also aid in the determination and monitoring of any contaminating homodimers post-purification (e.g., IEF gels, cIEF, and analytical IEX columns).

X. Biological and Biochemical Functionality of IL-12 Heterodimeric Immunomodulatory Fc Fusion Proteins

[00690] Generally the Fc fusion proteins of the invention are administered to patients with cancer, and efficacy is assessed, in a number of ways as described herein. Thus, while standard assays of efficacy can be run, such as cancer load, size of tumor, evaluation of presence or extent of metastasis, etc., immuno-oncology treatments can be assessed on the basis of immune status evaluations as well. This can be done in a number of ways, including both in vitro and in vivo assays. For example, evaluation of changes in immune status (e.g., presence of ICOS+ CD4+ T cells following ipi treatment) along with "old fashioned" measurements such as tumor burden, size, invasiveness, LN involvement, metastasis, etc. can be done. Thus, any or all of the following can be evaluated: the inhibitory effects of PVRIG on $CD4^+$ T cell activation or proliferation, $CD8^+$ T (CTL) cell activation or proliferation, $CD8^+$ T cell-mediated cytotoxic activity and/or CTL mediated cell depletion, NK cell activity and NK mediated cell depletion, the potentiating effects of PVRIG on Treg cell differentiation and proliferation and Treg- or myeloid derived suppressor cell (MDSC)- mediated immunosuppression or immune tolerance, and/or the effects of PVRIG on proinflammatory cytokine production by immune cells, e.g., IL-2, IFN-ɣ or TNF-α production by T or other immune cells.

[00691] In some embodiments, assessment of treatment is done by evaluating immune cell proliferation, using for example, CFSE dilution method, Ki67 intracellular staining of immune effector cells, and ^{3}H-thymidine incorporation method,

[00692] In some embodiments, assessment of treatment is done by evaluating the increase in gene expression or increased protein levels of activation-associated markers, including one or more of: CD25, CD69, CD137, ICOS, PD1, GITR, OX40, and cell degranulation measured by surface expression of CD107A.

[00693] In general, gene expression assays are done as is known in the art.

[00694] In general, protein expression measurements are also similarly done as is known in the art.

[00695] In some embodiments, assessment of treatment is done by assessing cytotoxic activity measured by target cell viability detection via estimating numerous cell parameters such as enzyme activity (including protease activity), cell membrane permeability, cell adherence, ATP production, co-enzyme production, and nucleotide uptake activity. Specific examples of these assays include, but are not limited to, Trypan Blue or PI staining, ^{51}Cr or ^{35}S release method, LDH activity, MTT and/or WST assays, Calcein-AM assay, Luminescent based assay, and others.

[00696] In some embodiments, assessment of treatment is done by assessing T cell activity measured by cytokine production, measure either intracellularly in culture supernatant using cytokines including, but not limited to, IFNγ, TNFα, GM-CSF, IL2, IL6, IL4, IL5, IL10, IL13 using well known techniques.

[00697] Accordingly, assessment of treatment can be done using assays that evaluate one or more of the following: (i) increases in immune response, (ii) increases in activation of αβ and/or γδ T cells, (iii) increases in cytotoxic T cell activity, (iv) increases in NK and/or NKT cell activity, (v) alleviation of αβ and/or γδ T-cell suppression, (vi) increases in pro-inflammatory cytokine secretion, (vii) increases in IL-2 secretion; (viii) increases in interferon-γ production, (ix)

increases in Th1 response, (x) decreases in Th2 response, (xi) decreases or eliminates cell number and/or activity of at least one of regulatory T cells (Tregs).

A. Assays to Measure Efficacy and Potency

[00698] In some embodiments, T cell activation is assessed using a Mixed Lymphocyte Reaction (MLR) assay as is known in the art. An increase in activity indicates immunostimulatory activity. Appropriate increases in activity are outlined below.

[00699] In one embodiment, the signaling pathway assay measures increases or decreases in immune response as measured for an example by phosphorylation or de-phosphorylation of different factors, or by measuring other post translational modifications. IL-12 mediates IFNγ expression and secretion through phosphorylation of STAT4 (Morinobu et al., 2002). Accordingly, in a preferred embodiment, the signaling pathway assay meaures increases or decreases in immune response as indicated by phosphorylation of STAT4. An increase in activity indicates immunostimulatory activity. Appropriate increases in activity are outlined below.

[00700] In one embodiment, the signaling pathway assay measures increases or decreases in activation of αβ and/or γδ T cells as measured for an example by cytokine secretion or by proliferation or by changes in expression of activation markers like for an example CD137, CD107a, PD1, etc. An increase in activity indicates immunostimulatory activity. Appropriate increases in activity are outlined below.

[00701] In one embodiment, the signaling pathway assay measures increases or decreases in cytotoxic T cell activity as measured for an example by direct killing of target cells like for an example cancer cells or by cytokine secretion or by proliferation or by changes in expression of activation markers like for an example CD137, CD107a, PD1, etc. An increase in activity indicates immunostimulatory activity. Appropriate increases in activity are outlined below.

[00702] In one embodiment, the signaling pathway assay measures increases or decreases in NK and/or NKT cell activity as measured for an example by direct killing of target cells like for an

example cancer cells or by cytokine secretion or by changes in expression of activation markers like for an example CD107a, etc. An increase in activity indicates immunostimulatory activity. Appropriate increases in activity are outlined below.

[00703] In one embodiment, the signaling pathway assay measures increases or decreases in αβ and/or γδ T-cell suppression, as measured for an example by cytokine secretion or by proliferation or by changes in expression of activation markers like for an example CD137, CD107a, PD1, etc. An increase in activity indicates immunostimulatory activity. Appropriate increases in activity are outlined below.

[00704] In one embodiment, the signaling pathway assay measures increases or decreases in pro-inflammatory cytokine secretion as measured for example by ELISA or by Luminex or by Multiplex bead based methods or by intracellular staining and FACS analysis or by Alispot etc. An increase in activity indicates immunostimulatory activity. Appropriate increases in activity are outlined below.

[00705] In one embodiment, the signaling pathway assay measures increases or decreases in IL-2 secretion as measured for example by ELISA or by Luminex or by Multiplex bead based methods or by intracellular staining and FACS analysis or by Alispot etc. An increase in activity indicates immunostimulatory activity. Appropriate increases in activity are outlined below.

[00706] In one embodiment, the signaling pathway assay measures increases or decreases in interferon-ɣ production as measured for example by ELISA or by Luminex or by Multiplex bead based methods or by intracellular staining and FACS analysis or by Alispot etc. An increase in activity indicates immunostimulatory activity. Appropriate increases in activity are outlined below.

[00707] In one embodiment, the signaling pathway assay measures increases or decreases in Th1 response as measured for an example by cytokine secretion or by changes in expression of activation markers. An increase in activity indicates immunostimulatory activity. Appropriate increases in activity are outlined below.

[00708] In one embodiment, the signaling pathway assay measures increases or decreases in Th2 response as measured for an example by cytokine secretion or by changes in expression of activation markers. An increase in activity indicates immunostimulatory activity. Appropriate increases in activity are outlined below.

[00709] In one embodiment, the signaling pathway assay measures increases or decreases cell number and/or activity of at least one of regulatory T cells (Tregs), as measured for example by flow cytometry or by IHC. A decrease in response indicates immunostimulatory activity. Appropriate decreases are the same as for increases, outlined below.

[00710] In one embodiment, the signaling pathway assay measures increases or decreases in M2 macrophages cell numbers, as measured for example by flow cytometry or by IHC. A decrease in response indicates immunostimulatory activity. Appropriate decreases are the same as for increases, outlined below.

[00711] In one embodiment, the signaling pathway assay measures increases or decreases in M2 macrophage pro-tumorigenic activity, as measured for an example by cytokine secretion or by changes in expression of activation markers. A decrease in response indicates immunostimulatory activity. Appropriate decreases are the same as for increases, outlined below.

[00712] In one embodiment, the signaling pathway assay measures increases or decreases in N2 neutrophils increase, as measured for example by flow cytometry or by IHC. A decrease in response indicates immunostimulatory activity. Appropriate decreases are the same as for increases, outlined below.

[00713] In one embodiment, the signaling pathway assay measures increases or decreases in N2 neutrophils pro-tumorigenic activity, as measured for an example by cytokine secretion or by changes in expression of activation markers. A decrease in response indicates immunostimulatory activity. Appropriate decreases are the same as for increases, outlined below.

[00714] In one embodiment, the signaling pathway assay measures increases or decreases in inhibition of T cell activation, as measured for an example by cytokine secretion or by proliferation or by changes in expression of activation markers like for an example CD137, CD107a, PD1, etc. An increase in activity indicates immunostimulatory activity. Appropriate increases in activity are outlined below.

[00715] In one embodiment, the signaling pathway assay measures increases or decreases in inhibition of CTL activation as measured for an example by direct killing of target cells like for an example cancer cells or by cytokine secretion or by proliferation or by changes in expression of activation markers like for an example CD137, CD107a, PD1, etc. An increase in activity indicates immunostimulatory activity. Appropriate increases in activity are outlined below.

[00716] In one embodiment, the signaling pathway assay measures increases or decreases in $\alpha\beta$ and/or $\gamma\delta$ T cell exhaustion as measured for an example by changes in expression of activation markers. A decrease in response indicates immunostimulatory activity. Appropriate decreases are the same as for increases, outlined below.

[00717] In one embodiment, the signaling pathway assay measures increases or decreases $\alpha\beta$ and/or $\gamma\delta$ T cell response as measured for an example by cytokine secretion or by proliferation or by changes in expression of activation markers like for an example CD137, CD107a, PD1, etc. An increase in activity indicates immunostimulatory activity. Appropriate increases in activity are outlined below.

[00718] In one embodiment, the signaling pathway assay measures increases or decreases in stimulation of antigen-specific memory responses as measured for an example by cytokine secretion or by proliferation or by changes in expression of activation markers like for an example CD45RA, CCR7 etc. An increase in activity indicates immunostimulatory activity. Appropriate increases in activity are outlined below. .

[00719] In one embodiment, the signaling pathway assay measures increases or decreases in apoptosis or lysis of cancer cells as measured for an example by cytotoxicity assays such as for

an example MTT, Cr release, Calcine AM, or by flow cytometry based assays like for an example CFSE dilution or propidium iodide staining etc. An increase in activity indicates immunostimulatory activity. Appropriate increases in activity are outlined below.

[00720] In one embodiment, the signaling pathway assay measures increases or decreases in stimulation of cytotoxic or cytostatic effect on cancer cells, as measured for an example by cytotoxicity assays such as for an example MTT, Cr release, Calcine AM, or by flow cytometry based assays like for an example CFSE dilution or propidium iodide staining etc. An increase in activity indicates immunostimulatory activity. Appropriate increases in activity are outlined below.

[00721] In one embodiment, the signaling pathway assay measures increases or decreases direct killing of cancer cells as measured for an example by cytotoxicity assays such as for an example MTT, Cr release, Calcine AM, or by flow cytometry based assays like for an example CFSE dilution or propidium iodide staining etc. An increase in activity indicates immunostimulatory activity. Appropriate increases in activity are outlined below.

[00722] In one embodiment, the signaling pathway assay measures increases or decreases Th17 activity as measured for an example by cytokine secretion or by proliferation or by changes in expression of activation markers. An increase in activity indicates immunostimulatory activity. Appropriate increases in activity are outlined below.

[00723] In one embodiment, the signaling pathway assay measures increases or decreases in induction of complement dependent cytotoxicity and/or antibody dependent cell-mediated cytotoxicity, as measured for an example by cytotoxicity assays such as for an example MTT, Cr release, Calcine AM, or by flow cytometry based assays like for an example CFSE dilution or propidium iodide staining etc. An increase in activity indicates immunostimulatory activity. Appropriate increases in activity are outlined below.

[00724] In one embodiment, T cell activation is measured for an example by direct killing of target cells like for an example cancer cells or by cytokine secretion or by proliferation or by

changes in expression of activation markers like for an example CD137, CD107a, PD1, etc. For T-cells, increases in proliferation, cell surface markers of activation (e.g., CD25, CD69, CD137, PD1), cytotoxicity (ability to kill target cells), and cytokine production (e.g., IL-2, IL-4, IL-6, IFNγ, TNF-a, IL-10, IL-17A) would be indicative of immune modulation that would be consistent with enhanced killing of cancer cells.

[00725] In one embodiment, NK cell activation is measured for example by direct killing of target cells like for an example cancer cells or by cytokine secretion or by changes in expression of activation markers like for an example CD107a, etc. For NK cells, increases in proliferation, cytotoxicity (ability to kill target cells and increases CD107a, granzyme, and perforin expression), cytokine production (e.g., IFNγ and TNF), and cell surface receptor expression (e.g. CD25) would be indicative of immune modulation that would be consistent with enhanced killing of cancer cells.

[00726] In one embodiment, γδ T cell activation is measured for example by cytokine secretion or by proliferation or by changes in expression of activation markers.

[00727] In one embodiment, Th1 cell activation is measured for example by cytokine secretion or by changes in expression of activation markers.

[00728] Appropriate increases in activity or response (or decreases, as appropriate as outlined above), are increases of 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90%, 95% or 98 to 99% percent over the signal in either a reference sample or in control samples, for example test samples that do not contain an IL-12 heterodimeric fusion protein of the invention. Similarly, increases of at least one-, two-, three-, four- or five-fold as compared to reference or control samples show efficacy.

XI. Combination Therapy

[00729] In some aspects, the IL-12-Fc fusion proteins described herein is administered in combination with another therapeutic agent. Administered "in combination", as used herein, means that two (or more) different treatments are delivered to the subject during the course of

the subject's affliction with the disorder, e.g., the two or more treatments are delivered after the subject has been diagnosed with the disorder and before the disorder has been cured or eliminated or treatment has ceased for other reasons. In some embodiments, the delivery of one treatment is still occurring when the delivery of the second begins, so that there is overlap in terms of administration. This is sometimes referred to herein as "simultaneous" or "concurrent delivery". In other embodiments, the delivery of one treatment ends before the delivery of the other treatment begins. In some embodiments of either case, the treatment is more effective because of combined administration. For example, the second treatment is more effective, e.g., an equivalent effect is seen with less of the second treatment, or the second treatment reduces symptoms to a greater extent, than would be seen if the second treatment were administered in the absence of the first treatment, or the analogous situation is seen with the first treatment. In some embodiments, delivery is such that the reduction in a symptom, or other parameter related to the disorder is greater than what would be observed with one treatment delivered in the absence of the other. The effect of the two treatments can be partially additive, wholly additive, or greater than additive. The delivery can be such that an effect of the first treatment delivered is still detectable when the second is delivered.

[00730] The IL-12-Fc fusion proteins (e.g., any XENP sequence described herein) described herein and the at least one additional therapeutic agent can be administered simultaneously, in the same or in separate compositions, or sequentially. For sequential administration, the IL-12-Fc fusion proteins (e.g., any XENP sequence described herein) described herein can be administered first, and the additional agent can be administered second, or the order of administration can be reversed.

[00731] The IL-12-Fc fusion proteins (e.g., any XENP sequence described herein) described herein and/or other therapeutic agents, procedures or modalities can be administered during periods of active disorder, or during a period of remission or less active disease. The IL-12-Fc fusion proteins (e.g., any XENP sequence described herein) can be administered before the other treatment, concurrently with the treatment, post-treatment, or during remission of the disorder.

A. Checkpoint Blockade Antibodies

[00732] In some embodiments, the IL-12-Fc fusion proteins described herein are combined with other therapeutic agents including checkpoint blockade antibodies, such as but not limited to, a PD-1 inhibitor, a TIM3 inhibitor, a CTLA4 inhibitor, a PD-L1 inhibitor, a TIGIT inhibitor, a LAG3 inhibitor, or a combination thereof.

1. Anti-PD-1 Antibodies

[00733] In some embodiments, an IL-12-Fc fusion protein described herein can be administered to a subject with cancer in combination with an anti-PD-1 antibody. In some cases, the anti-PD-1 antibody includes XENP16432 (a bivalent anti-PD-1 mAb, a checkpoint inhibitor which enhances anti-tumor activity by de-repressing the engrafted human T cells; sequences depicted in Figure 53).

[00734] In some embodiments, the Il-12-Fc fusion proteins of the invention are administered in combination with Keytruda® or Optivo®.

[00735] Exemplary non-limiting anti-PD-1 antibody molecules are disclosed in US 2015/0210769, published on July 30, 2015, entitled "Antibody Molecules to PD-1 and Uses Thereof," incorporated by reference in its entirety.

[00736] In one embodiment, the anti-PD-1 antibody molecule includes at least one or two heavy chain variable domain (optionally including a constant region), at least one or two light chain variable domain (optionally including a constant region), or both, comprising the amino acid sequence of BAP049-Clone-A, BAP049-Clone-B, BAP049-Clone-C, BAP049-Clone-D, or BAP049-Clone-E; or as described in Table 1 of US 2015/0210769, or encoded by the nucleotide sequence in Table 1; or a sequence substantially identical (e.g., at least 80%, 85%, 90%, 92%, 95%, 97%, 98%, 99% or higher identical) to any of the aforesaid sequences. The anti-PD-1 antibody molecule, optionally, comprises a leader sequence from a heavy chain, a light chain, or both, as shown in Table 4 of US 2015/0210769; or a sequence substantially identical thereto.

[00737] In yet another embodiment, the anti-PD-1 antibody molecule includes at least one, two, or three complementarity determining regions (CDRs) from a heavy chain variable region and/or a light chain variable region of an antibody described herein, e.g., an antibody chosen from any of BAP049-hum01, BAP049-hum02, BAP049-hum03, BAP049-hum04, BAP049-hum05, BAP049-hum06, BAP049-hum07, BAP049-hum08, BAP049-hum09, BAP049-hum10, BAP049-hum11, BAP049-hum12, BAP049-hum13, BAP049-hum14, BAP049-hum15, BAP049-hum16, BAP049-Clone-A, BAP049-Clone-B, BAP049-Clone-C, BAP049-Clone-D, or BAP049-Clone-E; or as described in Table 1, or encoded by the nucleotide sequence in Table 1; or a sequence substantially identical (e.g., at least 80%, 85%, 90%, 92%, 95%, 97%, 98%, 99% or higher identical) to any of the aforesaid sequences.

[00738] In yet another embodiment, the anti-PD-1 antibody molecule includes at least one, two, or three CDRs (or collectively all of the CDRs) from a heavy chain variable region comprising an amino acid sequence shown in Table 1 of US 2015/0210769, or encoded by a nucleotide sequence shown in Table 1. In one embodiment, one or more of the CDRs (or collectively all of the CDRs) have one, two, three, four, five, six or more changes, e.g., amino acid substitutions or deletions, relative to the amino acid sequence shown in Table 1, or encoded by a nucleotide sequence shown in Table 1.

[00739] In yet another embodiment, the anti-PD-1 antibody molecule includes at least one, two, or three CDRs (or collectively all of the CDRs) from a light chain variable region comprising an amino acid sequence shown in Table 1 of US 2015/0210769, or encoded by a nucleotide sequence shown in Table 1. In one embodiment, one or more of the CDRs (or collectively all of the CDRs) have one, two, three, four, five, six or more changes, e.g., amino acid substitutions or deletions, relative to the amino acid sequence shown in Table 1, or encoded by a nucleotide sequence shown in Table 1. In certain embodiments, the anti-PD-1 antibody molecule includes a substitution in a light chain CDR, e.g., one or more substitutions in a CDR1, CDR2 and/or CDR3 of the light chain. In one embodiment, the anti-PD-1 antibody molecule includes a substitution in the light chain CDR3 at position 102 of the light variable region, e.g., a substitution of a cysteine to tyrosine, or a cysteine to serine residue, at position 102 of the light variable region

according to Table 1 (e.g., SEQ ID NO: 16 or 24 for murine or chimeric, unmodified; or any of SEQ ID NOs: 34, 42, 46, 54, 58, 62, 66, 70, 74, or 78 for a modified sequence).

[00740] In another embodiment, the anti-PD-1 antibody molecule includes at least one, two, three, four, five or six CDRs (or collectively all of the CDRs) from a heavy and light chain variable region comprising an amino acid sequence shown in Table 1 of US 2015/0210769, or encoded by a nucleotide sequence shown in Table 1. In one embodiment, one or more of the CDRs (or collectively all of the CDRs) have one, two, three, four, five, six or more changes, e.g., amino acid substitutions or deletions, relative to the amino acid sequence shown in Table 1, or encoded by a nucleotide sequence shown in Table 1.

[00741] In one embodiment, the anti-PD-1 antibody molecule includes:

[00742] (a) a heavy chain variable region (VH) comprising a VHCDR1 amino acid sequence of SEQ ID NO: 4, a VHCDR2 amino acid sequence of SEQ ID NO: 5, and a VHCDR3 amino acid sequence of SEQ ID NO: 3; and a light chain variable region (VL) comprising a VLCDR1 amino acid sequence of SEQ ID NO: 13, a VLCDR2 amino acid sequence of SEQ ID NO: 14, and a VLCDR3 amino acid sequence of SEQ ID NO: 33, each disclosed in Table 1 of US 2015/0210769;

[00743] (b) a VH comprising a VHCDR1 amino acid sequence chosen from SEQ ID NO: 1; a VHCDR2 amino acid sequence of SEQ ID NO: 2; and a VHCDR3 amino acid sequence of SEQ ID NO: 3; and a VL comprising a VLCDR1 amino acid sequence of SEQ ID NO: 10, a VLCDR2 amino acid sequence of SEQ ID NO: 11, and a VLCDR3 amino acid sequence of SEQ ID NO: 32, each disclosed in Table 1 of US 2015/0210769;

[00744] (c) a VH comprising a VHCDR1 amino acid sequence of SEQ ID NO: 224, a VHCDR2 amino acid sequence of SEQ ID NO: 5, and a VHCDR3 amino acid sequence of SEQ ID NO: 3; and a VL comprising a VLCDR1 amino acid sequence of SEQ ID NO: 13, a VLCDR2 amino acid sequence of SEQ ID NO: 14, and a VLCDR3 amino acid sequence of SEQ ID NO: 33, each disclosed in Table 1 of US 2015/0210769; or

[00745] (d) a VH comprising a VHCDR1 amino acid sequence of SEQ ID NO: 224; a VHCDR2 amino acid sequence of SEQ ID NO: 2; and a VHCDR3 amino acid sequence of SEQ ID NO: 3; and a VL comprising a VLCDR1 amino acid sequence of SEQ ID NO: 10, a VLCDR2 amino acid sequence of SEQ ID NO: 11, and a VLCDR3 amino acid sequence of SEQ ID NO: 32, each disclosed in Table 1 of US 2015/0210769.

[00746] In another embodiment, the anti-PD-1 antibody molecule comprises (i) a heavy chain variable region (VH) comprising a VHCDR1 amino acid sequence chosen from SEQ ID NO: 1, SEQ ID NO: 4, or SEQ ID NO: 224; a VHCDR2 amino acid sequence of SEQ ID NO: 2 or SEQ ID NO: 5; and a VHCDR3 amino acid sequence of SEQ ID NO: 3; and (ii) a light chain variable region (VL) comprising a VLCDR1 amino acid sequence of SEQ ID NO: 10 or SEQ ID NO: 13, a VLCDR2 amino acid sequence of SEQ ID NO: 11 or SEQ ID NO: 14, and a VLCDR3 amino acid sequence of SEQ ID NO: 32 or SEQ ID NO: 33, each disclosed in Table 1 of US 2015/0210769.

[00747] In other embodiments, the PD-1 inhibitor is an anti-PD-1 antibody chosen from nivolumab, pembrolizumab, or pidilizumab.

[00748] In some embodiments, the anti-PD-1 antibody is nivolumab. Alternative names for nivolumab include MDX- 1106, MDX-1106-04, ONO-4538, or BMS-936558. In some embodiments, the anti-PD- 1 antibody is Nivolumab (CAS Registry Number: 946414-94-4). Nivolumab is a fully human IgG4 monoclonal antibody which specifically blocks PD1. Nivolumab (clone 5C4) and other human monoclonal antibodies that specifically bind to PD1 are disclosed in US 8,008,449 and WO2006/121168. In one embodiment, the inhibitor of PD-1 is nivolumab, and having a sequence disclosed herein (or a sequence substantially identical or similar thereto, e.g., a sequence at least 85%, 90%, 95% identical or higher to the sequence specified). In some embodiments, the anti-PD-1 antibody is pembrolizumab. Pembrolizumab (also referred to as lambrolizumab, MK-3475, MK03475, SCH-900475 or KEYTRUDA®; Merck) is a humanized IgG4 monoclonal antibody that binds to PD-1. Pembrolizumab and other humanized anti-PD-1 antibodies are disclosed in Hamid, O. et al. (2013) New England Journal of Medicine 369 (2): 134–44, US 8,354,509 and WO2009/114335.

[00749] In one embodiment, the inhibitor of PD-1 is pembrolizumab disclosed in, e.g., US 8,354,509 and WO 2009/114335, and having a sequence disclosed herein (or a sequence substantially identical or similar thereto, e.g., a sequence at least 85%, 90%, 95% identical or higher to the sequence specified).

[00750] In some embodiments, the anti-PD-1 antibody is pidilizumab. Pidilizumab (CT-011; Cure Tech) is a humanized IgG1k monoclonal antibody that binds to PD1. Pidilizumab and other humanized anti-PD-1 monoclonal antibodies are disclosed in US 8,747,847 and WO2009/101611.

[00751] Other anti-PD1 antibodies include AMP 514 (Amplimmune), among others, e.g., anti-PD1 antibodies disclosed in US 8,609,089, US 2010028330, and/or US 20120114649.

[00752] In some embodiments, the PD-1 inhibitor is an immunoadhesin (e.g., an immunoadhesin comprising an extracellular or PD-1 binding portion of PD-Ll or PD-L2 fused to a constant region (e.g., an Fc region of an immunoglobulin sequence). In some embodiments, the PD-1 inhibitor is AMP-224 (B7-DCIg; Amplimmune; e.g., disclosed in WO2010/027827 and WO2011/066342), is a PD-L2 Fc fusion soluble receptor that blocks the interaction between PD-1 and B7-H1.

[00753] In some enbodiments, anti-PD-1 antibodies can be used in combination with an IL-12 Fc fusion protein of the invention. There are several anti-PD-1 antibodies including, but not limited to, two currently FDA approved antibodies, pembrolizumab and nivolizumab, as well as those in clinical testing currently, including, but not limited to, tislelizumab, Sym021, REGN2810 (developed by Rengeneron), JNJ-63723283 (developed by J and J), SHR-1210, pidilizumab, AMP-224, MEDIo680, PDR001 and CT-001, as well as others outlined in Liu et al., J. Hemat. & Oncol. (2017)10:136, the antibodies therein expressly incorporated by reference.

[00754] In some embodiments, an IL-12-Fc fusion protein described herein can be used in combination with a PD-1 inhibitor (e.g., an anti-PD-1 antibody). In certain embodiments, an IL-

12-Fc fusion protein (e.g., any XENP sequence described herein) described herein is administered in combination with an anti-PD-1 antibody.

2.Anti-TIM3 Antibodies

[00755] In some embodiments, the IL-12-Fc fusion proteins of the invention are administered in combination with an anti-TIM3 antibody.

[00756] In some embodiments, the Il-12-Fc fusion proteins of the invention are administered in combination with LY3321367 (Eli Lilly and Company), MBG453 (Novartis Pharmaceuticals), and TSR-022 (Tesaro, Inc.).

[00757] Exemplary non-limiting anti-TIM-3 antibody molecules are disclosed in US 2015/0218274, published on August 6, 2015, entitled "Antibody Molecules to TIM-3 and Uses Thereof," incorporated by reference in its entirety.

[00758] In one embodiment, the anti-TIM-3 antibody molecule includes at least one or two heavy chain variable domain (optionally including a constant region), at least one or two light chain variable domain (optionally including a constant region), or both, comprising the amino acid sequence of ABTIM3, ABTIM3-hum01, ABTIM3-hum02, ABTIM3-hum03, ABTIM3-hum04, ABTIM3-hum05, ABTIM3-hum06, ABTIM3-hum07, ABTIM3-hum08, ABTIM3-hum09, ABTIM3-hum10, ABTIM3-hum11, ABTIM3-hum12, ABTIM3-hum13, ABTIM3-hum14, ABTIM3-hum15, ABTIM3-hum16, ABTIM3-hum17, ABTIM3-hum18, ABTIM3-hum19, ABTIM3-hum20, ABTIM3-hum21, ABTIM3-hum22, ABTIM3-hum23; or as described in Tables 1-4 of US 2015/0218274; or encoded by the nucleotide sequence in Tables 1-4; or a sequence substantially identical (e.g., at least 80%, 85%, 90%, 92%, 95%, 97%, 98%, 99% or higher identical) to any of the aforesaid sequences. The anti-TIM-3 antibody molecule, optionally, comprises a leader sequence from a heavy chain, a light chain, or both, as shown in US 2015/0218274; or a sequence substantially identical thereto.

[00759] In yet another embodiment, the anti-TIM-3 antibody molecule includes at least one, two, or three complementarity determining regions (CDRs) from a heavy chain variable region

and/or a light chain variable region of an antibody described herein, e.g., an antibody chosen from any of ABTIM3, ABTIM3-hum01, ABTIM3-hum02, ABTIM3-hum03, ABTIM3-hum04, ABTIM3-hum05, ABTIM3-hum06, ABTIM3-hum07, ABTIM3-hum08, ABTIM3-hum09, ABTIM3-hum10, ABTIM3-hum11, ABTIM3-hum12, ABTIM3-hum13, ABTIM3-hum14, ABTIM3-hum15, ABTIM3-hum16, ABTIM3-hum17, ABTIM3-hum18, ABTIM3-hum19, ABTIM3-hum20, ABTIM3-hum21, ABTIM3-hum22, ABTIM3-hum23; or as described in Tables 1-4 of US 2015/0218274; or encoded by the nucleotide sequence in Tables 1-4; or a sequence substantially identical (e.g., at least 80%, 85%, 90%, 92%, 95%, 97%, 98%, 99% or higher identical) to any of the aforesaid sequences.

[00760] In yet another embodiment, the anti-TIM-3 antibody molecule includes at least one, two, or three CDRs (or collectively all of the CDRs) from a heavy chain variable region comprising an amino acid sequence shown in Tables 1-4 of US 2015/0218274, or encoded by a nucleotide sequence shown in Tables 1-4. In one embodiment, one or more of the CDRs (or collectively all of the CDRs) have one, two, three, four, five, six or more changes, e.g., amino acid substitutions or deletions, relative to the amino acid sequence shown in Tables 1-4, or encoded by a nucleotide sequence shown in Table 1-4.

[00761] In yet another embodiment, the anti- TIM-3 antibody molecule includes at least one, two, or three CDRs (or collectively all of the CDRs) from a light chain variable region comprising an amino acid sequence shown in Tables 1-4 of US 2015/0218274, or encoded by a nucleotide sequence shown in Tables 1-4. In one embodiment, one or more of the CDRs (or collectively all of the CDRs) have one, two, three, four, five, six or more changes, e.g., amino acid substitutions or deletions, relative to the amino acid sequence shown in Tables 1-4, or encoded by a nucleotide sequence shown in Tables 1-4. In certain embodiments, the anti-TIM-3 antibody molecule includes a substitution in a light chain CDR, e.g., one or more substitutions in a CDR1, CDR2 and/or CDR3 of the light chain.

[00762] In another embodiment, the anti-TIM-3 antibody molecule includes at least one, two, three, four, five or six CDRs (or collectively all of the CDRs) from a heavy and light chain

variable region comprising an amino acid sequence shown in Tables 1-4 of US 2015/0218274, or encoded by a nucleotide sequence shown in Tables 1-4. In one embodiment, one or more of the CDRs (or collectively all of the CDRs) have one, two, three, four, five, six or more changes, e.g., amino acid substitutions or deletions, relative to the amino acid sequence shown in Tables 1-4, or encoded by a nucleotide sequence shown in Tables 1-4.

[00763] In one embodiment, the anti-TIM-3 antibody molecule includes:

[00764] (a) a heavy chain variable region (VH) comprising a VHCDR1 amino acid sequence chosen from SEQ ID NO: 9; a VHCDR2 amino acid sequence of SEQ ID NO: 10; and a VHCDR3 amino acid sequence of SEQ ID NO: 5; and a light chain variable region (VL) comprising a VLCDR1 amino acid sequence of SEQ ID NO: 12, a VLCDR2 amino acid sequence of SEQ ID NO: 13, and a VLCDR3 amino acid sequence of SEQ ID NO: 14, each disclosed in Tables 1-4 of US 2015/0218274;

[00765] (b) a VH comprising a VHCDR1 amino acid sequence chosen from SEQ ID NO: 3; a VHCDR2 amino acid sequence of SEQ ID NO: 4; and a VHCDR3 amino acid sequence of SEQ ID NO: 5; and a VL comprising a VLCDR1 amino acid sequence of SEQ ID NO: 6, a VLCDR2 amino acid sequence of SEQ ID NO: 7, and a VLCDR3 amino acid sequence of SEQ ID NO: 8, each disclosed in Tables 1-4 of US 2015/0218274;

[00766] (c) a VH comprising a VHCDR1 amino acid sequence chosen from SEQ ID NO: 9; a VHCDR2 amino acid sequence of SEQ ID NO: 25; and a VHCDR3 amino acid sequence of SEQ ID NO: 5; and a VL comprising a VLCDR1 amino acid sequence of SEQ ID NO: 12, a VLCDR2 amino acid sequence of SEQ ID NO: 13, and a VLCDR3 amino acid sequence of SEQ ID NO: 14, each disclosed in Tables 1-4 of US 2015/0218274;

[00767] (d) a VH comprising a VHCDR1 amino acid sequence chosen from SEQ ID NO: 3; a VHCDR2 amino acid sequence of SEQ ID NO: 24; and a VHCDR3 amino acid sequence of SEQ ID NO: 5; and a VL comprising a VLCDR1 amino acid sequence of SEQ ID NO: 6, a

VLCDR2 amino acid sequence of SEQ ID NO: 7, and a VLCDR3 amino acid sequence of SEQ ID NO: 8, each disclosed in Tables 1-4 of US 2015/0218274;

[00768] (e) a VH comprising a VHCDR1 amino acid sequence chosen from SEQ ID NO: 9; a VHCDR2 amino acid sequence of SEQ ID NO: 31; and a VHCDR3 amino acid sequence of SEQ ID NO: 5; and a VL comprising a VLCDR1 amino acid sequence of SEQ ID NO: 12, a VLCDR2 amino acid sequence of SEQ ID NO: 13, and a VLCDR3 amino acid sequence of SEQ ID NO: 14, each disclosed in Tables 1-4 of US 2015/0218274; or

[00769] (f) a VH comprising a VHCDR1 amino acid sequence chosen from SEQ ID NO: 3; a VHCDR2 amino acid sequence of SEQ ID NO: 30; and a VHCDR3 amino acid sequence of SEQ ID NO: 5; and a VL comprising a VLCDR1 amino acid sequence of SEQ ID NO: 6, a VLCDR2 amino acid sequence of SEQ ID NO: 7, and a VLCDR3 amino acid sequence of SEQ ID NO: 8, each disclosed in Tables 1-4 of US 2015/0218274.

[00770] Exemplary anti-TIM-3 antibodies are disclosed in U.S. Patent No.: 8,552,156, WO 2011/155607, EP 2581113 and U.S Publication No.: 2014/044728.

[00771] In some enbodiments, anti-TIM-3 antibodies can be used in combination an IL-12 Fc fusion protein of the invention. There are several TIM-3 antibodies in clinical development, including, but not limited to, MBG453 and TSR-022.

[00772] In some embodiments, an IL-12-Fc fusion protein described herein can be used in combination with a TIM-3 inhibitor (e.g., an anti-TIM3 antibody). In certain embodiments, an IL-12 Fc fusion protein (e.g., any XENP sequence described herein) described herein is administered in combination with an anti-TIM3 antibody.

3.Anti-CTLA4 Antibodies

[00773] Exemplary anti-CTLA4 antibodies include tremelimumab (IgG2 monoclonal antibody available from Pfizer, formerly known as ticilimumab, CP-675,206); and ipilimumab (CTLA-4 antibody, also known as MDX-010, CAS No. 477202-00-9). Other exemplary anti-CTLA-4 antibodies are disclosed, *e.g.*, in U.S. Pat. No. 5,811,097.

[00774] In one embodiment, the anti-CTLA4 antibody is ipilimumab disclosed in, *e.g.*, US 5,811,097, US 7,605,238, WO00/32231 and WO97/20574, and having a sequence disclosed herein (or a sequence substantially identical or similar thereto, *e.g.*, a sequence at least 85%, 90%, 95% identical or higher to the sequence specified).

[00775] In one embodiment, the anti-CTLA4 antibody is tremelimumab disclosed in, *e.g.*, US 6,682,736 and WO00/37504, and having a sequence disclosed herein (or a sequence substantially identical or similar thereto, *e.g.*, a sequence at least 85%, 90%, 95% identical or higher to the sequence specified).

[00776] In some embodiments, anti-CTLA-4 antibodies can be used in combination with an IL-12-Fc fusion protein of the invention. Thus, suitable anti-CTLA-4 antibodies for use in combination therapies as outlined herein include, but are not limited to, one currently FDA approved antibody ipilimumab, and several more in development, including CP-675,206 and AGEN-1884.

[00777] In some embodiments, an IL-12-Fc fusion protein described herein can be used in combination with a CTLA-4 inhibitor (e.g., an anti-CTLA-4 antibody). In certain embodiments, an IL-12-Fc fusion proteins (e.g., any XENP sequence described herein) described herein is administered in combination with an anti-CTLA-4 antibody.

4.Anti-PD-L1 Antibodies

[00778] Exemplary non-limiting anti-PD-L1 antibody molecules are disclosed in US 2016/0108123, published on April 21, 2016, entitled "Antibody Molecules to PD-L1 and Uses Thereof," incorporated by reference in its entirety.

[00779] In one embodiment, the anti-PD-L1 antibody molecule includes at least one or two heavy chain variable domain (optionally including a constant region), at least one or two light chain variable domain (optionally including a constant region), or both, comprising the amino acid sequence of any of BAP058-hum01, BAP058-hum02, BAP058-hum03, BAP058-hum04, BAP058-hum05, BAP058-hum06, BAP058-hum07, BAP058-hum08, BAP058-hum09, BAP058-

hum10, BAP058-hum11, BAP058-hum12, BAP058-hum13, BAP058-hum14, BAP058-hum15, BAP058-hum16, BAP058-hum17, BAP058-Clone-K, BAP058-Clone-L, BAP058-Clone-M, BAP058-Clone-N, or BAP058-Clone-O; or as described in Table 1 of US 2016/0108123, or encoded by the nucleotide sequence in Table 1; or a sequence substantially identical (e.g., at least 80%, 85%, 90%, 92%, 95%, 97%, 98%, 99% or higher identical) to any of the aforesaid sequences.

[00780] In yet another embodiment, the anti-PD-L1 antibody molecule includes at least one, two, or three complementarity determining regions (CDRs) from a heavy chain variable region and/or a light chain variable region of an antibody described herein, e.g., an antibody chosen from any of BAP058-hum01, BAP058-hum02, BAP058-hum03, BAP058-hum04, BAP058-hum05, BAP058-hum06, BAP058-hum07, BAP058-hum08, BAP058-hum09, BAP058-hum10, BAP058-hum11, BAP058-hum12, BAP058-hum13, BAP058-hum14, BAP058-hum15, BAP058-hum16, BAP058-hum17, BAP058-Clone-K, BAP058-Clone-L, BAP058-Clone-M, BAP058-Clone-N, or BAP058-Clone-O; or as described in Table 1 of US 2016/0108123, or encoded by the nucleotide sequence in Table 1; or a sequence substantially identical (e.g., at least 80%, 85%, 90%, 92%, 95%, 97%, 98%, 99% or higher identical) to any of the aforesaid sequences.

[00781] In yet another embodiment, the anti-PD-L1 antibody molecule includes at least one, two, or three CDRs (or collectively all of the CDRs) from a heavy chain variable region comprising an amino acid sequence shown in Table 1 of US 2016/0108123, or encoded by a nucleotide sequence shown in Table 1. In one embodiment, one or more of the CDRs (or collectively all of the CDRs) have one, two, three, four, five, six or more changes, e.g., amino acid substitutions or deletions, relative to the amino acid sequence shown in Table 1, or encoded by a nucleotide sequence shown in Table 1.

[00782] In yet another embodiment, the anti-PD-L1 antibody molecule includes at least one, two, or three CDRs (or collectively all of the CDRs) from a light chain variable region comprising an amino acid sequence shown in Table 1 of US 2016/0108123, or encoded by a nucleotide sequence shown in Table 1. In one embodiment, one or more of the CDRs (or collectively all of the CDRs)

have one, two, three, four, five, six or more changes, e.g., amino acid substitutions or deletions, relative to the amino acid sequence shown in Table 1, or encoded by a nucleotide sequence shown in Table 1. In certain embodiments, the anti-PD-L1 antibody molecule includes a substitution in a light chain CDR, e.g., one or more substitutions in a CDR1, CDR2 and/or CDR3 of the light chain.

[00783] In another embodiment, the anti-PD-L1 antibody molecule includes at least one, two, three, four, five or six CDRs (or collectively all of the CDRs) from a heavy and light chain variable region comprising an amino acid sequence shown in Table 1, or encoded by a nucleotide sequence shown in Table 1 of US 2016/0108123. In one embodiment, one or more of the CDRs (or collectively all of the CDRs) have one, two, three, four, five, six or more changes, e.g., amino acid substitutions or deletions, relative to the amino acid sequence shown in Table 1, or encoded by a nucleotide sequence shown in Table 1.

[00784] In one embodiment, the anti-PD-L1 antibody molecule includes:

(i) a heavy chain variable region (VH) including a VHCDR1 amino acid sequence chosen from SEQ ID NO: 1, SEQ ID NO: 4 or SEQ ID NO: 195; a VHCDR2 amino acid sequence of SEQ ID NO: 2; and a VHCDR3 amino acid sequence of SEQ ID NO: 3, each disclosed in Table 1 of US 2016/0108123; and

(ii) a light chain variable region (VL) including a VLCDR1 amino acid sequence of SEQ ID NO: 9, a VLCDR2 amino acid sequence of SEQ ID NO: 10, and a VLCDR3 amino acid sequence of SEQ ID NO: 11, each disclosed in Table 1 of US 2016/0108123.

