

HETERODIMERIC ANTIBODIES THAT BIND ENPP3 AND CD3

PRIORITY CLAIM

[0001] This application claims priority to U.S. Provisional Application Nos. 62/812,922, filed March 1, 2019 and 62/929,687, filed November 1, 2019, which are hereby incorporated by reference in their entirety.

BACKGROUND

[0002] Antibody-based therapeutics have been used successfully to treat a variety of diseases, including cancer. An increasingly prevalent avenue being explored is the engineering of single immunoglobulin molecules that co-engage two different antigens. Such alternate antibody formats that engage two different antigens are often referred to as bispecific antibodies. Because the considerable diversity of the antibody variable region (Fv) makes it possible to produce an Fv that recognizes virtually any molecule, the typical approach to bispecific antibody generation is the introduction of new variable regions into the antibody.

[0003] A particularly useful approach for bispecific antibodies is to engineer a first binding domain that engages CD3 and a second binding domain that engages an antigen associated with or upregulated on cancer cells so that the bispecific antibody redirects CD3⁺ T cells to destroy the cancer cells. Ectonucleotide pyrophosphatase/phosphodiesterase family member 3 (ENPP3) has previously been reported to be highly expressed in renal cell carcinoma and minimally expressed in normal tissue. In view of this, it is believed that anti-ENPP3 antibodies are useful, for example, for localizing anti-tumor therapeutics (e.g., chemotherapeutic agents and T cells) to such ENPP3 expressing tumors. Provided herein are novel bispecific antibodies to CD3 and ENPP3 that are capable of localizing CD3⁺ effector T cells to ENPP3 expressing tumors.

BRIEF SUMMARY

[0004] Accordingly, provided herein are ENPP3 antigen binding domains and anti-ENPP3 antibodies (e.g., bispecific antibodies).

[0005] In one aspect, provided herein is a composition that includes an Ectonucleotide pyrophosphatase/phosphodiesterase family member 3 (ENPP3) binding domain that includes the variable heavy complementary determining regions 1-3 (vhCDR1-3) and the variable

light complementary determining regions (vLCDR1-3) of any of the following ENPP3 binding domains: AN1[ENPP3] H1L1, AN1[ENPP3] H1 L1.33, AN1[ENPP3] H1 L1.77, AN1[ENPP3] H1.8 L1, AN1[ENPP3] H1.8 L1.33, AN1[ENPP3] H1 L1.77, H16-7.213, H16-9.69, H16-1.52, Ha16-1(1)23, H16-9.44, H16-1.67, Ha1 6-1 (3,5)36, H16-1.86, H16-9.10, H16-9.33, H16-1.68, Ha16-1(1)1, Ha1 6-1(3,5)18, Ha16-1(2,4)4, Ha16-1(3,5)56, H16-7.8, H16-1.93, Ha1 6-1(3,5)27.1, H16-1.61, H16-1(3,5)5, H16-7.200, H16-1(3,5)42, H1 6-9.65, Ha1-1(3,5)19, and Ha16-1.80, (Figures 12, 13A-13B, and 14A-14I). In some embodiments, the vhCDR1-3 and vLCDR1-3 are selected from the vhCDR1-3 and vLCDR1-3 sequences of an ENPP3 binding domain provided in Figures 12, 13A-13B, and 14A-14I.

[0006] In another aspect, provided herein is a composition that includes an Ectonucleotide pyrophosphatase/phosphodiesterase family member 3 (ENPP3) binding domain that includes a variable heavy domain and a variable light domain of any of the following ENPP3 binding domains: AN1[ENPP3] H1L1, AN1[ENPP3] H1 L1.33, AN1[ENPP3] H1 L1.77, AN1[ENPP3] H1.8 L1, AN1[ENPP3] H1.8 L1.33, AN1[ENPP3] H1 L1.77, H16-7.213, H16-9.69, H16-1.52, Ha16-1(1)23, H16-9.44, H16-1.67, Ha1 6-1 (3,5)36, H16-1.86, H16-9.10, H16-9.33, H16-1.68, Ha16-1(1)1, Ha1 6-1(3,5)18, Ha16-1(2,4)4, Ha16-1(3,5)56, H16-7.8, H16-1.93, Ha1 6-1(3,5)27.1, H16-1.61, H16-1(3,5)5, H16-7.200, H16-1(3,5)42, H1 6-9.65, Ha1-1(3,5)19, and Ha16-1.80, (Figures 12, 13A-13B, and 14A-14I).

[0007] In another aspect, the present invention provides a composition that includes a Ectonucleotide pyrophosphatase/phosphodiesterase family member 3 (ENPP3) binding domain selected from the following ENPP3 binding domains: AN1[ENPP3] H1L1, AN1[ENPP3] H1 L1.33, AN1[ENPP3] H1 L1.77, AN1[ENPP3] H1.8 L1, AN1[ENPP3] H1.8 L1.33, AN1[ENPP3] H1 L1.77, H16-7.213, H16-9.69, H16-1.52, Ha16-1(1)23, H16-9.44, H16-1.67, Ha1 6-1 (3,5)36, H16-1.86, H16-9.10, H16-9.33, H16-1.68, Ha16-1(1)1, Ha1 6-1(3,5)18, Ha16-1(2,4)4, Ha16-1(3,5)56, H16-7.8, H16-1.93, Ha1 6-1(3,5)27.1, H16-1.61, H16-1(3,5)5, H16-7.200, H16-1(3,5)42, H1 6-9.65, Ha1-1(3,5)19, and Ha16-1.80, (Figures 12, 13A-13B, and 14A-14I).

[0008] In another aspect, the present invention provides a nucleic acid composition that includes: a) a first nucleic acid encoding a variable heavy domain that includes the variable heavy complementary determining regions 1-3 (vhCDR1-3) of an ENPP3 binding domain; and b) a second nucleic acid encoding a variable light domain that includes the variable light complementary determining regions 1-3 (vLCDR1-3) of the ENPP3 binding domain, wherein

the ENPP3 binding domain is one of the following ENPP3 binding domains: AN1[ENPP3] H1L1, AN1[ENPP3] H1 L1.33, AN1[ENPP3] H1 L1.77, AN1[ENPP3] H1.8 L1, AN1[ENPP3] H1.8 L1.33, AN1[ENPP3] H1 L1.77, H16-7.213, H16-9.69, H16-1.52, Ha16-1(1)23, H16-9.44, H16-1.67, Ha1 6-1 (3,5)36, H16-1.86, H16-9.10, H16-9.33, H16-1.68, Ha16-1(1)1, Ha1 6-1(3,5)18, Ha16-1(2,4)4, Ha16-1(3,5)56, H16-7.8, H16-1.93, Ha1 6-1(3,5)27.1, H16-1.61, H16-1(3,5)5, H16-7.200, H16-1(3,5)42, H1 6-9.65, Ha1-1(3,5)19, and Ha16-1.80, (Figures 12, 13A-13B, and 14A-14I). In some embodiments, the vhCDR1-3 and vlCDR1-3 are selected from the vhCDR1-3 and vlCDR1-3 sequences provided in Figures 12, 13A-13B, and 14A-14I.

[0009] In another aspect, the present invention provides a nucleic acid composition that includes: a) a first nucleic acid encoding a variable heavy domain that includes the variable heavy domain of an ENPP3 binding domain; and b) a second nucleic acid encoding a variable light domain that includes the variable light domain of the ENPP3 binding domain, wherein the ENPP3 binding domain is any one of the following ENPP3 binding domains:

AN1[ENPP3] H1L1, AN1[ENPP3] H1 L1.33, AN1[ENPP3] H1 L1.77, AN1[ENPP3] H1.8 L1, AN1[ENPP3] H1.8 L1.33, AN1[ENPP3] H1 L1.77, H16-7.213, H16-9.69, H16-1.52, Ha16-1(1)23, H16-9.44, H16-1.67, Ha1 6-1 (3,5)36, H16-1.86, H16-9.10, H16-9.33, H16-1.68, Ha16-1(1)1, Ha1 6-1(3,5)18, Ha16-1(2,4)4, Ha16-1(3,5)56, H16-7.8, H16-1.93, Ha1 6-1(3,5)27.1, H16-1.61, H16-1(3,5)5, H16-7.200, H16-1(3,5)42, H1 6-9.65, Ha1-1(3,5)19, and Ha16-1.80, (Figures 12, 13A-13B, and 14A-14I).

[0010] In some embodiments, the present invention provides an expression vector composition that includes: a) a first expression vector that includes the first nucleic acid b) a second expression vector that includes a second nucleic acid. In further embodiments, the present invention provides a host cell that includes the expression vector composition.

[0011] In some embodiments, the present invention provides a method of making an Ectonucleotide pyrophosphatase/phosphodiesterase family member 3 (ENPP3) binding domain that includes culturing the host cell under conditions wherein the ENPP3 binding domain is expressed, and recovering the ENPP3 binding domain.

[0012] In another aspect, the present invention provides an anti-ENPP3 antibody that includes an Ectonucleotide pyrophosphatase/phosphodiesterase family member 3 (ENPP3) binding domain, the ENPP3 binding domain includes the variable heavy complementary determining regions 1-3 (vhCDR1-3) and the variable light complementary determining

regions (vLCDR1-3) of any of the following ENPP3 binding domains: AN1[ENPP3] H1L1, AN1[ENPP3] H1 L1.33, AN1[ENPP3] H1 L1.77, AN1[ENPP3] H1.8 L1, AN1[ENPP3] H1.8 L1.33, AN1[ENPP3] H1 L1.77, H16-7.213, H16-9.69, H16-1.52, Ha16-1(1)23, H16-9.44, H16-1.67, Ha1 6-1 (3,5)36, H16-1.86, H16-9.10, H16-9.33, H16-1.68, Ha16-1(1)1, Ha1 6-1(3,5)18, Ha16-1(2,4)4, Ha16-1(3,5)56, H16-7.8, H16-1.93, Ha1 6-1(3,5)27.1, H16-1.61, H16-1(3,5)5, H16-7.200, H16-1(3,5)42, H1 6-9.65, Ha1-1(3,5)19, and Ha16-1.80, (Figures 12, 13A-13B, and 14A-14I). In some embodiments, the vhCDR1-3 and vLCDR1-3 are selected from the vhCDR1-3 and vLCDR1-3 of any of the following ENPP3 binding domains in Figures 12, 13A-13B, and 14A-14I.

[0013] In another aspect, the present invention provides an anti-ENPP3 antibody that includes an Ectonucleotide pyrophosphatase/phosphodiesterase family member 3 (ENPP3) binding domain, the ENPP3 binding domain includes a variable heavy domain and a variable light domain of any of the following ENPP3 binding domains: AN1[ENPP3] H1L1, AN1[ENPP3] H1 L1.33, AN1[ENPP3] H1 L1.77, AN1[ENPP3] H1.8 L1, AN1[ENPP3] H1.8 L1.33, AN1[ENPP3] H1 L1.77, H16-7.213, H16-9.69, H16-1.52, Ha16-1(1)23, H16-9.44, H16-1.67, Ha1 6-1 (3,5)36, H16-1.86, H16-9.10, H16-9.33, H16-1.68, Ha16-1(1)1, Ha1 6-1(3,5)18, Ha16-1(2,4)4, Ha16-1(3,5)56, H16-7.8, H16-1.93, Ha1 6-1(3,5)27.1, H16-1.61, H16-1(3,5)5, H16-7.200, H16-1(3,5)42, H1 6-9.65, Ha1-1(3,5)19, and Ha16-1.80, (Figures 12, 13A-13B, and 14A-14I).

[0014] In another aspect, provided herein is an anti-ENPP3 antibody that includes an Ectonucleotide pyrophosphatase/phosphodiesterase family member 3 (ENPP3) binding domain selected from any one of the following ENPP3 binding domains: AN1[ENPP3] H1L1, AN1[ENPP3] H1 L1.33, AN1[ENPP3] H1 L1.77, AN1[ENPP3] H1.8 L1, AN1[ENPP3] H1.8 L1.33, AN1[ENPP3] H1 L1.77, H16-7.213, H16-9.69, H16-1.52, Ha16-1(1)23, H16-9.44, H16-1.67, Ha1 6-1 (3,5)36, H16-1.86, H16-9.10, H16-9.33, H16-1.68, Ha16-1(1)1, Ha1 6-1(3,5)18, Ha16-1(2,4)4, Ha16-1(3,5)56, H16-7.8, H16-1.93, Ha1 6-1(3,5)27.1, H16-1.61, H16-1(3,5)5, H16-7.200, H16-1(3,5)42, H1 6-9.65, Ha1-1(3,5)19, and Ha16-1.80, (Figures 12, 13A-13B, and 14A-14I).

[0015] In some embodiments, the antibody includes: a) a first monomer that includes a first antigen binding domain and a first constant domain; and b) a second monomer that includes a second antigen binding domain and a second constant domain, wherein either of the first antigen binding domain or second antigen binding domain is the ENPP3 binding domain. In

further embodiments, first antigen binding domain and the second antigen binding domain bind different antigens. In further embodiments, the first antigen binding domain is the ENPP3 binding domain and the second antigen binding domain is a CD3 binding domain. In further embodiments, the CD3 binding domain includes the vhCDR1-3 , and vlCDR1-3 of any of the following CD3 binding domains: H1.30_L1.47, H1.32_L1.47, H1.89_L1.47, H1.90_L1.47, H1.33_L1.47, H1.31_L1.47, L1.47_H1.30, L1.47_H1.30, L1.47_H1.32, L1.47_H1.89, L1.47_H1.90, L1.47_H1.33, and L1.47_H1.31 (Figures 10A-10F). In further embodiments, the vhCDR1-3 and vlCDR1-3 of the CD3 binding domain are selected from the vhCDR1-3 and vlCDR1-3 in Figures 10A-10F.

[0016] In some embodiments, the CD3 binding domain includes the variable heavy domain and variable light domain of any of the following CD3 binding domains: H1.30_L1.47, H1.32_L1.47, H1.89_L1.47, H1.90_L1.47, H1.33_L1.47, H1.31_L1.47, L1.47_H1.30, L1.47_H1.30, L1.47_H1.32, L1.47_H1.89, L1.47_H1.90, L1.47_H1.33, and L1.47_H1.31 (Figures 10A-10F).

[0017] In some embodiments, the CD3 binding domain is an anti-CD3 scFv.

[0018] In some embodiments, wherein the first and second constant domains each includes CH2-CH3.

[0019] In some embodiments, the first and second constant domains each includes CH1-hinge-CH2-CH3.

[0020] In some embodiments, the first and second constant domains each are a variant constant domain.

[0021] In some embodiments, the first and second monomers include a set of heterodimerization variants are any one of the variants depicted in Figures 1A-1E. In some embodiments, the set of heterodimerization variants includes one of the follow set of variants: S364K/E357Q : L368D/K370S; S364K : L368D/K370S; S364K : L368E/K370S; D401K: T411E/K360E/Q362E; and T366W : T366S/L368A/Y407V.

[0022] In some embodiments, the first and second monomers each further include an ablation variant. In further embodiments, the ablation variant is E233P/L234V/L235A/G236del/S267K.

[0023] In some embodiments, the at least one of the first or second monomer further includes a pI variant. In further embodiments, the pI variant is N208D/Q295E/N384D/Q418E/N421D. In some embodiments, the scFv includes a charged scFv linker.

[0024] In some embodiments, the present invention provides a nucleic acid composition including nucleic acids encoding the anti-ENPP3. In some embodiments, the composition including nucleic acids encoding first and second monomers. In some embodiments, the present invention provides expression vectors that include the nucleic acids. In some embodiments, the present invention provides a host cell transformed with the expression vector.

[0025] In some embodiments, the present invention provides a method of making an anti-ENPP3 antibody according to any one of claims B1 to B21. The method includes culturing the host cell according to claim B25 under conditions wherein the anti-ENPP3 antibody is expressed, and recovering the anti-ENPP3 antibody. In some embodiments, the present invention provides a method of treating a cancer that includes administering to a patient in need thereof the antibody.

[0026] In another aspect, the present invention provides a heterodimeric antibody that includes: a) a first monomer that includes: i) an anti-CD3 scFv that includes a first variable light domain, an scFv linker and a first variable heavy domain; and ii) a first Fc domain, wherein the scFv is covalently attached to the N-terminus of the first Fc domain using a domain linker; b) a second monomer that includes a VH2-CH1-hinge-CH2-CH3 monomer, wherein VH is a second variable heavy domain and CH2-CH3 is a second Fc domain; and c) a light chain that includes a second variable light domain, wherein the second variable heavy domain and the second variable light domain form an ENPP3 binding domain.

[0027] In some embodiments, the ENPP3 binding domain includes the vhCDR1-3 and vlCDR1-3 of any of the following ENPP3 binding domains: AN1[ENPP3] H1L1, AN1[ENPP3] H1 L1.33, AN1[ENPP3] H1 L1.77, AN1[ENPP3] H1.8 L1, AN1[ENPP3] H1.8 L1.33, AN1[ENPP3] H1 L1.77, H16-7.213, H16-9.69, H16-1.52, Ha16-1(1)23, H16-9.44, H16-1.67, Ha1 6-1 (3,5)36, H16-1.86, H16-9.10, H16-9.33, H16-1.68, Ha16-1(1)1, Ha1 6-1(3,5)18, Ha16-1(2,4)4, Ha16-1(3,5)56, H16-7.8, H16-1.93, Ha1 6-1(3,5)27.1, H16-1.61, H16-1(3,5)5, H16-7.200, H16-1(3,5)42, H1 6-9.65, Ha1-1(3,5)19, and Ha16-1.80, (Figures 12, 13A-13B, and 14A-14I).

[0028] In some embodiments, the vhCDR1-3 and vlCDR1-3 of the ENPP3 binding domain are selected from the vhCDR1-3 and vlCDR1-3 sequences of the ENPP3 binding domains provided in Figures 12, 13A-13B, and 14A-14I.

[0029] In some embodiments, the second heavy variable domain includes a heavy variable domain and the second light variable domain includes a variable light domain of any of the following ENPP3 binding domains: AN1[ENPP3] H1L1, AN1[ENPP3] H1 L1.33, AN1[ENPP3] H1 L1.77, AN1[ENPP3] H1.8 L1, AN1[ENPP3] H1.8 L1.33, AN1[ENPP3] H1 L1.77, H16-7.213, H16-9.69, H16-1.52, Ha16-1(1)23, H16-9.44, H16-1.67, Ha1 6-1 (3,5)36, H16-1.86, H16-9.10, H16-9.33, H16-1.68, Ha16-1(1)1, Ha1 6-1(3,5)18, Ha16-1(2,4)4, Ha16-1(3,5)56, H16-7.8, H16-1.93, Ha1 6-1(3,5)27.1, H16-1.61, H16-1(3,5)5, H16-7.200, H16-1(3,5)42, H1 6-9.65, Ha1-1(3,5)19, and Ha16-1.80, (Figures 12, 13A-13B, and 14A-14I).

[0030] In some embodiments, the anti-CD3 scFv includes the vhCDR1-3 and the vlCDR1-3 of any of the following CD3 binding domains: H1.30_L1.47, H1.32_L1.47, H1.89_L1.47, H1.90_L1.47, H1.33_L1.47, H1.31_L1.47, L1.47_H1.30, L1.47_H1.30, L1.47_H1.32, L1.47_H1.89, L1.47_H1.90, L1.47_H1.33, and L1.47_H1.31 (Figures 10A-10F).

[0031] In some embodiments, the vhCDR1-3 and vlCDR1-3 of the anti-CD3 scFv are selected from the vhCDR1-3 and vlCDR1-3 in Figures 10A-10F.

[0032] In some embodiments, the anti-CD3 scFv includes the variable heavy domain and variable light domain of any of the following CD3 binding domains: H1.30_L1.47, H1.32_L1.47, H1.89_L1.47, H1.90_L1.47, H1.33_L1.47, H1.31_L1.47, L1.47_H1.30, L1.47_H1.30, L1.47_H1.32, L1.47_H1.89, L1.47_H1.90, L1.47_H1.33, and L1.47_H1.31 (Figures 10A-10F).

[0033] In some embodiments, the first variable light domain is covalently attached to the N-terminus of the first Fc domain using a domain linker.

[0034] In some embodiments, the first variable heavy domain is covalently attached to the N-terminus of the first Fc domain using a domain linker.

[0035] In some embodiments, the scFv linker is a charged scFv linker.

[0036] In some embodiments, the first and second Fc domains are variant Fc domains.

[0037] In some embodiments, the first and second monomers includes a set of heterodimerization variants selected from any of the heterodimerization variants in Figures 1A-1E. In some embodiments, the set of heterodimerization variants selected is from following: S364K/E357Q : L368D/K370S; S364K : L368D/K370S; S364K : L368E/K370S; D401K: T411E/K360E/Q362E; and T366W : T366S/L368A/Y407V, wherein numbering is according to EU numbering.

[0038] In some embodiments, the first and second monomers further includes an ablation variant. In some embodiments, the ablation variant is E233P/L234V/L235A/G236del/S267K, wherein numbering is according to EU numbering.

[0039] In some embodiments, one of the first or second monomer includes a pI variant.

[0040] In some embodiments, the pI variant is N208D/Q295E/N384D/Q418E/N421D, wherein numbering is according to EU numbering.

[0041] In some embodiments, the first monomer includes amino acid variants S364K/E357Q/E233P/L234V/L235A/G236del/S267K, wherein the second monomer includes amino acid variants L368D/K370S/N208D/Q295E/N384D/Q418E/N421D/E233P/L234V/L235A/G236del/S267K, and wherein numbering is according to EU numbering.

[0042] In some embodiments, the scFv linker is a charged scFv linker having the amino acid sequence (GKPGS)₄.

[0043] In some embodiments, the first and second monomers each further include amino acid variants 428/434S.

[0044] In some embodiments, the heterodimeric antibody includes the following heterodimeric antibodies: XENP24804, XENP26820, XENP28287, XENP28925, XENP29516, XENP30262, XENP26821, XENP29436, XENP28390, XENP29463, and XENP30263.

[0045] In another aspect, the present invention provides a heterodimeric antibody that includes: a) a first monomer that includes from N-terminal to C-terminal, a scFv-linker-CH2-CH3, wherein scFv is an anti-CD3 scFV and CH2-CH3 is a first Fc domain; b) a second monomer that includes from N-terminal to C-terminal a VH-CH1-hinge-CH2-CH3, wherein CH2-CH3 is a second Fc domain; and c) a light chain that includes a VL-CL; wherein the

first variant Fc domain includes amino acid variants S364K/E357Q, wherein the second variant Fc domain includes amino acid variants L368D/K370S, wherein the first and second variant Fc domains each include amino acid variants E233P/L234V/L235A/G236del/S267K, wherein the hinge-CH2-CH3 of the second monomer includes amino acid variants N208D/Q295E/N384D/Q418E/N421D, wherein the VH and VL form an ENPP3 binding domain that includes the variable heavy domain and the variable light domain, respectively, of an ENPP3 binding domain selected from AN1[ENPP3] H1L1, AN1[ENPP3] H1 L1.33, AN1[ENPP3] H1 L1.77, AN1[ENPP3] H1.8 L1, AN1[ENPP3] H1.8 L1.33, AN1[ENPP3] H1 L1.77, H16-7.213, H16-9.69, H16-1.52, Ha16-1(1)23, H16-9.44, H16-1.67, Ha1 6-1(3,5)36, H16-1.86, H16-9.10, H16-9.33, H16-1.68, Ha16-1(1)1, Ha1 6-1(3,5)18, Ha16-1(2,4)4, Ha16-1(3,5)56, H16-7.8, H16-1.93, Ha1 6-1(3,5)27.1, H16-1.61, H16-1(3,5)5, H16-7.200, H16-1(3,5)42, H1 6-9.65, Ha1-1(3,5)19, and Ha16-1.80, (Figures 12, 13A-13B, and 14A-14I), wherein the anti-CD3 scFv includes the variable heavy domain and the variable light domain of a CD3 binding domain selected from H1.30_L1.47, H1.32_L1.47, H1.89_L1.47, H1.90_L1.47, H1.33_L1.47, H1.31_L1.47, L1.47_H1.30, L1.47_H1.30, L1.47_H1.32, L1.47_H1.89, L1.47_H1.90, L1.47_H1.33, and L1.47_H1.31 (Figures 10A-10F), and wherein numbering is according to EU numbering.

[0046] In some embodiments, the scFv includes a charged scFv linker having the amino acid sequence (GKPGS)₄.

[0047] In some embodiments, the first and second variant Fc domains each further include amino acid variants 428/434S, wherein numbering is according to EU numbering.

[0048] In some embodiments, the present invention provides a nucleic acid composition that includes nucleic acids encoding the first and second monomers and the light chain of the antibody.

[0049] In some embodiments, the present invention provides an expression vector that includes the nucleic acids. In some embodiments, the present invention provides a host cell transformed with the expression vector.

[0050] In some embodiments, the present invention provides a method of treating an ENPP3 associated cancer that includes administering to a patient in need thereof any one of the antibodies provided herein.

[0051] In another aspect, the present invention provides a heterodimeric antibody that includes: a) a first monomer that includes from N-terminal to C-terminal, a VH1-CH1-linker 1-scFv-linker 2-CH2-CH3, wherein VH1 is a first variable heavy domain, scFv is an anti-CD3 scFV, linker 1 and linker 2 are a first domain linker and second domain linker, respectively, and CH2-CH3 is a first Fc domain; b) a second monomer that includes from N-terminal to C-terminal a VH2-CH1-hinge-CH2-CH3, wherein VH2 is a second variable heavy domain and CH2-CH3 is a second Fc domain; and c) a common light chain that includes a variable light domain; wherein the first variable heavy domain and the variable light domain form a first ENPP3 binding domain, and the second variable heavy domain and the variable light domain form a second ENPP3 binding domain.

[0052] In some embodiments, the first and second ENPP3 binding domains each includes the vhCDR1-3 and vlCDR1-3 of any of the following ENPP3 binding domains: AN1[ENPP3] H1L1, AN1[ENPP3] H1 L1.33, AN1[ENPP3] H1 L1.77, AN1[ENPP3] H1.8 L1, AN1[ENPP3] H1.8 L1.33, AN1[ENPP3] H1 L1.77, H16-7.213, H16-9.69, H16-1.52, Ha16-1(1)23, H16-9.44, H16-1.67, Ha1 6-1 (3,5)36, H16-1.86, H16-9.10, H16-9.33, H16-1.68, Ha16-1(1)1, Ha1 6-1(3,5)18, Ha16-1(2,4)4, Ha16-1(3,5)56, H16-7.8, H16-1.93, Ha1 6-1(3,5)27.1, H16-1.61, H16-1(3,5)5, H16-7.200, H16-1(3,5)42, H1 6-9.65, Ha1-1(3,5)19, and Ha16-1.80, (Figures 12, 13A-13B, and 14A-14I).,

[0053] In some embodiments, the vhCDR1-3 and vlCDR1-3 of the first and second ENPP3 binding domains are selected from the vhCDR1-3 and vlCDR1-3 provided in Figures 14 and 45.

[0054] In some embodiments, the first and second variable heavy domain each include a variable heavy domain of a ENPP3 binding domain, and the first and second variable light domain each include a variable light domain of the ENPP3 binding domain, wherein the ENPP3 binding domain is any of the following ENPP3 binding domains: AN1[ENPP3] H1L1, AN1[ENPP3] H1 L1.33, AN1[ENPP3] H1 L1.77, AN1[ENPP3] H1.8 L1, AN1[ENPP3] H1.8 L1.33, AN1[ENPP3] H1 L1.77, H16-7.213, H16-9.69, H16-1.52, Ha16-1(1)23, H16-9.44, H16-1.67, Ha1 6-1 (3,5)36, H16-1.86, H16-9.10, H16-9.33, H16-1.68, Ha16-1(1)1, Ha1 6-1(3,5)18, Ha16-1(2,4)4, Ha16-1(3,5)56, H16-7.8, H16-1.93, Ha1 6-1(3,5)27.1, H16-1.61, H16-1(3,5)5, H16-7.200, H16-1(3,5)42, H1 6-9.65, Ha1-1(3,5)19, and Ha16-1.80, (Figures 12, 13A-13B, and 14A-14I).

[0055] In some embodiments, the scFv includes the vhCDR1-3 and the vlCDR1-3 of any of the following CD3 binding domains: H1.30_L1.47, H1.32_L1.47, H1.89_L1.47, H1.90_L1.47, H1.33_L1.47, H1.31_L1.47, L1.47_H1.30, L1.47_H1.30, L1.47_H1.32, L1.47_H1.89, L1.47_H1.90, L1.47_H1.33, and L1.47_H1.31 (Figures 10A-10F).

[0056] In some embodiments, the vhCDR1-3 and vlCDR1-3 of the scFv are selected from the vhCDR1-3 and vlCDR1-3 in Figures 10A-10F.

[0057] In some embodiments, the scFv includes the variable heavy domain and variable light domain of any of the following CD3 binding domains: H1.30_L1.47, H1.32_L1.47, H1.89_L1.47, H1.90_L1.47, H1.33_L1.47, H1.31_L1.47, L1.47_H1.30, L1.47_H1.30, L1.47_H1.32, L1.47_H1.89, L1.47_H1.90, L1.47_H1.33, and L1.47_H1.31 (Figures 10A-10F).

[0058] In some embodiments, the scFv includes an scFv variable heavy domain, an scFv variable light domain and an scFv linker that connects the scFv variable heavy domain and the scFv variable light domain.

[0059] In some embodiments, the scFv variable heavy domain is attached to the C-terminus of the CH1 of the first monomer using the first domain linker and the scFv variable light domain is covalently attached to the N-terminus of the first Fc domain using the second domain linker.

[0060] In some embodiments, the scFv variable light domain is attached to the C-terminus of the CH1 of the first monomer using the first domain linker and the scFv variable heavy domain is covalently attached to the N-terminus of the first Fc domain using the second domain linker.

[0061] In some embodiments, the scFv linker is a charged scFv linker.

[0062] In some embodiments, the first and second Fc domains are variant Fc domains.

[0063] In some embodiments, the first and second monomers includes a set of heterodimerization variants selected from those depicted in Figures 1A-1E.

[0064] In some embodiments, the set of heterodimerization variants selected is from following: S364K/E357Q : L368D/K370S; S364K : L368D/K370S; S364K : L368E/K370S; D401K: T411E/K360E/Q362E; and T366W : T366S/L368A/Y407V, wherein numbering is according to EU numbering.

[0065] In some embodiments, the first and second monomers further include an ablation variant.

[0066] In some embodiments, the ablation variant is E233P/L234V/L235A/G236del/S267K, wherein numbering is according to EU numbering.

[0067] In some embodiments, one of the first or second monomer further includes a pI variant.

[0068] In some embodiments, the pI variant is N208D/Q295E/N384D/Q418E/N421D, wherein numbering is according to EU numbering.

[0069] In some embodiments, first variant Fc domain includes amino acid variants S364K/E357Q/ E233P/L234V/L235A/G236del/S267K, wherein the second variant Fc domain includes amino acid variants L368D/K370S/N208D/Q295E/N384D/Q418E/N421D/E233P/L234V/L235A/G236del/S267K, and wherein numbering is according to EU numbering.

[0070] In some embodiments, the scFv linker is a charged scFv linker having the amino acid sequence (GKPGS)₄.

[0071] In some embodiments, the first and second variant Fc domains each further include amino acid variants 428/434S, wherein numbering is according to EU numbering.

[0072] In some embodiments, the heterodimeric antibody includes the following heterodimeric antibodies: XENP29437, XENP29520, XENP30264, XENP26822, XENP28438, XENP29438, XENP29467, XENP30469, XENP30470, XENP30819, XENP30821, XENP31148, XENP31149, XENP31150, XENP31419, and XENP31471.

