

and inducing IFN γ 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.

SUMMARY

[0006] In one aspect, the present disclosure 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 examples, the present disclosure 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 examples, the present disclosure 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 examples, the present disclosure 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: 326 (IL-12p40 (D34N/E59K)), l) SEQ ID NO: 325 (IL-12p40(E59K/K99E)), li) SEQ ID NO: 339 (IL-12p40(D18K/E59K/K99E)), lii) SEQ ID NO: 343 (IL-12p40 (E59K/K99E/K264E)), liii) SEQ ID NO: 336 (IL-12p40 (E59K/K99Y)), liv) SEQ ID NO: 335 (IL-12p40 (E59Y/K99E)), lv) SEQ ID NO: 338 (IL-12p40 (E45K/E59K/K99E)), lvi) SEQ ID NO: 340 (IL-12p40 (E59K/K99E/Q144E)), lvii) SEQ ID NO: 341 (IL-12p40 (E59K/K99E/Q144K)), lviii) SEQ ID NO: 342 (IL-12p40 (E59K/K99E/R159E)), lix) SEQ ID NO: 343 (IL-12p40 (E59K/K99E/K264E)), lx) SEQ ID NO: 344 (IL-12p40 (D18K/E59K/K99E/K264E)), lxi) SEQ ID NO: 360 (IL-12p40 (C252S)), lxii) SEQ ID NO: 361 (IL-12p40 (D18K/E59K/K99E/C252S)), lxiii) SEQ ID NO: 362 (IL-12p40 (D18K/E59K/K99E/C252S/K264E)), lxiv) SEQ ID NO: 363 (IL-12p40 (E59K/K99Y/C252S)), lxv) SEQ ID NO: 364 (IL-12p40 (E59K/K99E/C252S/K264E)), lxvi) SEQ ID NO: 365 (IL-12p40 (E59K/K99E/C252S)), lxvii) SEQ ID NO: 254 (IL-12p40 (N103D/N113D)), lxviii) SEQ ID NO: 255 (IL-12p40 (N103D/N200D)), lxix) SEQ ID NO: 256 (IL-12p40 (N103D/N281D)), lxx) SEQ ID NO: 257 (IL-12p40 (N113D/N200D)), lxxi) SEQ ID NO: 258 (IL-12p40 (N113D/N281D)), lxxii) SEQ ID NO: 259 (IL-12p40 (N200D/N281D)), lxxiii) SEQ ID NO: 260 (IL-12p40 (N103D/N113D/N200D)), lxxiv) SEQ ID NO: 261 (IL-12p40 (N103D/N113D/N281D)), lxxv) SEQ ID NO: 262 (IL-12p40 (N103D/N200D/N281D)), lxxvi) SEQ ID NO: 263 (IL-12p40 (N113D/N200D/N281D)), lxxvii) SEQ ID NO: 264 (IL-12p40 (N103Q)), lxxviii) SEQ ID NO: 265 (IL-12p40 (N113Q)), lxxix) SEQ ID NO: 266 (IL-12p40 (N200Q)), lxxx) SEQ ID NO: 267 (IL-12p40 (N281Q)), lxxxi) SEQ ID NO: 268 (IL-12p40 (N103Q/N113Q)), lxxxii) SEQ ID NO: 269 (IL-12p40 (N103Q/N200Q)), lxxxiii) SEQ

ID NO: 270 (IL-12p40 (N103Q/N281Q)), lxxxiv) SEQ ID NO: 271 (IL-12p40 (N113Q/N200Q)), lxxxv) SEQ ID NO: 272 (IL-12p40 (N113Q/N281Q)), lxxxvi) SEQ ID NO: 273 (IL-12p40 (N200Q/N281Q)), lxxxvii) SEQ ID NO: 274 (IL-12p40 (N103Q/N113Q/N200Q)), lxxxviii) SEQ ID NO: 275 (IL-12p40 (N103Q/N113Q/N281Q)), lxxxix) SEQ ID NO: 276 (IL-12p40 (N103Q/N200Q/N281Q)), xc) SEQ ID NO: 277 (IL-12p40 (N113Q/N200Q/N281Q)), xci) SEQ ID NO: 278 (IL-12p40 (N103Q/N113Q/N200Q/N281Q)), xcii) SEQ ID NO: 327 (IL-12p40 (D34N/E59K/K99E)), xciii) SEQ ID NO: 328 (IL-12p40 (D34K/E59K/K99E)), xciv) SEQ ID NO: 329 (IL-12p40 (E32Q/D34N/E59K/K99E)), xcv) SEQ ID NO: 331 (IL-12p40 (E32K/D34N/E59K/K99E)), xcvi) SEQ ID NO: 337 (IL-12p40 (E59Y/K99Y)), xcvii) SEQ ID NO: 366 (IL-12p40 (E59K/K99E/N103Q/C252S/K264E)), xcviii) SEQ ID NO: 367 (IL-12p40 (E59K/K99E/N113Q/C252S/K264E)), xcix) SEQ ID NO: 368 (IL-12p40 (E59K/K99E/N200Q/C252S/K264E)), c) SEQ ID NO: 369 (IL-12p40 (E59K/K99E/N281Q/C252S/K264E)), ci) SEQ ID NO: 370 (IL-12p40 (E59K/K99E/N103Q/N113Q/C252S/K264E)), cii) SEQ ID NO: 371 (IL-12p40 (E59K/K99E/N103Q/N200Q/C252S/K264E)), ciii) SEQ ID NO: 372 (IL-12p40 (E59K/K99E/N103Q/N281Q/C252S/K264E)), civ) SEQ ID NO: 373 (IL-12p40 (E59K/K99E/N113Q/N200Q/C252S/K264E)), cv) SEQ ID NO: 374 (IL-12p40 (E59K/K99E/N113Q/N281Q/C252S/K264E)), cvi) SEQ ID NO: 375 (IL-12p40 (E59K/K99E/N200Q/N281Q/C252S/K264E)), cvii) SEQ ID NO: 376 (IL-12p40 (E59K/K99E/N103Q/N113Q/N200Q/C252S/K264E)), cviii) SEQ ID NO: 377 (IL-12p40 (E59K/K99E/N103Q/N200Q/N281Q/C252S/K264E)), and cix) SEQ ID NO: 378 (IL-12p40 (E59K/K99E/N113Q/N200Q/N281Q/C252S/K264E)).