[00785] In another embodiment, the anti-PD-L1 antibody molecule includes:

(i) a heavy chain variable region (VH) including a VHCDR1 amino acid sequence chosen from SEQ ID NO: 1, SEQ ID NO: 4 or SEQ ID NO: 195; a VHCDR2 amino acid sequence of SEQ ID NO: 5, and a VHCDR3 amino acid sequence of SEQ ID NO: 3, each disclosed in Table 1 of US 2016/0108123; and

(ii) a light chain variable region (VL) including a VLCDR1 amino acid sequence of SEQ ID NO: 12, a VLCDR2 amino acid sequence of SEQ ID NO: 13, and a VLCDR3 amino acid sequence of SEQ ID NO: 14, each disclosed in Table 1 of US 2016/0108123.

[00786] In one embodiment, the anti-PD-L1 antibody molecule comprises the VHCDR1 amino acid sequence of SEQ ID NO: 1. In another embodiment, the anti-PD-L1 antibody molecule comprises the VHCDR1 amino acid sequence of SEQ ID NO: 4. In yet another embodiment, the anti-PD-L1 antibody molecule comprises the VHCDR1 amino acid sequence of SEQ ID NO: 195, each disclosed in Table 1 of US 2016/0108123.

[00787] In some embodiments, the PD-L1 inhibitor is an antibody molecule. In some embodiments, the anti-PD-Ll inhibitor is chosen from YW243.55.S70, MPDL3280A, MEDI-4736, MSB-0010718C, MDX-1105, atezolizumab, durbalumab, avelumab, or BMS936559.

[00788] In some embodiments, the anti-PD-L1 antibody is atezolizumab. Atezolizumab (also referred to as MPDL3280A and Atezo®; Roche) is a monoclonal antibody that binds to PD-L1. Atezolizumab and other humanized anti-PD-L1 antibodies are disclosed in US 8,217,149, and having a sequence disclosed herein (or a sequence substantially identical or similar thereto, e.g., a sequence at least 85%, 90%, 95% identical or higher to the sequence specified).

[00789] In some embodiments, the anti-PD-L1 antibody is avelumab. Avelumab (also referred to as A09-246-2; Merck Serono) is a monoclonal antibody that binds to PD-L1. Avelumab and other humanized anti-PD-L1 antibodies are disclosed in US 9,324,298 and WO2013/079174, and having a sequence disclosed herein (or a sequence substantially identical or similar thereto, e.g., a sequence at least 85%, 90%, 95% identical or higher to the sequence specified).

[00790] In some embodiments, the anti-PD-L1 antibody is durvalumab. Durvalumab (also referred to as MEDI4736; AstraZeneca) is a monoclonal antibody that binds to PD-L1. Durvalumab and other humanized anti-PD-L1 antibodies are disclosed in US 8,779,108, and having a sequence disclosed herein (or a sequence substantially identical or similar thereto, e.g., a sequence at least 85%, 90%, 95% identical or higher to the sequence specified).

[00791] In some embodiments, the anti-PD-L1 antibody is BMS-936559. BMS-936559 (also referred to as MDX-1105; BMS) is a monoclonal antibody that binds to PD-L1. BMS-936559 and other humanized anti-PD-L1 antibodies are disclosed in US 7,943,743 and WO2007005874, and having a sequence disclosed herein (or a sequence substantially identical or similar thereto, e.g., a sequence at least 85%, 90%, 95% identical or higher to the sequence specified).

[00792] In some enbodiments, anti-PD-L1 antibodies can be used in combination with an IL-12-Fc fusion protein of the invention. There are several anti-PD-L1 antibodies including three currently FDA approved antibodies, atezolizumab, avelumab, durvalumab, as well as those in clinical testing currently, including, but not limited to, LY33000054 and CS1001, as well as others outlined in Liu et al., J. Hemat. & Oncol. (2017)10:136, the antibodies therein expressly incorporated by reference.

[00793] In some embodiments, an IL-12-Fc fusion protein described herein can be used in combination with a PD-L1 or PD-L2 inhibitor (e.g., an anti-PD-L1 antibody).

5.Anti-TIGIT Antibodies

[00794] In some embodiments, the anti-TIGIT antibody is OMP-313M32. OMP-313M32 (OncoMed Pharmaceuticals) is a monoclonal antibody that binds to TIGIT. OMP-313M32 and other humanized anti- TIGIT antibodies are disclosed in US20160376365 and WO2016191643, and having a sequence disclosed herein (or a sequence substantially identical or similar thereto, e.g., a sequence at least 85%, 90%, 95% identical or higher to the sequence specified).

[00795] In some embodiments, the anti-TIGIT antibody is BMS-986207. BMS-986207 (also referred to as ONO-4686; Bristol-Myers Squibb) is a monoclonal antibody that binds to TIGIT. BMS-986207 and other humanized anti- TIGIT antibodies are disclosed in US20160176963 and WO2016106302, and having a sequence disclosed herein (or a sequence substantially identical or similar thereto, e.g., a sequence at least 85%, 90%, 95% identical or higher to the sequence specified).

[00796] In some embodiments, the anti-TIGIT antibody is MTIG7192. MTIG7192 (Genentech) is a monoclonal antibody that binds to TIGIT. MTIG7192 and other humanized anti-TIGIT antibodies are disclosed in US2017088613, WO2017053748, and WO2016011264, and having a sequence disclosed herein (or a sequence substantially identical or similar thereto, e.g., a sequence at least 85%, 90%, 95% identical or higher to the sequence specified).

[00797] In some enbodiments, anti-TIGIT antibodies can be used in combination with an IL-12-Fc fusion protein of the invention. There are several TIGIT antibodies in clinical development, BMS-986207, OMP-313M32 and MTIG7192A.

[00798] In some embodiments, an IL-12-Fc fusion protein described herein can be used in combination with a TIGIT inhibitor (e.g., an anti-TIGIT antibody). In certain embodiments, an IL-12-Fc fusion protein (e.g., any XENP sequence described herein) described herein is administered in combination with an anti-TIGIT antibody.

6.Anti-LAG3 Antibodies

[00799] Exemplary non-limiting anti- LAG-3 antibody molecules are disclosed in US 2015/0259420 published on September 17, 2015, entitled "Antibody Molecules to LAG-3 and Uses Thereof," incorporated by reference in its entirety.

[00800] In one embodiment, the anti-LAG-3 antibody molecule includes at least one or two heavy chain variable domain (optionally including a constant region), at least one or two light chain variable domain (optionally including a constant region), or both, comprising the amino acid sequence of any of BAP050-hum01, BAP050-hum02, BAP050-hum03, BAP050-hum04, BAP050-hum05, BAP050-hum06, BAP050-hum07, BAP050-hum08, BAP050-hum09, BAP050-hum10, BAP050-hum11, BAP050-hum12, BAP050-hum13, BAP050-hum14, BAP050-hum15, BAP050-hum16, BAP050-hum17, BAP050-hum18, BAP050-hum19, BAP050-hum20, huBAP050(Ser) (e.g., BAP050-hum01-Ser, BAP050-hum02-Ser, BAP050-hum03-Ser, BAP050-hum04-Ser, BAP050-hum05-Ser, BAP050-hum06-Ser, BAP050-hum07-Ser, BAP050-hum08-Ser, BAP050-hum09-Ser, BAP050-hum10-Ser, BAP050-hum11-Ser, BAP050-hum12-Ser, BAP050-hum13-Ser, BAP050-hum14-Ser, BAP050-hum15-Ser, BAP050-hum18-Ser, BAP050-hum19-Ser,

or BAP050-hum20-Ser), BAP050-Clone-F, BAP050-Clone-G, BAP050-Clone-H, BAP050-Clone-I, or BAP050-Clone-J; or as described in Table 1 of US 2015/0259420, or encoded by the nucleotide sequence in Table 1; or a sequence substantially identical (e.g., at least 80%, 85%, 90%, 92%, 95%, 97%, 98%, 99% or higher identical) to any of the aforesaid sequences.

[00801] In yet another embodiment, the anti- LAG-3 antibody molecule includes at least one, two, or three complementarity determining regions (CDRs) from a heavy chain variable region and/or a light chain variable region of an antibody described herein, e.g., an antibody chosen from any of BAP050-hum01, BAP050-hum02, BAP050-hum03, BAP050-hum04, BAP050-hum05, BAP050-hum06, BAP050-hum07, BAP050-hum08, BAP050-hum09, BAP050-hum10, BAP050-hum11, BAP050-hum12, BAP050-hum13, BAP050-hum14, BAP050-hum15, BAP050-hum16, BAP050-hum17, BAP050-hum18, BAP050-hum19, BAP050-hum20, huBAP050(Ser) (e.g., BAP050-hum01-Ser, BAP050-hum02-Ser, BAP050-hum03-Ser, BAP050-hum04-Ser, BAP050-hum05-Ser, BAP050-hum06-Ser, BAP050-hum07-Ser, BAP050-hum08-Ser, BAP050-hum09-Ser, BAP050-hum10-Ser, BAP050-hum11-Ser, BAP050-hum12-Ser, BAP050-hum13-Ser, BAP050-hum14-Ser, BAP050-hum15-Ser, BAP050-hum18-Ser, BAP050-hum19-Ser, or BAP050-hum20-Ser), BAP050-Clone-F, BAP050-Clone-G, BAP050-Clone-H, BAP050-Clone-I, or BAP050-Clone-J; or as described in Table 1 of US 2015/0259420, or encoded by the nucleotide sequence in Table 1; or a sequence substantially identical (e.g., at least 80%, 85%, 90%, 92%, 95%, 97%, 98%, 99% or higher identical) to any of the aforesaid sequences.

[00802] In yet another embodiment, the anti- LAG-3 antibody molecule includes at least one, two, or three CDRs (or collectively all of the CDRs) from a heavy chain variable region comprising an amino acid sequence shown in Table 1 of US 2015/0259420, or encoded by a nucleotide sequence shown in Table 1. In one embodiment, one or more of the CDRs (or collectively all of the CDRs) have one, two, three, four, five, six or more changes, e.g., amino acid substitutions or deletions, relative to the amino acid sequence shown in Table 1, or encoded by a nucleotide sequence shown in Table 1.

[00803] In yet another embodiment, the anti-LAG-3 antibody molecule includes at least one, two, or three CDRs (or collectively all of the CDRs) from a light chain variable region comprising an amino acid sequence shown in Table 1 of US 2015/0259420, or encoded by a nucleotide sequence shown in Table 1. In one embodiment, one or more of the CDRs (or collectively all of the CDRs) have one, two, three, four, five, six or more changes, e.g., amino acid substitutions or deletions, relative to the amino acid sequence shown in Table 1, or encoded by a nucleotide sequence shown in Table 1. In certain embodiments, the anti-PD-L1 antibody molecule includes a substitution in a light chain CDR, e.g., one or more substitutions in a CDR1, CDR2 and/or CDR3 of the light chain.

[00804] In another embodiment, the anti- LAG-3 antibody molecule includes at least one, two, three, four, five or six CDRs (or collectively all of the CDRs) from a heavy and light chain variable region comprising an amino acid sequence shown in Table 1, or encoded by a nucleotide sequence shown in Table 1 of US 2015/0259420. In one embodiment, one or more of the CDRs (or collectively all of the CDRs) have one, two, three, four, five, six or more changes, e.g., amino acid substitutions or deletions, relative to the amino acid sequence shown in Table 1, or encoded by a nucleotide sequence shown in Table 1.

[00805] In one embodiment, the anti- LAG-3 antibody molecule includes:

(i) a heavy chain variable region (VH) including a VHCDR1 amino acid sequence chosen from SEQ ID NO: 1, SEQ ID NO: 4 or SEQ ID NO: 286; a VHCDR2 amino acid sequence of SEQ ID NO: 2; and a VHCDR3 amino acid sequence of SEQ ID NO: 3, each disclosed in Table 1 of US 2015/0259420; and

(ii) a light chain variable region (VL) including a VLCDR1 amino acid sequence of SEQ ID NO: 10, a VLCDR2 amino acid sequence of SEQ ID NO: 11, and a VLCDR3 amino acid sequence of SEQ ID NO: 12, each disclosed in Table 1 of US 2015/0259420.

[00806] In another embodiment, the anti-LAG-3 antibody molecule includes:

(i) a heavy chain variable region (VH) including a VHCDR1 amino acid sequence chosen from SEQ ID NO: 1, SEQ ID NO: 4 or SEQ ID NO: 286; a VHCDR2 amino acid sequence of SEQ ID NO: 5, and a VHCDR3 amino acid sequence of SEQ ID NO: 3, each disclosed in Table 1 of US 2015/0259420; and

(ii) a light chain variable region (VL) including a VLCDR1 amino acid sequence of SEQ ID NO: 13, a VLCDR2 amino acid sequence of SEQ ID NO: 14, and a VLCDR3 amino acid sequence of SEQ ID NO: 15, each disclosed in Table 1 of US 2015/0259420.

[00807] In one embodiment, the anti-LAG-3 antibody molecule comprises the VHCDR1 amino acid sequence of SEQ ID NO: 1. In another embodiment, the anti-LAG-3 antibody molecule comprises the VHCDR1 amino acid sequence of SEQ ID NO: 4. In yet another embodiment, the anti-LAG-3 antibody molecule comprises the VHCDR1 amino acid sequence of SEQ ID NO: 286, each disclosed in Table 1 of US 2015/0259420.

[00808] In some embodiments, the anti-LAG-3 antibody is BMS-986016. BMS-986016 (also referred to as BMS986016; Bristol-Myers Squibb) is a monoclonal antibody that binds to LAG-3. BMS-986016 and other humanized anti-LAG-3 antibodies are disclosed in US 2011/0150892, WO2010/019570, and WO2014/008218.

[00809] In some embodiments, the anti-LAG3 antibody is LAG525. LAG525 (also referred to as IMP701; Novartis) is a monoclonal antibody that binds to LAG3. LAG525 and other humanized anti-LAG3 antibodies are disclosed in US 9,244,059 and WO2008132601, and having a sequence disclosed herein (or a sequence substantially identical or similar thereto, e.g., a sequence at least 85%, 90%, 95% identical or higher to the sequence specified).

[00810] Other exemplary anti-LAG3 antibodies are disclosed, *e.g.*, in US2011150892 and US2018066054.

[00811] In some embodiments, anti-LAG-3 antibodies can be used in combination with an IL-12-Fc fusion protein of the invention. There are several anti-LAG-3 antibodies in clinical

development including REGN3767, by Regeneron, BMS-986016 (Bristol-Myers Squibb), MK-4280 (Merck), LAG525 (Novartis), and TSR-033 (Tesaro).

[00812] In some embodiments, an IL-12-Fc fusion protein described herein can be used in combination with a LAG3 inhibitor (e.g., an anti-LAG3 antibody). In certain embodiments, an IL-12-Fc fusion protein (e.g., any XENP sequence described herein) described herein is administered in combination with an anti-LAG3 antibody.

XII. Treatments

[00813] Once made, the compositions of the invention find use in a number of oncology applications, by treating cancer, generally by promoting T cell activation (e.g., T cells are no longer suppressed) with the binding of the heterodimeric Fc fusion proteins of the invention.

[00814] Accordingly, the heterodimeric compositions of the invention find use in the treatment of these cancers.

A. Fusion Protein Compositions for *In Vivo* Administration

[00815] Formulations of the fusion proteins used in accordance with the present invention are prepared for storage by mixing a fusion protein having the desired degree of purity with optional pharmaceutically acceptable carriers, excipients or stabilizers (as generally outlined in Remington's Pharmaceutical Sciences 16th edition, Osol, A. Ed. [1980]), in the form of lyophilized formulations or aqueous solutions.

B. Administrative Modalities

[00816] The fusion proteins and chemotherapeutic agents of the invention are administered to a subject, in accord with known methods, such as intravenous administration as a bolus or by continuous infusion over a period of time.

[00817]

B. Treatment Modalities

[00818] In the methods of the invention, therapy is used to provide a positive therapeutic response with respect to a disease or condition. By "positive therapeutic response" is intended an improvement in the disease or condition, and/or an improvement in the symptoms associated with the disease or condition. For example, a positive therapeutic response would refer to one or more of the following improvements in the disease: (1) a reduction in the number of neoplastic cells; (2) an increase in neoplastic cell death; (3) inhibition of neoplastic cell survival; (5) inhibition (i.e., slowing to some extent, preferably halting) of tumor growth; (6) an increased patient survival rate; and (7) some relief from one or more symptoms associated with the disease or condition.

[00819] Positive therapeutic responses in any given disease or condition can be determined by standardized response criteria specific to that disease or condition. Tumor response can be assessed for changes in tumor morphology (i.e., overall tumor burden, tumor size, and the like) using screening techniques such as magnetic resonance imaging (MRI) scan, x-radiographic imaging, computed tomographic (CT) scan, bone scan imaging, endoscopy, and tumor biopsy sampling including bone marrow aspiration (BMA) and counting of tumor cells in the circulation.

[00820] In addition to these positive therapeutic responses, the subject undergoing therapy may experience the beneficial effect of an improvement in the symptoms associated with the disease.

[00821] Treatment according to the present invention includes a "therapeutically effective amount" of the medicaments used. A "therapeutically effective amount" refers to an amount effective, at dosages and for periods of time necessary, to achieve a desired therapeutic result.

[00822] A therapeutically effective amount may vary according to factors such as the disease state, age, sex, and weight of the individual, and the ability of the medicaments to elicit a desired response in the individual. A therapeutically effective amount is also one in which any

toxic or detrimental effects of the protein or protein portion are outweighed by the therapeutically beneficial effects.

[00823] A "therapeutically effective amount" for tumor therapy may also be measured by its ability to stabilize the progression of disease. The ability of a compound to inhibit cancer may be evaluated in an animal model system predictive of efficacy in human tumors.

[00824] Alternatively, this property of a composition may be evaluated by examining the ability of the compound to inhibit cell growth or to induce apoptosis by in vitro assays known to the skilled practitioner. A therapeutically effective amount of a therapeutic compound may decrease tumor size, or otherwise ameliorate symptoms in a subject. One of ordinary skill in the art would be able to determine such amounts based on such factors as the subject's size, the severity of the subject's symptoms, and the particular composition or route of administration selected.

[00825] Dosage regimens are adjusted to provide the optimum desired response (e.g., a therapeutic response). For example, a single bolus may be administered, several divided doses may be administered over time or the dose may be proportionally reduced or increased as indicated by the exigencies of the therapeutic situation. Parenteral compositions may be formulated in dosage unit form for ease of administration and uniformity of dosage. Dosage unit form as used herein refers to physically discrete units suited as unitary dosages for the subjects to be treated; each unit contains a predetermined quantity of active compound calculated to produce the desired therapeutic effect in association with the required pharmaceutical carrier.

[00826] The specification for the dosage unit forms of the present invention are dictated by and directly dependent on (a) the unique characteristics of the active compound and the particular therapeutic effect to be achieved, and (b) the limitations inherent in the art of compounding such an active compound for the treatment of sensitivity in individuals.

[00827] The efficient dosages and the dosage regimens for the heterodimeric proteins used in the present invention depend on the disease or condition to be treated and may be determined by the persons skilled in the art.

[00828] An exemplary, non-limiting range for a therapeutically effective amount of an heterodimeric proteins used in the present invention is about 0.1-100 mg/kg.

[00829] All cited references are herein expressly incorporated by reference in their entirety.

[00830] Whereas particular embodiments of the invention have been described above for purposes of illustration, it will be appreciated by those skilled in the art that numerous variations of the details may be made without departing from the invention as described in the appended claims.

EXAMPLES

[00831] Examples are provided below to illustrate the present invention. These examples are not meant to constrain the present invention to any particular application or theory of operation. For all constant region positions discussed in the present invention, numbering is according to the EU index as in Kabat (Kabat et al., 1991, Sequences of Proteins of Immunological Interest, 5th Ed., United States Public Health Service, National Institutes of Health, Bethesda, entirely incorporated by reference). Those skilled in the art of antibodies will appreciate that this convention consists of nonsequential numbering in specific regions of an immunoglobulin sequence, enabling a normalized reference to conserved positions in immunoglobulin families. Accordingly, the positions of any given immunoglobulin as defined by the EU index will not necessarily correspond to its sequential sequence.

[00832] General and specific scientific techniques are outlined in US Publications 2015/0307629, 2014/0288275 and WO2014/145806, all of which are expressly incorporated by reference in their entirety and particularly for the techniques outlined therein.

Example 1: IL-12-Fc Fusion Proteins

[00833] As with other cytokines, IL-12 has a short half-life, and high dose treatment to overcome the short half-life results in systemic toxicity. Additionally, it has also been reported that anti-tumor effect requires sustained induction of IFNγ production by IL-12 (Gollob, JA et al., 2000). Further, the IL-12p40 subunit, as either a monomer or a homodimer, has been reported to antagonize IL-12 activity by competing for binding to IL-12 receptors (Gillessen, S et al., 1995); accordingly, it is advantageous to pre-complex the IL-12p40 and IL-12p35 subunits. In order to address these two caveats, we engineered the IL-12 heterodimer as Fc fusion proteins (collectively referred to hereon as IL-12-Fc fusions) both to enhance circulation through FcRn-mediated recycling and to pre-complex the IL-12p40 and IL-12p35 subunits.

1A: Engineering IL-12-Fc fusions in various formats

[00834] We generated the N-terminal IL-12 heterodimeric Fc fusion or "IL-12-heteroFc" format which comprises the IL-12p40 subunit recombinantly fused to the N-terminus of one side of a heterodimeric Fc and the IL-12p35 subunit recombinantly fused to N-terminus of the other side of the heterodimeric Fc (Figure 8A). The IL-12p40 and IL-12p35 subunits may be linked to their respective Fc chains by a domain linker. An illustrative protein of this format is XENP27201, sequences for which are depicted in Figure 9.

[00835] We also generated the C-terminal IL-12 heterodimeric Fc fusion or "heteroFc-IL-12" format which comprises the IL-12p40 subunit recombinantly fused to the C-terminus of one side of a heterodimeric Fc and the IL-12p35 subunit recombinantly fused to the C-terminus of the other side of the heterodimeric Fc (Figure 8B). The IL-12p30 and IL-12p35 subunits may be linked to their respective Fc chains by a domain linker. An illustrative protein of this format is XENP27202, sequences for which are depicted in Figure 10.

[00836] We further generated the N-terminal single-chain IL-12-Fc fusion or "scIL-12-Fc" format which comprises a single-chain IL-12 complex (or "scIL-12 complex") recombinantly fused to the N-terminus of one side of a heterodimeric Fc (optionally via a domain linker), with the other

side of the molecule being a "Fc-only" or "empty-Fc" heterodimeric Fc (Figure 8C-D). The scIL-12 complex can comprise either IL-12p35 N-terminally linked to IL-12p40 or IL-12p40 N-terminally linked to IL-12p35, optionally with a domain linker. The order of the two subunits in the scIL-12 complex are designated herein as follows: "scIL-12(p40/p35)", wherein the IL-12p40 subunit is N-terminally linked to the IL-12p35 subunit, or "scIL-12(p35/p40)", wherein the IL-12p35 is N-terminally linked to the IL-12p35 subunit. Illustrative proteins of the scIL-12-Fc format include XENP27203 and XENP27204, sequences for which are depicted in Figure 11.

[00837] Cartoon schematics for additional IL-12-Fc formats contemplated for use are depicted in Figure 8.

1B: Production and physical characterization of illustrative IL-12-Fc fusions

1B(a): IL-12-heteroFc

[00838] Plasmids coding for the IL-12p35 and IL-12p40 subunits were constructed by standard gene synthesis, followed by subcloning into a pTT5 expression vector containing Fc fusion partners (e.g., constant regions as depicted in Figure 7). Proteins were produced by transient transfection in HEK293E cells and were purified by a two-step purification process comprising protein A chromatography (purification part 1) followed by anion exchange chromatography (purification part 2). Chromatogram depicting purification part 2 for illustrative IL-12-heteroFc XENP27201 is depicted in Figure 12A. The chromatogram shows the isolation of two peaks (peak A and peak B), which were further characterized by analytical size-exclusion chromatography with multi-angle light scattering (aSEC-MALS) and analytical anion-exchange chromatography (analytical AIEX) for identity, purity and homogeneity as generally described below.

[00839] Peaks A and B isolated from purification part 2 for XENP27201 were analyzed using aSEC-MALS to deduce their component protein species. The analysis was performed on an Agilent 1200 high-performance liquid chromatography (HPLC) system. Samples were injected onto a Superdex™ 200 10/300 GL column (GE Healthcare Life Sciences) at 1.0 mL/min using 1 x

PBS, pH 7.4 as the mobile phase at 4°C for 25 minutes with UV detection wavelength at 280 nM. MALS was performed on a miniDAWN® TREOS® with an Optilab® T-rEX Refractive Index Detector (Wyatt Technology, Santa Barbara, Cali.). Analysis was performed using Agilent OpenLab Chromatography Data System (CDS) ChemStation Edition AIC version C.01.07 and ASTRA version 6.1.7.15. Chromatograms depicting aSEC separation profiles for peaks A and B are depicted in Figure 12B along with MW of component species as determined by MALS. The profiles show that peak A comprises species with molecular weights of ~299kD and ~140kD, while peak B primarily comprises a species with molecular weight of ~118kD, which is consistent with the calculated molecular weight of XENP27201 (based on amino acid sequence) of 110.4 kDa taking into account additional mass contributed by glycans.

[00840] The peaks from purification part 2 were also analyzed using analytical AIEX to further assess the purity and homogeneity of peak B. The analysis was performed on an Agilent 1200 high-performance liquid chromatography (HPLC) system. Samples were injected onto a Proteomix SAX-NP5 5µM non-porous column (Sepax Technologies, Inc., Newark, Del.) at 1.0 mL/min using 0-40% NaCl gradient in 20 mM Tris, pH 8.5 buffer with UV detection wavelength at 280 nM. Analysis was performed using Agilent OpenLAB CDS ChemStation Edition AIC version C.01.07. Chromatograms depicting analytical AIEX separation of peaks A and B are depicted in Figure 12C. Consistent with the aSEC separation profile for peak B, the analytical AIEX separation profile for peak B illustrates the purity and homogeneity of species in peak B. From here on, XENP27201 refers to peak B as isolated from purification part 2 as depicted in Figure 12A.

1B(b): scIL-12(p40/p35)-Fc

[00841] Plasmids coding for IL-12p40 recombinantly fused to IL-12p35 via a linker were constructed by standard gene synthesis, followed by subcloning into a pTT5 expression vector containing Fc fusion partners (e.g., constant regions as depicted in Figure 7). Proteins were produced by transient transfection in HEK293E cells and were purified by a two-step

purification process comprising protein A chromatography (purification part 1) and anion exchange chromatography (purification part 2).

[00842] Chromatogram depicting purification part 2 for illustrative scIL-12(p40/p35)-Fc fusion XENP27203 is depicted in Figure 13A. As above, the chromatogram shows the isolation of two peaks, which were further characterized by aSEC-MALS and analytical AIEX for identity, purity and homogeneity as described in Example 1B(a), chromatograms for which are depicted in Figures 13B-C.

[00843] The aSEC separation profiles for peaks A and B isolated from purification part 2 of XENP27203 show that peak A comprises species with molecular weights of ~396kD, ~188kD, and ~118kD, while peak B primarily comprises a species with molecular weight of ~118kD, which is consistent with the calculated molecular weight of XENP27203 (based on amino acid sequence) of 111.3 kDa taking into account additional mass contributed by glycans. The peaks were also analyzed using analytical AIEX as described in Example 1B(a) to further investigate the purity and homogeneity of peak B. Consistent with the aSEC separation profile for peak B, the analytical AIEX separation profile for peak B (Figure 13C) illustrates the purity and homogeneity of species in peak B. From here on, XENP27203 refers to peak B as isolated from anion exchange as depicted in Figure 13A.

1C: In vitro activity of IL-12-Fc fusions in induction of STAT4 phosphorylation

[00844] Following binding of cytokines to their receptors, Janus kinases (JAKs) associated with the cytokine receptors phosphorylate STAT proteins which then translocate into the nucleus to regulate further downstream processes. In particular, IL-12 mediates IFNγ expression and secretion through phosphorylation of STAT4 (Morinobu et al., 2002). Accordingly, the ability of the above described IL-12-heteroFc (XENP27201) and scIL-12(p40/p35)-Fc (XENP27203) to induce STAT4 phosphorylation in various lymphocyte populations was investigated. Bivalent IL-12p35-Fc fusions and IL-12p40-Fc fusions (cartoon schematics and sequences for which are depicted in Figures 14-15) as well as recombinant IL-12 were used as controls.

[00845] Fresh PBMCs were activated by incubation with plate bound anti-CD3 (100 ng/ml) for 3 days. Following activation, PBMCs were then incubated with the indicated test articles at the indicated concentrations for 15 minutes at 37°C. Following incubation, PBMCs were first stained with anti-CD3-BUV395 (UCHT1), anti-CD4-BV605 (RPA-T4), anti-CD8-AF700 (SK1), anti-CD14-APC/Fire750 (M5E2), anti-CD20-PerCP/5.5 (2H7), anti-CD25-BV421 (M-A251), and anti-CD56-PE antibodies. Following the first stain, cells were permeabilized using PerFix EXPOSE (Beckman Coulter, Indianapolis, Ind.). Following permeabilization, cells were stained with anti-anti-CD45RA-BV510 (HI100), anti-FoxP3-AF488 (259D), and anti-pSTAT4-AF647 (38/p-Stat4) antibodies. Following the second staining, the cells were analyzed by flow cytometry to investigate STAT4 phosphorylation on various lymphocyte populations. Data depicting pSTAT4 MFI on various lymphocyte populations, indicating signaling by the IL-12-Fc fusions via IL-12 receptors, are depicted in Figure 16.

[00846] The data show that both XENP27201 and XENP27203 were active in inducing STAT4 phosphorylation in various lymphocyte populations to a similar level as that induced by recombinant IL-12, while the bivalent IL-12p40-Fc (XENP27560) and bivalent IL-12p35-Fc (XENP27561) fusions were inactive. Notably, the two IL-12-Fc fusion formats demonstrated similar potency. Additionally, the ability of the purified IL-12-Fc fusion proteins to induce STAT4 phosphorylation in comparison to XENP27560 and XENP27561 confirms that that peak B isolated from purification part 2 for both XENP27201 and XENP27203 (as described in Example 1B) consisted of the active species comprising the complete IL-12 heterodimer.

Example 2: IL-12 Variants Engineered for Reduced Potency

[00847] In order to further prolong half-life as well as reduce potential for toxicity, we engineered IL-12 variants with decreased binding affinity for IL-12 receptors as we reasoned that this would decrease the antigen sink as well as reduce potency.

2A(a): Engineering IL-12p40 variants

[00848] We first identified W15, P17, D18, A19, P20, G21, M23, L40, D41, Q42, S43, E45, L47, T54, I55, Q56, K58, E59, F60, G61, D62, Y66, and K84 as potential sites on IL-12p40 for introducing affinity-modulating substitutions (residues depicted in Figure 17).

[00849] As a second strategy, by using the QuaSAR package in MOE software, we identified highly exposed aspartic acid, glutamic acid, asparagine, and glutamine residues (according to the water accessible surface area calculated using a radius of 1.4 Å for the water molecule and a polyhedral representation for each atom) in the IL-12p40 crystal structure (PDB code 3HMX) reasoning that these residues may contribute to binding between IL-12p40 and the IL-12 receptors. In particular, we focused our search on residues at which we could incorporate isosteric substitutions (i.e. Asn and Asp; and Gln and Glu), with the aim to minimize potential for immunogenicity, Accordingly, we identified E3, D7, E12, D14, D18, E22, D29, E32, E33, D41, Q42, E45, Q56, E59, D62, Q65, E73, E86, D93, D97, E100, N103, E110, D129, D142, Q144, E156, D161, N162, E166, E170, Q172, D174, E187, N200, D209, D214, N218, Q220, N226, Q229, E231, E235, Q256, E262, D265, D270, N281, Q289, and E299 which have an ASA score of at least 19 (residues and ASA scores depicted in Figure 18).

[00850] As described above, the p40 subunit is shared by IL-12 and IL-23. Bloch et al. (2018) reported the crystal structure for IL-23 in complex with IL-23R and Nb22E11. As a third strategy, we reasoned that residues on the IL-12p40 subunit in contact with IL-23R may also be involved with binding the analogous receptor IL-12Rβ2 in the IL-12 receptor complex. Accordingly, using the crystal structure reported by Bloch et al. (PDB code 5MZV) and modeling in MOE software, we identified D87, G88, I89, W90, K104, and F106 as residues on IL-12p40 potentially in contact with IL-12Rβ2 (residues and predicted contact type depicted in Figure 19).

[00851] Finally, to ensure that we do not disturb the natural interaction between the IL-12p40 and IL-12p35 subunits, we identified the following residues on IL-12p40 potentially in contact with IL-12p35 based on the crystal structure reported by Luo et al. (2010): K102, Y114, A176,

C177, P178, A179, A180, E181, S183, P185, S204, F206, R208, T242, P243, S245, Y246, F247, S248, D290, R291, Y292, and Y293.

[00852] In view of the above we designed a number of IL-12p40 variants, in particular as described above, at residue at which isosteric substitutions could be introduced, with the aim to to reduce the affinity of the IL-12 heterodimer to IL-12 receptors. Substitutions to remove potential N-glycosylation sites in p40 were also designed to examine the impact of glycosylation on protein heterogeneity. Sequences for illustrative variants are depicted in Figure 20. Plasmids coding for the IL-12p40 variants were constructed by standard gene synthesis, followed by subcloning into a pTT5 expression vector containing Fc fusion partners (e.g., constant regions as depicted in Figure 7), corresponding amino acid sequences for which are depicted in Figure 21.

2A(b): Engineering IL-12p35 variants

[00853] As with the second strategy described above, we used the QuaSAR package in MOE software to identify highly exposed aspartic acid, glutamic acid, asparagine, and glutamine residues in the IL-12p35 crystal structure (PDB code 3HMX) reasoning that these residues may contribute to binding between the IL-12 heterodimeric complex and the IL-12 receptors. Again, we focused here, in particular, on residues at which we could incorporate isosteric substitutions, to minimize the potential for immunogenicity. We identified Q35, E38, E46, D55, E67, N71, N76, N85, Q135, Q146, N151, E153, E162, and E163 which have an ASA score of at least 103 (residues and ASA scores depicted in Figure 22).

[00854] As above, to ensure that we do not disturb the natural interaction between the IL-12p40 and IL-12p354 subunits, we identified the following residues on IL-12p35 potentially in contact with IL-12p40 based on the crystal structure reported by Luo et al. (2010): Q20, S44, E45, E46, H49, K54, T59, V60, E61, C63, L64, P65, E67, L68, S73, C74, R181, I182, R183, V185, T186, D188, R189, V190, S192, Y193, and A196.

In view of the above we designed a number of IL-12p35 variants with isosteric substitutions with the aim to reduce the affinity of the IL-12 heterodimer to IL-12 receptors. Substitutions to

remove potential N-glycosylation sites in p35 were also designed to examine the impact of glycosylation on protein heterogeneity. Sequences for illustrative variants are depicted in Figure 23. Plasmids coding for the IL-12p35 variants were constructed by standard gene synthesis, followed by subcloning into a pTT5 expression vector containing Fc fusion partners (e.g., constant regions as depicted in Figure 7), corresponding amino acid sequences for which are depicted in Figure 24.

2A(c): Screening IL-12-Fc fusions having reduced potency

[00855] Illustrative variant IL-12-Fc fusion proteins having the above described variant IL-12 subunits were designed with the view to generate IL-12-Fc fusions having reduced affinity for the IL-12 receptors in order to reduce potency, sequences for which are depicted in Figure 25. We produced and purified the IL-12-Fc fusions as generally described in Example 1B, and investigated their activity in a pSTAT4 assay.

[00856] Human PBMCs were activated with 100 ng/ml anti-CD3 (OKT3) for 2 days. Activated PBMCs were then incubated with the indicated test articles at 37°C for 15 minutes. Cells were then stained with anti-CD3-BUV395 (UCHT1), anti-CD4-BV605 (RPAT4), anti-CD8-AF700 (SK1), anti-CD25-BV510 (M-A251), anti-CD45RA-BV421 (HI100), anti-CD56-PE (N901), anti-FoxP3-AF488 (259D), and anti-pSTAT4-AF647 (38/p-Stat4) using PerFix EXPOSE kit (Beckman Coulter, Indianapolis, Ind.) and analyzed by flow cytometry.

[00857] Data showing induction of STAT4 phosphorylation on $CD4^+CD45RA^+CD25^+$ and $CD8^+CD45RA^+CD25^+$ T cells by IL-12-Fc fusion having IL-12p35 or IL-12p40 variants are depicted respectively in Figures 26 and 27. Figure 28 depicts the EC50 of the various variant IL-12-Fc fusions and the fold decrease in EC50 relative to WT IL-12-Fc XENP27201. The data show that most of the IL-12-Fc fusions comprising variant IL-12p40 subunits or IL-12p30 subunits exhibit decreased potency in inducing STAT4 phosphorylation. Notably, E59Q and E235Q substitutions in the IL-12p40 subunit, and E153Q and N151D substitutions in the IL-12p35 substitutions were able to individually provide over 2 -fold reduction in potency for the variant

IL-12-Fc fusions. Additionally, IL-12p40 double- and triple-mutants comprising E59Q provided up to 6.5-fold reduction in potency.

[00858] Surprisingly, two variant IL-12-Fc fusions comprising IL-12p40(D87N) or IL-12p40(E262Q) exhibited an increase in potency. While we are interested in decreasing potency with the aim to decrease antigen sink and thereby increase the half-life of IL-12-Fc fusions, IL-12-Fc fusions having increased potency may find use in other contexts.

2B: Engineering IL-12p40 and IL-12p35 variants (Round 2)

[00859] In order to engineer further IL-12p40 and IL-12p35 variants with the aim to reduce their affinity for the IL-12 receptors and reduce the potency of the biologically active IL-12 complex, we re-applied the strategies described in Examples 2A with expanded criteria. For example, we used a lower ASA score threshold than that defined in Examples 2A for identifying IL-12p40 and IL-12p35 residues at which to introduce substitutions. We also expanded our criteria to include additional amino acid residues, with a particular focus on larger residues such as lysine, at which introduction of substitutions was more likely to disrupt the interaction of IL-12p35, IL-12p40, and/or the IL-12 complex with the IL-12 receptors.

[00860] Similarly, we introduced non-isosteric substitutions, with a particular focus on larger residues such as lysine, to disrupt the interaction of IL-12p35, IL-12p40, and/or the IL-12 complex with the IL-12 receptors. Using these approaches, we further identified D34, K99, K163, K258, and K260 in the IL-12p40 subunit; and N21, E79, Q130, N136, E143, K158, and D165 in the IL-12p35 subunit as residues suitable for engineering substitutions. In additional, we generated additional combination variants incorporating substitutions identified in Example 2 which contributed the greatest reduction in potency.

[00861] In view of the above, we designed further IL-12p40 and IL-12p35 variants, illustrative sequences for which are depicted respectively in Figures 29 and 31. As above, plasmids coding for the IL-12p40 and IL-12p35 variants were constructed by standard gene synthesis, followed by subcloning into a pTT5 expression vector containing Fc fusion partners, corresponding

amino acid sequences for which are depicted respectively in Figures 30 and 32. IL-12-Fc fusion proteins were generated with the additional variant IL-12 subunits, sequences for which are depicted in Figure 33, and produced as generally described in Example 1B.

[00862] The activity of the additional variant IL-12-Fc fusions were investigated in a pSTAT4 assay as generally described above. In particular, human PBMCs were activated with 100 ng/ml anti-CD3 (OKT3) for 2 days. Activated PBMCs were then incubated with the indicated test articles at 37°C for 15 minutes. Cells were then stained with anti-CD3-BUV395 (UCHT1), anti-CD4-BV605 (RPAT4), anti-CD8-AF700 (SK1), anti-CD25-BV510 (M-A251), anti-CD45RA-BV421 (HI100), and anti-CD56-PE (N901). Next, cells were permeabilized using PerFix EXPOSE (Beckman Coulter, Indianopolis, Ind.). Following permeabilization, cells were stained with anti-FoxP3-AF488 (259D) and anti-pSTAT4-AF647 (38/p-Stat4) and analyzed by flow cytometry. Data depicting pSTAT4 MFI on $CD4^+CD45RA^+CD25^+$ and $CD8^+CD45RA^+CD25^+$ T cells are depicted in Figure 34-35, and data depicting the EC50 (and fold decrease relative to WT IL-12-Fc XENP27201) are shown in Figure 36. The data show that most of the IL-12-Fc fusions comprising variant IL-12p40 and/or IL-12p30 subunits exhibit decreased potency in inducing STAT4 phosphorylation.

2C: Engineering IL-12p40 and IL-12p35 variants (Round 3)

[00863] In Example 2B, we found that IL-12-Fc fusion XENP29952 comprising IL-12p40(E59K) enabled a ~12 fold reduction, in contrast to IL-12-Fc fusion XENP28825 comprising IL-12p40(E59Q) which had only about 2-fold reduction in potency. Additionally, we noted that several double mutants comprising E59Q in the IL-12p40 subunit demonstrated >3.5 fold reduction in potency (e.g. XENP29953, XENP29954, and XENP29959). Further, we noted that while XENP28846 comprising IL-12p35(N151D) enabled increased potency, IL-12p35(D55Q/N151D) actually enabled ~2-fold reduction in potency, which indicated to us that modification of D55 in the IL-12p35 subunit contributes greatly to a reduction in potency. Accordingly, to further reduce the potency of IL-12-Fc fusions, we generated additional combination variants incorporating substitutions in Example 2B which contributed the greatest

reduction in potency. In addition as in Example 2B, we further included non-isosteric substitutions, with a particular focus on larger residues such as lysine.

