heterodimerization domains like the Jun/Fos coiled-coil to scFv domains lead to a mixture of homo- and heterodimers and need to be assembled by refolding (de Kruif and Logtenberg, J. Biol. Chem. 271 : 7630-4, 1996). Fusion of scFv fragments to whole antibodies was also used as a dimerization device (Coloma and Morrison, Nat. Biotechnol. 15: 159-63, 1997). However, such fusion results in a large molecule with poor solid tissue penetration capabilities. Fusion of two scFv fragments together has also been used to generate bispecific proteins (*e.g.*, BITE® antibodies by Micromet Inc., Bethesda, MD, U.S. Patent No. 7,635,472). However, such proteins do not contain Fc regions, and thus do not allow manipulation of their activities via Fc regions. In addition, these proteins are small (~55 kDa) and thus have relatively short half lives in serum.

To date, immunoglobulin fusion technology has not provided commercially viable heterodimeric proteins or methods for making them. Thus, there remains a need in the art for alternative multispecific heterodimeric proteins as well as efficient methods for producing the same.

BRIEF SUMMARY

The present disclosure provides polypeptide heterodimers formed between two different single chain polypeptides via natural heterodimerization of an immunoglobulin CH1 region and an immunoglobulin light chain constant region (CL). The present disclosure also provides nucleic acids, vectors, host cells and methods for making polypeptide heterodimers as well as methods for using such polypeptide heterodimers, such as in directed T cell activation, inhibiting solid malignancy growth, treating autoimmune or inflammatory conditions, or treating B-cell associated disorders or diseases.

Disclosed is~~In one aspect, the present disclosure provides~~ a polypeptide heterodimer that comprises (a) a first single chain polypeptide (SCP-I) comprising from one to four binding domains that specifically bind from one to four targets, a hinge (H-I), an immunoglobulin heterodimerization domain (HD-I), and an Fc region portion (FRP-I); and (b) a second single chain polypeptide (SCP-II) comprising from zero to four binding domains that specifically bind from zero to four targets, a hinge (H-II), an immunoglobulin heterodimerization domain (HD-II), and an Fc region portion (FRP-II); wherein (i) the immunoglobulin HD-I and the immunoglobulin HD-II preferentially associate with each other to form a polypeptide heterodimer comprised of SCP-I and SCP-II, and (1) the immunoglobulin heterodimerization domain of the first single chain polypeptide (HD-I) comprises a first immunoglobulin CH1 region, and the immunoglobulin heterodimerization domain of the second single chain polypeptide

(HD-II) comprises a first immunoglobulin CL region, or (2) the immunoglobulin heterodimerization domain of the first single chain polypeptide (HD-I) comprises a first immunoglobulin CL region, and the immunoglobulin heterodimerization domain of the second single chain polypeptide (HD-II) comprises a first immunoglobulin CH1 region; and (ii) the Fc region portion of SCP-I and the Fc region portion of SCP-II comprise an immunoglobulin CH2 and CH3 domain of IgG1, IgG2, IgG3, IgG4, IgA1, IgA2, IgD, or any combination thereof; one or two immunoglobulin CH3 domains of IgG1, IgG2, IgG3, IgG4, IgA1, IgA2, IgD, IgE, IgM, or any combination thereof; or an immunoglobulin CH3 and CH4 domain of IgE, IgM, or any combination thereof, provided that the polypeptide heterodimer comprises at least two binding domains that specifically bind a target, for instance, at least two different targets.

In one aspect, the present invention provides a polypeptide heterodimer, comprising:

(a) a first single chain polypeptide (SCP-I) comprising from one to four binding domains that specifically bind from one to four targets, a hinge (H-I), an immunoglobulin heterodimerization domain (HD-I), and an immunoglobulin CH2 and CH3 domain of IgG1, IgG2, IgG3, IgG4, IgA1, IgA2, IgD, or any combination thereof (CH2CH3-I); and

