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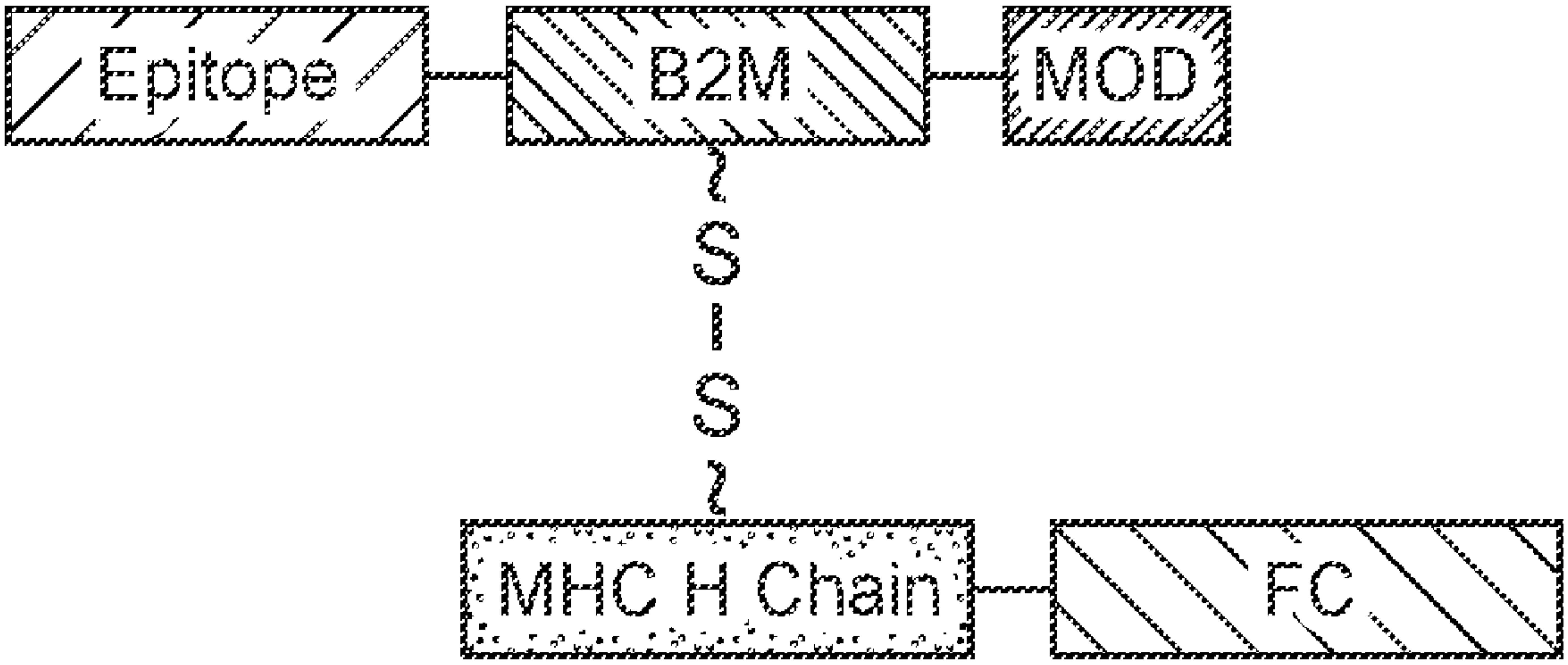
(54) **Titre : POLYPEPTIDES MULTIMERES MODULATEURS DES LYMPHOCYTES T ET LEURS PROCEDE D'UTILISATION**
(54) **Title: T-CELL MODULATORY MULTIMERIC POLYPEPTIDES AND METHODS OF USE THEREOF**

FIG. 1A



(57) **Abrégé/Abstract:**
The present disclosure provides variant immunomodulatory polypeptides, and fusion polypeptides comprising the variant immunomodulatory peptides. The present disclosure provides T-cell modulatory multimeric polypeptides, and compositions comprising same, where the T-cell modulatory multimeric polypeptides comprise a variant immunomodulatory polypeptide of the present disclosure. The present disclosure provides nucleic acids comprising nucleotide sequences encoding the T-cell modulatory multimeric polypeptides, and host cells comprising the nucleic acids. The present disclosure provides methods of modulating the activity of a T cell; the methods comprise contacting the T cell with a T-cell modulatory multimeric polypeptide of the present disclosure.

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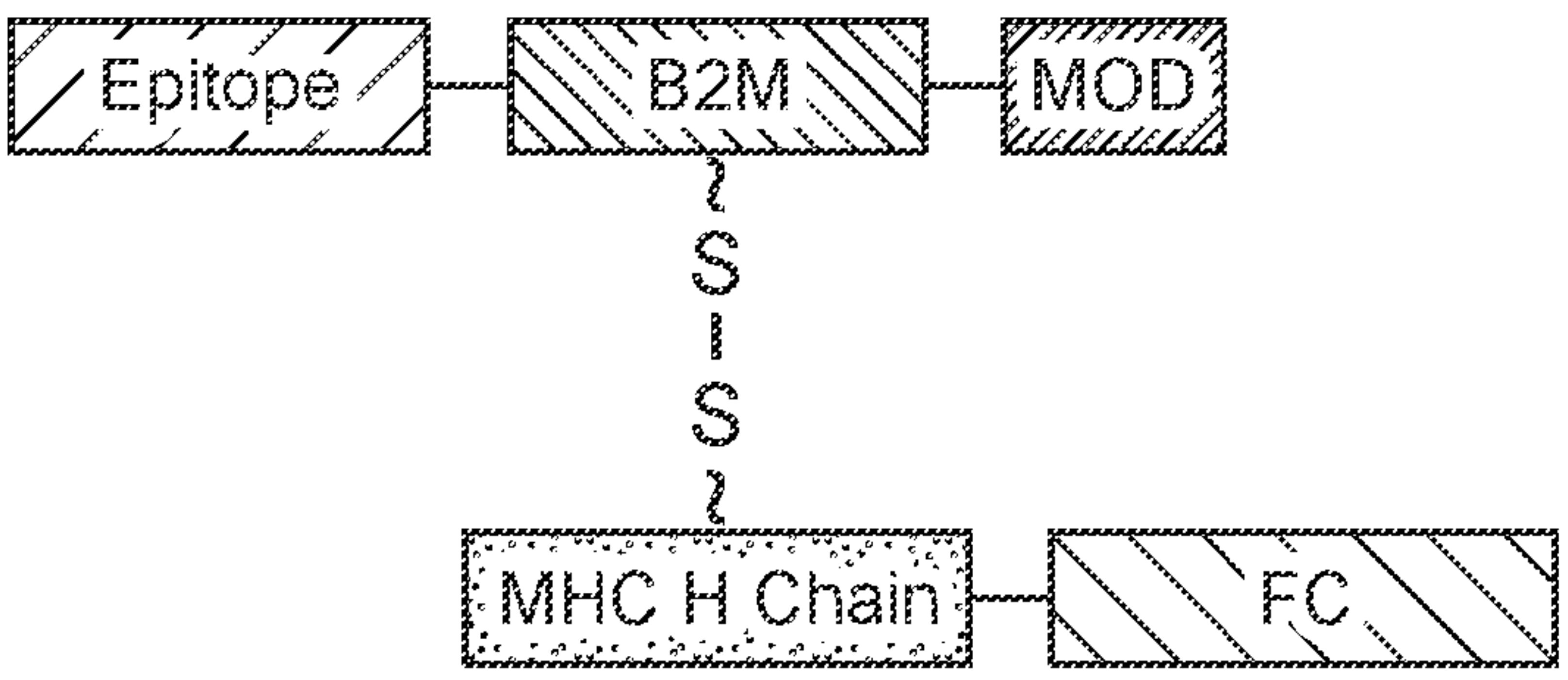
FIG. 1A



(57) Abstract: The present disclosure provides variant immunomodulatory polypeptides, and fusion polypeptides comprising the variant immunomodulatory peptides. The present disclosure provides T-cell modulatory multimeric polypeptides, and compositions comprising same, where the T-cell modulatory multimeric polypeptides comprise a variant immunomodulatory polypeptide of the present disclosure. The present disclosure provides nucleic acids comprising nucleotide sequences encoding the T-cell modulatory multimeric polypeptides, and host cells comprising the nucleic acids. The present disclosure provides methods of modulating the activity of a T cell; the methods comprise contacting the T cell with a T-cell modulatory multimeric polypeptide of the present disclosure.

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CECI EST LE TOME 1 DE 2
CONTENANT LES PAGES 1 À 242

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THIS SECTION OF THE APPLICATION/PATENT CONTAINS MORE THAN ONE VOLUME

THIS IS VOLUME 1 OF 2
CONTAINING PAGES 1 TO 242

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T-CELL MODULATORY MULTIMERIC POLYPEPTIDES AND METHODS OF USE THEREOF

CROSS-REFERENCE

[0001] This application claims the benefit of U.S. Provisional Patent Application No. 62/303,268, filed March 3, 2016, which application is incorporated herein by reference in its entirety.

INTRODUCTION

[0002] An adaptive immune response involves the engagement of the T cell receptor (TCR), present on the surface of a T cell, with a small peptide antigen non-covalently presented on the surface of an antigen presenting cell (APC) by a major histocompatibility complex (MHC; also referred to in humans as a human leukocyte antigen (HLA) complex). This engagement represents the immune system's targeting mechanism and is a requisite molecular interaction for T cell modulation (activation or inhibition) and effector function. Following epitope-specific cell targeting, the targeted T cells are activated through engagement of costimulatory proteins found on the APC with counterpart costimulatory proteins the T cells. Both signals – epitope/TCR binding and engagement of APC costimulatory proteins with T cell costimulatory proteins – are required to drive T cell specificity and activation or inhibition. The TCR is specific for a given epitope; however, the costimulatory protein not epitope specific and instead is generally expressed on all T cells or on large T cell subsets.

SUMMARY

[0003] The present disclosure provides variant immunomodulatory polypeptides, and fusion polypeptides comprising the variant immunomodulatory peptides. The present disclosure provides T-cell modulatory multimeric polypeptides, and compositions comprising same, where the T-cell modulatory multimeric polypeptides comprise a variant immunomodulatory polypeptide of the present disclosure. The present disclosure provides nucleic acids comprising nucleotide sequences encoding the T-cell modulatory multimeric polypeptides, and host cells comprising the nucleic acids. The present disclosure provides methods of modulating the activity of a T cell; the methods comprise contacting the T cell with a T-cell modulatory multimeric polypeptide of the present disclosure.

[0004] The present disclosure provides a variant 4-1BBL immunomodulatory polypeptide comprising an amino acid sequence having at least 85%, at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the 4-1BBL amino acid sequence depicted in FIG. 2A or to the 4-1BBL amino acid sequence set forth in one of SEQ ID NOs:1-3, wherein the variant 4-1BBL immunomodulatory polypeptide has one or more (e.g., 1, 2, 3, 4, 5, 6, 7, 8, 9, or 10, or more than 10) amino acid substitutions relative to the 4-1BBL amino acid sequence depicted in FIG. 2A or to the 4-1BBL amino acid sequence set forth in one of SEQ ID NOs:1-3; and wherein the variant 4-1BBL immunomodulatory polypeptide: i) exhibits reduced binding affinity to a 4-1BB polypeptide having an amino acid sequence depicted in FIG. 3 and set forth in SEQ ID NO:91, compared to the binding affinity of the 4-1BBL amino acid sequence depicted in FIG. 2A or set forth in one of SEQ ID NOs:1-3 for the 4-1BB polypeptide; and/or ii) wherein the variant 4-1BBL immunomodulatory polypeptide exhibits increased production levels by a mammalian cell, compared to the production levels of the 4-1BBL amino acid sequence depicted in FIG. 2A or set forth in one of SEQ ID NOs:1-3. In some cases, the polypeptide comprises a substitution of one of amino acids 91, 92, 94-115, 117-126, 128-132, 144-153, 155-158, 184-187, 189-191, 193-195, 197, 210-219, 221-224, 226, 228-231, 233, and 234 based on the amino acid numbering set out in FIG. 2A. In some cases, the variant immunomodulatory polypeptide exhibits less than 50% of binding affinity exhibited by to the 4-1BBL amino acid sequence depicted in FIG. 2A, or as set forth in one of SEQ ID NOs:1-3, for the 4-1BB polypeptide.

[0005] The present disclosure provides a multimeric polypeptide comprising: a) a first polypeptide comprising, in order from N-terminus to C-terminus: i) an epitope; ii) a first major histocompatibility complex (MHC) polypeptide; and b) a second polypeptide comprising, in order from N-terminus to C-terminus: i) a second MHC polypeptide; and ii) optionally an immunoglobulin (Ig) Fc polypeptide or a non-Ig scaffold, wherein the multimeric polypeptide comprises one or more immunomodulatory domains, wherein the one or more immunomodulatory domain is: A) at the C-terminus of the first polypeptide; B) at the N-terminus of the second polypeptide; C) at the C-terminus of the second polypeptide; or D) at the C-terminus of the first polypeptide and at the N-terminus of the second polypeptide, wherein the immunomodulatory domain is a variant 4-1BBL immunomodulatory polypeptide as described above or elsewhere herein; and wherein: i) the multimeric polypeptide exhibits reduced binding affinity to a 4-1BB polypeptide having an amino acid sequence depicted in FIG. 3 and set forth in SEQ ID NO:91, compared to the binding affinity of a control multimeric polypeptide comprising an immunomodulatory domain comprising the 4-1BBL amino acid sequence depicted in FIG. 2A or set forth in one of SEQ ID NOs:1-3 for the 4-1BB polypeptide; and/or ii) wherein the multimeric polypeptide exhibits increased production levels by a mammalian cell, compared to the production levels of a control multimeric polypeptide comprising an immunomodulatory domain comprising the 4-1BBL amino acid sequence depicted in FIG. 2A or set forth in one of SEQ ID NOs:1-3. In some cases, the multimeric polypeptide comprises: a) a first polypeptide comprising, in order from N-terminus to C-terminus: i) an epitope; ii) a first MHC polypeptide; and iii) an immunomodulatory domain; and b) a second polypeptide comprising, in order from N-terminus to C-terminus: i) a second MHC polypeptide; and ii) an Ig Fc polypeptide. In some cases, the multimeric polypeptide comprises: a) a first polypeptide comprising, in order from N-terminus to C-terminus: i) an epitope; and ii) a first MHC polypeptide; and b) a second polypeptide comprising, in order from N-terminus to C-terminus: i) an immunomodulatory domain; iii) a second MHC polypeptide; and iv) an immunoglobulin (Ig) Fc polypeptide. In some cases, the multimeric polypeptide comprises: a) a first polypeptide comprising, in order from N-terminus to C-terminus: i) an epitope; and ii) a first MHC polypeptide; and b) a second polypeptide comprising, in order from N-terminus to C-terminus: i) a second MHC polypeptide; and ii) an Ig Fc polypeptide; and iii) an immunomodulatory domain. In some cases, the multimeric polypeptide comprises: a) a first polypeptide comprising, in order from N-terminus to C-terminus: i) an epitope; and ii) a first MHC polypeptide; and b) a second polypeptide comprising, in order from N-terminus to C-terminus: i) a second MHC polypeptide; and ii) an immunomodulatory domain. In some cases, the multimeric polypeptide comprises: a) a first polypeptide comprising, in order from N-terminus to C-terminus: i) an epitope; and ii) a first MHC polypeptide; and b) a second polypeptide comprising, in order from N-terminus to C-terminus: i) an immunomodulatory domain; and ii) a second MHC polypeptide. In some cases, the multimeric polypeptide comprises: a) a first polypeptide comprising, in order from N-terminus to C-terminus: i) an epitope; ii) a first MHC polypeptide; and iii) an immunomodulatory domain; and b) a second polypeptide comprising, in order from N-terminus to C-terminus: i) a second MHC polypeptide. In some cases, the non-Ig scaffold is an XTEN polypeptide, a transferrin polypeptide, an elastin-like polypeptide, a silk-like polypeptide, or a silk-elastin-like polypeptide. In some cases, the first MHC polypeptide is a β2-microglobulin polypeptide; and wherein the second MHC polypeptide is an MHC class I heavy chain polypeptide. In some cases, the β2-microglobulin polypeptide comprises an amino acid sequence having at least 85% amino acid sequence identity to any one of the amino acid sequences set forth in SEQ ID NO:6. In some cases, the MHC class I heavy chain polypeptide is an HLA-A, an HLA-B, or an HLA-C heavy chain. In some cases, the MHC class I heavy chain polypeptide comprises an amino acid sequence having at least 85% amino acid sequence identity to the amino acid sequence depicted in any one of FIG. 5A-5C. In some cases, the first MHC polypeptide is an MHC Class II alpha chain polypeptide; and wherein the second MHC polypeptide is an MHC class II beta chain polypeptide. In some cases, the epitope is a T-cell epitope. In some cases, the multimeric polypeptide comprises an Fc polypeptide, and wherein the Ig Fc polypeptide is an IgG1 Fc polypeptide, an IgG2 Fc polypeptide, an IgG3 Fc polypeptide, an IgG4 Fc polypeptide, an IgA Fc polypeptide, or an IgM Fc polypeptide. In some cases, the Ig Fc polypeptide comprises an amino acid sequence having at least 85% amino acid sequence identity to an amino acid sequence depicted in FIG. 4A-4C. In some cases, the first polypeptide and the second polypeptide are non-covalently associated. In some cases, the first polypeptide and the second polypeptide are covalently linked. In some cases, the covalent linkage is via a disulfide bond. In some cases, the first MHC polypeptide or a linker between the epitope and the first MHC polypeptide comprises an amino acid substitution to provide a first Cys residue, and the second MHC polypeptide comprises an amino acid substitution to provide a second Cys residue, and wherein the disulfide linkage is between the first and the second Cys residues. In some cases, the multimeric polypeptide comprises a first linker interposed between the epitope and the first MHC polypeptide. In some cases, the variant 4-1BBL immunomodulatory polypeptide comprises a substitution of one of amino acids 91, 92, 94-115, 117-126, 128-132, 144-153, 155-158, 184-187, 189-191, 193-195, 197, 210-219, 221-224, 226, 228-231, 233, and 234 based on the amino acid numbering set out in FIG. 2A. In some cases, the multimeric polypeptide comprises 2 or more immunomodulatory polypeptides. In some cases, the 2 or more immunomodulatory polypeptides are in tandem. In some cases, the multimeric polypeptide comprises a third polypeptide, wherein the third polypeptide comprises an immunomodulatory polypeptide comprising an amino acid sequence having at least 90% amino acid sequence identity to the immunomodulatory polypeptide of the first polypeptide or the second polypeptide. In some cases, the third polypeptide is covalently linked to the first polypeptide. In some cases, the second polypeptide comprises, in order from N-terminus to C-terminus: i) the second MHC polypeptide; ii) the Ig Fc polypeptide; and iii) an affinity tag.

[0006] The present disclosure provides a nucleic acid comprising a nucleotide sequence encoding a recombinant polypeptide, i) wherein the recombinant polypeptide comprises, in order from N-terminus to C-terminus: a) an epitope; b) a first major histocompatibility complex (MHC) polypeptide; c) an immunomodulatory polypeptide; d) a proteolytically cleavable linker or a ribosome skipping signal; e) a second MHC polypeptide; and f) an immunoglobulin (Ig) Fc polypeptide; wherein the immunomodulatory polypeptide is a variant immunomodulatory polypeptide as described above or elsewhere herein; or ii) wherein the recombinant polypeptide comprises, in order from N-terminus to C-terminus: a) an epitope; b) a first MHC polypeptide;

[0007] c) a proteolytically cleavable linker or a ribosome skipping signal; d) an immunomodulatory polypeptide; e) a second MHC polypeptide; and f) an Ig Fc polypeptide, wherein the immunomodulatory polypeptide is a variant immunomodulatory polypeptide as described above or elsewhere herein. In some cases, the first MHC polypeptide is a β2-microglobulin polypeptide; and wherein the second MHC polypeptide is an MHC class I heavy chain polypeptide. In some cases, the β2-microglobulin polypeptide comprises an amino acid sequence having at least 85% amino acid sequence identity to any one of the amino acid sequences depicted in FIG. 6. In some cases, the MHC class I heavy chain polypeptide is an HLA-A, HLA-B, or HLA-C heavy chain. In some cases, the MHC class I heavy chain polypeptide comprises an amino acid sequence having at least 85% amino acid sequence identity to the amino acid sequence depicted in any one of FIG. 5A-5C. In some cases, the first MHC polypeptide is an MHC Class II alpha chain polypeptide; and wherein the second MHC polypeptide is an MHC class II beta chain polypeptide. In some cases, the epitope is a T-cell epitope. In some cases, the Ig Fc polypeptide is an IgG1 Fc polypeptide, an IgG2 Fc polypeptide, an IgG3 Fc polypeptide, an IgG4 Fc polypeptide, an IgA Fc polypeptide, or an IgM Fc polypeptide. In some cases, the Ig Fc polypeptide comprises an amino acid sequence having at least 85% amino acid sequence identity to an amino acid sequence depicted in Figures 4A-4C. In some cases, the variant 4-1BBL immunomodulatory polypeptide comprises a substitution of one of amino acids 91, 92, 94-115, 117-126, 128-132, 144-153, 155-158, 184-187, 189-191, 193-195, 197, 210-219, 221-224, 226, 228-231, 233, and 234 based on the amino acid numbering set out in FIG. 2A. In some cases, the multimeric polypeptide comprises a second immunomodulatory polypeptide selected from a CD7, CD30L, CD40, CD70, CD83, HLA-G, MICA, MICB, HVEM, lymphotoxin beta receptor, 3/TR6, ILT3, ILT4, and HVEM. In some cases, the proteolytically cleavable linker or ribosome skipping signal comprises an amino acid sequence selected from: a) LEVLFQGP (SEQ ID NO:116); b) ENLYTQS (SEQ ID NO:117); c) a furin cleavage site; d) LVPR (SEQ ID NO:118); e) GSGATNFSLLKQAGDVEENPGP (SEQ ID NO:119); f) GSGEGRGSLLTCGDVEENPGP (SEQ ID NO:120); g) GSGQCTNYALLKLAGDVESNPGP (SEQ ID NO:121); and h) GSGVKQTLNFDLLKLAGDVESNPGP (SEQ ID NO:122). In some cases, the recombinant polypeptide comprises, in order from N-terminus to C-terminus: a) a first leader peptide; b) the epitope; c) the first MHC polypeptide; d) the immunomodulatory polypeptide; e) the proteolytically cleavable linker or ribosome skipping signal; f) a second leader peptide; g) the second MHC polypeptide; and h) the immunoglobulin (Ig) Fc polypeptide. In some cases, the first leader peptide and the second leader peptide is a β2-M leader peptide. In some cases, the nucleotide sequence is operably linked to a transcriptional control element. In some cases, the transcriptional control element is a promoter that is functional in a eukaryotic cell. In some cases, the first MHC polypeptide or a linker between the epitope and the first MHC polypeptide comprises an amino acid substitution to provide a first Cys residue, and the second MHC polypeptide comprises an amino acid substitution to provide a second Cys residue, and wherein the first and the second Cys residues provide for a disulfide linkage between the first MHC polypeptide and the second MHC polypeptide. The present disclosure provides a recombinant expression vector comprising a nucleic acid as described above or elsewhere herein. In some cases, the vector is a viral vector or a non-viral vector. The present disclosure provides a host cell genetically modified with a recombinant expression vector as described above or elsewhere herein. In some cases, the host cell is *in vitro*. In some cases, the host cell is genetically modified such that the cell does not produce an endogenous MHC β2-microglobulin polypeptide. In some cases, the host cell is a T lymphocyte.

[0008] The present disclosure provides a composition comprising: a) a first nucleic acid comprising a nucleotide sequence encoding a first polypeptide comprising, in order from N-terminus to C-terminus: i) an epitope; ii) a first MHC polypeptide; and iii) an immunomodulatory domain, wherein the immunomodulatory domain is a variant immunomodulatory polypeptide as described above or elsewhere herein; and b) a first nucleic acid comprising a nucleotide sequence encoding a second polypeptide comprising, in order from N-terminus to C-terminus: i) a second MHC polypeptide; and ii) an Ig Fc polypeptide. The present disclosure provides a host cell genetically modified with a nucleic acid composition as described above or elsewhere herein.

[0009] The present disclosure provides a composition comprising: a) a first nucleic acid comprising a nucleotide sequence encoding a first polypeptide comprising, in order from N-terminus to C-terminus: i) an epitope; and ii) a first MHC polypeptide; and b) a first nucleic acid comprising a nucleotide sequence encoding a second polypeptide comprising, in order from N-terminus to C-terminus: i) an immunomodulatory domain, wherein the immunomodulatory domain is a variant immunomodulatory polypeptide as described above or elsewhere herein; ii) a second MHC polypeptide; and iii) an Ig Fc polypeptide. In some cases, the first and/or the second nucleic acid is present in a recombinant expression vector.

[0010] The present disclosure provides a host cell genetically modified with a nucleic acid composition as described above or elsewhere herein.

[0011] The present disclosure provides a method of producing a multimeric polypeptide as described above or elsewhere herein, the method comprising: a) culturing a host cell as described above or elsewhere herein *in vitro* in a culture medium under conditions such that the host cell synthesizes the multimeric polypeptide; and b) isolating the multimeric polypeptide from the host cell and/or from the culture medium. In some cases, the second polypeptide comprises an affinity tag, and wherein said isolating comprises contacting the multimeric polypeptide produced by the cell with a binding partner for the affinity tag, wherein the binding partner is immobilized, thereby immobilizing the multimeric polypeptide. In some cases, the method comprises eluting the immobilized multimeric polypeptide.

[0012] The present disclosure provides a method of selectively modulating the activity of an epitope-specific T cell, the method comprising contacting the T cell with a multimeric polypeptide as described above or elsewhere herein, wherein said contacting selectively modulates the activity of the epitope-specific T cell. In some cases, the immunomodulatory polypeptide is an activating polypeptide, and wherein the multimeric polypeptide activates the epitope-specific T cell. In some cases, the immunomodulatory polypeptide is an inhibiting polypeptide, and wherein the multimeric polypeptide inhibits the epitope-specific T cell. In some cases, said contacting is *in vitro*. In some cases, said contacting is *in vivo*.

[0013] The present disclosure provides a method of selectively modulating the activity of an epitope-specific T cell in an individual, the method comprising administering to the individual an effective amount of a multimeric polypeptide as described above or elsewhere herein effective to selectively modulate the activity of an epitope-specific T cell in an individual. In some cases, the immunomodulatory polypeptide is an activating polypeptide, and wherein the multimeric polypeptide activates the epitope-specific T cell. In some cases, the epitope is a cancer-associated epitope, and wherein said administering selectively increases the activity of a T cell specific for the cancer-associate epitope. In some cases, the immunomodulatory polypeptide is an inhibitory polypeptide, and wherein the multimeric polypeptide inhibits activity of the epitope-specific T cell. In some cases, the epitope is a self-epitope, and wherein said administering selectively inhibits the activity of a T cell specific for the self-epitope.

[0014] The present disclosure provides a method of treating an infection in an individual, the method comprising administering to the individual an effective amount of a) a multimeric polypeptide as described above or elsewhere herein; or b) one or more recombinant expression vectors comprising nucleotide sequences encoding a multimeric polypeptide as described above or elsewhere herein; or c) one or more mRNAs comprising nucleotide sequences encoding a multimeric polypeptide as described above or elsewhere herein, wherein the epitope is a pathogen-associated epitope, wherein the immunomodulatory polypeptide is an activating polypeptide, and wherein said administering effective to selectively modulate the activity of a pathogen-associated epitope-specific T cell in an individual. In some cases, the pathogen is a virus, a bacterium, or a protozoan. In some cases, said administering is subcutaneous. In some cases, said administering is intravenous. In some cases, said administering is intramuscular. In some cases, said administering is systemic. In some cases, said administering is distal to a treatment site. In some cases, said administering is local. In some cases, said administering is at or near a treatment site.

[0015] The present disclosure provides a composition comprising: a) a multimeric polypeptide as described above or elsewhere herein; and b) a pharmaceutically acceptable excipient.

[0016] The present disclosure provides a composition comprising: a) a nucleic acid as described above or elsewhere herein or a recombinant expression vector as described above or elsewhere herein; and b) a pharmaceutically acceptable excipient.

BRIEF DESCRIPTION OF THE DRAWINGS

[0017] FIG. 1A-1D schematically depict various embodiments of a T-cell modulatory multimeric polypeptide of the present disclosure. In these embodiments, disulfide bonds are formed between MHC (e.g., HLA) polypeptides present in separate polypeptides.

[0018] FIG. 2A-2IIII provide an amino acid sequence of a 4-1BBL (FIG. 2A) and examples of variant 4-1BBL polypeptides (FIG. 2B-2IIII).

[0019] FIG. 3 provides an amino acid sequence of 4-1BB (FIG. 3A-3C).

[0020] FIG. 4A-4C provide amino acid sequences of immunoglobulin Fc polypeptides.

[0021] FIG. 5A-5C provide amino acid sequences of human leukocyte antigen (HLA) Class I heavy chain polypeptides. Signal sequences are underlined.

[0022] FIG. 6 provides a multiple amino acid sequence alignment of beta-2 microglobulin (β2M) precursors (i.e., including the leader sequence) from *Homo sapiens* (NP_004039.1; SEQ ID NO:103), *Pan troglodytes* (NP_001009066.1; SEQ ID NO:104), *Macaca mulatta* (NP_001040602.1; SEQ ID NO:105), Bos Taurus (NP_776318.1; SEQ ID NO:106) and Mus musculus (NP_033865.2; SEQ ID NO:107). Amino acids 1-20 are a signal peptide.

[0023] FIG. 7A-7B provide amino acid sequences of PD-L1 polypeptides.

[0024] FIG. 8 provides an amino acid sequence of a CD80 polypeptide.

[0025] FIG. 9 provides an amino acid sequence of an ICOS-L polypeptide.

[0026] FIG. 10 provides an amino acid sequence of an OX40L polypeptide.

[0027] FIG. 11 provides an amino acid sequence of a PD-L2 polypeptide.

[0028] FIG. 12 provides an amino acid sequence of a CD86 (B7-2) polypeptide.

[0029] FIG. 13 provides an amino acid sequence of a Fas ligand (FAS-L) polypeptide.

[0030] FIG. 14A-14B depicts interferon-gamma (IFN-γ) secretion by target cells contacted with a synTac polypeptide for 3 days (FIG. 14A) or 5 days (FIG. 14B) according to an embodiment of the present disclosure.

[0031] FIG. 15A-15B depicts interleukin-2 (IL-2) secretion by target cells contacted with a synTac polypeptide for 3 days (FIG. 15A) or 5 days (FIG. 15B) according to an embodiment of the present disclosure.

[0032] FIG. 16A-16B depicts interleukin-6 (IL-6) secretion by target cells contacted with a synTac polypeptide for 3 days (FIG. 16A) or 5 days (FIG. 16B) according to an embodiment of the present disclosure.

[0033] FIG. 17A-17B depicts tumor necrosis factor-alpha (TNFα) secretion by target cells contacted with a synTac polypeptide for 3 days (FIG. 17A) or 5 days (FIG. 17B) according to an embodiment of the present disclosure.

[0034] FIG. 18A-18B depicts interleukin-10 (IL-10) secretion by target cells contacted with a synTac polypeptide for 3 days (FIG. 18A) or 5 days (FIG. 18B) according to an embodiment of the present disclosure.

[0035] FIG. 19A-19B depicts interleukin-17A (IL-17A) secretion by target cells contacted with a synTac polypeptide for 3 days (FIG. 19A) or 5 days (FIG. 19B) according to an embodiment of the present disclosure.

[0036] FIG. 20A-20B depicts interleukin-4 (IL-4) secretion by target cells contacted with a synTac polypeptide for 3 days (FIG. 20A) or 5 days (FIG. 20B) according to an embodiment of the present disclosure.

[0037] FIG. 21 depicts proliferation of target cells contacted with a synTac polypeptide according to an embodiment of the present disclosure.

[0038] FIG. 22 depicts viability of target cells contacted with a synTac polypeptide according to an embodiment of the present disclosure.

[0039] FIG. 23 depicts expression levels of various synTac polypeptides produced in CHO cells.

[0040] FIG. 24 depicts the *in vivo* effect of a synTac polypeptide of the present disclosure on tumor volume.

DEFINITIONS

[0041] The terms “polynucleotide” and “nucleic acid,” used interchangeably herein, refer to a polymeric form of nucleotides of any length, either ribonucleotides or deoxyribonucleotides. Thus, this term includes, but is not limited to, single-, double-, or multi-stranded DNA or RNA, genomic DNA, cDNA, DNA-RNA hybrids, or a polymer comprising purine and pyrimidine bases or other natural, chemically or biochemically modified, non-natural, or derivatized nucleotide bases.

[0042] The terms "peptide," "polypeptide," and "protein" are used interchangeably herein, and refer to a polymeric form of amino acids of any length, which can include coded and non-coded amino acids, chemically or biochemically modified or derivatized amino acids, and polypeptides having modified peptide backbones.

[0043] A polynucleotide or polypeptide has a certain percent "sequence identity" to another polynucleotide or polypeptide, meaning that, when aligned, that percentage of bases or amino acids are the same, and in the same relative position, when comparing the two sequences. Sequence identity can be determined in a number of different ways. To determine sequence identity, sequences can be aligned using various convenient methods and computer programs (e.g., BLAST, T-COFFEE, MUSCLE, MAFFT, etc.), available over the world wide web at sites including ncbi.nlm.nili.gov/BLAST, ebi.ac.uk/Tools/msa/tcoffee/, ebi.ac.uk/Tools/msa/muscle/, mafft.cbrc.jp/alignment/software/. See, e.g., Altschul et al. (1990), J. Mol. Bioi. 215:403-10.

[0044] The term "conservative amino acid substitution" refers to the interchangeability in proteins of amino acid residues having similar side chains. For example, a group of amino acids having aliphatic side chains consists of glycine, alanine, valine, leucine, and isoleucine; a group of amino acids having aliphatic-hydroxyl side chains consists of serine and threonine; a group of amino acids having amide containing side chains consisting of asparagine and glutamine; a group of amino acids having aromatic side chains consists of phenylalanine, tyrosine, and tryptophan; a group of amino acids having basic side chains consists of lysine, arginine, and histidine; a group of amino acids having acidic side chains consists of glutamate and aspartate; and a group of amino acids having sulfur containing side chains consists of cysteine and methionine. Exemplary conservative amino acid substitution groups are: valine-leucine-isoleucine, phenylalanine-tyrosine, lysine-arginine, alanine-valine-glycine, and asparagine-glutamine.

[0045] "Binding" as used herein (e.g. with reference to binding of a T-cell modulatory multimeric polypeptide of the present disclosure to a polypeptide (e.g., a T-cell receptor) on a T cell) refers to a non-covalent interaction between. Binding interactions are generally characterized by a dissociation constant (K_D) of less than 10^{-6} M, less than 10^{-7} M, less than 10^{-8} M, less than 10^{-9} M, less than 10^{-10} M, less than 10^{-11} M, less than 10^{-12} M, less than 10^{-13} M, less than 10^{-14} M, or less than 10^{-15} M. "Affinity" refers to the strength of binding, increased binding affinity being correlated with a lower K_D.

[0046] The term “immunological synapse” or “immune synapse” as used herein generally refers to the natural interface between two interacting immune cells of an adaptive immune response including, e.g., the interface between an antigen-presenting cell (APC) or target cell and an effector cell, e.g., a lymphocyte, an effector T cell, a natural killer cell, and the like. An immunological synapse between an APC and a T cell is generally initiated by the interaction of a T cell antigen receptor and major histocompatibility complex molecules, e.g., as described in Bromley et al., Annu Rev Immunol. 2001;19:375-96; the disclosure of which is incorporated herein by reference in its entirety.

[0047] “T cell” includes all types of immune cells expressing CD3, including T-helper cells ($CD4^+$ cells), cytotoxic T-cells ($CD8^+$ cells), T-regulatory cells (Treg), and NK-T cells.

[0048] "Co-stimulatory polypeptide," as the term is used herein, includes a polypeptide on an antigen presenting cell (APC) (e.g., a dendritic cell, a B cell, and the like) that specifically binds a cognate co-stimulatory polypeptide on a T cell, thereby providing a signal which, in addition to the primary signal provided by, for instance, binding of a TCR/CD3 complex with a major histocompatibility complex (MHC) polypeptide loaded with peptide, mediates a T cell response, including, but not limited to, proliferation, activation, differentiation, and the like. A co-stimulatory ligand can include, but is not limited to, CD7, B7-1 (CD80), B7-2 (CD86), PD-L1, PD-L2, 4-1BBL, OX40L, Fas ligand (FasL), inducible costimulatory ligand (ICOS-L), intercellular adhesion molecule (ICAM), CD30L, CD40, CD70, CD83, HLA-G, MICA, MICB, HVEM, lymphotoxin beta receptor, 3/TR6, ILT3, ILT4, HVEM, an agonist or antibody that binds Toll ligand receptor and a ligand that specifically binds with B7-H3. A co-stimulatory ligand also encompasses, inter alia, an antibody that specifically binds with a co-stimulatory molecule present on a T cell, such as, but not limited to, CD27, CD28, 4-1BB, OX40, CD30, CD40, PD-1, ICOS, lymphocyte function-associated antigen-1 (LFA-1), CD2, LIGHT, NKG2C, B7-H3, and a ligand that specifically binds to CD83.

[0049] A “modulatory domain” of a T-cell modulatory multimeric polypeptide of the present disclosure comprises a co-stimulatory polypeptide.

[0050] “Heterologous,” as used herein, means a nucleotide or polypeptide that is not found in the native nucleic acid or protein, respectively.

[0051] "Recombinant," as used herein, means that a particular nucleic acid (DNA or RNA) is the product of various combinations of cloning, restriction, polymerase chain reaction (PCR) and/or ligation steps resulting in a construct having a structural coding or non-coding sequence distinguishable from endogenous nucleic acids found in natural systems. DNA sequences encoding polypeptides can be assembled from cDNA fragments or from a series of synthetic oligonucleotides, to provide a synthetic nucleic acid which is capable of being expressed from a recombinant transcriptional unit contained in a cell or in a cell-free transcription and translation system.

[0052] The terms “recombinant expression vector,” or “DNA construct” are used interchangeably herein to refer to a DNA molecule comprising a vector and one insert. Recombinant expression vectors are usually generated for the purpose of expressing and/or propagating the insert(s), or for the construction of other recombinant nucleotide sequences. The insert(s) may or may not be operably linked to a promoter sequence and may or may not be operably linked to DNA regulatory sequences.

[0053] A cell has been “genetically modified” or "transformed" or "transfected" by exogenous DNA, e.g. a recombinant expression vector, when such DNA has been introduced inside the cell. The presence of the exogenous DNA results in permanent or transient genetic change. The transforming DNA may or may not be integrated (covalently linked) into the genome of the cell. In prokaryotes, yeast, and mammalian cells, for example, the transforming DNA may be maintained on an episomal element such as a plasmid. With respect to eukaryotic cells, a stably transformed cell is one in which the transforming DNA has become integrated into a chromosome so that it is inherited by daughter cells through chromosome replication.

[0054] A “host cell,” as used herein, denotes an *in vivo* or *in vitro* eukaryotic cell or a cell from a multicellular organism (e.g., a cell line) cultured as a unicellular entity, which eukaryotic cells can be, or have been, used as recipients for a nucleic acid (e.g., an expression vector that comprises a nucleotide sequence encoding a multimeric polypeptide of the present disclosure), and include the progeny of the original cell which has been genetically modified by the nucleic acid. It is understood that the progeny of a single cell may not necessarily be completely identical in morphology or in genomic or total DNA complement as the original parent, due to natural, accidental, or deliberate mutation. A “recombinant host cell” (also referred to as a “genetically modified host cell”) is a host cell into which has been introduced a heterologous nucleic acid, e.g., an expression vector. For example, a genetically modified eukaryotic host cell is genetically modified by virtue of introduction into a suitable eukaryotic host cell a heterologous nucleic acid, e.g., an exogenous nucleic acid that is foreign to the eukaryotic host cell, or a recombinant nucleic acid that is not normally found in the eukaryotic host cell.

[0055] The terms "treatment", "treating" and the like are used herein to generally mean obtaining a desired pharmacologic and/or physiologic effect. The effect may be prophylactic in terms of completely or partially preventing a disease or symptom thereof and/or may be therapeutic in terms of a partial or complete cure for a disease and/or adverse effect attributable to the disease. "Treatment" as used herein covers any treatment of a disease or symptom in a mammal, and includes: (a) preventing the disease or symptom from occurring in a subject which may be predisposed to acquiring the disease or symptom but has not yet been diagnosed as having it; (b) inhibiting the disease or symptom, i.e., arresting its development; or (c) relieving the disease, i.e., causing regression of the disease. The therapeutic agent may be administered before, during or after the onset of disease or injury. The treatment of ongoing disease, where the treatment stabilizes or reduces the undesirable clinical symptoms of the patient, is of particular interest. Such treatment is desirably performed prior to complete loss of function in the affected tissues.

The subject therapy will desirably be administered during the symptomatic stage of the disease, and in some cases after the symptomatic stage of the disease.

[0056] The terms "individual," "subject," "host," and "patient," are used interchangeably herein and refer to any mammalian subject for whom diagnosis, treatment, or therapy is desired. Mammals include, e.g., humans, non-human primates, rodents (e.g., rats; mice), lagomorphs (e.g., rabbits), ungulates (e.g., cows, sheep, pigs, horses, goats, and the like), etc.

[0057] Before the present invention is further described, it is to be understood that this invention is not limited to particular embodiments described, as such may, of course, vary. It is also to be understood that the terminology used herein is for the purpose of describing particular embodiments only, and is not intended to be limiting, since the scope of the present invention will be limited only by the appended claims.

[0058] Where a range of values is provided, it is understood that each intervening value, to the tenth of the unit of the lower limit unless the context clearly dictates otherwise, between the upper and lower limit of that range and any other stated or intervening value in that stated range, is encompassed within the invention. The upper and lower limits of these smaller ranges may independently be included in the smaller ranges, and are also encompassed within the invention, subject to any specifically excluded limit in the stated range. Where the stated range includes one or both of the limits, ranges excluding either or both of those included limits are also included in the invention.

[0059] Unless defined otherwise, 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 any methods and materials similar or equivalent to those described herein can also be used in the practice or testing of the present invention, the preferred methods and materials are now described. All publications mentioned herein are incorporated herein by reference to disclose and describe the methods and/or materials in connection with which the publications are cited.

[0060] It must be noted that as used herein and in the appended claims, the singular forms “a,” “an,” and “the” include plural referents unless the context clearly dictates otherwise. Thus, for example, reference to “a modulatory domain” includes a plurality of such modulatory domains and reference to “the HLA polypeptide” includes reference to one or more HLA polypeptides and equivalents thereof known to those skilled in the art, and so forth. It is further noted that the claims may be drafted to exclude any optional element. As such, this statement is intended to serve as antecedent basis for use of such exclusive terminology as “solely,” “only” and the like in connection with the recitation of claim elements, or use of a “negative” limitation.

[0061] It is appreciated that certain features of the invention, which are, for clarity, described in the context of separate embodiments, may also be provided in combination in a single embodiment. Conversely, various features of the invention, which are, for brevity, described in the context of a single embodiment, may also be provided separately or in any suitable sub-combination. All combinations of the embodiments pertaining to the invention are specifically embraced by the present invention and are disclosed herein just as if each and every combination was individually and explicitly disclosed. In addition, all sub-combinations of the various embodiments and elements thereof are also specifically embraced by the present invention and are disclosed herein just as if each and every such sub-combination was individually and explicitly disclosed herein.

[0062] The publications discussed herein are provided solely for their disclosure prior to the filing date of the present application. Nothing herein is to be construed as an admission that the present invention is not entitled to antedate such publication by virtue of prior invention. Further, the dates of publication provided may be different from the actual publication dates which may need to be independently confirmed.

DETAILED DESCRIPTION

[0063] The present disclosure provides variant immunomodulatory polypeptides, and fusion polypeptides comprising the variant immunomodulatory peptides. The present disclosure provides T-cell modulatory multimeric polypeptides, and compositions comprising same, where the T-cell modulatory multimeric polypeptides comprise a variant immunomodulatory polypeptide of the present disclosure. The present disclosure provides nucleic acids comprising nucleotide sequences encoding the T-cell modulatory multimeric polypeptides, and host cells comprising the nucleic acids. The present disclosure provides methods of modulating the activity of a T cell; the methods comprise contacting the T cell with a T-cell modulatory multimeric polypeptide of the present disclosure.

[0064] A T-cell modulatory multimeric polypeptide of the present disclosure is also referred to as a “synTac polypeptide.” A synTac polypeptide of the present disclosure comprises a variant modulatory domain, where the variant modulatory domain exhibits reduced binding affinity to an immunomodulatory polypeptide. A synTac polypeptide of the present disclosure can modulate the activity of a target T-cell. A synTac polypeptide of the present disclosure provides for enhanced target cell specificity.

VARIANT IMMUNOMODULATORY POLYPEPTIDES

[0065] The present disclosure provides variant 4-1BBL modulatory polypeptides. A wild-type human 4-1BBL amino acid sequence is provided in FIG. 2A. The tumor necrosis factor (TNF) homology domain (THD) of human 4-1BBL comprises amino acids 81-254, amino acids 80-254, or amino acids 80-246 of the amino acid sequence depicted in FIG. 2A. Thus, a wild-type amino acid sequence of the THD of human 4-1BBL can be, e.g., one of SEQ ID NOs:1-3, as follows:

[0066] PAGLLDLRQG MFAQLVAQNV LLIDGPLSWY SDPGLAGVSL TGGLSYKEDT KELVVAKAGV YYVFFQLELR RVVAGEGSGS VSLALHLQPL RSAAGAAALA LTVDLPPASS EARNSAFGFQ GRLLHLSAGQ RLGVHLHTEA RARHAWQLTQ GATVLGLFRV TPEIPAGLPS PRSE (SEQ ID NO:1).

[0067] D PAGLLDLRQG MFAQLVAQNV LLIDGPLSWY SDPGLAGVSL TGGLSYKEDT KELVVAKAGV YYVFFQLELR RVVAGEGSGS VSLALHLQPL RSAAGAAALA LTVDLPPASS EARNSAFGFQ GRLLHLSAGQ RLGVHLHTEA RARHAWQLTQ GATVLGLFRV TPEIPAGLPS PRSE (SEQ ID NO:2).

[0068] D PAGLLDLRQG MFAQLVAQNV LLIDGPLSWY SDPGLAGVSL TGGLSYKEDT KELVVAKAGV YYVFFQLELR RVVAGEGSGS VSLALHLQPL RSAAGAAALA LTVDLPPASS EARNSAFGFQ GRLLHLSAGQ RLGVHLHTEA RARHAWQLTQ GATVLGLFRV TPEIPA (SEQ ID NO:3).

[0069] Wild-type 4-1BBL binds to 4-1BB (CD137). An amino acid sequences of 4-1BB is provided in FIG. 3. A variant 4-1BBL polypeptide of the present disclosure binds to 4-1BB with reduced affinity compared to binding of wild-type 4-1BBL to 4-1BB.

[0070] In some cases, a variant 4-1BBL polypeptide of the present disclosure exhibits reduced binding affinity to 4-1BB, compared to the binding affinity of a 4-1BBL polypeptide comprising the amino acid sequence depicted in FIG. 2A. For example, in some cases, a variant 4-1BBL polypeptide of the present disclosure binds 4-1BB with a binding affinity that less than the binding affinity of a 4-1BBL polypeptide comprising the amino acid sequence depicted in FIG. 2A for a 4-1BB polypeptide comprising the amino acid sequence depicted in FIG. 3. For example, in some cases, a variant 4-1BBL polypeptide of the present disclosure binds 4-1BB with a binding affinity that is at least 10% less, at least 15% less, at least 20% less, at least 25%, at least 30% less, at least 35% less, at least 40% less, at least 45% less, at least 50% less, at least 55% less, at least 60% less, at least 65% less, at least 70% less, at least 75% less, at least 80% less, at least 85% less, at least 90% less, at least 95% less, or more than 95% less, than the binding affinity of a 4-1BBL polypeptide comprising the amino acid sequence depicted in FIG.

2A for a 4-1BB (e.g., a 4-1BB polypeptide comprising the amino acid sequence depicted in FIG. 3), when assayed under the same conditions.

[0071] In some cases, a variant 4-1BBL polypeptide of the present disclosure exhibits reduced binding affinity to 4-1BB, compared to the binding affinity of a 4-1BBL polypeptide comprising the amino acid sequence set forth in SEQ ID NO:1. For example, in some cases, a variant 4-1BBL polypeptide of the present disclosure binds 4-1BB with a binding affinity that less than the binding affinity of a 4-1BBL polypeptide comprising the amino acid sequence set forth in SEQ ID NO:1 for a 4-1BB polypeptide comprising the amino acid sequence depicted in FIG. 3. For example, in some cases, a variant 4-1BBL polypeptide of the present disclosure binds 4-1BB with a binding affinity that is is at least 10% less, at least 15% less, at least 20% less, at least 25%, at least 30% less, at least 35% less, at least 40% less, at least 45% less, at least 50% less, at least 55% less, at least 60% less, at least 65% less, at least 70% less, at least 75% less, at least 80% less, at least 85% less, at least 90% less, at least 95% less, or more than 95% less, than the binding affinity of a 4-1BBL polypeptide comprising the amino acid sequence set forth in SEQ ID NO:1 for a 4-1BB polypeptide (e.g., a 4-1BB polypeptide comprising the amino acid sequence depicted in FIG. 3), when assayed under the same conditions.

[0072] In some cases, a variant 4-1BBL polypeptide of the present disclosure has a binding affinity to 4-1BB that is from 100 nM to 100 μM. As another example, in some cases, a variant 4-1BBL polypeptide of the present disclosure has a binding affinity for 4-1BB (e.g., a 4-1BB polypeptide comprising the amino acid sequence depicted in FIG. 3) that is from about 100 nM to 150 nM, from about 150 nM to about 200 nM, from about 200 nM to about 250 nM, from about 250 nM to about 300 nM, from about 300 nM to about 350 nM, from about 350 nM to about 400 nM, from about 400 nM to about 500 nM, from about 500 nM to about 600 nM, from about 600 nM to about 700 nM, from about 700 nM to about 800 nM, from about 800 nM to about 900 nM, from about 900 nM to about 1 μM, to about 1 μM to about 5 μM, from about 5 μM to about 10 μM, from about 10 μM to about 15 μM, from about 15 μM to about 20 μM, from about 20 μM to about 25 μM, from about 25 μM to about 50 μM, from about 50 μM to about 75 μM, or from about 75 μM to about 100 μM.

[0073] In some cases, a variant 4-1BBL polypeptide of the present disclosure exhibits increased production in a mammalian host cell, compared to the production in the same mammalian host cell of a wild-type 4-1BBL polypeptide (e.g., a 4-1BBL polypeptide comprising the amino acid sequence depicted in FIG. 2A or as set forth in SEQ ID NO:1). For example, in some cases, a variant 4-1BBL polypeptide of the present disclosure, when expressed in a mammalian host cell, is produced in an amount that is from 25% higher to about 50% higher, from about 50% higher to about 75% higher, from about 75% higher to about 2-fold higher, from about 2-fold higher to about 5-fold higher, from about 5-fold higher to about 10-fold higher, from about 10-fold higher to about 20-fold higher, from about 20-fold higher to about 30-fold higher, from about 30-fold higher to about 40-fold higher, from about 40-fold higher to about 50-fold higher, from abouat 50-fold higher to about 75-fold higher, from about 75-fold higher to about 100-fold higher, or more than 100-fold higher, than the amount of a wild-type 4-1BBL polypeptide (e.g., a 4-1BBL polypeptide comprising the amino acid sequence depicted in FIG. 2A or as set forth in SEQ ID NO:1) produced in the same mammalian host cell.

[0074] In some cases, a variant 4-1BBL polypeptide of the present disclosure is produced in a mammalian host cell in an amount of from about 50 mg/L to about 75 mg/L, from about 75 mg/L to about 100 mg/L, from about 100 mg/L to about 150 mg/L, from about 150 mg/L to about 200 mg/L, from about 200 mg/L to about 250 mg/L, from about 250 mg/L to about 500 mg/L, or more than 500 mg/L. In some cases, a variant 4-1BBL polypeptide of the present disclosure is produced in a mammalian host cell in an amount of from about 10 mg/L to about 15 mg/L, from about 15 mg/L to about 20 mg/L, from about 20 mg/L to about 25 mg/L, from about 25 mg/L to about 30 mg/L, from about 35 mg/L to about 40 mg/L, from about 40 mg/L to about 45 mg/L, or from about 45 mg/L to about 50 mg/L.

[0075] A variant 4-1BBL polypeptide of the present disclosure can have a single amino acid substitution relative to a wild-type 4-1BBL polypeptide (e.g., a 4-1BBL polypeptide comprising the amino acid sequence depicted in FIG. 2A or as set forth in SEQ ID NO:1). In some cases, a variant 4-1BBL polypeptide of the present disclosure has from 2 to 10 amino acid substitutions relative to a wild-type 4-1BBL polypeptide (e.g., a 4-1BBL polypeptide comprising the amino acid sequence depicted in FIG. 2A or as set forth in SEQ ID NO:1). In some cases, a variant 4-1BBL polypeptide of the present disclosure has 2 amino acid substitutions relative to a wild-type 4-1BBL polypeptide (e.g., a 4-1BBL polypeptide comprising the amino acid sequence depicted in FIG. 2A or as set forth in SEQ ID NO:1). In some cases, a variant 4-1BBL polypeptide of the present disclosure has 3 amino acid substitutions relative to a wild-type 4-1BBL polypeptide (e.g., a 4-1BBL polypeptide comprising the amino acid sequence depicted in FIG. 2A or as set forth in SEQ ID NO:1). In some cases, a variant 4-1BBL polypeptide of the present disclosure has 4 amino acid substitutions relative to a wild-type 4-1BBL polypeptide (e.g., a 4-1BBL polypeptide comprising the amino acid sequence depicted in FIG. 2A or as set forth in SEQ ID NO:1). In some cases, a variant 4-1BBL polypeptide of the present disclosure has 5 amino acid substitutions relative to a wild-type 4-1BBL polypeptide (e.g., a 4-1BBL polypeptide comprising the amino acid sequence depicted in FIG. 2A or as set forth in SEQ ID NO:1). In some cases, a variant 4-1BBL polypeptide of the present disclosure has 6 amino acid substitutions relative to a wild-type 4-1BBL polypeptide (e.g., a 4-1BBL polypeptide comprising the amino acid sequence depicted in FIG. 2A or as set forth in SEQ ID NO:1). In some cases, a variant 4-1BBL polypeptide of the present disclosure has 7 amino acid substitutions relative to a wild-type 4-1BBL polypeptide (e.g., a 4-1BBL polypeptide comprising the amino acid sequence depicted in FIG. 2A or as set forth in SEQ ID NO:1). In some cases, a variant 4-1BBL polypeptide of the present disclosure has 8 amino acid substitutions relative to a wild-type 4-1BBL polypeptide (e.g., a 4-1BBL polypeptide comprising the amino acid sequence depicted in FIG. 2A or as set forth in SEQ ID NO:1). In some cases, a variant 4-1BBL polypeptide of the present disclosure has 9 amino acid substitutions relative to a wild-type 4-1BBL polypeptide (e.g., a 4-1BBL polypeptide comprising the amino acid sequence depicted in FIG. 2A or as set forth in SEQ ID NO:1). In some cases, a variant 4-1BBL polypeptide of the present disclosure has 10 amino acid substitutions relative to a wild-type 4-1BBL polypeptide (e.g., a 4-1BBL polypeptide comprising the amino acid sequence depicted in FIG. 2A or as set forth in SEQ ID NO:1).

[0076] In some cases, a variant 4-1BBL polypeptide of the present disclosure has from 11 to 50 amino acid substitutions relative to a wild-type 4-1BBL polypeptide (e.g., a 4-1BBL polypeptide comprising the amino acid sequence depicted in FIG. 2A or as set forth in SEQ ID NO:1). For example, in some cases, a variant 4-1BBL polypeptide of the present disclosure has from 11 to 15, from 15 to 20, from 20 to 25, from 25 to 30, from 30 to 35, from 35 to 40, from 40 to 45, or from 45 to 50, amino acid substitutions relative to a wild-type 4-1BBL polypeptide (e.g., a 4-1BBL polypeptide comprising the amino acid sequence depicted in FIG. 2A or as set forth in SEQ ID NO:1).

[0077] A variant 4-1BBL polypeptide of the present disclosure can have a length of from 200 amino acids to 254 amino acids. A variant 4-1BBL polypeptide of the present disclosure can have a length of from 150 amino acids to 200 amino acids. For example, in some cases, a variant 4-1BBL polypeptide of the present disclosure has a length of from 150 amino acids to 160 amino acids, from 160 amino acids to 170 amino acids, from 170 amino acids to 180 amino acids, from 180 amino acids to 190 amino acids, or from 190 amino acids to 200 amino acids. In some cases, a variant 4-1BBL polypeptide of the present disclosure has a length of from 160 amino acids to 175 amino acids. In some cases, a variant 4-1BBL polypeptide of the present disclosure has a length of 162 amino acids. In some cases, a variant 4-1BBL polypeptide of the present disclosure has a length of 167 amino acids. In some cases, a variant 4-1BBL polypeptide of the present disclosure has a length of 174 amino acids. In some cases, a variant 4-1BBL polypeptide of the present disclosure has a length of 175 amino acids.

[0078] Variant 4-1BBL polypeptides are described below in relation to the amino acid sequences depicted in FIG. 2A-2IIII, and in relation to the amino acid sequence depicted in SEQ ID NO:1. However, for each variant 4-1BBL polypeptide described below, a corresponding amino acid in

SEQ ID NO:2 or SEQ ID NO:3 could be substituted. For example, in some cases, a variant 4-1BBL polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence set forth in SEQ ID NO:2, with an amino acid substitution at K48. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises the amino acid sequence set forth in SEQ ID NO:2, with an amino acid substitution at K48. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence set forth in SEQ ID NO:3, with an amino acid substitution at K48. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises the amino acid sequence set forth in SEQ ID NO:3, with an amino acid substitution at K48.

K127 substitution

[0079] In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence depicted in FIG. 2B, where amino acid 127 (indicated by an “x”) is an amino acid other than a lysine, e.g., where amino acid 127 is Gly, Ala, Val, Leu, Ile, Pro, Phe, Tyr, Trp, Ser, Thr, Cys, Met, Asn, Gln, Arg, His, Asp, or Glu. The amino acid numbering in FIG. 2B retains the numbering of FIG. 2A. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence depicted in FIG. 2B, where amino acid 127 is Phe. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence depicted in FIG. 2B, where amino acid 127 is Leu. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence depicted in FIG. 2B, where amino acid 127 is Ile. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence depicted in FIG. 2B, where amino acid 127 is Met. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence depicted in FIG. 2B, where amino acid 127 is Val. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence depicted in FIG. 2B, where amino acid 127 is Ser. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence depicted in FIG. 2B, where amino acid 127 is Pro. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence depicted in FIG. 2B, where amino acid 127 is Thr. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence depicted in FIG. 2B, where amino acid 127 is Ala. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence depicted in FIG. 2B, where amino acid 127 is Tyr. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence depicted in FIG. 2B, where amino acid 127 is His. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence depicted in FIG. 2B, where amino acid 127 is Gln. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence depicted in FIG. 2B, where amino acid 127 is Asn. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence depicted in FIG. 2B, where amino acid 127 is Asp. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence depicted in FIG. 2B, where amino acid 127 is Glu. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence depicted in FIG. 2B, where amino acid 127 is Cys. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence depicted in FIG. 2B, where amino acid 127 is Trp. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence depicted in FIG. 2B, where amino acid 127 is Arg. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence depicted in FIG. 2B, where amino acid 127 is Gly. In some cases, the variant 4-1BBL polypeptide has a binding affinity to CD137 that is from about 100 nM to about 150 nM, from about 150 nM to about 200 nM, from about 200 nM to about 250 nM, from about 250 nM to about 300 nM, from about 300 nM to about 350 nM, from about 350 nM to about 400 nM, from about 400 nM to about 500 nM, from about 500 nM to about 600 nM, from about 600 nM to about 700 nM, from about 700 nM to about 800 nM, from about 800 nM to about 900 nM, from about 900 nM to about 1 μM, to about 1 μM to about 5 μM, from about 5 μM to about 10 μM, from about 10 μM to about 15 μM, from about 15 μM to about 20 μM, from about 20 μM to about 25 μM, from about 25 μM to about 50 μM, from about 50 μM to about 75 μM, or from about 75 μM to about 100 μM.

[0080] In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2A, with an amino acid substitution at K127. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence set forth in SEQ ID NO:1, with an amino acid substitution at K47. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises the amino acid sequence set forth in SEQ ID NO:1, with an amino acid substitution at K47. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence set forth in SEQ ID NO:2, with an amino acid substitution at K48. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises the amino acid sequence set forth in SEQ ID NO:2, with an amino acid substitution at K48. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence set forth in SEQ ID NO:3, with an amino acid substitution at K48. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises the amino acid sequence set forth in SEQ ID NO:3, with an amino acid substitution at K48.