[00864] In view of the above, we designed further IL-12p40 and IL-12p35 variants, illustrative sequences for which are depicted respectively in Figures 37 and 38. IL-12-Fc fusion proteins were generated with the additional variant IL-12 subunits, sequences for which are depicted in Figure 39, and produced as generally described in Example 1B. The activity of the additional variant IL-12-Fc fusions were investigated in a pSTAT4 assay as generally described above. Data depicting pSTAT4 MFI on $CD4^+CD45RA^+CD25^+$ and $CD8^+CD45RA^+CD25^+$ T cells are depicted in Figure 40, and data depicting the EC50 (and fold decrease relative to WT IL-12-Fc XENP27201) are shown in Figure 41. The data show that all of the new IL-12-Fc fusions comprising variant IL-12p40 and/or IL-12p30 subunits exhibited substantially decreased potency in inducing STAT4 phosphorylation. Notably, XENP30607 and XENP30608 exhibited ~100-fold decrease in potency in comparison to XENP27201.

2D: Engineering IL-12p40 and IL-12p35 variants (Round 4)

[00865] We further engineered the IL-12p40 and IL-12p35 subunits both by incorporating additional variants found in Example 2 contribute to a shift in potency, and by further using non-isosteric substitutions, with a particular focus on larger residues such as lysine and tyrosine, to disrupt the interaction of IL-12p35, IL-12p40, and/or the IL-12 complex with the IL-12 receptors.

[00866] In addition, we performed further structural analysis in the MOE software based on surface exposure to identify additional residues in the IL-12p40 and IL-12p35 subunits at which to introduce modifications. We further identified residues R159 and K264 in the IL-12p40 subunit; and residues L75, L89, F96, M97, L124, M125, and I171 in the IL-12p35 subunit. We used the earlier described approaches, as well as alanine substitutions (in line with the alanine scanning mutagenesis technique), to preliminarily investigate the effect of modifications at these additionally identified residues.

[00867] In view of the above, we designed further IL-12p40 and IL-12p35 variants, illustrative sequences for which are depicted respectively in Figures 42 and 43. IL-12-Fc fusion proteins were generated with the additional variant IL-12 subunits, sequences for which are depicted in Figure 44, and produced as generally described in Example 1B.

[00868] The activity of the additional variant IL-12-Fc fusions were investigated in a pSTAT4 assay as generally described above. Data depicting pSTAT4 MFI on $CD4^+CD45RA^+CD25^+$, $CD4^+CD45RA^-CD25^+$, $CD8^+CD45RA^+CD25^+$, and $CD8^+CD45RA^-CD25^+$ T cells are depicted in Figure 46, and data depicting the EC50 (and fold decrease relative to WT IL-12-Fc XENP27201) are shown in Figure 47. The data show that all of the new IL-12-Fc fusions comprising variant IL-12p40 and/or IL-12p35 subunits exhibited substantially decreased potency in inducing STAT4 phosphorylation.

Example 3: Bivalent IL-12-Fc Fusions

[00869] We also conceived bivalent IL-12-Fc fusions. One such format is the bivalent N-terminal IL-12-Fc fusion format (Figures 48A-B) which comprises two identical monomers each comprising a scIL-12 complex recombinant fused to the N-terminus of a homodimeric Fc chain (optionally via a domain linker). Another such format is the bivalent C-terminal IL-12-Fc fusion format (Figures 48C-D)which comprises two identical monomers each comprising a scIL-12 complex recombinant fused to the C-terminus of a homodimeric Fc chain (optionally via a domain linker).

Example 4: Reduced Potency IL-12 Variants in Alternative IL-12-Fc Fusion Formats

[00870] We also investigated the IL-12 variants in the context of additional IL-12-Fc fusion formats. Fusions in the scIL-12(p40/p35)-Fc and $(scIL\text{-}12(p40/p35))_2$-Fc formats were generated with either WT IL-12p40 subunits or variant IL-12p40(E59K/K99E) subunits and produced as generally described in Example 1B (and sequences for which are depicted in Figures 49-50).

[00871] The activity of the IL-12-Fc fusions were investigated in a pSTAT4 assay as generally described above. In particular, human PBMCs were activated with 100 ng/ml anti-CD3 (OKT3)

for 2 days at 37°C. Activated PBMCs were then incubated with the indicated test articles for 15 minutes at 37°C. Cells were then stained with anti-CD3-BUV395 (UCHT1), anti-CD4-BV605 (RPAT4), anti-CD8-AF700 (SK1), anti-CD25-BV711 (M-A251), anti-CD45RA-BV421 (HI100), and anti-CD56-PE (N901). Next, cells were permeabilized using PerFix EXPOSE (Beckman Coulter, Indianopolis, Ind.). Following permeabilization, cells were stained with anti-FoxP3-V450 (259D) and anti-pSTAT4-AF647 (38/p-Stat4) and analyzed by flow cytometry. Data depicting pSTAT4 MFI on $CD4^+CD45RA^+CD25^+$, $CD4^+CD45RA^-CD25^+$, $CD8^+CD45RA^+CD25^+$, and $CD8^+CD45RA^-CD25^+$ T cells are depicted in Figure 51, and data depicting the EC50 (and fold decrease relative to WT IL-12-Fc XENP27201) are shown in Figure 52. The data show that IL-12-Fc fusions in the various format demonstrated very similar potency, and the IL-12-Fc fusions in scIL-12(p40/p35)-Fc and $(scIL\text{-}12(p40/p35))_2$-Fc fusions comprising variant IL-12p40(E59K/K99E) subunits demonstrated very similar potency to IL-12-Fc fusions in the IL-12-heteroFc format comprising variant IL-12p40(E59K/K99E) subunit.

Example 5: IL-12-Fc Fusions enhance allogeneic anti-tumor effect of T cells in vivo

[00872] Next, we investigated *in vivo* anti-tumor effect of the IL-12-Fc fusions of the invention. NOD SCID gamma (NSG) mice were engrafted intradermally with 3×10^6 pp-65 expressing MCF-7 cells in the right flank on Day -15. On Day 0, mice were engrafted intraperitoneally with 1.5×10^6 human PBMCs. Mice (n = 15) were then treated on Days 0, 7, 14, and 21 with 0.03 mg/kg XENP29952, a reduced potency IL-12-Fc fusion in the IL-12-heteroFc format comprising variant IL-12p40(E59K). Controls (n = 10) used were PBS and XENP16432 (a bivalent anti-PD-1 mAb, a checkpoint inhibitor which enhances anti-tumor activity by de-repressing the engrafted human T cells; sequences depicted in Figure 53). Tumor volumes were monitored by caliper measurements, data for which are shown (days post 1st dose) in Figure 54. Blood and serum were drawn on Days 7, 14, and 21 and analyzed by flow cytometry to investigate expansion of human lymphocytes, data for which are depicted in Figure 55, as well as by U-PLEX Biomarker Group 1 Human Assays (Meso Scale, Rockville, Md.) for serum cytokine concentrations, data for which are depicted in Figure 56.

[00873] The data show that the IL-12-heteroFc fusion XENP29952 had significantly enhanced expansion of $CD45^+$, $CD3^+$ T cells, $CD4^+$ T cells, $CD8^+$ T cells, and NK cells by Day 14 in comparison to both PBS control and checkpoint blockade by XENP16432 (statistics performed on log-transformed data using unpaired t-test). Notably, XENP29952 significantly enhanced anti-tumor activity by Day 11 as indicated by change in tumor volume (statistics performed on baseline corrected data using unpaired t-test). Furthermore, XENP29952 significantly enhanced secretion of IFNγ and CD25 by Day 7 in comparison to checkpoint blockade by XENP16432 (statistics performed on log-transformed date using unpaired t-test).

[00874] In addition to anti-tumor activity, the engrafted human PBMCs develop an autoimmune response against mouse cells and subsequently graft-versus-host disease (GVHD). Accordingly, it should be noted that while all the animals treated with XENP29952 were dead by Day 19, this was likely due to their succumbing to GVHD exacerbated by significantly enhanced expansion of human lymphocytes. This highlights the importance of reduced potency IL-12 variants not just for improving pharmacokinetics but also for improving therapeutic index.

Example 6: IL-12-Fc Fusions Demonstrate Modulated Activity In Vivo Correlating to In Vitro Potency

[00875] As all the animals treated with XENP29952 in Example 5 were dead as a result of GVHD, we investigated the *in vivo* activity of IL-12-Fc fusions engineered with reduced potency IL-12 variants in a GVHD study.

[00876] NSG mice were engrafted with 10 x 10^6 human PBMCs via IV-OSP on Day -1 and dosed intraperitoneally on Days 0, 7, 14, and 21 with the following test articles: XENP29952 (a reduced potency IL-12-Fc fusion in the IL-12-heteroFc format comprising variant IL-12p40(E59K)), XENP30597 (a reduced potency IL-12-Fc fusion in the IL-12-heteroFc format comprising variant IL-12p40(E59K/K99E)), XENP31254 (a reduced potency IL-12-Fc fusion in the IL-12-heteroFc format comprising variant IL-12p40(D18K/E59K/K99E)), XENP31251 (a reduced potency IL-12-Fc fusion in the IL-12-heteroFc format comprising variant IL-12p40(E59K/K99Y)), or XENP31258 (a reduced potency IL-12-Fc fusion in the IL-12-heteroFc format comprising variant IL-

12p40(E59K/K99E/K264E)) at 0.3 or 0.03 mg/kg. Body weights were assessed twice per week as an indicator of GVHD, data for which are depicted in Figure 57 as a change in body weight (relative to initial body weight). Additionally, blood was drawn on Day 7, 10, 14, and 31 to investigate the activation of various T cell populations as indicated by PD-1 expression levels (data for which are depicted in Figures 58 and 59), and serum was drawn to investigate cytokine secretion (data for which are depicted in Figure 60). Notably, the data show a dose response for the test articles (i.e. enhanced GVHD, T cell activation, and IFNγ secretion by 0.3 mg/kg dose in comparison to 0.03 mg/kg dose).

[00877] It was surprising that XENP31251, which appeared to be one of the weakest variants in inducing STAT4 phosphorylation on $CD4^+$ T cells in vitro (see Figures 46A-46B), was one of the stronger inducers of GVHD. Accordingly, we re-investigated the *in vitro* activity of the same illustrative reduced potency IL-12-Fc in a STAT4 phosphorylation assay as generally described above using two separate PBMC donors. Human PBMCs were activated with 1 μg/ml anti-CD3 (OKT3) for 2 days at 37°C. Activated PBMCs were then incubated with the indicated test articles at 37°C for 15 minutes.

[00878] Cells were then stained with anti-CD3-BUV395 (UCHT1), anti-CD4-BV605 (RPAT4), anti-CD8-AF700 (SK1), anti-CD25-BV510 (M-A251), anti-CD45-BV785, anti-CD45RA-BV421 (HI100), anti-CD16-PE (B73), and anti-CD56-PE (N901). Next, cells were permeabilized using PerFix EXPOSE (Beckman Coulter, Indianapolis, Ind.). Following permeabilization, cells were stained with anti-FoxP3-AF488 (259D) and anti-pSTAT4-AF647 (38/p-Stat4) and analyzed by flow cytometry. Induction of STAT4 phosphorylation on various lymphocyte populations in PBMCs from the two donors are depicted in Figures 61 (data not shown for the second donor but incorporated by reference from Figure 62 in 62/848,512). The data from both donors and across the various lymphocyte populations show a potency ladder with XENP29952 as the most potent variant, XENP31254 and XENP31258 as the least potent variants, and XENP30597 and XENP31251 falling in between. Notably, the degree of GVHD, T cell activation, and IFNγ secretion as induced by the reduced potency IL-12-Fc fusion variants *in vivo* correlated with the *in vitro* potency. For example, at 0.03 mg/kg dose, XENP29952 induced greater GVHD, T cell

activation, and IFNγ secretion than all the reduced potency IL-12-Fc fusions, while XENP31254 and XENP31258 induced the least GVHD, T cell activation, and IFNγ secretion.

Example 7: IL-12-Fc Fusions Have Anti-Tumor Activity and Combine Productively with Checkpoint Blockade

[00879] Next, we investigated the *in vivo* anti-tumor effect of combining the additional IL-12-Fc fusions as well as the effect of combining the IL-12-Fc fusions with checkpoint blockade. NSG mice were engrafted intradermally with 3 x 10^6 pp-65 expressing MCF-7 cells in the right flank on Day -15. On Day 0, mice were engrafted intraperitoneally with 5 x 10^6 human PBMCs. Mice were then treated on Days 0, 7, 14, and 21 with 0.1 mg/kg XENP31251 alone or in combination with 3.0 mg/kg anti-PD-1 mAb XENP16432. Controls used were PBS, 3.0 mg/kg XENP16432 alone, and XENP31258. Tumor volumes were monitored by caliper measurements, data for which are shown (days post 1st dose) in Figure 62. Blood was drawn on Days 7, 14, and 21 and analyzed by flow cytometry to investigate expansion of human lymphocytes, data for which are depicted in Figure 63 for Day 14.

[00880] The data show that XENP31258 significantly enhanced anti-tumor activity by Day 14, and XENP31251 (alone or in combination with XENP16432) significantly enhanced anti-tumor activity (as indicated by change in tumor volume) by Day 16 in comparison to treatment with PBS (statistics performed on baseline corrected data using Mann-Whitney test). Notably, the data show that XENP31251 in combination with XENP16432 significantly enhanced anti-tumor activity by Day 21 in comparison to treatment with XENP16432 alone; and that treatment with XENP31251 in combination with XENP16432 significantly enhanced lymphocyte expansion in comparison to either XENP31251 or XENP16432 alone, indicating that IL-12-Fc fusions combine productively with checkpoint blockade.

Example 8: Removing Free Cysteine in IL-12p40 Subunit

[00881] The IL-12p40 subunit has a free cysteine at position 252 (numbered according to the Human IL-12 subunit beta (IL-12p40) mature form sequence as depicted in Figure 1) which may

bond with other free cysteines leading at least to heterogeneity and at worse to immunogenicity. Accordingly, IL-12p40 variants were engineered to remove the free cysteine, for example, by introducing C252S modification (although other substitutions may also be used). Modification of C252 (e.g. C252S) can be used alone or in combination with any other IL-12p40 variants, such as affinity or expression variants. Illustrative IL-12p40 variants comprising a modification at C252 to remove the free cysteine are depicted in Figure 65. Illustrative IL-12-Fc fusions proteins were generated with the additional variant IL-12p40 subunits, sequences for which are depicted in Figure 66, and produced as generally described in Example 1B.

[00882] The activity of the IL-12p40 variants engineered to remove the free cysteine were investigated in a pSTAT4 assay as generally described above in order to ascertain that removal of the free cysteine did not engender any change in activity. Activated PBMCs were incubated with the indicated test articles and pSTAT4 MFI on various populations were assessed, data for which are depicted in Figure 67. The data show that the variants comprising the C252S modification demonstrated similar activity to the counterpart variant which did not comprise the C252S modification. Notably, for 3 of the 4 variants tested for which we engineered versions with and without the C252S mutation (i.e. D18K/E59K/K99E; D18K/E59K/K99E/K264E; and E59K/K99E), the C252S mutation appeared to slightly increase the potency of the IL-12-Fc fusions.

[00883] The examples set forth above are provided to give those of ordinary skill in the art a complete disclosure and description of how to make and use the embodiments of the compositions, systems and methods of the invention, and are not intended to limit the scope of what the inventors regard as their invention. Modifications of the above-described modes for carrying out the invention that are obvious to persons of skill in the art are intended to be within the scope of the following claims. All patents and publications mentioned in the specification are indicative of the levels of skill of those skilled in the art to which the invention pertains. All references cited in this disclosure are incorporated by reference to the same extent as if each reference had been incorporated by reference in its entirety individually.

[00884] All headings and section designations are used for clarity and reference purposes only and are not to be considered limiting in any way. For example, those of skill in the art will appreciate the usefulness of combining various aspects from different headings and sections as appropriate according to the spirit and scope of the invention described herein.

[00885] All references cited herein are hereby incorporated by reference herein in their entireties and for all purposes to the same extent as if each individual publication or patent or patent application was specifically and individually indicated to be incorporated by reference in its entirety for all purposes.

[00886] Many modifications and variations of this application can be made without departing from its spirit and scope, as will be apparent to those skilled in the art. The specific embodiments and examples described herein are offered by way of example only, and the application is to be limited only by the terms of the appended claims, along with the full scope of equivalents to which the claims are entitled.

WHAT IS CLAIMED IS:

1. A heterodimeric Fc fusion protein comprising:

a) a first fusion protein comprising a variant IL-12p40 subunit domain and a first Fc domain, wherein said IL-12p40 subunit domain is covalently attached to the N-terminus of said first Fc domain; and

b) a second fusion protein comprising an IL-12p35 subunit domain and a second Fc domain, wherein said IL-12p35 subunit domain is covalently attached to the N-terminus of said Fc domain;

wherein said first and said second Fc domains comprise modifications promoting heterodimerization of said first and said second Fc domains.

2. The heterodimeric Fc fusion protein according to claim 1, wherein said IL-12p40 subunit has one or more amino acid substitutions selected from the group consisting of: E59K, E59Q, D18N, D18K, E32Q, E33Q, D34N, D34K, Q42E, S43E, S43K, E45Q, Q56E, D62N, E73Q, D87N, K99E, K99Y, E100Q, N103D, N103Q, N113D, N113Q, Q144E, D161N, R159E, K163E, E187Q, N200D, N200Q, N218Q, Q229E, E235Q, C252S, Q256N, K258E, K260E, E262Q, K264E, N281D, N281Q, and E299Q.

3. The heterodimeric Fc fusion protein according to claims 1 or 2, wherein said IL-12p40 subunit has amino acid substitutions selected from the group consisting of N103D/N113D/N200D/N281D, Q42E/E45Q, E45Q/Q56E, Q42E/E59Q, Q56E/E59Q, Q42E/E45Q/Q56E, E45Q/Q56E/E59Q, E32Q/E59Q, D34N/E59K, D34N/E59K/K99E, D34K/E59K/K99E, E32Q/D34N/E59K/K99E, E32K/D34N/E59K/K99E, D34N/E59Q, E59Q/E187Q, S43E/E59Q, S43K/E49Q, E59Q/K163E, E59Q/K99E, E59Q/K258E, E59Q/K260E, E59K/K99E, D18K/E59K/K99E, E59K/K99E/K264E, E59K/K99Y, E59Y/K99Y, E59Y/K99E, E45K/E59K/K99E, E59K/K99E/Q144E, E59K/K99E/Q144K, E59K/K99E/R159E, E59K/K99E/K264E, D18K/E59K/K99E/K264E, DI8K/E59K/K99E/C252S, D18K/E59K/K99E/C252S/K264E, E59K/K99Y/C252S, E59K/K99E/C252S/K264E, E59K/K99E/C252S, N103D/N113D,

N103D/N200D, N103D/N281D, N113D/N200D, N113D/N281D, N200D/N281D, N103D/N113D/N200D, N103D/N113D/N281D, N103D/N200D/N281D, N113D/N200D/N281D, N103Q/N113Q, N103Q/N200Q, N103Q/N281Q, N113Q/N200Q, N113Q/N281Q, N200Q/N281Q, N103Q/N113Q/N200Q, N103Q/N113Q/N281Q, N103Q/N200Q/N281Q, N113Q/N200Q/N281Q, N103Q/N113Q/N200Q/N281Q, E59K/K99E/N103Q/C252S/K264E, E59K/K99E/N113Q/C252S/K264E, E59K/K99E/N200Q/C252S/K264E, E59K/K99E/N281Q/C252S/K264E, E59K/K99E/N103Q/N113Q/C252S/K264E, E59K/K99E/N103Q/N200Q/C252S/K264E, E59K/K99E/N103Q/N281Q/C252S/K264E, E59K/K99E/N113Q/N200Q/C252S/K264E, E59K/K99E/N113Q/N281Q/C252S/K264E, E59K/K99E/N200Q/N281Q/C252S/K264E, E59K/K99E/N103Q/N113Q/N200Q/C252S/K264E, E59K/K99E/N103Q/N200Q/N281Q/C252S/K264E, E59K/K99E/N113Q/N200Q/N281Q/C252S/K264E, and E59K/K99E/N103Q/N113Q/N200Q/N281Q/C252S/K264E.

4. The heterodimeric Fc fusion protein according to any one of claims 1-3, wherein said IL-12p40 subunit has a polypeptide sequence selected from the group consisting of: i) SEQ ID NO:57 (IL-12p40(N103D)), ii) SEQ ID NO:58 (IL-12p40(N113D)), iii) SEQ ID NO:59 (IL-12p40(N200D)), iv) SEQ ID NO:60 (IL-12p40(N281D)), v) SEQ ID NO:61 (IL-12p40(N103D/N113D/N200D/N281D)), vi) SEQ ID NO:62 (IL-12p40(Q42E)), vii) SEQ ID NO:63 (IL-12p40(E45Q)), viii) SEQ ID NO:64 (IL-12p40(Q56E)), ix) SEQ ID NO:65 (IL-12p40(E59Q)), x) SEQ ID NO:66 (IL-12p40(D62N)), xi) SEQ ID NO:67 (IL-12p40(Q42E/E45Q)), xii) SEQ ID NO:68 (IL-12p40(E45Q/Q56E)), xiii) SEQ ID NO:69 (IL-12p40(Q42E/E59Q)), xiv) SEQ ID NO:70 (IL-12p40(Q56E/E59Q)), xv) SEQ ID NO:71 (IL-12p40(Q42E/E45Q/Q56E)), xvi) SEQ ID NO:72 (IL-12p40(E45Q/Q56E/E59Q)), xvii) SEQ ID NO:73 (IL-12p40(D161N)), xviii) SEQ ID NO:74 (IL-12p40(E73Q)), xix) SEQ ID NO:75 (IL-12p40(Q144E)), xx) SEQ ID NO:76 (IL-12p40(E262Q)), xxi) SEQ ID NO:77 (IL-12p40(E100Q)), xxii) SEQ ID NO:78 (IL-12p40(D18N)), xxiii) SEQ ID NO:79 (IL-12p40(E33Q)), xxiv) SEQ ID NO:80 (IL-12p40(Q229E)), xxv) SEQ ID NO:81 (IL-12p40(E235Q)), xxvi) SEQ ID NO:82 (IL-12p40(Q256N)), xxvii) SEQ ID NO:83 (IL-12p40(E299Q)), xxviii) SEQ ID NO:84 (IL-12p40(D87N)), xxix) IL-12p40(E32Q), xxx) IL-

12p40(D34N), xxxi) IL-12p40(S43E), xxxii) IL-12p40(S43K), xxxiii) SEQ ID NO:XXX (IL-12p40 (E59K/K99E/N103Q/N113Q/N200Q/N281Q/C252S/K264E)), xxxiv) SEQ ID NO:205 (IL-12p40(E59K)), xxxv) IL-12p40(K99E), xxxvi) IL-12p40(K163E), xxxvii) IL-12p40(E187Q), xxxviii) IL-12p40(K258E), xxxix) IL-12p40(K260E), xl) SEQ ID NO:206 (IL-12p40(E32Q/E59Q)), xli) SEQ ID NO:207 (IL-12p40(D34N/E59Q)), xlii) SEQ ID NO:208 (IL-12p40(E59Q/E187Q)), xliii) SEQ ID NO:209 (IL-12p40(S43E/E59Q)), xliv) SEQ ID NO:210 (IL-12p40(S43K/E49Q)), xlv) SEQ ID NO:211 (IL-12p40(E59Q/K163E)), xlvi) SEQ ID NO:212 (IL-12p40(E59Q/K99E)), xlvii) SEQ ID NO:213 (IL-12p40(E59Q/K258E)), xlviii) SEQ ID NO:214 (IL-12p40(E59Q/K260E)), xlix) SEQ ID NO: XXX (IL-12p40 (D34N/E59K)), l) SEQ ID NO: XXX (IL-12p40(E59K/K99E)), li) SEQ ID NO: XXX (IL-12p40(D18K/E59K/K99E)), lii) SEQ ID NO: XXX (IL-12p40 (E59K/K99E/K264E)), liii) SEQ ID NO: XXX (IL-12p40 (E59K/K99Y)), liv) SEQ ID NO: XXX (IL-12p40 (E59Y/K99E)), lv) SEQ ID NO: XXX (IL-12p40 (E45K/E59K/K99E)), lvi) SEQ ID NO: XXX (IL-12p40 (E59K/K99E/Q144E)), lvii) SEQ ID NO: XXX (IL-12p40 (E59K/K99E/Q144K)), lviii) SEQ ID NO: XXX (IL-12p40 (E59K/K99E/R159E)), lix) SEQ ID NO: XXX (IL-12p40 (E59K/K99E/K264E)), lx) SEQ ID NO: XXX (IL-12p40 (D18K/E59K/K99E/K264E)), lxi) SEQ ID NO: XXX (IL-12p40 (C252S)), lxii) SEQ ID NO: XXX (IL-12p40 (DI8K/E59K/K99E/C252S)), lxiii) SEQ ID NO: XXX (IL-12p40 (D18K/E59K/K99E/C252S/K264E)), lxiv) SEQ ID NO: XXX (IL-12p40 (E59K/K99Y/C252S)), lxv) SEQ ID NO: XXX (IL-12p40 (E59K/K99E/C252S/K264E)), lxvi) SEQ ID NO: XXX (IL-12p40 (E59K/K99E/C252S)), lxvii) SEQ ID NO: XXX (IL-12p40 (N103D/N113D)), lxviii) SEQ ID NO: XXX (IL-12p40 (N103D/N200D)), lxix) SEQ ID NO: XXX (IL-12p40 (N103D/N281D)), lxx) SEQ ID NO: XXX (IL-12p40 (N113D/N200D)), lxxi) SEQ ID NO: XXX (IL-12p40 (N113D/N281D)), lxxii) SEQ ID NO: XXX (IL-12p40 (N200D/N281D)), lxxiii) SEQ ID NO: XXX (IL-12p50 (N103D/N113D/N200D)), lxxiv) SEQ ID NO: XXX (IL-12p40 (N103D/N113D/N281D)), lxxv) SEQ ID NO: XXX (IL-12p40 (N103D/N200D/N281D)), lxxvi) SEQ ID NO: XXX (IL-12p40 (N113D/N200D/N281D)), lxxvii) SEQ ID NO: XXX (IL-12p40 (N103Q)), lxxviii) SEQ ID NO: XXX (IL-12p40 (N113Q)), lxxix) SEQ ID NO: XXX (IL-12p40 (N200Q)), lxxx) SEQ ID NO: XXX (IL-12p40 (N281Q)), lxxxi) SEQ ID NO: XXX (IL-12p40 (N103Q/N113Q)), lxxxii) SEQ ID NO: XXX (IL-12p40 (N103Q/N200Q)), lxxxiii) SEQ ID NO: XXX (IL-12p40 (N103Q/N281Q)), lxxxiv) SEQ ID NO: XXX (IL-12p40 (N113Q/N200Q)), lxxxv) SEQ ID

NO: XXX (IL-12p40 (N113Q/N281Q)), lxxxvi) SEQ ID NO: XXX (IL-12p40 (N200Q/N281Q)), lxxxvii) SEQ ID NO: XXX (IL-12p40 (N103Q/N113Q/N200Q)), lxxxviii) SEQ ID NO: XXX (IL-12p40 (N103Q/N113Q/N281Q)), lxxxix) SEQ ID NO: XXX (IL-12p40 (N103Q/N200Q/N281Q)), xc) SEQ ID NO: XXX (IL-12p40 (N113Q/N200Q/N281Q)), xci) SEQ ID NO:XXX (IL-12p40 (N103Q/N113Q/N200Q/N281Q)), xcii) SEQ ID NO:XXX (IL-12p40 (D34N/E59K/K99E)), xciii) SEQ ID NO:XXX (IL-12p40 (D34K/E59K/K99E)), xciv) SEQ ID NO:XXX (IL-12p40 (E32Q/D34N/E59K/K99E)), xcv) SEQ ID NO:XXX (IL-12p40 (E32K/D34N/E59K/K99E)), xcvi) SEQ ID NO: XXX (IL-12p40 (E59Y/K99Y)), xcvii) SEQ ID NO:XXX (IL-12p40 (E59K/K99E/N103Q/C252S/K264E)), xcviii) SEQ ID NO:XXX (IL-12p40 (E59K/K99E/N113Q/C252S/K264E)), xcix) SEQ ID NO:XXX (IL-12p40 (E59K/K99E/N200Q/C252S/K264E)), c) SEQ ID NO:XXX (IL-12p40 (E59K/K99E/N281Q/C252S/K264E)), ci) SEQ ID NO:XXX (IL-12p40 (E59K/K99E/N103Q/N113Q/C252S/K264E)), cii) SEQ ID NO:XXX (IL-12p40 (E59K/K99E/N103Q/N200Q/C252S/K264E)), ciii) SEQ ID NO:XXX (IL-12p40 (E59K/K99E/N103Q/N281Q/C252S/K264E)), civ) SEQ ID NO:XXX (IL-12p40 (E59K/K99E/N113Q/N200Q/C252S/K264E)), cv) SEQ ID NO:XXX (IL-12p40 (E59K/K99E/N113Q/N281Q/C252S/K264E)), cvi) SEQ ID NO:XXX (IL-12p40 (E59K/K99E/N200Q/N281Q/C252S/K264E)), cvii) SEQ ID NO:XXX (IL-12p40 (E59K/K99E/N103Q/N113Q/N200Q/C252S/K264E)), cviii) SEQ ID NO:XXX (IL-12p40 (E59K/K99E/N103Q/N200Q/N281Q/C252S/K264E)), and cix) SEQ ID NO:XXX (IL-12p40 (E59K/K99E/N113Q/N200Q/N281Q/C252S/K264E)).

5. The heterodimeric Fc fusion protein according to any one of claims 1-4, wherein said variant IL-12p40 subunit has at least 90% identity to a polypeptide sequence selected from the group consisting of SEQ ID NO:3 (human IL-12 subunit beta (IL-12p40) precursor sequence) and SEQ ID NO:4 (human IL-12 subunit beta (IL-12p40) mature form sequence), and/or said IL-12p35 subunit has a polypeptide sequence selected from the group consisting of SEQ ID NO:1 (human IL-12 subunit alpha (IL-12p35) precursor sequence) and SEQ ID NO:2 (human IL-12 subunit alpha (IL-12p35) mature form sequence).

6. The heterodimeric Fc fusion protein according to any one of claims 1-4, wherein said IL-12p35 subunit is a variant IL-12p35 subunit.

7. The heterodimeric Fc fusion protein according to any one of claims 1-4 and 6, wherein said IL-12p40 subunit is a variant IL-12p40 subunit having altered affinity for IL-12 receptor subunit beta-1 (IL-12Rß1), IL-12 receptor subunit beta-2 (IL-12Rß2), and/or IL-12 receptor complex; and/or said IL-12p35 subunit is a variant IL-12p35 subunit having altered affinity for IL-12 receptor subunit beta-1 (IL-12Rß1), IL-12 receptor subunit beta-2 (IL-12Rß2), and/or IL-12 receptor complex.

8. The heterodimeric Fc fusion protein according to any one of claims 6-7, wherein said variant IL-12p35 subunit has one or more amino acid substitutions selected from the group consisting of N21D, Q35D, E38Q, D55Q, D55K, N71D, N71Q, L75A, N76D, E79Q, N85D, N85Q, L89A, F96A, M97A, L124A, M125A, Q130E, Q135E, N136D, E143Q, Q146E, N151D, N151K, E153K, E153Q, K158E, E162Q, E163Q, D165N, I171A, N195D, and N195Q.

9. The heterodimeric Fc fusion protein according to any one of claims 6-8, wherein said variant IL-12p35 subunit has amino acid substitutions selected from the group consisting of: N71D/N85D/N195D, N151D/E153Q, N151D/D165N, Q130E/N151D, N151D/K158E, E79Q/N151D, D55Q/N151D, N136D/N151D, N21D/N151D, E143Q/N151D, N71Q/N85Q, N71Q/N195Q, N85Q/N195Q, N71Q/N85Q/N195Q, N71D/N85D, N71D/N195D, and N85D/N195D.

10. The heterodimeric Fc fusion protein according to any one of claims 6-9, wherein said variant IL-12p35 subunit has a polypeptide sequence selected from the group consisting of: i) SEQ ID NO:113 (IL-12p35(N71D)), ii) SEQ ID NO:114 (IL-12p35(N85D)), iii) SEQ ID NO:115 (IL-12p35(N195D)), iv) SEQ ID NO:116 (IL-12p35(N71D/N85D/N195D)), v) SEQ ID NO:117 (IL-12p35(E153Q)), vi) SEQ ID NO:118 (IL-12p35(E38Q)), vii) SEQ ID NO:119 (IL-12p35(N151D)), viii) SEQ ID NO:120 (IL-12p35(Q135E)), ix) SEQ ID NO:121 (IL-12p35(Q35D)), x) SEQ ID NO:122 (IL-12p35(Q146E)), xi) SEQ ID NO:123 (IL-12p35(N76D)), xii) SEQ ID NO:124 (IL-12p35(E162Q)), xiii) SEQ ID NO:125 (IL-12p35(E163Q)), xiv) IL-12p35(N21D), xv) SEQ ID

NO:XXX (IL-12p35(D55Q)), xvi) IL-12p35(E79Q), xvii) IL-12p35(Q130E), xviii) IL-12p35(N136D), xix) IL-12p35(E143Q), xx) SEQ ID NO:227 (IL-12p35(N151K)), xxi) SEQ ID NO:226 (IL-12p35(E153K)), xxii) IL-12p35(K158E), xxiii) IL-12p35(D165N), xxiv) SEQ ID NO:225 (IL-12p35(N151D/E153Q)), xxv) SEQ ID NO:228 (IL-12p35(N151D/D165N)), xxvi) SEQ ID NO:229 (IL-12p35(Q130E/N151D)), xxvii) SEQ ID NO:230 (IL-12p35(N151D/K158E)), xxviii) SEQ ID NO:231 (IL-12p35(E79Q/N151D)), xxix) SEQ ID NO:232 (IL-12p35(D55Q/N151D)), xxx) SEQ ID NO:233 (IL-12p35(N136D/N151D)), xxxi) SEQ ID NO:234 (IL-12p35(N21D/N151D)), xxxii) SEQ ID NO:235 (IL-12p35(E143Q/N151D)), xxxiii) SEQ ID NO: XXX (IL-12p35(F96A)), xxxiv) SEQ ID NO: XXX (IL-12p35(M97A)), xxxv) SEQ ID NO: XXX (IL-12p35(L89A)), xxxvi) SEQ ID NO: XXX (IL-12p35(L124A)), xxxvii) SEQ ID NO: XXX (IL-12p35(M125A)), xxxviii) SEQ ID NO: XXX (IL-12p35(L75A)), xxxiv) SEQ ID NO: XXX (IL-12p35(I171A)), xxxv) SEQ ID NO: XXX (IL-12p35 (N71Q)), xxxvi) SEQ ID NO: XXX (IL-12p35 (N85Q)), xxxvii) SEQ ID NO: XXX (IL-12p35 (N195Q)), xxxviii) SEQ ID NO: XXX (IL-12p35 (N71Q/N85Q)), xxxix) SEQ ID NO: XXX (IL-12p35 (N71Q/N195Q)), xl) SEQ ID NO: XXX (IL-12p35 (N85Q/N195Q), xli) SEQ ID NO: XXX (IL-12p35 (N71Q/N85Q/N195Q)), xlii) SEQ ID NO: XXX (IL-12p35 (N71D/N85D)), xliii) SEQ ID NO: XXX (IL-12p35 (N71D/N195D), xliv) SEQ ID NO: XXX (IL-12p35 (N85D/N195D)), xlv) SEQ ID NO: XXX (IL-12p35 (D55Q)), and xlvi) SEQ ID NO: XXX (IL-12p35 (D55K)).

11. The heterodimeric Fc fusion protein according to any one of claims 1-10, wherein said modifications promoting heterodimerization of said first and said second Fc domains are a set of amino acid substitutions selected from the group consisting of L368D/K370S and S364K; L368D/K370S and S364K/E357L; L368D/K370S and S364K/E357Q; T411E/K360E/Q362E and D401K; L368E/K370S and S364K; K370S and S364K/E357Q; T366S/L368A/Y407V and T366W; T366S/L368A/Y407V/Y349C and T366W/S354C, according to EU numbering.

12. The heterodimeric Fc fusion protein according to any one of claims 1-11, wherein said variant IL-12p40 subunit domain is attached to said first Fc domain using a first domain linker and/or said IL-12p35 subunit domain is attached to said second Fc domain using a second domain linker.

13. The heterodimeric Fc fusion protein according to any one of claims 1-12, wherein said first and/or said second Fc domains have an additional set of amino acid substitutions comprising Q295E/N384D/Q418E/N421D, according to EU numbering.

14. The heterodimeric Fc fusion protein according to any one of claims 1-13, wherein said first and/or said second Fc domains have an additional set of amino acid substitutions selected from the group consisting of G236R/L328R, E233P/L234V/L235A/G236_/S239K, E233P/L234V/L235A/G236_/S239K/A327G, E233P/L234V/L235A/G236_/S267K/A327G, E233P/L234V/L235A/G236_, and E233P/L234V/L235A/G236_/S267K, according to EU numbering.

15. The heterodimeric Fc fusion protein according to any one of claims 1-14, wherein said first and second Fc domains further comprise amino acid substitutions M428L/N424S.

16. The heterodimeric Fc fusion protein according to any one of claims 1-15, wherein said heterodimeric Fc fusion protein comprises:

a) said first fusion protein having a polypeptide sequence selected from the group consisting of: i) SEQ ID NO:47 (XENP27201 Chain 1), ii) SEQ ID NO:85 (XenD24752), iii) SEQ ID NO:86 (XenD24753), iv) SEQ ID NO:87 (XenD24754), v) SEQ ID NO:88 (XenD24755), vi) SEQ ID NO:89 (XenD24756), vii) SEQ ID NO:90 (XenD24757), viii) SEQ ID NO:91 (XenD24758), ix) SEQ ID NO:92 (XenD24759), x) SEQ ID NO:93 (XenD24760), xi) SEQ ID NO:94 (XenD24761), xii) SEQ ID NO:95 (XenD24762), xiii) SEQ ID NO:96 (XenD24763), xiv) SEQ ID NO:97 (XenD24764), xv) SEQ ID NO:98 (XenD24765), xvi) SEQ ID NO:99 (XenD24766), xvii) SEQ ID NO:100 (XenD24767), xviii) SEQ ID NO:101 (XenD24768), xix) SEQ ID NO:102 (XenD24769), xx) SEQ ID NO:103 (XenD24770), xxi) SEQ ID NO:104 (XenD24771), xxii) SEQ ID NO:105 (XenD24772), xxiii) SEQ ID NO:106 (XenD24773), xxiv) SEQ ID NO:107 (XenD24774), xxv) SEQ ID NO:108 (XenD24775), xxvi) SEQ ID NO:109 (XenD24776), xxvii) SEQ ID NO:110 (XenD24777), xxviii) SEQ ID NO:111 (XenD24778), xxix) SEQ ID NO:112 (XenD24792), xxx) SEQ ID NO:215 (XenD25922), xxxi) SEQ ID NO:216 (XenD25923), xxxii) SEQ ID NO:217 (XenD25924), xxxiii) SEQ ID NO:218 (XenD25925), xxxiv) SEQ ID NO:219

(XenD25926), xxxv) SEQ ID NO:220(XenD25927), xxxvi) SEQ ID NO:221 (XenD25928), xxxvii) SEQ ID NO:222 (XenD25929), xxxviii) SEQ ID NO:223 (XenD25930), xxxix) SEQ ID NO:224 (XenD25931), xl) SEQ ID NO:XXX (XenD26411), xli) SEQ ID NO:XXX (XenD26412), xlii) SEQ ID NO:XXX (XenD26413), xliii) SEQ ID NO:XXX (XenD26414), xliv) SEQ ID NO:XXX (XenD26415), xlv) SEQ ID NO:XXX (XenD26416), xlvi) SEQ ID NO:XXX (XenD26417), xlvii) SEQ ID NO:XXX (XenD26418), xlviii) SEQ ID NO:XXX (XenD27070), xlix) SEQ ID NO:XXX (XenD27071), l) SEQ ID NO:XXX (XenD27072), li) SEQ ID NO:XXX (XenD27073), lii) SEQ ID NO:XXX (XenD27074), liii) SEQ ID NO:XXX (XenD27075), liv) SEQ ID NO:XXX (XenD27076), lv) SEQ ID NO:XXX (XenD27077), lvi) SEQ ID NO:XXX (XenD27078), lvii) SEQ ID NO:XXX (XenD28173), lviii) SEQ ID NO:XXX (XenD24876), lix) SEQ ID NO:XXX (XenD27162), lx) SEQ ID NO:XXX (XenD27163), lxi) SEQ ID NO:XXX (XenD27164), lxii) SEQ ID NO:XXX (XenD27165) lxiii) SEQ ID NO:XXX (XENP31582 Chain 1), lxiv) SEQ ID NO:XXX (XENP31583 Chain 1), lxv) SEQ ID NO:XXX (XENP31584 Chain 1), lxvi) SEQ ID NO:XXX (XENP32187 Chain 1), lxvii) SEQ ID NO:XXX (XENP32188 Chain 1), lxviii) SEQ ID NO:XXX (XENP32189, Chain 1), lxix) SEQ ID NO:XXX (XENP32190 Chain 1), lxx) SEQ ID NO:XXX (XENP32191 Chain 1), lxxi) SEQ ID NO:XXX (XENP32991 Chain 1), lxxii) SEQ ID NO:XXX (XENP32992 Chain 1), lxxiii) SEQ ID NO:XXX (XENP32993 Chain 1), lxxiv) SEQ ID NO:XXX (XENP32994 Chain 1), lxxv) SEQ ID NO:XXX (XENP32995 Chain 1), lxxvi) SEQ ID NO:XXX (XENP32996 Chain 1), lxxvi) SEQ ID NO:XXX (XENP32997 Chain 1), lxxvii) SEQ ID NO:XXX (XENP32998 Chain 1), lxxvii) SEQ ID NO:XXX (XENP32999 Chain 1), lxxviii) SEQ ID NO:XXX (XENP33000 Chain 1), lxxix) SEQ ID NO:XXX (XENP33001 Chain 1), lxxx) SEQ ID NO:XXX (XENP33002 Chain 1), lxxxi) SEQ ID NO:XXX (XENP33003 Chain 1), lxxxii) SEQ ID NO:XXX (XENP33004 Chain 1), lxxxiii) SEQ ID NO:XXX (XENP33005 Chain 1), lxxxiv) SEQ ID NO:XXX (XENP33006 Chain 1), lxxxv) SEQ ID NO:XXX (XENP33007 Chain 1), lxxxvi) SEQ ID NO:XXX (XENP33008 Chain 1), lxxxvii) SEQ ID NO:XXX (XENP33008 Chain 1), lxxxviii) SEQ ID NO:XXX (XENP33009 Chain 1), lxxxix) SEQ ID NO:XXX (XENP33010 Chain 1), and xc) SEQ ID NO:XXX (XENP33011 Chain 1); and

b) said second fusion protein having a polypeptide sequence selected from the group consisting of: i) SEQ ID NO:48 (XENP27201 Chain 2), ii) SEQ ID NO:126 (XenD24779), iii) SEQ

ID NO:127 (XenD24780), iv) SEQ ID NO:128 (XenD24781), v) SEQ ID NO:129 (XenD24782), vi) SEQ ID NO:130 (XenD24783), vii) SEQ ID NO:131 (XenD24784), viii) SEQ ID NO:132 (XenD24785), ix) SEQ ID NO:133 (XenD24786), x) SEQ ID NO:134 (XenD24787), xi) SEQ ID NO:135 (XenD24788), xii) SEQ ID NO:136 (XenD24789), xiii) SEQ ID NO:137 (XenD24790), xiv) SEQ ID NO:138 (XenD24791), xv) SEQ ID NO:236 (XenD25911), xvi) SEQ ID NO:237 (XenD25912), xvii) SEQ ID NO:238 (XenD25913), xviii) SEQ ID NO:239 (XenD25914), xix) SEQ ID NO:240 (XenD25915), xx) SEQ ID NO:241 (XenD25916), xxi) SEQ ID NO:242 (XenD25917), xxii) SEQ ID NO:243 (XenD25918), xxiii) SEQ ID NO:244 (XenD25919), xxiv) SEQ ID NO:245 (XenD25920), xxv) SEQ ID NO:246 (XenD25921), xxvi) SEQ ID NO:XXX (XenD26427), xxvii) SEQ ID NO:XXX (XenD26428), xxviii) SEQ ID NO:XXX (XenD27089), xxix) SEQ ID NO:XXX (XenD27090), xxx) SEQ ID NO:XXX (XenD27091), xxxi) SEQ ID NO:XXX (XenD27092), xxxii) SEQ ID NO:XXX (XenD27093), xxxiii) SEQ ID NO:XXX (XenD27094), xxxix) SEQ ID NO:XXX (XenD24877), xl) SEQ ID NO:XXX (XenD27166), xli) SEQ ID NO:XXX (XENP31582 Chain 2), xlii) SEQ ID NO:XXX (XENP31583 Chain 2), xliii) SEQ ID NO:XXX (XENP31584 Chain 2), xliv) SEQ ID NO:XXX (XENP32187 Chain 2), xlv) SEQ ID NO:XXX (XENP32188 Chain 2), xlvi) SEQ ID NO:XXX (XENP32189, Chain 2), xlvii) SEQ ID NO:XXX (XENP32190 Chain 2), xlviii) SEQ ID NO:XXX (XENP32191 Chain 2), xlix) SEQ ID NO:XXX (XENP32991 Chain 2), l) SEQ ID NO:XXX (XENP32992 Chain 2), li) SEQ ID NO:XXX (XENP32993 Chain 2), lii) SEQ ID NO:XXX (XENP32994 Chain 2), liii) SEQ ID NO:XXX (XENP32995 Chain 2), liv) SEQ ID NO:XXX (XENP32996 Chain 2), lv) SEQ ID NO:XXX (XENP32997 Chain 2), lvi) SEQ ID NO:XXX (XENP32998 Chain 2), lvii) SEQ ID NO:XXX (XENP32999 Chain 2), lviii) SEQ ID NO:XXX (XENP33000 Chain 2), lix) SEQ ID NO:XXX (XENP33001 Chain 2), lx) SEQ ID NO:XXX (XENP33002 Chain 2), lxi) SEQ ID NO:XXX (XENP33003 Chain 2), lxii) SEQ ID NO:XXX (XENP33004 Chain 2), lxiii) SEQ ID NO:XXX (XENP33005 Chain 2), lxiv) SEQ ID NO:XXX (XENP33006 Chain 2), lxv) SEQ ID NO:XXX (XENP33007 Chain 2), lxvi) SEQ ID NO:XXX (XENP33008 Chain 2), lxvii) SEQ ID NO:XXX (XENP33008 Chain 2), lxviii) SEQ ID NO:XXX (XENP33009 Chain 2), lxix) SEQ ID NO:XXX (XENP33010 Chain 2), and lxx) SEQ ID NO:XXX (XENP33011 Chain 2).