[0009] In some examples, the present disclosure 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 examples, the present disclosure provides a heterodimeric Fc fusion protein, wherein said IL-12p35 subunit is a variant IL-12p35 subunit.

[0011] In some examples, the present disclosure 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-12R β 1), IL-12 receptor subunit beta-2 (IL-12R β 2), 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-12R β 1), IL-12 receptor subunit beta-2 (IL-12R β 2), and/or IL-12 receptor complex.

[0012] In some examples, the present disclosure 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 examples, the present disclosure 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 examples, the present disclosure 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)), xli) SEQ ID NO: XXX (IL-12p35(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 examples, the present disclosure 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 examples, the present disclosure 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 examples, the present disclosure 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 examples, the present disclosure 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 examples, the present disclosure provides a heterodimeric Fc fusion protein, wherein said first and second Fc domains further comprise amino acid substitutions M428L/N424S.

[0020] In some examples, the present disclosure 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), lxxvii) SEQ ID NO:XXX (XENP32997 Chain 1), lxxviii) SEQ ID NO:XXX (XENP32998 Chain 1), lxxviii) 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 examples, the present disclosure 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 examples, the present disclosure 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 examples, the present disclosure 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 another aspect, the present disclosure refers to a composition comprising a heterodimeric Fc fusion protein as disclosed herein for use in therapy. In some examples, the present disclosure provides a composition comprising a heterodimeric Fc fusion protein for use in treating cancer in a subject.

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

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

[0029] In some aspects, the present disclosure 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 aspects, the present disclosure 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 examples, the present disclosure 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 examples, the present disclosure 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 example, the present disclosure 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; 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.

[0034] In some examples, the present disclosure 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 examples, the present disclosure 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 examples, the present disclosure 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 examples, the present disclosure 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 examples, the present disclosure provides a heterodimeric Fc fusion protein wherein said IL-12p40 subunit has 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).

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

[0040] In some examples, the present disclosure 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 examples, the present disclosure 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-12R β 1), IL-12 receptor subunit beta-2 (IL-12R β 2), 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-12R β 1), IL-12 receptor subunit beta-2 (IL-12R β 2), and/or IL-12 receptor complex.

[0042] In some examples, the present disclosure 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 examples, the present disclosure 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 examples, the present disclosure 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 examples, the present disclosure 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.

[0046] In some examples, the present disclosure 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 (D18K/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 examples, the present disclosure 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 examples, the present disclosure 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 examples, the present disclosure 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 examples, the present disclosure 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 examples, the present disclosure 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 examples, the present disclosure provides a heterodimeric Fc fusion protein wherein said heterodimeric Fc fusion protein is XENP27202.

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

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

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

[0055] In some examples, the present disclosure 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 examples, the present disclosure 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.

[0057] In another example, the present disclosure 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 examples, the present disclosure 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 examples, the present disclosure 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 examples, the present disclosure 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 examples, the present disclosure 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 examples, the present disclosure provides a heterodimeric Fc fusion protein wherein said IL-12p40 subunit has 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).

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

[0064] In some examples, the present disclosure 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 examples, the present disclosure 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-12Rβ1), IL-12 receptor subunit beta-2 (IL-12Rβ2), 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-12Rβ1), IL-12 receptor subunit beta-2 (IL-12Rβ2), and/or IL-12 receptor complex.

[0066] In some examples, the present disclosure 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 examples, the present disclosure 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 examples, the present disclosure 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, D18K/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 examples, the present disclosure 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)), xcvi) 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 examples, the present disclosure 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 examples, the present disclosure 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 examples, the present disclosure 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 examples, the present disclosure 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 examples, the present disclosure 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 examples, the present disclosure provides a heterodimeric Fc fusion protein wherein said heterodimeric Fc fusion protein is XENP27203.