[0081] For example, in some cases, a variant 4-1BBL polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2B, where “x” is any amino acid other than lysine; for example, “x” can be Gly, Ala, Val, Leu, Ile, Pro, Phe, Tyr, Trp, Ser, Thr, Cys, Met, Asn, Gln, Arg, His, Asp, or Glu. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2B, where “x” is Phe. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2B, where “x” is Leu. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2B, where “x” is Ile. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2B, where “x” is Met. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2B, where “x” is Val. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2B, where “x” is Ser. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2B, where “x” is Pro. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2B, where “x” is Thr. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2B, where “x” is Ala. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2B, where “x” is Tyr. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2B, where “x” is His. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2B, where “x” is Gln. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2B, where “x” is Asn. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2B, where “x” is Asp. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2B, where “x” is Glu. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2B, where “x” is Cys. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2B, where “x” is Trp. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2B, where “x” is Arg. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2B, where “x” is Gly. For example, in some cases, a variant 4-1BBL polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2C. In some cases, the variant 4-1BBL polypeptide has a binding affinity to CD137 that is from about 100 nM to about 150 nM, from about 150 nM to about 200 nM, from about 200 nM to about 250 nM, from about 250 nM to about 300 nM, from about 300 nM to about 350 nM, from about 350 nM to about 400 nM, from about 400 nM to about 500 nM, from about 500 nM to about 600 nM, from about 600 nM to about 700 nM, from about 700 nM to about 800 nM, from about 800 nM to about 900 nM, from about 900 nM to about 1 µM, to about 1 µM to about 5 µM, from about 5 µM to about 10 µM, from about 10 µM to about 15 µM, from about 15 µM to about 20 µM, from about 20 µM to about 25 µM, from about 25 µM to about 50 µM, from about 50 µM to about 75 µM, or from about 75 µM to about 100 µM.

Q227 Substitution

[0082] In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence depicted in FIG. 2D, where amino acid 227 (indicated by an "x") is an amino acid other than a glutamine, e.g., where amino acid 227 is Gly, Ala, Val, Leu, Ile, Pro, Phe, Tyr, Trp, Ser, Thr, Cys, Met, Asn, Lys, Arg, His, Asp, or Glu. The amino acid numbering in FIG. 2D retains the numbering of FIG. 2A. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence depicted in FIG. 2D, where amino acid 227 is Phe. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence depicted in FIG. 2D, where amino acid 227 is Leu. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence depicted in FIG. 2D, where amino acid 227 is Ile. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence depicted in FIG. 2D, where amino acid 227 is Met. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence depicted in FIG. 2D, where amino acid 227 is Val. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence depicted in FIG. 2D, where amino acid 227 is Ser. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence depicted in FIG. 2D, where amino acid 227 is Pro. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence depicted in FIG. 2D, where amino acid 227 is Thr. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence depicted in FIG. 2D, where amino acid 227 is Ala. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence depicted in FIG. 2D, where amino acid 227 is Tyr. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence depicted in FIG. 2D, where amino acid 227 is His. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence depicted in FIG. 2D, where amino acid 227 is Asn. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence depicted in FIG. 2D, where amino acid 227 is Lys. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence depicted in FIG. 2D, where amino acid 227 is Asp. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence depicted in FIG. 2D, where amino acid 227 is Glu. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence depicted in FIG. 2D, where amino acid 227 is Cys. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence depicted in FIG. 2D, where amino acid 227 is Trp. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence depicted in FIG. 2D, where amino acid 227 is Arg. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence depicted in FIG. 2D, where amino acid 227 is Gly. In some cases, the variant 4-1BBL polypeptide has a binding affinity to CD137 that is from about 100 nM to about 150 nM, from about 150 nM to about 200 nM, from about 200 nM to about 250 nM, from about 250 nM to about 300 nM, from about 300 nM to about 350 nM, from about 350 nM to about 400 nM, from about 400 nM to about 500 nM, from about 500 nM to about 600 nM, from about 600 nM to about 700 nM, from about 700 nM to about 800 nM, from about 800 nM to about 900 nM, from about 900 nM to about 1 μM, to about 1 μM to about 5 μM, from about 5 μM to about 10 μM, from about 10 μM to about 15 μM, from about 15 μM to about 20 μM, from about 20 μM to about 25 μM, from about 25 μM to about 50 μM, from about 50 μM to about 75 μM, or from about 75 μM to about 100 μM.

[0083] In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2A, with an amino acid substitution at Q227. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence set forth in SEQ ID NO:1, with an amino acid substitution at Q147. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises the amino acid sequence set forth in SEQ ID NO:1, with an amino acid substitution at Q147. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence set forth in SEQ ID NO:2, with an amino acid substitution at Q148. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises the amino acid sequence set forth in SEQ ID NO:2, with an amino acid substitution at Q148. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence set forth in SEQ ID NO:3, with an amino acid substitution at Q148. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises the amino acid sequence set forth in SEQ ID NO:3, with an amino acid substitution at Q148.

[0084] For example, in some cases, a variant 4-1BBL polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2D, where “x” is any amino acid other than glutamine; for example, “x” can be Gly, Ala, Val, Leu, Ile, Pro, Phe, Tyr, Trp, Ser, Thr, Cys, Met, Asn, Gln, Arg, His, Asp, or Glu. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2D, where “x” is Phe. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2D, where “x” is Leu. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2D, where “x” is Ile. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2D, where “x” is Met. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2D, where “x” is Val. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2D, where “x” is Ser. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2D, where “x” is Pro. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2D, where “x” is Thr. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2D, where “x” is Ala. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2D, where “x” is Tyr. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2D, where “x” is His. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2D, where “x” is Asn. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2D, where “x” is Lys. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2D, where “x” is Asp. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2D, where “x” is Glu. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2D, where “x” is Cys. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2D, where “x” is Trp. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2D, where “x” is Arg. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2D, where “x” is Gly. In some cases, the variant 4-1BBL polypeptide has a binding affinity to CD137 that is from about 100 nM to about 150 nM, from about 150 nM to about 200 nM, from about 200 nM to about 250 nM, from about 250 nM to about 300 nM, from about 300 nM to about 350 nM, from about 350 nM to about 400 nM, from about 400 nM to about 500 nM, from about 500 nM to about 600 nM, from about 600 nM to about 700 nM, from about 700 nM to about 800 nM, from about 800 nM to about 900 nM, from about 900 nM to about 1 μM, to about 1 μM to about 5 μM, from about 5 μM to about 10 μM, from about 10 μM to about 15 μM, from about 15 μM to about 20 μM, from about 20 μM to about 25 μM, from about 25 μM to about 50 μM, from about 50 μM to about 75 μM, or from about 75 μM to about 100 μM.

M91 Substitution

[0085] In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence depicted in FIG. 2E, where amino acid 91 (indicated by an “x”) is an amino acid other than a methionine, e.g., where amino acid 91 is Gly, Ala, Val, Leu, Ile, Pro, Phe, Tyr, Trp, Ser, Thr, Cys, Asn, Gln, Lys, Arg, His, Asp, or Glu. The amino acid numbering in FIG. 2E retains the numbering of FIG. 2A. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence depicted in FIG. 2E, where amino acid 91 is Gly. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence depicted in FIG. 2E, where amino acid 91 is Ala. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence depicted in FIG. 2E, where amino acid 91 is Val. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence depicted in FIG. 2E, where amino acid 91 is Leu. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence depicted in FIG. 2E, where amino acid 91 is Ile. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence depicted in FIG. 2E, where amino acid 91 is Pro. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence depicted in FIG. 2E, where amino acid 91 is Phe. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence depicted in FIG. 2E, where amino acid 91 is Tyr. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence depicted in FIG. 2E, where amino acid 91 is Trp. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence depicted in FIG. 2E, where amino acid 91 is Ser. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence depicted in FIG. 2E, where amino acid 91 is Thr. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence depicted in FIG. 2E, where amino acid 91 is Cys. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence depicted in FIG. 2E, where amino acid 91 is Asn. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence depicted in FIG. 2E, where amino acid 91 is Gln. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence depicted in FIG. 2E, where amino acid 91 is Lys. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence depicted in FIG. 2E, where amino acid 91 is Arg. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence depicted in FIG. 2E, where amino acid 91 is His. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence depicted in FIG. 2E, where amino acid 91 is Asp. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence depicted in FIG. 2E, where amino acid 91 is Glu. In some cases, the variant 4-1BBL polypeptide has a binding affinity to CD137 that is from about 100 nM to about 150 nM, from about 150 nM to about 200 nM, from about 200 nM to about 250 nM, from about 250 nM to about 300 nM, from about 300 nM to about 350 nM, from about 350 nM to about 400 nM, from about 400 nM to about 500 nM, from about 500 nM to about 600 nM, from about 600 nM to about 700 nM, from about 700 nM to about 800 nM, from about 800 nM to about 900 nM, from about 900 nM to about 1 μM, to about 1 μM to about 5 μM, from about 5 μM to about 10 μM, from about 10 μM to about 15 μM, from about 15 μM to about 20 μM, from about 20 μM to about 25 μM, from about 25 μM to about 50 μM, from about 50 μM to about 75 μM, or from about 75 μM to about 100 μM.

[0086] In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2A, with an amino acid substitution at M91. In some cases, a variant

4-1BBL polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence set forth in SEQ ID NO:1, with an amino acid substitution at M11. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises the amino acid sequence set forth in SEQ ID NO:1, with an amino acid substitution at M11. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence set forth in SEQ ID NO:2, with an amino acid substitution at M12. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises the amino acid sequence set forth in SEQ ID NO:2, with an amino acid substitution at M12. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence set forth in SEQ ID NO:3, with an amino acid substitution at M12. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises the amino acid sequence set forth in SEQ ID NO:3, with an amino acid substitution at M12.

[0087] For example, in some cases, a variant 4-1BBL polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2E, where “x” is any amino acid other than methionine; for example, “x” can be Gly, Ala, Val, Leu, Ile, Pro, Phe, Tyr, Trp, Ser, Thr, Cys, Asn, Gln, Lys, Arg, His, Asp, or Glu. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2E, where “x” is Gly. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2E, where “x” is Ala. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2E, where “x” is Val. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2E, where “x” is Leu. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2E, where “x” is Ile. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2E, where “x” is Pro. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2E, where “x” is Phe. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2E, where “x” is Tyr. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2E, where “x” is Trp. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2E, where “x” is Ser. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2E, where “x” is Thr.

In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2E, where “x” is Cys. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2E, where “x” is Asn. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2E, where “x” is Gln. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2E, where “x” is Lys. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2E, where “x” is Arg. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2E, where “x” is His. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2E, where “x” is Asp. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2E, where “x” is Glu. In some cases, the variant 4-1BBL polypeptide has a binding affinity to CD137 that is from about 100 nM to about 150 nM, from about 150 nM to about 200 nM, from about 200 nM to about 250 nM, from about 250 nM to about 300 nM, from about 300 nM to about 350 nM, from about 350 nM to about 400 nM, from about 400 nM to about 500 nM, from about 500 nM to about 600 nM, from about 600 nM to about 700 nM, from about 700 nM to about 800 nM, from about 800 nM to about 900 nM, from about 900 nM to about 1 μM, to about 1 μM to about 5 μM, from about 5 μM to about 10 μM, from about 10 μM to about 15 μM, from about 15 μM to about 20 μM, from about 20 μM to about 25 μM, from about 25 μM to about 50 μM, from about 50 μM to about 75 μM, or from about 75 μM to about 100 μM.

F92 Substitution

[0088] In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence depicted in FIG. 2F, where amino acid 92 (indicated by an “x”) is an amino acid other than a phenylalanine, e.g., where amino acid 92 is Gly, Ala, Val, Leu, Ile, Pro, Tyr, Trp, Ser, Thr, Cys, Met, Asn, Gln, Lys, Arg, His, Asp, or Glu. The amino acid numbering in FIG. 2F retains the numbering of FIG. 2A. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence depicted in FIG. 2F, where amino acid 92 is Gly. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence depicted in FIG. 2F, where amino acid 92 is Ala. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence depicted in FIG. 2F, where amino acid 92 is Val. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence depicted in FIG. 2F, where amino acid 92 is Leu. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence depicted in FIG. 2F, where amino acid 92 is Ile. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence depicted in FIG. 2F, where amino acid 92 is Pro. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence depicted in FIG. 2F, where amino acid 92 is Tyr. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence depicted in FIG. 2F, where amino acid 92 is Trp. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence depicted in FIG. 2F, where amino acid 92 is Ser. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence depicted in FIG. 2F, where amino acid 92 is Thr. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence depicted in FIG. 2F, where amino acid 92 is Cys. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence depicted in FIG. 2F, where amino acid 92 is Met. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence depicted in FIG. 2F, where amino acid 92 is Asn. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence depicted in FIG. 2F, where amino acid 92 is Gln. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence depicted in FIG. 2F, where amino acid 92 is Lys. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence depicted in FIG. 2F, where amino acid 92 is Arg. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence depicted in FIG. 2F, where amino acid 92 is His. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence depicted in FIG. 2F, where amino acid 92 is Asp. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence depicted in FIG. 2F, where amino acid 92 is Glu. In some cases, the variant 4-1BBL polypeptide has a binding affinity to CD137 that is from about 100 nM to about 150 nM, from about 150 nM to about 200 nM, from about 200 nM to about 250 nM, from about 250 nM to about 300 nM, from about 300 nM to about 350 nM, from about 350 nM to about 400 nM, from about 400 nM to about 500 nM, from about 500 nM to about 600 nM, from about 600 nM to about 700 nM, from about 700 nM to about 800 nM, from about 800 nM to about 900 nM, from about 900 nM to about 1 μM, to about 1 μM to about 5 μM, from about 5 μM to about 10 μM, from about 10 μM to about 15 μM, from about 15 μM to about 20 μM, from about 20 μM to about 25 μM, from about 25 μM to about 50 μM, from about 50 μM to about 75 μM, or from about 75 μM to about 100 μM.

[0089] In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2A, with an amino acid substitution at F92. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence set forth in SEQ ID NO:1, with an amino acid substitution at F12. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises the amino acid sequence set forth in SEQ ID NO:1, with an amino acid substitution at F12. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence set forth in SEQ ID NO:2, with an amino acid substitution at F13. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises the amino acid sequence set forth in SEQ ID NO:2, with an amino acid substitution at F13. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence set forth in SEQ ID NO:3, with an amino acid substitution at F13. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises the amino acid sequence set forth in SEQ ID NO:3, with an amino acid substitution at F13.

[0090] For example, in some cases, a variant 4-1BBL polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2F, where “x” is any amino acid other than phenylalanine; for example, “x” can be Gly, Ala, Val, Leu, Ile, Pro, Tyr, Trp, Ser, Thr, Cys, Met, Asn, Gln, Lys, Arg, His, Asp, or Glu. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2F, where “x” is Gly. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2F, where “x” is Ala. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2F, where “x” is Val. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2F, where “x” is Leu. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2F, where “x” is Ile. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2F, where “x” is Pro. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2F, where “x” is Tyr. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2F, where “x” is Trp. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2F, where “x” is Ser. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2F, where “x” is Thr. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2F, where “x” is Cys. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2F, where “x” is Met. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2F, where “x” is Asn. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2F, where “x” is Gln. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2F, where “x” is Lys. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2F, where “x” is Arg. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2F, where “x” is His.

In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2F, where "x" is Asp. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2F, where "x" is Glu. In some cases, the variant 4-1BBL polypeptide has a binding affinity to CD137 that is from about 100 nM to about 150 nM, from about 150 nM to about 200 nM, from about 200 nM to about 250 nM, from about 250 nM to about 300 nM, from about 300 nM to about 350 nM, from about 350 nM to about 400 nM, from about 400 nM to about 500 nM, from about 500 nM to about 600 nM, from about 600 nM to about 700 nM, from about 700 nM to about 800 nM, from about 800 nM to about 900 nM, from about 900 nM to about 1 μM, to about 1 μM to about 5 μM, from about 5 μM to about 10 μM, from about 10 μM to about 15 μM, from about 15 μM to about 20 μM, from about 20 μM to about 25 μM, from about 25 μM to about 50 μM, from about 50 μM to about 75 μM, or from about 75 μM to about 100 μM.

Q94 Substitution

[0091] In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence depicted in FIG. 2G, where amino acid 94 (indicated by an "x") is an amino acid other than a glutamine, e.g., where amino acid 94 is Gly, Ala, Val, Leu, Ile, Pro, Phe, Tyr, Trp, Ser, Thr, Cys, Met, Asn, Lys, Arg, His, Asp, or Glu. The amino acid numbering in FIG. 2G retains the numbering of FIG. 2A. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence depicted in FIG. 2G, where amino acid 94 is Gly. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence depicted in FIG. 2G, where amino acid 94 is Ala. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence depicted in FIG. 2G, where amino acid 94 is Val. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence depicted in FIG. 2G, where amino acid 94 is Leu. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence depicted in FIG. 2G, where amino acid 94 is Ile. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence depicted in FIG. 2G, where amino acid 94 is Pro. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence depicted in FIG. 2G, where amino acid 94 is Phe. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence depicted in FIG. 2G, where amino acid 94 is Tyr. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence depicted in FIG. 2G, where amino acid 94 is Trp. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence depicted in FIG. 2G, where amino acid 94 is Ser. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence depicted in FIG. 2G, where amino acid 94 is Thr. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence depicted in FIG. 2G, where amino acid 94 is Cys. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence depicted in FIG. 2G, where amino acid 94 is Met. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence depicted in FIG. 2G, where amino acid 94 is Asn. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence depicted in FIG. 2G, where amino acid 94 is Lys. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence depicted in FIG. 2G, where amino acid 94 is Arg. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence depicted in FIG. 2G, where amino acid 94 is His. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence depicted in FIG. 2G, where amino acid 94 is Asp. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence depicted in FIG. 2G, where amino acid 94 is Glu. In some cases, the variant 4-1BBL polypeptide has a binding affinity to CD137 that is from about 100 nM to about 150 nM, from about 150 nM to about 200 nM, from about 200 nM to about 250 nM, from about 250 nM to about 300 nM, from about 300 nM to about 350 nM, from about 350 nM to about 400 nM, from about 400 nM to about 500 nM, from about 500 nM to about 600 nM, from about 600 nM to about 700 nM, from about 700 nM to about 800 nM, from about 800 nM to about 900 nM, from about 900 nM to about 1 μM, to about 1 μM to about 5 μM, from about 5 μM to about 10 μM, from about 10 μM to about 15 μM, from about 15 μM to about 20 μM, from about 20 μM to about 25 μM, from about 25 μM to about 50 μM, from about 50 μM to about 75 μM, or from about 75 μM to about 100 μM.

[0092] In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2A, with an amino acid substitution at Q94. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence set forth in SEQ ID NO:1, with an amino acid substitution at Q14. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises the amino acid sequence set forth in SEQ ID NO:1, with an amino acid substitution at Q14. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence set forth in SEQ ID NO:2, with an amino acid substitution at Q15. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises the amino acid sequence set forth in SEQ ID NO:2, with an amino acid substitution at Q15. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence set forth in SEQ ID NO:3, with an amino acid substitution at Q15. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises the amino acid sequence set forth in SEQ ID NO:3, with an amino acid substitution at Q15.

[0093] For example, in some cases, a variant 4-1BBL polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2G, where “x” is any amino acid other than methionine; for example, “x” can be Gly, Ala, Val, Leu, Ile, Pro, Phe, Tyr, Trp, Ser, Thr, Cys, Met, Asn, Lys, Arg, His, Asp, or Glu. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2G, where "x" is Gly. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2G, where "x" is Ala. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2G, where "x" is Val. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2G, where "x" is Leu. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2G, where "x" is Ile. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2G, where "x" is Pro. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2G, where "x" is Phe. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2G, where "x" is Tyr. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2G, where "x" is Trp. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2G, where "x" is Ser. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2G, where "x" is Thr. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2G, where "x" is Cys. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2G, where "x" is Met. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2G, where "x" is Asn. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2G, where "x" is Lys. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2G, where "x" is Arg. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2G, where "x" is His. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2G, where "x" is Asp. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2G, where "x" is Glu. In some cases, the variant 4-1BBL polypeptide has a binding affinity to CD137 that is from about 100 nM to about 150 nM, from about 150 nM to about 200 nM, from about 200 nM to about 250 nM, from about 250 nM to about 300 nM, from about 300 nM to about 350 nM, from about 350 nM to about 400 nM, from about 400 nM to about 500 nM, from about 500 nM to about 600 nM, from about 600 nM to about 700 nM, from about 700 nM to about 800 nM, from about 800 nM to about 900 nM, from about 900 nM to about 1 μM, to about 1 μM to about 5 µM, from about 5 µM to about 10 µM, from about 10 µM to about 15 µM, from about 15 µM to about 20 µM, from about 20 µM to about 25 µM, from about 25 µM to about 50 µM, from about 50 µM to about 75 µM, or from about 75 µM to about 100 µM.

L95 Substitution

[0094] In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence depicted in FIG. 2H, where amino acid 95 (indicated by an "x") is an amino acid other than a leucine, e.g., where amino acid 95 is Gly, Ala, Val, Ile, Pro, Phe, Tyr, Trp, Ser, Thr, Cys, Met, Asn, Gln, Lys, Arg, His, Asp, or Glu. The amino acid numbering in FIG. 2H retains the numbering of FIG. 2A. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence depicted in FIG. 2H, where amino acid 95 is Gly. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence depicted in FIG. 2H, where amino acid 95 is Ala. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence depicted in FIG. 2H, where amino acid 95 is Val. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence depicted in FIG. 2H, where amino acid 95 is Ile. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence depicted in FIG. 2H, where amino acid 95 is Pro. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence depicted in FIG. 2H, where amino acid 95 is Phe. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence depicted in FIG. 2H, where amino acid 95 is Tyr. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence depicted in FIG. 2H, where amino acid 95 is Trp. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence depicted in FIG. 2H, where amino acid 95 is Ser. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence depicted in FIG. 2H, where amino acid 95 is Thr. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence depicted in FIG. 2H, where amino acid 95 is Cys. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence depicted in FIG. 2H, where amino acid 95 is Met. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence depicted in FIG. 2H, where amino acid 95 is Asn. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence depicted in FIG. 2H, where amino acid 95 is Gln. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence depicted in FIG. 2H, where amino acid 95 is Lys. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence depicted in FIG. 2H, where amino acid 95 is Arg. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence depicted in FIG. 2H, where amino acid 95 is His. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence depicted in FIG. 2H, where amino acid 95 is Asp. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence depicted in FIG. 2H, where amino acid 95 is Glu. In some cases, the variant 4-1BBL polypeptide has a binding affinity to CD137 that is from about 100 nM to about 150 nM, from about 150 nM to about 200 nM, from about 200 nM to about 250 nM, from about 250 nM to about 300 nM, from about 300 nM to about 350 nM, from about 350 nM to about 400 nM, from about 400 nM to about 500 nM, from about 500 nM to about 600 nM, from about 600 nM to about 700 nM, from about 700 nM to about 800 nM, from about 800 nM to about 900 nM, from about 900 nM to about 1 μM, to about 1 μM to about 5 μM, from about 5 μM to about 10 μM, from about 10 μM to about 15 μM, from about 15 μM to about 20 μM, from about 20 μM to about 25 μM, from about 25 μM to about 50 μM, from about 50 μM to about 75 μM, or from about 75 μM to about 100 μM.

[0095] In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2A, with an amino acid substitution at L95. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence set forth in SEQ ID NO:1, with an amino acid substitution at L15. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises the amino acid sequence set forth in SEQ ID NO:1, with an amino acid substitution at L15. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence set forth in SEQ ID NO:2, with an amino acid substitution at L16. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises the amino acid sequence set forth in SEQ ID NO:2, with an amino acid substitution at L16. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence set forth in SEQ ID NO:3, with an amino acid substitution at L16. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises the amino acid sequence set forth in SEQ ID NO:3, with an amino acid substitution at L16.

[0096] For example, in some cases, a variant 4-1BBL polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2H, where “x” is any amino acid other than leucine; for example, “x” can be Gly, Ala, Val, Ile, Pro, Phe, Tyr, Trp, Ser, Thr, Cys, Met, Asn, Gln, Lys, Arg, His, Asp, or Glu. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2H, where “x” is Gly. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2H, where “x” is Ala. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2H, where “x” is Val. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2H, where “x” is Ile. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2H, where “x” is Pro. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2H, where “x” is Phe. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2H, where “x” is Tyr. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2H, where “x” is Trp. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2H, where “x” is Ser. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2H, where “x” is Thr. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2H, where “x” is Cys. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2H, where “x” is Met. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. v, where “x” is Asn. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2H, where “x” is Gln. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2H, where “x” is Lys. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2H, where “x” is Arg. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2H, where “x” is His. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2H, where “x” is Asp. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2H, where “x” is Glu. In some cases, the variant 4-1BBL polypeptide has a binding affinity to CD137 that is from about 100 nM to about 150 nM, from about 150 nM to about 200 nM, from about 200 nM to about 250 nM, from about 250 nM to about 300 nM, from about 300 nM to about 350 nM, from about 350 nM to about 400 nM, from about 400 nM to about 500 nM, from about 500 nM to about 600 nM, from about 600 nM to about 700 nM, from about 700 nM to about 800 nM, from about 800 nM to about 900 nM, from about 900 nM to about 1 μM, to about 1 μM to about 5 μM, from about 5 μM to about 10 μM, from about 10 μM to about 15 μM, from about 15 μM to about 20 μM, from about 20 μM to about 25 μM, from about 25 μM to about 50 μM, from about 50 μM to about 75 μM, or from about 75 μM to about 100 μM.

V96 Substitution

[0097] In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence depicted in FIG. 2I, where amino acid 96 (indicated by an “x”) is an amino acid other than a valine, e.g., where amino acid 96 is Gly, Ala, Leu, Ile, Pro, Phe, Tyr, Trp, Ser, Thr, Cys, Met, Asn, Gln, Lys, Arg, His, Asp, or Glu. The amino acid numbering in FIG. 2I retains the numbering of FIG. 2A. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence depicted in FIG. 2I, where amino acid 96 is Gly. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence depicted in FIG. 2I, where amino acid 96 is Ala. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence depicted in FIG. 2I, where amino acid 96 is Leu. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence depicted in FIG. 2I, where amino acid 96 is Ile. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence depicted in FIG. 2I, where amino acid 96 is Pro. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence depicted in FIG. 2I, where amino acid 96 is Phe. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence depicted in FIG. 2I, where amino acid 96 is Tyr. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence depicted in FIG. 2I, where amino acid 96 is Trp. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence depicted in FIG. 2I, where amino acid 96 is Ser. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence depicted in FIG. 2I, where amino acid 96 is Thr. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence depicted in FIG. 2I, where amino acid 96 is Cys. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence depicted in FIG. 2I, where amino acid 96 is Met. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence depicted in FIG. 2I, where amino acid 96 is Asn. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence depicted in FIG. 2I, where amino acid 96 is Gln. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence depicted in FIG. 2I, where amino acid 96 is Lys. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence depicted in FIG. 2I, where amino acid 96 is Arg. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence depicted in FIG. 2I, where amino acid 96 is His. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence depicted in FIG. 2I, where amino acid 96 is Asp. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence depicted in FIG. 2I, where amino acid 96 is Glu. In some cases, the variant 4-1BBL polypeptide has a binding affinity to CD137 that is from about 100 nM to about 150 nM, from about 150 nM to about 200 nM, from about 200 nM to about 250 nM, from about 250 nM to about 300 nM, from about 300 nM to about 350 nM, from about 350 nM to about 400 nM, from about 400 nM to about 500 nM, from about 500 nM to about 600 nM, from about 600 nM to about 700 nM, from about 700 nM to about 800 nM, from about 800 nM to about 900 nM, from about 900 nM to about 1 μM, to about 1 μM to about 5 μM, from about 5 μM to about 10 μM, from about 10 μM to about 15 μM, from about 15 μM to about 20 μM, from about 20 μM to about 25 μM, from about 25 μM to about 50 μM, from about 50 μM to about 75 μM, or from about 75 μM to about 100 μM.

[0098] In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2A, with an amino acid substitution at V96. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises an amino acid sequence having at least

90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence set forth in SEQ ID NO:1, with an amino acid substitution at V16. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises the amino acid sequence set forth in SEQ ID NO:1, with an amino acid substitution at V16. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence set forth in SEQ ID NO:2, with an amino acid substitution at V17. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises the amino acid sequence set forth in SEQ ID NO:2, with an amino acid substitution at V17. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence set forth in SEQ ID NO:3, with an amino acid substitution at V17. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises the amino acid sequence set forth in SEQ ID NO:3, with an amino acid substitution at V17.

[0099] For example, in some cases, a variant 4-1BBL polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2I, where “x” is any amino acid other than valine; for example, “x” can be Gly, Ala, Leu, Ile, Pro, Phe, Tyr, Trp, Ser, Thr, Cys, Met, Asn, Gln, Lys, Arg, His, Asp, or Glu. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2I, where “x” is Gly. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2I, where “x” is Ala. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2I, where “x” is Leu. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2I, where “x” is Ile. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2I, where “x” is Pro. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2I, where “x” is Phe. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2I, where “x” is Tyr. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2I, where “x” is Trp. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2I, where “x” is Ser. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2I, where “x” is Thr. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2I, where “x” is Cys. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2I, where "x" is Met. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2I, where "x" is Asn. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2I, where "x" is Gln. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2I, where "x" is Lys. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2I, where "x" is Arg. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2I, where "x" is His. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2I, where "x" is Asp. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2I, where "x" is Glu. In some cases, the variant 4-1BBL polypeptide has a binding affinity to CD137 that is from about 100 nM to about 150 nM, from about 150 nM to about 200 nM, from about 200 nM to about 250 nM, from about 250 nM to about 300 nM, from about 300 nM to about 350 nM, from about 350 nM to about 400 nM, from about 400 nM to about 500 nM, from about 500 nM to about 600 nM, from about 600 nM to about 700 nM, from about 700 nM to about 800 nM, from about 800 nM to about 900 nM, from about 900 nM to about 1 μM, to about 1 μM to about 5 μM, from about 5 μM to about 10 μM, from about 10 μM to about 15 μM, from about 15 μM to about 20 μM, from about 20 μM to about 25 μM, from about 25 μM to about 50 μM, from about 50 μM to about 75 μM, or from about 75 μM to about 100 μM.

Q98 Substitution

[00100] In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence depicted in FIG. 2J, where amino acid 98 (indicated by an "x") is an amino acid other than a glutamine, e.g., where amino acid 98 is Gly, Ala, Val, Leu, Ile, Pro, Phe, Tyr, Trp, Ser, Thr, Cys, Met, Asn, Lys, Arg, His, Asp, or Glu. The amino acid numbering in FIG. 2J retains the numbering of FIG. 2A. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence depicted in FIG. 2J, where amino acid 98 is Gly. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence depicted in FIG. 2J, where amino acid 98 is Ala. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence depicted in FIG. 2J, where amino acid 98 is Val. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence depicted in FIG. 2J, where amino acid 98 is Leu. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence depicted in FIG. 2J, where amino acid 98 is Ile. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence depicted in FIG. 2J, where amino acid 98 is Pro. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence depicted in FIG. 2J, where amino acid 98 is Phe. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence depicted in FIG. 2J, where amino acid 98 is Tyr. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence depicted in FIG. 2J, where amino acid 98 is Trp. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence depicted in FIG. 2J, where amino acid 98 is Ser. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence depicted in FIG. 2J, where amino acid 98 is Thr. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence depicted in FIG. 2J, where amino acid 98 is Cys. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence depicted in FIG. 2J, where amino acid 98 is Met. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence depicted in FIG. 2J, where amino acid 98 is Asn. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence depicted in FIG. 2J, where amino acid 98 is Lys. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence depicted in FIG. 2J, where amino acid 98 is Arg. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence depicted in FIG. 2J, where amino acid 98 is His. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence depicted in FIG. 2J, where amino acid 98 is Asp. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence depicted in FIG. 2J, where amino acid 98 is Glu. In some cases, the variant 4-1BBL polypeptide has a binding affinity to CD137 that is from about 100 nM to about 150 nM, from about 150 nM to about 200 nM, from about 200 nM to about 250 nM, from about 250 nM to about 300 nM, from about 300 nM to about 350 nM, from about 350 nM to about 400 nM, from about 400 nM to about 500 nM, from about 500 nM to about 600 nM, from about 600 nM to about 700 nM, from about 700 nM to about 800 nM, from about 800 nM to about 900 nM, from about 900 nM to about 1 μM, to about 1 μM to about 5 μM, from about 5 μM to about 10 μM, from about 10 μM to about 15 μM, from about 15 μM to about 20 μM, from about 20 μM to about 25 μM, from about 25 μM to about 50 μM, from about 50 μM to about 75 μM, or from about 75 μM to about 100 μM.

[00101] In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2A, with an amino acid substitution at Q98. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence set forth in SEQ ID NO:1, with an amino acid substitution at Q18. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises the amino acid sequence set forth in SEQ ID NO:1, with an amino acid substitution at Q18. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence set forth in SEQ ID NO:2, with an amino acid substitution at Q19. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises the amino acid sequence set forth in SEQ ID NO:2, with an amino acid substitution at Q19. In some cases, a variant 4-

1BBL polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence set forth in SEQ ID NO:3, with an amino acid substitution at Q19. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises the amino acid sequence set forth in SEQ ID NO:3, with an amino acid substitution at Q19.

[00102] For example, in some cases, a variant 4-1BBL polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2J, where “x” is any amino acid other than glutamine; for example, “x” can be Gly, Ala, Val, Leu, Ile, Pro, Phe, Tyr, Trp, Ser, Thr, Cys, Met, Asn, Lys, Arg, His, Asp, or Glu. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2J, where “x” is Gly. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2J, where “x” is Ala. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2J, where “x” is Val. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2J, where “x” is Leu. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2J, where “x” is Ile. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2J, where “x” is Pro. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2J, where “x” is Phe. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2J, where “x” is Tyr. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2J, where “x” is Trp. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2J, where “x” is Ser. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2J, where “x” is Thr. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2J, where “x” is Cys. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2J, where “x” is Met. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2J, where “x” is Asn. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2J, where “x” is Lys. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2J, where “x” is Arg. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2J, where “x” is His. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2J, where "x" is Asp. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2J, where "x" is Glu. In some cases, the variant 4-1BBL polypeptide has a binding affinity to CD137 that is from about 100 nM to about 150 nM, from about 150 nM to about 200 nM, from about 200 nM to about 250 nM, from about 250 nM to about 300 nM, from about 300 nM to about 350 nM, from about 350 nM to about 400 nM, from about 400 nM to about 500 nM, from about 500 nM to about 600 nM, from about 600 nM to about 700 nM, from about 700 nM to about 800 nM, from about 800 nM to about 900 nM, from about 900 nM to about 1 μM, to about 1 μM to about 5 μM, from about 5 μM to about 10 μM, from about 10 μM to about 15 μM, from about 15 μM to about 20 μM, from about 20 μM to about 25 μM, from about 25 μM to about 50 μM, from about 50 μM to about 75 μM, or from about 75 μM to about 100 μM.

N99 Substitution

[00103] In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence depicted in FIG. 2K, where amino acid 99 (indicated by an "x") is an amino acid other than an asparagine, e.g., where amino acid 99 is Gly, Ala, Val, Leu, Ile, Pro, Phe, Tyr, Trp, Ser, Thr, Cys, Met, Gln, Lys, Arg, His, Asp, or Glu. The amino acid numbering in FIG. 2K retains the numbering of FIG. 2A. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence depicted in FIG. 2K, where amino acid 99 is Gly. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence depicted in FIG. 2K, where amino acid 99 is Ala. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence depicted in FIG. 2K, where amino acid 99 is Val. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence depicted in FIG. 2K, where amino acid 99 is Leu. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence depicted in FIG. 2K, where amino acid 99 is Ile. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence depicted in FIG. 2K, where amino acid 99 is Pro. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence depicted in FIG. 2K, where amino acid 99 is Phe. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence depicted in FIG. 2K, where amino acid 99 is Tyr. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence depicted in FIG. 2K, where amino acid 99 is Trp. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence depicted in FIG. 2K, where amino acid 99 is Ser. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence depicted in FIG. 2K, where amino acid 99 is Thr. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence depicted in FIG. 2K, where amino acid 99 is Cys. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence depicted in FIG. 2K, where amino acid 99 is Met. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence depicted in FIG. 2K, where amino acid 99 is Gln. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence depicted in FIG. 2K, where amino acid 99 is Lys. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence depicted in FIG. 2K, where amino acid 99 is Arg. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence depicted in FIG. 2K, where amino acid 99 is His. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence depicted in FIG. 2K, where amino acid 99 is Asp. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence depicted in FIG. 2K, where amino acid 99 is Glu. In some cases, the variant 4-1BBL polypeptide has a binding affinity to CD137 that is from about 100 nM to about 150 nM, from about 150 nM to about 200 nM, from about 200 nM to about 250 nM, from about 250 nM to about 300 nM, from about 300 nM to about 350 nM, from about 350 nM to about 400 nM, from about 400 nM to about 500 nM, from about 500 nM to about 600 nM, from about 600 nM to about 700 nM, from about 700 nM to about 800 nM, from about 800 nM to about 900 nM, from about 900 nM to about 1 μM, to about 1 μM to about 5 μM, from about 5 μM to about 10 μM, from about 10 μM to about 15 μM, from about 15 μM to about 20 μM, from about 20 μM to about 25 μM, from about 25 μM to about 50 μM, from about 50 μM to about 75 μM, or from about 75 μM to about 100 μM.

[00104] In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2A, with an amino acid substitution at N99. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence set forth in SEQ ID NO:1, with an amino acid substitution at N19. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises the amino acid sequence set forth in SEQ ID NO:1, with an amino acid substitution at N19. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence set forth in SEQ ID NO:2, with an amino acid substitution at N20. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises the amino acid sequence set forth in SEQ ID NO:2, with an amino acid substitution at N20. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence set forth in SEQ ID NO:3, with an amino acid substitution at N20. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises the amino acid sequence set forth in SEQ ID NO:3, with an amino acid substitution at N20.

[00105] For example, in some cases, a variant 4-1BBL polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2K, where “x” is any amino acid other than asparagine; for example, “x” can be Gly, Ala, Val, Leu, Ile, Pro, Phe, Tyr, Trp, Ser, Thr, Cys, Met, Gln, Lys, Arg, His, Asp, or Glu. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2K, where "x" is Gly. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2K, where "x" is Ala. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2K, where "x" is Val. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2K, where "x" is Leu. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2K, where "x" is Ile. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2K, where "x" is Pro. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2K, where "x" is Phe. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2K, where "x" is Tyr. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2K, where "x" is Trp. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2K, where "x" is Ser. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2K, where "x" is Thr. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2K, where "x" is Cys. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2K, where "x" is Met. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2K, where "x" is Gln. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2K, where "x" is Lys. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2K, where "x" is Arg. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2K, where "x" is His. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2K, where "x" is Asp. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2K, where "x" is Glu. In some cases, the variant 4-1BBL polypeptide has a binding affinity to CD137 that is from about 100 nM to about 150 nM, from about 150 nM to about 200 nM, from about 200 nM to about 250 nM, from about 250 nM to about 300 nM, from about 300 nM to about 350 nM, from about 350 nM to about 400 nM, from about 400 nM to about 500 nM, from about 500 nM to about 600 nM, from about 600 nM to about 700 nM, from about 700 nM to about 800 nM, from about 800 nM to about 900 nM, from about 900 nM to about 1 μM, to about 1 μM to about 5 μM, from about 5 μM to about 10 μM, from about 10 μM to about 15 μM, from about 15 μM to

about 20 µM, from about 20 µM to about 25 µM, from about 25 µM to about 50 µM, from about 50 µM to about 75 µM, or from about 75 µM to about 100 µM.

V100 Substitution

[00106] In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence depicted in FIG. 2L, where amino acid 100 (indicated by an "x") is an amino acid other than a valine, e.g., where amino acid 100 is Gly, Ala, Leu, Ile, Pro, Phe, Tyr, Trp, Ser, Thr, Cys, Met, Asn, Gln, Lys, Arg, His, Asp, or Glu. The amino acid numbering in FIG. 2L retains the numbering of FIG. 2A. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence depicted in FIG. 2L, where amino acid 100 is Gly. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence depicted in FIG. 2L, where amino acid 100 is Ala. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence depicted in FIG. 2L, where amino acid 100 is Leu. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence depicted in FIG. 2L, where amino acid 100 is Ile. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence depicted in FIG. 2L, where amino acid 100 is Pro. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence depicted in FIG. 2L, where amino acid 100 is Phe. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence depicted in FIG. 2L, where amino acid 100 is Tyr. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence depicted in FIG. 2L, where amino acid 100 is Trp. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence depicted in FIG. 2L, where amino acid 100 is Ser. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence depicted in FIG. 2L, where amino acid 100 is Thr. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence depicted in FIG. 2L, where amino acid 100 is Cys. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence depicted in FIG. 2L, where amino acid 100 is Met. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence depicted in FIG. 2L, where amino acid 100 is Asn. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence depicted in FIG. 2L, where amino acid 100 is Gln. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence depicted in FIG. 2L, where amino acid 100 is Lys. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence depicted in FIG. 2L, where amino acid 100 is Arg. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence depicted in FIG. 2L, where amino acid 100 is His. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence depicted in FIG. 2L, where amino acid 100 is Asp. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence depicted in FIG. 2L, where amino acid 100 is Glu. In some cases, the variant 4-1BBL polypeptide has a binding affinity to CD137 that is from about from about 100 nM to about 150 nM, from about 150 nM to about 200 nM, from about 200 nM to about 250 nM, from about 250 nM to about 300 nM, from about 300 nM to about 350 nM, from about 350 nM to about 400 nM, from about 400 nM to about 500 nM, from about 500 nM to about 600 nM, from about 600 nM to about 700 nM, from about 700 nM to about 800 nM, from about 800 nM to about 900 nM, from about 900 nM to about 1 μM, to about 1 μM to about 5 μM, from about 5 μM to about 10 μM, from about 10 μM to about 15 μM, from about 15 μM to about 20 μM, from about 20 μM to about 25 μM, from about 25 μM to about 50 μM, from about 50 μM to about 75 μM, or from about 75 μM to about 100 μM.

[00107] In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2A, with an amino acid substitution at V100. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence set forth in SEQ ID NO:1, with an amino acid substitution at V20. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises the amino acid sequence set forth in SEQ ID NO:1, with an amino acid substitution at V20. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence set forth in SEQ ID NO:2, with an amino acid substitution at V21. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises the amino acid sequence set forth in SEQ ID NO:2, with an amino acid substitution at V21. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence set forth in SEQ ID NO:3, with an amino acid substitution at V21. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises the amino acid sequence set forth in SEQ ID NO:3, with an amino acid substitution at V21.

[00108] For example, in some cases, a variant 4-1BBL polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2L, where "x" is any amino acid other than valine; for example, "x" can be Gly, Ala, Leu, Ile, Pro, Phe, Tyr, Trp, Ser, Thr, Cys, Met, Asn, Gln, Lys, Arg, His, Asp, or Glu. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2L, where "x" is Gly. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2L, where "x" is Ala. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2L, where "x" is Leu. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2L, where "x" is Ile. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2L, where "x" is Pro. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2L, where "x" is Phe. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2L, where “x” is Tyr. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2L, where “x” is Trp. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2L, where “x” is Ser. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2L, where “x” is Thr. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2L, where “x” is Cys. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2L, where “x” is Met. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2L, where “x” is Asn. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2L, where “x” is Gln. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2L, where “x” is Lys. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2L, where “x” is Arg. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2L, where “x” is His. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2L, where “x” is Asp. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2L, where “x” is Glu. In some cases, the variant 4-1BBL polypeptide has a binding affinity to CD137 that is from about 100 nM to about 150 nM, from about 150 nM to about 200 nM, from about 200 nM to about 250 nM, from about 250 nM to about 300 nM, from about 300 nM to about 350 nM, from about 350 nM to about 400 nM, from about 400 nM to about 500 nM, from about 500 nM to about 600 nM, from about 600 nM to about 700 nM, from about 700 nM to about 800 nM, from about 800 nM to about 900 nM, from about 900 nM to about 1 μM, to about 1 μM to about 5 μM, from about 5 μM to about 10 μM, from about 10 μM to about 15 μM, from about 15 μM to about 20 μM, from about 20 μM to about 25 μM, from about 25 μM to about 50 μM, from about 50 μM to about 75 μM, or from about 75 μM to about 100 μM.

L101 Substitution

[00109] In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence depicted in FIG. 2M, where amino acid 101 (indicated by an “x”) is an amino acid other than a leucine, e.g., where amino acid 101 is Gly, Ala, Val, Ile, Pro, Phe, Tyr, Trp, Ser, Thr, Cys, Met, Asn, Gln, Lys, Arg, His, Asp, or Glu. The amino acid numbering in FIG. 2M retains the numbering of FIG. 2A. In some cases, a variant 4-

1BBL polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence depicted in FIG. 2M, where amino acid 101 is Gly. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence depicted in FIG. 2M, where amino acid 101 is Ala. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence depicted in FIG. 2M, where amino acid 101 is Val. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence depicted in FIG. 2M, where amino acid 101 is Ile. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence depicted in FIG. 2M, where amino acid 101 is Pro. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence depicted in FIG. 2M, where amino acid 101 is Phe. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence depicted in FIG. 2M, where amino acid 101 is Tyr. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence depicted in FIG. 2M, where amino acid 101 is Trp. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence depicted in FIG. 2M, where amino acid 101 is Ser. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence depicted in FIG. 2M, where amino acid 101 is Thr. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence depicted in FIG. 2M, where amino acid 101 is Cys. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence depicted in FIG. 2M, where amino acid 101 is Met. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence depicted in FIG. 2M, where amino acid 101 is Asn. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence depicted in FIG. 2M, where amino acid 101 is Gln. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence depicted in FIG. 2M, where amino acid 101 is Lys. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence depicted in FIG. 2M, where amino acid 101 is Arg. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence depicted in FIG. 2M, where amino acid 101 is His. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence depicted in FIG. 2M, where amino acid 101 is Asp. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence depicted in FIG. 2M, where amino acid 101 is Glu. In some cases, the variant 4-1BBL polypeptide has a binding affinity to CD137 that is from about 100 nM to about 150 nM, from about 150 nM to about 200 nM, from about 200 nM to about 250 nM, from about 250 nM to about 300 nM, from about 300 nM to about 350 nM, from about 350 nM to about 400 nM, from about 400 nM to about 500 nM, from about 500 nM to about 600 nM, from about 600 nM to about 700 nM, from about 700 nM to about 800 nM, from about 800 nM to about 900 nM, from about 900 nM to about 1 μM, to about 1 μM to about 5 μM, from about 5 μM to about 10 μM, from about 10 μM to about 15 μM, from about 15 μM to about 20 μM, from about 20 μM to about 25 μM, from about 25 μM to about 50 μM, from about 50 μM to about 75 μM, or from about 75 μM to about 100 μM.

[00110] In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2A, with an amino acid substitution at L101. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence set forth in SEQ ID NO:1, with an amino acid substitution at L21. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises the amino acid sequence set forth in SEQ ID NO:1, with an amino acid substitution at L21. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence set forth in SEQ ID NO:2, with an amino acid substitution at L22. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises the amino acid sequence set forth in SEQ ID NO:2, with an amino acid substitution at L22. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence set forth in SEQ ID NO:3, with an amino acid substitution at L22. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises the amino acid sequence set forth in SEQ ID NO:3, with an amino acid substitution at L22.

[00111] For example, in some cases, a variant 4-1BBL polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2M, where “x” is any amino acid other than leucine; for example, “x” can be Gly, Ala, Val, Ile, Pro, Phe, Tyr, Trp, Ser, Thr, Cys, Met, Asn, Gln, Lys, Arg, His, Asp, or Glu. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2M, where “x” is Gly. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2M, where “x” is Ala. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2M, where “x” is Val. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2M, where “x” is Ile. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2M, where “x” is Pro. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2M, where “x” is Phe. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2M, where “x” is Tyr. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2M, where “x” is Trp. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2M, where “x” is Ser. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2M, where “x” is Thr. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2M, where “x” is Cys. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2M, where “x” is Met. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2M, where “x” is Asn. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2M, where “x” is Gln. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2M, where “x” is Lys. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2M, where “x” is Arg. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2M, where “x” is His. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2M, where “x” is Asp. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2M, where “x” is Glu. In some cases, the variant 4-1BBL polypeptide has a binding affinity to CD137 that is from about 100 nM to about 150 nM, from about 150 nM to about 200 nM, from about 200 nM to about 250 nM, from about 250 nM to about 300 nM, from about 300 nM to about 350 nM, from about 350 nM to about 400 nM, from about 400 nM to about 500 nM, from about 500 nM to about 600 nM, from about 600 nM to about 700 nM, from about 700 nM to about 800 nM, from about 800 nM to about 900 nM, from about 900 nM to about 1 μM, to about 1 μM to about 5 μM, from about 5 μM to about 10 μM, from about 10 μM to about 15 μM, from about 15 μM to about 20 μM, from about 20 μM to about 25 μM, from about 25 μM to about 50 μM, from about 50 μM to about 75 μM, or from about 75 μM to about 100 μM.

L102 Substitution

[00112] In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence depicted in FIG. 2N, where amino acid 102 (indicated by an “x”) is an amino acid other than a leucine, e.g., where amino acid 102 is Gly, Ala, Val, Ile, Pro, Phe, Tyr, Trp, Ser, Thr, Cys, Met, Asn, Gln, Lys, Arg, His, Asp, or Glu. The amino acid numbering in FIG. 2N retains the numbering of FIG. 2A. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence depicted in FIG. 2N, where amino acid 102 is Gly. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence depicted in FIG. 2N, where amino acid 102 is Ala. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence depicted in FIG. 2N, where amino acid 102 is Val. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence depicted in FIG. 2N, where amino acid 102 is Ile. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence depicted in FIG. 2N, where amino acid 102 is Pro. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence depicted in FIG. 2N, where amino acid 102 is Phe. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence depicted in FIG. 2N, where amino acid 102 is Tyr. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence depicted in FIG. 2N, where amino acid 102 is Trp. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence depicted in FIG. 2N, where amino acid 102 is Ser. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence depicted in FIG. 2N, where amino acid 102 is Thr. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence depicted in FIG. 2N, where amino acid 102 is Cys. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence depicted in FIG. 2N, where amino acid 102 is Met. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence depicted in FIG. 2N, where amino acid 102 is Asn. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence depicted in FIG. 2N, where amino acid 102 is Gln. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence depicted in FIG. 2N, where amino acid 102 is Lys. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence depicted in FIG. 2N, where amino acid 102 is Arg. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence depicted in FIG. 2N, where amino acid 102 is His. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence depicted in FIG. 2N, where amino acid 102 is Asp. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence depicted in FIG. 2N, where amino acid 102 is Glu. In some cases, the variant 4-1BBL polypeptide has a binding affinity to CD137 that is from about from about 100 nM to about 150 nM, from about 150 nM to about 200 nM, from about 200 nM to about 250 nM, from about 250 nM to about 300 nM, from about 300 nM to about 350 nM, from about 350 nM to about 400 nM, from about 400 nM to about 500 nM, from about 500 nM to about 600 nM, from about 600 nM to about 700 nM, from about 700 nM to about 800 nM, from about 800 nM to about 900 nM, from about 900 nM to about 1 μM, to about 1 μM to about 5 μM, from about 5 μM to about 10 μM, from about 10 μM to about 15 μM, from about 15 μM to about 20 μM, from about 20 μM to about 25 μM, from about 25 μM to about 50 μM, from about 50 μM to about 75 μM, or from about 75 μM to about 100 μM.

[00113] In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2A, with an amino acid substitution at L102. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence set forth in SEQ ID NO:1, with an amino acid substitution at L22. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises the amino acid sequence set forth in SEQ ID NO:1, with an amino acid substitution at L22. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence set forth in SEQ ID NO:2, with an amino acid substitution at L23. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises the amino acid sequence set forth in SEQ ID NO:2, with an amino acid substitution at L23. In some cases, a variant 4-

1BBL polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence set forth in SEQ ID NO:3, with an amino acid substitution at L23. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises the amino acid sequence set forth in SEQ ID NO:3, with an amino acid substitution at L23.

[00114] For example, in some cases, a variant 4-1BBL polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2N, where “x” is any amino acid other than leucine; for example, “x” can be Gly, Ala, Val, Ile, Pro, Phe, Tyr, Trp, Ser, Thr, Cys, Met, Asn, Gln, Lys, Arg, His, Asp, or Glu. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2N, where “x” is Gly. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2N, where “x” is Ala. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2N, where “x” is Val. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2N, where “x” is Ile. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2N, where “x” is Pro. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2N, where “x” is Phe. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2N, where “x” is Tyr. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2N, where “x” is Trp. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2N, where “x” is Ser. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2N, where “x” is Thr. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2N, where “x” is Cys. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2N, where “x” is Met. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2N, where “x” is Asn. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2N, where “x” is Gln. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2N, where “x” is Lys. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2N, where “x” is Arg. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2N, where “x” is His. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2N, where “x” is Asp. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2N, where “x” is Glu. In some cases, the variant 4-1BBL polypeptide has a binding affinity to CD137 that is from about 100 nM to about 150 nM, from about 150 nM to about 200 nM, from about 200 nM to about 250 nM, from about 250 nM to about 300 nM, from about 300 nM to about 350 nM, from about 350 nM to about 400 nM, from about 400 nM to about 500 nM, from about 500 nM to about 600 nM, from about 600 nM to about 700 nM, from about 700 nM to about 800 nM, from about 800 nM to about 900 nM, from about 900 nM to about 1 μM, to about 1 μM to about 5 μM, from about 5 μM to about 10 μM, from about 10 μM to about 15 μM, from about 15 μM to about 20 μM, from about 20 μM to about 25 μM, from about 25 μM to about 50 μM, from about 50 μM to about 75 μM, or from about 75 μM to about 100 μM.

I103 Substitution

[00115] In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence depicted in FIG. 2O, where amino acid 103 (indicated by an “x”) is an amino acid other than an isoleucine, e.g., where amino acid 103 is Gly, Ala, Val, Leu, Pro, Phe, Tyr, Trp, Ser, Thr, Cys, Met, Asn, Gln, Lys, Arg, His, Asp, or Glu. The amino acid numbering in FIG. 2O retains the numbering of FIG. 2A. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence depicted in FIG. 2O, where amino acid 103 is Gly. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence depicted in FIG. 2O, where amino acid 103 is Ala. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence depicted in FIG. 2O, where amino acid 103 is Val. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence depicted in FIG. 2O, where amino acid 103 is Leu. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence depicted in FIG. 2O, where amino acid 103 is Pro. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence depicted in FIG. 2O, where amino acid 103 is Phe. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence depicted in FIG. 2O, where amino acid 103 is Tyr. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence depicted in FIG. 2O, where amino acid 103 is Trp. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence depicted in FIG. 2O, where amino acid 103 is Ser. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence depicted in FIG. 2O, where amino acid 103 is Thr. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence depicted in FIG. 2O, where amino acid 103 is Cys. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence depicted in FIG. 2O, where amino acid 103 is Met. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence depicted in FIG. 2O, where amino acid 103 is Asn. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence depicted in FIG. 2O, where amino acid 103 is Gln. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence depicted in FIG. 2O, where amino acid 103 is Lys. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence depicted in FIG. 2O, where amino acid 103 is Arg. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence depicted in FIG. 2O, where amino acid 103 is His. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence depicted in FIG. 2O, where amino acid 103 is Asp. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence depicted in FIG. 2O, where amino acid 103 is Glu. In some cases, the variant 4-1BBL polypeptide has a binding affinity to CD137 that is from about 100 nM to about 150 nM, from about 150 nM to about 200 nM, from about 200 nM to about 250 nM, from about 250 nM to about 300 nM, from about 300 nM to about 350 nM, from about 350 nM to about 400 nM, from about 400 nM to about 500 nM, from about 500 nM to about 600 nM, from about 600 nM to about 700 nM, from about 700 nM to about 800 nM, from about 800 nM to about 900 nM, from about 900 nM to about 1 μM, to about 1 μM to about 5 μM, from about 5 μM to about 10 μM, from about 10 μM to about 15 μM, from about 15 μM to about 20 μM, from about 20 μM to about 25 μM, from about 25 μM to about 50 μM, from about 50 μM to about 75 μM, or from about 75 μM to about 100 μM.

[00116] In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2A, with an amino acid substitution at I103. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence set forth in SEQ ID NO:1, with an amino acid substitution at I23. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises the amino acid sequence set forth in SEQ ID NO:1, with an amino acid substitution at I23. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence set forth in SEQ ID NO:2, with an amino acid substitution at I24. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises the amino acid sequence set forth in SEQ ID NO:2, with an amino acid substitution at I24. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence set forth in SEQ ID NO:3, with an amino acid substitution at I24. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises the amino acid sequence set forth in SEQ ID NO:3, with an amino acid substitution at I24.

[00117] For example, in some cases, a variant 4-1BBL polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2O, where “x” is any amino acid other than isoleucine; for example, “x” can be Gly, Ala, Val, Leu, Pro, Phe, Tyr, Trp, Ser, Thr, Cys, Met, Asn, Gln, Lys, Arg, His, Asp, or Glu. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2O, where “x” is Gly. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2O, where “x” is Ala. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2O, where “x” is Val. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2O, where “x” is Leu. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2O, where “x” is Pro. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2O, where “x” is Phe. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2O, where “x” is Tyr. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2O, where “x” is Trp. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2O, where “x” is Ser. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2O, where “x” is Thr. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2O, where “x” is Cys. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2O, where “x” is Met. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2O, where “x” is Asn. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2O, where “x” is Gln. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2O, where “x” is Lys. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2O, where “x” is Arg. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2O, where “x” is His. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2O, where “x” is Asp. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2O, where “x” is Glu. In some cases, the variant 4-1BBL polypeptide has a binding affinity to CD137 that is from about 100 nM to about 150 nM, from about 150 nM to about 200 nM, from about 200 nM to about 250 nM, from about 250 nM to about 300 nM, from about 300 nM to about 350 nM, from about 350 nM to about 400 nM, from about 400 nM to about 500 nM, from about 500 nM to about 600 nM, from about 600 nM to about 700 nM, from about 700 nM to about 800 nM, from about 800 nM to about 900 nM, from about 900 nM to about 1 μM, to about 1 μM to about 5 μM, from about 5 μM to about 10 μM, from about 10 μM to about 15 μM, from about 15 μM to

about 20 μM, from about 20 μM to about 25 μM, from about 25 μM to about 50 μM, from about 50 μM to about 75 μM, or from about 75 μM to about 100 μM.

D104 Substitution

[00118] In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence depicted in FIG. 2P, where amino acid 104 (indicated by an "x") is an amino acid other than an aspartic acid, e.g., where amino acid 104 is Gly, Ala, Val, Leu, Ile, Pro, Phe, Tyr, Trp, Ser, Thr, Cys, Met, Asn, Gln, Lys, Arg, His, or Glu. The amino acid numbering in FIG. 2P retains the numbering of FIG. 2A. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence depicted in FIG. 2P, where amino acid 104 is Gly. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence depicted in FIG. 2P, where amino acid 104 is Ala. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence depicted in FIG. 2P, where amino acid 104 is Val. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence depicted in FIG. 2P, where amino acid 104 is Leu. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence depicted in FIG. 2P, where amino acid 104 is Ile. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence depicted in FIG. 2P, where amino acid 104 is Pro. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence depicted in FIG. 2P, where amino acid 104 is Phe. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence depicted in FIG. 2P, where amino acid 104 is Tyr. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence depicted in FIG. 2P, where amino acid 104 is Trp. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence depicted in FIG. 2P, where amino acid 104 is Ser. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence depicted in FIG. 2P, where amino acid 104 is Thr. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence depicted in FIG. 2P, where amino acid 104 is Cys. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence depicted in FIG. 2P, where amino acid 104 is Met. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence depicted in FIG. 2P, where amino acid 104 is Asn. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence depicted in FIG. 2P, where amino acid 104 is Gln. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence depicted in FIG. 2P, where amino acid 104 is Lys. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence depicted in FIG. 2P, where amino acid 104 is Arg. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence depicted in FIG. 2P, where amino acid 104 is His. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence depicted in FIG. 2P, where amino acid 104 is Glu. In some cases, the variant 4-1BBL polypeptide has a binding affinity to CD137 that is from about 100 nM to about 150 nM, from about 150 nM to about 200 nM, from about 200 nM to about 250 nM, from about 250 nM to about 300 nM, from about 300 nM to about 350 nM, from about 350 nM to about 400 nM, from about 400 nM to about 500 nM, from about 500 nM to about 600 nM, from about 600 nM to

about 700 nM, from about 700 nM to about 800 nM, from about 800 nM to about 900 nM, from about 900 nM to about 1 μM, to about 1 μM to about 5 μM, from about 5 μM to about 10 μM, from about 10 μM to about 15 μM, from about 15 μM to about 20 μM, from about 20 μM to about 25 μM, from about 25 μM to about 50 μM, from about 50 μM to about 75 μM, or from about 75 μM to about 100 μM.

[00119] In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2A, with an amino acid substitution at D104. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence set forth in SEQ ID NO:1, with an amino acid substitution at D24. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises the amino acid sequence set forth in SEQ ID NO:1, with an amino acid substitution at D24. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence set forth in SEQ ID NO:2, with an amino acid substitution at D25. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises the amino acid sequence set forth in SEQ ID NO:2, with an amino acid substitution at D25. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence set forth in SEQ ID NO:3, with an amino acid substitution at D25. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises the amino acid sequence set forth in SEQ ID NO:3, with an amino acid substitution at D25.