17. The heterodimeric Fc fusion protein according to any one of claims 1-16, wherein said heterodimeric Fc fusion protein is selected from the group consisting of XENP27201, XENP28820, XENP28821, XENP28822, XENP28823, XENP28824, XENP28825, XENP28826, XENP28827, XENP28828, XENP28829, XENP28830, XENP28831, XENP28832, XENP28833, XENP28834, XENP28835, XENP28836, XENP28837, XENP28838, XENP28839, XENP28840, XENP28841, XENP28842, XENP28843, XENP28844, XENP28845, XENP28846, XENP28847, XENP28848, XENP28849, XENP28850, XENP28851, XENP28852, XENP29949, XENP29950, XENP29951, XENP29952, XENP30597, XENP30598, XENP30599, XENP30600, XENP30601, XENP30602, XENP30603, XENP30604, XENP30605, XENP30606. XENP30307, XENP30308, XENP30609, XENP31250, XENP31251, XENP31252, XENP31253, XENP31254, XENP31255, XENP31256, XENP31257, XENP31258, XENP31259, XENP31260, XENP31261, XENP31262, XENP31263, XENP31264, XENP31265, XENP31286, XENP31142, XENP31143, XENP31144, XENP31145, XENP31146, XENP31582, XENP31583, XENP31584, XENP32186, XENP32187, XENP32188, XENP32189, XENP32190, XENP32191, XENP32991, XENP32992, XENP32993, XENP32994, XENP32995, XENP32996, XENP32997, XENP32998, XENP32999, XENP33000, XENP33001, XENP33002, XENP33003, XENP33004, XENP33005, XENP33006, XENP33007, XENP33008, XENP33008, XENP33009, XENP33010, and XENP33011.

18. The heterodimeric Fc fusion according to any one of claims 1-17, wherein said heterodimeric Fc fusion protein is selected from the group consisting of: XENP31251, XENP31254, XENP31258, XENP32186, XENP32187, XENP32188, XENP32189, XENP32190, and XENP32191.

19. The heterodimeric Fc fusion protein according to any one of claims 17-18, further comprising one or more modifications to the IL-12p40 subunit selected from the group consisting of: N103Q, N113Q, N200Q, and N281Q.

20. A composition comprising a heterodimeric Fc fusion protein according to any one of claims 1-19 for use in treating cancer in a subject.

21. One or more nucleic acids encoding a heterodimeric Fc fusion protein according to any one of claims 1-19.

22. A host cell comprising said one or more nucleic acids of claim 21.

23. A method of making a heterodimeric Fc fusion protein, said method comprising culturing a host cell according to claim 22 under conditions whereby said heterodimeric Fc fusion protein is produced.

24. A method of purifying a heterodimeric Fc fusion protein according to any one of claims 1-19, said method comprising:

a) providing a composition comprising the heterodimeric Fc fusion protein;

b) loading said composition onto an ion exchange column; and

c) collecting a fraction containing said heterodimeric Fc fusion protein.

25. A heterodimeric Fc fusion protein comprising:

a) a first fusion protein comprising a first protein domain and a first Fc domain, wherein said first protein domain is covalently attached to the C-terminus of said first Fc domain; and

b) a second fusion protein comprising a second protein domain and a second Fc domain, wherein said second protein domain is covalently attached to the C-terminus of said Fc domain;

wherein said first and said second Fc domains comprise modifications promoting heterodimerization of said first and said second Fc domains and wherein said first protein domain comprises an IL-12p40 subunit and said second protein domain comprises an IL-12p35 subunit.

26. The heterodimeric Fc fusion protein according to claim 25, wherein said modifications promoting heterodimerization of said first and said second Fc domains are a set of amino acid substitutions selected from the group consisting of L368D/K370S and S364K; L368D/K370S and S364K/E357L; L368D/K370S and S364K/E357Q; T411E/K360E/Q362E and D401K; L368E/K370S and S364K; K370S and S364K/E357Q and T366S/L368A/Y407V : T366W (optionally including a bridging disulfide, T366S/L368A/Y407V/Y349C : T366W/S354C), according to EU numbering.

27. The heterodimeric Fc fusion protein according to claim 25 or 26, wherein said first protein domain is attached to said first Fc domain using a first domain linker and/or said second protein domain is attached to said second Fc domain using a second domain linker.

28. The heterodimeric Fc fusion protein according to any one of claims 25 to 27, wherein said first and/or said second Fc domains have an additional set of amino acid substitutions comprising Q295E/N384D/Q418E/N421D, according to EU numbering.

29. The heterodimeric Fc fusion protein according to any one of claims 25 to 28, wherein said first and/or said second Fc domains have an additional set of amino acid substitutions selected from the group consisting of G236R/L328R, E233P/L234V/L235A/G236_/S239K, E233P/L234V/L235A/G236_/S239K/A327G, E233P/L234V/L235A/G236_/S267K/A327G, E233P/L234V/L235A/G236_, and E233P/L234V/L235A/G236_/S267K, according to EU numbering.

30. The heterodimeric Fc fusion protein according to any one of claims 25-29, wherein said IL-12p40 subunit has a polypeptide sequence selected form the group consisting of SEQ ID NO:3 (human IL-12 subunit beta (IL-12p40) precursor sequence) and SEQ ID NO:4 (human IL-12 subunit beta (IL-12p40) mature form sequence), and/or said IL-12p35 subunit has a polypeptide sequence selected from the group consisting of SEQ ID NO:1 (human IL-12 subunit alpha (IL-12p35) precursor sequence) and SEQ ID NO:2 (human IL-12 subunit alpha (IL-12p35) mature form sequence).

31. The heterodimeric Fc fusion protein according to any of claims 25-30 wherein said first and second Fc domains further comprise amino acid substitutions M428L/N424S.

32. The heterodimeric Fc fusion protein according to any one of claims 25-31, wherein said IL-12p40 subunit is a variant IL-12p40 subunit and/or said IL-12p35 subunit is a variant IL-12p35 subunit.

33. The heterodimeric Fc fusion protein according to any one of claims 25-32, wherein said IL-12p40 subunit is a variant IL-12p40 subunit having altered affinity for IL-12 receptor subunit

beta-1 (IL-12Rß1), IL-12 receptor subunit beta-2 (IL-12Rß2), and/or IL-12 receptor complex; and/or said IL-12p35 subunit is a variant IL-12p35 subunit having altered affinity for IL-12 receptor subunit beta-1 (IL-12Rß1), IL-12 receptor subunit beta-2 (IL-12Rß2), and/or IL-12 receptor complex.

34. The heterodimeric Fc fusion protein according to any one of claims 25-33, wherein said IL-12p40 subunit has one or more amino acid modifications at amino acid residues selected from the group consisting of E3, D7, E12, D14, W15, P17, D18, A19, P20, G21, E22, M23, D29, E32, E33, D34, L40, D41, Q42, S43, E45, L47, T54, I55, Q56, K58, E59, F60, G61, D62, Q65, Y66, E73, K84, E86, D87, G88, I89, W90, D93, D97, K99, E100, K102, N103, K104, F106, E110, N113, Y114, D129, D142, Q144, E156, R159, D161, N162, K163, D166, D170, Q172, D174, A176, C177, P178, A179, A180, E181, S183, P185, E187, N200, S204, F206, R208, D209, D214, N218, Q220, N226, Q229, E231, E235, T242, P243, S245, Y246, F247, S248, C252, Q256, K258, K260, E262, K264, D265, D270, N281, Q289, D290, R291, Y292, Y293, and E299.

35. The heterodimeric Fc fusion protein according to any one of claims 25-34, wherein said IL-12p40 subunit has one or more amino acid substitutions selected from the group consisting of: D18N, D18K, E32Q, E33Q, D34N, D34K, Q42E, S43E, S43K, E45Q, Q56E, E59Q, E59K, D62N, E73Q, D87N, K99E, K99Y, E100Q, N103D, N103Q, N113D, N113Q, Q144E, D161N, R159E, K163E, E187Q, N200D, N200Q, N218Q, Q229E, E235Q, C252S, Q256N, K258E, K260E, E262Q, K264E, N281D, N281Q, and E299Q.

36. The heterodimeric Fc fusion protein according to any one of claims 25-35, wherein said IL-12p40 subunit has amino acid substitutions selected from the group consisting of: N103D/N113D/N200D/N281D, Q42E/E45Q, E45Q/Q56E, Q42E/E59Q, Q56E/E59Q, Q42E/E45Q/Q56E, E45Q/Q56E/E59Q, E32Q/E59Q, D34N/E59K, D34N/E59K/K99E, D34K/E59K/K99E, E32Q/D34N/E59K/K99E, E32K/D34N/E59K/K99E, D34N/E59Q, E59Q/E187Q, S43E/E59Q, S43K/E49Q, E59Q/K163E, E59Q/K99E, E59Q/K258E, E59Q/K260E, E59K/K99E, D18K/E59K/K99E, E59K/K99E/K264E, E59K/K99Y, E59Y/K99Y, E59Y/K99E, E45K/E59K/K99E, E59K/K99E/Q144E, E59K/K99E/Q144K, E59K/K99E/R159E, E59K/K99E/K264E,

D18K/E59K/K99E/K264E, DI8K/E59K/K99E/C252S, D18K/E59K/K99E/C252S/K264E, E59K/K99Y/C252S, E59K/K99E/C252S/K264E, E59K/K99E/C252S, N103D/N113D, N103D/N200D, N103D/N281D, N113D/N200D, N113D/N281D, N200D/N281D, N103D/N113D/N200D, N103D/N113D/N281D, N103D/N200D/N281D, N113D/N200D/N281D, N103Q/N113Q, N103Q/N200Q, N103Q/N281Q, N113Q/N200Q, N113Q/N281Q, N200Q/N281Q, N103Q/N113Q/N200Q, N103Q/N113Q/N281Q, N103Q/N200Q/N281Q, N113Q/N200Q/N281Q, N103Q/N113Q/N200Q/N281Q, E59K/K99E/N103Q/C252S/K264E, E59K/K99E/N113Q/C252S/K264E, E59K/K99E/N200Q/C252S/K264E, E59K/K99E/N281Q/C252S/K264E, E59K/K99E/N103Q/N113Q/C252S/K264E, E59K/K99E/N103Q/N200Q/C252S/K264E, E59K/K99E/N103Q/N281Q/C252S/K264E, E59K/K99E/N113Q/N200Q/C252S/K264E, E59K/K99E/N113Q/N281Q/C252S/K264E, E59K/K99E/N200Q/N281Q/C252S/K264E, E59K/K99E/N103Q/N113Q/N200Q/C252S/K264E, E59K/K99E/N103Q/N200Q/N281Q/C252S/K264E, E59K/K99E/N113Q/N200Q/N281Q/C252S/K264E, and E59K/K99E/N103Q/N113Q/N200Q/N281Q/C252S/K264E.

37. The heterodimeric Fc fusion protein according to any one of claims 25-36, wherein said IL-12p40 subunit has a polypeptide sequence selected from the group consisting of: i) SEQ ID NO:57 (IL-12p40(N103D)), ii) SEQ ID NO:58 (IL-12p40(N113D)), iii) SEQ ID NO:59 (IL-12p40(N200D)), iv) SEQ ID NO:60 (IL-12p40(N281D)), v) SEQ ID NO:61 (IL-12p40(N103D/N113D/N200D/N281D)), vi) SEQ ID NO:62 (IL-12p40(Q42E)), vii) SEQ ID NO:63 (IL-12p40(E45Q)), viii) SEQ ID NO:64 (IL-12p40(Q56E)), ix) SEQ ID NO:65 (IL-12p40(E59Q)), x) SEQ ID NO:66 (IL-12p40(D62N)), xi) SEQ ID NO:67 (IL-12p40(Q42E/E45Q)), xii) SEQ ID NO:68 (IL-12p40(E45Q/Q56E)), xiii) SEQ ID NO:69 (IL-12p40(Q42E/E59Q)), xiv) SEQ ID NO:70 (IL-12p40(Q56E/E59Q)), xv) SEQ ID NO:71 (IL-12p40(Q42E/E45Q/Q56E)), xvi) SEQ ID NO:72 (IL-12p40(E45Q/Q56E/E59Q)), xvii) SEQ ID NO:73 (IL-12p40(D161N)), xviii) SEQ ID NO:74 (IL-12p40(E73Q)), xix) SEQ ID NO:75 (IL-12p40(Q144E)), xx) SEQ ID NO:76 (IL-12p40(E262Q)), xxi) SEQ ID NO:77 (IL-12p40(E100Q)), xxii) SEQ ID NO:78 (IL-12p40(D18N)), xxiii) SEQ ID NO:79 (IL-12p40(E33Q)), xxiv) SEQ ID NO:80 (IL-12p40(Q229E)), xxv) SEQ ID NO:81 (IL-

12p40(E235Q)), xxvi) SEQ ID NO:82 (IL-12p40(Q256N)), xxvii) SEQ ID NO:83 (IL-12p40(E299Q)), xxviii) SEQ ID NO:84 (IL-12p40(D87N)), xxix) IL-12p40(E32Q), xxx) IL-12p40(D34N), xxxi) IL-12p40(S43E), xxxii) IL-12p40(S43K), xxxiii) SEQ ID NO:XXX (IL-12p40 (E59K/K99E/N103Q/N113Q/N200Q/N281Q/C252S/K264E)), xxxiv) SEQ ID NO:205 (IL-12p40(E59K)), xxxv) IL-12p40(K99E), xxxvi) IL-12p40(K163E), xxxvii) IL-12p40(E187Q), xxxviii) IL-12p40(K258E), xxxix) IL-12p40(K260E), xl) SEQ ID NO:206 (IL-12p40(E32Q/E59Q)), xli) SEQ ID NO:207 (IL-12p40(D34N/E59Q)), xlii) SEQ ID NO:208 (IL-12p40(E59Q/E187Q)), xliii) SEQ ID NO:209 (IL-12p40(S43E/E59Q)), xliv) SEQ ID NO:210 (IL-12p40(S43K/E49Q)), xlv) SEQ ID NO:211 (IL-12p40(E59Q/K163E)), xlvi) SEQ ID NO:212 (IL-12p40(E59Q/K99E)), xlvii) SEQ ID NO:213 (IL-12p40(E59Q/K258E)), xlviii) SEQ ID NO:214 (IL-12p40(E59Q/K260E)), xlix) SEQ ID NO: XXX (IL-12p40 (D34N/E59K)), l) SEQ ID NO: XXX (IL-12p40(E59K/K99E)), li) SEQ ID NO: XXX (IL-12p40(D18K/E59K/K99E)), lii) SEQ ID NO: XXX (IL-12p40 (E59K/K99E/K264E)), liii) SEQ ID NO: XXX (IL-12p40 (E59K/K99Y)), liv) SEQ ID NO: XXX (IL-12p40 (E59Y/K99E)), lv) SEQ ID NO: XXX (IL-12p40 (E45K/E59K/K99E)), lvi) SEQ ID NO: XXX (IL-12p40 (E59K/K99E/Q144E)), lvii) SEQ ID NO: XXX (IL-12p40 (E59K/K99E/Q144K)), lviii) SEQ ID NO: XXX (IL-12p40 (E59K/K99E/R159E)), lix) SEQ ID NO: XXX (IL-12p40 (E59K/K99E/K264E)), lx) SEQ ID NO: XXX (IL-12p40 (D18K/E59K/K99E/K264E)), lxi) SEQ ID NO: XXX (IL-12p40 (C252S)), lxii) SEQ ID NO: XXX (IL-12p40 (DI8K/E59K/K99E/C252S)), lxiii) SEQ ID NO: XXX (IL-12p40 (D18K/E59K/K99E/C252S/K264E)), lxiv) SEQ ID NO: XXX (IL-12p40 (E59K/K99Y/C252S)), lxv) SEQ ID NO: XXX (IL-12p40 (E59K/K99E/C252S/K264E)), lxvi) SEQ ID NO: XXX (IL-12p40 (E59K/K99E/C252S)), lxvii) SEQ ID NO: XXX (IL-12p40 (N103D/N113D)), lxviii) SEQ ID NO: XXX (IL-12p40 (N103D/N200D)), lxix) SEQ ID NO: XXX (IL-12p40 (N103D/N281D)), lxx) SEQ ID NO: XXX (IL-12p40 (N113D/N200D)), lxxi) SEQ ID NO: XXX (IL-12p40 (N113D/N281D)), lxxii) SEQ ID NO: XXX (IL-12p40 (N200D/N281D)), lxxiii) SEQ ID NO: XXX (IL-12p50 (N103D/N113D/N200D)), lxxiv) SEQ ID NO: XXX (IL-12p40 (N103D/N113D/N281D)), lxxv) SEQ ID NO: XXX (IL-12p40 (N103D/N200D/N281D)), lxxvi) SEQ ID NO: XXX (IL-12p40 (N113D/N200D/N281D)), lxxvii) SEQ ID NO: XXX (IL-12p40 (N103Q)), lxxviii) SEQ ID NO: XXX (IL-12p40 (N113Q)), lxxix) SEQ ID NO: XXX (IL-12p40 (N200Q)), lxxx) SEQ ID NO: XXX (IL-12p40 (N281Q)), lxxxi) SEQ ID NO: XXX (IL-12p40

(N103Q/N113Q)), lxxxii) SEQ ID NO: XXX (IL-12p40 (N103Q/N200Q)), lxxxiii) SEQ ID NO: XXX (IL-12p40 (N103Q/N281Q)), lxxxiv) SEQ ID NO: XXX (IL-12p40 (N113Q/N200Q)), lxxxv) SEQ ID NO: XXX (IL-12p40 (N113Q/N281Q)), lxxxvi) SEQ ID NO: XXX (IL-12p40 (N200Q/N281Q)), lxxxvii) SEQ ID NO: XXX (IL-12p40 (N103Q/N113Q/N200Q)), lxxxviii) SEQ ID NO: XXX (IL-12p40 (N103Q/N113Q/N281Q)), lxxxix) SEQ ID NO: XXX (IL-12p40 (N103Q/N200Q/N281Q)), xc) SEQ ID NO: XXX (IL-12p40 (N113Q/N200Q/N281Q)), xci) SEQ ID NO:XXX (IL-12p40 (N103Q/N113Q/N200Q/N281Q)), xcii) SEQ ID NO:XXX (IL-12p40 (D34N/E59K/K99E)), xciii) SEQ ID NO:XXX (IL-12p40 (D34K/E59K/K99E)), xciv) SEQ ID NO:XXX (IL-12p40 (E32Q/D34N/E59K/K99E)), xcv) SEQ ID NO:XXX (IL-12p40 (E32K/D34N/E59K/K99E)), xcvi) SEQ ID NO: XXX (IL-12p40 (E59Y/K99Y)), xcvii) SEQ ID NO:XXX (IL-12p40 (E59K/K99E/N103Q/C252S/K264E)), xcviii) SEQ ID NO:XXX (IL-12p40 (E59K/K99E/N113Q/C252S/K264E)), xcix) SEQ ID NO:XXX (IL-12p40 (E59K/K99E/N200Q/C252S/K264E)), c) SEQ ID NO:XXX (IL-12p40 (E59K/K99E/N281Q/C252S/K264E)), ci) SEQ ID NO:XXX (IL-12p40 (E59K/K99E/N103Q/N113Q/C252S/K264E)), cii) SEQ ID NO:XXX (IL-12p40 (E59K/K99E/N103Q/N200Q/C252S/K264E)), ciii) SEQ ID NO:XXX (IL-12p40 (E59K/K99E/N103Q/N281Q/C252S/K264E)), civ) SEQ ID NO:XXX (IL-12p40 (E59K/K99E/N113Q/N200Q/C252S/K264E)), cv) SEQ ID NO:XXX (IL-12p40 (E59K/K99E/N113Q/N281Q/C252S/K264E)), cvi) SEQ ID NO:XXX (IL-12p40 (E59K/K99E/N200Q/N281Q/C252S/K264E)), cvii) SEQ ID NO:XXX (IL-12p40 (E59K/K99E/N103Q/N113Q/N200Q/C252S/K264E)), cviii) SEQ ID NO:XXX (IL-12p40 (E59K/K99E/N103Q/N200Q/N281Q/C252S/K264E)), and cix) SEQ ID NO:XXX (IL-12p40 (E59K/K99E/N113Q/N200Q/N281Q/C252S/K264E)).

38. The heterodimeric Fc fusion protein according to any one of claims 25-37, wherein said IL-12p35 subunit has one or more amino acid modifications as amino acid residues selected from the group consisting of: Q20, N21, Q35, E38, S44, E45, E46, H49, K54, D55, T59, V60, E61, C63, L64, P65, E67, L68, N71, S73, C74, L75, N76, E79, N85, L89, F96, M97, L124, M125, Q130, Q135,

N136, E143, Q146, N151, E153, K158, E162, E163, D165, I171, R181, I182, R183, V185, T186, D188, R189, V190, S192, Y193, N195, and A196.

39. The heterodimeric Fc fusion protein according to any one of claims 25-38, wherein said IL-12p35 subunit has one or more amino acid substitutions selected from the group consisting of: N21D, Q35D, E38Q, D55Q, D55K, N71D, N71Q, L75A, N76D, E79Q, N85D, N85Q, L89A, F96A, M97A, L124A, M125A, Q130E, Q135E, N136D, E143Q, Q146E, N151D, N151K, E153K, E153Q, K158E, E162Q, E163Q, D165N, I171A, N195D, and N195Q.

40. The heterodimeric Fc fusion protein according to any one of claims 25-39, wherein said IL-12p35 subunit has amino acid substitutions selected from the group consisting of: N71D/N85D/N195D, N151D/E153Q, N151D/D165N, Q130E/N151D, N151D/K158E, E79Q/N151D, D55Q/N151D, N136D/N151D, N21D/N151D, E143Q/N151D, N71Q/N85Q, N71Q/N195Q, N85Q/N195Q, N71Q/N85Q/N195Q, N71D/N85D, N71D/N195D, N85D/N195D.

40. The heterodimeric Fc fusion protein according to any one of claims 25-40, wherein said IL-12p35 subunit has a polypeptide sequence selected from the group consisting of: i) SEQ ID NO:113 (IL-12p35(N71D)), ii) SEQ ID NO:114 (IL-12p35(N85D)), iii) SEQ ID NO:115 (IL-12p35(N195D)), iv) SEQ ID NO:116 (IL-12p35(N71D/N85D/N195D)), v) SEQ ID NO:117 (IL-12p35(E153Q)), vi) SEQ ID NO:118 (IL-12p35(E38Q)), vii) SEQ ID NO:119 (IL-12p35(N151D)), viii) SEQ ID NO:120 (IL-12p35(Q135E)), ix) SEQ ID NO:121 (IL-12p35(Q35D)), x) SEQ ID NO:122 (IL-12p35(Q146E)), xi) SEQ ID NO:123 (IL-12p35(N76D)), xii) SEQ ID NO:124 (IL-12p35(E162Q)), xiii) SEQ ID NO:125 (IL-12p35(E163Q)), xiv) IL-12p35(N21D), xv) SEQ ID NO:XXX (IL-12p35(D55Q)), xvi) IL-12p35(E79Q), xvii) IL-12p35(Q130E), xviii) IL-12p35(N136D), xix) IL-12p35(E143Q), xx) SEQ ID NO:227 (IL-12p35(N151K)), xxi) SEQ ID NO:226 (IL-12p35(E153K)), xxii) IL-12p35(K158E), xxiii) IL-12p35(D165N), xxiv) SEQ ID NO:225 (IL-12p35(N151D/E153Q)), xxv) SEQ ID NO:228 (IL-12p35(N151D/D165N)), xxvi) SEQ ID NO:229 (IL-12p35(Q130E/N151D)), xxvii) SEQ ID NO:230 (IL-12p35(N151D/K158E)), xxviii) SEQ ID NO:231 (IL-12p35(E79Q/N151D)), xxix) SEQ ID NO:232 (IL-12p35(D55Q/N151D)), xxx) SEQ ID NO:233 (IL-12p35(N136D/N151D)), xxxi) SEQ ID NO:234 (IL-12p35(N21D/N151D)),

xxxii) SEQ ID NO:235 (IL-12p35(E143Q/N151D)), xxxiii) SEQ ID NO: XXX (IL-12p35(F96A)), xxxiv) SEQ ID NO: XXX (IL-12p35(M97A)), xxxv) SEQ ID NO: XXX (IL-12p35(L89A)), xxxvi) SEQ ID NO: XXX (IL-12p35(L124A)), xxxvii) SEQ ID NO: XXX (IL-12p35(M125A)), xxxviii) SEQ ID NO: XXX (IL-12p35(L75A)), xxxiv) SEQ ID NO: XXX (IL-12p35(I171A)), xxxv) SEQ ID NO: XXX (IL-12p35 (N71Q)), xxxvi) SEQ ID NO: XXX (IL-12p35 (N85Q)), xxxvii) SEQ ID NO: XXX (IL-12p35 (N195Q)), xxxviii) SEQ ID NO: XXX (IL-12p35 (N71Q/N85Q)), xxxix) SEQ ID NO: XXX (IL-12p35 (N71Q/N195Q)), xl) SEQ ID NO: XXX (IL-12p35 (N85Q/N195Q), xli) SEQ ID NO: XXX (IL-12p35 (N71Q/N85Q/N195Q)), xlii) SEQ ID NO: XXX (IL-12p35 (N71D/N85D)), xliii) SEQ ID NO: XXX (IL-12p35 (N71D/N195D), xliv) SEQ ID NO: XXX (IL-12p35 (N85D/N195D)), xlv) SEQ ID NO: XXX (IL-12p35 (D55Q)), and xlvi) SEQ ID NO: XXX (IL-12p35 (D55K)).

41. The heterodimeric Fc fusion protein according to any one of claims 25-40, wherein said heterodimeric Fc fusion protein comprises:

a) said first fusion protein having a polypeptide sequence of SEQ ID NO:49 (XENP27202 Chain 1), and

b) said second fusion protein having a polypeptide sequence of SEQ ID NO:50 (XENP27202 Chain 2).

42. The heterodimeric Fc fusion protein according to any one of claims 25-41, wherein said heterodimeric Fc fusion protein is XENP27202.

43. A composition comprising a heterodimeric Fc fusion protein according to any one of claims 25-42 for use in treating cancer in a subject.

44. One or more nucleic acids encoding a heterodimeric Fc fusion protein according to any one of claims 25-42.

45. A host cell comprising said one or more nucleic acids of claim 44.

46. A method of making a heterodimeric Fc fusion protein, said method comprising culturing a host cell according to claim 45 under conditions whereby said heterodimeric Fc fusion protein is produced.

47. A method of purifying a heterodimeric Fc fusion protein according to any one of claims 25-42, said method comprising:

a) providing a composition comprising the heterodimeric Fc fusion protein;

b) loading said composition onto an ion exchange column; and

c) collecting a fraction containing said heterodimeric Fc fusion protein.

48. A heterodimeric Fc fusion protein comprising:

a) a fusion protein comprising a first protein domain, a second protein domain and a first Fc domain, wherein said first protein domain is covalently attached to said second protein domain, and wherein said second protein domain is covalently attached to the N-terminus of said first Fc domain; and

b) a second Fc domain;

wherein said first and said second Fc domains comprise modifications promoting heterodimerization of said first and said second Fc domains and wherein said first protein domain comprises an IL-12p40 subunit and said second protein domain comprises an IL-12p35 subunit.

49. The heterodimeric Fc fusion protein according to claim 48, wherein said modifications promoting heterodimerization of said first and said second Fc domains are a set of amino acid substitutions selected from the group consisting of L368D/K370S and S364K; L368D/K370S and S364K/E357L; L368D/K370S and S364K/E357Q; T411E/K360E/Q362E and D401K; L368E/K370S and S364K; K370S and S364K/E357Q and T366S/L368A/Y407V : T366W (optionally including a bridging disulfide, T366S/L368A/Y407V/Y349C : T366W/S354C), according to EU numbering.

50. The heterodimeric Fc fusion protein according to claim 48 or 49, wherein said first protein domain is attached to said second protein domain using a first domain linker and/or said second protein domain is attached to said first Fc domain using a second domain linker.

51. The heterodimeric Fc fusion protein according to any one of claims 48 to 50, wherein said first and/or said second Fc domains have an additional set of amino acid substitutions comprising Q295E/N384D/Q418E/N421D, according to EU numbering.

52. The heterodimeric Fc fusion protein according to any one of claims 48 to 51, wherein said first and/or said second Fc domains have an additional set of amino acid substitutions selected from the group consisting of G236R/L328R, E233P/L234V/L235A/G236_/S239K, E233P/L234V/L235A/G236_/S239K/A327G, E233P/L234V/L235A/G236_/S267K/A327G, E233P/L234V/L235A/G236_, and E233P/L234V/L235A/G236_/S267K, according to EU numbering.

53. The heterodimeric Fc fusion protein according to any one of claims 48-52, wherein said IL-12p40 subunit has a polypeptide sequence selected form the group consisting of SEQ ID NO:3 (human IL-12 subunit beta (IL-12p40) precursor sequence) and SEQ ID NO:4 (human IL-12 subunit beta (IL-12p40) mature form sequence), and/or said IL-12p35 subunit has a polypeptide sequence selected from the group consisting of SEQ ID NO:1 (human IL-12 subunit alpha (IL-12p35) precursor sequence) and SEQ ID NO:2 (human IL-12 subunit alpha (IL-12p35) mature form sequence).

54. The heterodimeric Fc fusion protein according to any one of claims 48-53, wherein said first and second Fc domains further comprise amino acid substitutions M428L/N424S.

55. The heterodimeric Fc fusion protein according to any one of claims 48-53, wherein said IL-12p40 subunit is a variant IL-12p40 subunit and/or said IL-12p35 subunit is a variant IL-12p35 subunit.

56. The heterodimeric Fc fusion protein according to any one of claims 48-55, wherein said IL-12p40 subunit is a variant IL-12p40 subunit having altered affinity for IL-12 receptor subunit

beta-1 (IL-12Rß1), IL-12 receptor subunit beta-2 (IL-12Rß2), and/or IL-12 receptor complex; and/or said IL-12p35 subunit is a variant IL-12p35 subunit having altered affinity for IL-12 receptor subunit beta-1 (IL-12Rß1), IL-12 receptor subunit beta-2 (IL-12Rß2), and/or IL-12 receptor complex.

57. The heterodimeric Fc fusion protein according to any one of claims 48-56, wherein said IL-12p40 subunit has one or more amino acid modifications at amino acid residues selected from the group consisting of E3, D7, E12, D14, W15, P17, D18, A19, P20, G21, E22, M23, D29, E32, E33, D34, L40, D41, Q42, S43, E45, L47, T54, I55, Q56, K58, E59, F60, G61, D62, Q65, Y66, E73, K84, E86, D87, G88, I89, W90, D93, D97, K99, E100, K102, N103, K104, F106, E110, N113, Y114, D129, D142, Q144, E156, R159, D161, N162, K163, D166, D170, Q172, D174, A176, C177, P178, A179, A180, E181, S183, P185, E187, N200, S204, F206, R208, D209, D214, N218, Q220, N226, Q229, E231, E235, T242, P243, S245, Y246, F247, S248, Q256, K158, C252, K260, E262, K264, D265, D270, N281, Q289, D290, R291, Y292, Y293, and E299.

58. The heterodimeric Fc fusion protein according to any one of claims 48-57, wherein said IL-12p40 subunit has one or more amino acid substitutions selected from the group consisting of D18N, D18K, E32Q, E33Q, D34N, D34K, Q42E, S43E, S43K, E45Q, Q56E, E59Q, E59K, D62N, E73Q, D87N, K99E, K99Y, E100Q, N103D, N103Q, N113D, N113Q, Q144E, D161N, R159E, K163E, E187Q, N200D, N200Q, N218Q, Q229E, E235Q, C252S, Q256N, K258E, K260E, E262Q, K264E, N281D, N281Q, and E299Q.

59. The heterodimeric Fc fusion protein according to any one of claims 48-58, wherein said IL-12p40 subunit has amino acid substitutions selected from the group consisting of N103D/N113D/N200D/N281D, Q42E/E45Q, E45Q/Q56E, Q42E/E59Q, Q56E/E59Q, Q42E/E45Q/Q56E, E45Q/Q56E/E59Q, E32Q/E59Q, D34N/E59K, D34N/E59K/K99E, D34K/E59K/K99E, E32Q/D34N/E59K/K99E, E32K/D34N/E59K/K99E, D34N/E59Q, E59Q/E187Q, S43E/E59Q, S43K/E49Q, E59Q/K163E, E59Q/K99E, E59Q/K258E, E59Q/K260E, E59K/K99E, D18K/E59K/K99E, E59K/K99E/K264E, E59K/K99Y, E59Y/K99Y, E59Y/K99E, E45K/E59K/K99E, E59K/K99E/Q144E, E59K/K99E/Q144K, E59K/K99E/R159E, E59K/K99E/K264E,

D18K/E59K/K99E/K264E, DI8K/E59K/K99E/C252S, D18K/E59K/K99E/C252S/K264E, E59K/K99Y/C252S, E59K/K99E/C252S/K264E, E59K/K99E/C252S, N103D/N113D, N103D/N200D, N103D/N281D, N113D/N200D, N113D/N281D, N200D/N281D, N103D/N113D/N200D, N103D/N113D/N281D, N103D/N200D/N281D, N113D/N200D/N281D, N103Q/N113Q, N103Q/N200Q, N103Q/N281Q, N113Q/N200Q, N113Q/N281Q, N200Q/N281Q, N103Q/N113Q/N200Q, N103Q/N113Q/N281Q, N103Q/N200Q/N281Q, N113Q/N200Q/N281Q, N103Q/N113Q/N200Q/N281Q, E59K/K99E/N103Q/C252S/K264E, E59K/K99E/N113Q/C252S/K264E, E59K/K99E/N200Q/C252S/K264E, E59K/K99E/N281Q/C252S/K264E, E59K/K99E/N103Q/N113Q/C252S/K264E, E59K/K99E/N103Q/N200Q/C252S/K264E, E59K/K99E/N103Q/N281Q/C252S/K264E, E59K/K99E/N113Q/N200Q/C252S/K264E, E59K/K99E/N113Q/N281Q/C252S/K264E, E59K/K99E/N200Q/N281Q/C252S/K264E, E59K/K99E/N103Q/N113Q/N200Q/C252S/K264E, E59K/K99E/N103Q/N200Q/N281Q/C252S/K264E, E59K/K99E/N113Q/N200Q/N281Q/C252S/K264E, and E59K/K99E/N103Q/N113Q/N200Q/N281Q/C252S/K264E.