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

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

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

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

[0080] In some examples, the present disclosure 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 examples, the present disclosure 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.

[0082] In another example, the present disclosure 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 examples, the present disclosure 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 examples, the present disclosure 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 examples, the present disclosure 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 examples, the present disclosure 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 examples, the present disclosure provides a heterodimeric Fc fusion protein wherein said IL-12p40 subunit has a polypeptide sequence selected from 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 examples, the present disclosure provides a heterodimeric Fc fusion protein wherein said first and second Fc domains further comprise amino acid substitutions M428L/N424S.

[0089] In some examples, the present disclosure 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 examples, the present disclosure 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-12R β 1), IL-12 receptor subunit beta-2 (IL-12R β 2), 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-12R β 1), IL-12 receptor subunit beta-2 (IL-12R β 2), and/or IL-12 receptor complex.

[0091] In some examples, the present disclosure 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 examples, the present disclosure 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 examples, the present disclosure 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, D18K/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 examples, the present disclosure 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)), xlvi) 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 (D18K/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)), xcvi) SEQ ID NO: XXX (IL-12p40
 (E59K/K99E/N103Q/C252S/K264E)), xcvi) 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 examples, the present disclosure 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 examples, the present disclosure 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 examples, the present disclosure 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 examples, the present disclosure 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)), xli) SEQ ID NO: XXX (IL-12p35(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 examples, the present disclosure 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 examples, the present disclosure provides a heterodimeric Fc fusion protein wherein said heterodimeric Fc fusion protein is XENP27204.

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

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

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

[00104] In some examples, the present disclosure 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 examples, the present disclosure 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.

[00106] In another example, the present disclosure 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 examples, the present disclosure 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 examples, the present disclosure 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 examples, the present disclosure 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 examples, the present disclosure 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 examples, the present disclosure provides a heterodimeric Fc fusion protein wherein said IL-12p40 subunit has 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).

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

[00113]In some examples, the present disclosure 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 examples, the present disclosure 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-12R β 1), IL-12 receptor subunit beta-2 (IL-12R β 2), 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-12R β 1), IL-12 receptor subunit beta-2 (IL-12R β 2), and/or IL-12 receptor complex.

[00115]In some examples, the present disclosure 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 examples, the present disclosure 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 examples, the present disclosure 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, D18K/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 examples, the present disclosure 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)), xcvi) SEQ ID NO:XXX (IL-12p40
 (E59K/K99E/N103Q/C252S/K264E)), xcvi) 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 examples, the present disclosure 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 examples, the present disclosure 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 examples, the present disclosure 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 examples, the present disclosure 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 examples, the present disclosure provides a composition comprising a heterodimeric Fc fusion protein for use in treating cancer in a subject.

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

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

[00126] In some examples, the present disclosure 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 examples, the present disclosure 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.

[00128] In another example, the present disclosure 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 examples, the present disclosure 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 examples, the present disclosure 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 examples, the present disclosure 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 examples, the present disclosure 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 examples, the present disclosure provides a heterodimeric Fc fusion protein wherein said IL-12p40 subunit has 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).

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

[00135] In some examples, the present disclosure 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 examples, the present disclosure 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-12R β 1), IL-12 receptor subunit beta-2 (IL-12R β 2), 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-12R β 1), IL-12 receptor subunit beta-2 (IL-12R β 2), and/or IL-12 receptor complex.

[00137] In some examples, the present disclosure 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 examples, the present disclosure 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 examples, the present disclosure 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, D18K/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 examples, the present disclosure 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 (D18K/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)), xcvi) SEQ ID NO:XXX (IL-12p40 (E59K/K99E/N103Q/C252S/K264E)), xcvi) 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 examples, the present disclosure 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 examples, the present disclosure 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 examples, the present disclosure 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 examples, the present disclosure 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 examples, the present disclosure provides a composition comprising a heterodimeric Fc fusion protein for use in treating cancer in a subject.

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

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

[00149] In some examples, the present disclosure 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 examples, the present disclosure 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.

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

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

[00153] In some examples, the present disclosure provides a heterodimeric complex wherein said IL-12p40 subunit has 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).

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

[00155] In some examples, the present disclosure 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 examples, the present disclosure 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-12R β 1), IL-12 receptor subunit beta-2 (IL-12R β 2), 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-12R β 1), IL-12 receptor subunit beta-2 (IL-12R β 2), and/or IL-12 receptor complex.

[00157] In some examples, the present disclosure 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 examples, the present disclosure 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 examples, the present disclosure 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, D18K/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 examples, the present disclosure 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 (D18K/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)), xcvi) SEQ ID NO: XXX (IL-12p40 (E59K/K99E/N103Q/C252S/K264E)), xcvi) 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 examples, the present disclosure 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 examples, the present disclosure 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 examples, the present disclosure 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 examples, the present disclosure 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)), xxxix) 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 examples, the present disclosure provides a composition comprising a heterodimeric complex for use in treating cancer in a subject.