[0073] In another aspect, the heterodimeric antibody includes: a) a first monomer that includes from N-terminal to C-terminal, a VH1-CH1-linker 1-scFv-linker 2-CH2-CH3, wherein scFv is an anti-CD3 scFV and CH2-CH3 is a first Fc domain; b) a second monomer that includes from N-terminal to C-terminal a VH1-CH1-hinge-CH2-CH3, wherein CH2-CH3 is a second Fc domain; and c) a common light chain that includes VL-CL; wherein the first variant Fc domain includes amino acid variants S364K/E357Q, wherein the second variant Fc domain includes amino acid variants L368D/K370S, wherein the first and second variant Fc domains each include amino acid variants E233P/L234V/L235A/G236del/S267K, wherein the hinge-CH2-CH3 of the second monomer includes amino acid variants

N208D/Q295E/N384D/Q418E/N421D, wherein said VH and VL include the variable heavy domain and the variable light domain of a ENPP3 binding domain selected from AN1[ENPP3] H1L1, AN1[ENPP3] H1 L1.33, AN1[ENPP3] H1 L1.77, AN1[ENPP3] H1.8 L1, AN1[ENPP3] H1.8 L1.33, AN1[ENPP3] H1 L1.77, H16-7.213, H16-9.69, H16-1.52, Ha16-1(1)23, H16-9.44, H16-1.67, Ha1 6-1 (3,5)36, H16-1.86, H16-9.10, H16-9.33, H16-1.68, Ha16-1(1)1, Ha1 6-1(3,5)18, Ha16-1(2,4)4, Ha16-1(3,5)56, H16-7.8, H16-1.93, Ha1 6-1(3,5)27.1, H16-1.61, H16-1(3,5)5, H16-7.200, H16-1(3,5)42, H1 6-9.65, Ha1-1(3,5)19, and Ha16-1.80, (Figures 12, 13A-13B, and 14A-14I), wherein the anti-CD3 scFv includes the variable heavy domain and the variable light domain of a CD3 binding domain selected from H1.30_L1.47, H1.32_L1.47, H1.89_L1.47, H1.90_L1.47, H1.33_L1.47, H1.31_L1.47, L1.47_H1.30, L1.47_H1.30, L1.47_H1.32, L1.47_H1.89, L1.47_H1.90, L1.47_H1.33, and L1.47_H1.31 (Figures 10A-10F), and wherein numbering is according to EU numbering.

[0074] In some embodiments, the scFv includes a charged scFv linker having the amino acid sequence (GKPGS)₄.

[0075] In some embodiments, the first and second variant Fc domains each further include amino acid variants 428/434S.

[0076] In some embodiments, the first and second monomers and the common light chain of the antibody. In some embodiments, the present invention provides an expression vector that includes the nucleic acids. In some embodiments, the present invention provides a host cell transformed with the expression vector. In some embodiments, the present invention provides treating an ENPP3 associated cancer includes administering to a patient in need thereof the antibody.

[0077] In another aspect, the present invention provides a heterodimeric antibody including the following heterodimeric antibodies: XENP24804, XENP26820, XENP28287, XENP28925, XENP29516, XENP30262, XENP26821, XENP29436, XENP28390, XENP29463, and XENP30263.

[0078] In another aspect, the present invention provides a heterodimeric antibody including the following heterodimeric antibodies: XENP29437, XENP29520, XENP30264, XENP26822, XENP28438, XENP29438, XENP29467, XENP30469, XENP30470, XENP30819, XENP30821, XENP31148, XENP31149, XENP31150, XENP31419, and XENP31471. In some embodiments, the present invention provides nucleic acid composition

that includes the nucleic acids encoding the heterodimeric antibody. In some embodiments, the present invention provides an expression vector includes the nucleic acids. In some embodiments, the present invention provides a host cell transformed with the expression vector.

[0079] In some embodiments, the present method provides a method of treating an ENPP3 related cancer that includes administering to a patient in need thereof any one of the heterodimeric antibodies provided herein.

BRIEF DESCRIPTION OF THE DRAWINGS

[0080] Figure 1A-1E depict useful pairs of Fc heterodimerization variant sets (including skew and pI variants). There are variants for which there are no corresponding “monomer 2” variants; these are pI variants which can be used alone on either monomer.

[0081] Figure 2 depicts a list of isosteric variant antibody constant regions and their respective substitutions. pI₋(-) indicates lower pI variants, while pI₊(+) indicates higher pI variants. These can be optionally and independently combined with other heterodimerization variants of the antibodies described herein (and other variant types as well, as outlined herein).

[0082] Figure 3 depicts useful ablation variants that ablate FcγR binding (sometimes referred to as “knock outs” or “KO” variants). Generally, ablation variants are found on both monomers, although in some cases they may be on only one monomer.

[0083] Figure 4 depicts particularly useful embodiments of “non-Fv” components of the antibodies described herein.

[0084] Figure 5 depicts a number of charged scFv linkers that find use in increasing or decreasing the pI of the subject heterodimeric bsAbs that utilize one or more scFv as a component, as described herein. The (+H) positive linker finds particular use herein, particularly with anti-CD3 V_L and V_H sequences shown herein. A single prior art scFv linker with a single charge is referenced as “Whitlow”, from Whitlow et al., Protein Engineering 6(8):989-995 (1993). It should be noted that this linker was used for reducing aggregation and enhancing proteolytic stability in scFvs. Such charged scFv linkers can be used in any of the subject antibody formats disclosed herein that include scFvs (e.g., 1 + 1 Fab-scFv-Fc and 2 + 1 Fab₂-scFv-Fc formats).

[0085] Figure 6 depicts a number of exemplary domain linkers. In some embodiments, these linkers find use linking a single-chain Fv to an Fc chain. In some embodiments, these linkers may be combined. For example, a GGGGS linker may be combined with a “half hinge” linker.

[0086] Figures 7A-7D depict the sequences of several useful 1 + 1 Fab-scFv-Fc bispecific antibody format heavy chain backbones based on human IgG1, without the Fv sequences (e.g. the scFv and the VH for the Fab side). Backbone 1 is based on human IgG1 (356E/358M allotype), and includes the S364K/E357Q : L368D/K370S skew variants, C220S on the chain with the S364K/E357Q skew variants, the N208D/Q295E/N384D/Q418E/N421D pI variants on the chain with L368D/K370S skew variants and the E233P/L234V/L235A/G236del/S267K ablation variants on both chains. Backbone 2 is based on human IgG1 (356E/358M allotype), and includes S364K : L368D/K370S skew variants, C220S on the chain with the S364K skew variant, the N208D/Q295E/N384D/Q418E/N421D pI variants on the chain with L368D/K370S skew variants, and the E233P/L234V/L235A/G236del/S267K ablation variants on both chains. Backbone 3 is based on human IgG1 (356E/358M allotype), and includes S364K : L368E/K370S skew variants, C220S on the chain with the S364K skew variant, the N208D/Q295E/N384D/Q418E/N421D pI variants on the chain with L368E/K370S skew variants and the E233P/L234V/L235A/G236del/S267K ablation variants on both chains. Backbone 4 is based on human IgG1 (356E/358M allotype), and includes D401K : K360E/Q362E/T411E skew variants, C220S on the chain with the D401K skew variant, the N208D/Q295E/N384D/Q418E/N421D pI variants on the chain with K360E/Q362E/T411E skew variants and the E233P/L234V/L235A/G236del/S267K ablation variants on both chains. Backbone 5 is based on human IgG1 (356D/358L allotype), and includes S364K/E357Q : L368D/K370S skew variants, C220S on the chain with the S364K/E357Q skew variants, the N208D/Q295E/N384D/Q418E/N421D pI variants on the chain with L368D/K370S skew variants and the E233P/L234V/L235A/G236del/S267K ablation variants on both chains. Backbone 6 is based on human IgG1 (356E/358M allotype), and includes S364K/E357Q : L368D/K370S skew variants, C220S on the chain with the S364K/E357Q skew variants, N208D/Q295E/N384D/Q418E/N421D pI variants on the chain with L368D/K370S skew variants and the E233P/L234V/L235A/G236del/S267K ablation variants on both chains, as well as an N297A variant on both chains. Backbone 7 is identical to 6

except the mutation is N297S. Backbone 8 is based on human IgG4, and includes the S364K/E357Q : L368D/K370S skew variants, the N208D/Q295E/N384D/Q418E/N421D pI variants on the chain with L368D/K370S skew variants, as well as a S228P (EU numbering, this is S241P in Kabat) variant on both chains that ablates Fab arm exchange as is known in the art. Backbone 9 is based on human IgG2, and includes the S364K/E357Q : L368D/K370S skew variants, the N208D/Q295E/N384D/Q418E/N421D pI variants on the chain with L368D/K370S skew variants. Backbone 10 is based on human IgG2, and includes the S364K/E357Q : L368D/K370S skew variants, the N208D/Q295E/N384D/Q418E/N421D pI variants on the chain with L368D/K370S skew variants as well as a S267K variant on both chains. Backbone 11 is identical to backbone 1, except it includes M428L/N434S Xtend mutations. Backbone 12 is based on human IgG1 (356E/358M allotype), and includes S364K/E357Q : L368D/K370S skew variants, C220S and the P217R/P229R/N276K pI variants on the chain with S364K/E357Q skew variants and the E233P/L234V/L235A/G236del/S267K ablation variants on both chains. Included within each of these backbones are sequences that are 90, 95, 98 and 99% identical (as defined herein) to the recited sequences, and/or contain from 1, 2, 3, 4, 5, 6, 7, 8, 9 or 10 additional amino acid substitutions (as compared to the “parent” of the Figure, which, as will be appreciated by those in the art, already contain a number of amino acid modifications as compared to the parental human IgG1 (or IgG2 or IgG4, depending on the backbone). That is, the recited backbones may contain additional amino acid modifications (generally amino acid substitutions) in addition to the skew, pI and ablation variants contained within the backbones of this figure.

[0087] Figures 8A-8C depict the sequences of several useful 2 + 1 Fab₂-scFv-Fc bispecific antibody format heavy chain backbones based on human IgG1, without the Fv sequences (e.g. the scFv and the VH for the Fab side). Backbone 1 is based on human IgG1 (356E/358M allotype), and includes the S364K/E357Q : L368D/K370S skew variants, the N208D/Q295E/N384D/Q418E/N421D pI variants on the chain with L368D/K370S skew variants and the E233P/L234V/L235A/G236del/S267K ablation variants on both chains. Backbone 2 is based on human IgG1 (356E/358M allotype), and includes S364K : L368D/K370S skew variants, the N208D/Q295E/N384D/Q418E/N421D pI variants on the chain with L368D/K370S skew variants, and the E233P/L234V/L235A/G236del/S267K ablation variants on both chains. Backbone 3 is based on human IgG1 (356E/358M allotype),

and includes S364K : L368E/K370S skew variants, the N208D/Q295E/N384D/Q418E/N421D pI variants on the chain with L368E/K370S skew variants and the E233P/L234V/L235A/G236del/S267K ablation variants on both chains. Backbone 4 is based on human IgG1 (356E/358M allotype), and includes D401K : K360E/Q362E/T411E skew variants, the N208D/Q295E/N384D/Q418E/N421D pI variants on the chain with K360E/Q362E/T411E skew variants and the E233P/L234V/L235A/G236del/S267K ablation variants on both chains. Backbone 5 is based on human IgG1 (356D/358L allotype), and includes S364K/E357Q : L368D/K370S skew variants, the N208D/Q295E/N384D/Q418E/N421D pI variants on the chain with L368D/K370S skew variants and the E233P/L234V/L235A/G236del/S267K ablation variants on both chains. Backbone 6 is based on human IgG1 (356E/358M allotype), and includes S364K/E357Q : L368D/K370S skew variants, N208D/Q295E/N384D/Q418E/N421D pI variants on the chain with L368D/K370S skew variants and the E233P/L234V/L235A/G236del/S267K ablation variants on both chains, as well as an N297A variant on both chains. Backbone 7 is identical to 6 except the mutation is N297S. Backbone 8 is identical to backbone 1, except it includes M428L/N434S Xtend mutations. Backbone 9 is based on human IgG1 (356E/358M allotype), and includes S364K/E357Q : L368D/K370S skew variants, the P217R/P229R/N276K pI variants on the chain with S364K/E357Q skew variants and the E233P/L234V/L235A/G236del/S267K ablation variants on both chains. Included within each of these backbones are sequences that are 90, 95, 98 and 99% identical (as defined herein) to the recited sequences, and/or contain from 1, 2, 3, 4, 5, 6, 7, 8, 9 or 10 additional amino acid substitutions (as compared to the “parent” of the Figure, which, as will be appreciated by those in the art, already contain a number of amino acid modifications as compared to the parental human IgG1 (or IgG2 or IgG4, depending on the backbone). That is, the recited backbones may contain additional amino acid modifications (generally amino acid substitutions) in addition to the skew, pI and ablation variants contained within the backbones of this figure.

[0088] Figure 9 depicts the sequences of several useful constant light domain backbones based on human IgG1, without the Fv sequences (e.g. the scFv or the Fab). Included herein are constant light backbone sequences that are 90, 95, 98 and 99% identical (as defined herein) to the recited sequences, and/or contain from 1, 2, 3, 4, 5, 6, 7, 8, 9 or 10 additional amino acid modifications.

[0089] Figures 10A-10F depict sequences for exemplary anti-CD3 scFvs suitable for use in the bispecific antibodies described herein. The CDRs are underlined, the scFv linker is double underlined (in the sequences, the scFv linker is a positively charged scFv (GKPGS)₄ linker (SEQ ID NO: XXX), although as will be appreciated by those in the art, this linker can be replaced by other linkers, including uncharged or negatively charged linkers, some of which are depicted in Figure 5), and the slashes indicate the border(s) of the variable domains. In addition, the naming convention illustrates the orientation of the scFv from N- to C-terminus. As noted herein and is true for every sequence herein containing CDRs, the exact identification of the CDR locations may be slightly different depending on the numbering used as is shown in Table 2, and thus included herein are not only the CDRs that are underlined but also CDRs included within the V_H and V_L domains using other numbering systems. Furthermore, as for all the sequences in the Figures, these V_H and V_L sequences can be used either in a scFv format or in a Fab format.

[0090] Figures 11A-11B depict the antigen sequences for a number of antigens of use in the antibodies described herein, including both human and cyno, to facilitate the development of antigen binding domains that bind to both for ease of clinical development.

[0091] Figure 12 depicts the variable heavy and variable light chain sequences for an exemplary humanized ENPP3 binding domain referred to herein as AN1, as well as the sequences for XENP28278 an anti-ENPP3 mAb based on AN1 and IgG1 backbone with E233P/L234V/L235A/G236del/S267K ablation variant. CDRs are underlined and slashes indicate the border(s) between the variable regions and constant domain. As noted herein and is true for every sequence herein containing CDRs, the exact identification of the CDR locations may be slightly different depending on the numbering used as is shown in Table 2 and thus included herein are not only the CDRs that are underlined but also CDRs included within the V_H and V_L domains using other numbering systems. Furthermore, as for all the sequences in the Figures, these V_H and V_L sequences can be used either in a scFv format or in a Fab format.

[0092] Figures 13A-13B depict the variable heavy and variable light chain sequences for AN1 variants engineered for improved purification and/or modulation of ENPP3 binding affinity and/or potency. CDRs are underlined and slashes indicate the border(s) between the variable regions and constant domain. As noted herein and is true for every sequence herein containing CDRs, the exact identification of the CDR locations may be slightly different

depending on the numbering used as is shown in Figure 12, and thus included herein are not only the CDRs that are underlined but also CDRs included within the V_H and V_L domains using other numbering systems. Further, as for all the sequences in the Figures, these V_H and V_L sequences can be used either in a scFv format or in a Fab format. Furthermore, each of the variable heavy domains depicted herein can be paired with any other α ENPP3 variable light domain; and each of the variable light domains depicted herein can be paired with any other α ENPP3 variable heavy domain.

[0093] Figures 14A-14I depicts the variable regions of additional ENPP3 antigen binding domains which may find use in the α ENPP3 x α CD3 antibodies. The CDRs are underlined. As noted herein and is true for every sequence herein containing CDRs, the exact identification of the CDR locations may be slightly different depending on the numbering used as is shown in Figure 12, and thus included herein are not only the CDRs that are underlined but also CDRs included within the V_H and V_L domains using other numbering systems. Furthermore, as for all the sequences in the Figures, these V_H and V_L sequences can be used either in a scFv format or in a Fab format.

[0094] Figure 15 depicts a couple of formats of the antibodies described herein. Figure 15A depicts the “1 + 1 Fab-scFv-Fc” format, with a first arm that includes a ENPP3 binding Fab and a second arm that includes a CD3 binding scFv. Figure 30B depicts the “2 + 1 Fab₂-scFv-Fc” format, with a first arm that includes an ENPP3 binding Fab and a second arm that includes a Fab and an scFv, wherein the Fab binds ENPP3 and the scFv binds CD3.

[0095] Figure 16 depicts the amino acid sequences of a control anti-RSV x anti-CD3 bispecific antibodies in the bottle-opener format (Fab-scFv-Fc). The antibody is named using the Fab variable region first and the scFv variable region second, separated by a dash. CDRs are underlined and slashes indicate the border(s) of the variable regions. The scFv domain has orientation (N- to C-terminus) of V_H-scFv linker-V_L, although this can be reversed. In addition, each sequence outlined herein can include or exclude the M428L/N434S variants in one or preferably both Fc domains, which results in longer half-life in serum.

[0096] Figures 17A-17C depict the sequences for illustrative α ENPP3 x α CD3 bsAbs in the 1 + 1 Fab-scFv-Fc format and comprising a H1.30_L1.47 anti-CD3 scFv (a.k.a. CD3 High[VHVL]). CDRs are underlined and slashes indicate the border(s) between the variable regions and other chain components (e.g. constant region and domain linkers). It should be noted that the α ENPP3 x α CD3 bsAbs can utilize variable region, Fc region, and constant

domain sequences that are 90, 95, 98 and 99% identical (as defined herein), and/or contain from 1, 2, 3, 4, 5, 6, 7, 8, 9 or 10 amino acid substitutions. In addition, each sequence outlined herein can include or exclude the M428L/N434S variants in one or preferably both Fc domains, which results in longer half-life in serum.

[0097] Figures 18A-18C depict the sequences for illustrative α ENPP3 x α CD3 bsAbs in the 1 + 1 Fab-scFv-Fc format and comprising a H1.32_L1.47 anti-CD3 scFv (a.k.a. CD3 High-Int #1[VHVL]). CDRs are underlined and slashes indicate the border(s) between the variable regions and other chain components (e.g. constant region and domain linkers). It should be noted that the α ENPP3 x α CD3 bsAbs can utilize variable region, Fc region, and constant domain sequences that are 90, 95, 98 and 99% identical (as defined herein), and/or contain from 1, 2, 3, 4, 5, 6, 7, 8, 9 or 10 amino acid substitutions. In addition, each sequence outlined herein can include or exclude the M428L/N434S variants in one or preferably both Fc domains, which results in longer half-life in serum.

[0098] Figures 19A-19C depict the sequences for illustrative α ENPP3 x α CD3 bsAbs in the 2 + 1 Fab₂-scFv-Fc format and comprising a H1.30_L1.47 anti-CD3 scFv (a.k.a. CD3 High[VHVL]). CDRs are underlined and slashes indicate the border(s) between the variable regions and other chain components (e.g. constant region and domain linkers). It should be noted that the α ENPP3 x α CD3 bsAbs can utilize variable region, Fc region, and constant domain sequences that are 90, 95, 98 and 99% identical (as defined herein), and/or contain from 1, 2, 3, 4, 5, 6, 7, 8, 9 or 10 amino acid substitutions. In addition, each sequence outlined herein can include or exclude the M428L/N434S variants in one or preferably both Fc domains, which results in longer half-life in serum.

[0099] Figures 20A-20D depict the sequences for illustrative α ENPP3 x α CD3 bsAbs in the 2 + 1 Fab₂-scFv-Fc format and comprising a H1.32_L1.47 anti-CD3 scFv (a.k.a. CD3 High-Int #1[VHVL]). CDRs are underlined and slashes indicate the border(s) between the variable regions and other chain components (e.g. constant region and domain linkers). It should be noted that the α ENPP3 x α CD3 bsAbs can utilize variable region, Fc region, and constant domain sequences that are 90, 95, 98 and 99% identical (as defined herein), and/or contain from 1, 2, 3, 4, 5, 6, 7, 8, 9 or 10 amino acid substitutions. In addition, each sequence outlined herein can include or exclude the M428L/N434S variants in one or preferably both Fc domains, which results in longer half-life in serum.

[00100] Figure 21 depicts the sequences for illustrative α ENPP3 x α CD3 bsAbs in the 2 + 1 Fab₂-scFv-Fc format and comprising a L1.47_H1.30 anti-CD3 scFv (a.k.a. CD3 High[VLVH]). CDRs are underlined and slashes indicate the border(s) between the variable regions and other chain components (e.g. constant region and domain linkers). It should be noted that the α ENPP3 x α CD3 bsAbs can utilize variable region, Fc region, and constant domain sequences that are 90, 95, 98 and 99% identical (as defined herein), and/or contain from 1, 2, 3, 4, 5, 6, 7, 8, 9 or 10 amino acid substitutions. In addition, each sequence outlined herein can include or exclude the M428L/N434S variants in one or preferably both Fc domains, which results in longer half-life in serum.

[00101] Figures 22A-22C depict the sequences for illustrative α ENPP3 x α CD3 bsAbs in the 2 + 1 Fab₂-scFv-Fc format and comprising a L1.47_H1.32 anti-CD3 scFv (a.k.a. CD3 High-Int #1[VLVH]). CDRs are underlined and slashes indicate the border(s) between the variable regions and other chain components (e.g. constant region and domain linkers). It should be noted that the α ENPP3 x α CD3 bsAbs can utilize variable region, Fc region, and constant domain sequences that are 90, 95, 98 and 99% identical (as defined herein), and/or contain from 1, 2, 3, 4, 5, 6, 7, 8, 9 or 10 amino acid substitutions. In addition, each sequence outlined herein can include or exclude the M428L/N434S variants in one or preferably both Fc domains, which results in longer half-life in serum.

[00102] Figures 23A-23E depict the sequences for illustrative α ENPP3 x α CD3 bsAbs in the 2 + 1 Fab₂-scFv-Fc format and comprising a L1.47_H1.89 anti-CD3 scFv (a.k.a. CD3 High-Int #2[VLVH]). CDRs are underlined and slashes indicate the border(s) between the variable regions and other chain components (e.g. constant region and domain linkers). It should be noted that the α ENPP3 x α CD3 bsAbs can utilize variable region, Fc region, and constant domain sequences that are 90, 95, 98 and 99% identical (as defined herein), and/or contain from 1, 2, 3, 4, 5, 6, 7, 8, 9 or 10 amino acid substitutions. In addition, each sequence outlined herein can include or exclude the M428L/N434S variants in one or preferably both Fc domains, which results in longer half-life in serum.

[00103] Figure 24 depicts induction of RTCC on CFSE-labeled KU812 cells A) as indicated by decrease in number of CFSE⁺ KU812 cells and B) as indicated by percentage of CFSE⁺ KU812 cells stained with Zombie Aqua after incubation of CFSE-labeled KU812 for 24 hours with human PBMCs (10:1 effector to target cell ratio) and α ENPP3 x α CD3 bispecific antibodies (XENP26820, XENP26821, XENP28287, and XENP28390). Controls

used were α RSV x α CD3 bispecific antibody (XENP13245), effector and target cells only, and target cells only. Collectively, the data show that the prototype α ENPP3 x α CD3 bsAbs dose-dependently induced redirected T-cell cytotoxicity (RTCC) on KU812 cells; CD3 binding affinity correlated with RTCC potency (i.e. bsAbs with CD3 High induced RTCC more potently than bsAbs with CD3 High-Int #1); and bsAbs with AN1 binding domain induced RTCC more potently than bsAbs with H16-7.8 binding domain.

[00104] Figure 25 depict activation CD4⁺ T cells as indicated by A) CD107a MFI on CD4⁺ T cells, B) CD25 MFI on CD4⁺ T cells, and C) CD69 MFI on CD4⁺ T cells after incubation of CFSE-labeled KU812 for 24 hours with human PBMCs (10:1 effector to target cell ratio) and α ENPP3 x α CD3 bispecific antibodies (XENP26820, XENP26821, XENP28287, and XENP28390). Controls used were α RSV x α CD3 bispecific antibody (XENP13245), effector and target cells only, and target cells only. Consistent with RTCC data, the α ENPP3 x α CD3 bsAbs dose-dependently induced activation of CD4⁺ T cells; CD3 binding affinity correlated with activation potency (i.e. bsAbs with CD3 High induced CD4⁺ T cell activation more potently than bsAbs with CD3 High-Int #1); and bsAbs with AN1 binding domain induced CD4⁺ T cell activation more potently than bsAbs with H16-7.8 binding domain.

[00105] Figure 26 depicts activation CD8⁺ T cells as indicated by A) CD107a MFI on CD8⁺ T cells, B) CD25 MFI on CD8⁺ T cells, and C) CD69 MFI on CD8⁺ T cells after incubation of CFSE-labeled KU812 for 24 hours with human PBMCs (10:1 effector to target cell ratio) and α ENPP3 x α CD3 bispecific antibodies (XENP26820, XENP26821, XENP28287, and XENP28390). Controls used were α RSV x α CD3 bispecific antibody (XENP13245), effector and target cells only, and target cells only. Consistent with RTCC data, the α ENPP3 x α CD3 bsAbs dose-dependently induced activation of CD8⁺ T cells; CD3 binding affinity correlated with activation potency (i.e. bsAbs with CD3 High induced CD8⁺ T cell activation more potently than bsAbs with CD3 High-Int #1); and bsAbs with AN1 binding domain induced CD8⁺ T cell activation more potently than bsAbs with H16-7.8 binding domain.

[00106] Figure 27 depicts induction of RTCC on CFSE-labeled RXF393 cells A) as indicated by decrease in number of CFSE⁺ RXF393 cells and B) as indicated by percentage of CFSE⁺ RXF393 cells stained with Zombie Aqua after incubation of CFSE-labeled RXF393 for 24 hours with human PBMCs (20:1 effector to target cell ratio) and α ENPP3 x

α CD3 bispecific antibodies (XENP26820, XENP26821, XENP28287, and XENP28390). Controls used were α RSV x α CD3 bispecific antibody (XENP13245), effector and target cells only, and target cells only. Consistent with the data for KU812 cells, the data show that the prototype α ENPP3 x α CD3 bsAbs dose-dependently induced redirected T-cell cytotoxicity (RTCC) on RXF393 cells; CD3 binding affinity correlated with RTCC potency (i.e. bsAbs with CD3 High induced RTCC more potently than bsAbs with CD3 High-Int #1); and bsAbs with AN1 binding domain induced RTCC more potently than bsAbs with H16-7.8 binding domain.

[00107] Figure 28 depict activation CD4⁺ T cells as indicated by A) CD107a MFI on CD4⁺ T cells, B) CD25 MFI on CD4⁺ T cells, and C) CD69 MFI on CD4⁺ T cells after incubation of CFSE-labeled RXF393 for 24 hours with human PBMCs (20:1 effector to target cell ratio) and α ENPP3 x α CD3 bispecific antibodies (XENP26820, XENP26821, XENP28287, and XENP28390). Controls used were α RSV x α CD3 bispecific antibody (XENP13245), effector and target cells only, and target cells only. Consistent with RTCC data, the α ENPP3 x α CD3 bsAbs dose-dependently induced activation of CD4⁺ T cells; CD3 binding affinity correlated with activation potency (i.e. bsAbs with CD3 High induced CD4⁺ T cell activation more potently than bsAbs with CD3 High-Int #1); and bsAbs with AN1 binding domain induced CD4⁺ T cell activation more potently than bsAbs with H16-7.8 binding domain.

[00108] Figure 29 depicts activation CD8⁺ T cells as indicated by A) CD107a MFI on CD8⁺ T cells, B) CD25 MFI on CD8⁺ T cells, and C) CD69 MFI on CD8⁺ T cells after incubation of CFSE-labeled RXF393 for 24 hours with human PBMCs (20:1 effector to target cell ratio) and α ENPP3 x α CD3 bispecific antibodies (XENP26820, XENP26821, XENP28287, and XENP28390). Controls used were α RSV x α CD3 bispecific antibody (XENP13245), effector and target cells only, and target cells only. Consistent with RTCC data, the α ENPP3 x α CD3 bsAbs dose-dependently induced activation of CD8⁺ T cells; CD3 binding affinity correlated with activation potency (i.e. bsAbs with CD3 High induced CD8⁺ T cell activation more potently than bsAbs with CD3 High-Int #1); and bsAbs with AN1 binding domain induced CD8⁺ T cell activation more potently than bsAbs with H16-7.8 binding domain.

[00109] Figure 30 depicts A) chromatogram illustrating purification part 2 of XENP28287 (cation exchange chromatography following protein A chromatography), and

the purity and homogeneity of peaks B and BC isolated from cation exchange separation as depicted in Figure 30A (as well as pre-purified material) by B) analytical size-exclusion chromatography with multi-angle light scattering (aSEC-MALS) and C) analytical cation exchange chromatography (aCIEX). Figure 30B also depicts the molecular weight of protein species in peaks as determined by multi-angle light scattering.

[00110] Figure 31 depicts A) chromatogram illustrating purification part 2 of XENP28925 (cation exchange chromatography following protein A chromatography), and the purity and homogeneity of peak B isolated from cation exchange separation as depicted in Figure 31A (as well as pre-purified material) by B) analytical size-exclusion chromatography with multi-angle light scattering (aSEC-MALS) and C) analytical cation exchange chromatography (aCIEX). Figure 31B also depicts the molecular weight of protein species in peaks as determined by multi-angle light scattering.

[00111] Figure 32 depicts A) chromatogram illustrating purification part 2 of XENP31149 (cation exchange chromatography following protein A chromatography), and B) the identity of peaks A and B as isolated from cation exchange separation as depicted in Figure 32A (as well as pre-purified material) by analytical size-exclusion chromatography with multi-angle light scattering (aSEC-MALS).

[00112] Figure 33 depicts A) chromatogram illustrating purification part 2 of XENP31419 (cation exchange chromatography following protein A chromatography), and B) the identity of peaks A and B as isolated from cation exchange separation as depicted in Figure 33A (as well as pre-purified material) by analytical size-exclusion chromatography with multi-angle light scattering (aSEC-MALS).

[00113] Figure 34 depicts induction of RTCC on CFSE-labeled KU812 (solid line, ENPP3^{high}) or CFSE-labeled RCC4 (dashed line, ENPP3^{low}) cells as indicated by percentage of CFSE⁺ cells stained with Zombie Aqua after incubation of CFSE-labeled target cells for 18 hours with human PBMCs (10:1 effector to target cell ratio) and α ENPP3 x α CD3 bispecific antibody XENP28925.

[00114] Figure 35 depicts binding of affinity-engineered α ENPP3 x α CD3 1 + 1 bsAbs to ENPP3^{high} KU812 cells.

[00115] Figure 36 depicts induction of RTCC on CFSE-labeled KU812 (solid line, ENPP3^{high}) or CFSE-labeled RCC4 (dashed line, ENPP3^{low}) cells as indicated by percentage

of CFSE⁺ cells stained with Zombie Aqua after incubation of CFSE-labeled target cells for 42 hours with human PBMCs (10:1 effector to target cell ratio) and α ENPP3 x α CD3 bispecific antibodies XENP28925 (WT high ENPP3 binding), XENP29516 (intermediate ENPP3 binding), or XENP30262 (low ENPP3 binding). The data show that both XENP29516 and XENP30262 demonstrated substantially less potent induction of RTCC on ENPP3^{low} RCC4 cells in comparison to XENP28925, with RTCC potency correlating with binding potency as shown above. XENP29516 and XENP30262 also demonstrated less potent induction of RTCC on ENPP3^{high} cells.