(b) a second single chain polypeptide (SCP-II) comprising from zero to four binding domains that specifically bind from zero to four targets, a hinge (H-II), an immunoglobulin heterodimerization domain (HD-II), and an immunoglobulin CH2 and CH3 domain of IgG1, IgG2, IgG3, IgG4, IgA1, IgA2, IgD, or any combination thereof (CH2CH3-II);

wherein

(i) the immunoglobulin heterodimerization domain of the first single chain polypeptide (HD-I) and the immunoglobulin heterodimerization domain of the second single chain polypeptide (HD-II) preferentially associate with each other to form a polypeptide heterodimer comprised of the first single chain polypeptide (SCP-I) and the second single chain polypeptide (SCP-II), and

(1) the immunoglobulin heterodimerization domain of the first single chain polypeptide (HD-I) comprises a first immunoglobulin CH1 region, and the immunoglobulin heterodimerization domain of the second single chain polypeptide (HD-II) comprises a first immunoglobulin CL region, or

(2) the immunoglobulin heterodimerization domain of the first single chain polypeptide (HD-I) comprises a first immunoglobulin CL region, and the immunoglobulin heterodimerization domain of the second single chain polypeptide (HD-II) comprises a first immunoglobulin CH1 region;

(ii) wherein the first immunoglobulin CL region is an altered human immunoglobulin Cκ region with one or more amino acids of a wild type human Cκ region substituted at N29, N30, Q52, V55, T56, S68, or T70,

provided that the polypeptide heterodimer comprises at least two binding domains that specifically bind at least two different targets.

In certain ~~embodiments~~examples, the binding domains of the polypeptide heterodimers are single chain Fv (scFv) polypeptides.

In certain ~~embodiments~~examples, the polypeptide heterodimer comprises two binding domains (BD1 and BD2). In one ~~embodiment~~example, the two binding domains (BDl and BD2) are both on the first single chain polypeptide (SCP-I) and wherein the HD-I and FRP-I are disposed between BD1 and BD2. In another ~~embodiment~~example, the first binding domain (BD1) is on the first single chain polypeptide (SCP-I) and the second binding domain (BD2) is on the second single chain polypeptide (SCP-II). For example, the first binding domain (BD1) may be amino terminal to the Fc region portion of the first single chain polypeptide (FRP-I), and the second binding domain (BD2) may amino terminal to the Fc region portion of the second single chain polypeptide (FRP-II). Alternatively, the first binding domain (BD1) may be amino terminal to the Fc region portion of the first single chain polypeptide (FRP-I), and the second binding domain (BD2) may be carboxyl terminal to the Fc region portion of the second single chain polypeptide (FRP-II). Also alternatively, the first binding domain (BD1) may be carboxyl terminal to the Fc region portion of the first single chain polypeptide (FRP-I), and the second binding domain (BD2) may be carboxyl terminal to the Fc region portion of the second single chain polypeptide (FRP-II).

In certain ~~embodiments~~examples, the polypeptide heterodimer comprises three binding domains (BD1, BD2 and BD3). In one ~~embodiment~~example, the HD-I and FRP-I are disposed between BD1 and BD2, and the third binding domain (BD3) is amino terminal to the Fc region portion of the second single chain polypeptide (FRP-II). In an alternative ~~embodiment~~example, the HD-I and FRP-I are disposed between BD1 and BD2, and the third binding domain (BD3) is carboxyl terminal to the Fc region portion of the second single chain polypeptide (FRP-II).

In certain ~~embodiments~~examples, the polypeptide heterodimer comprises four binding domains (BD1, BD2, BD3, and BD4). For instance, the HD-I and FRP-I may be disposed between BD1 and BD2, and the HD-II and FRP-II may be disposed between BD3 and BD4.

In certain ~~embodiments~~examples, the polypeptide heterodimer comprises five to eight binding domains (*e.g.*, 5, 6, 7 or 8 binding domains).