60. The heterodimeric Fc fusion protein according to any one of claims 48-59, wherein said IL-12p40 subunit has a polypeptide sequence selected from the group consisting of:

i) SEQ ID NO:57 (IL-12p40(N103D)), ii) SEQ ID NO:58 (IL-12p40(N113D)), iii) SEQ ID NO:59 (IL-12p40(N200D)), iv) SEQ ID NO:60 (IL-12p40(N281D)), v) SEQ ID NO:61 (IL-12p40(N103D/N113D/N200D/N281D)), vi) SEQ ID NO:62 (IL-12p40(Q42E)), vii) SEQ ID NO:63 (IL-12p40(E45Q)), viii) SEQ ID NO:64 (IL-12p40(Q56E)), ix) SEQ ID NO:65 (IL-12p40(E59Q)), x) SEQ ID NO:66 (IL-12p40(D62N)), xi) SEQ ID NO:67 (IL-12p40(Q42E/E45Q)), xii) SEQ ID NO:68 (IL-12p40(E45Q/Q56E)), xiii) SEQ ID NO:69 (IL-12p40(Q42E/E59Q)), xiv) SEQ ID NO:70 (IL-12p40(Q56E/E59Q)), xv) SEQ ID NO:71 (IL-12p40(Q42E/E45Q/Q56E)), xvi) SEQ ID NO:72 (IL-12p40(E45Q/Q56E/E59Q)), xvii) SEQ ID NO:73 (IL-12p40(D161N)), xviii) SEQ ID NO:74 (IL-12p40(E73Q)), xix) SEQ ID NO:75 (IL-12p40(Q144E)), xx) SEQ ID NO:76 (IL-12p40(E262Q)), xxi) SEQ ID NO:77 (IL-12p40(E100Q)), xxii) SEQ ID NO:78 (IL-12p40(D18N)), xxiii) SEQ ID NO:79 (IL-12p40(E33Q)), xxiv) SEQ ID NO:80 (IL-12p40(Q229E)), xxv) SEQ ID NO:81 (IL-

12p40(E235Q)), xxvi) SEQ ID NO:82 (IL-12p40(Q256N)), xxvii) SEQ ID NO:83 (IL-12p40(E299Q)), xxviii) SEQ ID NO:84 (IL-12p40(D87N)), xxix) IL-12p40(E32Q), xxx) IL-12p40(D34N), xxxi) IL-12p40(S43E), xxxii) IL-12p40(S43K), xxxiii) SEQ ID NO:XXX (IL-12p40 (E59K/K99E/N103Q/N113Q/N200Q/N281Q/C252S/K264E)), xxxiv) SEQ ID NO:205 (IL-12p40(E59K)), xxxv) IL-12p40(K99E), xxxvi) IL-12p40(K163E), xxxvii) IL-12p40(E187Q), xxxviii) IL-12p40(K258E), xxxix) IL-12p40(K260E), xl) SEQ ID NO:206 (IL-12p40(E32Q/E59Q)), xli) SEQ ID NO:207 (IL-12p40(D34N/E59Q)), xlii) SEQ ID NO:208 (IL-12p40(E59Q/E187Q)), xliii) SEQ ID NO:209 (IL-12p40(S43E/E59Q)), xliv) SEQ ID NO:210 (IL-12p40(S43K/E49Q)), xlv) SEQ ID NO:211 (IL-12p40(E59Q/K163E)), xlvi) SEQ ID NO:212 (IL-12p40(E59Q/K99E)), xlvii) SEQ ID NO:213 (IL-12p40(E59Q/K258E)), xlviii) SEQ ID NO:214 (IL-12p40(E59Q/K260E)), xlix) SEQ ID NO: XXX (IL-12p40 (D34N/E59K)), l) SEQ ID NO: XXX (IL-12p40(E59K/K99E)), li) SEQ ID NO: XXX (IL-12p40(D18K/E59K/K99E)), lii) SEQ ID NO: XXX (IL-12p40 (E59K/K99E/K264E)), liii) SEQ ID NO: XXX (IL-12p40 (E59K/K99Y)), liv) SEQ ID NO: XXX (IL-12p40 (E59Y/K99E)), lv) SEQ ID NO: XXX (IL-12p40 (E45K/E59K/K99E)), lvi) SEQ ID NO: XXX (IL-12p40 (E59K/K99E/Q144E)), lvii) SEQ ID NO: XXX (IL-12p40 (E59K/K99E/Q144K)), lviii) SEQ ID NO: XXX (IL-12p40 (E59K/K99E/R159E)), lix) SEQ ID NO: XXX (IL-12p40 (E59K/K99E/K264E)), lx) SEQ ID NO: XXX (IL-12p40 (D18K/E59K/K99E/K264E)), lxi) SEQ ID NO: XXX (IL-12p40 (C252S)), lxii) SEQ ID NO: XXX (IL-12p40 (DI8K/E59K/K99E/C252S)), lxiii) SEQ ID NO: XXX (IL-12p40 (D18K/E59K/K99E/C252S/K264E)), lxiv) SEQ ID NO: XXX (IL-12p40 (E59K/K99Y/C252S)), lxv) SEQ ID NO: XXX (IL-12p40 (E59K/K99E/C252S/K264E)), lxvi) SEQ ID NO: XXX (IL-12p40 (E59K/K99E/C252S)), lxvii) SEQ ID NO: XXX (IL-12p40 (N103D/N113D)), lxviii) SEQ ID NO: XXX (IL-12p40 (N103D/N200D)), lxix) SEQ ID NO: XXX (IL-12p40 (N103D/N281D)), lxx) SEQ ID NO: XXX (IL-12p40 (N113D/N200D)), lxxi) SEQ ID NO: XXX (IL-12p40 (N113D/N281D)), lxxii) SEQ ID NO: XXX (IL-12p40 (N200D/N281D)), lxxiii) SEQ ID NO: XXX (IL-12p50 (N103D/N113D/N200D)), lxxiv) SEQ ID NO: XXX (IL-12p40 (N103D/N113D/N281D)), lxxv) SEQ ID NO: XXX (IL-12p40 (N103D/N200D/N281D)), lxxvi) SEQ ID NO: XXX (IL-12p40 (N113D/N200D/N281D)), lxxvii) SEQ ID NO: XXX (IL-12p40 (N103Q)), lxxviii) SEQ ID NO: XXX (IL-12p40 (N113Q)), lxxix) SEQ ID NO: XXX (IL-12p40 (N200Q)), lxxx) SEQ ID NO: XXX (IL-12p40 (N281Q)), lxxxi) SEQ ID NO: XXX (IL-12p40

(N103Q/N113Q)), lxxxii) SEQ ID NO: XXX (IL-12p40 (N103Q/N200Q)), lxxxiii) SEQ ID NO: XXX (IL-12p40 (N103Q/N281Q)), lxxxiv) SEQ ID NO: XXX (IL-12p40 (N113Q/N200Q)), lxxxv) SEQ ID NO: XXX (IL-12p40 (N113Q/N281Q)), lxxxvi) SEQ ID NO: XXX (IL-12p40 (N200Q/N281Q)), lxxxvii) SEQ ID NO: XXX (IL-12p40 (N103Q/N113Q/N200Q)), lxxxviii) SEQ ID NO: XXX (IL-12p40 (N103Q/N113Q/N281Q)), lxxxix) SEQ ID NO: XXX (IL-12p40 (N103Q/N200Q/N281Q)), xc) SEQ ID NO: XXX (IL-12p40 (N113Q/N200Q/N281Q)), xci) SEQ ID NO:XXX (IL-12p40 (N103Q/N113Q/N200Q/N281Q)), xcii) SEQ ID NO:XXX (IL-12p40 (D34N/E59K/K99E)), xciii) SEQ ID NO:XXX (IL-12p40 (D34K/E59K/K99E)), xciv) SEQ ID NO:XXX (IL-12p40 (E32Q/D34N/E59K/K99E)), xcv) SEQ ID NO:XXX (IL-12p40 (E32K/D34N/E59K/K99E)), xcvi) SEQ ID NO: XXX (IL-12p40 (E59Y/K99Y)), xcvii) SEQ ID NO:XXX (IL-12p40 (E59K/K99E/N103Q/C252S/K264E)), xcviii) SEQ ID NO:XXX (IL-12p40 (E59K/K99E/N113Q/C252S/K264E)), xcix) SEQ ID NO:XXX (IL-12p40 (E59K/K99E/N200Q/C252S/K264E)), c) SEQ ID NO:XXX (IL-12p40 (E59K/K99E/N281Q/C252S/K264E)), ci) SEQ ID NO:XXX (IL-12p40 (E59K/K99E/N103Q/N113Q/C252S/K264E)), cii) SEQ ID NO:XXX (IL-12p40 (E59K/K99E/N103Q/N200Q/C252S/K264E)), ciii) SEQ ID NO:XXX (IL-12p40 (E59K/K99E/N103Q/N281Q/C252S/K264E)), civ) SEQ ID NO:XXX (IL-12p40 (E59K/K99E/N113Q/N200Q/C252S/K264E)), cv) SEQ ID NO:XXX (IL-12p40 (E59K/K99E/N113Q/N281Q/C252S/K264E)), cvi) SEQ ID NO:XXX (IL-12p40 (E59K/K99E/N200Q/N281Q/C252S/K264E)), cvii) SEQ ID NO:XXX (IL-12p40 (E59K/K99E/N103Q/N113Q/N200Q/C252S/K264E)), cviii) SEQ ID NO:XXX (IL-12p40 (E59K/K99E/N103Q/N200Q/N281Q/C252S/K264E)), and cix) SEQ ID NO:XXX (IL-12p40 (E59K/K99E/N113Q/N200Q/N281Q/C252S/K264E)).

61. The heterodimeric Fc fusion protein according to any one of claims 48-60, wherein said IL-12p35 subunit has one or more amino acid modifications as amino acid residues selected from the group consisting of Q20, N21, Q35, E38, S44, E45, E46, H49, K54, D55, T59, V60, E61, C63, L64, P65, E67, L68, N71, S73, C74, L75, N76, E79, N85, L89, F96, M97, L124, M125, Q130, Q135,

N136, E143, Q146, N151, E153, K158, E162, E163, D165, I171, R181, I182, R183, V185, T186, D188, R189, V190, S192, Y193, N195, and A196.

62. The heterodimeric Fc fusion protein according to any one of claims 48-61, wherein said IL-12p35 subunit has one or more amino acid substitutions selected from the group consisting of N21D, Q35D, E38Q, D55Q, D55K, N71D, N71Q, L75A, N76D, E79Q, N85D, N85Q, L89A, F96A, M97A, L124A, M125A, Q130E, Q135E, N136D, E143Q, Q146E, N151D, N151K, E153K, E153Q, K158E, E162Q, E163Q, D165N, I171A, N195D, and N195Q.

63. The heterodimeric Fc fusion protein according to any one of claims 48-62, wherein said IL-12p35 subunit has amino acid substitutions selected from the group consisting of: N71D/N85D/N195D, N151D/E153Q, N151D/D165N, Q130E/N151D, N151D/K158E, E79Q/N151D, D55Q/N151D, N136D/N151D, N21D/N151D, E143Q/N151D, N71Q/N85Q, N71Q/N195Q, N85Q/N195Q, N71Q/N85Q/N195Q, N71D/N85D, N71D/N195D, N85D/N195D.

64. The heterodimeric Fc fusion protein according to any one of claims 48-63, wherein said IL-12p35 subunit has a polypeptide sequence selected from the group consisting of:

i) SEQ ID NO:113 (IL-12p35(N71D)), ii) SEQ ID NO:114 (IL-12p35(N85D)), iii) SEQ ID NO:115 (IL-12p35(N195D)), iv) SEQ ID NO:116 (IL-12p35(N71D/N85D/N195D)), v) SEQ ID NO:117 (IL-12p35(E153Q)), vi) SEQ ID NO:118 (IL-12p35(E38Q)), vii) SEQ ID NO:119 (IL-12p35(N151D)), viii) SEQ ID NO:120 (IL-12p35(Q135E)), ix) SEQ ID NO:121 (IL-12p35(Q35D)), x) SEQ ID NO:122 (IL-12p35(Q146E)), xi) SEQ ID NO:123 (IL-12p35(N76D)), xii) SEQ ID NO:124 (IL-12p35(E162Q)), xiii) SEQ ID NO:125 (IL-12p35(E163Q)), xiv) IL-12p35(N21D), xv) SEQ ID NO:XXX (IL-12p35(D55Q)), xvi) IL-12p35(E79Q), xvii) IL-12p35(Q130E), xviii) IL-12p35(N136D), xix) IL-12p35(E143Q), xx) SEQ ID NO:227 (IL-12p35(N151K)), xxi) SEQ ID NO:226 (IL-12p35(E153K)), xxii) IL-12p35(K158E), xxiii) IL-12p35(D165N), xxiv) SEQ ID NO:225 (IL-12p35(N151D/E153Q)), xxv) SEQ ID NO:228 (IL-12p35(N151D/D165N)), xxvi) SEQ ID NO:229 (IL-12p35(Q130E/N151D)), xxvii) SEQ ID NO:230 (IL-12p35(N151D/K158E)), xxviii) SEQ ID NO:231 (IL-12p35(E79Q/N151D)), xxix) SEQ ID NO:232 (IL-12p35(D55Q/N151D)), xxx) SEQ ID NO:233 (IL-12p35(N136D/N151D)), xxxi) SEQ ID NO:234 (IL-12p35(N21D/N151D)),

xxxii) SEQ ID NO:235 (IL-12p35(E143Q/N151D)), xxxiii) SEQ ID NO: XXX (IL-12p35(F96A)), xxxiv) SEQ ID NO: XXX (IL-12p35(M97A)), xxxv) SEQ ID NO: XXX (IL-12p35(L89A)), xxxvi) SEQ ID NO: XXX (IL-12p35(L124A)), xxxvii) SEQ ID NO: XXX (IL-12p35(M125A)), xxxviii) SEQ ID NO: XXX (IL-12p35(L75A)), xxxiv) SEQ ID NO: XXX (IL-12p35(I171A)), xxxv) SEQ ID NO: XXX (IL-12p35 (N71Q)), xxxvi) SEQ ID NO: XXX (IL-12p35 (N85Q)), xxxvii) SEQ ID NO: XXX (IL-12p35 (N195Q)), xxxviii) SEQ ID NO: XXX (IL-12p35 (N71Q/N85Q)), xxxix) SEQ ID NO: XXX (IL-12p35 (N71Q/N195Q)), xl) SEQ ID NO: XXX (IL-12p35 (N85Q/N195Q), xli) SEQ ID NO: XXX (IL-12p35 (N71Q/N85Q/N195Q)), xlii) SEQ ID NO: XXX (IL-12p35 (N71D/N85D)), xliii) SEQ ID NO: XXX (IL-12p35 (N71D/N195D), xliv) SEQ ID NO: XXX (IL-12p35 (N85D/N195D)), xlv) SEQ ID NO: XXX (IL-12p35 (D55Q)), and xlvi) SEQ ID NO: XXX (IL-12p35 (D55K)).

65. The heterodimeric Fc fusion protein according to any one of claims 48-64, wherein said heterodimeric Fc fusion protein comprises:

a) said fusion protein having a polypeptide sequence of SEQ ID NO:51 (XENP27203 Chain 1), and

b) said second Fc domain having a polypeptide sequence of SEQ ID NO:52 (XENP27203 Chain 2).

66. The heterodimeric Fc fusion protein according to any one of claims 48-65, wherein said heterodimeric Fc fusion protein is XENP27203.

67. The heterodimeric Fc fusion protein according to any one of claims 48-65, wherein said heterodimeric Fc fusion protein is XENP31290.

68. A composition comprising a heterodimeric Fc fusion protein according to any one of claims 48-66 for use in treating cancer in a subject.

69. One or more nucleic acids encoding a heterodimeric Fc fusion protein according to any one of claims 48-67.

70. A host cell comprising said one or more nucleic acids of claim 69.

71. A method of making a heterodimeric Fc fusion protein, said method comprising culturing a host cell according to claim 70 under conditions whereby said heterodimeric Fc fusion protein is produced.

72. A method of purifying a heterodimeric Fc fusion protein according to any one of claims 48-67, said method comprising:

a) providing a composition comprising the heterodimeric Fc fusion protein;

b) loading said composition onto an ion exchange column; and

c) collecting a fraction containing said heterodimeric Fc fusion protein.

73. A heterodimeric Fc fusion protein comprising:

a) a fusion protein comprising a first protein domain, a second protein domain and a first Fc domain, wherein said first protein domain is covalently attached to said second protein domain, and wherein said second protein domain is covalently attached to the N-terminus of said first Fc domain; and

b) a second Fc domain;

wherein said first and said second Fc domains comprise modifications promoting heterodimerization of said first and said second Fc domains and wherein said first protein domain comprises an IL-12p35 subunit and said second protein domain comprises an IL-12p40 subunit.

74. The heterodimeric Fc fusion protein according to claim 73, wherein said modifications promoting heterodimerization of said first and said second Fc domains are a set of amino acid substitutions selected from the group consisting of L368D/K370S and S364K; L368D/K370S and S364K/E357L; L368D/K370S and S364K/E357Q; T411E/K360E/Q362E and D401K; L368E/K370S and S364K; K370S and S364K/E357Q and T366S/L368A/Y407V : T366W (optionally including a bridging disulfide, T366S/L368A/Y407V/Y349C : T366W/S354C), according to EU numbering.

75. The heterodimeric Fc fusion protein according to claim 73 or 74, wherein said first protein domain is attached to said second protein domain using a first domain linker and/or said second protein domain is attached to said first Fc domain using a second domain linker.

76. The heterodimeric Fc fusion protein according to any one of claims 73 to 75, wherein said first and/or said second Fc domains have an additional set of amino acid substitutions comprising Q295E/N384D/Q418E/N421D, according to EU numbering.

77. The heterodimeric Fc fusion protein according to any one of claims 73 to 76, wherein said first and/or said second Fc domains have an additional set of amino acid substitutions selected from the group consisting of G236R/L328R, E233P/L234V/L235A/G236_/S239K, E233P/L234V/L235A/G236_/S239K/A327G, E233P/L234V/L235A/G236_/S267K/A327G, E233P/L234V/L235A/G236_, and E233P/L234V/L235A/G236_/S267K, according to EU numbering.

78. The heterodimeric Fc fusion protein according to any one of claims 73-77, wherein said IL-12p40 subunit has a polypeptide sequence selected form the group consisting of SEQ ID NO:3 (human IL-12 subunit beta (IL-12p40) precursor sequence) and/or SEQ ID NO:4 (human IL-12 subunit beta (IL-12p40) mature form sequence), and said IL-12p35 subunit has a polypeptide sequence selected from the group consisting of SEQ ID NO:1 (human IL-12 subunit alpha (IL-12p35) precursor sequence) and SEQ ID NO:2 (human IL-12 subunit alpha (IL-12p35) mature form sequence).

79. The heterodimeric Fc fusion protein according to any one of claims 73-78, wherein said first and second Fc domains further comprise amino acid substitutions M428L/N424S.

80. The heterodimeric Fc fusion protein according to any one of claims 73-79, wherein said IL-12p40 subunit is a variant IL-12p40 subunit and/or said IL-12p35 subunit is a variant IL-12p35 subunit.

81. The heterodimeric Fc fusion protein according to any one of claims 73-80, wherein said IL-12p40 subunit is a variant IL-12p40 subunit having altered affinity for IL-12 receptor subunit

beta-1 (IL-12Rß1), IL-12 receptor subunit beta-2 (IL-12Rß2), and/or IL-12 receptor complex; and/or said IL-12p35 subunit is a variant IL-12p35 subunit having altered affinity for IL-12 receptor subunit beta-1 (IL-12Rß1), IL-12 receptor subunit beta-2 (IL-12Rß2), and/or IL-12 receptor complex.

82. The heterodimeric Fc fusion protein according to any one of claims 73-81, wherein said IL-12p40 subunit has one or more amino acid modifications at amino acid residues selected from the group consisting of: E3, D7, E12, D14, W15, P17, D18, A19, P20, G21, E22, M23, D29, E32, E33, D34, L40, D41, Q42, S43, E45, L47, T54, I55, Q56, K58, E59, F60, G61, D62, Q65, Y66, E73, K84, E86, D87, G88, I89, W90, D93, D97, K99, E100, K102, N103, K104, F106, E110, N113, Y114, D129, D142, Q144, E156, R159, D161, N162, K163, D166, D170, Q172, D174, A176, C177, P178, A179, A180, E181, S183, P185, E187, N200, S204, F206, R208, D209, D214, N218, Q220, N226, Q229, E231, E235, T242, P243, S245, Y246, F247, S248, C252, Q256, K258, K260, E262, K264, D265, D270, N281, Q289, D290, R291, Y292, Y293, and E299.

83. The heterodimeric Fc fusion protein according to any one of claims 73-82, wherein said IL-12p40 subunit has one or more amino acid substitutions selected from the group consisting of: D18N, D18K, E32Q, E33Q, D34N, D34K, Q42E, S43E, S43K, E45Q, Q56E, E59Q, E59K, D62N, E73Q, D87N, K99E, K99Y, E100Q, N103D, N103Q, N113D, N113Q, Q144E, D161N, R159E, K163E, E187Q, N200D, N200Q, N218Q, Q229E, E235Q, C252S, Q256N, K258E, K260E, E262Q, K264E, N281D, N281Q, and E299Q.

84. The heterodimeric Fc fusion protein according to any one of claims 73-83, wherein said IL-12p40 subunit has amino acid substitutions selected from the group consisting of: N103D/N113D/N200D/N281D, Q42E/E45Q, E45Q/Q56E, Q42E/E59Q, Q56E/E59Q, Q42E/E45Q/Q56E, E45Q/Q56E/E59Q, E32Q/E59Q, D34N/E59K, D34N/E59K/K99E, D34K/E59K/K99E, E32Q/D34N/E59K/K99E, E32K/D34N/E59K/K99E, D34N/E59Q, E59Q/E187Q, S43E/E59Q, S43K/E49Q, E59Q/K163E, E59Q/K99E, E59Q/K258E, E59Q/K260E, E59K/K99E, D18K/E59K/K99E, E59K/K99E/K264E, E59K/K99Y, E59Y/K99Y, E59Y/K99E, E45K/E59K/K99E, E59K/K99E/Q144E, E59K/K99E/Q144K, E59K/K99E/R159E, E59K/K99E/K264E,

D18K/E59K/K99E/K264E, DI8K/E59K/K99E/C252S, D18K/E59K/K99E/C252S/K264E, E59K/K99Y/C252S, E59K/K99E/C252S/K264E, E59K/K99E/C252S, N103D/N113D, N103D/N200D, N103D/N281D, N113D/N200D, N113D/N281D, N200D/N281D, N103D/N113D/N200D, N103D/N113D/N281D, N103D/N200D/N281D, N113D/N200D/N281D, N103Q/N113Q, N103Q/N200Q, N103Q/N281Q, N113Q/N200Q, N113Q/N281Q, N200Q/N281Q, N103Q/N113Q/N200Q, N103Q/N113Q/N281Q, N103Q/N200Q/N281Q, N113Q/N200Q/N281Q, N103Q/N113Q/N200Q/N281Q, E59K/K99E/N103Q/C252S/K264E, E59K/K99E/N113Q/C252S/K264E, E59K/K99E/N200Q/C252S/K264E, E59K/K99E/N281Q/C252S/K264E, E59K/K99E/N103Q/N113Q/C252S/K264E, E59K/K99E/N103Q/N200Q/C252S/K264E, E59K/K99E/N103Q/N281Q/C252S/K264E, E59K/K99E/N113Q/N200Q/C252S/K264E, E59K/K99E/N113Q/N281Q/C252S/K264E, E59K/K99E/N200Q/N281Q/C252S/K264E, E59K/K99E/N103Q/N113Q/N200Q/C252S/K264E, E59K/K99E/N103Q/N200Q/N281Q/C252S/K264E, E59K/K99E/N113Q/N200Q/N281Q/C252S/K264E, and E59K/K99E/N103Q/N113Q/N200Q/N281Q/C252S/K264E.

85. The heterodimeric Fc fusion protein according to any one of claims 73-84, wherein said IL-12p40 subunit has a polypeptide sequence selected from the group consisting of: i) SEQ ID NO:57 (IL-12p40(N103D)), ii) SEQ ID NO:58 (IL-12p40(N113D)), iii) SEQ ID NO:59 (IL-12p40(N200D)), iv) SEQ ID NO:60 (IL-12p40(N281D)), v) SEQ ID NO:61 (IL-12p40(N103D/N113D/N200D/N281D)), vi) SEQ ID NO:62 (IL-12p40(Q42E)), vii) SEQ ID NO:63 (IL-12p40(E45Q)), viii) SEQ ID NO:64 (IL-12p40(Q56E)), ix) SEQ ID NO:65 (IL-12p40(E59Q)), x) SEQ ID NO:66 (IL-12p40(D62N)), xi) SEQ ID NO:67 (IL-12p40(Q42E/E45Q)), xii) SEQ ID NO:68 (IL-12p40(E45Q/Q56E)), xiii) SEQ ID NO:69 (IL-12p40(Q42E/E59Q)), xiv) SEQ ID NO:70 (IL-12p40(Q56E/E59Q)), xv) SEQ ID NO:71 (IL-12p40(Q42E/E45Q/Q56E)), xvi) SEQ ID NO:72 (IL-12p40(E45Q/Q56E/E59Q)), xvii) SEQ ID NO:73 (IL-12p40(D161N)), xviii) SEQ ID NO:74 (IL-12p40(E73Q)), xix) SEQ ID NO:75 (IL-12p40(Q144E)), xx) SEQ ID NO:76 (IL-12p40(E262Q)), xxi) SEQ ID NO:77 (IL-12p40(E100Q)), xxii) SEQ ID NO:78 (IL-12p40(D18N)), xxiii) SEQ ID NO:79 (IL-12p40(E33Q)), xxiv) SEQ ID NO:80 (IL-12p40(Q229E)), xxv) SEQ ID NO:81 (IL-

12p40(E235Q)), xxvi) SEQ ID NO:82 (IL-12p40(Q256N)), xxvii) SEQ ID NO:83 (IL-12p40(E299Q)), xxviii) SEQ ID NO:84 (IL-12p40(D87N)), xxix) IL-12p40(E32Q), xxx) IL-12p40(D34N), xxxi) IL-12p40(S43E), xxxii) IL-12p40(S43K), xxxiii) SEQ ID NO:XXX (IL-12p40 (E59K/K99E/N103Q/N113Q/N200Q/N281Q/C252S/K264E)), xxxiv) SEQ ID NO:205 (IL-12p40(E59K)), xxxv) IL-12p40(K99E), xxxvi) IL-12p40(K163E), xxxvii) IL-12p40(E187Q), xxxviii) IL-12p40(K258E), xxxix) IL-12p40(K260E), xl) SEQ ID NO:206 (IL-12p40(E32Q/E59Q)), xli) SEQ ID NO:207 (IL-12p40(D34N/E59Q)), xlii) SEQ ID NO:208 (IL-12p40(E59Q/E187Q)), xliii) SEQ ID NO:209 (IL-12p40(S43E/E59Q)), xliv) SEQ ID NO:210 (IL-12p40(S43K/E49Q)), xlv) SEQ ID NO:211 (IL-12p40(E59Q/K163E)), xlvi) SEQ ID NO:212 (IL-12p40(E59Q/K99E)), xlvii) SEQ ID NO:213 (IL-12p40(E59Q/K258E)), xlviii) SEQ ID NO:214 (IL-12p40(E59Q/K260E)), xlix) SEQ ID NO: XXX (IL-12p40 (D34N/E59K)), l) SEQ ID NO: XXX (IL-12p40(E59K/K99E)), li) SEQ ID NO: XXX (IL-12p40(D18K/E59K/K99E)), lii) SEQ ID NO: XXX (IL-12p40 (E59K/K99E/K264E)), liii) SEQ ID NO: XXX (IL-12p40 (E59K/K99Y)), liv) SEQ ID NO: XXX (IL-12p40 (E59Y/K99E)), lv) SEQ ID NO: XXX (IL-12p40 (E45K/E59K/K99E)), lvi) SEQ ID NO: XXX (IL-12p40 (E59K/K99E/Q144E)), lvii) SEQ ID NO: XXX (IL-12p40 (E59K/K99E/Q144K)), lviii) SEQ ID NO: XXX (IL-12p40 (E59K/K99E/R159E)), lix) SEQ ID NO: XXX (IL-12p40 (E59K/K99E/K264E)), lx) SEQ ID NO: XXX (IL-12p40 (D18K/E59K/K99E/K264E)), lxi) SEQ ID NO: XXX (IL-12p40 (C252S)), lxii) SEQ ID NO: XXX (IL-12p40 (DI8K/E59K/K99E/C252S)), lxiii) SEQ ID NO: XXX (IL-12p40 (D18K/E59K/K99E/C252S/K264E)), lxiv) SEQ ID NO: XXX (IL-12p40 (E59K/K99Y/C252S)), lxv) SEQ ID NO: XXX (IL-12p40 (E59K/K99E/C252S/K264E)), lxvi) SEQ ID NO: XXX (IL-12p40 (E59K/K99E/C252S)), lxvii) SEQ ID NO: XXX (IL-12p40 (N103D/N113D)), lxviii) SEQ ID NO: XXX (IL-12p40 (N103D/N200D)), lxix) SEQ ID NO: XXX (IL-12p40 (N103D/N281D)), lxx) SEQ ID NO: XXX (IL-12p40 (N113D/N200D)), lxxi) SEQ ID NO: XXX (IL-12p40 (N113D/N281D)), lxxii) SEQ ID NO: XXX (IL-12p40 (N200D/N281D)), lxxiii) SEQ ID NO: XXX (IL-12p50 (N103D/N113D/N200D)), lxxiv) SEQ ID NO: XXX (IL-12p40 (N103D/N113D/N281D)), lxxv) SEQ ID NO: XXX (IL-12p40 (N103D/N200D/N281D)), lxxvi) SEQ ID NO: XXX (IL-12p40 (N113D/N200D/N281D)), lxxvii) SEQ ID NO: XXX (IL-12p40 (N103Q)), lxxviii) SEQ ID NO: XXX (IL-12p40 (N113Q)), lxxix) SEQ ID NO: XXX (IL-12p40 (N200Q)), lxxx) SEQ ID NO: XXX (IL-12p40 (N281Q)), lxxxi) SEQ ID NO: XXX (IL-12p40

(N103Q/N113Q)), lxxxii) SEQ ID NO: XXX (IL-12p40 (N103Q/N200Q)), lxxxiii) SEQ ID NO: XXX (IL-12p40 (N103Q/N281Q)), lxxxiv) SEQ ID NO: XXX (IL-12p40 (N113Q/N200Q)), lxxxv) SEQ ID NO: XXX (IL-12p40 (N113Q/N281Q)), lxxxvi) SEQ ID NO: XXX (IL-12p40 (N200Q/N281Q)), lxxxvii) SEQ ID NO: XXX (IL-12p40 (N103Q/N113Q/N200Q)), lxxxviii) SEQ ID NO: XXX (IL-12p40 (N103Q/N113Q/N281Q)), lxxxix) SEQ ID NO: XXX (IL-12p40 (N103Q/N200Q/N281Q)), xc) SEQ ID NO: XXX (IL-12p40 (N113Q/N200Q/N281Q)), xci) SEQ ID NO:XXX (IL-12p40 (N103Q/N113Q/N200Q/N281Q)), xcii) SEQ ID NO:XXX (IL-12p40 (D34N/E59K/K99E)), xciii) SEQ ID NO:XXX (IL-12p40 (D34K/E59K/K99E)), xciv) SEQ ID NO:XXX (IL-12p40 (E32Q/D34N/E59K/K99E)), xcv) SEQ ID NO:XXX (IL-12p40 (E32K/D34N/E59K/K99E)), xcvi) SEQ ID NO: XXX (IL-12p40 (E59Y/K99Y)), xcvii) SEQ ID NO:XXX (IL-12p40 (E59K/K99E/N103Q/C252S/K264E)), xcviii) SEQ ID NO:XXX (IL-12p40 (E59K/K99E/N113Q/C252S/K264E)), xcix) SEQ ID NO:XXX (IL-12p40 (E59K/K99E/N200Q/C252S/K264E)), c) SEQ ID NO:XXX (IL-12p40 (E59K/K99E/N281Q/C252S/K264E)), ci) SEQ ID NO:XXX (IL-12p40 (E59K/K99E/N103Q/N113Q/C252S/K264E)), cii) SEQ ID NO:XXX (IL-12p40 (E59K/K99E/N103Q/N200Q/C252S/K264E)), ciii) SEQ ID NO:XXX (IL-12p40 (E59K/K99E/N103Q/N281Q/C252S/K264E)), civ) SEQ ID NO:XXX (IL-12p40 (E59K/K99E/N113Q/N200Q/C252S/K264E)), cv) SEQ ID NO:XXX (IL-12p40 (E59K/K99E/N113Q/N281Q/C252S/K264E)), cvi) SEQ ID NO:XXX (IL-12p40 (E59K/K99E/N200Q/N281Q/C252S/K264E)), cvii) SEQ ID NO:XXX (IL-12p40 (E59K/K99E/N103Q/N113Q/N200Q/C252S/K264E)), cviii) SEQ ID NO:XXX (IL-12p40 (E59K/K99E/N103Q/N200Q/N281Q/C252S/K264E)), and cix) SEQ ID NO:XXX (IL-12p40 (E59K/K99E/N113Q/N200Q/N281Q/C252S/K264E)).

86. The heterodimeric Fc fusion protein according to any one of claims 73-85, wherein said IL-12p35 subunit has one or more amino acid modifications as amino acid residues selected from the group consisting of: Q20, N21, Q35, E38, S44, E45, E46, H49, K54, D55, T59, V60, E61, C63, L64, P65, E67, L68, N71, S73, C74, L75, N76, E79, N85, L89, F96, M97, L124, M125, Q130, Q135,

N136, E143, Q146, N151, E153, K158, E162, E163, D165, I171, R181, I182, R183, V185, T186, D188, R189, V190, S192, Y193, N195, and A196.

87. The heterodimeric Fc fusion protein according to any one of claims 73-86, wherein said IL-12p35 subunit has one or more amino acid substitutions selected from the group consisting of: N21D, Q35D, E38Q, D55Q, D55K, N71D, N71Q, L75A, N76D, E79Q, N85D, N85Q, L89A, F96A, M97A, L124A, M125A, Q130E, Q135E, N136D, E143Q, Q146E, N151D, N151K, E153K, E153Q, K158E, E162Q, E163Q, D165N, I171A, N195D, and N195Q.

88. The heterodimeric Fc fusion protein according to any one of claims 73-87, wherein said IL-12p35 subunit has amino acid substitutions selected from the group consisting of: N71D/N85D/N195D, N151D/E153Q, N151D/D165N, Q130E/N151D, N151D/K158E, E79Q/N151D, D55Q/N151D, N136D/N151D, N21D/N151D, E143Q/N151D, N71Q/N85Q, N71Q/N195Q, N85Q/N195Q, N71Q/N85Q/N195Q, N71D/N85D, N71D/N195D, and N85D/N195D.

89. The heterodimeric Fc fusion protein according to any one of claims 73-88, wherein said IL-12p35 subunit has a polypeptide sequence selected from the group consisting of: i) SEQ ID NO:113 (IL-12p35(N71D)), ii) SEQ ID NO:114 (IL-12p35(N85D)), iii) SEQ ID NO:115 (IL-12p35(N195D)), iv) SEQ ID NO:116 (IL-12p35(N71D/N85D/N195D)), v) SEQ ID NO:117 (IL-12p35(E153Q)), vi) SEQ ID NO:118 (IL-12p35(E38Q)), vii) SEQ ID NO:119 (IL-12p35(N151D)), viii) SEQ ID NO:120 (IL-12p35(Q135E)), ix) SEQ ID NO:121 (IL-12p35(Q35D)), x) SEQ ID NO:122 (IL-12p35(Q146E)), xi) SEQ ID NO:123 (IL-12p35(N76D)), xii) SEQ ID NO:124 (IL-12p35(E162Q)), xiii) SEQ ID NO:125 (IL-12p35(E163Q)), xiv) IL-12p35(N21D), xv) SEQ ID NO:XXX (IL-12p35(D55Q)), xvi) IL-12p35(E79Q), xvii) IL-12p35(Q130E), xviii) IL-12p35(N136D), xix) IL-12p35(E143Q), xx) SEQ ID NO:227 (IL-12p35(N151K)), xxi) SEQ ID NO:226 (IL-12p35(E153K)), xxii) IL-12p35(K158E), xxiii) IL-12p35(D165N), xxiv) SEQ ID NO:225 (IL-12p35(N151D/E153Q)), xxv) SEQ ID NO:228 (IL-12p35(N151D/D165N)), xxvi) SEQ ID NO:229 (IL-12p35(Q130E/N151D)), xxvii) SEQ ID NO:230 (IL-12p35(N151D/K158E)), xxviii) SEQ ID NO:231 (IL-12p35(E79Q/N151D)), xxix) SEQ ID NO:232 (IL-12p35(D55Q/N151D)), xxx)

SEQ ID NO:233 (IL-12p35(N136D/N151D)), xxxi) SEQ ID NO:234 (IL-12p35(N21D/N151D)), xxxii) SEQ ID NO:235 (IL-12p35(E143Q/N151D)), xxxiii) SEQ ID NO: XXX (IL-12p35(F96A)), xxxiv) SEQ ID NO: XXX (IL-12p35(M97A)), xxxv) SEQ ID NO: XXX (IL-12p35(L89A)), xxxvi) SEQ ID NO: XXX (IL-12p35(L124A)), xxxvii) SEQ ID NO: XXX (IL-12p35(M125A)), xxxviii) SEQ ID NO: XXX (IL-12p35(L75A)), xxxiv) SEQ ID NO: XXX (IL-12p35(I171A)), xxxv) SEQ ID NO: XXX (IL-12p35 (N71Q)), xxxvi) SEQ ID NO: XXX (IL-12p35 (N85Q)), xxxvii) SEQ ID NO: XXX (IL-12p35 (N195Q)), xxxviii) SEQ ID NO: XXX (IL-12p35 (N71Q/N85Q)), xxxix) SEQ ID NO: XXX (IL-12p35 (N71Q/N195Q)), xl) SEQ ID NO: XXX (IL-12p35 (N85Q/N195Q), xli) SEQ ID NO: XXX (IL-12p35 (N71Q/N85Q/N195Q)), xlii) SEQ ID NO: XXX (IL-12p35 (N71D/N85D)), xliii) SEQ ID NO: XXX (IL-12p35 (N71D/N195D), xliv) SEQ ID NO: XXX (IL-12p35 (N85D/N195D)), xlv) SEQ ID NO: XXX (IL-12p35 (D55Q)), and xlvi) SEQ ID NO: XXX (IL-12p35 (D55K)).

90. The heterodimeric Fc fusion protein according to any one of claims 73-89, wherein said heterodimeric Fc fusion protein comprises:

a) said fusion protein having a polypeptide sequence of SEQ ID NO:53 (XENP27204 Chain 1), and

b) said second Fc domain having a polypeptide sequence of SEQ ID NO:54 (XENP27204 Chain 2).

91. The heterodimeric Fc fusion protein according to any one of claims 73-90, wherein said heterodimeric Fc fusion protein is XENP27204.

92. A composition comprising a heterodimeric Fc fusion protein according to any one of claims 73-91 for use in treating cancer in a subject.

93. One or more nucleic acids encoding a heterodimeric Fc fusion protein according to any one of claims 73-91.

94. A host cell comprising said one or more nucleic acids of claim 93.

95. A method of making a heterodimeric Fc fusion protein, said method comprising culturing a host cell according to claim 94 under conditions whereby said heterodimeric Fc fusion protein is produced.

96. A method of purifying a heterodimeric Fc fusion protein according to any one of claims 73-91, said method comprising:

a) providing a composition comprising the heterodimeric Fc fusion protein;

b) loading said composition onto an ion exchange column; and

c) collecting a fraction containing said heterodimeric Fc fusion protein.

97. A heterodimeric Fc fusion protein comprising:

a) a fusion protein comprising a first protein domain, a second protein domain and a first Fc domain, wherein said first protein domain is attached to the C-terminus of said first Fc domain, and wherein said second protein domain is covalently attached to said first protein domain; and

b) a second Fc domain;

wherein said first and said second Fc domains comprise modifications promoting heterodimerization of said first and said second Fc domains and wherein said first protein domain comprises an IL-12p40 subunit and said second protein domain comprises an IL-12p35 subunit.

98. The heterodimeric Fc fusion protein according to claim 97, wherein said modifications promoting heterodimerization of said first and said second Fc domains are a set of amino acid substitutions selected from the group consisting of L368D/K370S and S364K; L368D/K370S and S364K/E357L; L368D/K370S and S364K/E357Q; T411E/K360E/Q362E and D401K; L368E/K370S and S364K; K370S and S364K/E357Q and T366S/L368A/Y407V : T366W (optionally including a bridging disulfide, T366S/L368A/Y407V/Y349C : T366W/S354C), according to EU numbering.

99. The heterodimeric Fc fusion protein according to claim 97 or 98, wherein said first protein domain is attached to said second protein domain using a first domain linker and/or said second protein domain is attached to said first Fc domain using a second domain linker.

100. The heterodimeric Fc fusion protein according to any one of claims 97 to 99, wherein said first and/or said second Fc domains have an additional set of amino acid substitutions comprising Q295E/N384D/Q418E/N421D, according to EU numbering.

101. The heterodimeric Fc fusion protein according to any one of claims 97 to 100, wherein said first and/or said second Fc domains have an additional set of amino acid substitutions selected from the group consisting of G236R/L328R, E233P/L234V/L235A/G236_/S239K, E233P/L234V/L235A/G236_/S239K/A327G, E233P/L234V/L235A/G236_/S267K/A327G, E233P/L234V/L235A/G236_, and E233P/L234V/L235A/G236_/S267K, according to EU numbering.

102. The heterodimeric Fc fusion protein according to any one of claims 97-101, wherein said IL-12p40 subunit has a polypeptide sequence selected form the group consisting of SEQ ID NO:3 (human IL-12 subunit beta (IL-12p40) precursor sequence) and SEQ ID NO:4 (human IL-12 subunit beta (IL-12p40) mature form sequence), and/or said IL-12p35 subunit has a polypeptide sequence selected from the group consisting of SEQ ID NO:1 (human IL-12 subunit alpha (IL-12p35) precursor sequence) and SEQ ID NO:2 (human IL-12 subunit alpha (IL-12p35) mature form sequence).

103. The heterodimeric Fc fusion protein according to one of claims 97-102, wherein said first and second Fc domains further comprise amino acid substitutions M428L/N424S.

104. The heterodimeric Fc fusion protein according to any one of claims 97-103, wherein said IL-12p40 subunit is a variant IL-12p40 subunit and/or said IL-12p35 subunit is a variant IL-12p35 subunit.

105. The heterodimeric Fc fusion protein according to any one of claims 97-104, wherein said IL-12p40 subunit is a variant IL-12p40 subunit having altered affinity for IL-12 receptor subunit

beta-1 (IL-12Rß1), IL-12 receptor subunit beta-2 (IL-12Rß2), and/or IL-12 receptor complex; and/or said IL-12p35 subunit is a variant IL-12p35 subunit having altered affinity for IL-12 receptor subunit beta-1 (IL-12Rß1), IL-12 receptor subunit beta-2 (IL-12Rß2), and/or IL-12 receptor complex.

106. The heterodimeric Fc fusion protein according to any one of claims 97-105, wherein said IL-12p40 subunit has one or more amino acid modifications at amino acid residues selected from the group consisting of: E3, D7, E12, D14, W15, P17, D18, A19, P20, G21, E22, M23, D29, E32, E33, D34, L40, D41, Q42, S43, E45, L47, T54, I55, Q56, K58, E59, F60, G61, D62, Q65, Y66, E73, K84, E86, D87, G88, I89, W90, D93, D97, K99, E100, K102, N103, K104, F106, E110, N113, Y114, D129, D142, Q144, E156, R159, D161, N162, K163, D166, D170, Q172, D174, A176, C177, P178, A179, A180, E181, S183, P185, E187, N200, S204, F206, R208, D209, D214, N218, Q220, N226, Q229, E231, E235, T242, P243, S245, Y246, F247, S248, C252, Q256, K258, K260, E262, K264, D265, D270, N281, Q289, D290, R291, Y292, Y293, and E299.

107. The heterodimeric Fc fusion protein according to any one of claims 97-106, wherein said IL-12p40 subunit has one or more amino acid substitutions selected from the group consisting of: D18N, D18K, E32Q, E33Q, D34N, D34K, Q42E, S43E, S43K, E45Q, Q56E, E59Q, E59K, D62N, E73Q, D87N, K99E, K99Y, E100Q, N103D, N103Q, N113D, N113Q, Q144E, D161N, R159E, K163E, E187Q, N200D, N200Q, N218Q, Q229E, E235Q, C252S, Q256N, K258E, K260E, E262Q, K264E, N281D, N281Q, and E299Q.