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

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

[00169] In some examples, the present disclosure 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 examples, the present disclosure 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 example, the present disclosure provides a variant IL-12p40 subunit having altered affinity for IL-12 receptor subunit beta-1 (IL-12R β 1), IL-12 receptor subunit beta-2 (IL-12R β 2), and/or IL-12 receptor complex.

[00172] In some examples, the present disclosure 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 examples, the present disclosure 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 examples, the present disclosure 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, D18K/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 examples, the present disclosure 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)), xliiii) 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)), xcvi) SEQ ID NO: XXX (IL-12p40 (E59K/K99E/N103Q/C252S/K264E)), xcvi) 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 examples, the present disclosure provides a composition comprising a variant IL-12p40 subunit.

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

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

[00178] In some examples, the present disclosure 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 example, the present disclosure provides a variant IL-12p35 subunit having altered affinity for IL-12 receptor subunit beta-1 (IL-12R β 1), IL-12 receptor subunit beta-2 (IL-12R β 2), and/or IL-12 receptor complex.

[00180] In some examples, the present disclosure 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 examples, the present disclosure 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 examples, the present disclosure 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 examples, the present disclosure provides a variant IL-12p35 subunit, wherein said variant IL-12p35 subunit has amino acid substitutions N71D/N85D/N195D.

[00184] In some examples, the present disclosure 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 examples, the present disclosure provides a composition comprising a variant IL-12p35 subunit.

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

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

[00188] In some examples, the present disclosure 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 example, the present disclosure 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 covalently attached to a second Fc domain; wherein said first and second Fc domains have 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 examples, the present disclosure 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 examples, the present disclosure 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 examples, the present disclosure 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 examples, the present disclosure provides a heterodimeric protein wherein said first and second Fc domain comprises the IgG1 hinge-CH2-CH3.

[00194] In some examples, the present disclosure 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 examples, the present disclosure 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 examples, the present disclosure 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)), xcvi) SEQ ID NO: XXX (IL-12p40 (E59K/K99E/N103Q/C252S/K264E)), xcvi) 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 examples, the present disclosure 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 example, the present disclosure 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 examples, the present disclosure 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 examples, the present disclosure 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 examples, the present disclosure 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 examples, the present disclosure 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 examples, the present disclosure 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 examples, the present disclosure 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 example, the present disclosure 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 examples, the present disclosure 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 examples, the present disclosure 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 examples, the present disclosure 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 examples, the present disclosure 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 examples, the present disclosure provides a homodimeric Fc fusion protein wherein said IL-12p40 subunit has a polypeptide sequence selected from 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 examples, the present disclosure provides a homodimeric Fc fusion protein wherein each of said Fc domain further comprises amino acid substitutions M428L/N424S.

[00212]In some examples, the present disclosure 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 examples, the present disclosure 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-12R β 1), IL-12 receptor subunit beta-2 (IL-12R β 2), 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-12R β 1), IL-12 receptor subunit beta-2 (IL-12R β 2), and/or IL-12 receptor complex.

[00214]In some examples, the present disclosure 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 examples, the present disclosure 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 examples, the present disclosure 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, D18K/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 examples, the present disclosure 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)), xcvi) SEQ ID NO:XXX (IL-12p40
 (E59K/K99E/N103Q/C252S/K264E)), xcvi) 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 examples, the present disclosure 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 examples, the present disclosure 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 examples, the present disclosure 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 examples, the present disclosure 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)), xxxix) SEQ ID NO: XXX (IL-12p35(I171A)), xl) SEQ ID NO: XXX (IL-12p35(N71Q)), xli) SEQ ID NO: XXX (IL-12p35(N85Q)), xlii) SEQ ID NO: XXX (IL-12p35(N195Q)), xliii) SEQ ID NO: XXX (IL-12p35(N71Q/N85Q)), xliv) SEQ ID NO: XXX (IL-12p35(N71Q/N195Q)), xlv) SEQ ID NO: XXX (IL-12p35(N85Q/N195Q)), xlvi) SEQ ID NO: XXX (IL-12p35(N71D/N85D)), xlvii) SEQ ID NO: XXX (IL-12p35(N71D/N195D)), xlviii) SEQ ID NO: XXX (IL-12p35(N85D/N195D)), xlvix) SEQ ID NO: XXX (IL-12p35(D55Q)), and xlvi) SEQ ID NO: XXX (IL-12p35(D55K)).

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

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

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

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

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

[00227] In some examples, the present disclosure 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 examples, the present disclosure 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 example, the present disclosure 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 examples, the present disclosure 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 examples, the present disclosure 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 examples, the present disclosure 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 examples, the present disclosure 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 examples, the present disclosure provides a homodimeric Fc fusion protein wherein said IL-12p40 subunit has 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 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 examples, the present disclosure provides a homodimeric Fc fusion protein wherein each of said Fc domain further comprises amino acid substitutions M428L/N424S.