In certain ~~embodiments~~examples, at least one of the binding domains of the polypeptide heterodimers provided herein specifically binds to, or is an antagonist of, TCRα, TCRβ, CD3γ, CD3δ, CD3ε, CD28, CD79b, hyperIL-6, monoIL-10 , CD86, CD20, PSMA, CD19, HLA-DR, Ron, c-Met, CEACAM-6, LIGHT, GITRL, CD40, PDL1, PDL2, HVEM, LTBR, EGFR, EGFRvIII, ErbB2, ErbB3, ErbB4, IGF1R, EphA2, PDGFR, VEGFR1-4, Angiopoietin 2, CD64, CD32A, CD 16, CD71, TNFR1, TNFR2, TWEAKR, TACI, BAFF-R, BCMA, FAS, CD32B, CD21, CD22, CD30, CD33, CD37, CD38, CD70, TNFα, IL-6, hyperIL-6, IL-2, IL-1, IL-7, IL-8, IL-17A/C, IP-10, IFNγ, IFNα, RANKL, FASL, TGFβ, IL10, IL17A/F, CSF2, IGF1, IGF2, BLyS/APRIL, , HGF, MSP, EGF (including epiregulin, herregulin, β-regulin, neuregulin), HIF-1α, VEGFA, VEGFB, VEGFC, VEGFD, TNFα, Wnt, sHH, TGFβ, PDGF, TWEAK, EpCAM, CEA, PCTA-1, STEAP-1, PSCA, ALCAM (CD166), EphA2, CD151, CA-125, MUC-1, MAGE-1, TROP2, CCR5, HER-3, HER-4, EGFR, CEA, MUC2, MUC3, MUC4, $MUC5_{AC}$, $MUC5_b$, MUC7, βhCG, Lewis-Y, ganglioside GD3, 9-O-Acetyl-GD3, GM2, Globo H, fucosyl GM1, Poly SA, GD2, Carboanhydrase IX (MN/CA IX), CD44v6, Sonic Hedgehog (Shh), Wue-1, Plasma Cell Antigen, (membrane-bound) IgE, Melanoma Chondroitin Sulfate Proteoglycan (MCSP), CCR8, TNF-alpha precursor, STEAP, mesothelin, A33 Antigen, Prostate Stem Cell Antigen (PSCA), Ly-6; desmoglein 4, E-cadherin neoepitope, Fetal Acetylcholine Receptor, CD25, CA19-9 marker, CA-125 marker and Muellerian Inhibitory Substance (MIS) Receptor type II, sTn (sialylated Tn antigen; TAG-72), FAP (fibroblast activation antigen), endosialin, EGFRvIII, LG, SAS, CD63, IGF1R, CD151, TGFBR2, GHRHR, GHR, IL-6R, gp130, TNFR2, OSMRβ, Patched-1, Frizzled, Robo1, CD80, CD81, CD86, OX40, CD40, CD137, LIFRβ, TLR7 or TLR9.

In certain other ~~embodiments~~examples, at least one of the binding domains of the polypeptide heterodimer is an agonist of IL-10, HLA-G, HGF, IL-35, PD-1, BTLA, TNFR1, TNFR2, DR4, DR5, TWEAKR, or FAS.

In certain polypeptide heterodimers provided herein, at least one binding domain specifically binds a TCR complex or a component thereof, and at least another binding domain specifically binds to PSMA, CD79b, CD19, HLA-DR, CD20, RON, c-Met, or CEACAM-6.

In certain other polypeptide heterodimers provided herein, at least one binding domain specifically binds to CD28, and at least another binding domain specifically binds to, or is an antagonist of, CD79b, hyperIL-6, PDL2, monoIL-10, CD86, LIGHT, GITRL, CD40, PDL1, HVEM, or LTBR.

In certain other polypeptide heterodimers provided herein, at least one binding domain specifically binds to CD28, and at least another binding domain is an agonist of IL-10, HLA-G, HGF, IL-35, PD-1, or BTLA.

In certain ~~embodiments~~examples, the immunoglobulin heterodimerization domain of the first single chain polypeptide (HD-I) comprises the first immunoglobulin CH1 region and the immunoglobulin heterodimerization domain of the second single chain polypeptide (HD-II) comprises the first immunoglobulin CL region. In one ~~embodiment~~example, the first CH1 region is amino terminal to the Fc region portion of the first single chain polypeptide, and the first CL region is amino terminal to the Fc region portion of the second single chain polypeptide. In another ~~embodiment~~example, the first CH1 region is carboxyl terminal to the Fc region portion of the first single chain polypeptide, and the first CL region is carboxyl terminal to the Fc region portion of the second single chain polypeptide.

