

IL-12 LOSS OF POTENCY MUTEINS

CROSS-REFERENCE TO RELATED APPLICATIONS

[0001] This application claims priority to U.S. Provisional Application Serial No. 63/611,673, filed December 18, 2023, which is incorporated herein by reference in its entirety.

BACKGROUND

[0002] Cytokines can be used to treat a variety of diseases or conditions, such as cancer, inflammatory conditions, autoimmune conditions, rheumatoid arthritis, multiple sclerosis, myasthenia gravis, systemic lupus erythematosus, Alzheimer's disease, Schizophrenia, viral infections, (e.g., chronic hepatitis C, AIDS), allergic asthma, retinal neurodegenerative processes, metabolic disorder, insulin resistance, and diabetic cardiomyopathy. IL-12 is a pleiotropic secreted cytokine composed of two subunits, namely p35 (IL-12p35), and p40 (IL-12p40). In the naturally occurring IL-12, the two subunits are linked through a disulfide bond to form the bioactive IL-12p70 cytokine. IL-12 is, *inter alia*, pro-inflammatory, and mediates its functions through binding to IL-12 receptors (IL-12R). The high affinity IL-12R is heterodimeric comprising a IL-12Rβ1 subunit and a IL-12Rβ2 subunit. IL-12 is produced primarily by cells of the immune system to signal and organize an immune response. Binding of IL-12 to IL-12R leads to activation of T-cells and NK cells, leading to increased production of IFN-γ, proliferation, and cytotoxic potential. IL-12 has been recognized as an extremely attractive anticancer candidate because it can activate innate and adaptive immunity, induce antiangiogenic chemokines, promote remodeling of the tumor extracellular matrix, and stimulate expression of MHC class I molecules.

[0003] However, the therapeutic utility of IL-12 is limited due its cellular toxicity and the potential to elicit undesired immune responses in patients. This limits the practical applications of IL-12 in a therapeutic setting. Although multiple clinical trials have been on-going since the first human clinical trial of IL-12 in 1996, an FDA-approved IL-12 product remains elusive.

[0004] This presents a significant unmet need for new strategies that can overcome the challenges for the therapeutic use of IL-12. If the potency of cytokines like IL-12 could be safely harnessed and the toxicity challenges could be controlled, these agents could serve as powerful therapeutics for potential use against a broad spectrum of cancers.

SUMMARY

[0005] Disclosed herein is an interleukin-12 (IL-12) mutein comprising a variant p35 subunit, a variant p40 subunit, or both a variant p35 subunit and a variant p40 subunit, wherein the variant p35 subunit comprises an amino acid sequence with one or more substitutions or deletions relative to SEQ ID NO: 1; wherein the variant p40 subunit comprises an amino acid sequence with one or more substitutions or deletions relative to SEQ ID NO: 2. In some embodiments, the IL-12 mutein comprises a variant p35 subunit, and wherein the variant p35 subunit comprises a substitution at amino acid residue L133 relative to SEQ ID NO: 1. In some embodiments, the L133 substitution of the variant p35 subunit is selected from the group consisting of L133A, L133R, L133H, L133K, L133D, L133E, L133S, L133T, L133N, L133Q, L133G, L133P, L133I, L133M, L133F, L133W, L133V, and L133Y. In some embodiments, the variant p35 subunit further comprises one or more amino acid substitutions relative to SEQ ID NO: 1.

[0006] In some embodiments, the variant p35 subunit comprises one, two, three, four, five, or more amino acid substitutions at a position selected from the group consisting of L37, F39, Y40, P41, D48, M125, D126, K128, R129, I131, L133, P164, D165, F166, Y167, K170, L173, L177, H178 relative to SEQ ID NO: 1. In some embodiments, the variant p35 subunit comprises a substitution at L37, optionally wherein the substitution is L37R, L37H, L37K, L37D, L37E, L37S, L37T, L37N, L37Q, L37G, L37P, L37A, L37V, L37I, L37M, L37F, L37Y, or L37W. In some embodiments, the variant p35 subunit comprises a substitution at Y40, optionally wherein the substitution is Y40R, Y40H, Y40K, Y40D, Y40E, Y40S, Y40T, Y40N, Y40Q, Y40G, Y40P, Y40A, Y40V, Y40I, Y40L, Y40M, Y40F, or Y40W. In some embodiments, the variant p35 subunit comprises a substitution at D48, optionally wherein the substitution is D48R, D48H, D48K, D48E, D48S, D48T, D48N, D48Q, D48G, D48P, D48A, D48V, D48I, D48L, D48M, D48F, D48Y, or D48W. In some embodiments, the variant p35 subunit comprises a substitution at K128, optionally wherein the substitution is K128R, K128H, K128K, K128D, K128E, K128S, K128T, K128N, K128Q, K128G, K128P, K128A, K128V, K128I, K128L, K128M, K128F, K128Y, or K128W.

[0007] In some embodiments, the variant p35 subunit comprises a substitution at M125, optionally wherein the substitution is M125R, M125H, M125K, M125D, M125E, M125S, M125T, M125N, M125Q, M125G, M125P, M125A, M125V, M125I, M125L, M125F, M125Y, or M125W. In some embodiments, the variant p35 subunit comprises a substitution at R129, optionally wherein the substitution is R129H, R129K, R129D, R129E, R129S, R129T, R129N, R129Q, R129G, R129P, R129A, R129V, R129I, R129L, R129M, R129F, R129Y, or R129W. In some embodiments, the variant p35 subunit comprises a substitution at L133, optionally wherein

the substitution is L133R, L133H, L133K, L133D, L133E, L133S, L133T, L133N, L133Q, L133G, L133P, L133A, L133V, L133I, L133M, L133F, L133Y, or L133W. In some embodiments, the variant p35 subunit comprises a substitution at L177, optionally wherein the substitution is L177R, L177H, L177K, L177D, L177E, L177S, L177T, L177N, L177Q, L177G, L177P, L177A, L177V, L177I, L177M, L177F, L177Y, or L177W. In some embodiments, the variant p35 subunit comprises a substitution at H178, optionally wherein the substitution is H178R, H178K, H178D, H178E, H178S, H178T, H178N, H178Q, H178G, H178P, H178A, H178V, H178I, H178L, H178M, H178F, H178Y, or H178W.

[0008] In some embodiments, the IL-12 mutein comprises a variant p35 subunit, wherein the variant p35 subunit comprises a substitution at L37, optionally wherein the substitution is L37A; and a substitution at Y40, optionally wherein the substitution is Y40S. In some embodiments, the variant p35 subunit comprises a substitution at L37, optionally wherein the substitution is L37A; a substitution at Y40, optionally wherein the substitution is Y40S; and a substitution at L133, optionally wherein the substitution is L133A. In some embodiments, the variant p35 subunit comprises a substitution at L37, optionally wherein the substitution is L37A; a substitution at Y40, optionally wherein the substitution is Y40S; and a substitution at K128, optionally wherein the substitution is K128N or K128Q. In some embodiments, the variant p35 subunit comprises a substitution at L37, optionally wherein the substitution is L37A; a substitution at Y40, optionally wherein the substitution is Y40S; a substitution at K128, optionally wherein the substitution is K128N or K128Q; and a substitution at L133, optionally wherein the substitution is L133A. In some embodiments, the variant p35 subunit comprises an amino acid sequence that is at least 80%, 85%, 90%, or 95% identical to SEQ ID NO: 1.

[0009] In some embodiments, the IL-12 mutein comprises a variant p40 subunit, wherein the variant p40 subunit comprises one or more amino acid substitutions relative to SEQ ID NO: 2, and wherein the one or more amino acid substitutions comprises a substitution at amino acid residue Y16 of SEQ ID NO: 2. In some embodiments, the Y16 substitution of the variant p40 subunit is selected from the group consisting of Y16A, Y16R, Y16H, Y16K, Y16D, Y16E, Y16S, Y16T, Y16N, Y16Q, Y16G, Y16P, Y16I, Y16L, Y16M, Y16F, Y16W, and Y16V.

[0010] In some embodiments, the IL-12 mutein comprises a variant p40 subunit further comprising one or more amino acid substitutions relative to SEQ ID NO: 2. In some embodiments, the variant p40 subunit further comprises one or more, two or more, three or more, four or more, or five or more amino acid substitutions at a position selected from the group consisting of W15, P17, D18, E59, F60, K84, E86, D93, H194, K195, and K197 relative to SEQ ID NO: 2. In some embodiments, the variant p40 subunit further comprises one or more, two or more, or three or

more amino acid substitutions at a position selected from E59, F60, K84, K195, and K197 relative to SEQ ID NO: 2. In some embodiments, the variant p40 subunit comprises an amino acid sequence that is at least 80%, 85%, 90%, or 95% identical to SEQ ID NO: 2.

[0011] In some embodiments, the interleukin-12 mutein comprising a variant p35 subunit and a variant p40 subunit, wherein the variant p35 subunit comprises one or more amino acid substitutions relative to SEQ ID NO: 1, wherein the one or more amino acid substitutions are at a position selected from A33, R34, L37, E38, F39, Y40, P41, E45, E46, I47, D48, H49, E50, D51, I52, T53, K54, K56, T57, D108, L109, K110, M111, Y112, Q113, V114, E115, F116, K117, T118, A121, L123, M125, D126, R129, I131, P156, S159, S160, L161, E162, E163, D165, F166, Y167, K168, T169, K170, I171, K172, L173, I175, H178, and R181 relative to SEQ ID NO: 1, and the variant p40 subunit comprises one or more amino acid substitutions relative to SEQ ID NO: 2, wherein the one or more amino acid substitutions are at a position selected from V10, V11, E12, L13, D14, W15, Y16, P17, D18, A19, P20, G21, W38, T39, L40, D41, S43, S44, E45, V46, L47, I55, V57, K58, E59, F60, D62, A63, G64, Q65, Y66, L81, L82, H83, K84, K85, E86, G88, I89, W90, S91, T92, D93, I94, L95, D97, Q98, K99, E100, N103, K104, T105, F106, L107, L123, K135, S136, S137, R138, G139, S140, S141, D142, N162, E181, S183, L184, P185, I186, E187, V188, V190, D191, H194, K195, L196, K197, Y198, E199, N200, T202, S203, S204, F206, and D209 relative to SEQ ID NO: 2.

[0012] In some embodiments, Y16 of SEQ ID NO: 2 is substituted, preferably with an alanine residue. In some embodiments, L133 of SEQ ID NO: 1 is substituted, preferably with an alanine residue. In some embodiments, the variant p35 further comprises one or more amino acid substitutions or substitutions at a position selected from: (a) wherein D126 of SEQ ID NO: 1 is substituted, preferably with an alanine residue; (b) wherein I131 of SEQ ID NO: 1 is substituted, preferably with an alanine residue; (c) wherein H178 of SEQ ID NO: 1 is substituted, preferably with an alanine residue; and (d) any combination thereof. In some embodiments, the IL-12 mutein comprises a variant p35 subunit and a variant p40 subunit, wherein: (a) the variant p35 subunit comprises a substitution of one or more amino acids as shown in Table 1; (b) the p40 moiety comprises a substitution of one or more amino acids as shown in Table 4; or (c) the p35 moiety comprises a substitution of one or more amino acids as shown in Table 1 and the p40 moiety comprises a substitution of one or more amino acids as shown in Table 4. In some embodiments, the interleukin-12 (IL-12) mutein comprises a variant p35 subunit and a variant p40 subunit, wherein: (a) the variant p35 subunit comprises a substitution of one or more amino acid residues corresponding to an amino acid residue in SEQ ID NO: 1, wherein the substitution of one or more amino acids is a substitution listed in Table 1; (b) the variant p40 subunit comprises a substitution

of one or more amino acid residues corresponding to an amino acid residue in SEQ ID NO: 2, wherein the substitution of one or more amino acids is a substitution listed in Table 4; or (c) the variant p35 subunit comprises a substitution of one or more amino acid residues corresponding to an amino acid residue in SEQ ID NO: 1, wherein the substitution of one or more amino acids is a substitution listed in Table 1, and the p40 moiety comprises a substitution of one or more amino acid residues corresponding to an amino acid residue in SEQ ID NO: 2, wherein the substitution of one or more amino acids is a substitution listed in Table 4.

[0013] In some embodiments, the IL-12 mutein is a human IL-12 mutein. In some embodiments, the variant p35 subunit, the variant p40 subunit, or the variant p35 and p40 subunit are variant of the respective human IL-12 subunits

[0014] Disclosed herein is an interleukin-12 (IL-12) mutein comprising a variant p35 subunit, a variant p40 subunit, or both a variant p35 subunit and a variant p40 subunit, wherein the variant p35 subunit, if present, comprises an amino acid sequence with one or more substitutions or deletions relative to SEQ ID NO: 1; and wherein the variant p40 subunit, if present, comprises an amino acid sequence with one or more substitutions or deletions relative to SEQ ID NO: 2. In some embodiments, the IL-12 mutein comprises the variant p35 subunit.

[0015] In some embodiments, the variant p35 subunit comprises a substitution at amino acid residue L133, optionally wherein the substitution is L133A, L133R, L133H, L133K, L133D, L133E, L133S, L133T, L133N, L133Q, L133G, L133P, L133I, L133M, L133F, L133W, L133V, or L133Y. In some embodiments, the variant p35 subunit comprises a substitution at amino acid residue L37, optionally wherein the substitution is L37R, L37H, L37K, L37D, L37E, L37S, L37T, L37N, L37Q, L37G, L37P, L37A, L37V, L37I, L37M, L37F, L37Y, or L37W. In some embodiments, the variant p35 subunit comprises a substitution at amino acid residue L37, wherein the substitution is L37A. In some embodiments, the variant p35 subunit comprises a substitution at amino acid residue L37, wherein the substitution at amino acid residue L37 is L37R, L37H, L37K, L37D, L37N, L37Q, L37P, L37A, L37V, L37M, L37F, L37Y, or L37W.

[0016] In some embodiments, the variant p35 subunit comprises a substitution at amino acid residue Y40, optionally wherein the substitution is Y40R, Y40H, Y40K, Y40D, Y40E, Y40S, Y40T, Y40N, Y40Q, Y40G, Y40P, Y40A, Y40V, Y40I, Y40L, Y40M, Y40F, or Y40W. In some embodiments, variant p35 subunit comprises a substitution at amino acid residue Y40, wherein the substitution at amino acid residue Y40 is Y40H, Y40E, Y40V, Y40I, Y40L, Y40M, or Y40F. In some embodiments, variant p35 subunit comprises a substitution at amino acid residue Y40, wherein the Y40 substitution is Y40S.

[0017] In some embodiments, the variant p35 subunit comprises a substitution at amino acid residue D48, optionally wherein the substitution is D48R, D48H, D48K, D48E, D48S, D48T, D48N, D48Q, D48G, D48P, D48A, D48V, D48I, D48L, D48M, D48F, D48Y, or D48W. In some embodiments, wherein the substitution at amino acid residue D48 is D48H, D48K, D48E, D48S, D48T, D48N, D48G, D48P, D48V, D48I, D48L, D48M, D48F, D48Y, or D48W, optionally wherein the substitution at amino acid residue D48 is D48K, D48E, D48T, D48N, D48G, D48P, D48V, D48I, D48L, D48M, or D48F.

[0018] In some embodiments, the variant p35 subunit comprises a substitution at amino acid residue K128, optionally wherein the substitution is K128R, K128H, K128K, K128D, K128E, K128S, K128T, K128N, K128Q, K128G, K128P, K128A, K128V, K128I, K128L, K128M, K128F, K128Y, or K128W. In some embodiments the variant p35 subunit comprises a substitution at amino acid residue K128, wherein the substitution at amino acid residue K128 is K128N or K128Q.

[0019] In some embodiments, the variant p35 subunit comprises a substitution at amino acid residue M125, optionally wherein the substitution is M125R, M125H, M125K, M125D, M125E, M125S, M125T, M125N, M125Q, M125G, M125P, M125A, M125V, M125I, M125L, M125F, M125Y, or M125W. In some embodiments, the variant p35 subunit comprises a substitution at amino acid residue M125, wherein the substitution at amino acid residue M125 is M125R, M125H, M125K, M125D, M125E, M125S, M125T, M125N, M125Q, M125G, M125P, M125L, M125F, M125Y, or M125W.

[0020] In some embodiments, wherein the variant p35 subunit comprises a substitution at amino acid residue R129, optionally wherein the substitution is R129H, R129K, R129D, R129E, R129S, R129T, R129N, R129Q, R129G, R129P, R129A, R129V, R129I, R129L, R129M, R129F, R129Y, or R129W.

[0021] In some embodiments, the variant p35 subunit comprises a substitution at amino acid residue L177, optionally wherein the substitution is L177R, L177H, L177K, L177D, L177E, L177S, L177T, L177N, L177Q, L177G, L177P, L177A, L177V, L177I, L177M, L177F, L177Y, or L177W. In some embodiments, the variant p35 subunit comprises a substitution at amino acid residue L177, wherein the substitution at amino acid residue L177 is L177D, L177S, L177T, L177N, L177G, L177P, L177V, L177I, L177M, or L177W.

[0022] In some embodiments, the variant p35 subunit comprises a substitution at amino acid residue H178, optionally wherein the substitution is H178R, H178K, H178D, H178E, H178S, H178T, H178N, H178Q, H178G, H178P, H178A, H178V, H178I, H178L, H178M, H178F, H178Y, or H178W.

[0023] In some embodiments, the variant p35 subunit comprises a substitution at amino acid residue K56, optionally wherein the substitution at amino acid residue K56 is K56R, K56H, K56D, K56E, K56S, K56T, K56N, K56Q, K56G, K56P, K56A, K56V, K56I, K56L, K56M, K56F, K56Y, or K56W. In some embodiments, the variant p35 subunit comprises a substitution at amino acid residue K56, wherein the K56 substitution is K56R, K56H, K56D, K56E, K56S, K56T, K56G, K56P, K56A, K56V, K56I, K56L, K56M, K56F, K56Y, or K56W, optionally wherein the substitution at amino acid residue K56 is K56E, K56S, K56T, K56G, K56A, K56V, K56I, or K56L. In some embodiments, the variant p35 subunit comprises a substitution at amino acid residue K56, wherein the substitution at amino acid residue K56 is K56N.

[0024] In some embodiments, wherein the variant p35 subunit comprises a substitution at amino acid residue K170, optionally wherein the substitution at amino acid residue K170 is K170R, K170H, K170D, K170E, K170S, K170T, K170N, K170Q, K170G, K170P, K170A, K170V, K170I, K170L, K170M, K170F, K170Y, or K170W.

[0025] In some embodiments, the variant p35 subunit comprises a substitution at amino acid residue I171, optionally wherein the substitution at amino acid residue I171 is I171R, I171K, I171H, I171D, I171E, I171S, I171T, I171N, I171Q, I171G, I171P, I171A, I171V, I171L, I171M, I171F, I171Y, or I171W.

[0026] In some embodiments, wherein the variant p35 subunit comprises a substitution at amino acid residue A33, R34, E38, F39, P41, E45, E46, I47, H49, E50, D51, I52, T53, K54, T57, D108, L109, K110, M111, Y112, Q113, V114, E115, F116, K117, T118, A121, L123, D126, I131, P156, S159, S160, L161, E162, E163, D165, F166, Y167, K168, T169, K172, L173, I175, or R181.

[0027] In some embodiments, the variant p35 subunit comprises one or more amino acid substitutions selected from Table 1a, Table 1b, or Table 1c. In some embodiments, the variant p35 subunit comprises a substitution at L37, optionally wherein the substitution is L37A; and a substitution at Y40, optionally wherein the substitution is Y40S. In some embodiments, the variant p35 subunit comprises a substitution at L37, optionally wherein the substitution is L37A; a substitution at Y40, optionally wherein the substitution is Y40S; and a substitution at L133, optionally wherein the substitution is L133A. In some embodiments, the variant p35 subunit comprises a substitution at L37, optionally wherein the substitution is L37A; a substitution at Y40, optionally wherein the substitution is Y40S; and a substitution at K128, optionally wherein the substitution is K128N or K128Q. In some embodiments, the variant p35 subunit comprises a substitution at L37, optionally wherein the substitution is L37A; a substitution at Y40, optionally wherein the substitution is Y40S; a substitution at K128, optionally wherein the substitution is K128N or K128Q; and a substitution at L133, optionally wherein the substitution is L133A.

[0028] In some embodiments, the variant p35 subunit comprises an amino acid sequence that is at least 80%, 85%, 90%, or 95% identical to SEQ ID NO: 1.

[0029] In some embodiments, the IL-12 mutein of the disclosure comprises the variant p40 subunit.

[0030] In some embodiments, the variant p40 subunit comprises a substitution at amino acid residue V10, optionally wherein the substitution is V10A, V10R, V10H, V10K, V10D, V10E, V10S, V10T, V10N, V10Q, V10G, V10P, V10I, V10L, V10M, V10F, V10W, or V10Y.

[0031] In some embodiments, the variant p40 subunit comprises a substitution at amino acid residue I94, optionally wherein the substitution is I94A, I94R, I94H, I94K, I94D, I94E, I94S, I94T, I94N, I94Q, I94G, I94P, I94L, I94M, I94F, I94W, I94V, or I94Y.

[0032] In some embodiments, the variant p40 subunit comprises a substitution at amino acid residue L95, optionally wherein the substitution is L95A, L95R, L95H, L95K, L95D, L95E, L95S, L95T, L95N, L95Q, L95G, L95P, L95I, L95M, L95F, L95W, L95V, or L95Y.

[0033] In some embodiments, the variant p40 subunit comprises a substitution at amino acid residue G64, optionally wherein the substitution is G64A, G64R, G64H, G64K, G64D, G64E, G64S, G64T, G64N, G64Q, G64P, G64I, G64L, G64M, G64F, G64W, G64V, or G64Y. In some embodiments, the variant p40 subunit comprises a substitution at amino acid residue G64, wherein the G64 substitution is G64R, G64H, G64K, G64D, G64E, G64S, G64T, G64N, G64Q, G64P, G64I, G64L, G64M, G64F, G64W, G64V, or G64Y, optionally wherein the G64 substitution is G64R, G64K, G64E, G64S, G64T, G64Q, G64P, G64I, G64L, G64M, G64F, G64W, G64V, or G64Y.

[0034] In some embodiments, the variant p40 subunit comprises a substitution at amino acid residue Y198, optionally wherein the substitution is Y198A, Y198R, Y198H, Y198K, Y198D, Y198E, Y198S, Y198T, Y198N, Y198Q, Y198G, Y198P, Y198I, Y198L, Y198M, Y198F, Y198W, or Y198V. In some embodiments, the variant p40 subunit comprises a substitution at amino acid residue Y198, wherein the Y198 substitution is Y198A, Y198R, Y198H, Y198K, Y198D, Y198E, Y198T, Y198N, Y198Q, Y198G, Y198P, Y198I, Y198L, Y198M, Y198F, Y198W, or Y198V, optionally wherein the Y198 substitution is Y198G, Y198E, Y198K, Y198Q, Y198R, Y198W.

[0035] In some embodiments, the variant p40 subunit comprises a substitution at amino acid residue Y16, optionally wherein the substitution is Y16A, Y16R, Y16H, Y16K, Y16D, Y16E, Y16S, Y16T, Y16N, Y16Q, Y16G, Y16P, Y16I, Y16L, Y16M, Y16F, Y16W, or Y16V.

[0036] In some embodiments, the variant p40 subunit comprises a substitution at amino acid residue V11, E12, L13, D14, W15, P17, D18, A19, P20, G21, W38, T39, L40, D41, S43, S44,

E45, V46, L47, I55, V57, K58, E59, F60, D62, A63, Q65, Y66, L81, L82, H83, K84, K85, E86, G88, I89, W90, S91, T92, D93, D97, Q98, K99, E100, N103, K104, T105, F106, L107, L123, K135, S136, S137, R138, G139, S140, S141, D142, N162, E181, S183, L184, P185, I186, E187, V188, V190, D191, H194, K195, L196, K197, E199, N200, T202, S203, S204, F206, or D209. In some embodiments, the variant p40 subunit comprises a substitution at amino acid residue W15, P17, D18, E59, F60, K84, E86, D93, H194, K195, or K197, optionally wherein the variant p40 subunit comprises a substitution at amino acid residue E59, F60, K84, K195, or K197. In some embodiments, the variant p40 subunit comprises one or more amino acid substitutions selected from Table 4a, Table 4b, or Table 4c. In some embodiments, the variant p40 subunit comprises an amino acid sequence that is at least 80%, 85%, 90%, or 95% identical to SEQ ID NO: 2.

[0037] In some embodiments, the invention comprises a fusion protein comprising any IL-12 mutein disclosed herein. In some embodiments, the invention comprises pharmaceutical composition comprising any IL-12 mutein disclosed herein and a pharmaceutically acceptable carrier or excipient. In some embodiments, the invention comprises a polynucleotide sequence that encodes any IL-12 mutein disclosed herein. In some embodiments, the invention comprises an expression vector comprising a polynucleotide encoding any of the IL-12 muteins disclosed herein. In some embodiments, the invention comprises a host cell comprising an expression comprising a polynucleotide encoding any of the IL-12 muteins disclosed herein.

[0038] In some embodiments, the invention comprises a kit comprising any IL-12 mutein disclosed herein.

[0039] In some embodiments, the invention is directed to a method of producing any of the IL-12 muteins disclosed herein, comprising culturing a host cell under conditions to express the IL-12 mutein. In some embodiments, the method further comprises isolating the IL-12 mutein from the host cell.

[0040] In some embodiments, the invention is directed to a method of treating cancer in a patient in need thereof, comprising administering to the patient a therapeutically effective amount of any IL-12 mutein disclosed herein. In some embodiments, the cancer comprises a solid tumor.

INCORPORATION BY REFERENCE

[0041] All publications, patents, and patent applications mentioned in this specification are herein incorporated by reference to the same extent as if each individual publication, patent, or patent application was specifically and individually indicated to be incorporated by reference.

BRIEF DESCRIPTION OF THE DRAWINGS

The features and advantages of the invention may be further explained by reference to the following detailed description and accompanying drawings that sets forth illustrative embodiments.

[0042] **FIG. 1** is a predicted model structure of IL-12 generated by AlphaFold 2 (AF2) from an initial ten angstrom cryoEM map of the IL-12 p35/p40 complex (grey cloud area), the crystal structure of the p40/IL-12Rb1 complex (ID No: 6WDQ in the Protein Data Bank), and the crystal structure of the IL-12 p35/p40 complex (ID No: 1F45 in the Protein Data Bank).

[0043] **FIG. 2** is a comparison of the starting model of IL-12/receptor interaction as generated by AlphaFold2 and the refined model after molecular dynamics simulation in the GROMACS system. The starting model is colored grey and the refined model yellow.

[0044] **FIG. 3** is a structure model of IL-12 (p35 and p40 subunits) interacting with the IL-12 receptor (IL-12Rb1 and IL-12Rb2) showing structure-predicted mutein effector regions.

[0045] **FIG. 4** is a graph showing surface plasmon resonance (SPR) binding data for an IL-12 mutein including single alanine mutants of the p35 subunit and the effect on binding to IL-12Rb1 and IL-12Rb2.

[0046] **FIG. 5** is a graph showing surface plasmon resonance (SPR) binding data for an IL-12 mutein including single alanine mutants of the p40 subunit and the effect on binding to IL-12Rb1 and IL-12Rb2.

DETAILED DESCRIPTION

[0047] While IL-12 has a tremendous therapeutic potential, it can produce undesirable side effects even at low concentrations. When administered systemically, IL-12 may activate immune cells in the bloodstream that express IL-12R, thereby creating a systemic inflammatory response that may elicit side effects. Thus, the practical application of IL-12 in a clinical setting is limited. The present disclosure provides IL-12 proteins including substitutions (e.g., substitution substitutions) within the p35 and/or p40 subunits of IL-12 to create mutant IL-12 muteins with attenuated IL-12 activity (e.g., decreased IL-12 receptor binding or activation relative to wild-type IL-12). Substitutions described herein limit IL-12 mutein activity to reduce or eliminate unwanted systemic effects and toxicity. IL-12 muteins and fusion proteins described herein are therefore candidates for therapeutic uses. The present disclosure provides, IL-12 muteins and fusion proteins containing the same for use in therapeutic compositions for the treatment of diseases. The present disclosure provides, IL-12 muteins and fusion proteins containing the same for use in therapeutic compositions for the prevention of diseases.

[0048] Before the embodiments of the invention are described, it is to be understood that such embodiments are provided by way of example only, and that various alternatives to the embodiments of the invention described herein may be employed in practicing the invention. Numerous variations, changes, and substitutions will now occur to those skilled in the art without departing from the invention.

[0049] Unless otherwise defined, all technical and scientific terms used herein have the same meaning as commonly understood by one of ordinary skill in the art to which this invention belongs. Although methods and materials similar or equivalent to those described herein can be used in the practice or testing of the present invention, suitable methods and materials are described below. In case of conflict, the patent specification, including definitions, will control. In addition, the materials, methods, and examples are illustrative only and not intended to be limiting. Numerous variations, changes, and substitutions will now occur to those skilled in the art without departing from the invention.

[0050] IL-12 is a disulfide-linked heterodimer of two separately encoded subunits (p35 and p40), which are linked covalently to give rise to the so-called bioactive heterodimeric (p70) molecule. Apart from forming heterodimers (IL-12 and IL-23), the p40 subunit is also secreted as a monomer (p40) and a homodimer (p40₂). It is known in the art that synthesis of the heterodimer as a single chain with a linker connecting the p35 to the p40 subunit preserves the full biological activity of the heterodimer. IL-12 plays a critical role in the early inflammatory response to infection and in the generation of Th1 cells, which favor cell-mediated immunity. It has been found that overproduction of IL-12 can be dangerous to the host because it is involved in the pathogenesis of a number of autoimmune inflammatory diseases (e.g. MS, arthritis, type 1 diabetes).

[0051] The IL-12 receptor (IL-12R) is a heterodimeric complex consisting of IL-12Rβ1 and IL-12Rβ2 chains expressed on the surface of activated T-cells and natural killer cells. The IL-12Rβ1 chain binds to the IL-12p40 subunit, whereas IL-12p35 in association with IL-12Rβ2 confers an intracellular signaling ability. Signal transduction through IL-12R induces phosphorylation of Janus kinase (Jak2) and tyrosine kinase (Tyk2), that phosphorylate and activate signal transducer and activator of transcription (STAT)1, STAT3, STAT4, and STATS. The specific cellular effects of IL-12 are due mainly to activation of STAT4. IL-12 induces natural killer and T-cells to produce cytokines, in particular interferon (IFN)γ, that mediate many of the proinflammatory activities of IL-12, including CD4+ T-cell differentiation toward the Th1 phenotype.

[0052] IL-12 is a pleiotropic cytokine, that creates an interconnection between the innate and adaptive immunity. IL-12 was first described as a factor secreted from PMA-induced EBV-transformed B-cell lines. Based on its actions, IL-12 has been designated as cytotoxic lymphocyte

maturation factor and natural killer cell stimulatory factor. Due to bridging the innate and adaptive immunity and potentially stimulating the production of IFN γ cytokine coordinating natural mechanisms of anticancer defense, IL-12 seemed like a promising candidate for tumor immunotherapy in humans. However, severe side effects associated with systemic administration of IL-12 in clinical investigations and the very narrow therapeutic index of this cytokine markedly hampered the use of this cytokine in cancer patients. Approaches to IL-12 therapy in which delivery of the cytokine is tumor-targeted, which may diminish some of the previous issues with IL-12 therapy, are currently in clinical trials for cancers.

[0053] The direct use of IL-12 as an agonist to bind the IL-12R and modulate immune responses therapeutically has been limited due its therapeutic risks, e.g., its short serum half-life and high toxicity. These risks have also limited the therapeutic development and use of other cytokines. New forms of cytokines that reduce these risks are needed. Disclosed herein are compositions and methods comprising IL-12 muteins designed to address the risks associated with conventional cytokine therapy and provide much needed immunomodulatory therapeutics.

Definitions

[0054] As used herein, the following terms have the meanings ascribed to them unless specified otherwise.

[0055] As used in the specification and claims, the singular forms “a”, “an” and “the” include plural references unless the context clearly dictates otherwise. For example, the term “a cell” includes a plurality of cells, including mixtures thereof.

[0056] The term “and/or” where used herein is to be taken as specific disclosure of each of the two specified features or components with or without the other. Thus, the term “and/or” as used in a phrase such as “A and/or B” herein is intended to include “A and B,” “A or B,” “A” (alone), and “B” (alone). Likewise, the term “and/or” as used in a phrase such as “A, B, and/or C” is intended to encompass each of the following embodiments: A, B, and C; A, B, or C; A or C; A or B; B or C; A and C; A and B; B and C; A (alone); B (alone); and C (alone).

[0057] As described herein, any concentration range, percentage range, ratio range or integer range is to be understood to include the value of any integer within the recited range and, when appropriate, fractions thereof (such as one tenth and one hundredth of an integer), unless otherwise indicated.

[0058] The term “about” refers to a value or composition that is within an acceptable error range for the particular value or composition as determined by one of ordinary skill in the art, which will depend in part on how the value or composition is measured or determined, i.e., the limitations of

the measurement system. When particular values or compositions are provided in the application and claims, unless otherwise stated, the meaning of “about” should be assumed to be within an acceptable error range for that particular value or composition. In some embodiments, “about” refers to a value of +/- 10% when compared to a reference value (e.g., about 100 includes a range of 90 to 110).

[0059] The term “interleukin 12” or “IL-12” refers to a human IL-12 protein or polypeptide. The term encompasses functional IL-12 protein complexes comprising an IL-12 p35 subunit and an IL-12 p40 subunit. An IL-12 described herein may be an IL-12 mutein comprising a variant p35 subunit (e.g., paired with a wild-type p40 subunit or a variant p40 subunit) and/or a variant p40 subunit (e.g., paired with a wild-type p35 subunit or a variant p35 subunit).

[0060] The term “p35” or “p35 subunit” as used, interchangeably, herein refers to the human alpha subunit of the heterodimeric human IL-12 protein. The amino acid sequence of an exemplary reference IL-12 p35 can be found under Uniprot Accession Number P29459, and herein set forth in SEQ ID NO: 1.

[0061] The term “p40” or “p40 subunit” as used, interchangeably, herein refers to the human beta subunit of the heterodimeric human IL-12 protein. The amino acid sequence of an exemplary reference IL-12 p40 can be found under Uniprot Accession Number P29460, and herein set forth in SEQ ID NO: 2.

[0062] By “wild type” or “WT” or “native” herein is meant an amino acid sequence or a nucleotide sequence that is found in nature, including allelic variations. A wild-type protein, polypeptide, antibody, immunoglobulin, IgG, etc. has an amino acid sequence or a nucleotide sequence that has not been modified by the hand of man.

[0063] As used herein, the term “variant” or “variants” is used to refer to a protein with a modified amino acid sequence when compared to a reference sequence. For example, a variant p35 subunit can refer to a p35 subunit with an amino acid sequence that has one or more substitutions or deletions when compared to a reference human p35 subunit protein sequence. Unless otherwise specified, variant proteins and subunits have an amino acid sequence modified when compared to a human amino acid sequence.

[0064] As used herein, the term “mutein” is used to refer to modified versions of wild type polypeptides comprising modifications to a recognized reference primary structure (i.e., amino acid sequence) of such polypeptide. The term mutein may refer to the polypeptide itself, a composition comprising the polypeptide, or a nucleic acid sequence that encodes it. A mutein may be at least about 90, 91, 92, 94, 94, 95, 96, 97, 98, or 99% identical to the parent polypeptide. In some instances, as used herein, the compositions comprise a variant p40 subunit, wherein the p40

comprises an amino acid sequence that is at least about 90, 91, 92, 94, 94, 95, 96, 97, 98, or 99% identical to the wild type p40 polypeptide (SEQ ID NO: 2). In some instances, as used herein, the compositions comprise a variant p35 subunit, wherein the p35 comprises an amino acid sequence that is at least about 90, 91, 92, 94, 94, 95, 96, 97, 98, or 99% identical to the wild type p40 polypeptide (SEQ ID NO: 1). As used herein with respect to IL-12, the term “mutein” refers to an IL-12 comprising a variant p35 subunit, variant p40 subunit, or both a variant p35 subunit and variant p40 subunit.

[0065] As used herein, the term “receptor” refers to a polypeptide having a domain that specifically binds a ligand that binding of the ligand results in a change to at least one biological property of the polypeptide. In some embodiments, the receptor is a cell membrane associated protein that comprises an extracellular domain (ECD) and a membrane associated domain which serves to anchor the ECD to the cell surface. In some embodiments of cell surface receptors, the receptor is a membrane spanning polypeptide comprising an intracellular domain (ICD) and extracellular domain (ECD) linked by a membrane spanning domain referred to as a transmembrane domain (TM). The binding of a ligand to the receptor results in a conformational change in the receptor resulting in a measurable biological effect. In some instances, where the receptor is a membrane spanning polypeptide comprising an ECD, TM and ICD, the binding of a ligand to the ECD results in a measurable intracellular biological effect mediated by one or more domains of the ICD in response to the binding of the ligand to the ECD. In some embodiments, a receptor is a component of a multi- component complex to facilitate intracellular signaling. For example, the ligand may bind a cell surface receptor that is not associated with any intracellular signaling alone but upon ligand binding facilitates the formation of a heteromultimeric (including heterodimeric, heterotrimeric, etc.) or homomultimeric (including homodimeric, homotrimeric, homotetrameric, etc.) complex that results in a measurable biological effect in the cell such as activation of an intracellular signaling cascade (e.g., the Jak/STAT pathway). In some embodiments, a receptor is a membrane spanning single chain polypeptide comprising ECD, TM and ICD domains wherein the ECD, TM and ICD domains are derived from the same or differing naturally occurring receptor variants or synthetic functional equivalents thereof.

[0066] The term “response,” for example, of a cell, tissue, organ, or organism, encompasses a quantitative or qualitative change in a evaluable biochemical or physiological parameter, (e.g., concentration, density, adhesion, proliferation, activation, phosphorylation, migration, enzymatic activity, level of gene expression, rate of gene expression, rate of energy consumption, level of or state of differentiation) where the change is correlated with the activation, stimulation, or treatment, with or contact with exogenous agents or internal mechanisms such as genetic

programming. In certain contexts, the terms “activation”, “stimulation”, and the like refer to cell activation as regulated by internal mechanisms, as well as by external or environmental factors; whereas the terms “inhibition”, “down-regulation” and the like refer to the opposite effects. A “response” may be evaluated in vitro such as through the use of assay systems, surface plasmon resonance, enzymatic activity, mass spectroscopy, amino acid or protein sequencing technologies. A “response” may be evaluated in vivo quantitatively by evaluation of objective physiological parameters such as body temperature, bodyweight, tumor volume, blood pressure, results of X-ray or other imaging technology or qualitatively through changes in reported subjective feelings of well-being, depression, agitation, or pain. In some embodiments, the level of activation of T cells in response to the administration of a test agent may be determined by flow cytometric methods. In some methods, a response can be measured by determining the level of STAT (e.g., STAT3, STAT4) phosphorylation, or IFN γ production, in accordance with methods well known in the art.

[0067] Where proteins and/or polypeptides are described herein, it is understood that polynucleotides (e.g., RNA (e.g., mRNA) or DNA polynucleotides) encoding the protein or polypeptide are also provided herein.

[0068] Where proteins, polypeptides, polynucleotides, cells, expression vectors, etc. are described herein, it is understood that isolated forms of the proteins, polypeptides, polynucleotides, cells, expression vectors, etc. are also provided herein.

[0069] The terms “polypeptide”, “peptide”, and “protein” are used interchangeably herein to refer to polymers of amino acids of any length. The polymer may be linear or branched, it may comprise modified amino acids, and it may be interrupted by non-amino acids. The terms also encompass an amino acid polymer that has been modified, for example, by disulfide bond formation, glycosylation, lipidation, acetylation, phosphorylation, or any other manipulation, such as conjugation with a labeling component.

[0070] As used herein the term “amino acid” refers to either natural and/or unnatural or synthetic amino acids, including but not limited to glycine and both the D or L optical isomers, and amino acid analogs and peptidomimetics. Standard single or three letter codes are used to designate amino acids.

[0071] The term “natural L-amino acid” means the L optical isomer forms of glycine (G), proline (P), alanine (A), valine (V), leucine (L), isoleucine (I), methionine (M), cysteine (C), phenylalanine (F), tyrosine (Y), tryptophan (W), histidine (H), lysine (K), arginine (R), glutamine (Q), asparagine (N), glutamic acid (E), aspartic acid (D), serine (S), and threonine (T).

[0072] The term “non-naturally occurring,” as applied to sequences and as used herein, means polypeptide or polynucleotide sequences that do not have a counterpart to, are not complementary to, or do not have a high degree of homology with a wild-type or naturally-occurring sequence found in a mammal. For example, a non-naturally occurring polypeptide may share no more than 99%, 98%, 95%, 90%, 80%, 70%, 60%, 50% or even less amino acid sequence identity as compared to a natural sequence when suitably aligned.

[0073] The terms “hydrophilic” and “hydrophobic” refer to the degree of affinity that a substance has with water. A hydrophilic substance has a strong affinity for water, tending to dissolve in, mix with, or be wetted by water, while a hydrophobic substance substantially lacks affinity for water, tending to repel and not absorb water and tending not to dissolve in or mix with or be wetted by water. Amino acids can be characterized based on their hydrophobicity. A number of scales have been developed. An example is a scale developed by Levitt, M, et al., J Mol Biol (1976) 104:59, which is listed in Hopp, TP, et al., Proc Natl Acad Sci U S A (1981) 78:3824. Examples of “hydrophilic amino acids” are arginine, lysine, threonine, alanine, asparagine, and glutamine. Of particular interest are the hydrophilic amino acids aspartate, glutamate, and serine, and glycine. Examples of “hydrophobic amino acids” are tryptophan, tyrosine, phenylalanine, methionine, leucine, isoleucine, and valine.

[0074] As used herein in the context of the structure of a polypeptide, “N-terminus” (or “amino terminus”) and “C-terminus” (or “carboxyl terminus”) refer to the extreme amino and carboxyl ends of the polypeptide, respectively, while the terms “N-terminal” and “C-terminal” refer to relative positions in the amino acid sequence of the polypeptide toward the N-terminus and the C-terminus, respectively, and can include the residues at the N-terminus and C-terminus, respectively. “Immediately N-terminal” refers to the position of a first amino acid residue relative to a second amino acid residue in a contiguous polypeptide sequence, the first amino acid being closer to the N-terminus of the polypeptide. “Immediately C-terminal” refers to the position of a first amino acid residue relative to a second amino acid residue in a contiguous polypeptide sequence, the first amino acid being closer to the C-terminus of the polypeptide. As used herein in the context of nucleic acids, the “5'-terminus” (or “five-prime terminus”) and “3'-terminus” (or “carboxyl terminus”) refer to the extreme ends of the nucleic acid sequence, respectively, while the terms “5'” and “3'” refer to relative positions in the nucleic acid sequence of the polypeptide toward the 5'-terminus and the 3'-terminus, respectively, and can include the residues at the 5'-terminus and 3'-terminus, respectively.

[0075] A “fragment” is a truncated form of a native biologically active protein that retains at least a portion of the therapeutic and/or biological activity. As used herein, the term “biologically

active protein moiety” includes proteins modified deliberately, as for example, by site directed mutagenesis, insertions, or accidentally through substitutions.

[0076] A “host cell” includes an individual cell or cell culture which can be or has been a recipient for the subject vectors. Host cells include progeny of a single host cell. The progeny may not necessarily be completely identical (in morphology or in genomic or total DNA complement) to the original parent cell due to natural, accidental, or deliberate substitution. A host cell includes cells transfected in vivo with a vector of this invention.

[0077] “Isolated,” when used to describe the various polypeptides disclosed herein, means polypeptide that has been identified and separated and/or recovered from a component of its natural environment. Contaminant components of its natural environment are materials that would typically interfere with diagnostic or therapeutic uses for the polypeptide, and may include enzymes, hormones, and other proteinaceous or non-proteinaceous solutes. As is apparent to those of skill in the art, a non-naturally occurring polynucleotide, peptide, polypeptide, protein, antibody, or fragments thereof, does not require “isolation” to distinguish it from its naturally occurring counterpart. In addition, a “concentrated”, “separated” or “diluted” polynucleotide, peptide, polypeptide, protein, antibody, or fragments thereof, is distinguishable from its naturally occurring counterpart in that the concentration or number of molecules per volume is generally greater than that of its naturally occurring counterpart. In general, a polypeptide made by recombinant means and expressed in a host cell is considered to be “isolated.”

[0078] An “isolated” polynucleotide or polypeptide-encoding nucleic acid or other polypeptide-encoding nucleic acid is a nucleic acid molecule that is identified and separated from at least one contaminant nucleic acid molecule with which it is ordinarily associated in the natural source of the polypeptide-encoding nucleic acid. An isolated polypeptide-encoding nucleic acid molecule is other than in the form or setting in which it is found in nature. Isolated polypeptide-encoding nucleic acid molecules therefore are distinguished from the specific polypeptide-encoding nucleic acid molecule as it exists in natural cells. However, an isolated polypeptide-encoding nucleic acid molecule includes polypeptide-encoding nucleic acid molecules contained in cells that ordinarily express the polypeptide where, for example, the nucleic acid molecule is in a chromosomal or extra-chromosomal location different from that of natural cells.

[0079] A “fusion protein” contains at least one polypeptide comprising regions in a different position in the sequence than that which occurs in nature. The regions may normally exist in separate proteins and are brought together in the fusion protein; or they may normally exist in the same protein but are placed in a new arrangement in the fusion protein. A fusion protein may be

created, for example, by chemical synthesis, or by creating and translating a polynucleotide in which the peptide regions are encoded in the desired relationship.

[0080] “Conjugated”, “linked,” “fused,” and “fusion” are used interchangeably herein. These terms refer to the joining together of two more chemical elements or components, by whatever means including chemical conjugation or recombinant means. For example, a promoter or enhancer is operably linked to a coding sequence if it affects the transcription of the sequence. Generally, “operably linked” means that the DNA sequences being linked are contiguous, and in reading phase or in-frame. An “in-frame fusion” refers to the joining of two or more open reading frames (ORFs) to form a continuous longer ORF, in a manner that maintains the correct reading frame of the original ORFs. Thus, the resulting recombinant fusion protein is a single protein containing two or more segments that correspond to polypeptides encoded by the original ORFs (which segments are not normally so joined in nature). The terms “link,” “linked,” and “linking” are used in the broadest sense, and are specifically intended to include both covalent and non-covalent attachment of a moiety of the therapeutic agent to another moiety of the therapeutic agent in a direct or indirect way. The term “linked directly,” as used herein in the context of a therapeutic agent, generally refers to a structure in which a moiety is connected with or attached to another moiety without an intervening tether. The term “linked indirectly,” as used herein in the context of a therapeutic agent, generally refers to a structure in which a moiety of the therapeutic agent is connected with, or attached to, another moiety of the therapeutic agent via an intervening tether.

[0081] In the context of polypeptides, a “linear sequence” or a “sequence” is an order of amino acids in a polypeptide in an amino to carboxyl terminus direction in which residues that neighbor each other in the sequence are contiguous in the primary structure of the polypeptide. A “partial sequence” is a linear sequence of part of a polypeptide that is known to comprise additional residues in one or both directions.

[0082] “Heterologous” means derived from a genotypically distinct entity from the rest of the entity to which it is being compared. For example, a glycine rich sequence removed from its native coding sequence and operatively linked to a coding sequence other than the native sequence is a heterologous glycine rich sequence. The term “heterologous” as applied to a polynucleotide, a polypeptide, means that the polynucleotide or polypeptide is derived from a genotypically distinct entity from that of the rest of the entity to which it is being compared.