[00120] For example, in some cases, a variant 4-1BBL polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2P, where “x” is any amino acid other than aspartic acid; for example, “x” can be Gly, Ala, Val, Leu, Ile, Pro, Phe, Tyr, Trp, Ser, Thr, Cys, Met, Asn, Gln, Lys, Arg, His, or Glu. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2P, where “x” is Gly. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2P, where “x” is Ala. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2P, where “x” is Val. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2P, where “x” is Leu. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2P, where “x” is Ile. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2P, where “x” is Pro. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2P, where “x” is Phe. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2P, where “x” is Tyr. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2P, where “x” is Trp. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2P, where “x” is Ser. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2P, where “x” is Thr. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2P, where “x” is Cys. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2P, where “x” is Met. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2P, where “x” is Asn. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2P, where “x” is Gln. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2P, where “x” is Lys. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2P, where “x” is Arg. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2P where “x” is His. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2P, where “x” is Glu. In some cases, the variant 4-1BBL polypeptide has a binding affinity to CD137 that is from about 100 nM to about 150 nM, from about 150 nM to about 200 nM, from about 200 nM to about 250 nM, from about 250 nM to about 300 nM, from about 300 nM to about 350 nM, from about 350 nM to about 400 nM, from about 400 nM to about 500 nM, from about 500 nM to about 600 nM, from about 600 nM to about 700 nM, from about 700 nM to about 800 nM, from about 800 nM to about 900 nM, from about 900 nM to about 1 μM, to about 1 μM to about 5 μM, from about 5 μM to about 10 μM, from about 10 μM to about 15 μM, from about 15 μM to about 20 μM, from about 20 μM to about 25 μM, from about 25 μM to about 50 μM, from about 50 μM to about 75 μM, or from about 75 μM to about 100 μM.

G105 Substitution

[00121] In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence depicted in FIG. 2Q, where amino acid 105 (indicated by an “x”) is an amino acid other than a glycine, e.g., where amino acid 105 is Ala, Val, Leu, Ile, Pro, Phe, Tyr, Trp, Ser, Thr, Cys, Met, Asn, Gln, Lys, Arg, His, Asp, or Glu. The amino acid numbering in FIG. 2Q retains the numbering of FIG. 2A. In some cases, a variant 4-

1BBL polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence depicted in FIG. 2Q, where amino acid 105 is Ala. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence depicted in FIG. 2Q, where amino acid 105 is Val. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence depicted in FIG. 2Q, where amino acid 105 is Leu. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence depicted in FIG. 2Q, where amino acid 105 is Ile. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence depicted in FIG. 2Q, where amino acid 105 is Pro. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence depicted in FIG. 2Q, where amino acid 105 is Phe. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence depicted in FIG. 2Q, where amino acid 105 is Tyr. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence depicted in FIG. 2Q, where amino acid 105 is Trp. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence depicted in FIG. 2Q, where amino acid 105 is Ser. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence depicted in FIG. 2Q, where amino acid 105 is Thr. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence depicted in FIG. 2Q, where amino acid 105 is Cys. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence depicted in FIG. 2Q, where amino acid 105 is Met. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence depicted in FIG. 2Q, where amino acid 105 is Asn. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence depicted in FIG. 2Q, where amino acid 105 is Gln. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence depicted in FIG. 2Q, where amino acid 105 is Lys. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence depicted in FIG. 2Q, where amino acid 105 is Arg. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence depicted in FIG. 2Q, where amino acid 105 is His. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence depicted in FIG. 2Q, where amino acid 105 is Asp. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence depicted in FIG. 2Q, where amino acid 105 is Glu. In some cases, the variant 4-1BBL polypeptide has a binding affinity to CD137 that is from about 100 nM to about 150 nM, from about 150 nM to about 200 nM, from about 200 nM to about 250 nM, from about 250 nM to about 300 nM, from about 300 nM to about 350 nM, from about 350 nM to about 400 nM, from about 400 nM to about 500 nM, from about 500 nM to about 600 nM, from about 600 nM to about 700 nM, from about 700 nM to about 800 nM, from about 800 nM to about 900 nM, from about 900 nM to about 1 μM, to about 1 μM to about 5 μM, from about 5 μM to about 10 μM, from about 10 μM to about 15 μM, from about 15 μM to about 20 μM, from about 20 μM to about 25 μM, from about 25 μM to about 50 μM, from about 50 μM to about 75 μM, or from about 75 μM to about 100 μM.

[00122] In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2A, with an amino acid substitution at G105. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence set forth in SEQ ID NO:1, with an amino acid substitution at G25. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises the amino acid sequence set forth in SEQ ID NO:1, with an amino acid substitution at G25. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence set forth in SEQ ID NO:2, with an amino acid substitution at G26. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises the amino acid sequence set forth in SEQ ID NO:2, with an amino acid substitution at G26. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence set forth in SEQ ID NO:3, with an amino acid substitution at G26. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises the amino acid sequence set forth in SEQ ID NO:3, with an amino acid substitution at G26.

[00123] For example, in some cases, a variant 4-1BBL polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2Q, where “x” is any amino acid other than glycine; for example, “x” can be Ala, Val, Leu, Ile, Pro, Phe, Tyr, Trp, Ser, Thr, Cys, Met, Asn, Gln, Lys, Arg, His, Asp, or Glu. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2Q, where “x” is Ala. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2Q, where “x” is Val. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2Q, where “x” is Leu. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2Q, where “x” is Ile. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2Q, where “x” is Pro. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2Q, where “x” is Phe. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2Q, where “x” is Tyr. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2Q, where “x” is Trp. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2Q, where “x” is Ser. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2Q, where “x” is Thr. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2Q, where “x” is Cys. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2Q, where “x” is Met. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2Q, where “x” is Asn. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2Q, where “x” is Gln. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2Q, where “x” is Lys. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2Q, where “x” is Arg. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2Q, where “x” is His. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2Q, where “x” is Asp. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2Q, where “x” is Glu. In some cases, the variant 4-1BBL polypeptide has a binding affinity to CD137 that is from about 100 nM to about 150 nM, from about 150 nM to about 200 nM, from about 200 nM to about 250 nM, from about 250 nM to about 300 nM, from about 300 nM to about 350 nM, from about 350 nM to about 400 nM, from about 400 nM to about 500 nM, from about 500 nM to about 600 nM, from about 600 nM to about 700 nM, from about 700 nM to about 800 nM, from about 800 nM to about 900 nM, from about 900 nM to about 1 μM, to about 1 μM to about 5 μM, from about 5 μM to about 10 μM, from about 10 μM to about 15 μM, from about 15 μM to about 20 μM, from about 20 μM to about 25 μM, from about 25 μM to about 50 μM, from about 50 μM to about 75 μM, or from about 75 μM to about 100 μM.

P106 Substitution

[00124] In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence depicted in FIG. 2R, where amino acid 106 (indicated by an “x”) is an amino acid other than a proline, e.g., where amino acid 106 is Gly, Ala, Val, Leu, Ile, Phe, Tyr, Trp, Ser, Thr, Cys, Met, Asn, Gln, Lys, Arg, His, Asp, or Glu. The amino acid numbering in FIG. 2R retains the numbering of FIG. 2A. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence depicted in FIG. 2R, where amino acid 106 is Gly. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence depicted in FIG. 2R, where amino acid 106 is Ala. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence depicted in FIG. 2R, where amino acid 106 is Val. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence depicted in FIG. 2R, where amino acid 106 is Leu. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence depicted in FIG. 2R, where amino acid 106 is Ile. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence depicted in FIG. 2R, where amino acid 106 is Phe. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence depicted in FIG. 2R, where amino acid 106 is Tyr. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence depicted in FIG. 2R, where amino acid 106 is Trp. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence depicted in FIG. 2R, where amino acid 106 is Ser. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence depicted in FIG. 2R, where amino acid 106 is Thr. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence depicted in FIG. 2R, where amino acid 106 is Cys. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence depicted in FIG. 2R, where amino acid 106 is Met. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence depicted in FIG. 2R, where amino acid 106 is Asn. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence depicted in FIG. 2R, where amino acid 106 is Gln. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence depicted in FIG. 2R, where amino acid 106 is Lys. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence depicted in FIG. 2R, where amino acid 106 is Arg. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence depicted in FIG. 2R, where amino acid 106 is His. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence depicted in FIG. 2R, where amino acid 106 is Asp. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence depicted in FIG. 2R, where amino acid 1106 is Glu. In some cases, the variant 4-1BBL polypeptide has a binding affinity to CD137 that is from about 100 nM to about 150 nM, from about 150 nM to about 200 nM, from about 200 nM to about 250 nM, from about 250 nM to about 300 nM, from about 300 nM to about 350 nM, from about 350 nM to about 400 nM, from about 400 nM to about 500 nM, from about 500 nM to about 600 nM, from about 600 nM to about 700 nM, from about 700 nM to about 800 nM, from about 800 nM to about 900 nM, from about 900 nM to about 1 μM, to about 1 μM to about 5 μM, from about 5 μM to about 10 μM, from about 10 μM to about 15 μM, from about 15 μM to about 20 μM, from about 20 μM to about 25 μM, from about 25 μM to about 50 μM, from about 50 μM to about 75 μM, or from about 75 μM to about 100 μM.

[00125] In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2A, with an amino acid substitution at P106. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence set forth in SEQ ID NO:1, with an amino acid substitution at P26. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises the amino acid sequence set forth in SEQ ID NO:1, with an amino acid substitution at P26. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence set forth in SEQ ID NO:2, with an amino acid substitution at P27. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises the amino acid sequence set forth in SEQ ID NO:2, with an amino acid substitution at P27. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises an amino acid sequence having at least

90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence set forth in SEQ ID NO:3, with an amino acid substitution at P27. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises the amino acid sequence set forth in SEQ ID NO:3, with an amino acid substitution at P27.

[00126] For example, in some cases, a variant 4-1BBL polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2R, where "x" is any amino acid other than proline; for example, "x" can be Gly, Ala, Val, Leu, Ile, Phe, Tyr, Trp, Ser, Thr, Cys, Met, Asn, Gln, Lys, Arg, His, Asp, or Glu. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2R, where "x" is Gly. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2R, where "x" is Ala. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2R, where "x" is Val. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2R, where "x" is Leu. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2R, where "x" is Ile. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2R, where "x" is Phe. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2R, where "x" is Tyr. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2R, where "x" is Trp. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2R, where "x" is Ser. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2R, where "x" is Thr. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2R, where "x" is Cys. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2R, where "x" is Met. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2R, where "x" is Asn. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2R, where "x" is Gln. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2R, where "x" is Lys. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2R, where "x" is Arg. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2R, where "x" is His. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2R, where "x" is Asp. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2R, where “x” is Glu. In some cases, the variant 4-1BBL polypeptide has a binding affinity to CD137 that is from about 100 nM to about 150 nM, from about 150 nM to about 200 nM, from about 200 nM to about 250 nM, from about 250 nM to about 300 nM, from about 300 nM to about 350 nM, from about 350 nM to about 400 nM, from about 400 nM to about 500 nM, from about 500 nM to about 600 nM, from about 600 nM to about 700 nM, from about 700 nM to about 800 nM, from about 800 nM to about 900 nM, from about 900 nM to about 1 μM, to about 1 μM to about 5 μM, from about 5 μM to about 10 μM, from about 10 μM to about 15 μM, from about 15 μM to about 20 μM, from about 20 μM to about 25 μM, from about 25 μM to about 50 μM, from about 50 μM to about 75 μM, or from about 75 μM to about 100 μM.

L107 Substitution

[00127] In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence depicted in FIG. 2S, where amino acid 107 (indicated by an “x”) is an amino acid other than a leucine, e.g., where amino acid 107 is Gly, Ala, Val, Ile, Pro, Phe, Tyr, Trp, Ser, Thr, Cys, Met, Asn, Gln, Lys, Arg, His, Asp, or Glu. The amino acid numbering in FIG. 2S retains the numbering of FIG. 2A. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence depicted in FIG. 2S, where amino acid 107 is Gly. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence depicted in FIG. 2S, where amino acid 107 is Ala. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence depicted in FIG. 2S, where amino acid 107 is Val. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence depicted in FIG. 2S, where amino acid 107 is Ile. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence depicted in FIG. 2S, where amino acid 107 is Pro. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence depicted in FIG. 2S, where amino acid 107 is Phe. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence depicted in FIG. 2S, where amino acid 107 is Tyr. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence depicted in FIG. 2S, where amino acid 107 is Trp. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence depicted in FIG. 2S, where amino acid 107 is Ser. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence depicted in FIG. 2S, where amino acid 107 is Thr. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence depicted in FIG. 2S, where amino acid 107 is Cys. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence depicted in FIG. 2S, where amino acid 107 is Met. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence depicted in FIG. 2S, where amino acid 107 is Asn. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence depicted in FIG. 2S, where amino acid 107 is Gln. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence depicted in FIG. 2S, where amino acid 107 is Lys. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence depicted in FIG. 2S, where amino acid 107 is Arg. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence depicted in FIG. 2S, where amino acid 107 is His. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence depicted in FIG. 2S, where amino acid 107 is Asp. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence depicted in FIG. 2S, where amino acid 107 is Glu. In some cases, the variant 4-1BBL polypeptide has a binding affinity to CD137 that is from about 100 nM to about 150 nM, from about 150 nM to about 200 nM, from about 200 nM to about 250 nM, from about 250 nM to about 300 nM, from about 300 nM to about 350 nM, from about 350 nM to about 400 nM, from about 400 nM to about 500 nM, from about 500 nM to about 600 nM, from about 600 nM to about 700 nM, from about 700 nM to about 800 nM, from about 800 nM to about 900 nM, from about 900 nM to about 1 μM, to about 1 μM to about 5 μM, from about 5 μM to about 10 μM, from about 10 μM to about 15 μM, from about 15 μM to about 20 μM, from about 20 μM to about 25 μM, from about 25 μM to about 50 μM, from about 50 μM to about 75 μM, or from about 75 μM to about 100 μM.

[00128] In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2A, with an amino acid substitution at L107. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence set forth in SEQ ID NO:1, with an amino acid substitution at L27. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises the amino acid sequence set forth in SEQ ID NO:1, with an amino acid substitution at L27. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence set forth in SEQ ID NO:2, with an amino acid substitution at L28. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises the amino acid sequence set forth in SEQ ID NO:2, with an amino acid substitution at L28. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence set forth in SEQ ID NO:3, with an amino acid substitution at L28. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises the amino acid sequence set forth in SEQ ID NO:3, with an amino acid substitution at L28.

[00129] For example, in some cases, a variant 4-1BBL polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2S, where “x” is any amino acid other than leucine; for example, “x” can be Gly, Ala, Val, Ile, Pro, Phe, Tyr, Trp, Ser, Thr, Cys, Met, Asn, Gln, Lys, Arg, His, Asp, or Glu. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2S, where “x” is Gly. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2S, where “x” is Ala. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2S, where “x” is Val. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2S, where “x” is Ile. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2S, where “x” is Pro. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2S, where “x” is Phe. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2S, where “x” is Tyr. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2S, where “x” is Trp. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2S, where “x” is Ser. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2S, where “x” is Thr. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2S, where “x” is Cys. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2S, where “x” is Met. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2S, where “x” is Asn. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2S, where “x” is Gln. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2S, where “x” is Lys. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2S, where “x” is Arg. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2S, where “x” is His. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2S, where “x” is Asp. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2S, where “x” is Glu. In some cases, the variant 4-1BBL polypeptide has a binding affinity to CD137 that is from about 100 nM to about 150 nM, from about 150 nM to about 200 nM, from about 200 nM to about 250 nM, from about 250 nM to about 300 nM, from about 300 nM to about 350 nM, from about 350 nM to about 400 nM, from about 400 nM to about 500 nM, from about 500 nM to about 600 nM, from about 600 nM to about 700 nM, from about 700 nM to about 800 nM, from about 800 nM to about 900 nM, from about 900 nM to about 1 μM, to about 1 μM to about 5 μM, from about 5 μM to about 10 μM, from about 10 μM to about 15 μM, from about 15 μM to

about 20 μM, from about 20 μM to about 25 μM, from about 25 μM to about 50 μM, from about 50 μM to about 75 μM, or from about 75 μM to about 100 μM.

S108 Substitution

[00130] In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence depicted in FIG. 2T, where amino acid 108 (indicated by an "x") is an amino acid other than a serine, e.g., where amino acid 108 is Gly, Ala, Val, Leu, Ile, Pro, Phe, Tyr, Trp, Thr, Cys, Met, Asn, Gln, Lys, Arg, His, Asp, or Glu. The amino acid numbering in FIG. 2T retains the numbering of FIG. 2A. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence depicted in FIG. 2T, where amino acid 108 is Gly. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence depicted in FIG. 2T, where amino acid 108 is Ala. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence depicted in FIG. 2T, where amino acid 108 is Val. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence depicted in FIG. 2T, where amino acid 108 is Leu. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence depicted in FIG. 2T, where amino acid 108 is Ile. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence depicted in FIG. 2T, where amino acid 108 is Pro. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence depicted in FIG. 2T, where amino acid 108 is Phe. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence depicted in FIG. 2T, where amino acid 108 is Tyr. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence depicted in FIG. 2T, where amino acid 108 is Trp. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence depicted in FIG. 2T, where amino acid 108 is Thr. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence depicted in FIG. 2T, where amino acid 108 is Cys. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence depicted in FIG. 2T, where amino acid 108 is Met. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence depicted in FIG. 2T, where amino acid 108 is Asn. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence depicted in FIG. 2T, where amino acid 108 is Gln. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence depicted in FIG. 2T, where amino acid 108 is Lys. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence depicted in FIG. 2T, where amino acid 108 is Arg. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence depicted in FIG. 2T, where amino acid 108 is His. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence depicted in FIG. 2T, where amino acid 108 is Asp. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence depicted in FIG. 2T, where amino acid 108 is Glu. In some cases, the variant 4-1BBL polypeptide has a binding affinity to CD137 that is from about 100 nM to about 150 nM, from about 150 nM to about 200 nM, from about 200 nM to about 250 nM, from about 250 nM to about 300 nM, from about 300 nM to about 350 nM, from about 350 nM to about 400 nM, from about 400 nM to about 500 nM, from about 500 nM to about 600 nM, from about 600 nM to

about 700 nM, from about 700 nM to about 800 nM, from about 800 nM to about 900 nM, from about 900 nM to about 1 μM, to about 1 μM to about 5 μM, from about 5 μM to about 10 μM, from about 10 μM to about 15 μM, from about 15 μM to about 20 μM, from about 20 μM to about 25 μM, from about 25 μM to about 50 μM, from about 50 μM to about 75 μM, or from about 75 μM to about 100 μM.

[00131] In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2A, with an amino acid substitution at S108. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence set forth in SEQ ID NO:1, with an amino acid substitution at S28. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises the amino acid sequence set forth in SEQ ID NO:1, with an amino acid substitution at S28. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence set forth in SEQ ID NO:2, with an amino acid substitution at S29. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises the amino acid sequence set forth in SEQ ID NO:2, with an amino acid substitution at S29. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence set forth in SEQ ID NO:3, with an amino acid substitution at S29. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises the amino acid sequence set forth in SEQ ID NO:3, with an amino acid substitution at S29.

[00132] For example, in some cases, a variant 4-1BBL polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2T, where “x” is any amino acid other than serine; for example, “x” can be Gly, Ala, Val, Leu, Ile, Pro, Phe, Tyr, Trp, Thr, Cys, Met, Asn, Gln, Lys, Arg, His, Asp, or Glu. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2T, where “x” is Gly. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2T, where “x” is Ala. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2T, where “x” is Val. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2T, where “x” is Leu. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2T, where “x” is Ile. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2T, where “x” is Pro. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2T, where “x” is Phe. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2T, where “x” is Tyr. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2T, where “x” is Trp. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2T, where “x” is Thr. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2T, where “x” is Cys. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2T, where “x” is Met. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2T, where “x” is Asn. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2T, where “x” is Gln. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2T, where “x” is Lys. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2T, where “x” is Arg. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2T, where “x” is His. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2T, where “x” is Asp. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2T, where “x” is Glu. In some cases, the variant 4-1BBL polypeptide has a binding affinity to CD137 that is from about 100 nM to about 150 nM, from about 150 nM to about 200 nM, from about 200 nM to about 250 nM, from about 250 nM to about 300 nM, from about 300 nM to about 350 nM, from about 350 nM to about 400 nM, from about 400 nM to about 500 nM, from about 500 nM to about 600 nM, from about 600 nM to about 700 nM, from about 700 nM to about 800 nM, from about 800 nM to about 900 nM, from about 900 nM to about 1 μM, to about 1 μM to about 5 μM, from about 5 μM to about 10 μM, from about 10 μM to about 15 μM, from about 15 μM to about 20 μM, from about 20 μM to about 25 μM, from about 25 μM to about 50 μM, from about 50 μM to about 75 μM, or from about 75 μM to about 100 μM.

W109 Substitution

[00133] In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence depicted in FIG. 2U, where amino acid 109 (indicated by an “x”) is an amino acid other than a tryptophan, e.g., where amino acid 109 is Gly, Ala, Val, Leu, Ile, Pro, Phe, Tyr, Ser, Thr, Cys, Met, Asn, Gln, Lys, Arg, His, Asp, or Glu. The amino acid numbering in FIG. 2U retains the numbering of FIG. 2A. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence depicted in FIG. 2U, where amino acid 109 is Gly. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence depicted in FIG. 2U, where amino acid 109 is Ala. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence depicted in FIG. 2U, where amino acid 109 is Val. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence depicted in FIG. 2U, where amino acid 109 is Leu. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence depicted in FIG. 2U, where amino acid 109 is Ile. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence depicted in FIG. 2U, where amino acid 109 is Pro. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence depicted in FIG. 2U, where amino acid 109 is Phe. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence depicted in FIG. 2U, where amino acid 109 is Tyr. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence depicted in FIG. 2U, where amino acid 109 is Ser. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence depicted in FIG. 2U, where amino acid 109 is Thr. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence depicted in FIG. 2U, where amino acid 109 is Cys. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence depicted in FIG. 2U, where amino acid 109 is Met. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence depicted in FIG. 2U, where amino acid 109 is Asn. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence depicted in FIG. 2U, where amino acid 109 is Gln. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence depicted in FIG. 2U, where amino acid 109 is Lys. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence depicted in FIG. 2U, where amino acid 109 is Arg. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence depicted in FIG. 2U, where amino acid 109 is His. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence depicted in FIG. 2U, where amino acid 109 is Asp. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence depicted in FIG. 2U, where amino acid 109 is Glu. In some cases, the variant 4-1BBL polypeptide has a binding affinity to CD137 that is from about 100 nM to about 150 nM, from about 150 nM to about 200 nM, from about 200 nM to about 250 nM, from about 250 nM to about 300 nM, from about 300 nM to about 350 nM, from about 350 nM to about 400 nM, from about 400 nM to about 500 nM, from about 500 nM to about 600 nM, from about 600 nM to about 700 nM, from about 700 nM to about 800 nM, from about 800 nM to about 900 nM, from about 900 nM to about 1 μM, to about 1 μM to about 5 μM, from about 5 μM to about 10 μM, from about 10 μM to about 15 μM, from about 15 μM to about 20 μM, from about 20 μM to about 25 μM, from about 25 μM to about 50 μM, from about 50 μM to about 75 μM, or from about 75 μM to about 100 μM.

[00134] In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2A, with an amino acid substitution at W109. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence set forth in SEQ ID NO:1, with an amino acid substitution at W29. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises the amino acid sequence set forth in SEQ ID NO:1, with an amino acid substitution at W29. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence set forth in SEQ ID NO:2, with an amino acid substitution at W30. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises the amino acid sequence set forth in SEQ ID NO:2, with an amino acid substitution at W30. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence set forth in SEQ ID NO:3, with an amino acid substitution at W30. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises the amino acid sequence set forth in SEQ ID NO:3, with an amino acid substitution at W30.

[00135] For example, in some cases, a variant 4-1BBL polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2U, where “x” is any amino acid other than tryptophan; for example, “x” can be Gly, Ala, Val, Leu, Ile, Pro, Phe, Tyr, Ser, Thr, Cys, Met, Asn, Gln, Lys, Arg, His, Asp, or Glu. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2U, where “x” is Gly. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2U, where “x” is Ala. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2U, where “x” is Val. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2U, where “x” is Leu. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2U, where “x” is Ile. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2U, where “x” is Pro. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2U, where “x” is Phe. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2U, where “x” is Tyr. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2U, where “x” is Ser. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2U, where “x” is Thr. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2U, where “x” is Cys. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2U, where “x” is Met. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2U, where “x” is Asn. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2U, where “x” is Gln. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2U, where “x” is Lys. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2U, where “x” is Arg. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2U, where “x” is His. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2U, where “x” is Asp. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2U, where “x” is Glu. In some cases, the variant 4-1BBL polypeptide has a binding affinity to CD137 that is from about 100 nM to about 150 nM, from about 150 nM to about 200 nM, from about 200 nM to about 250 nM, from about 250 nM to about 300 nM, from about 300 nM to about 350 nM, from about 350 nM to about 400 nM, from about 400 nM to about 500 nM, from about 500 nM to about 600 nM, from about 600 nM to about 700 nM, from about 700 nM to about 800 nM, from about 800 nM to about 900 nM, from about 900 nM to about 1 μM, to about 1 μM to about 5 μM, from about 5 μM to about 10 μM, from about 10 μM to about 15 μM, from about 15 μM to about 20 μM, from about 20 μM to about 25 μM, from about 25 μM to about 50 μM, from about 50 μM to about 75 μM, or from about 75 μM to about 100 μM.

Y110 Substitution

[00136] In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence depicted in FIG. 2V, where amino acid 110 (indicated by an “x”) is an amino acid other than a tyrosine, e.g., where amino acid 110 is Gly, Ala, Val, Leu, Ile, Pro, Phe, Trp, Ser, Thr, Cys, Met, Asn, Gln, Lys, Arg, His, Asp, or Glu. The amino acid numbering in FIG. 2V retains the numbering of FIG. 2A. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence depicted in FIG. 2V, where amino acid 110 is Gly. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence depicted in FIG. 2V, where amino acid 110 is Ala. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence depicted in FIG. 2V, where amino acid 110 is Val. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence depicted in FIG. 2V, where amino acid 110 is Leu. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence depicted in FIG. 2V, where amino acid 110 is Ile. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence depicted in FIG. 2V, where amino acid 110 is Pro. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence depicted in FIG. 2V, where amino acid 110 is Phe. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence depicted in FIG. 2V, where amino acid 110 is Trp. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence depicted in FIG. 2V, where amino acid 110 is Ser. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence depicted in FIG. 2V, where amino acid 110 is Thr. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence depicted in FIG. 2V, where amino acid 110 is Cys. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence depicted in FIG. 2V, where amino acid 110 is Met. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence depicted in FIG. 2V, where amino acid 110 is Asn. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence depicted in FIG. 2V, where amino acid 110 is Gln. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence depicted in FIG. 2V, where amino acid 110 is Lys. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence depicted in FIG. 2V, where amino acid 110 is Arg. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence depicted in FIG. 2V, where amino acid 110 is His. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence depicted in FIG. 2V, where amino acid 110 is Asp. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence depicted in FIG. 2V, where amino acid 110 is Glu. In some cases, the variant 4-1BBL polypeptide has a binding affinity to CD137 that is from about 100 nM to about 150 nM, from about 150 nM to about 200 nM, from about 200 nM to about 250 nM, from about 250 nM to about 300 nM, from about 300 nM to about 350 nM, from about 350 nM to about 400 nM, from about 400 nM to about 500 nM, from about 500 nM to about 600 nM, from about 600 nM to about 700 nM, from about 700 nM to about 800 nM, from about 800 nM to about 900 nM, from about 900 nM to about 1 μM, to about 1 μM to about 5 μM, from about 5 μM to about 10 μM, from about 10 μM to about 15 μM, from about 15 μM to about 20 μM, from about 20 μM to about 25 μM, from about 25 μM to about 50 μM, from about 50 μM to about 75 μM, or from about 75 μM to about 100 μM.

[00137] In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2A, with an amino acid substitution at Y110. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence set forth in SEQ ID NO:1, with an amino acid substitution at Y30. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises the amino acid sequence set forth in SEQ ID NO:1, with an amino acid substitution at Y30. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence set forth in SEQ ID NO:2, with an amino acid substitution at Y31. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises the amino acid sequence set forth in SEQ ID NO:2, with an amino acid substitution at Y31. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises an amino acid sequence having at least

90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence set forth in SEQ ID NO:3, with an amino acid substitution at Y31. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises the amino acid sequence set forth in SEQ ID NO:3, with an amino acid substitution at Y31.

[00138] For example, in some cases, a variant 4-1BBL polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2V, where “x” is any amino acid other than tyrosine; for example, “x” can be Gly, Ala, Val, Leu, Ile, Pro, Phe, Trp, Ser, Thr, Cys, Met, Asn, Gln, Lys, Arg, His, Asp, or Glu. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2V, where “x” is Gly. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2V, where “x” is Ala. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2V, where “x” is Val. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2V, where “x” is Leu. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2V, where “x” is Ile. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2V, where “x” is Pro. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2V, where “x” is Phe. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2V, where “x” is Trp. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2V, where “x” is Ser. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2V, where “x” is Thr. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2V, where “x” is Cys. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2V, where “x” is Met. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2V, where “x” is Asn. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2V, where “x” is Gln. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2V, where “x” is Lys. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2V, where “x” is Arg. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2V, where “x” is His. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2V, where “x” is Asp. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2V, where "x" is Glu. In some cases, the variant 4-1BBL polypeptide has a binding affinity to CD137 that is from about 100 nM to about 150 nM, from about 150 nM to about 200 nM, from about 200 nM to about 250 nM, from about 250 nM to about 300 nM, from about 300 nM to about 350 nM, from about 350 nM to about 400 nM, from about 400 nM to about 500 nM, from about 500 nM to about 600 nM, from about 600 nM to about 700 nM, from about 700 nM to about 800 nM, from about 800 nM to about 900 nM, from about 900 nM to about 1 μM, to about 1 μM to about 5 μM, from about 5 μM to about 10 μM, from about 10 μM to about 15 μM, from about 15 μM to about 20 μM, from about 20 μM to about 25 μM, from about 25 μM to about 50 μM, from about 50 μM to about 75 μM, or from about 75 μM to about 100 μM.

S111 Substitution

[00139] In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence depicted in FIG. 2W, where amino acid 111 (indicated by an "x") is an amino acid other than a serine, e.g., where amino acid 111 is Gly, Ala, Val, Leu, Ile, Pro, Phe, Tyr, Trp, Thr, Cys, Met, Asn, Gln, Lys, Arg, His, Asp, or Glu. The amino acid numbering in FIG. 2W retains the numbering of FIG. 2A. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence depicted in FIG. 2W, where amino acid 111 is Gly. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence depicted in FIG. 2W, where amino acid 111 is Ala. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence depicted in FIG. 2W, where amino acid 111 is Val. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence depicted in FIG. 2W, where amino acid 111 is Leu. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence depicted in FIG. 2W, where amino acid 111 is Ile. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence depicted in FIG. 2W, where amino acid 111 is Pro. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence depicted in FIG. 2W, where amino acid 111 is Phe. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence depicted in FIG. 2W, where amino acid 111 is Tyr. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence depicted in FIG. 2W, where amino acid 111 is Trp. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence depicted in FIG. 2W, where amino acid 111 is Thr. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence depicted in FIG. 2W, where amino acid 111 is Cys. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence depicted in FIG. 2W, where amino acid 111 is Met. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence depicted in FIG. 2W, where amino acid 111 is Asn. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence depicted in FIG. 2W, where amino acid 111 is Gln. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence depicted in FIG. 2W, where amino acid 111 is Lys. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence depicted in FIG. 2W, where amino acid 111 is Arg. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence depicted in FIG. 2W, where amino acid 111 is His. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence depicted in FIG. 2W, where amino acid 111 is Asp. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence depicted in FIG. 2W, where amino acid 111 is Glu. In some cases, the variant 4-1BBL polypeptide has a binding affinity to CD137 that is from about 100 nM to about 150 nM, from about 150 nM to about 200 nM, from about 200 nM to about 250 nM, from about 250 nM to about 300 nM, from about 300 nM to about 350 nM, from about 350 nM to about 400 nM, from about 400 nM to about 500 nM, from about 500 nM to about 600 nM, from about 600 nM to about 700 nM, from about 700 nM to about 800 nM, from about 800 nM to about 900 nM, from about 900 nM to about 1 μM, to about 1 μM to about 5 μM, from about 5 μM to about 10 μM, from about 10 μM to about 15 μM, from about 15 μM to about 20 μM, from about 20 μM to about 25 μM, from about 25 μM to about 50 μM, from about 50 μM to about 75 μM, or from about 75 μM to about 100 μM.

[00140] In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2A, with an amino acid substitution at S111. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence set forth in SEQ ID NO:1, with an amino acid substitution at S31. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises the amino acid sequence set forth in SEQ ID NO:1, with an amino acid substitution at S31. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence set forth in SEQ ID NO:2, with an amino acid substitution at S32. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises the amino acid sequence set forth in SEQ ID NO:2, with an amino acid substitution at S32. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence set forth in SEQ ID NO:3, with an amino acid substitution at S32. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises the amino acid sequence set forth in SEQ ID NO:3, with an amino acid substitution at S32.

[00141] For example, in some cases, a variant 4-1BBL polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2W, where “x” is any amino acid other than serine; for example, “x” can be Gly, Ala, Val, Leu, Ile, Pro, Phe, Tyr, Trp, Thr, Cys, Met, Asn, Gln, Lys, Arg, His, Asp, or Glu. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2W, where “x” is Gly. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2W, where “x” is Ala. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2W, where “x” is Val. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2W, where “x” is Leu. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2W, where “x” is Ile. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2W, where “x” is Pro. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2W, where “x” is Phe. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2W, where “x” is Tyr. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2W, where “x” is Trp. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2W, where “x” is Thr. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2W, where “x” is Cys. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2W, where “x” is Met. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2W, where “x” is Asn. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2W, where “x” is Gln. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2W, where “x” is Lys. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2W, where “x” is Arg. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2W, where “x” is His. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2W, where “x” is Asp. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2W, where “x” is Glu. In some cases, the variant 4-1BBL polypeptide has a binding affinity to CD137 that is from about 100 nM to about 150 nM, from about 150 nM to about 200 nM, from about 200 nM to about 250 nM, from about 250 nM to about 300 nM, from about 300 nM to about 350 nM, from about 350 nM to about 400 nM, from about 400 nM to about 500 nM, from about 500 nM to about 600 nM, from about 600 nM to about 700 nM, from about 700 nM to about 800 nM, from about 800 nM to about 900 nM, from about 900 nM to about 1 μM, to about 1 μM to about 5 μM, from about 5 μM to about 10 μM, from about 10 μM to about 15 μM, from about 15 μM to about 20 μM, from about 20 μM to about 25 μM, from about 25 μM to about 50 μM, from about 50 μM to about 75 μM, or from about 75 μM to about 100 μM.

D112 Substitution

[00142] In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence depicted in FIG. 2X, where amino acid 112 (indicated by an "x") is an amino acid other than an aspartic acid, e.g., where amino acid 112 is Gly, Ala, Val, Leu, Ile, Pro, Phe, Tyr, Trp, Ser, Thr, Cys, Met, Asn, Gln, Lys, Arg, His, or Glu. The amino acid numbering in FIG. 2X retains the numbering of FIG. 2A. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence depicted in FIG. 2X, where amino acid 112 is Gly. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence depicted in FIG. 2X, where amino acid 112 is Ala. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence depicted in FIG. 2X, where amino acid 112 is Val. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence depicted in FIG. 2X, where amino acid 112 is Leu. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence depicted in FIG. 2X, where amino acid 112 is Ile. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence depicted in FIG. 2X, where amino acid 112 is Pro. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence depicted in FIG. 2X, where amino acid 112 is Phe. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence depicted in FIG. 2X, where amino acid 112 is Tyr. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence depicted in FIG. 2X, where amino acid 112 is Trp. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence depicted in FIG. 2X, where amino acid 112 is Ser. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence depicted in FIG. 2X, where amino acid 112 is Thr. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence depicted in FIG. 2X, where amino acid 112 is Cys. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence depicted in FIG. 2X, where amino acid 112 is Met. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence depicted in FIG. 2X, where amino acid 112 is Asn. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence depicted in FIG. 2X, where amino acid 112 is Gln. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence depicted in FIG. 2X, where amino acid 112 is Lys. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence depicted in FIG. 2X, where amino acid 112 is Arg. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence depicted in FIG. 2X, where amino acid 112 is His. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence depicted in FIG. 2X, where amino acid 2X is Glu. In some cases, the variant 4-1BBL polypeptide has a binding affinity to CD137 that is from about 100 nM to about 150 nM, from about 150 nM to about 200 nM, from about 200 nM to about 250 nM, from about 250 nM to about 300 nM, from about 300 nM to about 350 nM, from about 350 nM to about 400 nM, from about 400 nM to about 500 nM, from about 500 nM to about 600 nM, from about 600 nM to

about 700 nM, from about 700 nM to about 800 nM, from about 800 nM to about 900 nM, from about 900 nM to about 1 μM, to about 1 μM to about 5 μM, from about 5 μM to about 10 μM, from about 10 μM to about 15 μM, from about 15 μM to about 20 μM, from about 20 μM to about 25 μM, from about 25 μM to about 50 μM, from about 50 μM to about 75 μM, or from about 75 μM to about 100 μM.

[00143] In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2A, with an amino acid substitution at D112. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence set forth in SEQ ID NO:1, with an amino acid substitution at D32. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises the amino acid sequence set forth in SEQ ID NO:1, with an amino acid substitution at D32. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence set forth in SEQ ID NO:2, with an amino acid substitution at D33. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises the amino acid sequence set forth in SEQ ID NO:2, with an amino acid substitution D33. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence set forth in SEQ ID NO:3, with an amino acid substitution at D33. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises the amino acid sequence set forth in SEQ ID NO:3, with an amino acid substitution at D33.

[00144] For example, in some cases, a variant 4-1BBL polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2X, where “x” is any amino acid other than aspartic acid; for example, “x” can be Gly, Ala, Val, Leu, Ile, Pro, Phe, Tyr, Trp, Ser, Thr, Cys, Met, Asn, Gln, Lys, Arg, His, or Glu. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2X, where “x” is Gly. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2X, where “x” is Ala. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2X, where “x” is Val. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2X, where “x” is Leu. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2X, where “x” is Ile. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2X, where “x” is Pro. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2X, where “x” is Phe. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2X, where “x” is Tyr. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2X, where “x” is Trp. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2X, where “x” is Ser. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2X, where “x” is Thr. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2X, where “x” is Cys. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2X, where “x” is Met. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2X, where “x” is Asn. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2X, where “x” is Gln. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2X, where “x” is Lys. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2X, where “x” is Arg. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2X, where “x” is His. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2X, where “x” is Glu. In some cases, the variant 4-1BBL polypeptide has a binding affinity to CD137 that is from about 100 nM to about 150 nM, from about 150 nM to about 200 nM, from about 200 nM to about 250 nM, from about 250 nM to about 300 nM, from about 300 nM to about 350 nM, from about 350 nM to about 400 nM, from about 400 nM to about 500 nM, from about 500 nM to about 600 nM, from about 600 nM to about 700 nM, from about 700 nM to about 800 nM, from about 800 nM to about 900 nM, from about 900 nM to about 1 μM, to about 1 μM to about 5 μM, from about 5 μM to about 10 μM, from about 10 μM to about 15 μM, from about 15 μM to about 20 μM, from about 20 μM to about 25 μM, from about 25 μM to about 50 μM, from about 50 μM to about 75 μM, or from about 75 μM to about 100 μM.

P113 Substitution

[00145] In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence depicted in FIG. 2Y, where amino acid 113 (indicated by an “x”) is an amino acid other than a proline, e.g., where amino acid 113 is Gly, Ala, Val, Leu, Ile, Phe, Tyr, Trp, Ser, Thr, Cys, Met, Asn, Gln, Lys, Arg, His, Asp, or Glu. The amino acid numbering in FIG. 2Y retains the numbering of FIG. 2A. In some cases, a variant 4-

1BBL polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence depicted in FIG. 2Y, where amino acid 113 is Gly. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence depicted in FIG. 2Y, where amino acid 113 is Ala. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence depicted in FIG. 2Y, where amino acid 113 is Val. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence depicted in FIG. 2Y, where amino acid 113 is Leu. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence depicted in FIG. 2Y, where amino acid 113 is Ile. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence depicted in FIG. 2Y, where amino acid 113 is Phe. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence depicted in FIG. 2Y, where amino acid 113 is Tyr. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence depicted in FIG. 2Y, where amino acid 113 is Trp. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence depicted in FIG. 2Y, where amino acid 113 is Ser. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence depicted in FIG. 2Y, where amino acid 113 is Thr. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence depicted in FIG. 2Y, where amino acid 113 is Cys. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence depicted in FIG. 2Y, where amino acid 1113 is Met. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence depicted in FIG. 2Y, where amino acid 113 is Asn. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence depicted in FIG. 2Y, where amino acid 113 is Gln. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence depicted in FIG. 2Y, where amino acid 113 is Lys. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence depicted in FIG. 2Y, where amino acid 113 is Arg. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence depicted in FIG. 2Y, where amino acid 113 is His. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence depicted in FIG. 2Y, where amino acid 113 is Asp. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence depicted in FIG. 2Y, where amino acid 113 is Glu. In some cases, the variant 4-1BBL polypeptide has a binding affinity to CD137 that is from about 100 nM to about 150 nM, from about 150 nM to about 200 nM, from about 200 nM to about 250 nM, from about 250 nM to about 300 nM, from about 300 nM to about 350 nM, from about 350 nM to about 400 nM, from about 400 nM to about 500 nM, from about 500 nM to about 600 nM, from about 600 nM to about 700 nM, from about 700 nM to about 800 nM, from about 800 nM to about 900 nM, from about 900 nM to about 1 μM, to about 1 μM to about 5 μM, from about 5 μM to about 10 μM, from about 10 μM to about 15 μM, from about 15 μM to about 20 μM, from about 20 μM to about 25 μM, from about 25 μM to about 50 μM, from about 50 μM to about 75 μM, or from about 75 μM to about 100 μM.

[00146] In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2A, with an amino acid substitution at P113. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence set forth in SEQ ID NO:1, with an amino acid substitution at P33. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises the amino acid sequence set forth in SEQ ID NO:1, with an amino acid substitution at P33. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence set forth in SEQ ID NO:2, with an amino acid substitution at P34. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises the amino acid sequence set forth in SEQ ID NO:2, with an amino acid substitution P34. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence set forth in SEQ ID NO:3, with an amino acid substitution at P34. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises the amino acid sequence set forth in SEQ ID NO:3, with an amino acid substitution at P34.

[00147] For example, in some cases, a variant 4-1BBL polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2Y, where “x” is any amino acid other than methionine; for example, “x” can be Gly, Ala, Val, Leu, Ile, Phe, Tyr, Trp, Ser, Thr, Cys, Met, Asn, Gln, Lys, Arg, His, Asp, or Glu. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2Y, where “x” is Gly. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2Y, where “x” is Ala. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2Y, where “x” is Val. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2Y, where “x” is Leu. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2Y, where “x” is Ile. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2Y, where “x” is Phe. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2Y, where “x” is Tyr. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2Y, where “x” is Trp. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2Y, where “x” is Ser. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2Y, where “x” is Thr. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2Y, where “x” is Cys. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2Y, where “x” is Met. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2Y, where “x” is Asn. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2Y, where “x” is Gln. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2Y, where “x” is Lys. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2Y, where “x” is Arg. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2Y, where “x” is His. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2Y, where “x” is Asp. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2Y, where “x” is Glu. In some cases, the variant 4-1BBL polypeptide has a binding affinity to CD137 that is from about 100 nM to about 150 nM, from about 150 nM to about 200 nM, from about 200 nM to about 250 nM, from about 250 nM to about 300 nM, from about 300 nM to about 350 nM, from about 350 nM to about 400 nM, from about 400 nM to about 500 nM, from about 500 nM to about 600 nM, from about 600 nM to about 700 nM, from about 700 nM to about 800 nM, from about 800 nM to about 900 nM, from about 900 nM to about 1 μM, to about 1 μM to about 5 μM, from about 5 μM to about 10 μM, from about 10 μM to about 15 μM, from about 15 μM to about 20 μM, from about 20 μM to about 25 μM, from about 25 μM to about 50 μM, from about 50 μM to about 75 μM, or from about 75 μM to about 100 μM.

G114 Substitution

[00148] In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence depicted in FIG. 2Z, where amino acid 114 (indicated by an “x”) is an amino acid other than a glycine, e.g., where amino acid 114 is Ala, Val, Leu, Ile, Pro, Phe, Tyr, Trp, Ser, Thr, Cys, Met, Asn, Gln, Lys, Arg, His, Asp, or Glu. The amino acid numbering in FIG. 2Z retains the numbering of FIG. 2A. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence depicted in FIG. 2Z, where amino acid 114 is Ala. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence depicted in FIG. 2Z, where amino acid 114 is Val. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence depicted in FIG. 2Z, where amino acid 114 is Leu. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence depicted in FIG. 2Z, where amino acid 114 is Ile. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence depicted in FIG. 2Z, where amino acid 114 is Pro. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence depicted in FIG. 2Z, where amino acid 114 is Phe. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence depicted in FIG. 2Z, where amino acid 114 is Tyr. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence depicted in FIG. 2Z, where amino acid 114 is Trp. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence depicted in FIG. 2Z, where amino acid 114 is Ser. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence depicted in FIG. 2Z, where amino acid 114 is Thr. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence depicted in FIG. 2Z, where amino acid 114 is Cys. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence depicted in FIG. 2Z, where amino acid 114 is Met. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence depicted in FIG. 2Z, where amino acid 114 is Asn. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence depicted in FIG. 2Z, where amino acid 114 is Gln. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence depicted in FIG. 2Z, where amino acid 114 is Lys. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence depicted in FIG. 2Z, where amino acid 114 is Arg. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence depicted in FIG. 2Z, where amino acid 114 is His. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence depicted in FIG. 2Z, where amino acid 114 is Asp. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence depicted in FIG. 2Z, where amino acid 114 is Glu. In some cases, the variant 4-1BBL polypeptide has a binding affinity to CD137 that is from about 100 nM to about 150 nM, from about 150 nM to about 200 nM, from about 200 nM to about 250 nM, from about 250 nM to about 300 nM, from about 300 nM to about 350 nM, from about 350 nM to about 400 nM, from about 400 nM to about 500 nM, from about 500 nM to about 600 nM, from about 600 nM to about 700 nM, from about 700 nM to about 800 nM, from about 800 nM to about 900 nM, from about 900 nM to about 1 μM, to about 1 μM to about 5 μM, from about 5 μM to about 10 μM, from about 10 μM to about 15 μM, from about 15 μM to about 20 μM, from about 20 μM to about 25 μM, from about 25 μM to about 50 μM, from about 50 μM to about 75 μM, or from about 75 μM to about 100 μM.

[00149] In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2A, with an amino acid substitution at G114. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence set forth in SEQ ID NO:1, with an amino acid substitution at G34. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises the amino acid sequence set forth in SEQ ID NO:1, with an amino acid substitution at G34. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence set forth in SEQ ID NO:2, with an amino acid substitution at G35. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises the amino acid sequence set forth in SEQ ID NO:2, with an amino acid substitution G35. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence set forth in SEQ ID NO:3, with an amino acid substitution at G35. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises the amino acid sequence set forth in SEQ ID NO:3, with an amino acid substitution at G35.

[00150] For example, in some cases, a variant 4-1BBL polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2Z, where "x" is any amino acid other than glycine; for example, "x" can be Ala, Val, Leu, Ile, Pro, Phe, Tyr, Trp, Ser, Thr, Cys, Met, Asn, Gln, Lys, Arg, His, Asp, or Glu. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2Z, where "x" is Ala. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2Z, where "x" is Val. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2Z, where "x" is Leu. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2Z, where "x" is Ile. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2Z, where "x" is Pro. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2Z, where "x" is Phe. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2Z, where "x" is Tyr. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2Z, where "x" is Trp. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2Z, where "x" is Ser. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2Z, where "x" is Thr. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2Z, where "x" is Cys. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2Z, where "x" is Met. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2Z, where "x" is Asn. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2Z, where "x" is Gln. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2Z, where "x" is Lys. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2Z, where "x" is Arg. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2Z, where "x" is His. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2Z, where "x" is Asp. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2Z, where “x” is Glu. In some cases, the variant 4-1BBL polypeptide has a binding affinity to CD137 that is from about 100 nM to about 150 nM, from about 150 nM to about 200 nM, from about 200 nM to about 250 nM, from about 250 nM to about 300 nM, from about 300 nM to about 350 nM, from about 350 nM to about 400 nM, from about 400 nM to about 500 nM, from about 500 nM to about 600 nM, from about 600 nM to about 700 nM, from about 700 nM to about 800 nM, from about 800 nM to about 900 nM, from about 900 nM to about 1 μM, to about 1 μM to about 5 μM, from about 5 μM to about 10 μM, from about 10 μM to about 15 μM, from about 15 μM to about 20 μM, from about 20 μM to about 25 μM, from about 25 μM to about 50 μM, from about 50 μM to about 75 μM, or from about 75 μM to about 100 μM.

L115

[00151] In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence depicted in FIG. 2AA, where amino acid 115 (indicated by an “x”) is an amino acid other than a leucine, e.g., where amino acid 115 is Gly, Ala, Val, Ile, Pro, Phe, Tyr, Trp, Ser, Thr, Cys, Met, Asn, Gln, Lys, Arg, His, Asp, or Glu. The amino acid numbering in FIG. 2AA retains the numbering of FIG. 2A. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence depicted in FIG. 2AA, where amino acid 115 is Gly. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence depicted in FIG. 2AA, where amino acid 115 is Ala. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence depicted in FIG. 2AA, where amino acid 115 is Val. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence depicted in FIG. 2AA, where amino acid 115 is Ile. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence depicted in FIG. 2AA, where amino acid 115 is Pro. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence depicted in FIG. 2AA, where amino acid 115 is Phe. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence depicted in FIG. 2AA, where amino acid 115 is Tyr. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence depicted in FIG. 2AA, where amino acid 115 is Trp. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence depicted in FIG. 2AA, where amino acid 115 is Ser. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence depicted in FIG. 2AA, where amino acid 115 is Thr. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence depicted in FIG. 2AA, where amino acid 115 is Cys. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence depicted in FIG. 2AA, where amino acid 115 is Met. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence depicted in FIG. 2AA, where amino acid 115 is Asn. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence depicted in FIG. 2AA, where amino acid 115 is Gln. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence depicted in FIG. 2AA, where amino acid 115 is Lys. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence depicted in FIG. 2AA, where amino acid 115 is Arg. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence depicted in FIG. 2AA, where amino acid 115 is His. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence depicted in FIG. 2AA, where amino acid 115 is Asp. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence depicted in FIG. 2AA, where amino acid 115 is Glu. In some cases, the variant 4-1BBL polypeptide has a binding affinity to CD137 that is from about 100 nM to about 150 nM, from about 150 nM to about 200 nM, from about 200 nM to about 250 nM, from about 250 nM to about 300 nM, from about 300 nM to about 350 nM, from about 350 nM to about 400 nM, from about 400 nM to about 500 nM, from about 500 nM to about 600 nM, from about 600 nM to about 700 nM, from about 700 nM to about 800 nM, from about 800 nM to about 900 nM, from about 900 nM to about 1 μM, to about 1 μM to about 5 μM, from about 5 μM to about 10 μM, from about 10 μM to about 15 μM, from about 15 μM to about 20 μM, from about 20 μM to about 25 μM, from about 25 μM to about 50 μM, from about 50 μM to about 75 μM, or from about 75 μM to about 100 μM.

[00152] In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2A, with an amino acid substitution at L115. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence set forth in SEQ ID NO:1, with an amino acid substitution at L35. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises the amino acid sequence set forth in SEQ ID NO:1, with an amino acid substitution at L35. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence set forth in SEQ ID NO:2, with an amino acid substitution at L36. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises the amino acid sequence set forth in SEQ ID NO:2, with an amino acid substitution L36. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence set forth in SEQ ID NO:3, with an amino acid substitution at L36. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises the amino acid sequence set forth in SEQ ID NO:3, with an amino acid substitution at L36.

[00153] For example, in some cases, a variant 4-1BBL polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2AA, where “x” is any amino acid other than leucine; for example, “x” can be Gly, Ala, Val, Ile, Pro, Phe, Tyr, Trp, Ser, Thr, Cys, Met, Asn, Gln, Lys, Arg, His, Asp, or Glu. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2AA, where “x” is Gly.

In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2AA, where “x” is Ala. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2AA, where “x” is Val. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2AA, where “x” is Ile. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2AA, where “x” is Pro. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2AA, where “x” is Phe. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2AA, where “x” is Tyr. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2AA, where “x” is Trp. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2AA, where “x” is Ser. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2AA, where “x” is Thr. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2AA, where “x” is Cys. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2AA, where “x” is Met. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2AA, where “x” is Asn. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2AA, where “x” is Gln. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2AA, where “x” is Lys. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2AA, where “x” is Arg. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2AA, where “x” is His. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2AA, where “x” is Asp. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2AA, where “x” is Glu. In some cases, the variant 4-1BBL polypeptide has a binding affinity to CD137 that is from about 100 nM to about 150 nM, from about 150 nM to about 200 nM, from about 200 nM to about 250 nM, from about 250 nM to about 300 nM, from about 300 nM to about 350 nM, from about 350 nM to about 400 nM, from about 400 nM to about 500 nM, from about 500 nM to about 600 nM, from about 600 nM to about 700 nM, from about 700 nM to about 800 nM, from about 800 nM to about 900 nM, from about 900 nM to about 1 μM, to about 1 μM to about 5 μM, from about 5 μM to about 10 μM, from about 10 μM to about 15 μM, from about 15 μM to

about 20 μM, from about 20 μM to about 25 μM, from about 25 μM to about 50 μM, from about 50 μM to about 75 μM, or from about 75 μM to about 100 μM.

G117 Substitution

[00154] In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence depicted in FIG. 2BB, where amino acid 117 (indicated by an "x") is an amino acid other than a glycine, e.g., where amino acid 117 is Ala, Val, Leu, Ile, Pro, Phe, Tyr, Trp, Ser, Thr, Cys, Met, Asn, Gln, Lys, Arg, His, Asp, or Glu. The amino acid numbering in FIG. 2BB retains the numbering of FIG. 2A. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence depicted in FIG. 2BB, where amino acid 117 is Ala. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence depicted in FIG. 2BB, where amino acid 117 is Val. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence depicted in FIG. 2BB, where amino acid 117 is Leu. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence depicted in FIG. 2BB, where amino acid 117 is Ile. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence depicted in FIG. 2BB, where amino acid 117 is Pro. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence depicted in FIG. 2BB, where amino acid 117 is Phe. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence depicted in FIG. 2BB, where amino acid 117 is Tyr. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence depicted in FIG. 2BB, where amino acid 117 is Trp. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence depicted in FIG. 2BB, where amino acid 117 is Ser. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence depicted in FIG. 2BB, where amino acid 117 is Thr. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence depicted in FIG. 2BB, where amino acid 117 is Cys. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence depicted in FIG. 2BB, where amino acid 117 is Met. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence depicted in FIG. 2BB, where amino acid 117 is Asn. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence depicted in FIG. 2BB, where amino acid 117 is Gln. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence depicted in FIG. 2BB, where amino acid 117 is Lys. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence depicted in FIG. 2BB, where amino acid 117 is Arg. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence depicted in FIG. 2BB, where amino acid 117 is His. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence depicted in FIG. 2BB, where amino acid 117 is Asp. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence depicted in FIG. 2BB, where amino acid 117 is Glu. In some cases, the variant 4-1BBL polypeptide has a binding affinity to CD137 that is from about 100 nM to about 150 nM, from about 150 nM to about 200 nM, from about 200 nM to about 250 nM, from about 250 nM to about 300 nM, from about 300 nM to about 350 nM, from about 350 nM to about 400 nM, from about 400 nM to about 500 nM, from about 500 nM to about 600 nM, from about 600 nM to

about 700 nM, from about 700 nM to about 800 nM, from about 800 nM to about 900 nM, from about 900 nM to about 1 μM, to about 1 μM to about 5 μM, from about 5 μM to about 10 μM, from about 10 μM to about 15 μM, from about 15 μM to about 20 μM, from about 20 μM to about 25 μM, from about 25 μM to about 50 μM, from about 50 μM to about 75 μM, or from about 75 μM to about 100 μM.

[00155] In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2A, with an amino acid substitution at G117. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence set forth in SEQ ID NO:1, with an amino acid substitution at G37. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises the amino acid sequence set forth in SEQ ID NO:1, with an amino acid substitution at G37. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence set forth in SEQ ID NO:2, with an amino acid substitution at G38. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises the amino acid sequence set forth in SEQ ID NO:2, with an amino acid substitution G38. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence set forth in SEQ ID NO:3, with an amino acid substitution at G38. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises the amino acid sequence set forth in SEQ ID NO:3, with an amino acid substitution at G38.

[00156] For example, in some cases, a variant 4-1BBL polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2BB, where "x" is any amino acid other than glycine; for example, "x" can be Ala, Val, Leu, Ile, Pro, Phe, Tyr, Trp, Ser, Thr, Cys, Met, Asn, Gln, Lys, Arg, His, Asp, or Glu. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2BB, where "x" is Ala. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2BB, where "x" is Val. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2BB, where "x" is Leu. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2BB, where "x" is Ile. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2BB, where "x" is Pro. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2BB, where "x" is Phe. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2BB, where “x” is Tyr. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2BB, where “x” is Trp. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2BB, where “x” is Ser. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2BB, where “x” is Thr. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2BB, where “x” is Cys. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2BB, where “x” is Met. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2BB, where “x” is Asn. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2BB, where “x” is Gln. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2BB, where “x” is Lys. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2BB, where “x” is Arg. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2BB, where “x” is His. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2BB, where “x” is Asp. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2BB, where “x” is Glu. In some cases, the variant 4-1BBL polypeptide has a binding affinity to CD137 that is from about 100 nM to about 150 nM, from about 150 nM to about 200 nM, from about 200 nM to about 250 nM, from about 250 nM to about 300 nM, from about 300 nM to about 350 nM, from about 350 nM to about 400 nM, from about 400 nM to about 500 nM, from about 500 nM to about 600 nM, from about 600 nM to about 700 nM, from about 700 nM to about 800 nM, from about 800 nM to about 900 nM, from about 900 nM to about 1 μM, to about 1 μM to about 5 μM, from about 5 μM to about 10 μM, from about 10 μM to about 15 μM, from about 15 μM to about 20 μM, from about 20 μM to about 25 μM, from about 25 μM to about 50 μM, from about 50 μM to about 75 μM, or from about 75 μM to about 100 μM.

V118

[00157] In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence depicted in FIG. 2CC, where amino acid 118 (indicated by an “x”) is an amino acid other than a valine, e.g., where amino acid 118 is Gly, Ala, Leu, Ile, Pro, Phe, Tyr, Trp, Ser, Thr, Cys, Met, Asn, Gln, Lys, Arg, His, Asp, or Glu. The amino acid numbering in FIG. 2CC retains the numbering of FIG. 2A. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence depicted in FIG. 2CC, where amino acid 118 is Gly. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence depicted in FIG. 2CC, where amino acid 118 is Ala. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence depicted in FIG. 2CC, where amino acid 118 is Leu. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence depicted in FIG. 2CC, where amino acid 118 is Ile. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence depicted in FIG. 2CC, where amino acid 118 is Pro. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence depicted in FIG. 2CC, where amino acid 118 is Phe. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence depicted in FIG. 2CC, where amino acid 118 is Tyr. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence depicted in FIG. 2CC, where amino acid 118 is Trp. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence depicted in FIG. 2CC, where amino acid 118 is Ser. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence depicted in FIG. 2CC, where amino acid 118 is Thr. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence depicted in FIG. 2CC, where amino acid 118 is Cys. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence depicted in FIG. 2CC, where amino acid 118 is Met. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence depicted in FIG. 2CC, where amino acid 118 is Asn. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence depicted in FIG. 2CC, where amino acid 118 is Gln. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence depicted in FIG. 2CC, where amino acid 118 is Lys. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence depicted in FIG. 2CC, where amino acid 118 is Arg. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence depicted in FIG. 2CC, where amino acid 118 is His. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence depicted in FIG. 2CC, where amino acid 118 is Asp. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence depicted in FIG. 2CC, where amino acid 118 is Glu. In some cases, the variant 4-1BBL polypeptide has a binding affinity to CD137 that is from about 100 nM to about 150 nM, from about 150 nM to about 200 nM, from about 200 nM to about 250 nM, from about 250 nM to about 300 nM, from about 300 nM to about 350 nM, from about 350 nM to about 400 nM, from about 400 nM to about 500 nM, from about 500 nM to about 600 nM, from about 600 nM to about 700 nM, from about 700 nM to about 800 nM, from about 800 nM to about 900 nM, from about 900 nM to about 1 μM, to about 1 μM to about 5 μM, from about 5 μM to about 10 μM, from about 10 μM to about 15 μM, from about 15 μM to about 20 μM, from about 20 μM to about 25 μM, from about 25 μM to about 50 μM, from about 50 μM to about 75 μM, or from about 75 μM to about 100 μM.