In certain ~~embodiments~~examples in which the immunoglobulin heterodimerization domain of the first single chain polypeptide (HD-I) comprises the first immunoglobulin CH1 region and the immunoglobulin heterodimerization domain of the second single chain polypeptide (HD-II) comprises the first immunoglobulin CL region, the first single chain polypeptide further comprises a second CH1 region and the second single chain polypeptide further comprises a second CL region, and the second CH1 region of the first single chain polypeptide and the second CL region of the second single chain polypeptide associate with each other in the polypeptide heterodimer. For instance, the Fc region portion of the first single chain polypeptide may be disposed between the first and second CH1 regions, and the Fc region portion of the second single chain polypeptide may be disposed between the first and second CL regions. Alternatively, both the first and second CH1 regions may be amino terminal to the Fc region portion of the first single chain polypeptide, and both the first and second CL regions may be amino terminal to the Fc region portion of the second single chain polypeptide. Also alternatively, both the first and second CH1 regions may be carboxyl terminal to the Fc region portion of the first single chain polypeptide, and both the first and second CL regions may be carboxyl terminal to the Fc region portion of the second single chain polypeptide.

In certain other ~~embodiments~~examples in which the immunoglobulin heterodimerization domain of the first single chain polypeptide (HD-I) comprises the

first immunoglobulin CH1 region and the immunoglobulin heterodimerization domain of the second single chain polypeptide (HD-II) comprises the first immunoglobulin CL region, the first single chain polypeptide further comprises a second CL region and the second single chain polypeptide further comprises a second CH1 region, and the second CL region of the first single chain polypeptide and the second CH1 region of the second single chain polypeptide associate with each other in the polypeptide heterodimer. For instance, in one ~~embodiment~~example, in the first single chain polypeptide, the first CH1 region is amino terminal to the Fc region portion and the second CL region is carboxyl terminal to the Fc region portion; and in the second single chain polypeptide, the first CL region is amino terminal to the Fc region portion, and the second CH1 region is carboxyl terminal to the Fc region portion. In another ~~embodiment~~example, in the first single chain polypeptide, the first CH1 region is carboxyl terminal to the Fc region portion, and the second CL region is amino terminal to the Fc region portion; and in the second single chain polypeptide, the first CL region is carboxyl terminal to the Fc region portion, and the second CH1 region is amino terminal to the Fc region portion. In yet another ~~embodiment~~example, in the first single chain polypeptide, both the first CH1 region and the second CL regions are amino terminal to the Fc region portion, and the first CH1 region is amino terminal to the second CL region; and in the second single chain polypeptide, both the first CL region and the second CH1 region are amino terminal to the Fc region portion, and the first CL region is amino terminal to the second CH1 region. In yet another ~~embodiment~~example, in the first single chain polypeptide, both the first CH1 region and the second CL regions are amino terminal to the Fc region portion, and the second CL region is amino terminal to the first CH1 region; and in the second single chain polypeptide, both the first CL region and the second CH1 region are amino terminal to the Fc region portion, and the second CH1 region is amino terminal to the first CL region. In a further ~~embodiment~~example, in the first single chain polypeptide, both the first CH1 region and the second CL regions are carboxyl terminal to the Fc region portion, and the first CH1 region is amino terminal to the second CL region; and in the second single chain polypeptide, both the first CL region and the second CH1 region are carboxyl terminal to the Fc region portion, and the first CL region is amino terminal to the second CH1 region. In another ~~embodiment~~example, in the first single chain polypeptide, both the first CH1 region and the second CL regions are carboxyl terminal to the Fc region portion, and the second CL region is amino terminal to the first CH1 region; and in the second single chain polypeptide, both the first CL region and the second CH1 region are carboxyl terminal to the Fc region portion, and the second CH1 region is amino terminal to the first CL region.