[00236] In some examples, the present disclosure 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 examples, the present disclosure 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-12R β 1), IL-12 receptor subunit beta-2 (IL-12R β 2), 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-12R β 1), IL-12 receptor subunit beta-2 (IL-12R β 2), and/or IL-12 receptor complex.

[00238] In some examples, the present disclosure 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 examples, the present disclosure 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 examples, the present disclosure 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, D18K/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 examples, the present disclosure 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 (D18K/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 examples, the present disclosure 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 examples, the present disclosure 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.

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 examples, the present disclosure provides a homodimeric Fc fusion protein wherein said first and second monomers are XENP31289.

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

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

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

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

[00250] In some examples, the present disclosure 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 examples, the present disclosure 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 example, the present disclosure 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 examples, the present disclosure 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 examples, the present disclosure 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 examples, the present disclosure 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 examples, the present disclosure 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 examples, the present disclosure provides a homodimeric Fc fusion protein wherein said IL-12p40 subunit has 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 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 examples, the present disclosure provides a homodimeric Fc fusion protein wherein each of said Fc domain further comprises amino acid substitutions M428L/N424S.

[00259]In some examples, the present disclosure 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 examples, the present disclosure 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-12R β 1), IL-12 receptor subunit beta-2 (IL-12R β 2), 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-12R β 1), IL-12 receptor subunit beta-2 (IL-12R β 2), and/or IL-12 receptor complex.

[00261]In some examples, the present disclosure 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 examples, the present disclosure 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 examples, the present disclosure 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, D18K/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 examples, the present disclosure 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 (D18K/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 examples, the present disclosure 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 examples, the present disclosure 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 examples, the present disclosure 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 examples, the present disclosure 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 examples, the present disclosure provides a homodimeric Fc fusion protein wherein said first and second monomers are XENP31289.

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

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

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

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

[00274] In some examples, the present disclosure 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 examples, the present disclosure 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 example, the present disclosure 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 examples, the present disclosure 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 examples, the present disclosure 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 examples, the present disclosure 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 examples, the present disclosure 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 examples, the present disclosure provides a homodimeric Fc fusion protein wherein said IL-12p40 subunit has 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 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 examples, the present disclosure provides a homodimeric Fc fusion protein wherein each of said Fc domain further comprises amino acid substitutions M428L/N424S.

[00283] In some examples, the present disclosure 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 examples, the present disclosure 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-12R β 1), IL-12 receptor subunit beta-2 (IL-12R β 2), 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-12R β 1), IL-12 receptor subunit beta-2 (IL-12R β 2), and/or IL-12 receptor complex.

[00285] In some examples, the present disclosure 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 examples, the present disclosure 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 examples, the present disclosure 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, D18K/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 examples, the present disclosure 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 (D18K/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 examples, the present disclosure 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 examples, the present disclosure 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 examples, the present disclosure 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 examples, the present disclosure 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)), xxxix) 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 examples, the present disclosure provides a homodimeric Fc fusion protein wherein said first and second monomers are XENP31289.

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

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

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

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

[00298] In some examples, the present disclosure 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 examples, the present disclosure 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 example, the present disclosure 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 examples, the present disclosure 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 examples, the present disclosure 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 examples, the present disclosure 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 examples, the present disclosure 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 examples, the present disclosure provides a homodimeric Fc fusion protein wherein said IL-12p40 subunit has 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 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 examples, the present disclosure provides a homodimeric Fc fusion protein wherein each of said Fc domain further comprises amino acid substitutions M428L/N424S.

[00307] In some examples, the present disclosure 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 examples, the present disclosure 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-12R β 1), IL-12 receptor subunit beta-2 (IL-12R β 2), 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-12R β 1), IL-12 receptor subunit beta-2 (IL-12R β 2), and/or IL-12 receptor complex.

[00309]In some examples, the present disclosure 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 examples, the present disclosure 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 examples, the present disclosure 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, D18K/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 examples, the present disclosure 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 (D18K/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)), xcvi) SEQ ID NO:XXX (IL-12p40 (E59K/K99E/N103Q/C252S/K264E)), xcvi) 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 examples, the present disclosure 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 examples, the present disclosure 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 examples, the present disclosure 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 examples, the present disclosure 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 examples, the present disclosure provides a homodimeric Fc fusion protein wherein said first and second monomers are XENP31289.

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

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

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

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

[00321] In some examples, the present disclosure 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 examples, the present disclosure 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 example, the present disclosure 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 examples, the present disclosure 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 examples, the present disclosure 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 examples, the present disclosure 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 examples, the present disclosure 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 examples, the present disclosure provides a homodimeric Fc fusion protein wherein said IL-12p40 subunit has 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 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 examples, the present disclosure provides a homodimeric Fc fusion protein wherein each of said Fc domain further comprises amino acid substitutions M428L/N424S.