108. The heterodimeric Fc fusion protein according to any one of claims 97-107, wherein said IL-12p40 subunit has amino acid substitutions selected from the group consisting of: N103D/N113D/N200D/N281D, Q42E/E45Q, E45Q/Q56E, Q42E/E59Q, Q56E/E59Q, Q42E/E45Q/Q56E, E45Q/Q56E/E59Q, E32Q/E59Q, D34N/E59K, D34N/E59K/K99E, D34K/E59K/K99E, E32Q/D34N/E59K/K99E, E32K/D34N/E59K/K99E, D34N/E59Q, E59Q/E187Q, S43E/E59Q, S43K/E49Q, E59Q/K163E, E59Q/K99E, E59Q/K258E, E59Q/K260E, E59K/K99E, D18K/E59K/K99E, E59K/K99E/K264E, E59K/K99Y, E59Y/K99Y, E59Y/K99E, E45K/E59K/K99E, E59K/K99E/Q144E, E59K/K99E/Q144K, E59K/K99E/R159E, E59K/K99E/K264E,

D18K/E59K/K99E/K264E, DI8K/E59K/K99E/C252S, D18K/E59K/K99E/C252S/K264E, E59K/K99Y/C252S, E59K/K99E/C252S/K264E, E59K/K99E/C252S, N103D/N113D, N103D/N200D, N103D/N281D, N113D/N200D, N113D/N281D, N200D/N281D, N103D/N113D/N200D, N103D/N113D/N281D, N103D/N200D/N281D, N113D/N200D/N281D, N103Q/N113Q, N103Q/N200Q, N103Q/N281Q, N113Q/N200Q, N113Q/N281Q, N200Q/N281Q, N103Q/N113Q/N200Q, N103Q/N113Q/N281Q, N103Q/N200Q/N281Q, N113Q/N200Q/N281Q, N103Q/N113Q/N200Q/N281Q, E59K/K99E/N103Q/C252S/K264E, E59K/K99E/N113Q/C252S/K264E, E59K/K99E/N200Q/C252S/K264E, E59K/K99E/N281Q/C252S/K264E, E59K/K99E/N103Q/N113Q/C252S/K264E, E59K/K99E/N103Q/N200Q/C252S/K264E, E59K/K99E/N103Q/N281Q/C252S/K264E, E59K/K99E/N113Q/N200Q/C252S/K264E, E59K/K99E/N113Q/N281Q/C252S/K264E, E59K/K99E/N200Q/N281Q/C252S/K264E, E59K/K99E/N103Q/N113Q/N200Q/C252S/K264E, E59K/K99E/N103Q/N200Q/N281Q/C252S/K264E, E59K/K99E/N113Q/N200Q/N281Q/C252S/K264E, and E59K/K99E/N103Q/N113Q/N200Q/N281Q/C252S/K264E.

109. The heterodimeric Fc fusion protein according to any one of claims 97-1081, wherein said IL-12p40 subunit has a polypeptide sequence selected from the group consisting of i) SEQ ID NO:57 (IL-12p40(N103D)), ii) SEQ ID NO:58 (IL-12p40(N113D)), iii) SEQ ID NO:59 (IL-12p40(N200D)), iv) SEQ ID NO:60 (IL-12p40(N281D)), v) SEQ ID NO:61 (IL-12p40(N103D/N113D/N200D/N281D)), vi) SEQ ID NO:62 (IL-12p40(Q42E)), vii) SEQ ID NO:63 (IL-12p40(E45Q)), viii) SEQ ID NO:64 (IL-12p40(Q56E)), ix) SEQ ID NO:65 (IL-12p40(E59Q)), x) SEQ ID NO:66 (IL-12p40(D62N)), xi) SEQ ID NO:67 (IL-12p40(Q42E/E45Q)), xii) SEQ ID NO:68 (IL-12p40(E45Q/Q56E)), xiii) SEQ ID NO:69 (IL-12p40(Q42E/E59Q)), xiv) SEQ ID NO:70 (IL-12p40(Q56E/E59Q)), xv) SEQ ID NO:71 (IL-12p40(Q42E/E45Q/Q56E)), xvi) SEQ ID NO:72 (IL-12p40(E45Q/Q56E/E59Q)), xvii) SEQ ID NO:73 (IL-12p40(D161N)), xviii) SEQ ID NO:74 (IL-12p40(E73Q)), xix) SEQ ID NO:75 (IL-12p40(Q144E)), xx) SEQ ID NO:76 (IL-12p40(E262Q)), xxi) SEQ ID NO:77 (IL-12p40(E100Q)), xxii) SEQ ID NO:78 (IL-12p40(D18N)), xxiii) SEQ ID NO:79 (IL-12p40(E33Q)), xxiv) SEQ ID NO:80 (IL-12p40(Q229E)), xxv) SEQ ID NO:81 (IL-

12p40(E235Q)), xxvi) SEQ ID NO:82 (IL-12p40(Q256N)), xxvii) SEQ ID NO:83 (IL-12p40(E299Q)), xxviii) SEQ ID NO:84 (IL-12p40(D87N)), xxix) IL-12p40(E32Q), xxx) IL-12p40(D34N), xxxi) IL-12p40(S43E), xxxii) IL-12p40(S43K), xxxiii) SEQ ID NO:XXX (IL-12p40 (E59K/K99E/N103Q/N113Q/N200Q/N281Q/C252S/K264E)), xxxiv) SEQ ID NO:205 (IL-12p40(E59K)), xxxv) IL-12p40(K99E), xxxvi) IL-12p40(K163E), xxxvii) IL-12p40(E187Q), xxxviii) IL-12p40(K258E), xxxix) IL-12p40(K260E), xl) SEQ ID NO:206 (IL-12p40(E32Q/E59Q)), xli) SEQ ID NO:207 (IL-12p40(D34N/E59Q)), xlii) SEQ ID NO:208 (IL-12p40(E59Q/E187Q)), xliii) SEQ ID NO:209 (IL-12p40(S43E/E59Q)), xliv) SEQ ID NO:210 (IL-12p40(S43K/E49Q)), xlv) SEQ ID NO:211 (IL-12p40(E59Q/K163E)), xlvi) SEQ ID NO:212 (IL-12p40(E59Q/K99E)), xlvii) SEQ ID NO:213 (IL-12p40(E59Q/K258E)), xlviii) SEQ ID NO:214 (IL-12p40(E59Q/K260E)), xlix) SEQ ID NO: XXX (IL-12p40 (D34N/E59K)), l) SEQ ID NO: XXX (IL-12p40(E59K/K99E)), li) SEQ ID NO: XXX (IL-12p40(D18K/E59K/K99E)), lii) SEQ ID NO: XXX (IL-12p40 (E59K/K99E/K264E)), liii) SEQ ID NO: XXX (IL-12p40 (E59K/K99Y)), liv) SEQ ID NO: XXX (IL-12p40 (E59Y/K99E)), lv) SEQ ID NO: XXX (IL-12p40 (E45K/E59K/K99E)), lvi) SEQ ID NO: XXX (IL-12p40 (E59K/K99E/Q144E)), lvii) SEQ ID NO: XXX (IL-12p40 (E59K/K99E/Q144K)), lviii) SEQ ID NO: XXX (IL-12p40 (E59K/K99E/R159E)), lix) SEQ ID NO: XXX (IL-12p40 (E59K/K99E/K264E)), lx) SEQ ID NO: XXX (IL-12p40 (D18K/E59K/K99E/K264E)), lxi) SEQ ID NO: XXX (IL-12p40 (C252S)), lxii) SEQ ID NO: XXX (IL-12p40 (DI8K/E59K/K99E/C252S)), lxiii) SEQ ID NO: XXX (IL-12p40 (D18K/E59K/K99E/C252S/K264E)), lxiv) SEQ ID NO: XXX (IL-12p40 (E59K/K99Y/C252S)), lxv) SEQ ID NO: XXX (IL-12p40 (E59K/K99E/C252S/K264E)), lxvi) SEQ ID NO: XXX (IL-12p40 (E59K/K99E/C252S)), lxvii) SEQ ID NO: XXX (IL-12p40 (N103D/N113D)), lxviii) SEQ ID NO: XXX (IL-12p40 (N103D/N200D)), lxix) SEQ ID NO: XXX (IL-12p40 (N103D/N281D)), lxx) SEQ ID NO: XXX (IL-12p40 (N113D/N200D)), lxxi) SEQ ID NO: XXX (IL-12p40 (N113D/N281D)), lxxii) SEQ ID NO: XXX (IL-12p40 (N200D/N281D)), lxxiii) SEQ ID NO: XXX (IL-12p50 (N103D/N113D/N200D)), lxxiv) SEQ ID NO: XXX (IL-12p40 (N103D/N113D/N281D)), lxxv) SEQ ID NO: XXX (IL-12p40 (N103D/N200D/N281D)), lxxvi) SEQ ID NO: XXX (IL-12p40 (N113D/N200D/N281D)), lxxvii) SEQ ID NO: XXX (IL-12p40 (N103Q)), lxxviii) SEQ ID NO: XXX (IL-12p40 (N113Q)), lxxix) SEQ ID NO: XXX (IL-12p40 (N200Q)), lxxx) SEQ ID NO: XXX (IL-12p40 (N281Q)), lxxxi) SEQ ID NO: XXX (IL-12p40

(N103Q/N113Q)), lxxxii) SEQ ID NO: XXX (IL-12p40 (N103Q/N200Q)), lxxxiii) SEQ ID NO: XXX (IL-12p40 (N103Q/N281Q)), lxxxiv) SEQ ID NO: XXX (IL-12p40 (N113Q/N200Q)), lxxxv) SEQ ID NO: XXX (IL-12p40 (N113Q/N281Q)), lxxxvi) SEQ ID NO: XXX (IL-12p40 (N200Q/N281Q)), lxxxvii) SEQ ID NO: XXX (IL-12p40 (N103Q/N113Q/N200Q)), lxxxviii) SEQ ID NO: XXX (IL-12p40 (N103Q/N113Q/N281Q)), lxxxix) SEQ ID NO: XXX (IL-12p40 (N103Q/N200Q/N281Q)), xc) SEQ ID NO: XXX (IL-12p40 (N113Q/N200Q/N281Q)), xci) SEQ ID NO:XXX (IL-12p40 (N103Q/N113Q/N200Q/N281Q)), xcii) SEQ ID NO:XXX (IL-12p40 (D34N/E59K/K99E)), xciii) SEQ ID NO:XXX (IL-12p40 (D34K/E59K/K99E)), xciv) SEQ ID NO:XXX (IL-12p40 (E32Q/D34N/E59K/K99E)), xcv) SEQ ID NO:XXX (IL-12p40 (E32K/D34N/E59K/K99E)), xcvi) SEQ ID NO: XXX (IL-12p40 (E59Y/K99Y)), xcvii) SEQ ID NO:XXX (IL-12p40 (E59K/K99E/N103Q/C252S/K264E)), xcviii) SEQ ID NO:XXX (IL-12p40 (E59K/K99E/N113Q/C252S/K264E)), xcix) SEQ ID NO:XXX (IL-12p40 (E59K/K99E/N200Q/C252S/K264E)), c) SEQ ID NO:XXX (IL-12p40 (E59K/K99E/N281Q/C252S/K264E)), ci) SEQ ID NO:XXX (IL-12p40 (E59K/K99E/N103Q/N113Q/C252S/K264E)), cii) SEQ ID NO:XXX (IL-12p40 (E59K/K99E/N103Q/N200Q/C252S/K264E)), ciii) SEQ ID NO:XXX (IL-12p40 (E59K/K99E/N103Q/N281Q/C252S/K264E)), civ) SEQ ID NO:XXX (IL-12p40 (E59K/K99E/N113Q/N200Q/C252S/K264E)), cv) SEQ ID NO:XXX (IL-12p40 (E59K/K99E/N113Q/N281Q/C252S/K264E)), cvi) SEQ ID NO:XXX (IL-12p40 (E59K/K99E/N200Q/N281Q/C252S/K264E)), cvii) SEQ ID NO:XXX (IL-12p40 (E59K/K99E/N103Q/N113Q/N200Q/C252S/K264E)), cviii) SEQ ID NO:XXX (IL-12p40 (E59K/K99E/N103Q/N200Q/N281Q/C252S/K264E)), and cix) SEQ ID NO:XXX (IL-12p40 (E59K/K99E/N113Q/N200Q/N281Q/C252S/K264E)).

110. The heterodimeric Fc fusion protein according to any one of claims 97-109, wherein said IL-12p35 subunit has one or more amino acid modifications as amino acid residues selected from the group consisting of: Q20, N21, Q35, E38, S44, E45, E46, H49, K54, D55, T59, V60, E61, C63, L64, P65, E67, L68, N71, S73, C74, L75, N76, E79, N85, L89, F96, M97, L124, M125, Q130, Q135,

N136, E143, Q146, N151, E153, K158, E162, E163, D165, I171, R181, I182, R183, V185, T186, D188, R189, V190, S192, Y193, N195, and A196.

111. The heterodimeric Fc fusion protein according to any one of claims 97-110, wherein said IL-12p35 subunit has one or more amino acid substitutions selected from the group consisting of: N21D, Q35D, E38Q, D55Q, D55K, N71D, N71Q, L75A, N76D, E79Q, N85D, N85Q, L89A, F96A, M97A, L124A, M125A, Q130E, Q135E, N136D, E143Q, Q146E, N151D, N151K, E153K, E153Q, K158E, E162Q, E163Q, D165N, I171A, N195D, and N195Q.

112. The heterodimeric Fc fusion protein according to any one of claims 97-111, wherein said IL-12p35 subunit has amino acid substitutions selected from the group consisting of: N71D/N85D/N195D, N151D/E153Q, N151D/D165N, Q130E/N151D, N151D/K158E, E79Q/N151D, D55Q/N151D, N136D/N151D, N21D/N151D, E143Q/N151D, N71Q/N85Q, N71Q/N195Q, N85Q/N195Q, N71Q/N85Q/N195Q, N71D/N85D, N71D/N195D, and N85D/N195D.

113. The heterodimeric Fc fusion protein according to any one of claims 97-112, wherein said IL-12p35 subunit has a polypeptide sequence selected from the group consisting of: i) SEQ ID NO:113 (IL-12p35(N71D)), ii) SEQ ID NO:114 (IL-12p35(N85D)), iii) SEQ ID NO:115 (IL-12p35(N195D)), iv) SEQ ID NO:116 (IL-12p35(N71D/N85D/N195D)), v) SEQ ID NO:117 (IL-12p35(E153Q)), vi) SEQ ID NO:118 (IL-12p35(E38Q)), vii) SEQ ID NO:119 (IL-12p35(N151D)), viii) SEQ ID NO:120 (IL-12p35(Q135E)), ix) SEQ ID NO:121 (IL-12p35(Q35D)), x) SEQ ID NO:122 (IL-12p35(Q146E)), xi) SEQ ID NO:123 (IL-12p35(N76D)), xii) SEQ ID NO:124 (IL-12p35(E162Q)), xiii) SEQ ID NO:125 (IL-12p35(E163Q)), xiv) IL-12p35(N21D), xv) SEQ ID NO:XXX (IL-12p35(D55Q)), xvi) IL-12p35(E79Q), xvii) IL-12p35(Q130E), xviii) IL-12p35(N136D), xix) IL-12p35(E143Q), xx) SEQ ID NO:227 (IL-12p35(N151K)), xxi) SEQ ID NO:226 (IL-12p35(E153K)), xxii) IL-12p35(K158E), xxiii) IL-12p35(D165N), xxiv) SEQ ID NO:225 (IL-12p35(N151D/E153Q)), xxv) SEQ ID NO:228 (IL-12p35(N151D/D165N)), xxvi) SEQ ID NO:229 (IL-12p35(Q130E/N151D)), xxvii) SEQ ID NO:230 (IL-12p35(N151D/K158E)), xxviii) SEQ ID NO:231 (IL-12p35(E79Q/N151D)), xxix) SEQ ID NO:232 (IL-12p35(D55Q/N151D)), xxx)

SEQ ID NO:233 (IL-12p35(N136D/N151D)), xxxi) SEQ ID NO:234 (IL-12p35(N21D/N151D)), xxxii) SEQ ID NO:235 (IL-12p35(E143Q/N151D)), xxxiii) SEQ ID NO: XXX (IL-12p35(F96A)), xxxiv) SEQ ID NO: XXX (IL-12p35(M97A)), xxxv) SEQ ID NO: XXX (IL-12p35(L89A)), xxxvi) SEQ ID NO: XXX (IL-12p35(L124A)), xxxvii) SEQ ID NO: XXX (IL-12p35(M125A)), xxxviii) SEQ ID NO: XXX (IL-12p35(L75A)), xxxiv) SEQ ID NO: XXX (IL-12p35(I171A)), xxxv) SEQ ID NO: XXX (IL-12p35 (N71Q)), xxxvi) SEQ ID NO: XXX (IL-12p35 (N85Q)), xxxvii) SEQ ID NO: XXX (IL-12p35 (N195Q)), xxxviii) SEQ ID NO: XXX (IL-12p35 (N71Q/N85Q)), xxxix) SEQ ID NO: XXX (IL-12p35 (N71Q/N195Q)), xl) SEQ ID NO: XXX (IL-12p35 (N85Q/N195Q), xli) SEQ ID NO: XXX (IL-12p35 (N71Q/N85Q/N195Q)), xlii) SEQ ID NO: XXX (IL-12p35 (N71D/N85D)), xliii) SEQ ID NO: XXX (IL-12p35 (N71D/N195D), xliv) SEQ ID NO: XXX (IL-12p35 (N85D/N195D)), xlv) SEQ ID NO: XXX (IL-12p35 (D55Q)), and xlvi) SEQ ID NO: XXX (IL-12p35 (D55K)).

114. A composition comprising a heterodimeric Fc fusion protein according to any one of claims 97-113 for use in treating cancer in a subject.

115. One or more nucleic acids encoding a heterodimeric Fc fusion protein according to any one of claims 97-113.

116. A host cell comprising said one or more nucleic acids of claim 115.

117. A method of making a heterodimeric Fc fusion protein, said method comprising culturing a host cell according to claim 116 under conditions whereby said heterodimeric Fc fusion protein is produced.

118. A method of purifying a heterodimeric Fc fusion protein according to any one of claims 97-113, said method comprising:

a) providing a composition comprising the heterodimeric Fc fusion protein;

b) loading said composition onto an ion exchange column; and

c) collecting a fraction containing said heterodimeric Fc fusion protein.

119. A heterodimeric Fc fusion protein comprising:

a) a fusion protein comprising a first protein domain, a second protein domain and a first Fc domain, wherein said first protein domain is attached to the C-terminus of said first Fc domain, and wherein said second protein domain is covalently attached to said first protein domain; and

b) a second Fc domain;

wherein said first and said second Fc domains comprise modifications promoting heterodimerization of said first and said second Fc domains and wherein said first protein domain comprises an IL-12p35 subunit and said second protein domain comprises an IL-12p40 subunit.

120. The heterodimeric Fc fusion protein according to claim 119, wherein said modifications promoting heterodimerization of said first and said second Fc domains are a set of amino acid substitutions selected from the group consisting of L368D/K370S and S364K; L368D/K370S and S364K/E357L; L368D/K370S and S364K/E357Q; T411E/K360E/Q362E and D401K; L368E/K370S and S364K; K370S and S364K/E357Q and T366S/L368A/Y407V : T366W (optionally including a bridging disulfide, T366S/L368A/Y407V/Y349C : T366W/S354C), according to EU numbering.

121. The heterodimeric Fc fusion protein according to claim 119 or 120, wherein said first protein domain is attached to said second protein domain using a first domain linker and/or said second protein domain is attached to said first Fc domain using a second domain linker.

122. The heterodimeric Fc fusion protein according to any one of claims 119 to 121, wherein said first and/or said second Fc domains have an additional set of amino acid substitutions comprising Q295E/N384D/Q418E/N421D, according to EU numbering.

123. The heterodimeric Fc fusion protein according to any one of claims 119 to 122, wherein said first and/or said second Fc domains have an additional set of amino acid substitutions selected from the group consisting of G236R/L328R, E233P/L234V/L235A/G236_/S239K,

E233P/L234V/L235A/G236_/S239K/A327G, E233P/L234V/L235A/G236_/S267K/A327G, E233P/L234V/L235A/G236_, and E233P/L234V/L235A/G236_/S267K, according to EU numbering.

124. The heterodimeric Fc fusion protein according to any one of claims 119-123, wherein said IL-12p40 subunit has a polypeptide sequence selected form the group consisting of SEQ ID NO:3 (human IL-12 subunit beta (IL-12p40) precursor sequence) and SEQ ID NO:4 (human IL-12 subunit beta (IL-12p40) mature form sequence), and/or said IL-12p35 subunit has a polypeptide sequence selected from the group consisting of SEQ ID NO:1 (human IL-12 subunit alpha (IL-12p35) precursor sequence) and SEQ ID NO:2 (human IL-12 subunit alpha (IL-12p35) mature form sequence).

125. The heterodimeric Fc fusion protein according to any one of claims 119-124, wherein said IL-12p40 subunit is a variant IL-12p40 subunit and/or said IL-12p35 subunit is a variant IL-12p35 subunit.

126. The heterodimeric Fc fusion protein according to any one of claims 119-125, wherein said IL-12p40 subunit is a variant IL-12p40 subunit having altered affinity for IL-12 receptor subunit beta-1 (IL-12Rß1), IL-12 receptor subunit beta-2 (IL-12Rß2), and/or IL-12 receptor complex; and/or said IL-12p35 subunit is a variant IL-12p35 subunit having altered affinity for IL-12 receptor subunit beta-1 (IL-12Rß1), IL-12 receptor subunit beta-2 (IL-12Rß2), and/or IL-12 receptor complex.

127. The heterodimeric Fc fusion protein according to any one of claims 119-126, wherein said IL-12p40 subunit has one or more amino acid modifications at amino acid residues selected from the group consisting of E3, D7, E12, D14, W15, P17, D18, A19, P20, G21, E22, M23, D29, E32, E33, D34, L40, D41, Q42, S43, E45, L47, T54, I55, Q56, K58, E59, F60, G61, D62, Q65, Y66, E73, K84, E86, D87, G88, I89, W90, D93, D97, K99, E100, K102, N103, K104, F106, E110, N113, Y114, D129, D142, Q144, E156, R159, D161, N162, K163, D166, D170, Q172, D174, A176, C177, P178, A179, A180, E181, S183, P185, E187, N200, S204, F206, R208, D209, D214, N218, Q220,

N226, Q229, E231, E235, T242, P243, S245, Y246, F247, S248, C252, Q256, K258, K260, E262, K264, D265, D270, N281, Q289, D290, R291, Y292, Y293, and E299.

128. The heterodimeric Fc fusion protein according to any one of claims 119-127, wherein said IL-12p40 subunit has one or more amino acid substitutions selected from the group consisting of: D18N, D18K, E32Q, E33Q, D34N, D34K, Q42E, S43E, S43K, E45Q, Q56E, E59Q, E59K, D62N, E73Q, D87N, K99E, K99Y, E100Q, N103D, N103Q, N113D, N113Q, Q144E, D161N, R159E, K163E, E187Q, N200D, N200Q, N218Q, Q229E, E235Q, C252S, Q256N, K258E, K260E, E262Q, K264E, N281D, N281Q, and E299Q.

129. The heterodimeric Fc fusion protein according to any one of claims 119-128, wherein said IL-12p40 subunit has amino acid substitutions selected from the group consisting of: N103D/N113D/N200D/N281D, Q42E/E45Q, E45Q/Q56E, Q42E/E59Q, Q56E/E59Q, Q42E/E45Q/Q56E, E45Q/Q56E/E59Q, E32Q/E59Q, D34N/E59K, D34N/E59K/K99E, D34K/E59K/K99E, E32Q/D34N/E59K/K99E, E32K/D34N/E59K/K99E, D34N/E59Q, E59Q/E187Q, S43E/E59Q, S43K/E49Q, E59Q/K163E, E59Q/K99E, E59Q/K258E, E59Q/K260E, E59K/K99E, D18K/E59K/K99E, E59K/K99E/K264E, E59K/K99Y, E59Y/K99Y, E59Y/K99E, E45K/E59K/K99E, E59K/K99E/Q144E, E59K/K99E/Q144K, E59K/K99E/R159E, E59K/K99E/K264E, D18K/E59K/K99E/K264E, DI8K/E59K/K99E/C252S, D18K/E59K/K99E/C252S/K264E, E59K/K99Y/C252S, E59K/K99E/C252S/K264E, E59K/K99E/C252S, N103D/N113D, N103D/N200D, N103D/N281D, N113D/N200D, N113D/N281D, N200D/N281D, N103D/N113D/N200D, N103D/N113D/N281D, N103D/N200D/N281D, N113D/N200D/N281D, N103Q/N113Q, N103Q/N200Q, N103Q/N281Q, N113Q/N200Q, N113Q/N281Q, N200Q/N281Q, N103Q/N113Q/N200Q, N103Q/N113Q/N281Q, N103Q/N200Q/N281Q, N113Q/N200Q/N281Q, N103Q/N113Q/N200Q/N281Q, E59K/K99E/N103Q/C252S/K264E, E59K/K99E/N113Q/C252S/K264E, E59K/K99E/N200Q/C252S/K264E, E59K/K99E/N281Q/C252S/K264E, E59K/K99E/N103Q/N113Q/C252S/K264E, E59K/K99E/N103Q/N200Q/C252S/K264E, E59K/K99E/N103Q/N281Q/C252S/K264E, E59K/K99E/N113Q/N200Q/C252S/K264E, E59K/K99E/N113Q/N281Q/C252S/K264E, E59K/K99E/N200Q/N281Q/C252S/K264E, E59K/K99E/N103Q/N113Q/N200Q/C252S/K264E,

E59K/K99E/N103Q/N200Q/N281Q/C252S/K264E,
E59K/K99E/N113Q/N200Q/N281Q/C252S/K264E, and
E59K/K99E/N103Q/N113Q/N200Q/N281Q/C252S/K264E.

130. The heterodimeric Fc fusion protein according to any one of claims 119-129, wherein said IL-12p40 subunit has a polypeptide sequence selected from the group consisting of: i) SEQ ID NO:57 (IL-12p40(N103D)), ii) SEQ ID NO:58 (IL-12p40(N113D)), iii) SEQ ID NO:59 (IL-12p40(N200D)), iv) SEQ ID NO:60 (IL-12p40(N281D)), v) SEQ ID NO:61 (IL-12p40(N103D/N113D/N200D/N281D)), vi) SEQ ID NO:62 (IL-12p40(Q42E)), vii) SEQ ID NO:63 (IL-12p40(E45Q)), viii) SEQ ID NO:64 (IL-12p40(Q56E)), ix) SEQ ID NO:65 (IL-12p40(E59Q)), x) SEQ ID NO:66 (IL-12p40(D62N)), xi) SEQ ID NO:67 (IL-12p40(Q42E/E45Q)), xii) SEQ ID NO:68 (IL-12p40(E45Q/Q56E)), xiii) SEQ ID NO:69 (IL-12p40(Q42E/E59Q)), xiv) SEQ ID NO:70 (IL-12p40(Q56E/E59Q)), xv) SEQ ID NO:71 (IL-12p40(Q42E/E45Q/Q56E)), xvi) SEQ ID NO:72 (IL-12p40(E45Q/Q56E/E59Q)), xvii) SEQ ID NO:73 (IL-12p40(D161N)), xviii) SEQ ID NO:74 (IL-12p40(E73Q)), xix) SEQ ID NO:75 (IL-12p40(Q144E)), xx) SEQ ID NO:76 (IL-12p40(E262Q)), xxi) SEQ ID NO:77 (IL-12p40(E100Q)), xxii) SEQ ID NO:78 (IL-12p40(D18N)), xxiii) SEQ ID NO:79 (IL-12p40(E33Q)), xxiv) SEQ ID NO:80 (IL-12p40(Q229E)), xxv) SEQ ID NO:81 (IL-12p40(E235Q)), xxvi) SEQ ID NO:82 (IL-12p40(Q256N)), xxvii) SEQ ID NO:83 (IL-12p40(E299Q)), xxviii) SEQ ID NO:84 (IL-12p40(D87N)), xxix) IL-12p40(E32Q), xxx) IL-12p40(D34N), xxxi) IL-12p40(S43E), xxxii) IL-12p40(S43K), xxxiii) SEQ ID NO:XXX (IL-12p40 (E59K/K99E/N103Q/N113Q/N200Q/N281Q/C252S/K264E)), xxxiv) SEQ ID NO:205 (IL-12p40(E59K)), xxxv) IL-12p40(K99E), xxxvi) IL-12p40(K163E), xxxvii) IL-12p40(E187Q), xxxviii) IL-12p40(K258E), xxxix) IL-12p40(K260E), xl) SEQ ID NO:206 (IL-12p40(E32Q/E59Q)), xli) SEQ ID NO:207 (IL-12p40(D34N/E59Q)), xlii) SEQ ID NO:208 (IL-12p40(E59Q/E187Q)), xliii) SEQ ID NO:209 (IL-12p40(S43E/E59Q)), xliv) SEQ ID NO:210 (IL-12p40(S43K/E49Q)), xlv) SEQ ID NO:211 (IL-12p40(E59Q/K163E)), xlvi) SEQ ID NO:212 (IL-12p40(E59Q/K99E)), xlvii) SEQ ID NO:213 (IL-12p40(E59Q/K258E)), xlviii) SEQ ID NO:214 (IL-12p40(E59Q/K260E)), xlix) SEQ ID NO: XXX (IL-12p40 (D34N/E59K)), l) SEQ ID NO: XXX (IL-12p40(E59K/K99E)), li) SEQ ID NO: XXX (IL-12p40(D18K/E59K/K99E)), lii) SEQ ID NO: XXX (IL-12p40 (E59K/K99E/K264E)),

liii) SEQ ID NO: XXX (IL-12p40 (E59K/K99Y)), liv) SEQ ID NO: XXX (IL-12p40 (E59Y/K99E)), lv) SEQ ID NO: XXX (IL-12p40 (E45K/E59K/K99E)), lvi) SEQ ID NO: XXX (IL-12p40 (E59K/K99E/Q144E)), lvii) SEQ ID NO: XXX (IL-12p40 (E59K/K99E/Q144K)), lviii) SEQ ID NO: XXX (IL-12p40 (E59K/K99E/R159E)), lix) SEQ ID NO: XXX (IL-12p40 (E59K/K99E/K264E)), lx) SEQ ID NO: XXX (IL-12p40 (D18K/E59K/K99E/K264E)), lxi) SEQ ID NO: XXX (IL-12p40 (C252S)), lxii) SEQ ID NO: XXX (IL-12p40 (DI8K/E59K/K99E/C252S)), lxiii) SEQ ID NO: XXX (IL-12p40 (D18K/E59K/K99E/C252S/K264E)), lxiv) SEQ ID NO: XXX (IL-12p40 (E59K/K99Y/C252S)), lxv) SEQ ID NO: XXX (IL-12p40 (E59K/K99E/C252S/K264E)), lxvi) SEQ ID NO: XXX (IL-12p40 (E59K/K99E/C252S)), lxvii) SEQ ID NO: XXX (IL-12p40 (N103D/N113D)), lxviii) SEQ ID NO: XXX (IL-12p40 (N103D/N200D)), lxix) SEQ ID NO: XXX (IL-12p40 (N103D/N281D)), lxx) SEQ ID NO: XXX (IL-12p40 (N113D/N200D)), lxxi) SEQ ID NO: XXX (IL-12p40 (N113D/N281D)), lxxii) SEQ ID NO: XXX (IL-12p40 (N200D/N281D)), lxxiii) SEQ ID NO: XXX (IL-12p50 (N103D/N113D/N200D)), lxxiv) SEQ ID NO: XXX (IL-12p40 (N103D/N113D/N281D)), lxxv) SEQ ID NO: XXX (IL-12p40 (N103D/N200D/N281D)), lxxvi) SEQ ID NO: XXX (IL-12p40 (N113D/N200D/N281D)), lxxvii) SEQ ID NO: XXX (IL-12p40 (N103Q)), lxxviii) SEQ ID NO: XXX (IL-12p40 (N113Q)), lxxix) SEQ ID NO: XXX (IL-12p40 (N200Q)), lxxx) SEQ ID NO: XXX (IL-12p40 (N281Q)), lxxxi) SEQ ID NO: XXX (IL-12p40 (N103Q/N113Q)), lxxxii) SEQ ID NO: XXX (IL-12p40 (N103Q/N200Q)), lxxxiii) SEQ ID NO: XXX (IL-12p40 (N103Q/N281Q)), lxxxiv) SEQ ID NO: XXX (IL-12p40 (N113Q/N200Q)), lxxxv) SEQ ID NO: XXX (IL-12p40 (N113Q/N281Q)), lxxxvi) SEQ ID NO: XXX (IL-12p40 (N200Q/N281Q)), lxxxvii) SEQ ID NO: XXX (IL-12p40 (N103Q/N113Q/N200Q)), lxxxviii) SEQ ID NO: XXX (IL-12p40 (N103Q/N113Q/N281Q)), lxxxix) SEQ ID NO: XXX (IL-12p40 (N103Q/N200Q/N281Q)), xc) SEQ ID NO: XXX (IL-12p40 (N113Q/N200Q/N281Q)), xci) SEQ ID NO:XXX (IL-12p40 (N103Q/N113Q/N200Q/N281Q)), xcii) SEQ ID NO:XXX (IL-12p40 (D34N/E59K/K99E)), xciii) SEQ ID NO:XXX (IL-12p40 (D34K/E59K/K99E)), xciv) SEQ ID NO:XXX (IL-12p40 (E32Q/D34N/E59K/K99E)), xcv) SEQ ID NO:XXX (IL-12p40 (E32K/D34N/E59K/K99E)), xcvi) SEQ ID NO: XXX (IL-12p40 (E59Y/K99Y)), xcvii) SEQ ID NO:XXX (IL-12p40 (E59K/K99E/N103Q/C252S/K264E)), xcviii) SEQ ID NO:XXX (IL-12p40 (E59K/K99E/N113Q/C252S/K264E)), xcix) SEQ ID NO:XXX (IL-12p40

(E59K/K99E/N200Q/C252S/K264E)), c) SEQ ID NO:XXX (IL-12p40 (E59K/K99E/N281Q/C252S/K264E)), ci) SEQ ID NO:XXX (IL-12p40 (E59K/K99E/N103Q/N113Q/C252S/K264E)), cii) SEQ ID NO:XXX (IL-12p40 (E59K/K99E/N103Q/N200Q/C252S/K264E)), ciii) SEQ ID NO:XXX (IL-12p40 (E59K/K99E/N103Q/N281Q/C252S/K264E)), civ) SEQ ID NO:XXX (IL-12p40 (E59K/K99E/N113Q/N200Q/C252S/K264E)), cv) SEQ ID NO:XXX (IL-12p40 (E59K/K99E/N113Q/N281Q/C252S/K264E)), cvi) SEQ ID NO:XXX (IL-12p40 (E59K/K99E/N200Q/N281Q/C252S/K264E)), cvii) SEQ ID NO:XXX (IL-12p40 (E59K/K99E/N103Q/N113Q/N200Q/C252S/K264E)), cviii) SEQ ID NO:XXX (IL-12p40 (E59K/K99E/N103Q/N200Q/N281Q/C252S/K264E)), and cix) SEQ ID NO:XXX (IL-12p40 (E59K/K99E/N113Q/N200Q/N281Q/C252S/K264E)).

131. The heterodimeric Fc fusion protein according to any one of claims 119-130, wherein said IL-12p35 subunit has one or more amino acid modifications as amino acid residues selected from the group consisting of: Q20, N21, Q35, E38, S44, E45, E46, H49, K54, D55, T59, V60, E61, C63, L64, P65, E67, L68, N71, S73, C74, L75, N76, E79, N85, L89, F96, M97, L124, M125, Q130, Q135, N136, E143, Q146, N151, E153, K158, E162, E163, D165, I171, R181, I182, R183, V185, T186, D188, R189, V190, S192, Y193, N195, and A196.

132. The heterodimeric Fc fusion protein according to any one of claims 119-131, wherein said IL-12p35 subunit has one or more amino acid substitutions selected from the group consisting of: N21D, Q35D, E38Q, D55Q, D55K, N71D, N71Q, L75A, N76D, E79Q, N85D, N85Q, L89A, F96A, M97A, L124A, M125A, Q130E, Q135E, N136D, E143Q, Q146E, N151D, N151K, E153K, E153Q, K158E, E162Q, E163Q, D165N, I171A, N195D, and N195Q.

133. The heterodimeric Fc fusion protein according to any one of claims 119-132, wherein said IL-12p35 subunit has amino acid substitutions selected from the group consisting of: N71D/N85D/N195D, N151D/E153Q, N151D/D165N, Q130E/N151D, N151D/K158E, E79Q/N151D, D55Q/N151D, N136D/N151D, N21D/N151D, E143Q/N151D, N71Q/N85Q,

N71Q/N195Q, N85Q/N195Q, N71Q/N85Q/N195Q, N71D/N85D, N71D/N195D, and N85D/N195D.

134. The heterodimeric Fc fusion protein according to any one of claims 119-133, wherein said IL-12p35 subunit has a polypeptide sequence selected from the group consisting of: i) SEQ ID NO:113 (IL-12p35(N71D)), ii) SEQ ID NO:114 (IL-12p35(N85D)), iii) SEQ ID NO:115 (IL-12p35(N195D)), iv) SEQ ID NO:116 (IL-12p35(N71D/N85D/N195D)), v) SEQ ID NO:117 (IL-12p35(E153Q)), vi) SEQ ID NO:118 (IL-12p35(E38Q)), vii) SEQ ID NO:119 (IL-12p35(N151D)), viii) SEQ ID NO:120 (IL-12p35(Q135E)), ix) SEQ ID NO:121 (IL-12p35(Q35D)), x) SEQ ID NO:122 (IL-12p35(Q146E)), xi) SEQ ID NO:123 (IL-12p35(N76D)), xii) SEQ ID NO:124 (IL-12p35(E162Q)), xiii) SEQ ID NO:125 (IL-12p35(E163Q)), xiv) IL-12p35(N21D), xv) SEQ ID NO:XXX (IL-12p35(D55Q)), xvi) IL-12p35(E79Q), xvii) IL-12p35(Q130E), xviii) IL-12p35(N136D), xix) IL-12p35(E143Q), xx) SEQ ID NO:227 (IL-12p35(N151K)), xxi) SEQ ID NO:226 (IL-12p35(E153K)), xxii) IL-12p35(K158E), xxiii) IL-12p35(D165N), xxiv) SEQ ID NO:225 (IL-12p35(N151D/E153Q)), xxv) SEQ ID NO:228 (IL-12p35(N151D/D165N)), xxvi) SEQ ID NO:229 (IL-12p35(Q130E/N151D)), xxvii) SEQ ID NO:230 (IL-12p35(N151D/K158E)), xxviii) SEQ ID NO:231 (IL-12p35(E79Q/N151D)), xxix) SEQ ID NO:232 (IL-12p35(D55Q/N151D)), xxx) SEQ ID NO:233 (IL-12p35(N136D/N151D)), xxxi) SEQ ID NO:234 (IL-12p35(N21D/N151D)), xxxii) SEQ ID NO:235 (IL-12p35(E143Q/N151D)), xxxiii) SEQ ID NO: XXX (IL-12p35(F96A)), xxxiv) SEQ ID NO: XXX (IL-12p35(M97A)), xxxv) SEQ ID NO: XXX (IL-12p35(L89A)), xxxvi) SEQ ID NO: XXX (IL-12p35(L124A)), xxxvii) SEQ ID NO: XXX (IL-12p35(M125A)), xxxviii) SEQ ID NO: XXX (IL-12p35(L75A)), xxxiv) SEQ ID NO: XXX (IL-12p35(I171A)), xxxv) SEQ ID NO: XXX (IL-12p35 (N71Q)), xxxvi) SEQ ID NO: XXX (IL-12p35 (N85Q)), xxxvii) SEQ ID NO: XXX (IL-12p35 (N195Q)), xxxviii) SEQ ID NO: XXX (IL-12p35 (N71Q/N85Q)), xxxix) SEQ ID NO: XXX (IL-12p35 (N71Q/N195Q)), xl) SEQ ID NO: XXX (IL-12p35 (N85Q/N195Q), xli) SEQ ID NO: XXX (IL-12p35 (N71Q/N85Q/N195Q)), xlii) SEQ ID NO: XXX (IL-12p35 (N71D/N85D)), xliii) SEQ ID NO: XXX (IL-12p35 (N71D/N195D), xliv) SEQ ID NO: XXX (IL-12p35 (N85D/N195D)), xlv) SEQ ID NO: XXX (IL-12p35 (D55Q)), and xlvi) SEQ ID NO: XXX (IL-12p35 (D55K)).

135. A composition comprising a heterodimeric Fc fusion protein according to any one of claims 119-134 for use in treating cancer in a subject.

136. One or more nucleic acids encoding a heterodimeric Fc fusion protein according to any one of claims 119-134.

137. A host cell comprising said one or more nucleic acids of claim 136.

138. A method of making a heterodimeric Fc fusion protein, said method comprising culturing a host cell according to claim 137 under conditions whereby said heterodimeric Fc fusion protein is produced.

139. A method of purifying a heterodimeric Fc fusion protein according to any one of claims 119-134, said method comprising:

a) providing a composition comprising the heterodimeric Fc fusion protein;

b) loading said composition onto an ion exchange column; and

c) collecting a fraction containing said heterodimeric Fc fusion protein.

140. A heterodimeric complex comprising:

a) an IL-12p40 subunit, and

b) an IL-12p35 subunit.

141. The heterodimeric complex according to claim 140, wherein said IL-12p40 subunit is covalently attached to said IL-12p35 subunit using domain linker.

142. The heterodimeric complex according to claim 140 or 141, wherein said IL-12p40 subunit has a polypeptide sequence selected form the group consisting of SEQ ID NO:3 (human IL-12 subunit beta (IL-12p40) precursor sequence) and SEQ ID NO:4 (human IL-12 subunit beta (IL-12p40) mature form sequence), and/or said IL-12p35 subunit has a polypeptide sequence

selected from the group consisting of SEQ ID NO:1 (human IL-12 subunit alpha (IL-12p35) precursor sequence) and SEQ ID NO:2 (human IL-12 subunit alpha (IL-12p35) mature form sequence).

143. The heterodimeric complex according to any one of claims 140-142, wherein said first and second Fc domains further comprise amino acid substitutions M428L/N424S.

144. The heterodimeric complex according to any one of claims 140-143, wherein said IL-12p40 subunit is a variant IL-12p40 subunit and/or said IL-12p35 subunit is a variant IL-12p35 subunit.