In certain ~~embodiments~~examples, the immunoglobulin heterodimerization domain of the first single chain polypeptide (HD-I) comprises a first immunoglobulin CL region, and the immunoglobulin heterodimerization domain of the second single chain polypeptide (HD-II) comprises a first immunoglobulin CH1 region. In one ~~embodiment~~example, the first CL region is amino terminal to the Fc region portion of the first single chain polypeptide, and the first CH1 region is amino terminal to the Fc region portion of the second single chain polypeptide. In another ~~embodiment~~example, the first CL region is carboxyl terminal to the Fc region portion of the first single chain polypeptide, and the first CH1 region is carboxyl terminal to the Fc region portion of the second single chain polypeptide. In yet another ~~embodiment~~example, the first single chain polypeptide further comprises a second CL region and the second single chain polypeptide further comprises a second CH1 region, and the second CL region of the first single chain polypeptide and the second CH1 region of the second single chain polypeptide associate with each other in the polypeptide heterodimer. For instance, the Fc region portion of the first single chain polypeptide may be disposed between the first and second CL regions, and the Fc region portion of the second single chain polypeptide may be disposed between the first and second CH1 regions. Alternatively, both the first and second CL regions may be amino terminal to the Fc region portion of the first single chain polypeptide, and both the first and second CH1 regions may be amino terminal to the Fc region portion of the second single chain polypeptide. Also alternatively, both the first and second CL regions may be carboxyl terminal to the Fc region portion of the first single chain polypeptide, and both the first and second CH1 regions may be carboxyl terminal to the Fc region portion of the second single chain polypeptide.

In certain ~~embodiments~~examples, the first CL region is a Cκ region. In other ~~embodiments~~examples, the first CL region is a Cλ region.

In certain ~~embodiments~~examples, the second CL region is a Cκ region. In other ~~embodiments~~examples, the second CL region is a Cλ region.

In certain ~~embodiments~~examples, the Cκ region is a wild type human immunoglobulin Cκ region. In certain other ~~embodiments~~examples, the Cκ region is an altered human immunoglobulin Cκ region with one or more amino acids of a wild type human Cκ region substituted at N29, N30, Q52, V55, T56, T56, S68, or T70. For example, the one or more amino acid substitutions may be selected from Ala (A), Arg (R), Trp (W), Tyr (Y), Glu (E), Gin (Q), Lys (K), Asp (D), Met (M), Ser (S), and Phe (F).

In certain ~~embodiments~~examples, the CH1 region is an altered human immunoglobulin CH1 region comprising an amino acid substitution by which Val (V) at position 68 is substituted by Lys (K), Arg (R) or His (H), and wherein the Ck region is an altered human immunoglobulin Ck region comprising an amino acid substitution

by which Leu (L) at position 27 is substituted by Asp (D) or Glu (E). In certain other ~~embodiments~~examples, the CH1 region is an altered human immunoglobulin CH1 region comprising an amino acid substitution by which Val (V) at position 68 is changed to Asp (D) or Glu (E), and wherein the Ck region is an altered human immunoglobulin Ck region comprising an amino acid substitution by which Leu (L) at position 27 is changed to Lys (K), Arg (R) or His (H).

In certain ~~embodiments~~examples, the Cλ region is a wild type human immunoglobulin Cλ region.

In certain ~~embodiments~~examples, the first CH1 region or the second CH1 region when present is a wild type human immunoglobulin CH1 region, such as a wild type human IgG1 CH1 region.

In certain ~~embodiments~~examples, the first CH1 region or the second CH1 region when present is an altered human immunoglobulin CH1 region, such as an altered human IgG1 CH1 region, with the cysteine of a wild type human immunoglobulin CH1 region that is involved in forming a disulfide bond with a wild type human immunoglobulin CL region deleted or substituted.

In certain ~~embodiments~~examples, the Cκ region is an altered human immunoglobulin Ck region, such as an with the cysteine residue of a wild type human Cκ region that is involved in forming a disulfide bond with a wild type human immunoglobulin CH1 region deleted or substituted.

In certain ~~embodiments~~examples, the Cλ region is an altered human immunoglobulin Cλ region with the cysteine residue of a wild type human Cλ region that is involved in forming a disulfide bond with a wild type human immunoglobulin CH1 region deleted or substituted.