[0083] The terms “polynucleotides”, “nucleic acids”, “nucleotides” and “oligonucleotides” are used interchangeably. They refer to a polymeric form of nucleotides of any length, either deoxyribonucleotides or ribonucleotides, or analogs thereof. Polynucleotides may have any three-dimensional structure, and may perform any function, known or unknown. The following are non-

limiting examples of polynucleotides: coding or non-coding regions of a gene or gene fragment, loci (locus) defined from linkage analysis, exons, introns, messenger RNA (mRNA), transfer RNA, ribosomal RNA, ribozymes, cDNA, recombinant polynucleotides, branched polynucleotides, plasmids, vectors, isolated DNA of any sequence, isolated RNA of any sequence, nucleic acid probes, and primers. A polynucleotide may comprise modified nucleotides, such as methylated nucleotides and nucleotide analogs. If present, modifications to the nucleotide structure may be imparted before or after assembly of the polymer. The sequence of nucleotides may be interrupted by non-nucleotide components. A polynucleotide may be further modified after polymerization, such as by conjugation with a labeling component.

[0084] The term “complement of a polynucleotide” denotes a polynucleotide molecule having a complementary base sequence and reverse orientation as compared to a reference sequence, such that it could hybridize with a reference sequence with complete fidelity.

[0085] “Recombinant” as applied to a polynucleotide means that the polynucleotide is the product of various combinations of *in vitro* cloning, restriction and/or ligation steps, and other procedures that result in a construct that can potentially be expressed in a host cell.

[0086] The terms “gene” or “gene fragment” are used interchangeably herein. They refer to a polynucleotide containing at least one open reading frame that is capable of encoding a particular protein after being transcribed and translated. A gene or gene fragment may be genomic or cDNA, as long as the polynucleotide contains at least one open reading frame, which may cover the entire coding region or a segment thereof. A “fusion gene” is a gene composed of at least two heterologous polynucleotides that are linked together.

[0087] “Homology” or “homologous” refers to sequence similarity or interchangeability between two or more polynucleotide sequences or two or more polypeptide sequences. When using a program such as BestFit to determine sequence identity, similarity or homology between two different amino acid sequences, the default settings may be used, or an appropriate scoring matrix, such as blosum45 or blosum80, may be selected to optimize identity, similarity or homology scores. Preferably, polynucleotides that are homologous are those which hybridize under stringent conditions as defined herein and have at least 70%, preferably at least 80%, more preferably at least 90%, more preferably 95%, more preferably 97%, more preferably 98%, and even more preferably 99% sequence identity to those sequences.

[0088] The terms “percent identity” and “% identity,” as applied to polynucleotide sequences, refer to the percentage of residue matches between at least two polynucleotide sequences aligned using a standardized algorithm. Such an algorithm may insert, in a standardized and reproducible way, gaps in the sequences being compared in order to optimize alignment between two sequences,

and therefore achieve a more meaningful comparison of the two sequences. Percent identity may be measured over the length of an entire defined polynucleotide sequence, for example, as defined by a particular SEQ ID number, or may be measured over a shorter length, for example, over the length of a fragment taken from a larger, defined polynucleotide sequence, for instance, a fragment of at least 45, at least 60, at least 90, at least 120, at least 150, at least 210 or at least 450 contiguous residues. Such lengths are exemplary only, and it is understood that any fragment length supported by the sequences shown herein, in the tables, figures or Sequence Listing, may be used to describe a length over which percentage identity may be measured.

[0089] “Percent (%) amino acid sequence identity,” with respect to the polypeptide sequences identified herein, is defined as the percentage of amino acid residues in a query sequence that are identical with the amino acid residues of a second, reference polypeptide sequence or a portion thereof, after aligning the sequences and introducing gaps, if necessary, to achieve the maximum percent sequence identity, and not considering any conservative substitutions as part of the sequence identity. Alignment for purposes of determining percent amino acid sequence identity can be achieved in various ways that are within the skill in the art, for instance, using publicly available computer software such as BLAST, BLAST-2, ALIGN or Megalign (DNASTAR) software. Those skilled in the art can determine appropriate parameters for measuring alignment, including any algorithms needed to achieve maximal alignment over the full length of the sequences being compared. Percent identity may be measured over the length of an entire defined polypeptide sequence, for example, as defined by a particular SEQ ID number, or may be measured over a shorter length, for example, over the length of a fragment taken from a larger, defined polypeptide sequence, for instance, a fragment of at least 15, at least 20, at least 30, at least 40, at least 50, at least 70 or at least 150 contiguous residues. Such lengths are exemplary only, and it is understood that any fragment length supported by the sequences shown herein, in the tables, figures or Sequence Listing, may be used to describe a length over which percentage identity may be measured.

[0090] A “vector” is a nucleic acid molecule, preferably self-replicating in an appropriate host, which transfers an inserted nucleic acid molecule into and/or between host cells. The term includes vectors that function primarily for insertion of DNA or RNA into a cell, replication of vectors that function primarily for the replication of DNA or RNA, and expression vectors that function for transcription and/or translation of the DNA or RNA. Also included are vectors that provide more than one of the above functions. An “expression vector” is a polynucleotide which, when introduced into an appropriate host cell, can be transcribed and translated into a polypeptide(s).

An “expression system” usually connotes a suitable host cell comprised of an expression vector that can function to yield a desired expression product.

[0091] “Physiological conditions” refer to a set of conditions in a living host as well as *in vitro* conditions, including temperature, salt concentration, pH, that mimic those conditions of a living subject. A host of physiologically relevant conditions for use in *in vitro* assays have been established. Generally, a physiological buffer contains a physiological concentration of salt and is adjusted to a neutral pH ranging from about 6.5 to about 7.8, and preferably from about 7.0 to about 7.5. A variety of physiological buffers is listed in Sambrook et al. (1989). Physiologically relevant temperature ranges from about 25°C to about 38°C, and preferably from about 35°C to about 37°C.

[0092] The terms “antigen”, “target antigen” or “immunogen” are used interchangeably herein to refer to the structure or binding determinant that an antibody fragment or an antibody fragment-based therapeutic binds to or has specificity against.

[0093] The term “antagonist”, as used herein, includes any molecule that partially or fully blocks, inhibits, or neutralizes a biological activity of a native polypeptide disclosed herein. Methods for identifying antagonists of a polypeptide may comprise contacting a native polypeptide with a candidate antagonist molecule and measuring a detectable change in one or more biological activities normally associated with the native polypeptide. In the context of the present invention, antagonists may include proteins, nucleic acids, carbohydrates, antibodies or any other molecules that decrease the effect of a biologically active protein.

[0094] The term “agonist” is used in the broadest sense and includes any molecule that mimics a biological activity of a native polypeptide disclosed herein. Suitable agonist molecules specifically include agonist antibodies or antibody fragments, fragments or amino acid sequence variants of native polypeptides, peptides, small organic molecules, etc. Methods for identifying agonists of a native polypeptide may comprise contacting a native polypeptide with a candidate agonist molecule and measuring a detectable change in one or more biological activities normally associated with the native polypeptide.

[0095] “Activity” for the purposes herein refers to an action or effect of a component of a protein consistent with that of the corresponding native biologically active protein, wherein “biological activity” refers to an *in vitro* or *in vivo* biological function or effect, including but not limited to receptor binding, antagonist activity, agonist activity, or a cellular or physiologic response.

[0096] As used herein, “treatment” or “treating,” refer to an approach for obtaining beneficial or desired results including but not limited to a therapeutic benefit. By therapeutic benefit is meant eradication, amelioration, or a delay in the progression of the underlying disorder being treated.

Thus, for example, treatment refers to a method of reducing the effects of a disease or condition or symptom of the disease or condition. Thus, in the disclosed method, treatment can refer to at least about 10%, at least about 20%, at least about 30%, at least about 40%, at least about 50%, at least about 60%, at least about 70%, at least about 80%, at least about 90%, or substantially complete reduction in the severity of an established disease or condition or symptom of the disease or condition. For example, a method for treating a disease is considered to be a treatment if there is a 10% reduction in one or more symptoms of the disease in a subject as compared to a control. Thus, the reduction can be a 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90%, 100%, or any percent reduction in between 10% and 100% as compared to native or control levels. Also, a therapeutic benefit is achieved with the eradication or amelioration of one or more of the physiological symptoms associated with the underlying disease condition such that an improvement is observed in the subject, notwithstanding that the subject may still be afflicted with the underlying disorder. It is understood that treatment does not necessarily refer to a cure or complete ablation of the disease, condition, or symptoms of the disease or condition.

[0097] A “therapeutic effect”, as used herein, refers to a physiologic effect, including but not limited to the cure, mitigation, amelioration, or delay in progression of a disease or condition in humans or other animals, or to otherwise enhance physical or mental wellbeing of humans or animals, caused by a polypeptide of the invention other than the ability to induce the production of an antibody against an antigenic epitope possessed by the biologically active protein. Determination of a therapeutically effective amount is within the capability of those skilled in the art.

[0098] The terms “therapeutically effective amount” and “therapeutically effective dose”, as used herein, refers to an amount of a biologically active protein, either alone, as a part of a fusion protein composition, or as part of a therapeutic regimen, that is capable of having any detectable, beneficial effect on any symptom, aspect, measured parameter or characteristics of a disease state or condition when administered in one or repeated doses to a subject. Such effect need not be absolute to be beneficial. The disease or condition can refer to a disorder or a disease.

[0099] The term “therapeutically effective dose regimen”, as used herein, refers to a schedule for consecutively administered doses of a biologically active protein, either alone or as a part of a fusion protein composition, wherein the doses are given in therapeutically effective amounts to result in sustained beneficial effect on any symptom, aspect, measured parameter or characteristics of a disease state or condition.

[0100] As used herein, the terms “prevent”, “preventing”, and “prevention” of a disease or disorder refers to an action, for example, administration of the fusion protein or nucleic acid

sequence encoding the fusion protein, that occurs before or at about the same time a subject begins to show one or more symptoms of the disease or disorder, which inhibits or delays onset or exacerbation of one or more symptoms of the disease or disorder.

[0101] As used herein, references to “decreasing”, “reducing”, or “inhibiting” include a change of at least about 10%, of at least about 20%, of at least about 30%, of at least about 40%, of at least about 50%, of at least about 60%, of at least about 70%, of at least about 80%, of at least about 90% or greater as compared to a suitable control level. Such terms can include but do not necessarily include complete elimination of a function or property, such as agonist activity.

[0102] The terms “cancer” and “tumor” are used interchangeably herein and refer to a broad group of various diseases characterized by the uncontrolled growth of abnormal cells in the body. Unregulated cell division and growth results in the formation of malignant tumors that can invade neighboring tissues and may also metastasize to distant parts of the body through, e.g., the lymphatic system or bloodstream.

IL-12 Protein

[0103] IL-12 is a covalently disulfide linked heterodimeric protein comprising two subunits, p35 and p40. In humans, the naturally occurring form of IL-12 comprises an interchain disulfide linkage between residue C74 of p35 (numbered in accordance with SEQ ID NO:1, Uniprot ID: P29459) and residue C177 of p40 (numbered in accordance with SEQ ID NO:2, Uniprot ID: P29459).

[0104] In some embodiments, the IL-12 mutein comprises a heterodimer comprising p35 and p40 subunits. In some embodiments the two subunits of the IL-12 heterodimer are covalently linked. In some embodiments, the covalent linkage is a disulfide bond. Alternatively, the IL-12 mutein may be single chain IL-12 comprising a single polypeptide comprising both the p35 and p40 subunit moieties. In some embodiments, the single chain IL-12 may further comprise additional domains, for example, a half-life extending moiety.

[0105] In some embodiments, the present disclosure provides IL-12 muteins with one or more amino acid substitutions in p35. In some embodiments, IL-12 muteins comprise one or more amino acid substitutions in p35. In some embodiments, IL-12 muteins comprise one more amino acid substitutions in both p35 and p40.

[0106] In some embodiments, the IL-12 mutein has decreased affinity for an IL-12 receptor (e.g., relative to wild-type human IL-12 having a p35 comprising SEQ ID NO:1 and a p40 comprising SEQ ID NO: 2). In some embodiments, the IL-12 mutein has a decreased affinity for the IL-12 receptor beta 1. In some embodiments, the IL-12 mutein has a decreased affinity for the IL-12

receptor beta 2. In some embodiments, decreased affinity is measured by surface plasmon resonance (SPR).

[0107] In some embodiments, the IL-12 protein has a 10% decrease in affinity for IL-12 receptor beta 1 when measured by SPR. In some embodiments, the IL-12 protein has a 15% decrease in affinity for IL-12 receptor beta 1 when measured by SPR. In some embodiments, the IL-12 protein has a 20% decrease in affinity for IL-12 receptor beta 1 when measured by SPR. In some embodiments, the IL-12 protein has a 25% decrease in affinity for IL-12 receptor beta 1 when measured by SPR. In some embodiments, the IL-12 protein has a 30% decrease in affinity for IL-12 receptor beta 1 when measured by SPR. In some embodiments, the IL-12 protein has a 35% decrease in affinity for IL-12 receptor beta 1 when measured by SPR. In some embodiments, the IL-12 protein has a 40% decrease in affinity for IL-12 receptor beta 1 when measured by SPR. In some embodiments, the IL-12 protein has a 45% decrease in affinity for IL-12 receptor beta 1 when measured by SPR. In some embodiments, the IL-12 protein has a 50% decrease in affinity for IL-12 receptor beta 1 when measured by SPR. In some embodiments, the IL-12 protein has a 55% decrease in affinity for IL-12 receptor beta 1 when measured by SPR. In some embodiments, the IL-12 protein has a 60% decrease in affinity for IL-12 receptor beta 1 when measured by SPR. In some embodiments, the IL-12 protein has a 65% decrease in affinity for IL-12 receptor beta 1 when measured by SPR. In some embodiments, the IL-12 protein has a 70% decrease in affinity for IL-12 receptor beta 1 when measured by SPR. In some embodiments, the IL-12 protein has a 75% decrease in affinity for IL-12 receptor beta 1 when measured by SPR. In some embodiments, the IL-12 protein has a 80% decrease in affinity for IL-12 receptor beta 1 when measured by SPR. In some embodiments, the IL-12 protein has a 85% decrease in affinity for IL-12 receptor beta 1 when measured by SPR.

[0108] In some embodiments, the IL-12 protein has a 10% decrease in affinity for IL-12 receptor beta 2 when measured by SPR. In some embodiments, the IL-12 protein has a 15% decrease in affinity for IL-12 receptor beta 2 when measured by SPR. In some embodiments, the IL-12 protein has a 20% decrease in affinity for IL-12 receptor beta 2 when measured by SPR. In some embodiments, the IL-12 protein has a 25% decrease in affinity for IL-12 receptor beta 2 when measured by SPR. In some embodiments, the IL-12 protein has a 30% decrease in affinity for IL-12 receptor beta 2 when measured by SPR. In some embodiments, the IL-12 protein has a 35% decrease in affinity for IL-12 receptor beta 2 when measured by SPR. In some embodiments, the IL-12 protein has a 40% decrease in affinity for IL-12 receptor beta 2 when measured by SPR. In some embodiments, the IL-12 protein has a 45% decrease in affinity for IL-12 receptor beta 2 when measured by SPR. In some embodiments, the IL-12 protein has a 50% decrease in affinity

for IL-12 receptor beta 2 when measured by SPR. In some embodiments, the IL-12 protein has a 55% decrease in affinity for IL-12 receptor beta 2 when measured by SPR. In some embodiments, the IL-12 protein has a 60% decrease in affinity for IL-12 receptor beta 2 when measured by SPR. In some embodiments, the IL-12 protein has a 65% decrease in affinity for IL-12 receptor beta 2 when measured by SPR. In some embodiments, the IL-12 protein has a 70% decrease in affinity for IL-12 receptor beta 2 when measured by SPR. In some embodiments, the IL-12 protein has a 75% decrease in affinity for IL-12 receptor beta 2 when measured by SPR. In some embodiments, the IL-12 protein has a 80% decrease in affinity for IL-12 receptor beta 2 when measured by SPR. In some embodiments, the IL-12 protein has a 85% decrease in affinity for IL-12 receptor beta 1 when measured by SPR.

[0109] The IL-12 protein of the invention can comprise mutein-effector regions. Mutein-effector regions are regions that may interact with other proteins and in which a substitution can alter a proteins interaction with IL-12. The IL-12 protein of the invention can comprise a mutein-effector region in the p35 subunit, the p40 subunit, or in both the p35 and p40 subunits. In some embodiments, a mutein effector region comprises at least one, at least two, at least three, at least four, or at least five amino acid substitutions such as substitutions or deletions (e.g., at least one, at least two, at least three, at least four, or at least five amino acid residues within the mutein effector region are substituted or deleted). In some embodiments, the IL-12 protein comprises multiple mutein effector regions, wherein each mutein effector region comprises at least one, at least two, at least three, at least four, or at least five amino acid substitutions such as substitutions or deletions (e.g., at least one, at least two, at least three, at least four, or at least five amino acid residues within each mutein effector region are substituted or deleted).

[0110] In some embodiments, the IL-12 protein comprises a p35 subunit which comprises a mutein-effector region. In some embodiments, the variant p35 subunit-effector region comprises amino acid residues 36-52, 124-133, or 163-178 of SEQ ID NO: 1. In some embodiments, the variant p35 subunit-effector region comprises amino acid residues 36-52 of SEQ ID NO: 1. In some embodiments, the variant p35 subunit-effector region comprises amino acid residues 37-48 of SEQ ID NO: 1. In some embodiments, the variant p35 subunit-effector region comprises amino acid residues 37-41 of SEQ ID NO: 1. In some embodiments, the variant p35 subunit-effector region comprises amino acid residues 124-133 of SEQ ID NO: 1. In some embodiments, the variant p35 subunit-effector region comprises amino acid residues 163-178 of SEQ ID NO: 1. In some embodiments, the variant p35 subunit-effector region comprises amino acid residues 164-178 of SEQ ID NO: 1.

[0111] In some embodiments, the IL-12 mutein comprises a p40 subunit which comprises a mutein-effector region. In some embodiments, the variant p40 subunit-effector region comprises amino acid residues 14-18, 41-45, 58-62, 84-107, 195-197, 201-202 of SEQ ID NO: 2. In some embodiments, the variant p40 subunit-effector region comprises amino acid residues 14-18 of SEQ ID NO: 2. In some embodiments, the variant p35 subunit-effector region comprises amino acid residues 41-45 of SEQ ID NO: 2. In some embodiments, the variant p40 subunit-effector region comprises amino acid residues 58-62 of SEQ ID NO: 2. In some embodiments, the variant p40 subunit-effector region comprises amino acid residues 84-107 of SEQ ID NO: 2. In some embodiments, the variant p40 subunit-effector region comprises amino acid residues 195-197 of SEQ ID NO: 2. In some embodiments, the variant p40 subunit-effector region comprises amino acid residues 201-202 of SEQ ID NO: 2.

[0112] A number of IL-12 mutants have been produced, such as those disclosed in patent publications WO2020072821, WO2021189139, WO2021212083, WO2022140797, WO2022192898, WO2023004282, WO2023043978, WO2023050006, WO2023070038, WO2023070056, WO2023133540, U.S. Patent No. 8,114,839, WO2016172703, WO2022094046, WO2022155263, WO2023279085, WO2023004282, and WO2023133540, each of which is incorporated herein by reference. In some embodiments, the IL-12 muteins described herein comprise one or more IL-12 substitutions described in the patent publications above.

IL 12 p35 Subunit and Muteins

[0113] A reference p35 monomer (p35) is expressed as a 219 amino acid pro-protein (SEQ ID NO: 3) comprising a 22 amino acid signal sequence which is post-translationally removed to render a 197 amino acid mature protein (SEQ ID NO:1).

[0114] In some embodiments, the IL-12 muteins of the present disclosure comprises a modified p35 sequence comprising one or more amino acid substitutions and/or deletions at the interface with the extracellular domain of IL-12R β 2. In one aspect, provided herein are variant p35 subunits comprising or consisting of an amino acid sequence at least 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% identical to the amino acid sequence set forth in SEQ ID NO: 1.

[0115] In some embodiments, the variant p35 subunit comprises one or more amino acid substitutions and/or deletions relative to SEQ ID NO: 1. In some embodiments, the variant p35 subunit comprises one amino acid substitution/substitution or deletion relative to SEQ ID NO: 1. In some embodiments, the variant p35 subunit comprises two amino acid substitutions/substitutions and/or deletions relative to SEQ ID NO: 1. In some embodiments, the variant p35 subunit comprises three amino acid substitutions/substitutions and/or deletions

relative to SEQ ID NO: 1. In some embodiments, the variant p35 subunit comprises four amino acid substitutions/substitutions and/or deletions relative to SEQ ID NO: 1. In some embodiments, the variant p35 subunit comprises five amino acid substitutions/substitutions and/or deletions relative to SEQ ID NO: 1. In some embodiments, the variant p35 subunit comprises six amino acid substitutions/substitutions and/or deletions relative to SEQ ID NO: 1. In some embodiments, the variant p35 subunit comprises seven amino acid substitutions/substitutions and/or deletions relative to SEQ ID NO: 1. In some embodiments, the variant p35 subunit comprises eight amino acid substitutions/substitutions and/or deletions relative to SEQ ID NO: 1. In some embodiments, the variant p35 subunit comprises nine amino acid substitutions/substitutions and/or deletions relative to SEQ ID NO: 1. In some embodiments, the variant p35 subunit comprises ten amino acid substitutions/substitutions and/or deletions relative to SEQ ID NO: 1.

[0116] In some embodiments, the variant p35 subunit comprises an amino acid sequence that is at least 80%, 85%, 90%, or 95% identical to SEQ ID NO: 1.

[0117] In some embodiments, the variant p35 subunit comprises an amino acid substitution and/or deletion at an amino acid residue corresponding to amino acid residue A33, R34, L37, E38, F39, Y40, P41, T43, S44, E45, E46, I47, D48, H49, E50, D51, I52, T53, K54, K56, T57, D108, L109, K110, M111, Y112, Q113, V114, E115, F116, K117, T118, A121, L123, M125, D126, K128, R129, Q130, I131, L133, P156, S159, S160, L161, E162, E163, P164, D165, F166, Y167, K168, T169, K170, I171, K172, L173, I175, L177, H178, or R181 with respect to the position in the sequence of mature p35 (SEQ ID NO: 1). In some embodiments, the variant p35 subunit comprises one, two, three, four, five, or more substitutions selected from the positions of the foregoing list. In some embodiments, the variant p35 subunit comprises an amino acid substitution, wherein the amino acid substitution is any naturally occurring amino acid other than the corresponding wild-type residue in SEQ ID NO: 1. In some embodiments, the variant p35 subunit comprises an amino acid substitution, wherein the amino acid substitution is any naturally occurring amino acid other than the corresponding wild-type residue in SEQ ID NO: 1 or cysteine.

[0118] In some embodiments, the variant p35 subunit comprises a substitution at amino acid residue L133, optionally wherein the substitution is L133A, L133R, L133H, L133K, L133D, L133E, L133S, L133T, L133N, L133Q, L133G, L133P, L133I, L133M, L133F, L133W, L133V, or L133Y.

[0119] In some embodiments, the variant p35 subunit comprises a substitution at amino acid residue L37, optionally wherein the substitution is L37R, L37H, L37K, L37D, L37E, L37S, L37T, L37N, L37Q, L37G, L37P, L37A, L37V, L37I, L37M, L37F, L37Y, or L37W. In some embodiments, the substitution at amino acid residue L37 is L37R, L37H, L37K, L37D, L37N,

L37Q, L37P, L37A, L37V, L37M, L37F, L37Y, or L37W. In some embodiments, the L37 substitution is L37A.

[0120] In some embodiments, the variant p35 subunit comprises a substitution at amino acid residue Y40, optionally wherein the substitution is Y40R, Y40H, Y40K, Y40D, Y40E, Y40S, Y40T, Y40N, Y40Q, Y40G, Y40P, Y40A, Y40V, Y40I, Y40L, Y40M, Y40F, or Y40W. In some embodiments, the substitution at amino acid residue Y40 is Y40H, Y40E, Y40V, Y40I, Y40L, Y40M, or Y40F. In some embodiments, the substitution at amino acid residue Y40 is Y40S.

[0121] In some embodiments, the variant p35 subunit comprises a substitution at amino acid residue D48, optionally wherein the substitution is D48R, D48H, D48K, D48E, D48S, D48T, D48N, D48Q, D48G, D48P, D48A, D48V, D48I, D48L, D48M, D48F, D48Y, or D48W. In some embodiments, the substitution at amino acid residue D48 is D48H, D48K, D48E, D48S, D48T, D48N, D48G, D48P, D48V, D48I, D48L, D48M, D48F, D48Y, or D48W, optionally wherein the substitution at amino acid residue D48 is D48K, D48E, D48T, D48N, D48G, D48P, D48V, D48I, D48L, D48M, or D48F.

[0122] In some embodiments, the variant p35 subunit comprises a substitution at amino acid residue M125, optionally wherein the substitution is M125R, M125H, M125K, M125D, M125E, M125S, M125T, M125N, M125Q, M125G, M125P, M125A, M125V, M125I, M125L, M125F, M125Y, or M125W. In some embodiments, the substitution at amino acid residue M125 is M125R, M125H, M125K, M125D, M125E, M125S, M125T, M125N, M125Q, M125G, M125P, M125L, M125F, M125Y, or M125W.

[0123] In some embodiments, the variant p35 subunit comprises a substitution at amino acid residue K128, optionally wherein the substitution is K128R, K128H, K128K, K128D, K128E, K128S, K128T, K128N, K128Q, K128G, K128P, K128A, K128V, K128I, K128L, K128M, K128F, K128Y, or K128W. In some embodiments, the substitution at amino acid residue K128 is K128N or K128Q.

[0124] In some embodiments, the variant p35 subunit comprises a substitution at amino acid residue R129, optionally wherein the substitution is R129H, R129K, R129D, R129E, R129S, R129T, R129N, R129Q, R129G, R129P, R129A, R129V, R129I, R129L, R129M, R129F, R129Y, or R129W.

[0125] In some embodiments, the variant p35 subunit comprises a substitution at amino acid residue L177, optionally wherein the substitution is L177R, L177H, L177K, L177D, L177E, L177S, L177T, L177N, L177Q, L177G, L177P, L177A, L177V, L177I, L177M, L177F, L177Y, or L177W. In some embodiments, the substitution at amino acid residue L177 is L177D, L177S, L177T, L177N, L177G, L177P, L177V, L177I, L177M, or L177W.

[0126] In some embodiments, wherein the variant p35 subunit comprises a substitution at amino acid residue H178, optionally wherein the substitution is H178R, H178K, H178D, H178E, H178S, H178T, H178N, H178Q, H178G, H178P, H178A, H178V, H178I, H178L, H178M, H178F, H178Y, or H178W.

[0127] In some embodiments, the variant p35 subunit comprises a substitution at amino acid residue K56, optionally wherein the substitution at amino acid residue K56 is K56R, K56H, K56D, K56E, K56S, K56T, K56N, K56Q, K56G, K56P, K56A, K56V, K56I, K56L, K56M, K56F, K56Y, or K56W. In some embodiments, the K56 substitution is K56R, K56H, K56D, K56E, K56S, K56T, K56G, K56P, K56A, K56V, K56I, K56L, K56M, K56F, K56Y, or K56W, optionally wherein the substitution at amino acid residue K56 is K56E, K56S, K56T, K56G, K56A, K56V, K56I, or K56L. In some embodiments, the substitution at amino acid residue K56 is K56N.

[0128] In some embodiments, the variant p35 subunit comprises a substitution at amino acid residue K170, optionally wherein the substitution at amino acid residue K170 is K170R, K170H, K170D, K170E, K170S, K170T, K170N, K170Q, K170G, K170P, K170A, K170V, K170I, K170L, K170M, K170F, K170Y, or K170W.

[0129] In some embodiments, the variant p35 subunit comprises a substitution at amino acid residue I171, optionally wherein the substitution at amino acid residue I171 is I171R, I171K, I171H, I171D, I171E, I171S, I171T, I171N, I171Q, I171G, I171P, I171A, I171V, I171L, I171M, I171F, I171Y, or I171W.

[0130] In some embodiments, the variant p35 subunit comprises a substitution at amino acid residue A33, R34, E38, F39, P41, E45, E46, I47, H49, E50, D51, I52, T53, K54, T57, D108, L109, K110, M111, Y112, Q113, V114, E115, F116, K117, T118, A121, L123, D126, I131, P156, S159, S160, L161, E162, E163, D165, F166, Y167, K168, T169, K172, L173, I175, or R181.

[0131] In some embodiments, the variant p35 subunit comprises an amino acid substitution and/or deletion at an amino acid residue corresponding to amino acid residue 33 with respect to the position in mature p35 sequence of SEQ ID NO: 1. In some embodiments, the variant p35 subunit comprises an amino acid substitution and/or deletion at an amino acid residue corresponding to amino acid residue 34 with respect to the position in mature p35 sequence of SEQ ID NO: 1. In some embodiments, the variant p35 subunit comprises an amino acid substitution and/or deletion at an amino acid residue corresponding to amino acid residue 37 with respect to the position in mature p35 sequence of SEQ ID NO: 1. In some embodiments, the variant p35 subunit comprises an amino acid substitution and/or deletion at an amino acid residue corresponding to amino acid residue 38 with respect to the position in mature p35 sequence of SEQ ID NO: 1. In some embodiments, the variant p35 subunit comprises an amino acid substitution and/or deletion at an

[illegible]

[illegible]

[illegible]

p35 sequence of SEQ ID NO: 1. In some embodiments, the variant p35 subunit comprises an amino acid substitution and/or deletion at an amino acid residue corresponding to amino acid residue 181 with respect to the position in mature p35 sequence of SEQ ID NO: 1. Exemplary p35 substitution substitutions are provided in Table 1a, Table 1b, and Table 1c. In some embodiments, the variant p35 subunit comprises one or more amino acid substitutions (e.g., one, two, three, four, five, or more amino acid substitutions) selected from Table 1a, Table 1b, or Table 1c.

Table 1a. p35 Substitutions

p35 Substitution	Substitution selected from
A33	G, S, P, V, T, I, L, N, D, K, Q, E, M, H, F, R, Y, or W
R34	A, G, S, P, V, T, I, L, N, D, K, Q, E, M, H, F, Y, or W
L37	A, G, S, P, V, T, I, N, D, K, Q, E, M, H, F, R, Y, or W
E38	A, G, S, P, V, T, I, L, N, D, K, Q, M, H, F, R, Y, or W
F39	A, G, S, P, V, T, I, L, N, D, K, Q, E, M, H, R, Y, or W
Y40	A, G, S, P, V, T, I, L, N, D, K, Q, E, M, H, F, R, or W
P41	A, G, S, V, T, I, L, N, D, K, Q, E, M, H, F, R, Y, or W
T43	A, G, S, P, V, I, L, N, D, K, Q, E, M, H, F, R, Y, or W
S44	A, G, P, V, T, I, L, N, D, K, Q, E, M, H, F, R, Y, or W
E45	A, G, S, P, V, T, I, L, N, D, K, Q, M, H, F, R, Y, or W
E46	A, G, S, P, V, T, I, L, N, D, K, Q, M, H, F, R, Y, or W
I47	A, G, S, P, V, T, L, N, D, K, Q, E, M, H, F, R, Y, or W
D48	A, G, S, P, V, T, I, L, N, K, Q, E, M, H, F, R, Y, or W
H49	A, G, S, P, V, T, I, L, N, D, K, Q, E, M, F, R, Y, or W
E50	A, G, S, P, V, T, I, L, N, D, K, Q, M, H, F, R, Y, or W
D51	A, G, S, P, V, T, I, L, N, K, Q, E, M, H, F, R, Y, or W
I52	A, G, S, P, V, T, L, N, D, K, Q, E, M, H, F, R, Y, or W
T53	A, G, S, P, V, I, L, N, D, K, Q, E, M, H, F, R, Y, or W
K54	A, G, S, P, V, T, I, L, N, D, Q, E, M, H, F, R, Y, or W
K56	A, G, S, P, V, T, I, L, N, D, Q, E, M, H, F, R, Y, or W
T57	A, G, S, P, V, I, L, N, D, K, Q, E, M, H, F, R, Y, or W
D108	A, G, S, P, V, T, I, L, N, K, Q, E, M, H, F, R, Y, or W
L109	A, G, S, P, V, T, I, N, D, K, Q, E, M, H, F, R, Y, or W

K110	A, G, S, P, V, T, I, L, N, D, Q, E, M, H, F, R, Y, or W
M111	A, G, S, P, V, T, I, L, N, D, K, Q, E, H, F, R, Y, or W
Y112	A, G, S, P, V, T, I, L, N, D, K, Q, E, M, H, F, R, or W
Q113	A, G, S, P, V, T, I, L, N, D, K, E, M, H, F, R, Y, or W
V114	A, G, S, P, T, I, L, N, D, K, Q, E, M, H, F, R, Y, or W
E115	A, G, S, P, V, T, I, L, N, D, K, Q, M, H, F, R, Y, or W
F116	A, G, S, P, V, T, I, L, N, D, K, Q, E, M, H, R, Y, or W
K117	A, G, S, P, V, T, I, L, N, D, Q, E, M, H, F, R, Y, or W
T118	A, G, S, P, V, I, L, N, D, K, Q, E, M, H, F, R, Y, or W
A121	G, S, P, V, T, I, L, N, D, K, Q, E, M, H, F, R, Y, or W
L123	A, G, S, P, V, T, I, N, D, K, Q, E, M, H, F, R, Y, or W
M125	A, G, S, P, V, T, I, L, N, D, K, Q, E, H, F, R, Y, or W
D126	A, G, S, P, V, T, I, L, N, K, Q, E, M, H, F, R, Y, or W
K128	A, G, S, P, V, T, I, L, N, D, Q, E, M, H, F, R, Y, or W
R129	A, G, S, P, V, T, I, L, N, D, K, Q, E, M, H, F, Y, or W
Q130	A, G, S, P, V, T, I, L, N, D, K, E, M, H, F, R, Y, or W
I131	A, G, S, P, V, T, L, N, D, K, Q, E, M, H, F, R, Y, or W
L133	A, G, S, P, V, T, I, N, D, K, Q, E, M, H, F, R, Y, or W
P156	A, G, S, P, V, T, I, L, N, D, K, Q, E, M, H, F, R, Y, or W
S159	A, G, P, V, T, I, L, N, D, K, Q, E, M, H, F, R, Y, or W
S160	A, G, P, V, T, I, L, N, D, K, Q, E, M, H, F, R, Y, or W
L161	A, G, S, P, V, T, I, N, D, K, Q, E, M, H, F, R, Y, or W
E162	A, G, S, P, V, T, I, L, N, D, K, Q, M, H, F, R, Y, or W
E163	A, G, S, P, V, T, I, L, N, D, K, Q, M, H, F, R, Y, or W
P164	A, G, S, V, T, I, L, N, D, K, Q, E, M, H, F, R, Y, or W
D165	A, G, S, P, V, T, I, L, N, K, Q, E, M, H, F, R, Y, or W
F166	A, G, S, P, V, T, I, L, N, D, K, Q, E, M, H, R, Y, or W
Y167	A, G, S, P, V, T, I, L, N, D, K, Q, E, M, H, F, R, or W
K168	A, G, S, P, V, T, I, L, N, D, Q, E, M, H, F, R, Y, or W
T169	A, G, S, P, V, I, L, N, D, K, Q, E, M, H, F, R, Y, or W
K170	A, G, S, P, V, T, I, L, N, D, Q, E, M, H, F, R, Y, or W
I171	A, G, S, P, V, T, L, N, D, K, Q, E, M, H, F, R, Y, or W
K172	A, G, S, P, V, T, I, L, N, D, Q, E, M, H, F, R, Y, or W

L173	A, G, S, P, V, T, I, N, D, K, Q, E, M, H, F, R, Y, or W
I175	A, G, S, P, V, T, L, N, D, K, Q, E, M, H, F, R, Y, or W
L177	A, G, S, P, V, T, I, N, D, K, Q, E, M, H, F, R, Y, or W
H178	A, G, S, P, V, T, I, L, N, D, K, Q, E, M, F, R, Y, or W
R181	A, G, S, P, V, T, I, L, N, D, K, Q, E, M, H, F, Y, or W

Table 1b. p35 Substitutions

p35 Substitution	Substitution selected from
A33	H, M, T
R34	A
E38	M
Y40	A, D, E, F, G, H, I, K, L, M, N, P, Q, R, S, T, V, W
P41	A, G, S, T
E45	D, F, H, I, K, L, M, N, Q, R, T, V, W, Y
E46	A, D, G, H, K, N, S, V
I47	F, K, L, Q, R, S, T, W, Y
D48	E, F, G, I, K, L, M, N, P, Q, T, V
H49	A, E, F, G, I, K, L, M, Q, R, S, T, V, W, Y
E50	A, D, F, G, H, I, K, L, M, N, P, Q, R, S, T, V, W, Y,
D51	G
I52	A, D, E, G, K, L, N, P, T, Y,
T53	A, D, H, P, S, Y
K54	D, E, G, W
K56	A, E, G, I, L, N, S, T, V
T57	A, D, E, G, N, Q, R, V, W
D108	R
L109	Y
K110	Y
M111	A, G, H, K, L, N, P, Q, R, V
Y112	H, K, P, R
Q113	A, F, G, K, L, M, N, P, R, S, T, V, W, Y
V114	A, D, E, F, G, H, I, K, L, M, N, P, Q, R, S, T, W, Y

E115	G, H, K, P, Q, R, S, T, V, W
F116	A, E, H, K, L, M, N, P, Q, R, S, T, V, W
K117	E, P
T118	D
A121	K
L123	D, E, G, Q, S, T
D126	P
I131	D, E, Q
P156	W
S159	A, E, G, P
S160	D, E, K, R, V,
L161	K, N, R, T
E162	A, G
E163	A, D, F, G, H, I, K, L, M, N, P, Q, R, S, T, Y
D165	A, E, F, G, H, I, L, M, N, S, T, V
F166	A, D, E, G, H, L, M, N, P, S, T, V
Y167	A, D, E, F, G, H, I, K, L, M, N, P, Q, R, S, T, V
K168	A, D, E, F, G, H, I, L, M, N, P, Q, S, T, V, W, Y
T169	M, N, P, Q, S, W
K170	E, F, G, L, P, Q, R, W, Y
I171	D, F, G, H, L, N, P, V
K172	D, E, F, G, I, L, M, P, Q, T, V, W, Y
L173	D, E, F, G, P, V, W
I175	D, E, G
R181	D, G, V

Table 1c. p35 Substitutions

p35 Substitution	Substitution selected from
Y40	K, P, G, Q, D
D48	Q, T
I52	D
K56	N

L109	Y
F116	L
E163	K, L
D165	T
F166	S
Y167	T, I, K, H, S, P, E, Q, L, V, D, R, N
K170	P, F, E, G
I171	P
K172	I, F

[0132] In some embodiments, the IL-12 mutein comprises a substitution or deletion of an amino acid in the p35 subunit corresponding to an amino acid shown in the first column of the Table 2 below, where the amino acid residue to be mutated corresponds to an amino acid residue in SEQ ID NO: 1. In some embodiments, the IL-12 mutein comprises a substitution or deletion of an amino acid in the p35 subunit corresponding to the amino acids shown in the first column of the Table 2 below, in combination with one, two, three, four, five or more additional p35 substitutions shown in the corresponding second column in Table 2 below.

Table 2. p35 Substitution Combinations

p35 Substitution	Additional p35 Substitutions
A33	R34, L37, E38, F39, Y40, P41, T43, S44, E45, E46, I47, D48, H49, E50, D51, I52, T53, K54, K56, T57, D108, L109, K110, M111, Y112, Q113, V114, E115, F116, K117, T118, A121, L123, M125, D126, K128, R129, Q130, I131, L133, P156, S159, S160, L161, E162, E163, P164, D165, F166, Y167, K168, T169, K170, I171, K172, L173, I175, L177, H178, and/or R181
R34	A33, L37, E38, F39, Y40, P41, T43, S44, E45, E46, I47, D48, H49, E50, D51, I52, T53, K54, K56, T57, D108, L109, K110, M111, Y112, Q113, V114, E115, F116, K117, T118, A121, L123, M125, D126, K128, R129, Q130, I131, L133, P156, S159, S160, L161, E162, E163, P164, D165, F166, Y167, K168, T169, K170, I171, K172, L173, I175, L177, H178, and/or R181
L37	A33, R34, E38, F39, Y40, P41, T43, S44, E45, E46, I47, D48, H49, E50, D51, I52, T53, K54, K56, T57, D108, L109, K110, M111, Y112, Q113, V114, E115, F116, K117, T118, A121, L123, M125, D126, K128, R129, Q130, I131, L133, P156, S159, S160, L161, E162, E163, P164, D165, F166, Y167, K168, T169, K170, I171, K172, L173, I175, L177, H178, and/or R181
E38	A33, R34, L37, F39, Y40, P41, T43, S44, E45, E46, I47, D48, H49, E50, D51, I52, T53, K54, K56, T57, D108, L109, K110, M111, Y112, Q113, V114, E115, F116, K117, T118, A121, L123, M125, D126, K128, R129, Q130, I131, L133, P156, S159, S160, L161, E162, E163, P164, D165, F166, Y167, K168, T169, K170, I171, K172, L173, I175, L177, H178, and/or R181

F39	A33, R34, L37, E38, Y40, P41, T43, S44, E45, E46, I47, D48, H49, E50, D51, I52, T53, K54, K56, T57, D108, L109, K110, M111, Y112, Q113, V114, E115, F116, K117, T118, A121, L123, M125, D126, K128, R129, Q130, I131, L133, P156, S159, S160, L161, E162, E163, P164, D165, F166, Y167, K168, T169, K170, I171, K172, L173, I175, L177, H178, and/or R181
Y40	A33, R34, L37, E38, F39, P41, T43, S44, E45, E46, I47, D48, H49, E50, D51, I52, T53, K54, K56, T57, D108, L109, K110, M111, Y112, Q113, V114, E115, F116, K117, T118, A121, L123, M125, D126, K128, R129, Q130, I131, L133, P156, S159, S160, L161, E162, E163, P164, D165, F166, Y167, K168, T169, K170, I171, K172, L173, I175, L177, H178, and/or R181
P41	A33, R34, L37, E38, F39, Y40, T43, S44, E45, E46, I47, D48, H49, E50, D51, I52, T53, K54, K56, T57, D108, L109, K110, M111, Y112, Q113, V114, E115, F116, K117, T118, A121, L123, M125, D126, K128, R129, Q130, I131, L133, P156, S159, S160, L161, E162, E163, P164, D165, F166, Y167, K168, T169, K170, I171, K172, L173, I175, L177, H178, and/or R181
T43	A33, R34, L37, E38, F39, Y40, P41, S44, E45, E46, I47, D48, H49, E50, D51, I52, T53, K54, K56, T57, D108, L109, K110, M111, Y112, Q113, V114, E115, F116, K117, T118, A121, L123, M125, D126, K128, R129, Q130, I131, L133, P156, S159, S160, L161, E162, E163, P164, D165, F166, Y167, K168, T169, K170, I171, K172, L173, I175, L177, H178, and/or R181
S44	A33, R34, L37, E38, F39, Y40, P41, T43, E45, E46, I47, D48, H49, E50, D51, I52, T53, K54, K56, T57, D108, L109, K110, M111, Y112, Q113, V114, E115, F116, K117, T118, A121, L123, M125, D126, K128, R129, Q130, I131, L133, P156, S159, S160, L161, E162, E163, P164, D165, F166, Y167, K168, T169, K170, I171, K172, L173, I175, L177, H178, and/or R181
E45	A33, R34, L37, E38, F39, Y40, P41, T43, S44, E46, I47, D48, H49, E50, D51, I52, T53, K54, K56, T57, D108, L109, K110, M111, Y112, Q113, V114, E115, F116, K117, T118, A121, L123, M125, D126, K128, R129, Q130, I131, L133, P156, S159, S160, L161, E162, E163, P164, D165, F166, Y167, K168, T169, K170, I171, K172, L173, I175, L177, H178, and/or R181
E46	A33, R34, L37, E38, F39, Y40, P41, T43, S44, E45, I47, D48, H49, E50, D51, I52, T53, K54, K56, T57, D108, L109, K110, M111, Y112, Q113, V114, E115, F116, K117, T118, A121, L123, M125, D126, K128, R129, Q130, I131, L133, P156, S159, S160, L161, E162, E163, P164, D165, F166, Y167, K168, T169, K170, I171, K172, L173, I175, L177, H178, and/or R181
I47	A33, R34, L37, E38, F39, Y40, P41, T43, S44, E45, E46, D48, H49, E50, D51, I52, T53, K54, K56, T57, D108, L109, K110, M111, Y112, Q113, V114, E115, F116, K117, T118, A121, L123, M125, D126, K128, R129, Q130, I131, L133, P156, S159, S160, L161, E162, E163, P164, D165, F166, Y167, K168, T169, K170, I171, K172, L173, I175, L177, H178, and/or R181
D48	A33, R34, L37, E38, F39, Y40, P41, T43, S44, E45, E46, I47, H49, E50, D51, I52, T53, K54, K56, T57, D108, L109, K110, M111, Y112, Q113, V114, E115, F116, K117, T118, A121, L123, M125, D126, K128, R129, Q130, I131, L133, P156, S159, S160, L161, E162, E163, P164, D165, F166, Y167, K168, T169, K170, I171, K172, L173, I175, L177, H178, and/or R181
H49	A33, R34, L37, E38, F39, Y40, P41, T43, S44, E45, E46, I47, D48, E50, D51, I52, T53, K54, K56, T57, D108, L109, K110, M111, Y112, Q113, V114, E115, F116, K117, T118, A121, L123, M125, D126, K128, R129, Q130, I131, L133, P156, S159, S160, L161, E162, E163, P164, D165, F166, Y167, K168, T169, K170, I171, K172, L173, I175, L177, H178, and/or R181