145. The heterodimeric complex according to any one of claims 140-144, wherein said IL-12p40 subunit is a variant IL-12p40 subunit having altered affinity for IL-12 receptor subunit beta-1 (IL-12Rß1), IL-12 receptor subunit beta-2 (IL-12Rß2), and/or IL-12 receptor complex; and/or said IL-12p35 subunit is a variant IL-12p35 subunit having altered affinity for IL-12 receptor subunit beta-1 (IL-12Rß1), IL-12 receptor subunit beta-2 (IL-12Rß2), and/or IL-12 receptor complex.

146. The heterodimeric complex according to any one of claims 140-145, wherein said IL-12p40 subunit has one or more amino acid modifications at amino acid residues selected from the group consisting of: E3, D7, E12, D14, W15, P17, D18, A19, P20, G21, E22, M23, D29, E32, E33, D34, L40, D41, Q42, S43, E45, L47, T54, I55, Q56, K58, E59, F60, G61, D62, Q65, Y66, E73, K84, E86, D87, G88, I89, W90, D93, D97, K99, E100, K102, N103, K104, F106, E110, N113, Y114, D129, D142, Q144, E156, R159, D161, N162, K163, D166, D170, Q172, D174, A176, C177, P178, A179, A180, E181, S183, P185, E187, N200, S204, F206, R208, D209, D214, N218, Q220, N226, Q229, E231, E235, T242, P243, S245, Y246, F247, S248, C252, Q256, K258, K260, E262, K264, D265, D270, N281, Q289, D290, R291, Y292, Y293, and E299.

147. The heterodimeric complex according to any one of claims 140-146, wherein said IL-12p40 subunit has one or more amino acid substitutions selected from the group consisting of: D18N, D18K, E32Q, E33Q, D34N, D34K, Q42E, S43E, S43K, E45Q, Q56E, E59Q, E59K, D62N, E73Q, D87N, K99E, K99Y, E100Q, N103D, N103Q, N113D, N113Q, Q144E, D161N, R159E, K163E,

E187Q, N200D, N200Q, N218Q, Q229E, E235Q, C252S, Q256N, K258E, K260E, E262Q, K264E, N281D, N281Q, and E299Q.

148. The heterodimeric complex according to any one of claims 140-147, wherein said IL-12p40 subunit has amino acid substitutions selected from the group consisting of: N103D/N113D/N200D/N281D, Q42E/E45Q, E45Q/Q56E, Q42E/E59Q, Q56E/E59Q, Q42E/E45Q/Q56E, E45Q/Q56E/E59Q, E32Q/E59Q, D34N/E59K, D34N/E59K/K99E, D34K/E59K/K99E, E32Q/D34N/E59K/K99E, E32K/D34N/E59K/K99E, D34N/E59Q, E59Q/E187Q, S43E/E59Q, S43K/E49Q, E59Q/K163E, E59Q/K99E, E59Q/K258E, E59Q/K260E, E59K/K99E, D18K/E59K/K99E, E59K/K99E/K264E, E59K/K99Y, E59Y/K99Y, E59Y/K99E, E45K/E59K/K99E, E59K/K99E/Q144E, E59K/K99E/Q144K, E59K/K99E/R159E, E59K/K99E/K264E, D18K/E59K/K99E/K264E, DI8K/E59K/K99E/C252S, D18K/E59K/K99E/C252S/K264E, E59K/K99Y/C252S, E59K/K99E/C252S/K264E, E59K/K99E/C252S, N103D/N113D, N103D/N200D, N103D/N281D, N113D/N200D, N113D/N281D, N200D/N281D, N103D/N113D/N200D, N103D/N113D/N281D, N103D/N200D/N281D, N113D/N200D/N281D, N103Q/N113Q, N103Q/N200Q, N103Q/N281Q, N113Q/N200Q, N113Q/N281Q, N200Q/N281Q, N103Q/N113Q/N200Q, N103Q/N113Q/N281Q, N103Q/N200Q/N281Q, N113Q/N200Q/N281Q, N103Q/N113Q/N200Q/N281Q, E59K/K99E/N103Q/C252S/K264E, E59K/K99E/N113Q/C252S/K264E, E59K/K99E/N200Q/C252S/K264E, E59K/K99E/N281Q/C252S/K264E, E59K/K99E/N103Q/N113Q/C252S/K264E, E59K/K99E/N103Q/N200Q/C252S/K264E, E59K/K99E/N103Q/N281Q/C252S/K264E, E59K/K99E/N113Q/N200Q/C252S/K264E, E59K/K99E/N113Q/N281Q/C252S/K264E, E59K/K99E/N200Q/N281Q/C252S/K264E, E59K/K99E/N103Q/N113Q/N200Q/C252S/K264E, E59K/K99E/N103Q/N200Q/N281Q/C252S/K264E, E59K/K99E/N113Q/N200Q/N281Q/C252S/K264E, and E59K/K99E/N103Q/N113Q/N200Q/N281Q/C252S/K264E.

149. The heterodimeric complex according to any one of claims 140-148, wherein said IL-12p40 subunit has a polypeptide sequence selected from the group consisting of i) SEQ ID NO:57 (IL-12p40(N103D)), ii) SEQ ID NO:58 (IL-12p40(N113D)), iii) SEQ ID NO:59 (IL-12p40(N200D)),

iv) SEQ ID NO:60 (IL-12p40(N281D)), v) SEQ ID NO:61 (IL-12p40(N103D/N113D/N200D/N281D)), vi) SEQ ID NO:62 (IL-12p40(Q42E)), vii) SEQ ID NO:63 (IL-12p40(E45Q)), viii) SEQ ID NO:64 (IL-12p40(Q56E)), ix) SEQ ID NO:65 (IL-12p40(E59Q)), x) SEQ ID NO:66 (IL-12p40(D62N)), xi) SEQ ID NO:67 (IL-12p40(Q42E/E45Q)), xii) SEQ ID NO:68 (IL-12p40(E45Q/Q56E)), xiii) SEQ ID NO:69 (IL-12p40(Q42E/E59Q)), xiv) SEQ ID NO:70 (IL-12p40(Q56E/E59Q)), xv) SEQ ID NO:71 (IL-12p40(Q42E/E45Q/Q56E)), xvi) SEQ ID NO:72 (IL-12p40(E45Q/Q56E/E59Q)), xvii) SEQ ID NO:73 (IL-12p40(D161N)), xviii) SEQ ID NO:74 (IL-12p40(E73Q)), xix) SEQ ID NO:75 (IL-12p40(Q144E)), xx) SEQ ID NO:76 (IL-12p40(E262Q)), xxi) SEQ ID NO:77 (IL-12p40(E100Q)), xxii) SEQ ID NO:78 (IL-12p40(D18N)), xxiii) SEQ ID NO:79 (IL-12p40(E33Q)), xxiv) SEQ ID NO:80 (IL-12p40(Q229E)), xxv) SEQ ID NO:81 (IL-12p40(E235Q)), xxvi) SEQ ID NO:82 (IL-12p40(Q256N)), xxvii) SEQ ID NO:83 (IL-12p40(E299Q)), xxviii) SEQ ID NO:84 (IL-12p40(D87N)), xxix) IL-12p40(E32Q), xxx) IL-12p40(D34N), xxxi) IL-12p40(S43E), xxxii) IL-12p40(S43K), xxxiii) SEQ ID NO:XXX (IL-12p40 (E59K/K99E/N103Q/N113Q/N200Q/N281Q/C252S/K264E)), xxxiv) SEQ ID NO:205 (IL-12p40(E59K)), xxxv) IL-12p40(K99E), xxxvi) IL-12p40(K163E), xxxvii) IL-12p40(E187Q), xxxviii) IL-12p40(K258E), xxxix) IL-12p40(K260E), xl) SEQ ID NO:206 (IL-12p40(E32Q/E59Q)), xli) SEQ ID NO:207 (IL-12p40(D34N/E59Q)), xlii) SEQ ID NO:208 (IL-12p40(E59Q/E187Q)), xliii) SEQ ID NO:209 (IL-12p40(S43E/E59Q)), xliv) SEQ ID NO:210 (IL-12p40(S43K/E49Q)), xlv) SEQ ID NO:211 (IL-12p40(E59Q/K163E)), xlvi) SEQ ID NO:212 (IL-12p40(E59Q/K99E)), xlvii) SEQ ID NO:213 (IL-12p40(E59Q/K258E)), xlviii) SEQ ID NO:214 (IL-12p40(E59Q/K260E)), xlix) SEQ ID NO: XXX (IL-12p40 (D34N/E59K)), l) SEQ ID NO: XXX (IL-12p40(E59K/K99E)), li) SEQ ID NO: XXX (IL-12p40(D18K/E59K/K99E)), lii) SEQ ID NO: XXX (IL-12p40 (E59K/K99E/K264E)), liii) SEQ ID NO: XXX (IL-12p40 (E59K/K99Y)), liv) SEQ ID NO: XXX (IL-12p40 (E59Y/K99E)), lv) SEQ ID NO: XXX (IL-12p40 (E45K/E59K/K99E)), lvi) SEQ ID NO: XXX (IL-12p40 (E59K/K99E/Q144E)), lvii) SEQ ID NO: XXX (IL-12p40 (E59K/K99E/Q144K)), lviii) SEQ ID NO: XXX (IL-12p40 (E59K/K99E/R159E)), lix) SEQ ID NO: XXX (IL-12p40 (E59K/K99E/K264E)), lx) SEQ ID NO: XXX (IL-12p40 (D18K/E59K/K99E/K264E)), lxi) SEQ ID NO: XXX (IL-12p40 (C252S)), lxii) SEQ ID NO: XXX (IL-12p40 (DI8K/E59K/K99E/C252S)), lxiii) SEQ ID NO: XXX (IL-12p40 (D18K/E59K/K99E/C252S/K264E)), lxiv) SEQ ID NO: XXX (IL-12p40

(E59K/K99Y/C252S)), lxv) SEQ ID NO: XXX (IL-12p40 (E59K/K99E/C252S/K264E)), lxvi) SEQ ID NO: XXX (IL-12p40 (E59K/K99E/C252S)), lxvii) SEQ ID NO: XXX (IL-12p40 (N103D/N113D)), lxviii) SEQ ID NO: XXX (IL-12p40 (N103D/N200D)), lxix) SEQ ID NO: XXX (IL-12p40 (N103D/N281D)), lxx) SEQ ID NO: XXX (IL-12p40 (N113D/N200D)), lxxi) SEQ ID NO: XXX (IL-12p40 (N113D/N281D)), lxxii) SEQ ID NO: XXX (IL-12p40 (N200D/N281D)), lxxiii) SEQ ID NO: XXX (IL-12p50 (N103D/N113D/N200D)), lxxiv) SEQ ID NO: XXX (IL-12p40 (N103D/N113D/N281D)), lxxv) SEQ ID NO: XXX (IL-12p40 (N103D/N200D/N281D)), lxxvi) SEQ ID NO: XXX (IL-12p40 (N113D/N200D/N281D)), lxxvii) SEQ ID NO: XXX (IL-12p40 (N103Q)), lxxviii) SEQ ID NO: XXX (IL-12p40 (N113Q)), lxxix) SEQ ID NO: XXX (IL-12p40 (N200Q)), lxxx) SEQ ID NO: XXX (IL-12p40 (N281Q)), lxxxi) SEQ ID NO: XXX (IL-12p40 (N103Q/N113Q)), lxxxii) SEQ ID NO: XXX (IL-12p40 (N103Q/N200Q)), lxxxiii) SEQ ID NO: XXX (IL-12p40 (N103Q/N281Q)), lxxxiv) SEQ ID NO: XXX (IL-12p40 (N113Q/N200Q)), lxxxv) SEQ ID NO: XXX (IL-12p40 (N113Q/N281Q)), lxxxvi) SEQ ID NO: XXX (IL-12p40 (N200Q/N281Q)), lxxxvii) SEQ ID NO: XXX (IL-12p40 (N103Q/N113Q/N200Q)), lxxxviii) SEQ ID NO: XXX (IL-12p40 (N103Q/N113Q/N281Q)), lxxxix) SEQ ID NO: XXX (IL-12p40 (N103Q/N200Q/N281Q)), xc) SEQ ID NO: XXX (IL-12p40 (N113Q/N200Q/N281Q)), xci) SEQ ID NO:XXX (IL-12p40 (N103Q/N113Q/N200Q/N281Q)), xcii) SEQ ID NO:XXX (IL-12p40 (D34N/E59K/K99E)), xciii) SEQ ID NO:XXX (IL-12p40 (D34K/E59K/K99E)), xciv) SEQ ID NO:XXX (IL-12p40 (E32Q/D34N/E59K/K99E)), xcv) SEQ ID NO:XXX (IL-12p40 (E32K/D34N/E59K/K99E)), xcvi) SEQ ID NO: XXX (IL-12p40 (E59Y/K99Y)), xcvii) SEQ ID NO:XXX (IL-12p40 (E59K/K99E/N103Q/C252S/K264E)), xcviii) SEQ ID NO:XXX (IL-12p40 (E59K/K99E/N113Q/C252S/K264E)), xcix) SEQ ID NO:XXX (IL-12p40 (E59K/K99E/N200Q/C252S/K264E)), c) SEQ ID NO:XXX (IL-12p40 (E59K/K99E/N281Q/C252S/K264E)), ci) SEQ ID NO:XXX (IL-12p40 (E59K/K99E/N103Q/N113Q/C252S/K264E)), cii) SEQ ID NO:XXX (IL-12p40 (E59K/K99E/N103Q/N200Q/C252S/K264E)), ciii) SEQ ID NO:XXX (IL-12p40 (E59K/K99E/N103Q/N281Q/C252S/K264E)), civ) SEQ ID NO:XXX (IL-12p40 (E59K/K99E/N113Q/N200Q/C252S/K264E)), cv) SEQ ID NO:XXX (IL-12p40 (E59K/K99E/N113Q/N281Q/C252S/K264E)), cvi) SEQ ID NO:XXX (IL-12p40

(E59K/K99E/N200Q/N281Q/C252S/K264E)), cvii) SEQ ID NO:XXX (IL-12p40 (E59K/K99E/N103Q/N113Q/N200Q/C252S/K264E)), cviii) SEQ ID NO:XXX (IL-12p40 (E59K/K99E/N103Q/N200Q/N281Q/C252S/K264E)), and cix) SEQ ID NO:XXX (IL-12p40 (E59K/K99E/N113Q/N200Q/N281Q/C252S/K264E)).

150. The heterodimeric complex according to any one of claims 140-149, wherein said IL-12p35 subunit has one or more amino acid modifications as amino acid residues selected from the group consisting of: Q20, N21, Q35, E38, S44, E45, E46, H49, K54, D55, T59, V60, E61, C63, L64, P65, E67, L68, N71, S73, C74, L75, N76, E79, N85, L89, F96, M97, L124, M125, Q130, Q135, N136, E143, Q146, N151, E153, K158, E162, E163, D165, I171, R181, I182, R183, V185, T186, D188, R189, V190, S192, Y193, N195, and A196.

151. The heterodimeric complex according to any one of claims 140-150, wherein said IL-12p35 subunit has one or more amino acid substitutions selected from the group consisting of: N21D, Q35D, E38Q, D55Q, D55K, N71D, N71Q, L75A, N76D, E79Q, N85D, N85Q, L89A, F96A, M97A, L124A, M125A, Q130E, Q135E, N136D, E143Q, Q146E, N151D, N151K, E153K, E153Q, K158E, E162Q, E163Q, D165N, I171A, N195D, and N195Q.

152. The heterodimeric complex according to any one of claims 140-151, wherein said IL-12p35 subunit has amino acid substitutions selected from the group consisting of: N71D/N85D/N195D, N151D/E153Q, N151D/D165N, Q130E/N151D, N151D/K158E, E79Q/N151D, D55Q/N151D, N136D/N151D, N21D/N151D, E143Q/N151D, N71Q/N85Q, N71Q/N195Q, N85Q/N195Q, N71Q/N85Q/N195Q, N71D/N85D, N71D/N195D, and N85D/N195D.

153. The heterodimeric complex according to any one of claims 140-152, wherein said IL-12p35 subunit has a polypeptide sequence selected from the group consisting of: i) SEQ ID NO:113 (IL-12p35(N71D)), ii) SEQ ID NO:114 (IL-12p35(N85D)), iii) SEQ ID NO:115 (IL-12p35(N195D)), iv) SEQ ID NO:116 (IL-12p35(N71D/N85D/N195D)), v) SEQ ID NO:117 (IL-12p35(E153Q)), vi) SEQ ID NO:118 (IL-12p35(E38Q)), vii) SEQ ID NO:119 (IL-12p35(N151D)), viii) SEQ ID NO:120 (IL-12p35(Q135E)), ix) SEQ ID NO:121 (IL-12p35(Q35D)), x) SEQ ID

NO:122 (IL-12p35(Q146E)), xi) SEQ ID NO:123 (IL-12p35(N76D)), xii) SEQ ID NO:124 (IL-12p35(E162Q)), xiii) SEQ ID NO:125 (IL-12p35(E163Q)), xiv) IL-12p35(N21D), xv) SEQ ID NO:XXX (IL-12p35(D55Q)), xvi) IL-12p35(E79Q), xvii) IL-12p35(Q130E), xviii) IL-12p35(N136D), xix) IL-12p35(E143Q), xx) SEQ ID NO:227 (IL-12p35(N151K)), xxi) SEQ ID NO:226 (IL-12p35(E153K)), xxii) IL-12p35(K158E), xxiii) IL-12p35(D165N), xxiv) SEQ ID NO:225 (IL-12p35(N151D/E153Q)), xxv) SEQ ID NO:228 (IL-12p35(N151D/D165N)), xxvi) SEQ ID NO:229 (IL-12p35(Q130E/N151D)), xxvii) SEQ ID NO:230 (IL-12p35(N151D/K158E)), xxviii) SEQ ID NO:231 (IL-12p35(E79Q/N151D)), xxix) SEQ ID NO:232 (IL-12p35(D55Q/N151D)), xxx) SEQ ID NO:233 (IL-12p35(N136D/N151D)), xxxi) SEQ ID NO:234 (IL-12p35(N21D/N151D)), xxxii) SEQ ID NO:235 (IL-12p35(E143Q/N151D)), xxxiii) SEQ ID NO: XXX (IL-12p35(F96A)), xxxiv) SEQ ID NO: XXX (IL-12p35(M97A)), xxxv) SEQ ID NO: XXX (IL-12p35(L89A)), xxxvi) SEQ ID NO: XXX (IL-12p35(L124A)), xxxvii) SEQ ID NO: XXX (IL-12p35(M125A)), xxxviii) SEQ ID NO: XXX (IL-12p35(L75A)), xxxiv) SEQ ID NO: XXX (IL-12p35(I171A)), xxxv) SEQ ID NO: XXX (IL-12p35 (N71Q)), xxxvi) SEQ ID NO: XXX (IL-12p35 (N85Q)), xxxvii) SEQ ID NO: XXX (IL-12p35 (N195Q)), xxxviii) SEQ ID NO: XXX (IL-12p35 (N71Q/N85Q)), xxxix) SEQ ID NO: XXX (IL-12p35 (N71Q/N195Q)), xl) SEQ ID NO: XXX (IL-12p35 (N85Q/N195Q), xli) SEQ ID NO: XXX (IL-12p35 (N71Q/N85Q/N195Q)), xlii) SEQ ID NO: XXX (IL-12p35 (N71D/N85D)), xliii) SEQ ID NO: XXX (IL-12p35 (N71D/N195D), xliv) SEQ ID NO: XXX (IL-12p35 (N85D/N195D)), xlv) SEQ ID NO: XXX (IL-12p35 (D55Q)), and xlvi) SEQ ID NO: XXX (IL-12p35 (D55K)).

154. A composition comprising a heterodimeric complex according to any one of claims 140-153 for use in treating cancer in a subject.

155. One or more nucleic acids encoding a heterodimeric complex according to any one of claims 140-153.

156. A host cell comprising said one or more nucleic acids of claim 155.

157. A method of making a heterodimeric complex, said method comprising culturing a host cell according to claim 156 under conditions whereby said heterodimeric complex is produced.

158. A variant IL-12p40 subunit having altered affinity for IL-12 receptor subunit beta-1 (IL-12Rß1), IL-12 receptor subunit beta-2 (IL-12Rß2), and/or IL-12 receptor complex.

159. The variant IL-12p40 subunit according to claim 1, wherein said variant IL-12p40 subunit has one or more amino acid modifications at amino acid residues selected from the group consisting of: E3, D7, E12, D14, W15, P17, D18, A19, P20, G21, E22, M23, D29, E32, E33, D34, L40, D41, Q42, S43, E45, L47, T54, I55, Q56, K58, E59, F60, G61, D62, Q65, Y66, E73, K84, E86, D87, G88, I89, W90, D93, D97, K99, E100, K102, N103, K104, F106, E110, N113, Y114, D129, D142, Q144, E156, R159, D161, N162, K163, D166, D170, Q172, D174, A176, C177, P178, A179, A180, E181, S183, P185, E187, N200, S204, F206, R208, D209, D214, N218, Q220, N226, Q229, E231, E235, T242, P243, S245, Y246, F247, S248, Q256, C252, K258, K260, E262, K264, D265, D270, N281, Q289, D290, R291, Y292, Y293, and E299.

160. The variant Il-12p40 subunit according to claim 158 or 159, wherein said variant IL-12p40 subunit has one or more amino acid substitutions selected from the group consisting of: D18N, D18K, E32Q, E33Q, D34N, D34K, Q42E, S43E, S43K, E45Q, Q56E, E59Q, E59K, D62N, E73Q, D87N, K99E, K99Y, E100Q, N103D, N103Q, N113D, N113Q, Q144E, D161N, R159E, K163E, E187Q, N200D, N200Q, N218Q, Q229E, E235Q, C252S, Q256N, K258E, K260E, E262Q, K264E, N281D, N281Q, and E299Q.

161. The variant IL-12p40 subunit according to any one of claims 158-160, wherein said variant IL-12p40 subunit has amino acid substitutions selected from the group consisting of: N103D/N113D/N200D/N281D, Q42E/E45Q, E45Q/Q56E, Q42E/E59Q, Q56E/E59Q, Q42E/E45Q/Q56E, E45Q/Q56E/E59Q, E32Q/E59Q, D34N/E59K, D34N/E59K/K99E, D34K/E59K/K99E, E32Q/D34N/E59K/K99E, E32K/D34N/E59K/K99E, D34N/E59Q, E59Q/E187Q, S43E/E59Q, S43K/E49Q, E59Q/K163E, E59Q/K99E, E59Q/K258E, E59Q/K260E, E59K/K99E, D18K/E59K/K99E, E59K/K99E/K264E, E59K/K99Y, E59Y/K99Y, E59Y/K99E, E45K/E59K/K99E, E59K/K99E/Q144E, E59K/K99E/Q144K, E59K/K99E/R159E, E59K/K99E/K264E, D18K/E59K/K99E/K264E, DI8K/E59K/K99E/C252S, D18K/E59K/K99E/C252S/K264E, E59K/K99Y/C252S, E59K/K99E/C252S/K264E, E59K/K99E/C252S, N103D/N113D,

N103D/N200D, N103D/N281D, N113D/N200D, N113D/N281D, N200D/N281D, N103D/N113D/N200D, N103D/N113D/N281D, N103D/N200D/N281D, N113D/N200D/N281D, N103Q/N113Q, N103Q/N200Q, N103Q/N281Q, N113Q/N200Q, N113Q/N281Q, N200Q/N281Q, N103Q/N113Q/N200Q, N103Q/N113Q/N281Q, N103Q/N200Q/N281Q, N113Q/N200Q/N281Q, N103Q/N113Q/N200Q/N281Q, E59K/K99E/N103Q/C252S/K264E, E59K/K99E/N113Q/C252S/K264E, E59K/K99E/N200Q/C252S/K264E, E59K/K99E/N281Q/C252S/K264E, E59K/K99E/N103Q/N113Q/C252S/K264E, E59K/K99E/N103Q/N200Q/C252S/K264E, E59K/K99E/N103Q/N281Q/C252S/K264E, E59K/K99E/N113Q/N200Q/C252S/K264E, E59K/K99E/N113Q/N281Q/C252S/K264E, E59K/K99E/N200Q/N281Q/C252S/K264E, E59K/K99E/N103Q/N113Q/N200Q/C252S/K264E, E59K/K99E/N103Q/N200Q/N281Q/C252S/K264E, E59K/K99E/N113Q/N200Q/N281Q/C252S/K264E, and E59K/K99E/N103Q/N113Q/N200Q/N281Q/C252S/K264E.

162. The variant IL-12p40 according to any one of claims 158-161, wherein said variant IL-12p40 subunit has a polypeptide sequence selected from the group consisting of: i) SEQ ID NO:57 (IL-12p40(N103D)), ii) SEQ ID NO:58 (IL-12p40(N113D)), iii) SEQ ID NO:59 (IL-12p40(N200D)), iv) SEQ ID NO:60 (IL-12p40(N281D)), v) SEQ ID NO:61 (IL-12p40(N103D/N113D/N200D/N281D)), vi) SEQ ID NO:62 (IL-12p40(Q42E)), vii) SEQ ID NO:63 (IL-12p40(E45Q)), viii) SEQ ID NO:64 (IL-12p40(Q56E)), ix) SEQ ID NO:65 (IL-12p40(E59Q)), x) SEQ ID NO:66 (IL-12p40(D62N)), xi) SEQ ID NO:67 (IL-12p40(Q42E/E45Q)), xii) SEQ ID NO:68 (IL-12p40(E45Q/Q56E)), xiii) SEQ ID NO:69 (IL-12p40(Q42E/E59Q)), xiv) SEQ ID NO:70 (IL-12p40(Q56E/E59Q)), xv) SEQ ID NO:71 (IL-12p40(Q42E/E45Q/Q56E)), xvi) SEQ ID NO:72 (IL-12p40(E45Q/Q56E/E59Q)), xvii) SEQ ID NO:73 (IL-12p40(D161N)), xviii) SEQ ID NO:74 (IL-12p40(E73Q)), xix) SEQ ID NO:75 (IL-12p40(Q144E)), xx) SEQ ID NO:76 (IL-12p40(E262Q)), xxi) SEQ ID NO:77 (IL-12p40(E100Q)), xxii) SEQ ID NO:78 (IL-12p40(D18N)), xxiii) SEQ ID NO:79 (IL-12p40(E33Q)), xxiv) SEQ ID NO:80 (IL-12p40(Q229E)), xxv) SEQ ID NO:81 (IL-12p40(E235Q)), xxvi) SEQ ID NO:82 (IL-12p40(Q256N)), xxvii) SEQ ID NO:83 (IL-12p40(E299Q)), xxviii) SEQ ID NO:84 (IL-12p40(D87N)), xxix) IL-12p40(E32Q), xxx) IL-

12p40(D34N), xxxi) IL-12p40(S43E), xxxii) IL-12p40(S43K), xxxiii) SEQ ID NO:XXX (IL-12p40 (E59K/K99E/N103Q/N113Q/N200Q/N281Q/C252S/K264E)), xxxiv) SEQ ID NO:205 (IL-12p40(E59K)), xxxv) IL-12p40(K99E), xxxvi) IL-12p40(K163E), xxxvii) IL-12p40(E187Q), xxxviii) IL-12p40(K258E), xxxix) IL-12p40(K260E), xl) SEQ ID NO:206 (IL-12p40(E32Q/E59Q)), xli) SEQ ID NO:207 (IL-12p40(D34N/E59Q)), xlii) SEQ ID NO:208 (IL-12p40(E59Q/E187Q)), xliii) SEQ ID NO:209 (IL-12p40(S43E/E59Q)), xliv) SEQ ID NO:210 (IL-12p40(S43K/E49Q)), xlv) SEQ ID NO:211 (IL-12p40(E59Q/K163E)), xlvi) SEQ ID NO:212 (IL-12p40(E59Q/K99E)), xlvii) SEQ ID NO:213 (IL-12p40(E59Q/K258E)), xlviii) SEQ ID NO:214 (IL-12p40(E59Q/K260E)), xlix) SEQ ID NO: XXX (IL-12p40 (D34N/E59K)), l) SEQ ID NO: XXX (IL-12p40(E59K/K99E)), li) SEQ ID NO: XXX (IL-12p40(D18K/E59K/K99E)), lii) SEQ ID NO: XXX (IL-12p40 (E59K/K99E/K264E)), liii) SEQ ID NO: XXX (IL-12p40 (E59K/K99Y)), liv) SEQ ID NO: XXX (IL-12p40 (E59Y/K99E)), lv) SEQ ID NO: XXX (IL-12p40 (E45K/E59K/K99E)), lvi) SEQ ID NO: XXX (IL-12p40 (E59K/K99E/Q144E)), lvii) SEQ ID NO: XXX (IL-12p40 (E59K/K99E/Q144K)), lviii) SEQ ID NO: XXX (IL-12p40 (E59K/K99E/R159E)), lix) SEQ ID NO: XXX (IL-12p40 (E59K/K99E/K264E)), lx) SEQ ID NO: XXX (IL-12p40 (D18K/E59K/K99E/K264E)), lxi) SEQ ID NO: XXX (IL-12p40 (C252S)), lxii) SEQ ID NO: XXX (IL-12p40 (DI8K/E59K/K99E/C252S)), lxiii) SEQ ID NO: XXX (IL-12p40 (D18K/E59K/K99E/C252S/K264E)), lxiv) SEQ ID NO: XXX (IL-12p40 (E59K/K99Y/C252S)), lxv) SEQ ID NO: XXX (IL-12p40 (E59K/K99E/C252S/K264E)), lxvi) SEQ ID NO: XXX (IL-12p40 (E59K/K99E/C252S)), lxvii) SEQ ID NO: XXX (IL-12p40 (N103D/N113D)), lxviii) SEQ ID NO: XXX (IL-12p40 (N103D/N200D)), lxix) SEQ ID NO: XXX (IL-12p40 (N103D/N281D)), lxx) SEQ ID NO: XXX (IL-12p40 (N113D/N200D)), lxxi) SEQ ID NO: XXX (IL-12p40 (N113D/N281D)), lxxii) SEQ ID NO: XXX (IL-12p40 (N200D/N281D)), lxxiii) SEQ ID NO: XXX (IL-12p50 (N103D/N113D/N200D)), lxxiv) SEQ ID NO: XXX (IL-12p40 (N103D/N113D/N281D)), lxxv) SEQ ID NO: XXX (IL-12p40 (N103D/N200D/N281D)), lxxvi) SEQ ID NO: XXX (IL-12p40 (N113D/N200D/N281D)), lxxvii) SEQ ID NO: XXX (IL-12p40 (N103Q)), lxxviii) SEQ ID NO: XXX (IL-12p40 (N113Q)), lxxix) SEQ ID NO: XXX (IL-12p40 (N200Q)), lxxx) SEQ ID NO: XXX (IL-12p40 (N281Q)), lxxxi) SEQ ID NO: XXX (IL-12p40 (N103Q/N113Q)), lxxxii) SEQ ID NO: XXX (IL-12p40 (N103Q/N200Q)), lxxxiii) SEQ ID NO: XXX (IL-12p40 (N103Q/N281Q)), lxxxiv) SEQ ID NO: XXX (IL-12p40 (N113Q/N200Q)), lxxxv) SEQ ID

NO: XXX (IL-12p40 (N113Q/N281Q)), lxxxvi) SEQ ID NO: XXX (IL-12p40 (N200Q/N281Q)), lxxxvii) SEQ ID NO: XXX (IL-12p40 (N103Q/N113Q/N200Q)), lxxxviii) SEQ ID NO: XXX (IL-12p40 (N103Q/N113Q/N281Q)), lxxxix) SEQ ID NO: XXX (IL-12p40 (N103Q/N200Q/N281Q)), xc) SEQ ID NO: XXX (IL-12p40 (N113Q/N200Q/N281Q)), xci) SEQ ID NO:XXX (IL-12p40 (N103Q/N113Q/N200Q/N281Q)), xcii) SEQ ID NO:XXX (IL-12p40 (D34N/E59K/K99E)), xciii) SEQ ID NO:XXX (IL-12p40 (D34K/E59K/K99E)), xciv) SEQ ID NO:XXX (IL-12p40 (E32Q/D34N/E59K/K99E)), xcv) SEQ ID NO:XXX (IL-12p40 (E32K/D34N/E59K/K99E)), xcvi) SEQ ID NO: XXX (IL-12p40 (E59Y/K99Y)), xcvii) SEQ ID NO:XXX (IL-12p40 (E59K/K99E/N103Q/C252S/K264E)), xcviii) SEQ ID NO:XXX (IL-12p40 (E59K/K99E/N113Q/C252S/K264E)), xcix) SEQ ID NO:XXX (IL-12p40 (E59K/K99E/N200Q/C252S/K264E)), c) SEQ ID NO:XXX (IL-12p40 (E59K/K99E/N281Q/C252S/K264E)), ci) SEQ ID NO:XXX (IL-12p40 (E59K/K99E/N103Q/N113Q/C252S/K264E)), cii) SEQ ID NO:XXX (IL-12p40 (E59K/K99E/N103Q/N200Q/C252S/K264E)), ciii) SEQ ID NO:XXX (IL-12p40 (E59K/K99E/N103Q/N281Q/C252S/K264E)), civ) SEQ ID NO:XXX (IL-12p40 (E59K/K99E/N113Q/N200Q/C252S/K264E)), cv) SEQ ID NO:XXX (IL-12p40 (E59K/K99E/N113Q/N281Q/C252S/K264E)), cvi) SEQ ID NO:XXX (IL-12p40 (E59K/K99E/N200Q/N281Q/C252S/K264E)), cvii) SEQ ID NO:XXX (IL-12p40 (E59K/K99E/N103Q/N113Q/N200Q/C252S/K264E)), cviii) SEQ ID NO:XXX (IL-12p40 (E59K/K99E/N103Q/N200Q/N281Q/C252S/K264E)), and cix) SEQ ID NO:XXX (IL-12p40 (E59K/K99E/N113Q/N200Q/N281Q/C252S/K264E)).

163. A composition comprising a variant IL-12p40 subunit according to any one of claims 158-162.

164. A nucleic acid encoding a variant IL-12p40 subunit according to any one of claims 158-162.

165. A host cell comprising said nucleic acid of claim 164.

166. A method of making a variant IL-12p40 subunit, said method comprising culturing a host cell according to claim 165 under conditions whereby said variant IL-12p40 subunit is produced.

167. A variant IL-12p35 subunit having altered affinity for IL-12 receptor subunit beta-1 (IL-12Rß1), IL-12 receptor subunit beta-2 (IL-12Rß2), and/or IL-12 receptor complex.

168. The variant IL-12p35 subunit according to claim 167, wherein said variant IL-12p35 subunit has one or more amino acid modifications as amino acid residues selected from the group consisting of Q20, N21, Q35, E38, S44, E45, E46, H49, K54, D55, T59, V60, E61, C63, L64, P65, E67, L68, N71, S73, C74, L75, N76, E79, N85, L89, F96, M97, L124, M125, Q130, Q135, N136, E143, Q146, N151, E153, K158, E162, E163, D165, I171, R181, I182, R183, V185, T186, D188, R189, V190, S192, Y193, N195, and A196.

169. The variant IL-12p35 subunit according to claim 167 or 168, wherein said variant IL-12p35 subunit has one or more amino acid substitutions selected from the group consisting of: N21D, Q35D, E38Q, D55Q, D55K, N71D, N71Q, L75A, N76D, E79Q, N85D, N85Q, L89A, F96A, M97A, L124A, M125A, Q130E, Q135E, N136D, E143Q, Q146E, N151D, N151K, E153K, E153Q, K158E, E162Q, E163Q, D165N, I171A, N195D, and N195Q.

170. The variant IL-12p35 subunit according to any one of claims 167-169, wherein said variant IL-12p35 subunit amino acid substitutions selected from the group consisting of: N71D/N85D/N195D, N151D/E153Q, N151D/D165N, Q130E/N151D, N151D/K158E, E79Q/N151D, D55Q/N151D, N136D/N151D, N21D/N151D, E143Q/N151D, N71Q/N85Q, N71Q/N195Q, N85Q/N195Q, N71Q/N85Q/N195Q, N71D/N85D, N71D/N195D, and N85D/N195D.

171. The variant IL-12p35 subunit according to any one of claims 167-170, wherein said variant IL-12p35 subunit has amino acid substitutions N71D/N85D/N195D.

172. The variant IL-12p35 subunit according to any one of claims 167-171, wherein said variant IL-12p35 subunit has a polypeptide sequence selected from the group consisting of:

i) SEQ ID NO:113 (IL-12p35(N71D)), ii) SEQ ID NO:114 (IL-12p35(N85D)), iii) SEQ ID NO:115 (IL-12p35(N195D)), iv) SEQ ID NO:116 (IL-12p35(N71D/N85D/N195D)), v) SEQ ID NO:117 (IL-12p35(E153Q)), vi) SEQ ID NO:118 (IL-12p35(E38Q)), vii) SEQ ID NO:119 (IL-12p35(N151D)), viii) SEQ ID NO:120 (IL-12p35(Q135E)), ix) SEQ ID NO:121 (IL-12p35(Q35D)), x) SEQ ID NO:122 (IL-12p35(Q146E)), xi) SEQ ID NO:123 (IL-12p35(N76D)), xii) SEQ ID NO:124 (IL-12p35(E162Q)), xiii) SEQ ID NO:125 (IL-12p35(E163Q)), xiv) IL-12p35(N21D), xv) SEQ ID NO:XXX (IL-12p35(D55Q)), xvi) IL-12p35(E79Q), xvii) IL-12p35(Q130E), xviii) IL-12p35(N136D), xix) IL-12p35(E143Q), xx) SEQ ID NO:227 (IL-12p35(N151K)), xxi) SEQ ID NO:226 (IL-12p35(E153K)), xxii) IL-12p35(K158E), xxiii) IL-12p35(D165N), xxiv) SEQ ID NO:225 (IL-12p35(N151D/E153Q)), xxv) SEQ ID NO:228 (IL-12p35(N151D/D165N)), xxvi) SEQ ID NO:229 (IL-12p35(Q130E/N151D)), xxvii) SEQ ID NO:230 (IL-12p35(N151D/K158E)), xxviii) SEQ ID NO:231 (IL-12p35(E79Q/N151D)), xxix) SEQ ID NO:232 (IL-12p35(D55Q/N151D)), xxx) SEQ ID NO:233 (IL-12p35(N136D/N151D)), xxxi) SEQ ID NO:234 (IL-12p35(N21D/N151D)), xxxii) SEQ ID NO:235 (IL-12p35(E143Q/N151D)), xxxiii) SEQ ID NO: XXX (IL-12p35(F96A)), xxxiv) SEQ ID NO: XXX (IL-12p35(M97A)), xxxv) SEQ ID NO: XXX (IL-12p35(L89A)), xxxvi) SEQ ID NO: XXX (IL-12p35(L124A)), xxxvii) SEQ ID NO: XXX (IL-12p35(M125A)), xxxviii) SEQ ID NO: XXX (IL-12p35(L75A)), xxxiv) SEQ ID NO: XXX (IL-12p35(I171A)), xxxv) SEQ ID NO: XXX (IL-12p35 (N71Q)), xxxvi) SEQ ID NO: XXX (IL-12p35 (N85Q)), xxxvii) SEQ ID NO: XXX (IL-12p35 (N195Q)), xxxviii) SEQ ID NO: XXX (IL-12p35 (N71Q/N85Q)), xxxix) SEQ ID NO: XXX (IL-12p35 (N71Q/N195Q)), xl) SEQ ID NO: XXX (IL-12p35 (N85Q/N195Q), xli) SEQ ID NO: XXX (IL-12p35 (N71Q/N85Q/N195Q)), xlii) SEQ ID NO: XXX (IL-12p35 (N71D/N85D)), xliii) SEQ ID NO: XXX (IL-12p35 (N71D/N195D), xliv) SEQ ID NO: XXX (IL-12p35 (N85D/N195D)), xlv) SEQ ID NO: XXX (IL-12p35 (D55Q)), and xlvi) SEQ ID NO: XXX (IL-12p35 (D55K)).

173. A composition comprising a variant IL-12p35 subunit according to any one of claims 167-172.

174. A nucleic acid encoding a variant IL-12p35 subunit according to any one of claims 167-172.

175. A host cell comprising said nucleic acid of claim 174.

176. A method of making a variant IL-12p35 subunit, said method comprising culturing a host cell according to claim 175 under conditions whereby said variant IL-12p35 subunit is produced.

177. A heterodimeric protein comprising:

a) a first fusion protein comprising an IL-12p40 subunit domain covalently attached to a first Fc domain; and

b) a second fusion protein comprising an IL-12p35 subunit domain covalent attached to a second Fc domain;

wherein said first and second Fc domains a set of amino acid substitutions selected from the group consisting of L368D/K370S and S364K; L368D/K370S and S364K/E357L; L368D/K370S and S364K/E357Q; T411E/K360E/Q362E and D401K; L368E/K370S and S364K; K370S and S364K/E357Q; T366S/L368A/Y407V and T366W; and T366S/L368A/Y407V/Y349C and T366W/S354C; according to EU numbering.

178. A heterodimeric protein according to claim 177, wherein said IL-12p40 subunit domain is attached N-terminal to said first Fc domain and said IL-12p35 subunit domain is attached N-terminal to said second Fc domain.

179. A heterodimeric protein according to claim 177, wherein said IL-12p40 subunit domain is attached C-terminal to said first Fc domain and said IL-12p35 subunit domain is attached C-terminal to said second Fc domain.

180. A heterodimeric protein according to any of claims 177 to 179, wherein said Il-12p40 subunit domain is attached to said first Fc domain using a domain linker and said IL-12p35 subunit domain is attached to said second Fc domain using a domain linker.

181. A heterodimeric protein according to any of claims 177 to 180 wherein said first and second Fc domain comprises the IgG1 hinge-CH2-CH3.

182. A heterodimeric protein according to claim 177 wherein said first fusion protein comprises, from N- to C-terminal, an IL-12p40 subunit domain-domain linker-hinge-CH2-CH3 and said second fusion protein comprises, from N- to C-terminal, an IL-12p35 subunit domain-domain linker-hinge-CH2-CH3.