In certain ~~embodiments~~examples, the first CH1 region and the second CH1 region when present is a polypeptide comprising SEQ ID NO: 114, 844 or 845.

In certain ~~embodiments~~examples, the Ck region when present is selected from any one of the polypeptides comprising SEQ ID NOS: 141-178, 202, and 838-843.

In certain ~~embodiments~~examples, the Cλ region when present is a polypeptide comprising SEQ ID NO: 140.

In certain ~~embodiments~~examples, the Fc region portion of the first single chain polypeptide (FRP-I) and the Fc region portion of the second single chain polypeptide (FRP-II) each comprise an immunoglobulin CH2 domain, such as an IgG1 CH2 domain or an IgG2, IgG3, IgG4, IgA1, IgA2, or IgD CH2 domain.

In certain ~~embodiments~~examples, the Fc region portion of the first single chain polypeptide (FRP-I) and the Fc region portion of the second single chain polypeptide

(FRP-II) each comprise an immunoglobulin CH3 domain, such as an IgG1 CH3 domain or an IgG2, IgG3, IgG4, IgA1, IgA2, IgD, IgE or IgM CH3 domain.

In certain ~~embodiments~~examples, the Fc region portion of the first single chain polypeptide (FRP-I) and the Fc region portion of the second single chain polypeptide (FRP-II) each comprise an immunoglobulin CH2 domain and an immunoglobulin CH3 domain, such as are IgG1, IgG2, IgG3, IgG4, IgA1, IgA2, or IgD CH2 and CH3 domains.

In some ~~embodiments~~examples in which the Fc region portion comprises an immunoglobulin CH3 domain that is immediately amino terminal to an immunoglobulin heterodimerization domain (*e.g.*, a CH1 domain or a Ck domain), the immunoglobulin CH3 domain is linked to the CH1 domain immediately carboxyl terminal to the immunoglobulin CH3 domain in one single chain polypeptide via a peptide comprising SEQ ID NOS:846, 847, 848, or 849; and the immunoglobulin CH3 domain is linked to the Ck domain immediately carboxyl terminal to the immunoglobulin CH3 domain in the other single chain polypeptide via a peptide comprising SEQ ID NOs:846, 850, 951 , or 852.

In certain ~~embodiments~~examples, the Fc region portion of the first single chain polypeptide (FRP-I) and the Fc region portion of the second chain polypeptide (FRP-II) comprise IgM or IgE CH3 and CH4 domains.

In certain ~~embodiments~~examples in which the Fc region portion comprises an immunoglobulin CH2 domain, the CH2 domain may be an altered human IgG1, IgG2, IgG3, or IgG4 CH2 domain. Exemplary altered human IgG1, IgG2, IgG3 or IgG4 CH2 domain include those that comprise (a) an amino acid substitution at position 297 and at least one additional substitution or deletion at positions 234 to 238; (b) one or more amino acid mutations at positions 234-238 and at least one substitution at position 253, 310, 318, 320, 322, or 331; or (c) an amino acid substitution at the asparagine of position 297, one or more substitutions or deletions at positions 234 to 238, and at least one substitution at position 253, 310, 318, 320, 322, or 331. Another exemplary CH2 domain is an altered human IgG1 CH2 domain that comprises amino acid substitutions at positions L234, L235, G237, E318, K320 and K322.

In certain ~~embodiments~~examples, the CH3 domain of the first single chain polypeptide is an altered human IgG1, IgG2, IgG3, or IgG4 CH3 domain that comprises a T366W, and the CH3 domain of the second single chain polypeptide is an altered human IgG1, IgG2, IgG3 or IgG4 CH3 domain that comprises a Y407A substitution. In certain other ~~embodiments~~examples, the CH3 domain of the first single chain polypeptide is an altered human IgG1, IgG2, IgG3, or IgG4 CH3 domain that comprises a T366Y substitution, and the CH3 domain of the second single chain polypeptide is an altered