E50	A33, R34, L37, E38, F39, Y40, P41, T43, S44, E45, E46, I47, D48, H49, D51, I52, T53, K54, K56, T57, D108, L109, K110, M111, Y112, Q113, V114, E115, F116, K117, T118, A121, L123, M125, D126, K128, R129, Q130, I131, L133, P156, S159, S160, L161, E162, E163, P164, D165, F166, Y167, K168, T169, K170, I171, K172, L173, I175, L177, H178, and/or R181
D51	A33, R34, L37, E38, F39, Y40, P41, T43, S44, E45, E46, I47, D48, H49, E50, I52, T53, K54, K56, T57, D108, L109, K110, M111, Y112, Q113, V114, E115, F116, K117, T118, A121, L123, M125, D126, K128, R129, Q130, I131, L133, P156, S159, S160, L161, E162, E163, P164, D165, F166, Y167, K168, T169, K170, I171, K172, L173, I175, L177, H178, and/or R181
I52	A33, R34, L37, E38, F39, Y40, P41, T43, S44, E45, E46, I47, D48, H49, E50, D51, T53, K54, K56, T57, D108, L109, K110, M111, Y112, Q113, V114, E115, F116, K117, T118, A121, L123, M125, D126, K128, R129, Q130, I131, L133, P156, S159, S160, L161, E162, E163, P164, D165, F166, Y167, K168, T169, K170, I171, K172, L173, I175, L177, H178, and/or R181
T53	A33, R34, L37, E38, F39, Y40, P41, T43, S44, E45, E46, I47, D48, H49, E50, D51, I52, K54, K56, T57, D108, L109, K110, M111, Y112, Q113, V114, E115, F116, K117, T118, A121, L123, M125, D126, K128, R129, Q130, I131, L133, P156, S159, S160, L161, E162, E163, P164, D165, F166, Y167, K168, T169, K170, I171, K172, L173, I175, L177, H178, and/or R181
K54	A33, R34, L37, E38, F39, Y40, P41, T43, S44, E45, E46, I47, D48, H49, E50, D51, I52, T53, K56, T57, D108, L109, K110, M111, Y112, Q113, V114, E115, F116, K117, T118, A121, L123, M125, D126, K128, R129, Q130, I131, L133, P156, S159, S160, L161, E162, E163, P164, D165, F166, Y167, K168, T169, K170, I171, K172, L173, I175, L177, H178, and/or R181
E56	A33, R34, L37, E38, F39, Y40, P41, T43, S44, E45, I47, D48, H49, E50, D51, I52, T53, K54, K56, T57, D108, L109, K110, M111, Y112, Q113, V114, E115, F116, K117, T118, A121, L123, M125, D126, K128, R129, Q130, I131, L133, P156, S159, S160, L161, E162, E163, P164, D165, F166, Y167, K168, T169, K170, I171, K172, L173, I175, L177, H178, and/or R181
T57	A33, R34, L37, E38, F39, Y40, P41, T43, S44, E45, E46, I47, D48, H49, E50, D51, I52, T53, K54, K56, D108, L109, K110, M111, Y112, Q113, V114, E115, F116, K117, T118, A121, L123, M125, D126, K128, R129, Q130, I131, L133, P156, S159, S160, L161, E162, E163, P164, D165, F166, Y167, K168, T169, K170, I171, K172, L173, I175, L177, H178, and/or R181
D108	A33, R34, L37, E38, F39, Y40, P41, T43, S44, E45, E46, I47, D48, H49, E50, D51, I52, T53, K54, K56, T57, L109, K110, M111, Y112, Q113, V114, E115, F116, K117, T118, A121, L123, M125, D126, K128, R129, Q130, I131, L133, P156, S159, S160, L161, E162, E163, P164, D165, F166, Y167, K168, T169, K170, I171, K172, L173, I175, L177, H178, and/or R181
L109	A33, R34, L37, E38, F39, Y40, P41, T43, S44, E45, E46, I47, D48, H49, E50, D51, I52, T53, K54, K56, T57, D108, K110, M111, Y112, Q113, V114, E115, F116, K117, T118, A121, L123, M125, D126, K128, R129, Q130, I131, L133, P156, S159, S160, L161, E162, E163, P164, D165, F166, Y167, K168, T169, K170, I171, K172, L173, I175, L177, H178, and/or R181
K110	A33, R34, L37, E38, F39, Y40, P41, T43, S44, E45, E46, I47, D48, H49, E50, D51, I52, T53, K54, K56, T57, D108, L109, M111, Y112, Q113, V114, E115, F116, K117, T118, A121, L123, M125, D126, K128, R129, Q130, I131, L133, P156, S159, S160, L161, E162, E163, P164, D165, F166, Y167, K168, T169, K170, I171, K172, L173, I175, L177, H178, and/or R181

M111	A33, R34, L37, E38, F39, Y40, P41, T43, S44, E45, E46, I47, D48, H49, E50, D51, I52, T53, K54, K56, T57, D108, L109, K110, Y112, Q113, V114, E115, F116, K117, T118, A121, L123, M125, D126, K128, R129, Q130, I131, L133, P156, S159, S160, L161, E162, E163, P164, D165, F166, Y167, K168, T169, K170, I171, K172, L173, I175, L177, H178, and/or R181
Y112	A33, R34, L37, E38, F39, Y40, P41, T43, S44, E45, E46, I47, D48, H49, E50, D51, I52, T53, K54, K56, T57, D108, L109, K110, M111, Q113, V114, E115, F116, K117, T118, A121, L123, M125, D126, K128, R129, Q130, I131, L133, P156, S159, S160, L161, E162, E163, P164, D165, F166, Y167, K168, T169, K170, I171, K172, L173, I175, L177, H178, and/or R181
Q113	A33, R34, L37, E38, F39, Y40, P41, T43, S44, E45, E46, I47, D48, H49, E50, D51, I52, T53, K54, K56, T57, D108, L109, K110, M111, Y112, V114, E115, F116, K117, T118, A121, L123, M125, D126, K128, R129, Q130, I131, L133, P156, S159, S160, L161, E162, E163, P164, D165, F166, Y167, K168, T169, K170, I171, K172, L173, I175, L177, H178, and/or R181
V114	A33, R34, L37, E38, F39, Y40, P41, T43, S44, E45, E46, I47, D48, H49, E50, D51, I52, T53, K54, K56, T57, D108, L109, K110, M111, Y112, Q113, E115, F116, K117, T118, A121, L123, M125, D126, K128, R129, Q130, I131, L133, P156, S159, S160, L161, E162, E163, P164, D165, F166, Y167, K168, T169, K170, I171, K172, L173, I175, L177, H178, and/or R181
E115	A33, R34, L37, E38, F39, Y40, P41, T43, S44, E45, E46, I47, D48, H49, E50, D51, I52, T53, K54, K56, T57, D108, L109, K110, M111, Y112, Q113, V114, F116, K117, T118, A121, L123, M125, D126, K128, R129, Q130, I131, L133, P156, S159, S160, L161, E162, E163, P164, D165, F166, Y167, K168, T169, K170, I171, K172, L173, I175, L177, H178, and/or R181
F116	A33, R34, L37, E38, F39, Y40, P41, T43, S44, E45, E46, I47, D48, H49, E50, D51, I52, T53, K54, K56, T57, D108, L109, K110, M111, Y112, Q113, V114, E115, K117, T118, A121, L123, M125, D126, K128, R129, Q130, I131, L133, P156, S159, S160, L161, E162, E163, P164, D165, F166, Y167, K168, T169, K170, I171, K172, L173, I175, L177, H178, and/or R181
K117	A33, R34, L37, E38, F39, Y40, P41, T43, S44, E45, E46, I47, D48, H49, E50, D51, I52, T53, K54, K56, T57, D108, L109, K110, M111, Y112, Q113, V114, E115, F116, T118, A121, L123, M125, D126, K128, R129, Q130, I131, L133, P156, S159, S160, L161, E162, E163, P164, D165, F166, Y167, K168, T169, K170, I171, K172, L173, I175, L177, H178, and/or R181
T118	A33, R34, L37, E38, F39, Y40, P41, T43, S44, E45, E46, I47, D48, H49, E50, D51, I52, T53, K54, K56, T57, D108, L109, K110, M111, Y112, Q113, V114, E115, F116, K117, A121, L123, M125, D126, K128, R129, Q130, I131, L133, P156, S159, S160, L161, E162, E163, P164, D165, F166, Y167, K168, T169, K170, I171, K172, L173, I175, L177, H178, and/or R181
A121	A33, R34, L37, E38, F39, Y40, P41, T43, S44, E45, E46, I47, D48, H49, E50, D51, I52, T53, K54, K56, T57, D108, L109, K110, M111, Y112, Q113, V114, E115, F116, K117, T118, L123, M125, D126, K128, R129, Q130, I131, L133, P156, S159, S160, L161, E162, E163, P164, D165, F166, Y167, K168, T169, K170, I171, K172, L173, I175, L177, H178, and/or R181
L123	A33, R34, L37, E38, F39, Y40, P41, T43, S44, E45, E46, I47, D48, H49, E50, D51, I52, T53, K54, K56, T57, D108, L109, K110, M111, Y112, Q113, V114, E115, F116, K117, T118, A121, M125, D126, K128, R129, Q130, I131, L133, P156, S159, S160, L161, E162, E163, P164, D165, F166, Y167, K168, T169, K170, I171, K172, L173, I175, L177, H178, and/or R181

M125	A33, R34, L37, E38, F39, Y40, P41, T43, S44, E45, E46, I47, D48, H49, E50, D51, I52, T53, K54, K56, T57, D108, L109, K110, M111, Y112, Q113, V114, E115, F116, K117, T118, A121, L123, D126, K128, R129, Q130, I131, L133, P156, S159, S160, L161, E162, E163, P164, D165, F166, Y167, K168, T169, K170, I171, K172, L173, I175, L177, H178, and/or R181
D126	A33, R34, L37, E38, F39, Y40, P41, T43, S44, E45, E46, I47, D48, H49, E50, D51, I52, T53, K54, K56, T57, D108, L109, K110, M111, Y112, Q113, V114, E115, F116, K117, T118, A121, L123, M125, K128, R129, Q130, I131, L133, P156, S159, S160, L161, E162, E163, P164, D165, F166, Y167, K168, T169, K170, I171, K172, L173, I175, L177, H178, and/or R181
K128	A33, R34, L37, E38, F39, Y40, P41, T43, S44, E45, E46, I47, D48, H49, E50, D51, I52, T53, K54, K56, T57, D108, L109, K110, M111, Y112, Q113, V114, E115, F116, K117, T118, A121, L123, M125, D126, R129, Q130, I131, L133, P156, S159, S160, L161, E162, E163, P164, D165, F166, Y167, K168, T169, K170, I171, K172, L173, I175, L177, H178, and/or R181
R129	A33, R34, L37, E38, F39, Y40, P41, T43, S44, E45, E46, I47, D48, H49, E50, D51, I52, T53, K54, K56, T57, D108, L109, K110, M111, Y112, Q113, V114, E115, F116, K117, T118, A121, L123, M125, D126, K128, Q130, I131, L133, P156, S159, S160, L161, E162, E163, P164, D165, F166, Y167, K168, T169, K170, I171, K172, L173, I175, L177, H178, and/or R181
Q130	A33, R34, L37, E38, F39, Y40, P41, T43, S44, E45, E46, I47, D48, H49, E50, D51, I52, T53, K54, K56, T57, D108, L109, K110, M111, Y112, Q113, V114, E115, F116, K117, T118, A121, L123, M125, D126, K128, R129, I131, L133, P156, S159, S160, L161, E162, E163, P164, D165, F166, Y167, K168, T169, K170, I171, K172, L173, I175, L177, H178, and/or R181
I131	A33, R34, L37, E38, F39, Y40, P41, T43, S44, E45, E46, I47, D48, H49, E50, D51, I52, T53, K54, K56, T57, D108, L109, K110, M111, Y112, Q113, V114, E115, F116, K117, T118, A121, L123, M125, D126, K128, R129, Q130, L133, P156, S159, S160, L161, E162, E163, P164, D165, F166, Y167, K168, T169, K170, I171, K172, L173, I175, L177, H178, and/or R181
L133	A33, R34, L37, E38, F39, Y40, P41, T43, S44, E45, E46, I47, D48, H49, E50, D51, I52, T53, K54, K56, T57, D108, L109, K110, M111, Y112, Q113, V114, E115, F116, K117, T118, A121, L123, M125, D126, K128, R129, Q130, I131, P156, S159, S160, L161, E162, E163, P164, D165, F166, Y167, K168, T169, K170, I171, K172, L173, I175, L177, H178, and/or R181
P156	A33, R34, L37, E38, F39, Y40, P41, T43, S44, E45, E46, I47, D48, H49, E50, D51, I52, T53, K54, K56, T57, D108, L109, K110, M111, Y112, Q113, V114, E115, F116, K117, T118, A121, L123, M125, D126, K128, R129, Q130, I131, L133, S159, S160, L161, E162, E163, P164, D165, F166, Y167, K168, T169, K170, I171, K172, L173, I175, L177, H178, and/or R181
S159	A33, R34, L37, E38, F39, Y40, P41, T43, S44, E45, E46, I47, D48, H49, E50, D51, I52, T53, K54, K56, T57, D108, L109, K110, M111, Y112, Q113, V114, E115, F116, K117, T118, A121, L123, M125, D126, K128, R129, Q130, I131, L133, P156, S160, L161, E162, E163, P164, D165, F166, Y167, K168, T169, K170, I171, K172, L173, I175, L177, H178, and/or R181
S160	A33, R34, L37, E38, F39, Y40, P41, T43, S44, E45, E46, I47, D48, H49, E50, D51, I52, T53, K54, K56, T57, D108, L109, K110, M111, Y112, Q113, V114, E115, F116, K117, T118, A121, L123, M125, D126, K128, R129, Q130, I131, L133, P156, S159, L161, E162, E163, P164, D165, F166, Y167, K168, T169, K170, I171, K172, L173, I175, L177, H178, and/or R181

L161	A33, R34, L37, E38, F39, Y40, P41, T43, S44, E45, E46, I47, D48, H49, E50, D51, I52, T53, K54, K56, T57, D108, L109, K110, M111, Y112, Q113, V114, E115, F116, K117, T118, A121, L123, M125, D126, K128, R129, Q130, I131, L133, P156, S159, S160, E162, E163, P164, D165, F166, Y167, K168, T169, K170, I171, K172, L173, I175, L177, H178, and/or R181
E162	A33, R34, L37, E38, F39, Y40, P41, T43, S44, E45, E46, I47, D48, H49, E50, D51, I52, T53, K54, K56, T57, D108, L109, K110, M111, Y112, Q113, V114, E115, F116, K117, T118, A121, L123, M125, D126, K128, R129, Q130, I131, L133, P156, S159, S160, L161, E163, P164, D165, F166, Y167, K168, T169, K170, I171, K172, L173, I175, L177, H178, and/or R181
E163	A33, R34, L37, E38, F39, Y40, P41, T43, S44, E45, E46, I47, D48, H49, E50, D51, I52, T53, K54, K56, T57, D108, L109, K110, M111, Y112, Q113, V114, E115, F116, K117, T118, A121, L123, M125, D126, K128, R129, Q130, I131, L133, P156, S159, S160, L161, E162, P164, D165, F166, Y167, K168, T169, K170, I171, K172, L173, I175, L177, H178, and/or R181
P164	A33, R34, L37, E38, F39, Y40, P41, T43, S44, E45, E46, I47, D48, H49, E50, D51, I52, T53, K54, K56, T57, D108, L109, K110, M111, Y112, Q113, V114, E115, F116, K117, T118, A121, L123, M125, D126, K128, R129, Q130, I131, L133, P156, S159, S160, L161, E162, E163, D165, F166, Y167, K168, T169, K170, I171, K172, L173, I175, L177, H178, and/or R181
D165	A33, R34, L37, E38, F39, Y40, P41, T43, S44, E45, E46, I47, D48, H49, E50, D51, I52, T53, K54, K56, T57, D108, L109, K110, M111, Y112, Q113, V114, E115, F116, K117, T118, A121, L123, M125, D126, K128, R129, Q130, I131, L133, P156, S159, S160, L161, E162, E163, P164, F166, Y167, K168, T169, K170, I171, K172, L173, I175, L177, H178, and/or R181
F166	A33, R34, L37, E38, F39, Y40, P41, T43, S44, E45, E46, I47, D48, H49, E50, D51, I52, T53, K54, K56, T57, D108, L109, K110, M111, Y112, Q113, V114, E115, F116, K117, T118, A121, L123, M125, D126, K128, R129, Q130, I131, L133, P156, S159, S160, L161, E162, E163, P164, D165, Y167, K168, T169, K170, I171, K172, L173, I175, L177, H178, and/or R181
Y167	A33, R34, L37, E38, F39, Y40, P41, T43, S44, E45, E46, I47, D48, H49, E50, D51, I52, T53, K54, K56, T57, D108, L109, K110, M111, Y112, Q113, V114, E115, F116, K117, T118, A121, L123, M125, D126, K128, R129, Q130, I131, L133, P156, S159, S160, L161, E162, E163, P164, D165, F166, K168, T169, K170, I171, K172, L173, I175, L177, H178, and/or R181
K168	A33, R34, L37, E38, F39, Y40, P41, T43, S44, E45, E46, I47, D48, H49, E50, D51, I52, T53, K54, K56, T57, D108, L109, K110, M111, Y112, Q113, V114, E115, F116, K117, T118, A121, L123, M125, D126, K128, R129, Q130, I131, L133, P156, S159, S160, L161, E162, E163, P164, D165, F166, Y167, T169, K170, I171, K172, L173, I175, L177, H178, and/or R181
T169	A33, R34, L37, E38, F39, Y40, P41, T43, S44, E45, E46, I47, D48, H49, E50, D51, I52, T53, K54, K56, T57, D108, L109, K110, M111, Y112, Q113, V114, E115, F116, K117, T118, A121, L123, M125, D126, K128, R129, Q130, I131, L133, P156, S159, S160, L161, E162, E163, P164, D165, F166, Y167, K168, K170, I171, K172, L173, I175, L177, H178, and/or R181
K170	A33, R34, L37, E38, F39, Y40, P41, T43, S44, E45, E46, I47, D48, H49, E50, D51, I52, T53, K54, K56, T57, D108, L109, K110, M111, Y112, Q113, V114, E115, F116, K117, T118, A121, L123, M125, D126, K128, R129, Q130, I131, L133, P156, S159, S160, L161, E162, E163, P164, D165, F166, Y167, K168, T169, I171, K172, L173, I175, L177, H178, and/or R181

I171	A33, R34, L37, E38, F39, Y40, P41, T43, S44, E45, E46, I47, D48, H49, E50, D51, I52, T53, K54, K56, T57, D108, L109, K110, M111, Y112, Q113, V114, E115, F116, K117, T118, A121, L123, M125, D126, K128, R129, Q130, I131, L133, P156, S159, S160, L161, E162, E163, P164, D165, F166, Y167, K168, T169, K170, K172, L173, I175, L177, H178, and/or R181
K172	A33, R34, L37, E38, F39, Y40, P41, T43, S44, E45, E46, I47, D48, H49, E50, D51, I52, T53, K54, K56, T57, D108, L109, K110, M111, Y112, Q113, V114, E115, F116, K117, T118, A121, L123, M125, D126, K128, R129, Q130, I131, L133, P156, S159, S160, L161, E162, E163, P164, D165, F166, Y167, K168, T169, K170, I171, L173, I175, L177, H178, and/or R181
L173	A33, R34, L37, E38, F39, Y40, P41, T43, S44, E45, E46, I47, D48, H49, E50, D51, I52, T53, K54, K56, T57, D108, L109, K110, M111, Y112, Q113, V114, E115, F116, K117, T118, A121, L123, M125, D126, K128, R129, Q130, I131, L133, P156, S159, S160, L161, E162, E163, P164, D165, F166, Y167, K168, T169, K170, I171, K172, I175, L177, H178, and/or R181
I175	A33, R34, L37, E38, F39, Y40, P41, T43, S44, E45, E46, I47, D48, H49, E50, D51, I52, T53, K54, K56, T57, D108, L109, K110, M111, Y112, Q113, V114, E115, F116, K117, T118, A121, L123, M125, D126, K128, R129, Q130, I131, L133, P156, S159, S160, L161, E162, E163, P164, D165, F166, Y167, K168, T169, K170, I171, K172, L173, L177, H178, and/or R181
L177	A33, R34, L37, E38, F39, Y40, P41, T43, S44, E45, E46, I47, D48, H49, E50, D51, I52, T53, K54, K56, T57, D108, L109, K110, M111, Y112, Q113, V114, E115, F116, K117, T118, A121, L123, M125, D126, K128, R129, Q130, I131, L133, P156, S159, S160, L161, E162, E163, P164, D165, F166, Y167, K168, T169, K170, I171, K172, L173, I175, H178, and/or R181
H178	A33, R34, L37, E38, F39, Y40, P41, T43, S44, E45, E46, I47, D48, H49, E50, D51, I52, T53, K54, K56, T57, D108, L109, K110, M111, Y112, Q113, V114, E115, F116, K117, T118, A121, L123, M125, D126, K128, R129, Q130, I131, L133, P156, S159, S160, L161, E162, E163, P164, D165, F166, Y167, K168, T169, K170, I171, K172, L173, I175, L177, and/or R181
R181	A33, R34, L37, E38, F39, Y40, P41, T43, S44, E45, E46, I47, D48, H49, E50, D51, I52, T53, K54, K56, T57, D108, L109, K110, M111, Y112, Q113, V114, E115, F116, K117, T118, A121, L123, M125, D126, K128, R129, Q130, I131, L133, P156, S159, S160, L161, E162, E163, P164, D165, F166, Y167, K168, T169, K170, I171, K172, L173, I175, L177, and/or H178

[0133] In some embodiments, the modified IL-12 protein comprises a p35 subunit comprising a substitution at an amino acid corresponding to an amino acid at position L133 of SEQ ID NO: 1. In some embodiments, the modified IL-12 protein comprises a p35 subunit comprising a substitution or deletion of an amino acid corresponding to amino acid L133 of SEQ ID NO: 1, and the modified IL-12 protein further comprises a p40 subunit with one more substitutions. For exemplary combinations, see Table 3.

Table 3. Exemplary Combinations

p35 Substitution	p40 Substitution
L133	V10
L133	V11

L133	E12
L133	L13
L133	D14
L133	W15
L133	Y16
L133	P17
L133	D18
L133	A19
L133	P20
L133	G21
L133	W38
L133	T39
L133	L40
L133	D41
L133	Q42
L133	S43
L133	S44
L133	E45
L133	V46
L133	L47
L133	I55
L133	V57
L133	K58
L133	E59
L133	F60
L133	D62
L133	A63
L133	G64
L133	Q65
L133	Y66
L133	L81
L133	L82
L133	H83
L133	K84
L133	K85
L133	E86
L133	G88
L133	I89
L133	W90
L133	S91
L133	T92
L133	D93
L133	I94
L133	L95
L133	D97
L133	Q98
L133	K99
L133	E100
L133	P101

L133	N103
L133	K104
L133	T105
L133	F106
L133	L107
L133	L123
L133	I126
L133	T128
L133	L130
L133	K135
L133	S136
L133	S137
L133	R138
L133	G139
L133	S140
L133	S141
L133	D142
L133	R157
L133	R159
L133	D161
L133	N162
L133	K163
L133	E181
L133	S183
L133	L184
L133	P185
L133	I186
L133	E187
L133	V188
L133	V190
L133	D191
L133	K195
L133	L196
L133	K197
L133	Y198
L133	E199
L133	N200
L133	T202
L133	S203
L133	S204
L133	F206
L133	D209

IL-12 p40

[0134] A reference p40 monomer (p40) is expressed as a 328 amino acid pro-protein (SEQ ID NO: 4) comprising a 22 amino acid signal sequence which is post-translationally removed to render a 306 amino acid mature protein (SEQ ID NO:2).

[0135] The IL-12 muteins of the present disclosure comprise a modified p40 comprising one or more amino acid substitutions and/or deletions at the interface with the extracellular domain of IL-12R β 1. In one aspect, provided herein are variant p40 subunits comprising or consisting of an amino acid sequence at least 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% identical to the amino acid sequence set forth in SEQ ID NO: 2.

[0136] In some embodiments, the variant p40 subunit comprises one or more amino acid substitutions and/or deletions (e.g., one, two, three, four, five, or more substitutions and/or deletions). In some embodiments, the variant p40 subunit comprises an amino acid substitution and/or deletion at amino position V10, V11, E12, L13, D14, W15, Y16, P17, D18, A19, P20, G21, W38, T39, L40, D41, Q42, S43, S44, E45, V46, L47, I55, V57, K58, E59, F60, D62, A63, G64, Q65, Y66, L81, L82, H83, K84, K85, E86, G88, I89, W90, S91, T92, D93, I94, L95, D97, Q98, K99, E100, P101, N103, K104, T105, F106, L107, L123, I126, T128, L130, K135, S136, S137, R138, G139, S140, S141, D142, R157, R159, D161, N162, K163, E181, S183, L184, P185, I186, E187, V188, V190, D191, K195, L196, K197, Y198, E199, N200, T202, S203, S204, F206, or D209 with respect to the position in mature p40 (SEQ ID NO: 2). In some embodiments, the variant p40 subunit comprises one, two, three, four, five, or more substitutions selected from the positions of the foregoing list. In some embodiments, the variant p40 subunit comprises an amino acid substitution, wherein the amino acid substitution is any naturally occurring amino acid other than the corresponding wild-type residue in SEQ ID NO: 2. In some embodiments, the variant p40 subunit comprises an amino acid substitution, wherein the amino acid substitution is any naturally occurring amino acid other than the corresponding wild-type residue in SEQ ID NO: 2 or cysteine.

[0137] In some embodiments, the variant p40 subunit comprises a substitution at amino acid residue V10, optionally wherein the substitution is V10A, V10R, V10H, V10K, V10D, V10E, V10S, V10T, V10N, V10Q, V10G, V10P, V10I, V10L, V10M, V10F, V10W, or V10Y.

[0138] In some embodiments, the variant p40 subunit comprises a substitution at amino acid residue I94, optionally wherein the substitution is I94A, I94R, I94H, I94K, I94D, I94E, I94S, I94T, I94N, I94Q, I94G, I94P, I94L, I94M, I94F, I94W, I94V, or I94Y. In some embodiments, the variant p40 subunit comprises a substitution at amino acid residue L95, optionally wherein the substitution is L95A, L95R, L95H, L95K, L95D, L95E, L95S, L95T, L95N, L95Q, L95G, L95P, L95I, L95M, L95F, L95W, L95V, or L95Y.

[0139] In some embodiments, variant p40 subunit comprises a substitution at amino acid residue G64, optionally wherein the substitution is G64A, G64R, G64H, G64K, G64D, G64E, G64S, G64T, G64N, G64Q, G64P, G64I, G64L, G64M, G64F, G64W, G64V, or G64Y. In some embodiments, variant p40 subunit comprises a substitution at amino acid residue G64, wherein

the G64 substitution is G64R, G64H, G64K, G64D, G64E, G64S, G64T, G64N, G64Q, G64P, G64I, G64L, G64M, G64F, G64W, G64V, or G64Y, optionally wherein the G64 substitution is G64R, G64K, G64E, G64S, G64T, G64Q, G64P, G64I, G64L, G64M, G64F, G64W, G64V, or G64Y.

[0140] In some embodiments, the variant p40 subunit comprises a substitution at amino acid residue W15, P17, D18, E59, F60, K84, E86, D93, H194, K195, or K197, optionally wherein the variant p40 subunit comprises a substitution at amino acid residue E59, F60, K84, K195, or K197. In some embodiments, the variant p40 subunit comprises a substitution at amino acid residue V11, E12, L13, D14, W15, P17, D18, A19, P20, G21, W38, T39, L40, D41, S43, S44, E45, V46, L47, I55, V57, K58, E59, F60, D62, A63, Q65, Y66, L81, L82, H83, K84, K85, E86, G88, I89, W90, S91, T92, D93, D97, Q98, K99, E100, N103, K104, T105, F106, L107, L123, K135, S136, S137, R138, G139, S140, S141, D142, N162, E181, S183, L184, P185, I186, E187, V188, V190, D191, H194, K195, L196, K197, E199, N200, T202, S203, S204, F206, or D209. In some embodiments, the variant p40 subunit comprises a substitution at amino acid residue Y198, optionally wherein the substitution is Y198A, Y198R, Y198H, Y198K, Y198D, Y198E, Y198S, Y198T, Y198N, Y198Q, Y198G, Y198P, Y198I, Y198L, Y198M, Y198F, Y198W, or Y198V. In some embodiments, the variant p40 subunit comprises a substitution at amino acid residue Y198, wherein the Y198 substitution is Y198A, Y198R, Y198H, Y198K, Y198D, Y198E, Y198T, Y198N, Y198Q, Y198G, Y198P, Y198I, Y198L, Y198M, Y198F, Y198W, or Y198V, optionally wherein the Y198 substitution is Y198G, Y198E, Y198K, Y198Q, Y198R, Y198W. In some embodiments, the variant p40 subunit comprises a substitution at amino acid residue Y16, optionally wherein the substitution is Y16A, Y16R, Y16H, Y16K, Y16D, Y16E, Y16S, Y16T, Y16N, Y16Q, Y16G, Y16P, Y16I, Y16L, Y16M, Y16F, Y16W, or Y16V.

[0141] In some embodiments, the variant p40 subunit comprises an amino acid substitution and/or deletion at an amino acid residue corresponding to amino acid residue 10 with respect to the position in mature p40 sequence of SEQ ID NO: 2. In some embodiments, the variant p40 subunit comprises an amino acid substitution and/or deletion at an amino acid residue corresponding to amino acid residue 11 with respect to the position in mature p40 sequence of SEQ ID NO: 2. In some embodiments, the variant p40 subunit comprises an amino acid substitution and/or deletion at an amino acid residue corresponding to amino acid residue 12 with respect to the position in mature p40 sequence of SEQ ID NO: 2. In some embodiments, the variant p40 subunit comprises an amino acid substitution and/or deletion at an amino acid residue corresponding to amino acid residue 13 with respect to the position in mature p40 sequence of SEQ ID NO: 2. In some embodiments, the variant p40 subunit comprises an amino acid substitution and/or deletion at an

[illegible]

embodiments, the variant p40 subunit comprises an amino acid substitution and/or deletion at an amino acid residue corresponding to amino acid residue 45 with respect to the position in mature p40 sequence of SEQ ID NO: 2. In some embodiments, the variant p40 subunit comprises an amino acid substitution and/or deletion at an amino acid residue corresponding to amino acid residue 46 with respect to the position in mature p40 sequence of SEQ ID NO: 2. In some embodiments, the variant p40 subunit comprises an amino acid substitution and/or deletion at an amino acid residue corresponding to amino acid residue 47 with respect to the position in mature p40 sequence of SEQ ID NO: 2. In some embodiments, the variant p40 subunit comprises an amino acid substitution and/or deletion at an amino acid residue corresponding to amino acid residue 55 with respect to the position in mature p40 sequence of SEQ ID NO: 2. In some embodiments, the variant p40 subunit comprises an amino acid substitution and/or deletion at an amino acid residue corresponding to amino acid residue 57 with respect to the position in mature p40 sequence of SEQ ID NO: 2. In some embodiments, the variant p40 subunit comprises an amino acid substitution and/or deletion at an amino acid residue corresponding to amino acid residue 59 with respect to the position in mature p40 sequence of SEQ ID NO: 2. In some embodiments, the variant p40 subunit comprises an amino acid substitution and/or deletion at an amino acid residue corresponding to amino acid residue 60 with respect to the position in mature p40 sequence of SEQ ID NO: 2. In some embodiments, the variant p40 subunit comprises an amino acid substitution and/or deletion at an amino acid residue corresponding to amino acid residue 62 with respect to the position in mature p40 sequence of SEQ ID NO: 2. In some embodiments, the variant p40 subunit comprises an amino acid substitution and/or deletion at an amino acid residue corresponding to amino acid residue 63 with respect to the position in mature p40 sequence of SEQ ID NO: 2. In some embodiments, the variant p40 subunit comprises an amino acid substitution and/or deletion at an amino acid residue corresponding to amino acid residue 64 with respect to the position in mature p40 sequence of SEQ ID NO: 2. In some embodiments, the variant p40 subunit comprises an amino acid substitution and/or deletion at an amino acid residue corresponding to amino acid residue 65 with respect to the position in mature p40 sequence of SEQ ID NO: 2. In some embodiments, the variant p40 subunit comprises an amino acid substitution and/or deletion at an amino acid residue corresponding to amino acid residue 66 with respect to the position in mature p40 sequence of SEQ ID NO: 2. In some embodiments, the variant p40 subunit comprises an amino acid substitution and/or deletion at an amino acid residue corresponding to amino acid

[illegible]

[illegible]

[illegible]

[illegible]

embodiments, the variant p40 subunit comprises an amino acid substitution and/or deletion at an amino acid residue corresponding to amino acid residue 200 with respect to the position in mature p40 sequence of SEQ ID NO: 2. In some embodiments, the variant p40 subunit comprises an amino acid substitution and/or deletion at an amino acid residue corresponding to amino acid residue 202 with respect to the position in mature p40 sequence of SEQ ID NO: 2. In some embodiments, the variant p40 subunit comprises an amino acid substitution and/or deletion at an amino acid residue corresponding to amino acid residue 203 with respect to the position in mature p40 sequence of SEQ ID NO: 2. In some embodiments, the variant p40 subunit comprises an amino acid substitution and/or deletion at an amino acid residue corresponding to amino acid residue 204 with respect to the position in mature p40 sequence of SEQ ID NO: 2. In some embodiments, the variant p40 subunit comprises an amino acid substitution and/or deletion at an amino acid residue corresponding to amino acid residue 206 with respect to the position in mature p40 sequence of SEQ ID NO: 2. In some embodiments, the variant p40 subunit comprises an amino acid substitution and/or deletion at an amino acid residue corresponding to amino acid residue 209 with respect to the position in mature p40 sequence of SEQ ID NO: 2. Exemplary p40 substitution substitutions are provided in Table 4a, Table 4c, and Table 4c. In some embodiments, the variant p40 subunit comprises one or more amino acid substitutions (e.g., one, two, three, four, five, or more amino acid substitutions) selected from Table 4a, Table 4b, or Table 4c.

Table 4a. p40 Substitutions

P40 Substitution	Substitution selected from
V10	A, G, S, P, T, I, L, N, D, K, Q, E, M, H, F, R, Y, or W
V11	A, G, S, P, T, I, L, N, D, K, Q, E, M, H, F, R, Y, or W
E12	A, G, S, P, V, T, I, L, N, D, K, Q, M, H, F, R, Y, or W
L13	A, G, S, P, V, T, I, N, D, K, Q, E, M, H, F, R, Y, or W
D14	A, G, S, P, V, T, I, L, N, K, Q, E, M, H, F, R, Y, or W
W15	A, G, S, P, V, T, I, L, N, D, K, Q, E, M, H, F, R, or Y
Y16	A, G, S, P, V, T, I, L, N, D, K, Q, E, M, H, F, R, or W
P17	A, G, S, V, T, I, L, N, D, K, Q, E, M, H, F, R, Y, or W
D18	A, G, S, P, V, T, I, L, N, K, Q, E, M, H, F, R, Y, or W
A19	G, S, P, V, T, I, L, N, D, K, Q, E, M, H, F, R, Y, or W
P20	A, G, S, V, T, I, L, N, D, K, Q, E, M, H, F, R, Y, or W

G21	A, S, P, V, T, I, L, N, D, K, Q, E, M, H, F, R, Y, or W
W38	A, G, S, P, V, T, I, L, N, D, K, Q, E, M, H, F, R, or Y
T39	A, G, S, P, V, I, L, N, D, K, Q, E, M, H, F, R, Y, or W
L40	A, G, S, P, V, T, I, N, D, K, Q, E, M, H, F, R, Y, or W
D41	A, G, S, P, V, T, I, L, N, K, Q, E, M, H, F, R, Y, or W
Q42	A, G, S, P, V, T, I, L, N, D, K, E, M, H, F, R, Y, or W
S43	A, G, P, V, T, I, L, N, D, K, Q, E, M, H, F, R, Y, or W
S44	A, G, P, V, T, I, L, N, D, K, Q, E, M, H, F, R, Y, or W
E45	A, G, S, P, V, T, I, L, N, D, K, Q, M, H, F, R, Y, or W
V46	A, G, S, P, T, I, L, N, D, K, Q, E, M, H, F, R, Y, or W
L47	A, G, S, P, V, T, I, N, D, K, Q, E, M, H, F, R, Y, or W
I55	A, G, S, P, V, T, L, N, D, K, Q, E, M, H, F, R, Y, or W
V57	A, G, S, P, T, I, L, N, D, K, Q, E, M, H, F, R, Y, or W
K58	A, G, S, P, V, T, I, L, N, D, Q, E, M, H, F, R, Y, or W
E59	A, G, S, P, V, T, I, L, N, D, K, Q, M, H, F, R, Y, or W
F60	A, G, S, P, V, T, I, L, N, D, K, Q, E, M, H, R, Y, or W
D62	A, G, S, P, V, T, I, L, N, K, Q, E, M, H, F, R, Y, or W
A63	G, S, P, V, T, I, L, N, D, K, Q, E, M, H, F, R, Y, or W
G64	A, S, P, V, T, I, L, N, D, K, Q, E, M, H, F, R, Y, or W
Q65	A, G, S, P, V, T, I, L, N, D, K, E, M, H, F, R, Y, or W
Y66	A, G, S, P, V, T, I, L, N, D, K, Q, E, M, H, F, R, or W
L81	A, G, S, P, V, T, I, N, D, K, Q, E, M, H, F, R, Y, or W
L82	A, G, S, P, V, T, I, N, D, K, Q, E, M, H, F, R, Y, or W
H83	A, G, S, P, V, T, I, L, N, D, K, Q, E, M, F, R, Y, or W
K84	A, G, S, P, V, T, I, L, N, D, Q, E, M, H, F, R, Y, or W
K85	A, G, S, P, V, T, I, L, N, D, Q, E, M, H, F, R, Y, or W
E86	A, G, S, P, V, T, I, L, N, D, K, Q, M, H, F, R, Y, or W
G88	A, S, P, V, T, I, L, N, D, K, Q, E, M, H, F, R, Y, or W
I89	A, G, S, P, V, T, L, N, D, K, Q, E, M, H, F, R, Y, or W
W90	A, G, S, P, V, T, I, L, N, D, K, Q, E, M, H, F, R, or Y
S91	A, G, P, V, T, I, L, N, D, K, Q, E, M, H, F, R, Y, or W
T92	A, G, S, P, V, I, L, N, D, K, Q, E, M, H, F, R, Y, or W
D93	A, G, S, P, V, T, I, L, N, K, Q, E, M, H, F, R, Y, or W

I94	A, G, S, P, V, T, L, N, D, K, Q, E, M, H, F, R, Y, or W
L95	A, G, S, P, V, T, I, N, D, K, Q, E, M, H, F, R, Y, or W
D97	A, G, S, P, V, T, I, L, N, K, Q, E, M, H, F, R, Y, or W
Q98	A, G, S, P, V, T, I, L, N, D, K, E, M, H, F, R, Y, or W
K99	A, G, S, P, V, T, I, L, N, D, Q, E, M, H, F, R, Y, or W
E100	A, G, S, P, V, T, I, L, N, D, K, Q, M, H, F, R, Y, or W
P101	A, G, S, V, T, I, L, N, D, K, Q, E, M, H, F, R, Y, or W
N103	A, G, S, P, V, T, I, L, D, K, Q, E, M, H, F, R, Y, or W
K104	A, G, S, P, V, T, I, L, N, D, Q, E, M, H, F, R, Y, or W
T105	A, G, S, P, V, I, L, N, D, K, Q, E, M, H, F, R, Y, or W
F106	A, G, S, P, V, T, I, L, N, D, K, Q, E, M, H, R, Y, or W
L107	A, G, S, P, V, T, I, N, D, K, Q, E, M, H, F, R, Y, or W
L123	A, G, S, P, V, T, I, N, D, K, Q, E, M, H, F, R, Y, or W
I126	A, G, S, P, V, T, L, N, D, K, Q, E, M, H, F, R, Y, or W
T128	A, G, S, P, V, I, L, N, D, K, Q, E, M, H, F, R, Y, or W
L130	A, G, S, P, V, T, I, N, D, K, Q, E, M, H, F, R, Y, or W
K135	A, G, S, P, V, T, I, L, N, D, Q, E, M, H, F, R, Y, or W
S136	A, G, P, V, T, I, L, N, D, K, Q, E, M, H, F, R, Y, or W
S137	A, G, P, V, T, I, L, N, D, K, Q, E, M, H, F, R, Y, or W
R138	A, G, S, P, V, T, I, L, N, D, K, Q, E, M, H, F, Y, or W
G139	A, S, P, V, T, I, L, N, D, K, Q, E, M, H, F, R, Y, or W
S140	A, G, P, V, T, I, L, N, D, K, Q, E, M, H, F, R, Y, or W
S141	A, G, P, V, T, I, L, N, D, K, Q, E, M, H, F, R, Y, or W
D142	A, G, S, P, V, T, I, L, N, K, Q, E, M, H, F, R, Y, or W
R157	A, G, S, P, V, T, I, L, N, D, K, Q, E, M, H, F, Y, or W
R159	A, G, S, P, V, T, I, L, N, D, K, Q, E, M, H, F, Y, or W
D161	A, G, S, P, V, T, I, L, N, K, Q, E, M, H, F, R, Y, or W
N162	A, G, S, P, V, T, I, L, D, K, Q, E, M, H, F, R, Y, or W
K163	A, G, S, P, V, T, I, L, N, D, Q, E, M, H, F, R, Y, or W
E181	A, G, S, P, V, T, I, L, N, D, K, Q, M, H, F, R, Y, or W
S183	A, G, P, V, T, I, L, N, D, K, Q, E, M, H, F, R, Y, or W
L184	A, G, S, P, V, T, I, N, D, K, Q, E, M, H, F, R, Y, or W
P185	A, G, S, V, T, I, L, N, D, K, Q, E, M, H, F, R, Y, or W

I186	A, G, S, P, V, T, L, N, D, K, Q, E, M, H, F, R, Y, or W
E187	A, G, S, P, V, T, I, L, N, D, K, Q, M, H, F, R, Y, or W
V188	A, G, S, P, T, I, L, N, D, K, Q, E, M, H, F, R, Y, or W
V190	A, G, S, P, T, I, L, N, D, K, Q, E, M, H, F, R, Y, or W
D191	A, G, S, P, V, T, I, L, N, K, Q, E, M, H, F, R, Y, or W
K195	A, G, S, P, V, T, I, L, N, D, Q, E, M, H, F, R, Y, or W
L196	A, G, S, P, V, T, I, N, D, K, Q, E, M, H, F, R, Y, or W
K197	A, G, S, P, V, T, I, L, N, D, Q, E, M, H, F, R, Y, or W
Y198	A, G, S, P, V, T, I, L, N, D, K, Q, E, M, H, F, R, or W
E199	A, G, S, P, V, T, I, L, N, D, K, Q, M, H, F, R, Y, or W
N200	A, G, S, P, V, T, I, L, D, K, Q, E, M, H, F, R, Y, or W
T202	A, G, S, P, V, I, L, N, D, K, Q, E, M, H, F, R, Y, or W
S203	A, G, P, V, T, I, L, N, D, K, Q, E, M, H, F, R, Y, or W
S204	A, G, P, V, T, I, L, N, D, K, Q, E, M, H, F, R, Y, or W
F206	A, G, S, P, V, T, I, L, N, D, K, Q, E, M, H, R, Y, or W
D209	A, G, S, P, V, T, I, L, N, K, Q, E, M, H, F, R, Y, or W

Table 4b. p40 Substitutions

P40 Substitution	Substitution selected from
V10	E
V11	Y
E12	K, L, R, V, W, Y
L13	F, H, I, L, M, N, P, Q, R, T, W, Y
D14	A, E, F, G, H, I, K, L, M, N, P, Q, R, S, T, V, W, Y
W15	A, D, E, G, H, I, K, L, M, N, P, Q, R, S, T, V
Y16	A, G, P, S
P17	E, G
D18	A, F, G, H, I, L, S
A19	H, K, L, M, Q, R
P20	A, D, E, G, H, L, M, Q, R
G21	A, D, E, G, K, L, M, N, Q, R
W38	A, D, E, G, H, M, N, P, Q, S, T, V, W

T39	E, G, H, T
L40	D, E, G, Q, R, S, T
D41	P, Y
S43	G, H, L
S44	A, G, S
E45	A, E, F, I, L, P, Q, S, T, V, Y
V46	D, E, F, G, L, N, Q, S, T, W
L47	A, D, H, P, Q, S, T
I55	D
V57	A, E, G, I, L, M, P, Q, S
K58	E, G, I, V
E59	A, D, E, N, S
F60	A, D, E, F, G, H, I, K, L, M, N, Q, R, S, T, V, Y
D62	N
A63	D, E, G, K, L, N, P, S, T
G64	A, E, F, G, I, K, L, M, P, Q, R, S, T, V, W, Y
Q65	D, K, M
Y66	A, G, P, R, S, T, Y
L81	N
L82	G
H83	A, F, G, H, N, P, R, S, V, W, Y
K84	A, G, I, L, N, P, S, T, V
K85	A, G, S
E86	A, D, E, F, H, K, L, M, N, P, S, T, V, Y
G88	F, G, I, N
I89	A, E, F, G, H, I, K, M, N, P, R, S, T, V, W, Y
W90	A, D, E, F, G, H, I, K, L, M, N, Q, R, S, T, V, W
S91	E, F, G, M, N, P, Q, W, Y
T92	A
D93	A, E, F, G, H, I, K, M, N, P, Q, S, T, V, Y
I94	E, P, Y
L95	A, D, E, F, G, H, I, L, N, Q, R, S, T, V, Y
D97	E

Q98	H, K, Y
K99	A, D, E, F, G, H, I, K, L, N, P, Q, S, Y
E100	D, H, W
N103	A, D, G
K104	A, G, H, Q, S, T
T105	D, F, H, P, R, T, Y
F106	A, D, F, G, H, I, K, L, M, N, Q, R, W
L107	I, N, P, T
L123	A, E, G, I, M
K135	E, G, L, R, W
S136	A, D, G, I, L, R, V, W
S137	E, G, L, S, Y
R138	H, K, N
G139	K, N, R, T, Y
S140	G
S141	A, D, I, L, N, P, Q, S, V
D142	A, E, G, I, K, L, M, N, P, Q, R, S, T, V, W
N162	D, W
E181	A, D, F, H, K, L, N, P, T, V, W, Y
S183	D, E, K, L, M, Q, R, W, Y
L184	E
P185	A, D, E, F, G, I, K, N, P, R, T, V
I186	D, E, Q, W, Y
E187	A, E, K, N, R, S, T
V188	A, D, G, H, K, N, R, S
V190	K
D191	W
K195	A, D, E, F, G, H, K, N, P, Q, R, S, T
L196	A, D, E, G, I, L, N, P, Q, S, T, V
K197	A, G, I, P, S, V
Y198	E, G, K, Q, R, W
E199	E, P
N200	D, E, G, P, Q

T202	A, G, S
S203	D, F, G, W
S204	A, H, M, V
F206	A, D, E, H, I, K, L, M, N, Q, R, S, T, V, W, Y
D209	A, G, H, I, K, L, M, P, Q, R, S, T, V, W, Y

Table 4c. p40 Substitutions

P40 Substitution	Substitution selected from
V10	E
E12	R
D14	W, G
W15	K, N, G, D, P, Q, R
G21	E
L47	A
V57	M, Q
F60	E, D, P, G, T, A, Q, K, N
A63	T, D
G64	Y, P, E, F
Q65	D
Y66	G
W90	N
I94	Y, P, E
L95	Q, Y, S
F106	H, I
L107	N, T
S136	W
R138	N
S183	R, W, M
P185	V
E187	T
K195	P
L196	Q

Y198	G, K, Q
S203	D
S204	M
D209	G

[0142] In some embodiments, IL-12 muteins comprise one or more p40 substitutions shown in the first column of the Table 5 below. In some embodiments, IL-12 muteins comprise a substitution at an amino acid residue selected from the left column of Table 5 below in combination with one, two, three, four, five or more additional p40 substitutions shown in the corresponding second column in Table 5 below.