[00158] In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2A, with an amino acid substitution at V118. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence set forth in SEQ ID NO:1, with an amino acid substitution at V38. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises the amino acid sequence set forth in SEQ ID NO:1, with an amino acid substitution at V38. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence set forth in SEQ ID NO:2, with an amino acid substitution at V39. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises the amino acid sequence set forth in SEQ ID NO:2, with an amino acid substitution V39. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence set forth in SEQ ID NO:3, with an amino acid substitution at V39. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises the amino acid sequence set forth in SEQ ID NO:3, with an amino acid substitution at V39.

[00159] For example, in some cases, a variant 4-1BBL polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2CC, where “x” is any amino acid other than valine; for example, “x” can be Gly, Ala, Leu, Ile, Pro, Phe, Tyr, Trp, Ser, Thr, Cys, Met, Asn, Gln, Lys, Arg, His, Asp, or Glu. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2CC, where “x” is Gly. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2CC, where “x” is Ala. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2CC, where “x” is Leu. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2CC, where “x” is Ile. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2CC, where “x” is Pro. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2CC, where “x” is Phe. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2CC, where “x” is Tyr. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2CC, where “x” is Trp. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2CC, where “x” is Ser. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2CC, where “x” is Thr. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2CC, where “x” is Cys. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2CC, where "x" is Met. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2CC, where "x" is Asn. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2CC, where "x" is Gln. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2CC, where "x" is Lys. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2CC, where "x" is Arg. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2CC, where "x" is His. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2CC, where "x" is Asp. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2CC, where "x" is Glu. In some cases, the variant 4-1BBL polypeptide has a binding affinity to CD137 that is from about 100 nM to about 150 nM, from about 150 nM to about 200 nM, from about 200 nM to about 250 nM, from about 250 nM to about 300 nM, from about 300 nM to about 350 nM, from about 350 nM to about 400 nM, from about 400 nM to about 500 nM, from about 500 nM to about 600 nM, from about 600 nM to about 700 nM, from about 700 nM to about 800 nM, from about 800 nM to about 900 nM, from about 900 nM to about 1 μM, to about 1 μM to about 5 μM, from about 5 μM to about 10 μM, from about 10 μM to about 15 μM, from about 15 μM to about 20 μM, from about 20 μM to about 25 μM, from about 25 μM to about 50 μM, from about 50 μM to about 75 μM, or from about 75 μM to about 100 μM.

S119 Substitution

[00160] In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence depicted in FIG. 2DD, where amino acid 119 (indicated by an "x") is an amino acid other than a serine, e.g., where amino acid 119 is Gly, Ala, Val, Leu, Ile, Pro, Phe, Tyr, Trp, Thr, Cys, Met, Asn, Gln, Lys, Arg, His, Asp, or Glu. The amino acid numbering in FIG. 2DD retains the numbering of FIG. 2A. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence depicted in FIG. 2DD, where amino acid 119 is Gly. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence depicted in FIG. 2DD, where amino acid 119 is Ala. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence depicted in FIG. 2DD, where amino acid 119 is Val. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence depicted in FIG. 2DD, where amino acid 119 is Leu. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence depicted in FIG. 2DD, where amino acid 119 is Ile. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence depicted in FIG. 2DD, where amino acid 119 is Pro. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence depicted in FIG. 2DD, where amino acid 119 is Phe. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence depicted in FIG. 2DD, where amino acid 119 is Tyr. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence depicted in FIG. 2DD, where amino acid 119 is Trp. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence depicted in FIG. 2DD, where amino acid 119 is Thr. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence depicted in FIG. 2DD, where amino acid 119 is Cys. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence depicted in FIG. 2DD, where amino acid 119 is Met. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence depicted in FIG. 2DD, where amino acid 119 is Asn. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence depicted in FIG. 2DD, where amino acid 119 is Gln. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence depicted in FIG. 2DD, where amino acid 119 is Lys. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence depicted in FIG. 2DD, where amino acid 119 is Arg. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence depicted in FIG. 2DD, where amino acid 119 is His. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence depicted in FIG. 2DD, where amino acid 119 is Asp. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence depicted in FIG. 2DD, where amino acid 119 is Glu. In some cases, the variant 4-1BBL polypeptide has a binding affinity to CD137 that is from about 100 nM to about 150 nM, from about 150 nM to about 200 nM, from about 200 nM to about 250 nM, from about 250 nM to about 300 nM, from about 300 nM to about 350 nM, from about 350 nM to about 400 nM, from about 400 nM to about 500 nM, from about 500 nM to about 600 nM, from about 600 nM to about 700 nM, from about 700 nM to about 800 nM, from about 800 nM to about 900 nM, from about 900 nM to about 1 μM, to about 1 μM to about 5 μM, from about 5 μM to about 10 μM, from about 10 μM to about 15 μM, from about 15 μM to about 20 μM, from about 20 μM to about 25 μM, from about 25 μM to about 50 μM, from about 50 μM to about 75 μM, or from about 75 μM to about 100 μM.

[00161] In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2A, with an amino acid substitution at S119. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence set forth in SEQ ID NO:1, with an amino acid substitution at S39. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises the amino acid sequence set forth in SEQ ID NO:1, with an amino acid substitution at S39. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence set forth in SEQ ID NO:2, with an amino acid substitution at S40. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises the amino acid sequence set forth in SEQ ID NO:2, with an amino acid substitution S40. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence set forth in SEQ ID NO:3, with an amino acid substitution at S40. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises the amino acid sequence set forth in SEQ ID NO:3, with an amino acid substitution at S40.

[00162] For example, in some cases, a variant 4-1BBL polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2DD, where “x” is any amino acid other than serine; for example, “x” can be Gly, Ala, Val, Leu, Ile, Pro, Phe, Tyr, Trp, Thr, Cys, Met, Asn, Gln, Lys, Arg, His, Asp, or Glu. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2DD, where “x” is Gly. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2DD, where “x” is Ala. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2DD, where “x” is Val. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2DD, where “x” is Leu. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2DD, where “x” is Ile. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2DD, where “x” is Pro. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2DD, where “x” is Phe. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2DD, where “x” is Tyr. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2DD, where “x” is Trp. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2DD, where “x” is Thr. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2DD, where “x” is Cys. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2DD, where “x” is Met. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2DD, where “x” is Asn. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2DD, where “x” is Gln. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2DD, where “x” is Lys. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2DD, where “x” is Arg. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2DD, where "x" is His. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2DD, where "x" is Asp. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2DD, where "x" is Glu. In some cases, the variant 4-1BBL polypeptide has a binding affinity to CD137 that is from about 100 nM to about 150 nM, from about 150 nM to about 200 nM, from about 200 nM to about 250 nM, from about 250 nM to about 300 nM, from about 300 nM to about 350 nM, from about 350 nM to about 400 nM, from about 400 nM to about 500 nM, from about 500 nM to about 600 nM, from about 600 nM to about 700 nM, from about 700 nM to about 800 nM, from about 800 nM to about 900 nM, from about 900 nM to about 1 μM, to about 1 μM to about 5 μM, from about 5 μM to about 10 μM, from about 10 μM to about 15 μM, from about 15 μM to about 20 μM, from about 20 μM to about 25 μM, from about 25 μM to about 50 μM, from about 50 μM to about 75 μM, or from about 75 μM to about 100 μM.

L120 Substitution

[00163] In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence depicted in FIG. 2EE, where amino acid 120 (indicated by an "x") is an amino acid other than a leucine, e.g., where amino acid 120 is Gly, Ala, Val, Ile, Pro, Phe, Tyr, Trp, Ser, Thr, Cys, Met, Asn, Gln, Lys, Arg, His, Asp, or Glu. The amino acid numbering in FIG. 2EE retains the numbering of FIG. 2A. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence depicted in FIG. 2EE, where amino acid 120 is Gly. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence depicted in FIG. 2EE, where amino acid 120 is Ala. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence depicted in FIG. 2EE, where amino acid 120 is Val. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence depicted in FIG. 2EE, where amino acid 120 is Ile. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence depicted in FIG. 2EE, where amino acid 120 is Pro. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence depicted in FIG. 2EE, where amino acid 120 is Phe. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence depicted in FIG. 2EE, where amino acid 120 is Tyr. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence depicted in FIG. 2EE, where amino acid 120 is Trp. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence depicted in FIG. 2EE, where amino acid 120 is Ser. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence depicted in FIG. 2EE, where amino acid 120 is Thr. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence depicted in FIG. 2EE, where amino acid 120 is Cys. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence depicted in FIG. 2EE, where amino acid 120 is Met. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence depicted in FIG. 2EE, where amino acid 120 is Asn. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence depicted in FIG. 2EE, where amino acid 120 is Gln. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence depicted in FIG. 2EE, where amino acid 120 is Lys. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence depicted in FIG. 2EE, where amino acid 120 is Arg. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence depicted in FIG. 2EE, where amino acid 120 is His. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence depicted in FIG. 2EE, where amino acid 120 is Asp. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence depicted in FIG. 2EE, where amino acid 120 is Glu. In some cases, the variant 4-1BBL polypeptide has a binding affinity to CD137 that is from about 100 nM to about 150 nM, from about 150 nM to about 200 nM, from about 200 nM to about 250 nM, from about 250 nM to about 300 nM, from about 300 nM to about 350 nM, from about 350 nM to about 400 nM, from about 400 nM to about 500 nM, from about 500 nM to about 600 nM, from about 600 nM to about 700 nM, from about 700 nM to about 800 nM, from about 800 nM to about 900 nM, from about 900 nM to about 1 μM, to about 1 μM to about 5 μM, from about 5 μM to about 10 μM, from about 10 μM to about 15 μM, from about 15 μM to about 20 μM, from about 20 μM to about 25 μM, from about 25 μM to about 50 μM, from about 50 μM to about 75 μM, or from about 75 μM to about 100 μM.

[00164] In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2A, with an amino acid substitution at L120. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence set forth in SEQ ID NO:1, with an amino acid substitution at L40. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises the amino acid sequence set forth in SEQ ID NO:1, with an amino acid substitution at L40. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence set forth in SEQ ID NO:2, with an amino acid substitution at L41. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises the amino acid sequence set forth in SEQ ID NO:2, with an amino acid substitution L41. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence set forth in SEQ ID NO:3, with an amino acid substitution at L41. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises the amino acid sequence set forth in SEQ ID NO:3, with an amino acid substitution at L41.

[00165] For example, in some cases, a variant 4-1BBL polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2EE, where "x" is any amino acid other than leucine; for example, “x” can be Gly, Ala, Val, Ile, Pro, Phe, Tyr, Trp, Ser, Thr, Cys, Met, Asn, Gln, Lys, Arg, His, Asp, or Glu. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2EE, where “x” is Gly. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2EE, where “x” is Ala. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2EE, where “x” is Val. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2EE, where “x” is Ile. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2EE, where “x” is Pro. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2EE, where “x” is Phe. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2EE, where “x” is Tyr. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2EE, where “x” is Trp. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2EE, where “x” is Ser. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2EE, where “x” is Thr. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2EE, where “x” is Cys. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2EE, where “x” is Met. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2EE, where “x” is Asn. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2EE, where “x” is Gln. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2EE, where “x” is Lys. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2EE, where “x” is Arg. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2EE, where “x” is His. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2EE, where “x” is Asp. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2EE, where “x” is Glu. In some cases, the variant 4-1BBL polypeptide has a binding affinity to CD137 that is from about 100 nM to about 150 nM, from about 150 nM to about 200 nM, from about 200 nM to about 250 nM, from about 250 nM to about 300 nM, from about 300 nM to about 350 nM, from about 350 nM to about 400 nM, from about 400 nM to about 500 nM, from about 500 nM to about 600 nM, from about 600 nM to about 700 nM, from about 700 nM to about 800 nM, from about 800 nM to about 900 nM, from about 900 nM to about 1 μM, to about 1 μM to about 5 μM, from about 5 μM to about 10 μM, from about 10 μM to about 15 μM, from about 15 μM to about 20 μM, from about 20 μM to about 25 μM, from about 25 μM to about 50 μM, from about 50 μM to about 75 μM, or from about 75 μM to about 100 μM.

T121 Substitution

[00166] In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence depicted in FIG. 2FF, where amino acid 121 (indicated by an "x") is an amino acid other than a threonine, e.g., where amino acid 121 is Gly, Ala, Val, Leu, Ile, Pro, Phe, Tyr, Trp, Ser, Cys, Met, Asn, Gln, Lys, Arg, His, Asp, or Glu. The amino acid numbering in FIG. 2FF retains the numbering of FIG. 2A. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence depicted in FIG. 2FF, where amino acid 121 is Gly. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence depicted in FIG. 2FF, where amino acid 121 is Ala. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence depicted in FIG. 2FF, where amino acid 121 is Val. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence depicted in FIG. 2FF, where amino acid 121 is Leu. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence depicted in FIG. 2FF, where amino acid 121 is Ile. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence depicted in FIG. 2FF, where amino acid 121 is Pro. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence depicted in FIG. 2FF, where amino acid 121 is Phe. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence depicted in FIG. 2FF, where amino acid 121 is Tyr. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence depicted in FIG. 2FF, where amino acid 121 is Trp. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence depicted in FIG. 2FF, where amino acid 121 is Ser. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence depicted in FIG. 2FF, where amino acid 121 is Cys. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence depicted in FIG. 2FF, where amino acid 121 is Met. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence depicted in FIG. 2FF, where amino acid 121 is Asn. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence depicted in FIG. 2FF, where amino acid 121 is Gln. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence depicted in FIG. 2FF, where amino acid 121 is Lys. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence depicted in FIG. 2FF, where amino acid 121 is Arg. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence depicted in FIG. 2FF, where amino acid 121 is His. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence depicted in FIG. 2FF, where amino acid 121 is Asp. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence depicted in FIG. 2FF, where amino acid 121 is Glu. In some cases, the variant 4-1BBL polypeptide has a binding affinity to CD137 that is from about 100 nM to about 150 nM, from about 150 nM to about 200 nM, from about 200 nM to about 250 nM, from about 250 nM to about 300 nM, from about 300 nM to about 350 nM, from about 350 nM to about 400 nM, from about 400 nM to about 500 nM, from about 500 nM to about 600 nM, from about 600 nM to about 700 nM, from about 700 nM to about 800 nM, from about 800 nM to about 900 nM, from about 900 nM to about 1 μM, to about 1 μM to about 5 μM, from about 5 μM to about 10 μM, from about 10 μM to about 15 μM, from about 15 μM to about 20 μM, from about 20 μM to about 25 μM, from about 25 μM to about 50 μM, from about 50 μM to about 75 μM, or from about 75 μM to about 100 μM.

[00167] In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2A, with an amino acid substitution at T121. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence set forth in SEQ ID NO:1, with an amino acid substitution at T41. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises the amino acid sequence set forth in SEQ ID NO:1, with an amino acid substitution at T41. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence set forth in SEQ ID NO:2, with an amino acid substitution at T42. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises the amino acid sequence set forth in SEQ ID NO:2, with an amino acid substitution T42. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence set forth in SEQ ID NO:3, with an amino acid substitution at T42. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises the amino acid sequence set forth in SEQ ID NO:3, with an amino acid substitution at T42.

[00168] For example, in some cases, a variant 4-1BBL polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2FF, where "x" is any amino acid other than threonine; for example, "x" can be Gly, Ala, Val, Leu, Ile, Pro, Phe, Tyr, Trp, Ser, Cys, Met, Asn, Gln, Lys, Arg, His, Asp, or Glu. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2FF, where "x" is Gly. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2FF, where "x" is Ala. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2FF, where "x" is Val. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2FF, where "x" is Leu. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2FF, where “x” is Ile. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2FF, where “x” is Pro. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2FF, where “x” is Phe. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2FF, where “x” is Tyr. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2FF, where “x” is Trp. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2FF, where “x” is Ser. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2FF, where “x” is Cys. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2FF, where “x” is Met. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2FF, where “x” is Asn. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2FF, where “x” is Gln. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2FF, where “x” is Lys. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2FF, where “x” is Arg. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2FF, where “x” is His. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2FF, where “x” is Asp. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2FF, where “x” is Glu. In some cases, the variant 4-1BBL polypeptide has a binding affinity to CD137 that is from about 100 nM to about 150 nM, from about 150 nM to about 200 nM, from about 200 nM to about 250 nM, from about 250 nM to about 300 nM, from about 300 nM to about 350 nM, from about 350 nM to about 400 nM, from about 400 nM to about 500 nM, from about 500 nM to about 600 nM, from about 600 nM to about 700 nM, from about 700 nM to about 800 nM, from about 800 nM to about 900 nM, from about 900 nM to about 1 μM, to about 1 μM to about 5 μM, from about 5 μM to about 10 μM, from about 10 μM to about 15 μM, from about 15 μM to about 20 μM, from about 20 μM to about 25 μM, from about 25 μM to about 50 μM, from about 50 μM to about 75 μM, or from about 75 μM to about 100 μM.

G122 Substitution

[00169] In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence depicted in FIG. 2GG, where amino acid 122 (indicated by an "x") is an amino acid other than a glycine, e.g., where amino acid 122 is Ala, Val, Leu, Ile, Pro, Phe, Tyr, Trp, Ser, Thr, Cys, Asn, Gln, Lys, Arg, His, Asp, or Glu. The amino acid numbering in FIG. 2GG retains the numbering of FIG. 2A. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence depicted in FIG. 2GG, where amino acid 122 is Ala. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence depicted in FIG. 2GG, where amino acid 122 is Val. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence depicted in FIG. 2GG, where amino acid 122 is Leu. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence depicted in FIG. 2GG, where amino acid 122 is Ile. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence depicted in FIG. 2GG, where amino acid 122 is Pro. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence depicted in FIG. 2GG, where amino acid 122 is Phe. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence depicted in FIG. 2GG, where amino acid 122 is Tyr. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence depicted in FIG. 2GG, where amino acid 122 is Trp. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence depicted in FIG. 2GG, where amino acid 122 is Ser. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence depicted in FIG. 2GG, where amino acid 122 is Thr. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence depicted in FIG. 2GG, where amino acid 122 is Cys. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence depicted in FIG. 2GG, where amino acid 122 is Met. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence depicted in FIG. 2GG, where amino acid 122 is Asn. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence depicted in FIG. 2GG, where amino acid 122 is Gln. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence depicted in FIG. 2GG, where amino acid 122 is Lys. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence depicted in FIG. 2GG, where amino acid 122 is Arg. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence depicted in FIG. 2GG, where amino acid 122 is His. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence depicted in FIG. 2GG, where amino acid 122 is Asp. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence depicted in FIG. 2GG, where amino acid 122 is Glu. In some cases, the variant 4-1BBL polypeptide has a binding affinity to CD137 that is from about 100 nM to about 150 nM, from about 150 nM to about 200 nM, from about 200 nM to about 250 nM, from about 250 nM to about 300 nM, from about 300 nM to about 350 nM, from about 350 nM to about 400 nM, from about 400 nM to about 500 nM, from about 500 nM to about 600 nM, from about 600 nM to about 700 nM, from about 700 nM to about 800 nM, from about 800 nM to about 900 nM, from about 900 nM to about 1 μM, to about 1 μM to about 5 μM, from about 5 μM to about 10 μM, from about 10 μM to about 15 μM, from about 15 μM to about 20 μM, from about 20 μM to about 25 μM, from about 25 μM to about 50 μM, from about 50 μM to about 75 μM, or from about 75 μM to about 100 μM.

[00170] In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2A, with an amino acid substitution at G122. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence set forth in SEQ ID NO:1, with an amino acid substitution at G42. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises the amino acid sequence set forth in SEQ ID NO:1, with an amino acid substitution at G42. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence set forth in SEQ ID NO:2, with an amino acid substitution at G43. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises the amino acid sequence set forth in SEQ ID NO:2, with an amino acid substitution G43. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence set forth in SEQ ID NO:3, with an amino acid substitution at G43. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises the amino acid sequence set forth in SEQ ID NO:3, with an amino acid substitution at G43.

[00171] For example, in some cases, a variant 4-1BBL polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2GG, where “x” is any amino acid other than glycine; for example, “x” can be Ala, Val, Leu, Ile, Pro, Phe, Tyr, Trp, Ser, Thr, Cys, Asn, Gln, Lys, Arg, His, Asp, or Glu. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2GG, where “x” is Ala. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2GG, where “x” is Val. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2GG, where “x” is Leu. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2GG, where “x” is Ile. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2GG, where “x” is Pro. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2GG, where “x” is Phe. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2GG, where “x” is Tyr. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2GG, where “x” is Trp. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2GG, where “x” is Ser. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2GG, where “x” is Thr. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2GG, where “x” is Cys. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2GG, where “x” is Met. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2GG, where “x” is Asn. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2GG, where “x” is Gln. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2GG, where “x” is Lys. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2GG, where “x” is Arg. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2GG, where “x” is His. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2GG, where “x” is Asp. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2GG, where “x” is Glu. In some cases, the variant 4-1BBL polypeptide has a binding affinity to CD137 that is from about 100 nM to about 150 nM, from about 150 nM to about 200 nM, from about 200 nM to about 250 nM, from about 250 nM to about 300 nM, from about 300 nM to about 350 nM, from about 350 nM to about 400 nM, from about 400 nM to about 500 nM, from about 500 nM to about 600 nM, from about 600 nM to about 700 nM, from about 700 nM to about 800 nM, from about 800 nM to about 900 nM, from about 900 nM to about 1 μM, to about 1 μM to about 5 μM, from about 5 μM to about 10 μM, from about 10 μM to about 15 μM, from about 15 μM to about 20 μM, from about 20 μM to about 25 μM, from about 25 μM to about 50 μM, from about 50 μM to about 75 μM, or from about 75 μM to about 100 μM.

G123 Substitution

[00172] In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence depicted in FIG. 2HH, where amino acid 123 (indicated by an “x”) is an amino acid other than a glycine, e.g., where amino acid 123 is Ala, Val, Leu, Ile, Pro, Phe, Tyr, Trp, Ser, Thr, Cys, Asn, Gln, Lys, Arg, His, Asp, or Glu. The amino acid numbering in FIG. 2HH retains the numbering of FIG. 2A. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence depicted in FIG. 2HH, where amino acid 123 is Ala. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at

least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence depicted in FIG. 2HH, where amino acid 123 is Val. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence depicted in FIG. 2HH, where amino acid 123 is Leu. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence depicted in FIG. 2HH, where amino acid 123 is Ile. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence depicted in FIG. 2HH, where amino acid 123 is Pro. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence depicted in FIG. 2HH, where amino acid 123 is Phe. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence depicted in FIG. 2HH, where amino acid 123 is Tyr. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence depicted in FIG. 2HH, where amino acid 123 is Trp. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence depicted in FIG. 2HH, where amino acid 123 is Ser. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence depicted in FIG. 2HH, where amino acid 123 is Thr. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence depicted in FIG. 2HH, where amino acid 123 is Cys. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence depicted in FIG. 2HH, where amino acid 123 is Met. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence depicted in FIG. 2HH, where amino acid 123 is Asn. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence depicted in FIG. 2HH, where amino acid 123 is Gln. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence depicted in FIG. 2HH, where amino acid 123 is Lys. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence depicted in FIG. 2HH, where amino acid 123 is Arg. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence depicted in FIG. 2HH, where amino acid 123 is His. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence depicted in FIG. 2HH, where amino acid 123 is Asp. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence depicted in FIG. 2HH, where amino acid 123 is Glu. In some cases, the variant 4-1BBL polypeptide has a binding affinity to CD137 that is from about 100 nM to about 150 nM, from about 150 nM to about 200 nM, from about 200 nM to about 250 nM, from about 250 nM to about 300 nM, from about 300 nM to about 350 nM, from about 350 nM to about 400 nM, from about 400 nM to about 500 nM, from about 500 nM to about 600 nM, from about 600 nM to about 700 nM, from about 700 nM to about 800 nM, from about 800 nM to about 900 nM, from about 900 nM to about 1 μM, to about 1 μM to about 5 μM, from about 5 μM to about 10 μM, from about 10 μM to about 15 μM, from about 15 μM to about 20 μM, from about 20 μM to about 25 μM, from about 25 μM to about 50 μM, from about 50 μM to about 75 μM, or from about 75 μM to about 100 μM.

[00173] In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2A, with an amino acid substitution at G123. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence set forth in SEQ ID NO:1, with an amino acid substitution at G43. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises the amino acid sequence set forth in SEQ ID NO:1, with an amino acid substitution at G43. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence set forth in SEQ ID NO:2, with an amino acid substitution at G44. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises the amino acid sequence set forth in SEQ ID NO:2, with an amino acid substitution G44. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence set forth in SEQ ID NO:3, with an amino acid substitution at G44. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises the amino acid sequence set forth in SEQ ID NO:3, with an amino acid substitution at G44.

[00174] For example, in some cases, a variant 4-1BBL polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2HH, where “x” is any amino acid other than glycine; for example, “x” can be Ala, Val, Leu, Ile, Pro, Phe, Tyr, Trp, Ser, Thr, Cys, Asn, Gln, Lys, Arg, His, Asp, or Glu. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2HH, where “x” is Ala. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2HH, where “x” is Val. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2HH, where “x” is Leu. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2HH, where “x” is Ile. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2HH, where “x” is Pro. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2HH, where “x” is Phe. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2HH, where “x” is Tyr. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2HH, where “x” is Trp. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2HH, where “x” is Ser. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2HH, where “x” is Thr. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2HH, where “x” is Cys. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2HH, where “x” is Met. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2HH, where “x” is Asn. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2HH, where “x” is Gln. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2HH, where “x” is Lys. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2HH, where “x” is Arg. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2HH, where “x” is His. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2HH, where “x” is Asp. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2HH, where “x” is Glu. In some cases, the variant 4-1BBL polypeptide has a binding affinity to CD137 that is from about 100 nM to about 150 nM, from about 150 nM to about 200 nM, from about 200 nM to about 250 nM, from about 250 nM to about 300 nM, from about 300 nM to about 350 nM, from about 350 nM to about 400 nM, from about 400 nM to about 500 nM, from about 500 nM to about 600 nM, from about 600 nM to about 700 nM, from about 700 nM to about 800 nM, from about 800 nM to about 900 nM, from about 900 nM to about 1 μM, to about 1 μM to about 5 μM, from about 5 μM to about 10 μM, from about 10 μM to about 15 μM, from about 15 μM to about 20 μM, from about 20 μM to about 25 μM, from about 25 μM to about 50 μM, from about 50 μM to about 75 μM, or from about 75 μM to about 100 μM.

L124 Substitution

[00175] In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence depicted in FIG. 2II, where amino acid 124 (indicated by an “x”) is an amino acid other than a leucine, e.g., where amino acid 124 is Gly, Ala, Val, Ile, Pro, Phe, Tyr, Trp, Ser, Thr, Cys, Met, Asn, Gln, Lys, Arg, His, Asp, or Glu. The amino acid numbering in FIG. 2II retains the numbering of FIG. 2A. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence depicted in FIG. 2II, where amino acid 124 is Gly. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence depicted in FIG. 2II, where amino acid 124 is Ala. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence depicted in FIG. 2II, where amino acid 124 is Val. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence depicted in FIG. 2II, where amino acid 124 is Ile. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence depicted in FIG. 2II, where amino acid 124 is Pro. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence depicted in FIG. 2II, where amino acid 124 is Phe. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence depicted in FIG. 2II, where amino acid 124 is Tyr. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence depicted in FIG. 2II, where amino acid 124 is Trp. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence depicted in FIG. 2II, where amino acid 124 is Ser. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence depicted in FIG. 2II, where amino acid 124 is Thr. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence depicted in FIG. 2II, where amino acid 124 is Cys. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence depicted in FIG. 2II, where amino acid 124 is Met. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence depicted in FIG. 2II, where amino acid 124 is Asn. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence depicted in FIG. 2II, where amino acid 124 is Gln. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence depicted in FIG. 2II, where amino acid 124 is Lys. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence depicted in FIG. 2II, where amino acid 124 is Arg. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence depicted in FIG. 2II, where amino acid 124 is His. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence depicted in FIG. 2II, where amino acid 124 is Asp. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence depicted in FIG. 2II, where amino acid 124 is Glu. In some cases, the variant 4-1BBL polypeptide has a binding affinity to CD137 that is from about 100 nM to about 150 nM, from about 150 nM to about 200 nM, from about 200 nM to about 250 nM, from about 250 nM to about 300 nM, from about 300 nM to about 350 nM, from about 350 nM to about 400 nM, from about 400 nM to about 500 nM, from about 500 nM to about 600 nM, from about 600 nM to about 700 nM, from about 700 nM to about 800 nM, from about 800 nM to about 900 nM, from about 900 nM to about 1 µM, to about 1 µM to about 5 µM, from about 5 µM to about 10 µM, from about 10 µM to about 15 µM, from about 15 µM to about 20 µM, from about 20 µM to about 25 µM, from about 25 µM to about 50 µM, from about 50 µM to about 75 µM, or from about 75 µM to about 100 µM.

[00176] In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2A, with an amino acid substitution at L124. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence set forth in SEQ ID NO:1, with an amino acid substitution at L44. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises the amino acid sequence set forth in SEQ ID NO:1, with an amino acid substitution at L44. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence set forth in SEQ ID NO:2, with an amino acid substitution at L45. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises the amino acid sequence set forth in SEQ ID NO:2, with an amino acid substitution L45. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence set forth in SEQ ID NO:3, with an amino acid substitution at L45. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises the amino acid sequence set forth in SEQ ID NO:3, with an amino acid substitution at L45.

[00177] For example, in some cases, a variant 4-1BBL polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2II, where “x” is any amino acid other than leucine; for example, “x” can be Gly, Ala, Val, Ile, Pro, Phe, Tyr, Trp, Ser, Thr, Cys, Met, Asn, Gln, Lys, Arg, His, Asp, or Glu. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2II, where “x” is Gly. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2II, where “x” is Ala. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2II, where “x” is Val. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2II, where “x” is Ile. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2II, where “x” is Pro. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2II, where “x” is Phe. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2II, where “x” is Tyr. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2II, where “x” is Trp. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2II, where “x” is Ser. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2II, where “x” is Thr. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2II, where “x” is Cys. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2II, where “x” is Met. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2II, where “x” is Asn. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2II, where “x” is Gln. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2II, where “x” is Lys. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2II, where “x” is Arg. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2II, where “x” is His. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2II, where “x” is Asp. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2II, where “x” is Glu. In some cases, the variant 4-1BBL polypeptide has a binding affinity to CD137 that is from about 100 nM to about 150 nM, from about 150 nM to about 200 nM, from about 200 nM to about 250 nM, from about 250 nM to about 300 nM, from about 300 nM to about 350 nM, from about 350 nM to about 400 nM, from about 400 nM to about 500 nM, from about 500 nM to about 600 nM, from about 600 nM to about 700 nM, from about 700 nM to about 800 nM, from about 800 nM to about 900 nM, from about 900 nM to about 1 μM, to about 1 μM to about 5 μM, from about 5 μM to about 10 μM, from about 10 μM to about 15 μM, from about 15 μM to about 20 μM, from about 20 μM to about 25 μM, from about 25 μM to about 50 μM, from about 50 μM to about 75 μM, or from about 75 μM to about 100 μM.

S125 Substitution

[00178] In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence depicted in FIG. 2JJ, where amino acid 125 (indicated by an “x”) is an amino acid other than a serine, e.g., where amino acid 125 is Gly, Ala, Val, Leu, Ile, Pro, Phe, Tyr, Trp, Thr, Cys, Met, Asn, Gln, Lys, Arg, His, Asp, or Glu. The amino acid numbering in FIG. 2JJ retains the numbering of FIG. 2A. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence depicted in FIG. 2JJ, where amino acid 125 is Gly. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence depicted in FIG. 2JJ, where amino acid 125 is Ala. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence depicted in FIG. 2DD, where amino acid 125 is Val. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence depicted in FIG. 2DD, where amino acid 125 is Leu. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence depicted in FIG. 2JJ, where amino acid 125 is Ile. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence depicted in FIG. 2JJ, where amino acid 125 is Pro. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence depicted in FIG. 2JJ, where amino acid 125 is Phe. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence depicted in FIG. 2JJ, where amino acid 125 is Tyr. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence depicted in FIG. 2JJ, where amino acid 125 is Trp. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence depicted in FIG. 2JJ, where amino acid 125 is Thr. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence depicted in FIG. 2JJ, where amino acid 125 is Cys. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence depicted in FIG. 2JJ, where amino acid 125 is Met. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence depicted in FIG. 2JJ, where amino acid 125 is Asn. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence depicted in FIG. 2JJ, where amino acid 125 is Gln. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence depicted in FIG. 2JJ, where amino acid 125 is Lys. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence depicted in FIG. 2JJ, where amino acid 125 is Arg. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence depicted in FIG. 22JJ DD, where amino acid 125 is His. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence depicted in FIG. 2JJ, where amino acid 125 is Asp. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence depicted in FIG. 2JJ, where amino acid 125 is Glu. In some cases, the variant 4-1BBL polypeptide has a binding affinity to CD137 that is from about 100 nM to about 150 nM, from about 150 nM to about 200 nM, from about 200 nM to about 250 nM, from about 250 nM to about 300 nM, from about 300 nM to about 350 nM, from about 350 nM to about 400 nM, from about 400 nM to about 500 nM, from about 500 nM to about 600 nM, from about 600 nM to about 700 nM, from about 700 nM to about 800 nM, from about 800 nM to about 900 nM, from about 900 nM to about 1 μM, to about 1 μM to about 5 μM, from about 5 μM to about 10 μM, from about 10 μM to about 15 μM, from about 15 μM to about 20 μM, from about 20 μM to about 25 μM, from about 25 μM to about 50 μM, from about 50 μM to about 75 μM, or from about 75 μM to about 100 μM.

[00179] In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2A, with an amino acid substitution at S125. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence set forth in SEQ ID NO:1, with an amino acid substitution at S45. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises the amino acid sequence set forth in SEQ ID NO:1, with an amino acid substitution at S45. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence set forth in SEQ ID NO:2, with an amino acid substitution at S46. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises the amino acid sequence set forth in SEQ ID NO:2, with an amino acid substitution S46. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence set forth in SEQ ID NO:3, with an amino acid substitution at S46. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises the amino acid sequence set forth in SEQ ID NO:3, with an amino acid substitution at S46.

[00180] For example, in some cases, a variant 4-1BBL polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2JJ, where "x" is any amino acid other than serine; for example, "x" can be Gly, Ala, Val, Leu, Ile, Pro, Phe, Tyr, Trp, Thr, Cys, Met, Asn, Gln, Lys, Arg, His, Asp, or Glu. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2JJ, where "x" is Gly. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2JJ, where "x" is Ala. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2JJ, where “x” is Val. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2JJ, where “x” is Leu. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2JJ, where “x” is Ile. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2JJ, where “x” is Pro. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2JJ, where “x” is Phe. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2JJ, where “x” is Tyr. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2JJ, where “x” is Trp. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2JJ, where “x” is Thr. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2JJ, where “x” is Cys. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2JJ, where “x” is Met. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2JJ, where “x” is Asn. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2JJ, where “x” is Gln. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2JJ, where “x” is Lys. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2JJ, where “x” is Arg. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2JJ, where “x” is His. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2JJ, where “x” is Asp. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2JJ, where “x” is Glu. In some cases, the variant 4-1BBL polypeptide has a binding affinity to CD137 that is from about 100 nM to about 150 nM, from about 150 nM to about 200 nM, from about 200 nM to about 250 nM, from about 250 nM to about 300 nM, from about 300 nM to about 350 nM, from about 350 nM to about 400 nM, from about 400 nM to about 500 nM, from about 500 nM to about 600 nM, from about 600 nM to about 700 nM, from about 700 nM to about 800 nM, from about 800 nM to about 900 nM, from about 900 nM to about 1 μM, to about 1 μM to about 5 μM, from about 5 μM to about 10 μM, from about 10 μM to about 15 μM, from about 15 μM to about 20 μM, from about 20 μM to about 25 μM, from about 25 μM to about 50 μM, from about 50 μM to about 75 μM, or from about 75 μM to about 100 μM.

Y126 Substitution

[00181] In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence depicted in FIG. 2KK, where amino acid 126 (indicated by an “x”) is an amino acid other than a tyrosine, e.g., where amino acid 126 is Gly, Ala, Val, Leu, Ile, Pro, Phe, Trp, Ser, Thr, Cys, Met, Asn, Gln, Lys, Arg, His, Asp, or Glu. The amino acid numbering in FIG. 2KK retains the numbering of FIG. 2A. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence depicted in FIG. 2KK, where amino acid 126 is Gly. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence depicted in FIG. 2KK, where amino acid 126 is Ala. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence depicted in FIG. 2KK, where amino acid 126 is Val. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence depicted in FIG. 2KK, where amino acid 126 is Leu. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence depicted in FIG. 2KK, where amino acid 126 is Ile. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence depicted in FIG. 2KK, where amino acid 126 is Pro. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence depicted in FIG. 2KK, where amino acid 126 is Phe. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence depicted in FIG. 2KK, where amino acid 126 is Trp. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence depicted in FIG. 2KK, where amino acid 126 is Ser. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence depicted in FIG. 2KK, where amino acid 126 is Thr. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence depicted in FIG. 2KK, where amino acid 126 is Cys. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence depicted in FIG. 2KK, where amino acid 126 is Met. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence depicted in FIG. 2KK, where amino acid 126 is Asn. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence depicted in FIG. 2KK, where amino acid 126 is Gln. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence depicted in FIG. 2KK, where amino acid 126 is Lys. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence depicted in FIG. 2KK, where amino acid 126 is Arg. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence depicted in FIG. 2KK, where amino acid 126 is His. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence depicted in FIG. 2KK, where amino acid 126 10 is Asp. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence depicted in FIG. 2KK, where amino acid 126 is Glu. In some cases, the variant 4-1BBL polypeptide has a binding affinity to CD137 that is from about 100 nM to about 150 nM, from about 150 nM to about 200 nM, from about 200 nM to about 250 nM, from about 250 nM to about 300 nM, from about 300 nM to about 350 nM, from about 350 nM to about 400 nM, from about 400 nM to about 500 nM, from about 500 nM to about 600 nM, from about 600 nM to about 700 nM, from about 700 nM to about 800 nM, from about 800 nM to about 900 nM, from about 900 nM to about 1 μM, to about 1 μM to about 5 μM, from about 5 μM to about 10 μM, from about 10 μM to about 15 μM, from about 15 μM to about 20 μM, from about 20 μM to about 25 μM, from about 25 μM to about 50 μM, from about 50 μM to about 75 μM, or from about 75 μM to about 100 μM.

[00182] In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2A, with an amino acid substitution at Y126. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence set forth in SEQ ID NO:1, with an amino acid substitution at Y46. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises the amino acid sequence set forth in SEQ ID NO:1, with an amino acid substitution at Y46. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence set forth in SEQ ID NO:2, with an amino acid substitution at Y47. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises the amino acid sequence set forth in SEQ ID NO:2, with an amino acid substitution Y47. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence set forth in SEQ ID NO:3, with an amino acid substitution at Y47. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises the amino acid sequence set forth in SEQ ID NO:3, with an amino acid substitution at Y47.

[00183] For example, in some cases, a variant 4-1BBL polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2KK, where “x” is any amino acid other than tyrosine; for example, “x” can be Gly, Ala, Val, Leu, Ile, Pro, Phe, Trp, Ser, Thr, Cys, Met, Asn, Gln, Lys, Arg, His, Asp, or Glu. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2KK, where “x” is Gly. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2KK, where “x” is Ala. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2KK, where “x” is Val. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2KK, where “x” is Leu. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2KK, where “x” is Ile. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2KK, where “x” is Pro. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2KK, where “x” is Phe. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2KK, where “x” is Trp. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2KK, where “x” is Ser. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2KK, where “x” is Thr. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2KK, where “x” is Cys. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2KK, where “x” is Met. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2KK, where “x” is Asn. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2KK, where “x” is Gln. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2KK, where “x” is Lys. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2KK, where “x” is Arg. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2KK, where “x” is His. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2V2KK where “x” is Asp. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2KK, where “x” is Glu. In some cases, the variant 4-1BBL polypeptide has a binding affinity to CD137 that is from about 100 nM to about 150 nM, from about 150 nM to about 200 nM, from about 200 nM to about 250 nM, from about 250 nM to about 300 nM, from about 300 nM to about 350 nM, from about 350 nM to about 400 nM, from about 400 nM to about 500 nM, from about 500 nM to about 600 nM, from about 600 nM to about 700 nM, from about 700 nM to about 800 nM, from about 800 nM to about 900 nM, from about 900 nM to about 1 μM, to about 1 μM to about 5 μM, from about 5 μM to about 10 μM, from about 10 μM to about 15 μM, from about 15 μM to about 20 μM, from about 20 μM to about 25 μM, from about 25 μM to about 50 μM, from about 50 μM to about 75 μM, or from about 75 μM to about 100 μM.

E128 Substitution

[00184] In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence depicted in FIG. 2LL, where amino acid 128 (indicated by an “x”) is an amino acid other than a glutamic acid, e.g., where amino acid 128 is Gly, Ala, Val, Leu, Ile, Pro, Phe, Tyr, Trp, Ser, Thr, Cys, Met, Asn, Gln, Lys, Arg, His, or Asp. The amino acid numbering in FIG. 2LL retains the numbering of FIG. 2A. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence depicted in FIG. 2LL, where amino acid 128 is Gly. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence depicted in FIG. 2LL, where amino acid 128 is Ala. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence depicted in FIG. 2LL, where amino acid 128 is Val. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence depicted in FIG. 2LL, where amino acid 128 is Leu. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence depicted in FIG. 2LL, where amino acid 128 is Ile. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence depicted in FIG. 2LL, where amino acid 128 is Pro. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence depicted in FIG. 2LL, where amino acid 128 is Phe. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence depicted in FIG. 2LL, where amino acid 128 is Tyr. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence depicted in FIG. 2LL, where amino acid 128 is Trp. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence depicted in FIG. 2LL, where amino acid 128 is Ser. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence depicted in FIG. 2LL, where amino acid 128 is Thr. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence depicted in FIG. 2LL, where amino acid 128 is Cys. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence depicted in FIG. 2LL, where amino acid 128 is Met. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence depicted in FIG. 2LL, where amino acid 128 is Asn. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence depicted in FIG. 2LL, where amino acid 128 is Gln. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence depicted in FIG. 2LL, where amino acid 128 is Lys. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence depicted in FIG. 2LL, where amino acid 128 is Arg. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence depicted in FIG. 2LL, where amino acid 128 is His. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence depicted in FIG. 2LL, where amino acid 128 is Asp. In some cases, the variant 4-1BBL polypeptide has a binding affinity to CD137 that is from about 100 nM to about 150 nM, from about 150 nM to about 200 nM, from about 200 nM to about 250 nM, from about 250 nM to about 300 nM, from about 300 nM to about 350 nM, from about 350 nM to about 400 nM, from about 400 nM to about 500 nM, from about 500 nM to about 600 nM, from about 600 nM to about 700 nM, from about 700 nM to about 800 nM, from about 800 nM to about 900 nM, from about 900 nM to about 1 μM, to about 1 μM to about 5 μM, from about 5 μM to about 10 μM, from about 10 μM to about 15 μM, from about 15 μM to about 20 μM, from about 20 μM to about 25 μM, from about 25 μM to about 50 μM, from about 50 μM to about 75 μM, or from about 75 μM to about 100 μM.

[00185] In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2A, with an amino acid substitution at E128. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence set forth in SEQ ID NO:1, with an amino acid substitution at E48. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises the amino acid sequence set forth in SEQ ID NO:1, with an amino acid substitution at E48. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence set forth in SEQ ID NO:2, with an amino acid substitution at E49. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises the amino acid sequence set forth in SEQ ID NO:2, with an amino acid substitution E49. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence set forth in SEQ ID NO:3, with an amino acid substitution at E49. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises the amino acid sequence set forth in SEQ ID NO:3, with an amino acid substitution at E49.

[00186] For example, in some cases, a variant 4-1BBL polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2LL, where “x” is any amino acid other than glutamic acid; for example, “x” can be Gly, Ala, Val, Leu, Ile, Pro, Phe, Tyr, Trp, Ser, Thr, Cys, Met, Asn, Gln, Lys, Arg, His, or Asp. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2LL, where “x” is Gly. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2LL, where “x” is Ala. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2LL, where “x” is Val. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2LL, where “x” is Leu. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2LL, where “x” is Ile. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2LL, where “x” is Pro. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2LL, where “x” is Phe. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2LL, where “x” is Tyr. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2LL, where “x” is Trp. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2LL, where “x” is Ser. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2LL, where “x” is Thr. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2LL, where “x” is Cys. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2LL, where "x" is Met. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2LL, where "x" is Asn. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2LL, where "x" is Gln. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2LL, where "x" is Lys. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2LL, where "x" is Arg. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2LL, where "x" is His. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2LL, where "x" is Asp. In some cases, the variant 4-1BBL polypeptide has a binding affinity to CD137 that is from about 100 nM to about 150 nM, from about 150 nM to about 200 nM, from about 200 nM to about 250 nM, from about 250 nM to about 300 nM, from about 300 nM to about 350 nM, from about 350 nM to about 400 nM, from about 400 nM to about 500 nM, from about 500 nM to about 600 nM, from about 600 nM to about 700 nM, from about 700 nM to about 800 nM, from about 800 nM to about 900 nM, from about 900 nM to about 1 μM, to about 1 μM to about 5 μM, from about 5 μM to about 10 μM, from about 10 μM to about 15 μM, from about 15 μM to about 20 μM, from about 20 μM to about 25 μM, from about 25 μM to about 50 μM, from about 50 μM to about 75 μM, or from about 75 μM to about 100 μM.

D129 Substitution

[00187] In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence depicted in FIG. 2MM, where amino acid 129 (indicated by an "x") is an amino acid other than an aspartic acid, e.g., where amino acid 129 is Gly, Ala, Val, Leu, Ile, Pro, Phe, Tyr, Trp, Ser, Thr, Cys, Met, Asn, Gln, Lys, Arg, His, or Glu. The amino acid numbering in FIG. 2MM retains the numbering of FIG. 2A. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence depicted in FIG. 2MM, where amino acid 129 is Gly. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence depicted in FIG. 2MM, where amino acid 129 is Ala. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence depicted in FIG. 2MM, where amino acid 129 is Val. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence depicted in FIG. 2MM, where amino acid 129 is Leu. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence depicted in FIG. 2MM, where amino acid 129 is Ile. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence depicted in FIG. 2MM, where amino acid 129 is Pro. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence depicted in FIG. 2MM, where amino acid 129 is Phe. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence depicted in FIG. 2MM, where amino acid 129 is Tyr. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence depicted in FIG. 2MM, where amino acid 129 is Trp. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence depicted in FIG. 2MM, where amino acid 129 is Ser. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence depicted in FIG. 2MM, where amino acid 129 is Thr. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence depicted in FIG. 2MM, where amino acid 129 is Cys. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence depicted in FIG. 2MM, where amino acid 129 is Met. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence depicted in FIG. 2MM, where amino acid 129 is Asn. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence depicted in FIG. 2MM, where amino acid 129 is Gln. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence depicted in FIG. 2MM, where amino acid 129 is Lys. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence depicted in FIG. 2MM, where amino acid 129 is Arg. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence depicted in FIG. 2MM, where amino acid 129 is His. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence depicted in FIG. 2MM, where amino acid 2MM is Glu. In some cases, the variant 4-1BBL polypeptide has a binding affinity to CD137 that is from about 100 nM to about 150 nM, from about 150 nM to about 200 nM, from about 200 nM to about 250 nM, from about 250 nM to about 300 nM, from about 300 nM to about 350 nM, from about 350 nM to about 400 nM, from about 400 nM to about 500 nM, from about 500 nM to about 600 nM, from about 600 nM to about 700 nM, from about 700 nM to about 800 nM, from about 800 nM to about 900 nM, from about 900 nM to about 1 μM, to about 1 μM to about 5 μM, from about 5 μM to about 10 μM, from about 10 μM to about 15 μM, from about 15 μM to about 20 μM, from about 20 μM to about 25 μM, from about 25 μM to about 50 μM, from about 50 μM to about 75 μM, or from about 75 μM to about 100 μM.

[00188] In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2A, with an amino acid substitution at D129. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence set forth in SEQ ID NO:1, with an amino acid substitution at D49. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises the amino acid sequence set forth in SEQ ID NO:1, with an amino acid substitution at D49. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence set forth in SEQ ID NO:2, with an amino acid substitution at D50. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises the amino acid sequence set forth in SEQ ID NO:2, with an amino acid substitution at D50. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises an amino acid sequence having at least

90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence set forth in SEQ ID NO:3, with an amino acid substitution at D50. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises the amino acid sequence set forth in SEQ ID NO:3, with an amino acid substitution at D50.

[00189] For example, in some cases, a variant 4-1BBL polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2MM, where “x” is any amino acid other than aspartic acid; for example, “x” can be Gly, Ala, Val, Leu, Ile, Pro, Phe, Tyr, Trp, Ser, Thr, Cys, Met, Asn, Gln, Lys, Arg, His, or Glu. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2MM, where “x” is Gly. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2MM, where “x” is Ala. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2MM, where “x” is Val. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2MM, where “x” is Leu. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2MM, where “x” is Ile. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2MM, where “x” is Pro. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2MM, where “x” is Phe. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2MM, where “x” is Tyr. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2MM, where “x” is Trp. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2MM, where “x” is Ser. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2MM, where “x” is Thr. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2MM, where “x” is Cys. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2MM, where “x” is Met. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2MM, where “x” is Asn. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2MM, where “x” is Gln. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2MM, where “x” is Lys. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2MM, where “x” is Arg. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2MM, where “x” is His. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2MM, where “x” is Glu. In some cases, the variant 4-1BBL polypeptide has a binding affinity to CD137 that is from about 100 nM to about 150 nM, from about 150 nM to about 200 nM, from about 200 nM to about 250 nM, from about 250 nM to about 300 nM, from about 300 nM to about 350 nM, from about 350 nM to about 400 nM, from about 400 nM to about 500 nM, from about 500 nM to about 600 nM, from about 600 nM to about 700 nM, from about 700 nM to about 800 nM, from about 800 nM to about 900 nM, from about 900 nM to about 1 μM, to about 1 μM to about 5 μM, from about 5 μM to about 10 μM, from about 10 μM to about 15 μM, from about 15 μM to about 20 μM, from about 20 μM to about 25 μM, from about 25 μM to about 50 μM, from about 50 μM to about 75 μM, or from about 75 μM to about 100 μM.

T130

[00190] In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence depicted in FIG. 2NN, where amino acid 130 (indicated by an “x”) is an amino acid other than a threonine, e.g., where amino acid 130 is Gly, Ala, Val, Leu, Ile, Pro, Phe, Tyr, Trp, Ser, Cys, Met, Asn, Gln, Lys, Arg, His, Asp, or Glu. The amino acid numbering in FIG. 2NN retains the numbering of FIG. 2A. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence depicted in FIG. 2NN, where amino acid 130 is Gly. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence depicted in FIG. 2NN, where amino acid 130 is Ala. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence depicted in FIG. 2NN, where amino acid 130 is Val. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence depicted in FIG. 2NN, where amino acid 130 is Leu. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence depicted in FIG. 2NN, where amino acid 130 is Ile. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence depicted in FIG. 2NN, where amino acid 130 is Pro. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence depicted in FIG. 2NN, where amino acid 130 is Phe. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence depicted in FIG. 2NN, where amino acid 130 is Tyr. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence depicted in FIG. 2NN, where amino acid 130 is Trp. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence depicted in FIG. 2NN, where amino acid 130 is Ser. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence depicted in FIG. 2NN, where amino acid 130 is Cys. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence depicted in FIG. 2NN, where amino acid 130 is Met. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence depicted in FIG. 2NN, where amino acid 130 is Asn. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence depicted in FIG. 2NN, where amino acid 130 is Gln. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence depicted in FIG. 2NN, where amino acid 130 is Lys. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence depicted in FIG. 2NN, where amino acid 130 is Arg. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence depicted in FIG. 2NN, where amino acid 130 is His. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence depicted in FIG. 2NN, where amino acid 130 is Asp. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence depicted in FIG. 2NN, where amino acid 130 is Glu. In some cases, the variant 4-1BBL polypeptide has a binding affinity to CD137 that is from about 100 nM to about 150 nM, from about 150 nM to about 200 nM, from about 200 nM to about 250 nM, from about 250 nM to about 300 nM, from about 300 nM to about 350 nM, from about 350 nM to about 400 nM, from about 400 nM to about 500 nM, from about 500 nM to about 600 nM, from about 600 nM to about 700 nM, from about 700 nM to about 800 nM, from about 800 nM to about 900 nM, from about 900 nM to about 1 μM, to about 1 μM to about 5 μM, from about 5 μM to about 10 μM, from about 10 μM to about 15 μM, from about 15 μM to about 20 μM, from about 20 μM to about 25 μM, from about 25 μM to about 50 μM, from about 50 μM to about 75 μM, or from about 75 μM to about 100 μM.

[00191] In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2A, with an amino acid substitution at T130. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence set forth in SEQ ID NO:1, with an amino acid substitution at T50. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises the amino acid sequence set forth in SEQ ID NO:1, with an amino acid substitution at T50. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence set forth in SEQ ID NO:2, with an amino acid substitution at T51. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises the amino acid sequence set forth in SEQ ID NO:2, with an amino acid substitution at T51. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence set forth in SEQ ID NO:3, with an amino acid substitution at T51. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises the amino acid sequence set forth in SEQ ID NO:3, with an amino acid substitution at T51.

[00192] For example, in some cases, a variant 4-1BBL polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2NN, where “x” is any amino acid other than threonine; for example, “x” can be Gly, Ala, Val, Leu, Ile, Pro, Phe, Tyr, Trp, Ser, Cys, Met, Asn, Gln, Lys, Arg, His, Asp, or Glu. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2NN, where “x” is Gly. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2NN, where “x” is Ala. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2NN, where “x” is Val. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2NN, where “x” is Leu. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2NN, where “x” is Ile. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2NN, where “x” is Pro. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2NN, where “x” is Phe. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2NN, where “x” is Tyr. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2NN, where “x” is Trp. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2NN, where “x” is Ser. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2NN, where “x” is Cys. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2NN, where “x” is Met. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2NN, where “x” is Asn. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2NN, where “x” is Gln. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2NN, where “x” is Lys. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2NN, where “x” is Arg. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2NN, where “x” is His. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2NN, where “x” is Asp. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2NN, where “x” is Glu. In some cases, the variant 4-1BBL polypeptide has a binding affinity to CD137 that is from about 100 nM to about 150 nM, from about 150 nM to about 200 nM, from about 200 nM to about 250 nM, from about 250 nM to about 300 nM, from about 300 nM to about 350 nM, from about 350 nM to about 400 nM, from about 400 nM to about 500 nM, from about 500 nM to about 600 nM, from about 600 nM to about 700 nM, from about 700 nM to about 800 nM, from about 800 nM to about 900 nM, from about 900 nM to about 1 μM, to about 1 μM to about 5 μM, from about 5 μM to about 10 μM, from about 10 μM to about 15 μM, from about 15 μM to about 20 μM, from about 20 μM to about 25 μM, from about 25 μM to about 50 μM, from about 50 μM to about 75 μM, or from about 75 μM to about 100 μM.

K131 Substitution

[00193] In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence depicted in FIG. 2OO, where amino acid 131 (indicated by an "x") is an amino acid other than a lysine, e.g., where amino acid 131 is Gly, Ala, Val, Leu, Ile, Pro, Phe, Tyr, Trp, Ser, Thr, Cys, Met, Asn, Gln, Arg, His, Asp, or Glu. The amino acid numbering in FIG. 2OO retains the numbering of FIG. 2A. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence depicted in FIG. 2OO, where amino acid 131 is Gly. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence depicted in FIG. 2OO, where amino acid 131 is Ala. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence depicted in FIG. 2OO, where amino acid 131 is Val. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence depicted in FIG. 2OO, where amino acid 131 is Leu. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence depicted in FIG. 2OO, where amino acid 131 is Ile. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence depicted in FIG. 2OO, where amino acid 131 is Pro. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence depicted in FIG. 2OO, where amino acid 131 is Phe. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence depicted in FIG. 2OO, where amino acid 131 is Tyr. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence depicted in FIG. 2OO, where amino acid 131 is Trp. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence depicted in FIG. 2OO, where amino acid 131 is Ser. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence depicted in FIG. 2E, where amino acid 131 is Thr. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence depicted in FIG. 2OO, where amino acid 131 is Cys. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence depicted in FIG. 2OO, where amino acid 131 is Met. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence depicted in FIG. 2OO, where amino acid 131 is Asn. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence depicted in FIG. 2OO, where amino acid 131 is Gln. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence depicted in FIG. 2OO, where amino acid 131 is Arg. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence depicted in FIG. 2OO, where amino acid 131 is His. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence depicted in FIG. 2OO, where amino acid 131 is Asp. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence depicted in FIG. 2OO, where amino acid 131 is Glu. In some cases, the variant 4-1BBL polypeptide has a binding affinity to CD137 that is from about 100 nM to about 150 nM, from about 150 nM to about 200 nM, from about 200 nM to about 250 nM, from about 250 nM to about 300 nM, from about 300 nM to about 350 nM, from about 350 nM to about 400 nM, from about 400 nM to about 500 nM, from about 500 nM to about 600 nM, from about 600 nM to about 700 nM, from about 700 nM to about 800 nM, from about 800 nM to about 900 nM, from about 900 nM to about 1 μM, to about 1 μM to about 5 μM, from about 5 μM to about 10 μM, from about 10 μM to about 15 μM, from about 15 μM to about 20 μM, from about 20 μM to about 25 μM, from about 25 μM to about 50 μM, from about 50 μM to about 75 μM, or from about 75 μM to about 100 μM.

[00194] In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2A, with an amino acid substitution at K131. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence set forth in SEQ ID NO:1, with an amino acid substitution at K51. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises the amino acid sequence set forth in SEQ ID NO:1, with an amino acid substitution at K51. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence set forth in SEQ ID NO:2, with an amino acid substitution at K52. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises the amino acid sequence set forth in SEQ ID NO:2, with an amino acid substitution at K52. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence set forth in SEQ ID NO:3, with an amino acid substitution at K52. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises the amino acid sequence set forth in SEQ ID NO:3, with an amino acid substitution at K52.

[00195] For example, in some cases, a variant 4-1BBL polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2OO, where “x” is any amino acid other than lysine; for example, “x” can be Gly, Ala, Val, Leu, Ile, Pro, Phe, Tyr, Trp, Ser, Thr, Cys, Met, Asn, Gln, Arg, His, Asp, or Glu. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2OO, where “x” is Gly. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2OO, where “x” is Ala. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2OO, where “x” is Val. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2OO, where “x” is Leu. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2OO, where “x” is Ile. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2OO, where “x” is Pro. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2OO, where “x” is Phe. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2OO, where “x” is Tyr. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2OO, where “x” is Trp. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2OO, where “x” is Ser. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2OO, where “x” is Thr. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2OO, where “x” is Cys. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2OO, where “x” is Met. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2OO, where “x” is Asn. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2OO, where “x” is Gln. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2OO, where “x” is Arg. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2OO, where “x” is His. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2OO, where “x” is Asp. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2OO, where “x” is Glu. In some cases, the variant 4-1BBL polypeptide has a binding affinity to CD137 that is from about 100 nM to about 150 nM, from about 150 nM to about 200 nM, from about 200 nM to about 250 nM, from about 250 nM to about 300 nM, from about 300 nM to about 350 nM, from about 350 nM to about 400 nM, from about 400 nM to about 500 nM, from about 500 nM to about 600 nM, from about 600 nM to about 700 nM, from about 700 nM to about 800 nM, from about 800 nM to about 900 nM, from about 900 nM to about 1 μM, to about 1 μM to about 5 μM, from about 5 μM to about 10 μM, from about 10 μM to about 15 μM, from about 15 μM to about 20 μM, from about 20 μM to about 25 μM, from about 25 μM to about 50 μM, from about 50 μM to about 75 μM, or from about 75 μM to about 100 μM.