[00330]In some examples, the present disclosure 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 examples, the present disclosure 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-12R β 1), IL-12 receptor subunit beta-2 (IL-12R β 2), 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-12R β 1), IL-12 receptor subunit beta-2 (IL-12R β 2), and/or IL-12 receptor complex.

[00332]In some examples, the present disclosure 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 examples, the present disclosure 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 examples, the present disclosure 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, D18K/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,
E59K/K99E/N103Q/N113Q/N200Q/N281Q/C252S/K264E.

and

In some examples, the present disclosure 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 (D18K/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)), xcvi) SEQ ID NO: XXX (IL-12p40 (E59K/K99E/N103Q/C252S/K264E)), xcvi) 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 examples, the present disclosure 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 examples, the present disclosure 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 examples, the present disclosure 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 examples, the present disclosure 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 examples, the present disclosure provides a homodimeric Fc fusion protein wherein said first and second monomers are XENP31289.

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

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

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

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

[00344] In some examples, the present disclosure 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 examples, the present disclosure 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 example, the present disclosure 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 examples, the present disclosure 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 examples, the present disclosure 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 examples, the present disclosure 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 examples, the present disclosure 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 examples, the present disclosure provides a homodimeric Fc fusion protein wherein said IL-12p40 subunit has 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 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 examples, the present disclosure provides a homodimeric Fc fusion protein wherein each of said Fc domain further comprises amino acid substitutions M428L/N424S.

[00353] In some examples, the present disclosure 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 examples, the present disclosure 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-12R β 1), IL-12 receptor subunit beta-2 (IL-12R β 2), 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-12R β 1), IL-12 receptor subunit beta-2 (IL-12R β 2), and/or IL-12 receptor complex.

[00355] In some examples, the present disclosure 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 examples, the present disclosure 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 examples, the present disclosure 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, D18K/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 examples, the present disclosure 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)), xcvi) SEQ ID NO:XXX (IL-12p40 (E59K/K99E/N103Q/C252S/K264E)), xcvi) 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 examples, the present disclosure 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 examples, the present disclosure 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 examples, the present disclosure 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 examples, the present disclosure 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 xlv) SEQ ID NO: XXX (IL-12p35 (D55K)).

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

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

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

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

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

[00367] In some examples, the present disclosure 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 examples, the present disclosure 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 example, the present disclosure 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 examples, the present disclosure 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 examples, the present disclosure 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 examples, the present disclosure 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 examples, the present disclosure 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 examples, the present disclosure provides a homodimeric Fc fusion protein wherein said IL-12p40 subunit has 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 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 examples, the present disclosure provides a homodimeric Fc fusion protein wherein each of said Fc domain further comprises amino acid substitutions M428L/N424S.

[00376]In some examples, the present disclosure 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 examples, the present disclosure 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-12R β 1), IL-12 receptor subunit beta-2 (IL-12R β 2), 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-12R β 1), IL-12 receptor subunit beta-2 (IL-12R β 2), and/or IL-12 receptor complex.

[00378]In some examples, the present disclosure 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 examples, the present disclosure 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 examples, the present disclosure 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, D18K/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,
E59K/K99E/N103Q/N113Q/N200Q/N281Q/C252S/K264E.

and

In some examples, the present disclosure 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 (D18K/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)), xcvi) SEQ ID NO: XXX (IL-12p40
 (E59K/K99E/N103Q/C252S/K264E)), xcvi) 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 examples, the present disclosure 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 examples, the present disclosure 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 examples, the present disclosure 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 examples, the present disclosure 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)), xxxix) 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 examples, the present disclosure provides a homodimeric Fc fusion protein wherein said first and second monomers are XENP31289.

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

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

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

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

[00391] In some examples, the present disclosure 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 examples, the present disclosure 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 disclosure refers to the use of a heterodimeric Fc fusion protein as disclosed herein in the manufacture of a medicament for treating cancer in a patient in need thereof, wherein said protein is to be administered to said patient. In another example, the present disclosure 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 examples, the present disclosure 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 examples, the present disclosure 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 examples, the present disclosure 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 examples, the present disclosure 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 examples, the present disclosure 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 examples, the present disclosure 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 examples, the present disclosure 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 examples, the present disclosure 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 disclosure refers to the use of a heterodimeric Fc fusion protein as disclosed herein in the manufacture of a medicament for inducing T cell expansion in a patient in need thereof, wherein said protein is to be administered to said patient. In another example, the present disclosure 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 examples, the present disclosure 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 examples, the present disclosure 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 examples, the present disclosure 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 examples, the present disclosure provides a method of inducing T cell expansion in a patient in need thereof, wherein said anti-PD-L1 antibody is atezolizumab, avelumab, or durvalumab.

[00407] In some further examples, the present disclosure 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 examples, the present disclosure 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 examples, the present disclosure 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 example the present disclosure provides an IL-12p40 subunit. In another example the present disclosure provides an IL-12p35 subunit. In a further example the present disclosure 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.