Table 5 – p40 Mutant Combinations

P40 Substitution	Additional p40 Substitutions
V10	V11, E12, L13, D14, W15, Y16, P17, D18, A19, P20, G21, W38, T39, L40, D41, Q42, S43, S44, E45, V46, L47, I55, V57, K58, E59, F60, D62, A63, G64, Q65, Y66, L81, L82, H83, K84, K85, E86, G88, I89, W90, S91, T92, D93, I94, L95, D97, Q98, K99, E100, P101, N103, K104, T105, F106, L107, L123, I126, T128, L130, K135, S136, S137, R138, G139, S140, S141, D142, R157, R159, D161, N162, K163, E181, S183, L184, P185, I186, E187, V188, V190, D191, K195, L196, K197, Y198, E199, N200, T202, S203, S204, F206, and/or D209
V11	V10, E12, L13, D14, W15, Y16, P17, D18, A19, P20, G21, W38, T39, L40, D41, Q42, S43, S44, E45, V46, L47, I55, V57, K58, E59, F60, D62, A63, G64, Q65, Y66, L81, L82, H83, K84, K85, E86, G88, I89, W90, S91, T92, D93, I94, L95, D97, Q98, K99, E100, P101, N103, K104, T105, F106, L107, L123, I126, T128, L130, K135, S136, S137, R138, G139, S140, S141, D142, R157, R159, D161, N162, K163, E181, S183, L184, P185, I186, E187, V188, V190, D191, K195, L196, K197, Y198, E199, N200, T202, S203, S204, F206, and/or D209
E12	V10, V11, L13, D14, W15, Y16, P17, D18, A19, P20, G21, W38, T39, L40, D41, Q42, S43, S44, E45, V46, L47, I55, V57, K58, E59, F60, D62, A63, G64, Q65, Y66, L81, L82, H83, K84, K85, E86, G88, I89, W90, S91, T92, D93, I94, L95, D97, Q98, K99, E100, P101, N103, K104, T105, F106, L107, L123, I126, T128, L130, K135, S136, S137, R138, G139, S140, S141, D142, R157, R159, D161, N162, K163, E181, S183, L184, P185, I186, E187, V188, V190, D191, K195, L196, K197, Y198, E199, N200, T202, S203, S204, F206, and/or D209
L13	V10, V11, E12, D14, W15, Y16, P17, D18, A19, P20, G21, W38, T39, L40, D41, Q42, S43, S44, E45, V46, L47, I55, V57, K58, E59, F60, D62, A63, G64, Q65, Y66, L81, L82, H83, K84, K85, E86, G88, I89, W90, S91, T92, D93, I94, L95, D97, Q98, K99, E100, P101, N103, K104, T105, F106, L107, L123, I126, T128, L130, K135, S136, S137, R138, G139, S140, S141, D142, R157, R159, D161, N162, K163, E181, S183, L184, P185, I186, E187, V188, V190,

	D191, K195, L196, K197, Y198, E199, N200, T202, S203, S204, F206, and/or D209
D14	V10, V11, E12, L13, W15, Y16, P17, D18, A19, P20, G21, W38, T39, L40, D41, Q42, S43, S44, E45, V46, L47, I55, V57, K58, E59, F60, D62, A63, G64, Q65, Y66, L81, L82, H83, K84, K85, E86, G88, I89, W90, S91, T92, D93, I94, L95, D97, Q98, K99, E100, P101, N103, K104, T105, F106, L107, L123, I126, T128, L130, K135, S136, S137, R138, G139, S140, S141, D142, R157, R159, D161, N162, K163, E181, S183, L184, P185, I186, E187, V188, V190, D191, K195, L196, K197, Y198, E199, N200, T202, S203, S204, F206, and/or D209
W15	V10, V11, E12, L13, D14, Y16, P17, D18, A19, P20, G21, W38, T39, L40, D41, Q42, S43, S44, E45, V46, L47, I55, V57, K58, E59, F60, D62, A63, G64, Q65, Y66, L81, L82, H83, K84, K85, E86, G88, I89, W90, S91, T92, D93, I94, L95, D97, Q98, K99, E100, P101, N103, K104, T105, F106, L107, L123, I126, T128, L130, K135, S136, S137, R138, G139, S140, S141, D142, R157, R159, D161, N162, K163, E181, S183, L184, P185, I186, E187, V188, V190, D191, K195, L196, K197, Y198, E199, N200, T202, S203, S204, F206, and/or D209
Y16	V10, V11, E12, L13, D14, W15, P17, D18, A19, P20, G21, W38, T39, L40, D41, Q42, S43, S44, E45, V46, L47, I55, V57, K58, E59, F60, D62, A63, G64, Q65, Y66, L81, L82, H83, K84, K85, E86, G88, I89, W90, S91, T92, D93, I94, L95, D97, Q98, K99, E100, P101, N103, K104, T105, F106, L107, L123, I126, T128, L130, K135, S136, S137, R138, G139, S140, S141, D142, R157, R159, D161, N162, K163, E181, S183, L184, P185, I186, E187, V188, V190, D191, K195, L196, K197, Y198, E199, N200, T202, S203, S204, F206, and/or D209
P17	V10, V11, E12, L13, D14, W15, Y16, D18, A19, P20, G21, W38, T39, L40, D41, Q42, S43, S44, E45, V46, L47, I55, V57, K58, E59, F60, D62, A63, G64, Q65, Y66, L81, L82, H83, K84, K85, E86, G88, I89, W90, S91, T92, D93, I94, L95, D97, Q98, K99, E100, P101, N103, K104, T105, F106, L107, L123, I126, T128, L130, K135, S136, S137, R138, G139, S140, S141, D142, R157, R159, D161, N162, K163, E181, S183, L184, P185, I186, E187, V188, V190, D191, K195, L196, K197, Y198, E199, N200, T202, S203, S204, F206, and/or D209
D18	V10, V11, E12, L13, D14, W15, Y16, P17, A19, P20, G21, W38, T39, L40, D41, Q42, S43, S44, E45, V46, L47, I55, V57, K58, E59, F60, D62, A63, G64, Q65, Y66, L81, L82, H83, K84, K85, E86, G88, I89, W90, S91, T92, D93, I94, L95, D97, Q98, K99, E100, P101, N103, K104, T105, F106, L107, L123, I126, T128, L130, K135, S136, S137, R138, G139, S140, S141, D142, R157, R159, D161, N162, K163, E181, S183, L184, P185, I186, E187, V188, V190, D191, K195, L196, K197, Y198, E199, N200, T202, S203, S204, F206, and/or D209
A19	V10, V11, E12, L13, D14, W15, Y16, P17, D18, P20, G21, W38, T39, L40, D41, Q42, S43, S44, E45, V46, L47, I55, V57, K58, E59, F60, D62, A63, G64, Q65, Y66, L81, L82, H83, K84, K85, E86, G88, I89, W90, S91, T92, D93, I94, L95, D97, Q98, K99, E100, P101, N103, K104, T105, F106, L107, L123, I126, T128, L130, K135, S136, S137, R138, G139, S140, S141, D142, R157, R159, D161, N162, K163, E181, S183, L184, P185, I186, E187, V188, V190, D191, K195, L196, K197, Y198, E199, N200, T202, S203, S204, F206, and/or D209

P20	V10, V11, E12, L13, D14, W15, Y16, P17, D18, A19, G21, W38, T39, L40, D41, Q42, S43, S44, E45, V46, L47, I55, V57, K58, E59, F60, D62, A63, G64, Q65, Y66, L81, L82, H83, K84, K85, E86, G88, I89, W90, S91, T92, D93, I94, L95, D97, Q98, K99, E100, P101, N103, K104, T105, F106, L107, L123, I126, T128, L130, K135, S136, S137, R138, G139, S140, S141, D142, R157, R159, D161, N162, K163, E181, S183, L184, P185, I186, E187, V188, V190, D191, K195, L196, K197, Y198, E199, N200, T202, S203, S204, F206, and/or D209
G21	V10, V11, E12, L13, D14, W15, Y16, P17, D18, A19, P20, W38, T39, L40, D41, Q42, S43, S44, E45, V46, L47, I55, V57, K58, E59, F60, D62, A63, G64, Q65, Y66, L81, L82, H83, K84, K85, E86, G88, I89, W90, S91, T92, D93, I94, L95, D97, Q98, K99, E100, P101, N103, K104, T105, F106, L107, L123, I126, T128, L130, K135, S136, S137, R138, G139, S140, S141, D142, R157, R159, D161, N162, K163, E181, S183, L184, P185, I186, E187, V188, V190, D191, K195, L196, K197, Y198, E199, N200, T202, S203, S204, F206, and/or D209
W38	V10, V11, E12, L13, D14, W15, Y16, P17, D18, A19, P20, G21, T39, L40, D41, Q42, S43, S44, E45, V46, L47, I55, V57, K58, E59, F60, D62, A63, G64, Q65, Y66, L81, L82, H83, K84, K85, E86, G88, I89, W90, S91, T92, D93, I94, L95, D97, Q98, K99, E100, P101, N103, K104, T105, F106, L107, L123, I126, T128, L130, K135, S136, S137, R138, G139, S140, S141, D142, R157, R159, D161, N162, K163, E181, S183, L184, P185, I186, E187, V188, V190, D191, K195, L196, K197, Y198, E199, N200, T202, S203, S204, F206, and/or D209
T39	V10, V11, E12, L13, D14, W15, Y16, P17, D18, A19, P20, G21, W38, L40, D41, Q42, S43, S44, E45, V46, L47, I55, V57, K58, E59, F60, D62, A63, G64, Q65, Y66, L81, L82, H83, K84, K85, E86, G88, I89, W90, S91, T92, D93, I94, L95, D97, Q98, K99, E100, P101, N103, K104, T105, F106, L107, L123, I126, T128, L130, K135, S136, S137, R138, G139, S140, S141, D142, R157, R159, D161, N162, K163, E181, S183, L184, P185, I186, E187, V188, V190, D191, K195, L196, K197, Y198, E199, N200, T202, S203, S204, F206, and/or D209
L40	V10, V11, E12, L13, D14, W15, Y16, P17, D18, A19, P20, G21, W38, T39, D41, Q42, S43, S44, E45, V46, L47, I55, V57, K58, E59, F60, D62, A63, G64, Q65, Y66, L81, L82, H83, K84, K85, E86, G88, I89, W90, S91, T92, D93, I94, L95, D97, Q98, K99, E100, P101, N103, K104, T105, F106, L107, L123, I126, T128, L130, K135, S136, S137, R138, G139, S140, S141, D142, R157, R159, D161, N162, K163, E181, S183, L184, P185, I186, E187, V188, V190, D191, K195, L196, K197, Y198, E199, N200, T202, S203, S204, F206, and/or D209
D41	V10, V11, E12, L13, D14, W15, Y16, P17, D18, A19, P20, G21, W38, T39, L40, Q42, S43, S44, E45, V46, L47, I55, V57, K58, E59, F60, D62, A63, G64, Q65, Y66, L81, L82, H83, K84, K85, E86, G88, I89, W90, S91, T92, D93, I94, L95, D97, Q98, K99, E100, P101, N103, K104, T105, F106, L107, L123, I126, T128, L130, K135, S136, S137, R138, G139, S140, S141, D142, R157, R159, D161, N162, K163, E181, S183, L184, P185, I186, E187, V188, V190, D191, K195, L196, K197, Y198, E199, N200, T202, S203, S204, F206, and/or D209
Q42	V10, V11, E12, L13, D14, W15, Y16, P17, D18, A19, P20, G21, W38, T39, L40, D41, S43, S44, E45, V46, L47, I55, V57, K58, E59, F60, D62, A63, G64,

	Q65, Y66, L81, L82, H83, K84, K85, E86, G88, I89, W90, S91, T92, D93, I94, L95, D97, Q98, K99, E100, P101, N103, K104, T105, F106, L107, L123, I126, T128, L130, K135, S136, S137, R138, G139, S140, S141, D142, R157, R159, D161, N162, K163, E181, S183, L184, P185, I186, E187, V188, V190, D191, K195, L196, K197, Y198, E199, N200, T202, S203, S204, F206, and/or D209
S43	V10, V11, E12, L13, D14, W15, Y16, P17, D18, A19, P20, G21, W38, T39, L40, D41, Q42, S44, E45, V46, L47, I55, V57, K58, E59, F60, D62, A63, G64, Q65, Y66, L81, L82, H83, K84, K85, E86, G88, I89, W90, S91, T92, D93, I94, L95, D97, Q98, K99, E100, P101, N103, K104, T105, F106, L107, L123, I126, T128, L130, K135, S136, S137, R138, G139, S140, S141, D142, R157, R159, D161, N162, K163, E181, S183, L184, P185, I186, E187, V188, V190, D191, K195, L196, K197, Y198, E199, N200, T202, S203, S204, F206, and/or D209
S44	V10, V11, E12, L13, D14, W15, Y16, P17, D18, A19, P20, G21, W38, T39, L40, D41, Q42, S43, E45, V46, L47, I55, V57, K58, E59, F60, D62, A63, G64, Q65, Y66, L81, L82, H83, K84, K85, E86, G88, I89, W90, S91, T92, D93, I94, L95, D97, Q98, K99, E100, P101, N103, K104, T105, F106, L107, L123, I126, T128, L130, K135, S136, S137, R138, G139, S140, S141, D142, R157, R159, D161, N162, K163, E181, S183, L184, P185, I186, E187, V188, V190, D191, K195, L196, K197, Y198, E199, N200, T202, S203, S204, F206, and/or D209
E45	V10, V11, E12, L13, D14, W15, Y16, P17, D18, A19, P20, G21, W38, T39, L40, D41, Q42, S43, S44, V46, L47, I55, V57, K58, E59, F60, D62, A63, G64, Q65, Y66, L81, L82, H83, K84, K85, E86, G88, I89, W90, S91, T92, D93, I94, L95, D97, Q98, K99, E100, P101, N103, K104, T105, F106, L107, L123, I126, T128, L130, K135, S136, S137, R138, G139, S140, S141, D142, R157, R159, D161, N162, K163, E181, S183, L184, P185, I186, E187, V188, V190, D191, K195, L196, K197, Y198, E199, N200, T202, S203, S204, F206, and/or D209
V46	V10, V11, E12, L13, D14, W15, Y16, P17, D18, A19, P20, G21, W38, T39, L40, D41, Q42, S43, S44, E45, L47, I55, V57, K58, E59, F60, D62, A63, G64, Q65, Y66, L81, L82, H83, K84, K85, E86, G88, I89, W90, S91, T92, D93, I94, L95, D97, Q98, K99, E100, P101, N103, K104, T105, F106, L107, L123, I126, T128, L130, K135, S136, S137, R138, G139, S140, S141, D142, R157, R159, D161, N162, K163, E181, S183, L184, P185, I186, E187, V188, V190, D191, K195, L196, K197, Y198, E199, N200, T202, S203, S204, F206, and/or D209
L47	V10, V11, E12, L13, D14, W15, Y16, P17, D18, A19, P20, G21, W38, T39, L40, D41, Q42, S43, S44, E45, V46, I55, V57, K58, E59, F60, D62, A63, G64, Q65, Y66, L81, L82, H83, K84, K85, E86, G88, I89, W90, S91, T92, D93, I94, L95, D97, Q98, K99, E100, P101, N103, K104, T105, F106, L107, L123, I126, T128, L130, K135, S136, S137, R138, G139, S140, S141, D142, R157, R159, D161, N162, K163, E181, S183, L184, P185, I186, E187, V188, V190, D191, K195, L196, K197, Y198, E199, N200, T202, S203, S204, F206, and/or D209
I55	V10, V11, E12, L13, D14, W15, Y16, P17, D18, A19, P20, G21, W38, T39, L40, D41, Q42, S43, S44, E45, V46, L47, V57, K58, E59, F60, D62, A63, G64, Q65, Y66, L81, L82, H83, K84, K85, E86, G88, I89, W90, S91, T92, D93, I94, L95, D97, Q98, K99, E100, P101, N103, K104, T105, F106, L107,

	L123, I126, T128, L130, K135, S136, S137, R138, G139, S140, S141, D142, R157, R159, D161, N162, K163, E181, S183, L184, P185, I186, E187, V188, V190, D191, K195, L196, K197, Y198, E199, N200, T202, S203, S204, F206, and/or D209
V57	V10, V11, E12, L13, D14, W15, Y16, P17, D18, A19, P20, G21, W38, T39, L40, D41, Q42, S43, S44, E45, V46, L47, I55, K58, E59, F60, D62, A63, G64, Q65, Y66, L81, L82, H83, K84, K85, E86, G88, I89, W90, S91, T92, D93, I94, L95, D97, Q98, K99, E100, P101, N103, K104, T105, F106, L107, L123, I126, T128, L130, K135, S136, S137, R138, G139, S140, S141, D142, R157, R159, D161, N162, K163, E181, S183, L184, P185, I186, E187, V188, V190, D191, K195, L196, K197, Y198, E199, N200, T202, S203, S204, F206, and/or D209
K58	V10, V11, E12, L13, D14, W15, Y16, P17, D18, A19, P20, G21, W38, T39, L40, D41, Q42, S43, S44, E45, V46, L47, I55, V57, E59, F60, D62, A63, G64, Q65, Y66, L81, L82, H83, K84, K85, E86, G88, I89, W90, S91, T92, D93, I94, L95, D97, Q98, K99, E100, P101, N103, K104, T105, F106, L107, L123, I126, T128, L130, K135, S136, S137, R138, G139, S140, S141, D142, R157, R159, D161, N162, K163, E181, S183, L184, P185, I186, E187, V188, V190, D191, K195, L196, K197, Y198, E199, N200, T202, S203, S204, F206, and/or D209
E59	V10, V11, E12, L13, D14, W15, Y16, P17, D18, A19, P20, G21, W38, T39, L40, D41, Q42, S43, S44, E45, V46, L47, I55, V57, K58, F60, D62, A63, G64, Q65, Y66, L81, L82, H83, K84, K85, E86, G88, I89, W90, S91, T92, D93, I94, L95, D97, Q98, K99, E100, P101, N103, K104, T105, F106, L107, L123, I126, T128, L130, K135, S136, S137, R138, G139, S140, S141, D142, R157, R159, D161, N162, K163, E181, S183, L184, P185, I186, E187, V188, V190, D191, K195, L196, K197, Y198, E199, N200, T202, S203, S204, F206, and/or D209
F60	V10, V11, E12, L13, D14, W15, Y16, P17, D18, A19, P20, G21, W38, T39, L40, D41, Q42, S43, S44, E45, V46, L47, I55, V57, K58, E59, D62, A63, G64, Q65, Y66, L81, L82, H83, K84, K85, E86, G88, I89, W90, S91, T92, D93, I94, L95, D97, Q98, K99, E100, P101, N103, K104, T105, F106, L107, L123, I126, T128, L130, K135, S136, S137, R138, G139, S140, S141, D142, R157, R159, D161, N162, K163, E181, S183, L184, P185, I186, E187, V188, V190, D191, K195, L196, K197, Y198, E199, N200, T202, S203, S204, F206, and/or D209
D62	V10, V11, E12, L13, D14, W15, Y16, P17, D18, A19, P20, G21, W38, T39, L40, D41, Q42, S43, S44, E45, V46, L47, I55, V57, K58, E59, F60, A63, G64, Q65, Y66, L81, L82, H83, K84, K85, E86, G88, I89, W90, S91, T92, D93, I94, L95, D97, Q98, K99, E100, P101, N103, K104, T105, F106, L107, L123, I126, T128, L130, K135, S136, S137, R138, G139, S140, S141, D142, R157, R159, D161, N162, K163, E181, S183, L184, P185, I186, E187, V188, V190, D191, K195, L196, K197, Y198, E199, N200, T202, S203, S204, F206, and/or D209
A63	V10, V11, E12, L13, D14, W15, Y16, P17, D18, A19, P20, G21, W38, T39, L40, D41, Q42, S43, S44, E45, V46, L47, I55, V57, K58, E59, F60, D62, G64, Q65, Y66, L81, L82, H83, K84, K85, E86, G88, I89, W90, S91, T92, D93, I94, L95, D97, Q98, K99, E100, P101, N103, K104, T105, F106, L107, L123, I126, T128, L130, K135, S136, S137, R138, G139, S140, S141, D142, R157, R159, D161, N162, K163, E181, S183, L184, P185, I186, E187, V188, V190,

	D191, K195, L196, K197, Y198, E199, N200, T202, S203, S204, F206, and/or D209
G64	V10, V11, E12, L13, D14, W15, Y16, P17, D18, A19, P20, G21, W38, T39, L40, D41, Q42, S43, S44, E45, V46, L47, I55, V57, K58, E59, F60, D62, A63, Q65, Y66, L81, L82, H83, K84, K85, E86, G88, I89, W90, S91, T92, D93, I94, L95, D97, Q98, K99, E100, P101, N103, K104, T105, F106, L107, L123, I126, T128, L130, K135, S136, S137, R138, G139, S140, S141, D142, R157, R159, D161, N162, K163, E181, S183, L184, P185, I186, E187, V188, V190, D191, K195, L196, K197, Y198, E199, N200, T202, S203, S204, F206, and/or D209
Q65	V10, V11, E12, L13, D14, W15, Y16, P17, D18, A19, P20, G21, W38, T39, L40, D41, Q42, S43, S44, E45, V46, L47, I55, V57, K58, E59, F60, D62, A63, G64, Y66, L81, L82, H83, K84, K85, E86, G88, I89, W90, S91, T92, D93, I94, L95, D97, Q98, K99, E100, P101, N103, K104, T105, F106, L107, L123, I126, T128, L130, K135, S136, S137, R138, G139, S140, S141, D142, R157, R159, D161, N162, K163, E181, S183, L184, P185, I186, E187, V188, V190, D191, K195, L196, K197, Y198, E199, N200, T202, S203, S204, F206, and/or D209
Y66	V10, V11, E12, L13, D14, W15, Y16, P17, D18, A19, P20, G21, W38, T39, L40, D41, Q42, S43, S44, E45, V46, L47, I55, V57, K58, E59, F60, D62, A63, G64, Q65, L81, L82, H83, K84, K85, E86, G88, I89, W90, S91, T92, D93, I94, L95, D97, Q98, K99, E100, P101, N103, K104, T105, F106, L107, L123, I126, T128, L130, K135, S136, S137, R138, G139, S140, S141, D142, R157, R159, D161, N162, K163, E181, S183, L184, P185, I186, E187, V188, V190, D191, K195, L196, K197, Y198, E199, N200, T202, S203, S204, F206, and/or D209
L81	V10, V11, E12, L13, D14, W15, Y16, P17, D18, A19, P20, G21, W38, T39, L40, D41, Q42, S43, S44, E45, V46, L47, I55, V57, K58, E59, F60, D62, A63, G64, Q65, Y66, L82, H83, K84, K85, E86, G88, I89, W90, S91, T92, D93, I94, L95, D97, Q98, K99, E100, P101, N103, K104, T105, F106, L107, L123, I126, T128, L130, K135, S136, S137, R138, G139, S140, S141, D142, R157, R159, D161, N162, K163, E181, S183, L184, P185, I186, E187, V188, V190, D191, K195, L196, K197, Y198, E199, N200, T202, S203, S204, F206, and/or D209
L82	V10, V11, E12, L13, D14, W15, Y16, P17, D18, A19, P20, G21, W38, T39, L40, D41, Q42, S43, S44, E45, V46, L47, I55, V57, K58, E59, F60, D62, A63, G64, Q65, Y66, L81, H83, K84, K85, E86, G88, I89, W90, S91, T92, D93, I94, L95, D97, Q98, K99, E100, P101, N103, K104, T105, F106, L107, L123, I126, T128, L130, K135, S136, S137, R138, G139, S140, S141, D142, R157, R159, D161, N162, K163, E181, S183, L184, P185, I186, E187, V188, V190, D191, K195, L196, K197, Y198, E199, N200, T202, S203, S204, F206, and/or D209
H83	V10, V11, E12, L13, D14, W15, Y16, P17, D18, A19, P20, G21, W38, T39, L40, D41, Q42, S43, S44, E45, V46, L47, I55, V57, K58, E59, F60, D62, A63, G64, Q65, Y66, L81, L82, K84, K85, E86, G88, I89, W90, S91, T92, D93, I94, L95, D97, Q98, K99, E100, P101, N103, K104, T105, F106, L107, L123, I126, T128, L130, K135, S136, S137, R138, G139, S140, S141, D142, R157, R159, D161, N162, K163, E181, S183, L184, P185, I186, E187, V188, V190, D191, K195, L196, K197, Y198, E199, N200, T202, S203, S204, F206, and/or D209

K84	V10, V11, E12, L13, D14, W15, Y16, P17, D18, A19, P20, G21, W38, T39, L40, D41, Q42, S43, S44, E45, V46, L47, I55, V57, K58, E59, F60, D62, A63, G64, Q65, Y66, L81, L82, H83, K85, E86, G88, I89, W90, S91, T92, D93, I94, L95, D97, Q98, K99, E100, P101, N103, K104, T105, F106, L107, L123, I126, T128, L130, K135, S136, S137, R138, G139, S140, S141, D142, R157, R159, D161, N162, K163, E181, S183, L184, P185, I186, E187, V188, V190, D191, K195, L196, K197, Y198, E199, N200, T202, S203, S204, F206, and/or D209
K85	V10, V11, E12, L13, D14, W15, Y16, P17, D18, A19, P20, G21, W38, T39, L40, D41, Q42, S43, S44, E45, V46, L47, I55, V57, K58, E59, F60, D62, A63, G64, Q65, Y66, L81, L82, H83, K84, E86, G88, I89, W90, S91, T92, D93, I94, L95, D97, Q98, K99, E100, P101, N103, K104, T105, F106, L107, L123, I126, T128, L130, K135, S136, S137, R138, G139, S140, S141, D142, R157, R159, D161, N162, K163, E181, S183, L184, P185, I186, E187, V188, V190, D191, K195, L196, K197, Y198, E199, N200, T202, S203, S204, F206, and/or D209
E86	V10, V11, E12, L13, D14, W15, Y16, P17, D18, A19, P20, G21, W38, T39, L40, D41, Q42, S43, S44, E45, V46, L47, I55, V57, K58, E59, F60, D62, A63, G64, Q65, Y66, L81, L82, H83, K84, K85, G88, I89, W90, S91, T92, D93, I94, L95, D97, Q98, K99, E100, P101, N103, K104, T105, F106, L107, L123, I126, T128, L130, K135, S136, S137, R138, G139, S140, S141, D142, R157, R159, D161, N162, K163, E181, S183, L184, P185, I186, E187, V188, V190, D191, K195, L196, K197, Y198, E199, N200, T202, S203, S204, F206, and/or D209
G88	V10, V11, E12, L13, D14, W15, Y16, P17, D18, A19, P20, G21, W38, T39, L40, D41, Q42, S43, S44, E45, V46, L47, I55, V57, K58, E59, F60, D62, A63, G64, Q65, Y66, L81, L82, H83, K84, K85, E86, I89, W90, S91, T92, D93, I94, L95, D97, Q98, K99, E100, P101, N103, K104, T105, F106, L107, L123, I126, T128, L130, K135, S136, S137, R138, G139, S140, S141, D142, R157, R159, D161, N162, K163, E181, S183, L184, P185, I186, E187, V188, V190, D191, K195, L196, K197, Y198, E199, N200, T202, S203, S204, F206, and/or D209
I89	V10, V11, E12, L13, D14, W15, Y16, P17, D18, A19, P20, G21, W38, T39, L40, D41, Q42, S43, S44, E45, V46, L47, I55, V57, K58, E59, F60, D62, A63, G64, Q65, Y66, L81, L82, H83, K84, K85, E86, G88, W90, S91, T92, D93, I94, L95, D97, Q98, K99, E100, P101, N103, K104, T105, F106, L107, L123, I126, T128, L130, K135, S136, S137, R138, G139, S140, S141, D142, R157, R159, D161, N162, K163, E181, S183, L184, P185, I186, E187, V188, V190, D191, K195, L196, K197, Y198, E199, N200, T202, S203, S204, F206, and/or D209
W90	V10, V11, E12, L13, D14, W15, Y16, P17, D18, A19, P20, G21, W38, T39, L40, D41, Q42, S43, S44, E45, V46, L47, I55, V57, K58, E59, F60, D62, A63, G64, Q65, Y66, L81, L82, H83, K84, K85, E86, G88, I89, S91, T92, D93, I94, L95, D97, Q98, K99, E100, P101, N103, K104, T105, F106, L107, L123, I126, T128, L130, K135, S136, S137, R138, G139, S140, S141, D142, R157, R159, D161, N162, K163, E181, S183, L184, P185, I186, E187, V188, V190, D191, K195, L196, K197, Y198, E199, N200, T202, S203, S204, F206, and/or D209
S91	V10, V11, E12, L13, D14, W15, Y16, P17, D18, A19, P20, G21, W38, T39, L40, D41, Q42, S43, S44, E45, V46, L47, I55, V57, K58, E59, F60, D62, A63, G64, Q65, Y66, L81, L82, H83, K84, K85, E86, G88, I89, W90, T92, D93,

	I94, L95, D97, Q98, K99, E100, P101, N103, K104, T105, F106, L107, L123, I126, T128, L130, K135, S136, S137, R138, G139, S140, S141, D142, R157, R159, D161, N162, K163, E181, S183, L184, P185, I186, E187, V188, V190, D191, K195, L196, K197, Y198, E199, N200, T202, S203, S204, F206, and/or D209
T92	V10, V11, E12, L13, D14, W15, Y16, P17, D18, A19, P20, G21, W38, T39, L40, D41, Q42, S43, S44, E45, V46, L47, I55, V57, K58, E59, F60, D62, A63, G64, Q65, Y66, L81, L82, H83, K84, K85, E86, G88, I89, W90, S91, D93, I94, L95, D97, Q98, K99, E100, P101, N103, K104, T105, F106, L107, L123, I126, T128, L130, K135, S136, S137, R138, G139, S140, S141, D142, R157, R159, D161, N162, K163, E181, S183, L184, P185, I186, E187, V188, V190, D191, K195, L196, K197, Y198, E199, N200, T202, S203, S204, F206, and/or D209
D93	V10, V11, E12, L13, D14, W15, Y16, P17, D18, A19, P20, G21, W38, T39, L40, D41, Q42, S43, S44, E45, V46, L47, I55, V57, K58, E59, F60, D62, A63, G64, Q65, Y66, L81, L82, H83, K84, K85, E86, G88, I89, W90, S91, T92, I94, L95, D97, Q98, K99, E100, P101, N103, K104, T105, F106, L107, L123, I126, T128, L130, K135, S136, S137, R138, G139, S140, S141, D142, R157, R159, D161, N162, K163, E181, S183, L184, P185, I186, E187, V188, V190, D191, K195, L196, K197, Y198, E199, N200, T202, S203, S204, F206, and/or D209
I94	V10, V11, E12, L13, D14, W15, Y16, P17, D18, A19, P20, G21, W38, T39, L40, D41, Q42, S43, S44, E45, V46, L47, I55, V57, K58, E59, F60, D62, A63, G64, Q65, Y66, L81, L82, H83, K84, K85, E86, G88, I89, W90, S91, T92, D93, L95, D97, Q98, K99, E100, P101, N103, K104, T105, F106, L107, L123, I126, T128, L130, K135, S136, S137, R138, G139, S140, S141, D142, R157, R159, D161, N162, K163, E181, S183, L184, P185, I186, E187, V188, V190, D191, K195, L196, K197, Y198, E199, N200, T202, S203, S204, F206, and/or D209
L95	V10, V11, E12, L13, D14, W15, Y16, P17, D18, A19, P20, G21, W38, T39, L40, D41, Q42, S43, S44, E45, V46, L47, I55, V57, K58, E59, F60, D62, A63, G64, Q65, Y66, L81, L82, H83, K84, K85, E86, G88, I89, W90, S91, T92, D93, I94, D97, Q98, K99, E100, P101, N103, K104, T105, F106, L107, L123, I126, T128, L130, K135, S136, S137, R138, G139, S140, S141, D142, R157, R159, D161, N162, K163, E181, S183, L184, P185, I186, E187, V188, V190, D191, K195, L196, K197, Y198, E199, N200, T202, S203, S204, F206, and/or D209
D97	V10, V11, E12, L13, D14, W15, Y16, P17, D18, A19, P20, G21, W38, T39, L40, D41, Q42, S43, S44, E45, V46, L47, I55, V57, K58, E59, F60, D62, A63, G64, Q65, Y66, L81, L82, H83, K84, K85, E86, G88, I89, W90, S91, T92, D93, I94, L95, Q98, K99, E100, P101, N103, K104, T105, F106, L107, L123, I126, T128, L130, K135, S136, S137, R138, G139, S140, S141, D142, R157, R159, D161, N162, K163, E181, S183, L184, P185, I186, E187, V188, V190, D191, K195, L196, K197, Y198, E199, N200, T202, S203, S204, F206, and/or D209
Q98	V10, V11, E12, L13, D14, W15, Y16, P17, D18, A19, P20, G21, W38, T39, L40, D41, Q42, S43, S44, E45, V46, L47, I55, V57, K58, E59, F60, D62, A63, G64, Q65, Y66, L81, L82, H83, K84, K85, E86, G88, I89, W90, S91, T92, D93, I94, L95, D97, K99, E100, P101, N103, K104, T105, F106, L107, L123, I126, T128, L130, K135, S136, S137, R138, G139, S140, S141, D142, R157,

	R159, D161, N162, K163, E181, S183, L184, P185, I186, E187, V188, V190, D191, K195, L196, K197, Y198, E199, N200, T202, S203, S204, F206, and/or D209
K99	V10, V11, E12, L13, D14, W15, Y16, P17, D18, A19, P20, G21, W38, T39, L40, D41, Q42, S43, S44, E45, V46, L47, I55, V57, K58, E59, F60, D62, A63, G64, Q65, Y66, L81, L82, H83, K84, K85, E86, G88, I89, W90, S91, T92, D93, I94, L95, D97, Q98, K99, E100, P101, N103, K104, T105, F106, L107, L123, I126, T128, L130, K135, S136, S137, R138, G139, S140, S141, D142, R157, R159, D161, N162, K163, E181, S183, L184, P185, I186, E187, V188, V190, D191, K195, L196, K197, Y198, E199, N200, T202, S203, S204, F206, and/or D209
E100	V10, V11, E12, L13, D14, W15, Y16, P17, D18, A19, P20, G21, W38, T39, L40, D41, Q42, S43, S44, E45, V46, L47, I55, V57, K58, E59, F60, D62, A63, G64, Q65, Y66, L81, L82, H83, K84, K85, E86, G88, I89, W90, S91, T92, D93, I94, L95, D97, Q98, K99, P101, N103, K104, T105, F106, L107, L123, I126, T128, L130, K135, S136, S137, R138, G139, S140, S141, D142, R157, R159, D161, N162, K163, E181, S183, L184, P185, I186, E187, V188, V190, D191, K195, L196, K197, Y198, E199, N200, T202, S203, S204, F206, and/or D209
P101	V10, V11, E12, L13, D14, W15, Y16, P17, D18, A19, P20, G21, W38, T39, L40, D41, Q42, S43, S44, E45, V46, L47, I55, V57, K58, E59, F60, D62, A63, G64, Q65, Y66, L81, L82, H83, K84, K85, E86, G88, I89, W90, S91, T92, D93, I94, L95, D97, Q98, K99, E100, N103, K104, T105, F106, L107, L123, I126, T128, L130, K135, S136, S137, R138, G139, S140, S141, D142, R157, R159, D161, N162, K163, E181, S183, L184, P185, I186, E187, V188, V190, D191, K195, L196, K197, Y198, E199, N200, T202, S203, S204, F206, and/or D209
N103	V10, V11, E12, L13, D14, W15, Y16, P17, D18, A19, P20, G21, W38, T39, L40, D41, Q42, S43, S44, E45, V46, L47, I55, V57, K58, E59, F60, D62, A63, G64, Q65, Y66, L81, L82, H83, K84, K85, E86, G88, I89, W90, S91, T92, D93, I94, L95, D97, Q98, K99, E100, P101, K104, T105, F106, L107, L123, I126, T128, L130, K135, S136, S137, R138, G139, S140, S141, D142, R157, R159, D161, N162, K163, E181, S183, L184, P185, I186, E187, V188, V190, D191, K195, L196, K197, Y198, E199, N200, T202, S203, S204, F206, and/or D209
K104	V10, V11, E12, L13, D14, W15, Y16, P17, D18, A19, P20, G21, W38, T39, L40, D41, Q42, S43, S44, E45, V46, L47, I55, V57, K58, E59, F60, D62, A63, G64, Q65, Y66, L81, L82, H83, K84, K85, E86, G88, I89, W90, S91, T92, D93, I94, L95, D97, Q98, K99, E100, P101, N103, T105, F106, L107, L123, I126, T128, L130, K135, S136, S137, R138, G139, S140, S141, D142, R157, R159, D161, N162, K163, E181, S183, L184, P185, I186, E187, V188, V190, D191, K195, L196, K197, Y198, E199, N200, T202, S203, S204, F206, and/or D209
T105	V10, V11, E12, L13, D14, W15, Y16, P17, D18, A19, P20, G21, W38, T39, L40, D41, Q42, S43, S44, E45, V46, L47, I55, V57, K58, E59, F60, D62, A63, G64, Q65, Y66, L81, L82, H83, K84, K85, E86, G88, I89, W90, S91, T92, D93, I94, L95, D97, Q98, K99, E100, P101, N103, K104, F106, L107, L123, I126, T128, L130, K135, S136, S137, R138, G139, S140, S141, D142, R157, R159, D161, N162, K163, E181, S183, L184, P185, I186, E187, V188, V190,

	D191, K195, L196, K197, Y198, E199, N200, T202, S203, S204, F206, and/or D209
F106	V10, V11, E12, L13, D14, W15, Y16, P17, D18, A19, P20, G21, W38, T39, L40, D41, Q42, S43, S44, E45, V46, L47, I55, V57, K58, E59, F60, D62, A63, G64, Q65, Y66, L81, L82, H83, K84, K85, E86, G88, I89, W90, S91, T92, D93, I94, L95, D97, Q98, K99, E100, P101, N103, K104, T105, L107, L123, I126, T128, L130, K135, S136, S137, R138, G139, S140, S141, D142, R157, R159, D161, N162, K163, E181, S183, L184, P185, I186, E187, V188, V190, D191, K195, L196, K197, Y198, E199, N200, T202, S203, S204, F206, and/or D209
L107	V10, V11, E12, L13, D14, W15, Y16, P17, D18, A19, P20, G21, W38, T39, L40, D41, Q42, S43, S44, E45, V46, L47, I55, V57, K58, E59, F60, D62, A63, G64, Q65, Y66, L81, L82, H83, K84, K85, E86, G88, I89, W90, S91, T92, D93, I94, L95, D97, Q98, K99, E100, P101, N103, K104, T105, F106, L123, I126, T128, L130, K135, S136, S137, R138, G139, S140, S141, D142, R157, R159, D161, N162, K163, E181, S183, L184, P185, I186, E187, V188, V190, D191, K195, L196, K197, Y198, E199, N200, T202, S203, S204, F206, and/or D209
L123	V10, V11, E12, L13, D14, W15, Y16, P17, D18, A19, P20, G21, W38, T39, L40, D41, Q42, S43, S44, E45, V46, L47, I55, V57, K58, E59, F60, D62, A63, G64, Q65, Y66, L81, L82, H83, K84, K85, E86, G88, I89, W90, S91, T92, D93, I94, L95, D97, Q98, K99, E100, P101, N103, K104, T105, F106, L107, I126, T128, L130, K135, S136, S137, R138, G139, S140, S141, D142, R157, R159, D161, N162, K163, E181, S183, L184, P185, I186, E187, V188, V190, D191, K195, L196, K197, Y198, E199, N200, T202, S203, S204, F206, and/or D209
I126	V10, V11, E12, L13, D14, W15, Y16, P17, D18, A19, P20, G21, W38, T39, L40, D41, Q42, S43, S44, E45, V46, L47, I55, V57, K58, E59, F60, D62, A63, G64, Q65, Y66, L81, L82, H83, K84, K85, E86, G88, I89, W90, S91, T92, D93, I94, L95, D97, Q98, K99, E100, P101, N103, K104, T105, F106, L107, L123, T128, L130, K135, S136, S137, R138, G139, S140, S141, D142, R157, R159, D161, N162, K163, E181, S183, L184, P185, I186, E187, V188, V190, D191, K195, L196, K197, Y198, E199, N200, T202, S203, S204, F206, and/or D209
T128	V10, V11, E12, L13, D14, W15, Y16, P17, D18, A19, P20, G21, W38, T39, L40, D41, Q42, S43, S44, E45, V46, L47, I55, V57, K58, E59, F60, D62, A63, G64, Q65, Y66, L81, L82, H83, K84, K85, E86, G88, I89, W90, S91, T92, D93, I94, L95, D97, Q98, K99, E100, P101, N103, K104, T105, F106, L107, L123, I126, L130, K135, S136, S137, R138, G139, S140, S141, D142, R157, R159, D161, N162, K163, E181, S183, L184, P185, I186, E187, V188, V190, D191, K195, L196, K197, Y198, E199, N200, T202, S203, S204, F206, and/or D209
L130	V10, V11, E12, L13, D14, W15, Y16, P17, D18, A19, P20, G21, W38, T39, L40, D41, Q42, S43, S44, E45, V46, L47, I55, V57, K58, E59, F60, D62, A63, G64, Q65, Y66, L81, L82, H83, K84, K85, E86, G88, I89, W90, S91, T92, D93, I94, L95, D97, Q98, K99, E100, P101, N103, K104, T105, F106, L107, L123, I126, T128, K135, S136, S137, R138, G139, S140, S141, D142, R157, R159, D161, N162, K163, E181, S183, L184, P185, I186, E187, V188, V190, D191, K195, L196, K197, Y198, E199, N200, T202, S203, S204, F206, and/or D209

K135	V10, V11, E12, L13, D14, W15, Y16, P17, D18, A19, P20, G21, W38, T39, L40, D41, Q42, S43, S44, E45, V46, L47, I55, V57, K58, E59, F60, D62, A63, G64, Q65, Y66, L81, L82, H83, K84, K85, E86, G88, I89, W90, S91, T92, D93, I94, L95, D97, Q98, K99, E100, P101, N103, K104, T105, F106, L107, L123, I126, T128, L130, S136, S137, R138, G139, S140, S141, D142, R157, R159, D161, N162, K163, E181, S183, L184, P185, I186, E187, V188, V190, D191, K195, L196, K197, Y198, E199, N200, T202, S203, S204, F206, and/or D209
S136	V10, V11, E12, L13, D14, W15, Y16, P17, D18, A19, P20, G21, W38, T39, L40, D41, Q42, S43, S44, E45, V46, L47, I55, V57, K58, E59, F60, D62, A63, G64, Q65, Y66, L81, L82, H83, K84, K85, E86, G88, I89, W90, S91, T92, D93, I94, L95, D97, Q98, K99, E100, P101, N103, K104, T105, F106, L107, L123, I126, T128, L130, K135, S137, R138, G139, S140, S141, D142, R157, R159, D161, N162, K163, E181, S183, L184, P185, I186, E187, V188, V190, D191, K195, L196, K197, Y198, E199, N200, T202, S203, S204, F206, and/or D209
S137	V10, V11, E12, L13, D14, W15, Y16, P17, D18, A19, P20, G21, W38, T39, L40, D41, Q42, S43, S44, E45, V46, L47, I55, V57, K58, E59, F60, D62, A63, G64, Q65, Y66, L81, L82, H83, K84, K85, E86, G88, I89, W90, S91, T92, D93, I94, L95, D97, Q98, K99, E100, P101, N103, K104, T105, F106, L107, L123, I126, T128, L130, K135, S136, R138, G139, S140, S141, D142, R157, R159, D161, N162, K163, E181, S183, L184, P185, I186, E187, V188, V190, D191, K195, L196, K197, Y198, E199, N200, T202, S203, S204, F206, and/or D209
R138	V10, V11, E12, L13, D14, W15, Y16, P17, D18, A19, P20, G21, W38, T39, L40, D41, Q42, S43, S44, E45, V46, L47, I55, V57, K58, E59, F60, D62, A63, G64, Q65, Y66, L81, L82, H83, K84, K85, E86, G88, I89, W90, S91, T92, D93, I94, L95, D97, Q98, K99, E100, P101, N103, K104, T105, F106, L107, L123, I126, T128, L130, K135, S136, S137, G139, S140, S141, D142, R157, R159, D161, N162, K163, E181, S183, L184, P185, I186, E187, V188, V190, D191, K195, L196, K197, Y198, E199, N200, T202, S203, S204, F206, and/or D209
G139	V10, V11, E12, L13, D14, W15, Y16, P17, D18, A19, P20, G21, W38, T39, L40, D41, Q42, S43, S44, E45, V46, L47, I55, V57, K58, E59, F60, D62, A63, G64, Q65, Y66, L81, L82, H83, K84, K85, E86, G88, I89, W90, S91, T92, D93, I94, L95, D97, Q98, K99, E100, P101, N103, K104, T105, F106, L107, L123, I126, T128, L130, K135, S136, S137, R138, S140, S141, D142, R157, R159, D161, N162, K163, E181, S183, L184, P185, I186, E187, V188, V190, D191, K195, L196, K197, Y198, E199, N200, T202, S203, S204, F206, and/or D209
S140	V10, V11, E12, L13, D14, W15, Y16, P17, D18, A19, P20, G21, W38, T39, L40, D41, Q42, S43, S44, E45, V46, L47, I55, V57, K58, E59, F60, D62, A63, G64, Q65, Y66, L81, L82, H83, K84, K85, E86, G88, I89, W90, S91, T92, D93, I94, L95, D97, Q98, K99, E100, P101, N103, K104, T105, F106, L107, L123, I126, T128, L130, K135, S136, S137, R138, G139, S141, D142, R157, R159, D161, N162, K163, E181, S183, L184, P185, I186, E187, V188, V190, D191, K195, L196, K197, Y198, E199, N200, T202, S203, S204, F206, and/or D209
S141	V10, V11, E12, L13, D14, W15, Y16, P17, D18, A19, P20, G21, W38, T39, L40, D41, Q42, S43, S44, E45, V46, L47, I55, V57, K58, E59, F60, D62, A63,

	G64, Q65, Y66, L81, L82, H83, K84, K85, E86, G88, I89, W90, S91, T92, D93, I94, L95, D97, Q98, K99, E100, P101, N103, K104, T105, F106, L107, L123, I126, T128, L130, K135, S136, S137, R138, G139, S140, D142, R157, R159, D161, N162, K163, E181, S183, L184, P185, I186, E187, V188, V190, D191, K195, L196, K197, Y198, E199, N200, T202, S203, S204, F206, and/or D209
D142	V10, V11, E12, L13, D14, W15, Y16, P17, D18, A19, P20, G21, W38, T39, L40, D41, Q42, S43, S44, E45, V46, L47, I55, V57, K58, E59, F60, D62, A63, G64, Q65, Y66, L81, L82, H83, K84, K85, E86, G88, I89, W90, S91, T92, D93, I94, L95, D97, Q98, K99, E100, P101, N103, K104, T105, F106, L107, L123, I126, T128, L130, K135, S136, S137, R138, G139, S140, S141, R157, R159, D161, N162, K163, E181, S183, L184, P185, I186, E187, V188, V190, D191, K195, L196, K197, Y198, E199, N200, T202, S203, S204, F206, and/or D209
R157	V10, V11, E12, L13, D14, W15, Y16, P17, D18, A19, P20, G21, W38, T39, L40, D41, Q42, S43, S44, E45, V46, L47, I55, V57, K58, E59, F60, D62, A63, G64, Q65, Y66, L81, L82, H83, K84, K85, E86, G88, I89, W90, S91, T92, D93, I94, L95, D97, Q98, K99, E100, P101, N103, K104, T105, F106, L107, L123, I126, T128, L130, K135, S136, S137, R138, G139, S140, S141, D142, R157, D161, N162, K163, E181, S183, L184, P185, I186, E187, V188, V190, D191, K195, L196, K197, Y198, E199, N200, T202, S203, S204, F206, and/or D209
R159	V10, V11, E12, L13, D14, W15, Y16, P17, D18, A19, P20, G21, W38, T39, L40, D41, Q42, S43, S44, E45, V46, L47, I55, V57, K58, E59, F60, D62, A63, G64, Q65, Y66, L81, L82, H83, K84, K85, E86, G88, I89, W90, S91, T92, D93, I94, L95, D97, Q98, K99, E100, P101, N103, K104, T105, F106, L107, L123, I126, T128, L130, K135, S136, S137, R138, G139, S140, S141, D142, R157, D161, N162, K163, E181, S183, L184, P185, I186, E187, V188, V190, D191, K195, L196, K197, Y198, E199, N200, T202, S203, S204, F206, and/or D209
D161	V10, V11, E12, L13, D14, W15, Y16, P17, D18, A19, P20, G21, W38, T39, L40, D41, Q42, S43, S44, E45, V46, L47, I55, V57, K58, E59, F60, D62, A63, G64, Q65, Y66, L81, L82, H83, K84, K85, E86, G88, I89, W90, S91, T92, D93, I94, L95, D97, Q98, K99, E100, P101, N103, K104, T105, F106, L107, L123, I126, T128, L130, K135, S136, S137, R138, G139, S140, S141, D142, R157, R159, N162, K163, E181, S183, L184, P185, I186, E187, V188, V190, D191, K195, L196, K197, Y198, E199, N200, T202, S203, S204, F206, and/or D209
N162	V10, V11, E12, L13, D14, W15, Y16, P17, D18, A19, P20, G21, W38, T39, L40, D41, Q42, S43, S44, E45, V46, L47, I55, V57, K58, E59, F60, D62, A63, G64, Q65, Y66, L81, L82, H83, K84, K85, E86, G88, I89, W90, S91, T92, D93, I94, L95, D97, Q98, K99, E100, P101, N103, K104, T105, F106, L107, L123, I126, T128, L130, K135, S136, S137, R138, G139, S140, S141, D142, R157, R159, D161, K163, E181, S183, L184, P185, I186, E187, V188, V190, D191, K195, L196, K197, Y198, E199, N200, T202, S203, S204, F206, and/or D209
K163	V10, V11, E12, L13, D14, W15, Y16, P17, D18, A19, P20, G21, W38, T39, L40, D41, Q42, S43, S44, E45, V46, L47, I55, V57, K58, E59, F60, D62, A63, G64, Q65, Y66, L81, L82, H83, K84, K85, E86, G88, I89, W90, S91, T92, D93, I94, L95, D97, Q98, K99, E100, P101, N103, K104, T105, F106, L107,