183. A heterodimeric protein according to claim 177 wherein said first fusion protein comprises, from N- to C-terminal, hinge-CH2-CH3-domain linker-IL-12p40 subunit domain and said second fusion protein comprises, from N- to C-terminal, hinge-CH2-CH3-domain linker-IL-12p35 subunit domain.

184. A heterodimeric protein comprising:

a) a first Fc domain; and

b) a fusion protein comprising:

i) a second Fc domain;

ii) an IL-12p40 subunit domain; and

iii) an IL-12p35 subunit domain;

wherein said first and second Fc domains a set of amino acid substitutions selected from the group consisting of L368D/K370S and S364K; L368D/K370S and S364K/E357L; L368D/K370S and S364K/E357Q; T411E/K360E/Q362E and D401K; L368E/K370S and S364K; K370S and S364K/E357Q; T366S/L368A/Y407V and T366W; and T366S/L368A/Y407V/Y349C and T366W/S354C; according to EU numbering.

185. A heterodimeric protein according to claim 184 wherein said fusion protein comprises, from N- to C-terminal: IL-12p40 subunit domain-domain linker-IL-12p35 subunit domain-domain linker-hinge-CH2-CH3.

186. A heterodimeric protein according to claim 184 wherein said fusion protein comprises, from N- to C-terminal: IL-12p35 subunit domain-domain linker-IL-12p40 subunit domain-domain linker-hinge-CH2-CH3.

187. A heterodimeric protein according to claim 184 wherein said fusion protein comprises, from N- to C-terminal: hinge-CH2-CH3-domain linker-IL-12p35 subunit domain-domain linker-IL-12p40 subunit domain.

188. A heterodimeric protein according to claim 184 wherein said fusion protein comprises, from N- to C-terminal: hinge-CH2-CH3-domain linker-IL-12p40 subunit domain-domain linker-IL-12p35 subunit domain.

189. A heterodimeric protein according to any of claim 177 to 188 wherein said IL-12p40 subunit has a sequence selected from the group consisting of: i) SEQ ID NO:57 (IL-12p40(N103D)), ii) SEQ ID NO:58 (IL-12p40(N113D)), iii) SEQ ID NO:59 (IL-12p40(N200D)), iv) SEQ ID NO:60 (IL-12p40(N281D)), v) SEQ ID NO:61 (IL-12p40(N103D/N113D/N200D/N281D)), vi) SEQ ID NO:62 (IL-12p40(Q42E)), vii) SEQ ID NO:63 (IL-12p40(E45Q)), viii) SEQ ID NO:64 (IL-12p40(Q56E)), ix) SEQ ID NO:65 (IL-12p40(E59Q)), x) SEQ ID NO:66 (IL-12p40(D62N)), xi) SEQ ID NO:67 (IL-12p40(Q42E/E45Q)), xii) SEQ ID NO:68 (IL-12p40(E45Q/Q56E)), xiii) SEQ ID NO:69 (IL-12p40(Q42E/E59Q)), xiv) SEQ ID NO:70 (IL-12p40(Q56E/E59Q)), xv) SEQ ID NO:71 (IL-12p40(Q42E/E45Q/Q56E)), xvi) SEQ ID NO:72 (IL-12p40(E45Q/Q56E/E59Q)), xvii) SEQ ID NO:73 (IL-12p40(D161N)), xviii) SEQ ID NO:74 (IL-12p40(E73Q)), xix) SEQ ID NO:75 (IL-12p40(Q144E)), xx) SEQ ID NO:76 (IL-12p40(E262Q)), xxi) SEQ ID NO:77 (IL-12p40(E100Q)), xxii) SEQ ID NO:78 (IL-12p40(D18N)), xxiii) SEQ ID NO:79 (IL-12p40(E33Q)), xxiv) SEQ ID NO:80 (IL-12p40(Q229E)), xxv) SEQ ID NO:81 (IL-12p40(E235Q)), xxvi) SEQ ID NO:82 (IL-12p40(Q256N)), xxvii) SEQ ID NO:83 (IL-12p40(E299Q)), xxviii) SEQ ID NO:84 (IL-12p40(D87N)), xxix) IL-12p40(E32Q), xxx) IL-12p40(D34N), xxxi) IL-12p40(S43E), xxxii) IL-12p40(S43K), xxxiii) SEQ ID NO:XXX (IL-12p40(E59K/K99E/N103Q/N113Q/N200Q/N281Q/C252S/K264E)), xxxiv) SEQ ID NO:205 (IL-12p40(E59K)), xxxv) IL-12p40(K99E), xxxvi) IL-12p40(K163E), xxxvii) IL-12p40(E187Q),

xxxviii) IL-12p40(K258E), xxxix) IL-12p40(K260E), xl) SEQ ID NO:206 (IL-12p40(E32Q/E59Q)), xli) SEQ ID NO:207 (IL-12p40(D34N/E59Q)), xlii) SEQ ID NO:208 (IL-12p40(E59Q/E187Q)), xliii) SEQ ID NO:209 (IL-12p40(S43E/E59Q)), xliv) SEQ ID NO:210 (IL-12p40(S43K/E49Q)), xlv) SEQ ID NO:211 (IL-12p40(E59Q/K163E)), xlvi) SEQ ID NO:212 (IL-12p40(E59Q/K99E)), xlvii) SEQ ID NO:213 (IL-12p40(E59Q/K258E)), xlviii) SEQ ID NO:214 (IL-12p40(E59Q/K260E)), xlix) SEQ ID NO: XXX (IL-12p40 (D34N/E59K)), l) SEQ ID NO: XXX (IL-12p40(E59K/K99E)), li) SEQ ID NO: XXX (IL-12p40(D18K/E59K/K99E)), lii) SEQ ID NO: XXX (IL-12p40 (E59K/K99E/K264E)), liii) SEQ ID NO: XXX (IL-12p40 (E59K/K99Y)), liv) SEQ ID NO: XXX (IL-12p40 (E59Y/K99E)), lv) SEQ ID NO: XXX (IL-12p40 (E45K/E59K/K99E)), lvi) SEQ ID NO: XXX (IL-12p40 (E59K/K99E/Q144E)), lvii) SEQ ID NO: XXX (IL-12p40 (E59K/K99E/Q144K)), lviii) SEQ ID NO: XXX (IL-12p40 (E59K/K99E/R159E)), lix) SEQ ID NO: XXX (IL-12p40 (E59K/K99E/K264E)), lx) SEQ ID NO: XXX (IL-12p40 (D18K/E59K/K99E/K264E)), lxi) SEQ ID NO: XXX (IL-12p40 (C252S)), lxii) SEQ ID NO: XXX (IL-12p40 (DI8K/E59K/K99E/C252S)), lxiii) SEQ ID NO: XXX (IL-12p40 (D18K/E59K/K99E/C252S/K264E)), lxiv) SEQ ID NO: XXX (IL-12p40 (E59K/K99Y/C252S)), lxv) SEQ ID NO: XXX (IL-12p40 (E59K/K99E/C252S/K264E)), lxvi) SEQ ID NO: XXX (IL-12p40 (E59K/K99E/C252S)), lxvii) SEQ ID NO: XXX (IL-12p40 (N103D/N113D)), lxviii) SEQ ID NO: XXX (IL-12p40 (N103D/N200D)), lxix) SEQ ID NO: XXX (IL-12p40 (N103D/N281D)), lxx) SEQ ID NO: XXX (IL-12p40 (N113D/N200D)), lxxi) SEQ ID NO: XXX (IL-12p40 (N113D/N281D)), lxxii) SEQ ID NO: XXX (IL-12p40 (N200D/N281D)), lxxiii) SEQ ID NO: XXX (IL-12p50 (N103D/N113D/N200D)), lxxiv) SEQ ID NO: XXX (IL-12p40 (N103D/N113D/N281D)), lxxv) SEQ ID NO: XXX (IL-12p40 (N103D/N200D/N281D)), lxxvi) SEQ ID NO: XXX (IL-12p40 (N113D/N200D/N281D)), lxxvii) SEQ ID NO: XXX (IL-12p40 (N103Q)), lxxviii) SEQ ID NO: XXX (IL-12p40 (N113Q)), lxxix) SEQ ID NO: XXX (IL-12p40 (N200Q)), lxxx) SEQ ID NO: XXX (IL-12p40 (N281Q)), lxxxi) SEQ ID NO: XXX (IL-12p40 (N103Q/N113Q)), lxxxii) SEQ ID NO: XXX (IL-12p40 (N103Q/N200Q)), lxxxiii) SEQ ID NO: XXX (IL-12p40 (N103Q/N281Q)), lxxxiv) SEQ ID NO: XXX (IL-12p40 (N113Q/N200Q)), lxxxv) SEQ ID NO: XXX (IL-12p40 (N113Q/N281Q)), lxxxvi) SEQ ID NO: XXX (IL-12p40 (N200Q/N281Q)), lxxxvii) SEQ ID NO: XXX (IL-12p40 (N103Q/N113Q/N200Q)), lxxxviii) SEQ ID NO: XXX (IL-12p40 (N103Q/N113Q/N281Q)), lxxxix) SEQ ID NO: XXX (IL-12p40 (N103Q/N200Q/N281Q)),

xc) SEQ ID NO: XXX (IL-12p40 (N113Q/N200Q/N281Q)), xci) SEQ ID NO:XXX (IL-12p40 (N103Q/N113Q/N200Q/N281Q)), xcii) SEQ ID NO:XXX (IL-12p40 (D34N/E59K/K99E)), xciii) SEQ ID NO:XXX (IL-12p40 (D34K/E59K/K99E)), xciv) SEQ ID NO:XXX (IL-12p40 (E32Q/D34N/E59K/K99E)), xcv) SEQ ID NO:XXX (IL-12p40 (E32K/D34N/E59K/K99E)), xcvi) SEQ ID NO: XXX (IL-12p40 (E59Y/K99Y)), xcvii) SEQ ID NO:XXX (IL-12p40 (E59K/K99E/N103Q/C252S/K264E)), xcviii) SEQ ID NO:XXX (IL-12p40 (E59K/K99E/N113Q/C252S/K264E)), xcix) SEQ ID NO:XXX (IL-12p40 (E59K/K99E/N200Q/C252S/K264E)), c) SEQ ID NO:XXX (IL-12p40 (E59K/K99E/N281Q/C252S/K264E)), ci) SEQ ID NO:XXX (IL-12p40 (E59K/K99E/N103Q/N113Q/C252S/K264E)), cii) SEQ ID NO:XXX (IL-12p40 (E59K/K99E/N103Q/N200Q/C252S/K264E)), ciii) SEQ ID NO:XXX (IL-12p40 (E59K/K99E/N103Q/N281Q/C252S/K264E)), civ) SEQ ID NO:XXX (IL-12p40 (E59K/K99E/N113Q/N200Q/C252S/K264E)), cv) SEQ ID NO:XXX (IL-12p40 (E59K/K99E/N113Q/N281Q/C252S/K264E)), cvi) SEQ ID NO:XXX (IL-12p40 (E59K/K99E/N200Q/N281Q/C252S/K264E)), cvii) SEQ ID NO:XXX (IL-12p40 (E59K/K99E/N103Q/N113Q/N200Q/C252S/K264E)), cviii) SEQ ID NO:XXX (IL-12p40 (E59K/K99E/N103Q/N200Q/N281Q/C252S/K264E)), and cix) SEQ ID NO:XXX (IL-12p40 (E59K/K99E/N113Q/N200Q/N281Q/C252S/K264E)).

190. A heterodimeric protein according to any of claim 177 to 188 wherein said IL-12p35 subunit has a sequence selected from the group consisting of: i) SEQ ID NO:113 (IL-12p35(N71D)), ii) SEQ ID NO:114 (IL-12p35(N85D)), iii) SEQ ID NO:115 (IL-12p35(N195D)), iv) SEQ ID NO:116 (IL-12p35(N71D/N85D/N195D)), v) SEQ ID NO:117 (IL-12p35(E153Q)), vi) SEQ ID NO:118 (IL-12p35(E38Q)), vii) SEQ ID NO:119 (IL-12p35(N151D)), viii) SEQ ID NO:120 (IL-12p35(Q135E)), ix) SEQ ID NO:121 (IL-12p35(Q35D)), x) SEQ ID NO:122 (IL-12p35(Q146E)), xi) SEQ ID NO:123 (IL-12p35(N76D)), xii) SEQ ID NO:124 (IL-12p35(E162Q)), xiii) SEQ ID NO:125 (IL-12p35(E163Q)), xiv) IL-12p35(N21D), xv) SEQ ID NO:XXX (IL-12p35(D55Q)), xvi) IL-12p35(E79Q), xvii) IL-12p35(Q130E), xviii) IL-12p35(N136D), xix) IL-12p35(E143Q), xx) SEQ ID NO:227 (IL-12p35(N151K)), xxi) SEQ ID NO:226 (IL-12p35(E153K)), xxii) IL-12p35(K158E), xxiii)

IL-12p35(D165N), xxiv) SEQ ID NO:225 (IL-12p35(N151D/E153Q)), xxv) SEQ ID NO:228 (IL-12p35(N151D/D165N)), xxvi) SEQ ID NO:229 (IL-12p35(Q130E/N151D)), xxvii) SEQ ID NO:230 (IL-12p35(N151D/K158E)), xxviii) SEQ ID NO:231 (IL-12p35(E79Q/N151D)), xxix) SEQ ID NO:232 (IL-12p35(D55Q/N151D)), xxx) SEQ ID NO:233 (IL-12p35(N136D/N151D)), xxxi) SEQ ID NO:234 (IL-12p35(N21D/N151D)), xxxii) SEQ ID NO:235 (IL-12p35(E143Q/N151D)),xxxiii) SEQ ID NO: XXX (IL-12p35(F96A)),xxxiv) SEQ ID NO: XXX (IL-12p35(M97A)), xxxv) SEQ ID NO: XXX (IL-12p35(L89A)),xxxvi) SEQ ID NO: XXX (IL-12p35(L124A)),xxxvii) SEQ ID NO: XXX (IL-12p35(M125A)),xxxviii) SEQ ID NO: XXX (IL-12p35(L75A)), xxxiv) SEQ ID NO: XXX (IL-12p35(I171A)),xxxv) SEQ ID NO: XXX (IL-12p35 (N71Q)), xxxvi) SEQ ID NO: XXX (IL-12p35 (N85Q)), xxxvii) SEQ ID NO: XXX (IL-12p35 (N195Q)), xxxviii) SEQ ID NO: XXX (IL-12p35 (N71Q/N85Q)),xxxix) SEQ ID NO: XXX (IL-12p35 (N71Q/N195Q)),lx) SEQ ID NO: XXX (IL-12p35 (N85Q/N195Q),lxi) SEQ ID NO: XXX (IL-12p35 (N71Q/N85Q/N195Q)),lxii) SEQ ID NO: XXX (IL-12p35 (N71D/N85D)), lxiii) SEQ ID NO: XXX (IL-12p35 (N71D/N195D),lxiv) SEQ ID NO: XXX (IL-12p35 (N85D/N195D)), lxv) SEQ ID NO: XXX (IL-12p35 (D55Q)), and lxvi) SEQ ID NO: XXX (IL-12p35 (D55K)).

191. The heterodimeric Fc fusion protein according to any one of claims 177-190, wherein said first and second Fc domains further comprise amino acid substitutions M428L/N424S.

192. A homodimeric Fc fusion protein comprising a first monomer and a second monomer each comprising, from N- to C-terminal, an IL-12p40 subunit domain- an optional first domain linker- an IL-12p35 subunit domain-an optional second domain linker- an Fc domain.

193. A homodimeric Fc fusion protein comprising a first monomer and a second monomer each comprising, from N- to C-terminal, an IL-12p35 subunit domain-an optional first domain linker- an IL-12p40 subunit domain-an optional second domain linker-an Fc domain.

194. A homodimeric Fc fusion protein comprising a first monomer and a second monomer each comprising, from N- to C-terminal, an Fc domain-an optional first domain linker-an IL-12p40 subunit domain-an optional second domain linker-an IL-12p35 subunit domain .

195. A homodimeric Fc fusion protein comprising a first monomer and a second monomer each comprising, from N- to C-terminal, an Fc domain-an optional first domain linker-an IL-12p35 subunit domain –an optional second domain linker-an IL-12p40 subunit domain.

196. A homodimeric Fc fusion protein comprising a first monomer and a second monomer each comprising, from N- to C-terminal, an IL-12p40 subunit domain- a first domain linker-an IL-12p35 subunit domain – a second domain linker-Fc domain.

197. A homodimeric Fc fusion protein comprising a first monomer and a second monomer each comprising, from N- to C-terminal, an IL-12p35 subunit domain – a first domain linker-an IL-12p40 subunit domain-a second domain linker-Fc domain.

198. A homodimeric Fc fusion protein comprising a first monomer and a second monomer each comprising, from N- to C-terminal, a Fc domain-a first domain linker-an IL-12p40 subunit domain-a second domain linker- an IL-12p35 subunit domain.

199. A homodimeric Fc fusion protein comprising a first monomer and a second monomer each comprising, from N- to C-terminal, a Fc domain-a first domain linker-an IL-12p35 subunit domain- a second domain linker-an IL-12p40 subunit domain.

200. The homodimeric Fc fusion protein according to claims 192 to 199, wherein said modifications promoting homodimerization of said Fc domain are a set of amino acid substitutions selected from the group consisting of L368D/K370S; S364K; S364K/E357L; S364K/E357Q; T411E/K360E/Q362E; D401K; T366S/L368A/Y407V; T366W; T366S/L368A/Y407V/Y349C; and T366W/S354C, according to EU numbering.

201. The homodimeric Fc fusion protein according to claim 192 to 200, wherein said first domain linker and said second domain linker have the same amino acid sequence.

202. The homodimeric Fc fusion protein according to any one of claims 192 to 201, wherein said Fc domain has an additional set of amino acid substitutions comprising Q295E/N384D/Q418E/N421D, according to EU numbering.

203. The homodimeric Fc fusion protein according to any one of claims 192 to 202, wherein said Fc domain has an additional set of amino acid substitutions selected from the group consisting of G236R/L328R, E233P/L234V/L235A/G236_/S239K, E233P/L234V/L235A/G236_/S239K/A327G, E233P/L234V/L235A/G236_/S267K/A327G, E233P/L234V/L235A/G236_, and E233P/L234V/L235A/G236_/S267K, according to EU numbering.

204. The homodimeric Fc fusion protein according to any one of claims 192 to 203, wherein said IL-12p40 subunit has a polypeptide sequence selected form the group consisting of SEQ ID NO:3 (human IL-12 subunit beta (IL-12p40) precursor sequence) and SEQ ID NO:4 (human IL-12 subunit beta (IL-12p40) mature form sequence), and said IL-12p35 subunit has a polypeptide sequence selected from the group consisting of SEQ ID NO:1 (human IL-12 subunit alpha (IL-12p35) precursor sequence) and SEQ ID NO:2 (human IL-12 subunit alpha (IL-12p35) mature form sequence).

205. The homodimeric Fc fusion protein according to any one of claims 192-204, wherein each of said Fc domain further comprises amino acid substitutions M428L/N424S.

206. The homodimeric Fc fusion protein according to any one of claims 192 to 205, wherein said IL-12p40 subunit is a variant IL-12p40 subunit and/or said IL-12p35 subunit is a variant IL-12p35 subunit.

207. The homodimeric Fc fusion protein according to any one of claims 192 to 206, wherein said IL-12p40 subunit is a variant IL-12p40 subunit having altered affinity for IL-12 receptor subunit beta-1 (IL-12Rß1), IL-12 receptor subunit beta-2 (IL-12Rß2), and/or IL-12 receptor complex; and/or said IL-12p35 subunit is a variant IL-12p35 subunit having altered affinity for IL-12 receptor subunit beta-1 (IL-12Rß1), IL-12 receptor subunit beta-2 (IL-12Rß2), and/or IL-12 receptor complex.

208. The homodimeric Fc fusion protein according to any one of claims 192 to 207, wherein said IL-12p40 subunit has one or more amino acid modifications at amino acid residues selected from the group consisting of: E3, D7, E12, D14, W15, P17, D18, A19, P20, G21, E22, M23, D29,

E32, E33, D34, L40, D41, Q42, S43, E45, L47, T54, I55, Q56, K58, E59, F60, G61, D62, Q65, Y66, E73, K84, E86, D87, G88, I89, W90, D93, D97, K99, E100, K102, N103, K104, F106, E110, N113, Y114, D129, D142, Q144, E156, R159, D161, N162, K163, D166, D170, Q172, D174, A176, C177, P178, A179, A180, E181, S183, P185, E187, N200, S204, F206, R208, D209, D214, N218, Q220, N226, Q229, E231, E235, T242, P243, S245, Y246, F247, S248, C252S, Q256, K158, K260, E262, K264, D265, D270, N281, Q289, D290, R291, Y292, Y293, and E299.

209. The homodimeric Fc fusion protein according to any one of claims 192 to 208, wherein said IL-12p40 subunit has one or more amino acid substitutions selected from the group consisting of: D18N, D18K, E32Q, E33Q, D34N, D34K, Q42E, S43E, S43K, E45Q, Q56E, E59Q, E59K, D62N, E73Q, D87N, K99E, K99Y, E100Q, N103D, N103Q, N113D, N113Q, Q144E, D161N, R159E, K163E, E187Q, N200D, N200Q, N218Q, Q229E, E235Q, C252S, Q256N, K258E, K260E, E262Q, K264E, N281D, N281Q, and E299Q.

210. The homodimeric Fc fusion protein according to any one of claims 192 to 209, wherein said IL-12p40 subunit has amino acid substitutions selected from the group consisting of: N103D/N113D/N200D/N281D, Q42E/E45Q, E45Q/Q56E, Q42E/E59Q, Q56E/E59Q, Q42E/E45Q/Q56E, E45Q/Q56E/E59Q, E32Q/E59Q, D34N/E59K, D34N/E59K/K99E, D34K/E59K/K99E, E32Q/D34N/E59K/K99E, E32K/D34N/E59K/K99E, D34N/E59Q, E59Q/E187Q, S43E/E59Q, S43K/E49Q, E59Q/K163E, E59Q/K99E, E59Q/K258E, E59Q/K260E, E59K/K99E, D18K/E59K/K99E, E59K/K99E/K264E, E59K/K99Y, E59Y/K99Y, E59Y/K99E, E45K/E59K/K99E, E59K/K99E/Q144E, E59K/K99E/Q144K, E59K/K99E/R159E, E59K/K99E/K264E, D18K/E59K/K99E/K264E, DI8K/E59K/K99E/C252S, D18K/E59K/K99E/C252S/K264E, E59K/K99Y/C252S, E59K/K99E/C252S/K264E, E59K/K99E/C252S, N103D/N113D, N103D/N200D, N103D/N281D, N113D/N200D, N113D/N281D, N200D/N281D, N103D/N113D/N200D, N103D/N113D/N281D, N103D/N200D/N281D, N113D/N200D/N281D, N103Q/N113Q, N103Q/N200Q, N103Q/N281Q, N113Q/N200Q, N113Q/N281Q, N200Q/N281Q, N103Q/N113Q/N200Q, N103Q/N113Q/N281Q, N103Q/N200Q/N281Q, N113Q/N200Q/N281Q, N103Q/N113Q/N200Q/N281Q, E59K/K99E/N103Q/C252S/K264E, E59K/K99E/N113Q/C252S/K264E, E59K/K99E/N200Q/C252S/K264E,

E59K/K99E/N281Q/C252S/K264E, E59K/K99E/N103Q/N113Q/C252S/K264E, E59K/K99E/N103Q/N200Q/C252S/K264E, E59K/K99E/N103Q/N281Q/C252S/K264E, E59K/K99E/N113Q/N200Q/C252S/K264E, E59K/K99E/N113Q/N281Q/C252S/K264E, E59K/K99E/N200Q/N281Q/C252S/K264E, E59K/K99E/N103Q/N113Q/N200Q/C252S/K264E, E59K/K99E/N103Q/N200Q/N281Q/C252S/K264E, E59K/K99E/N113Q/N200Q/N281Q/C252S/K264E, and E59K/K99E/N103Q/N113Q/N200Q/N281Q/C252S/K264E.

211. The homodimeric Fc fusion protein according to any one of claims 192 to 210, wherein said IL-12p40 subunit has a polypeptide sequence selected from the group consisting of: i) SEQ ID NO:57 (IL-12p40(N103D)), ii) SEQ ID NO:58 (IL-12p40(N113D)), iii) SEQ ID NO:59 (IL-12p40(N200D)), iv) SEQ ID NO:60 (IL-12p40(N281D)), v) SEQ ID NO:61 (IL-12p40(N103D/N113D/N200D/N281D)), vi) SEQ ID NO:62 (IL-12p40(Q42E)), vii) SEQ ID NO:63 (IL-12p40(E45Q)), viii) SEQ ID NO:64 (IL-12p40(Q56E)), ix) SEQ ID NO:65 (IL-12p40(E59Q)), x) SEQ ID NO:66 (IL-12p40(D62N)), xi) SEQ ID NO:67 (IL-12p40(Q42E/E45Q)), xii) SEQ ID NO:68 (IL-12p40(E45Q/Q56E)), xiii) SEQ ID NO:69 (IL-12p40(Q42E/E59Q)), xiv) SEQ ID NO:70 (IL-12p40(Q56E/E59Q)), xv) SEQ ID NO:71 (IL-12p40(Q42E/E45Q/Q56E)), xvi) SEQ ID NO:72 (IL-12p40(E45Q/Q56E/E59Q)), xvii) SEQ ID NO:73 (IL-12p40(D161N)), xviii) SEQ ID NO:74 (IL-12p40(E73Q)), xix) SEQ ID NO:75 (IL-12p40(Q144E)), xx) SEQ ID NO:76 (IL-12p40(E262Q)), xxi) SEQ ID NO:77 (IL-12p40(E100Q)), xxii) SEQ ID NO:78 (IL-12p40(D18N)), xxiii) SEQ ID NO:79 (IL-12p40(E33Q)), xxiv) SEQ ID NO:80 (IL-12p40(Q229E)), xxv) SEQ ID NO:81 (IL-12p40(E235Q)), xxvi) SEQ ID NO:82 (IL-12p40(Q256N)), xxvii) SEQ ID NO:83 (IL-12p40(E299Q)), xxviii) SEQ ID NO:84 (IL-12p40(D87N)), xxix) IL-12p40(E32Q), xxx) IL-12p40(D34N), xxxi) IL-12p40(S43E), xxxii) IL-12p40(S43K), xxxiii) SEQ ID NO:XXX (IL-12p40 (E59K/K99E/N103Q/N113Q/N200Q/N281Q/C252S/K264E)), xxxiv) SEQ ID NO:205 (IL-12p40(E59K)), xxxv) IL-12p40(K99E), xxxvi) IL-12p40(K163E), xxxvii) IL-12p40(E187Q), xxxviii) IL-12p40(K258E), xxxix) IL-12p40(K260E), xl) SEQ ID NO:206 (IL-12p40(E32Q/E59Q)), xli) SEQ ID NO:207 (IL-12p40(D34N/E59Q)), xlii) SEQ ID NO:208 (IL-12p40(E59Q/E187Q)), xliii) SEQ ID NO:209 (IL-12p40(S43E/E59Q)), xliv) SEQ ID NO:210 (IL-12p40(S43K/E49Q)), xlv)

SEQ ID NO:211 (IL-12p40(E59Q/K163E)), xlvi) SEQ ID NO:212 (IL-12p40(E59Q/K99E)), xlvii) SEQ ID NO:213 (IL-12p40(E59Q/K258E)), xlviii) SEQ ID NO:214 (IL-12p40(E59Q/K260E)), xlix) SEQ ID NO: XXX (IL-12p40 (D34N/E59K)), l) SEQ ID NO: XXX (IL-12p40(E59K/K99E)), li) SEQ ID NO: XXX (IL-12p40(D18K/E59K/K99E)), lii) SEQ ID NO: XXX (IL-12p40 (E59K/K99E/K264E)), liii) SEQ ID NO: XXX (IL-12p40 (E59K/K99Y)), liv) SEQ ID NO: XXX (IL-12p40 (E59Y/K99E)), lv) SEQ ID NO: XXX (IL-12p40 (E45K/E59K/K99E)), lvi) SEQ ID NO: XXX (IL-12p40 (E59K/K99E/Q144E)), lvii) SEQ ID NO: XXX (IL-12p40 (E59K/K99E/Q144K)), lviii) SEQ ID NO: XXX (IL-12p40 (E59K/K99E/R159E)), lix) SEQ ID NO: XXX (IL-12p40 (E59K/K99E/K264E)), lx) SEQ ID NO: XXX (IL-12p40 (D18K/E59K/K99E/K264E)), lxi) SEQ ID NO: XXX (IL-12p40 (C252S)), lxii) SEQ ID NO: XXX (IL-12p40 (DI8K/E59K/K99E/C252S)), lxiii) SEQ ID NO: XXX (IL-12p40 (D18K/E59K/K99E/C252S/K264E)), lxiv) SEQ ID NO: XXX (IL-12p40 (E59K/K99Y/C252S)), lxv) SEQ ID NO: XXX (IL-12p40 (E59K/K99E/C252S/K264E)), lxvi) SEQ ID NO: XXX (IL-12p40 (E59K/K99E/C252S)), lxvii) SEQ ID NO: XXX (IL-12p40 (N103D/N113D)), lxviii) SEQ ID NO: XXX (IL-12p40 (N103D/N200D)), lxix) SEQ ID NO: XXX (IL-12p40 (N103D/N281D)), lxx) SEQ ID NO: XXX (IL-12p40 (N113D/N200D)), lxxi) SEQ ID NO: XXX (IL-12p40 (N113D/N281D)), lxxii) SEQ ID NO: XXX (IL-12p40 (N200D/N281D)), lxxiii) SEQ ID NO: XXX (IL-12p50 (N103D/N113D/N200D)), lxxiv) SEQ ID NO: XXX (IL-12p40 (N103D/N113D/N281D)), lxxv) SEQ ID NO: XXX (IL-12p40 (N103D/N200D/N281D)), lxxvi) SEQ ID NO: XXX (IL-12p40 (N113D/N200D/N281D)), lxxvii) SEQ ID NO: XXX (IL-12p40 (N103Q)), lxxviii) SEQ ID NO: XXX (IL-12p40 (N113Q)), lxxix) SEQ ID NO: XXX (IL-12p40 (N200Q)), lxxx) SEQ ID NO: XXX (IL-12p40 (N281Q)), lxxxi) SEQ ID NO: XXX (IL-12p40 (N103Q/N113Q)), lxxxii) SEQ ID NO: XXX (IL-12p40 (N103Q/N200Q)), lxxxiii) SEQ ID NO: XXX (IL-12p40 (N103Q/N281Q)), lxxxiv) SEQ ID NO: XXX (IL-12p40 (N113Q/N200Q)), lxxxv) SEQ ID NO: XXX (IL-12p40 (N113Q/N281Q)), lxxxvi) SEQ ID NO: XXX (IL-12p40 (N200Q/N281Q)), lxxxvii) SEQ ID NO: XXX (IL-12p40 (N103Q/N113Q/N200Q)), lxxxviii) SEQ ID NO: XXX (IL-12p40 (N103Q/N113Q/N281Q)), lxxxix) SEQ ID NO: XXX (IL-12p40 (N103Q/N200Q/N281Q)), xc) SEQ ID NO: XXX (IL-12p40 (N113Q/N200Q/N281Q)), xci) SEQ ID NO:XXX (IL-12p40 (N103Q/N113Q/N200Q/N281Q)), xcii) SEQ ID NO:XXX (IL-12p40 (D34N/E59K/K99E)), xciii) SEQ ID NO:XXX (IL-12p40 (D34K/E59K/K99E)), xciv) SEQ ID NO:XXX (IL-12p40

(E32Q/D34N/E59K/K99E)), xcv) SEQ ID NO:XXX (IL-12p40 (E32K/D34N/E59K/K99E)), xcvi) SEQ ID NO: XXX (IL-12p40 (E59Y/K99Y)), xcvii) SEQ ID NO:XXX (IL-12p40 (E59K/K99E/N103Q/C252S/K264E)), xcviii) SEQ ID NO:XXX (IL-12p40 (E59K/K99E/N113Q/C252S/K264E)), xcix) SEQ ID NO:XXX (IL-12p40 (E59K/K99E/N200Q/C252S/K264E)), c) SEQ ID NO:XXX (IL-12p40 (E59K/K99E/N281Q/C252S/K264E)), ci) SEQ ID NO:XXX (IL-12p40 (E59K/K99E/N103Q/N113Q/C252S/K264E)), cii) SEQ ID NO:XXX (IL-12p40 (E59K/K99E/N103Q/N200Q/C252S/K264E)), ciii) SEQ ID NO:XXX (IL-12p40 (E59K/K99E/N103Q/N281Q/C252S/K264E)), civ) SEQ ID NO:XXX (IL-12p40 (E59K/K99E/N113Q/N200Q/C252S/K264E)), cv) SEQ ID NO:XXX (IL-12p40 (E59K/K99E/N113Q/N281Q/C252S/K264E)), cvi) SEQ ID NO:XXX (IL-12p40 (E59K/K99E/N200Q/N281Q/C252S/K264E)), cvii) SEQ ID NO:XXX (IL-12p40 (E59K/K99E/N103Q/N113Q/N200Q/C252S/K264E)), cviii) SEQ ID NO:XXX (IL-12p40 (E59K/K99E/N103Q/N200Q/N281Q/C252S/K264E)), and cix) SEQ ID NO:XXX (IL-12p40 (E59K/K99E/N113Q/N200Q/N281Q/C252S/K264E)).

212. The homodimeric Fc fusion protein according to any one of claims 192 to 211, wherein said IL-12p35 subunit has one or more amino acid modifications as amino acid residues selected from the group consisting of: Q20, N21, Q35, E38, S44, E45, E46, H49, K54, D55, T59, V60, E61, C63, L64, P65, E67, L68, N71, S73, C74, L75, N76, E79, N85, L89, F96, M97, L124, M125, Q130, Q135, N136, E143, Q146, N151, E153, K158, E162, E163, D165, I171, R181, I182, R183, V185, T186, D188, R189, V190, S192, Y193, N195, and A196.

213. The homodimeric Fc fusion protein according to any one of claims 192 to 212, wherein said IL-12p35 subunit has one or more amino acid substitutions selected from the group consisting of: N21D, Q35D, E38Q, D55Q, D55K, N71D, N71Q, L75A, N76D, E79Q, N85D, N85Q, L89A, F96A, M97A, L124A, M125A, Q130E, Q135E, N136D, E143Q, Q146E, N151D, N151K, E153K, E153Q, K158E, E162Q, E163Q, D165N, I171A, N195D, and N195Q.

214. The homodimeric Fc fusion protein according to any one of claims 192-213, wherein said IL-12p35 subunit has amino acid substitutions selected from the group consisting of: N71D/N85D/N195D, N151D/E153Q, N151D/D165N, Q130E/N151D, N151D/K158E, E79Q/N151D, D55Q/N151D, N136D/N151D, N21D/N151D, E143Q/N151D, N71Q/N85Q, N71Q/N195Q, N85Q/N195Q, N71Q/N85Q/N195Q, N71D/N85D, N71D/N195D, and N85D/N195D.

215. The homodimeric Fc fusion protein according to any one of claims 192-214, wherein said IL-12p35 subunit has a polypeptide sequence selected from the group consisting of: i) SEQ ID NO:113 (IL-12p35(N71D)), ii) SEQ ID NO:114 (IL-12p35(N85D)), iii) SEQ ID NO:115 (IL-12p35(N195D)), iv) SEQ ID NO:116 (IL-12p35(N71D/N85D/N195D)), v) SEQ ID NO:117 (IL-12p35(E153Q)), vi) SEQ ID NO:118 (IL-12p35(E38Q)), vii) SEQ ID NO:119 (IL-12p35(N151D)), viii) SEQ ID NO:120 (IL-12p35(Q135E)), ix) SEQ ID NO:121 (IL-12p35(Q35D)), x) SEQ ID NO:122 (IL-12p35(Q146E)), xi) SEQ ID NO:123 (IL-12p35(N76D)), xii) SEQ ID NO:124 (IL-12p35(E162Q)), xiii) SEQ ID NO:125 (IL-12p35(E163Q)), xiv) IL-12p35(N21D), xv) SEQ ID NO:XXX (IL-12p35(D55Q)), xvi) IL-12p35(E79Q), xvii) IL-12p35(Q130E), xviii) IL-12p35(N136D), xix) IL-12p35(E143Q), xx) SEQ ID NO:227 (IL-12p35(N151K)), xxi) SEQ ID NO:226 (IL-12p35(E153K)), xxii) IL-12p35(K158E), xxiii) IL-12p35(D165N), xxiv) SEQ ID NO:225 (IL-12p35(N151D/E153Q)), xxv) SEQ ID NO:228 (IL-12p35(N151D/D165N)), xxvi) SEQ ID NO:229 (IL-12p35(Q130E/N151D)), xxvii) SEQ ID NO:230 (IL-12p35(N151D/K158E)), xxviii) SEQ ID NO:231 (IL-12p35(E79Q/N151D)), xxix) SEQ ID NO:232 (IL-12p35(D55Q/N151D)), xxx) SEQ ID NO:233 (IL-12p35(N136D/N151D)), xxxi) SEQ ID NO:234 (IL-12p35(N21D/N151D)), xxxii) SEQ ID NO:235 (IL-12p35(E143Q/N151D)), xxxiii) SEQ ID NO: XXX (IL-12p35(F96A)), xxxiv) SEQ ID NO: XXX (IL-12p35(M97A)), xxxv) SEQ ID NO: XXX (IL-12p35(L89A)), xxxvi) SEQ ID NO: XXX (IL-12p35(L124A)), xxxvii) SEQ ID NO: XXX (IL-12p35(M125A)), xxxviii) SEQ ID NO: XXX (IL-12p35(L75A)), xxxiv) SEQ ID NO: XXX (IL-12p35(I171A)), xxxv) SEQ ID NO: XXX (IL-12p35 (N71Q)), xxxvi) SEQ ID NO: XXX (IL-12p35 (N85Q)), xxxvii) SEQ ID NO: XXX (IL-12p35 (N195Q)), xxxviii) SEQ ID NO: XXX (IL-12p35 (N71Q/N85Q)), xxxix) SEQ ID NO: XXX (IL-12p35 (N71Q/N195Q)), xl) SEQ ID NO: XXX (IL-12p35 (N85Q/N195Q), xli) SEQ ID NO:

XXX (IL-12p35 (N71Q/N85Q/N195Q)), xlii) SEQ ID NO: XXX (IL-12p35 (N71D/N85D)), xliii) SEQ ID NO: XXX (IL-12p35 (N71D/N195D), xliv) SEQ ID NO: XXX (IL-12p35 (N85D/N195D)), xlv) SEQ ID NO: XXX (IL-12p35 (D55Q)), and xlvi) SEQ ID NO: XXX (IL-12p35 (D55K)).

216. The homodimeric Fc fusion protein according to claims 192 or 196 , wherein said first and second monomers are XENP31289.

217. The homodimeric Fc fusion protein according to any one of claims 192 or 196, wherein said first and second monomers are XENP31291.

218. A composition comprising a homodimeric Fc fusion protein according to any one of claims 192-217 for use in treating cancer in a subject.

219. One or more nucleic acids encoding a homodimeric Fc fusion protein according to any one of claims 192-217.

220. A host cell comprising said one or more nucleic acids of claim 219.

221.A method of making a homodimeric Fc fusion protein, said method comprising culturing a host cell according to claim 220 under conditions whereby said homodimeric Fc fusion protein is produced.

222. A method of purifying a homodimeric Fc fusion protein according to any one of claims 192 to 217, said method comprising:

a) providing a composition comprising the homodimeric Fc fusion protein;

b) loading said composition onto an ion exchange column; and

c) collecting a fraction containing said homodimeric Fc fusion protein.

223. A method of treating cancer in a patient in need thereof, the method comprising administering a therapeutically effective amount of a heterodimeric or homodimeric Fc fusion protein according to any of the previous claims to said patient.

224. The method according to claim 223, further comprising administering a therapeutically effective amount of a checkpoint blockade antibody.

225. The method according to claim 224, wherein said checkpoint blockade antibody is selected from an anti-PD-1 antibody, an anti-PD-L1 antibody, an anti-TIM3 antibody, an anti-TIGIT antibody, an anti-LAG3 antibody, and an anti-CTLA-4 antibody.

226. The method according to claim225, wherein said anti-PD-1 antibody is nivolumab, pembrolizumab, or pidilizumab.

227. The method according to claim 225, wherein said anti-PD-L1 antibody is atezolizumab, avelumab, or durbalumab.

228. The method according to any one of claims 223 to 227, wherein the patient exhibits an increase in lymphocytes following administration.

229. The method according to any one of claims 223 to 228, wherein the patient exhibits an increase in peripheral CD8+ T cells following administration.

230. A method of inducing T cell expansion in a patient in need thereof comprising administering a therapeutically effective amount of a heterodimeric or homodimeric Fc fusion protein according any of the previous claims to said patient.

231. The method according to claim 230, further comprising administering a therapeutically effective amount of a checkpoint blockade antibody.

232. The method according to claim 231, wherein said checkpoint blockade antibody is selected from an anti-PD-1 antibody, an anti-PD-L1 antibody, an anti-TIM3 antibody, an anti-TIGIT antibody, an anti-LAG3 antibody, and an anti-CTLA-4 antibody.

233. The method according to claim 232, wherein said anti-PD-1 antibody is nivolumab, pembrolizumab, or pidilizumab.

234. The method according to claim 232, wherein said anti-PD-L1 antibody is atezolizumab, avelumab, or durbalumab.

235. The method according to any one of claims 230 to 234, wherein the T cell expansion is at least a 2-fold increase in T cells.

236. The method according to any one of claims 224-229 and 230-235 wherein the IL-12 Fc fusion protein is administered before the checkpoint inhibitor to increase checkpoint expression prior to treatment.

237. The method according to any one of claims 226, 228, 229, 233, and 235, wherein the IL-12 Fc fusion protein is administered before the checkpoint inhibitor to increase checkpoint expression prior to treatment.

238. The heterodimeric Fc fusion protein according to any one of claims 119-124, wherein said first and second Fc domains further comprise amino acid substitutions M428L/N424S.