E132 Substitution

[00196] In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence depicted in FIG. 2PP, where amino acid 132 (indicated by an “x”) is an amino acid other than a glutamic acid, e.g., where amino acid 132 is

Gly, Ala, Val, Leu, Ile, Pro, Phe, Tyr, Trp, Ser, Thr, Cys, Met, Asn, Gln, Lys, Arg, His, or Asp. The amino acid numbering in FIG. 2PP retains the numbering of FIG. 2A. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence depicted in FIG. 2PP, where amino acid 132 is Gly. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence depicted in FIG. 2PP, where amino acid 132 is Ala. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence depicted in FIG. 2PP, where amino acid 132 is Val. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence depicted in FIG. 2PP, where amino acid 132 is Leu. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence depicted in FIG. 2PP, where amino acid 132 is Ile. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence depicted in FIG. 2PP, where amino acid 132 is Pro. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence depicted in FIG. 2PP, where amino acid 132 is Phe. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence depicted in FIG. 2PP, where amino acid 132 is Tyr. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence depicted in FIG. 2PP, where amino acid 132 is Trp. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence depicted in FIG. 2PP, where amino acid 132 is Ser. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence depicted in FIG. 2PP, where amino acid 132 is Thr. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence depicted in FIG. 2PP, where amino acid 132 is Cys. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence depicted in FIG. 2PP, where amino acid 132 is Met. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence depicted in FIG. 2PP, where amino acid 132 is Asn. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence depicted in FIG. 2PP, where amino acid 132 is Gln. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence depicted in FIG. 2PP, where amino acid 132 is Lys. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence depicted in FIG. 2PP, where amino acid 132 is Arg. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence depicted in FIG. 2PP, where amino acid 132 is His. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence depicted in FIG. 2PP, where amino acid 132 is Asp. In some cases, the variant 4-1BBL polypeptide has a binding affinity to CD137 that is from about 100 nM to about 150 nM, from about 150 nM to about 200 nM, from about 200 nM to about 250 nM, from about 250 nM to about 300 nM, from about 300 nM to about 350 nM, from about 350 nM to about 400 nM, from about 400 nM to about 500 nM, from about 500 nM to about 600 nM, from about 600 nM to about 700 nM, from about 700 nM to about 800 nM, from about 800 nM to about 900 nM, from about 900 nM to about 1 μM, to about 1 μM to about 5 μM, from about 5 μM to about 10 μM, from about 10 μM to about 15 μM, from about 15 μM to about 20 μM, from about 20 μM to about 25 μM, from about 25 μM to about 50 μM, from about 50 μM to about 75 μM, or from about 75 μM to about 100 μM.

[00197] In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2A, with an amino acid substitution at E132. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence set forth in SEQ ID NO:1, with an amino acid substitution at E52. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises the amino acid sequence set forth in SEQ ID NO:1, with an amino acid substitution at E52. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence set forth in SEQ ID NO:2, with an amino acid substitution at E53. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises the amino acid sequence set forth in SEQ ID NO:2, with an amino acid substitution at E53. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence set forth in SEQ ID NO:3, with an amino acid substitution at E53. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises the amino acid sequence set forth in SEQ ID NO:3, with an amino acid substitution at E53.

[00198] For example, in some cases, a variant 4-1BBL polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2PP, where “x” is any amino acid other than glutamic acid; for example, “x” can be Gly, Ala, Val, Leu, Ile, Pro, Phe, Tyr, Trp, Ser, Thr, Cys, Met, Asn, Gln, Lys, Arg, His, or Asp. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2PP, where “x” is Gly. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2PP, where “x” is Ala. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2L2PP L, where “x” is Val. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2PP, where “x” is Leu. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2PP, where “x” is Ile. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2PP, where “x” is Pro. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2PP, where “x” is Phe. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2PP, where “x” is Tyr. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2PP, where “x” is Trp. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2PP, where “x” is Ser. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2PP, where "x" is Thr. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2PP, where "x" is Cys. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2PP, where "x" is Met. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2PP, where "x" is Asn. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2PP, where "x" is Gln. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2PP, where "x" is Lys. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2PP, where "x" is Arg. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2PP, where "x" is His. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2PP, where "x" is Asp. In some cases, the variant 4-1BBL polypeptide has a binding affinity to CD137 that is from about 100 nM to about 150 nM, from about 150 nM to about 200 nM, from about 200 nM to about 250 nM, from about 250 nM to about 300 nM, from about 300 nM to about 350 nM, from about 350 nM to about 400 nM, from about 400 nM to about 500 nM, from about 500 nM to about 600 nM, from about 600 nM to about 700 nM, from about 700 nM to about 800 nM, from about 800 nM to about 900 nM, from about 900 nM to about 1 μM, to about 1 μM to about 5 μM, from about 5 μM to about 10 μM, from about 10 μM to about 15 μM, from about 15 μM to about 20 μM, from about 20 μM to about 25 μM, from about 25 μM to about 50 μM, from about 50 μM to about 75 μM, or from about 75 μM to about 100 μM.

F144 Substitution

[00199] In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence depicted in FIG. 2QQ, where amino acid 144 (indicated by an "x") is an amino acid other than a phenylalanine, e.g., where amino acid 144 is Gly, Ala, Val, Leu, Ile, Pro, Tyr, Trp, Ser, Thr, Cys, Met, Asn, Gln, Lys, Arg, His, Asp, or Glu. The amino acid numbering in FIG. 2QQ retains the numbering of FIG. 2A. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence depicted in FIG. 2QQ, where amino acid 144 is Gly. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence depicted in FIG. 2QQ, where amino acid 144 is Ala. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence depicted in FIG. 2QQ, where amino acid 144 is Val. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence depicted in FIG. 2QQ, where amino acid 144 is Leu. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence depicted in FIG. 2QQ, where amino acid 144 is Ile. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence depicted in FIG. 2QQ, where amino acid 144 is Pro. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence depicted in FIG. 2QQ, where amino acid 144 is Tyr. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence depicted in FIG. 2QQ, where amino acid 144 is Trp. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence depicted in FIG. 2QQ, where amino acid 144 is Ser. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence depicted in FIG. 2QQ, where amino acid 144 is Thr. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence depicted in FIG. 2QQ, where amino acid 144 is Cys. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence depicted in FIG. 2QQ, where amino acid 144 is Met. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence depicted in FIG. 2QQ, where amino acid 144 is Asn. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence depicted in FIG. 2QQ, where amino acid 144 is Gln. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence depicted in FIG. 2QQ, where amino acid 144 is Lys. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence depicted in FIG. 2QQ, where amino acid 144 is Arg. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence depicted in FIG. 2QQ, where amino acid 144 is His. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence depicted in FIG. 2QQ, where amino acid 144 is Asp. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence depicted in FIG. 2QQ, where amino acid 144 is Glu. In some cases, the variant 4-1BBL polypeptide has a binding affinity to CD137 that is from about 100 nM to about 150 nM, from about 150 nM to about 200 nM, from about 200 nM to about 250 nM, from about 250 nM to about 300 nM, from about 300 nM to about 350 nM, from about 350 nM to about 400 nM, from about 400 nM to about 500 nM, from about 500 nM to about 600 nM, from about 600 nM to about 700 nM, from about 700 nM to about 800 nM, from about 800 nM to about 900 nM, from about 900 nM to about 1 μM, to about 1 μM to about 5 μM, from about 5 μM to about 10 μM, from about 10 μM to about 15 μM, from about 15 μM to about 20 μM, from about 20 μM to about 25 μM, from about 25 μM to about 50 μM, from about 50 μM to about 75 μM, or from about 75 μM to about 100 μM.

[00200] In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2A, with an amino acid substitution at F144. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence set forth in SEQ ID NO:1, with an amino acid substitution at F64. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises the amino acid sequence set forth in SEQ ID NO:1, with an amino acid substitution at F64. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence set forth in SEQ ID NO:2, with an amino acid substitution at F65. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises the amino acid sequence set forth in SEQ ID NO:2, with an amino acid substitution at F65. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence set forth in SEQ ID NO:3, with an amino acid substitution at F65. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises the amino acid sequence set forth in SEQ ID NO:3, with an amino acid substitution at F65.

[00201] For example, in some cases, a variant 4-1BBL polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2QQ, where “x” is any amino acid other than phenylalanine; for example, “x” can be Gly, Ala, Val, Leu, Ile, Pro, Tyr, Trp, Ser, Thr, Cys, Met, Asn, Gln, Lys, Arg, His, Asp, or Glu. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2QQ, where “x” is Gly. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2QQ, where “x” is Ala. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2QQ, where “x” is Val. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2QQ, where “x” is Leu. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2QQ, where “x” is Ile. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2QQ, where “x” is Pro. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2QQ, where “x” is Tyr. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2QQ, where “x” is Trp. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2QQ, where “x” is Ser. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2QQ, where “x” is Thr. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2QQ, where “x” is Cys. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2QQ, where “x” is Met. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2QQ, where “x” is Asn. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2QQ, where “x” is Gln. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2QQ, where “x” is Lys. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2QQ, where "x" is Arg. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2QQ, where "x" is His. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2QQ, where "x" is Asp. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2QQ, where "x" is Glu. In some cases, the variant 4-1BBL polypeptide has a binding affinity to CD137 that is from about 100 nM to about 150 nM, from about 150 nM to about 200 nM, from about 200 nM to about 250 nM, from about 250 nM to about 300 nM, from about 300 nM to about 350 nM, from about 350 nM to about 400 nM, from about 400 nM to about 500 nM, from about 500 nM to about 600 nM, from about 600 nM to about 700 nM, from about 700 nM to about 800 nM, from about 800 nM to about 900 nM, from about 900 nM to about 1 μM, to about 1 μM to about 5 μM, from about 5 μM to about 10 μM, from about 10 μM to about 15 μM, from about 15 μM to about 20 μM, from about 20 μM to about 25 μM, from about 25 μM to about 50 μM, from about 50 μM to about 75 μM, or from about 75 μM to about 100 μM.

F145 Substitution

[00202] In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence depicted in FIG. 2RR, where amino acid 145 (indicated by an "x") is an amino acid other than a phenylalanine, e.g., where amino acid 145 is Gly, Ala, Val, Leu, Ile, Pro, Tyr, Trp, Ser, Thr, Cys, Met, Asn, Gln, Lys, Arg, His, Asp, or Glu. The amino acid numbering in FIG. 2RR retains the numbering of FIG. 2A. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence depicted in FIG. 2RR, where amino acid 145 is Gly. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence depicted in FIG. 2RR, where amino acid 145 is Ala. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence depicted in FIG. 2RR, where amino acid 145 is Val. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence depicted in FIG. 2RR, where amino acid 145 is Leu. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence depicted in FIG. 2RR, where amino acid 145 is Ile. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence depicted in FIG. 2RR, where amino acid 145 is Pro. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence depicted in FIG. 2RR, where amino acid 145 is Tyr. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence depicted in FIG. 2RR, where amino acid 145 is Trp. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence depicted in FIG. 2RR, where amino acid 145 is Ser. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence depicted in FIG. 2RR, where amino acid 145 is Thr. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence depicted in FIG. 2RR, where amino acid 145 is Cys. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence depicted in FIG. 2RR, where amino acid 145 is Met. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence depicted in FIG. 2RR, where amino acid 145 is Asn. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence depicted in FIG. 2RR, where amino acid 145 is Gln. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence depicted in FIG. 2RR, where amino acid 145 is Lys. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence depicted in FIG. 2RR, where amino acid 145 is Arg. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence depicted in FIG. 2RR, where amino acid 145 is His. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence depicted in FIG. 2RR, where amino acid 145 is Asp. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence depicted in FIG. 2QQ, where amino acid 145 is Glu. In some cases, the variant 4-1BBL polypeptide has a binding affinity to CD137 that is from about 100 nM to about 150 nM, from about 150 nM to about 200 nM, from about 200 nM to about 250 nM, from about 250 nM to about 300 nM, from about 300 nM to about 350 nM, from about 350 nM to about 400 nM, from about 400 nM to about 500 nM, from about 500 nM to about 600 nM, from about 600 nM to about 700 nM, from about 700 nM to about 800 nM, from about 800 nM to about 900 nM, from about 900 nM to about 1 μM, to about 1 μM to about 5 μM, from about 5 μM to about 10 μM, from about 10 μM to about 15 μM, from about 15 μM to about 20 μM, from about 20 μM to about 25 μM, from about 25 μM to about 50 μM, from about 50 μM to about 75 μM, or from about 75 μM to about 100 μM.

[00203] In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2A, with an amino acid substitution at F145. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence set forth in SEQ ID NO:1, with an amino acid substitution at F65. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises the amino acid sequence set forth in SEQ ID NO:1, with an amino acid substitution at F65. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence set forth in SEQ ID NO:2, with an amino acid substitution at F56. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises the amino acid sequence set forth in SEQ ID NO:2, with an amino acid substitution at F56. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence set forth in SEQ ID NO:3, with an amino acid substitution at F56. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises the amino acid sequence set forth in SEQ ID NO:3, with an amino acid substitution at F56.

[00204] For example, in some cases, a variant 4-1BBL polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2RR, where “x” is any amino acid other than phenylalanine; for example, “x” can be Gly, Ala, Val, Leu, Ile, Pro, Tyr, Trp, Ser, Thr, Cys, Met, Asn, Gln, Lys, Arg, His, Asp, or Glu. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2RR, where “x” is Gly. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2RR, where “x” is Ala. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2RR, where “x” is Val. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2RR, where “x” is Leu. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2RR, where “x” is Ile. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2RR, where “x” is Pro. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2RR, where “x” is Tyr. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2RR, where “x” is Trp. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2RR, where “x” is Ser. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2RR, where “x” is Thr. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2RR, where “x” is Cys. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2RR, where “x” is Met. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2RR, where “x” is Asn. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2RR, where “x” is Gln. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2RR, where “x” is Lys. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2RR, where “x” is Arg. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2RR, where “x” is His. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2RR, where “x” is Asp. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2RR, where “x” is Glu. In some cases, the variant 4-1BBL polypeptide has a binding affinity to CD137 that is from about 100 nM to about 150 nM, from about 150 nM to about 200 nM, from about 200 nM to about 250 nM, from about 250 nM to about 300 nM, from about 300 nM to about 350 nM, from about 350 nM to about 400 nM, from about 400 nM to about 500 nM, from about 500 nM to about 600 nM, from about 600 nM to about 700 nM, from about 700 nM to about 800 nM, from about 800 nM to about 900 nM, from about 900 nM to about 1 μM, to about 1 μM to about 5 μM, from about 5 μM to about 10 μM, from about 10 μM to about 15 μM, from about 15 μM to about 20 μM, from about 20 μM to about 25 μM, from about 25 μM to about 50 μM, from about 50 μM to about 75 μM, or from about 75 μM to about 100 μM.

Q146

[00205] In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence depicted in FIG. 2SS, where amino acid 146 (indicated by an "x") is an amino acid other than a glutamine, e.g., where amino acid 146 is Gly, Ala, Val, Leu, Ile, Pro, Phe, Tyr, Trp, Ser, Thr, Cys, Met, Asn, Lys, Arg, His, Asp, or Glu. The amino acid numbering in FIG. 2SS retains the numbering of FIG. 2A. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence depicted in FIG. 2SS, where amino acid 146 is Phe. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence depicted in FIG. 2SS, where amino acid 146 is Leu. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence depicted in FIG. 2SS, where amino acid 146 is Ile. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence depicted in FIG. 2SS, where amino acid 146 is Met. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence depicted in FIG. 2SS, where amino acid 146 is Val. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence depicted in FIG. 2SS, where amino acid 146 is Ser. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence depicted in FIG. 2SS, where amino acid 146 is Pro. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence depicted in FIG. 2SS, where amino acid 146 is Thr. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence depicted in FIG. 2SS, where amino acid 146 is Ala. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence depicted in FIG. 2SS, where amino acid 146 is Tyr. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence depicted in FIG. 2SS, where amino acid 146 is His. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence depicted in FIG. 2SS, where amino acid 146 is Asn. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence depicted in FIG. 2SS, where amino acid 146 is Lys. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence depicted in FIG. 2SS, where amino acid 146 is Asp. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence depicted in FIG. 2SS, where amino acid 146 is Glu. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence depicted in FIG. 2SS, where amino acid 146 is Cys. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence depicted in FIG. 2SS, where amino acid 146 is Trp. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence depicted in FIG. 2SS, where amino acid 146 is Arg. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence depicted in FIG. 2SS, where amino acid 146 is Gly. In some cases, the variant 4-1BBL polypeptide has a binding affinity to CD137 that is from about 100 nM to about 150 nM, from about 150 nM to about 200 nM, from about 200 nM to about 250 nM, from about 250 nM to about 300 nM, from about 300 nM to about 350 nM, from about 350 nM to about 400 nM, from about 400 nM to about 500 nM, from about 500 nM to about 600 nM, from about 600 nM to about 700 nM, from about 700 nM to about 800 nM, from about 800 nM to about 900 nM, from about 900 nM to about 1 μM, to about 1 μM to about 5 μM, from about 5 μM to about 10 μM, from about 10 μM to about 15 μM, from about 15 μM to about 20 μM, from about 20 μM to about 25 μM, from about 25 μM to about 50 μM, from about 50 μM to about 75 μM, or from about 75 μM to about 100 μM.

[00206] In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2A, with an amino acid substitution at Q146. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence set forth in SEQ ID NO:1, with an amino acid substitution at Q66. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises the amino acid sequence set forth in SEQ ID NO:1, with an amino acid substitution at Q66. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence set forth in SEQ ID NO:2, with an amino acid substitution at Q67. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises the amino acid sequence set forth in SEQ ID NO:2, with an amino acid substitution at Q67. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence set forth in SEQ ID NO:3, with an amino acid substitution at Q67. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises the amino acid sequence set forth in SEQ ID NO:3, with an amino acid substitution at Q67.

[00207] For example, in some cases, a variant 4-1BBL polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2SS, where “x” is any amino acid other than glutamine; for example, “x” can be Gly, Ala, Val, Leu, Ile, Pro, Phe, Tyr, Trp, Ser, Thr, Cys, Met, Asn, Gln, Arg, His, Asp, or Glu. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2SS, where “x” is Phe. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2SS, where “x” is Leu. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2SS, where “x” is

Ile. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2SS, where “x” is Met. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2SS, where “x” is Val. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2SS, where “x” is Ser. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2SS, where “x” is Pro. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2SS, where “x” is Thr. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2SS, where “x” is Ala. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2SS, where “x” is Tyr. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2SS, where “x” is His. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2SS, where “x” is Asn. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2SS, where “x” is Lys. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2SS, where “x” is Asp. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2SS, where “x” is Glu. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2SS, where “x” is Cys. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2SS, where “x” is Trp. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2SS, where “x” is Arg. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2SS where “x” is Gly. In some cases, the variant 4-1BBL polypeptide has a binding affinity to CD137 that is from about 100 nM to about 150 nM, from about 150 nM to about 200 nM, from about 200 nM to about 250 nM, from about 250 nM to about 300 nM, from about 300 nM to about 350 nM, from about 350 nM to about 400 nM, from about 400 nM to about 500 nM, from about 500 nM to about 600 nM, from about 600 nM to about 700 nM, from about 700 nM to about 800 nM, from about 800 nM to about 900 nM, from about 900 nM to about 1 μM, to about 1 μM to about 5 μM, from about 5 μM to about 10 μM, from about 10 μM to about 15 μM, from about 15 μM to about 20 μM, from about 20 μM to about 25 μM, from about 25 μM to about 50 μM, from about 50 μM to about 75 μM, or from about 75 μM to about 100 μM.

L147 Substitution

[00208] In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence depicted in FIG. 2TT, where amino acid 147 (indicated by an “x”) is an amino acid other than a leucine, e.g., where amino acid 147 is Gly, Ala, Val, Ile, Pro, Phe, Tyr, Trp, Ser, Thr, Cys, Met, Asn, Gln, Lys, Arg, His, Asp, or Glu. The amino acid numbering in FIG. 2TT retains the numbering of FIG. 2A. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence depicted in FIG. 2TT, where amino acid 147 is Gly. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence depicted in FIG. 2TT, where amino acid 147 is Ala. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence depicted in FIG. 2TT, where amino acid 147 is Val. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence depicted in FIG. 2TT, where amino acid 147 is Ile. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence depicted in FIG. 2TT, where amino acid 147 is Pro. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence depicted in FIG. 2TT, where amino acid 147 is Phe. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence depicted in FIG. 2TT, where amino acid 147 is Tyr. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence depicted in FIG. 2TT, where amino acid 147 is Trp. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence depicted in FIG. 2TT, where amino acid 147 is Ser. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence depicted in FIG. 2TT, where amino acid 147 is Thr. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence depicted in FIG. 2TT, where amino acid 147 is Cys. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence depicted in FIG. 2TT, where amino acid 147 is Met. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence depicted in FIG. 2TT, where amino acid 147 is Asn. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence depicted in FIG. 2TT, where amino acid 147 is Gln. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence depicted in FIG. 2TT, where amino acid 147 is Lys. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence depicted in FIG. 2TT, where amino acid 147 is Arg. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence depicted in FIG. 2TT, where amino acid 147 is His. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence depicted in FIG. 2TT, where amino acid 147 is Asp. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence depicted in FIG. 2TT, where amino acid 147 is Glu. In some cases, the variant 4-1BBL polypeptide has a binding affinity to CD137 that is from about 100 nM to about 150 nM, from about 150 nM to about 200 nM, from about 200 nM to about 250 nM, from about 250 nM to about 300 nM, from about 300 nM to about 350 nM, from about 350 nM to about 400 nM, from about 400 nM to about 500 nM, from about 500 nM to about 600 nM, from about 600 nM to about 700 nM, from about 700 nM to about 800 nM, from about 800 nM to about 900 nM, from about 900 nM to about 1 μM, to about 1 μM to about 5 μM, from about 5 μM to about 10 μM, from about 10 μM to about 15 μM, from about 15 μM to about 20 μM, from about 20 μM to about 25 μM, from about 25 μM to about 50 μM, from about 50 μM to about 75 μM, or from about 75 μM to about 100 μM.

[00209] In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2A, with an amino acid substitution at L147. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence set forth in SEQ ID NO:1, with an amino acid substitution at L67. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises the amino acid sequence set forth in SEQ ID NO:1, with an amino acid substitution at L67. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence set forth in SEQ ID NO:2, with an amino acid substitution at L68. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises the amino acid sequence set forth in SEQ ID NO:2, with an amino acid substitution at L68. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence set forth in SEQ ID NO:3, with an amino acid substitution at L68. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises the amino acid sequence set forth in SEQ ID NO:3, with an amino acid substitution at L68.

[00210] For example, in some cases, a variant 4-1BBL polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2TT, where “x” is any amino acid other than leucine; for example, “x” can be Gly, Ala, Val, Ile, Pro, Phe, Tyr, Trp, Ser, Thr, Cys, Met, Asn, Gln, Lys, Arg, His, Asp, or Glu. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2TT, where “x” is Gly. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2TT, where “x” is Ala. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2TT, where “x” is Val. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2TT, where “x” is Ile. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2TT, where “x” is Pro. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2TT, where “x” is Phe. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2TT, where “x” is Tyr. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2TT, where “x” is Trp. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2TT, where “x” is Ser. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2TT, where “x” is Thr. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2TT, where “x” is Cys. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2TT, where “x” is Met. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2TT, where “x” is Asn. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2TT, where “x” is Gln. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2TT, where “x” is Lys. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2TT, where “x” is Arg. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2TT, where “x” is His. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2TT, where “x” is Asp. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2TT, where “x” is Glu. In some cases, the variant 4-1BBL polypeptide has a binding affinity to CD137 that is from about 100 nM to about 150 nM, from about 150 nM to about 200 nM, from about 200 nM to about 250 nM, from about 250 nM to about 300 nM, from about 300 nM to about 350 nM, from about 350 nM to about 400 nM, from about 400 nM to about 500 nM, from about 500 nM to about 600 nM, from about 600 nM to about 700 nM, from about 700 nM to about 800 nM, from about 800 nM to about 900 nM, from about 900 nM to about 1 μM, to about 1 μM to about 5 μM, from about 5 μM to about 10 μM, from about 10 μM to about 15 μM, from about 15 μM to about 20 μM, from about 20 μM to about 25 μM, from about 25 μM to about 50 μM, from about 50 μM to about 75 μM, or from about 75 μM to about 100 μM.

E148 Substitution

[00211] In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence depicted in FIG. 2UU, where amino acid 148 (indicated by an “x”) is an amino acid other than a glutamic acid, e.g., where amino acid 148 is Gly, Ala, Val, Leu, Ile, Pro, Phe, Tyr, Trp, Ser, Thr, Cys, Met, Asn, Gln, Lys, Arg, His, or Asp. The amino acid numbering in FIG. 2UU retains the numbering of FIG. 2A. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence depicted in FIG. 2UU, where amino acid 148 is Gly. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence depicted in FIG. 2UU, where amino acid 148 is Ala. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence depicted in FIG. 2UU, where amino acid 148 is Val. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence depicted in FIG. 2UU, where amino acid 148 is Leu. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence depicted in FIG. 2UU, where amino acid 148 is Ile. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence depicted in FIG. 2UU, where amino acid 148 is Pro. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence depicted in FIG. 2UU, where amino acid 148 is Phe. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence depicted in FIG. 2UU, where amino acid 148 is Tyr. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence depicted in FIG. 2UU, where amino acid 148 is Trp. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence depicted in FIG. 2UU, where amino acid 148 is Ser. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence depicted in FIG. 2UU, where amino acid 148 is Thr. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence depicted in FIG. 2UU, where amino acid 148 is Cys. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence depicted in FIG. 2UU, where amino acid 148 is Met. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence depicted in FIG. 2UU, where amino acid 148 is Asn. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence depicted in FIG. 2UU, where amino acid 148 is Gln. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence depicted in FIG. 2UU, where amino acid 148 is Lys. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence depicted in FIG. 2UU, where amino acid 148 is Arg. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence depicted in FIG. 2UU, where amino acid 148 is His. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence depicted in FIG. 2UU, where amino acid 148 is Asp. In some cases, the variant 4-1BBL polypeptide has a binding affinity to CD137 that is from about 100 nM to about 150 nM, from about 150 nM to about 200 nM, from about 200 nM to about 250 nM, from about 250 nM to about 300 nM, from about 300 nM to about 350 nM, from about 350 nM to about 400 nM, from about 400 nM to about 500 nM, from about 500 nM to about 600 nM, from about 600 nM to about 700 nM, from about 700 nM to about 800 nM, from about 800 nM to about 900 nM, from about 900 nM to about 1 μM, to about 1 μM to about 5 μM, from about 5 μM to about 10 μM, from about 10 μM to about 15 μM, from about 15 μM to about 20 μM, from about 20 μM to about 25 μM, from about 25 μM to about 50 μM, from about 50 μM to about 75 μM, or from about 75 μM to about 100 μM.

[00212] In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2A, with an amino acid substitution at E148. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence set forth in SEQ ID NO:1, with an amino acid substitution at E68. In

some cases, a variant 4-1BBL polypeptide of the present disclosure comprises the amino acid sequence set forth in SEQ ID NO:1, with an amino acid substitution at E68. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence set forth in SEQ ID NO:2, with an amino acid substitution at E69. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises the amino acid sequence set forth in SEQ ID NO:2, with an amino acid substitution at E69. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence set forth in SEQ ID NO:3, with an amino acid substitution at E69. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises the amino acid sequence set forth in SEQ ID NO:3, with an amino acid substitution at E69.

[00213] For example, in some cases, a variant 4-1BBL polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2UU, where “x” is any amino acid other than glutamic acid; for example, “x” can be Gly, Ala, Val, Leu, Ile, Pro, Phe, Tyr, Trp, Ser, Thr, Cys, Met, Asn, Gln, Lys, Arg, His, or Asp. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2UU, where “x” is Gly. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2UU, where “x” is Ala. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2UU L, where “x” is Val. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2UU, where “x” is Leu. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2UU, where “x” is Ile. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2UU, where “x” is Pro. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2UU, where “x” is Phe. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2UU, where “x” is Tyr. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2UU, where “x” is Trp. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2UU, where “x” is Ser. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2UU, where “x” is Thr. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2UU, where “x” is Cys. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2UU, where “x” is Met. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2UU, where “x” is Asn. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2UU, where “x” is Gln. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2UU, where “x” is Lys. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2UU, where “x” is Arg. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2UU, where “x” is His. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2UU, where “x” is Asp. In some cases, the variant 4-1BBL polypeptide has a binding affinity to CD137 that is from about 100 nM to about 150 nM, from about 150 nM to about 200 nM, from about 200 nM to about 250 nM, from about 250 nM to about 300 nM, from about 300 nM to about 350 nM, from about 350 nM to about 400 nM, from about 400 nM to about 500 nM, from about 500 nM to about 600 nM, from about 600 nM to about 700 nM, from about 700 nM to about 800 nM, from about 800 nM to about 900 nM, from about 900 nM to about 1 μM, to about 1 μM to about 5 μM, from about 5 μM to about 10 μM, from about 10 μM to about 15 μM, from about 15 μM to about 20 μM, from about 20 μM to about 25 μM, from about 25 μM to about 50 μM, from about 50 μM to about 75 μM, or from about 75 μM to about 100 μM.

L149 Substitution

[00214] In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence depicted in FIG. 2VV, where amino acid 149 (indicated by an “x”) is an amino acid other than a leucine, e.g., where amino acid 149 is Gly, Ala, Val, Ile, Pro, Phe, Tyr, Trp, Ser, Thr, Cys, Met, Asn, Gln, Lys, Arg, His, Asp, or Glu. The amino acid numbering in FIG. 2VV retains the numbering of FIG. 2A. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence depicted in FIG. 2VV, where amino acid 149 is Gly. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence depicted in FIG. 2VV, where amino acid 149 is Ala. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence depicted in FIG. 2VV, where amino acid 149 is Val. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence depicted in FIG. 2VV, where amino acid 149 is Ile. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence depicted in FIG. 2VV, where amino acid 149 is Pro. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence depicted in FIG. 2VV, where amino acid 149 is Phe. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence depicted in FIG. 2VV, where amino acid 149 is Tyr. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence depicted in FIG. 2VV, where amino acid 149 is Trp. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence depicted in FIG. 2VV, where amino acid 149 is Ser. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence depicted in FIG. 2VV, where amino acid 149 is Thr. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence depicted in FIG. 2VV, where amino acid 149 is Cys. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence depicted in FIG. 2VV, where amino acid 149 is Met. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence depicted in FIG. 2VV, where amino acid 149 is Asn. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence depicted in FIG. 2VV, where amino acid 149 is Gln. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence depicted in FIG. 2VV, where amino acid 149 is Lys. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence depicted in FIG. 2VV, where amino acid 149 is Arg. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence depicted in FIG. 2VV, where amino acid 149 is His. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence depicted in FIG. 2VV, where amino acid 149 is Asp. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence depicted in FIG. 2VV, where amino acid 149 is Glu. In some cases, the variant 4-1BBL polypeptide has a binding affinity to CD137 that is from about 100 nM to about 150 nM, from about 150 nM to about 200 nM, from about 200 nM to about 250 nM, from about 250 nM to about 300 nM, from about 300 nM to about 350 nM, from about 350 nM to about 400 nM, from about 400 nM to about 500 nM, from about 500 nM to about 600 nM, from about 600 nM to about 700 nM, from about 700 nM to about 800 nM, from about 800 nM to about 900 nM, from about 900 nM to about 1 μM, to about 1 μM to about 5 μM, from about 5 μM to about 10 μM, from about 10 μM to about 15 μM, from about 15 μM to about 20 μM, from about 20 μM to about 25 μM, from about 25 μM to about 50 μM, from about 50 μM to about 75 μM, or from about 75 μM to about 100 μM.

[00215] In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2A, with an amino acid substitution at L149. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence set forth in SEQ ID NO:1, with an amino acid substitution at L69. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises the amino acid sequence set forth in SEQ ID NO:1, with an amino acid substitution at L69. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence set forth in SEQ ID NO:2, with an amino acid substitution at L70. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises the amino acid sequence set forth in SEQ ID NO:2, with an amino acid substitution at L70. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises an amino acid sequence having at least

90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence set forth in SEQ ID NO:3, with an amino acid substitution at L70. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises the amino acid sequence set forth in SEQ ID NO:3, with an amino acid substitution at L70.

[00216] For example, in some cases, a variant 4-1BBL polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2VV, where "x" is any amino acid other than leucine; for example, "x" can be Gly, Ala, Val, Ile, Pro, Phe, Tyr, Trp, Ser, Thr, Cys, Met, Asn, Gln, Lys, Arg, His, Asp, or Glu. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2VV, where "x" is Gly. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2VV, where "x" is Ala. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2VV, where "x" is Val. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2VV, where "x" is Ile. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2VV, where "x" is Pro. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2VV, where "x" is Phe. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2VV, where "x" is Tyr. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2VV, where "x" is Trp. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2VV, where "x" is Ser. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2VV, where "x" is Thr. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2VV, where "x" is Cys. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2VV, where "x" is Met. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2VV, where "x" is Asn. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2VV, where "x" is Gln. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2VV, where "x" is Lys. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2VV, where "x" is Arg. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2VV, where "x" is His. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2VV, where "x" is Asp. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2VV, where "x" is Glu. In some cases, the variant 4-1BBL polypeptide has a binding affinity to CD137 that is from about 100 nM to about 150 nM, from about 150 nM to about 200 nM, from about 200 nM to about 250 nM, from about 250 nM to about 300 nM, from about 300 nM to about 350 nM, from about 350 nM to about 400 nM, from about 400 nM to about 500 nM, from about 500 nM to about 600 nM, from about 600 nM to about 700 nM, from about 700 nM to about 800 nM, from about 800 nM to about 900 nM, from about 900 nM to about 1 μM, to about 1 μM to about 5 μM, from about 5 μM to about 10 μM, from about 10 μM to about 15 μM, from about 15 μM to about 20 μM, from about 20 μM to about 25 μM, from about 25 μM to about 50 μM, from about 50 μM to about 75 μM, or from about 75 μM to about 100 μM.

R150 Substitution

[00217] In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence depicted in FIG. 2WW, where amino acid 150 (indicated by an "x") is an amino acid other than an arginine, e.g., where amino acid 150 is Gly, Ala, Val, Leu, Ile, Pro, Phe, Tyr, Trp, Ser, Thr, Cys, Met, Asn, Gln, Lys, His, Asp, or Glu. The amino acid numbering in FIG. 2WW retains the numbering of FIG. 2A. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence depicted in FIG. 2WW, where amino acid 150 is Gly. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence depicted in FIG. 2WW, where amino acid 150 is Ala. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence depicted in FIG. 2WW, where amino acid 150 is Val. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence depicted in FIG. 2WW, where amino acid 150 is Leu. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence depicted in FIG. 2WW, where amino acid 150 is Ile. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence depicted in FIG. 2WW, where amino acid 150 is Pro. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence depicted in FIG. 2WW, where amino acid 150 is Phe. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence depicted in FIG. 2WW, where amino acid 150 is Tyr. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence depicted in FIG. 2WW, where amino acid 150 is Trp. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence depicted in FIG. 2WW, where amino acid 150 is Ser. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence depicted in FIG. 2WW, where amino acid 150 is Thr. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence depicted in FIG. 2WW, where amino acid 150 is Cys. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence depicted in FIG. 2WW, where amino acid 150 is Met. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence depicted in FIG. 2WW, where amino acid 150 is Asn. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence depicted in FIG. 2WW, where amino acid 150 is Gln. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence depicted in FIG. 2WW, where amino acid 150 is Lys. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence depicted in FIG. 2WW, where amino acid 150 is His. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence depicted in FIG. 2WW, where amino acid 150 is Asp. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence depicted in FIG. 2WW, where amino acid 150 is Glu. In some cases, the variant 4-1BBL polypeptide has a binding affinity to CD137 that is from about 100 nM to about 150 nM, from about 150 nM to about 200 nM, from about 200 nM to about 250 nM, from about 250 nM to about 300 nM, from about 300 nM to about 350 nM, from about 350 nM to about 400 nM, from about 400 nM to about 500 nM, from about 500 nM to about 600 nM, from about 600 nM to about 700 nM, from about 700 nM to about 800 nM, from about 800 nM to about 900 nM, from about 900 nM to about 1 μM, to about 1 μM to about 5 μM, from about 5 μM to about 10 μM, from about 10 μM to about 15 μM, from about 15 μM to about 20 μM, from about 20 μM to about 25 μM, from about 25 μM to about 50 μM, from about 50 μM to about 75 μM, or from about 75 μM to about 100 μM.

[00218] In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2A, with an amino acid substitution at R150. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence set forth in SEQ ID NO:1, with an amino acid substitution at R70. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises the amino acid sequence set forth in SEQ ID NO:1, with an amino acid substitution at R70. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence set forth in SEQ ID NO:2, with an amino acid substitution at R71. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises the amino acid sequence set forth in SEQ ID NO:2, with an amino acid substitution at R71. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence set forth in SEQ ID NO:3, with an amino acid substitution at R71. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises the amino acid sequence set forth in SEQ ID NO:3, with an amino acid substitution at R71.

[00219] For example, in some cases, a variant 4-1BBL polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2WW, where “x” is any amino acid other than arginine; for example, “x” can be Gly, Ala, Val, Leu, Ile, Pro, Phe, Tyr, Trp, Ser, Thr, Cys, Met, Asn, Gln, Lys, His, Asp, or Glu. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2WW, where “x” is Gly. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2WW, where “x” is Ala. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2WW, where “x” is Val. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2WW, where “x” is Leu. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2WW, where “x” is Ile. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2WW, where “x” is Pro. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2WW, where “x” is Phe. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2WW, where “x” is Tyr. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2WW, where “x” is Trp. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2WW, where “x” is Ser. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2WW, where “x” is Thr. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2WW, where “x” is Cys. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2WW, where “x” is Met. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2WW, where “x” is Asn. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2WW, where “x” is Gln. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2WW, where “x” is Lys. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2WW, where “x” is His. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2WW, where “x” is Asp. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2WW, where “x” is Glu. In some cases, the variant 4-1BBL polypeptide has a binding affinity to CD137 that is from about 100 nM to about 150 nM, from about 150 nM to about 200 nM, from about 200 nM to about 250 nM, from about 250 nM to about 300 nM, from about 300 nM to about 350 nM, from about 350 nM to about 400 nM, from about 400 nM to about 500 nM, from about 500 nM to about 600 nM, from about 600 nM to about 700 nM, from about 700 nM to about 800 nM, from about 800 nM to about 900 nM, from about 900 nM to about 1 μM, to about

1 μM to about 5 μM, from about 5 μM to about 10 μM, from about 10 μM to about 15 μM, from about 15 μM to about 20 μM, from about 20 μM to about 25 μM, from about 25 μM to about 50 μM, from about 50 μM to about 75 μM, or from about 75 μM to about 100 μM.

R151 Substitution

[00220] In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence depicted in FIG. 2XX, where amino acid 151 (indicated by an "x") is an amino acid other than an arginine, e.g., where amino acid 150 is Gly, Ala, Val, Leu, Ile, Pro, Phe, Tyr, Trp, Ser, Thr, Cys, Met, Asn, Gln, Lys, His, Asp, or Glu. The amino acid numbering in FIG. 2XX retains the numbering of FIG. 2A. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence depicted in FIG. 2XX, where amino acid 151 is Gly. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence depicted in FIG. 2XX, where amino acid 151 is Ala. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence depicted in FIG. 2XX, where amino acid 151 is Val. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence depicted in FIG. 2XX, where amino acid 151 is Leu. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence depicted in FIG. 2XX, where amino acid 151 is Ile. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence depicted in FIG. 2XX, where amino acid 151 is Pro. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence depicted in FIG. 2XX, where amino acid 151 is Phe. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence depicted in FIG. 2XX, where amino acid 151 is Tyr. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at

least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence depicted in FIG. 2XX, where amino acid 151 is Trp. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence depicted in FIG. 2XX, where amino acid 151 is Ser. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence depicted in FIG. 2XX, where amino acid 151 is Thr. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence depicted in FIG. 2XX, where amino acid 151 is Cys. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence depicted in FIG. 2XX, where amino acid 151 is Met. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence depicted in FIG. 2XX, where amino acid 151 is Asn. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence depicted in FIG. 2XX, where amino acid 151 is Gln. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence depicted in FIG. 2XX, where amino acid 151 is Lys. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence depicted in FIG. 2XX, where amino acid 151 is His. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence depicted in FIG. 2XX, where amino acid 151 is Asp. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence depicted in FIG. 2XX, where amino acid 151 is Glu. In some cases, the variant 4-1BBL polypeptide has a binding affinity to CD137 that is from about 100 nM to about 150 nM, from about 150 nM to about 200 nM, from about 200 nM to about 250 nM, from about 250 nM to about 300 nM, from about 300 nM to about 350 nM, from about 350 nM to about 400 nM, from about 400 nM to about 500 nM, from about 500 nM to about 600 nM, from about 600 nM to about 700 nM, from about 700 nM to about 800 nM, from about 800 nM to about 900 nM, from about 900 nM to about 1 μM, to about 1 μM to about 5 μM, from about 5 μM to about 10 μM, from about 10 μM to about 15 μM, from about 15 μM to about 20 μM, from about 20 μM to about 25 μM, from about 25 μM to about 50 μM, from about 50 μM to about 75 μM, or from about 75 μM to about 100 μM.

[00221] In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2A, with an amino acid substitution at R151. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence set forth in SEQ ID NO:1, with an amino acid substitution at R71. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises the amino acid sequence set forth in SEQ ID NO:1, with an amino acid substitution at R71. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence set forth in SEQ ID NO:2, with an amino acid substitution at R72. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises the amino acid sequence set forth in SEQ ID NO:2, with an amino acid substitution at R72. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence set forth in SEQ ID NO:3, with an amino acid substitution at R72. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises the amino acid sequence set forth in SEQ ID NO:3, with an amino acid substitution at R72.

[00222] For example, in some cases, a variant 4-1BBL polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2XX, where “x” is any amino acid other than arginine; for example, “x” can be Gly, Ala, Val, Leu, Ile, Pro, Phe, Tyr, Trp, Ser, Thr, Cys, Met, Asn, Gln, Lys, His, Asp, or Glu. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2XX, where “x” is Gly. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2XX, where “x” is Ala. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2XX, where “x” is Val. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2XX, where “x” is Leu. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2XX, where “x” is Ile. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2XX, where “x” is Pro. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2XX, where “x” is Phe. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2XX, where “x” is Tyr. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2XX, where “x” is Trp. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2XX where “x” is Ser. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2XX, where “x” is Thr. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2XX, where “x” is Cys. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2XX, where “x” is Met. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2XX, where “x” is Asn. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2XX, where “x” is Gln. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2XX, where “x” is Lys. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2XX, where “x” is His. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2XX, where “x” is Asp. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2XX, where “x” is Glu. In some cases, the variant 4-1BBL polypeptide has a binding affinity to CD137 that is from about 100 nM to about 150 nM, from about 150 nM to about 200 nM, from about 200 nM to about 250 nM, from about 250 nM to about 300 nM, from about 300 nM to about 350 nM, from about 350 nM to about 400 nM, from about 400 nM to about 500 nM, from about 500 nM to about 600 nM, from about 600 nM to about 700 nM, from about 700 nM to about 800 nM, from about 800 nM to about 900 nM, from about 900 nM to about 1 μM, to about 1 μM to about 5 μM, from about 5 μM to about 10 μM, from about 10 μM to about 15 μM, from about 15 μM to about 20 μM, from about 20 μM to about 25 μM, from about 25 μM to about 50 μM, from about 50 μM to about 75 μM, or from about 75 μM to about 100 μM.

V152 Substitution

[00223] In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence depicted in FIG. 2YY, where amino acid 152 (indicated by an “x”) is an amino acid other than a valine, e.g., where amino acid 152 is Gly, Ala, Leu, Ile, Pro, Phe, Tyr, Trp, Ser, Thr, Cys, Met, Asn, Gln, Lys, Arg, His, Asp, or Glu. The amino acid numbering in FIG. 2YY retains the numbering of FIG. 2A. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence depicted in FIG. 2YY, where amino acid 152 is Gly. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence depicted in FIG. 2YY, where amino acid 152 is Ala. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence depicted in FIG. 2YY, where amino acid 152 is Leu. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence depicted in FIG. 2YY, where amino acid 152 is Ile. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence depicted in FIG. 2YY, where amino acid 152 is Pro. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence depicted in FIG. 2YY, where amino acid 152 is Phe. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence depicted in FIG. 2YY, where amino acid 152 is Tyr. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence depicted in FIG. 2YY, where amino acid 152 is Trp. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence depicted in FIG. 2YY, where amino acid 152 is Ser. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence depicted in FIG. 2YY, where amino acid 152 is Thr. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence depicted in FIG. 2YY, where amino acid 152 is Cys. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence depicted in FIG. 2YY, where amino acid 152 is Met. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence depicted in FIG. 2YY, where amino acid 152 is Asn. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence depicted in FIG. 2YY, where amino acid 152 is Gln. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence depicted in FIG. 2YY, where amino acid 152 is Lys. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence depicted in FIG. 2YY, where amino acid 152 is Arg. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence depicted in FIG. 2YY, where amino acid 152 is His. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence depicted in FIG. 2YY, where amino acid 152 is Asp. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence depicted in FIG. 2YY, where amino acid 152 is Glu. In some cases, the variant 4-1BBL polypeptide has a binding affinity to CD137 that is from about 100 nM to about 150 nM, from about 150 nM to about 200 nM, from about 200 nM to about 250 nM, from about 250 nM to about 300 nM, from about 300 nM to about 350 nM, from about 350 nM to about 400 nM, from about 400 nM to about 500 nM, from about 500 nM to about 600 nM, from about 600 nM to about 700 nM, from about 700 nM to about 800 nM, from about 800 nM to about 900 nM, from about 900 nM to about 1 μM, to about 1 μM to about 5 μM, from about 5 μM to about 10 μM, from about 10 μM to about 15 μM, from about 15 μM to about 20 μM, from about 20 μM to about 25 μM, from about 25 μM to about 50 μM, from about 50 μM to about 75 μM, or from about 75 μM to about 100 μM.

[00224] In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2A, with an amino acid substitution at V152. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence set forth in SEQ ID NO:1, with an amino acid substitution at V72. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises the amino acid sequence set forth in SEQ ID NO:1, with an amino acid substitution at V72. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence set forth in SEQ ID NO:2, with an amino acid substitution at V73. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises the amino acid sequence set forth in SEQ ID NO:2, with an amino acid substitution at V73. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence set forth in SEQ ID NO:3, with an amino acid substitution at V73. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises the amino acid sequence set forth in SEQ ID NO:3, with an amino acid substitution at V73.

[00225] For example, in some cases, a variant 4-1BBL polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2YY, where “x” is any amino acid other than valine; for example, “x” can be Gly, Ala, Leu, Ile, Pro, Phe, Tyr, Trp, Ser, Thr, Cys, Met, Asn, Gln, Lys, Arg, His, Asp, or Glu. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2YY, where “x” is Gly. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2YY, where “x” is Ala. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2YY, where “x” is Leu. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2YY, where “x” is Ile. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2YY, where “x” is Pro. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2YY, where “x” is Phe. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2YY, where “x” is Tyr. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2YY, where “x” is Trp. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2YY, where “x” is Ser. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2YY, where “x” is Thr. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2YY, where “x” is Cys. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2YY, where “x” is Met. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2YY, where “x” is Asn. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2YY, where “x” is Gln. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2YY, where “x” is Lys. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2YY, where “x” is Arg. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2YY, where “x” is His. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2YY, where “x” is Asp. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2YY, where “x” is Glu. In some cases, the variant 4-1BBL polypeptide has a binding affinity to CD137 that is from about 100 nM to about 150 nM, from about 150 nM to about 200 nM, from about 200 nM to about 250 nM, from about 250 nM to about 300 nM, from about 300 nM to about 350 nM, from about 350 nM to about 400 nM, from about 400 nM to about 500 nM, from about 500 nM to about 600 nM, from about 600 nM to about 700 nM, from about 700 nM to about 800 nM, from about 800 nM to about 900 nM, from about 900 nM to about 1 μM, to about 1 μM to about 5 μM, from about 5 μM to about 10 μM, from about 10 μM to about 15 μM, from about 15 μM to about 20 μM, from about 20 μM to about 25 μM, from about 25 μM to about 50 μM, from about 50 μM to about 75 μM, or from about 75 μM to about 100 μM.

V153 Substitution

[00226] In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence depicted in FIG. 2ZZ, where amino acid 153 (indicated by an “x”) is an amino acid other than a valine, e.g., where amino acid 153 is Gly, Ala, Leu, Ile, Pro, Phe, Tyr, Trp, Ser, Thr, Cys, Met, Asn, Gln, Lys, Arg, His, Asp, or Glu. The amino acid numbering in FIG. 2ZZ retains the numbering of FIG. 2A. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence depicted in FIG. 2ZZ, where amino acid 152 is Gly. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence depicted in FIG. 2ZZ, where amino acid 152 is Ala. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence depicted in FIG. 2ZZ, where amino acid 152 is Leu. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence depicted in FIG. 2ZZ, where amino acid 152 is Ile. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence depicted in FIG. 2ZZ, where amino acid 152 is Pro. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence depicted in FIG. 2ZZ, where amino acid 152 is Phe. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence depicted in FIG. 2ZZ, where amino acid 152 is Tyr. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence depicted in FIG. 2ZZ, where amino acid 152 is Trp. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence depicted in FIG. 2ZZ, where amino acid 152 is Ser. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence depicted in FIG. 2ZZ, where amino acid 152 is Thr. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence depicted in FIG. 2ZZ, where amino acid 152 is Cys. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence depicted in FIG. 2ZZ, where amino acid 152 is Met. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence depicted in FIG. 2ZZ, where amino acid 152 is Asn. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence depicted in FIG. 2ZZ, where amino acid 152 is Gln. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence depicted in FIG. 2ZZ, where amino acid 152 is Lys. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence depicted in FIG. 2ZZ, where amino acid 152 is Arg. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence depicted in FIG. 2ZZ, where amino acid 152 is His. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence depicted in FIG. 2ZZ, where amino acid 152 is Asp. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence depicted in FIG. 2ZZ, where amino acid 152 is Glu. In some cases, the variant 4-1BBL polypeptide has a binding affinity to CD137 that is from about 100 nM to about 150 nM, from about 150 nM to about 200 nM, from about 200 nM to about 250 nM, from about 250 nM to about 300 nM, from about 300 nM to about 350 nM, from about 350 nM to about 400 nM, from about 400 nM to about 500 nM, from about 500 nM to about 600 nM, from about 600 nM to about 700 nM, from about 700 nM to about 800 nM, from about 800 nM to about 900 nM, from about 900 nM to about 1 μM, to about 1 μM to about 5 μM, from about 5 μM to about 10 μM, from about 10 μM to about 15 μM, from about 15 μM to about 20 μM, from about 20 μM to about 25 μM, from about 25 μM to about 50 μM, from about 50 μM to about 75 μM, or from about 75 μM to about 100 μM.

[00227] In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2A, with an amino acid substitution at V153. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence set forth in SEQ ID NO:1, with an amino acid substitution at V73. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises the amino acid sequence set forth in SEQ ID NO:1, with an amino acid substitution at V73. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence set forth in SEQ ID NO:2, with an amino acid substitution at V74. In some

cases, a variant 4-1BBL polypeptide of the present disclosure comprises the amino acid sequence set forth in SEQ ID NO:2, with an amino acid substitution at V74. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence set forth in SEQ ID NO:3, with an amino acid substitution at V74. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises the amino acid sequence set forth in SEQ ID NO:3, with an amino acid substitution at V74.

[00228] For example, in some cases, a variant 4-1BBL polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2ZZ, where “x” is any amino acid other than valine; for example, “x” can be Gly, Ala, Leu, Ile, Pro, Phe, Tyr, Trp, Ser, Thr, Cys, Met, Asn, Gln, Lys, Arg, His, Asp, or Glu. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2ZZ, where “x” is Gly. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2ZZ, where “x” is Ala. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2ZZ, where “x” is Leu. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2ZZ, where “x” is Ile. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2ZZ, where “x” is Pro. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2ZZ, where “x” is Phe. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2ZZ, where “x” is Tyr. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2ZZ, where “x” is Trp. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2ZZ, where “x” is Ser. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2ZZ, where “x” is Thr. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2ZZ, where “x” is Cys. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2ZZ, where “x” is Met. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2ZZ, where “x” is Asn. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2ZZ, where “x” is Gln. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2ZZ, where “x” is Lys. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2ZZ, where “x” is Arg. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2ZZ, where “x” is His. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2ZZ, where “x” is Asp. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2ZZ, where “x” is Glu. In some cases, the variant 4-1BBL polypeptide has a binding affinity to CD137 that is from about 100 nM to about 150 nM, from about 150 nM to about 200 nM, from about 200 nM to about 250 nM, from about 250 nM to about 300 nM, from about 300 nM to about 350 nM, from about 350 nM to about 400 nM, from about 400 nM to about 500 nM, from about 500 nM to about 600 nM, from about 600 nM to about 700 nM, from about 700 nM to about 800 nM, from about 800 nM to about 900 nM, from about 900 nM to about 1 μM, to about 1 μM to about 5 μM, from about 5 μM to about 10 μM, from about 10 μM to about 15 μM, from about 15 μM to about 20 μM, from about 20 μM to about 25 μM, from about 25 μM to about 50 μM, from about 50 μM to about 75 μM, or from about 75 μM to about 100 μM.

G155 Substitution

[00229] In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence depicted in FIG. 2AAA, where amino acid 155 (indicated by an “x”) is an amino acid other than a glycine, e.g., where amino acid 155 is Ala, Val, Leu, Ile, Pro, Phe, Tyr, Trp, Ser, Thr, Cys, Met, Asn, Gln, Lys, Arg, His, Asp, or Glu. The amino acid numbering in FIG. 2AAA retains the numbering of FIG. 2A. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence depicted in FIG. 2AAA, where amino acid 155 is Ala. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence depicted in FIG. 2AAA, where amino 155 is Val. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence depicted in FIG. 2AAA, where amino acid 155 is Leu. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence depicted in FIG. 2AAA, where amino acid 155 is Ile. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence depicted in FIG. 2AAA, where amino acid 155 is Pro. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence depicted in FIG. 2AAA, where amino acid 155 is Phe. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence depicted in FIG. 2AAA, where amino acid 155 is Tyr. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence depicted in FIG. 2AAA, where amino acid 155 is Trp. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence depicted in FIG. 2AAA, where amino acid 155 is Ser. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence depicted in FIG. 2AAA, where amino acid 155 is Thr. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence depicted in FIG. 2AAA, where amino acid 155 is Cys. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence depicted in FIG. 2AAA, where amino acid 155 is Met. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence depicted in FIG. 2AAA, where amino acid 155 is Asn. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence depicted in FIG. 2AAA, where amino acid 155 is Gln. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence depicted in FIG. 2AAA, where amino acid 155 is Lys. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence depicted in FIG. 2AAA, where amino acid 155 is Arg. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises an amino acid sequence having at least

90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence depicted in FIG. 2AAA, where amino acid 155 is His. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence depicted in FIG. 2AAA, where amino acid 155 is Asp. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence depicted in FIG. 2AAA, where amino acid 155 is Glu. In some cases, the variant 4-1BBL polypeptide has a binding affinity to CD137 that is from about 100 nM to about 150 nM, from about 150 nM to about 200 nM, from about 200 nM to about 250 nM, from about 250 nM to about 300 nM, from about 300 nM to about 350 nM, from about 350 nM to about 400 nM, from about 400 nM to about 500 nM, from about 500 nM to about 600 nM, from about 600 nM to about 700 nM, from about 700 nM to about 800 nM, from about 800 nM to about 900 nM, from about 900 nM to about 1 μM, to about 1 μM to about 5 μM, from about 5 μM to about 10 μM, from about 10 μM to about 15 μM, from about 15 μM to about 20 μM, from about 20 μM to about 25 μM, from about 25 μM to about 50 μM, from about 50 μM to about 75 μM, or from about 75 μM to about 100 μM.

[00230] In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2A, with an amino acid substitution at G155. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence set forth in SEQ ID NO:1, with an amino acid substitution at G75. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises the amino acid sequence set forth in SEQ ID NO:1, with an amino acid substitution at G75. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence set forth in SEQ ID NO:2, with an amino acid substitution at G76. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises the amino acid sequence set forth in SEQ ID NO:2, with an amino acid substitution at G76. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence set forth in SEQ ID NO:3, with an amino acid substitution at G76. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises the amino acid sequence set forth in SEQ ID NO:3, with an amino acid substitution at G76.

[00231] For example, in some cases, a variant 4-1BBL polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2AAA, where “x” is any amino acid other than glycine; for example, “x” can be Ala, Val, Leu, Ile, Pro, Phe, Tyr, Trp, Ser, Thr, Cys, Met, Asn, Gln, Lys, Arg, His, Asp, or Glu. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2AAA, where “x” is Ala. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2AAA, where “x” is Val. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2AAA, where “x” is Leu. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2AAA, where “x” is Ile. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2AAA, where “x” is Pro. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2AAA, where “x” is Phe. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2AAA, where “x” is Tyr. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2AAA, where “x” is Trp. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2AAA, where “x” is Ser. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2AAA, where “x” is Thr. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2AAA, where “x” is Cys. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2AAA, where “x” is Met. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2AAA, where “x” is Asn. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2AAA, where “x” is Gln. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2AAA, where “x” is Lys. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2AAA, where “x” is Arg. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2AAA, where “x” is His. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2AAA, where “x” is Asp. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2AAA, where “x” is Glu. In some cases, the variant 4-1BBL polypeptide has a binding affinity to CD137 that is from about 100 nM to about 150 nM, from about 150 nM to about 200 nM, from about 200 nM to about 250 nM, from about 250 nM to about 300 nM, from about 300 nM to about 350 nM, from about 350 nM to about 400 nM, from about 400 nM to about 500 nM, from about 500 nM to about 600 nM, from about 600 nM to about 700 nM, from about 700 nM to about 800 nM, from about 800 nM to about 900 nM, from about 900 nM to about 1 μM, to about 1 μM to about 5 μM, from about 5 μM to about 10 μM, from about 10 μM to about 15 μM, from about 15 μM to about 20 μM, from about 20 μM to about 25 μM, from about 25 μM to about 50 μM, from about 50 μM to about 75 μM, or from about 75 μM to about 100 μM.

E156 Substitution

[00232] In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence depicted in FIG. 2BBB, where amino acid 156 (indicated by an "x") is an amino acid other than a glutamic acid, e.g., where amino acid 156 is Gly, Ala, Val, Leu, Ile, Pro, Phe, Tyr, Trp, Ser, Thr, Cys, Met, Asn, Gln, Lys, Arg, His, or Asp. The amino acid numbering in FIG. 2BBB retains the numbering of FIG. 2A. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence depicted in FIG. 2BBB, where amino acid 156 is Gly. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence depicted in FIG. 2BBB, where amino acid 156 is Ala. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence depicted in FIG. 2BBB, where amino acid 156 is Val. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence depicted in FIG. 2BBB, where amino acid 156 is Leu. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence depicted in FIG. 2BBB, where amino acid 156 is Ile. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence depicted in FIG. 2BBB, where amino acid 156 is Pro. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence depicted in FIG. 2BBB, where amino acid 156 is Phe. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence depicted in FIG. 2BBB, where amino acid 156 is Tyr. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence depicted in FIG. 2BBB, where amino acid 156 is Trp. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence depicted in FIG. 2BBB, where amino acid 156 is Ser. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence depicted in FIG. 2BBB, where amino acid 156 is Thr. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence depicted in FIG. 2BBB, where amino acid 156 is Cys. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence depicted in FIG. 2BBB, where amino acid 156 is Met. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence depicted in FIG. 2BBB, where amino acid 156 is Asn. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence depicted in FIG. 2BBB, where amino acid 156 is Gln. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence depicted in FIG. 2BBB, where amino acid 156 is Lys. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence depicted in FIG. 2BBB, where amino acid 156 is Arg. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence depicted in FIG. 2BBB, where amino acid 156 is His. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence depicted in FIG. 2BBB, where amino acid 156 is Asp. In some cases, the variant 4-1BBL polypeptide has a binding affinity to CD137 that is from about 100 nM to about 150 nM, from about 150 nM to about 200 nM, from about 200 nM to about 250 nM, from about 250 nM to about 300 nM, from about 300 nM to about 350 nM, from about 350 nM to about 400 nM, from about 400 nM to about 500 nM, from about 500 nM to about 600 nM, from about 600 nM to about 700 nM, from about 700 nM to about 800 nM, from about 800 nM to about 900 nM, from about 900 nM to about 1 μM, to about 1 μM to about 5 μM, from about 5 μM to about 10 μM, from about 10 μM to about 15 μM, from about 15 μM to about 20 μM, from about 20 μM to about 25 μM, from about 25 μM to about 50 μM, from about 50 μM to about 75 μM, or from about 75 μM to about 100 μM.

[00233] In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2A, with an amino acid substitution at E156. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence set forth in SEQ ID NO:1, with an amino acid substitution at E76. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises the amino acid sequence set forth in SEQ ID NO:1, with an amino acid substitution at E76. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence set forth in SEQ ID NO:2, with an amino acid substitution at E77. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises the amino acid sequence set forth in SEQ ID NO:2, with an amino acid substitution at E77. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence set forth in SEQ ID NO:3, with an amino acid substitution at E77. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises the amino acid sequence set forth in SEQ ID NO:3, with an amino acid substitution at E77.