	L123, I126, T128, L130, K135, S136, S137, R138, G139, S140, S141, D142, R157, R159, D161, N162, E181, S183, L184, P185, I186, E187, V188, V190, D191, K195, L196, K197, Y198, E199, N200, T202, S203, S204, F206, and/or D209
E181	V10, V11, E12, L13, D14, W15, Y16, P17, D18, A19, P20, G21, W38, T39, L40, D41, Q42, S43, S44, E45, V46, L47, I55, V57, K58, E59, F60, D62, A63, G64, Q65, Y66, L81, L82, H83, K84, K85, E86, G88, I89, W90, S91, T92, D93, I94, L95, D97, Q98, K99, E100, P101, N103, K104, T105, F106, L107, L123, I126, T128, L130, K135, S136, S137, R138, G139, S140, S141, D142, R157, R159, D161, N162, K163, S183, L184, P185, I186, E187, V188, V190, D191, K195, L196, K197, Y198, E199, N200, T202, S203, S204, F206, and/or D209
S183	V10, V11, E12, L13, D14, W15, Y16, P17, D18, A19, P20, G21, W38, T39, L40, D41, Q42, S43, S44, E45, V46, L47, I55, V57, K58, E59, F60, D62, A63, G64, Q65, Y66, L81, L82, H83, K84, K85, E86, G88, I89, W90, S91, T92, D93, I94, L95, D97, Q98, K99, E100, P101, N103, K104, T105, F106, L107, L123, I126, T128, L130, K135, S136, S137, R138, G139, S140, S141, D142, R157, R159, D161, N162, K163, E181, L184, P185, I186, E187, V188, V190, D191, K195, L196, K197, Y198, E199, N200, T202, S203, S204, F206, and/or D209
L184	V10, V11, E12, L13, D14, W15, Y16, P17, D18, A19, P20, G21, W38, T39, L40, D41, Q42, S43, S44, E45, V46, L47, I55, V57, K58, E59, F60, D62, A63, G64, Q65, Y66, L81, L82, H83, K84, K85, E86, G88, I89, W90, S91, T92, D93, I94, L95, D97, Q98, K99, E100, P101, N103, K104, T105, F106, L107, L123, I126, T128, L130, K135, S136, S137, R138, G139, S140, S141, D142, R157, R159, D161, N162, K163, E181, S183, P185, I186, E187, V188, V190, D191, K195, L196, K197, Y198, E199, N200, T202, S203, S204, F206, and/or D209
P185	V10, V11, E12, L13, D14, W15, Y16, P17, D18, A19, P20, G21, W38, T39, L40, D41, Q42, S43, S44, E45, V46, L47, I55, V57, K58, E59, F60, D62, A63, G64, Q65, Y66, L81, L82, H83, K84, K85, E86, G88, I89, W90, S91, T92, D93, I94, L95, D97, Q98, K99, E100, P101, N103, K104, T105, F106, L107, L123, I126, T128, L130, K135, S136, S137, R138, G139, S140, S141, D142, R157, R159, D161, N162, K163, E181, S183, L184, I186, E187, V188, V190, D191, K195, L196, K197, Y198, E199, N200, T202, S203, S204, F206, and/or D209
I186	V10, V11, E12, L13, D14, W15, Y16, P17, D18, A19, P20, G21, W38, T39, L40, D41, Q42, S43, S44, E45, V46, L47, I55, V57, K58, E59, F60, D62, A63, G64, Q65, Y66, L81, L82, H83, K84, K85, E86, G88, I89, W90, S91, T92, D93, I94, L95, D97, Q98, K99, E100, P101, N103, K104, T105, F106, L107, L123, I126, T128, L130, K135, S136, S137, R138, G139, S140, S141, D142, R157, R159, D161, N162, K163, E181, S183, L184, P185, E187, V188, V190, D191, K195, L196, K197, Y198, E199, N200, T202, S203, S204, F206, and/or D209
E187	V10, V11, E12, L13, D14, W15, Y16, P17, D18, A19, P20, G21, W38, T39, L40, D41, Q42, S43, S44, E45, V46, L47, I55, V57, K58, E59, F60, D62, A63, G64, Q65, Y66, L81, L82, H83, K84, K85, E86, G88, I89, W90, S91, T92, D93, I94, L95, D97, Q98, K99, E100, P101, N103, K104, T105, F106, L107, L123, I126, T128, L130, K135, S136, S137, R138, G139, S140, S141, D142, R157, R159, D161, N162, K163, E181, S183, L184, P185, I186, V188, V190,

	D191, K195, L196, K197, Y198, E199, N200, T202, S203, S204, F206, and/or D209
V188	V10, V11, E12, L13, D14, W15, Y16, P17, D18, A19, P20, G21, W38, T39, L40, D41, Q42, S43, S44, E45, V46, L47, I55, V57, K58, E59, F60, D62, A63, G64, Q65, Y66, L81, L82, H83, K84, K85, E86, G88, I89, W90, S91, T92, D93, I94, L95, D97, Q98, K99, E100, P101, N103, K104, T105, F106, L107, L123, I126, T128, L130, K135, S136, S137, R138, G139, S140, S141, D142, R157, R159, D161, N162, K163, E181, S183, L184, P185, I186, E187, V190, D191, K195, L196, K197, Y198, E199, N200, T202, S203, S204, F206, and/or D209
V190	V10, V11, E12, L13, D14, W15, Y16, P17, D18, A19, P20, G21, W38, T39, L40, D41, Q42, S43, S44, E45, V46, L47, I55, V57, K58, E59, F60, D62, A63, G64, Q65, Y66, L81, L82, H83, K84, K85, E86, G88, I89, W90, S91, T92, D93, I94, L95, D97, Q98, K99, E100, P101, N103, K104, T105, F106, L107, L123, I126, T128, L130, K135, S136, S137, R138, G139, S140, S141, D142, R157, R159, D161, N162, K163, E181, S183, L184, P185, I186, E187, V188, D191, K195, L196, K197, Y198, E199, N200, T202, S203, S204, F206, and/or D209
D191	V10, V11, E12, L13, D14, W15, Y16, P17, D18, A19, P20, G21, W38, T39, L40, D41, Q42, S43, S44, E45, V46, L47, I55, V57, K58, E59, F60, D62, A63, G64, Q65, Y66, L81, L82, H83, K84, K85, E86, G88, I89, W90, S91, T92, D93, I94, L95, D97, Q98, K99, E100, P101, N103, K104, T105, F106, L107, L123, I126, T128, L130, K135, S136, S137, R138, G139, S140, S141, D142, R157, R159, D161, N162, K163, E181, S183, L184, P185, I186, E187, V188, V190, K195, L196, K197, Y198, E199, N200, T202, S203, S204, F206, and/or D209
K195	V10, V11, E12, L13, D14, W15, Y16, P17, D18, A19, P20, G21, W38, T39, L40, D41, Q42, S43, S44, E45, V46, L47, I55, V57, K58, E59, F60, D62, A63, G64, Q65, Y66, L81, L82, H83, K84, K85, E86, G88, I89, W90, S91, T92, D93, I94, L95, D97, Q98, K99, E100, P101, N103, K104, T105, F106, L107, L123, I126, T128, L130, K135, S136, S137, R138, G139, S140, S141, D142, R157, R159, D161, N162, K163, E181, S183, L184, P185, I186, E187, V188, V190, D191, L196, K197, Y198, E199, N200, T202, S203, S204, F206, and/or D209
L196	V10, V11, E12, L13, D14, W15, Y16, P17, D18, A19, P20, G21, W38, T39, L40, D41, Q42, S43, S44, E45, V46, L47, I55, V57, K58, E59, F60, D62, A63, G64, Q65, Y66, L81, L82, H83, K84, K85, E86, G88, I89, W90, S91, T92, D93, I94, L95, D97, Q98, K99, E100, P101, N103, K104, T105, F106, L107, L123, I126, T128, L130, K135, S136, S137, R138, G139, S140, S141, D142, R157, R159, D161, N162, K163, E181, S183, L184, P185, I186, E187, V188, V190, D191, K195, K197, Y198, E199, N200, T202, S203, S204, F206, and/or D209
K197	V10, V11, E12, L13, D14, W15, Y16, P17, D18, A19, P20, G21, W38, T39, L40, D41, Q42, S43, S44, E45, V46, L47, I55, V57, K58, E59, F60, D62, A63, G64, Q65, Y66, L81, L82, H83, K84, K85, E86, G88, I89, W90, S91, T92, D93, I94, L95, D97, Q98, K99, E100, P101, N103, K104, T105, F106, L107, L123, I126, T128, L130, K135, S136, S137, R138, G139, S140, S141, D142, R157, R159, D161, N162, K163, E181, S183, L184, P185, I186, E187, V188, V190, D191, K195, L196, Y198, E199, N200, T202, S203, S204, F206, and/or D209

Y198	V10, V11, E12, L13, D14, W15, Y16, P17, D18, A19, P20, G21, W38, T39, L40, D41, Q42, S43, S44, E45, V46, L47, I55, V57, K58, E59, F60, D62, A63, G64, Q65, Y66, L81, L82, H83, K84, K85, E86, G88, I89, W90, S91, T92, D93, I94, L95, D97, Q98, K99, E100, P101, N103, K104, T105, F106, L107, L123, I126, T128, L130, K135, S136, S137, R138, G139, S140, S141, D142, R157, R159, D161, N162, K163, E181, S183, L184, P185, I186, E187, V188, V190, D191, K195, L196, K197, E199, N200, T202, S203, S204, F206, and/or D209
E199	V10, V11, E12, L13, D14, W15, Y16, P17, D18, A19, P20, G21, W38, T39, L40, D41, Q42, S43, S44, E45, V46, L47, I55, V57, K58, E59, F60, D62, A63, G64, Q65, Y66, L81, L82, H83, K84, K85, E86, G88, I89, W90, S91, T92, D93, I94, L95, D97, Q98, K99, E100, P101, N103, K104, T105, F106, L107, L123, I126, T128, L130, K135, S136, S137, R138, G139, S140, S141, D142, R157, R159, D161, N162, K163, E181, S183, L184, P185, I186, E187, V188, V190, D191, K195, L196, K197, Y198, N200, T202, S203, S204, F206, and/or D209
N200	V10, V11, E12, L13, D14, W15, Y16, P17, D18, A19, P20, G21, W38, T39, L40, D41, Q42, S43, S44, E45, V46, L47, I55, V57, K58, E59, F60, D62, A63, G64, Q65, Y66, L81, L82, H83, K84, K85, E86, G88, I89, W90, S91, T92, D93, I94, L95, D97, Q98, K99, E100, P101, N103, K104, T105, F106, L107, L123, I126, T128, L130, K135, S136, S137, R138, G139, S140, S141, D142, R157, R159, D161, N162, K163, E181, S183, L184, P185, I186, E187, V188, V190, D191, K195, L196, K197, Y198, E199, T202, S203, S204, F206, and/or D209
T202	V10, V11, E12, L13, D14, W15, Y16, P17, D18, A19, P20, G21, W38, T39, L40, D41, Q42, S43, S44, E45, V46, L47, I55, V57, K58, E59, F60, D62, A63, G64, Q65, Y66, L81, L82, H83, K84, K85, E86, G88, I89, W90, S91, T92, D93, I94, L95, D97, Q98, K99, E100, P101, N103, K104, T105, F106, L107, L123, I126, T128, L130, K135, S136, S137, R138, G139, S140, S141, D142, R157, R159, D161, N162, K163, E181, S183, L184, P185, I186, E187, V188, V190, D191, K195, L196, K197, Y198, E199, N200, S203, S204, F206, and/or D209
D203	V10, V11, E12, L13, D14, W15, Y16, P17, D18, A19, P20, G21, W38, T39, L40, D41, Q42, S43, S44, E45, V46, L47, I55, V57, K58, E59, F60, D62, A63, G64, Q65, Y66, L81, L82, H83, K84, K85, E86, G88, I89, W90, S91, T92, D93, I94, L95, D97, Q98, K99, E100, P101, N103, K104, T105, F106, L107, L123, I126, T128, L130, K135, S136, S137, R138, G139, S140, S141, D142, R157, R159, D161, N162, K163, E181, S183, L184, P185, I186, E187, V188, V190, D191, K195, L196, K197, Y198, E199, N200, T202, S204, F206, and/or D209
S204	V10, V11, E12, L13, D14, W15, Y16, P17, D18, A19, P20, G21, W38, T39, L40, D41, Q42, S43, S44, E45, V46, L47, I55, V57, K58, E59, F60, D62, A63, G64, Q65, Y66, L81, L82, H83, K84, K85, E86, G88, I89, W90, S91, T92, D93, I94, L95, D97, Q98, K99, E100, P101, N103, K104, T105, F106, L107, L123, I126, T128, L130, K135, S136, S137, R138, G139, S140, S141, D142, R157, R159, D161, N162, K163, E181, S183, L184, P185, I186, E187, V188, V190, D191, K195, L196, K197, Y198, E199, N200, T202, S203, F206, and/or D209
F206	V10, V11, E12, L13, D14, W15, Y16, P17, D18, A19, P20, G21, W38, T39, L40, D41, Q42, S43, S44, E45, V46, L47, I55, V57, K58, E59, F60, D62, A63,

	G64, Q65, Y66, L81, L82, H83, K84, K85, E86, G88, I89, W90, S91, T92, D93, I94, L95, D97, Q98, K99, E100, P101, N103, K104, T105, F106, L107, L123, I126, T128, L130, K135, S136, S137, R138, G139, S140, S141, D142, R157, R159, D161, N162, K163, E181, S183, L184, P185, I186, E187, V188, V190, D191, K195, L196, K197, Y198, E199, N200, T202, S203, S204, and/or D209
D209	V10, V11, E12, L13, D14, W15, Y16, P17, D18, A19, P20, G21, W38, T39, L40, D41, Q42, S43, S44, E45, V46, L47, I55, V57, K58, E59, F60, D62, A63, G64, Q65, Y66, L81, L82, H83, K84, K85, E86, G88, I89, W90, S91, T92, D93, I94, L95, D97, Q98, K99, E100, P101, N103, K104, T105, F106, L107, L123, I126, T128, L130, K135, S136, S137, R138, G139, S140, S141, D142, R157, R159, D161, N162, K163, E181, S183, L184, P185, I186, E187, V188, V190, D191, K195, L196, K197, Y198, E199, N200, T202, S203, S204, and/or F206

[0143] In one aspect of the invention, the modified IL-12 protein comprises a p40 subunit with a substitution or deletion at an amino acid residue corresponding to amino acid Y16 of SEQ ID NO: 2. In some embodiments, the modified IL-12 protein comprises a p40 subunit comprising a substitution at an amino acid corresponding to amino acid residue position Y16 of SEQ ID NO: 2 and a p35 subunit with one more substitutions. For exemplary combinations, see Table 6.

Table 6. Exemplary Combinations

p40 Substitution	p35 Substitution
V10	A33
V10	R34
V10	L37
V10	E38
V10	F39
V10	Y40
V10	P41
V10	T43
V10	S44
V10	E45
V10	E46
V10	I47
V10	D48
V10	H49
V10	E50
V10	D51
V10	I52
V10	T53
V10	K54
V10	K56
V10	T57
V10	D108
V10	L109
V10	K110

V10	M111
V10	Y112
V10	Q113
V10	V114
V10	E115
V10	F116
V10	K117
V10	T118
V10	A121
V10	L123
V10	M125
V10	D126
V10	K128
V10	R129
V10	Q130
V10	I131
V10	L133
V10	P156
V10	S159
V10	S160
V10	L161
V10	E162
V10	E163
V10	P164
V10	D165
V10	F166
V10	Y167
V10	K168
V10	T169
V10	K170
V10	I171
V10	K172
V10	L173
V10	I175
V10	L177
V10	H178
V10	R181
Y16	A33
Y16	R34
Y16	L37
Y16	E38
Y16	F39
Y16	Y40
Y16	P41
Y16	T43
Y16	S44
Y16	E45
Y16	E46
Y16	I47

Y16	D48
Y16	H49
Y16	E50
Y16	D51
Y16	I52
Y16	T53
Y16	K54
Y16	K56
Y16	T57
Y16	D108
Y16	L109
Y16	K110
Y16	M111
Y16	Y112
Y16	Q113
Y16	V114
Y16	E115
Y16	F116
Y16	K117
Y16	T118
Y16	A121
Y16	L123
Y16	M125
Y16	D126
Y16	K128
Y16	R129
Y16	Q130
Y16	I131
Y16	L133
Y16	P156
Y16	S159
Y16	S160
Y16	L161
Y16	E162
Y16	E163
Y16	P164
Y16	D165
Y16	F166
Y16	Y167
Y16	K168
Y16	T169
Y16	K170
Y16	I171
Y16	K172
Y16	L173
Y16	I175
Y16	L177
Y16	H178
Y16	R181

I94	A33
I94	R34
I94	L37
I94	E38
I94	F39
I94	Y40
I94	P41
I94	T43
I94	S44
I94	E45
I94	E46
I94	I47
I94	D48
I94	H49
I94	E50
I94	D51
I94	I52
I94	T53
I94	K54
I94	K56
I94	T57
I94	D108
I94	L109
I94	K110
I94	M111
I94	Y112
I94	Q113
I94	V114
I94	E115
I94	F116
I94	K117
I94	T118
I94	A121
I94	L123
I94	M125
I94	D126
I94	K128
I94	R129
I94	Q130
I94	I131
I94	L133
I94	P156
I94	S159
I94	S160
I94	L161
I94	E162
I94	E163
I94	P164
I94	D165

I94	F166
I94	Y167
I94	K168
I94	T169
I94	K170
I94	I171
I94	K172
I94	L173
I94	I175
I94	L177
I94	H178
I94	R181
L95	A33
L95	R34
L95	L37
L95	E38
L95	F39
L95	Y40
L95	P41
L95	T43
L95	S44
L95	E45
L95	E46
L95	I47
L95	D48
L95	H49
L95	E50
L95	D51
L95	I52
L95	T53
L95	K54
L95	K56
L95	T57
L95	D108
L95	L109
L95	K110
L95	M111
L95	Y112
L95	Q113
L95	V114
L95	E115
L95	F116
L95	K117
L95	T118
L95	A121
L95	L123
L95	M125
L95	D126
L95	K128

L95	R129
L95	Q130
L95	I131
L95	L133
L95	P156
L95	S159
L95	S160
L95	L161
L95	E162
L95	E163
L95	P164
L95	D165
L95	F166
L95	Y167
L95	K168
L95	T169
L95	K170
L95	I171
L95	K172
L95	L173
L95	I175
L95	L177
L95	H178
L95	R181

IL-12 Mutant Combinations

In some embodiments, the IL-12 mutein comprises one or more substitutions in the p35 subunit and one or more substitutions in the p40 subunit. In some embodiments, the IL-12 mutein comprises a p35 subunit and a p40 subunit, wherein the p35 subunit comprises an amino acid substitution and/or deletion at an amino acid residue corresponding to amino acid residue A33, R34, L37, E38, F39, Y40, P41, T43, S44, E45, E46, I47, D48, H49, E50, D51, I52, T53, K54, K56, T57, D108, L109, K110, M111, Y112, Q113, V114, E115, F116, K117, T118, A121, L123, M125, D126, K128, R129, Q130, I131, L133, P156, S159, S160, L161, E162, E163, P164, D165, F166, Y167, K168, T169, K170, I171, K172, L173, I175, L177, H178, or R181 with respect to the position in the sequence of mature p35 (SEQ ID NO: 1), and wherein the p40 subunit comprises one substitution selected from the p40 substitution column of Table 5, and optionally wherein the p40 subunit comprises two or more substitutions selected from the corresponding additional substitutions column of Table 5. In some embodiments, the IL-12 mutein comprises a p35 subunit and a p40 subunit, wherein the p40 subunit comprises an amino acid substitution and/or deletion at amino position V10, V11, E12, L13, D14, W15, Y16, P17, D18, A19, P20, G21, W38, T39, L40, D41, Q42, S43, S44, E45, V46, L47, I55, V57, K58, E59, F60, D62, A63, G64,

Q65, Y66, L81, L82, H83, K84, K85, E86, G88, I89, W90, S91, T92, D93, I94, L95, D97, Q98, K99, E100, P101, N103, K104, T105, F106, L107, L123, I126, T128, L130, K135, S136, S137, R138, G139, S140, S141, D142, R157, R159, D161, N162, K163, E181, S183, L184, P185, I186, E187, V188, V190, D191, K195, L196, K197, Y198, E199, N200, T202, S203, S204, F206, and/or D209 with respect to the position in mature p40 (SEQ ID NO: 2), and wherein the p35 subunit comprises one substitution selected from the p35 substitution column of Table 2, and optionally wherein the p35 subunit comprises two or more substitutions selected from the corresponding additional substitutions column of Table 2.

Fusion Proteins and Methods of use

[0144] In certain embodiments, it is contemplated that the IL-12 muteins of the present invention also may be fused with other proteins or peptides to create fusion proteins. In some embodiments, an IL-12 mutein may be fused to a half-life extending moiety.

[0145] In certain embodiments, it is contemplated that the IL-12 muteins of the present invention may be used in a method for preventing a disease or disorder.

Polynucleotides, Vectors, and Host Cells

[0146] Disclosed herein are also polynucleotides, vectors, and host cells encoding any IL-12 mutein disclosed herein, and/or the reverse complements of such polynucleotides. A polynucleotide of the invention comprises a nucleic acid encoding an IL-12 mutein comprising a p35 subunit and a p40 subunit, wherein the p35 subunit comprises an amino acid sequence with one or more substitutions or deletions relative to SEQ ID NO: 1, and wherein the p40 subunit comprises an amino acid sequence with one or more substitutions or deletions relative to SEQ ID NO: 2. In some embodiments, the nucleic acid encodes an IL-12 mutein comprising a p35 subunit and a p40 subunit, wherein the p35 subunit comprises an amino acid substitution and/or deletion at an amino acid residue corresponding to amino acid residue A33, R34, L37, E38, F39, Y40, P41, T43, S44, E45, E46, I47, D48, H49, E50, D51, I52, T53, K54, K56, T57, D108, L109, K110, M111, Y112, Q113, V114, E115, F116, K117, T118, A121, L123, M125, D126, K128, R129, Q130, I131, L133, P156, S159, S160, L161, E162, E163, P164, D165, F166, Y167, K168, T169, K170, I171, K172, L173, I175, L177, H178, or R181 with respect to the position in the sequence of mature p35 (SEQ ID NO: 1), and wherein the p40 subunit comprises two or more substitutions selected from Table 5. In some embodiments, the nucleic acid encodes an IL-12 mutein comprising a p35 subunit and a p40 subunit, wherein the p40 subunit comprises an amino acid substitution and/or deletion at amino position V10, V11, E12, L13, D14, W15, Y16, P17, D18, A19, P20, G21, W38, T39, L40, D41, Q42, S43, S44, E45, V46, L47, I55, V57, K58, E59, F60, D62, A63, G64, Q65, Y66, L81, L82, H83, K84, K85, E86, G88, I89, W90, S91,

T92, D93, I94, L95, D97, Q98, K99, E100, P101, N103, K104, T105, F106, L107, L123, I126, T128, L130, K135, S136, S137, R138, G139, S140, S141, D142, R157, R159, D161, N162, K163, E181, S183, L184, P185, I186, E187, V188, V190, D191, K195, L196, K197, Y198, E199, N200, T202, S203, S204, F206, and/or D209 with respect to the position in mature p40 (SEQ ID NO: 2), and wherein the p35 subunit comprises two or more substitutions selected from Table 2.

[0147] Expression vectors of the invention comprise a nucleic acid or a set of nucleic acids encoding any IL-12 mutein disclosed herein, and a regulatory sequence operably linked to the nucleic acid or the set of nucleic acids.

[0148] Host cells of the invention may be prokaryotic or eukaryotic in origin. In some embodiments, IL-12 muteins of the instant invention are produced in eukaryotic cells. In some embodiments, the eukaryotic cell is a mammalian cell. In some embodiments, the mammalian cell is a human cell. In some embodiments, the human cell is a HEK293 cell, a CHO cell, or a genetic variant thereof. In some embodiments, IL-12 muteins of the instant invention are produced in prokaryotic cells. In some embodiments, the prokaryotic cell is *E. coli* or a genetic variant thereof.

EXAMPLES

General Techniques

[0149] The practice of the present invention employs, unless otherwise indicated, conventional techniques of immunology, biochemistry, chemistry, molecular biology, microbiology, cell biology, genomics and recombinant DNA, which are within the skill of the art. *See* Sambrook, J. *et al.*, “Molecular Cloning: A Laboratory Manual,” 3rd edition, Cold Spring Harbor Laboratory Press, 2001; “Current protocols in molecular biology”, F. M. Ausubel, *et al.* eds., 1987; the series “Methods in Enzymology,” Academic Press, San Diego, CA.; “PCR 2: a practical approach”, M.J. MacPherson, B.D. Hames and G.R. Taylor eds., Oxford University Press, 1995; “Antibodies, a laboratory manual” Harlow, E. and Lane, D. eds., Cold Spring Harbor Laboratory, 1988; “Goodman & Gilman’s The Pharmacological Basis of Therapeutics,” 11th Edition, McGraw-Hill, 2005; and Freshney, R.I., “Culture of Animal Cells: A Manual of Basic Technique,” 4th edition, John Wiley & Sons, Somerset, NJ, 2000, the contents of which are incorporated in their entirety herein by reference.

Example 1: Structure Modeling of IL-12 Interactions

IL-12 interacts with IL-12Rb1 and IL-12Rb2. To identify amino acid residues that, when mutated, may result in an altered binding affinity with these two receptors, a predictive model was

developed. To obtain high resolution information, AlphaFold2 and molecular dynamic (MD) simulation techniques were used to model the IL-12-IL-12R interactions from the low-resolution ($\sim 10\text{\AA}$) cryoEM map. Firstly, the structure of IL-12Rb2 was modeled by AlphaFold2 from its primary sequences. Secondly, the crystal structure of IL-12 (PDB 1F45), the crystal structure of IL-12 p40- IL-12-Rb1 (PDB 6WDQ), and the AlphaFold2 model of IL-12Rb2 were rigid-body fitted into the cryoEM map (EMD-21645) by Chimera (Pettersen EF, et al. J Comput Chem. 2004 Oct;25(13):1605-12.) (Fig. 1).

Thirdly, the missing loops in IL-12 p35 were modeled by AlphaFold2. Finally, the resulting model was subjected to 200ns MD simulation by GROMACS to optimize and stabilize the structure and interactions. The IL-12-IL-12R complex remains stable during the 200 ns simulation time, and only minor conformational changes happened comparing to the starting model (Fig. 2). The structure model at the 200ns snapshot was used for downstream design. Based on above, region of putative receptor interaction region were identified (Table 7 and Fig. 3).

TABLE 7. IL-12 / IL-12R Interaction Regions Identified by Structure Modeling

p35 substitution regions identified	p40 substitution regions identified
Amino acids 36-52; 124-133; 163-178	Amino acids 14-18; 41-45; 58-62; 84-107; 195-197; 201-202

Example 2: In Silico Screening of IL-12 Single Mutants

[0150] In addition to the structure modeling described above, IL-12 p35 and p40 single mutants were screened *in silico* using a Molecular Operating Environment (MOE) modeling system using the structure model described above to predict change in affinity (MOE δ Affinity) and change in stability (MOE δ Stability) relative to wild-type IL-12. In total, (306 p40 residues + 178 p35 residues) x 19 amino acid substitutions = 9196 single point substitutions were modeled and then computed for both affinity changes for binding IL-12 receptors, and stability changes. The substitution sites identified by this *in silico* screen are shown in Table 8 and were selected according to the following criteria: MOE δ Affinity of > 0.05 kcal/mol (where an increase in MOE δ Affinity predicts a decrease in receptor affinity) and MOE δ Stability of < 6 kcal/mol (where an increase in MOE δ Stability predicts a decrease in stability). Thus the substitutions in Table 8 predict IL-12 having decreased affinity relative to wild-type IL-12, while maintain the stability of IL-12. The predicted immunogenicity of the single mutants according to a proprietary putative T cell epitope score algorithm was also considered in the selection of IL-12

mutants. The MOE δ Affinity and MOE δ Stability values of selected p35 mutants are provided in Table 9 and of selected p40 mutants are provided in Table 10.

Table 8. Identified Substitution Sites

p35 substitution sites identified in silico	p40 substitution sites identified in silico
A33, R34, E38, Y40, P41, E45, E46, I47, D48, H49, E50, D51, I52, T53, K54, K56, T57, D108, L109, K110, M111, Y112, Q113, V114, E115, F116, K117, T118, A121, L123, D126, I131, P156, S159, S160, L161, E162, E163, D165, F166, Y167, K168, T169, K170, I171, K172, L173, I175, R181	V10, V11, E12, L13, D14, W15, Y16, P17, D18, A19, P20, G21, W38, T39, L40, D41, S43, S44, E45, V46, L47, I55, V57, K58, E59, F60, D62, A63, G64, Q65, Y66, L81, L82, H83, K84, K85, E86, G88, I89, W90, S91, T92, D93, I94, L95, D97, Q98, K99, E100, N103, K104, T105, F106, L107, L123, K135, S136, S137, R138, G139, S140, S141, D142, N162, E181, S183, L184, P185, I186, E187, V188, V190, D191, K195, L196, K197, Y198, E199, N200, T202, S203, S204, F206, D209

Table 9. Predicted Binding Dynamics for Additional Amino Acid Substitutions in p35 Subunit

Substitution	MOE Affinity	MOE δ Affinity	MOE Stability	MOE δ Stability
33H	-197.24368	0.07706748	-3188.81955	0.43393749
33M	-197.264988	0.05575886	-3189.08725	0.16623547
33T	-197.233815	0.08693182	-3188.93702	0.31646011
34A	-197.265095	0.05725721	-3175.17811	1.37185627
38M	-197.096291	0.09611362	-3183.76422	0.24929748
40A	-196.933023	0.19509972	-3187.89059	2.63824896
40D	-196.794234	0.33388875	-3187.75432	2.77451277
40E	-196.905781	0.22234225	-3188.48228	2.0465535
40F	-196.987019	0.14110435	-3189.44516	1.08367903
40G	-196.928443	0.19967975	-3186.78942	3.73941991
40H	-196.802718	0.32540502	-3188.43763	2.09120233
40I	-196.80928	0.31884277	-3189.45345	1.07538706
40K	-196.832135	0.29598818	-3188.25158	2.27725667
40L	-196.781178	0.34694534	-3189.65051	0.87832858
40M	-196.855545	0.27257837	-3189.0804	1.44843242
40N	-196.919234	0.20888925	-3188.10791	2.42093152
40P	-196.822998	0.30512526	-3188.17864	2.35019721
40Q	-196.866168	0.26195536	-3188.62494	1.90389773
40R	-196.807929	0.32019403	-3188.97021	1.55862539
40S	-196.776641	0.35148159	-3187.61238	2.9164564
40T	-196.923353	0.2047702	-3188.15349	2.3753469
40V	-196.748823	0.37930022	-3188.89647	1.63237115
40W	-196.994071	0.13405151	-3189.56884	0.95999368
41A	-196.991243	0.35973134	-3189.3983	1.15722867
41G	-196.725424	0.62555067	-3188.57084	1.98469588
41S	-196.799107	0.55186759	-3189.20825	1.34728017
41T	-197.256139	0.09483544	-3189.36735	1.18817886
45D	-196.825952	0.1659472	-3182.83931	0.59615865
45F	-196.450546	0.54135296	-3184.05509	-0.61961615
45H	-196.204808	0.78709157	-3182.99064	0.44483064
45I	-196.727767	0.26413253	-3183.27256	0.16290754

45K	-196.094433	0.89746636	-3183.25383	0.18163706
45L	-196.673476	0.31842287	-3183.42646	0.00900594
45M	-196.520334	0.47156564	-3183.07617	0.3593036
45N	-196.670375	0.32152468	-3182.22983	1.2056428
45Q	-196.173158	0.81874157	-3182.4765	0.9589734
45R	-195.868728	1.12317099	-3183.79415	-0.35867662
45T	-195.611594	1.38030555	-3183.26234	0.1731265
45V	-196.47599	0.51590937	-3182.80717	0.62830379
45W	-196.349442	0.64245711	-3184.28226	-0.84678987
45Y	-196.446951	0.54494824	-3183.33602	0.09945122
46A	-198.336331	0.72760514	-3183.5369	0.63927002
46D	-196.651108	2.41282737	-3182.83707	1.33909928
46G	-197.183692	1.88024339	-3183.00833	1.16784568
46H	-197.920157	1.14377793	-3183.77635	0.39982004
46K	-196.911328	2.15260799	-3183.77092	0.40525236
46N	-198.927778	0.13615736	-3183.39576	0.78041676
46S	-198.718377	0.34555874	-3183.33554	0.84063516
46V	-198.291665	0.77227095	-3184.60841	-0.43223688
47F	-195.583268	0.1707344	-3188.49645	0.7625365
47K	-195.536134	0.21786775	-3187.98238	1.27660927
47L	-195.689745	0.0642573	-3188.7186	0.5403912
47Q	-195.124488	0.62951393	-3187.8647	1.39428463
47R	-195.309268	0.44473386	-3189.18514	0.0738443
47S	-195.512466	0.24153564	-3187.6919	1.56708681
47T	-195.228725	0.52527699	-3187.85107	1.40792058
47W	-195.172933	0.58106894	-3189.08779	0.17120326
47Y	-195.521684	0.23231823	-3188.65567	0.60331389
48E	-196.873215	0.16375753	-3182.95349	0.30245853
48F	-194.255471	2.78150205	-3183.32793	-0.07197609
48G	-192.778592	4.25838131	-3182.49795	0.75799957
48I	-196.329747	0.70722601	-3183.91178	-0.65582258
48K	-195.356822	1.68015057	-3184.46158	-1.20562431
48L	-196.557498	0.47947527	-3184.49397	-1.23801968
48M	-196.936046	0.10092728	-3184.40334	-1.14738264
48N	-196.98236	0.05461335	-3183.09988	0.15607431
48P	-195.437174	1.5997994	-3182.68305	0.57290358
48Q	-195.999416	1.03755676	-3183.218	0.03795676
48T	-196.087434	0.94953911	-3182.98758	0.26836955
48V	-196.383167	0.65380558	-3184.17627	-0.92031189
49A	-195.269558	0.82075663	-3186.77471	1.16156243
49E	-193.76565	2.32466491	-3188.05641	-0.12013702
49F	-195.573003	0.5173121	-3188.20432	-0.26804351
49G	-194.20929	1.88102487	-3186.09708	1.83919811
49I	-195.811427	0.27888783	-3188.18166	-0.24538755
49K	-195.770737	0.31957798	-3187.38107	0.55521049
49L	-195.736416	0.35389833	-3188.39332	-0.45704687
49M	-195.919749	0.17056568	-3187.63342	0.30285633
49Q	-195.713685	0.37662936	-3187.28311	0.65316781
49R	-194.594365	1.49594941	-3188.12396	-0.18768664
49S	-193.826874	2.26344107	-3186.8171	1.11917324
49T	-193.982881	2.10743332	-3187.49874	0.43753591
49V	-194.982254	1.10806116	-3187.79991	0.13636733
49W	-195.47577	0.61454422	-3188.45321	-0.51693551
49Y	-195.022809	1.06750621	-3188.66916	-0.73288029
50A	-192.030971	7.70258445	-3184.11235	1.91030867
50D	-196.314549	3.4190069	-3184.26304	1.75961856
50F	-197.320133	2.41342271	-3185.62408	0.3985778
50G	-189.742859	9.99069607	-3183.23072	2.79193938

50H	-194.272229	5.46132661	-3184.61943	1.4032276
50I	-199.575277	0.15827845	-3185.7481	0.27455879
50K	-192.299182	7.43437342	-3183.5418	2.48085104
50L	-193.717415	6.01614039	-3184.87153	1.15113037
50M	-195.576109	4.15744682	-3184.88577	1.13688427
50N	-194.398287	5.3352682	-3184.30207	1.72058159
50P	-192.789116	6.94443916	-3184.33831	1.68434096
50Q	-196.799112	2.93444306	-3185.24867	0.77398236
50R	-192.95092	6.78263557	-3185.11759	0.90506062
50S	-193.214793	6.5187625	-3184.07994	1.94271078
50T	-193.669989	6.06356688	-3183.79513	2.22752666
50V	-194.679458	5.05409791	-3185.25178	0.77088013
50W	-194.837343	4.89621208	-3186.35445	-0.33179804
50Y	-197.034502	2.69905334	-3185.87182	0.15083304
51G	-193.215515	0.80934263	-3183.09451	0.66570062
52A	-198.043498	0.92259	-3189.05026	2.32956535
52D	-196.377421	2.58866733	-3188.20359	3.17623605
52E	-198.202852	0.76323651	-3189.00989	2.36993361
52G	-197.559778	1.40630952	-3188.3214	3.05841962
52K	-197.6419	1.32418849	-3190.31602	1.06380605
52L	-198.827581	0.1385072	-3190.54613	0.83369214
52N	-198.14156	0.82452817	-3189.18759	2.19222813
52P	-198.003818	0.96226975	-3189.62898	1.75083946
52T	-198.348211	0.61787661	-3189.41787	1.96195735
52Y	-197.907746	1.05834249	-3189.64164	1.7381802
53A	-193.460507	0.15905492	-3186.91523	0.88294844
53D	-192.751954	0.86760753	-3186.35268	1.44550322
53H	-192.894206	0.72535519	-3184.82171	2.9764716
53P	-192.512898	1.10666361	-3186.14316	1.65502249
53S	-193.333255	0.28630685	-3186.43471	1.36346768
53Y	-192.92567	0.69389157	-3187.8079	-0.00972167
54D	-188.653917	0.45341281	-3184.58804	1.67248604
54E	-188.713868	0.39346089	-3185.52021	0.74031615
54G	-188.201433	0.90589644	-3185.13681	1.12371857
54W	-189.025075	0.08225444	-3187.62931	-1.36878456
56A	-193.048777	0.72656447	-3185.78734	1.56118373
56E	-193.303783	0.47155894	-3185.91688	1.43164256
56G	-192.745321	1.03002058	-3185.23814	2.11038632
56I	-193.510479	0.26486207	-3187.40761	-0.05908241
56L	-193.454953	0.3203882	-3186.21688	1.13164683
56N	-193.602656	0.17268523	-3186.20628	1.14224224
56S	-192.788498	0.98684353	-3185.88069	1.46783288
56T	-193.155899	0.6194427	-3186.05445	1.29407621
56V	-193.321724	0.45361723	-3186.51413	0.83439353
57A	-193.045494	0.30163747	-3185.01748	1.66805454
57D	-193.270698	0.07643355	-3185.24923	1.43630335
57E	-193.198246	0.14888517	-3186.81541	-0.12988338
57G	-193.249025	0.09810635	-3183.81751	2.86802155
57N	-192.70338	0.64375148	-3185.76893	0.9165996
57Q	-192.046648	1.30048387	-3186.20116	0.48436681
57R	-192.458745	0.88838639	-3186.66501	0.02052115
57V	-193.286251	0.06088085	-3186.16477	0.52076186
57W	-193.08447	0.2626615	-3186.18646	0.4990699
108R	-197.034045	0.18713871	-3184.6839	-0.33468277
109Y	-197.230036	0.06230429	-3187.45168	1.667667
110Y	-197.139173	0.08279859	-3187.25766	0.73724111
111A	-197.612189	0.20248342	-3188.81003	1.60999862
111G	-197.748469	0.06620257	-3187.78799	2.6320368

111H	-197.32931	0.48536192	-3189.88559	0.53443452
111K	-197.162075	0.65259707	-3189.74004	0.67999274
111L	-197.566293	0.24837927	-3190.93456	-0.51453581
111N	-197.465654	0.34901759	-3189.71717	0.70285796
111P	-197.528062	0.28660991	-3188.74336	1.67666414
111Q	-197.63531	0.1793615	-3188.87555	1.54447987
111R	-196.719969	1.09470339	-3190.8172	-0.39716645
111V	-197.740138	0.07453389	-3190.79521	-0.37518345
112H	-197.365197	0.15094167	-3187.40389	1.91203336
112K	-196.948658	0.56748001	-3188.0165	1.29942921
112P	-197.27696	0.23917869	-3185.53822	3.77770296
112R	-197.164516	0.3516226	-3187.10265	2.21327062
113A	-197.11734	0.21356886	-3183.34437	1.20805569
113F	-197.271878	0.05903126	-3184.67726	-0.12483048
113G	-197.191573	0.13933637	-3182.84271	1.7097125
113K	-197.161471	0.16943758	-3183.97998	0.57244304
113L	-197.270785	0.06012409	-3184.73399	-0.18156519
113M	-197.241489	0.08941963	-3184.36414	0.18829
113N	-197.216382	0.11452683	-3183.53283	1.01959311
113P	-197.164485	0.16642408	-3182.26168	2.29074544
113R	-197.217113	0.11379599	-3184.37823	0.17419599
113S	-197.196256	0.13465305	-3183.27792	1.27450313
113T	-197.116738	0.21417097	-3183.43896	1.11346044
113V	-197.072812	0.25809688	-3183.88388	0.66854206
113W	-197.229022	0.10188641	-3184.06311	0.48931635
113Y	-197.22866	0.10224905	-3184.63424	-0.08181912
114A	-197.389415	1.41916667	-3187.70079	1.4994669
114D	-198.058218	0.75036447	-3187.17169	2.02856314
114E	-198.594278	0.21430359	-3187.87237	1.32789171
114F	-198.00842	0.8001621	-3188.56711	0.63314412
114G	-197.577004	1.23157813	-3186.6763	2.52395366
114H	-197.516098	1.29248424	-3186.91572	2.28454038
114I	-196.766198	2.04238403	-3189.18605	0.01420923
114K	-196.765346	2.04323625	-3187.51573	1.68452277
114L	-197.486087	1.32249526	-3188.32017	0.88009163
114M	-197.939998	0.86858411	-3188.38648	0.81377953
114N	-198.215706	0.59287631	-3187.57461	1.62564496
114P	-197.621853	1.18672862	-3187.77597	1.42429204
114Q	-198.141387	0.6671952	-3188.14734	1.05291724
114R	-197.551367	1.25721524	-3188.79371	0.40654669
114S	-196.889231	1.91935118	-3187.61513	1.58512684
114T	-196.731296	2.07728649	-3187.86399	1.33627034
114W	-197.556211	1.25237113	-3187.6569	1.54335729
114Y	-197.362419	1.44616272	-3187.98457	1.21568535
115G	-197.590506	3.35937348	-3183.1213	2.81290234
115H	-199.979294	0.97058579	-3184.52463	1.40957015
115K	-197.494674	3.45520548	-3184.84295	1.0912564
115P	-197.082917	3.86696267	-3183.87721	2.05698927
115Q	-199.373527	1.57635299	-3185.07414	0.86006255
115R	-196.533162	4.41671733	-3186.02677	-0.0925671
115S	-197.563359	3.38652057	-3183.78938	2.14482397
115T	-198.406658	2.54322157	-3184.56695	1.36725448
115V	-197.97357	2.97630971	-3185.13807	0.79613862
115W	-198.140273	2.80960621	-3185.58394	0.35026213
116A	-197.840245	0.10142145	-3189.25468	2.64869551
116E	-197.821933	0.1197335	-3189.447	2.45637276
116H	-197.87584	0.06582677	-3189.56185	2.34152155
116K	-197.522794	0.41887248	-3189.33575	2.56762361

116L	-197.857018	0.08464852	-3190.46444	1.4389387
116M	-197.885583	0.05608415	-3190.34597	1.55740852
116N	-197.865383	0.07628364	-3188.9441	2.9592757
116P	-197.525975	0.41569219	-3188.69636	3.20701529
116Q	-197.792597	0.14907011	-3189.35333	2.5500502
116R	-197.653615	0.2880524	-3190.0591	1.84427787
116S	-197.859507	0.08216012	-3189.00503	2.89834793
116T	-197.716434	0.22523321	-3189.164	2.73937222
116V	-197.832204	0.1094634	-3189.8613	2.04207314
116W	-197.6667	0.27496656	-3189.8796	2.02377152
117E	-197.783129	0.13610198	-3185.98867	1.16313991
117P	-197.820991	0.09824015	-3185.51747	1.63433466
118D	-196.808569	0.29549723	-3186.71555	1.38420248
121K	-197.448925	0.21733155	-3188.49372	1.22130086
123D	-197.399609	1.83154908	-3187.40312	2.46078526
123E	-198.951319	0.27983858	-3187.54585	2.31805202
123G	-196.148727	3.08243041	-3186.38263	3.48127482
123Q	-198.80541	0.42574753	-3188.06206	1.8018441
123S	-197.945296	1.28586209	-3187.13251	2.73139175
123T	-198.523972	0.70718594	-3187.74231	2.12159249
126P	-193.305938	0.98829397	-3184.00895	1.40110472
131D	-195.667057	0.24707543	-3188.70607	2.37312396
131E	-195.75869	0.15544235	-3189.42851	1.65068387
131Q	-195.766592	0.14754014	-3189.18125	1.89794527
156W	-197.14581	0.09469158	-3188.87728	0.03544636
159A	-198.630486	0.33691745	-3188.68373	0.3534827
159E	-195.218514	3.74888898	-3188.69996	0.33725353
159G	-198.097819	0.86958362	-3187.48987	1.54734278
159P	-198.05024	0.91716343	-3188.15406	0.8831558
160D	-195.407411	0.38489789	-3186.70773	0.80690451
160E	-195.338396	0.45391383	-3186.29432	1.22031088
160K	-194.83849	0.95381933	-3185.79511	1.7195165
160R	-195.024904	0.76740548	-3186.62107	0.89355533
160V	-195.191097	0.60121219	-3187.01211	0.50252376
161K	-195.278732	0.27296049	-3185.52034	1.15911884
161N	-195.366165	0.18552688	-3185.7428	0.93666451
161R	-195.348542	0.20314972	-3185.6364	1.04306032
161T	-195.449692	0.10200043	-3185.8266	0.85286591
162A	-193.659223	0.20592939	-3183.88969	0.55875098
162G	-193.256897	0.60825539	-3182.99541	1.45303538
163A	-197.237371	1.61807764	-3183.75536	1.06875566
163D	-198.698579	0.15686994	-3183.63308	1.19103322
163F	-198.288373	0.56707631	-3184.63289	0.19122768
163G	-197.38393	1.47151912	-3183.35345	1.4706631
163H	-198.403791	0.45165747	-3183.38575	1.43836728
163I	-197.651331	1.2041182	-3185.07785	-0.25373508
163K	-196.77349	2.08195943	-3182.50478	2.31933491
163L	-197.234572	1.62087689	-3184.28382	0.54029178
163M	-197.05578	1.79966846	-3184.0067	0.81741733
163N	-198.45666	0.39878855	-3183.87289	0.95122338
163P	-197.528333	1.32711636	-3183.66332	1.16079128
163Q	-197.191578	1.66387097	-3183.90062	0.92348956
163R	-196.667544	2.18790516	-3183.46725	1.35686847
163S	-196.764709	2.09074017	-3183.73899	1.08512531
163T	-198.617943	0.23750634	-3184.14213	0.68198484
163Y	-198.63	0.22544943	-3184.40552	0.41859037
165A	-197.011105	2.45145046	-3184.04629	0.72944919
165E	-198.63684	0.82571569	-3184.49563	0.28011236