[00116] Figures 37A-37C depict induction of A) IFN γ , B) IL-6, and C) TNF α release by human PBMCs incubated with KU812 cells (10:1 effector to target cell ratio) and α ENPP3 x α CD3 bispecific antibodies XENP28925 (CD3 High) or XENP29436 (CD3 High-Int #1) for 18 hours. The data show that XENP29436 demonstrated substantially less potent induction of cytokine release in comparison to XENP28925.

[00117] Figure 38 depicts induction of IFN γ release by human PBMCs incubated with RCC4 cells (10:1 effector to target cell ratio) and α ENPP3 x α CD3 bispecific antibodies XENP28925 (CD3 High) or XENP29436 (CD3 High-Int #1) for 18 hours. The data show that XENP29436 demonstrated negligible induction of cytokine release in comparison to XENP28925 in the presence of ENPP3^{low} RCC4 cells.

[00118] Figure 39 depicts induction of RTCC on CFSE-labeled KU812 (solid line, ENPP3^{high}) or CFSE-labeled RCC4 (dashed line, RCC4^{low}) cells as indicated by percentage of CFSE⁺ cells stained with Zombie Aqua after incubation of CFSE-labeled target cells for 42 hours with human PBMCs (10:1 effector to target cell ratio) and α ENPP3 x α CD3 bispecific antibodies XENP28925 (CD3 High) or XENP29436 (CD3 High-Int #1). The data show that XENP29436 demonstrated substantially less potent induction of RTCC on ENPP3^{low} cells in comparison to XENP28925; however, XENP29436 also demonstrated reduced potency in induction of RTCC on ENPP3^{high} cells.

[00119] Figure 40 depicts the induction of IFN γ release by human PBMCs incubated with KU812 cells (10:1 effector to target cell ratio) and α ENPP3 x α CD3 bispecific antibodies XENP28925 (ENPP3 High; CD3 High), XENP29436 (ENPP3 High; CD3 High-Int #1), XENP29518 (ENPP3 Intermediate; CD3 High), XENP29463 (ENPP3 Intermediate; CD3 High-Int #1), XENP30262 (ENPP3 Low; CD3 High), or XENP30263 (ENPP3 Low; CD3 High-Int #1). The data show that reducing either CD3 or ENPP3 binding potency

reduces induction of cytokine release. Notably, reducing CD3 and ENPP3 binding potency further reduces induction of cytokine release.

[00120] Figure 41 depicts induction of RTCC on CFSE-labeled KU812 (solid line, ENPP3^{high}) or CFSE-labeled RCC4 (dashed line, ENPP3^{low}) cells as indicated by percentage of CFSE⁺ cells stained with Zombie Aqua after incubation of CFSE-labeled target cells for 42 hours with human PBMCs (10:1 effector to target cell ratio) and α ENPP3 x α CD3 bispecific antibodies XENP28925 (WT high ENPP3 binding; CD3 High; monovalent ENPP3 binding), XENP29516 (intermediate ENPP3 binding; CD3 High; monovalent ENPP3 binding), or XENP29520 (intermediate ENPP3 binding; CD3 High; bivalent ENPP3 binding). The data show that bivalent binding (with intermediate ENPP3 binding) maintained reduced RTCC potency on ENPP3^{low} cells, but restored RTCC potency on ENPP3^{high} cells close to that demonstrated by XENP28925.

[00121] Figure 42 depicts induction of RTCC on CFSE-labeled KU812 (solid line, ENPP3^{high}) or CFSE-labeled RCC4 (dashed line, ENPP3^{low}) cells as indicated by percentage of CFSE⁺ cells stained with Zombie Aqua after incubation of CFSE-labeled target cells for 42 hours with human PBMCs (10:1 effector to target cell ratio) and α ENPP3 x α CD3 bispecific antibodies XENP28925 (WT high ENPP3 binding; CD3 High; monovalent ENPP3 binding), XENP30262 (low ENPP3 binding; CD3 High; monovalent ENPP3 binding), or XENP30264 (low ENPP3 binding; CD3 High; bivalent ENPP3 binding). The data show that bivalent binding (with low ENPP3 binding) further reduced RTCC potency on ENPP3^{low} cells, and restored some RTCC potency on ENPP3^{high} cells.

[00122] Figure 43 depicts induction of RTCC on CFSE-labeled KU812 as indicated by percentage of CFSE⁺ cells stained with Zombie Aqua after incubation of CFSE-labeled target cells for 44 hours with human PBMCs (10:1 effector to target cell ratio) and α ENPP3 x α CD3 bispecific antibodies XENP28925 (CD3 High; monovalent ENPP3 binding), XENP29437 (CD3 High; bivalent ENPP3 binding), XENP29436 (CD3 High-Int #1; monovalent ENPP3 binding), or XENP29438 (CD3 High-Int #1; bivalent ENPP3 binding). Unexpectedly, XENP29438 was unable to induce RTCC on KU812 cells.

[00123] Figure 44 depicts induction of RTCC on CFSE-labeled KU812 (solid line, ENPP3^{high}) or CFSE-labeled RCC4 (dashed line, ENPP3^{low}) as indicated by percentage of CFSE⁺ cells stained with Zombie Aqua after incubation of CFSE-labeled target cells for 24 hours with human PBMCs (10:1 effector to target cell ratio) and α ENPP3 x α CD3 bispecific

antibodies XENP29437 (CD3 High VH/VL; bivalent ENPP3 binding), XENP30469 (CD3 High VL/VH; bivalent ENPP3 binding), XENP29428 (CD3 High-Int #1 VH/VL; bivalent ENPP3 binding), or XENP30470 (CD3 High-Int #2 VL/VH; bivalent ENPP3 binding). The data showed that swapping the orientation of the variable heavy and variable light domains in the CD3 High-Int #1 scFv restored its activity in the context of 2 + 1 Fab₂-scFv-Fc bsAb format (XENP29438 vs. XENP30470). Swapping the orientation of the variable heavy and variable light domains in the CD3 High scFv enabled much more modest improvement in RTCC potency in the context of 2 + 1 Fab₂-scFv-Fc bsAb format (XENP29437 vs. XENP30469).

[00124] Figure 45 depicts induction of RTCC on CFSE-labeled KU812 (solid line, ENPP3^{high}) or CFSE-labeled RCC4 (dashed line, ENPP3^{low}) as indicated by percentage of CFSE⁺ cells stained with Zombie Aqua after incubation of CFSE-labeled target cells for 40 hours with human PBMCs (10:1 effector to target cell ratio) and αENPP3 x αCD3 bispecific antibodies XENP29520 (CD3 High[VH/VL]; bivalent ENPP3 intermediate binding), XENP30819 (CD3 High-Int #1[VL/VHL]; bivalent ENPP3 intermediate binding), XENP31149 (CD3 High-Int #2[VL/VHL]; bivalent ENPP3 intermediate binding), XENP30264 (CD3 High[VH/VL]; bivalent ENPP3 low binding), XENP30821 (CD3 High-Int #1[VL/VHL]; bivalent ENPP3 low binding), or XENP31150 (CD3 High-Int #2[VL/VHL]; bivalent ENPP3 low binding).

[00125] Figure 46 depicts the sequences for XENP16432, anti-PD-1 mAb based on nivolumab and IgG1 backbone with E233P/L234V/L235A/G236del/S267K ablation variant; and XENP21461 (pembrolizumab).

[00126] Figure 47 depicts the change in tumor volume (as determined by caliper measurements) over time in KU812 and huPBMC-engrafted NSG mice dosed with PBS, XENP16432 (a bivalent anti-PD-1 mAb), or with illustrative αENPP3 x αCD3 2 + 1 bsAbs (XENP30819, XENP30821, or XENP31149) alone or in combination with XENP16432. Each of the αENPP3 x αCD3 bsAbs, at low and/or higher dose treatment, were able to enhance allogeneic anti-tumor effect of T cells on KU812 cells, and combined well with PD-1 blockade.

[00127] Figures 48A-48D depict the expansion of A) CD45⁺ lymphocytes, B) CD8⁺ T cells, and C) CD4⁺ T cells by Day 14 in blood of KU812 and huPBMC-engrafted NSG mice dosed with PBS, XENP16432 (a bivalent anti-PD-1 mAb), or with illustrative αENPP3 x

α CD3 2 + 1 bsAbs (XENP30819, XENP30821, or XENP31419) alone or in combination with XENP16432. In all cases, combining with PD-1 blockade enhanced lymphocyte expansion.

[00128] Figure 49 depicts the change in tumor volume (as determined by caliper measurements) over time in RXF-393 and huPBMC-engrafted NSG mice dosed with PBS, XENP16432 (a bivalent anti-PD-1 mAb), or with illustrative α ENPP3 x α CD3 2 + 1 bsAbs (XENP30819 or XENP31419) alone or in combination with XENP16432. Each of the α ENPP3 x α CD3 bsAbs, at low, mid and/or high dose treatment, were able to enhance allogeneic anti-tumor effect of T cells on KU812 cells, and combined well with PD-1 blockade.

[00129] Figures 50A-50N depict the change in tumor volume (as determined by caliper measurements) over time in individual KU812 and huPBMC-engrafted NSG mice dosed with A) PBS, B) XENP16432 (a bivalent anti-PD-1 mAb), or with illustrative α ENPP3 x α CD3 2 + 1 bsAbs (XENP30819, XENP30821, or XENP31419) alone or in combination with XENP16432. Each of the α ENPP3 x α CD3 bsAbs, at low and/or higher dose treatment, were able to enhance allogeneic anti-tumor effect of T cells on KU812 cells, and combined well with PD-1 blockade.

[00130] Figures 51A-51L depict the change in tumor volume (as determined by caliper measurements) over time in individual RXF-393 and huPBMC-engrafted NSG mice dosed with A) PBS, B) XENP16432 (a bivalent anti-PD-1 mAb), or with illustrative α ENPP3 x α CD3 2 + 1 bsAbs (XENP30819 or XENP31419) alone or in combination with XENP16432. Each of the α ENPP3 x α CD3 bsAbs, at low, mid and/or high dose treatment, were able to enhance allogeneic anti-tumor effect of T cells on KU812 cells, and combined well with PD-1 blockade.

[00131] Figures 52A-52K depict several formats for use in the anti-ENPP3 x anti-CD3 bispecific antibodies disclosed herein. The first is the “1 + 1 Fab-scFv-Fc” format (also referred to as the “bottle opener” or “Triple F” format), with a first antigen binding domain that is a Fab domain and a second anti-antigen binding domain that is an scFv domain (Figure 1A). Additionally, “mAb-Fv,” “mAb-scFv,” “2+1 Fab2-scFv-Fc” (also referred to as the “central scFv” or “central-scFv” format), “central-Fv,” “one armed central-scFv,” “one scFv-mAb,” “scFv-mAb,” “dual scFv,” “trident,” and non-heterodimeric bispecific formats are all shown. The scFv domains depicted in Figure 49 can be either, from N- to C-terminus orientation: variable heavy-(optional linker)-variable light, or variable light-(optional linker)-

variable heavy. In addition, for the one armed scFv-mAb, the scFv can be attached either to the N-terminus of a heavy chain monomer or to the N-terminus of the light chain. In certain embodiments, “Anti-antigen 1” in Figure 52 refers to a ENPP3 binding domain. In certain embodiments, “Anti-antigen 1” in Figure 52 refers to a CD3 binding domain. In certain embodiments, “Anti-antigen 2” in Figure 52 refers to a ENPP3 binding domain. In certain embodiments “Anti-antigen 2” in Figure 52 refers to a CD3 binding domain. In some embodiments, “Anti-antigen 1” in Figure 52 refers to a ENPP3 binding domain and “Anti-antigen 2” in Figure 52 refers to a CD3 binding domain. In some embodiments, “Anti-antigen 1” in Figure 52 refers to a CD3 binding domain and “Anti-antigen 2” in Figure 52 refers to a ENPP3 binding domain. Any of the ENPP3 binding domains and CD3 binding domains disclosed can be included in the bispecific formats of Figure 52.

[00132] Figure 53 provide schematics of heterodimeric Fc proteins described herein including 2:1 Fab₂-scFv-Fc, 1:1 Fab=scFv-Fc, Y/Z-Fc (e.g., untargeted interleukin-Fc), anti-X x Y/Z-F (e.g., targeted interleukin-Fc)c, and one arm Fc proteins.

[00133] Figure 54 provides structural models of CH3-CH3 interface built using MOE based on Protein Data Bank entry 3AVE. Novel set of Fc substitutions are capable of achieving heterodimer yields over 95% with little change in thermostability.

[00134] Figure 55 depict isosteric substitutions used to minimize impact to tertiary structure. Engineered isoelectric point differences in the Fc region allow or facilitate straightforward purification of Fc heterodimers.

[00135] Figure 56 depict hinge and CH2 substitutions abolish FcγR binding.

[00136] Figures 57A-57C show that the 2:1 Fab₂-scFv-Fc format enables targeting of tumor antigens with low density on normal cells. Tuning TAA valency and TAA/CD3 affinities enables selective cytotoxicity of cell lines mimicking cancer tissue and normal tissue (high/low antigen density). Tuned 2:1 bispecifics also have reduced interference from soluble antigen and reduced cytokine release.

[00137] Figure 57A shows that tuning FAP valency and FAP/CD3 affinities enables selective cytotoxicity of cell lines mimicking cancer tissue and normal tissue (high/low antigen density). XENP23535 represents a tuned 1:1 format targeting FAP. XENP25967 represents a tuned 2:1 format targeting FAP.

[00138] Figure 57B shows that tuning SSTR2 valency and SSTR2/CD3 affinities enables selective cytotoxicity of cell lines mimicking cancer tissue and normal tissue (high/low antigen density). XENP18087 represents a tuned 1:1 format targeting SSTR2. XENP30458 represents a tuned 2:1 format targeting SSTR2.

[00139] Figure 57C shows that tuning ENPP3 valency and ENPP3/CD3 affinities enables selective cytotoxicity of cell lines mimicking cancer tissue and normal tissue (high/low antigen density). XENP28925 represents a tuned 1:1 format targeting ENPP3. XENP31149 represents a tuned 2:1 format targeting ENPP3.

[00140] Figure 58 depicts advantages of research scale production of heterodimeric Fc proteins using the method described herein. The method is useful for straightforward production of heterodimeric Fc proteins.

[00141] Figure 59 shows stable cell line development results in clones with high titer and high heterodimer prevalence. Top clones have shake flask yields of 1-2 g/L with about 90% heterodimer content. The data was obtained after only a standard protein A purification step.

[00142] Figure 60 depicts induction of RTCC on A549 cells transfected with SSTR2 (at high, medium, and low densities) by A) XENP18087 or B) XENP30458.

[00143] Figure 61 depicts A) reduction in number of target cells and release of B) IL-6, C) TNF α , D) IFN γ , and E) IL-1 β by effector cells following incubation of CFSE-labeled SSTR2+ COR-L279 target cells with human PBMCs (effector:target ratio of 20:1) for 48 hours in the presence of XENP18087 or XENP30458

[00144] Figure 62A-Figure 62D. Sequences for illustrative 1:1 tuned format and 2:1 tuned format TAA x CD3 bispecifics described herein. Anti-TTA (e.g., anti-FAP, anti-SSTR2, and anti-ENPP3) components such as variable regions, anti-CD3 components such as variable regions, constant/Fc regions, and linkers are shown. Linkers are double underlined (although as will be appreciated by those in the art, the linkers can be replaced by other linkers), slashes (/) indicate border(s) between the variable regions, constant/Fc regions, and linkers. The CDRs are underlined. In some embodiments, the 1:1 format TAA x CD3 bispecifics is XENP23535, XENP18087, or XENP28925. In some embodiments, the 2:1 format TAA x CD3 bispecifics is XENP25967, XENP30458, XENP31149.

[00145] Figure 63 depicts the sequences for SSTR2 binding domain [αSSTR2]_H1.24_L1.30.

DETAILED DESCRIPTION OF THE INVENTION

I. Overview

[00146] Anti-bispecific antibodies that co-engage CD3 and a tumor antigen target are used to redirect T cells to attack and lyse targeted tumor cells. Examples include the BiTE® and DART formats, which monovalently engage CD3 and a tumor antigen. While the CD3-targeting approach has shown considerable promise, a common side effect of such therapies is the associated production of cytokines, often leading to toxic cytokine release syndrome. Because the anti-CD3 binding domain of the bispecific antibody engages all T cells, the high cytokine-producing CD4 T cell subset is recruited. Moreover, the CD4 T cell subset includes regulatory T cells, whose recruitment and expansion can potentially lead to immune suppression and have a negative impact on long-term tumor suppression. In addition, these formats do not contain Fc domains and show very short serum half-lives in patients.

[00147] Provided herein are novel anti-CD3 x anti-ENPP3 (also referred to as anti-ENPP3 x anti-CD3, αCD3 x αENPP3, or αENPP3 x αCD3) heterodimeric bispecific antibodies and methods of using such antibodies for the treatment of cancers. In particular, provided herein are anti-CD3, anti-ENPP3 bispecific antibodies in a variety of formats such as those depicted in Figures 15A and 15B. These bispecific antibodies are useful for the treatment of cancers, particularly those with increased ENPP3 expression such as renal cell carcinoma. Such antibodies are used to direct CD3+ effector T cells to ENPP3+ tumors, thereby allowing the CD3+ effector T cells to attack and lyse the ENPP3+ tumors.

[00148] Additionally, in some embodiments, the disclosure provides bispecific antibodies that have different binding affinities to human CD3 that can alter or reduce the potential side effects of anti-CD3 therapy. That is, in some embodiments the antibodies described herein provide antibody constructs comprising anti-CD3 antigen binding domains that are “strong” or “high affinity” binders to CD3 (e.g. one example are heavy and light variable domains depicted as H1.30_L1.47 (optionally including a charged linker as appropriate)) and also bind to ENPP3. In other embodiments, the antibodies described herein provide antibody constructs comprising anti-CD3 antigen binding domains that are “lite” or “lower affinity” binders to CD3. Additional embodiments provides antibody constructs

comprising anti-CD3 antigen binding domains that have intermediate or “medium” affinity to CD3 that also bind to ENPP3. While a very large number of anti-CD3 antigen binding domains (ABDs) can be used, particularly useful embodiments use 6 different anti-CD3 ABDs, although they can be used in two scFv orientations as discussed herein. Affinity is generally measured using a Biacore assay.

[00149] It should be appreciated that the “high, medium, low” anti-CD3 sequences provided herein can be used in a variety of heterodimerization formats as depicted in Figures 15A, 15B, and . In general, due to the potential side effects of T cell recruitment, exemplary embodiments utilize formats that only bind CD3 monovalently, such as depicted in Figure 15A and 15B, and in the formats depicted herein, it is the CD3 ABD that is a scFv as more fully described herein. In contrast, the subject bispecific antibodies can bind ENPP3 either monovalently (e.g. Figure 15A) or bivalently (e.g. Figure 15B).

[00150] Provided herein are compositions that include ENPP3 binding domains, including antibodies with such ENPP binding domains (e.g., ENPP3 x CD3 bispecific antibodies). Subject antibodies that include such ENPP3 binding domains advantageously elicit a range of different immune responses, depending on the particular ENPP3 binding domain used. For example, the subject antibodies exhibit differences in selectivity for cells with different ENPP3 expression, potencies for ENPP3 expressing cells, ability to elicit cytokine release, and sensitivity to soluble ENPP3. Such ENPP3 binding domains and related antibodies find use, for example, in the treatment of ENPP3 associated cancers.

[00151] Accordingly, in one aspect, provided herein are heterodimeric antibodies that bind to two different antigens, e.g. the antibodies are “bispecific”, in that they bind two different target antigens, generally ENPP3 and CD3 as described herein. These heterodimeric antibodies can bind these target antigens either monovalently (e.g. there is a single antigen binding domain such as a variable heavy and variable light domain pair) or bivalently (there are two antigen binding domains that each independently bind the antigen). In some embodiments, the heterodimeric antibody provided herein includes one CD3 binding domain and one ENPP3 binding domain (e.g., heterodimeric antibodies in the “1 + 1 Fab-scFv-Fc” format described herein). In other embodiments, the heterodimeric antibody provided herein includes one CD3 binding domain and two ENPP3 binding domains (e.g., heterodimeric antibodies in the “2 + 1 Fab2-scFv-Fc” formats described herein). The heterodimeric antibodies provided herein are based on the use different monomers which

contain amino acid substitutions that “skew” formation of heterodimers over homodimers, as is more fully outlined below, coupled with “pI variants” that allow simple purification of the heterodimers away from the homodimers, as is similarly outlined below. The heterodimeric bispecific antibodies provided generally rely on the use of engineered or variant Fc domains that can self-assemble in production cells to produce heterodimeric proteins, and methods to generate and purify such heterodimeric proteins.

II. Nomenclature

[00152] The antibodies provided herein are listed in several different formats. In some instances, each monomer of a particular antibody is given a unique “XENP” number, although as will be appreciated in the art, a longer sequence might contain a shorter one. For example, a “scFv-Fc” monomer of a 1 + 1 Fab-scFv-Fc format antibody may have a first XENP number, while the scFv domain itself will have a different XENP number. Some molecules have three polypeptides, so the XENP number, with the components, is used as a name. Thus, the molecule XENP29520, which is in 2 + 1 Fab₂-scFv-Fc format, comprises three sequences (see Figure 19A) a “Fab-Fc Heavy Chain” monomer; 2) a “Fab-scFv-Fc Heavy Chain” monomer; and 3) a “Light Chain” monomer or equivalents, although one of skill in the art would be able to identify these easily through sequence alignment. These XENP numbers are in the sequence listing as well as identifiers, and used in the Figures. In addition, one molecule, comprising the three components, gives rise to multiple sequence identifiers. For example, the listing of the Fab includes, the full heavy chain sequence, the variable heavy domain sequence and the three CDRs of the variable heavy domain sequence, the full light chain sequence, a variable light domain sequence and the three CDRs of the variable light domain sequence. A Fab-scFv-Fc monomer includes a full length sequence, a variable heavy domain sequence, 3 heavy CDR sequences, and an scFv sequence (include scFv variable heavy domain sequence, scFv variable light domain sequence and scFv linker). Note that some molecules herein with a scFv domain use a single charged scFv linker (+H), although others can be used. In addition, the naming nomenclature of particular antigen binding domains (e.g., ENPP3 and CD3 binding domains) use a “Hx.xx_Ly.yy” type of format, with the numbers being unique identifiers to particular variable chain sequences. Thus, the variable domain of the Fab side of CD3 binding domain AN1[ENPP3] H1L1 (e.g., Figure 12) is “H1 L1”, which indicates that the variable heavy domain, H1, was combined

with the light domain L1. In the case that these sequences are used as scFvs, the designation “H1 L1”, indicates that the variable heavy domain, H1 is combined with the light domain, L1, and is in VH-linker-VL orientation, from N- to C-terminus. This molecule with the identical sequences of the heavy and light variable domains but in the reverse order (VL-linker-VH orientation, from N- to C-terminus) would be designated “L1_H1.1”. Similarly, different constructs may “mix and match” the heavy and light chains as will be evident from the sequence listing and the figures.

III. Definitions

[00153] In order that the application may be more completely understood, several definitions are set forth below. Such definitions are meant to encompass grammatical equivalents.

[00154] By “ENPP3” or “Ectonucleotide pyrophosphatase/phosphodiesterase family member 3” (e.g., Genebank Accession Number NP 005012.2) herein is meant a protein belonging to a series of ectoenzymes that are involved in hydrolysis of extracellular nucleotides. ENPP3 sequences are depicted, for example, in Figures 11A and 11B. ENPP3 is expressed in particular cancers, including renal cell carcinomas.

[00155] By “ablation” herein is meant a decrease or removal of activity. Thus for example, “ablating FcγR binding” means the Fc region amino acid variant has less than 50% starting binding as compared to an Fc region not containing the specific variant, with more than 70-80-90-95-98% loss of activity being preferred, and in general, with the activity being below the level of detectable binding in a Biacore, SPR or BLI assay. Of particular use in the ablation of FcγR binding are those shown in Figure 5, which generally are added to both monomers.

[00156] By “ADCC” or “antibody dependent cell-mediated cytotoxicity” as used herein is meant the cell-mediated reaction wherein nonspecific cytotoxic cells that express FcγRs recognize bound antibody on a target cell and subsequently cause lysis of the target cell. ADCC is correlated with binding to FcγRIIIa; increased binding to FcγRIIIa leads to an increase in ADCC activity.

[00157] By “ADCP” or antibody dependent cell-mediated phagocytosis as used herein is meant the cell-mediated reaction wherein nonspecific phagocytic cells that express FcγRs

recognize bound antibody on a target cell and subsequently cause phagocytosis of the target cell.

[00158] As used herein, term “antibody” is used generally. Antibodies described herein can take on a number of formats as described herein, including traditional antibodies as well as antibody derivatives, fragments and mimetics, described herein.

[00159] Traditional immunoglobulin (Ig) antibodies are “Y” shaped tetramers. Each tetramer is typically composed of two identical pairs of polypeptide chains, each pair having one “light chain” monomer (typically having a molecular weight of about 25 kDa) and one “heavy chain” monomer (typically having a molecular weight of about 50-70 kDa).

[00160] Other useful antibody formats include, but are not limited to, the 1 + 1 Fab-scFv-Fc format and 2 + 1 Fab-scFv-Fc antibody formats described herein, as well as “mAb-Fv,” “mAb-scFv,” “central-Fv,” “one armed scFv-mAb,” “scFv-mAb,” “dual scFv,” and “trident” format antibodies, as shown in Figure 49.

[00161] Antibody heavy chains typically include a variable heavy (VH) domain, which includes vhCDR1-3, and an Fc domain, which includes a CH2-CH3 monomer. In some embodiments, antibody heavy chains include a hinge and CH1 domain. Traditional antibody heavy chains are monomers that are organized, from N- to C-terminus: VH-CH1-hinge-CH2-CH3. The CH1-hinge-CH2-CH3 is collectively referred to as the heavy chain “constant domain” or “constant region” of the antibody, of which there are five different categories or “isotypes”: IgA, IgD, IgG, IgE and IgM. Thus, “isotype” as used herein is meant any of the subclasses of immunoglobulins defined by the chemical and antigenic characteristics of their constant regions. It should be understood that therapeutic antibodies can also comprise hybrids of isotypes and/or subclasses. For example, as shown in US Publication 2009/0163699, incorporated by reference, the antibodies described herein include the use of human IgG1/G2 hybrids.

[00162] In some embodiments, the antibodies provided herein include IgG isotype constant domains, which has several subclasses, including, but not limited to IgG1, IgG2, IgG3, and IgG4. In the IgG subclass of immunoglobulins, there are several immunoglobulin domains in the heavy chain. By “immunoglobulin (Ig) domain” herein is meant a region of an immunoglobulin having a distinct tertiary structure. Of interest in the antibodies described herein are the heavy chain domains, including, the constant heavy (CH) domains and the

hinge domains. In the context of IgG antibodies, the IgG isotypes each have three CH regions. Accordingly, “CH” domains in the context of IgG are as follows: “CH1” refers to positions 118-220 according to the EU index as in Kabat. “CH2” refers to positions 237-340 according to the EU index as in Kabat, and “CH3” refers to positions 341-447 according to the EU index as in Kabat. As shown herein and described below, the pI variants can be in one or more of the CH regions, as well as the hinge region, discussed below.

[00163] It should be noted that IgG1 has different allotypes with polymorphisms at 356 (D or E) and 358 (L or M). The sequences depicted herein use the 356D/358M allotype, however the other allotype is included herein. That is, any sequence inclusive of an IgG1 Fc domain included herein can have 356E/358L replacing the 356D/358M allotype. It should be understood that therapeutic antibodies can also comprise hybrids of isotypes and/or subclasses. For example, as shown in US Publication 2009/0163699, incorporated by reference, the present antibodies, in some embodiments, include IgG1/IgG2 hybrids.

[00164] By “Fc” or “Fc region” or “Fc domain” as used herein is meant the polypeptide comprising the constant region of an antibody, in some instances, excluding all of the first constant region immunoglobulin domain (e.g., CH1) or a portion thereof, and in some cases, optionally including all or part of the hinge. For IgG, the Fc domain comprises immunoglobulin domains CH2 and CH3 (C γ 2 and C γ 3), and optionally all or a portion of the hinge region between CH1 (C γ 1) and CH2 (C γ 2). Thus, in some cases, the Fc domain includes, from N- to C-terminal, CH2-CH3 and hinge-CH2-CH3. In some embodiments, the Fc domain is that from IgG1, IgG2, IgG3 or IgG4, with IgG1 hinge-CH2-CH3 and IgG4 hinge-CH2-CH3 finding particular use in many embodiments. Additionally, in the case of human IgG1 Fc domains, frequently the hinge includes a C220S amino acid substitution. Furthermore, in the case of human IgG4 Fc domains, frequently the hinge includes a S228P amino acid substitution. Although the boundaries of the Fc region may vary, the human IgG heavy chain Fc region is usually defined to include residues E216, C226, or A231 to its carboxyl-terminal, wherein the numbering is according to the EU index as in Kabat. In some embodiments, as is more fully described below, amino acid modifications are made to the Fc region, for example to alter binding to one or more Fc γ R or to the FcRn.

[00165] By “heavy chain constant region” herein is meant the CH1-hinge-CH2-CH3 portion of an antibody (or fragments thereof), excluding the variable heavy domain; in EU numbering of human IgG1 this is amino acids 118-447. By “heavy chain constant region

fragment” herein is meant a heavy chain constant region that contains fewer amino acids from either or both of the N- and C-termini but still retains the ability to form a dimer with another heavy chain constant region.

[00166] Another type of Ig domain of the heavy chain is the hinge region. By “hinge” or “hinge region” or “antibody hinge region” or “hinge domain” herein is meant the flexible polypeptide comprising the amino acids between the first and second constant domains of an antibody. Structurally, the IgG CH1 domain ends at EU position 215, and the IgG CH2 domain begins at residue EU position 231. Thus for IgG the antibody hinge is herein defined to include positions 216 (E216 in IgG1) to 230 (p230 in IgG1), wherein the numbering is according to the EU index as in Kabat. In some cases, a “hinge fragment” is used, which contains fewer amino acids at either or both of the N- and C-termini of the hinge domain. As noted herein, pl variants can be made in the hinge region as well. Many of the antibodies herein have at least one the cysteines at position 220 according to EU numbering (hinge region) replaced by a serine. Generally, this modification is on the “scFv monomer” side for most of the sequences depicted herein, although it can also be on the “Fab monomer” side, or both, to reduce disulfide formation. Specifically included within the sequences herein are one or both of these cysteines replaced (C220S).

[00167] As will be appreciated by those in the art, the exact numbering and placement of the heavy constant region domains can be different among different numbering systems. A useful comparison of heavy constant region numbering according to EU and Kabat is as below, see Edelman et al., 1969, Proc Natl Acad Sci USA 63:78-85 and Kabat et al., 1991, Sequences of Proteins of Immunological Interest, 5th Ed., United States Public Health Service, National Institutes of Health, Bethesda, entirely incorporated by reference.

TABLE 1

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

[00168] The antibody light chain generally comprises two domains: the variable light domain (VL), which includes light chain CDRs vLCDR1-3, and a constant light chain region (often referred to as CL or Cκ). The antibody light chain is typically organized from N- to C-terminus: VL-CL.