[00234] For example, in some cases, a variant 4-1BBL polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2BBB, where “x” is any amino acid other than glutamic acid; for example, “x” can be Gly, Ala, Val, Leu, Ile, Pro, Phe, Tyr, Trp, Ser, Thr, Cys, Met, Asn, Gln, Lys, Arg, His, or Asp. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2BBB, where “x” is Gly. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2BBB, where “x” is Ala. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2BBB, where “x” is Val. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2BBB, where “x” is Leu. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2BBB, where “x” is Ile. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2BBB, where “x” is Pro. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2BBB, where “x” is Phe. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2BBB, where “x” is Tyr. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2BBB, where “x” is Trp. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2BBB, where “x” is Ser. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2BBB, where “x” is Thr. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2BBB, where “x” is Cys. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2BBB, where “x” is Met. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2BBB, where “x” is Asn. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2BBB, where “x” is Gln. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2BBB, where “x” is Lys. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2BBB, where “x” is Arg. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2BBB, where “x” is His. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2BBB, where “x” is Asp. In some cases, the variant 4-1BBL polypeptide has a binding affinity to CD137 that is from about 100 nM to about 150 nM, from about 150 nM to about 200 nM, from about 200 nM to about 250 nM, from about 250 nM to about 300 nM, from about 300 nM to about 350 nM, from about 350 nM to about 400 nM, from about 400 nM to about 500 nM, from about 500 nM to about 600 nM, from about 600 nM to about 700 nM, from about 700 nM to about 800 nM, from about 800 nM to about 900 nM, from about 900 nM to about 1 μM, to about 1 μM to about 5 μM, from about 5 μM to about 10 μM, from about 10 μM to about 15 μM, from about 15 μM to about 20 μM, from about 20 μM to about 25 μM, from about 25 μM to about 50 μM, from about 50 μM to about 75 μM, or from about 75 μM to about 100 μM.

G157 Substitution

[00235] In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence depicted in FIG. 2CCC, where amino acid 157 (indicated by an "x") is an amino acid other than a glycine, e.g., where amino acid 157 is Ala, Val, Leu, Ile, Pro, Phe, Tyr, Trp, Ser, Thr, Cys, Met, Asn, Gln, Lys, Arg, His, Asp, or Glu. The amino acid numbering in FIG. 2CCC retains the numbering of FIG. 2A. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence depicted in FIG. 2CCC, where amino acid 157 is Ala. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence depicted in FIG. 2CCC, where amino acid 157 is Val. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence depicted in FIG. 2CCC, where amino acid 157 is Leu. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence depicted in FIG. 2CCC, where amino acid 157 is Ile. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence depicted in FIG. 2CCC, where amino acid 157 is Pro. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence depicted in FIG. 2CCC, where amino acid 157 is Phe. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence depicted in FIG. 2CCC, where amino acid 157 is Tyr. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence depicted in FIG. 2CCC, where amino acid 157 is Trp. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence depicted in FIG. 2CCC, where amino acid 157 is Ser. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence depicted in FIG. 2CCC, where amino acid 157 is Thr. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence depicted in FIG. 2CCC, where amino acid 157 is Cys. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence depicted in FIG. 2CCC, where amino acid 157 is Met. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence depicted in FIG. 2CCC, where amino acid 157 is Asn. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence depicted in FIG. 2CCC, where amino acid 157 is Gln. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence depicted in FIG. 2CCC, where amino acid 157 is Lys. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence depicted in FIG. 2CCC, where amino acid 157 is Arg. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence depicted in FIG. 2CCC, where amino acid 157 is His. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence depicted in FIG. 2CCC, where amino acid 157 is Asp. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence depicted in FIG. 2CCC, where amino acid 157 is Glu. In some cases, the variant 4-1BBL polypeptide has a binding affinity to CD137 that is from about 100 nM to about 150 nM, from about 150 nM to about 200 nM, from about 200 nM to about 250 nM, from about 250 nM to about 300 nM, from about 300 nM to about 350 nM, from about 350 nM to about 400 nM, from about 400 nM to about 500 nM, from about 500 nM to about 600 nM, from about 600 nM to about 700 nM, from about 700 nM to about 800 nM, from about 800 nM to about 900 nM, from about 900 nM to about 1 μM, to about 1 μM to about 5 μM, from about 5 μM to about 10 μM, from about 10 μM to about 15 μM, from about 15 μM to about 20 μM, from about 20 μM to about 25 μM, from about 25 μM to about 50 μM, from about 50 μM to about 75 μM, or from about 75 μM to about 100 μM.

[00236] In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2A, with an amino acid substitution at G157. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence set forth in SEQ ID NO:1, with an amino acid substitution at G77. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises the amino acid sequence set forth in SEQ ID NO:1, with an amino acid substitution at G77. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence set forth in SEQ ID NO:2, with an amino acid substitution at G78. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises the amino acid sequence set forth in SEQ ID NO:2, with an amino acid substitution at G78. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence set forth in SEQ ID NO:3, with an amino acid substitution at G78. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises the amino acid sequence set forth in SEQ ID NO:3, with an amino acid substitution at G78.

[00237] For example, in some cases, a variant 4-1BBL polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2CCC, where “x” is any amino acid other than methionine; for example, “x” can be Ala, Val, Leu, Ile, Pro, Phe, Tyr, Trp, Ser, Thr, Cys, Met, Asn, Gln, Lys, Arg, His, Asp, or Glu. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2CCC, where “x” is Ala. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2CCC, where “x” is Val. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2CCC, where “x” is Leu. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2CCC, where “x” is Ile. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2CCC, where “x” is Pro. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2CCC, where “x” is Phe. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2CCC, where “x” is Tyr. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2CCC, where “x” is Trp. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2CCC, where “x” is Ser. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2CCC, where “x” is Thr. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2CCC, where “x” is Cys. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2CCC, where “x” is Met. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2CCC, where “x” is Asn. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2CCC, where “x” is Gln. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2CCC, where “x” is Lys. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2CCC, where “x” is Arg. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2CCC, where “x” is His. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2CCC, where “x” is Asp. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2CCC, where “x” is Glu. In some cases, the variant 4-1BBL polypeptide has a binding affinity to CD137 that is from about 100 nM to about 150 nM, from about 150 nM to about 200 nM, from about 200 nM to about 250 nM, from about 250 nM to about 300 nM, from about 300 nM to about 350 nM, from about 350 nM to about 400 nM, from about 400 nM to about 500 nM, from about 500 nM to about 600 nM, from about 600 nM to about 700 nM, from about 700 nM to about 800 nM, from about 800 nM to about 900 nM, from about 900 nM to about 1 μM, to about 1 μM to about 5 μM, from about 5 μM to about 10 μM, from about 10 μM to about 15 μM, from about 15 μM to about 20 μM, from about 20 μM to about 25 μM, from about 25 μM to about 50 μM, from about 50 μM to about 75 μM, or from about 75 μM to about 100 μM.

S158 Substitution

[00238] In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence depicted in FIG. 2DDD, where amino acid 158 (indicated by an “x”) is an amino acid other than a serine, e.g., where amino acid 158 is Gly, Ala, Val, Leu, Ile, Pro, Phe, Tyr, Trp, Thr, Cys, Met, Asn, Gln, Lys, Arg, His, Asp, or Glu. The amino acid numbering in FIG. 2DDD retains the numbering of FIG. 2A. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises an amino acid sequence having at least

90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence depicted in FIG. 2DDD, where amino acid 158 is Gly. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence depicted in FIG. 2DDD, where amino acid 158 is Ala. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence depicted in FIG. 2DDD, where amino acid 158 is Val. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence depicted in FIG. 2DDD, where amino acid 158 is Leu. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence depicted in FIG. 2DDD, where amino acid 158 is Ile. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence depicted in FIG. 2DDD, where amino acid 158 is Pro. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence depicted in FIG. 2DDD, where amino acid 158 is Phe. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence depicted in FIG. 2DDD, where amino acid 158 is Tyr. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence depicted in FIG. 2DDD, where amino acid 158 is Trp. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence depicted in FIG. 2DDD, where amino acid 158 is Thr. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence depicted in FIG. 2DDD, where amino acid 158 is Cys. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence depicted in FIG. 2DDD, where amino acid 158 is Met. In some cases, a variant 4-

1BBL polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence depicted in FIG. 2DDD, where amino acid 158 is Asn. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence depicted in FIG. 2DDD, where amino acid 158 is Gln. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence depicted in FIG. 2DDD, where amino acid 158 is Lys. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence depicted in FIG. 2DDD, where amino acid 158 is Arg. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence depicted in FIG. 2DDD, where amino acid 158 is His. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence depicted in FIG. 2DDD, where amino acid 158 is Asp. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence depicted in FIG. 2DDD, where amino acid 158 is Glu. In some cases, the variant 4-1BBL polypeptide has a binding affinity to CD137 that is from about 100 nM to about 150 nM, from about 150 nM to about 200 nM, from about 200 nM to about 250 nM, from about 250 nM to about 300 nM, from about 300 nM to about 350 nM, from about 350 nM to about 400 nM, from about 400 nM to about 500 nM, from about 500 nM to about 600 nM, from about 600 nM to about 700 nM, from about 700 nM to about 800 nM, from about 800 nM to about 900 nM, from about 900 nM to about 1 μM, to about 1 μM to about 5 μM, from about 5 μM to about 10 μM, from about 10 μM to about 15 μM, from about 15 μM to about 20 μM, from about 20 μM to about 25 μM, from about 25 μM to about 50 μM, from about 50 μM to about 75 μM, or from about 75 μM to about 100 μM.

[00239] In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2A, with an amino acid substitution at S158. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence set forth in SEQ ID NO:1, with an amino acid substitution at S78. In

some cases, a variant 4-1BBL polypeptide of the present disclosure comprises the amino acid sequence set forth in SEQ ID NO:1, with an amino acid substitution at S78. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence set forth in SEQ ID NO:2, with an amino acid substitution at S79. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises the amino acid sequence set forth in SEQ ID NO:2, with an amino acid substitution at S79. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence set forth in SEQ ID NO:3, with an amino acid substitution at S79. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises the amino acid sequence set forth in SEQ ID NO:3, with an amino acid substitution at S79.

[00240] For example, in some cases, a variant 4-1BBL polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2DDD, where “x” is any amino acid other than methionine; for example, “x” can be Gly, Ala, Val, Leu, Ile, Pro, Phe, Tyr, Trp, Ser, Thr, Cys, Asn, Gln, Lys, Arg, His, Asp, or Glu. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2DDD, where “x” is Gly. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2DDD, where “x” is Ala. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2DDD, where “x” is Val. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2DDD, where “x” is Leu. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2DDD, where “x” is Ile. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2DDD, where “x” is Pro. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2DDD, where “x” is Phe. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2DDD, where “x” is Tyr. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2DDD, where “x” is Trp. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2DDD, where “x” is Thr. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2DDD, where “x” is Cys. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2DDD, where “x” is Met. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2DDD, where “x” is Asn. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2DDD, where “x” is Gln. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2DDD, where “x” is Lys. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2DDD, where “x” is Arg. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2DDD, where “x” is His. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2DDD, where “x” is Asp. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2DDD, where “x” is Glu. In some cases, the variant 4-1BBL polypeptide has a binding affinity to CD137 that is from about 100 nM to about 150 nM, from about 150 nM to about 200 nM, from about 200 nM to about 250 nM, from about 250 nM to about 300 nM, from about 300 nM to about 350 nM, from about 350 nM to about 400 nM, from about 400 nM to about 500 nM, from about 500 nM to about 600 nM, from about 600 nM to about 700 nM, from about 700 nM to about 800 nM, from about 800 nM to about 900 nM, from about 900 nM to about 1 μM, to about 1 μM to about 5 μM, from about 5 μM to about 10 μM, from about 10 μM to about 15 μM, from about 15 μM to about 20 μM, from about 20 μM to about 25 μM, from about 25 μM to about 50 μM, from about 50 μM to about 75 μM, or from about 75 μM to about 100 μM.

D184 Substitution

[00241] In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence depicted in FIG. 2EEE, where amino acid 184 (indicated by an “x”) is an amino acid other than an aspartic acid, e.g., where amino acid 184 is Gly, Ala, Val, Leu, Ile, Pro, Phe, Tyr, Trp, Ser, Thr, Cys, Met, Asn, Gln, Lys, Arg, His, or Glu. The amino acid numbering in FIG. 2EEE retains the numbering of FIG. 2A. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence depicted in FIG. 2EEE, where amino acid 155 is Gly. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence depicted in FIG. 2EEE, where amino acid 184 is Ala. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence depicted in FIG. 2EEE, where amino acid 184 is Val. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence depicted in FIG. 2EEE, where amino acid 184 is Leu. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence depicted in FIG. 2EEE, where amino acid 184 is Ile. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence depicted in FIG. 2EEE, where amino acid 184 is Pro. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence depicted in FIG. 2EEE, where amino acid 184 is Phe. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence depicted in FIG. 2EEE, where amino acid 184 is Tyr. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence depicted in FIG. 2EEE, where amino acid 184 is Trp. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence depicted in FIG. 2EEE, where amino acid 184 is Ser. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence depicted in FIG. 2EEE, where amino acid 184 is Thr. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence depicted in FIG. 2EEE, where amino acid 184 is Cys. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence depicted in FIG. 2EEE, where amino acid 184 is Met. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence depicted in FIG. 2EEE, where amino acid 184 is Asn. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence depicted in FIG. 2EEE, where amino acid 184 is Gln. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence depicted in FIG. 2EEE, where amino acid 184 is Lys. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence depicted in FIG. 2EEE, where amino acid 184 is Arg. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence depicted in FIG. 2EEE, where amino acid 184 is His. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence depicted in FIG. 2EEE, where amino acid 184 is Glu. In some cases, the variant 4-1BBL polypeptide has a binding affinity to CD137 that is from about 100 nM to about 150 nM, from about 150 nM to about 200 nM, from about 200 nM to about 250 nM, from about 250 nM to about 300 nM, from about 300 nM to about 350 nM, from about 350 nM to about 400 nM, from about 400 nM to about 500 nM, from about 500 nM to about 600 nM, from about 600 nM to about 700 nM, from about 700 nM to about 800 nM, from about 800 nM to about 900 nM, from about 900 nM to about 1 μM, to about 1 μM to about 5 μM, from about 5 μM to about 10 μM, from about 10 μM to about 15 μM, from about 15 μM to about 20 μM, from about 20 μM to about 25 μM, from about 25 μM to about 50 μM, from about 50 μM to about 75 μM, or from about 75 μM to about 100 μM.

[00242] In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2A, with an amino acid substitution at D184. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence set forth in SEQ ID NO:1, with an amino acid substitution at D104. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises the amino acid sequence set forth in SEQ ID NO:1, with an amino acid substitution at D104. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence set forth in SEQ ID NO:2, with an amino acid substitution at D105. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises the amino acid sequence set forth in SEQ ID NO:2, with an amino acid substitution at D105. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence set forth in SEQ ID NO:3, with an amino acid substitution at D105. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises the amino acid sequence set forth in SEQ ID NO:3, with an amino acid substitution at D105.

[00243] For example, in some cases, a variant 4-1BBL polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2EEE, where “x” is any amino acid other than aspartic acid; for example, “x” can be Gly, Ala, Val, Leu, Ile, Pro, Phe, Tyr, Trp, Ser, Thr, Cys, Met, Asn, Gln, Lys, Arg, His, or Glu. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2EEE, where “x” is Gly. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2EEE, where “x” is Ala. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2EEE, where “x” is Val. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2EEE, where “x” is Leu. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2EEE, where “x” is Ile. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2EEE, where “x” is Pro. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2EEE, where “x” is Phe. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2EEE, where “x” is Tyr. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2EEE, where “x” is Trp. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2EEE, where “x” is Ser. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2EEE, where “x” is Thr. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2EEE, where “x” is Cys. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2EEE, where “x” is Met. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2EEE, where “x” is Asn. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2EEE, where “x” is Gln. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2EEE, where “x” is Lys. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2EEE, where “x” is

Arg. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2EEE, where “x” is His. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2EEE, where “x” is Glu. In some cases, the variant 4-1BBL polypeptide has a binding affinity to CD137 that is from about 100 nM to about 150 nM, from about 150 nM to about 200 nM, from about 200 nM to about 250 nM, from about 250 nM to about 300 nM, from about 300 nM to about 350 nM, from about 350 nM to about 400 nM, from about 400 nM to about 500 nM, from about 500 nM to about 600 nM, from about 600 nM to about 700 nM, from about 700 nM to about 800 nM, from about 800 nM to about 900 nM, from about 900 nM to about 1 μM, to about 1 μM to about 5 μM, from about 5 μM to about 10 μM, from about 10 μM to about 15 μM, from about 15 μM to about 20 μM, from about 20 μM to about 25 μM, from about 25 μM to about 50 μM, from about 50 μM to about 75 μM, or from about 75 μM to about 100 μM.

L185 Substitution

[00244] In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence depicted in FIG. 2FFF, where amino acid 185 (indicated by an “x”) is an amino acid other than a leucine, e.g., where amino acid 185 is Gly, Ala, Val, Ile, Pro, Phe, Tyr, Trp, Ser, Thr, Cys, Met, Asn, Gln, Lys, Arg, His, Asp, or Glu. The amino acid numbering in FIG. 2FFF retains the numbering of FIG. 2A. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence depicted in FIG. 2FFF, where amino acid 185 is Gly. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence depicted in FIG. 2FFF, where amino acid 184 is Ala. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence depicted in FIG. 2FFF, where amino acid 185 is Val. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence depicted in FIG. 2FFF, where amino acid 185 is Ile. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence depicted in FIG. 2FFF, where amino acid 185 is Pro. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence depicted in FIG. 2FFF, where amino acid 185 is Phe. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence depicted in FIG. 2FFF, where amino acid 185 is Tyr. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence depicted in FIG. 2FFF, where amino acid 185 is Trp. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence depicted in FIG. 2FFF, where amino acid 185 is Ser. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence depicted in FIG. 2FFF, where amino acid 185 is Thr. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence depicted in FIG. 2FFF, where amino acid 185 is Cys. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence depicted in FIG. 2FFF, where amino acid 185 is Met. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence depicted in FIG. 2FFF, where amino acid 185 is Asn. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence depicted in FIG. 2FFF, where amino acid 185 is Gln. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence depicted in FIG. 2FFF, where amino acid 185 is Lys. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence depicted in FIG. 2FFF, where amino acid 185 is Arg. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence depicted in FIG. 2FFF, where amino acid 185 is His. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence depicted in FIG. 2FFF, where amino acid 185 is Asp. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence depicted in FIG. 2FFF, where amino acid 185 is Glu. In some cases, the variant 4-1BBL polypeptide has a binding affinity to CD137 that is from about 100 nM to about 150 nM, from about 150 nM to about 200 nM, from about 200 nM to about 250 nM, from about 250 nM to about 300 nM, from about 300 nM to about 350 nM, from about 350 nM to about 400 nM, from about 400 nM to about 500 nM, from about 500 nM to about 600 nM, from about 600 nM to about 700 nM, from about 700 nM to about 800 nM, from about 800 nM to about 900 nM, from about 900 nM to about 1 μM, to about 1 μM to about 5 μM, from about 5 μM to about 10 μM, from about 10 μM to about 15 μM, from about 15 μM to about 20 μM, from about 20 μM to about 25 μM, from about 25 μM to about 50 μM, from about 50 μM to about 75 μM, or from about 75 μM to about 100 μM.

[00245] In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2A, with an amino acid substitution at L185. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence set forth in SEQ ID NO:1, with an amino acid substitution at L105. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises the amino acid sequence set forth in SEQ ID NO:1, with an amino acid substitution at L105. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence set forth in SEQ ID NO:2, with an amino acid substitution at L106. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises the amino acid sequence set forth in SEQ ID NO:2, with an amino acid substitution at L106. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence set forth in SEQ ID NO:3, with an amino acid substitution at L106. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises the amino acid sequence set forth in SEQ ID NO:3, with an amino acid substitution at L106.

[00246] For example, in some cases, a variant 4-1BBL polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2FFF, where "x" is any amino acid other than methionine; for example, "x" can be Gly, Ala, Val, Ile, Pro, Phe, Tyr, Trp, Ser, Thr, Cys, Met, Asn, Gln, Lys, Arg, His, Asp, or Glu. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2FFF, where “x” is Gly. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2FFF, where “x” is Ala. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2FFF, where “x” is Val. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2FFF, where “x” is Ile. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2FFF, where “x” is Pro. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2FFF, where “x” is Phe. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2FFF, where “x” is Tyr. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2FFF, where “x” is Trp. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2FFF, where “x” is Ser. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2FFF, where “x” is Thr. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2FFF, where “x” is Cys. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2FFF, where “x” is Met. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2FFF, where “x” is Asn. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2FFF, where “x” is Gln. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2FFF, where “x” is Lys. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2FFF, where “x” is Arg. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2FFF, where “x” is His. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2FFF, where “x” is Asp. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2FFF, where “x” is Glu. In some cases, the variant 4-1BBL polypeptide has a binding affinity to CD137 that is from about 100 nM to about 150 nM, from about 150 nM to about 200 nM, from about 200 nM to about 250 nM, from about 250 nM to about 300 nM, from about 300 nM to about 350 nM, from about 350 nM to about 400 nM, from about 400 nM to about 500 nM, from about 500 nM to about 600 nM, from about 600 nM to about 700 nM, from about 700 nM to about 800 nM, from about 800 nM to about 900 nM, from about 900 nM to about 1 μM, to about 1 μM to about 5 μM, from about 5 μM to about 10 μM, from about 10 μM to about 15 μM, from about 15 μM to about 20 μM, from about 20 μM to about 25 μM, from about 25 μM to about 50 μM, from about 50 μM to about 75 μM, or from about 75 μM to about 100 μM.

P186 Substitution

[00247] In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence depicted in FIG. 2GGG, where amino acid 186 (indicated by an "x") is an amino acid other than a proline, e.g., where amino acid 186 is Gly, Ala, Val, Leu, Ile, Phe, Tyr, Trp, Ser, Thr, Cys, Met, Asn, Gln, Lys, Arg, His, Asp, or Glu. The amino acid numbering in FIG. 2DDD retains the numbering of FIG. 2A. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence depicted in FIG. 2GGG, where amino acid 186 is Gly. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence depicted in FIG. 2GGG, where amino acid 186 is Ala. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence depicted in FIG. 2GGG, where amino acid 186 is Val. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence depicted in FIG. 2GGG, where amino acid 186 is Leu. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence depicted in FIG. 2GGG, where amino acid 186 is Ile. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence depicted in FIG. 2GGG, where amino acid 186 is Phe. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence depicted in FIG. 2GGG, where amino acid 186 is Tyr. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence depicted in FIG. 2GGG, where amino acid 186 is Trp. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence depicted in FIG. 2GGG, where amino acid 186 is Ser. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence depicted in FIG. 2GGG, where amino acid 186 is Thr. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence depicted in FIG. 2GGG, where amino acid 186 is Cys. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence depicted in FIG. 2GGG, where amino acid 186 is Met. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence depicted in FIG. 2GGG, where amino acid 186 is Asn. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence depicted in FIG. 2GGG, where amino acid 186 is Gln. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence depicted in FIG. 2GGG, where amino acid 186 is Lys. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence depicted in FIG. 2GGG, where amino acid 186 is Arg. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence depicted in FIG. 2GGG, where amino acid 186 is His. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence depicted in FIG. 2GGG, where amino acid 186 is Asp. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence depicted in FIG. 2GGG, where amino acid 186 is Glu. In some cases, the variant 4-1BBL polypeptide has a binding affinity to CD137 that is from about 100 nM to about 150 nM, from about 150 nM to about 200 nM, from about 200 nM to about 250 nM, from about 250 nM to about 300 nM, from about 300 nM to about 350 nM, from about 350 nM to about 400 nM, from about 400 nM to about 500 nM, from about 500 nM to about 600 nM, from about 600 nM to about 700 nM, from about 700 nM to about 800 nM, from about 800 nM to about 900 nM, from about 900 nM to about 1 μM, to about 1 μM to about 5 μM, from about 5 μM to about 10 μM, from about 10 μM to about 15 μM, from about 15 μM to about 20 μM, from about 20 μM to about 25 μM, from about 25 μM to about 50 μM, from about 50 μM to about 75 μM, or from about 75 μM to about 100 μM.

[00248] In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2A, with an amino acid substitution at P186. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence set forth in SEQ ID NO:1, with an amino acid substitution at P106. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises the amino acid sequence set forth in SEQ ID NO:1, with an amino acid substitution at P106. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence set forth in SEQ ID NO:2, with an amino acid substitution at P107. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises the amino acid sequence set forth in SEQ ID NO:2, with an amino acid substitution at P107. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence set forth in SEQ ID NO:3, with an amino acid substitution at P107. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises the amino acid sequence set forth in SEQ ID NO:3, with an amino acid substitution at P107.

[00249] For example, in some cases, a variant 4-1BBL polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2GGG, where “x” is any amino acid other than proline; for example, “x” can be Gly, Ala, Val, Leu, Ile, Phe, Tyr, Trp, Ser, Thr, Cys, Met, Asn, Gln, Lys, Arg, His, Asp, or Glu. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2GGG, where “x” is Gly. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2GGG, where “x” is Ala. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2GGG, where “x” is Val. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2GGG, where “x” is Leu. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2GGG, where “x” is Ile. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2GGG, where “x” is Phe. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2GGG, where “x” is Tyr. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2GGG, where “x” is Trp. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2GGG, where “x” is Ser. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2GGG, where “x” is Thr. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2GGG, where “x” is Cys. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2GGG, where “x” is Met. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2GGG, where “x” is Asn. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2GGG, where “x” is Gln. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2GGG, where “x” is Lys. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2GGG, where “x” is Arg. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2GGG, where “x” is His. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2GGG, where “x” is Asp. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2GGG, where “x” is Glu. In some cases, the variant 4-1BBL polypeptide has a binding affinity to CD137 that is from about 100 nM to about 150 nM, from about 150 nM to about 200 nM, from about 200 nM to about 250 nM, from about 250 nM to about 300 nM, from about 300 nM to about 350 nM, from about 350 nM to about 400 nM, from about 400 nM to about 500 nM, from about 500 nM to about 600 nM, from about 600 nM to about 700 nM, from about 700 nM to about 800 nM, from about 800 nM to about 900 nM, from about 900 nM to about 1 μM, to about 1 μM to about 5 μM, from about 5 μM to about 10 μM, from about 10 μM to about 15 μM, from about 15 μM to about 20 μM, from about 20 μM to about 25 μM, from about 25 μM to about 50 μM, from about 50 μM to about 75 μM, or from about 75 μM to about 100 μM.

P187 Substitution

[00250] In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence depicted in FIG. 2HHH, where amino acid 187 (indicated by an “x”) is an amino acid other than a proline, e.g., where amino acid 187 is Gly, Ala, Val, Leu, Ile, Phe, Tyr, Trp, Ser, Thr, Cys, Met, Asn, Gln, Lys, Arg, His, Asp, or Glu. The amino acid numbering in FIG. 2DDD retains the numbering of FIG. 2A. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence depicted in FIG. 2HHH, where amino acid 187 is Gly. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence depicted in FIG. 2HHH, where amino acid 187 is Ala. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence depicted in FIG. 2HHH, where amino acid 187 is Val. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence depicted in FIG. 2HHH, where amino acid 187 is Leu. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence depicted in FIG. 2HHH, where amino acid 187 is Ile. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence depicted in FIG. 2HHH, where amino acid 187 is Phe. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence depicted in FIG. 2HHH, where amino acid 187 is Tyr. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence depicted in FIG. 2HHH, where amino acid 187 is Trp. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence depicted in FIG. 2HHH, where amino acid 187 is Ser. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence depicted in FIG. 2HHH, where amino acid 187 is Thr. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence depicted in FIG. 2HHH, where amino acid 187 is Cys. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence depicted in FIG. 2HHH, where amino acid 187 is Met. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence depicted in FIG. 2HHH, where amino acid 187 is Asn. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence depicted in FIG. 2HHH, where amino acid 187 is Gln. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence depicted in FIG. 2HHH, where amino acid 187 is Lys. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence depicted in FIG. 2HHH, where amino acid 187 is Arg. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence depicted in FIG. 2HHH, where amino acid 187 is His. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence depicted in FIG. 2HHH, where amino acid 187 is Asp. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence depicted in FIG. 2HHH, where amino acid 187 is Glu. In some cases, the variant 4-1BBL polypeptide has a binding affinity to CD137 that is from about 100 nM to about 150 nM, from about 150 nM to about 200 nM, from about 200 nM to about 250 nM, from about 250 nM to about 300 nM, from about 300 nM to about 350 nM, from about 350 nM to about 400 nM, from about 400 nM to about 500 nM, from about 500 nM to about 600 nM, from about 600 nM to about 700 nM, from about 700 nM to about 800 nM, from about 800 nM to about 900 nM, from about 900 nM to about 1 μM, to about 1 μM to about 5 μM, from about 5 μM to about 10 μM, from about 10 μM to about 15 μM, from about 15 μM to about 20 μM, from about 20 μM to about 25 μM, from about 25 μM to about 50 μM, from about 50 μM to about 75 μM, or from about 75 μM to about 100 μM.

[00251] In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2A, with an amino acid substitution at P187. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence set forth in SEQ ID NO:1, with an amino acid substitution at P107. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises the amino acid sequence set forth in SEQ ID NO:1, with an amino acid substitution at P107. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence set forth in SEQ ID NO:2, with an amino acid substitution at P108. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises the amino acid sequence set forth in SEQ ID NO:2, with an amino acid substitution at P108. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence set forth in SEQ ID NO:3, with an amino acid substitution at P108. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises the amino acid sequence set forth in SEQ ID NO:3, with an amino acid substitution at P108.

[00252] For example, in some cases, a variant 4-1BBL polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2HHH, where “x” is any amino acid other than proline; for example, “x” can be Gly, Ala, Val, Leu, Ile, Phe, Tyr, Trp, Ser, Thr, Cys, Met, Asn, Gln, Lys, Arg, His, Asp, or Glu. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2HHH, where “x” is Gly. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2HHH, where “x” is Ala. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2HHH, where “x” is Val. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2HHH, where “x” is Leu. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2HHH, where “x” is Ile. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2HHH, where “x” is Phe. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2HHH, where “x” is Tyr. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2HHH, where “x” is Trp. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2HHH, where “x” is Ser. In some cases, a

variant 4-1BBL polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2HHH, where “x” is Thr. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2HHH, where “x” is Cys. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2HHH, where “x” is Met. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2HHH, where “x” is Asn. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2HHH, where “x” is Gln. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2HHH, where “x” is Lys. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2HHH, where “x” is Arg. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2HHH, where “x” is His. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2HHH, where “x” is Asp. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2HHH, where “x” is Glu. In some cases, the variant 4-1BBL polypeptide has a binding affinity to CD137 that is from about 100 nM to about 150 nM, from about 150 nM to about 200 nM, from about 200 nM to about 250 nM, from about 250 nM to about 300 nM, from about 300 nM to about 350 nM, from about 350 nM to about 400 nM, from about 400 nM to about 500 nM, from about 500 nM to about 600 nM, from about 600 nM to about 700 nM, from about 700 nM to about 800 nM, from about 800 nM to about 900 nM, from about 900 nM to about 1 μM, to about 1 μM to about 5 μM, from about 5 μM to about 10 μM, from about 10 μM to about 15 μM, from about 15 μM to about 20 μM, from about 20 μM to about 25 μM, from about 25 μM to about 50 μM, from about 50 μM to about 75 μM, or from about 75 μM to about 100 μM.

S189 Substitution

[00253] In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence depicted in FIG. 2III, where amino acid 189 (indicated by an “x”) is an amino acid other than a serine, e.g., where amino acid 189 is Gly, Ala, Val, Leu, Ile, Pro, Phe, Tyr, Trp, Thr, Cys, Met, Asn, Gln, Lys, Arg, His, Asp, or Glu. The amino acid numbering in FIG. 2III retains the numbering of FIG. 2A. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence depicted in FIG. 2III, where amino acid 189 is Gly. In some cases, a variant 4-1BBL

polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence depicted in FIG. 2III, where amino acid 189 is Ala. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence depicted in FIG. 2III, where amino acid 189 is Val. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence depicted in FIG. 2III, where amino acid 189 is Leu. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence depicted in FIG. 2III, where amino acid 189 is Ile. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence depicted in FIG. 2III, where amino acid 189 is Pro. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence depicted in FIG. 2III, where amino acid 189 is Phe. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence depicted in FIG. 2III, where amino acid 189 is Tyr. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence depicted in FIG. 2III, where amino acid 189 is Trp. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence depicted in FIG. 2III, where amino acid 189 is Thr. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence depicted in FIG. 2III, where amino acid 189 is Cys. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence depicted in FIG. 2III, where amino acid 189 is Met. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence depicted in FIG. 2III, where amino acid 189 is Asn. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence depicted in FIG. 2III, where amino acid 189 is Gln. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence depicted in FIG. 2III, where amino acid 189 is Lys. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence depicted in FIG. 2III, where amino acid 189 is Arg. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence depicted in FIG. 2III, where amino acid 189 is His. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence depicted in FIG. 2III, where amino acid 189 is Asp. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence depicted in FIG. 2III, where amino acid 189 is Glu. In some cases, the variant 4-1BBL polypeptide has a binding affinity to CD137 that is from about 100 nM to about 150 nM, from about 150 nM to about 200 nM, from about 200 nM to about 250 nM, from about 250 nM to about 300 nM, from about 300 nM to about 350 nM, from about 350 nM to about 400 nM, from about 400 nM to about 500 nM, from about 500 nM to about 600 nM, from about 600 nM to about 700 nM, from about 700 nM to about 800 nM, from about 800 nM to about 900 nM, from about 900 nM to about 1 μM, to about 1 μM to about 5 μM, from about 5 μM to about 10 μM, from about 10 μM to about 15 μM, from about 15 μM to about 20 μM, from about 20 μM to about 25 μM, from about 25 μM to about 50 μM, from about 50 μM to about 75 μM, or from about 75 μM to about 100 μM.

[00254] In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2A, with an amino acid substitution at S190. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence set forth in SEQ ID NO:1, with an amino acid substitution at S109. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises the amino acid sequence set forth in SEQ ID NO:1, with an amino acid substitution at S109. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence set forth in SEQ ID NO:2, with an amino acid substitution at S110. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises the amino acid sequence set forth in SEQ ID NO:2, with an amino acid substitution at S110. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence set forth in SEQ ID NO:3, with an amino acid substitution at S110. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises the amino acid sequence set forth in SEQ ID NO:3, with an amino acid substitution at S110.

[00255] For example, in some cases, a variant 4-1BBL polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2III, where “x” is any amino acid other than serine; for example, “x” can be Gly, Ala, Val, Leu, Ile, Pro, Phe, Tyr, Trp, Thr, Cys, Met, Asn, Gln, Lys, Arg, His, Asp, or Glu. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2III, where “x” is Gly. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2III, where “x” is Ala. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2III, where “x” is Val. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2III, where “x” is Leu. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2III, where “x” is Ile. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2III, where “x” is Pro. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2III, where “x” is Phe. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2III, where “x” is Tyr. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2III, where “x” is Trp. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2III, where “x” is Thr. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2III, where “x” is Cys. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2III, where “x” is Met. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2III, where “x” is Asn. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2III, where “x” is

Gln. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2III, where “x” is Lys. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2III, where “x” is Arg. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2III, where “x” is His. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2III, where “x” is Asp. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises the amino acid sequence set forth in FIG. 2III, where “x” is Glu. In some cases, the variant 4-1BBL polypeptide has a binding affinity to CD137 that is from about 100 nM to about 150 nM, from about 150 nM to about 200 nM, from about 200 nM to about 250 nM, from about 250 nM to about 300 nM, from about 300 nM to about 350 nM, from about 350 nM to about 400 nM, from about 400 nM to about 500 nM, from about 500 nM to about 600 nM, from about 600 nM to about 700 nM, from about 700 nM to about 800 nM, from about 800 nM to about 900 nM, from about 900 nM to about 1 μM, to about 1 μM to about 5 μM, from about 5 μM to about 10 μM, from about 10 μM to about 15 μM, from about 15 μM to about 20 μM, from about 20 μM to about 25 μM, from about 25 μM to about 50 μM, from about 50 μM to about 75 μM, or from about 75 μM to about 100 μM.

S190 Substitution

[00256] In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence depicted in FIG. 2JJJ, where amino acid 190 (indicated by an “x”) is an amino acid other than a serine, e.g., where amino acid 190 is Gly, Ala, Val, Leu, Ile, Pro, Phe, Tyr, Trp, Thr, Cys, Met, Asn, Gln, Lys, Arg, His, Asp, or Glu. The amino acid numbering in FIG. 2JJJ retains the numbering of FIG. 2A. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence depicted in FIG. 2JJJ, where amino acid 190 is Gly. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence depicted in FIG. 2JJJ, where amino acid 190 is Ala. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence depicted in FIG. 2JJJ, where amino acid 190 is Val. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence depicted in FIG. 2JJJ, where amino acid 190 is Leu. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence depicted in FIG. 2JJJ, where amino acid 190 is Ile. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence depicted in FIG. 2JJJ, where amino acid 190 is Pro. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence depicted in FIG. 2JJJ, where amino acid 190 is Phe. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence depicted in FIG. 2JJJ, where amino acid 190 is Tyr. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence depicted in FIG. 2JJJ, where amino acid 190 is Trp. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence depicted in FIG. 2JJJ, where amino acid 190 is Thr. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence depicted in FIG. 2JJJ, where amino acid 190 is Cys. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence depicted in FIG. 2JJJ, where amino acid 190 is Met. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence depicted in FIG. 2JJJ, where amino acid 190 is Asn. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence depicted in FIG. 2JJJ, where amino acid 190 is Gln. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence depicted in FIG. 2JJJ, where amino acid 190 is Lys. In some cases, a variant 4-1BBL polypeptide of the present disclosure comprises an amino acid sequence having at least 90%, at

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CONTAINING PAGES 1 TO 242

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CLAIMS

What is claimed is:

1. A variant 4-1BBL immunomodulatory polypeptide comprising an amino acid sequence having at least 85% amino acid sequence identity to the 4-1BBL amino acid sequence depicted in FIG. 2A or to the 4-1BBL amino acid sequence set forth in one of SEQ ID NOs:1-3,

wherein the variant 4-1BBL immunomodulatory polypeptide has one or more amino acid substitutions relative to the 4-1BBL amino acid sequence depicted in FIG. 2A or to the 4-1BBL amino acid sequence set forth in one of SEQ ID NOs:1-3; and

wherein the variant 4-1BBL immunomodulatory polypeptide: i) exhibits reduced binding affinity to a 4-1BB polypeptide having an amino acid sequence depicted in FIG. 3 and set forth in SEQ ID NO:91, compared to the binding affinity of the 4-1BBL amino acid sequence depicted in FIG. 2A or set forth in one of SEQ ID NOs:1-3 for the 4-1BB polypeptide; and/or ii) wherein the variant 4-1BBL immunomodulatory polypeptide exhibits increased production levels by a mammalian cell, compared to the production levels of the 4-1BBL amino acid sequence depicted in FIG. 2A or set forth in one of SEQ ID NOs:1-3.

2. The variant immunomodulatory polypeptide of claim 1, wherein the polypeptide comprises a substitution of one of amino acids 91, 92, 94-115, 117-126, 128-132, 144-153, 155-158, 184-187, 189-191, 193-195, 197, 210-219, 221-224, 226, 228-231, 233, and 234 based on the amino acid numbering set out in FIG. 2A.

3. The variant immunomodulatory polypeptide of claim 1, wherein the variant immunomodulatory polypeptide exhibits less than 50% of binding affinity exhibited by to the 4-1BBL amino acid sequence depicted in FIG. 2A, or as set forth in one of SEQ ID NOs:1-3, for the 4-1BB polypeptide.

4. A multimeric polypeptide comprising:

a) a first polypeptide comprising, in order from N-terminus to C-terminus:

i) an epitope;

ii) a first major histocompatibility complex (MHC) polypeptide; and

b) a second polypeptide comprising, in order from N-terminus to C-terminus:

i) a second MHC polypeptide; and

ii) optionally an immunoglobulin (Ig) Fc polypeptide or a non-Ig scaffold, wherein the multimeric polypeptide comprises one or more immunomodulatory domains, wherein the one or more immunomodulatory domain is:

A) at the C-terminus of the first polypeptide;

B) at the N-terminus of the second polypeptide;

C) at the C-terminus of the second polypeptide; or

D) at the C-terminus of the first polypeptide and at the N-terminus of the second polypeptide,

wherein the immunomodulatory domain is a variant immunomodulatory polypeptide of any one of claims 1-3; and

wherein: i) the multimeric polypeptide exhibits reduced binding affinity to a 4-1BB polypeptide having an amino acid sequence depicted in FIG. 3 and set forth in SEQ ID NO:91, compared to the binding affinity of a control multimeric polypeptide comprising an immunomodulatory domain comprising the 4-1BBL amino acid sequence depicted in FIG. 2A or set forth in one of SEQ ID NOs:1-3 for the 4-1BB polypeptide; and/or ii) wherein the multimeric polypeptide exhibits increased production levels by a mammalian cell, compared to the production levels of a control multimeric polypeptide comprising an immunomodulatory domain comprising the 4-1BBL amino acid sequence depicted in FIG. 2A or set forth in one of SEQ ID NOs:1-3.

5. The multimeric polypeptide of claim 4, wherein the multimeric polypeptide comprises:

a) a first polypeptide comprising, in order from N-terminus to C-terminus:

i) an epitope;

ii) a first MHC polypeptide; and

iii) an immunomodulatory domain; and

b) a second polypeptide comprising, in order from N-terminus to C-terminus:

i) a second MHC polypeptide; and

ii) an Ig Fc polypeptide.

6. The multimeric polypeptide of claim 4, wherein the multimeric polypeptide comprises:

a) a first polypeptide comprising, in order from N-terminus to C-terminus:

i) an epitope; and

ii) a first MHC polypeptide; and

b) a second polypeptide comprising, in order from N-terminus to C-terminus:

i) an immunomodulatory domain;

iii) a second MHC polypeptide; and

ii) an immunoglobulin (Ig) Fc polypeptide.

7. The multimeric polypeptide of claim 4, wherein the multimeric polypeptide comprises:
a) a first polypeptide comprising, in order from N-terminus to C-terminus:
  i) an epitope; and
  ii) a first MHC polypeptide; and
b) a second polypeptide comprising, in order from N-terminus to C-terminus:
  i) a second MHC polypeptide; and
  ii) an Ig Fc polypeptide; and
  iii) an immunomodulatory domain.

8. The multimeric polypeptide of claim 4, wherein the multimeric polypeptide comprises:
a) a first polypeptide comprising, in order from N-terminus to C-terminus:
  i) an epitope; and
  ii) a first MHC polypeptide; and
b) a second polypeptide comprising, in order from N-terminus to C-terminus:
  i) a second MHC polypeptide; and
  ii) an immunomodulatory domain.

9. The multimeric polypeptide of claim 4, wherein the multimeric polypeptide comprises:
a) a first polypeptide comprising, in order from N-terminus to C-terminus:
  i) an epitope; and
  ii) a first MHC polypeptide; and
b) a second polypeptide comprising, in order from N-terminus to C-terminus:
  i) an immunomodulatory domain; and
  ii) a second MHC polypeptide.

10. The multimeric polypeptide of claim 4, wherein the multimeric polypeptide comprises:
a) a first polypeptide comprising, in order from N-terminus to C-terminus:
  i) an epitope;
  ii) a first MHC polypeptide; and
  iii) an immunomodulatory domain; and
b) a second polypeptide comprising, in order from N-terminus to C-terminus:
  i) a second MHC polypeptide.

11. The multimeric polypeptide of claim 4, wherein the non-Ig scaffold is an XTEN polypeptide, a transferrin polypeptide, an elastin-like polypeptide, a silk-like polypeptide, or a silk-elastin-like polypeptide.

12. The multimeric polypeptide of any one of claims 4-11, wherein the first MHC polypeptide is a β2-microglobulin polypeptide; and wherein the second MHC polypeptide is an MHC class I heavy chain polypeptide.

13. The multimeric polypeptide of claim 12, wherein the β2-microglobulin polypeptide comprises an amino acid sequence having at least 85% amino acid sequence identity to any one of the amino acid sequences set forth in SEQ ID NO:6.

14. The multimeric polypeptide of claim 11, wherein the MHC class I heavy chain polypeptide is an HLA-A, an HLA-B, or an HLA-C heavy chain.

15. The multimeric polypeptide of claim 12, wherein the MHC class I heavy chain polypeptide comprises an amino acid sequence having at least 85% amino acid sequence identity to the amino acid sequence depicted in any one of FIG. 5A-5C.

16. The multimeric polypeptide of any one of claims 4-11, wherein the first MHC polypeptide is an MHC Class II alpha chain polypeptide; and wherein the second MHC polypeptide is an MHC class II beta chain polypeptide.

17. The multimeric polypeptide of any one of claims 4-16, wherein the epitope is a T-cell epitope.

18. The multimeric polypeptide of any one of claims 4-10 and 12-17, wherein multimeric polypeptide comprises an Fc polypeptide, and wherein the Ig Fc polypeptide is an IgG1 Fc polypeptide, an IgG2 Fc polypeptide, an IgG3 Fc polypeptide, an IgG4 Fc polypeptide, an IgA Fc polypeptide, or an IgM Fc polypeptide.

19. The multimeric polypeptide of claim 18, wherein the Ig Fc polypeptide comprises an amino acid sequence having at least 85% amino acid sequence identity to an amino acid sequence depicted in FIG. 4A-4C.

20. The multimeric polypeptide of any one of claims 4-19, wherein the first polypeptide and the second polypeptide are non-covalently associated.

21. The multimeric polypeptide of any one of claims 4-19, wherein the first polypeptide and the second polypeptide are covalently linked.

22. The multimeric polypeptide of claim 21, wherein the covalent linkage is via a disulfide bond.

23. The multimeric polypeptide of claim 22, wherein the first MHC polypeptide or a linker between the epitope and the first MHC polypeptide comprises an amino acid substitution to provide a first Cys residue, and the second MHC polypeptide comprises an amino acid substitution to provide a second Cys residue, and wherein the disulfide linkage is between the first and the second Cys residues.

24. The multimeric polypeptide of any one of claims 4-11, comprising a first linker interposed between the epitope and the first MHC polypeptide.

25. The multimeric polypeptide of any one of claims 4-11, wherein the variant 4-1BBL immunomodulatory polypeptide comprises a substitution of one of amino acids 91, 92, 94-115, 117-126, 128-132, 144-153, 155-158, 184-187, 189-191, 193-195, 197, 210-219, 221-224, 226, 228-231, 233, and 234 based on the amino acid numbering set out in FIG. 2A.

26. The multimeric polypeptide of any one of claims 4-25, comprising 2 or more immunomodulatory polypeptides.

27. The multimeric polypeptide of claim 26, wherein the 2 or more immunomodulatory polypeptides are in tandem.

28. The multimeric polypeptide of any one of claims 26 and 27, wherein the multimeric polypeptide comprises a third polypeptide, wherein the third polypeptide comprises an immunomodulatory polypeptide comprising an amino acid sequence having at least 90% amino acid sequence identity to the immunomodulatory polypeptide of the first polypeptide or the second polypeptide.

29. The multimeric polypeptide of claim 28, wherein the third polypeptide is covalently linked to the first polypeptide.

30. The multimeric polypeptide of any one of claims 4-10 and 12-29, wherein the second polypeptide comprises, in order from N-terminus to C-terminus:

i) the second MHC polypeptide;
ii) the Ig Fc polypeptide; and
iii) an affinity tag.

31. A nucleic acid comprising a nucleotide sequence encoding a recombinant polypeptide,

i) wherein the recombinant polypeptide comprises, in order from N-terminus to C-terminus:

a) an epitope;
b) a first major histocompatibility complex (MHC) polypeptide;
c) an immunomodulatory polypeptide;
d) a proteolytically cleavable linker or a ribosome skipping signal;
e) a second MHC polypeptide; and
f) an immunoglobulin (Ig) Fc polypeptide;

wherein the immunomodulatory polypeptide is a variant immunomodulatory polypeptide of any one of claims 1-3; or

ii) wherein the recombinant polypeptide comprises, in order from N-terminus to C-terminus:

a) an epitope;
b) a first MHC polypeptide;
c) a proteolytically cleavable linker or a ribosome skipping signal;
d) an immunomodulatory polypeptide
e) a second MHC polypeptide; and
f) an Ig Fc polypeptide,

wherein the immunomodulatory polypeptide is a variant immunomodulatory polypeptide of any one of claims 1-3.

32. The nucleic acid of claim 31, wherein the first MHC polypeptide is a β2-microglobulin polypeptide; and wherein the second MHC polypeptide is an MHC class I heavy chain polypeptide.

33. The nucleic acid of claim 32, wherein the β2-microglobulin polypeptide comprises an amino acid sequence having at least 85% amino acid sequence identity to any one of the amino acid sequences depicted in FIG. 6

34. The nucleic acid of claim 31, wherein the MHC class I heavy chain polypeptide is an HLA-A, HLA-B, or HLA-C heavy chain.

35. The nucleic acid of claim 34, wherein the MHC class I heavy chain polypeptide comprises an amino acid sequence having at least 85% amino acid sequence identity to the amino acid sequence depicted in any one of FIG. 5A-5C.

36. The nucleic acid of claim 31, wherein the first MHC polypeptide is an MHC Class II alpha chain polypeptide; and wherein the second MHC polypeptide is an MHC class II beta chain polypeptide.

37. The nucleic acid of claim 31, wherein the epitope is a T-cell epitope.

38. The nucleic acid of claim 31, wherein the Ig Fc polypeptide is an IgG1 Fc polypeptide, an IgG2 Fc polypeptide, an IgG3 Fc polypeptide, an IgG4 Fc polypeptide, an IgA Fc polypeptide, or an IgM Fc polypeptide.

39. The nucleic acid of claim 38, wherein the Ig Fc polypeptide comprises an amino acid sequence having at least 85% amino acid sequence identity to an amino acid sequence depicted in Figures 4A-4C.

40. The nucleic acid of claim 31, wherein the variant 4-1BBL immunomodulatory polypeptide comprises a substitution of one of amino acids 91, 92, 94-115, 117-126, 128-132, 144-153, 155-158, 184-187, 189-191, 193-195, 197, 210-219, 221-224, 226, 228-231, 233, and 234 based on the amino acid numbering set out in FIG. 2A.

41. The nucleic acid of claim 31, wherein the multimeric polypeptide comprises a second immunomodulatory polypeptide selected from a CD7, CD30L, CD40, CD70, CD83, HLA-G, MICA, MICB, HVEM, lymphotoxin beta receptor, 3/TR6, ILT3, ILT4, and HVEM.

42. The nucleic acid of claim 31, wherein the proteolytically cleavable linker or ribosome skipping signal comprises an amino acid sequence selected from:

a) LEVLFQGP (SEQ ID NO:116);

b) ENLYTQS (SEQ ID NO:117);

c) a furin cleavage site;
d) LVPR (SEQ ID NO:118);
e) GSGATNFSLLKQAGDVEENPGP (SEQ ID NO:119);
f) GSGEGRGSLLTCGDVEENPGP (SEQ ID NO:120);
g) GSGQCTNYALLKLAGDVESNPGP (SEQ ID NO:121); and
h) GSGVKQTLNFDLLKLAGDVESNPGP (SEQ ID NO:122).

43. The nucleic acid of claim 31, wherein the recombinant polypeptide comprises, in order from N-terminus to C-terminus:

a) a first leader peptide;
b) the epitope;
c) the first MHC polypeptide;
d) the immunomodulatory polypeptide;
e) the proteolytically cleavable linker or ribosome skipping signal;
f) a second leader peptide;
g) the second MHC polypeptide; and
h) the immunoglobulin (Ig) Fc polypeptide.

44. The nucleic acid of claim 43, wherein the first leader peptide and the second leader peptide is a β2-M leader peptide.

45. The nucleic acid of claim 31, wherein the nucleotide sequence is operably linked to a transcriptional control element.

46. The nucleic acid of claim 45, wherein the transcriptional control element is a promoter that is functional in a eukaryotic cell.

47. The nucleic acid of claim 31, wherein the first MHC polypeptide or a linker between the epitope and the first MHC polypeptide comprises an amino acid substitution to provide a first Cys residue, and the second MHC polypeptide comprises an amino acid substitution to provide a second Cys residue, and wherein the first and the second Cys residues provide for a disulfide linkage between the first MHC polypeptide and the second MHC polypeptide.

48. A recombinant expression vector comprising the nucleic acid of any one of claims 31-47.

49. The recombinant expression vector of claim 48, wherein the vector is a viral vector or a non-viral vector.

50. A host cell genetically modified with the recombinant expression vector of claim 48 or claim 49.

51. The host cell of claim 50, wherein the host cell is *in vitro*.

52. The host cell of claim 50, wherein the host cell is genetically modified such that the cell does not produce an endogenous MHC β2-microglobulin polypeptide.

53. The host cell of claim 50, wherein the host cell is a T lymphocyte.

54. A composition comprising:

a) a first nucleic acid comprising a nucleotide sequence encoding a first polypeptide comprising, in order from N-terminus to C-terminus:

i) an epitope;

ii) a first MHC polypeptide; and

iii) an immunomodulatory domain,

wherein the immunomodulatory domain is a variant immunomodulatory polypeptide of any one of claims 1-3; and

b) a first nucleic acid comprising a nucleotide sequence encoding a second polypeptide comprising, in order from N-terminus to C-terminus:

i) a second MHC polypeptide; and

ii) an Ig Fc polypeptide.

55. A composition comprising:

a) a first nucleic acid comprising a nucleotide sequence encoding a first polypeptide comprising, in order from N-terminus to C-terminus:

i) an epitope; and

ii) a first MHC polypeptide; and

b) a first nucleic acid comprising a nucleotide sequence encoding a second polypeptide comprising, in order from N-terminus to C-terminus:

i) an immunomodulatory domain, wherein the immunomodulatory domain is a variant immunomodulatory polypeptide of any one of claims 1-3;

ii) a second MHC polypeptide; and

iii) an Ig Fc polypeptide.

56. The composition of claim 54 or 55, wherein the first and/or the second nucleic acid is present in a recombinant expression vector.

57. A host cell genetically modified with the composition of any one of claims 54-56.

58. A method of producing the multimeric polypeptide of any one of claims 4-30, the method comprising:

a) culturing the host cell of 48, 49, or 57 *in vitro* in a culture medium under conditions such that the host cell synthesizes the multimeric polypeptide; and

b) isolating the multimeric polypeptide from the host cell and/or from the culture medium.

59. The method of claim 58, wherein the second polypeptide comprises an affinity tag, and wherein said isolating comprises contacting the multimeric polypeptide produced by the cell with a binding partner for the affinity tag, wherein the binding partner is immobilized, thereby immobilizing the multimeric polypeptide.

60. The method of claim 58, comprising eluting the immobilized multimeric polypeptide.

61. A method of selectively modulating the activity of an epitope-specific T cell, the method comprising contacting the T cell with the multimeric polypeptide of any one of claims 4-30, wherein said contacting selectively modulates the activity of the epitope-specific T cell.

62. The method of claim 61, wherein the immunomodulatory polypeptide is an activating polypeptide, and wherein the multimeric polypeptide activates the epitope-specific T cell.

63. The method of claim 61, wherein the immunomodulatory polypeptide is an inhibiting polypeptide, and wherein the multimeric polypeptide inhibits the epitope-specific T cell.

64. The method of claim 61, wherein said contacting is *in vitro*.

65. The method of claim 61, wherein said contacting is *in vivo*.

66. A method of selectively modulating the activity of an epitope-specific T cell in an individual, the method comprising administering to the individual an effective amount of the multimeric polypeptide of any one of claims 4-30 effective to selectively modulate the activity of an epitope-specific T cell in an individual.

67. The method of claim 66, wherein the immunomodulatory polypeptide is an activating polypeptide, and wherein the multimeric polypeptide activates the epitope-specific T cell.

68. The method of claim 67, wherein the epitope is a cancer-associated epitope, and wherein said administering selectively increases the activity of a T cell specific for the cancer-associate epitope.

69. The method of claim 66, wherein the immunomodulatory polypeptide is an inhibitory polypeptide, and wherein the multimeric polypeptide inhibits activity of the epitope-specific T cell.

70. The method of claim 69, wherein the epitope is a self-epitope, and wherein said administering selectively inhibits the activity of a T cell specific for the self-epitope.

71. A method of treating an infection in an individual, the method comprising administering to the individual an effective amount of

a) the multimeric polypeptide of any one of claims 4-30; or

b) one or more recombinant expression vectors comprising nucleotide sequences encoding the multimeric polypeptide of any one of claims 4-30; or

c) one or more mRNAs comprising nucleotide sequences encoding the multimeric polypeptide of any one of claims 4-30.

wherein the epitope is a pathogen-associated epitope, wherein the immunomodulatory polypeptide is an activating polypeptide, and wherein said administering effective to selectively modulate the activity of a pathogen-associated epitope-specific T cell in an individual.

72. The method of claim 71, wherein the pathogen is a virus, a bacterium, or a protozoan.

73. The method of any one of claims 66-71, wherein said administering is subcutaneous.

74. The method of any one of claims 66-71, wherein said administering is intravenous.

75. The method of any one of claims 66-71, wherein said administering is intramuscular.

76. The method of any one of claims 66-71, wherein said administering is systemic.

77. The method of any one of claims 66-71, wherein said administering is distal to a treatment site.

78. The method of any one of claims 66-71, wherein said administering is local.

79. The method of any one of claims 66-71, wherein said administering is at or near a treatment site.

80. A composition comprising:
a) the multimeric polypeptide of any one of claims 4-30; and
b) a pharmaceutically acceptable excipient.

81. A composition comprising:
a) the nucleic acid of any one of claims 31-47 or the recombinant expression vector of claim 48 or 49; and
b) a pharmaceutically acceptable excipient.