165F	-196.84811	2.61444592	-3184.83663	-0.06088789
165G	-197.359709	2.10284723	-3183.44074	1.3350032
165H	-197.531551	1.93100487	-3183.88723	0.88851736
165I	-197.119519	2.3430365	-3185.26214	-0.48639718
165L	-198.496313	0.96624277	-3185.30604	-0.53029837
165M	-196.763956	2.69859953	-3184.37949	0.39624855
165N	-196.616474	2.8460814	-3184.0064	0.76934407
165S	-197.098299	2.36425703	-3184.28456	0.49118134
165T	-197.741156	1.72139977	-3184.9217	-0.14595647
165V	-198.053819	1.4087373	-3184.66134	0.11440621
166A	-196.001425	2.9703729	-3189.17062	2.4221685
166D	-196.259199	2.71259903	-3188.99618	2.59660314
166E	-196.757816	2.21398263	-3189.14933	2.44345657
166G	-196.592436	2.3793626	-3188.37651	3.21627181
166H	-198.80996	0.16183816	-3189.77905	1.81373748
166L	-197.0739	1.89789832	-3190.38674	1.20604449
166M	-198.604945	0.36685369	-3190.32643	1.26635353
166N	-197.001066	1.97073255	-3189.07522	2.51756223
166P	-198.394333	0.5774654	-3188.97784	2.61494717
166S	-196.999667	1.97213092	-3189.14355	2.44923197
166T	-197.602408	1.3693905	-3189.48399	2.10879405
166V	-198.596006	0.37579192	-3189.98944	1.60334595
167A	-193.68877	6.23167983	-3188.19396	2.1032582
167D	-195.129411	4.79103874	-3188.31723	1.9799943
167E	-195.917647	4.00280307	-3188.39416	1.9030658
167F	-199.280996	0.63945401	-3189.8833	0.41391906
167G	-191.795916	8.12453391	-3187.3276	2.96962089
167H	-199.044912	0.8755381	-3188.45742	1.83980348
167I	-195.012773	4.9076766	-3189.29937	0.997852
167K	-197.051471	2.86897848	-3188.22873	2.06848845
167L	-197.406319	2.5141307	-3189.36675	0.93046875
167M	-199.412139	0.50831111	-3188.9982	1.2990188
167N	-198.25341	1.66704016	-3188.20743	2.0897915
167P	-193.630779	6.28967092	-3187.96823	2.32899094
167Q	-196.471745	3.44870496	-3188.48473	1.81249181
167R	-199.636129	0.28432108	-3188.46188	1.8353418
167S	-195.331789	4.58866051	-3188.01182	2.28539858
167T	-195.570886	4.34956403	-3188.50413	1.79308856
167V	-194.975009	4.94544093	-3188.33864	1.95858
168A	-190.927203	7.65217797	-3186.39307	2.36126253
168D	-192.928222	5.65098619	-3186.36221	2.39212341
168E	-194.800875	3.77833369	-3186.43975	2.31458301
168F	-195.477673	3.10153502	-3187.71128	1.04305215
168G	-190.171411	8.40779703	-3185.56351	3.19082701
168H	-197.013746	1.5654621	-3186.71729	2.03704843
168I	-192.335685	6.24352291	-3187.18916	1.56517279
168L	-194.721268	3.85794088	-3187.8573	0.89703126
168M	-195.40285	3.17635842	-3187.42007	1.33426627
168N	-194.048566	4.53064266	-3187.07082	1.68351276
168P	-194.484607	4.09460149	-3186.91452	1.83981795
168Q	-197.120088	1.45911993	-3187.16556	1.58877471
168S	-191.110942	7.4682668	-3186.014	2.74033729
168T	-192.100155	6.47905291	-3186.82804	1.92629222
168V	-192.196382	6.3828265	-3187.06109	1.69324955
168W	-194.61441	3.96479827	-3187.3486	1.40573909
168Y	-197.140909	1.43829934	-3187.77348	0.98085708
169M	-196.718751	1.48260762	-3188.15482	0.43086716
169N	-197.646188	0.5551706	-3186.71214	1.87354618

169P	-191.283025	6.91833356	-3184.98104	3.6046518
169Q	-196.824913	1.37644616	-3187.88597	0.69972287
169S	-197.101249	1.10011012	-3186.31103	2.27466012
169W	-197.903422	0.29793715	-3185.28365	3.30204064
170E	-196.82049	0.73144144	-3187.47803	1.30221743
170F	-194.728783	2.8231485	-3188.96084	-0.18058812
170G	-196.261047	1.29088462	-3185.67834	3.10190788
170L	-194.825626	2.72630594	-3188.46647	0.31378293
170P	-194.753609	2.79832321	-3186.16066	2.61958779
170Q	-196.605104	0.94682814	-3187.70384	1.07641007
170R	-196.09697	1.45496217	-3188.62807	0.15217498
170W	-196.619059	0.93287268	-3188.7891	-0.00884723
170Y	-195.928142	1.62378965	-3188.95703	-0.17678469
171D	-195.983745	0.41753234	-3188.79319	2.32165328
171F	-195.887915	0.5133621	-3190.38148	0.73336252
171G	-192.861573	3.53970473	-3187.99945	3.11539476
171H	-194.682139	1.71913863	-3188.92448	2.19036344
171L	-195.799719	0.60155852	-3189.9827	1.1321448
171N	-193.878881	2.52239614	-3188.71473	2.40011108
171P	-194.70953	1.69174713	-3188.0342	3.08064729
171V	-195.026905	1.37437286	-3189.86077	1.25407771
172D	-192.770733	4.17497479	-3186.16439	2.57552738
172E	-195.149001	1.79670677	-3186.73022	2.0096986
172F	-195.304502	1.64120547	-3188.71389	0.02603496
172G	-191.636754	5.30895311	-3185.69002	3.0499002
172I	-192.953014	3.99269404	-3187.95973	0.78019114
172L	-195.594625	1.35108304	-3188.30638	0.43354306
172M	-195.769276	1.17643197	-3188.3665	0.37342489
172P	-194.362422	2.58328528	-3186.06505	2.67486744
172Q	-194.920071	2.02563645	-3187.15723	1.58268904
172T	-193.974683	2.9710248	-3187.25769	1.48222996
172V	-191.750075	5.19563293	-3187.47066	1.26925775
172W	-195.323939	1.62176811	-3187.43876	1.30115805
172Y	-194.186898	2.75880978	-3187.48768	1.25224076
173D	-196.226664	0.76277098	-3188.35704	2.03271836
173E	-196.683296	0.30613893	-3188.42094	1.9688211
173F	-196.876694	0.1127409	-3189.08612	1.30364066
173G	-196.746989	0.24244577	-3186.82518	3.56458154
173P	-196.491568	0.49786642	-3186.27596	4.11379692
173V	-196.609851	0.37958353	-3189.09966	1.29009871
173W	-196.361921	0.62751314	-3189.04404	1.34571674
175D	-193.113326	4.32212713	-3186.82059	3.65368484
175E	-197.144546	0.29090626	-3188.83901	1.63526281
175G	-191.102586	6.33286689	-3186.78493	3.68934522
181D	-197.27798	0.05578351	-3175.59555	1.06717442
181G	-197.250167	0.08359709	-3173.92625	2.73647815
181V	-197.266216	0.06754787	-3176.11578	0.54695208

Table 10. Predicted Binding Dynamics for Additional Amino Acid Substitutions in p40 Subunit

Substitution	MOE Affinity	MOE δ Affinity	MOE Stability	MOE δ Stability
10E	-197.206818	0.07025301	-3188.20413	2.13126785
11Y	-197.237071	0.05023713	-3188.6914	1.68169871
12K	-194.707919	0.19350615	-3183.23532	1.58561819
12L	-194.83229	0.06913507	-3184.36373	0.45720207
12R	-194.785347	0.1160783	-3183.97255	0.84838725

12V	-194.737191	0.16423392	-3184.77484	0.04609053
12W	-194.291503	0.60992191	-3183.73656	1.08437137
12Y	-194.758359	0.14306674	-3183.30339	1.51754909
13F	-199.642117	0.75103342	-3188.56543	1.03227547
13H	-196.617258	3.77589281	-3188.87238	0.72532853
13I	-196.611015	3.78213617	-3189.20987	0.38784069
13L	-196.639301	3.75384941	-3188.53349	1.06422146
13M	-200.02817	0.36498061	-3188.02038	1.57733288
13N	-196.59343	3.79972021	-3187.57028	2.02743136
13P	-196.528697	3.86445344	-3187.77374	1.8239694
13Q	-195.668921	4.72422948	-3188.14825	1.44945849
13R	-198.166251	2.22690006	-3189.30816	0.28955233
13T	-196.317569	4.07558174	-3187.97453	1.62317979
13W	-198.842094	1.55105674	-3188.25576	1.34194866
13Y	-198.084338	2.30881274	-3187.73396	1.8637466
14A	-193.07042	3.73321287	-3184.31822	0.56577796
14E	-193.854915	2.94871735	-3184.13225	0.75175133
14F	-190.356016	6.44761661	-3184.97192	-0.08792638
14G	-192.066746	4.73688626	-3183.93722	0.94677199
14H	-193.851709	2.95192351	-3183.96016	0.92383799
14I	-195.051137	1.75249569	-3185.64115	-0.75714861
14K	-192.883735	3.91989733	-3184.03951	0.84448506
14L	-194.984531	1.81910142	-3185.30517	-0.42117789
14M	-193.833627	2.97000547	-3184.69539	0.18860904
14N	-193.355044	3.4485887	-3183.96381	0.92019013
14P	-191.565563	5.23806993	-3184.68151	0.20248153
14Q	-191.560432	5.24320003	-3184.47238	0.4116194
14R	-192.790054	4.01357881	-3185.32365	-0.43965606
14S	-192.310586	4.49304662	-3183.54367	1.34032694
14T	-192.774219	4.02941336	-3184.26416	0.61983484
14V	-196.021843	0.78178992	-3184.40537	0.47862648
14W	-193.548499	3.25513362	-3184.90507	-0.02107243
14Y	-194.084271	2.71936165	-3184.80117	0.08282824
15A	-184.615813	10.6365655	-3187.51337	3.30034611
15D	-186.503374	8.74900416	-3186.91471	3.89900019
15E	-192.587951	2.66442756	-3188.20521	2.60849989
15G	-183.876292	11.3760868	-3186.68581	4.12790729
15H	-194.085594	1.16678457	-3188.20189	2.61182553
15I	-191.578656	3.67372272	-3188.98041	1.83330711
15K	-193.059321	2.19305716	-3188.0014	2.81231108
15L	-192.128824	3.12355455	-3188.51326	2.30045001
15M	-191.973219	3.2791598	-3188.91463	1.89908202
15N	-187.16031	8.09206891	-3187.53371	3.27999996
15P	-189.569339	5.68303962	-3187.26688	3.54683282
15Q	-186.913411	8.3389676	-3188.32455	2.48916496
15R	-193.570758	1.68162015	-3189.40183	1.41188267
15S	-189.615128	5.63725044	-3187.18956	3.62415115
15T	-189.62916	5.62321818	-3187.80783	3.00588656
15V	-187.318232	7.93414654	-3188.47535	2.33836312
16A	-189.854842	0.70734171	-3186.70993	1.20533279
16G	-188.437299	2.12488514	-3185.97353	1.94173111
16P	-189.032959	1.52922473	-3186.55	1.36526765
16S	-189.576939	0.98524485	-3186.80145	1.11381197
17E	-191.725028	1.98566776	-3188.14577	1.47443462
17G	-190.905725	2.80497083	-3188.35869	1.26151693
18A	-197.983636	0.58402915	-3182.44551	0.63229468
18F	-198.184262	0.38340273	-3181.28337	1.79444152
18G	-198.46655	0.10111515	-3182.49226	0.58554527

18H	-198.149486	0.4181788	-3181.07977	1.99803549
18I	-197.743093	0.82457224	-3182.31996	0.75784678
18L	-197.959727	0.60793772	-3182.63303	0.44477335
18S	-197.72466	0.84300451	-3182.11711	0.96069755
19H	-193.391312	2.90756525	-3189.51818	0.35615793
19K	-193.152846	3.14603166	-3188.72376	1.15057865
19L	-194.937371	1.36150626	-3189.79973	0.07461633
19M	-195.787316	0.51156176	-3190.19902	-0.32468166
19Q	-193.665625	2.63325182	-3189.8466	0.02774108
19R	-192.810201	3.48867674	-3189.38455	0.48979217
20A	-196.600446	0.15163663	-3188.17536	0.82387438
20D	-196.496222	0.25586087	-3188.92241	0.07682786
20E	-196.530898	0.22118455	-3188.91209	0.08714837
20G	-196.673429	0.0786539	-3188.33451	0.66472703
20H	-196.616593	0.13548976	-3189.08281	-0.08357533
20L	-196.623593	0.12848988	-3189.5947	-0.59546087
20M	-196.680995	0.07108754	-3189.32705	-0.32781199
20Q	-196.554439	0.19764355	-3188.73208	0.2671561
20R	-196.106177	0.64590577	-3189.79007	-0.79082667
21A	-197.197773	0.11420369	-3188.75124	0.31996096
21D	-197.22684	0.08513661	-3187.95455	1.11665546
21E	-197.205413	0.10656402	-3187.98016	1.0910428
21G	-197.166123	0.14585338	-3187.93478	1.13642536
21K	-197.245508	0.06646908	-3187.81496	1.25623603
21L	-197.232156	0.07982039	-3188.7968	0.27440375
21M	-197.234434	0.07754251	-3189.00774	0.06345979
21N	-197.072007	0.23997024	-3188.23053	0.84066932
21Q	-197.245732	0.06624431	-3188.42883	0.64236768
21R	-197.090317	0.22165995	-3187.65139	1.41981535
38A	-197.824528	0.84073654	-3187.68183	4.24381644
38D	-197.393212	1.27205262	-3187.89953	4.02611543
38E	-197.730068	0.93519624	-3188.33546	3.59018773
38G	-198.335926	0.3293383	-3186.50197	5.42367106
38H	-197.747944	0.91732043	-3188.79616	3.12948423
38M	-197.837137	0.82812697	-3189.31837	2.60727412
38N	-197.448648	1.2166164	-3188.13271	3.7929358
38P	-197.78725	0.87801477	-3187.4329	4.49274396
38Q	-197.777434	0.88783051	-3188.60882	3.31682527
38S	-196.93418	1.73108484	-3187.59974	4.32590099
38T	-197.420684	1.24458026	-3188.28358	3.64205957
38V	-197.666438	0.99882647	-3189.12354	2.80210882
38W	-198.382199	0.28306567	-3190.17249	1.75314959
39E	-197.339681	3.10416852	-3186.39714	1.31882539
39G	-198.940187	1.50366247	-3185.88841	1.8275585
39H	-198.011235	2.4326144	-3186.64217	1.07379503
39T	-197.194714	3.24913584	-3187.83789	-0.12192444
40D	-196.54123	0.41143034	-3186.96635	2.32001241
40E	-196.164769	0.78789121	-3187.20048	2.08588215
40G	-194.773305	2.1793549	-3186.84023	2.44613034
40Q	-196.044895	0.90776498	-3188.158	1.12836197
40R	-196.776161	0.17649881	-3188.54576	0.7405991
40S	-195.813415	1.13924492	-3187.0506	2.23576149
40T	-196.275348	0.6773122	-3187.56438	1.72197797
41P	-195.755191	0.160485	-3181.80058	1.63425393
41Y	-195.081453	0.83422369	-3182.58932	0.84551872
43G	-195.39063	2.40040023	-3187.45482	1.21587338
43H	-197.688805	0.10222457	-3188.0686	0.60209115
43L	-196.968786	0.82224425	-3189.54876	-0.87806429

44A	-198.458053	0.8127292	-3187.7752	0.43371917
44G	-197.053102	2.21768045	-3187.51439	0.69452112
44S	-198.292408	0.97837406	-3187.73224	0.47667692
45A	-196.577742	2.49544492	-3183.94286	0.88237917
45E	-196.433168	2.64001828	-3183.40997	1.41526983
45F	-197.315633	1.75755319	-3184.00174	0.82350645
45I	-197.293491	1.779696	-3185.01306	-0.18781383
45L	-197.967982	1.10520405	-3184.75923	0.06601529
45P	-196.929019	2.14416702	-3183.89759	0.92765263
45Q	-198.120598	0.95258857	-3184.04805	0.7771915
45S	-196.733175	2.34001163	-3183.91075	0.91449542
45T	-197.538942	1.53424486	-3184.41898	0.40626166
45V	-196.915984	2.15720258	-3184.78965	0.03558887
45Y	-197.615736	1.45745075	-3183.95761	0.86762988
46D	-200.14691	0.21254698	-3186.8399	1.81598505
46E	-199.99648	0.36297676	-3186.97732	1.67856092
46F	-200.302373	0.05708348	-3187.7206	0.9352876
46G	-200.243329	0.11612752	-3187.1251	1.530783
46L	-199.386075	0.973382	-3187.76155	0.89433631
46N	-200.134879	0.22457766	-3187.08072	1.57515863
46Q	-199.893866	0.46559114	-3187.6194	1.03648164
46S	-199.372912	0.98654446	-3186.81741	1.83846841
46T	-200.278601	0.08085546	-3187.24641	1.40947083
46W	-200.254198	0.10525901	-3187.39079	1.26509622
47A	-198.802376	0.07183549	-3187.2152	1.97317964
47D	-198.792723	0.08148833	-3187.00014	2.18823976
47H	-198.76295	0.11126108	-3187.49744	1.69093916
47P	-198.699298	0.17491374	-3186.86385	2.32452894
47Q	-198.623636	0.25057547	-3187.69192	1.49645692
47S	-198.815848	0.05836349	-3186.91569	2.27268872
47T	-198.773514	0.10069688	-3187.26367	1.9247022
55D	-197.243939	0.05857478	-3188.42122	2.01134149
57A	-197.1735	0.0740035	-3188.14912	1.56774916
57E	-197.096689	0.15081455	-3188.32372	1.393155
57G	-197.077289	0.17021455	-3187.00416	2.71271244
57I	-197.124618	0.12288499	-3189.98944	-0.27256802
57L	-197.069733	0.17777002	-3189.47033	0.24653828
57M	-197.178184	0.06931928	-3189.36034	0.35653272
57P	-197.158463	0.08904032	-3188.06711	1.64976308
57Q	-197.134712	0.11279119	-3188.36831	1.34856105
57S	-197.123676	0.123827	-3187.82107	1.89580716
58E	-195.028227	0.55606652	-3186.15909	0.90158468
58G	-195.422531	0.16176193	-3185.72686	1.33381404
58I	-195.498611	0.08568172	-3187.54268	-0.48200546
58V	-195.373547	0.2107465	-3187.08327	-0.02259616
59A	-190.151381	3.77708764	-3182.85239	1.76671441
59D	-192.746956	1.18151273	-3183.60617	1.01293142
59E	-190.49329	3.43517834	-3182.13357	2.48554024
59N	-192.707757	1.22071125	-3183.12112	1.49798279
59S	-192.553473	1.37499537	-3182.88239	1.73671424
60A	-195.94188	3.98174789	-3187.57453	2.38999613
60D	-194.272702	5.65092608	-3186.91023	3.0542919
60E	-196.809227	3.11440178	-3187.99571	1.96881653
60F	-196.224205	3.69942325	-3187.68266	2.28187006
60G	-193.833716	6.08991225	-3186.99147	2.97305328
60H	-196.245063	3.67856563	-3188.10048	1.86405064
60I	-197.91485	2.00877816	-3189.08219	0.88233637
60K	-197.686047	2.23758129	-3188.23881	1.72571625

60L	-197.239556	2.6840724	-3188.69374	1.2707841
60M	-198.258938	1.66469004	-3188.23341	1.73111433
60N	-197.846609	2.07701898	-3187.89173	2.07280147
60Q	-197.552503	2.37112542	-3188.1384	1.82612942
60R	-199.55398	0.36964836	-3188.69216	1.27236262
60S	-196.046119	3.87750937	-3187.64678	2.31774175
60T	-195.652228	4.27139989	-3187.35545	2.60907805
60V	-195.671265	4.25236303	-3188.91914	1.0453817
60Y	-196.666327	3.25730086	-3188.94923	1.01529932
62N	-196.843041	0.06248272	-3183.75507	0.69064588
63D	-195.213111	0.23358086	-3188.85764	1.66217497
63E	-195.015159	0.43153222	-3189.11738	1.40244212
63G	-195.025112	0.42157939	-3188.83555	1.68426509
63K	-195.325405	0.12128686	-3189.80449	0.71532948
63L	-195.225493	0.22119885	-3190.42282	0.09700069
63N	-194.933606	0.51308587	-3189.45338	1.06644357
63P	-195.323524	0.12316708	-3189.8902	0.62961464
63S	-195.195304	0.25138732	-3189.63594	0.88388118
63T	-195.340776	0.1059159	-3189.85956	0.66026154
64A	-198.508733	0.18992383	-3188.6146	0.6353297
64E	-197.028909	1.6697486	-3187.62802	1.62191183
64F	-196.324325	2.37433241	-3188.91893	0.33100293
64G	-194.837871	3.86078599	-3188.30345	0.94648084
64I	-194.447474	4.25118333	-3187.6997	1.55023043
64K	-195.676262	3.02239539	-3187.6498	1.60012788
64L	-189.839804	8.8588535	-3186.92933	2.3205935
64M	-197.186024	1.51263333	-3188.73051	0.51941492
64P	-195.970293	2.72836427	-3187.84816	1.40176747
64Q	-195.497421	3.20123638	-3187.52504	1.7248874
64R	-196.569622	2.12903493	-3187.86165	1.38827421
64S	-198.258998	0.43965877	-3188.05416	1.19576692
64T	-198.079617	0.61904001	-3188.73419	0.51573529
64V	-195.234452	3.46420463	-3188.31158	0.93835112
64W	-196.304661	2.39399578	-3189.35716	-0.10723108
64Y	-194.479698	4.21895929	-3189.03498	0.21495169
65D	-197.333501	0.07203491	-3183.25662	1.63677809
65K	-197.178481	0.22705486	-3183.37121	1.52218791
65M	-197.352848	0.05268869	-3184.37301	0.52038935
66A	-197.343126	0.08058898	-3187.21115	2.75860332
66G	-197.341451	0.0822647	-3186.20103	3.76871721
66P	-197.323086	0.10062908	-3187.25137	2.71837861
66R	-197.329092	0.09462285	-3188.23141	1.73834412
66S	-197.342545	0.08117049	-3187.03422	2.93553224
66T	-197.339588	0.08412736	-3187.80591	2.16384182
66Y	-197.337801	0.0859141	-3187.99442	1.97532866
81N	-197.212404	0.13943153	-3187.34254	1.89272868
82G	-196.52994	0.17492127	-3185.88615	3.75988838
83A	-195.899799	1.19724757	-3187.05776	1.00787734
83F	-196.052749	1.0442978	-3188.81847	-0.75283496
83G	-194.094169	3.00287785	-3186.40997	1.65566429
83H	-196.897092	0.19995427	-3187.95295	0.11268174
83N	-196.735032	0.36201397	-3186.94199	1.12364389
83P	-195.831701	1.2653458	-3186.73532	1.33031233
83R	-196.978884	0.11816246	-3187.857	0.20863131
83S	-196.544794	0.55225202	-3186.71809	1.34753835
83V	-196.755209	0.34183746	-3187.30908	0.75655491
83W	-196.739744	0.35730218	-3187.92528	0.14034775
83Y	-195.821766	1.27528088	-3187.9064	0.15923726

84A	-197.846631	0.32399556	-3186.59413	0.15759786
84G	-196.780902	1.38972469	-3184.62361	2.12811463
84I	-196.207513	1.96311367	-3186.90381	-0.15208239
84L	-197.235939	0.93468805	-3187.53433	-0.78260172
84N	-197.948267	0.2223602	-3185.83694	0.91478584
84P	-197.560938	0.60968844	-3186.16472	0.5870063
84S	-198.08859	0.08203703	-3186.24055	0.51118198
84T	-195.511212	2.65941448	-3186.9462	-0.19447515
84V	-196.420773	1.74985351	-3187.264	-0.51226844
85A	-193.807812	1.10162336	-3187.00557	0.01634107
85G	-190.754284	4.15515175	-3185.81108	1.21082802
85S	-194.30947	0.5999658	-3186.80029	0.22162372
86A	-192.545733	1.1590326	-3182.61126	1.39824702
86D	-193.530538	0.17422764	-3182.655	1.35450153
86E	-192.308117	1.39664925	-3181.97275	2.03675182
86F	-192.363081	1.34168522	-3183.90605	0.10344917
86H	-192.853389	0.85137691	-3183.09133	0.91817258
86K	-192.833624	0.87114148	-3183.28191	0.7275926
86L	-192.591247	1.11351853	-3184.23738	-0.22787726
86M	-192.7762	0.92856622	-3183.40126	0.60824264
86N	-192.638222	1.06654444	-3182.90977	1.09972895
86P	-192.405556	1.29920959	-3182.37812	1.63138353
86S	-192.444452	1.26031377	-3182.23814	1.77135734
86T	-191.809785	1.89498083	-3182.99266	1.01684023
86V	-193.267086	0.43767959	-3183.53869	0.47081566
86Y	-192.756663	0.94810262	-3183.62543	0.38407285
88F	-192.067201	0.57211942	-3187.78545	1.06181494
88G	-189.950363	2.68895783	-3187.67831	1.16895283
88I	-186.956864	5.68245678	-3188.22146	0.62580318
88N	-192.474485	0.16483506	-3187.62307	1.22419317
89A	-190.338803	6.15962904	-3188.7167	2.10327898
89E	-195.687034	0.81139824	-3188.29863	2.52135087
89F	-195.360732	1.13769987	-3189.87086	0.94911587
89G	-189.001309	7.49712268	-3187.5622	3.25777801
89H	-192.314171	4.18426035	-3188.728	2.09197751
89I	-192.008055	4.49037708	-3190.16797	0.65200785
89K	-191.698056	4.80037617	-3189.06768	1.75230229
89M	-193.815195	2.68323632	-3189.38515	1.4348311
89N	-195.079577	1.41885507	-3188.76772	2.05225727
89P	-191.39048	5.10795206	-3188.69432	2.12565515
89R	-195.570412	0.92801982	-3190.05446	0.76551506
89S	-191.531035	4.96739637	-3188.39408	2.42589902
89T	-191.766367	4.73206432	-3189.23292	1.58705704
89V	-193.921858	2.5765739	-3189.72121	1.09877129
89W	-195.97541	0.52302132	-3190.21972	0.60025595
89Y	-194.845332	1.65310007	-3189.88648	0.93349952
90A	-192.188545	2.41084148	-3187.90576	2.20369181
90D	-191.003	3.59638697	-3188.25552	1.85393152
90E	-191.899895	2.69949158	-3187.77227	2.33718341
90F	-194.241146	0.35824071	-3188.91647	1.192989
90G	-191.696567	2.90281911	-3187.30225	2.80720438
90H	-192.258129	2.34125785	-3188.12968	1.97977917
90I	-192.472389	2.12699766	-3189.26112	0.84833129
90K	-193.17258	1.42680611	-3188.86227	1.24718017
90L	-192.646345	1.9530414	-3189.03855	1.07090518
90M	-191.83431	2.76507617	-3188.98428	1.125178
90N	-191.830279	2.76910786	-3187.915	2.19445216
90Q	-191.788916	2.81047078	-3188.5583	1.55115609

90R	-191.823202	2.77618504	-3188.53689	1.57256162
90S	-191.471895	3.12749139	-3187.59074	2.5187134
90T	-192.554514	2.04487302	-3188.62386	1.48559976
90V	-192.845501	1.75388561	-3189.25283	0.85662159
90W	-192.860215	1.73917167	-3188.9034	1.20605094
91E	-196.89065	1.44488822	-3188.39272	-0.31046179
91F	-196.475123	1.86041477	-3188.85187	-0.76961179
91G	-198.045794	0.28974391	-3186.55649	1.52576986
91M	-197.70095	0.63458816	-3188.75361	-0.67135295
91N	-195.680793	2.65474464	-3188.26565	-0.18338903
91P	-197.95464	0.38089744	-3187.61945	0.46281183
91Q	-197.66678	0.66875736	-3187.34052	0.74173734
91W	-197.340294	0.99524387	-3188.32771	-0.24545615
91Y	-196.382765	1.95277299	-3188.50117	-0.41891198
92A	-196.038745	0.34482011	-3186.33803	1.15432425
93A	-195.71453	0.7737071	-3181.9728	0.8220625
93E	-196.330087	0.15814997	-3182.22937	0.56549333
93F	-196.20117	0.28706719	-3182.2461	0.54876009
93G	-196.094274	0.3939626	-3182.17737	0.61749394
93H	-196.012675	0.47556244	-3182.54266	0.25220248
93I	-196.141105	0.34713256	-3183.61872	-0.8238593
93K	-196.289192	0.19904477	-3182.26775	0.5271108
93M	-195.863924	0.62431272	-3182.97209	-0.17723076
93N	-195.829246	0.6589913	-3182.38584	0.4090216
93P	-195.94531	0.5429271	-3181.8538	0.94106182
93Q	-195.819373	0.66886361	-3182.42794	0.36692054
93S	-196.014576	0.47366093	-3182.61533	0.17952903
93T	-195.922368	0.5658689	-3182.17344	0.62142244
93V	-195.963109	0.52512791	-3183.28559	-0.49072566
93Y	-196.133841	0.35439611	-3181.34952	1.44534658
94E	-197.230232	0.0655422	-3187.61761	2.35717872
94P	-197.049353	0.24642116	-3187.01353	2.96126225
94Y	-196.929764	0.36601052	-3189.16924	0.80554764
95A	-197.587303	0.19953545	-3187.07008	1.86869254
95D	-197.201677	0.58516146	-3186.72408	2.2146912
95E	-197.494221	0.29261708	-3187.73737	1.2013953
95F	-197.433512	0.35332655	-3188.28504	0.65372925
95G	-197.321604	0.46523399	-3186.32446	2.61431156
95H	-197.487947	0.29889107	-3187.46877	1.46999606
95I	-197.24466	0.54217819	-3188.74487	0.19390178
95L	-197.257222	0.52961585	-3188.11479	0.82398255
95N	-197.379984	0.40685376	-3187.22245	1.71632085
95Q	-197.465734	0.321104	-3187.9798	0.95897043
95R	-197.213005	0.57383291	-3188.14287	0.79590102
95S	-197.415339	0.37149954	-3186.85861	2.08016065
95T	-197.198604	0.58823375	-3187.38954	1.54922739
95V	-197.191455	0.59538311	-3187.70602	1.23274466
95Y	-197.459336	0.32750223	-3188.33214	0.60662641
97E	-197.066867	0.21998512	-3183.5778	0.99431986
98H	-198.429493	0.35307792	-3183.47851	0.64871203
98K	-197.866159	0.91641155	-3183.46849	0.65873948
98Y	-197.462502	1.32006895	-3183.87827	0.24895394
99A	-195.74761	0.90323628	-3186.03192	0.72252727
99D	-194.71414	1.93670628	-3185.33713	1.41732644
99E	-195.471341	1.17950574	-3184.90175	1.85269827
99F	-196.113245	0.53760103	-3186.45723	0.29722389
99G	-195.527312	1.12353462	-3185.8438	0.91064692
99H	-194.527835	2.12301179	-3186.06361	0.69083943

99I	-195.61225	1.03859596	-3186.14228	0.61217426
99K	-195.802626	0.84821995	-3186.2009	0.55355394
99L	-195.634372	1.01647442	-3186.01594	0.7385126
99N	-194.870524	1.78032197	-3186.03409	0.7203658
99P	-195.637799	1.01304779	-3185.67217	1.08228095
99Q	-196.167232	0.48361402	-3185.63315	1.12130071
99S	-195.215333	1.43551296	-3185.81257	0.94188683
99Y	-195.388292	1.26255446	-3185.41815	1.33630153
100D	-195.115294	1.17232951	-3183.19486	0.35056041
100H	-196.178879	0.10874525	-3182.01378	1.53163729
100W	-195.487411	0.80021293	-3182.7272	0.81821731
103A	-197.726057	1.40190841	-3182.75082	0.03606763
103D	-197.867758	1.26020822	-3182.51172	0.2751761
103G	-197.539167	1.58879888	-3182.34905	0.43783683
104A	-193.734344	3.09914315	-3186.13537	1.55617452
104G	-195.30988	1.5236076	-3185.09207	2.5994696
104H	-195.793201	1.0402858	-3185.66505	2.02649304
104Q	-196.734736	0.09875172	-3186.07131	1.62023289
104S	-195.032592	1.80089485	-3185.84264	1.84889649
104T	-195.653683	1.17980469	-3186.53245	1.15908925
105D	-197.690537	0.46653141	-3185.90759	0.85922898
105F	-197.225044	0.93202428	-3186.59945	0.16736663
105H	-194.666284	3.49078477	-3185.51961	1.24720924
105P	-197.290399	0.86666991	-3185.35377	1.41304799
105R	-196.926785	1.23028401	-3186.16473	0.6020919
105T	-195.535989	2.62107939	-3185.50783	1.2589898
105Y	-195.661246	2.49582259	-3185.99092	0.77590196
106A	-196.463182	1.01340862	-3187.92034	2.43344206
106D	-196.482685	0.99390566	-3188.01675	2.33703006
106F	-197.058745	0.41784636	-3188.38313	1.97064346
106G	-196.433509	1.04308183	-3186.7971	3.55668122
106H	-196.865435	0.61115639	-3188.14512	2.20865502
106I	-197.168789	0.30780231	-3189.03167	1.32210779
106K	-197.360594	0.11599679	-3188.48372	1.87005724
106L	-197.054166	0.4224252	-3189.10549	1.24828687
106M	-197.276094	0.2004975	-3188.84982	1.50396053
106N	-197.053349	0.42324232	-3187.91778	2.43599965
106Q	-196.961974	0.51461692	-3188.40815	1.94563239
106R	-197.364441	0.11215028	-3188.75448	1.59929848
106W	-196.489456	0.98713482	-3189.58456	0.76922241
107I	-197.264453	0.0601653	-3188.86817	0.82538834
107N	-197.258058	0.0665603	-3187.48626	2.20729589
107P	-197.248685	0.07593274	-3187.24593	2.44762401
107T	-197.260641	0.06397688	-3187.57918	2.11437832
123A	-197.224071	0.06258754	-3186.87631	2.32102923
123E	-197.20651	0.08014841	-3186.53802	2.65932113
123G	-197.231945	0.0547135	-3185.53553	3.66181495
123I	-197.225424	0.06123435	-3188.72522	0.47212022
123M	-197.220145	0.06651328	-3186.99333	2.20400731
135E	-197.175072	0.14125797	-3187.36318	1.24126568
135G	-197.260255	0.05607523	-3185.82691	2.77753181
135L	-196.806678	0.50965212	-3187.74679	0.85765631
135R	-196.70059	0.61573979	-3187.80287	0.80156785
135W	-197.002983	0.31334704	-3188.08363	0.52080759
136A	-197.13271	0.35843541	-3188.0672	0.55745112
136D	-196.786743	0.70440246	-3187.74766	0.87698653
136G	-197.351813	0.13933194	-3186.69394	1.93070637
136I	-197.348537	0.1426079	-3188.61978	0.00487036

136L	-197.36772	0.12342562	-3188.8437	-0.21904971
136R	-197.000833	0.49031208	-3188.64594	-0.02129019
136V	-197.321825	0.16931978	-3188.25738	0.36727192
136W	-197.392739	0.09840579	-3189.04367	-0.41901695
137E	-196.629765	0.35727707	-3186.59714	0.95726155
137G	-196.503873	0.48316826	-3186.41808	1.13631741
137L	-196.474447	0.51259445	-3187.00413	0.55026966
137S	-196.31997	0.66707129	-3186.89076	0.66364355
137Y	-196.78258	0.20446143	-3187.17223	0.38217618
138H	-197.225993	0.08081786	-3174.75974	2.07046586
138K	-197.250681	0.05613015	-3175.75492	1.0752821
138N	-197.246274	0.06053706	-3175.08513	1.74506895
139K	-196.304977	0.89350387	-3186.94318	0.76153058
139N	-196.940285	0.25819623	-3186.67149	1.03322343
139R	-196.590766	0.60771486	-3186.11989	1.58482044
139T	-197.077718	0.12076256	-3186.61306	1.09165356
139Y	-197.101903	0.09657823	-3186.59767	1.10703735
140G	-195.833145	0.75954555	-3186.39716	0.55762487
141A	-196.186069	0.99263663	-3188.05168	0.97962136
141D	-196.892821	0.28588548	-3187.81758	1.21372168
141I	-197.081245	0.09746055	-3189.29989	-0.26858173
141L	-196.084836	1.09387017	-3188.75327	0.27804014
141N	-196.88979	0.28891617	-3187.35597	1.67533479
141P	-195.687468	1.49123839	-3188.00651	1.02479663
141Q	-196.854461	0.3242453	-3188.39432	0.6369817
141S	-196.933559	0.24514697	-3188.55996	0.47134539
141V	-196.683921	0.49478538	-3189.50715	-0.47584951
142A	-197.161334	0.16531419	-3183.41515	0.60890988
142E	-197.15667	0.16997876	-3182.94804	1.07601239
142G	-197.207351	0.11929721	-3183.26445	0.75960964
142I	-197.111357	0.21529127	-3183.84273	0.18132306
142K	-197.091849	0.23479932	-3182.42923	1.59482146
142L	-197.258072	0.06857653	-3183.97143	0.0526255
142M	-197.114839	0.21180916	-3183.6053	0.41875652
142N	-197.191095	0.13555332	-3182.6435	1.38055954
142P	-196.960103	0.36654566	-3183.71033	0.31372797
142Q	-197.061895	0.2647532	-3183.0345	0.98955565
142R	-197.107362	0.21928637	-3183.15304	0.8710157
142S	-197.030734	0.29591392	-3183.39413	0.62992616
142T	-197.092955	0.23369318	-3183.69658	0.32747571
142V	-197.039549	0.28709953	-3183.9885	0.03555376
142W	-197.202703	0.1239455	-3183.59708	0.42697964
162D	-197.262005	0.29740132	-3182.07907	0.00813044
162W	-197.273315	0.28609043	-3181.96113	0.12606495
181A	-197.251011	0.07881471	-3181.54423	1.78175323
181D	-197.269308	0.06051749	-3182.15849	1.16749676
181F	-197.249163	0.080662	-3183.27678	0.04920465
181H	-197.250337	0.07948874	-3181.53839	1.78759372
181K	-197.1996	0.13022501	-3182.4961	0.82988699
181L	-197.233247	0.09657806	-3183.40376	-0.07777969
181N	-197.186431	0.14339395	-3181.86909	1.45689474
181P	-197.256229	0.07359603	-3181.34419	1.98179239
181T	-197.275573	0.0542521	-3182.09985	1.22613464
181V	-197.265448	0.06437694	-3182.47946	0.84652786
181W	-197.252499	0.07732639	-3184.12503	-0.79904803
181Y	-197.270449	0.05937658	-3184.03856	-0.71258134
183D	-197.154644	0.25007152	-3187.24637	0.96939968
183E	-197.218154	0.18656215	-3187.62908	0.58668254

183K	-197.227888	0.17682767	-3187.81856	0.39721006
183L	-197.3	0.10471589	-3188.21352	0.00224571
183M	-196.781872	0.62284385	-3188.18394	0.03182759
183Q	-197.061875	0.34284136	-3187.7373	0.47847083
183R	-197.31278	0.09193601	-3188.72461	-0.50884276
183W	-197.301809	0.10290739	-3189.04267	-0.82689849
183Y	-196.227123	1.17759334	-3188.72597	-0.51020207
184E	-197.130113	0.0579845	-3187.2627	0.66110004
185A	-197.926218	0.06365563	-3187.8934	0.75146161
185D	-197.264419	0.72545483	-3187.65545	0.98940561
185E	-197.386237	0.60363647	-3187.50355	1.14131137
185F	-197.853439	0.13643511	-3189.33623	-0.69136823
185G	-197.849638	0.14023559	-3186.96312	1.68174002
185I	-197.819171	0.17070278	-3189.22816	-0.58330304
185K	-196.320192	1.66968195	-3188.22306	0.42179607
185N	-197.776336	0.21353803	-3188.02656	0.61829735
185P	-197.923487	0.0663872	-3187.72404	0.92082124
185R	-197.442737	0.54713711	-3188.92279	-0.27793558
185T	-197.780153	0.20972037	-3188.10419	0.54066542
185V	-197.890434	0.09943925	-3188.98451	-0.33965445
186D	-196.377093	0.87353344	-3188.01276	3.21977981
186E	-196.779819	0.47080691	-3187.6724	3.56013706
186Q	-196.691174	0.55945248	-3188.4652	2.76734068
186W	-197.141964	0.10866189	-3190.72031	0.51222241
186Y	-196.938698	0.31192798	-3190.04594	1.18659164
187A	-197.085358	0.55614182	-3184.00331	0.88956795
187E	-197.113126	0.52837379	-3183.12949	1.7633876
187K	-197.390265	0.25123533	-3183.98289	0.90998321
187N	-196.636136	1.00536451	-3183.79183	1.10104423
187R	-196.857136	0.7843638	-3183.9772	0.9156805
187S	-197.156068	0.48543192	-3183.97062	0.92225974
187T	-197.565504	0.07599628	-3184.41599	0.47688267
188A	-197.978991	0.37476068	-3187.99249	1.83911629
188D	-197.069348	1.28440293	-3187.99996	1.83163791
188G	-197.931362	0.42238932	-3187.02271	2.80889236
188H	-198.143329	0.21042192	-3187.847	1.98460297
188K	-197.171837	1.18191384	-3188.6547	1.17690774
188N	-197.140677	1.21307408	-3187.86572	1.96587847
188R	-197.482373	0.87137856	-3189.60013	0.23146999
188S	-198.162802	0.19094894	-3187.76252	2.06908415
190K	-197.036618	0.12578409	-3188.17482	1.41836628
191W	-197.16724	0.06129822	-3183.69287	0.04983104
195A	-197.057976	0.37606415	-3186.45218	0.77080325
195D	-197.077538	0.35650223	-3185.43295	1.79002973
195E	-197.109367	0.32467313	-3186.66374	0.55923932
195F	-197.267517	0.16652348	-3187.69139	-0.46841337
195G	-197.132018	0.30202231	-3185.95187	1.27110786
195H	-197.318738	0.11530284	-3186.85648	0.36649749
195K	-197.209205	0.22483536	-3187.46856	-0.24557813
195N	-197.156099	0.27794126	-3186.73207	0.49091338
195P	-197.07388	0.3601608	-3185.74581	1.47717381
195Q	-197.020424	0.41361632	-3186.5953	0.62767967
195R	-197.077299	0.35674101	-3187.17685	0.0461325
195S	-197.050124	0.38391693	-3186.16982	1.05316104
195T	-197.13725	0.29678999	-3186.80987	0.41310897
196A	-199.64692	0.26034389	-3187.18026	2.55210938
196D	-199.520756	0.38650789	-3187.67876	2.0536155
196E	-199.526406	0.38085723	-3187.57768	2.15469492

196G	-199.565592	0.34167193	-3186.30064	3.43173058
196I	-199.835038	0.07222516	-3188.91326	0.81910763
196L	-198.888087	1.01917622	-3187.40096	2.33140867
196N	-199.547863	0.35940006	-3187.1844	2.54796664
196P	-199.589733	0.31753073	-3186.80218	2.93018701
196Q	-199.818391	0.08887227	-3187.69198	2.04039626
196S	-199.638606	0.26865712	-3187.13603	2.59634154
196T	-199.645953	0.26131078	-3187.47966	2.2527084
196V	-199.725843	0.18142008	-3187.80725	1.92512039
197A	-197.091681	0.06544323	-3185.40134	2.52083164
197G	-197.084951	0.0721734	-3184.55327	3.36890158
197I	-196.87138	0.28574431	-3187.35452	0.56764803
197P	-197.036027	0.12109743	-3184.14606	3.776112
197S	-197.09395	0.06317478	-3185.37325	2.54891763
197V	-197.027203	0.1299213	-3186.53658	1.38558661
198E	-197.117271	0.13253664	-3187.1964	2.52897598
198G	-197.19143	0.05837745	-3186.37219	3.35318156
198K	-197.173979	0.07582834	-3187.57671	2.14866676
198Q	-197.175117	0.07469041	-3187.74066	1.98471613
198R	-197.149133	0.10067447	-3188.25573	1.46964577
198W	-197.194792	0.05501548	-3189.10485	0.62052013
199E	-196.90428	0.21414871	-3181.96085	2.73127816
199P	-196.976568	0.14186086	-3183.10009	1.59203313
200D	-196.913997	0.13710898	-3181.66171	1.04916164
200E	-195.25713	1.79397638	-3182.6284	0.08247046
200G	-196.664468	0.38663791	-3181.56158	1.14928624
200P	-196.889981	0.16112569	-3182.41786	0.29301222
200Q	-196.99715	0.05395591	-3182.62895	0.08192089
202A	-196.329909	0.18775508	-3185.95886	0.64313053
202G	-196.281119	0.23654516	-3185.29223	1.30975828
202S	-196.152259	0.36540557	-3185.79992	0.80207447
203D	-196.647835	0.38609458	-3187.94609	0.73874755
203F	-196.897017	0.13691242	-3189.93665	-1.25180809
203G	-196.754644	0.27928528	-3187.41811	1.26673343
203W	-196.947764	0.08616543	-3189.78584	-1.10100376
204A	-195.553259	0.56909421	-3188.1161	0.41685056
204H	-196.042626	0.07972686	-3187.32826	1.20468934
204M	-196.023261	0.09909267	-3188.3272	0.20574988
204V	-195.7748	0.34755374	-3188.79675	-0.26379818
206A	-198.225638	0.28939352	-3188.05894	2.33115865
206D	-198.208563	0.30646835	-3187.21352	3.17657294
206E	-198.33046	0.18457228	-3187.44969	2.9404088
206H	-198.190575	0.32445691	-3188.53257	1.85752335
206I	-197.75448	0.76055151	-3189.90502	0.48507776
206K	-197.9069	0.60813162	-3188.69499	1.695109
206L	-198.263326	0.25170583	-3189.32796	1.06213052
206M	-198.251089	0.26394311	-3188.96759	1.42250382
206N	-197.776067	0.73896506	-3188.19699	2.19310503
206Q	-197.834036	0.68099567	-3188.4834	1.9066972
206R	-198.382911	0.13212118	-3189.46986	0.92023676
206S	-197.949993	0.56503887	-3188.15781	2.23228649
206T	-198.193675	0.32135654	-3188.3129	2.07719873
206V	-198.186797	0.32823486	-3188.90004	1.49005493
206W	-198.094121	0.42091069	-3189.73131	0.6587856
206Y	-197.818908	0.69612374	-3189.97575	0.41434847
209A	-197.249172	0.11115138	-3182.88427	0.22073637
209G	-197.244319	0.11600365	-3182.33283	0.77218194
209H	-197.24467	0.11565355	-3183.29156	-0.18655498

209I	-197.246953	0.11336995	-3183.90301	-0.79800292
209K	-197.245906	0.11441709	-3183.00094	0.10406338
209L	-197.240822	0.11950102	-3183.78792	-0.68291276
209M	-197.244558	0.11576481	-3183.87743	-0.77241752
209P	-197.244208	0.11611554	-3182.24865	0.85636144
209Q	-197.248483	0.11183986	-3183.08796	0.01705247
209R	-197.234659	0.12566433	-3183.2113	-0.1062918
209S	-197.249265	0.11105805	-3182.76684	0.33816544
209T	-197.247744	0.11257877	-3183.00296	0.10205005
209V	-197.251903	0.10841995	-3183.43157	-0.32656532
209W	-197.239756	0.12056746	-3183.94642	-0.84141348
209Y	-197.249995	0.11032784	-3183.17543	-0.07041962

Example 3: Alanine-Scanning Analysis of Structure-Predicted Mutein Effector Regions

[0151] Based on the structural analysis of predicted mutein effector regions, the effect of substitution of single sites was determined by alanine-scanning substitution and analyzed by Surface Plasmon Resonance (SPR). Analysis of IL-12 variant binding to recombinant human IL12R was performed on a Biacore 8K instrument using Fc capture. A series S CM5 chip was directly immobilized with anti-human IgG (Fc specific) according to the protocol provided by GE Healthcare in the Human Antibody Capture Kit. Recombinant human IL12Rβ1 Fc and IL12Rβ2 Fc (R&D Systems) were diluted in PBSP pH 7.4 running buffer and captured to the anti-huFc surface for 36 at 10 μL/min flowrate to obtain capture levels between 90 and 125 RU. Wild-type and IL12 variants were diluted to 500 nM in running buffer and injected over the captured receptors for 150 sec followed by 300 sec dissociation in buffer at 30 μL/min at 22°C. The surface was regenerated with 10 mM glycine pH 1.5 for 30 sec. Sensorgrams were processed using the Insight Evaluation Software (GE Healthcare). A response point was taken at the end of IL12 association phase and normalized to the capture level of IL12Rβ1 Fc or IL12Rβ2 Fc for the comparison plots. Results of the SPR analysis assay in WT and mutated IL-12 variants is shown in Table 11, Fig. 4 (p35) and Fig. 5 (p40).