human IgG1, IgG2, IgG3 or IgG4 CH3 domain that comprises a Y407T substitution. In certain other ~~embodiments~~examples, the CH3 domain of the first single chain polypeptide is an altered human IgG1, IgG2, IgG3, or IgG4 CH3 domain that comprises a T366W substitution, and the CH3 domain of the second single chain polypeptide is an altered human IgG1, IgG2, IgG3 or IgG4 CH3 domain that comprises T366S, L368A and Y407V substitutions. In certain other ~~embodiments~~examples, the CH3 domain of the first single chain polypeptide is an altered human IgG1, IgG2, IgG3, or IgG4 CH3 domain that comprises a Y407A substitution, and the CH3 domain of the second single chain polypeptide is an altered human IgG1, IgG2, IgG3 or IgG4 CH3 domain that comprises a T366W substitution. In certain other ~~embodiments~~examples, the CH3 domain of the first single chain polypeptide is an altered human IgG1, IgG2, IgG3, or IgG4 CH3 domain that comprises a Y407T substitution, and the CH3 domain of the second single chain polypeptide is an altered human IgG1, IgG2, IgG3 or IgG4 CH3 domain that comprises a T366Y substitution. In certain other ~~embodiments~~examples, the CH3 domain of the first single chain polypeptide is an altered human IgG1, IgG2, IgG3, or IgG4 CH3 domain that comprises T366S, L368A and Y407W substitutions, and the CH3 domain of the second single chain polypeptide is an altered human IgG1, IgG2, IgG3 or IgG4 CH3 domain that comprises a T366W substitution.

In certain ~~embodiments~~examples, the hinge of both the first and second single chain polypeptides is an immunoglobulin hinge region, such as an IgG1, IgG2, IgG3, IgG4, IgA1, IgA2, IgD, or IgE hinge. In certain ~~embodiments~~examples, the immunoglobulin hinge is a wild type immunoglobulin hinge. In certain other ~~embodiments~~examples, the immunoglobulin hinge is an altered immunoglobulin hinge selected from SEQ ID NOS:232, 234, 240, 664-673, 675 and 676.

In certain ~~embodiments~~examples, the hinge region is (a) at the amino terminal to the Fc region portion, (b) disposed between the binding domain and the immunoglobulin heterodimerization domain, (c) disposed between the immunoglobulin heterodimerization domain and the Fc region portion, (d) at the amino terminus of the first or second single chain polypeptide, (e) disposed between the Fc region portion and a binding domain, or (f) at the carboxyl terminus of the first or second single chain polypeptide.

In certain ~~embodiments~~examples, at least one of the first and second single chain polypeptide hinges is a C-type lectin hinge region, such as an NKg2A or NKg2D peptide, or a derivative thereof.

In certain ~~embodiments~~examples, the hinges of the first and second single chain polypeptides are identical. In certain other ~~embodiments~~examples, the hinges of the first and second single chain polypeptides are different.

In certain ~~embodiments~~examples, the first single chain polypeptide comprises a binding domain that specifically binds a TCR complex or a component thereof, and the second single chain polypeptide comprises a binding domain that specifically binds CD19, CD79b, HLA-DR or CD20.

In certain ~~embodiments~~examples, the first single chain polypeptide comprises a binding domain that specifically binds CD28, and the second single chain polypeptide comprises a binding domain (a) that specifically binds CD79b, hyperIL-6, or CD86 or (b) that comprises a PDL ectodomain or a monoIL-10.

In certain ~~embodiments~~examples, the first single chain polypeptide comprises a binding domain that specifically binds c-Met, and the second single chain polypeptide comprises a binding domain that specifically binds RON.