[00169] By “antigen binding domain” or “ABD” herein is meant a set of six Complementary Determining Regions (CDRs) that, when present as part of a polypeptide sequence, specifically binds a target antigen (e.g., ENPP3 or CD3) as discussed herein. As is known in the art, these CDRs are generally present as a first set of variable heavy CDRs (vhCDRs or VHCDRs) and a second set of variable light CDRs (vLCDRs or VLCDRs), each comprising three CDRs: vhCDR1, vhCDR2, vhCDR3 variable heavy CDRs and vLCDR1, vLCDR2 and vLCDR3 variable light CDRs. The CDRs are present in the variable heavy domain (vhCDR1-3) and variable light domain (vLCDR1-3). The variable heavy domain and variable light domain form an Fv region.

[00170] The antibodies described herein provide a large number of different CDR sets. In this case, a “full CDR set” comprises the three variable light and three variable heavy CDRs, e.g., a vLCDR1, vLCDR2, vLCDR3, vhCDR1, vhCDR2 and vhCDR3. These can be part of a larger variable light or variable heavy domain, respectfully. In addition, as more fully outlined herein, the variable heavy and variable light domains can be on separate polypeptide chains, when a heavy and light chain is used (for example when Fabs are used), or on a single polypeptide chain in the case of scFv sequences.

[00171] As will be appreciated by those in the art, the exact numbering and placement of the CDRs can be different among different numbering systems. However, it should be understood that the disclosure of a variable heavy and/or variable light sequence includes the disclosure of the associated (inherent) CDRs. Accordingly, the disclosure of each variable heavy region is a disclosure of the vhCDRs (e.g., vhCDR1, vhCDR2 and vhCDR3) and the disclosure of each variable light region is a disclosure of the vLCDRs (e.g., vLCDR1, vLCDR2 and vLCDR3). A useful comparison of CDR numbering is as below, *see Lafranc et al., Dev. Comp. Immunol.* 27(1):55-77 (2003):

TABLE 2

	Kabat+ Chothia	IMGT	Kabat	AbM	Chothia	Contact	Xencor
vhCDR1	26-35	27-38	31-35	26-35	26-32	30-35	27-35
vhCDR2	50-65	56-65	50-65	50-58	52-56	47-58	54-61
vhCDR3	95-102	105-117	95-102	95-102	95-102	93-101	103-116
vlCDR1	24-34	27-38	24-34	24-34	24-34	30-36	27-38
vlCDR2	50-56	56-65	50-56	50-56	50-56	46-55	56-62
vlCDR3	89-97	105-117	89-97	89-97	89-97	89-96	97-105

[00172] Throughout the present specification, the Kabat numbering system is generally used when referring to a residue in the variable domain (approximately, residues 1-107 of the light chain variable region and residues 1-113 of the heavy chain variable region) and the EU numbering system for Fc regions (e.g., Kabat et al., supra (1991)).

[00173] The CDRs contribute to the formation of the antigen-binding, or more specifically, epitope binding site of the antigen binding domains and antibodies. “Epitope” refers to a determinant that interacts with a specific antigen binding site in the variable region of an antibody molecule known as a paratope. Epitopes are groupings of molecules such as amino acids or sugar side chains and usually have specific structural characteristics, as well as specific charge characteristics. A single antigen may have more than one epitope.

[00174] The epitope may comprise amino acid residues directly involved in the binding (also called immunodominant component of the epitope) and other amino acid residues, which are not directly involved in the binding, such as amino acid residues which are effectively blocked by the specifically antigen binding peptide; in other words, the amino acid residue is within the footprint of the specifically antigen binding peptide.

[00175] Epitopes may be either conformational or linear. A conformational epitope is produced by spatially juxtaposed amino acids from different segments of the linear polypeptide chain. A linear epitope is one produced by adjacent amino acid residues in a

polypeptide chain. Conformational and nonconformational epitopes may be distinguished in that the binding to the former but not the latter is lost in the presence of denaturing solvents.

[00176] An epitope typically includes at least 3, and more usually, at least 5 or 8-10 amino acids in a unique spatial conformation. Antibodies that recognize the same epitope can be verified in a simple immunoassay showing the ability of one antibody to block the binding of another antibody to a target antigen, for example “binning.” As outlined below, the disclosure not only includes the enumerated antigen binding domains and antibodies herein, but those that compete for binding with the epitopes bound by the enumerated antigen binding domains.

[00177] In some embodiments, the six CDRs of the antigen binding domain are contributed by a variable heavy and a variable light domain. In a “Fab” format, the set of 6 CDRs are contributed by two different polypeptide sequences, the variable heavy domain (vh or VH; containing the vhCDR1, vhCDR2 and vhCDR3) and the variable light domain (vl or VL; containing the vlCDR1, vlCDR2 and vlCDR3), with the C-terminus of the vh domain being attached to the N-terminus of the CH1 domain of the heavy chain and the C-terminus of the vl domain being attached to the N-terminus of the constant light domain (and thus forming the light chain). In a scFv format, the vh and vl domains are covalently attached, generally through the use of a linker (a “scFv linker”) as outlined herein, into a single polypeptide sequence, which can be either (starting from the N-terminus) vh-linker-vl or vl-linker-vh, with the former being generally preferred (including optional domain linkers on each side, depending on the format used (e.g., from Figure 1). In general, the C-terminus of the scFv domain is attached to the N-terminus of the hinge in the second monomer.

[00178] By “variable region” or “variable domain” as used herein is meant the region of an immunoglobulin that comprises one or more Ig domains substantially encoded by any of the V κ , V λ , and/or VH genes that make up the kappa, lambda, and heavy chain immunoglobulin genetic loci respectively, and contains the CDRs that confer antigen specificity. Thus, a “variable heavy domain” pairs with a “variable light domain” to form an antigen binding domain (“ABD”). In addition, each variable domain comprises three hypervariable regions (“complementary determining regions,” “CDRs”) (VHCDR1, VHCDR2 and VHCDR3 for the variable heavy domain and VLCDR1, VLCDR2 and VLCDR3 for the variable light domain) and four framework (FR) regions, arranged from amino-terminus to carboxy-terminus in the following order: FR1-CDR1-FR2-CDR2-FR3-

CDR3-FR4. The hypervariable region generally encompasses amino acid residues from about amino acid residues 24-34 (LCDR1; "L" denotes light chain), 50-56 (LCDR2) and 89-97 (LCDR3) in the light chain variable region and around about 31-35B (HCDR1; "H" denotes heavy chain), 50-65 (HCDR2), and 95-102 (HCDR3) in the heavy chain variable region; Kabat et al., SEQUENCES OF PROTEINS OF IMMUNOLOGICAL INTEREST, 5th Ed. Public Health Service, National Institutes of Health, Bethesda, Md. (1991) and/or those residues forming a hypervariable loop (e.g. residues 26-32 (LCDR1), 50-52 (LCDR2) and 91-96 (LCDR3) in the light chain variable region and 26-32 (HCDR1), 53-55 (HCDR2) and 96-101 (HCDR3) in the heavy chain variable region; Chothia and Lesk (1987) J. Mol. Biol. 196:901-917. Specific CDRs of the invention are described in Table 2.

[00179] By "Fab" or "Fab region" as used herein is meant the polypeptide that comprises the VH, CH1, VL, and CL immunoglobulin domains, generally on two different polypeptide chains (e.g. VH-CH1 on one chain and VL-CL on the other). Fab may refer to this region in isolation, or this region in the context of a bispecific antibody described herein. In the context of a Fab, the Fab comprises an Fv region in addition to the CH1 and CL domains.

[00180] By "Fv" or "Fv fragment" or "Fv region" as used herein is meant a polypeptide that comprises the VL and VH domains of an ABD. Fv regions can be formatted as both Fabs (as discussed above, generally two different polypeptides that also include the constant regions as outlined above) and scFvs, where the VL and VH domains are combined (generally with a linker as discussed herein) to form an scFv.

[00181] By "single chain Fv" or "scFv" herein is meant a variable heavy domain covalently attached to a variable light domain, generally using a scFv linker as discussed herein, to form a scFv or scFv domain. A scFv domain can be in either orientation from N- to C-terminus (VH-linker-VL or VL-linker-VH). In the sequences depicted in the sequence listing and in the figures, the order of the VH and VL domain is indicated in the name, e.g. H.X_L.Y means N- to C-terminal is VH-linker-VL, and L.Y_H.X is VL-linker-VH.

[00182] Some embodiments of the subject antibodies provided herein comprise at least one scFv domain, which, while not naturally occurring, generally includes a variable heavy domain and a variable light domain, linked together by a scFv linker. As outlined herein, while the scFv domain is generally from N- to C-terminus oriented as VH-scFv linker-VL, this can be reversed for any of the scFv domains (or those constructed using vh and vl

sequences from Fabs), to VL-scFv linker-VH, with optional linkers at one or both ends depending on the format.

[00183] By "modification" herein is meant an amino acid substitution, insertion, and/or deletion in a polypeptide sequence or an alteration to a moiety chemically linked to a protein. For example, a modification may be an altered carbohydrate or PEG structure attached to a protein. By "amino acid modification" herein is meant an amino acid substitution, insertion, and/or deletion in a polypeptide sequence. For clarity, unless otherwise noted, the amino acid modification is always to an amino acid coded for by DNA, e.g. the 20 amino acids that have codons in DNA and RNA.

[00184] By "amino acid substitution" or "substitution" herein is meant the replacement of an amino acid at a particular position in a parent polypeptide sequence with a different amino acid. In particular, in some embodiments, the substitution is to an amino acid that is not naturally occurring at the particular position, either not naturally occurring within the organism or in any organism. For example, the substitution E272Y refers to a variant polypeptide, in this case an Fc variant, in which the glutamic acid at position 272 is replaced with tyrosine. For clarity, a protein which has been engineered to change the nucleic acid coding sequence but not change the starting amino acid (for example exchanging CGG (encoding arginine) to CGA (still encoding arginine) to increase host organism expression levels) is not an "amino acid substitution"; that is, despite the creation of a new gene encoding the same protein, if the protein has the same amino acid at the particular position that it started with, it is not an amino acid substitution.

[00185] By "amino acid insertion" or "insertion" as used herein is meant the addition of an amino acid sequence at a particular position in a parent polypeptide sequence. For example, -233E or 233E designates an insertion of glutamic acid after position 233 and before position 234. Additionally, -233ADE or A233ADE designates an insertion of AlaAspGlu after position 233 and before position 234.

[00186] By "amino acid deletion" or "deletion" as used herein is meant the removal of an amino acid sequence at a particular position in a parent polypeptide sequence. For example, E233- or E233#, E233() or E233del designates a deletion of glutamic acid at position 233. Additionally, EDA233- or EDA233# designates a deletion of the sequence GluAspAla that begins at position 233.

[00187] By "variant protein" or "protein variant", or "variant" as used herein is meant a protein that differs from that of a parent protein by virtue of at least one amino acid modification. The protein variant has at least one amino acid modification compared to the parent protein, yet not so many that the variant protein will not align with the parental protein using an alignment program such as that described below. In general, variant proteins (such as variant Fc domains, etc., outlined herein, are generally at least 75, 80, 85, 90, 91, 92, 93, 94, 95, 96, 97, 98 or 99% identical to the parent protein, using the alignment programs described below, such as BLAST. "Variant" as used herein also refers to particular amino acid modifications that confer particular function (e.g., a "heterodimerization variant," "pI variant," "ablation variant," etc.).

[00188] As described below, in some embodiments the parent polypeptide, for example an Fc parent polypeptide, is a human wild type sequence, such as the heavy constant domain or Fc region from IgG1, IgG2, IgG3 or IgG4, although human sequences with variants can also serve as "parent polypeptides", for example the IgG1/2 hybrid of US Publication 2006/0134105 can be included. The protein variant sequence herein will preferably possess at least about 80% identity with a parent protein sequence, and most preferably at least about 90% identity, more preferably at least about 95-98-99% identity. Accordingly, by "antibody variant" or "variant antibody" as used herein is meant an antibody that differs from a parent antibody by virtue of at least one amino acid modification, "IgG variant" or "variant IgG" as used herein is meant an antibody that differs from a parent IgG (again, in many cases, from a human IgG sequence) by virtue of at least one amino acid modification, and "immunoglobulin variant" or "variant immunoglobulin" as used herein is meant an immunoglobulin sequence that differs from that of a parent immunoglobulin sequence by virtue of at least one amino acid modification. "Fc variant" or "variant Fc" as used herein is meant a protein comprising an amino acid modification in an Fc domain as compared to an Fc domain of human IgG1, IgG2 or IgG4.

[00189] "Fc variant" or "variant Fc" as used herein is meant a protein comprising an amino acid modification in an Fc domain. The modification can be an addition, deletion, or substitution. The Fc variants are defined according to the amino acid modifications that compose them. Thus, for example, N434S or 434S is an Fc variant with the substitution for serine at position 434 relative to the parent Fc polypeptide, wherein the numbering is according to the EU index. Likewise, M428L/N434S defines an Fc variant with the

substitutions M428L and N434S relative to the parent Fc polypeptide. The identity of the WT amino acid may be unspecified, in which case the aforementioned variant is referred to as 428L/434S. It is noted that the order in which substitutions are provided is arbitrary, that is to say that, for example, 428L/434S is the same Fc variant as 434S/428L, and so on. For all positions discussed herein that relate to antibodies or derivatives and fragments thereof (e.g., Fc domains), unless otherwise noted, amino acid position numbering is according to the EU index. The "EU index" or "EU index as in Kabat" or "EU numbering" scheme refers to the numbering of the EU antibody (Edelman et al., 1969, Proc Natl Acad Sci USA 63:78-85, hereby entirely incorporated by reference).

[00190] In general, variant Fc domains have at least about 80, 85, 90, 95, 97, 98 or 99 percent identity to the corresponding parental human IgG Fc domain (using the identity algorithms discussed below, with one embodiment utilizing the BLAST algorithm as is known in the art, using default parameters). Alternatively, the variant Fc domains can have from 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19 or 20 amino acid modifications as compared to the parental Fc domain. Alternatively, the variant Fc domains can have up to 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19 or 20 amino acid modifications as compared to the parental Fc domain. Additionally, as discussed herein, the variant Fc domains described herein still retain the ability to form a dimer with another Fc domain as measured using known techniques as described herein, such as non-denaturing gel electrophoresis.

[00191] By "protein" herein is meant at least two covalently attached amino acids, which includes proteins, polypeptides, oligopeptides and peptides. In addition, polypeptides that make up the antibodies described herein may include synthetic derivatization of one or more side chains or termini, glycosylation, PEGylation, circular permutation, cyclization, linkers to other molecules, fusion to proteins or protein domains, and addition of peptide tags or labels.

[00192] By "residue" as used herein is meant a position in a protein and its associated amino acid identity. For example, Asparagine 297 (also referred to as Asn297 or N297) is a residue at position 297 in the human antibody IgG1.

[00193] By "IgG subclass modification" or "isotype modification" as used herein is meant an amino acid modification that converts one amino acid of one IgG isotype to the corresponding amino acid in a different, aligned IgG isotype. For example, because IgG1

comprises a tyrosine and IgG2 a phenylalanine at EU position 296, a F296Y substitution in IgG2 is considered an IgG subclass modification.

[00194] By "non-naturally occurring modification" as used herein is meant an amino acid modification that is not isotypic. For example, because none of the human IgGs comprise a serine at position 434, the substitution 434S in IgG1, IgG2, IgG3, or IgG4 (or hybrids thereof) is considered a non-naturally occurring modification.

[00195] By "amino acid" and "amino acid identity" as used herein is meant one of the 20 naturally occurring amino acids that are coded for by DNA and RNA.

[00196] By "effector function" as used herein is meant a biochemical event that results from the interaction of an antibody Fc region with an Fc receptor or ligand. Effector functions include but are not limited to ADCC, ADCP, and CDC.

[00197] By "IgG Fc ligand" as used herein is meant a molecule, preferably a polypeptide, from any organism that binds to the Fc region of an IgG antibody to form an Fc/Fc ligand complex. Fc ligands include but are not limited to Fc γ RI, Fc γ RII, Fc γ RIII, FcRn, C1q, C3, mannan binding lectin, mannose receptor, staphylococcal protein A, streptococcal protein G, and viral Fc γ R. Fc ligands also include Fc receptor homologs (FcRH), which are a family of Fc receptors that are homologous to the Fc γ Rs (Davis et al., 2002, Immunological Reviews 190:123-136, entirely incorporated by reference). Fc ligands may include undiscovered molecules that bind Fc. Particular IgG Fc ligands are FcRn and Fc gamma receptors. By "Fc ligand" as used herein is meant a molecule, preferably a polypeptide, from any organism that binds to the Fc region of an antibody to form an Fc/Fc ligand complex.

[00198] By "Fc gamma receptor", "Fc γ R" or "FcgammaR" as used herein is meant any member of the family of proteins that bind the IgG antibody Fc region and is encoded by an Fc γ R gene. In humans this family includes but is not limited to Fc γ RI (CD64), including isoforms Fc γ RIa, Fc γ RIb, and Fc γ RIc; Fc γ RII (CD32), including isoforms Fc γ RIIa (including allotypes H131 and R131), Fc γ RIIb (including Fc γ RIIb-1 and Fc γ RIIb-2), and Fc γ RIIc; and Fc γ RIII (CD16), including isoforms Fc γ RIIIa (including allotypes V158 and F158) and Fc γ RIIIb (including allotypes Fc γ RIIb-NA1 and Fc γ RIIb-NA2) (Jefferis et al., 2002, Immunol Lett 82:57-65, entirely incorporated by reference), as well as any undiscovered human Fc γ Rs or Fc γ R isoforms or allotypes. An Fc γ R may be from any

organism, including but not limited to humans, mice, rats, rabbits, and monkeys. Mouse FcγRs include but are not limited to FcγRI (CD64), FcγRII (CD32), FcγRIII (CD16), and FcγRIII-2 (CD16-2), as well as any undiscovered mouse FcγRs or FcγR isoforms or allotypes.

[00199] By "FcRn" or "neonatal Fc Receptor" as used herein is meant a protein that binds the IgG antibody Fc region and is encoded at least in part by an FcRn gene. The FcRn may be from any organism, including but not limited to humans, mice, rats, rabbits, and monkeys. As is known in the art, the functional FcRn protein comprises two polypeptides, often referred to as the heavy chain and light chain. The light chain is beta-2-microglobulin and the heavy chain is encoded by the FcRn gene. Unless otherwise noted herein, FcRn or an FcRn protein refers to the complex of FcRn heavy chain with beta-2-microglobulin. A variety of FcRn variants used to increase binding to the FcRn receptor, and in some cases, to increase serum half-life. An "FcRn variant" is one that increases binding to the FcRn receptor, and suitable FcRn variants are shown below.

[00200] By "parent polypeptide" as used herein is meant a starting polypeptide that is subsequently modified to generate a variant. The parent polypeptide may be a naturally occurring polypeptide, or a variant or engineered version of a naturally occurring polypeptide. Accordingly, by "parent immunoglobulin" as used herein is meant an unmodified immunoglobulin polypeptide that is modified to generate a variant, and by "parent antibody" as used herein is meant an unmodified antibody that is modified to generate a variant antibody. It should be noted that "parent antibody" includes known commercial, recombinantly produced antibodies as outlined below. In this context, a "parent Fc domain" will be relative to the recited variant; thus, a "variant human IgG1 Fc domain" is compared to the parent Fc domain of human IgG1, a "variant human IgG4 Fc domain" is compared to the parent Fc domain human IgG4, etc.

[00201] By "position" as used herein is meant a location in the sequence of a protein. Positions may be numbered sequentially, or according to an established format, for example the EU index for antibody numbering.

[00202] By "target antigen" as used herein is meant the molecule that is bound specifically by the antigen binding domain comprising the variable regions of a given antibody.

[00203] By “strandedness” in the context of the monomers of the heterodimeric antibodies described herein is meant that, similar to the two strands of DNA that “match”, heterodimerization variants are incorporated into each monomer so as to preserve the ability to “match” to form heterodimers. For example, if some pI variants are engineered into monomer A (e.g. making the pI higher) then steric variants that are “charge pairs” that can be utilized as well do not interfere with the pI variants, e.g. the charge variants that make a pI higher are put on the same “strand” or “monomer” to preserve both functionalities. Similarly, for “skew” variants that come in pairs of a set as more fully outlined below, the skilled artisan will consider pI in deciding into which strand or monomer one set of the pair will go, such that pI separation is maximized using the pI of the skews as well.

[00204] By “target cell” as used herein is meant a cell that expresses a target antigen.

[00205] By “host cell” in the context of producing a bispecific antibody according to the antibodies described herein is meant a cell that contains the exogenous nucleic acids encoding the components of the bispecific antibody and is capable of expressing the bispecific antibody under suitable conditions. Suitable host cells are discussed below.

[00206] By “wild type or WT” herein is meant an amino acid sequence or a nucleotide sequence that is found in nature, including allelic variations. A WT protein has an amino acid sequence or a nucleotide sequence that has not been intentionally modified.

[00207] Provided herein are a number of antibody domains that have sequence identity to human antibody domains. Sequence identity between two similar sequences (e.g., antibody variable domains) can be measured by algorithms such as that of Smith, T.F. & Waterman, M.S. (1981) “Comparison Of Biosequences,” Adv. Appl. Math. 2:482 [local homology algorithm]; Needleman, S.B. & Wunsch, CD. (1970) “A General Method Applicable To The Search For Similarities In The Amino Acid Sequence Of Two Proteins,” J. Mol. Biol. 48:443 [homology alignment algorithm], Pearson, W.R. & Lipman, D.J. (1988) “Improved Tools For Biological Sequence Comparison,” Proc. Natl. Acad. Sci. (U.S.A.) 85:2444 [search for similarity method]; or Altschul, S.F. et al, (1990) “Basic Local Alignment Search Tool,” J. Mol. Biol. 215:403-10, the “BLAST” algorithm, see <https://blast.ncbi.nlm.nih.gov/Blast.cgi>. When using any of the aforementioned algorithms, the default parameters (for Window length, gap penalty, etc) are used. In one embodiment, sequence identity is done using the BLAST algorithm, using default parameters

[00208] The antibodies described herein are generally isolated or recombinant. “Isolated,” when used to describe the various polypeptides disclosed herein, means a polypeptide that has been identified and separated and/or recovered from a cell or cell culture from which it was expressed. Ordinarily, an isolated polypeptide will be prepared by at least one purification step. An “isolated antibody,” refers to an antibody which is substantially free of other antibodies having different antigenic specificities. “Recombinant” means the antibodies are generated using recombinant nucleic acid techniques in exogenous host cells, and they can be isolated as well.

[00209] “Specific binding” or “specifically binds to” or is “specific for” a particular antigen or an epitope means binding that is measurably different from a non-specific interaction. Specific binding can be measured, for example, by determining binding of a molecule compared to binding of a control molecule, which generally is a molecule of similar structure that does not have binding activity. For example, specific binding can be determined by competition with a control molecule that is similar to the target.

[00210] Specific binding for a particular antigen or an epitope can be exhibited, for example, by an antibody having a K_D for an antigen or epitope of at least about 10^{-4} M, at least about 10^{-5} M, at least about 10^{-6} M, at least about 10^{-7} M, at least about 10^{-8} M, at least about 10^{-9} M, alternatively at least about 10^{-10} M, at least about 10^{-11} M, at least about 10^{-12} M, or greater, where K_D refers to a dissociation rate of a particular antibody-antigen interaction. Typically, an antibody that specifically binds an antigen will have a K_D that is 20-, 50-, 100-, 500-, 1000-, 5,000-, 10,000- or more times greater for a control molecule relative to the antigen or epitope.

[00211] Also, specific binding for a particular antigen or an epitope can be exhibited, for example, by an antibody having a K_A or K_a for an antigen or epitope of at least 20-, 50-, 100-, 500-, 1000-, 5,000-, 10,000- or more times greater for the epitope relative to a control, where K_A or K_a refers to an association rate of a particular antibody-antigen interaction. Binding affinity is generally measured using a Biacore, SPR or BLI assay.

IV. ENPP3 Binding Domains

[00212] In one aspect, provided herein are ENPP3 antigen binding domains (ABDs) and compositions that include such ENPP3 antigen binding domains (ABDs), including anti-

ENPP3 antibodies. Subject antibodies that include such ENPP3 antigen binding domains (e.g., anti-ENPP3 x anti-CD3 bispecific antibodies) advantageously elicit a range of different immune responses (see Examples 5 and 6). Such ENPP3 binding domains and related antibodies find use, for example, in the treatment of ENPP3 associated cancers.

[00213] As will be appreciated by those in the art, suitable ENPP3 binding domains can comprise a set of 6 CDRs as depicted in the sequence listing and Figures 12, 13A-13B, and 14A-14I, either as the CDRs are underlined or, in the case where a different numbering scheme is used as described herein and as shown in Table 2, as the CDRs that are identified using other alignments within the variable heavy (VH) domain and variable light domain (VL) sequences of those depicted in Figures 12, 13A-13B, and 14A-14I and the Sequence Listing (see Table 2). Suitable ENPP3 ABDs can also include the entire VH and VL sequences as depicted in these sequences and figures, used as scFvs or as Fab domains.

[00214] In one embodiment, the ENPP3 antigen binding domain includes the 6 CDRs (i.e., vhCDR1-3 and vlCDR1-3) of a ENPP3 ABD described herein, including the figures and sequence listing. In exemplary embodiments, the ENPP3 ABD is one of the following ENPP3 ABDs: AN1[ENPP3] H1L1, AN1[ENPP3] H1 L1.33, AN1[ENPP3] H1 L1.77, AN1[ENPP3] H1.8 L1, AN1[ENPP3] H1.8 L1.33, AN1[ENPP3] H1 L1.77, H16-7.213, H16-9.69, H16-1.52, Ha16-1(1)23, H16-9.44, H16-1.67, Ha1 6-1 (3,5)36, H16-1.86, H16-9.10, H16-9.33, H16-1.68, Ha16-1(1)1, Ha1 6-1(3,5)18, Ha16-1(2,4)4, Ha16-1(3,5)56, H16-7.8, H16-1.93, Ha1 6-1(3,5)27.1, H16-1.61, H16-1(3,5)5, H16-7.200, H16-1(3,5)42, H1 6-9.65, Ha1-1(3,5)19, and Ha16-1.80(Figures 12, 13A-13B, and 14A-14I).

[00215] In addition to the parental CDR sets disclosed in the figures and sequence listing that form an ABD to ENPP3, provided herein are variant ENPP3 ABDs having CDRs that include at least one modification of the ENPP3 ABD CDRs disclosed herein. In one embodiment, the ENPP3 ABD includes a set of 6 CDRs with 1, 2, 3, 4, 5, 6, 7, 8, 9, 10 amino acid modifications as compared to the 6 CDRs of a ENPP3 ABD described herein, including the figures and sequence listing. In exemplary embodiments, the ENPP3 ABD includes a set of 6 CDRs with 1, 2, 3, 4, 5, 6, 7, 8, 9, 10 amino acid modifications as compared to the 6 CDRs of one of the following ENPP3 ABDs: AN1[ENPP3] H1L1, AN1[ENPP3] H1 L1.33, AN1[ENPP3] H1 L1.77, AN1[ENPP3] H1.8 L1, AN1[ENPP3] H1.8 L1.33, AN1[ENPP3] H1 L1.77, H16-7.213, H16-9.69, H16-1.52, Ha16-1(1)23, H16-9.44, H16-1.67, Ha1 6-1 (3,5)36, H16-1.86, H16-9.10, H16-9.33, H16-1.68, Ha16-1(1)1, Ha1 6-1(3,5)18, Ha16-

1(2,4)4, Ha16-1(3,5)56, H16-7.8, H16-1.93, Ha1 6-1(3,5)27.1, H16-1.61, H16-1(3,5)5, H16-7.200, H16-1(3,5)42, H1 6-9.65, Ha1-1(3,5)19, and Ha16-1.80(Figures 12, 13A-13B, and 14A-14I). In certain embodiments, the variant ENPP3 ABD is capable of binding ENPP3 antigen, as measured by at least one of a Biacore, surface plasmon resonance (SPR) and/or BLI (biolayer interferometry, e.g., Octet assay) assay, with the latter finding particular use in many embodiments. In particular embodiments, the ENPP3 ABD is capable of binding human ENPP3 antigen (see Example 5).

[00216] In one embodiment, the ENPP3 ABD includes 6 CDRs that are at least 90, 95, 97, 98 or 99% identical to the 6 CDRs of a ENPP3 ABD as described herein, including the figures and sequence listing. In exemplary embodiments, the ENPP3 ABD includes 6 CDRs that are at least 90, 95, 97, 98 or 99% identical to the 6 CDRs of one of the following ENPP3 ABDs: AN1[ENPP3] H1L1, AN1[ENPP3] H1 L1.33, AN1[ENPP3] H1 L1.77, AN1[ENPP3] H1.8 L1, AN1[ENPP3] H1.8 L1.33, AN1[ENPP3] H1 L1.77, H16-7.213, H16-9.69, H16-1.52, Ha16-1(1)23, H16-9.44, H16-1.67, Ha1 6-1 (3,5)36, H16-1.86, H16-9.10, H16-9.33, H16-1.68, Ha16-1(1)1, Ha1 6-1(3,5)18, Ha16-1(2,4)4, Ha16-1(3,5)56, H16-7.8, H16-1.93, Ha1 6-1(3,5)27.1, H16-1.61, H16-1(3,5)5, H16-7.200, H16-1(3,5)42, H1 6-9.65, Ha1-1(3,5)19, and Ha16-1.80(Figures 12, 13A-13B, and 14A-14I). In certain embodiments, the ENPP3 ABD is capable of binding to ENPP3 antigen, as measured by at least one of a Biacore, surface plasmon resonance (SPR) and/or BLI (biolayer interferometry, e.g., Octet assay) assay, with the latter finding particular use in many embodiments. In particular embodiments, the ENPP3 ABD is capable of binding human ENPP3 antigen (see Figure 2).

[00217] In another exemplary embodiment, the ENPP3 ABD include the variable heavy (VH) domain and variable light (VL) domain of any one of the ENPP3 ABDs described herein, including the figures and sequence listing. In exemplary embodiments, the ENPP3 ABD is one of the following ENPP3 ABDs: AN1[ENPP3] H1L1, AN1[ENPP3] H1 L1.33, AN1[ENPP3] H1 L1.77, AN1[ENPP3] H1.8 L1, AN1[ENPP3] H1.8 L1.33, AN1[ENPP3] H1 L1.77, H16-7.213, H16-9.69, H16-1.52, Ha16-1(1)23, H16-9.44, H16-1.67, Ha1 6-1 (3,5)36, H16-1.86, H16-9.10, H16-9.33, H16-1.68, Ha16-1(1)1, Ha1 6-1(3,5)18, Ha16-1(2,4)4, Ha16-1(3,5)56, H16-7.8, H16-1.93, Ha1 6-1(3,5)27.1, H16-1.61, H16-1(3,5)5, H16-7.200, H16-1(3,5)42, H1 6-9.65, Ha1-1(3,5)19, and Ha16-1.80(Figures 12, 13A-13B, and 14A-14I).