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, D18K/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: 326 (IL-12p40 (D34N/E59K)), l) SEQ ID NO: 325 (IL-12p40(E59K/K99E)), li) SEQ ID NO: 339 (IL-12p40(D18K/E59K/K99E)), lii) SEQ ID NO: 343 (IL-12p40 (E59K/K99E/K264E)), liii) SEQ ID NO: 336 (IL-12p40 (E59K/K99Y)), liv) SEQ ID NO: 335 (IL-12p40 (E59Y/K99E)), lv) SEQ ID NO: 338 (IL-12p40 (E45K/E59K/K99E)), lvi) SEQ ID NO: 340 (IL-12p40 (E59K/K99E/Q144E)), lvii) SEQ ID NO: 341 (IL-12p40 (E59K/K99E/Q144K)), lviii) SEQ ID NO: 342 (IL-12p40 (E59K/K99E/R159E)), lix) SEQ ID NO: 343 (IL-12p40 (E59K/K99E/K264E)), lx) SEQ ID NO: 344 (IL-12p40 (D18K/E59K/K99E/K264E)), lxi) SEQ ID NO: 360 (IL-12p40 (C252S)), lxii) SEQ ID NO: 361 (IL-12p40 (D18K/E59K/K99E/C252S)), lxiii) SEQ ID NO: 362 (IL-12p40 (D18K/E59K/K99E/C252S/K264E)), lxiv) SEQ ID NO: 363 (IL-12p40 (E59K/K99Y/C252S)), lxv) SEQ ID NO: 364 (IL-12p40 (E59K/K99E/C252S/K264E)), lxvi) SEQ ID NO: 365 (IL-12p40 (E59K/K99E/C252S)), lxvii) SEQ ID NO: 254 (IL-12p40 (N103D/N113D)), lxviii) SEQ ID NO: 255 (IL-12p40 (N103D/N200D)), lxix) SEQ ID NO: 256 (IL-12p40 (N103D/N281D)), lxx) SEQ ID NO: 257 (IL-12p40 (N113D/N200D)), lxxi) SEQ ID NO: 258 (IL-12p40 (N113D/N281D)), lxxii) SEQ ID NO: 259 (IL-12p40 (N200D/N281D)), lxxiii) SEQ ID NO: 260 (IL-12p40 (N103D/N113D/N200D)), lxxiv) SEQ ID NO: 261 (IL-12p40 (N103D/N113D/N281D)), lxxv) SEQ ID NO: 262 (IL-12p40 (N103D/N200D/N281D)), lxxvi) SEQ ID NO: 263 (IL-12p40 (N113D/N200D/N281D)), lxxvii) SEQ ID NO: 264 (IL-12p40 (N103Q)), lxxviii) SEQ ID NO: 265 (IL-12p40 (N113Q)), lxxix) SEQ ID NO: 266 (IL-12p40 (N200Q)), lxxx) SEQ ID NO: 267 (IL-12p40 (N281Q)), lxxxi) SEQ ID NO: 268 (IL-12p40 (N103Q/N113Q)), lxxxii) SEQ ID NO: 269 (IL-12p40 (N103Q/N200Q)), lxxxiii) SEQ ID NO: 270 (IL-12p40 (N103Q/N281Q)), lxxxiv) SEQ ID NO: 271 (IL-12p40 (N113Q/N200Q)),