FIG. 1A

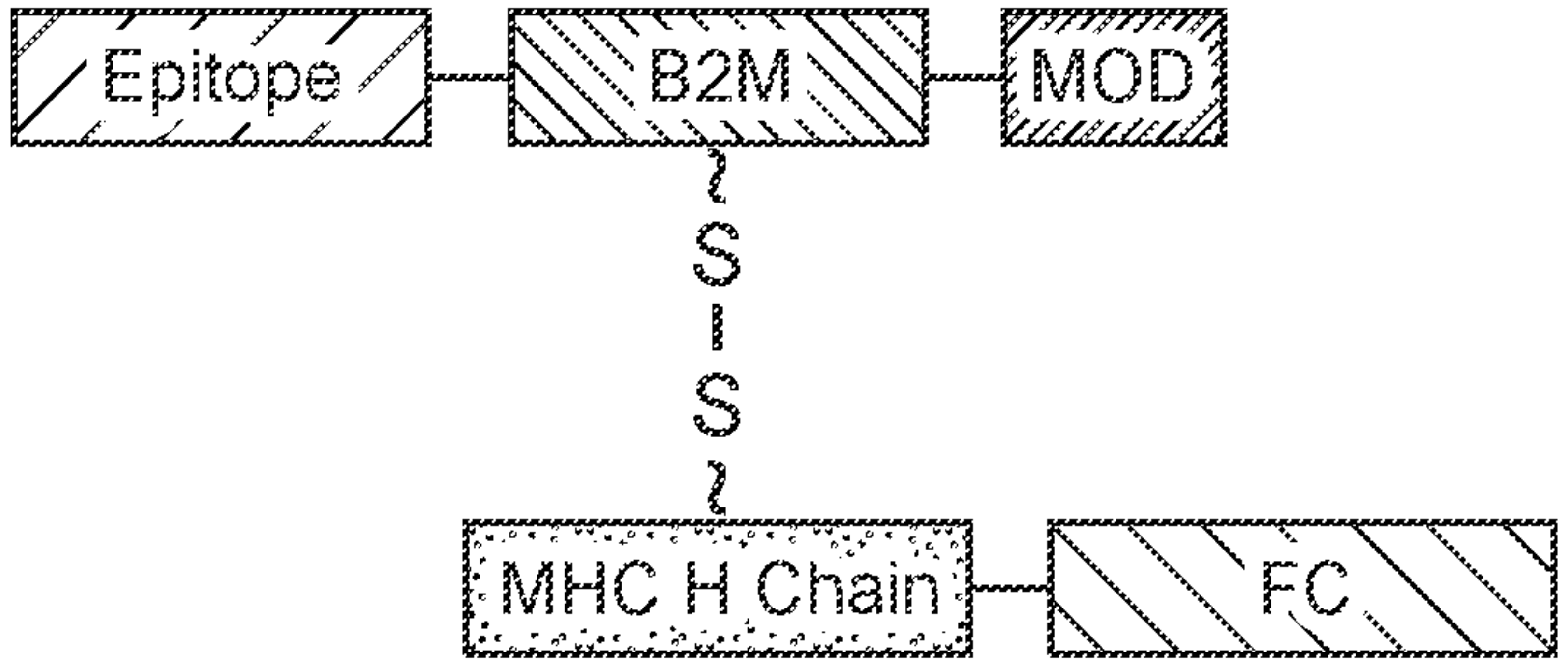

FIG. 1B

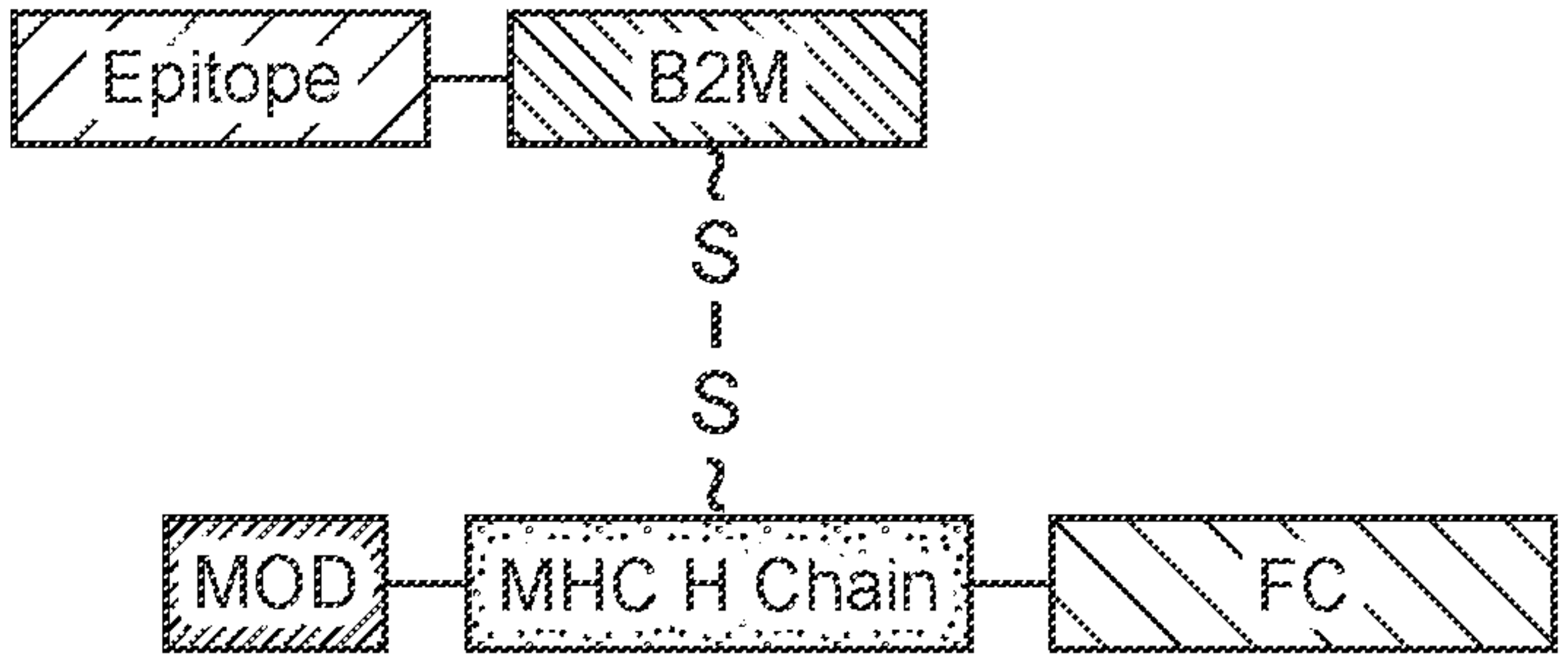

FIG. 1C

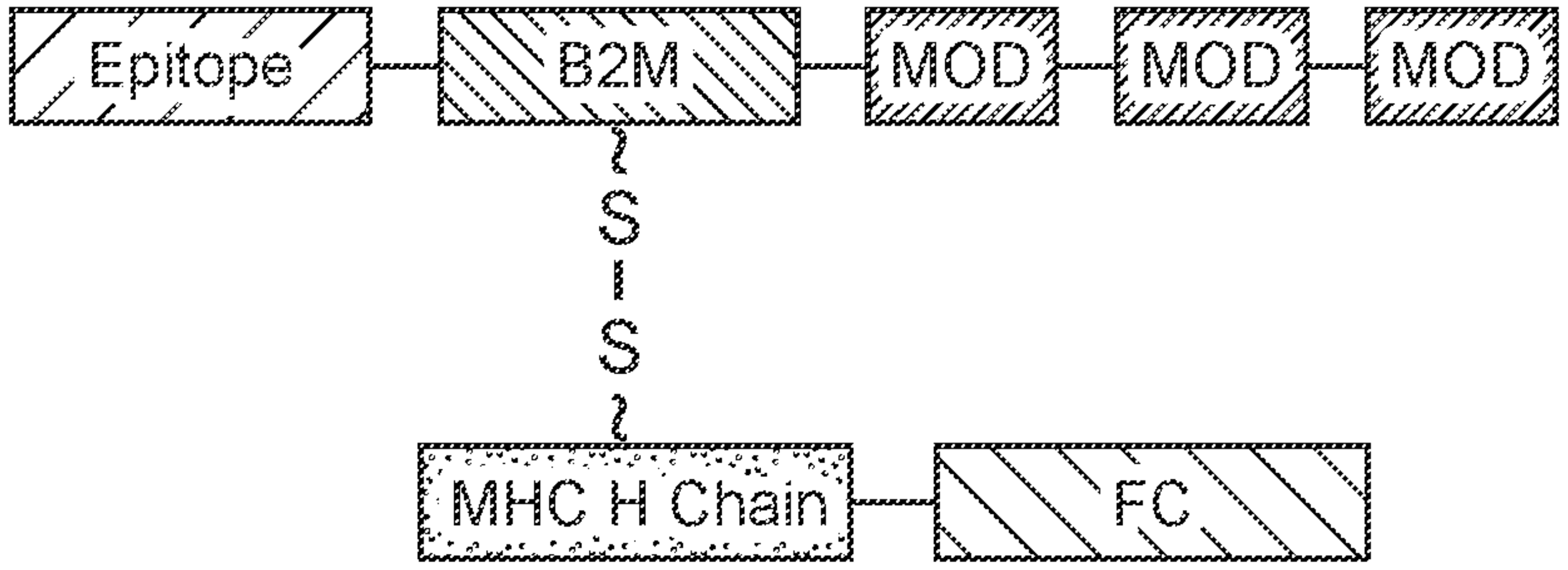

FIG. 1D

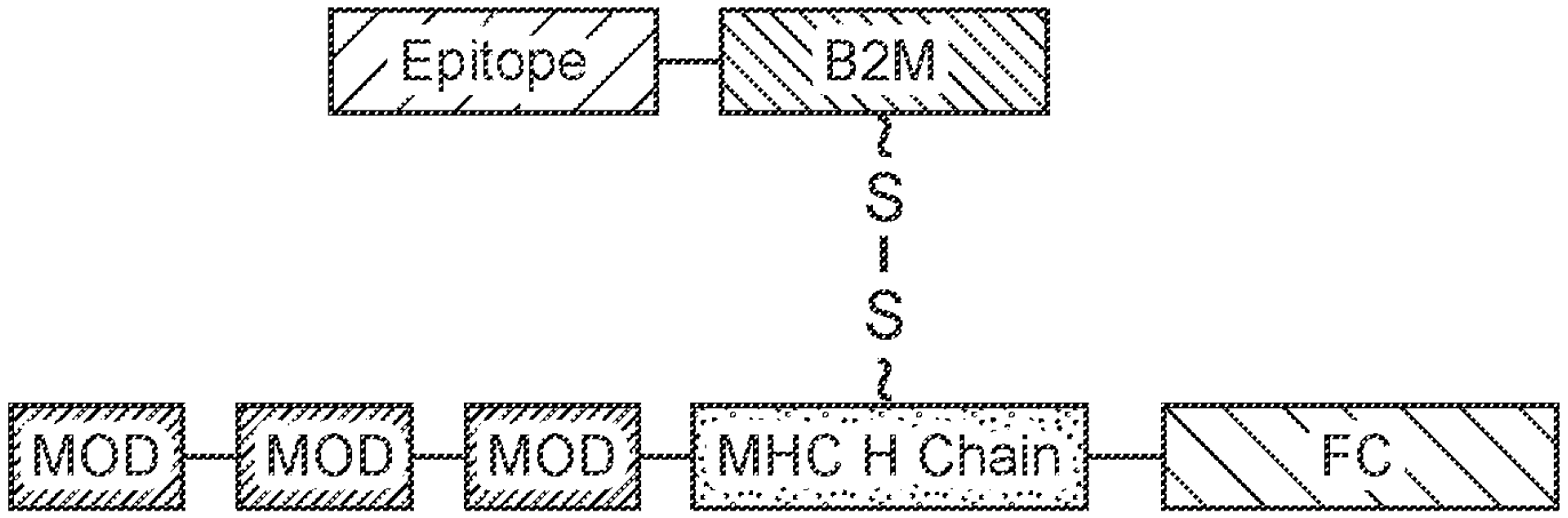

FIG. 2A

4-1-BBL
Homo sapiens
GenBank NP_003802
Cytoplasmic domain = 1-25
Transmembrane domain = 26-48
Ectodomain = 49-254
TNF homology domain = 80-254, 81-254, or 80-246

```
  1 MEYASDASLD PEAPWPPAPR ARACRVLPWA LVAGLLLLLL LAAACAVFLA CPWAVSGARA
 61 SPGSAASPRL REGPELSPDD PAGLLDLRQG MFAQLVAQNV LLIDGPLSWY SDPGLAGVSL
121 TGGLSYKEDT KELVVAKAGV YYVFFQLELR RVVAGEGSGS VSLALHLQPL RSAAGAAALA
181 LTVDLPPASS EARNSAFGFQ GRLLHLSAGQ RLGVHLHTEA RARHAWQLTQ GATVLGLFRV
241 TPEIPAGLPS PRSE (SEQ ID NO:4)
```

FIG. 2B
K127

81 PAGLLDLRQG MFAQLVAQNV LLIDGPLSWY SDPGLAGVSL
121 TGGLSY**X**EDT KELVVAKAGV YYVFFQLELR RVVAGEGSGS VSLALHLQPL RSAAGAAALA
181 LTVDLPPASS EARNSAFGFQ GRLLHLSAGQ RLGVHLHTEA RARHAWQLTQ GATVLGLFRV
241 TPEIPAGLPS PRSE (SEQ ID NO:5)

FIG. 2C
K127

81 PAGLLDLRQG MFAQLVAQNV LLIDGPLSWY SDPGLAGVSL
121 TGGLSY**A**EDT KELVVAKAGV YYVFFQLELR RVVAGEGSGS VSLALHLQPL RSAAGAAALA
181 LTVDLPPASS EARNSAFGFQ GRLLHLSAGQ RLGVHLHTEA RARHAWQLTQ GATVLGLFRV
241 TPEIPAGLPS PRSE (SEQ ID NO:6)

FIG. 2D
Q227

81 PAGLLDLRQG MFAQLVAQNV LLIDGPLSWY SDPGLAGVSL
121 TGGLSYKEDT KELVVAKAGV YYVFFQLELR RVVAGEGSGS VSLALHLQPL RSAAGAAALA
181 LTVDLPPASS EARNSAFGFQ GRLLHLSAGQ RLGVHLHTEA RARHAW**X**LTQ GATVLGLFRV
241 TPEIPAGLPS PRSE (SEQ ID NO:7)

FIG. 2E
M91

81 PAGLLDLRQG **X**FAQLVAQNV LLIDGPLSWY SDPGLAGVSL
121 TGGLSYKEDT KELVVAKAGV YYVFFQLELR RVVAGEGSGS VSLALHLQPL RSAAGAAALA
181 LTVDLPPASS EARNSAFGFQ GRLLHLSAGQ RLGVHLHTEA RARHAWQLTQ GATVLGLFRV
241 TPEIPAGLPS PRSE (SEQ ID NO:8)

FIG. 2F
F92

81 PAGLLDLRQG M**X**AQLVAQNV LLIDGPLSWY SDPGLAGVSL
121 TGGLSYKEDT KELVVAKAGV YYVFFQLELR RVVAGEGSGS VSLALHLQPL RSAAGAAALA
181 LTVDLPPASS EARNSAFGFQ GRLLHLSAGQ RLGVHLHTEA RARHAWQLTQ GATVLGLFRV
241 TPEIPAGLPS PRSE (SEQ ID NO:9)

FIG. 2G

Q94

```
 81                       PAGLLDLRQG MFAXLVAQNV LLIDGPLSWY SDPGLAGVSL
121 TGGLSYKEDT KELVVAKAGV YYVFFQLELR RVVAGEGSGS VSLALHLQPL RSAAGAAALA
181 LTVDLPPASS EARNSAFGFQ GRLLHLSAGQ RLGVHLHTEA RARHAWQLTQ GATVLGLFRV
241 TPEIPAGLPS PRSE (SEQ ID NO:10)
```

FIG. 2H

L95

```
 81                       PAGLLDLRQG MFAQXVAQNV LLIDGPLSWY SDPGLAGVSL
121 TGGLSYKEDT KELVVAKAGV YYVFFQLELR RVVAGEGSGS VSLALHLQPL RSAAGAAALA
181 LTVDLPPASS EARNSAFGFQ GRLLHLSAGQ RLGVHLHTEA RARHAWQLTQ GATVLGLFRV
241 TPEIPAGLPS PRSE (SEQ ID NO:11)
```

FIG. 2I

V96

```
 81                       PAGLLDLRQG MFAQLXAQNV LLIDGPLSWY SDPGLAGVSL
121 TGGLSYKEDT KELVVAKAGV YYVFFQLELR RVVAGEGSGS VSLALHLQPL RSAAGAAALA
181 LTVDLPPASS EARNSAFGFQ GRLLHLSAGQ RLGVHLHTEA RARHAWQLTQ GATVLGLFRV
241 TPEIPAGLPS PRSE (SEQ ID NO:12)
```

FIG. 2J
Q98

81 PAGLLDLRQG MFAQLVA**X**NV LLIDGPLSWY SDPGLAGVSL
121 TGGLSYKEDT KELVVAKAGV YYVFFQLELR RVVAGEGSGS VSLALHLQPL RSAAGAAALA
181 LTVDLPPASS EARNSAFGFQ GRLLHLSAGQ RLGVHLHTEA RARHAWQLTQ GATVLGLFRV
241 TPEIPAGLPS PRSE (SEQ ID NO:13)

FIG. 2K
N99

81 PAGLLDLRQG MFAQLVAQ**X**V LLIDGPLSWY SDPGLAGVSL
121 TGGLSYKEDT KELVVAKAGV YYVFFQLELR RVVAGEGSGS VSLALHLQPL RSAAGAAALA
181 LTVDLPPASS EARNSAFGFQ GRLLHLSAGQ RLGVHLHTEA RARHAWQLTQ GATVLGLFRV
241 TPEIPAGLPS PRSE (SEQ ID NO:14)

FIG. 2L
V100

81 PAGLLDLRQG MFAQLVAQN**X** LLIDGPLSWY SDPGLAGVSL
121 TGGLSYKEDT KELVVAKAGV YYVFFQLELR RVVAGEGSGS VSLALHLQPL RSAAGAAALA
181 LTVDLPPASS EARNSAFGFQ GRLLHLSAGQ RLGVHLHTEA RARHAWQLTQ GATVLGLFRV
241 TPEIPAGLPS PRSE (SEQ ID NO:15)

FIG. 2M

L101

```
 81                       PAGLLDLRQG MFAQLVAQNV XLIDGPLSWY SDPGLAGVSL
121 TGGLSYKEDT KELVVAKAGV YYVFFQLELR RVVAGEGSGS VSLALHLQPL RSAAGAAALA
181 LTVDLPPASS EARNSAFGFQ GRLLHLSAGQ RLGVHLHTEA RARHAWQLTQ GATVLGLFRV
241 TPEIPAGLPS PRSE (SEQ ID NO:16)
```

FIG. 2N

L102

```
 81                       PAGLLDLRQG MFAQLVAQNV LXIDGPLSWY SDPGLAGVSL
121 TGGLSYKEDT KELVVAKAGV YYVFFQLELR RVVAGEGSGS VSLALHLQPL RSAAGAAALA
181 LTVDLPPASS EARNSAFGFQ GRLLHLSAGQ RLGVHLHTEA RARHAWQLTQ GATVLGLFRV
241 TPEIPAGLPS PRSE (SEQ ID NO:17)
```

FIG. 2O

I103

```
 81                       PAGLLDLRQG MFAQLVAQNV LLXDGPLSWY SDPGLAGVSL
121 TGGLSYKEDT KELVVAKAGV YYVFFQLELR RVVAGEGSGS VSLALHLQPL RSAAGAAALA
181 LTVDLPPASS EARNSAFGFQ GRLLHLSAGQ RLGVHLHTEA RARHAWQLTQ GATVLGLFRV
241 TPEIPAGLPS PRSE (SEQ ID NO:18)
```

FIG. 2P
D104

```
 81                        PAGLLDLRQG MFAQLVAQNV LLIXGPLSWY SDPGLAGVSL
121 TGGLSYKEDT KELVVAKAGV YYVFFQLELR RVVAGEGSGS VSLALHLQPL RSAAGAAALA
181 LTVDLPPASS EARNSAFGFQ GRLLHLSAGQ RLGVHLHTEA RARHAWQLTQ GATVLGLFRV
241 TPEIPAGLPS PRSE (SEQ ID NO:19)
```

FIG. 2Q
G105

```
 81                        PAGLLDLRQG MFAQLVAQNV LLIDXPLSWY SDPGLAGVSL
121 TGGLSYKEDT KELVVAKAGV YYVFFQLELR RVVAGEGSGS VSLALHLQPL RSAAGAAALA
181 LTVDLPPASS EARNSAFGFQ GRLLHLSAGQ RLGVHLHTEA RARHAWQLTQ GATVLGLFRV
241 TPEIPAGLPS PRSE (SEQ ID NO:20)
```

FIG. 2R
P106

```
 81                        PAGLLDLRQG MFAQLVAQNV LLIDGXLSWY SDPGLAGVSL
121 TGGLSYKEDT KELVVAKAGV YYVFFQLELR RVVAGEGSGS VSLALHLQPL RSAAGAAALA
181 LTVDLPPASS EARNSAFGFQ GRLLHLSAGQ RLGVHLHTEA RARHAWQLTQ GATVLGLFRV
241 TPEIPAGLPS PRSE (SEQ ID NO:21)
```

FIG. 2S
L107

81 PAGLLDLRQG MFAQLVAQNV LLIDGP**X**SWY SDPGLAGVSL
121 TGGLSYKEDT KELVVAKAGV YYVFFQLELR RVVAGEGSGS VSLALHLQPL RSAAGAAALA
181 LTVDLPPASS EARNSAFGFQ GRLLHLSAGQ RLGVHLHTEA RARHAWQLTQ GATVLGLFRV
241 TPEIPAGLPS PRSE (SEQ ID NO:22)

FIG. 2T
S108

81 PAGLLDLRQG MFAQLVAQNV LLIDGPL**X**WY SDPGLAGVSL
121 TGGLSYKEDT KELVVAKAGV YYVFFQLELR RVVAGEGSGS VSLALHLQPL RSAAGAAALA
181 LTVDLPPASS EARNSAFGFQ GRLLHLSAGQ RLGVHLHTEA RARHAWQLTQ GATVLGLFRV
241 TPEIPAGLPS PRSE (SEQ ID NO:23)

FIG. 2U
W109

81 PAGLLDLRQG MFAQLVAQNV LLIDGPLS**X**Y SDPGLAGVSL
121 TGGLSYKEDT KELVVAKAGV YYVFFQLELR RVVAGEGSGS VSLALHLQPL RSAAGAAALA
181 LTVDLPPASS EARNSAFGFQ GRLLHLSAGQ RLGVHLHTEA RARHAWQLTQ GATVLGLFRV
241 TPEIPAGLPS PRSE (SEQ ID NO:24)

FIG. 2V
Y110

```
 81                        PAGLLDLRQG MFAQLVAQNV LLIDGPLSWX SDPGLAGVSL
121 TGGLSYKEDT KELVVAKAGV YYVFFQLELR RVVAGEGSGS VSLALHLQPL RSAAGAAALA
181 LTVDLPPASS EARNSAFGFQ GRLLHLSAGQ RLGVHLHTEA RARHAWQLTQ GATVLGLFRV
241 TPEIPAGLPS PRSE (SEQ ID NO:25)
```

FIG. 2W
S111

```
 81                        PAGLLDLRQG MFAQLVAQNV LLIDGPLSWY XDPGLAGVSL
121 TGGLSYKEDT KELVVAKAGV YYVFFQLELR RVVAGEGSGS VSLALHLQPL RSAAGAAALA
181 LTVDLPPASS EARNSAFGFQ GRLLHLSAGQ RLGVHLHTEA RARHAWQLTQ GATVLGLFRV
241 TPEIPAGLPS PRSE (SEQ ID NO:26)
```

FIG. 2X
D112

```
 81                        PAGLLDLRQG MFAQLVAQNV LLIDGPLSWY SXPGLAGVSL
121 TGGLSYKEDT KELVVAKAGV YYVFFQLELR RVVAGEGSGS VSLALHLQPL RSAAGAAALA
181 LTVDLPPASS EARNSAFGFQ GRLLHLSAGQ RLGVHLHTEA RARHAWQLTQ GATVLGLFRV
241 TPEIPAGLPS PRSE (SEQ ID NO:27)
```

FIG. 2Y
P113

81 PAGLLDLRQG MFAQLVAQNV LLIDGPLSWY SD**X**GLAGVSL
121 TGGLSYKEDT KELVVAKAGV YYVFFQLELR RVVAGEGSGS VSLALHLQPL RSAAGAAALA
181 LTVDLPPASS EARNSAFGFQ GRLLHLSAGQ RLGVHLHTEA RARHAWQLTQ GATVLGLFRV
241 TPEIPAGLPS PRSE (SEQ ID NO:28)

FIG. 2Z
G114

81 PAGLLDLRQG MFAQLVAQNV LLIDGPLSWY SDP**X**LAGVSL
121 TGGLSYKEDT KELVVAKAGV YYVFFQLELR RVVAGEGSGS VSLALHLQPL RSAAGAAALA
181 LTVDLPPASS EARNSAFGFQ GRLLHLSAGQ RLGVHLHTEA RARHAWQLTQ GATVLGLFRV
241 TPEIPAGLPS PRSE (SEQ ID NO:29)

FIG. 2AA
L115

81 PAGLLDLRQG MFAQLVAQNV LLIDGPLSWY SDPG**X**AGVSL
121 TGGLSYKEDT KELVVAKAGV YYVFFQLELR RVVAGEGSGS VSLALHLQPL RSAAGAAALA
181 LTVDLPPASS EARNSAFGFQ GRLLHLSAGQ RLGVHLHTEA RARHAWQLTQ GATVLGLFRV
241 TPEIPAGLPS PRSE (SEQ ID NO:30)

FIG. 2BB
G117

81 PAGLLDLRQG MFAQLVAQNV LLIDGPLSWY SDPGLA**X**VSL
121 TGGLSYKEDT KELVVAKAGV YYVFFQLELR RVVAGEGSGS VSLALHLQPL RSAAGAAALA
181 LTVDLPPASS EARNSAFGFQ GRLLHLSAGQ RLGVHLHTEA RARHAWQLTQ GATVLGLFRV
241 TPEIPAGLPS PRSE (SEQ ID NO:31)

FIG. 2CC
V118

```
 81                       PAGLLDLRQG MFAQLVAQNV LLIDGPLSWY SDPGLAGXSL
121 TGGLSYKEDT KELVVAKAGV YYVFFQLELR RVVAGEGSGS VSLALHLQPL RSAAGAAALA
181 LTVDLPPASS EARNSAFGFQ GRLLHLSAGQ RLGVHLHTEA RARHAWQLTQ GATVLGLFRV
241 TPEIPAGLPS PRSE (SEQ ID NO:32)
```

FIG. 2DD
S119

```
 81                       PAGLLDLRQG MFAQLVAQNV LLIDGPLSWY SDPGLAGVXL
121 TGGLSYKEDT KELVVAKAGV YYVFFQLELR RVVAGEGSGS VSLALHLQPL RSAAGAAALA
181 LTVDLPPASS EARNSAFGFQ GRLLHLSAGQ RLGVHLHTEA RARHAWQLTQ GATVLGLFRV
241 TPEIPAGLPS PRSE (SEQ ID NO:33)
```

FIG. 2EE
L120

```
 81                       PAGLLDLRQG MFAQLVAQNV LLIDGPLSWY SDPGLAGVSX
121 TGGLSYKEDT KELVVAKAGV YYVFFQLELR RVVAGEGSGS VSLALHLQPL RSAAGAAALA
181 LTVDLPPASS EARNSAFGFQ GRLLHLSAGQ RLGVHLHTEA RARHAWQLTQ GATVLGLFRV
241 TPEIPAGLPS PRSE (SEQ ID NO:34)
```

FIG. 2FF
T121

```
 81                       PAGLLDLRQG MFAQLVAQNV LLIDGPLSWY SDPGLAGVSL
121 XGGLSYKEDT KELVVAKAGV YYVFFQLELR RVVAGEGSGS VSLALHLQPL RSAAGAAALA
181 LTVDLPPASS EARNSAFGFQ GRLLHLSAGQ RLGVHLHTEA RARHAWQLTQ GATVLGLFRV
241 TPEIPAGLPS PRSE (SEQ ID NO:35)
```

FIG. 2GG

G122

```
 81                        PAGLLDLRQG MFAQLVAQNV LLIDGPLSWY SDPGLAGVSL
121 TXGLSYKEDT KELVVAKAGV YYVFFQLELR RVVAGEGSGS VSLALHLQPL RSAAGAAALA
181 LTVDLPPASS EARNSAFGFQ GRLLHLSAGQ RLGVHLHTEA RARHAWQLTQ GATVLGLFRV
241 TPEIPAGLPS PRSE (SEQ ID NO:36)
```

FIG. 2HH

G123

```
 81                        PAGLLDLRQG MFAQLVAQNV LLIDGPLSWY SDPGLAGVSL
121 TGXLSYKEDT KELVVAKAGV YYVFFQLELR RVVAGEGSGS VSLALHLQPL RSAAGAAALA
181 LTVDLPPASS EARNSAFGFQ GRLLHLSAGQ RLGVHLHTEA RARHAWQLTQ GATVLGLFRV
241 TPEIPAGLPS PRSE (SEQ ID NO:37)
```

FIG. 2II

L124

```
 81                        PAGLLDLRQG MFAQLVAQNV LLIDGPLSWY SDPGLAGVSL
121 TGGXSYKEDT KELVVAKAGV YYVFFQLELR RVVAGEGSGS VSLALHLQPL RSAAGAAALA
181 LTVDLPPASS EARNSAFGFQ GRLLHLSAGQ RLGVHLHTEA RARHAWQLTQ GATVLGLFRV
241 TPEIPAGLPS PRSE (SEQ ID NO:38)
```

FIG. 2JJ
S125

81 PAGLLDLRQG MFAQLVAQNV LLIDGPLSWY SDPGLAGVSL
121 TGGL**X**YKEDT KELVVAKAGV YYVFFQLELR RVVAGEGSGS VSLALHLQPL RSAAGAAALA
181 LTVDLPPASS EARNSAFGFQ GRLLHLSAGQ RLGVHLHTEA RARHAWQLTQ GATVLGLFRV
241 TPEIPAGLPS PRSE (SEQ ID NO:39)

FIG. 2KK
Y126

81 PAGLLDLRQG MFAQLVAQNV LLIDGPLSWY SDPGLAGVSL
121 TGGLS**X**KEDT KELVVAKAGV YYVFFQLELR RVVAGEGSGS VSLALHLQPL RSAAGAAALA
181 LTVDLPPASS EARNSAFGFQ GRLLHLSAGQ RLGVHLHTEA RARHAWQLTQ GATVLGLFRV
241 TPEIPAGLPS PRSE (SEQ ID NO:40)

FIG. 2LL
E128

81 PAGLLDLRQG MFAQLVAQNV LLIDGPLSWY SDPGLAGVSL
121 TGGLSYK**X**DT KELVVAKAGV YYVFFQLELR RVVAGEGSGS VSLALHLQPL RSAAGAAALA
181 LTVDLPPASS EARNSAFGFQ GRLLHLSAGQ RLGVHLHTEA RARHAWQLTQ GATVLGLFRV
241 TPEIPAGLPS PRSE (SEQ ID NO:41)

FIG. 2MM
D129

81 PAGLLDLRQG MFAQLVAQNV LLIDGPLSWY SDPGLAGVSL
121 TGGLSYKE**X**T KELVVAKAGV YYVFFQLELR RVVAGEGSGS VSLALHLQPL RSAAGAAALA
181 LTVDLPPASS EARNSAFGFQ GRLLHLSAGQ RLGVHLHTEA RARHAWQLTQ GATVLGLFRV
241 TPEIPAGLPS PRSE (SEQ ID NO:42)

FIG. 2NN

T130

```
 81                       PAGLLDLRQG MFAQLVAQNV LLIDGPLSWY SDPGLAGVSL
121 TGGLSYKEDX KELVVAKAGV YYVFFQLELR RVVAGEGSGS VSLALHLQPL RSAAGAAALA
181 LTVDLPPASS EARNSAFGFQ GRLLHLSAGQ RLGVHLHTEA RARHAWQLTQ GATVLGLFRV
241 TPEIPAGLPS PRSE (SEQ ID NO:43)
```

FIG. 2OO

K131

```
 81                       PAGLLDLRQG MFAQLVAQNV LLIDGPLSWY SDPGLAGVSL
121 TGGLSYKEDT XELVVAKAGV YYVFFQLELR RVVAGEGSGS VSLALHLQPL RSAAGAAALA
181 LTVDLPPASS EARNSAFGFQ GRLLHLSAGQ RLGVHLHTEA RARHAWQLTQ GATVLGLFRV
241 TPEIPAGLPS PRSE (SEQ ID NO:44)
```

FIG. 2PP

E132

```
 81                       PAGLLDLRQG MFAQLVAQNV LLIDGPLSWY SDPGLAGVSL
121 TGGLSYKEDT KXLVVAKAGV YYVFFQLELR RVVAGEGSGS VSLALHLQPL RSAAGAAALA
181 LTVDLPPASS EARNSAFGFQ GRLLHLSAGQ RLGVHLHTEA RARHAWQLTQ GATVLGLFRV
241 TPEIPAGLPS PRSE (SEQ ID NO:45)
```

FIG. 2QQ

F144

```
 81                       PAGLLDLRQG MFAQLVAQNV LLIDGPLSWY SDPGLAGVSL
121 TGGLSYKEDT KELVVAKAGV YYVXFQLELR RVVAGEGSGS VSLALHLQPL RSAAGAAALA
181 LTVDLPPASS EARNSAFGFQ GRLLHLSAGQ RLGVHLHTEA RARHAWQLTQ GATVLGLFRV
241 TPEIPAGLPS PRSE (SEQ ID NO:46)
```

FIG. 2RR
F145

```
 81                            PAGLLDLRQG MFAQLVAQNV LLIDGPLSWY SDPGLAGVSL
121 TGGLSYKEDT KELVVAKAGV YYVFXQLELR RVVAGEGSGS VSLALHLQPL RSAAGAAALA
181 LTVDLPPASS EARNSAFGFQ GRLLHLSAGQ RLGVHLHTEA RARHAWQLTQ GATVLGLFRV
241 TPEIPAGLPS PRSE (SEQ ID NO:47)
```

FIG. 2SS
Q146

```
 81                            PAGLLDLRQG MFAQLVAQNV LLIDGPLSWY SDPGLAGVSL
121 TGGLSYKEDT KELVVAKAGV YYVFFXLELR RVVAGEGSGS VSLALHLQPL RSAAGAAALA
181 LTVDLPPASS EARNSAFGFQ GRLLHLSAGQ RLGVHLHTEA RARHAWQLTQ GATVLGLFRV
241 TPEIPAGLPS PRSE (SEQ ID NO:48)
```

FIG. 2TT
L147

```
 81                            PAGLLDLRQG MFAQLVAQNV LLIDGPLSWY SDPGLAGVSL
121 TGGLSYKEDT KELVVAKAGV YYVFFQXELR RVVAGEGSGS VSLALHLQPL RSAAGAAALA
181 LTVDLPPASS EARNSAFGFQ GRLLHLSAGQ RLGVHLHTEA RARHAWQLTQ GATVLGLFRV
241 TPEIPAGLPS PRSE (SEQ ID NO:49)
```

FIG. 2UU
E148

```
 81                            PAGLLDLRQG MFAQLVAQNV LLIDGPLSWY SDPGLAGVSL
121 TGGLSYKEDT KELVVAKAGV YYVFFQLXLR RVVAGEGSGS VSLALHLQPL RSAAGAAALA
181 LTVDLPPASS EARNSAFGFQ GRLLHLSAGQ RLGVHLHTEA RARHAWQLTQ GATVLGLFRV
241 TPEIPAGLPS PRSE (SEQ ID NO:50)
```

FIG. 2VV

L149

81 PAGLLDLRQG MFAQLVAQNV LLIDGPLSWY SDPGLAGVSL
121 TGGLSYKEDT KELVVAKAGV YYVFFQLE**X**R RVVAGEGSGS VSLALHLQPL RSAAGAAALA
181 LTVDLPPASS EARNSAFGFQ GRLLHLSAGQ RLGVHLHTEA RARHAWQLTQ GATVLGLFRV
241 TPEIPAGLPS PRSE (SEQ ID NO:51)

FIG. 2WW

R150

81 PAGLLDLRQG MFAQLVAQNV LLIDGPLSWY SDPGLAGVSL
121 TGGLSYKEDT KELVVAKAGV YYVFFQLEL**X** RVVAGEGSGS VSLALHLQPL RSAAGAAALA
181 LTVDLPPASS EARNSAFGFQ GRLLHLSAGQ RLGVHLHTEA RARHAWQLTQ GATVLGLFRV
241 TPEIPAGLPS PRSE (SEQ ID NO:52)

FIG. 2XX

R151

81 PAGLLDLRQG MFAQLVAQNV LLIDGPLSWY SDPGLAGVSL
121 TGGLSYKEDT KELVVAKAGV YYVFFQLELR **X**VVAGEGSGS VSLALHLQPL RSAAGAAALA
181 LTVDLPPASS EARNSAFGFQ GRLLHLSAGQ RLGVHLHTEA RARHAWQLTQ GATVLGLFRV
241 TPEIPAGLPS PRSE (SEQ ID NO:53)

FIG. 2YY

V152

81 PAGLLDLRQG MFAQLVAQNV LLIDGPLSWY SDPGLAGVSL
121 TGGLSYKEDT KELVVAKAGV YYVFFQLELR R**X**VAGEGSGS VSLALHLQPL RSAAGAAALA
181 LTVDLPPASS EARNSAFGFQ GRLLHLSAGQ RLGVHLHTEA RARHAWQLTQ GATVLGLFRV
241 TPEIPAGLPS PRSE (SEQ ID NO:54)

FIG. 2ZZ

V153

```
 81                       PAGLLDLRQG MFAQLVAQNV LLIDGPLSWY SDPGLAGVSL
121 TGGLSYKEDT KELVVAKAGV YYVFFQLELR RVXAGEGSGS VSLALHLQPL RSAAGAAALA
181 LTVDLPPASS EARNSAFGFQ GRLLHLSAGQ RLGVHLHTEA RARHAWQLTQ GATVLGLFRV
241 TPEIPAGLPS PRSE (SEQ ID NO:55)
```

FIG. 2AAA

G155

```
 81                       PAGLLDLRQG MFAQLVAQNV LLIDGPLSWY SDPGLAGVSL
121 TGGLSYKEDT KELVVAKAGV YYVFFQLELR RVVAXEGSGS VSLALHLQPL RSAAGAAALA
181 LTVDLPPASS EARNSAFGFQ GRLLHLSAGQ RLGVHLHTEA RARHAWQLTQ GATVLGLFRV
241 TPEIPAGLPS PRSE (SEQ ID NO:56)
```

FIG. 2BBB

E156

```
 81                       PAGLLDLRQG MFAQLVAQNV LLIDGPLSWY SDPGLAGVSL
121 TGGLSYKEDT KELVVAKAGV YYVFFQLELR RVVAGXGSGS VSLALHLQPL RSAAGAAALA
181 LTVDLPPASS EARNSAFGFQ GRLLHLSAGQ RLGVHLHTEA RARHAWQLTQ GATVLGLFRV
241 TPEIPAGLPS PRSE (SEQ ID NO:57)
```

FIG. 2CCC

G157

```
 81                       PAGLLDLRQG MFAQLVAQNV LLIDGPLSWY SDPGLAGVSL
121 TGGLSYKEDT KELVVAKAGV YYVFFQLELR RVVAGEXSGS VSLALHLQPL RSAAGAAALA
181 LTVDLPPASS EARNSAFGFQ GRLLHLSAGQ RLGVHLHTEA RARHAWQLTQ GATVLGLFRV
241 TPEIPAGLPS PRSE (SEQ ID NO:58)
```

FIG. 2DDD

S158

```
 81                       PAGLLDLRQG MFAQLVAQNV LLIDGPLSWY SDPGLAGVSL
121 TGGLSYKEDT KELVVAKAGV YYVFFQLELR RVVAGEGXGS VSLALHLQPL RSAAGAAALA
181 LTVDLPPASS EARNSAFGFQ GRLLHLSAGQ RLGVHLHTEA RARHAWQLTQ GATVLGLFRV
241 TPEIPAGLPS PRSE (SEQ ID NO:59)
```

FIG. 2EEE

D184

```
 81                       PAGLLDLRQG MFAQLVAQNV LLIDGPLSWY SDPGLAGVSL
121 TGGLSYKEDT KELVVAKAGV YYVFFQLELR RVVAGEGSGS VSLALHLQPL RSAAGAAALA
181 LTVXLPPASS EARNSAFGFQ GRLLHLSAGQ RLGVHLHTEA RARHAWQLTQ GATVLGLFRV
241 TPEIPAGLPS PRSE (SEQ ID NO:60)
```

FIG. 2FFF
L185

```
 81                       PAGLLDLRQG MFAQLVAQNV LLIDGPLSWY SDPGLAGVSL
121 TGGLSYKEDT KELVVAKAGV YYVFFQLELR RVVAGEGSGS VSLALHLQPL RSAAGAAALA
181 LTVDXPPASS EARNSAFGFQ GRLLHLSAGQ RLGVHLHTEA RARHAWQLTQ GATVLGLFRV
241 TPEIPAGLPS PRSE (SEQ ID NO:61)
```

FIG. 2GGG
P186

```
 81                       PAGLLDLRQG MFAQLVAQNV LLIDGPLSWY SDPGLAGVSL
121 TGGLSYKEDT KELVVAKAGV YYVFFQLELR RVVAGEGSGS VSLALHLQPL RSAAGAAALA
181 LTVDLXPASS EARNSAFGFQ GRLLHLSAGQ RLGVHLHTEA RARHAWQLTQ GATVLGLFRV
241 TPEIPAGLPS PRSE (SEQ ID NO:62)
```

FIG. 2HHH
P187

```
 81                       PAGLLDLRQG MFAQLVAQNV LLIDGPLSWY SDPGLAGVSL
121 TGGLSYKEDT KELVVAKAGV YYVFFQLELR RVVAGEGSGS VSLALHLQPL RSAAGAAALA
181 LTVDLPXASS EARNSAFGFQ GRLLHLSAGQ RLGVHLHTEA RARHAWQLTQ GATVLGLFRV
241 TPEIPAGLPS PRSE (SEQ ID NO:63)
```

FIG. 2III

S189

81 PAGLLDLRQG MFAQLVAQNV LLIDGPLSWY SDPGLAGVSL

121 TGGLSYKEDT KELVVAKAGV YYVFFQLELR RVVAGEGSGS VSLALHLQPL RSAAGAAALA

181 LTVDLPPA**X**S EARNSAFGFQ GRLLHLSAGQ RLGVHLHTEA RARHAWQLTQ GATVLGLFRV

241 TPEIPAGLPS PRSE (SEQ ID NO:64)

FIG. 2JJJ

S190

81 PAGLLDLRQG MFAQLVAQNV LLIDGPLSWY SDPGLAGVSL

121 TGGLSYKEDT KELVVAKAGV YYVFFQLELR RVVAGEGSGS VSLALHLQPL RSAAGAAALA

181 LTVDLPPAS**X** EARNSAFGFQ GRLLHLSAGQ RLGVHLHTEA RARHAWQLTQ GATVLGLFRV

241 TPEIPAGLPS PRSE (SEQ ID NO:65)

FIG. 2KKK

E191

81 PAGLLDLRQG MFAQLVAQNV LLIDGPLSWY SDPGLAGVSL

121 TGGLSYKEDT KELVVAKAGV YYVFFQLELR RVVAGEGSGS VSLALHLQPL RSAAGAAALA

181 LTVDLPPASS **X**ARNSAFGFQ GRLLHLSAGQ RLGVHLHTEA RARHAWQLTQ GATVLGLFRV

241 TPEIPAGLPS PRSE (SEQ ID NO:66)

FIG. 2LLL

R193

81 PAGLLDLRQG MFAQLVAQNV LLIDGPLSWY SDPGLAGVSL

121 TGGLSYKEDT KELVVAKAGV YYVFFQLELR RVVAGEGSGS VSLALHLQPL RSAAGAAALA

181 LTVDLPPASS EA**X**NSAFGFQ GRLLHLSAGQ RLGVHLHTEA RARHAWQLTQ GATVLGLFRV

241 TPEIPAGLPS PRSE (SEQ ID NO:67)

FIG. 2MMM
N194

81 PAGLLDLRQG MFAQLVAQNV LLIDGPLSWY SDPGLAGVSL
121 TGGLSYKEDT KELVVAKAGV YYVFFQLELR RVVAGEGSGS VSLALHLQPL RSAAGAAALA
181 LTVDLPPASS EAR**X**SAFGFQ GRLLHLSAGQ RLGVHLHTEA RARHAWQLTQ GATVLGLFRV
241 TPEIPAGLPS PRSE (SEQ ID NO:68)

FIG. 2NNN
S195

81 PAGLLDLRQG MFAQLVAQNV LLIDGPLSWY SDPGLAGVSL
121 TGGLSYKEDT KELVVAKAGV YYVFFQLELR RVVAGEGSGS VSLALHLQPL RSAAGAAALA
181 LTVDLPPASS EARN**X**AFGFQ GRLLHLSAGQ RLGVHLHTEA RARHAWQLTQ GATVLGLFRV
241 TPEIPAGLPS PRSE (SEQ ID NO:69)

FIG. 2OOO
F197

81 PAGLLDLRQG MFAQLVAQNV LLIDGPLSWY SDPGLAGVSL
121 TGGLSYKEDT KELVVAKAGV YYVFFQLELR RVVAGEGSGS VSLALHLQPL RSAAGAAALA
181 LTVDLPPASS EARNSA**X**GFQ GRLLHLSAGQ RLGVHLHTEA RARHAWQLTQ GATVLGLFRV
241 TPEIPAGLPS PRSE (SEQ ID NO:70)

FIG. 2PPP
Q210

81 PAGLLDLRQG MFAQLVAQNV LLIDGPLSWY SDPGLAGVSL
121 TGGLSYKEDT KELVVAKAGV YYVFFQLELR RVVAGEGSGS VSLALHLQPL RSAAGAAALA
181 LTVDLPPASS EARNSAFGFQ GRLLHLSAG**X** RLGVHLHTEA RARHAWQLTQ GATVLGLFRV
241 TPEIPAGLPS PRSE (SEQ ID NO:71)

FIG. 2QQQ
R211

81 PAGLLDLRQG MFAQLVAQNV LLIDGPLSWY SDPGLAGVSL
121 TGGLSYKEDT KELVVAKAGV YYVFFQLELR RVVAGEGSGS VSLALHLQPL RSAAGAAALA
181 LTVDLPPASS EARNSAFGFQ GRLLHLSAGQ **X**LGVHLHTEA RARHAWQLTQ GATVLGLFRV
241 TPEIPAGLPS PRSE (SEQ ID NO:72)

FIG. 2RRR
L212

81 PAGLLDLRQG MFAQLVAQNV LLIDGPLSWY SDPGLAGVSL
121 TGGLSYKEDT KELVVAKAGV YYVFFQLELR RVVAGEGSGS VSLALHLQPL RSAAGAAALA
181 LTVDLPPASS EARNSAFGFQ GRLLHLSAGQ R**X**GVHLHTEA RARHAWQLTQ GATVLGLFRV
241 TPEIPAGLPS PRSE (SEQ ID NO:73)

FIG. 2SSS
G213

81 PAGLLDLRQG MFAQLVAQNV LLIDGPLSWY SDPGLAGVSL
121 TGGLSYKEDT KELVVAKAGV YYVFFQLELR RVVAGEGSGS VSLALHLQPL RSAAGAAALA
181 LTVDLPPASS EARNSAFGFQ GRLLHLSAGQ RL**X**VHLHTEA RARHAWQLTQ GATVLGLFRV
241 TPEIPAGLPS PRSE (SEQ ID NO:74)

FIG. 2TTT
V214

```
 81                       PAGLLDLRQG MFAQLVAQNV LLIDGPLSWY SDPGLAGVSL
121 TGGLSYKEDT KELVVAKAGV YYVFFQLELR RVVAGEGSGS VSLALHLQPL RSAAGAAALA
181 LTVDLPPASS EARNSAFGFQ GRLLHLSAGQ RLGXHLHTEA RARHAWQLTQ GATVLGLFRV
241 TPEIPAGLPS PRSE (SEQ ID NO:75)
```

FIG. 2UUU
H215

```
 81                       PAGLLDLRQG MFAQLVAQNV LLIDGPLSWY SDPGLAGVSL
121 TGGLSYKEDT KELVVAKAGV YYVFFQLELR RVVAGEGSGS VSLALHLQPL RSAAGAAALA
181 LTVDLPPASS EARNSAFGFQ GRLLHLSAGQ RLGVXLHTEA RARHAWQLTQ GATVLGLFRV
241 TPEIPAGLPS PRSE (SEQ ID NO:76)
```

FIG. 2VVV
L216

```
 81                       PAGLLDLRQG MFAQLVAQNV LLIDGPLSWY SDPGLAGVSL
121 TGGLSYKEDT KELVVAKAGV YYVFFQLELR RVVAGEGSGS VSLALHLQPL RSAAGAAALA
181 LTVDLPPASS EARNSAFGFQ GRLLHLSAGQ RLGVHXHTEA RARHAWQLTQ GATVLGLFRV
241 TPEIPAGLPS PRSE (SEQ ID NO:77)
```

FIG. 2WWW
H217

```
 81                       PAGLLDLRQG MFAQLVAQNV LLIDGPLSWY SDPGLAGVSL
121 TGGLSYKEDT KELVVAKAGV YYVFFQLELR RVVAGEGSGS VSLALHLQPL RSAAGAAALA
181 LTVDLPPASS EARNSAFGFQ GRLLHLSAGQ RLGVHLXTEA RARHAWQLTQ GATVLGLFRV
241 TPEIPAGLPS PRSE (SEQ ID NO:78)
```

FIG. 2XXX
T218

```
 81                       PAGLLDLRQG MFAQLVAQNV LLIDGPLSWY SDPGLAGVSL
121 TGGLSYKEDT KELVVAKAGV YYVFFQLELR RVVAGEGSGS VSLALHLQPL RSAAGAAALA
181 LTVDLPPASS EARNSAFGFQ GRLLHLSAGQ RLGVHLHXEA RARHAWQLTQ GATVLGLFRV
241 TPEIPAGLPS PRSE (SEQ ID NO:79)
```

FIG. 2YYY
E219

```
 81                       PAGLLDLRQG MFAQLVAQNV LLIDGPLSWY SDPGLAGVSL
121 TGGLSYKEDT KELVVAKAGV YYVFFQLELR RVVAGEGSGS VSLALHLQPL RSAAGAAALA
181 LTVDLPPASS EARNSAFGFQ GRLLHLSAGQ RLGVHLHTXA RARHAWQLTQ GATVLGLFRV
241 TPEIPAGLPS PRSE (SEQ ID NO:80)
```

FIG. 2ZZZ
R221

81 PAGLLDLRQG MFAQLVAQNV LLIDGPLSWY SDPGLAGVSL
121 TGGLSYKEDT KELVVAKAGV YYVFFQLELR RVVAGEGSGS VSLALHLQPL RSAAGAAALA
181 LTVDLPPASS EARNSAFGFQ GRLLHLSAGQ RLGVHLHTEA **X**ARHAWQLTQ GATVLGLFRV
241 TPEIPAGLPS PRSE (SEQ ID NO:81)

FIG. 2AAAA
R223

81 PAGLLDLRQG MFAQLVAQNV LLIDGPLSWY SDPGLAGVSL
121 TGGLSYKEDT KELVVAKAGV YYVFFQLELR RVVAGEGSGS VSLALHLQPL RSAAGAAALA
181 LTVDLPPASS EARNSAFGFQ GRLLHLSAGQ RLGVHLHTEA RA**X**HAWQLTQ GATVLGLFRV
241 TPEIPAGLPS PRSE (SEQ ID NO:82)

FIG. 2BBBB
H224

81 PAGLLDLRQG MFAQLVAQNV LLIDGPLSWY SDPGLAGVSL
121 TGGLSYKEDT KELVVAKAGV YYVFFQLELR RVVAGEGSGS VSLALHLQPL RSAAGAAALA
181 LTVDLPPASS EARNSAFGFQ GRLLHLSAGQ RLGVHLHTEA RAR**X**AWQLTQ GATVLGLFRV
241 TPEIPAGLPS PRSE (SEQ ID NO:83)

FIG. 2CCCC
W226

```
 81                       PAGLLDLRQG MFAQLVAQNV LLIDGPLSWY SDPGLAGVSL
121 TGGLSYKEDT KELVVAKAGV YYVFFQLELR RVVAGEGSGS VSLALHLQPL RSAAGAAALA
181 LTVDLPPASS EARNSAFGFQ GRLLHLSAGQ RLGVHLHTEA RARHAXQLTQ GATVLGLFRV
241 TPEIPAGLPS PRSE (SEQ ID NO:84)
```

FIG. 2DDDD
L228

```
 81                       PAGLLDLRQG MFAQLVAQNV LLIDGPLSWY SDPGLAGVSL
121 TGGLSYKEDT KELVVAKAGV YYVFFQLELR RVVAGEGSGS VSLALHLQPL RSAAGAAALA
181 LTVDLPPASS EARNSAFGFQ GRLLHLSAGQ RLGVHLHTEA RARHAWQXTQ GATVLGLFRV
241 TPEIPAGLPS PRSE (SEQ ID NO:85)
```

FIG. 2EEEE
T229

```
 81                       PAGLLDLRQG MFAQLVAQNV LLIDGPLSWY SDPGLAGVSL
121 TGGLSYKEDT KELVVAKAGV YYVFFQLELR RVVAGEGSGS VSLALHLQPL RSAAGAAALA
181 LTVDLPPASS EARNSAFGFQ GRLLHLSAGQ RLGVHLHTEA RARHAWQLXQ GATVLGLFRV
241 TPEIPAGLPS PRSE (SEQ ID NO:86)
```

FIG. 2FFFF

Q230

```
 81                       PAGLLDLRQG MFAQLVAQNV LLIDGPLSWY SDPGLAGVSL
121 TGGLSYKEDT KELVVAKAGV YYVFFQLELR RVVAGEGSGS VSLALHLQPL RSAAGAAALA
181 LTVDLPPASS EARNSAFGFQ GRLLHLSAGQ RLGVHLHTEA RARHAWQLTX GATVLGLFRV
241 TPEIPAGLPS PRSE (SEQ ID NO:87)
```

FIG. 2GGGG

G231

```
 81                       PAGLLDLRQG MFAQLVAQNV LLIDGPLSWY SDPGLAGVSL
121 TGGLSYKEDT KELVVAKAGV YYVFFQLELR RVVAGEGSGS VSLALHLQPL RSAAGAAALA
181 LTVDLPPASS EARNSAFGFQ GRLLHLSAGQ RLGVHLHTEA RARHAWQLTQ XATVLGLFRV
241 TPEIPAGLPS PRSE (SEQ ID NO:88)
```

FIG. 2HHHH

T233

```
 81                       PAGLLDLRQG MFAQLVAQNV LLIDGPLSWY SDPGLAGVSL
121 TGGLSYKEDT KELVVAKAGV YYVFFQLELR RVVAGEGSGS VSLALHLQPL RSAAGAAALA
181 LTVDLPPASS EARNSAFGFQ GRLLHLSAGQ RLGVHLHTEA RARHAWQLTQ GAXVLGLFRV
241 TPEIPAGLPS PRSE (SEQ ID NO:89)
```

FIG. 2IIII

V234

81 PAGLLDLRQG MFAQLVAQNV LLIDGPLSWY SDPGLAGVSL
121 TGGLSYKEDT KELVVAKAGV YYVFFQLELR RVVAGEGSGS VSLALHLQPL RSAAGAAALA
181 LTVDLPPASS EARNSAFGFQ GRLLHLSAGQ RLGVHLHTEA RARHAWQLTQ GAT**X**LGLFRV
241 TPEIPAGLPS PRSE (SEQ ID NO:90)

FIG. 3

Homo sapiens
4-1BB

```
  1 mgnscyniva tlllvlnfer trslqdpcsn cpagtfcdnn rnqicspcpp nsfssaggqr
 61 tcdicrqckg vfrtrkecss tsnaecdctp gfhclgagcs mceqdckqgq eltkkgckdc
121 cfgtfndqkr gicrpwtncs ldgksvlvng tkerdvvcgp spadlspgas svtppapare
181 pghspqiisf flaltstall fllffltlrf svvkrgrkkl lyifkqpfmr pvqttqeedg
241 cscrfpeeee ggcel (SEQ ID NO:91)
```

Figure 4A
GenBank 3S7G_A
Homo sapiens **IgG1** Fc (SEQ ID NO:92)
227 aa

1 dkthtcppcp apellggpsv flfppkpkdt lmisrtpevt cvvvdvshed pevkfnwyvd
61 gvevhnaktk preeqy**n**sty rvvsvltvlh qdwlngkeyk ckvsnkalpa piektiskak
121 gqprepqvyt lppsrdeltk nqvsltclvk gfypsdiave wesngqpenn ykttppvlds
181 dgsfflyskl tvdksrwqqg nvfscsvmhe alhnhytqks lslspgk

GenBank AAN76044
Homo sapiens **IgG2** Fc (amino acids 99-325) (SEQ ID NO:93)
227 aa

1 stkgpsvfpl apcsrstses taalgclvkd yfpepvtvsw nsgaltsgvh tfpavlqssg
61 lyslssvvtv pssnfgtqty tcnvdhkpsn tkvdktverk ccvecppcpa ppvagpsvfl
121 fppkpkdtlm isrtpevtcv vvdvshedpe vqfnwyvdgv evhnaktkpr eeqfnstfrv
181 vsvltvvhqd wlngkeykck vsnkglpapi ektisktkgq prepqvytlp psreemtknq
241 vsltclvkgf ypsdiavewe sngqpennyk ttppmldsdg sfflyskltv dksrwqqgnv
301 fscsvmheal hnhytqksls lspgk

GenBank AAW65947
Homo sapiens **IgG3** Fc (amino acids 19-246) (SEQ ID NO:94)
238 aa

1 hkpsntkvdk rvelktplgd tthtcppcpa pellggpsvf lfppkpkdtl misrtpevtc
61 vvvdvshedp evkfnwyvdg vevhnaktkp reeqynstyr vvsvltvlhq dwlngkeykc
121 kvsnkalpap iektiskakg qprepqvytl ppsrdeltkn qvsltclvkg fypsdiavew
181 esngqpenny kttppvldsd gsfflysklt vdksrwqqgn vfscsvmhea lhnhytqksl
241 slspgk

Figure 4B

GenBank AAA52770
Homo sapiens **IgD** Fc (amino acids 162-383) (SEQ ID NO:95)
222 aa

```
  1 ptkapdvfpi isgcrhpkdn spvvlaclit gyhptsvtvt wymgtqsqpq rtfpeiqrrd
 61 syymtssqls tplqqwrqge ykcvvqhtas kskkeifrwp espkaqassv ptaqpqaegs
121 lakattapat trntgrggee kkkekekeeq eeretktpec pshtqplgvy lltpavqdlw
181 lrdkatftcf vvgsdlkdah ltwevagkvp tggveeglle rhsngsqsqh srltlprslw
241 nagtsvtctl nhpslppqrl malrepaaqa pvklslnlla ssdppeaasw llcevsgfsp
301 pnillmwled qrevntsgfa parpppqprs ttfwawsvlr vpappspqpa tytcvvshed
361 srtllnasrs levsyvtdhg pmk
```

GenBank 0308221A
Homo sapiens **IgM** Fc (SEQ ID NO:96)
276 aa

```
  1 vtstltikzs dwlgesmftc rvdhrgltfq qnassmcvpd qdtairvfai ppsfasiflt
 61 kstkltclvt dlttybsvti swtreengav kthtnisesh pnatfsavge asicedbdws
121 gerftctvth tdlpsplkqt isrpkgvalh rpbvyllppa rzzlnlresa titclvtgfs
181 padvfvewmq rgeplspqky vtsapmpepq apgryfahsi ltvseeewnt ggtytcvvah
241 ealpnrvter tvdkstgkpt lynvslvmsd tagtcy
```

Figure 4C

GenBank P01876
Homo sapiens **IgA** Fc (amino acids 120-353) (SEQ ID NO:97)
234 aa

```
  1 asptspkvfp lslcstqpdg nvviaclvqg ffpqeplsvt wsesgqgvta rnfppsqdas
 61 gdlyttssql tlpatqclag ksvtchvkhy tnpsqdvtvp cpvpstpptp spstpptpsp
121 scchprlslh rpaledlllg seanltctlt glrdasgvtf twtpssgksa vqgpperdlc
181 gcysvsssvlp gcaepwnhgk tftctaaype sktpltatls ksgntfrpev hllpppseel
241 alnelvtltc largfspkdv lvrwlqgsqe lprekyltwa srqepsqgtt tfavtsilrv
301 aaedwkkgdt fscmvgheal plaftqktid rlagkpthvn vsvvmaevdg tcy
```

GenBank 1F6A_B
Homo sapiens **IgE** Fc (amino acids 6-222) (SEQ ID NO:98)
212 aa

```
  1 adpcdsnprg vsaylsrpsp fdlfirkspt itclvvdlap skgtvnltws rasgkpvnhs
 61 trkeekqrng tltvtstlpv gtrdwieget yqcrvthphl pralmrsttk tsgpraapev
121 yafatpewpg srdkrtlacl iqnfmpedis vqwlhnevql pdarhsttqp rktkgsgffv
181 fsrlevtrae weqkdeficr avheaaspsq tvqravsvnp gk
```

GenBank P01861
Homo sapiens **IgG4** Fc (amino acids 100-327) (SEQ ID NO:99)
228 aa

```
  1 astkgpsvfp lapcsrstse staalgclvk dyfpepvtvs wnsgaltsgv htfpavlqss
 61 glyslssvvt vpssslgtkt ytcnvdhkps ntkvdkrves kygppcpscp apeflggpsv
121 flfppkpkdt lmisrtpevt cvvvdvsqed pevqfnwyvd gvevhnaktk preeqfnsty
181 rvvsvltvlh qdwlngkeyk ckvsnkglps siektiskak gqprepqvyt lppsqeemtk
241 nqvsltclvk gfypsdiave wesngqpenn ykttppvlds dgsfflysrl tvdksrwqeg
301 nvfscsvmhe alhnhytqks lslslgk
```

Figure 5A
Homo sapiens
GenBank NP_001229687
HLA-A
Amino acids 25-365

1 mavmaprtll lllsgalalt qtwagshsmr yfftsvsrpg rgeprfiavg yvddtqfvrf
61 dsdaasqkme prapwieqeg peywdqetrn mkahsqtdra nlgtlrgyyn qsedgshtiq
121 imygcdvgpd grflrgyrqd aydgkdyial nedlrswtaa dmaaqitkrk weavhaaeqr
181 rvylegrcvd glrrylengk etlqrtdppk thmthhpisd heatlrcwal gfypaeitlt
241 wqrdgedqtq dtelvetrp**a** gdgtfqkwaa vvvpsgeeqr ytchvqhegl pkpltlrwel
301 ssqptipivg iiaglvllga vitgavvaav mwrrkssdrk ggsytqaass dsaqgsdvsl
361 tackv
(SEQ ID NO:100)

Figure 5B
Homo sapiens
GenBank NP_005505
HLA-B
Amino acids 25-362

1 mlvmaprtvl lllsaalalt etwagshsmr yfytsvsrpg rgeprfisvg yvddtqfvrf
61 dsdaaspree prapwieqeg peywdrntqi ykaqaqtdre slrnlrgyyn qseagshtlq
121 smygcdvgpd grllrghdqy aydgkdyial nedlrswtaa dtaaqitqrk weaareaeqr
181 raylegecve wlrrylengk dkleradppk thvthhpisd heatlrcwal gfypaeitlt
241 wqrdgedqtq dtelvetrp**a** gdrtfqkwaa vvvpsgeeqr ytchvqhegl pkpltlrwep
301 ssqstvpivg ivaglavlav vvigavvaav mcrrkssggk ggsysqaacs dsaqgsdvsl
361 ta
(SEQ ID NO:101)

Figure 5C
Homo sapiens
GenBank NP_001229971
HLA-C
Amino acids 25-366

1 mrvmaprall lllsgglalt etwacshsmr yfdtavsrpg rgeprfisvg yvddtqfvrf
61 dsdaasprge prapwveqeg peywdretqn ykrqaqadrv slrnlrgyyn qsedgshtlq
121 rmygcdlgpd grllrgydqs aydgkdyial nedlrswtaa dtaaqitqrk leaaraaeql
181 raylegtcve wlrrylengk etlqraeppk thvthhplsd heatlrcwal gfypaeitlt
241 wqrdgedqtq dtelvetrp**a** gdgtfqkwaa vvvpsgqeqr ytchmqhegl qepltlswep
301 ssqptipimg ivaglavlvv lavlgavvta mmcrrkssgg kggscsqaac snsaqgsdes
361 litcka
(SEQ ID NO:102)