Table 11.

Identifier	p35 substitutions	p40 substitutions	IL-12Rb1 (p40)	IL-12Rb2 (p35)
PPB-54404 WT	-----	-----	154	114.5
PPB-54376	L37A	-----	169.6	13.43
PPB-54386	E38A	-----	184.7	100.9
PPB-54396	F39A	-----	165.5	50.58
PPB-54406	Y40A	-----	227.9	6.445
PPB-54423	T43A	-----	210.2	113.1
PPB-54414	S44A	-----	202.9	109.8
PPB-54415	E46A	-----	225.7	96.31
PPB-54424	D48A	-----	190.7	95.13

PPB-54433	H49A	-----	191.6	125.1
PPB-54442	E50A	-----	202.1	91.92
PPB-54377	M125A	-----	209.5	88.96
PPB-54387	D126A	-----	134.7	89.21
PPB-54395	R129A	-----	168.5	86.92
PPB-54397	Q130A	-----	159.2	108.5
PPB-54407	I131A	-----	196.8	76.22
PPB-54416	S160A	-----	171	118.7
PPB-54425	L161A	-----	192.9	123.4
PPB-54405	E162A	-----	182.3	122.6
PPB-54434	E163A	-----	211.6	114.6
PPB-54432	D165A	-----	166.3	89.93
PPB-54441	Y167A	-----	190.2	1.417
PPB-54443	T169A	-----	205.5	106.1
PPB-54378	I171A	-----	158.1	136.8
PPB-54388	K172A	-----	165.6	119.9
PPB-54398	L173A	-----	164.5	98.08
PPB-54408	I175A	-----	198.4	116.2
PPB-54417	H178A	-----	186.6	86.18
PPB-54436	-----	Y16A	49.25	124
PPB-54426	-----	P17A	228	114.1
PPB-54435	-----	D18A	121.6	117.7
PPB-54445	-----	P20A	146.3	128.4
PPB-54380	-----	Q42A	161.1	95.88
PPB-54390	-----	S44A	192.3	105.9
PPB-54400	-----	V46A	168.8	120.1
PPB-54444	-----	E59A	7.937	127.2
PPB-54379	-----	F60A	1.351	110.2
PPB-54389	-----	K84A	2.344	109.5
PPB-54410	-----	E86A	192.4	107.3
PPB-54419	-----	I89A	211	108.8
PPB-54428	-----	T92A	166	116.2
PPB-54437	-----	L95A	162.9	112.5
PPB-54446	-----	Q98A	213.3	115.7
PPB-54381	-----	P101A	135.2	107.3
PPB-54391	-----	K104A	115.9	111.5
PPB-54401	-----	I126A	137.8	122.2
PPB-54411	-----	T128A	190.6	111.6
PPB-54420	-----	L130A	137.3	124.9
PPB-54429	-----	R157A	174.7	127.8
PPB-54438	-----	R159A	194.3	104.9
PPB-54447	-----	D161A	210	124.6
PPB-54382	-----	K163A	158.2	116.2
PPB-54399	-----	K195A	78.11	110.7
PPB-54392	-----	K197A	10.8	116

Example 4: Analysis of Mutant Combinations

[0152] Following identification of potential substitution sites within the p35 and p40 subunits, a number of combination mutants were designed and tested. An IL-12 fusion protein including the

below-described mutant combinations was expressed and purified. Combinations were evaluated in an IL-12 activity reporter assay for monitoring IL-12 triggered STAT4 activation. HEK-Blue IL-12 reporter cells (InvivoGen) were generated by stably introducing the human genes for the IL-12 receptor and the genes of the IL-12 signaling pathway into HEK293 cells. These cells express a STAT4-inducible secreted embryonic alkaline phosphatase (SEAP) reporter gene. Cells were cultured and frozen stocks were prepared according to the protocol described by InvivoGen. Frozen stocks with 1E+07 /ml /vial were prepared as Thaw-And-Use cells for the assay. At Day1 frozen cells were thawed and adjusted to 6E+06 cells/ml with DMEM w/o phenol red supplemented with 8% of heat-inactivated fetal bovine serum (FBS). Cells were seeded at 15000 cells/well/25 µl into 384-well tissue culture-treated white/flat bottom tissue culture plate (# 781080, Greiner Bio-One Germany) by using a Multidrop Combi. For dose response a series of 3-fold dilutions of test samples were prepared in DMEM containing 1% of heat-inactivated FBS in 384-well plate with non-binding surface (#3640, Corning). 5 µl/well of the diluted samples were transferred into the test plate containing seeded cells. The final sample concentrations in the wells were in the range from 100nM to 0.0051nM with assays run in 10-point concentration response in duplicate. The assay plates were incubated for 24 h at 37 °C/5%CO₂/95% humidity. For signal detection 5 µl/well of cells supernatant were transferred into a new 384-well plate, followed by addition of 20 µl of QUANTI-Blue solution (InvivoGen) prepared as manufacture's description. SEAP levels were determined by absorption at OD 620nm after 2 h incubation at 37°C using a PheraStar plate reader (BMG Labtech, Germany). As for IL-12 reporter assay data normalization, the readings of wells without any test samples were used as 0% activation (E%).

The results of the IL-12 reporter assay for panels of mutant combinations are provided in Table 12 and Table 13.

Table 12. IL-12 Reporter assay of combination mutants (panel 1)

Protein Position	p35					p40						%E _{max}	EC ₅₀ nM
	37	39	40	131	167	16	59	60	84	195	197		
WT Residue	L	F	Y	I	Y	Y	E	F	K	K	K	-	-
WT FF-22- 1876-1	---	---	---	---	---	---	---	---	---	---	---	589.3	0.13
Variant #1 FF-22- 1877-1	A	A	A	A	A	A	A	A	A	A	A	549.5	1.08
Variant #2 FF-22- 1878-1	A	A	A	A	A	---	---	---	---	---	---	129.8	95.40

Variant #3 FF-22- 1879-1	----	----	----	----	----	A	A	A	A	A	A	112.1	9.71
Variant #4 FF-22- 1880-1	A	----	A	----	A	----	A	A	A	----	A	4.2	NoVal
Variant #5 FF-22- 1881-1	A	----	A	----	A	----	----	----	----	----	----	157.3	36.00
Variant #6 FF-22- 1882-1	----	----	----	----	----	----	A	A	A	----	A	194.9	7.35
Variant #7 FF-22- 1883-1	----	A	----	A	----	A	----	----	----	A	----	475.5	0.27
Variant #8 FF-22- 1884-1	A	----	A	----	A	A	----	----	----	A	----	56.5	82.40
Variant #9 FF-22- 1885-1	----	A	----	A	----	----	A	A	A	----	A	75.8	85.50
Variant #10 FF-22- 1886-1	----	----	----	----	A	----	----	----	----	----	----	474.8	0.13
Variant #11 FF-22- 1887-1	----	----	----	----	----	----	----	A	----	----	----	417.9	0.28
Variant #12 FF-22- 1888-1	----	A	----	----	----	----	----	----	----	----	----	599.2	0.14
Variant #13 FF-22- 1889-1	----	----	----	----	----	A	----	----	----	----	----	560.9	0.12
Variant #14 FF-22- 1890-1	----	----	----	----	A	----	----	A	----	----	----	353.1	98.50
Variant #15 FF-22- 1891-1	----	----	A	----	A	----	----	A	A	----	----	9.5	NoVal
Variant #16 FF-22- 1892-1	A	----	A	----	A	----	A	A	A	----	----	2.6	NoVal
Variant #17 FF-22- 1893-1	A	A	A	A	A	A	----	----	----	----	----	11.3	NoVal
Variant #18 FF-22- 1894-1	----	A	----	A	----	A	----	----	----	----	----	453.8	0.13
Variant #19	----	----	A	A	A	A	----	A	A	----	A	-5.4	NoVal

FF-22-1895-1													
Variant #20 FF-22-1896-1	A	---	---	A	A	A	A	---	A	A	---	-6.5	NoVal
Variant #21 FF-22-1897-1	---	A	---	A	A	A	---	A	A	A	---	-5.9	NoVal
Variant #22 FF-22-1898-1	A	---	---	A	A	A	A	---	A	---	A	-1.0	NoVal

Table 13. IL-12 Reporter assay of combination mutants (panel 2)

Protein Position	p35												p40 16	%Emax	EC50 nM
	37	40	41	126	128	131	133	164	166	170	173	177	16		
WT Residue	L	Y	P	D	K	I	L	P	F	K	L	L	Y		
WT FF-23-825-1														1292	0.0316
Variant #23 FF-23-826-1	A													1123	0.0393
Variant #24 FF-23-827-1						A								1245	0.0318
Variant #25 FF-23-828-1													A	1210	0.0284
Variant #26 FF-23-829-1		S												1010	0.0906
Variant #27 FF-23-830-1	A					A								1017	0.11
Variant #28 FF-23-831-1	A												A	821	0.0976
Variant #29 FF-23-832-1	A	S												977	1.05
Variant #30 FF-23-833-1						A								1216	0.0969
Variant #31 FF-23-834-1		S				A								1132	0.743
Variant #32 FF-23-835-1		S											A	1175	0.644
Variant #33 FF-23-836-1	A					A							A	1172	0.461
Variant #34 FF-23-837-1		S				A							A	1113	2.22

Variant #35 FF-23-838-1	A	S											A	1128	3.4
Variant #36 FF-23-839-1	A	S				A								1173	1.63
Variant #37 FF-23-840-1	A	S				A							A	1061	4.94
Variant #38 FF-23-841-1	A	S				A	A						A	1015	11.2
Variant #39 FF-23-842-1	A	S				A			P				A	867	18.7
Variant #40 FF-23-843-1	A	S			A	A							A	869	10.8
Variant #41 FF-23-844-1	A	S				A	A				A	A	A	603	13.4
Variant #42 FF-23-845-1	A	S		A		A				A			A	786	14.5
Variant #43 FF-23-846-1	A	S		A		A	A						A	592	16
Variant #44 FF-23-847-1	A	S		A		A	A			A	A	A	A	687	17.5
Variant #45 FF-23-848-1	A	S		A	A	A	A			A	A	A	A	394	22.4
Variant #46 FF-23-849-1	A	S		A		A	A		P	A			A	276	18.9
Variant #47 FF-23-850-1	A	S		A		A	A		P	A	A	A	A	301	29
Variant #48 FF-23-851-1	A	S		A	A	A	A		P	A			A	169	50.9
Variant #49 FF-23-852-1	A	S	R			A							A	882	7.55
Variant #50 FF-23-853-1	A	S				A		R					A	673	9.36
Variant #51 FF-23-854-1	A	S	R			A		R					A	736	8.16
Variant #52 FF-23-855-1	A	S	R	A		A		R		A			A	352	16.8
Variant #53 FF-23-856-1	A	S	R	A	A	A	A	R					A	160	21.4
Variant #54 FF-23-857-1	A	S	R	A	A	A	A	R		A	A	A	A	158	>1.00E+02
Variant #55 FF-23-858-1	A	S	R	A	A	A	A	R	P	A	A	A	A	53	>1.00E+02

Table 15. IL-12 Reporter assay of combination mutants (panel 3)

Protein	p35				p40	%E _{max}	EC ₅₀ nM
Position	37	40	128	133	16		
WT Residue	L	Y	K	L	Y		
WT FF-23-825-1						1671.4	0.061
Variant #56 FF-24-018-1	A	S			A	1742.2	7.93
Variant #57 FF-24-026-1	A	S		A	A	1599.8	7.65
Variant #58 FF-24-034-1	A	S	N		A	1843.8	10.30
Variant #59 FF-24-042-1	A	S	N	A	A	1958.4	13.00
Variant #60 FF-24-050-1	A				A	1811.3	0.672

Example 5: Analysis of IL-12 muteins

[0153] Potential substitution sites within the p35 and p40 subunits were identified by structural analysis of IL-12 in complex with IL-12R as described in Example 1 and *in silico* screening as described in Example 2. Of the full single-mutant substitutional space (9196 possible single mutants across p35 and p40), 965 amino acid positions corresponding to 368 p35 single mutants (Table 9) and 597 p40 single mutants (Table 10) were selected and produced. Among those, 905 IL-12 fusion proteins were successfully expressed, purified, and tested in the IL-12 reporter assay (InvivoGen), as described in Example 4. The following selection criteria were used to select mutants of interest: protein mass concentration > 0.1 mg/mL, good protein quality (as assessed by LabChip QC) and a fold-change EC₅₀ >50 over WT IL-12 controls. Selected mutants were tested in an IL12RB1/IL12RB2 dimerization assay. Briefly, PathHunter® U2OS IL12RB1/IL12RB2 cells (Discover X, Eurofins) were transduced to overexpress human IL12RB1 and IL12RB2 genes. Binding of IL-12 (or IL-12 fusion protein) to one receptor subunit and subsequent dimerization with the other receptor subunit, leads to the complementation of two enzyme fragments, resulting in the formation of a functional enzyme

that hydrolyzes a substrate to generate a chemiluminescent signal. Cells were thawed and cultured following recommendations and reagents provided by the manufacturer. For the assay, cells were seeded at 10000 cells/well/90 μ l into 96-well white flat bottom tissue culture-treated plates and incubated at 37 °C/5%CO₂/95% humidity for 4h before treatment. For dose response, a series of 5-fold dilutions of compounds were prepared in test medium and 20 μ l/well of the diluted compounds were transferred into the test plate containing seeded cells. The final sample concentrations in the wells were in the range from 100 nM to 0.0013 nM with assays run in 8-point concentration response in triplicate. The assay plates were incubated for 18h to 24h at 37 °C/5%CO₂/95% humidity. For signal detection, 110 μ l/well of PathHunter Flash Detection Reagent (prepared following manufacturer's protocol) were added directly onto the cells. After 1h incubation at room temperature in the dark, luminescence values were determined using a Spark[®] microplate reader (Tecan). For data normalization, the readings of wells without any compounds were used as 0% activation (E%). Results (%Emax and EC50) obtained for 93 selected single mutants are provided in Tables 16, 17 and 18.

Table 16. IL-12RB1/B2 dimerization assay of single mutants (assay 1)

Protein	p35	p40	%Emax	EC50 nM
WT PPB-79488	-----	-----	227.9	0.012
WT PPB-80311	-----	-----	250.3	0.009
Variant #1 PPB-79485	K170P	-----	78.2	8.396
Variant #2 PPB-79503	Y167T	-----	93.9	6.165
Variant #3 PPB-79524	Y167I	-----	131.8	21.36
Variant #4 PPB-79528	Y167K	-----	126.1	18.50
Variant #5 PPB-79550	K170F	-----	207.7	0.059
Variant #6 PPB-80343	-----	D14W	177.8	0.013
Variant #7 PPB-80344	F116L	-----	213.9	0.017
Variant #8 PPB-80756	-----	F60E	154.4	4.005
Variant #9	-----	W15K	184.3	1.916

PPB-80783				
Variant #10 PPB-81123	-----	I94Y	156.3	0.058
Variant #11 PPB-81142	-----	I94P	176.8	0.211
Variant #12 PPB-82380	-----	Y198G	122.8	31.55
Variant #13 PPB-82451	-----	G21E	174.1	0.012
Variant #14 PPB-82462	-----	A63T	179.7	0.009
Variant #15 PPB-82469	-----	L107N	214.6	0.013
Variant #16 PPB-82474	-----	P185V	193.6	0.010
Variant #17 PPB-82475	-----	S183R	212.0	0.010
Variant #18 PPB-82478	-----	E187T	219.1	0.008
Variant #19 PPB-82479	-----	Q65D	194.6	0.009
Variant #20 PPB-82485	-----	S204M	235.0	0.010
Variant #21 PPB-82490	-----	L47A	220.1	0.011
Variant #22 PPB-82492	-----	E12R	223.8	0.032
Variant #23 PPB-82495	-----	S183W	189.3	0.009
Variant #24 PPB-82497	-----	L196Q	185.2	0.011
Variant #25 PPB-82500	-----	Y198K	181.3	0.104
Variant #26 PPB-82503	-----	D209G	184.7	0.010
Variant #27 PPB-82507	-----	S136W	194.0	0.011
Variant #28 PPB-82512	-----	V10E	185.2	0.330
Variant #29 PPB-82523	-----	V57M	195.2	0.011
Variant #30	F166S	-----	225.0	0.011

PPB-82798				
Variant #31 PPB-82800	K172F	-----	224.6	0.011
Variant #32 PPB-82821	D165T	-----	236.2	0.009
Variant #33 PPB-82827	D48Q	-----	251.7	0.002
Variant #34 PPB-82828	D48T	-----	207.1	0.010
Variant #35 PPB-82829	Y167H	-----	155.7	0.766
Variant #36 PPB-82830	E163K	-----	198.3	0.012
Variant #37 PPB-82833	E163L	-----	202.1	0.011
Variant #38 PPB-82843	I171P	-----	116.5	6.090

Table 17. IL-12RB1/B2 dimerization assay of single mutants (assay 2)

Protein	p35	p40	%Emax	EC50 nM
WT PPB-79488	-----	-----	118.9	0.015
WT PPB-80311	-----	-----	184.5	0.011
Variant #39 PPB-79470	Y167S	-----	78.0	4.096
Variant #40 PPB-79479	Y167P	-----	49.8	18.29
Variant #41 PPB-79482	Y167E	-----	46.7	21.83
Variant #42 PPB-79486	I52D	-----	130.0	0.023
Variant #43 PPB-79493	K172I	-----	126.5	0.018
Variant #44 PPB-79494	Y167Q	-----	61.8	20.22
Variant #45 PPB-79508	Y167L	-----	81.5	4.004
Variant #46 PPB-79513	Y167V	-----	104.2	5.332
Variant #47 PPB-79546	Y167D	-----	67.5	19.19

Variant #48 PPB-79968	Y40K	-----	49.2	1.136
Variant #49 PPB-79995	K56N	-----	83.7	6.660
Variant #50 PPB-80022	Y40P	-----	89.3	1.183
Variant #51 PPB-80027	Y40G	-----	137.0	2.251
Variant #52 PPB-80034	Y167R	-----	125.4	12.49
Variant #53 PPB-80046	Y40Q	-----	133.5	0.401
Variant #54 PPB-80293	-----	W15N	135.1	0.475
Variant #55 PPB-80294	-----	F60D	168.1	2.570
Variant #56 PPB-80295	-----	D14G	163.1	0.011
Variant #57 PPB-80296	-----	G64Y	200.1	0.117
Variant #58 PPB-80309	-----	F60P	168.1	0.152
Variant #59 PPB-80313	L109Y	-----	194.0	0.001
Variant #60 PPB-80325	-----	W15G	177.8	0.929
Variant #61 PPB-80348	-----	F60G	192.4	0.340
Variant #62 PPB-80350	-----	F60T	190.2	0.258
Variant #63 PPB-80358	-----	W15D	181.9	1.034
Variant #64 PPB-80359	-----	W15P	209.5	1.070
Variant #65 PPB-80362	-----	F60A	138.1	0.394
Variant #66 PPB-80369	-----	W15Q	143.6	0.073

Table 18. IL-12RB1/B2 dimerization assay of single mutants (assay 3)

Protein	p35	p40	%Emax	EC50 nM
WT	-----	-----	149.3	0.009

PPB-79488				
WT PPB-80311	-----	-----	131.4	0.007
Variant #67 PPB-80706	-----	F60Q	146.9	0.592
Variant #68 PPB-80725	-----	W90N	153.0	0.041
Variant #69 PPB-80747	-----	G64P	125.4	0.057
Variant #70 PPB-80750	-----	F60K	104.4	0.391
Variant #71 PPB-80754	-----	W15R	115.5	0.217
Variant #72 PPB-80765	-----	G64E	133.1	0.094
Variant #73 PPB-80782	-----	G64F	149.2	0.119
Variant #74 PPB-80784	-----	F60N	140.1	0.338
Variant #75 PPB-81015	-----	F106H	196.9	0.009
Variant #76 PPB-81069	-----	S183M	174.0	0.008
Variant #77 PPB-81100	-----	S203D	158.6	0.006
Variant #78 PPB-81105	-----	L95Q	143.9	0.163
Variant #79 PPB-81126	-----	L95Y	188.2	0.169
Variant #80 PPB-81149	-----	F106I	166.0	0.007
Variant #81 PPB-81177	-----	L95S	110.9	0.179
Variant #82 PPB-81178	-----	K195P	134.2	0.216
Variant #83 PPB-82406	-----	R138N	138.8	0.024
Variant #84 PPB-82413	-----	L107T	110.8	0.011
Variant #85 PPB-82476	-----	Y66G	123.1	0.050
Variant #86	-----	V57Q	103.7	0.040

PPB-82504				
Variant #87 PPB-82511	-----	Y198Q	99.7	0.612
Variant #88 PPB-82524	-----	I94E	99.3	0.084
Variant #89 PPB-82569	-----	A63D	89.0	0.087
Variant #90 PPB-82642	Y40D	-----	27.2	2.314
Variant #91 PPB-82699	K170E	-----	19.1	1.084
Variant #92 PPB-82825	K170G	-----	100.5	0.170
Variant #93 PPB-82865	Y167N	-----	41.4	3.755

[0154] 21 mutants of interest were tested again in the IL-12 activity reporter assay (Invivogen) for monitoring IL-12 triggered STAT4 activation. Cells were cultured and frozen stocks were prepared according to the protocol described by InvivoGen. Cells were thawed and passaged twice in complete growth medium (DMEM 4.5 g/L glucose, 2 mM L-glutamine, 10% heat-inactivated fetal bovine serum (FBS)) before adding selection antibiotics (blasticidin, hygromycin B and Zeocin™) at concentrations recommended by Invivogen. After a minimum of two passages in the selection medium, cells were prepared for the assay as follows: cells were resuspended in test medium (complete medium without selection antibiotics) and seeded at 50000 cells/well/160 µl into 96-well clear flat bottom tissue culture-treated plates (# 353072, Falcon®). For dose response, a series of 5-fold dilutions of compounds were prepared in test medium and 40 µl/well of the diluted compounds were transferred into the test plate containing seeded cells. The final sample concentrations in the wells were in the range from 100 nM to 0.0013 nM with assays run in 8-point concentration response in triplicate. The assay plates were incubated for 18h to 24h at 37 °C/5%CO₂/95% humidity. For signal detection, 20 µl/well of cells supernatant were transferred into a new 96-well plate, followed by addition of 180 µl of QUANTI-Blue solution (InvivoGen) prepared as manufacturer's description. SEAP levels were determined by absorption at OD 630nm after 3h incubation at 37°C using a Spark® microplate reader (Tecan). As for IL-12 reporter assay data normalization, the readings of wells without any compounds were used as 0% activation (E%). Results (%Emax and EC50) are provided in Table 19.

Table 19. IL-12 Reporter assay of single mutants

Protein	p35	p40	%E_{max}	EC₅₀ nM
WT PPB-79488	-----	-----	1454.1	0.020
WT PPB-80311	-----	-----	1485.3	0.017
WT PPB-82459	-----	-----	1582.0	0.013
Variant #1 PPB-79995	K56N	-----	1227.9	0.305
Variant #2 PPB-80296	-----	G64Y	1351.6	0.097
Variant #3 PPB-80725	-----	W90N	1668.8	0.078
Variant #4 PPB-80747	-----	G64P	1320.4	0.128
Variant #5 PPB-80765	-----	G64E	1411.5	0.068
Variant #6 PPB-80782	-----	G64F	1327.9	0.129
Variant #7 PPB-81105	-----	L95Q	1482.5	0.156
Variant #8 PPB-81123	-----	I94Y	1342.3	0.044
Variant #9 PPB-81126	-----	L95Y	1394.6	0.120
Variant #10 PPB-81142	-----	I94P	1272.6	0.210
Variant #11 PPB-81177	-----	L95S	1193.9	0.120
Variant #12 PPB-82380	-----	Y198G	1205.4	7.450
Variant #13 PPB-82476	-----	Y66G	1555.3	0.048
Variant #14 PPB-82492	-----	E12R	1345.2	0.023
Variant #15 PPB-82500	-----	Y198K	1253.5	0.078
Variant #16 PPB-82504	-----	V57Q	1587.0	0.056
Variant #17 PPB-82511	-----	Y198Q	1573.7	0.427

Variant #18 PPB-82512	-----	V10E	1399.2	0.291
Variant #19 PPB-82524	-----	I94E	1243.3	0.126
Variant #20 PPB-82569	-----	A63D	1614.5	0.076
Variant #21 PPB-82843	I171P	-----	1331.7	0.593

EMBODIMENTS

E1. An interleukin-12 (IL12) mutein comprising a variant p35 subunit, a variant p40 subunit, or both a variant p35 subunit and a variant p40 subunit,

wherein the variant p35 subunit comprises an amino acid sequence with one or more substitutions or deletions relative to SEQ ID NO: 1;

wherein the variant p40 subunit comprises an amino acid sequence with one or more substitutions or deletions relative to SEQ ID NO: 2.

E2. The IL-12 mutein of embodiment E1, comprising a variant p35 subunit, wherein the variant p35 subunit comprises a substitution at amino acid residue L133 relative to SEQ ID NO: 1.

E3. The IL-12 mutein of embodiment E2, wherein the L133 substitution of the variant p35 subunit is selected from the group consisting of L133A, L133R, L133H, L133K, L133D, L133E, L133S, L133T, L133N, L133Q, L133G, L133P, L133I, L133M, L133F, L133W, L133V, and L133Y.

E4. The IL-12 mutein of embodiment E2 or E3, wherein the variant p35 subunit further comprises one or more amino acid substitutions relative to SEQ ID NO: 1.

E5. The IL-12 mutein of embodiment E4, wherein the variant p35 subunit comprises one, two, three, four, five, or more amino acid substitutions at a position selected from the group consisting of L37, F39, Y40, P41, D48, M125, D126, K128, R129, I131, L133, P164, D165, F166, Y167, K170, L173, L177, H178 relative to SEQ ID NO: 1.

E6. The IL-12 mutein of embodiment E4 or E5, wherein the variant p35 subunit comprises a substitution at L37, optionally wherein the substitution is L37R, L37H, L37K, L37D, L37E, L37S, L37T, L37N, L37Q, L37G, L37P, L37A, L37V, L37I, L37M, L37F, L37Y, or L37W.

E7. The IL-12 mutein of any one of embodiments E4 to E6, wherein the variant p35 subunit comprises a substitution at Y40, optionally wherein the substitution is Y40R, Y40H, Y40K, Y40D, Y40E, Y40S, Y40T, Y40N, Y40Q, Y40G, Y40P, Y40A, Y40V, Y40I, Y40L, Y40M, Y40F, or Y40W.

- E8. The IL-12 mutein of any one of embodiments E4 to E7, wherein the variant p35 subunit comprises a substitution at D48, optionally wherein the substitution is D48R, D48H, D48K, D48E, D48S, D48T, D48N, D48Q, D48G, D48P, D48A, D48V, D48I, D48L, D48M, D48F, D48Y, or D48W.
- E9. The IL-12 mutein of any one of embodiments E4 to E8, wherein the variant p35 subunit comprises a substitution at K128, optionally wherein the substitution is K128R, K128H, K128K, K128D, K128E, K128S, K128T, K128N, K128Q, K128G, K128P, K128A, K128V, K128I, K128L, K128M, K128F, K128Y, or K128W.
- E10. The IL-12 mutein of any one of embodiments E4 to E9, wherein the variant p35 subunit comprises a substitution at M125, optionally wherein the substitution is M125R, M125H, M125K, M125D, M125E, M125S, M125T, M125N, M125Q, M125G, M125P, M125A, M125V, M125I, M125L, M125F, M125Y, or M125W.
- E11. The IL-12 mutein of any one of embodiments E4 to E10, wherein the variant p35 subunit comprises a substitution at R129, optionally wherein the substitution is R129H, R129K, R129D, R129E, R129S, R129T, R129N, R129Q, R129G, R129P, R129A, R129V, R129I, R129L, R129M, R129F, R129Y, or R129W.
- E12. The IL-12 mutein of any one of embodiments E4 to E11, wherein the variant p35 subunit comprises a substitution at L133, optionally wherein the substitution is L133R, L133H, L133K, L133D, L133E, L133S, L133T, L133N, L133Q, L133G, L133P, L133A, L133V, L133I, L133M, L133F, L133Y, or L133W.
- E13. The IL-12 mutein of any one of embodiments E4 to E12, wherein the variant p35 subunit comprises a substitution at L177, optionally wherein the substitution is L177R, L177H, L177K, L177D, L177E, L177S, L177T, L177N, L177Q, L177G, L177P, L177A, L177V, L177I, L177M, L177F, L177Y, or L177W.
- E14. The IL-12 mutein of any one of embodiments E4 to E13, wherein the variant p35 subunit comprises a substitution at H178, optionally wherein the substitution is H178R, H178K, H178D, H178E, H178S, H178T, H178N, H178Q, H178G, H178P, H178A, H178V, H178I, H178L, H178M, H178F, H178Y, or H178W.
- E15. The IL-12 mutein of embodiment E1, wherein the variant p35 subunit comprises
a substitution at L37, optionally wherein the substitution is L37A; and
a substitution at Y40, optionally wherein the substitution is Y40S.
- E16. The IL-12 mutein of embodiment E1, wherein the variant p35 subunit comprises
a substitution at L37, optionally wherein the substitution is L37A;
a substitution at Y40, optionally wherein the substitution is Y40S; and

a substitution at L133, optionally wherein the substitution is L133A.

E17. The IL-12 mutein of embodiment E1, wherein the variant p35 subunit comprises

a substitution at L37, optionally wherein the substitution is L37A;

a substitution at Y40, optionally wherein the substitution is Y40S; and

a substitution at K128, optionally wherein the substitution is K128N or K128Q.

E18. The IL-12 mutein of embodiment E1, wherein the variant p35 subunit comprises

a substitution at L37, optionally wherein the substitution is L37A;

a substitution at Y40, optionally wherein the substitution is Y40S;

a substitution at K128, optionally wherein the substitution is K128N or K128Q; and

a substitution at L133, optionally wherein the substitution is L133A.

E19. The IL-12 mutein of any one of embodiments E1 to E18, wherein the variant p35 subunit comprises an amino acid sequence that is at least 80%, 85%, 90%, or 95% identical to SEQ ID NO: 1.

E20. The IL-12 mutein of any one of embodiments E1 to E19, comprising a variant p40 subunit, wherein the variant p40 subunit comprises a substitution at amino acid residue Y16 relative to SEQ ID NO: 2.

E21. The IL-12 mutein of embodiment E20, wherein the Y16 substitution of the variant p40 subunit is selected from the group consisting of Y16A, Y16R, Y16H, Y16K, Y16D, Y16E, Y16S, Y16T, Y16N, Y16Q, Y16G, Y16P, Y16I, Y16L, Y16M, Y16F, Y16W, and Y16V.

E22. The IL-12 mutein of embodiment E20 or E21, wherein the variant p40 subunit further comprises one or more amino acid substitutions relative to SEQ ID NO: 2.

E23. The IL-12 mutein of any one of embodiments E1 to E22, wherein the variant p40 subunit comprises one, two, three, four, five, or more amino acid substitutions at a position selected from the group consisting of W15, P17, D18, E59, F60, K84, E86, D93, H194, K195, and K197 relative to SEQ ID NO: 2.

E24. The IL-12 mutein of any one of embodiments E1 to E23, wherein the variant p40 subunit further comprises one or more, two or more, or three or more amino acid substitutions at a position selected from E59, F60, K84, K195, and K197 relative to SEQ ID NO: 2.

E25. The IL-12 mutein of any one of embodiments E1 to E24, wherein the variant p40 subunit comprises an amino acid sequence that is at least 80%, 85%, 90%, or 95% identical to SEQ ID NO: 2.

E26. An interleukin-12 (IL-12) mutein comprising a variant p35 subunit and a variant p40 subunit, wherein: the variant p35 subunit comprises one or more amino acid substitutions

relative to SEQ ID NO: 1, wherein the one or more amino acid substitutions are at a position selected from A33, R34, L37, E38, F39, Y40, P41, E45, E46, I47, D48, H49, E50, D51, I52, T53, K54, K56, T57, D108, L109, K110, M111, Y112, Q113, V114, E115, F116, K117, T118, A121, L123, M125, D126, R129, I131, P156, S159, S160, L161, E162, E163, D165, F166, Y167, K168, T169, K170, I171, K172, L173, I175, H178, and R181 relative to SEQ ID NO: 1, and the variant p40 subunit comprises one or more amino acid substitutions relative to SEQ ID NO: 2, wherein the one or more amino acid substitutions are at a position selected from V10, V11, E12, L13, D14, W15, Y16, P17, D18, A19, P20, G21, W38, T39, L40, D41, S43, S44, E45, V46, L47, I55, V57, K58, E59, F60, D62, A63, G64, Q65, Y66, L81, L82, H83, K84, K85, E86, G88, I89, W90, S91, T92, D93, I94, L95, D97, Q98, K99, E100, N103, K104, T105, F106, L107, L123, K135, S136, S137, R138, G139, S140, S141, D142, N162, E181, S183, L184, P185, I186, E187, V188, V190, D191, H194, K195, L196, K197, Y198, E199, N200, T202, S203, S204, F206, and D209 relative to SEQ ID NO: 2.

E27. The IL-12 mutein of embodiment E26, wherein Y16 of SEQ ID NO: 2 is substituted, preferably with an alanine residue.

E28. The IL-12 mutein of embodiment E26 or E27, wherein L133 of SEQ ID NO: 1 is substituted, preferably with an alanine residue.

E29. The IL-12 mutein of any one of embodiment E26 to E28, wherein the variant p35 further comprises one or more amino acid substitutions or substitutions at a position selected from:

- (a) wherein D126 of SEQ ID NO: 1 is substituted, preferably with an alanine residue;
- (b) wherein I131 of SEQ ID NO: 1 is substituted, preferably with an alanine residue;
- (c) wherein H178 of SEQ ID NO: 1 is substituted, preferably with an alanine residue;

and

- (d) any combination thereof.

E30. An interleukin-12 (IL-12) mutein comprising a variant p35 subunit and a variant p40 subunit, wherein:

(a) the variant p35 subunit comprises a substitution of one or more amino acids as shown in Table 1;

(b) the p40 moiety comprises a substitution of one or more amino acids as shown in Table 4; or

(c) the p35 moiety comprises a substitution of one or more amino acids as shown in Table 1 and the p40 moiety comprises a substitution of one or more amino acids as shown in Table 4.

E31. An interleukin-12 (IL-12) mutein comprising a variant p35 subunit and a variant p40 subunit, wherein:

(a) the variant p35 subunit comprises a substitution of one or more amino acid residues corresponding to an amino acid residue in SEQ ID NO: 1, wherein the substitution of one or more amino acids is a substitution listed in Table 1;

(b) the variant p40 subunit comprises a substitution of one or more amino acid residues corresponding to an amino acid residue in SEQ ID NO: 2, wherein the substitution of one or more amino acids is a substitution listed in Table 4; or

(c) the variant p35 subunit comprises a substitution of one or more amino acid residues corresponding to an amino acid residue in SEQ ID NO: 1, wherein the substitution of one or more amino acids is a substitution listed in Table 1, and the p40 moiety comprises a substitution of one or more amino acid residues corresponding to an amino acid residue in SEQ ID NO: 2, wherein the substitution of one or more amino acids is a substitution listed in Table 4.

E32. The IL-12 mutein of any one of embodiments E1-E31, wherein the IL-12 mutein is a human IL-12 mutein.

E33. A fusion protein comprising the IL-12 mutein of any one of embodiments E1-E32.

E34. A pharmaceutical composition comprising the IL-12 mutein of any one of embodiment E1 to E32 or the fusion protein of embodiment E33, and a pharmaceutically acceptable carrier or excipient.

E35. A polynucleotide sequence that encodes the IL-12 mutein according to any one of embodiments E1 to E32 or the fusion protein of embodiment E33.

E36. An expression vector comprising the polynucleotide sequence of embodiment E35.

E37. A host cell comprising the expression vector of embodiment E36.

E38. A kit comprising the IL-12 mutein according to any one of c embodiments E1 to E32 or the fusion protein of embodiment E33 and optionally packaging material.

E39. A method of producing the IL-12 mutein of any one of embodiments E1 to E32 or the fusion protein of embodiment E33, comprising culturing the host cell of embodiment E35 under conditions to express the IL-12 mutein or the fusion protein.

E40. The method of embodiment E39, further comprising isolating the IL-12 mutein from the host cell.

E41. A method of treating cancer in a patient in need thereof comprising administering to the patient a therapeutically effective amount of the IL-12 mutein of any one of embodiments E1 to E32, the fusion protein of embodiment E33, or the pharmaceutical composition of embodiment E34.

E42. The method of embodiment E41, wherein the cancer comprises a solid tumor.

E43. An interleukin-12 (IL-12) mutein comprising a variant p35 subunit, a variant p40 subunit, or both a variant p35 subunit and a variant p40 subunit,

wherein the variant p35 subunit, if present, comprises an amino acid sequence with one or more substitutions or deletions relative to SEQ ID NO: 1; and

wherein the variant p40 subunit, if present, comprises an amino acid sequence with one or more substitutions or deletions relative to SEQ ID NO: 2.

E44. The IL-12 mutein of embodiment E43, comprising the variant p35 subunit.

E45. The IL-12 mutein of embodiment E44, wherein the variant p35 subunit comprises a substitution at amino acid residue L133, optionally wherein the substitution is L133A, L133R, L133H, L133K, L133D, L133E, L133S, L133T, L133N, L133Q, L133G, L133P, L133I, L133M, L133F, L133W, L133V, or L133Y.

E46. The IL-12 mutein of embodiment E44 or E45, wherein the variant p35 subunit comprises a substitution at amino acid residue L37, optionally wherein the substitution is L37R, L37H, L37K, L37D, L37E, L37S, L37T, L37N, L37Q, L37G, L37P, L37A, L37V, L37I, L37M, L37F, L37Y, or L37W.

E47. The IL-12 mutein of embodiment E46, wherein, the substitution at amino acid residue L37 is L37R, L37H, L37K, L37D, L37N, L37Q, L37P, L37A, L37V, L37M, L37F, L37Y, or L37W.

E48. The IL-12 mutein of embodiment E46, wherein the L37 substitution is L37A.

E49. The IL-12 mutein of any one of embodiments E44 to E48, wherein the variant p35 subunit comprises a substitution at amino acid residue Y40, optionally wherein the substitution is Y40R, Y40H, Y40K, Y40D, Y40E, Y40S, Y40T, Y40N, Y40Q, Y40G, Y40P, Y40A, Y40V, Y40I, Y40L, Y40M, Y40F, or Y40W.

E50. The IL-12 mutein of embodiment E49, wherein the substitution at amino acid residue Y40 is Y40H, Y40E, Y40V, Y40I, Y40L, Y40M, or Y40F.

E51. The IL-12 mutein of embodiment E49, wherein the Y40 substitution is Y40S.

E52. The IL-12 mutein of any one of embodiments E44 to E51, wherein the variant p35 subunit comprises a substitution at amino acid residue D48, optionally wherein the substitution is D48R, D48H, D48K, D48E, D48S, D48T, D48N, D48Q, D48G, D48P, D48A, D48V, D48I, D48L, D48M, D48F, D48Y, or D48W.

E53. The IL-12 mutein of embodiment E52, wherein the D48 substitution is D48H, D48K, D48E, D48S, D48T, D48N, D48G, D48P, D48V, D48I, D48L, D48M, D48F, D48Y, or D48W, optionally wherein the D48 substitution is D48K, D48E, D48T, D48N, D48G, D48P, D48V, D48I, D48L, D48M, or D48F.

E54. The IL-12 mutein of any one of embodiments E44 to E53, wherein the variant p35 subunit comprises a substitution at amino acid residue K128, optionally wherein the substitution is K128R, K128H, K128K, K128D, K128E, K128S, K128T, K128N, K128Q, K128G, K128P, K128A, K128V, K128I, K128L, K128M, K128F, K128Y, or K128W.

E55. The IL-12 mutein of embodiment E54, wherein the K128 substitution is K128N or K128Q.

E56. The IL-12 mutein of any one of embodiments E44 to E55, wherein the variant p35 subunit comprises a substitution at amino acid residue M125, optionally wherein the substitution is M125R, M125H, M125K, M125D, M125E, M125S, M125T, M125N, M125Q, M125G, M125P, M125A, M125V, M125I, M125L, M125F, M125Y, or M125W.

E57. The IL-12 mutein of embodiment E56, wherein the M125 substitution is M125R, M125H, M125K, M125D, M125E, M125S, M125T, M125N, M125Q, M125G, M125P, M125L, M125F, M125Y, or M125W.

E58. The IL-12 mutein of any one of embodiments E44 to E57, wherein the variant p35 subunit comprises a substitution at amino acid residue R129, optionally wherein the substitution is R129H, R129K, R129D, R129E, R129S, R129T, R129N, R129Q, R129G, R129P, R129A, R129V, R129I, R129L, R129M, R129F, R129Y, or R129W.

E59. The IL-12 mutein of any one of embodiments E44 to E58, wherein the variant p35 subunit comprises a substitution at amino acid residue L177, optionally wherein the substitution is L177R, L177H, L177K, L177D, L177E, L177S, L177T, L177N, L177Q, L177G, L177P, L177A, L177V, L177I, L177M, L177F, L177Y, or L177W.

E60. The IL-12 mutein of embodiment E59, wherein the L177 substitution is L177D, L177S, L177T, L177N, L177G, L177P, L177V, L177I, L177M, or L177W.

E61. The IL-12 mutein of any one of embodiments E44 to E60, wherein the variant p35 subunit comprises a substitution at amino acid residue H178, optionally wherein the substitution is H178R, H178K, H178D, H178E, H178S, H178T, H178N, H178Q, H178G, H178P, H178A, H178V, H178I, H178L, H178M, H178F, H178Y, or H178W.

E62. The IL-12 mutein of any one of embodiments E44 to E61, wherein the variant p35 subunit comprises a substitution at amino acid residue K56, optionally wherein the K56 substitution is K56R, K56H, K56D, K56E, K56S, K56T, K56N, K56Q, K56G, K56P, K56A, K56V, K56I, K56L, K56M, K56F, K56Y, or K56W.

E63. The IL-12 mutein of embodiment E62, wherein the K56 substitution is K56R, K56H, K56D, K56E, K56S, K56T, K56G, K56P, K56A, K56V, K56I, K56L, K56M, K56F, K56Y, or K56W, optionally wherein the K56 substitution is K56E, K56S, K56T, K56G, K56A, K56V, K56I, or K56L.

- E64. The IL-12 mutein of embodiment E62, wherein the K56 substitution is K56N.
- E65. The IL-12 mutein of any one of embodiments E44 to E64, wherein the variant p35 subunit comprises a substitution at amino acid residue K170, optionally wherein the K170 substitution is K170R, K170H, K170D, K170E, K170S, K170T, K170N, K170Q, K170G, K170P, K170A, K170V, K170I, K170L, K170M, K170F, K170Y, or K170W.
- E66. The IL-12 mutein of any one of embodiments E44 to E65, wherein the variant p35 subunit comprises a substitution at amino acid residue I171, optionally wherein the I171 substitution is I171R, I171K, I171H, I171D, I171E, I171S, I171T, I171N, I171Q, I171G, I171P, I171A, I171V, I171L, I171M, I171F, I171Y, or I171W.
- E67. The IL-12 mutein of any one of embodiments E44 to E66, wherein the variant p35 subunit comprises a substitution at amino acid residue A33, R34, E38, F39, P41, E45, E46, I47, H49, E50, D51, I52, T53, K54, T57, D108, L109, K110, M111, Y112, Q113, V114, E115, F116, K117, T118, A121, L123, D126, I131, P156, S159, S160, L161, E162, E163, D165, F166, Y167, K168, T169, K172, L173, I175, and R181.
- E68. The IL-12 mutein of embodiment E44, wherein the variant p35 subunit comprises one or more amino acid substitutions selected from Table 1a, Table 1b, or Table 1c.
- E69. The IL-12 mutein of embodiment E44, wherein the variant p35 subunit comprises
a substitution at L37, optionally wherein the substitution is L37A; and
a substitution at Y40, optionally wherein the substitution is Y40S.
- E70. The IL-12 mutein of embodiment E44, wherein the variant p35 subunit comprises
a substitution at L37, optionally wherein the substitution is L37A;
a substitution at Y40, optionally wherein the substitution is Y40S; and
a substitution at L133, optionally wherein the substitution is L133A.
- E71. The IL-12 mutein of embodiment E44, wherein the variant p35 subunit comprises
a substitution at L37, optionally wherein the substitution is L37A;
a substitution at Y40, optionally wherein the substitution is Y40S; and
a substitution at K128, optionally wherein the substitution is K128N or K128Q.
- E72. The IL-12 mutein of embodiment E44, wherein the variant p35 subunit comprises
a substitution at L37, optionally wherein the substitution is L37A;
a substitution at Y40, optionally wherein the substitution is Y40S;
a substitution at K128, optionally wherein the substitution is K128N or K128Q; and
a substitution at L133, optionally wherein the substitution is L133A.

E73. The IL-12 mutein of any one of embodiments E44 to E72, wherein the variant p35 subunit comprises an amino acid sequence that is at least 80%, 85%, 90%, or 95% identical to SEQ ID NO: 1.

E74. The IL-12 mutein of any one of embodiments E43 to E73, comprising the variant p40 subunit.

E75. The IL-12 mutein of embodiment E74, wherein the variant p40 subunit comprises a substitution at amino acid residue V10, optionally wherein the substitution is V10A, V10R, V10H, V10K, V10D, V10E, V10S, V10T, V10N, V10Q, V10G, V10P, V10I, V10L, V10M, V10F, V10W, or V10Y.

E76. The IL-12 mutein of embodiment E74 or E75, wherein the variant p40 subunit comprises a substitution at amino acid residue I94, optionally wherein the substitution is I94A, I94R, I94H, I94K, I94D, I94E, I94S, I94T, I94N, I94Q, I94G, I94P, I94L, I94M, I94F, I94W, I94V, or I94Y.

E77. The IL-12 mutein of any one of embodiments E74 to E76, wherein the variant p40 subunit comprises a substitution at amino acid residue L95, optionally wherein the substitution is L95A, L95R, L95H, L95K, L95D, L95E, L95S, L95T, L95N, L95Q, L95G, L95P, L95I, L95M, L95F, L95W, L95V, or L95Y.