[00218] In addition to the parental ENPP3 variable heavy and variable light domains disclosed herein, provided herein are ENPP3 ABDs that include a variable heavy domain and/or a variable light domain that are variants of a ENPP3 ABD VH and VL domain disclosed herein. In one embodiment, the variant VH domain and/or VL domain has from 1, 2, 3, 4, 5, 6, 7, 8, 9 or 10 amino acid changes from a VH and/or VL domain of a ENPP3 ABD described herein, including the figures and sequence listing. In exemplary embodiments, the variant VH domain and/or VL domain has from 1, 2, 3, 4, 5, 6, 7, 8, 9 or 10 amino acid changes from a VH and/or VL domain of one of the following ENPP3 ABDs: AN1[ENPP3] H1L1, AN1[ENPP3] H1 L1.33, AN1[ENPP3] H1 L1.77, AN1[ENPP3] H1.8 L1, AN1[ENPP3] H1.8 L1.33, AN1[ENPP3] H1 L1.77, H16-7.213, H16-9.69, H16-1.52, Ha16-1(1)23, H16-9.44, H16-1.67, Ha1 6-1 (3,5)36, H16-1.86, H16-9.10, H16-9.33, H16-1.68, Ha16-1(1)1, Ha1 6-1(3,5)18, Ha16-1(2,4)4, Ha16-1(3,5)56, H16-7.8, H16-1.93, Ha1 6-1(3,5)27.1, H16-1.61, H16-1(3,5)5, H16-7.200, H16-1(3,5)42, H1 6-9.65, Ha1-1(3,5)19, and Ha16-1.80(Figures 12, 13A-13B, and 14A-14I). In certain embodiments, the ENPP3 ABD is capable of binding to ENPP3, as measured at least one of a Biacore, surface plasmon resonance (SPR) and/or BLI (biolayer interferometry, e.g., Octet assay) assay, with the latter finding particular use in many embodiments. In particular embodiments, the ENPP3 ABD is capable of binding human ENPP3 antigen (see Example 5).

[00219] In one embodiment, the variant VH and/or VL domain is at least 90, 95, 97, 98 or 99% identical to the VH and/or VL of a ENPP3 ABD as described herein, including the figures and sequence listing. In exemplary embodiments, the variant VH and/or VL domain is at least 90, 95, 97, 98 or 99% identical to the VH and/or VL of one of the following ENPP3 ABDs: AN1[ENPP3] H1L1, AN1[ENPP3] H1 L1.33, AN1[ENPP3] H1 L1.77, AN1[ENPP3] H1.8 L1, AN1[ENPP3] H1.8 L1.33, AN1[ENPP3] H1 L1.77, H16-7.213, H16-9.69, H16-1.52, Ha16-1(1)23, H16-9.44, H16-1.67, Ha1 6-1 (3,5)36, H16-1.86, H16-9.10, H16-9.33, H16-1.68, Ha16-1(1)1, Ha1 6-1(3,5)18, Ha16-1(2,4)4, Ha16-1(3,5)56, H16-7.8, H16-1.93, Ha1 6-1(3,5)27.1, H16-1.61, H16-1(3,5)5, H16-7.200, H16-1(3,5)42, H1 6-9.65, Ha1-1(3,5)19, and Ha16-1.80 (Figures 12, 13A-13B, and 14A-14I). In certain embodiments, the ENPP3 ABD is capable of binding to the ENPP3, as measured by at least one of a Biacore, surface plasmon resonance (SPR) and/or BLI (biolayer interferometry, e.g., Octet assay) assay, with the latter finding particular use in many embodiments. In particular

embodiments, the ENPP3 ABD is capable of binding human ENPP3 antigen (see Example 5).

V. Antibodies

[00220] In one aspect, provided herein are antibodies that bind to ENPP3 (e.g., anti-ENPP3 antibodies). In certain embodiments, the antibody binds to human ENPP3 (Figure 11A). Subject anti-ENPP3 antibodies include monospecific ENPP3 antibodies, as well as multi-specific (e.g., bispecific) anti-ENPP3 antibodies. In certain embodiments, the anti-ENPP3 antibody has a format according to any one of the antibody formats depicted in Figure 15A, 15B, and 52A-52K.

[00221] In some embodiments, the subject compositions include an ENPP3 binding domain. In some embodiments, the composition includes an antibody having an ENPP3 binding domain. Antibodies provided herein include one, two, three, four, and five or more ENPP3 binding domains. In certain embodiments, the ENPP3 binding domain includes any one of the vhCDR1, vhCDR2, vhCDR3, vlCDR1, vlCDR2 and vlCDR3 sequences of an ENPP3 binding domain selected from those depicted in Figures 12, 13A-13B, and 14A-14I. In some embodiments, the ENPP3 binding domain includes the underlined vhCDR1, vhCDR2, vhCDR3, vlCDR1, vlCDR2 and vlCDR3 sequences of a ENPP3 binding domain selected from those depicted in Figures 12, 13A-13B, and 14A-14I. In some embodiments, the ENPP3 binding domain includes the variable heavy domain and variable light domain of a ENPP3 binding domain selected from those depicted in Figures 12, 13A-13B, and 14A-14I. ENPP3 binding domains depicted in Figures 12, 13A-13B, and 14A-14I include:

AN1[ENPP3] H1L1, AN1[ENPP3] H1 L1.33, AN1[ENPP3] H1 L1.77, AN1[ENPP3] H1.8 L1, AN1[ENPP3] H1.8 L1.33, AN1[ENPP3] H1 L1.77, H16-7.213, H16-9.69, H16-1.52, Ha16-1(1)23, H16-9.44, H16-1.67, Ha1 6-1 (3,5)36, H16-1.86, H16-9.10, H16-9.33, H16-1.68, Ha16-1(1)1, Ha1 6-1(3,5)18, Ha16-1(2,4)4, Ha16-1(3,5)56, H16-7.8, H16-1.93, Ha1 6-1(3,5)27.1, H16-1.61, H16-1(3,5)5, H16-7.200, H16-1(3,5)42, H1 6-9.65, Ha1-1(3,5)19, and Ha16-1.80.

[00222] In one aspect, provided herein are bispecific antibodies that bind to ENPP3 and CD3, in various formats as outlined below, and generally depicted in Figures 15A and 15B. These bispecific, heterodimeric antibodies include a ENPP3 binding domain. In certain embodiments, the ENPP3 binding domain includes the VHCDR1, VHCDR2, VHCDR3,

VLCDR1, VLCDR2 and VLCDR3 sequences of an ENPP3 binding domain selected from the group consisting of those depicted in Figures 12, 13A-13B, and 14A-14I. In some embodiments, the ENPP3 binding domain includes the underlined VHCDR1, VHCDR2, VHCDR3, VLCDR1, VLCDR2 and VLCDR3 sequences of an ENPP3 binding domain selected from those depicted in Figures 12, 13A-13B, and 14A-14I.

[00223] These bispecific heterodimeric antibodies bind ENPP3 and CD3. Such antibodies include a CD3 binding domain and at least one ENPP3 binding domain. Any suitable ENPP3 binding domain can be included in the anti-ENPP3 X anti-CD3 bispecific antibody. In some embodiments, the anti-ENPP3 X anti-CD3 bispecific antibody includes one, two, three, four or more ENPP3 binding domains, including but not limited to those depicted in Figures 12, 13A-13B, and 14A-14I. In certain embodiments, the anti-ENPP3 X anti-CD3 antibody includes an ENPP3 binding domain that includes the VHCDR1, VHCDR2, VHCDR3, VLCDR1, VLCDR2 and VLCDR3 sequences of an ENPP3 binding domain selected from the group consisting of those depicted in Figures 12, 13A-13B, and 14A-14I. In some embodiments, the anti-ENPP3 X anti-CD3 antibody includes a ENPP3 binding domain that includes the underlined VHCDR1, VHCDR2, VHCDR3, VLCDR1, VLCDR2 and VLCDR3 sequences of an ENPP3 binding domain selected from the group consisting of those depicted in Figures 12, 13A-13B, and 14A-14I. In some embodiments, the anti-ENPP3 X anti-CD3 antibody includes a ENPP3 binding domain that includes the variable heavy domain and variable light domain of an ENPP3 binding domain selected from the group consisting of those depicted in Figures 12, 13A-13B, and 14A-14I. In an exemplary embodiment, the anti-ENPP3 X anti-CD3 antibody includes an anti-ENPP3 AN1[ENPP3]_H1L1 binding domain.

[00224] The anti-ENPP3 x anti-CD3 antibody provided herein can include any suitable CD3 binding domain. In certain embodiments, the anti-ENPP3 X anti-CD3 antibody includes a CD3 binding domain that includes the VHCDR1, VHCDR2, VHCDR3, VLCDR1, VLCDR2 and VLCDR3 sequences of a CD3 binding domain selected from the group consisting of those depicted in Figure 10A-F. In some embodiments, the anti-ENPP3 X anti-CD3 antibody includes a CD3 binding domain that includes the underlined VHCDR1, VHCDR2, VHCDR3, VLCDR1, VLCDR2 and VLCDR3 sequences of a CD3 binding domain selected from the group consisting of those depicted in Figure 10A-10F. In some embodiments, the anti-ENPP3 X anti-CD3 antibody includes a CD3 binding domain that

includes the variable heavy domain and variable light domain of a CD3 binding domain selected from the group consisting of those depicted in Figure 10A-10F. In some embodiments, the CD3 binding domain is selected from anti-CD3 H1.30_L1.47, anti-CD3 H1.32_L1.47; anti-CD3 H1.89_L1.48; anti-CD3 H1.90_L1.47; Anti-CD3 H1.33_L1.47; and anti-CD3 H1.31_L1.47. As outlined herein, these anti-CD3 antigen binding domains (CD3-ABDs) can be used in scFv formats in either orientation (e.g. from N- to C-terminal, VH-scFv linker-VL or VL-scFv linker-VH).

[00225] The antibodies provided herein include different antibody domains. As described herein and known in the art, the antibodies described herein include different domains within the heavy and light chains, which can be overlapping as well. These domains include, but are not limited to, the Fc domain, the CH1 domain, the CH2 domain, the CH3 domain, the hinge domain, the heavy constant domain (CH1-hinge-Fc domain or CH1-hinge-CH2-CH3), the variable heavy domain, the variable light domain, the light constant domain, Fab domains and scFv domains.

[00226] As shown herein, there are a number of suitable linkers (for use as either domain linkers or scFv linkers) that can be used to covalently attach the recited domains (e.g., scFvs, Fabs, Fc domains, etc.), including traditional peptide bonds, generated by recombinant techniques. Exemplary linkers to attach domains of the subject antibody to each other are depicted in Figure 6. In some embodiments, the linker peptide may predominantly include the following amino acid residues: Gly, Ser, Ala, or Thr. The linker peptide should have a length that is adequate to link two molecules in such a way that they assume the correct conformation relative to one another so that they retain the desired activity. In one embodiment, the linker is from about 1 to 50 amino acids in length, preferably about 1 to 30 amino acids in length. In one embodiment, linkers of 1 to 20 amino acids in length may be used, with from about 5 to about 10 amino acids finding use in some embodiments. Useful linkers include glycine-serine polymers, including for example (GS)_n, (GSGGS)_n, (GGGGS)_n, and (GGGS)_n, where n is an integer of at least one (and generally from 3 to 4), glycine-alanine polymers, alanine-serine polymers, and other flexible linkers, some of which are shown in Figure 5 and Figure 6. Alternatively, a variety of nonproteinaceous polymers, including but not limited to polyethylene glycol (PEG), polypropylene glycol, polyoxyalkylenes, or copolymers of polyethylene glycol and polypropylene glycol, may find use as linkers.

[00227] Other linker sequences may include any sequence of any length of CL/CH1 domain but not all residues of CL/CH1 domain; for example the first 5-12 amino acid residues of the CL/CH1 domains. Linkers can be derived from immunoglobulin light chain, for example C κ or C λ . Linkers can be derived from immunoglobulin heavy chains of any isotype, including for example C γ 1, C γ 2, C γ 3, C γ 4, C α 1, C α 2, C δ , C ϵ , and C μ . Linker sequences may also be derived from other proteins such as Ig-like proteins (e.g. TCR, FcR, KIR), hinge region-derived sequences, and other natural sequences from other proteins.

[00228] In some embodiments, the linker is a “domain linker”, used to link any two domains as outlined herein together. For example, in Figure 15B, there may be a domain linker that attaches the C-terminus of the CH1 domain of the Fab to the N-terminus of the scFv, with another optional domain linker attaching the C-terminus of the scFv to the CH2 domain (although in many embodiments the hinge is used as this domain linker). While any suitable linker can be used, many embodiments utilize a glycine-serine polymer as the domain linker, including for example (GS) $_n$, (GSGGS) $_n$, (GGGGS) $_n$, and (GGGS) $_n$, where n is an integer of at least one (and generally from 3 to 4 to 5) as well as any peptide sequence that allows for recombinant attachment of the two domains with sufficient length and flexibility to allow each domain to retain its biological function. In some cases, and with attention being paid to “strandedness”, as outlined below, charged domain linkers, as used in some embodiments of scFv linkers can be used. Exemplary useful domain linkers are depicted in Figure 6.

[00229] With particular reference to the domain linker used to attach the scFv domain to the Fc domain in the “2+1” format, there are several domain linkers that find particular use, including “full hinge C220S variant,” “flex half hinge,” “charged half hinge 1,” and “charged half hinge 2” as shown in Figure 6.

[00230] In some embodiments, the linker is a “scFv linker”, used to covalently attach the VH and VL domains as discussed herein. In many cases, the scFv linker is a charged scFv linker, a number of which are shown in Figure 5. Accordingly, in some embodiments, the antibodies described herein further provide charged scFv linkers, to facilitate the separation in pI between a first and a second monomer. That is, by incorporating a charged scFv linker, either positive or negative (or both, in the case of scaffolds that use scFvs on different monomers), this allows the monomer comprising the charged linker to alter the pI without making further changes in the Fc domains. These charged linkers can be substituted

into any scFv containing standard linkers. Again, as will be appreciated by those in the art, charged scFv linkers are used on the correct “strand” or monomer, according to the desired changes in pI. For example, as discussed herein, to make 1+1 Fab-scFv-Fc format heterodimeric antibody, the original pI of the Fv region for each of the desired antigen binding domains are calculated, and one is chosen to make an scFv, and depending on the pI, either positive or negative linkers are chosen.

[00231] Charged domain linkers can also be used to increase the pI separation of the monomers of the antibodies described herein as well, and thus those included in Figure 5 can be used in any embodiment herein where a linker is utilized.

[00232] In particular, the formats depicted in Figure 15A and 15B are antibodies, usually referred to as “heterodimeric antibodies”, meaning that the protein has at least two associated Fc sequences self-assembled into a heterodimeric Fc domain and at least two Fv regions, whether as Fabs or as scFvs.

[00233] The ENPP3 binding domains provided can be included in any useful antibody format including, for example, canonical immunoglobulin, as well as the 1 + 1 Fab-scFv-Fc and 2 + 1 Fab2-scFv-Fv formats provided herein. Other useful antibody formats include, but are not limited to, “mAb-Fv,” “mAb-scFv,” “central-Fv,” “one armed scFv-mAb,” “scFv-mAb,” “dual scFv,” and “trident” format antibodies, as disclosed in Figures 52A-52K.

[00234] In some embodiments, the subject antibody includes one or more of the ENPP3 ABDs provided herein. In some embodiments, the antibody includes one ENPP3 ABD. In other embodiments, the antibody includes two ENPP3 ABDs. In exemplary embodiments, the ENPP3 ABD includes the variable heavy domain and variable light domain of one of the following ENPP3 ABDs: AN1[ENPP3] H1L1, AN1[ENPP3] H1 L1.33, AN1[ENPP3] H1 L1.77, AN1[ENPP3] H1.8 L1, AN1[ENPP3] H1.8 L1.33, AN1[ENPP3] H1 L1.77, H16-7.213, H16-9.69, H16-1.52, Ha16-1(1)23, H16-9.44, H16-1.67, Ha1 6-1 (3,5)36, H16-1.86, H16-9.10, H16-9.33, H16-1.68, Ha16-1(1)1, Ha1 6-1(3,5)18, Ha16-1(2,4)4, Ha16-1(3,5)56, H16-7.8, H16-1.93, Ha1 6-1(3,5)27.1, H16-1.61, H16-1(3,5)5, H16-7.200, H16-1(3,5)42, H1 6-9.65, Ha1-1(3,5)19, and Ha16-1.80 (Figures 12, 13A-13B, and 14A-14I). In some embodiments, the ENPO3 ABD is one of the following ENPP3 ABDs: AN1[ENPP3] H1L1, AN1[ENPP3] H1 L1.33, AN1[ENPP3] H1 L1.77, AN1[ENPP3] H1.8 L1, AN1[ENPP3] H1.8 L1.33, AN1[ENPP3] H1 L1.77, H16-7.213, H16-9.69, H16-1.52, Ha16-1(1)23, H16-9.44, H16-1.67, Ha1 6-1 (3,5)36, H16-1.86, H16-9.10, H16-9.33, H16-

1.68, Ha16-1(1)1, Ha1 6-1(3,5)18, Ha16-1(2,4)4, Ha16-1(3,5)56, H16-7.8, H16-1.93, Ha1 6-1(3,5)27.1, H16-1.61, H16-1(3,5)5, H16-7.200, H16-1(3,5)42, H1 6-9.65, Ha1-1(3,5)19, and Ha16-1.80 (Figures 12, 13A-13B, and 14A-14I).

[00235] In an exemplary embodiment, the antibody is a bispecific antibody that includes one or two ENPP3 ABDs, including any of the ENPP3 ABDs provided herein. Bispecific antibody that include such ENPP3 ABDs include, for example, 1 + 1 Fab-scFv-Fc and 2 + 1 Fab₂-scFv-Fc bispecifics format antibodies. In exemplary embodiments, the ENPP3 ABD is one of the following B7H3 ABDs: AN1[ENPP3] H1L1, AN1[ENPP3] H1 L1.33, AN1[ENPP3] H1 L1.77, AN1[ENPP3] H1.8 L1, AN1[ENPP3] H1.8 L1.33, AN1[ENPP3] H1 L1.77, H16-7.213, H16-9.69, H16-1.52, Ha16-1(1)23, H16-9.44, H16-1.67, Ha1 6-1 (3,5)36, H16-1.86, H16-9.10, H16-9.33, H16-1.68, Ha16-1(1)1, Ha1 6-1(3,5)18, Ha16-1(2,4)4, Ha16-1(3,5)56, H16-7.8, H16-1.93, Ha1 6-1(3,5)27.1, H16-1.61, H16-1(3,5)5, H16-7.200, H16-1(3,5)42, H1 6-9.65, Ha1-1(3,5)19, and Ha16-1.80(Figures 12, 13A-13B, and 14A-14I). In exemplary embodiments the ENPP3 binding domains is a Fab. In some embodiments, such bispecific antibodies are heterodimeric bispecific antibodies that include any of the heterodimerization skew variants, pI variants and/or ablation variants described herein.

A. Chimeric and Humanized Antibodies

[00236] In certain embodiments, the antibodies described herein comprise a heavy chain variable region from a particular germline heavy chain immunoglobulin gene and/or a light chain variable region from a particular germline light chain immunoglobulin gene. For example, such antibodies may comprise or consist of a human antibody comprising heavy or light chain variable regions that are "the product of" or "derived from" a particular germline sequence. A human antibody that is "the product of" or "derived from" a human germline immunoglobulin sequence can be identified as such by comparing the amino acid sequence of the human antibody to the amino acid sequences of human germline immunoglobulins and selecting the human germline immunoglobulin sequence that is closest in sequence (i.e., greatest % identity) to the sequence of the human antibody (using the methods outlined herein). A human antibody that is "the product of" or "derived from" a particular human germline immunoglobulin sequence may contain amino acid differences as compared to the germline sequence, due to, for example, naturally-occurring somatic mutations or intentional

introduction of site-directed mutation. However, a humanized antibody typically is at least 90% identical in amino acids sequence to an amino acid sequence encoded by a human germline immunoglobulin gene and contains amino acid residues that identify the antibody as being derived from human sequences when compared to the germline immunoglobulin amino acid sequences of other species (e.g., murine germline sequences). In certain cases, a humanized antibody may be at least 95, 96, 97, 98 or 99%, or even at least 96%, 97%, 98%, or 99% identical in amino acid sequence to the amino acid sequence encoded by the germline immunoglobulin gene. Typically, a humanized antibody derived from a particular human germline sequence will display no more than 10-20 amino acid differences from the amino acid sequence encoded by the human germline immunoglobulin gene (prior to the introduction of any skew, pI and ablation variants herein; that is, the number of variants is generally low, prior to the introduction of the variants described herein). In certain cases, the humanized antibody may display no more than 5, or even no more than 4, 3, 2, or 1 amino acid difference from the amino acid sequence encoded by the germline immunoglobulin gene (again, prior to the introduction of any skew, pI and ablation variants herein; that is, the number of variants is generally low, prior to the introduction of the variants described herein).

[00237] In one embodiment, the parent antibody has been affinity matured, as is known in the art. Structure-based methods may be employed for humanization and affinity maturation, for example as described in USSN 11/004,590. Selection based methods may be employed to humanize and/or affinity mature antibody variable regions, including but not limited to methods described in Wu et al., 1999, J. Mol. Biol. 294:151-162; Baca et al., 1997, J. Biol. Chem. 272(16):10678-10684; Rosok et al., 1996, J. Biol. Chem. 271(37): 22611-22618; Rader et al., 1998, Proc. Natl. Acad. Sci. USA 95: 8910-8915; Krauss et al., 2003, Protein Engineering 16(10):753-759, all entirely incorporated by reference. Other humanization methods may involve the grafting of only parts of the CDRs, including but not limited to methods described in USSN 09/810,510; Tan et al., 2002, J. Immunol. 169:1119-1125; De Pascalis et al., 2002, J. Immunol. 169:3076-3084, all entirely incorporated by reference.

B. Heterodimeric Antibodies

[00238] In exemplary embodiments, the bispecific antibodies provided herein are heterodimeric bispecific antibodies that include two variant Fc domain sequences. Such variant Fc domains include amino acid modifications to facilitate the self-assembly and/or purification of the heterodimeric antibodies.

[00239] An ongoing problem in antibody technologies is the desire for “bispecific” antibodies that bind to two different antigens simultaneously, in general thus allowing the different antigens to be brought into proximity and resulting in new functionalities and new therapies. In general, these antibodies are made by including genes for each heavy and light chain into the host cells. This generally results in the formation of the desired heterodimer (A-B), as well as the two homodimers (A-A and B-B (not including the light chain heterodimeric issues)). However, a major obstacle in the formation of bispecific antibodies is the difficulty in biasing the formation of the desired heterodimeric antibody over the formation of the homodimers and/or purifying the heterodimeric antibody away from the homodimers.

[00240] There are a number of mechanisms that can be used to generate the subject heterodimeric antibodies. In addition, as will be appreciated by those in the art, these different mechanisms can be combined to ensure high heterodimerization. Amino acid modifications that facilitate the production and purification of heterodimers are collectively referred to generally as “heterodimerization variants.” As discussed below, heterodimerization variants include “skew” variants (e.g., the “knobs and holes” and the “charge pairs” variants described below) as well as “pI variants,” which allow purification of heterodimers from homodimers. As is generally described in US Patent No. US 9,605,084, hereby incorporated by reference in its entirety and specifically as below for the discussion of heterodimerization variants, useful mechanisms for heterodimerization include “knobs and holes” (“KIH”) as described in US Patent No. US 9,605,084, “electrostatic steering” or “charge pairs” as described in US Patent No. US 9,605,084, pI variants as described in US Patent No. US 9,605,084, and general additional Fc variants as outlined in US Patent No. US 9,605,084 and below.

[00241] Heterodimerization variants that are useful for the formation and purification of the subject heterodimeric antibody (e.g., bispecific antibodies) are further discussed in detailed below.

1. Skew Variants

[00242] In some embodiments, the heterodimeric antibody includes skew variants which are one or more amino acid modifications in a first Fc domain (A) and/or a second Fc domain (B) that favor the formation of Fc heterodimers (Fc dimers that include the first and the second Fc domain; (A-B) over Fc homodimers (Fc dimers that include two of the first Fc domain or two of the second Fc domain; A-A or B-B). Suitable skew variants are included in the Figure 29 of US Publ. App. No. 2016/0355608, hereby incorporated by reference in its entirety and specifically for its disclosure of skew variants, as well as in Figures 1A-1E and Figure 4.

[00243] One mechanism is generally referred to in the art as “knobs and holes”, referring to amino acid engineering that creates steric influences to favor heterodimeric formation and disfavor homodimeric formation can also optionally be used; this is sometimes referred to as “knobs and holes”, as described in USSN 61/596,846, Ridgway et al., Protein Engineering 9(7):617 (1996); Atwell et al., J. Mol. Biol. 1997 270:26; US Patent No. 8,216,805, all of which are hereby incorporated by reference in their entirety. The Figures identify a number of “monomer A – monomer B” pairs that rely on “knobs and holes”. In addition, as described in Merchant et al., Nature Biotech. 16:677 (1998), these “knobs and hole” mutations can be combined with disulfide bonds to skew formation to heterodimerization.

[00244] An additional mechanism that finds use in the generation of heterodimers is sometimes referred to as “electrostatic steering” as described in Gunasekaran et al., J. Biol. Chem. 285(25):19637 (2010), hereby incorporated by reference in its entirety. This is sometimes referred to herein as “charge pairs”. In this embodiment, electrostatics are used to skew the formation towards heterodimerization. As those in the art will appreciate, these may also have an effect on pI, and thus on purification, and thus could in some cases also be considered pI variants. However, as these were generated to force heterodimerization and were not used as purification tools, they are classified as “steric variants”. These include, but are not limited to, D221E/P228E/L368E paired with D221R/P228R/K409R (e.g. these are “monomer corresponding sets) and C220E/P228E/368E paired with C220R/E224R/P228R/K409R.

[00245] In some embodiments, the skew variants advantageously and simultaneously favor heterodimerization based on both the “knobs and holes” mechanism as well as the

“electrostatic steering” mechanism. In some embodiments, the heterodimeric antibody includes one or more sets of such heterodimerization skew variants. These variants come in “pairs” of “sets”. That is, one set of the pair is incorporated into the first monomer and the other set of the pair is incorporated into the second monomer. It should be noted that these sets do not necessarily behave as “knobs in holes” variants, with a one-to-one correspondence between a residue on one monomer and a residue on the other. That is, these pairs of sets may instead form an interface between the two monomers that encourages heterodimer formation and discourages homodimer formation, allowing the percentage of heterodimers that spontaneously form under biological conditions to be over 90%, rather than the expected 50% (25 % homodimer A/A:50% heterodimer A/B:25% homodimer B/B). Exemplary heterodimerization “skew” variants are depicted in Figure 4. In exemplary embodiments, the heterodimeric antibody includes a S364K/E357Q : L368D/K370S; L368D/K370S : S364K; L368E/K370S : S364K; T411T/E360E/Q362E : D401K; L368D/K370S : S364K/E357L; K370S : S364K/E357Q; or a T366S/L368A/Y407V : T366W (optionally including a bridging disulfide, T366S/L368A/Y407V/Y349C : T366W/S354C) “skew” variant amino acid substitution set. In an exemplary embodiment, the heterodimeric antibody includes a “S364K/E357Q : L368D/K370S” amino acid substitution set. In terms of nomenclature, the pair “S364K/E357Q : L368D/K370S” means that one of the monomers includes an Fc domain that includes the amino acid substitutions S364K and E357Q and the other monomer includes an Fc domain that includes the amino acid substitutions L368D and K370S; as above, the “strandedness” of these pairs depends on the starting pI.

[00246] In some embodiments, the skew variants provided herein can be optionally and independently incorporated with any other modifications, including, but not limited to, other skew variants (see, e.g., in Figure 37 of US Publ. App. No. 2012/0149876, herein incorporated by reference, particularly for its disclosure of skew variants), pI variants, isotypic variants, FcRn variants, ablation variants, etc. into one or both of the first and second Fc domains of the heterodimeric antibody. Further, individual modifications can also independently and optionally be included or excluded from the subject the heterodimeric antibody.

[00247] Additional monomer A and monomer B variants that can be combined with other variants, optionally and independently in any amount, such as pI variants outlined

herein or other steric variants that are shown in Figure 37 of US 2012/0149876, the figure and legend and SEQ ID NOs of which are incorporated expressly by reference herein.

[00248] In some embodiments, the steric variants outlined herein can be optionally and independently incorporated with any pI variant (or other variants such as Fc variants, FcRn variants, etc.) into one or both monomers, and can be independently and optionally included or excluded from the proteins of the antibodies described herein.

[00249] A list of suitable skew variants is found in Figures 1A-1E, with Figure 4 showing some pairs of particular utility in many embodiments. Of particular use in many embodiments are the pairs of sets including, but not limited to, S364K/E357Q : L368D/K370S; L368D/K370S : S364K; L368E/K370S : S364K; T411T/E360E/Q362E : D401K; L368D/K370S : S364K/E357L and K370S : S364K/E357Q. In terms of nomenclature, the pair “S364K/E357Q : L368D/K370S” means that one of the monomers has the double variant set S364K/E357Q and the other has the double variant set L368D/K370S.

2. pI (Isoelectric point) Variants for Heterodimers

[00250] In some embodiments, the heterodimeric antibody includes purification variants that advantageously allow for the separation of heterodimeric antibody (e.g., anti-ENPP3 x anti-CD3 bispecific antibody) from homodimeric proteins.

[00251] There are several basic mechanisms that can lead to ease of purifying heterodimeric antibodies. For example, modifications to one or both of the antibody heavy chain monomers A and B such that each monomer has a different pI allows for the isoelectric purification of heterodimeric A-B antibody from monomeric A-A and B-B proteins. Alternatively, some scaffold formats, such as the “1 + 1 Fab-scFv-Fc” format and the “2 + 1 Fab₂-scFv-Fc” format, also allows separation on the basis of size. As described above, it is also possible to “skew” the formation of heterodimers over homodimers using skew variants. Thus, a combination of heterodimerization skew variants and pI variants find particular use in the heterodimeric antibodies provided herein.

[00252] Additionally, as more fully outlined below, depending on the format of the heterodimeric antibody, pI variants either contained within the constant region and/or Fc domains of a monomer, and/or domain linkers can be used. In some embodiments, the

heterodimeric antibody includes additional modifications for alternative functionalities that can also create pI changes, such as Fc, FcRn and KO variants.

[00253] In some embodiments, the subject heterodimeric antibodies provided herein include at least one monomer with one or more modifications that alter the pI of the monomer (i.e., a “pI variant”). In general, as will be appreciated by those in the art, there are two general categories of pI variants: those that increase the pI of the protein (basic changes) and those that decrease the pI of the protein (acidic changes). As described herein, all combinations of these variants can be done: one monomer may be wild type, or a variant that does not display a significantly different pI from wild-type, and the other can be either more basic or more acidic. Alternatively, each monomer is changed, one to more basic and one to more acidic.

[00254] Depending on the format of the heterodimer antibody, pI variants can be either contained within the constant and/or Fc domains of a monomer, or charged linkers, either domain linkers or scFv linkers, can be used. That is, antibody formats that utilize scFv(s) such as “1 + 1 Fab-scFv-Fc”, format can include charged scFv linkers (either positive or negative), that give a further pI boost for purification purposes. As will be appreciated by those in the art, some 1 + 1 Fab-scFv-Fc formats are useful with just charged scFv linkers and no additional pI adjustments, although the antibodies described herein do provide pI variants that are on one or both of the monomers, and/or charged domain linkers as well. In addition, additional amino acid engineering for alternative functionalities may also confer pI changes, such as Fc, FcRn and KO variants.