lxxxv) SEQ ID NO: 272 (IL-12p40 (N113Q/N281Q)), lxxxvi) SEQ ID NO: 273 (IL-12p40 (N200Q/N281Q)), lxxxvii) SEQ ID NO: 274 (IL-12p40 (N103Q/N113Q/N200Q)), lxxxviii) SEQ ID NO: 275 (IL-12p40 (N103Q/N113Q/N281Q)), lxxxix) SEQ ID NO: 276 (IL-12p40 (N103Q/N200Q/N281Q)), xc) SEQ ID NO: 277 (IL-12p40 (N113Q/N200Q/N281Q)), xci) SEQ ID NO: 278 (IL-12p40 (N103Q/N113Q/N200Q/N281Q)), xcii) SEQ ID NO: 327 (IL-12p40 (D34N/E59K/K99E)), xciii) SEQ ID NO: 328 (IL-12p40 (D34K/E59K/K99E)), xciv) SEQ ID NO: 329 (IL-12p40 (E32Q/D34N/E59K/K99E)), xcv) SEQ ID NO: 331 (IL-12p40 (E32K/D34N/E59K/K99E)), xcvi) SEQ ID NO: 337 (IL-12p40 (E59Y/K99Y)), xcvi) SEQ ID NO: 366 (IL-12p40 (E59K/K99E/N103Q/C252S/K264E)), xcvi) SEQ ID NO: 367 (IL-12p40 (E59K/K99E/N113Q/C252S/K264E)), xcix) SEQ ID NO: 368 (IL-12p40 (E59K/K99E/N200Q/C252S/K264E)), c) SEQ ID NO: 369 (IL-12p40 (E59K/K99E/N281Q/C252S/K264E)), ci) SEQ ID NO: 370 (IL-12p40 (E59K/K99E/N103Q/N113Q/C252S/K264E)), cii) SEQ ID NO: 371 (IL-12p40 (E59K/K99E/N103Q/N200Q/C252S/K264E)), ciii) SEQ ID NO: 372 (IL-12p40 (E59K/K99E/N103Q/N281Q/C252S/K264E)), civ) SEQ ID NO: 373 (IL-12p40 (E59K/K99E/N113Q/N200Q/C252S/K264E)), cv) SEQ ID NO: 374 (IL-12p40 (E59K/K99E/N113Q/N281Q/C252S/K264E)), cvi) SEQ ID NO: 375 (IL-12p40 (E59K/K99E/N200Q/N281Q/C252S/K264E)), cvii) SEQ ID NO: 376 (IL-12p40 (E59K/K99E/N103Q/N113Q/N200Q/C252S/K264E)), cviii) SEQ ID NO: 377 (IL-12p40 (E59K/K99E/N103Q/N200Q/N281Q/C252S/K264E)), and cix) SEQ ID NO: 378 (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 1-5, wherein said IL-12p40 subunit is a variant IL-12p40 subunit having altered affinity for IL-12 receptor subunit beta-1 (IL-12Rβ1), IL-12 receptor subunit beta-2 (IL-12Rβ2), and/or IL-12 receptor complex.
7. The heterodimeric Fc fusion according to any one of claims 1-6, wherein said heterodimeric Fc fusion protein is selected from the group consisting of: XENP31251 (SEQ ID NOS:302 and 452), XENP31254 (SEQ ID NOS:305 and 455), XENP31258 (SEQ ID NOS:309 and 460), XENP32186 (SEQ ID NOS:317 and 461), XENP32187 (SEQ ID NOS:380 and 402), XENP32188 (SEQ ID NOS:381 and 403), XENP32189 (SEQ ID NOS:382 and 404), XENP32190 (SEQ ID NOS:425 and 405), and XENP32191 (SEQ ID NOS:384 and 406).
8. The heterodimeric Fc fusion protein according to any one of claims 1-7, further comprising one or more modifications to the IL-12p40 subunit selected from the group consisting of: N103Q, N113Q, N200Q, and N281Q.
9. A composition comprising a heterodimeric Fc fusion protein according to any one of claims 1-8 for use in therapy.
10. One or more nucleic acids encoding a heterodimeric Fc fusion protein according to any one of claims 1-8.
11. A host cell comprising said one or more nucleic acids of claim 10.
12. A method of making a heterodimeric Fc fusion protein, said method comprising culturing a host cell according to claim 11 under conditions whereby said heterodimeric Fc fusion protein is produced.
13. A method of purifying a heterodimeric Fc fusion protein according to any one of claims 1-8, 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.

14. Use of a heterodimeric Fc fusion protein according to claims 1-8 in the manufacture of a medicament for treating cancer in a patient in need thereof, wherein said protein is to be administered to said patient.

15. The use according to claim 14, wherein a checkpoint blockade antibody is to be further administered.

16. The use according to claim 15, 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.

17. The use according to claim 16, wherein said anti-PD-1 antibody is nivolumab, pembrolizumab, or pidilizumab.

18. The use according to claim 16, wherein said anti-PD-L1 antibody is atezolizumab, avelumab, or durbalumab.

19. The use according to any one of claims 14 to 18, wherein the patient exhibits an increase in lymphocytes following administration.

20. The use according to any one of claims 14 to 19, wherein the patient exhibits an increase in peripheral CD8+ T cells following administration.

21. Use of a heterodimeric Fc fusion protein according to claims 1-8 in the manufacture of a medicament for inducing T cell expansion in a patient in need thereof, wherein said protein is to be administered to said patient.

22. The use according to claim 21, wherein a checkpoint blockade antibody is to be further administered.

23. The use according to claim 22, 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.
24. The use according to claim 23, wherein said anti-PD-1 antibody is nivolumab, pembrolizumab, or pidilizumab.
25. The use according to claim 23, wherein said anti-PD-L1 antibody is atezolizumab, avelumab, or durbalumab.
26. The use according to any one of claims 21 to 25, wherein the T cell expansion is at least a 2-fold increase in T cells.
27. The use according to any one of claims 15 to 20 and 22 to 26 wherein the heterodimeric Fc fusion protein is to be administered before the checkpoint blockade antibody to increase checkpoint expression prior to treatment.
28. The heterodimeric Fc fusion protein according to any one of claims 1-8, wherein said first and second Fc domains further comprise amino acid substitutions M428L/N424S.
29. The heterodimeric fusion protein according to claim 1 wherein said first fusion protein comprises the amino acid sequence of SEQ ID NO:382 further comprising the amino acid substitutions M428L/N434S, and wherein said second fusion protein comprises the amino acid sequence of SEQ ID NO:404 further comprising the amino acid substitutions M428L/N434S.