FIG. 6

```
NP_004039.1        MSRSVALAVLALLSLSGLEAIQRTPKIQVYSRHPAENGKSNFLNCYVSGFHPSDIEVDLL 60
NP_001009066.1     MSRSVALAVLALLSLSGLEAIQRTPKIQVYSRHPAENGKSNFLNCYVSGFHPSDIEVDLL 60
NP_001040602.1     MSRSVALAVLALLSLSGLEAIQRTPKIQVYSRHPPENGKPNFLNCYVSGFHPSDIEVDLL 60
NP_776318.1        MARFVALVLLGLLSLSGLDAIQRPPKIQVYSRHPPEDGKPNYLNCYVYGFHPPQIEIDLL 60
NP_033865.2        MARSVTLVFLVLVSLTGLYAIQKTPQIQVYSRHPPENGKPNILNCYVTQFHPPHIEIQML 60
                   *:* *:*..* *:**:** ***:.*:********.*:**.* *****  ***..**:::*

NP_004039.1        KNGERIEKVEHSDLSFSKDWSFYLLYYTEFTPTEKDEYACRVNHVTLSQPKIVKWDRDM 119
NP_001009066.1     KNGERIEKVEHSDLSFSKDWSFYLLYYTEFTPTEKDEYACRVNHVTLSQPKIVKWDRDM 119
NP_001040602.1     KNGEKMGKVEHSDLSFSKDWSFYLLYYTEFTPNEKDEYACRVNHVTLSGPRTVKWDRDM 119
NP_776318.1        KNGEKI-KSEQSDLSFSKDWSFYLLSHAEFTPNSKDQYSCRVKHVTLEQPRIVKWDRDL 118
NP_033865.2        KNGKKIPKVEMSDMSFSKDWSFYILAHTEFTPTETDTYACRVKHASMAEPKTVYWDRDM 119
                   ***::: * * **:*********:* ::****...* *:***:*.::  *: * ****:
```

Figure 7A
PD-L1
Mus musculus
NP_068693
Amino acids 19-290

```
  1 mrifagiift acchllraft itapkdlyvv eygsnvtmec rfpvereldl lalvvyweke
 61 deqviqfvag eedlkpqhsn frgraslpkd qllkgnaalq itdvklqdag vycciisygg
121 adykritlkv napyrkinqr isvdpatseh elicqaegyp eaeviwtnsd hqpvsgkrsv
181 ttsrtegmll nvtsslrvna tandvfyctf wrsqpgqnht aeliipelpa thppqnrthw
241 vllgsillfl ivvstvllfl rkqvrmldve kcgvedtssk nrndtqfeet
```

(SEQ ID NO:108)

Figure 7B
PD-L1
Homo sapiens
NP_054852
Amino acids 19-290

```
  1 mrifavfifm tywhllnaft vtvpkdlyvv eygsnmtiec kfpvekqldl aalivyweme
 61 dkniiqfvhg eedlkvqhss yrqrarllkd qlslgnaalq itdvklqdag vyrcmisygg
121 adykritvkv napynkinqr ilvvdpvtse heltcqaegy pkaeviwtss dhqvlsgktt
181 ttnskreekl fnvtstlrin tttneifyct frrldpeenh taelvipelp lahppnerth
241 lvilgaillc lgvaltfifr lrkgrmmdvk kcgiqdtnsk kqsdthleet
```

(SEQ ID NO:109)

Figure 8

CD80 (B7-1) Ectodomain

vihvtk evkevatlsc ghnvsveela

qtriywqkek kmvltmmsgd mniwpeyknr tifditnnls ivilalrpsd egtyecvvlk

yekdafkreh laevtlsvka dfptpsisdf eiptsnirri icstsggfpe phlswlenge

elnainttvs qdpetelyav sskldfnmtt nhsfmcliky ghlrvnqtfn wnttkqehfp

dn (SEQ ID NO:110)

Figure 9
Homo sapiens
ICOS-L
GenBank NP_056074
Amino acids 19-302

```
  1 mrlgspgllf llfsslradt qekevramvg sdvelscacp egsrfdlndv yvywqtsesk
 61 tvvtyhipqn sslenvdsry rnralmspag mlrgdfslrl fnvtpqdeqk fhclvlsqsl
121 gfqevlsvev tlhvaanfsv pvvsaphsps qdeltftcts ingyprpnvy winktdnsll
181 dqalqndtvf lnmrglydvv svlriartps vnigccienv llqqnltvgs qtgndigerd
241 kitenpvstg eknaatwsil avlcllvvva vaigwvcrdr clqhsyagaw avspeteltg
301 hv
```

(SEQ ID NO:111)

Figure 10
Homo sapiens
GenBank NP_003317
OX4L

```
  1 mervqpleen vgnaarprfe rnklllvasv iqglglllcf tyiclhfsal qvshrypriq
 61 sikvqfteyk kekgfiltsq kedeimkvqn nsviincdgf ylislkgyfs qevnislhyq
121 kdeeplfqlk kvrsvnslmv asltykdkvy lnvttdntsl ddfhvnggel ilihqnpgef
181 cvl
```

(SEQ ID NO:112)

Figure 11
Homo sapiens
GenBank NP_079515
PD-L2
Amino acids 20-273

```
  1 miflllmlsl elqlhqiaal ftvtvpkely iiehgsnvtl ecnfdtgshv nlgaitaslq
 61 kvendtsphr eratlleeql plgkasfhip qvqvrdegqy qciiiygvaw dykyltlkvk
121 asyrkinthi lkvpetdeve ltcqatgypl aevswpnvsv pantshsrtp eglyqvtsvl
181 rlkpppgrnf scvfwnthvr eltlasidlq sqmeprthpt wllhifipfc iiafifiatv
241 ialrkqlcqk lysskdttkr pvtttkrevn sai
```

(SEQ ID NO:113)

Figure 12
Homo sapiens
GenBank NP_787058
CD86 (B7-2)
Amino acids 31-329

```
  1 mdpqctmgls nilfvmafll sgaaplkiqa yfnetadlpc qfansqnqsl selvvfwqdq
 61 enlvlnevyl gkekfdsvhs kymgrtsfds dswtlrlhnl qikdkglyqc iihhkkptgm
121 irihqmnsel svlanfsqpe ivpisniten vyinltcssi hgypepkkms vllrtknsti
181 eydgimqksq dnvtelydvs islsvsfpdv tsnmtifcil etdktrllss pfsieledpq
241 pppdhipwit avlptviicv mvfclilwkw kkkkrprnsy kcgtntmere eseqtkkrek
301 ihipersdea qrvfksskts scdksdtcf
```

(SEQ ID NO:114)

FIG. 13

Fas ligand (FasL)
Homo sapiens
GenBank NP_000630
Amino acids 1-281

```
  1 mqqpfnypyp qiywvdssas spwappgtvl pcptsvprrp gqrrpppppp ppplpppppp
 61 pplpplplpp lkkrgnhstg lcllvmffmv lvalvglglg mfqlfhlqke laelrestsq
121 mhtasslekq ighpspppek kelrkvahlt gksnsrsmpl ewedtygivl lsgvkykkgg
181 lvinetglyf vyskvyfrgq scnnlplshk vymrnskypq dlvmmegkmm sycttgqmwa
241 rssylgavfn ltsadhlyvn vselslvnfe esqtffglyk l
```

(SEQ ID NO:115)

FIG. 14A

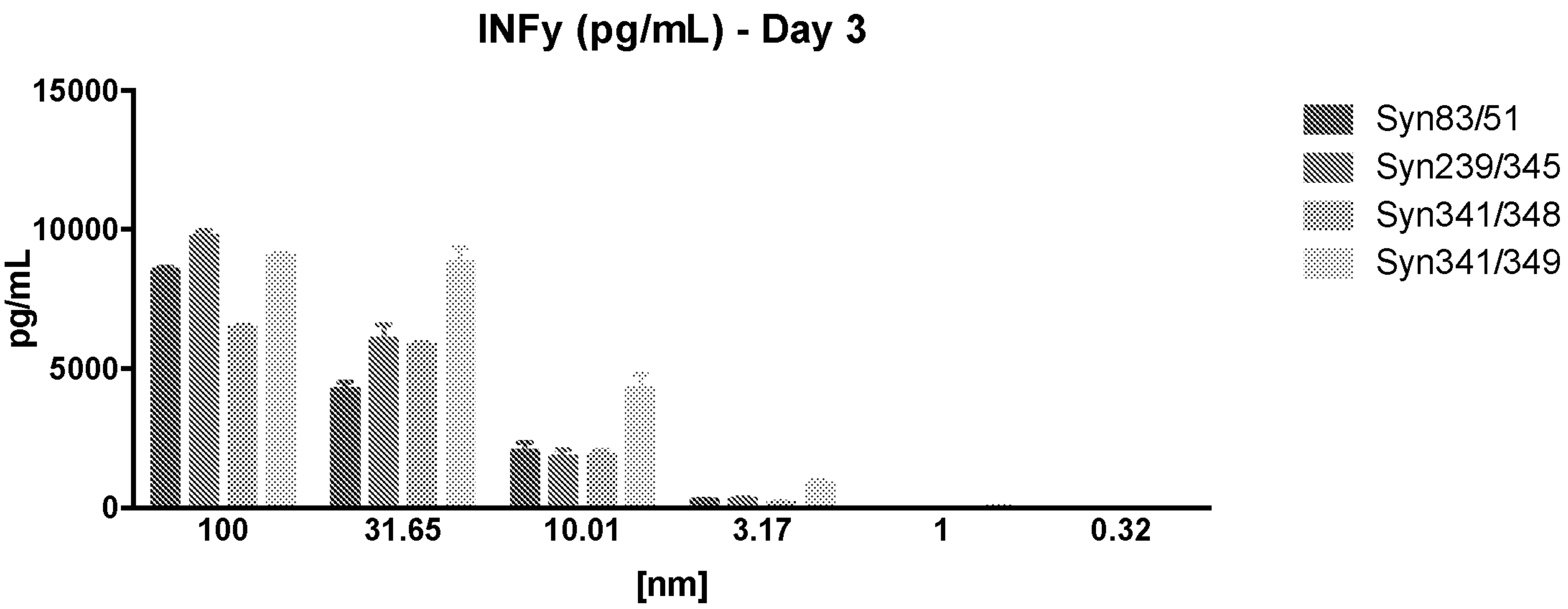

FIG. 14B

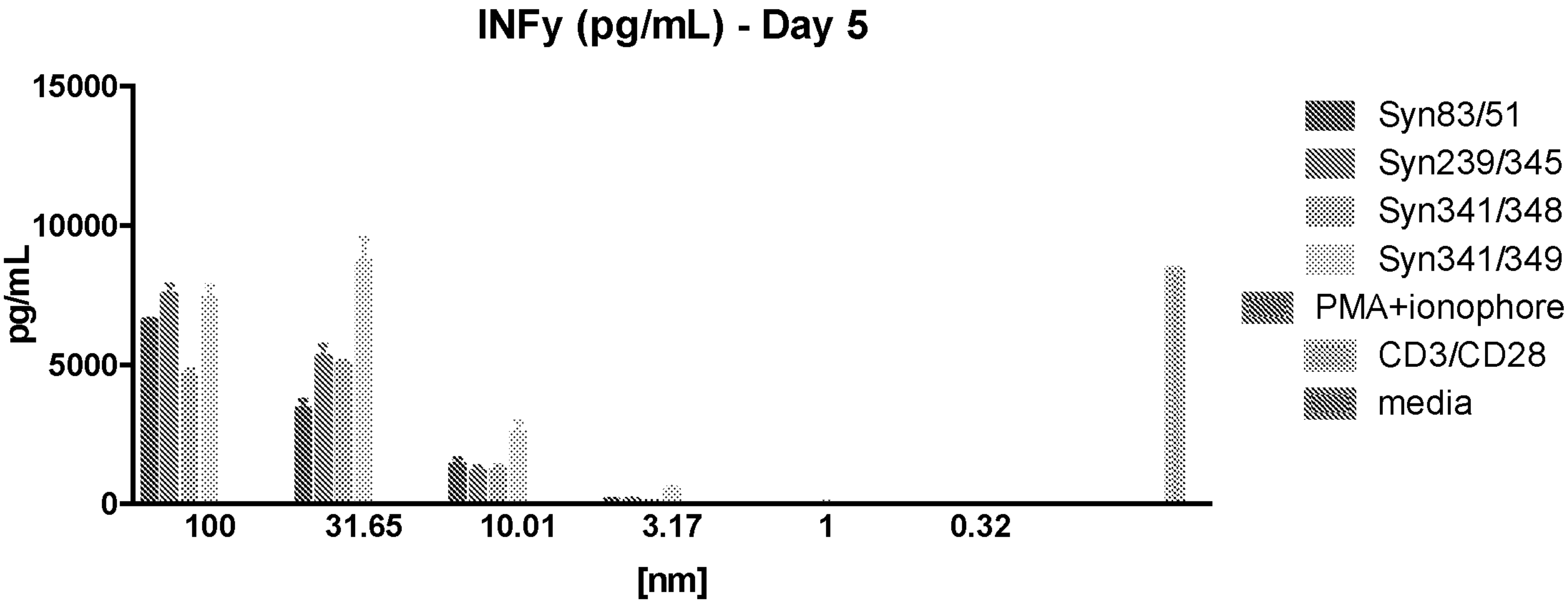

FIG. 15A

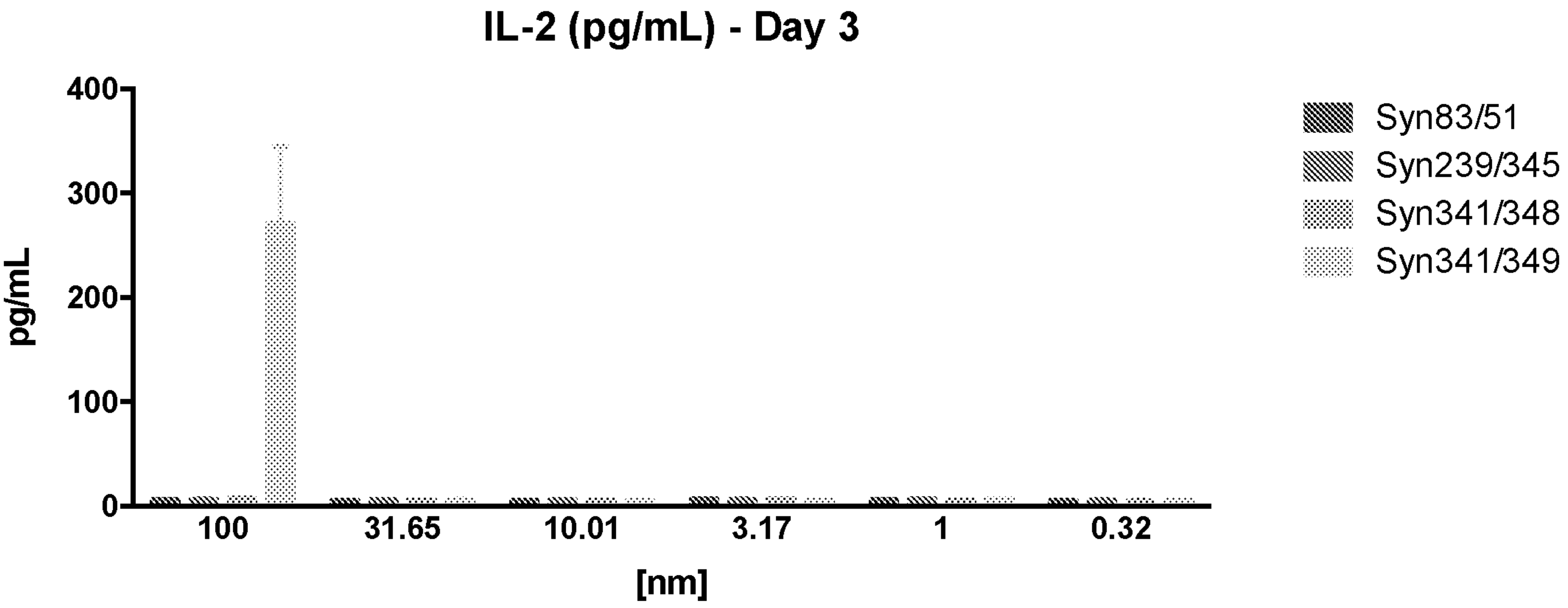

FIG. 15B

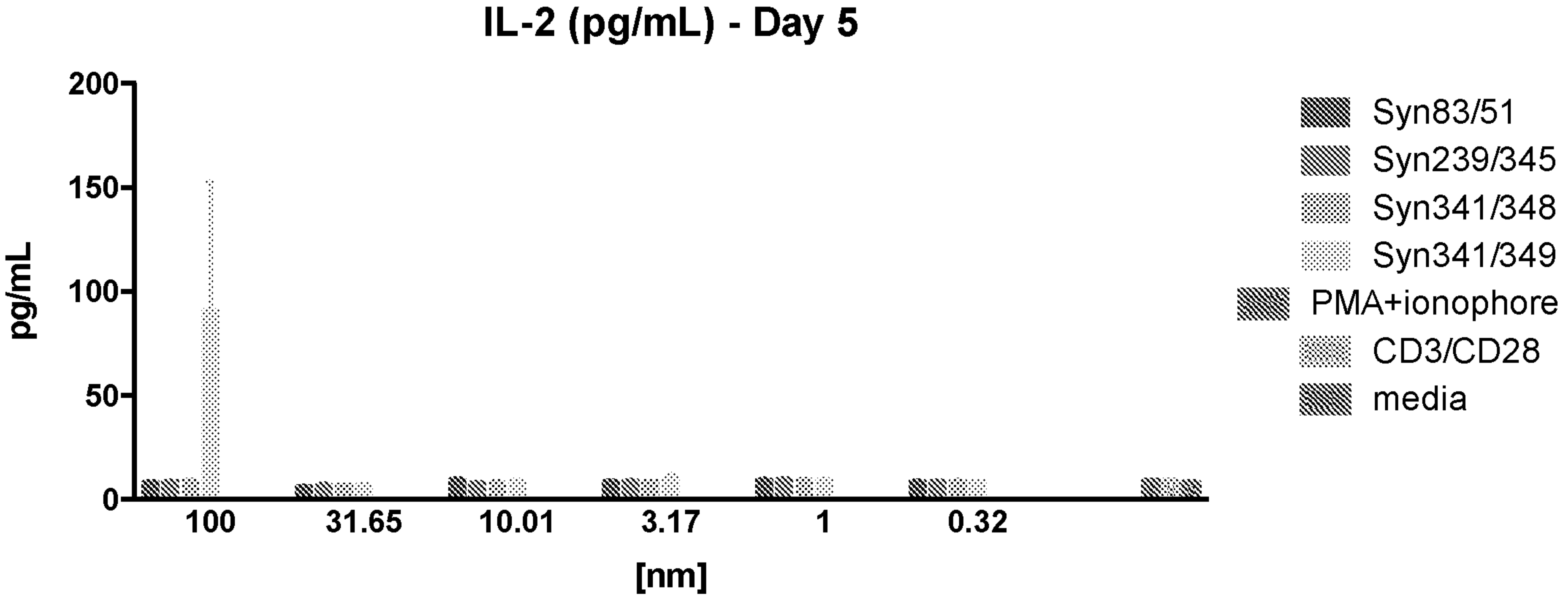

FIG. 16A

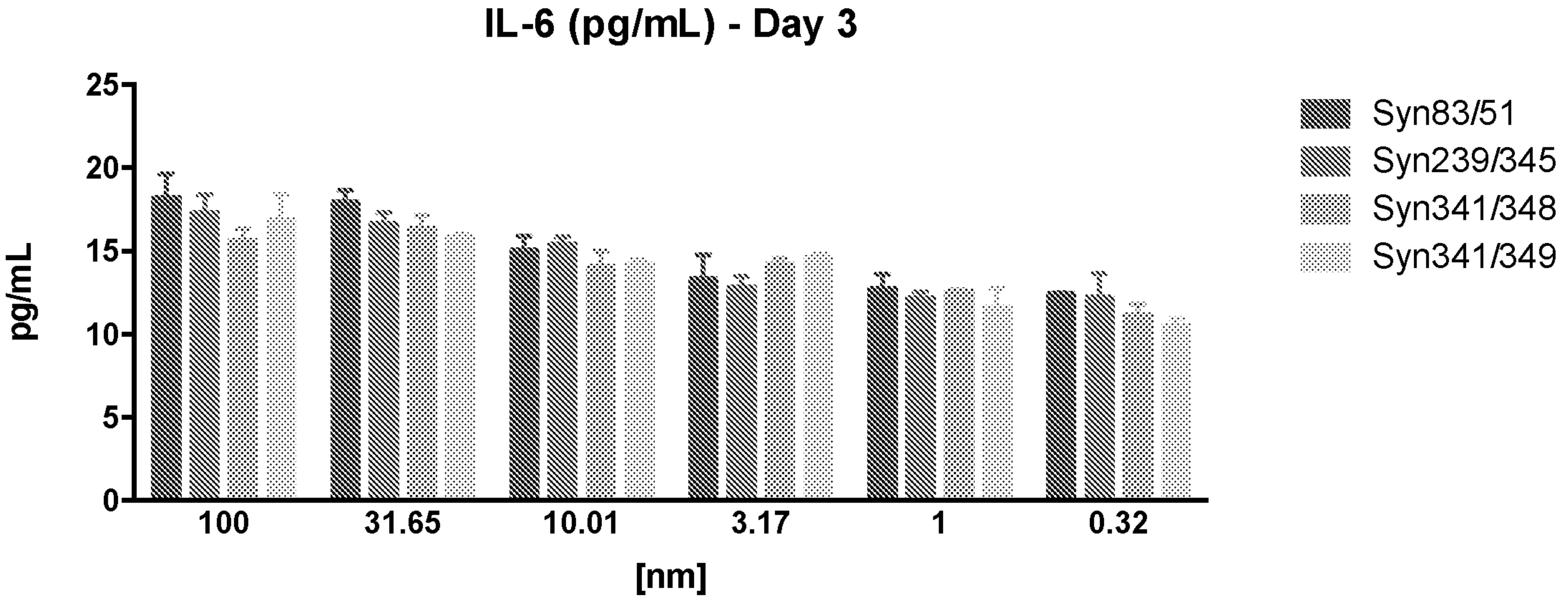

FIG. 16B

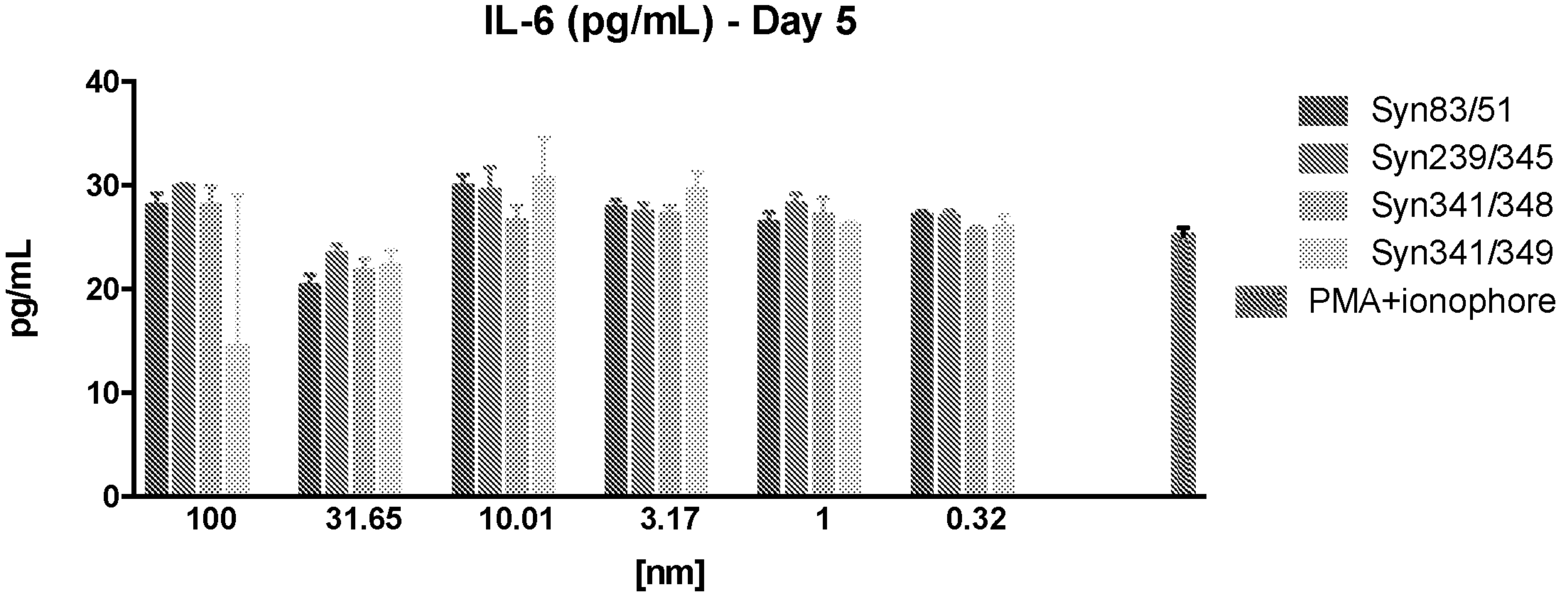

FIG. 17A

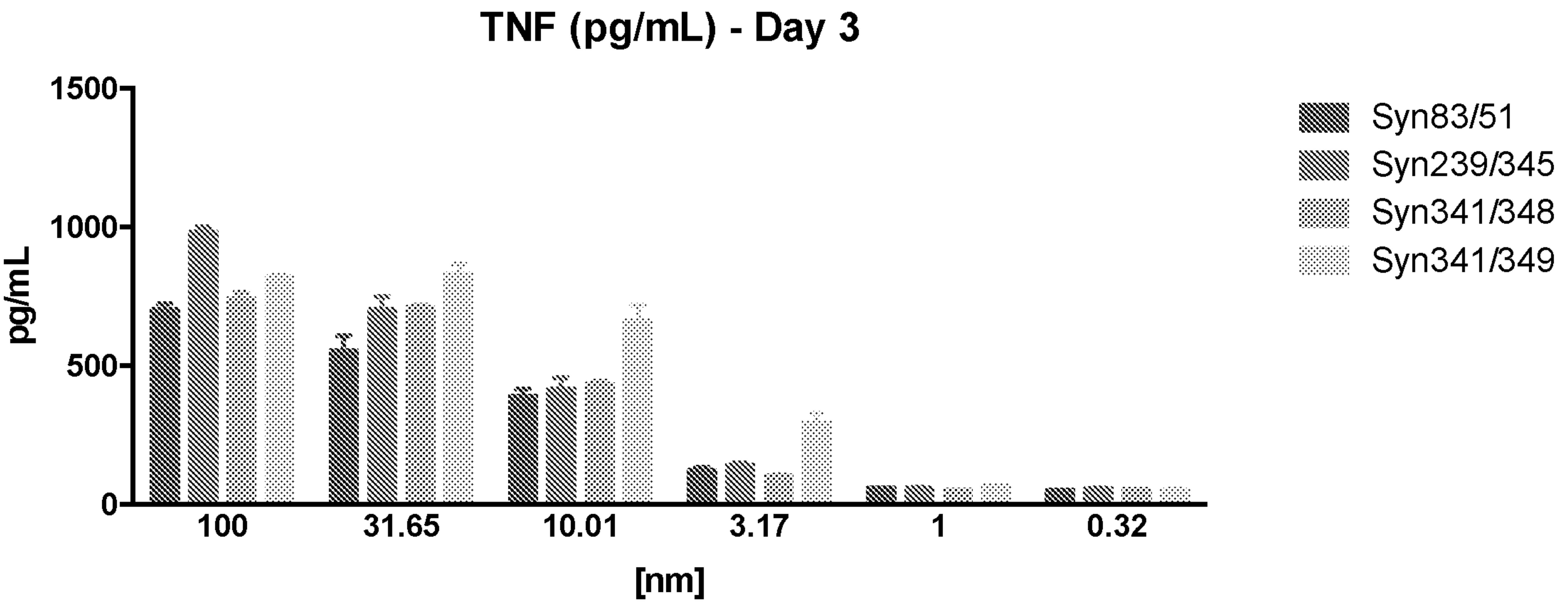

FIG. 17B

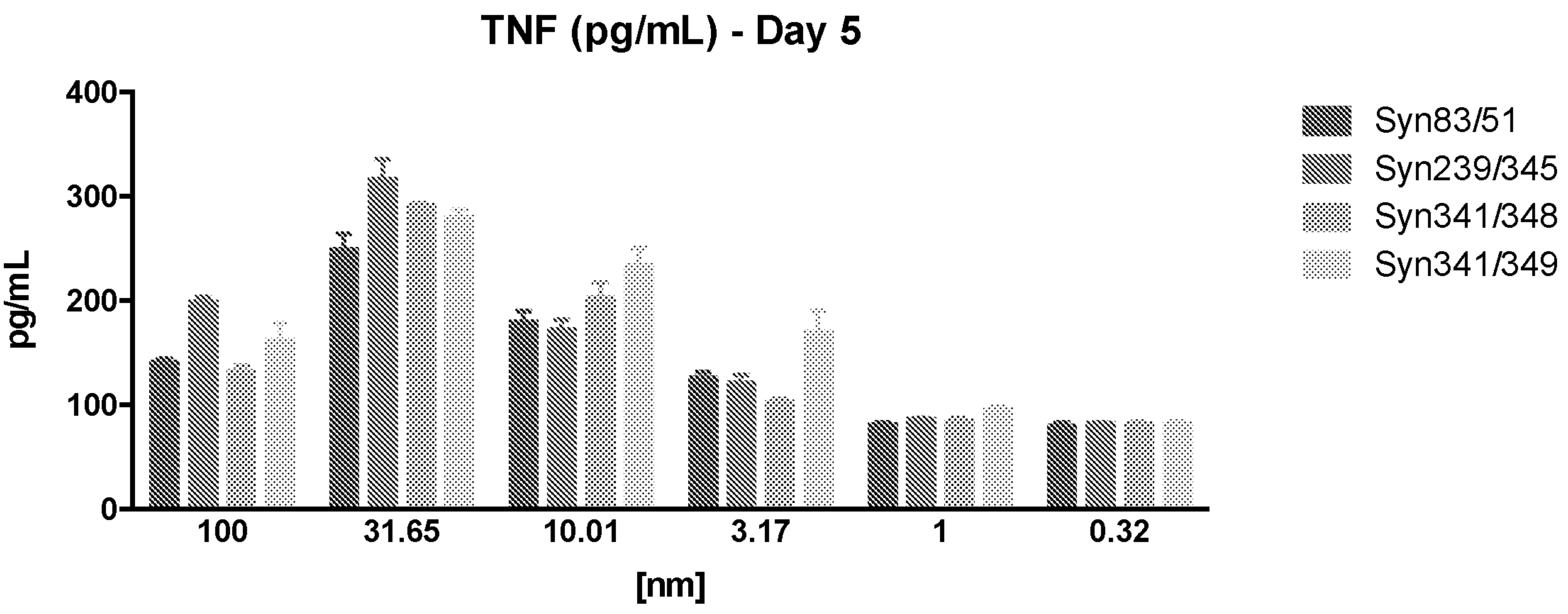

FIG. 18A

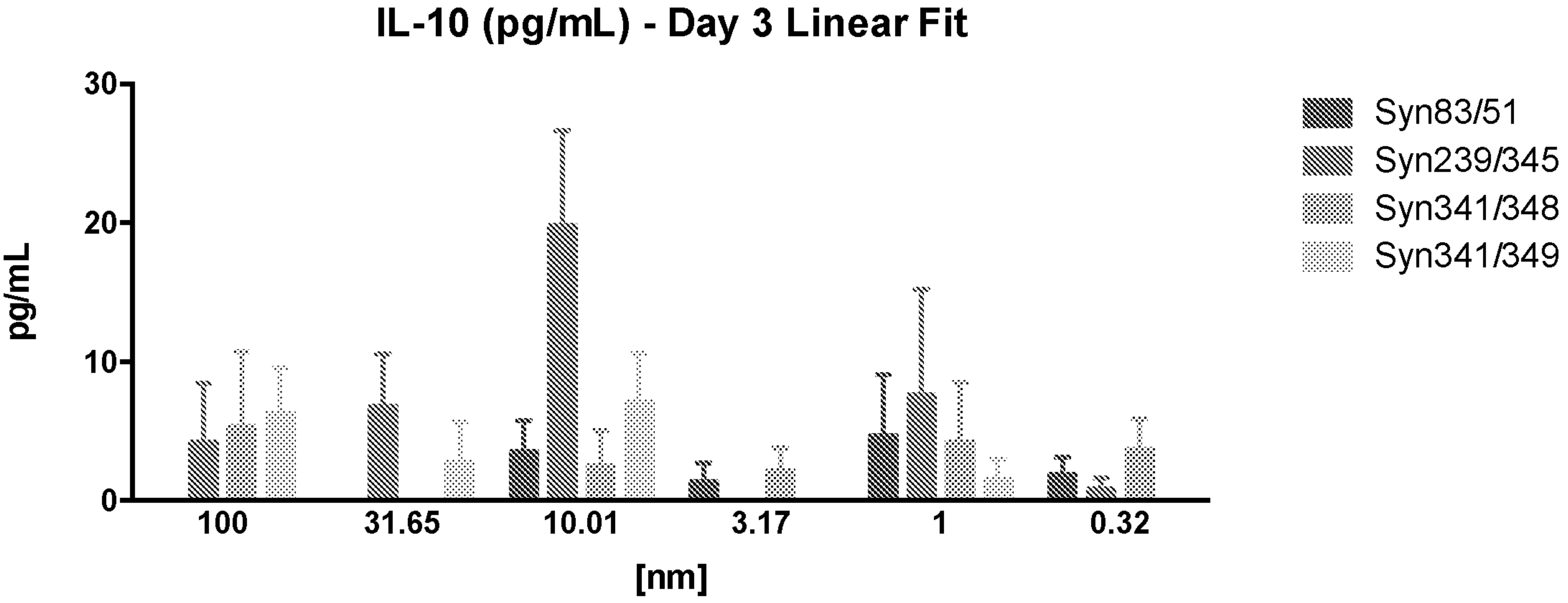

FIG. 18B

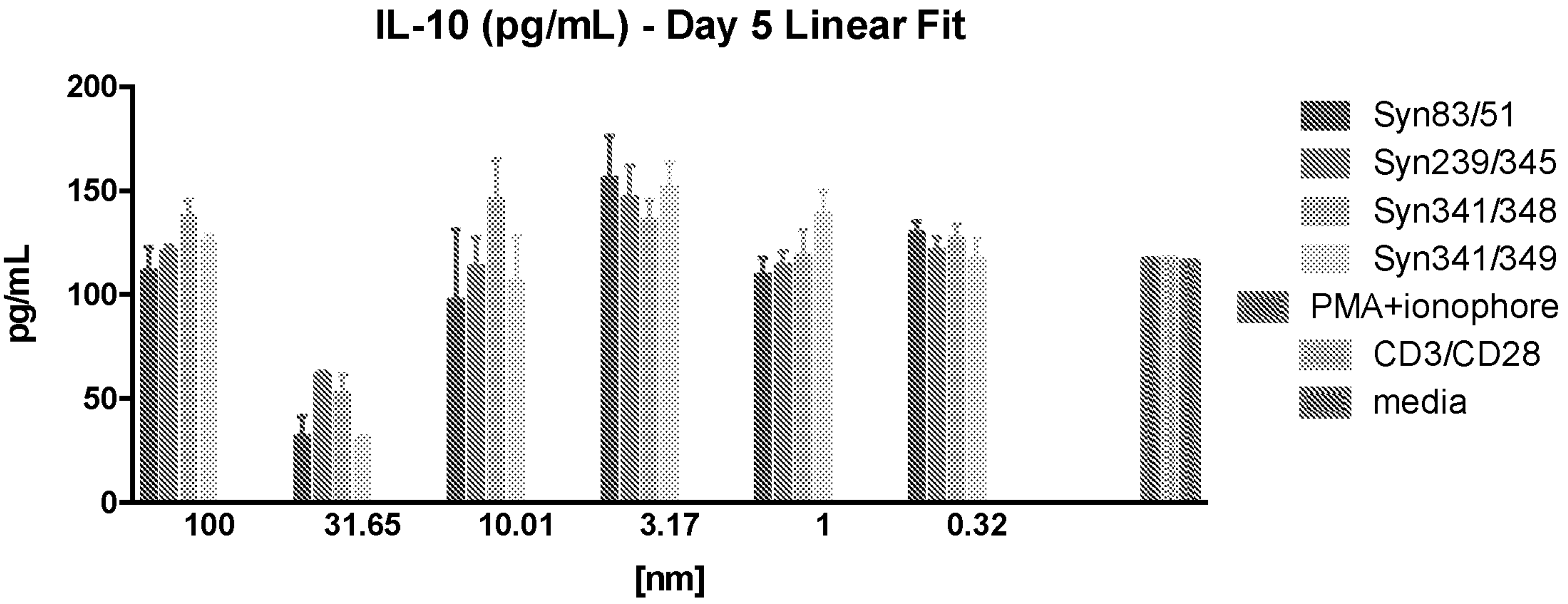

FIG. 19A

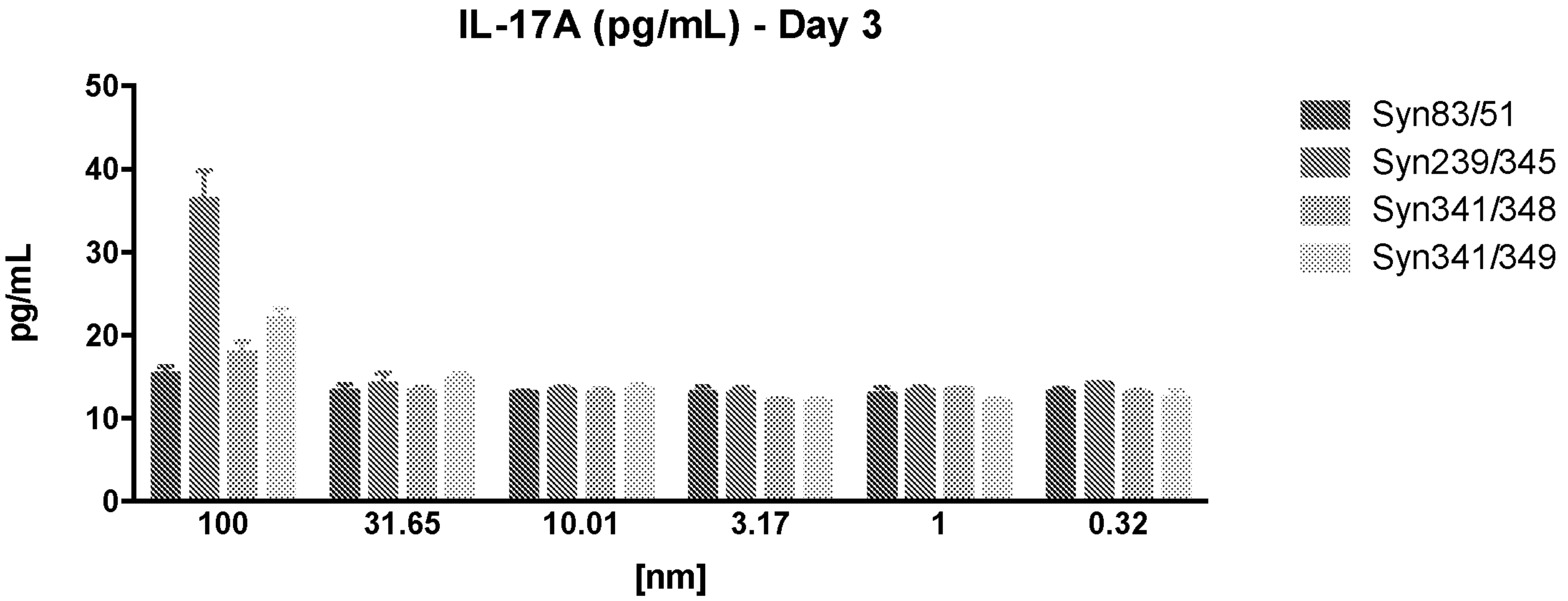

FIG. 19B

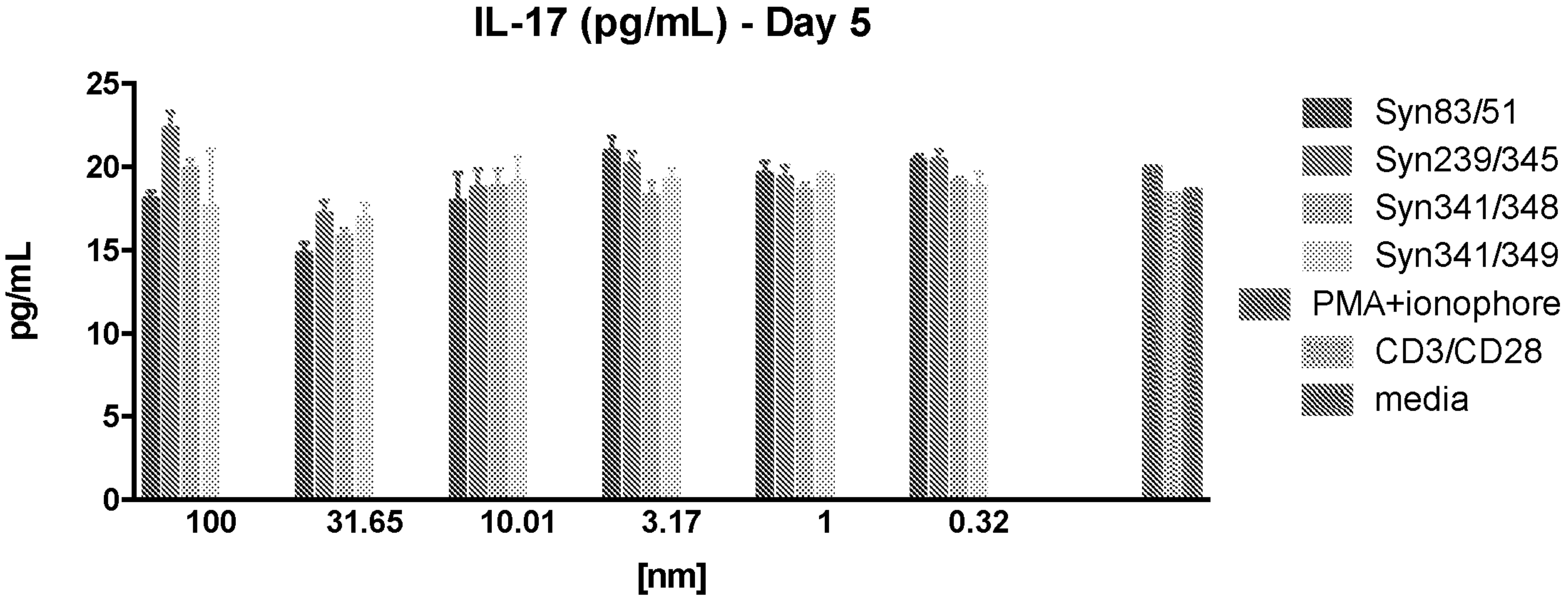

FIG. 20A

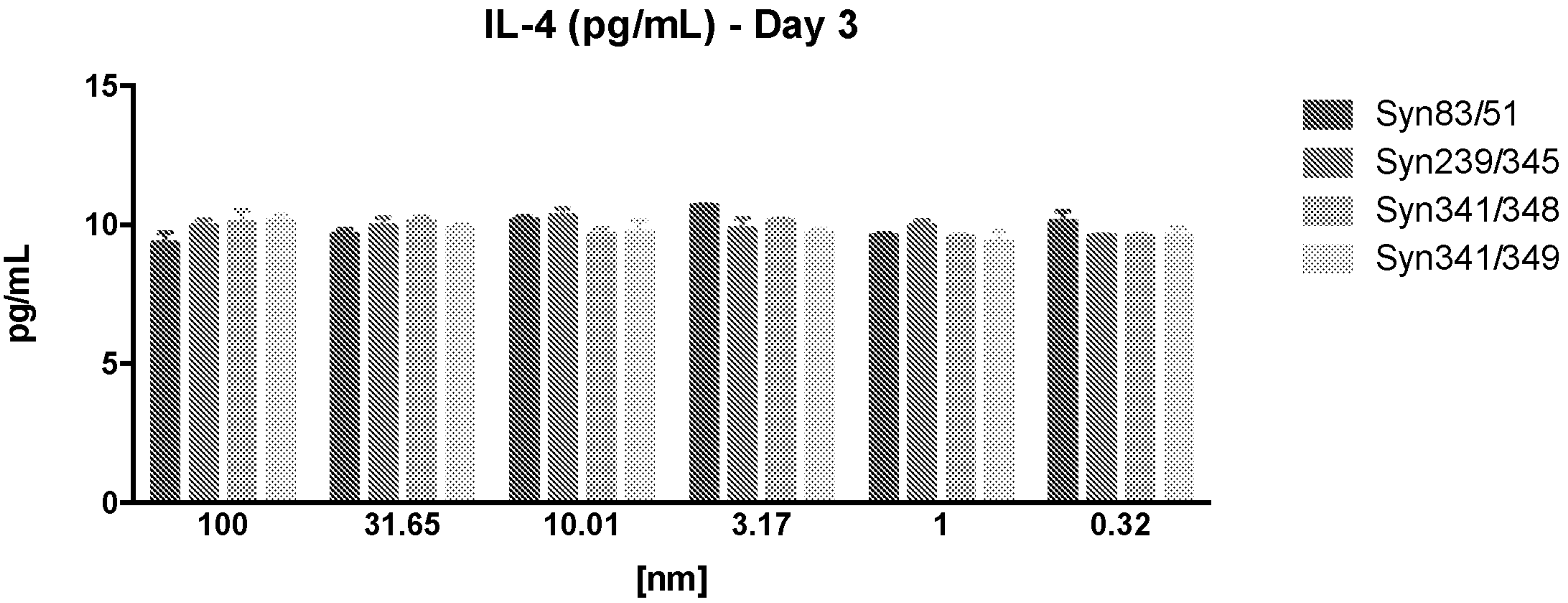

FIG. 20B

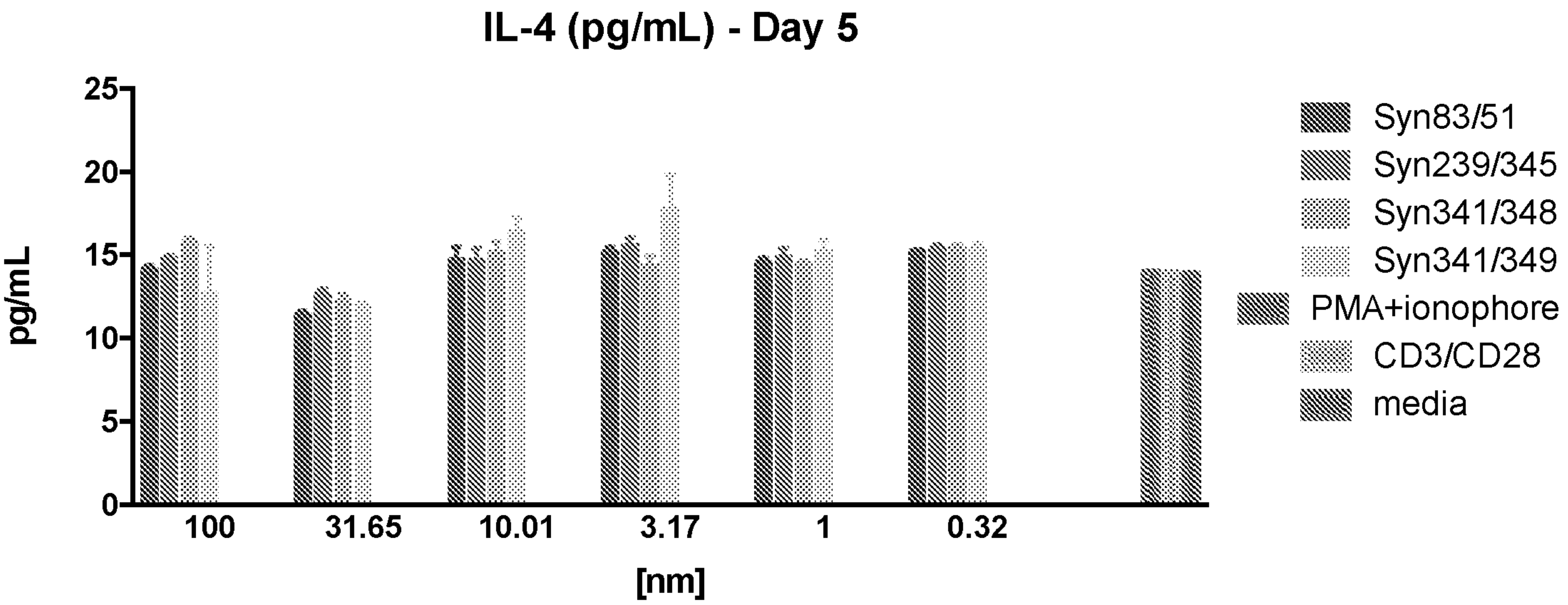

FIG. 21

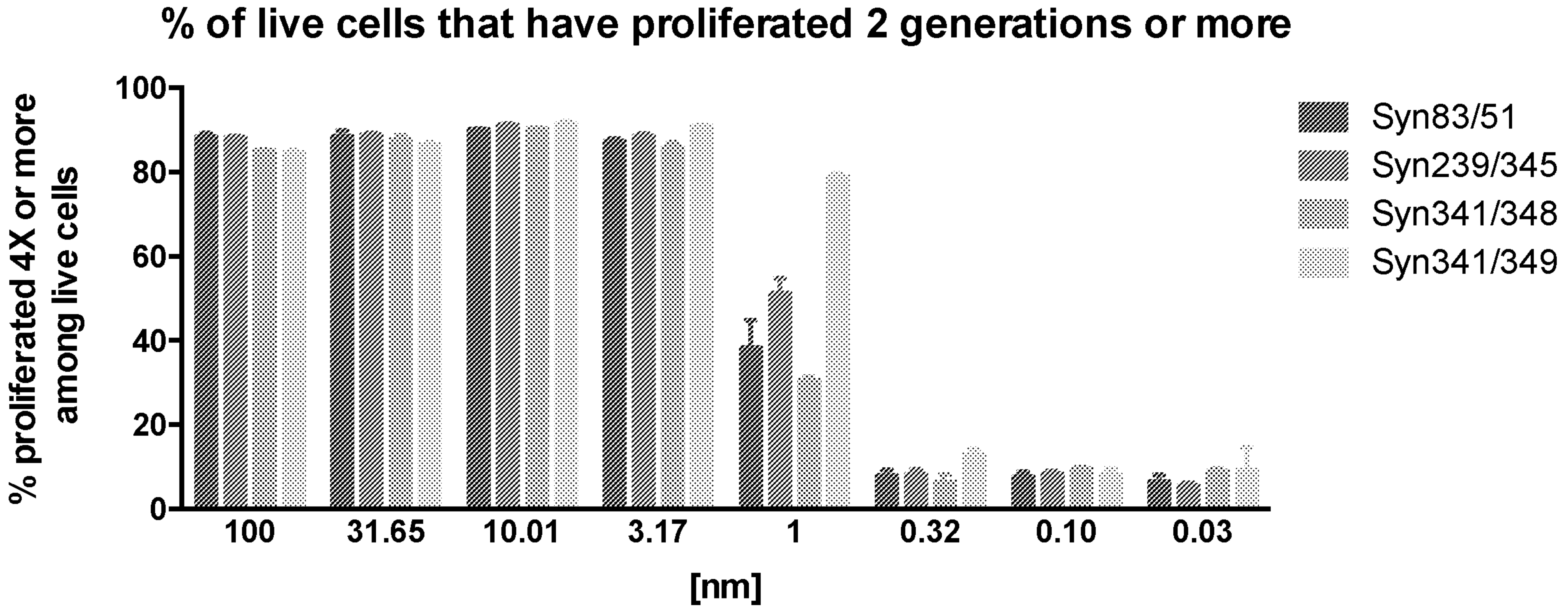
% of live cells that have proliferated 2 generations or more
% proliferated 4X or more among live cells
100
80
60
40
20
0
100
31.65
10.01
3.17
1
0.32
0.10
0.03
[nm]
Syn83/51
Syn239/345
Syn341/348
Syn341/349

FIG. 22

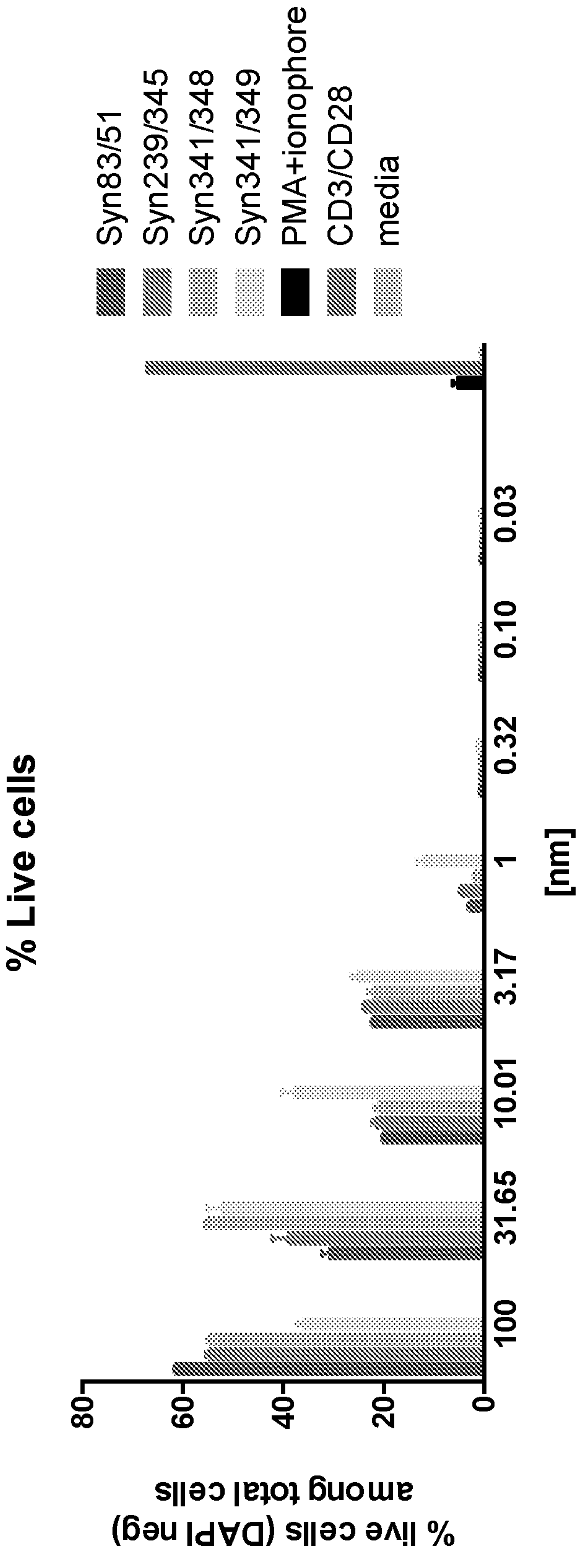
% Live cells
% live cells (DAPI neg) among total cells
80
60
40
20
0
100
31.65
10.01
3.17
1
0.32
0.10
0.03
[nm]
Syn83/51
Syn239/345
Syn341/348
Syn341/349
PMA+ionophore
CD3/CD28
media

FIG. 23

4-1BBL variant	Expression level mg/L	Expression level mg/L	Fold over wild-type
M91A	105.9	115.9	14
F92A	48.9	41.6	5.8
Q94A	13.8	23.1	2.3
L95A	81.8	63.3	9.2
V96A	16.2	24.4	2.6
Q98A	43.0	43.0	5.5
N99A	35.3	53.3	5.6
V100A	37.6	42.6	5.1
L101A	137.8	203.2	21.7
L102A	148.0	184.4	21.2
I103A	48.0	68.0	7.4
D104A	70.5	65.1	3.9
G105A	23.8	37.6	2.6
P106A	81.2	66.0	9.4
L107A	13.8	13.4	1.7
S108A	66.2	72.1	8.8
W109A	15.6	30.8	2.9
Y110A	107.0	110.1	13.8
S111A	104.0	109.0	13.6
D112A	28.0	32.1	3.8
P113A	60.1	60.4	7.7
G114A	94.8	81.7	3.9
L115A	23.0	26.4	3.1
G117A	4.4	12.5	
V118A	4.2	5.3	
S119A	4.6	5.6	
L120A	4.6	4.6	
T121A	4.9	6.3	
G122A	9.8	9.5	
G123A	2.5	10.4	
L124A	3.1	8.5	
S125A	8.9	8.3	
Y126A	2.3	0.6	
E128A	6.1	14.6	
D129A	2.2	0.0	

FIG. 23 (cont.)

4-1BBL variant	Expression level mg/L	Expression level mg/L	Fold over wild-type
T130A	1.9	2.6	
K131A	7.0	15.3	
E132A	2.3	6.8	
F144A	1.5	0.0	
F145A	8.2	6.3	
Q146A	5.7	10.5	
L147A	10.3	16.8	
E148A	5.7	4.4	
L149A	9.9	12.9	
R150A	10.3	4.7	
R151A	1.8	0.0	
V152A	2.9	6.7	
V153A	3.7	7.9	
G155A	6.9	13.1	
E156A	4.3	4.0	
G157A	12.3	18.7	
S158A	6.7	6.3	
D184A	3.6	5.0	
L185A	2.2	0.0	
P186A	4.3	2.2	
P187A	2.9	0.0	
S189A	3.8	6.1	
S190A	2.4	3.1	
E191A	1.8	4.1	
R193A	6.6	7.5	
N194A	4.3	0.1	
S195A	3.2	1.6	
F197A	3.1	6.5	
Q210A	5.1	3.9	
R211A	1.6	3.5	
L212A	2.0	9.8	
G213A	5.0	2.9	
V214A	2.7	7.5	
H215A	3.3	2.4	
L216A	3.4	10.2	

FIG. 23 (cont.)

4-1BBL variant	Expression level mg/L	Expression level mg/L	Fold over wild-type
H217A	8.6	3.2	
T218A	6.6	9.9	
E219A	2.8	5.2	
R221A	3.3	8.7	
R223A	6.2	9.7	
H224A	4.1	6.0	
W226A	1.9	0.0	
L228A	3.1	0.0	
T229A	6.0	7.8	
Q230A	2.7	4.7	
G231A	1.9	2.4	
T233A	1.8	0.0	
V234A	1.9	0.0	
wt	3.8	11.9	

FIG. 24

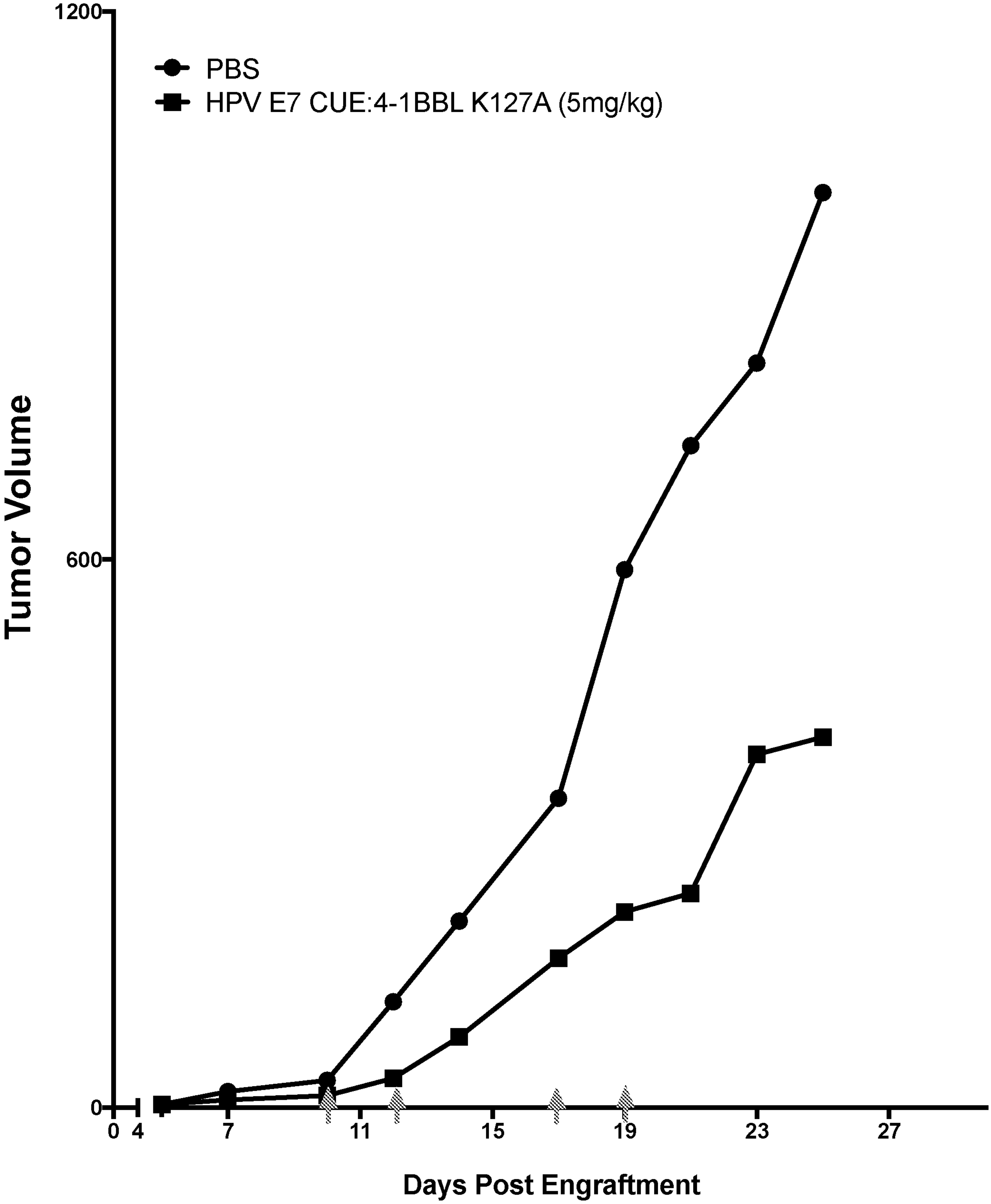
1200
600
0
Tumor Volume
PBS
HPV E7 CUE:4-1BBL K127A (5mg/kg)
0
4
7
11
15
19
23
27
Days Post Engraftment

FIG. 1A