[00255] In subject heterodimeric antibodies that utilizes pI as a separation mechanism to allow the purification of heterodimeric proteins, amino acid variants are introduced into one or both of the monomer polypeptides. That is, the pI of one of the monomers (referred to herein for simplicity as “monomer A”) can be engineered away from monomer B, or both monomer A and B change be changed, with the pI of monomer A increasing and the pI of monomer B decreasing. As is outlined more fully below, the pI changes of either or both monomers can be done by removing or adding a charged residue (e.g., a neutral amino acid is replaced by a positively or negatively charged amino acid residue, e.g., glycine to glutamic acid), changing a charged residue from positive or negative to the opposite charge (aspartic acid to lysine) or changing a charged residue to a neutral residue (e.g., loss of a charge; lysine to serine.). A number of these variants are shown in the Figures 3 and 4.

[00256] Thus, in some embodiments, the subject heterodimeric antibody includes amino acid modifications in the constant regions that alter the isoelectric point (pI) of at least one, if not both, of the monomers of a dimeric protein to form “pI antibodies”) by incorporating amino acid substitutions (“pI variants” or “pI substitutions”) into one or both of the monomers. As shown herein, the separation of the heterodimers from the two homodimers can be accomplished if the pIs of the two monomers differ by as little as 0.1 pH unit, with 0.2, 0.3, 0.4 and 0.5 or greater all finding use in the antibodies described herein.

[00257] As will be appreciated by those in the art, the number of pI variants to be included on each or both monomer(s) to get good separation will depend in part on the starting pI of the components, for example in the 1 + 1 Fab-scFv-Fc and 2 + 1 Fab₂-scFv-Fc formats, the starting pI of the scFv and Fab(s) of interest. That is, to determine which monomer to engineer or in which “direction” (e.g., more positive or more negative), the Fv sequences of the two target antigens are calculated and a decision is made from there. As is known in the art, different Fvs will have different starting pIs which are exploited in the antibodies described herein. In general, as outlined herein, the pIs are engineered to result in a total pI difference of each monomer of at least about 0.1 logs, with 0.2 to 0.5 being preferred as outlined herein.

[00258] In the case where pI variants are used to achieve heterodimerization, by using the constant region(s) of the heavy chain(s), a more modular approach to designing and purifying bispecific proteins, including antibodies, is provided. Thus, in some embodiments, heterodimerization variants (including skew and pI heterodimerization variants) are not included in the variable regions, such that each individual antibody must be engineered. In addition, in some embodiments, the possibility of immunogenicity resulting from the pI variants is significantly reduced by importing pI variants from different IgG isotypes such that pI is changed without introducing significant immunogenicity. Thus, an additional problem to be solved is the elucidation of low pI constant domains with high human sequence content, e.g., the minimization or avoidance of non-human residues at any particular position. Alternatively or in addition to isotypic substitutions, the possibility of immunogenicity resulting from the pI variants is significantly reduced by utilizing isosteric substitutions (e.g. Asn to Asp; and Gln to Glu).

[00259] As discussed below, a side benefit that can occur with this pI engineering is also the extension of serum half-life and increased FcRn binding. That is, as described in US

Publ. App. No. US 2012/0028304 (incorporated by reference in its entirety), lowering the pI of antibody constant domains (including those found in antibodies and Fc fusions) can lead to longer serum retention in vivo. These pI variants for increased serum half-life also facilitate pI changes for purification.

[00260] In addition, it should be noted that the pI variants give an additional benefit for the analytics and quality control process of bispecific antibodies, as the ability to either eliminate, minimize and distinguish when homodimers are present is significant. Similarly, the ability to reliably test the reproducibility of the heterodimeric antibody production is important.

[00261] In general, embodiments of particular use rely on sets of variants that include skew variants, which encourage heterodimerization formation over homodimerization formation, coupled with pI variants, which increase the pI difference between the two monomers to facilitate purification of heterodimers away from homodimers.

[00262] Exemplary combinations of pI variants are shown in Figures 4 and 5, and Figure 30 of US Publ. App. No. 2016/0355608, all of which are herein incorporated by reference in its entirety and specifically for the disclosure of pI variants. Preferred combinations of pI variants are shown in Figures 1 and 2. As outlined herein and shown in the figures, these changes are shown relative to IgG1, but all isotypes can be altered this way, as well as isotype hybrids. In the case where the heavy chain constant domain is from IgG2-4, R133E and R133Q can also be used.

[00263] In one embodiment, a preferred combination of pI variants has one monomer (the negative Fab side) comprising 208D/295E/384D/418E/421D variants (N208D/Q295E/N384D/Q418E/N421D when relative to human IgG1) and a second monomer (the positive scFv side) comprising a positively charged scFv linker, including (GKPGS)₄ (SEQ ID NO: XX). However, as will be appreciated by those in the art, the first monomer includes a CH1 domain, including position 208. Accordingly, in constructs that do not include a CH1 domain (for example for antibodies that do not utilize a CH1 domain on one of the domains), a preferred negative pI variant Fc set includes 295E/384D/418E/421D variants (Q295E/N384D/Q418E/N421D when relative to human IgG1).

[00264] Accordingly, in some embodiments, one monomer has a set of substitutions from Figure 2 and the other monomer has a charged linker (either in the form of a charged

scFv linker because that monomer comprises an scFv or a charged domain linker, as the format dictates, which can be selected from those depicted in Figure 5).

[00265] In some embodiments, modifications are made in the hinge of the Fc domain, including positions 216, 217, 218, 219, 220, 221, 222, 223, 224, 225, 226, 227, 228, 229, and 230 based on EU numbering. Thus, pI mutations and particularly substitutions can be made in one or more of positions 216-230, with 1, 2, 3, 4 or 5 mutations finding use. Again, all possible combinations are contemplated, alone or with other pI variants in other domains.

[00266] Specific substitutions that find use in lowering the pI of hinge domains include, but are not limited to, a deletion at position 221, a non-native valine or threonine at position 222, a deletion at position 223, a non-native glutamic acid at position 224, a deletion at position 225, a deletion at position 235 and a deletion or a non-native alanine at position 236. In some cases, only pI substitutions are done in the hinge domain, and in others, these substitution(s) are added to other pI variants in other domains in any combination.

[00267] In some embodiments, mutations can be made in the CH2 region, including positions 233, 234, 235, 236, 274, 296, 300, 309, 320, 322, 326, 327, 334 and 339, based on EU numbering. It should be noted that changes in 233-236 can be made to increase effector function (along with 327A) in the IgG2 backbone. Again, all possible combinations of these 14 positions can be made; e.g., = may include a variant Fc domain with 1, 2, 3, 4, 5, 6, 7, 8, 9 or 10 CH2 pI substitutions.

[00268] Specific substitutions that find use in lowering the pI of CH2 domains include, but are not limited to, a non-native glutamine or glutamic acid at position 274, a non-native phenylalanine at position 296, a non-native phenylalanine at position 300, a non-native valine at position 309, a non-native glutamic acid at position 320, a non-native glutamic acid at position 322, a non-native glutamic acid at position 326, a non-native glycine at position 327, a non-native glutamic acid at position 334, a non-native threonine at position 339, and all possible combinations within CH2 and with other domains.

[00269] In this embodiment, the modifications can be independently and optionally selected from position 355, 359, 362, 384, 389, 392, 397, 418, 419, 444 and 447 (EU numbering) of the CH3 region. Specific substitutions that find use in lowering the pI of CH3 domains include, but are not limited to, a non-native glutamine or glutamic acid at position 355, a non-native serine at position 384, a non-native asparagine or glutamic acid at position

392, a non-native methionine at position 397, a non-native glutamic acid at position 419, a non-native glutamic acid at position 359, a non-native glutamic acid at position 362, a non-native glutamic acid at position 389, a non-native glutamic acid at position 418, a non-native glutamic acid at position 444, and a deletion or non-native aspartic acid at position 447.

[00270] In general, as will be appreciated by those in the art, there are two general categories of pI variants: those that increase the pI of the protein (basic changes) and those that decrease the pI of the protein (acidic changes). As described herein, all combinations of these variants can be done: one monomer may be wild type, or a variant that does not display a significantly different pI from wild-type, and the other can be either more basic or more acidic. Alternatively, each monomer is changed, one to more basic and one to more acidic.

[00271] Preferred combinations of pI variants are shown in Figure 4. As outlined herein and shown in the figures, these changes are shown relative to IgG1, but all isotypes can be altered this way, as well as isotype hybrids. In the case where the heavy chain constant domain is from IgG2-4, R133E and R133Q can also be used.

[00272] In one embodiment, for example in the Figures 15A and 15B formats, a preferred combination of pI variants has one monomer (the negative Fab side) comprising 208D/295E/384D/418E/421D variants (N208D/Q295E/N384D/Q418E/N421D when relative to human IgG1) and a second monomer (the positive scFv side) comprising a positively charged scFv linker, including (GKPGS)₄ (SEQ ID NO: XXX). However, as will be appreciated by those in the art, the first monomer includes a CH1 domain, including position 208. Accordingly, in constructs that do not include a CH1 domain (for example for antibodies that do not utilize a CH1 domain on one of the domains, for example in a dual scFv format or a “one armed” format such as those depicted in Figure 42B, C or D), a preferred negative pI variant Fc set includes 295E/384D/418E/421D variants (Q295E/N384D/Q418E/N421D when relative to human IgG1).

[00273] Accordingly, in some embodiments, one monomer has a set of substitutions from Figure 4 and the other monomer has a charged linker (either in the form of a charged scFv linker because that monomer comprises an scFv or a charged domain linker, as the format dictates, which can be selected from those depicted in Figure 5).

3. Isotypic Variants

[00274] In addition, many embodiments of the antibodies described herein rely on the “importation” of pI amino acids at particular positions from one IgG isotype into another, thus reducing or eliminating the possibility of unwanted immunogenicity being introduced into the variants. A number of these are shown in Figure 21 of US Publ. 2014/0370013, hereby incorporated by reference. That is, IgG1 is a common isotype for therapeutic antibodies for a variety of reasons, including high effector function. However, the heavy constant region of IgG1 has a higher pI than that of IgG2 (8.10 versus 7.31). By introducing IgG2 residues at particular positions into the IgG1 backbone, the pI of the resulting monomer is lowered (or increased) and additionally exhibits longer serum half-life. For example, IgG1 has a glycine (pI 5.97) at position 137, and IgG2 has a glutamic acid (pI 3.22); importing the glutamic acid will affect the pI of the resulting protein. As is described below, a number of amino acid substitutions are generally required to significantly affect the pI of the variant antibody. However, it should be noted as discussed below that even changes in IgG2 molecules allow for increased serum half-life.

[00275] In other embodiments, non-isotypic amino acid changes are made, either to reduce the overall charge state of the resulting protein (e.g. by changing a higher pI amino acid to a lower pI amino acid), or to allow accommodations in structure for stability, etc. as is more further described below.

[00276] In addition, by pI engineering both the heavy and light constant domains, significant changes in each monomer of the heterodimer can be seen. As discussed herein, having the pIs of the two monomers differ by at least 0.5 can allow separation by ion exchange chromatography or isoelectric focusing, or other methods sensitive to isoelectric point.

4. Calculating pI

[00277] The pI of each monomer can depend on the pI of the variant heavy chain constant domain and the pI of the total monomer, including the variant heavy chain constant domain and the fusion partner. Thus, in some embodiments, the change in pI is calculated on the basis of the variant heavy chain constant domain, using the chart in the Figure 19 of US Pub. 2014/0370013. As discussed herein, which monomer to engineer is generally decided

by the inherent pI of the Fv and scaffold regions. Alternatively, the pI of each monomer can be compared.

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

[00278] In the case where the pI variant decreases the pI of the monomer, they can have the added benefit of improving serum retention in vivo.

[00279] Although still under examination, Fc regions are believed to have longer half-lives in vivo, because binding to FcRn at pH 6 in an endosome sequesters the Fc (Ghetie and Ward, 1997 Immunol Today. 18(12): 592-598, entirely incorporated by reference). The endosomal compartment then recycles the Fc to the cell surface. Once the compartment opens to the extracellular space, the higher pH, ~7.4, induces the release of Fc back into the blood. In mice, Dall'Acqua et al. showed that Fc mutants with increased FcRn binding at pH 6 and pH 7.4 actually had reduced serum concentrations and the same half life as wild-type Fc (Dall'Acqua et al. 2002, J. Immunol. 169:5171-5180, entirely incorporated by reference). The increased affinity of Fc for FcRn at pH 7.4 is thought to forbid the release of the Fc back into the blood. Therefore, the Fc mutations that will increase Fc's half-life in vivo will ideally increase FcRn binding at the lower pH while still allowing release of Fc at higher pH. The amino acid histidine changes its charge state in the pH range of 6.0 to 7.4. Therefore, it is not surprising to find His residues at important positions in the Fc/FcRn complex.

[00280] Recently it has been suggested that antibodies with variable regions that have lower isoelectric points may also have longer serum half-lives (Igawa et al., 2010 PEDS. 23(5): 385-392, entirely incorporated by reference). However, the mechanism of this is still poorly understood. Moreover, variable regions differ from antibody to antibody. Constant region variants with reduced pI and extended half-life would provide a more modular approach to improving the pharmacokinetic properties of antibodies, as described herein.

C. Additional Fc Variants for Additional Functionality

[00281] In addition to the heterodimerization variants discussed above, there are a number of useful Fc amino acid modification that can be made for a variety of reasons, including, but not limited to, altering binding to one or more FcγR receptors, altered binding to FcRn receptors, etc, as discussed below.

[00282] Accordingly, the antibodies provided herein (heterodimeric, as well as homodimeric) can include such amino acid modifications with or without the heterodimerization variants outlined herein (e.g., the pI variants and steric variants). Each set of variants can be independently and optionally included or excluded from any particular heterodimeric protein.

1. FcγR Variants

[00283] Accordingly, there are a number of useful Fc substitutions that can be made to alter binding to one or more of the FcγR receptors. In certain embodiments, the subject antibody includes modifications that alter the binding to one or more FcγR receptors (i.e., “FcγR variants”). Substitutions that result in increased binding as well as decreased binding can be useful. For example, it is known that increased binding to FcγRIIIa generally results in increased ADCC (antibody dependent cell-mediated cytotoxicity; the cell-mediated reaction wherein nonspecific cytotoxic cells that express FcγRs recognize bound antibody on a target cell and subsequently cause lysis of the target cell). Similarly, decreased binding to FcγRIIb (an inhibitory receptor) can be beneficial as well in some circumstances. Amino acid substitutions that find use in the antibodies described herein include those listed in US Patent Nos. 8,188,321 (particularly Figure 41) and 8,084,582, and US Publ. App. Nos. 20060235208 and 20070148170, all of which are expressly incorporated herein by reference in their entirety and specifically for the variants disclosed therein. Particular variants that find use include, but are not limited to, 236A, 239D, 239E, 332E, 332D, 239D/332E, 267D, 267E, 328F, 267E/328F, 236A/332E, 239D/332E/330Y, 239D/332E/330L, 243A, 243L, 264A, 264V and 299T.

[00284] In addition, there are additional Fc substitutions that find use in increased binding to the FcRn receptor and increased serum half-life, as specifically disclosed in USSN 12/341,769, hereby incorporated by reference in its entirety, including, but not limited to, 434S, 434A, 428L, 308F, 259I, 428L/434S, 259I/308F, 436I/428L, 436I or V/434S, 436V/428L and 259I/308F/428L. Such modification may be included in one or both Fc domains of the subject antibody.

2. Ablation Variants

[00285] Similarly, another category of functional variants are "FcγR ablation variants" or "Fc knock out (FcKO or KO)" variants. In these embodiments, for some therapeutic applications, it is desirable to reduce or remove the normal binding of the Fc domain to one or more or all of the Fcγ receptors (e.g. FcγR1, FcγRIIa, FcγRIIb, FcγRIIIa, etc.) to avoid additional mechanisms of action. That is, for example, in many embodiments, particularly in the use of bispecific antibodies that bind CD3 monovalently it is generally desirable to ablate FcγRIIIa binding to eliminate or significantly reduce ADCC activity, wherein one of the Fc domains comprises one or more Fcγ receptor ablation variants. These ablation variants are depicted in Figure 14, and each can be independently and optionally included or excluded, with preferred aspects utilizing ablation variants selected from the group consisting of G236R/L328R, E233P/L234V/L235A/G236del/S239K, E233P/L234V/L235A/G236del/S267K, E233P/L234V/L235A/G236del/S239K/A327G, E233P/L234V/L235A/G236del/S267K/A327G and E233P/L234V/L235A/G236del. It should be noted that the ablation variants referenced herein ablate FcγR binding but generally not FcRn binding.

[00286] As is known in the art, the Fc domain of human IgG1 has the highest binding to the Fcγ receptors, and thus ablation variants can be used when the constant domain (or Fc domain) in the backbone of the heterodimeric antibody is IgG1. Alternatively, or in addition to ablation variants in an IgG1 background, mutations at the glycosylation position 297 (generally to A or S) can significantly ablate binding to FcγRIIIa, for example. Human IgG2 and IgG4 have naturally reduced binding to the Fcγ receptors, and thus those backbones can be used with or without the ablation variants.

D. Combination of Heterodimeric and Fc Variants

[00287] As will be appreciated by those in the art, all of the recited heterodimerization variants (including skew and/or pI variants) can be optionally and independently combined in any way, as long as they retain their "strandedness" or "monomer partition". In some embodiments, the heterodimeric antibodies provided herein include the combination of heterodimerization skew variants, isosteric pI substitutions and FcKO variants as depicted in Figure 4. In addition, all of these variants can be combined into any of the heterodimerization formats.

[00288] In the case of pI variants, while embodiments finding particular use are shown in the Figures, other combinations can be generated, following the basic rule of altering the pI difference between two monomers to facilitate purification.

[00289] In addition, any of the heterodimerization variants, skew and pI, are also independently and optionally combined with Fc ablation variants, Fc variants, FcRn variants, as generally outlined herein.

[00290] Exemplary combination of variants that are included in some embodiments of the heterodimeric 1+1 Fab-scFv-Fc and 2 + 1 Fab₂-scFv-Fc format antibodies are included in Figure 4. In certain embodiments, the antibody is a heterodimeric 1+1 Fab-scFv-Fc or 2 + 1 Fab₂-scFv-Fc format antibody as shown in Figures 15A and 15B.

E. Anti-ENPP3 x Anti-CD3 Bispecific Antibodies

[00291] In another aspect, provided herein are anti-ENPP3 x anti-CD3 (also referred to herein as “αENPP3 x αCD3”) bispecific antibodies. Such antibodies include at least one ENPP3 binding domain and at least one CD3 binding domain. In some embodiments, bispecific αENPP3 x αCD3 provided herein immune responses selectively in tumor sites that express ENPP3.

[00292] Note that unless specified herein, the order of the antigen list in the name does not confer structure; that is a ENPP3 X CD3 1 + 1 Fab-scFv-Fc antibody can have the scFv bind to ENPP3 or CD3, although in some cases, the order specifies structure as indicated.

[00293] As is more fully outlined herein, these combinations of ABDs can be in a variety of formats, as outlined below, generally in combinations where one ABD is in a Fab format and the other is in an scFv format. Exemplary formats that are used in the bispecific antibodies provided herein include the 1 + 1 Fab-scFv-Fc and 2 + 1 Fab₂-scFv-Fv formats (see, e.g., Figures 15A and 15B). Other useful antibody formats include, but are not limited to, “mAb-Fv,” “mAb-scFv,” “central-Fv,” “one armed scFv-mAb,” “scFv-mAb,” “dual scFv,” and “trident” format antibodies, as disclosed in Figure 52A-52K.

[00294] In addition, in general, one of the ABDs comprises a scFv as outlined herein, in an orientation from N- to C-terminus of VH-scFv linker-VL or VL-scFv linker-VH. One or both of the other ABDs, according to the format, generally is a Fab, comprising a VH

domain on one protein chain (generally as a component of a heavy chain) and a VL on another protein chain (generally as a component of a light chain).

[00295] As will be appreciated by those in the art, any set of 6 CDRs or VH and VL domains can be in the scFv format or in the Fab format, which is then added to the heavy and light constant domains, where the heavy constant domains comprise variants (including within the CH1 domain as well as the Fc domain). The scFv sequences contained in the sequence listing utilize a particular charged linker, but as outlined herein, uncharged or other charged linkers can be used, including those depicted in Figure 5 and Figure 6.

[00296] In addition, as discussed above, the numbering used in the Sequence Listing for the identification of the CDRs is Kabat, however, different numbering can be used, which will change the amino acid sequences of the CDRs as shown in Table 2.

[00297] For all of the variable heavy and light domains listed herein, further variants can be made. As outlined herein, in some embodiments the set of 6 CDRs can have from 0, 1, 2, 3, 4 or 5 amino acid modifications (with amino acid substitutions finding particular use), as well as changes in the framework regions of the variable heavy and light domains, as long as the frameworks (excluding the CDRs) retain at least about 80, 85 or 90% identity to a human germline sequence selected from those listed in Figure 1 of U.S. Patent No. 7,657,380, which Figure and Legend is incorporated by reference in its entirety herein. Thus, for example, the identical CDRs as described herein can be combined with different framework sequences from human germline sequences, as long as the framework regions retain at least 80, 85 or 90% identity to a human germline sequence selected from those listed in Figure 1 of U.S. Patent No. 7,657,380. Alternatively, the CDRs can have amino acid modifications (e.g., from 1, 2, 3, 4 or 5 amino acid modifications in the set of CDRs (that is, the CDRs can be modified as long as the total number of changes in the set of 6 CDRs is less than 6 amino acid modifications, with any combination of CDRs being changed; e.g., there may be one change in vICDR1, two in vhCDR2, none in vhCDR3, etc.)), as well as having framework region changes, as long as the framework regions retain at least 80, 85 or 90% identity to a human germline sequence selected from those listed in Figure 1 of U.S. Patent No. 7,657,380.

[00298] The anti-ENPP3 x anti-CD3 bispecific antibody can include any suitable CD3 ABD, including those described herein (see, e.g., Figures 10A-10F). In some embodiments, the CD3 ABD of the anti-ENPP3 x anti-CD3 bispecific antibody includes the variable heavy domain and variable light domain of a CD3 ABD provided herein, including those described

in Figures 10A-10F and the sequence listing. In some embodiments, the CD3 ABD includes the variable heavy domain and variable light domain of one of the following CD3 ABDs: H1.30_L1.47, H1.32_L1.47, H1.89_L1.47, H1.90_L1.47, H1.33_L1.47, H1.31_L1.47, L1.47_H1.30, L1.47_H1.30, L1.47_H1.32, L1.47_H1.89, L1.47_H1.90, L1.47_H1.33, and L1.47_H1.31 (Figures 10A-10F). In exemplary embodiments, the CD3 ABD is one of the following CD3 ABDs: H1.30_L1.47, H1.32_L1.47, H1.89_L1.47, H1.90_L1.47, H1.33_L1.47, H1.31_L1.47, L1.47_H1.30, L1.47_H1.30, L1.47_H1.32, L1.47_H1.89, L1.47_H1.90, L1.47_H1.33, and L1.47_H1.31 (Figures 10A-10F) or a variant thereof. The anti-ENPP3 x anti-CD3 bispecific antibody can include any suitable ENPP3 ABD, including those described herein (see, e.g., Figures 12, 13A-13B, and 14A-14I). In some embodiments, the ENPP3 ABD of the anti-ENPP3 x anti-CD3 bispecific antibody includes the variable heavy domain and variable light domain of a ENPP3 ABD provided herein, including those described in Figures 12, 13A-13B, and 14A-14I and the sequence listing. In some embodiments, the ENPP3 ABD includes the variable heavy domain and variable light domain of one of the following ENPP3 ABDs: ENPP3 ABDs: AN1[ENPP3] H1L1, AN1[ENPP3] H1 L1.33, AN1[ENPP3] H1 L1.77, AN1[ENPP3] H1.8 L1, AN1[ENPP3] H1.8 L1.33, AN1[ENPP3] H1 L1.77, H16-7.213, H16-9.69, H16-1.52, Ha16-1(1)23, H16-9.44, H16-1.67, Ha1 6-1 (3,5)36, H16-1.86, H16-9.10, H16-9.33, H16-1.68, Ha16-1(1)1, Ha1 6-1(3,5)18, Ha16-1(2,4)4, Ha16-1(3,5)56, H16-7.8, H16-1.93, Ha1 6-1(3,5)27.1, H16-1.61, H16-1(3,5)5, H16-7.200, H16-1(3,5)42, H1 6-9.65, Ha1-1(3,5)19, and Ha16-1.80, (Figures 12, 13A-13B, and 14A-14I). In exemplary embodiments, the ENPP3 ABD is one of the following ENPP3 ABDs: ENPP3 ABDs: AN1[ENPP3] H1L1, AN1[ENPP3] H1 L1.33, AN1[ENPP3] H1 L1.77, AN1[ENPP3] H1.8 L1, AN1[ENPP3] H1.8 L1.33, AN1[ENPP3] H1 L1.77, H16-7.213, H16-9.69, H16-1.52, Ha16-1(1)23, H16-9.44, H16-1.67, Ha1 6-1 (3,5)36, H16-1.86, H16-9.10, H16-9.33, H16-1.68, Ha16-1(1)1, Ha1 6-1(3,5)18, Ha16-1(2,4)4, Ha16-1(3,5)56, H16-7.8, H16-1.93, Ha1 6-1(3,5)27.1, H16-1.61, H16-1(3,5)5, H16-7.200, H16-1(3,5)42, H1 6-9.65, Ha1-1(3,5)19, and Ha16-1.80, (Figures 12, 13A-13B, and 14A-14I) or variants thereof.

F. Anti-SSTR2 x Anti-CD3 Bispecific Antibodies

[00299] In another aspect, provided herein are anti-SSTR2 x anti-CD3 (also referred to herein as “ α SSTR2 x α CD3”) bispecific antibodies. Such antibodies include at least one SSTR2 binding domain and at least one CD3 binding domain. In some embodiments, the

bispecific α SSTR2 x α CD3 provided herein immune responses selectively in tumor sites that express SSTR2.

[00300] Note that unless specified herein, the order of the antigen list in the name does not confer structure; that is a SSTR2 X CD3 1 + 1 Fab-scFv-Fc antibody can have the scFv bind to SSTR2 or CD3, although in some cases, the order specifies structure as indicated.

[00301] As is more fully outlined herein, these combinations of ABDs can be in a variety of formats, as outlined below, generally in combinations where one ABD is in a Fab format and the other is in an scFv format. Exemplary formats that are used in the bispecific antibodies provided herein include the 1 + 1 Fab-scFv-Fc and 2 + 1 Fab2-scFv-Fv formats (see, e.g., Figures 15A and 15B). Other useful antibody formats include, but are not limited to, “mAb-Fv,” “mAb-scFv,” “central-Fv,” “one armed scFv-mAb,” “scFv-mAb,” “dual scFv,” and “trident” format antibodies, as disclosed in Figure 52A-52K.

[00302] In addition, in general, one of the ABDs comprises a scFv as outlined herein, in an orientation from N- to C-terminus of VH-scFv linker-VL or VL-scFv linker-VH. One or both of the other ABDs, according to the format, generally is a Fab, comprising a VH domain on one protein chain (generally as a component of a heavy chain) and a VL on another protein chain (generally as a component of a light chain).

[00303] As will be appreciated by those in the art, any set of 6 CDRs or VH and VL domains can be in the scFv format or in the Fab format, which is then added to the heavy and light constant domains, where the heavy constant domains comprise variants (including within the CH1 domain as well as the Fc domain). The scFv sequences contained in the sequence listing utilize a particular charged linker, but as outlined herein, uncharged or other charged linkers can be used, including those depicted in Figure 5 and Figure 6.

[00304] In addition, as discussed above, the numbering used in the Sequence Listing for the identification of the CDRs is Kabat, however, different numbering can be used, which will change the amino acid sequences of the CDRs as shown in Table 2.

[00305] For all of the variable heavy and light domains listed herein, further variants can be made. As outlined herein, in some embodiments the set of 6 CDRs can have from 0, 1, 2, 3, 4 or 5 amino acid modifications (with amino acid substitutions finding particular use), as well as changes in the framework regions of the variable heavy and light domains, as long as the frameworks (excluding the CDRs) retain at least about 80, 85 or 90% identity to a

human germline sequence selected from those listed in Figure 1 of U.S. Patent No. 7,657,380, which Figure and Legend is incorporated by reference in its entirety herein. Thus, for example, the identical CDRs as described herein can be combined with different framework sequences from human germline sequences, as long as the framework regions retain at least 80, 85 or 90% identity to a human germline sequence selected from those listed in Figure 1 of U.S. Patent No. 7,657,380. Alternatively, the CDRs can have amino acid modifications (e.g., from 1, 2, 3, 4 or 5 amino acid modifications in the set of CDRs (that is, the CDRs can be modified as long as the total number of changes in the set of 6 CDRs is less than 6 amino acid modifications, with any combination of CDRs being changed; e.g., there may be one change in vLCDR1, two in vhCDR2, none in vhCDR3, etc.)), as well as having framework region changes, as long as the framework regions retain at least 80, 85 or 90% identity to a human germline sequence selected from those listed in Figure 1 of U.S. Patent No. 7,657,380.

[00306] The anti-SSTR2 x anti-CD3 bispecific antibody can include any suitable CD3 ABD, including those described herein (see, e.g., Figures 10A-10F). In some embodiments, the CD3 ABD of the anti-SSTR2 x anti-CD3 bispecific antibody includes the variable heavy domain and variable light domain of a CD3 ABD provided herein, including those described in Figures 10A-10F and the sequence listing. In some embodiments, the CD3 ABD includes the variable heavy domain and variable light domain of one of the following CD3 ABDs: H1.30_L1.47, H1.32_L1.47, H1.89_L1.47, H1.90_L1.47, H1.33_L1.47, H1.31_L1.47, L1.47_H1.30, L1.47_H1.30, L1.47_H1.32, L1.47_H1.89, L1.47_H1.90, L1.47_H1.33, and L1.47_H1.31 (Figures 10A-10F). In exemplary embodiments, the CD3 ABD is one of the following CD3 ABDs: H1.30_L1.47, H1.32_L1.47, H1.89_L1.47, H1.90_L1.47, H1.33_L1.47, H1.31_L1.47, L1.47_H1.30, L1.47_H1.30, L1.47_H1.32, L1.47_H1.89, L1.47_H1.90, L1.47_H1.33, and L1.47_H1.31 (Figures 10A-10F) or a variant thereof.

The anti-SSTR2 x anti-CD3 bispecific antibody can include the variable heavy domain and variable light domain of [α SSTR2] H1.24_L1.30 (Figure 63), or variants thereof.

G. Useful Formats of the Invention

[00307] As will be appreciated by those in the art and discussed more fully below, the heterodimeric bispecific antibodies provided herein can take on a wide variety of configurations, as are generally depicted in Figure 1. Some figures depict “single ended” configurations, where there is one type of specificity on one “arm” of the molecule and a

different specificity on the other “arm”. Other figures depict “dual ended” configurations, where there is at least one type of specificity at the “top” of the molecule and one or more different specificities at the “bottom” of the molecule. Thus, in some embodiments, the antibodies described herein are directed to novel immunoglobulin compositions that co-engage a different first and a second antigen.

[00308] As will be appreciated by those in the art, the heterodimeric formats of the antibodies described herein can have different valencies as well as be bispecific. That is, heterodimeric antibodies of the antibodies described herein can be bivalent and bispecific, wherein one target tumor antigen (e.g. CD3) is bound by one binding domain and the other target tumor antigen (e.g. ENPP3) is bound by a second binding domain. The heterodimeric antibodies can also be trivalent and bispecific, wherein the first antigen is bound by two binding domains and the second antigen by a second binding domain. As is outlined herein, when CD3 is one of the target antigens, it is preferable that the CD3 is bound only monovalently, to reduce potential side effects.