E78. The IL-12 mutein of any one of embodiments E74 to E77, wherein the variant p40 subunit comprises a substitution at amino acid residue G64, optionally wherein the substitution is G64A, G64R, G64H, G64K, G64D, G64E, G64S, G64T, G64N, G64Q, G64P, G64I, G64L, G64M, G64F, G64W, G64V, or G64Y.

E79. The IL-12 mutein of embodiment E78, wherein the G64 substitution is G64R, G64H, G64K, G64D, G64E, G64S, G64T, G64N, G64Q, G64P, G64I, G64L, G64M, G64F, G64W, G64V, or G64Y, optionally wherein the G64 substitution is G64R, G64K, G64E, G64S, G64T, G64Q, G64P, G64I, G64L, G64M, G64F, G64W, G64V, or G64Y.

E80. The IL-12 mutein of any one of embodiments E74 to E79, wherein the variant p40 subunit comprises a substitution at amino acid residue Y198, optionally wherein the substitution is Y198A, Y198R, Y198H, Y198K, Y198D, Y198E, Y198S, Y198T, Y198N, Y198Q, Y198G, Y198P, Y198I, Y198L, Y198M, Y198F, Y198W, or Y198V.

E81. The IL-12 mutein of embodiment E80, wherein the Y198 substitution is Y198A, Y198R, Y198H, Y198K, Y198D, Y198E, Y198T, Y198N, Y198Q, Y198G, Y198P, Y198I, Y198L, Y198M, Y198F, Y198W, or Y198V, optionally wherein the Y198 substitution is Y198G, Y198E, Y198K, Y198Q, Y198R, Y198W.

E82. The IL-12 mutein of any one of embodiments E74 to E81, wherein the variant p40 subunit comprises a substitution at amino acid residue Y16, optionally wherein the substitution is Y16A,

Y16R, Y16H, Y16K, Y16D, Y16E, Y16S, Y16T, Y16N, Y16Q, Y16G, Y16P, Y16I, Y16L, Y16M, Y16F, Y16W, or Y16V.

E83. The IL-12 mutein of any one of embodiments E74 to E82, wherein the variant p40 subunit comprises a substitution at amino acid residue V11, E12, L13, D14, W15, P17, D18, A19, P20, G21, W38, T39, L40, D41, S43, S44, E45, V46, L47, I55, V57, K58, E59, F60, D62, A63, Q65, Y66, L81, L82, H83, K84, K85, E86, G88, I89, W90, S91, T92, D93, D97, Q98, K99, E100, N103, K104, T105, F106, L107, L123, K135, S136, S137, R138, G139, S140, S141, D142, N162, E181, S183, L184, P185, I186, E187, V188, V190, D191, H194, K195, L196, K197, E199, N200, T202, S203, S204, F206, or D209.

E84. The IL-12 mutein of embodiment E83, wherein the variant p40 subunit comprises a substitution at amino acid residue W15, P17, D18, E59, F60, K84, E86, D93, H194, K195, or K197, optionally wherein the variant p40 subunit comprises a substitution at amino acid residue E59, F60, K84, K195, or K197.

E85. The IL-12 mutein of embodiment E74, wherein the variant p40 subunit comprises one or more amino acid substitutions selected from Table 4a, Table 4b, or Table 4c.

E86. The IL-12 mutein of any one of embodiments E74 to E85, wherein the variant p40 subunit comprises an amino acid sequence that is at least 80%, 85%, 90%, or 95% identical to SEQ ID NO: 2.

E87. The IL-12 mutein of any one of embodiments E43 to E86, wherein the IL-12 mutein is a human IL-12 mutein.

E88. A fusion protein comprising the IL-12 mutein of any one of embodiments E43- E87.

E89. A pharmaceutical composition comprising the IL-12 mutein of any one of embodiments E43 to E87 or the fusion protein of embodiment E88, and a pharmaceutically acceptable carrier or excipient.

E90. A polynucleotide sequence that encodes the IL-12 mutein according to any one of embodiments E43 to E87 or the fusion protein of embodiment E88.

E91. An expression vector comprising the polynucleotide sequence of embodiment E90.

E92. A host cell comprising the expression vector of embodiment E91.

E93. A kit comprising the IL-12 mutein according to any one of embodiments E43 to E87 or the fusion protein of embodiment E88 and optionally packaging material.

E94. A method of producing the IL-12 mutein of any one of embodiments E43 to E87 or the fusion protein of embodiment E88, comprising culturing the host cell of embodiment E92 under conditions to express the IL-12 mutein or the fusion protein.

- E95. The method of embodiment E94, further comprising isolating the IL-12 mutein from the host cell.
- E96. A method of treating cancer in a patient in need thereof comprising administering to the patient a therapeutically effective amount of the IL-12 mutein of any one of embodiments E43 to E87, the fusion protein of embodiment E88, or the pharmaceutical composition of embodiment E89.
- E97. The method of embodiment E96, wherein the cancer comprises a solid tumor.
- E98. The IL-12 mutein of embodiment E46, wherein the L37 substitution is L37R.
- E99. The IL-12 mutein of embodiment E46, wherein the L37 substitution is L37H.
- E100. The IL-12 mutein of embodiment E46, wherein the L37 substitution is L37K.
- E101. The IL-12 mutein of embodiment E46, wherein the L37 substitution is L37D.
- E102. The IL-12 mutein of embodiment E46, wherein the L37 substitution is L37N.
- E103. The IL-12 mutein of embodiment E46, wherein the L37 substitution is L37Q.
- E104. The IL-12 mutein of embodiment E46, wherein the L37 substitution is L37P.
- E105. The IL-12 mutein of embodiment E46, wherein the L37 substitution is L37V.
- E106. The IL-12 mutein of embodiment E46, wherein the L37 substitution is L37M.
- E107. The IL-12 mutein of embodiment E46, wherein the L37 substitution is L37F.
- E108. The IL-12 mutein of embodiment E46, wherein the L37 substitution is L37Y.
- E109. The IL-12 mutein of embodiment E46, wherein the L37 substitution is L37W.
- E110. The IL-12 mutein of embodiment E45, wherein the L133 substitution is L133A.
- E111. The IL-12 mutein of embodiment E45, wherein the L133 substitution is L133R.
- E112. The IL-12 mutein of embodiment E45, wherein the L133 substitution is L133H.
- E113. The IL-12 mutein of embodiment E45, wherein the L133 substitution is L133K.
- E114. The IL-12 mutein of embodiment E45, wherein the L133 substitution is L133D.
- E115. The IL-12 mutein of embodiment E45, wherein the L133 substitution is L133E.
- E116. The IL-12 mutein of embodiment E45, wherein the L133 substitution is L133S.
- E117. The IL-12 mutein of embodiment E45, wherein the L133 substitution is L133T.
- E118. The IL-12 mutein of embodiment E45, wherein the L133 substitution is L133N.
- E119. The IL-12 mutein of embodiment E45, wherein the L133 substitution is L133Q.
- E120. The IL-12 mutein of embodiment E45, wherein the L133 substitution is L133G.
- E121. The IL-12 mutein of embodiment E45, wherein the L133 substitution is L133P.
- E122. The IL-12 mutein of embodiment E45, wherein the L133 substitution is L133I.
- E123. The IL-12 mutein of embodiment E45, wherein the L133 substitution is L133M.
- E124. The IL-12 mutein of embodiment E45, wherein the L133 substitution is L133F.

E125. The IL-12 mutein of embodiment E45, wherein the L133 substitution is L133W.
E126. The IL-12 mutein of embodiment E45, wherein the L133 substitution is L133V.
E127. The IL-12 mutein of embodiment E45, wherein the L133 substitution is L133Y.
E128. The IL-12 mutein of embodiment E50, wherein the Y40 substitution is Y40H.
E129. The IL-12 mutein of embodiment E50, wherein the Y40 substitution is Y40E.
E130. The IL-12 mutein of embodiment E50, wherein the Y40 substitution is Y40V.
E131. The IL-12 mutein of embodiment E50, wherein the Y40 substitution is Y40I.
E132. The IL-12 mutein of embodiment E50, wherein the Y40 substitution is Y40L.
E133. The IL-12 mutein of embodiment E50, wherein the Y40 substitution is Y40M.
E134. The IL-12 mutein of embodiment E50, wherein the Y40 substitution is Y40F.
E135. The IL-12 mutein of embodiment E53, wherein the D48 substitution is D48K.
E136. The IL-12 mutein of embodiment E53, wherein the D48 substitution is D48E.
E137. The IL-12 mutein of embodiment E53, wherein the D48 substitution is D48T.
E138. The IL-12 mutein of embodiment E53, wherein the D48 substitution is D48N.
E139. The IL-12 mutein of embodiment E53, wherein the D48 substitution is D48G.
E140. The IL-12 mutein of embodiment E53, wherein the D48 substitution is D48P.
E141. The IL-12 mutein of embodiment E53, wherein the D48 substitution is D48V.
E142. The IL-12 mutein of embodiment E53, wherein the D48 substitution is D48I.
E143. The IL-12 mutein of embodiment E53, wherein the D48 substitution is D48L.
E144. The IL-12 mutein of embodiment E53, wherein the D48 substitution is D48M.
E145. The IL-12 mutein of embodiment E53, wherein the D48 substitution is D48F.
E146. The IL-12 mutein of embodiment E55, wherein the K128 substitution is K128N.
E147. The IL-12 mutein of embodiment E55, wherein the K128 substitution is K128Q.
E148. The IL-12 mutein of embodiment E57, wherein the M125 substitution is M125R.
E149. The IL-12 mutein of embodiment E57, wherein the M125 substitution is M125H.
E150. The IL-12 mutein of embodiment E57, wherein the M125 substitution is M125K.
E151. The IL-12 mutein of embodiment E57, wherein the M125 substitution is M125D.
E152. The IL-12 mutein of embodiment E57, wherein the M125 substitution is M125E.
E153. The IL-12 mutein of embodiment E57, wherein the M125 substitution is M125S.
E154. The IL-12 mutein of embodiment E57, wherein the M125 substitution is M125T.
E155. The IL-12 mutein of embodiment E57, wherein the M125 substitution is M125N.
E156. The IL-12 mutein of embodiment E57, wherein the M125 substitution is M125Q.
E157. The IL-12 mutein of embodiment E57, wherein the M125 substitution is M125G.
E158. The IL-12 mutein of embodiment E57, wherein the M125 substitution is M125P.

E159. The IL-12 mutein of embodiment E57, wherein the M125 substitution is M125L.
E160. The IL-12 mutein of embodiment E57, wherein the M125 substitution is M125F.
E161. The IL-12 mutein of embodiment E57, wherein the M125 substitution is M125Y.
E162. The IL-12 mutein of embodiment E57, wherein the M125 substitution is M125W.
E163. The IL-12 mutein of embodiment E58, wherein the R129 substitution is R129H.
E164. The IL-12 mutein of embodiment E58, wherein the R129 substitution is R129K.
E165. The IL-12 mutein of embodiment E58, wherein the R129 substitution is R129D.
E166. The IL-12 mutein of embodiment E58, wherein the R129 substitution is R129E.
E167. The IL-12 mutein of embodiment E58, wherein the R129 substitution is R129S.
E168. The IL-12 mutein of embodiment E58, wherein the R129 substitution is R129T.
E169. The IL-12 mutein of embodiment E58, wherein the R129 substitution is R129N.
E170. The IL-12 mutein of embodiment E58, wherein the R129 substitution is R129Q.
E171. The IL-12 mutein of embodiment E58, wherein the R129 substitution is R129G.
E172. The IL-12 mutein of embodiment E58, wherein the R129 substitution is R129P.
E173. The IL-12 mutein of embodiment E58, wherein the R129 substitution is R129A.
E174. The IL-12 mutein of embodiment E58, wherein the R129 substitution is R129V.
E175. The IL-12 mutein of embodiment E58, wherein the R129 substitution is R129I.
E176. The IL-12 mutein of embodiment E58, wherein the R129 substitution is R129L.
E177. The IL-12 mutein of embodiment E58, wherein the R129 substitution is R129M.
E178. The IL-12 mutein of embodiment E58, wherein the R129 substitution is R129F.
E179. The IL-12 mutein of embodiment E58, wherein the R129 substitution is R129Y.
E180. The IL-12 mutein of embodiment E58, wherein the R129 substitution is R129W.
E181. The IL-12 mutein of embodiment E60, wherein the L177 substitution is L177D.
E182. The IL-12 mutein of embodiment E60, wherein the L177 substitution is L177S.
E183. The IL-12 mutein of embodiment E60, wherein the L177 substitution is L177T.
E184. The IL-12 mutein of embodiment E60, wherein the L177 substitution is L177N.
E185. The IL-12 mutein of embodiment E60, wherein the L177 substitution is L177G.
E186. The IL-12 mutein of embodiment E60, wherein the L177 substitution is L177P.
E187. The IL-12 mutein of embodiment E60, wherein the L177 substitution is L177V.
E188. The IL-12 mutein of embodiment E60, wherein the L177 substitution is L177I.
E189. The IL-12 mutein of embodiment E60, wherein the L177 substitution is L177M.
E190. The IL-12 mutein of embodiment E60, wherein the L177 substitution is L177W.
E191. The IL-12 mutein of embodiment E61, wherein the H178 substitution is H178R.
E192. The IL-12 mutein of embodiment E61, wherein the H178 substitution is H178K.

E193. The IL-12 mutein of embodiment E61, wherein the H178 substitution is H178D.
E194. The IL-12 mutein of embodiment E61, wherein the H178 substitution is H178E.
E195. The IL-12 mutein of embodiment E61, wherein the H178 substitution is H178S.
E196. The IL-12 mutein of embodiment E61, wherein the H178 substitution is H178T.
E197. The IL-12 mutein of embodiment E61, wherein the H178 substitution is H178N.
E198. The IL-12 mutein of embodiment E61, wherein the H178 substitution is H178Q.
E199. The IL-12 mutein of embodiment E61, wherein the H178 substitution is H178G.
E200. The IL-12 mutein of embodiment E61, wherein the H178 substitution is H178P.
E201. The IL-12 mutein of embodiment E61, wherein the H178 substitution is H178A.
E202. The IL-12 mutein of embodiment E61, wherein the H178 substitution is H178V.
E203. The IL-12 mutein of embodiment E61, wherein the H178 substitution is H178I.
E204. The IL-12 mutein of embodiment E61, wherein the H178 substitution is H178L.
E205. The IL-12 mutein of embodiment E61, wherein the H178 substitution is H178M.
E206. The IL-12 mutein of embodiment E61, wherein the H178 substitution is H178F.
E207. The IL-12 mutein of embodiment E61, wherein the H178 substitution is H178Y.
E208. The IL-12 mutein of embodiment E61, wherein the H178 substitution is H178W.
E209. The IL-12 mutein of embodiment E64, wherein the K56 substitution is K56N.
E210. The IL-12 mutein of embodiment E65, wherein the K170 substitution is K170R.
E211. The IL-12 mutein of embodiment E65, wherein the K170 substitution is K170H.
E212. The IL-12 mutein of embodiment E65, wherein the K170 substitution is K170D.
E213. The IL-12 mutein of embodiment E65, wherein the K170 substitution is K170E.
E214. The IL-12 mutein of embodiment E65, wherein the K170 substitution is K170S.
E215. The IL-12 mutein of embodiment E65, wherein the K170 substitution is K170T.
E216. The IL-12 mutein of embodiment E65, wherein the K170 substitution is K170N.
E217. The IL-12 mutein of embodiment E65, wherein the K170 substitution is K170Q.
E218. The IL-12 mutein of embodiment E65, wherein the K170 substitution is K170G.
E219. The IL-12 mutein of embodiment E65, wherein the K170 substitution is K170P.
E220. The IL-12 mutein of embodiment E65, wherein the K170 substitution is K170A.
E221. The IL-12 mutein of embodiment E65, wherein the K170 substitution is K170V.
E222. The IL-12 mutein of embodiment E65, wherein the K170 substitution is K170I.
E223. The IL-12 mutein of embodiment E65, wherein the K170 substitution is K170L.
E224. The IL-12 mutein of embodiment E65, wherein the K170 substitution is K170M.
E225. The IL-12 mutein of embodiment E65, wherein the K170 substitution is K170F.
E226. The IL-12 mutein of embodiment E65, wherein the K170 substitution is K170Y.

E227. The IL-12 mutein of embodiment E65, wherein the K170 substitution is K170W.
E228. The IL-12 mutein of embodiment E66, wherein the I171 substitution is I171R.
E229. The IL-12 mutein of embodiment E66, wherein the I171 substitution is I171K.
E230. The IL-12 mutein of embodiment E66, wherein the I171 substitution is I171H.
E231. The IL-12 mutein of embodiment E66, wherein the I171 substitution is I171D.
E232. The IL-12 mutein of embodiment E66, wherein the I171 substitution is I171E.
E233. The IL-12 mutein of embodiment E66, wherein the I171 substitution is I171S.
E234. The IL-12 mutein of embodiment E66, wherein the I171 substitution is I171T.
E235. The IL-12 mutein of embodiment E66, wherein the I171 substitution is I171N.
E236. The IL-12 mutein of embodiment E66, wherein the I171 substitution is I171Q.
E237. The IL-12 mutein of embodiment E66, wherein the I171 substitution is I171G.
E238. The IL-12 mutein of embodiment E66, wherein the I171 substitution is I171P.
E239. The IL-12 mutein of embodiment E66, wherein the I171 substitution is I171A.
E240. The IL-12 mutein of embodiment E66, wherein the I171 substitution is I171V.
E241. The IL-12 mutein of embodiment E66, wherein the I171 substitution is I171L.
E242. The IL-12 mutein of embodiment E66, wherein the I171 substitution is I171M.
E243. The IL-12 mutein of embodiment E66, wherein the I171 substitution is I171F.
E244. The IL-12 mutein of embodiment E66, wherein the I171 substitution is I171Y.
E245. The IL-12 mutein of embodiment E66, wherein the I171 substitution is I171W.
E246. The IL-12 mutein of embodiment E75, wherein the V10 substitution is V10A.
E247. The IL-12 mutein of embodiment E75, wherein the V10 substitution is V10R.
E248. The IL-12 mutein of embodiment E75, wherein the V10 substitution is V10H.
E249. The IL-12 mutein of embodiment E75, wherein the V10 substitution is V10K.
E250. The IL-12 mutein of embodiment E75, wherein the V10 substitution is V10D.
E251. The IL-12 mutein of embodiment E75, wherein the V10 substitution is V10E.
E252. The IL-12 mutein of embodiment E75, wherein the V10 substitution is V10S.
E253. The IL-12 mutein of embodiment E75, wherein the V10 substitution is V10T.
E254. The IL-12 mutein of embodiment E75, wherein the V10 substitution is V10N.
E255. The IL-12 mutein of embodiment E75, wherein the V10 substitution is V10Q.
E256. The IL-12 mutein of embodiment E75, wherein the V10 substitution is V10G.
E257. The IL-12 mutein of embodiment E75, wherein the V10 substitution is V10P.
E258. The IL-12 mutein of embodiment E75, wherein the V10 substitution is V10I.
E259. The IL-12 mutein of embodiment E75, wherein the V10 substitution is V10L.
E260. The IL-12 mutein of embodiment E75, wherein the V10 substitution is V10M.

E261. The IL-12 mutein of embodiment E75, wherein the V10 substitution is V10F.
E262. The IL-12 mutein of embodiment E75, wherein the V10 substitution is V10W.
E263. The IL-12 mutein of embodiment E75, wherein the V10 substitution is V10Y.
E264. The IL-12 mutein of embodiment E76, wherein the I94 substitution is I94A.
E265. The IL-12 mutein of embodiment E76, wherein the I94 substitution is I94R.
E266. The IL-12 mutein of embodiment E76, wherein the I94 substitution is I94H.
E267. The IL-12 mutein of embodiment E76, wherein the I94 substitution is I94K.
E268. The IL-12 mutein of embodiment E76, wherein the I94 substitution is I94D.
E269. The IL-12 mutein of embodiment E76, wherein the I94 substitution is I94E.
E270. The IL-12 mutein of embodiment E76, wherein the I94 substitution is I94S.
E271. The IL-12 mutein of embodiment E76, wherein the I94 substitution is I94T.
E272. The IL-12 mutein of embodiment E76, wherein the I94 substitution is I94N.
E273. The IL-12 mutein of embodiment E76, wherein the I94 substitution is I94Q.
E274. The IL-12 mutein of embodiment E76, wherein the I94 substitution is I94G.
E275. The IL-12 mutein of embodiment E76, wherein the I94 substitution is I94P.
E276. The IL-12 mutein of embodiment E76, wherein the I94 substitution is I94L.
E277. The IL-12 mutein of embodiment E76, wherein the I94 substitution is I94M.
E278. The IL-12 mutein of embodiment E76, wherein the I94 substitution is I94F.
E279. The IL-12 mutein of embodiment E76, wherein the I94 substitution is I94W.
E280. The IL-12 mutein of embodiment E76, wherein the I94 substitution is I94V.
E281. The IL-12 mutein of embodiment E76, wherein the I94 substitution is I94Y.
E282. The IL-12 mutein of embodiment E77, wherein the L95 substitution is L95A.
E283. The IL-12 mutein of embodiment E77, wherein the L95 substitution is L95R.
E284. The IL-12 mutein of embodiment E77, wherein the L95 substitution is L95H.
E285. The IL-12 mutein of embodiment E77, wherein the L95 substitution is L95K.
E286. The IL-12 mutein of embodiment E77, wherein the L95 substitution is L95D.
E287. The IL-12 mutein of embodiment E77, wherein the L95 substitution is L95E.
E288. The IL-12 mutein of embodiment E77, wherein the L95 substitution is L95S.
E289. The IL-12 mutein of embodiment E77, wherein the L95 substitution is L95T.
E290. The IL-12 mutein of embodiment E77, wherein the L95 substitution is L95N.
E291. The IL-12 mutein of embodiment E77, wherein the L95 substitution is L95Q.
E292. The IL-12 mutein of embodiment E77, wherein the L95 substitution is L95G.
E293. The IL-12 mutein of embodiment E77, wherein the L95 substitution is L95P.
E294. The IL-12 mutein of embodiment E77, wherein the L95 substitution is L95I.

E295. The IL-12 mutein of embodiment E77, wherein the L95 substitution is L9M.
E296. The IL-12 mutein of embodiment E77, wherein the L95 substitution is L95F.
E297. The IL-12 mutein of embodiment E77, wherein the L95 substitution is L95W.
E298. The IL-12 mutein of embodiment E77, wherein the L95 substitution is L95V.
E299. The IL-12 mutein of embodiment E77, wherein the L95 substitution is L95Y.
E300. The IL-12 mutein of embodiment E79, wherein the G64 substitution is G64R.
E301. The IL-12 mutein of embodiment E79, wherein the G64 substitution is G64K.
E302. The IL-12 mutein of embodiment E79, wherein the G64 substitution is G64E.
E303. The IL-12 mutein of embodiment E79, wherein the G64 substitution is G64S.
E304. The IL-12 mutein of embodiment E79, wherein the G64 substitution is G64T.
E305. The IL-12 mutein of embodiment E79, wherein the G64 substitution is G64Q.
E306. The IL-12 mutein of embodiment E79, wherein the G64 substitution is G64P.
E307. The IL-12 mutein of embodiment E79, wherein the G64 substitution is G64I.
E308. The IL-12 mutein of embodiment E79, wherein the G64 substitution is G64L.
E309. The IL-12 mutein of embodiment E79, wherein the G64 substitution is G64M.
E310. The IL-12 mutein of embodiment E79, wherein the G64 substitution is G64F.
E311. The IL-12 mutein of embodiment E79, wherein the G64 substitution is G64W.
E312. The IL-12 mutein of embodiment E79, wherein the G64 substitution is G64V.
E313. The IL-12 mutein of embodiment E79, wherein the G64 substitution is G64Y.
E314. The IL-12 mutein of embodiment E81, wherein the Y198 substitution is Y198G.
E315. The IL-12 mutein of embodiment E81, wherein the Y198 substitution is Y198E.
E316. The IL-12 mutein of embodiment E81, wherein the Y198 substitution is Y198K.
E317. The IL-12 mutein of embodiment E81, wherein the Y198 substitution is Y198Q.
E318. The IL-12 mutein of embodiment E81, wherein the Y198 substitution is Y198R.
E319. The IL-12 mutein of embodiment E81, wherein the Y198 substitution is Y198W.
E320. The IL-12 mutein of embodiment E82, wherein the Y16 substitution is Y16A.
E321. The IL-12 mutein of embodiment E82, wherein the Y16 substitution is Y16R.
E322. The IL-12 mutein of embodiment E82, wherein the Y16 substitution is Y16H.
E323. The IL-12 mutein of embodiment E82, wherein the Y16 substitution is Y16K.
E324. The IL-12 mutein of embodiment E82, wherein the Y16 substitution is Y16D.
E325. The IL-12 mutein of embodiment E82, wherein the Y16 substitution is Y16E.
E326. The IL-12 mutein of embodiment E82, wherein the Y16 substitution is Y16S.
E327. The IL-12 mutein of embodiment E82, wherein the Y16 substitution is Y16T.
E328. The IL-12 mutein of embodiment E82, wherein the Y16 substitution is Y16N.

- E329. The IL-12 mutein of embodiment E82, wherein the Y16 substitution is Y16Q.
E330. The IL-12 mutein of embodiment E82, wherein the Y16 substitution is Y16G.
E331. The IL-12 mutein of embodiment E82, wherein the Y16 substitution is Y16P.
E332. The IL-12 mutein of embodiment E82, wherein the Y16 substitution is Y16I.
E333. The IL-12 mutein of embodiment E82, wherein the Y16 substitution is Y16L.
E334. The IL-12 mutein of embodiment E82, wherein the Y16 substitution is Y16M.
E335. The IL-12 mutein of embodiment E82, wherein the Y16 substitution is Y16F.
E336. The IL-12 mutein of embodiment E82, wherein the Y16 substitution is Y16W.
E337. The IL-12 mutein of embodiment E82, wherein the Y16 substitution is Y16V.

Table 20: Polypeptide sequences of subunits of wild-type IL-12 and IL-12 receptor

SEQ NAME	SEQ ID NO:	Sequence
IL-12 p35 Mature form (UniProt ID: P29459)	1	RNLPVATPDP GMFPC LHHSQ NLLRAVS NML 30 QKARQTLEFY PCTSEEIDHE DITKDKTSTV 60 EACLPLELTK NESCLNSRET SFITNGSCLA 90 SRKTSFMMAL CLSSIIYEDLK MYQVEFKTMN 120 AKLLMDPKRQ IFLDQNMLAV IDELMQALNF 150 NSETVPQKSS LEEPDFYKTK IKLCILLHAF 180 RIRAVTIDRV MSYLNAS 197
IL-12 p40 Mature form (UniProt ID: P29460)	2	IWELKKDVYV VELDWYPDAP GEMVVLTCDT 30 PEEDGITWTL DQSSEVLGSG KTLTIQVKEF 60 GDAGQYTCHK GGEVLSHSL LLHKKEDGIW 90 STDILKDQKE PKNKTF LRCE AKNYSGRFTC 120 WWLTTISTDL TFSVKSSRG S DPQGVTCGA 150 ATLSAERVRG DNKEYEYSVE CQEDSACPAA 180 EESLPIEVMV DAVHKLKYEN YTSSFFIRDI 210 IKPDPPKNLQ LKPLKNSRQV EVSWEYPDTW 240 STPHSYFSLT FCVQVQGKSK REKKDRVFTD 270 KTSATVICRK NASISVRAQD RYSSSSWSEW 300 ASVPCS 306
IL-12 p35 Pro-protein (UniProt ID: P29459) Signal Sequence in Bold/Underline	3	<u>MCPARSLLLV</u> <u>ATLVLLDHLS</u> <u>LARNLPVATP</u> 30 DPGMFPC LHH SQNLLRAVS N MLQKARQ TLE 60 FY PCTSEEID HEDITKDKTS TVEACLPLEL 90 TKNESCLNSR ETSFITNGSC LASRKTSFMM 120 ALCLSSIIYED LKMYQVEFKT MNAKLLMDPK 150 RQIFLDQNML AVIDELMQAL NFNSETVPQK 180 SSLEEPDFYK TKIKLCILH AFRIRAVTID 210 RVMSYLNAS 219
IL-12 p40 Pro-protein (UniProt ID: P29460) Signal Sequence in Bold	4	<u>MCHQQLVISW</u> <u>FSLVFLASPL</u> <u>VAIWELKKDV</u> 30 YVVELDWYPD APGEMVVLTC DTPEEDGITW 60 TLDQSSEVLG SGKTLTIQVK EFGDAGQYTC 90 HKGGEVLSHS LLLLHKKEDG IWSTDILKDQ 120 KEPKNKTF LR CEAKNYSGRF TCWWLTTIST 150 DLTFSVKSSR GSSDPQGVTC GAATLSAERV 180 RGDNKEYEYS VECQEDSACP AAESLPIEV 210 MVDAVHKLKY ENYTSSFFIR DI IKPDPPKN 240

SEQ NAME	SEQ ID NO:	Sequence			
		LQLKPLKNSR	QVEVSWEYPD	TWSTPHSYFS	270
		LTFCVQVQGK	SKREKKDRVF	TDKTSATVIC	300
		RKNASISVRA	QDRYYSSWS	EWASVPCS	328
IL-12R β 1 (UniProt ID: P42701)	5	MEPLVTWVVP	LLFLFLLSRQ	GAACRTSECC	30
		FQDPYPAD	SGSASGPRDL	RCYRISSDRY	60
		ECSWQYEGPT	AGVSHFLRCC	LSSGRCCYFA	90
		AGSATRLQFS	DQAGVSVLYT	VTLWVESWAR	120
		NQTEKSPEVT	LQLYNSVKYE	PPLGDIKVS	150
		LAGQLRMEWE	TPDNQVGAEV	QFRHRTSPSSP	180
		WKLGDGCPQD	DDTESCLCPL	EMNVAQEFQL	210
		RRRQLGSQGS	SWSKWSSPVC	VPPENPPQPQ	240
		VRFSVEQLGQ	DGRRRLTLKE	QPTQLELPEG	270
		CQGLAPGTEV	TYRLQLHMLS	CPCAKATRT	300
		LHLGKMPYLS	GAAYNVAVIS	SNQFGPGLNQ	330
		TWHIPADTHT	EPVALNISVG	TNGTTMYWPA	360
		RAQSMTYCIE	WQPVGQDGGL	ATCSLTAPQD	390
		PDPAGMATYS	WSRESGAMGQ	EKCYIITIFA	420
		SAHPEKLTW	STVLSTYHFG	GNASAAGTPH	450
		HVSVKNHSLD	SVSVDWAPSL	LSTCPGVLKE	480
		YVVRCDDEDS	KQVSEHPVQP	TETQVTLSGL	510
		RAGVAYTVQV	RADTAWLRGV	WSQPQRFSIE	540
		VQVSDWLIFF	ASLGSFLSIL	LVGVVLGYLGL	570
		NRAARHLCP	LPTPCASSAI	EPFGKETWQ	600
		WINPVDFQEE	ASLQEALVE	MSWDKGERTE	630
		PLEKTELPEG	APELALDTEL	SLEDGDRCKA	660
		KM			662
IL-12R β 2 (UniProt ID: Q99665)	6	MAHTFRGCSL	AFMFIITWLL	IKAKIDACKR	30
		GDVTVKPSHV	ILLGSTVNIT	CSLKPRQGCF	60
		HYSRRNKLIL	YKFDRRINFH	HGHSLSNSQVT	90
		GLPLGTTLFV	CKLACINSDE	IQICGAEIFV	120
		GVAPEQPQNL	SCIQKGEQGT	VACTWERGRD	150
		THLYTEYTLQ	LSGPKNLTWQ	KQCKDIYCDY	180
		LDGGINLTPE	SPESNFTAKV	TAVNSLGSSS	210
		SLPSTFTFLD	IVRPLPPWDI	RIKFQKASVS	240
		RCTLYWRDEG	LVLLNRLRYR	PSNSRLWNMV	270
		NVTKAKGRHD	LLDLKPFTEY	EFQISSKLHL	300
		YKGSWSDWSE	SLRAQTPEEE	PTGMLDVWYM	330
		KRHIDYSRQQ	ISLFWKNLSV	SEARGKILHY	360
		QVTLQELTGG	KAMTQNITGH	TSWTTVIPRT	390
		GNWAVAVSAA	NSKGSSLPTR	INIMNLCEAG	420
		LLAPRQVSAN	SEGMDNILVT	WQPPRKDP	450
		VQEYVVEWRE	LHPGGDTQVP	LNWLRSRPYN	480
		VSALISENIK	SYICYEIRVY	ALSGDQGGCS	510
		SILGNSKHKA	PLSGPHINAI	TEEKGSILIS	540
		WNSIPVQEQM	GCLLHYRIYW	KERDSNSQPQ	570
		LCEIPYRVSQ	NSHPINSLQP	RVTYVLWMTA	600
		LTAAGESSHG	NEREFCLQGK	ANWMAFVAPS	630
		ICIAIIMVGI	FSTHYFQQKV	FVLLAALRPQ	660
		WCSREIPDPA	NSTCAKKYPI	AEEKTQLPLD	690
		RLIDWPTPE	DPEPLVISEV	LHQVTPVFRH	720
		PPCSNWPQRE	KGIQGHQASE	KDMMHSASSP	750
		PPRALQAES	RQLVDLYKVL	ESRGSDPKPE	780
		NPACPWTVLP	AGDLPTHG	LPSNIDDLPS	810
		HEAPLADSLE	ELEPQHISLS	VFPSSSLHPL	840

SEQ NAME	SEQ ID NO:	Sequence
		TFSCGDKLTL DQLKMRCDSL ML 862

CLAIMS

WHAT IS CLAIMED IS:

1. An interleukin-12 (IL-12) mutein comprising a variant p35 subunit, a variant p40 subunit, or both a variant p35 subunit and a variant p40 subunit,
wherein the variant p35 subunit, if present, comprises an amino acid sequence with one or more substitutions or deletions relative to SEQ ID NO: 1; and
wherein the variant p40 subunit, if present, comprises an amino acid sequence with one or more substitutions or deletions relative to SEQ ID NO: 2.
2. The IL-12 mutein of claim 1, comprising the variant p35 subunit.
3. The IL-12 mutein of claim 2, wherein the variant p35 subunit comprises a substitution at amino acid residue L133, optionally wherein the substitution is L133A, L133R, L133H, L133K, L133D, L133E, L133S, L133T, L133N, L133Q, L133G, L133P, L133I, L133M, L133F, L133W, L133V, or L133Y.
4. The IL-12 mutein of claim 2 or 3, wherein the variant p35 subunit comprises a substitution at amino acid residue L37, optionally wherein the substitution is L37R, L37H, L37K, L37D, L37E, L37S, L37T, L37N, L37Q, L37G, L37P, L37A, L37V, L37I, L37M, L37F, L37Y, or L37W.
5. The IL-12 mutein of claim 4, wherein the substitution at amino acid residue L37 is L37R, L37H, L37K, L37D, L37N, L37Q, L37P, L37A, L37V, L37M, L37F, L37Y, or L37W.
6. The IL-12 mutein of claim 4, wherein the L37 substitution is L37A.
7. The IL-12 mutein of any one of claims 2 to 6, wherein the variant p35 subunit comprises a substitution at amino acid residue Y40, optionally wherein the substitution is Y40R, Y40H, Y40K, Y40D, Y40E, Y40S, Y40T, Y40N, Y40Q, Y40G, Y40P, Y40A, Y40V, Y40I, Y40L, Y40M, Y40F, or Y40W.
8. The IL-12 mutein of claim 7, wherein the substitution at amino acid residue Y40 is Y40H, Y40E, Y40V, Y40I, Y40L, Y40M, or Y40F.
9. The IL-12 mutein of claim 7, wherein the substitution at amino acid residue Y40 is Y40S.

10. The IL-12 mutein of any one of claims 2 to 9, wherein the variant p35 subunit comprises a substitution at amino acid residue D48, optionally wherein the substitution is D48R, D48H, D48K, D48E, D48S, D48T, D48N, D48Q, D48G, D48P, D48A, D48V, D48I, D48L, D48M, D48F, D48Y, or D48W.

11. The IL-12 mutein of claim 10, wherein the substitution at amino acid residue D48 is D48H, D48K, D48E, D48S, D48T, D48N, D48G, D48P, D48V, D48I, D48L, D48M, D48F, D48Y, or D48W, optionally wherein the substitution at amino acid residue D48 is D48K, D48E, D48T, D48N, D48G, D48P, D48V, D48I, D48L, D48M, or D48F.

12. The IL-12 mutein of any one of claims 2 to 11, wherein the variant p35 subunit comprises a substitution at amino acid residue K128, optionally wherein the substitution is K128R, K128H, K128K, K128D, K128E, K128S, K128T, K128N, K128Q, K128G, K128P, K128A, K128V, K128I, K128L, K128M, K128F, K128Y, or K128W.

13. The IL-12 mutein of claim 12, wherein the substitution at amino acid residue K128 is K128N or K128Q.

14. The IL-12 mutein of any one of claims 2 to 13, wherein the variant p35 subunit comprises a substitution at amino acid residue M125, optionally wherein the substitution is M125R, M125H, M125K, M125D, M125E, M125S, M125T, M125N, M125Q, M125G, M125P, M125A, M125V, M125I, M125L, M125F, M125Y, or M125W.

15. The IL-12 mutein of claim 14, wherein the substitution at amino acid residue M125 is M125R, M125H, M125K, M125D, M125E, M125S, M125T, M125N, M125Q, M125G, M125P, M125L, M125F, M125Y, or M125W.

16. The IL-12 mutein of any one of claims 2 to 15, wherein the variant p35 subunit comprises a substitution at amino acid residue R129, optionally wherein the substitution is R129H, R129K, R129D, R129E, R129S, R129T, R129N, R129Q, R129G, R129P, R129A, R129V, R129I, R129L, R129M, R129F, R129Y, or R129W.

17. The IL-12 mutein of any one of claims 2 to 16, wherein the variant p35 subunit comprises a substitution at amino acid residue L177, optionally wherein the substitution is L177R, L177H, L177K, L177D, L177E, L177S, L177T, L177N, L177Q, L177G, L177P, L177A, L177V, L177I, L177M, L177F, L177Y, or L177W.

18. The IL-12 mutein of claim 17, wherein the substitution at amino acid residue L177 is L177D, L177S, L177T, L177N, L177G, L177P, L177V, L177I, L177M, or L177W.

19. The IL-12 mutein of any one of claims 2 to 18, wherein the variant p35 subunit comprises a substitution at amino acid residue H178, optionally wherein the substitution is H178R, H178K, H178D, H178E, H178S, H178T, H178N, H178Q, H178G, H178P, H178A, H178V, H178I, H178L, H178M, H178F, H178Y, or H178W.

20. The IL-12 mutein of any one of claims 2 to 19, wherein the variant p35 subunit comprises a substitution at amino acid residue K56, optionally wherein the substitution at amino acid residue K56 is K56R, K56H, K56D, K56E, K56S, K56T, K56N, K56Q, K56G, K56P, K56A, K56V, K56I, K56L, K56M, K56F, K56Y, or K56W.

21. The IL-12 mutein of claim 20, wherein the K56 substitution is K56R, K56H, K56D, K56E, K56S, K56T, K56G, K56P, K56A, K56V, K56I, K56L, K56M, K56F, K56Y, or K56W, optionally wherein the substitution at amino acid residue K56 is K56E, K56S, K56T, K56G, K56A, K56V, K56I, or K56L.

22. The IL-12 mutein of claim 20, wherein the substitution at amino acid residue K56 is K56N.

23. The IL-12 mutein of any one of claims 2 to 22, wherein the variant p35 subunit comprises a substitution at amino acid residue K170, optionally wherein the substitution at amino acid residue K170 is K170R, K170H, K170D, K170E, K170S, K170T, K170N, K170Q, K170G, K170P, K170A, K170V, K170I, K170L, K170M, K170F, K170Y, or K170W.

24. The IL-12 mutein of any one of claims 2 to 23, wherein the variant p35 subunit comprises a substitution at amino acid residue I171, optionally wherein the substitution at amino acid residue I171 is I171R, I171K, I171H, I171D, I171E, I171S, I171T, I171N, I171Q, I171G, I171P, I171A, I171V, I171L, I171M, I171F, I171Y, or I171W.

25. The IL-12 mutein of any one of claims 2 to 24, wherein the variant p35 subunit comprises a substitution at amino acid residue A33, R34, E38, F39, P41, E45, E46, I47, H49, E50, D51, I52, T53, K54, T57, D108, L109, K110, M111, Y112, Q113, V114, E115, F116, K117, T118, A121, L123, D126, I131, P156, S159, S160, L161, E162, E163, D165, F166, Y167, K168, T169, K172, L173, I175, or R181.
26. The IL-12 mutein of claim 2, wherein the variant p35 subunit comprises one or more amino acid substitutions selected from Table 1a, Table 1b, or Table 1c.
27. The IL-12 mutein of claim 2, wherein the variant p35 subunit comprises
a substitution at L37, optionally wherein the substitution is L37A; and
a substitution at Y40, optionally wherein the substitution is Y40S.
28. The IL-12 mutein of claim 2, wherein the variant p35 subunit comprises
a substitution at L37, optionally wherein the substitution is L37A;
a substitution at Y40, optionally wherein the substitution is Y40S; and
a substitution at L133, optionally wherein the substitution is L133A.
29. The IL-12 mutein of claim 2, wherein the variant p35 subunit comprises
a substitution at L37, optionally wherein the substitution is L37A;
a substitution at Y40, optionally wherein the substitution is Y40S; and
a substitution at K128, optionally wherein the substitution is K128N or K128Q.
30. The IL-12 mutein of claim 2, wherein the variant p35 subunit comprises
a substitution at L37, optionally wherein the substitution is L37A;
a substitution at Y40, optionally wherein the substitution is Y40S;
a substitution at K128, optionally wherein the substitution is K128N or K128Q; and
a substitution at L133, optionally wherein the substitution is L133A.
31. The IL-12 mutein of any one of claims 2 to 30, wherein the variant p35 subunit comprises an amino acid sequence that is at least 80%, 85%, 90%, or 95% identical to SEQ ID NO: 1.
32. The IL-12 mutein of any one of claims 1 to 31, comprising the variant p40 subunit.

33. The IL-12 mutein of claim 32, wherein the variant p40 subunit comprises a substitution at amino acid residue V10, optionally wherein the substitution is V10A, V10R, V10H, V10K, V10D, V10E, V10S, V10T, V10N, V10Q, V10G, V10P, V10I, V10L, V10M, V10F, V10W, or V10Y.

34. The IL-12 mutein of claim 32 or 33, wherein the variant p40 subunit comprises a substitution at amino acid residue I94, optionally wherein the substitution is I94A, I94R, I94H, I94K, I94D, I94E, I94S, I94T, I94N, I94Q, I94G, I94P, I94L, I94M, I94F, I94W, I94V, or I94Y.

35. The IL-12 mutein of any one of claims 32 to 34, wherein the variant p40 subunit comprises a substitution at amino acid residue L95, optionally wherein the substitution is L95A, L95R, L95H, L95K, L95D, L95E, L95S, L95T, L95N, L95Q, L95G, L95P, L95I, L95M, L95F, L95W, L95V, or L95Y.

36. The IL-12 mutein of any one of claims 32 to 35, wherein the variant p40 subunit comprises a substitution at amino acid residue G64, optionally wherein the substitution is G64A, G64R, G64H, G64K, G64D, G64E, G64S, G64T, G64N, G64Q, G64P, G64I, G64L, G64M, G64F, G64W, G64V, or G64Y.

37. The IL-12 mutein of claim 36, wherein the G64 substitution is G64R, G64H, G64K, G64D, G64E, G64S, G64T, G64N, G64Q, G64P, G64I, G64L, G64M, G64F, G64W, G64V, or G64Y, optionally wherein the G64 substitution is G64R, G64K, G64E, G64S, G64T, G64Q, G64P, G64I, G64L, G64M, G64F, G64W, G64V, or G64Y.

38. The IL-12 mutein of any one of claims 32 to 37, wherein the variant p40 subunit comprises a substitution at amino acid residue Y198, optionally wherein the substitution is Y198A, Y198R, Y198H, Y198K, Y198D, Y198E, Y198S, Y198T, Y198N, Y198Q, Y198G, Y198P, Y198I, Y198L, Y198M, Y198F, Y198W, or Y198V.

39. The IL-12 mutein of claim 38, wherein the Y198 substitution is Y198A, Y198R, Y198H, Y198K, Y198D, Y198E, Y198T, Y198N, Y198Q, Y198G, Y198P, Y198I, Y198L, Y198M, Y198F, Y198W, or Y198V, optionally wherein the Y198 substitution is Y198G, Y198E, Y198K, Y198Q, Y198R, Y198W.

40. The IL-12 mutein of any one of claims 32 to 39, wherein the variant p40 subunit comprises a substitution at amino acid residue Y16, optionally wherein the substitution is Y16A, Y16R, Y16H, Y16K, Y16D, Y16E, Y16S, Y16T, Y16N, Y16Q, Y16G, Y16P, Y16I, Y16L, Y16M, Y16F, Y16W, or Y16V.

41. The IL-12 mutein of any one of claims 32 to 40, wherein the variant p40 subunit comprises a substitution at amino acid residue V11, E12, L13, D14, W15, P17, D18, A19, P20, G21, W38, T39, L40, D41, S43, S44, E45, V46, L47, I55, V57, K58, E59, F60, D62, A63, Q65, Y66, L81, L82, H83, K84, K85, E86, G88, I89, W90, S91, T92, D93, D97, Q98, K99, E100, N103, K104, T105, F106, L107, L123, K135, S136, S137, R138, G139, S140, S141, D142, N162, E181, S183, L184, P185, I186, E187, V188, V190, D191, H194, K195, L196, K197, E199, N200, T202, S203, S204, F206, or D209.

42. The IL-12 mutein of claim 41, wherein the variant p40 subunit comprises a substitution at amino acid residue W15, P17, D18, E59, F60, K84, E86, D93, H194, K195, or K197, optionally wherein the variant p40 subunit comprises a substitution at amino acid residue E59, F60, K84, K195, or K197.

43. The IL-12 mutein of claim 32, wherein the variant p40 subunit comprises one or more amino acid substitutions selected from Table 4a, Table 4b, or Table 4c.

44. The IL-12 mutein of any one of claims 32 to 43, wherein the variant p40 subunit comprises an amino acid sequence that is at least 80%, 85%, 90%, or 95% identical to SEQ ID NO: 2.

45. The IL-12 mutein of any one of claims 1 to 44, wherein the IL-12 mutein is a mutein of the human IL-12 sequence.

46. A fusion protein comprising the IL-12 mutein of any one of claim 1 to 45.

47. A pharmaceutical composition comprising the IL-12 mutein of any one of claims 1 to 45 or the fusion protein of claim 46, and a pharmaceutically acceptable carrier or excipient.

48. A polynucleotide sequence that encodes the IL-12 mutein according to any one of claims 1 to 45 or the fusion protein of claim 46.
49. An expression vector comprising the polynucleotide sequence of claim 48.
50. A host cell comprising the expression vector of claim 49.
51. A kit comprising the IL-12 mutein according to any one of claims 1 to 45 or the fusion protein of claim 46 and optionally packaging material.
52. A method of producing the IL-12 mutein of any one of claims 1 to 45 or the fusion protein of claim 46, comprising culturing the host cell of claim 50 under conditions to express the IL-12 mutein or the fusion protein.
53. The method of claim 52, further comprising isolating the IL-12 mutein from the host cell.
54. A method of treating cancer in a patient in need thereof comprising administering to the patient a therapeutically effective amount of the IL-12 mutein of any one of claims 1 to 45, the fusion protein of claim 46, or the pharmaceutical composition of claim 47.
55. The method of claim 54, wherein the cancer comprises a solid tumor.