In certain ~~embodiments~~examples, the first and second single chain polypeptides comprise SEQ ID NOS: SEQ ID NOS:2 and 4, SEQ ID NOS:6 and 8, SEQ ID NOS: 10 and 12, SEQ ID NOS: 14 and 16, SEQ ID NOS: 18 and 20, SEQ ID NOS:20 and 22, SEQ ID NOS:20 and 24, SEQ ID NOS:30 and 32, SEQ ID NOS:29 and 31, SEQ ID NOS:29 and 32, SEQ ID NOS:30 and 72, SEQ ID NOS:53 and 72, SEQ ID NOS:54 and 72, SEQ ID NOS:55 and 72, SEQ ID NOS:70 and 72, SEQ ID NOS:71 and 72, SEQ ID NOS:63 and 56, SEQ ID NOS:64 and 57, SEQ ID NOS:65 and 60, SEQ ID NOS:66 and 58, SEQ ID NOS:67 and 59, SEQ ID NOS:68 and 61, SEQ ID NOS:69 and 62, SEQ ID NOS:54 and 811, SEQ ID NOS:54 and 812, SEQ ID NOS:54 and 813, SEQ ID NOS:814 and 818, SEQ ID NOS:815 and 818, SEQ ID NOS:816 and 818, SEQ ID NOS:817 and 818, SEQ ID NOS:814 and 820, SEQ ID NOS:814 and 821, SEQ ID NOS:54 and 819, SEQ ID NOS:814 and 826, SEQ ID NOS:814 and 822, SEQ ID NOS:814 and 823, SEQ ID NOS:814 and 824, SEQ ID NOS:859 and 862, SEQ ID NOS: 860 and 863, SEQ ID NOS: 861 and 864, SEQ ID NOS: 874 and 825, SEQ ID NOS:875 and 879, SEQ ID NOS:876 and 880, SEQ ID NOS:877 and 881, or SEQ ID NOS:878 and 882.

In another aspect, the present disclosure provides a composition comprising a polypeptide heterodimer provided herein and a pharmaceutically acceptable excipient.

In another aspect, the present disclosure provides an expression vector capable of expressing a polypeptide heterodimer provided herein, comprising a first polynucleotide encoding the first single chain polypeptide and a second polynucleotide encoding the second single chain polypeptide.

In another aspect, the present disclosure provides a host cell comprising the above expression vector.

In another aspect, the present disclosure provides a host cell comprising first and second expression vectors capable of expressing the first and second single chain polypeptides, respectively, of the polypeptide heterodimer provided herein.

In another aspect, the present disclosure provides a method for making a polypeptide heterodimer, comprising (a) culturing a host cell provided herein under conditions suitable to express first and second single chain polypeptides, and (b) optionally isolating or purifying the heterodimers formed from the first and second single chain polypeptides from the culture.

Also disclosed is~~In another aspect, the present disclosure provides~~ a method for directing T cell activation, comprising administering to a patient in need thereof an effective amount of a polypeptide heterodimer that comprises a binding domain that specifically binds TCRα, TCRβ, CD3γ, CD3δ, CD3ε, or a combination thereof, and a second binding domain that specifically binds a different target, for instance, a tumor-specific antigen or other antigen of choice at a site or cell where T cell activation is desired.

Also disclosed is~~In another aspect, the present disclosure provides~~ a method for inhibiting growth, metastatis, or metastatic growth of a malignancy, comprising administering to a patient in need thereof an effective amount of a polypeptide heterodimer that comprises a binding domain that specifically binds TCRα, TCRβ, CD3γ, CD3δ, CD3ε, c-Met, RON, or a combination thereof. In certain ~~embodiments~~examples, the method further comprises administering to a patient in need thereof a chemotherapeutic agent or ionizing radiation.

Also disclosed is~~In another aspect, the present disclosure provides~~ a method for treating an autoimmune or inflammatory condition, comprising administering to a patient in need thereof an effective amount of a polypeptide heterodimer that comprises a binding domain that specifically binds TCRα, TCRβ, CD3γ, CD3δ, CD3ε, or CD28.

Also disclosed is~~In another aspect, the present disclosure provides~~ a method for treating a B-cell associated disorder or disease comprising administering to a patient in need thereof an effective amount of a polypeptide heterodimer that comprises a binding domain that specifically binds TCRα, TCRβ, CD3γ, CD3δ, or CD3ε, and a second binding domain that specifically binds to CD19, CD20, CD79b or HLA-DR.

In certain ~~embodiments~~examples, the methods for using the polypeptide heterodimers provided herein may further comprise administering to a patient in need thereof a second active agent, such as a second polypeptide heterodimer, a monoclonal antibody, or an immunoglobulin-derived fusion protein.