[00309] The antibodies described herein utilize anti-CD3 antigen binding domains in combination with anti-ENPP3 binding domains. As will be appreciated by those in the art, any collection of anti-CD3 CDRs, anti-CD3 variable light and variable heavy domains, Fabs and scFvs as depicted in any of the Figures can be used. Similarly, any of the anti-ENPP3 antigen binding domains can be used, whether CDRs, variable light and variable heavy domains, Fabs and scFvs as depicted in any of the Figures (e.g., Figures 12, 13A-13B, and 14A-14I) can be used, optionally and independently combined in any combination.

1. 1+1 Fab-scFv-Fc Format

[00310] One heterodimeric scaffold that finds particular use in the antibodies described herein is the “1+1 Fab-scFv-Fc” or “bottle-opener” format as shown in Figure 15A with an exemplary combination of a CD3 binding domain and a tumor target antigen (ENPP3) binding domain. In this embodiment, one heavy chain monomer of the antibody contains a single chain Fv (“scFv”, as defined below) and an Fc domain. The scFv includes a variable heavy domain (VH1) and a variable light domain (VL1), wherein the VH1 is attached to the VL1 using an scFv linker that can be charged (see, e.g., Figure 5). The scFv is attached to the heavy chain using a domain linker (see, e.g., Figure 6). The other heavy chain monomer is a “regular” heavy chain (VH-CH1-hinge-CH2-CH3). The 1 + 1 Fab-scFv-Fc also includes a

light chain that interacts with the VH-CH1 to form a Fab. This structure is sometimes referred to herein as the “bottle-opener” format, due to a rough visual similarity to a bottle-opener. The two heavy chain monomers are brought together by the use of amino acid variants (e.g., heterodimerization variants, discussed above) in the constant regions (e.g., the Fc domain, the CH1 domain and/or the hinge region) that promote the formation of heterodimeric antibodies as is described more fully below.

[00311] There are several distinct advantages to the present “1+1 Fab-scFv-Fc” format. As is known in the art, antibody analogs relying on two scFv constructs often have stability and aggregation problems, which can be alleviated in the antibodies described herein by the addition of a “regular” heavy and light chain pairing. In addition, as opposed to formats that rely on two heavy chains and two light chains, there is no issue with the incorrect pairing of heavy and light chains (e.g. heavy 1 pairing with light 2, etc.).

[00312] Many of the embodiments outlined herein rely in general on the 1+1 Fab-scFv-Fc or “bottle opener” format antibody that comprises a first monomer comprising an scFv, comprising a variable heavy and a variable light domain, covalently attached using an scFv linker (charged, in many but not all instances), where the scFv is covalently attached to the N-terminus of a first Fc domain usually through a domain linker. The domain linker can be either charged or uncharged and exogenous or endogenous (e.g., all or part of the native hinge domain). Any suitable linker can be used to attach the scFv to the N-terminus of the first Fc domain. In some embodiments, the domain linker is chosen from the domain linkers in Figure 6. The second monomer of the 1 + 1 Fab-scFv-Fc format or “bottle opener” format is a heavy chain, and the composition further comprises a light chain.

[00313] In general, in many preferred embodiments, the scFv is the domain that binds to the CD3, and the Fab forms an ENPP3 binding domain. An exemplary anti-ENPP3 x anti-CD3 bispecific antibody in the 1 + 1 Fab-scFv-Fc format is depicted in Figure 15A. Exemplary anti-ENPP3 x anti-CD3 bispecific antibody in the 1+1 Fab-scFv-Fc format is depicted in Figures 17A-17C and Figures 18A-18C.

[00314] In addition, the Fc domains of the antibodies described herein generally include skew variants (e.g. a set of amino acid substitutions as shown in Figures 3 and 9, with particularly useful skew variants being selected from the group consisting of S364K/E357Q : L368D/K370S; L368D/K370S : S364K; L368E/K370S : S364K; T411T/E360E/Q362E : D401K; L368D/K370S : S364K/E357L; K370S : S364K/E357Q; T366S/L368A/Y407V :

T366W and T366S/L368A/Y407V/Y349C : T366W/S354C), optionally ablation variants (including those shown in Figure 3), optionally charged scFv linkers (including those shown in Figure 5) and the heavy chain comprises pI variants (including those shown in Figure 4).

[00315] In certain embodiments, the 1 + 1 Fab-scFv-Fc scaffold format includes a first monomer that includes a scFv-domain linker-CH2-CH3 monomer, a second monomer that includes a first variable heavy domain-CH1-hinge-CH2-CH3 monomer and a third monomer that includes a first variable light domain. In some embodiments, the CH2-CH3 of the first monomer is a first variant Fc domain and the CH2-CH3 of the second monomer is a second variant Fc domain. In some embodiments, the scFv includes a scFv variable heavy domain and a scFv variable light domain that form a CD3 binding moiety. In certain embodiments, the scFv variable heavy domain and scFv variable light domain are covalently attached using an scFv linker (charged, in many but not all instances. See, e.g., Figure 5). In some embodiments, the first variable heavy domain and first variable light domain form a ENPP3 binding domain. Particularly useful ENPP3 and CD3 combinations for use in the 1+1 Fab-scFv-Fc ENPP3 x CD3 bispecific antibody format are disclosed in Figures 17A-17C and Figures 18A-18C and include: ENPP3 H16-1.93 x CD3 H1.30 L1.47, ENPP3 H16-7.8 x CD3 H1.30 L1.47, ENPP3 AN1 [ENPP3] H1L1 x CD3 H1.30 L1.47, ENPP3 AN1[ENPP3] H1.8 L1 x CD3 H1.30 L1.47, ENPP3 AN1[ENPP3] H1.8 L1.33 x CD3 H1.30 L1.47, and ENPP3 H1.8 L1.77 x CD3 H.130 L1.47. In some embodiments, the 1 + 1 Fab-scFv-Fc format includes skew variants, pI variants, and ablation variants. Accordingly, some embodiments include 1 + 1 Fab-scFv-Fc formats that comprise: a) a first monomer (the “scFv monomer”) that comprises a charged scFv linker (with the +H sequence of Figure 5 being preferred in some embodiments), the skew variants S364K/E357Q, the ablation variants E233P/L234V/L235A/G236del/S267K, and an scFv that binds to CD3 as outlined herein; b) a second monomer (the “Fab monomer”) that comprises the skew variants L368D/K370S, the pI variants N208D/Q295E/N384D/Q418E/N421D, the ablation variants E233P/L234V/L235A/ G236del/S267K, and a variable heavy domain; and c) a light chain that includes a variable light domain light domain (VL) and a constant light domain (CL), wherein numbering is according to EU numbering. The variable heavy domain and variable light domain make up an ENPP3 binding moiety. CD3 binding domain sequences finding particular use in these embodiments include, but are not limited to, H1.30_L1.47, H1.32_L1.47, H1.89_L1.47, H1.90_L1.47, H1.33_L1.47, H1.31_L1.47, L1.47_H1.30,

L1.47_H1.30, L1.47_H1.32, L1.47_H1.89, L1.47_H1.90, L1.47_H1.33, and L1.47_H1.31 as well as those depicted in Figures 10A-10F. ENPP3 binding domain sequences that are of particular use in these embodiments include, but are not limited to, AN1[ENPP3] H1L1, AN1[ENPP3] H1 L1.33, AN1[ENPP3] H1 L1.77, AN1[ENPP3] H1.8 L1, AN1[ENPP3] H1.8 L1.33, AN1[ENPP3] H1 L1.77, H16-7.213, H16-9.69, H16-1.52, Ha16-1(1)23, H16-9.44, H16-1.67, Ha1 6-1 (3,5)36, H16-1.86, H16-9.10, H16-9.33, H16-1.68, Ha16-1(1)1, Ha1 6-1(3,5)18, Ha16-1(2,4)4, Ha16-1(3,5)56, H16-7.8, H16-1.93, Ha1 6-1(3,5)27.1, H16-1.61, H16-1(3,5)5, H16-7.200, H16-1(3,5)42, H1 6-9.65, Ha1-1(3,5)19, and Ha16-1.80, (Figures 12, 13A-13B, and 14A-14I). Particularly useful ENPP3 and CD3 sequence combinations for use with the 1 + 1 Fab₂-scFv-Fc format antibody include, for example, ENPP3 H16-1.93 x CD3 H1.30 L1.47, ENPP3 H16-7.8 x CD3 H1.30 L1.47, ENPP3 AN1[ENPP3] H1L1 x CD3 H1.30 L1.47, ENPP3 AN1[ENPP3] H1.8 L1 x CD3 H1.30 L1.47, ENPP3 AN1[ENPP3] H1.8 L1.33 x CD3 H1.30 L1.47, and ENPP3 H1.8 L1.77 x CD3 H.130 L1.47.

[00316] Exemplary variable heavy and light domains of the scFv that binds to CD3 are included in Figure 10A-10F. Exemplary variable heavy and light domains of the Fv that binds to ENPP3 are included in Figures 12, 13A-13B, and 14A-14I. In an exemplary embodiment, the ENPP3 binding domain of the 1 + 1 Fab-scFv-Fc ENPP3 x CD3 bispecific antibody includes the VH and VL of one of the following ENPP3 binding domains: AN1[ENPP3] H1L1, AN1[ENPP3] H1 L1.33, AN1[ENPP3] H1 L1.77, AN1[ENPP3] H1.8 L1, AN1[ENPP3] H1.8 L1.33, AN1[ENPP3] H1 L1.77, H16-7.213, H16-9.69, H16-1.52, Ha16-1(1)23, H16-9.44, H16-1.67, Ha1 6-1 (3,5)36, H16-1.86, H16-9.10, H16-9.33, H16-1.68, Ha16-1(1)1, Ha1 6-1(3,5)18, Ha16-1(2,4)4, Ha16-1(3,5)56, H16-7.8, H16-1.93, Ha1 6-1(3,5)27.1, H16-1.61, H16-1(3,5)5, H16-7.200, H16-1(3,5)42, H1 6-9.65, Ha1-1(3,5)19, and Ha16-1.80, (Figures 12, 13A-13B, and 14A-14I). In one embodiment, the CD3 binding domain of the 1 + 1 Fab-scFv-Fc ENPP3 x CD3 bispecific antibody includes the VH and VL of one of the following CD3 binding domains: H1.30_L1.47, H1.32_L1.47, H1.89_L1.47, H1.90_L1.47, H1.33_L1.47, H1.31_L1.47, L1.47_H1.30, L1.47_H1.30, L1.47_H1.32, L1.47_H1.89, L1.47_H1.90, L1.47_H1.33, and L1.47_H1.31 (Figures 10A-10F). Particularly useful ENPP3 and CD3 combinations for use in the 1+1 Fab-scFv-Fc ENPP3 x CD3 bispecific antibody format are disclosed in Figures 17A-17C and Figures 18A-18C and include: ENPP3 H16-1.93 x CD3 H1.30 L1.47, ENPP3 H16-7.8 x CD3 H1.30 L1.47, ENPP3

AN1 [ENPP3] H1L1 x CD3 H1.30 L1.47, ENPP3 AN1[ENPP3] H1.8 L1 x CD3 H1.30 L1.47, ENPP3 AN1[ENPP3] H1.8 L1.33 x CD3 H1.30 L1.47, and ENPP3 H1.8 L1.77 x CD3 H.130 L1.47.

[00317] In some embodiments, the 1 + 1 Fab-scFv-Fc format includes skew variants, pI variants, ablation variants and FcRn variants. Accordingly, some embodiments include 1 + 1 Fab-scFv-Fc formats that comprise: a) a first monomer (the “scFv monomer”) that comprises a charged scFv linker (with the +H sequence of Figure 6 being preferred in some embodiments), the skew variants S364K/E357Q, the ablation variants E233P/L234V/L235A/G236del/S267K, the FcRn variants M428L/N434S and an scFv that binds to CD3 as outlined herein; b) a second monomer (the “Fab monomer”) that comprises the skew variants L368D/K370S, the pI variants N208D/Q295E/N384D/Q418E/N421D, the ablation variants E233P/L234V/L235A/ G236del/S267K, the FcRn variants M428L/N434S, and a variable heavy domain; and c) a light chain that includes a variable light domain(VL) and a constant light domain (CL), wherein numbering is according to EU numbering. The variable heavy domain and variable light domain make up an ENPP3 binding domain. CD3 binding domain sequences finding particular use in these embodiments include, but are not limited to, H1.30_L1.47, H1.32_L1.47, H1.89_L1.47, H1.90_L1.47, H1.33_L1.47, H1.31_L1.47, L1.47_H1.30, L1.47_H1.30, L1.47_H1.32, L1.47_H1.89, L1.47_H1.90, L1.47_H1.33, and L1.47_H1.31 as well as those depicted in Figures 10A-10F. ENPP3 binding domain sequences that are of particular use in these embodiments include, but are not limited to, AN1[ENPP3] H1L1, AN1[ENPP3] H1 L1.33, AN1[ENPP3] H1 L1.77, AN1[ENPP3] H1.8 L1, AN1[ENPP3] H1.8 L1.33, AN1[ENPP3] H1 L1.77, H16-7.213, H16-9.69, H16-1.52, Ha16-1(1)23, H16-9.44, H16-1.67, Ha1 6-1 (3,5)36, H16-1.86, H16-9.10, H16-9.33, H16-1.68, Ha16-1(1)1, Ha1 6-1(3,5)18, Ha16-1(2,4)4, Ha16-1(3,5)56, H16-7.8, H16-1.93, Ha1 6-1(3,5)27.1, H16-1.61, H16-1(3,5)5, H16-7.200, H16-1(3,5)42, H1 6-9.65, Ha1-1(3,5)19, and Ha16-1.80, as depicted in Figures 12, 13A-13B, and 14A-14I. Particularly useful ENPP3 and CD3 sequence combinations for use with the 1 + 1 Fab₂-scFv-Fc format antibody include, for example, are disclosed in Figures 17A-17C and Figures 18A-18C and include: ENPP3 H16-1.93 x CD3 H1.30 L1.47, ENPP3 H16-7.8 x CD3 H1.30 L1.47, ENPP3 AN1 [ENPP3] H1L1 x CD3 H1.30 L1.47, ENPP3 AN1[ENPP3] H1.8 L1 x CD3 H1.30 L1.47, ENPP3 AN1[ENPP3] H1.8 L1.33 x CD3 H1.30 L1.47, and ENPP3 H1.8 L1.77 x CD3 H.130 L1.47.

[00318] Figures 7A-7D show some exemplary Fc domain sequences that are useful in the 1 + 1 Fab-scFv-Fc format antibodies. The “monomer 1” sequences depicted in Figures 7A-7D typically refer to the Fc domain of the “Fab-Fc heavy chain” and the “monomer 2” sequences refer to the Fc domain of the “scFv-Fc heavy chain.” Further, Figure 9 provides useful CL sequences that can be used with this format.

[00319] In some embodiments, any of the VH and VL sequences depicted herein (including all VH and VL sequences depicted in the Figures and Sequence Listings, including those directed to ENPP3) can be added to the bottle opener backbone formats of Figure 7A-7D as the “Fab side”, using any of the anti-CD3 scFv sequences shown in the Figures and Sequence Listings.

[00320] For bottle opener backbone 1 from Figure 7A, (optionally including the 428L/434S variants), CD binding domain sequences finding particular use in these embodiments include, but are not limited to, CD3 binding domain anti-CD3 H1.30_L1.47, anti-CD3 H1.32_L1.47, anti-CD3 H1.89_L1.47, anti-CD3 H1.90_L1.47, anti-CD3 H1.33_L1.47 and anti-CD3 H1.31_L1.47, as well as those depicted in Figure 10A-10F, attached as the scFv side of the backbones shown in Figures 7A-7D.

[00321] Particularly useful ENPP3 and CD3 sequence combinations for use (optionally including the 428L/434S variants), are disclosed in Figures 17A-17C and Figures 18A-18C.

2. mAb-Fv

[00322] One heterodimeric scaffold that finds particular use in the antibodies described herein is the mAb-Fv format. In this embodiment, the format relies on the use of a C-terminal attachment of an “extra” variable heavy domain to one monomer and the C-terminal attachment of an “extra” variable light domain to the other monomer, thus forming a third antigen binding domain, wherein the Fab portions of the two monomers bind a ENPP3 and the “extra” scFv domain binds CD3.

[00323] In this embodiment, the first monomer comprises a first heavy chain, comprising a first variable heavy domain and a first constant heavy domain comprising a first Fc domain, with a first variable light domain covalently attached to the C-terminus of the first Fc domain using a domain linker (VH1-CH1-hinge-CH2-CH3-[optional linker]-VL2). The second monomer comprises a second variable heavy domain of the second constant heavy

domain comprising a second Fc domain, and a third variable heavy domain covalently attached to the C-terminus of the second Fc domain using a domain linker (vh1-CH1-hinge-CH2-CH3-[optional linker]-VH2). The two C-terminally attached variable domains make up a Fv that binds CD3 (as it is less preferred to have bivalent CD3 binding). This embodiment further utilizes a common light chain comprising a variable light domain and a constant light domain that associates with the heavy chains to form two identical Fabs that bind a ENPP3. As for many of the embodiments herein, these constructs include skew variants, pI variants, ablation variants, additional Fc variants, etc. as desired and described herein.

[00324] The antibodies described herein provide mAb-Fv formats where the CD3 binding domain sequences are as shown in Figure 10A-10F. The antibodies described herein provide mAb-Fv formats wherein the ENPP3 binding domain sequences are as shown in Figures 12, 13A-13B, and 14A-14I.

[00325] In addition, the Fc domains of the mAb-Fv format comprise skew variants (e.g. a set of amino acid substitutions as shown in Figures 3 and 8, with particularly useful skew variants being selected from the group consisting of S364K/E357Q : L368D/K370S; L368D/K370S : S364K; L368E/K370S : S364K; T411T/E360E/Q362E : D401K; L368D/K370S : S364K/E357L, K370S : S364K/E357Q, T366S/L368A/Y407V : T366W and T366S/L368A/Y407V/Y349C : T366W/S354C), optionally ablation variants (including those shown in Figure 3), optionally charged scFv linkers (including those shown in Figure 5) and the heavy chain comprises pI variants (including those shown in Figure 2).

[00326] In some embodiments, the mAb-Fv format includes skew variants, pI variants, and ablation variants. Accordingly, some embodiments include mAb-Fv formats that comprise: a) a first monomer that comprises the skew variants S364K/E357Q, the ablation variants E233P/L234V/L235A/G236del/S267K, and a first variable heavy domain that, with the first variable light domain of the light chain, makes up an Fv that binds to ENPP3, and a second variable heavy domain; b) a second monomer that comprises the skew variants L368D/K370S, the pI variants N208D/Q295E/N384D/ Q418E/N421D, the ablation variants E233P/L234V/L235A/G236del/S267K, and a first variable heavy domain that, with the first variable light domain, makes up the Fv that binds to ENPP3 as outlined herein, and a second variable light chain, that together with the second variable heavy domain forms an Fv (ABD) that binds to CD3; and c) a light chain comprising a first variable light domain and a constant light domain.

[00327] In some embodiments, the mAb-Fv format includes skew variants, pI variants, ablation variants and FcRn variants. Accordingly, some embodiments include mAb-Fv formats that comprise: a) a first monomer that comprises the skew variants S364K/E357Q, the ablation variants E233P/L234V/L235A/G236del/S267K, the FcRn variants M428L/N434S and a first variable heavy domain that, with the first variable light domain of the light chain, makes up an Fv that binds to ENPP3, and a second variable heavy domain; b) a second monomer that comprises the skew variants L368D/K370S, the pI variants N208D/Q295E/N384D/Q418E/N421D, the ablation variants E233P/L234V/L235A/G236del/S267K, the FcRn variants M428L/N434S and a first variable heavy domain that, with the first variable light domain, makes up the Fv that binds to ENPP3 as outlined herein, and a second variable light chain, that together with the second variable heavy domain of the first monomer forms an Fv (ABD) that binds CD3; and c) a light chain comprising a first variable light domain and a constant light domain.

3. mAb-scFv

[00328] One heterodimeric scaffold that finds particular use in the antibodies described herein is the mAb-scFv format. In this embodiment, the format relies on the use of a C-terminal attachment of a scFv to one of the monomers, thus forming a third antigen binding domain, wherein the Fab portions of the two monomers bind ENPP3 and the “extra” scFv domain binds CD3. Thus, the first monomer comprises a first heavy chain (comprising a variable heavy domain and a constant domain), with a C-terminally covalently attached scFv comprising a scFv variable light domain, an scFv linker and a scFv variable heavy domain in either orientation (VH1-CH1-hinge-CH2-CH3-[optional linker]-VH2-scFv linker-VL2 or VH1-CH1-hinge-CH2-CH3-[optional linker]-VL2-scFv linker-VH2). This embodiment further utilizes a common light chain comprising a variable light domain and a constant light domain, that associates with the heavy chains to form two identical Fabs that bind ENPP3. As for many of the embodiments herein, these constructs include skew variants, pI variants, ablation variants, additional Fc variants, etc. as desired and described herein.

[00329] The antibodies described herein provide mAb-scFv formats where the CD binding domain sequences are as shown in Figure 10A-10F and the ENPP3 binding domain sequences are as shown in Figures 12, 13A-13B, and 14A-14I.

[00330] In addition, the Fc domains of the mAb-scFv format comprise skew variants (e.g. a set of amino acid substitutions as shown in Figure 1, with particularly useful skew variants being selected from the group consisting of S364K/E357Q : L368D/K370S; L368D/K370S : S364K; L368E/K370S : S364K; T411T/E360E/Q362E : D401K; L368D/K370S : S364K/E357L, K370S : S364K/E357Q, T366S/L368A/Y407V : T366W and T366S/L368A/Y407V/Y349C : T366W/S354C), optionally ablation variants (including those shown in Figure 3), optionally charged scFv linkers (including those shown in Figure 5) and the heavy chain comprises pI variants (including those shown in Figure 2).

[00331] In some embodiments, the mAb-scFv format includes skew variants, pI variants, and ablation variants. Accordingly, some embodiments include mAb-scFv formats that comprise: a) a first monomer that comprises the skew variants S364K/E357Q, the ablation variants E233P/L234V/L235A/G236del/S267K, and a variable heavy domain that, with the variable light domain of the common light chain, makes up an Fv that binds to ENPP3 as outlined herein, and a scFv domain that binds to CD3; b) a second monomer that comprises the skew variants L368D/K370S, the pI variants N208D/Q295E/N384D/Q418E/N421D, the ablation variants E233P/L234V/L235A/G236del/S267K, and a variable heavy domain that, with the variable light domain of the common light chain, makes up an Fv that binds to ENPP3 as outlined herein; and c) a common light chain comprising a variable light domain and a constant light domain.

[00332] In some embodiments, the mAb-scFv format includes skew variants, pI variants, ablation variants and FcRn variants. Accordingly, some embodiments include mAb-scFv formats that comprise: a) a first monomer that comprises the skew variants S364K/E357Q, the ablation variants E233P/L234V/L235A/G236del/S267K, the FcRn variants M428L/N434S and a variable heavy domain that, with the variable light domain of the common light chain, makes up an Fv that binds to ENPP3 as outlined herein, and a scFv domain that binds to CD3; b) a second monomer that comprises the skew variants L368D/K370S, the pI variants N208D/Q295E/N384D/Q418E/N421D, the ablation variants E233P/L234V/L235A/G236del/S267K, the FcRn variants M428L/N434S and a variable heavy domain that, with the variable light domain of the common light chain, makes up an Fv that binds to ENPP3 as outlined herein; and c) a common light chain comprising a variable light domain and a constant light domain.

4. 2+1 Fab₂-scFv-Fc Format

[00333] One heterodimeric scaffold that finds particular use in the antibodies described herein is the “2+1 Fab₂-scFv-Fc” format (also referred to in previous related filings as “Central-scFv format”) shown in Figure 15B with an exemplary combination of a CD3 binding domain and two tumor target antigen (ENPP3) binding domains. In this embodiment, the format relies on the use of an inserted scFv domain thus forming a third antigen binding domain, wherein the Fab portions of the two monomers bind ENPP3 and the “extra” scFv domain binds CD3. The scFv domain is inserted between the Fc domain and the CH1-Fv region of one of the monomers, thus providing a third antigen binding domain. As described, ENPP3 x CD3 bispecific antibodies having the 2 + 1 Fab₂-scFv-Fc format are potent in inducing redirected T cell cytotoxicity in cellular environments that express low levels of ENPP3. Moreover, as shown in the examples, ENPP3 x CD3 bispecific antibodies having the 2 + 1 Fab₂-scFv-Fc format allow for the “fine tuning” of immune responses as such antibodies exhibit a wide variety of different properties, depending on the ENPP3 and/or CD3 binding domains used. For example, such antibodies exhibit differences in selectivity for cells with different ENPP3 expression, potencies for ENPP3 expressing cells, ability to elicit cytokine release, and sensitivity to soluble ENPP3. These ENPP3 antibodies find use, for example, in the treatment of ENPP3 associated cancers.

[00334] In this embodiment, one monomer comprises a first heavy chain comprising a first variable heavy domain, a CH1 domain (and optional hinge) and Fc domain, with a scFv comprising a scFv variable light domain, an scFv linker and a scFv variable heavy domain. The scFv is covalently attached between the C-terminus of the CH1 domain of the heavy constant domain and the N-terminus of the first Fc domain using optional domain linkers (VH1-CH1-[optional linker]-VH2-scFv linker-VL2-[optional linker including the hinge]-CH2-CH3, or the opposite orientation for the scFv, VH1-CH1-[optional linker]-VL2-scFv linker-VH2-[optional linker including the hinge]-CH2-CH3). The optional linkers can be any suitable peptide linkers, including, for example, the domain linkers included in Figure 6. In some embodiments, the optional linker is a hinge or a fragment thereof. The other monomer is a standard Fab side (i.e., VH1-CH1-hinge-CH2-CH3). This embodiment further utilizes a common light chain comprising a variable light domain and a constant light domain, that associates with the heavy chains to form two identical Fabs that bind ENPP3. As for many of

the embodiments herein, these constructs include skew variants, pI variants, ablation variants, additional Fc variants, etc. as desired and described herein.

[00335] In one embodiment, the 2 + 1 Fab2-scFv-Fc format antibody includes an scFv with the VH and VL of a CD3 binding domain sequence depicted in Figure 10A-10F or the Sequence Listing. In one embodiment, the 2 + 1 Fab2-scFv-Fc format antibody includes two Fabs having the VH and VL of a ENPP3 binding domain as shown in Figures 12, 13A-13B, and 14A-14I and the Sequence Listing. In an exemplary embodiment, the ENPP3 binding domain of the 2 + 1 Fab2-scFv-Fc ENPP3 x CD3 bispecific antibody includes the VH and VL of one of the following ENPP3 binding domains: AN1[ENPP3] H1L1, AN1[ENPP3] H1 L1.33, AN1[ENPP3] H1 L1.77, AN1[ENPP3] H1.8 L1, AN1[ENPP3] H1.8 L1.33, AN1[ENPP3] H1 L1.77, H16-7.213, H16-9.69, H16-1.52, Ha16-1(1)23, H16-9.44, H16-1.67, Ha1 6-1 (3,5)36, H16-1.86, H16-9.10, H16-9.33, H16-1.68, Ha16-1(1)1, Ha1 6-1(3,5)18, Ha16-1(2,4)4, Ha16-1(3,5)56, H16-7.8, H16-1.93, Ha1 6-1(3,5)27.1, H16-1.61, H16-1(3,5)5, H16-7.200, H16-1(3,5)42, H1 6-9.65, Ha1-1(3,5)19, and Ha16-1.80, (Figures 12, 13A-13B, and 14A-14I). In one embodiment, the CD3 binding domain of the 2 + 1 Fab2-scFv-Fc format antibody includes the VH and VL of one of the following CD3 binding domains: H1.30_L1.47, H1.32_L1.47, H1.89_L1.47, H1.90_L1.47, H1.33_L1.47, H1.31_L1.47, L1.47_H1.30, L1.47_H1.30, L1.47_H1.32, L1.47_H1.89, L1.47_H1.90, L1.47_H1.33, and L1.47_H1.31 (Figures 10A-10F). Particularly useful ENPP3 and CD3 combinations for use in the 2 + 1 Fab2-scFv-Fc format antibody format are disclosed in Figures 19A-19C, 20A-D, 21, 22A-22C, 23A-E and include: ENPP3 H1.8 L1 x CD3 H1.30 L1.47, ENPP3 H1.8 L1.33 x CD3 H1.30 L1.47, ENPP3 H1.8 L1.77 x CD3 H1.30 L1.47, ENPP3 H16-7.8 x CD3 H1.32 L1.47, ENPP3 AN[ENPP3] H1L1 x CD3 H1.32 L1.47, ENPP3 H1.8 L1 x CD3 H1.32 L1.47, ENPP3 H1.8 L1.33 x CD3 H1.32 L1.47, ENPP3 H1.8 L1 x CD3 L1.47 H1.30, ENPP3 H1.8 L1 x CD3 L1.47 H1.32, ENPP3 H1.8 L1.33 x CD3 L1.47 H1.32, ENPP3 H1.8 L1.77 x CD3 L1.47 H1.32, ENPP3 H1.8 L1 x CD3 L1.47 H1.89, ENPP3 H1.8 L1.33 x CD3 L1.47 H1.89, ENPP3 H1.8 L1.77 x CD3 L1.47 H1.89, ENPP3 H1.8 L1.33 x CD3 L1.47 H1.89, and ENPP3 H1.8 L1.77 x CD3 L1.47 H1.89.

[00336] In addition, the Fc domains of the 2 + 1 Fab2-scFv-Fc format comprise skew variants (e.g. a set of amino acid substitutions as shown in Figure 1, with particularly useful skew variants being selected from the group consisting of S364K/E357Q : L368D/K370S; L368D/K370S : S364K; L368E/K370S : S364K; T411T/E360E/Q362E : D401K;

L368D/K370S : S364K/E357L, K370S : S364K/E357Q, T366S/L368A/Y407V : T366W and T366S/L368A/Y407V/Y349C : T366W/S354C), optionally ablation variants (including those shown in Figure 3), optionally charged scFv linkers (including those shown in Figure 5) and the heavy chain comprises pI variants (including those shown in Figure 2).

[00337] In some embodiments, the 2 + 1 Fab₂-scFv-Fc format antibody includes skew variants, pI variants, and ablation variants. Accordingly, some embodiments include 2 + 1 Fab₂-scFv-Fc formats that comprise: a) a first monomer (the Fab-scFv-Fc side) that comprises the skew variants S364K/E357Q, the ablation variants E233P/L234V/L235A/G236del/S267K, and a variable heavy domain that, with the variable light domain of the common light chain, makes up an Fv that binds to ENPP3 as outlined herein, and an scFv domain that binds to CD3; b) a second monomer (the Fab-Fc side) that comprises the skew variants L368D/K370S, the pI variants N208D/Q295E/N384D/Q418E/N421D, the ablation variants E233P/L234V/L235A/G236del/S267K, and a variable heavy domain that, with variable light domain of the common light chain, makes up an Fv that binds to ENPP3 as outlined herein; and c) a common light chain comprising the variable light domain and a constant light domain, where numbering is according to EU numbering. In some embodiments, the common light chain and variable heavy domains on each monomer for ENPP3 binding domains. CD3 binding domain sequences finding particular use in these embodiments include, but are not limited to, H1.30_L1.47, H1.32_L1.47, H1.89_L1.47, H1.90_L1.47, H1.33_L1.47, H1.31_L1.47, L1.47_H1.30, L1.47_H1.30, L1.47_H1.32, L1.47_H1.89, L1.47_H1.90, L1.47_H1.33, and L1.47_H1.31 as well as those depicted in Figures 10A-10F. ENPP3 binding domain sequences that are of particular use in these embodiments include, but are not limited to, AN1[ENPP3] H1L1, AN1[ENPP3] H1L1.33, AN1[ENPP3] H1 L1.77, AN1[ENPP3] H1.8 L1, AN1[ENPP3] H1.8 L1.33, AN1[ENPP3] H1 L1.77, H16-7.213, H16-9.69, H16-1.52, Ha16-1(1)23, H16-9.44, H16-1.67, Ha1 6-1 (3,5)36, H16-1.86, H16-9.10, H16-9.33, H16-1.68, Ha16-1(1)1, Ha1 6-1(3,5)18, Ha16-1(2,4)4, Ha16-1(3,5)56, H16-7.8, H16-1.93, Ha1 6-1(3,5)27.1, H16-1.61, H16-1(3,5)5, H16-7.200, H16-1(3,5)42, H1 6-9.65, Ha1-1(3,5)19, and Ha16-1.80, as depicted in Figures 12, 13A-13B, and 14A-14I.

[00338] In some embodiments, the 2 + 1 Fab₂-scFv-Fc format antibody includes skew variants, pI variants, ablation variants and FcRn variants. Accordingly, some embodiments include 2 + 1 Fab₂-scFv-Fc formats that comprise: a) a first monomer (the Fab-scFv-Fc side)

that comprises the skew variants S364K/E357Q, the ablation variants E233P/L234V/L235A/G236del/S267K, the FcRn variants M428L/N434S and a variable heavy domain that, with the variable light domain of the common light chain, makes up an Fv that binds to ENPP3 as outlined herein, and an scFv domain that binds to CD3; b) a second monomer (the Fab-Fc side) that comprises the skew variants L368D/K370S, the pI variants N208D/Q295E/N384D/ Q418E/N421D, the ablation variants E233P/L234V/L235A/G236del/S267K, the FcRn variants M428L/N434S and a variable heavy domain that, with variable light domain of the common light chain, makes up an Fv that binds to ENPP3 as outlined herein; and c) a common light chain comprising a variable light domain and a constant light domain, where numbering is according to EU numbering. In some embodiments, the common light chain and variable heavy domains on each monomer for ENPP3 binding domains. CD3 binding domain sequences finding particular use in these embodiments include, but are not limited to, H1.30_L1.47, H1.32_L1.47, H1.89_L1.47, H1.90_L1.47, H1.33_L1.47, H1.31_L1.47, L1.47_H1.30, L1.47_H1.30, L1.47_H1.32, L1.47_H1.89, L1.47_H1.90, L1.47_H1.33, and L1.47_H1.31 as well as those depicted in Figures 10A-10F. ENPP3 binding domain sequences that are of particular use in these embodiments include but are not limited to, AN1[ENPP3] H1L1, AN1[ENPP3] H1 L1.33, AN1[ENPP3] H1 L1.77, AN1[ENPP3] H1.8 L1, AN1[ENPP3] H1.8 L1.33, AN1[ENPP3] H1 L1.77, H16-7.213, H16-9.69, H16-1.52, Ha16-1(1)23, H16-9.44, H16-1.67, Ha1 6-1 (3,5)36, H16-1.86, H16-9.10, H16-9.33, H16-1.68, Ha16-1(1)1, Ha1 6-1(3,5)18, Ha16-1(2,4)4, Ha16-1(3,5)56, H16-7.8, H16-1.93, Ha1 6-1(3,5)27.1, H16-1.61, H16-1(3,5)5, H16-7.200, H16-1(3,5)42, H1 6-9.65, Ha1-1(3,5)19, and Ha16-1.80, as depicted in Figures 12, 13A-13B, and 14A-14I..

[00339] Figures 8A-8C shows some exemplary Fc domain sequences that are useful with the 2 + 1 Fab₂-scFv-Fc format. The “monomer 1” sequences depicted in Figures 8A-8C typically refer to the Fc domain of the “Fab-Fc heavy chain” and the “monomer 2” sequences refer to the Fc domain of the “Fab-scFv-Fc heavy chain.” Further, Figure 9 provides useful CL sequences that can be used with this format.

5. Central-Fv

[00340] One heterodimeric scaffold that finds particular use in the antibodies described herein is the Central-Fv format.. In this embodiment, the format relies on the use of an

inserted Fv domain (i.e., the central Fv domain) thus forming an “extra” third antigen binding domain, wherein the Fab portions of the two monomers bind a ENPP3 and the “extra” central Fv domain binds CD3. The “extra” central Fv domain is inserted between the Fc domain and the CH1-Fv region of the monomers, thus providing a third antigen binding domain (i.e., the “extra” central Fv domain), wherein each monomer contains a component of the “extra” central Fv domain (i.e., one monomer comprises the variable heavy domain and the other a variable light domain of the “extra” central Fv domain).

[00341] In this embodiment, one monomer comprises a first heavy chain comprising a first variable heavy domain, a CH1 domain, and Fc domain and an additional variable light domain. The light domain is covalently attached between the C-terminus of the CH1 domain of the heavy constant domain and the N-terminus of the first Fc domain using domain linkers (VH1-CH1-[optional linker]-VL2-hinge-CH2-CH3). The other monomer comprises a first heavy chain comprising a first variable heavy domain, a CH1 domain and Fc domain and an additional variable heavy domain (VH1-CH1-[optional linker]-VH2-hinge-CH2-CH3). The light domain is covalently attached between the C-terminus of the CH1 domain of the heavy constant domain and the N-terminus of the first Fc domain using domain linkers.

[00342] This embodiment further utilizes a common light chain comprising a variable light domain and a constant light domain, that associates with the heavy chains to form two identical Fabs that each bind an ENPP3. As for many of the embodiments herein, these constructs include skew variants, pI variants, ablation variants, additional Fc variants, etc. as desired and described herein.

[00343] The antibodies described herein provide central-Fv formats where the CD3 binding domain sequences are as shown in 10A-10F and the ENPP3 binding domain sequences are as shown in Figures 12, 13A-13B, and 14A-14I.

6. One armed central-scFv

[00344] One heterodimeric scaffold that finds particular use in the antibodies described herein is the one armed central-scFv format. In this embodiment, one monomer comprises just an Fc domain, while the other monomer includes a Fab domain (a first antigen binding domain), a scFv domain (a second antigen binding domain) and an Fc domain, where the scFv domain is inserted between the Fc domain and the Fc domain. In this format, the Fab

portion binds one receptor target and the scFv binds another. In this format, either the Fab portion binds a ENPP3 and the scFv binds CD3 or vice versa.

[00345] In this embodiment, one monomer comprises a first heavy chain comprising a first variable heavy domain, a CH1 domain and Fc domain, with a scFv comprising a scFv variable light domain, an scFv linker and a scFv variable heavy domain. The scFv is covalently attached between the C-terminus of the CH1 domain of the heavy constant domain and the N-terminus of the first Fc domain using domain linkers, in either orientation, VH1-CH1-[optional domain linker]-VH2-scFv linker-VL2-[optional domain linker]-CH2-CH3 or VH1-CH1-[optional domain linker]-VL2-scFv linker-VH2-[optional domain linker]-CH2-CH3. The second monomer comprises an Fc domain (CH2-CH3). This embodiment further utilizes a light chain comprising a variable light domain and a constant light domain that associates with the heavy chain to form a Fab.

[00346] As for many of the embodiments herein, these constructs include skew variants, pI variants, ablation variants, additional Fc variants, etc. as desired and described herein.

[00347] The antibodies described herein provide central-Fv formats where the CD3 binding domain sequences are as shown in Figure 10A-10F and the ENPP3 binding domain sequences are as shown in Figures 12, 13A-13B, and 14A-14I.

[00348] In addition, the Fc domains of the one armed central-scFv format generally include skew variants (e.g. a set of amino acid substitutions as shown in Figure 1, with particularly useful skew variants being selected from the group consisting of S364K/E357Q : L368D/K370S; L368D/K370S : S364K; L368E/K370S : S364K; T411T/E360E/Q362E : D401K; L368D/K370S : S364K/E357L, K370S : S364K/E357Q, T366S/L368A/Y407V : T366W and T366S/L368A/Y407V/Y349C : T366W/S354C), optionally ablation variants (including those shown in Figure 3), optionally charged scFv linkers (including those shown in Figure 5) and the heavy chain comprises pI variants (including those shown in Figure 2).

[00349] In some embodiments, the one armed central-scFv format includes skew variants, pI variants, and ablation variants. Accordingly, some embodiments of the one armed central-scFv formats comprise: a) a first monomer that comprises the skew variants S364K/E357Q, the ablation variants E233P/L234V/L235A/G236del/S267K, and a variable heavy domain that, with the variable light domain of the light chain, makes up an Fv that

binds to ENPP3 as outlined herein, and a scFv domain that binds to CD3; b) a second monomer that includes an Fc domain having the skew variants L368D/K370S, the pI variants N208D/Q295E/N384D/ Q418E/N421D, the ablation variants E233P/L234V/L235A/G236del/S267K; and c) a light chain comprising a variable light domain and a constant light domain.

[00350] In some embodiments, the one armed central-scFv format includes skew variants, pI variants, ablation variants and FcRn variants. Accordingly, some embodiments of the one armed central-scFv formats comprise: a) a first monomer that comprises the skew variants S364K/E357Q, the ablation variants E233P/L234V/L235A/G236del/S267K, the FcRn variants M428L/N434S and a variable heavy domain that, with the variable light domain of the light chain, makes up an Fv that binds to ENPP3 as outlined herein, and a scFv domain that binds to CD3; b) a second monomer that includes an Fc domain having the skew variants L368D/K370S, the pI variants N208D/Q295E/N384D/ Q418E/N421D, the ablation variants E233P/L234V/L235A/G236del/S267K, and the FcRn variants M428L/N434S; and c) a light chain comprising a variable light domain and a constant light domain.

7. One armed scFv-mAb

[00351] One heterodimeric scaffold that finds particular use in the antibodies described herein is the one armed scFv-mAb format. In this embodiment, one monomer comprises just an Fc domain, while the other monomer uses a scFv domain attached at the N-terminus of the heavy chain, generally through the use of a linker: VH-scFv linker-VL-[optional domain linker]-CH1-hinge-CH2-CH3 or (in the opposite orientation) VL-scFv linker-VH-[optional domain linker]-CH1-hinge-CH2-CH3. In this format, the Fab portions each bind ENPP3 and the scFv binds CD3. This embodiment further utilizes a light chain comprising a variable light domain and a constant light domain, that associates with the heavy chain to form a Fab. As for many of the embodiments herein, these constructs include skew variants, pI variants, ablation variants, additional Fc variants, etc. as desired and described herein.

[00352] The antibodies described herein provide one armed scFv-mAb formats where the CD3 binding domain sequences are as shown in 10A-10F and wherein the ENPP3 binding domain sequences are as shown in Figures 12, 13A-13B, and 14A-14I.

[00353] In addition, the Fc domains of the one armed scFv-mAb format generally include skew variants (e.g. a set of amino acid substitutions as shown in Figures 3 and 8, with particularly useful skew variants being selected from the group consisting of S364K/E357Q : L368D/K370S; L368D/K370S : S364K; L368E/K370S : S364K; T411T/E360E/Q362E : D401K; L368D/K370S : S364K/E357L, K370S : S364K/E357Q, T366S/L368A/Y407V : T366W and T366S/L368A/Y407V/Y349C : T366W/S354C), optionally ablation variants (including those shown in Figure 3), optionally charged scFv linkers (including those shown in Figure 5) and the heavy chain comprises pI variants (including those shown in Figure 2).

[00354] In some embodiments, the one armed scFv-mAb format includes skew variants, pI variants, and ablation variants. Accordingly, some embodiments of the one armed scFv-mAb formats comprise: a) a first monomer that comprises the skew variants S364K/E357Q, the ablation variants E233P/L234V/L235A/G236del/S267K, and a variable heavy domain that, with the variable light domain of the light chain, makes up an Fv that binds to ENPP3 as outlined herein, and a scFv domain that binds to CD3; b) a second monomer that includes an Fc domain having the skew variants L368D/K370S, the pI variants N208D/Q295E/N384D/ Q418E/N421D, the ablation variants E233P/L234V/L235A/G236del/S267K; and c) a light chain comprising a variable light domain and a constant light domain.

[00355] In some embodiments, the one armed scFv-mAb format includes skew variants, pI variants, ablation variants and FcRn variants. Accordingly, some embodiments one armed scFv-mAb formats comprise: a) a first monomer that comprises the skew variants S364K/E357Q, the ablation variants E233P/L234V/L235A/G236del/S267K, the FcRn variants M428L/N434S and a variable heavy domain that, with the variable light domain of the light chain, makes up an Fv that binds to ENPP3 as outlined herein, and a scFv domain that binds to CD3; b) a second monomer that includes an Fc domain having the skew variants L368D/K370S, the pI variants N208D/Q295E/N384D/ Q418E/N421D, the ablation variants E233P/L234V/L235A/G236del/S267K, and the FcRn variants M428L/N434S; and c) a light chain comprising a variable light domain and a constant light domain.

8. scFv-mAb

[00356] One heterodimeric scaffold that finds particular use in the antibodies described herein is the mAb-scFv format. In this embodiment, the format relies on the use of a N-

terminal attachment of a scFv to one of the monomers, thus forming a third antigen binding domain, wherein the Fab portions of the two monomers bind ENPP3 and the “extra” scFv domain binds CD3.

[00357] In this embodiment, the first monomer comprises a first heavy chain (comprising a variable heavy domain and a constant domain), with a N-terminally covalently attached scFv comprising a scFv variable light domain, an scFv linker and a scFv variable heavy domain in either orientation ((VH1-scFv linker-VL1-[optional domain linker]- VH2-CH1-hinge-CH2-CH3) or (with the scFv in the opposite orientation) ((VL1-scFv linker-VH1-[optional domain linker]-VH2-CH1-hinge-CH2-CH3)). This embodiment further utilizes a common light chain comprising a variable light domain and a constant light domain that associates with the heavy chains to form two identical Fabs that bind ENPP3. As for many of the embodiments herein, these constructs include skew variants, pI variants, ablation variants, additional Fc variants, etc. as desired and described herein.

[00358] The antibodies described herein provide scFv-mAb formats where the CD3 binding domain sequences are as shown in 10A-10F and wherein the ENPP3 binding domain sequences are as shown in Figures 12, 13A-13B, and 14A-14I.

[00359] In addition, the Fc domains of the scFv-mAb format generally include skew variants (e.g. a set of amino acid substitutions as shown in Figure 1, with particularly useful skew variants being selected from the group consisting of S364K/E357Q : L368D/K370S; L368D/K370S : S364K; L368E/K370S : S364K; T411T/E360E/Q362E : D401K; L368D/K370S : S364K/E357L, K370S : S364K/E357Q, T366S/L368A/Y407V : T366W and T366S/L368A/Y407V/Y349C : T366W/S354C), optionally ablation variants (including those shown in Figure 3), optionally charged scFv linkers (including those shown in Figure 5) and the heavy chain comprises pI variants (including those shown in Figure 2).

[00360] In some embodiments, the scFv-mAb format includes skew variants, pI variants, and ablation variants. Accordingly, some embodiments include scFv-mAb formats that comprise: a) a first monomer that comprises the skew variants S364K/E357Q, the ablation variants E233P/L234V/L235A/G236del/S267K, and a variable heavy domain that, with the variable light domain of the common light chain, makes up an Fv that binds to ENPP3 as outlined herein, and a scFv domain that binds to CD3; b) a second monomer that comprises the skew variants L368D/K370S, the pI variants N208D/Q295E/N384D/Q418E/N421D, the ablation variants E233P/L234V/L235A/G236del/S267K, and a variable

heavy domain that, with the variable light domain of the common light chain, makes up an Fv that binds to ENPP3 as outlined herein; and c) a common light chain comprising a variable light domain and a constant light domain.

[00361] In some embodiments, the scFv-mAb format includes skew variants, pI variants, ablation variants and FcRn variants. Accordingly, some embodiments include scFv-mAb formats that comprise: a) a first monomer that comprises the skew variants S364K/E357Q, the ablation variants E233P/L234V/L235A/G236del/S267K, the FcRn variants M428L/N434S and a variable heavy domain that, with the variable light domain of the common light chain, makes up an Fv that binds to ENPP3 as outlined herein, and a scFv domain that binds to CD3; b) a second monomer that comprises the skew variants L368D/K370S, the pI variants N208D/Q295E/N384D/ Q418E/N421D, the ablation variants E233P/L234V/L235A/G236del/S267K, the FcRn variants M428L/N434S and a variable heavy domain that, with the variable light domain of the common light chain, makes up an Fv that binds to ENPP3 as outlined herein; and c) a common light chain comprising a variable light domain and a constant light domain.

9. Dual scFv formats

[00362] The antibodies described herein also provide dual scFv formats as are known in the art. In this embodiment, the ENPP3 x CD3 heterodimeric bispecific antibody is made up of two scFv-Fc monomers (both in either (VH-scFv linker-VL-[optional domain linker]-CH2-CH3) format or (VL-scFv linker-VH-[optional domain linker]-CH2-CH3) format, or with one monomer in one orientation and the other in the other orientation.

[00363] The antibodies described herein provide dual scFv formats where the CD3 binding domain sequences are as shown in Figure 10A-10F and wherein the ENPP3 binding domain sequences are as shown in Figures 12, 13A-13B, and 14A-14I. In some embodiments, the dual scFv format includes skew variants, pI variants, and ablation variants. Accordingly, some embodiments include dual scFv formats that comprise: a) a first monomer that comprises the skew variants S364K/E357Q, the ablation variants E233P/L234V/L235A/G236del/S267K, and a first scFv that binds either CD3 or ENPP3; and b) a second monomer that comprises the skew variants L368D/K370S, the pI variants N208D/Q295E/N384D/ Q418E/N421D, the ablation variants

[00364] E233P/L234V/L235A/G236del/S267K, and a second scFv that binds either CD3 or ENPP3. In some embodiments, the dual scFv format includes skew variants, pI variants, ablation variants and FcRn variants. In some embodiments, the dual scFv format includes skew variants, pI variants, and ablation variants. Accordingly, some embodiments include dual scFv formats that comprise: a) a first monomer that comprises the skew variants S364K/E357Q, the ablation variants E233P/L234V/L235A/G236del/S267K, the FcRn variants M428L/N434S and a first scFv that binds either CD3 or ENPP3; and b) a second monomer that comprises the skew variants L368D/K370S, the pI variants N208D/Q295E/N384D/ Q418E/N421D, the ablation variants E233P/L234V/L235A/G236del/S267K, the FcRn variants M428L/N434S and a second scFv that binds either CD3 or ENPP3.

10. Non-heterodimeric bispecific antibodies

[00365] As will be appreciated by those in the art, the ENPP3 and CD3 Fv sequences outlined herein can also be used in both monospecific antibodies (e.g., “traditional monoclonal antibodies”) or non-heterodimeric bispecific formats.

[00366] CD3 binding domain sequences finding particular use include, but are not limited to H1.30_L1.47, H1.32_L1.47, H1.89_L1.47, H1.90_L1.47, H1.33_L1.47, H1.31_L1.47, L1.47_H1.30, L1.47_H1.30, L1.47_H1.32, L1.47_H1.89, L1.47_H1.90, L1.47_H1.33, and L1.47_H1.31 (Figures 10A-10F).

[00367] ENPP3 binding domain sequences that are of particular use include, but are not limited to: AN1[ENPP3] H1L1, AN1[ENPP3] H1 L1.33, AN1[ENPP3] H1 L1.77, AN1[ENPP3] H1.8 L1, AN1[ENPP3] H1.8 L1.33, AN1[ENPP3] H1 L1.77, H16-7.213, H16-9.69, H16-1.52, Ha16-1(1)23, H16-9.44, H16-1.67, Ha1 6-1 (3,5)36, H16-1.86, H16-9.10, H16-9.33, H16-1.68, Ha16-1(1)1, Ha1 6-1(3,5)18, Ha16-1(2,4)4, Ha16-1(3,5)56, H16-7.8, H16-1.93, Ha1 6-1(3,5)27.1, H16-1.61, H16-1(3,5)5, H16-7.200, H16-1(3,5)42, H1 6-9.65, Ha1-1(3,5)19, and Ha16-1.80, (Figures 12, 13A-13B, and 14A-14I).

[00368] Suitable non-heterodimeric bispecific formats are known in the art, and include a number of different formats as generally depicted in Spiess et al., *Molecular Immunology* (67):95-106 (2015) and Kontermann, *mAbs* 4:2, 182-197 (2012), both of which

are expressly incorporated by reference and in particular for the figures, legends and citations to the formats therein.

11. Trident Format

[00369] In some embodiments, the bispecific antibodies described herein are in the “Trident” format as generally described in WO2015/184203, hereby expressly incorporated by reference in its entirety and in particular for the Figures, Legends, definitions and sequences of “Heterodimer-Promoting Domains” or “HPDs”, including “K-coil” and “E-coil” sequences. Tridents rely on using two different HPDs that associate to form a heterodimeric structure as a component of the structure, see Figure 1K. In this embodiment, the Trident format include a “traditional” heavy and light chain (e.g., VH1-CH1-hinge-CH2-CH3 and VL1-CL), a third chain comprising a first “diabody-type binding domain” or “DART®”, VH2-(linker)-VL3-HPD1 and a fourth chain comprising a second DART®, VH3-(linker)-(linker)-VL2-HPD2. The VH1 and VL1 form a first ABD, the VH2 and VL2 form a second ABD, and the VH3 and VL3 form a third ABD. In some cases, as is shown in Figure 1K, the second and third ABDs bind the same antigen, in this instance generally ENPP3, e.g., bivalently, with the first ABD binding a CD3 monovalently.

12. Monospecific, Monoclonal antibodies

[00370] As will be appreciated by those in the art, the novel Fv sequences outlined herein can also be used in both monospecific antibodies (e.g., “traditional monoclonal antibodies”) or non-heterodimeric bispecific formats. Accordingly, in some embodiments, the antibodies described herein provide monoclonal (monospecific) antibodies comprising the 6 CDRs and/or the vh and vl sequences from the figures, generally with IgG1, IgG2, IgG3 or IgG4 constant regions, with IgG1, IgG2 and IgG4 (including IgG4 constant regions comprising a S228P amino acid substitution) finding particular use in some embodiments. That is, any sequence herein with a “H_L” designation can be linked to the constant region of a human IgG1 antibody.

[00371] In some embodiments, the monospecific antibody is an ENPP3 monospecific antibody. In certain embodiments, the monospecific anti-ENPP3 antibody includes the 6 CDRs of any of the anti-ENPP3 antibodies selected from: AN1[ENPP3] H1L1, AN1[ENPP3] H1 L1.33, AN1[ENPP3] H1 L1.77, AN1[ENPP3] H1.8 L1, AN1[ENPP3] H1.8 L1.33, AN1[ENPP3] H1 L1.77, H16-7.213, H16-9.69, H16-1.52, Ha16-1(1)23, H16-9.44, H16-

1.67, Ha1 6-1 (3,5)36, H16-1.86, H16-9.10, H16-9.33, H16-1.68, Ha16-1(1)1, Ha1 6-1(3,5)18, Ha16-1(2,4)4, Ha16-1(3,5)56, H16-7.8, H16-1.93, Ha1 6-1(3,5)27.1, H16-1.61, H16-1(3,5)5, H16-7.200, H16-1(3,5)42, H1 6-9.65, Ha1-1(3,5)19, and Ha16-1.80, (Figures 12, 13A-13B, and 14A-14I).

H. Antigen Binding Domains

[00372] As discussed herein, the subject heterodimeric antibodies include two antigen binding domains (ABDs), each of which bind to ENPP3 or CD3. As outlined herein, these heterodimeric antibodies can be bispecific and bivalent (each antigen is bound by a single ABD, for example, in the format depicted in Figure 15A), or bispecific and trivalent (one antigen is bound by a single ABD and the other is bound by two ABDs, for example as depicted in Figure 15B).

[00373] In addition, in general, one of the ABDs comprises a scFv as outlined herein, in an orientation from N- to C-terminus of VH-scFv linker-VL or VL-scFv linker-VH. One or both of the other ABDs, according to the format, generally is a Fab, comprising a VH domain on one protein chain (generally as a component of a heavy chain) and a VL on another protein chain (generally as a component of a light chain).

[00374] The disclosure provides a number of ABDs that bind to a number of different checkpoint proteins, as outlined below. As will be appreciated by those in the art, any set of 6 CDRs or VH and VL domains can be in the scFv format or in the Fab format, which is then added to the heavy and light constant domains, where the heavy constant domains comprise variants (including within the CH1 domain as well as the Fc domain). The scFv sequences contained in the sequence listing utilize a particular charged linker, but as outlined herein, uncharged or other charged linkers can be used, including those depicted in Figure 7.

[00375] In addition, as discussed above, the numbering used in the Sequence Listing for the identification of the CDRs is Kabat, however, different numbering can be used, which will change the amino acid sequences of the CDRs as shown in Table 2.

[00376] For all of the variable heavy and light domains listed herein, further variants can be made. As outlined herein, in some embodiments the set of 6 CDRs can have from 0, 1, 2, 3, 4 or 5 amino acid modifications (with amino acid substitutions finding particular use), as well as changes in the framework regions of the variable heavy and light domains, as long

as the frameworks (excluding the CDRs) retain at least about 80, 85 or 90% identity to a human germline sequence selected from those listed in Figure 1 of U.S. Patent No. 7,657,380, which Figure and Legend is incorporated by reference in its entirety herein. Thus, for example, the identical CDRs as described herein can be combined with different framework sequences from human germline sequences, as long as the framework regions retain at least 80, 85 or 90% identity to a human germline sequence selected from those listed in Figure 1 of U.S. Patent No. 7,657,380. Alternatively, the CDRs can have amino acid modifications (e.g. from 1, 2, 3, 4 or 5 amino acid modifications in the set of CDRs (that is, the CDRs can be modified as long as the total number of changes in the set of 6 CDRs is less than 6 amino acid modifications, with any combination of CDRs being changed; e.g. there may be one change in VLCDR1, two in VHCDR2, none in VHCDR3, etc.)), as well as having framework region changes, as long as the framework regions retain at least 80, 85 or 90% identity to a human germline sequence selected from those listed in Figure 1 of U.S. Patent No. 7,657,380.

1. ENPP3 Antigen Binding Domains

[00377] In some embodiments, one of the ABDs binds ENPP3. Suitable sets of 6 CDRs and/or VH and VL domains are depicted in Figures 12, 13A-13B, and 14A-14I. In some embodiments, the heterodimeric antibody is a 1 + 1 Fab-scFv-Fc or 2 + 1 Fab2-scFv-Fv format antibody (see, e.g., Figures 15A and 15B).

[00378] In one embodiment, the ENPP3 antigen binding domain includes the 6 CDRs (i.e., vhCDR1-3 and vlCDR1-3) of a ENPP3 ABD described herein, including the figures and sequence listing. In exemplary embodiments, the ENPP3 ABD is one of the following ENPP3 ABDs: AN1[ENPP3] H1L1, AN1[ENPP3] H1 L1.33, AN1[ENPP3] H1 L1.77, AN1[ENPP3] H1.8 L1, AN1[ENPP3] H1.8 L1.33, AN1[ENPP3] H1 L1.77, H16-7.213, H16-9.69, H16-1.52, Ha16-1(1)23, H16-9.44, H16-1.67, Ha1 6-1 (3,5)36, H16-1.86, H16-9.10, H16-9.33, H16-1.68, Ha16-1(1)1, Ha1 6-1(3,5)18, Ha16-1(2,4)4, Ha16-1(3,5)56, H16-7.8, H16-1.93, Ha1 6-1(3,5)27.1, H16-1.61, H16-1(3,5)5, H16-7.200, H16-1(3,5)42, H1 6-9.65, Ha1-1(3,5)19, and Ha16-1.80(Figures 12, 13A-13B, and 14A-14I).

[00379] As will be appreciated by those in the art, suitable ENPP3 binding domains can comprise a set of 6 CDRs as depicted in the Figures, either as they are underlined or, in the case where a different numbering scheme is used as described herein and as shown in Table 2, as the CDRs that are identified using other alignments within the VH and VL

sequences of those depicted in Figures 12, 13A-13B, and 14A-14I. Suitable ABDs can also include the entire VH and VL sequences as depicted in these sequences and Figures, used as scFvs or as Fabs. In many of the embodiments herein that contain an Fv to ENPP3, it is the Fab monomer that binds ENPP3.

[00380] In addition to the parental CDR sets disclosed in the figures and sequence listing that form an ABD to ENPP3, the disclosure provides variant CDR sets. In one embodiment, a set of 6 CDRs can have 1, 2, 3, 4 or 5 amino acid changes from the parental CDRs, as long as the ENPP3 ABD is still able to bind to the target antigen, as measured by at least one of a Biacore, surface plasmon resonance (SPR) and/or BLI (biolayer interferometry, e.g. Octet assay) assay, with the latter finding particular use in many embodiments.

[00381] In addition to the parental variable heavy and variable light domains disclosed herein that form an ABD to ENPP3, the disclosure provides variant VH and VL domains. In one embodiment, the variant VH and VL domains each can have from 1, 2, 3, 4, 5, 6, 7, 8, 9 or 10 amino acid changes from the parental VH and VL domain, as long as the ABD is still able to bind to the target antigen, as measured at least one of a Biacore, surface plasmon resonance (SPR) and/or BLI (biolayer interferometry, e.g. Octet assay) assay, with the latter finding particular use in many embodiments. In another embodiment, the variant VH and VL are at least 90, 95, 97, 98 or 99% identical to the respective parental VH or VL, as long as the ABD is still able to bind to the target antigen, as measured by at least one of a Biacore, surface plasmon resonance (SPR) and/or BLI (biolayer interferometry, e.g. Octet assay) assay, with the latter finding particular use in many embodiments.

2. CD3 Antigen Binding Domains

[00382] In some embodiments, one of the ABDs binds CD3. Suitable sets of 6 CDRs and/or VH and VL domains, as well as scFv sequences, are depicted in Figures 10A-10F and the Sequence Listing. CD3 binding domain sequences that are of particular use include, but are not limited to, anti-CD3 H1.30_L1.47, anti-CD3 H1.32, anti-CD3 L1.47, anti-CD3 H1.89_L1.47, anti-CD3 H1.90_L1.47, anti-CD3 H1.33_L1.47, anti-CD3 H1.31_L1.47, anti-CD3 L1.47_H1.30, anti-CD3 L1.47_H1.30, anti-CD3 L1.47_H1.32, anti-CD3 L1.47_H1.89, anti-CD3 L1.47_H1.90, anti-CD3 L1.47_H1.33, and anti-CD3 L1.47_H1.31 as depicted in Figures 10A-10F.

[00383] As will be appreciated by those in the art, suitable CD3 binding domains can comprise a set of 6 CDRs as depicted in Figures 10A-10F, either as they are underlined or, in the case where a different numbering scheme is used as described herein and as shown in Table 2, as the CDRs that are identified using other alignments within the VH and VL sequences of those depicted in Figures 10A-10F. Suitable ABDs can also include the entire VH and VL sequences as depicted in these sequences and Figures, used as scFvs or as Fabs. In many of the embodiments herein that contain an Fv to CD3, it is the scFv monomer that binds CD3.

[00384] In addition to the parental CDR sets disclosed in the figures and sequence listing that form an ABD to CD3, the disclosure provides variant CDR sets. In one embodiment, a set of 6 CDRs can have 1, 2, 3, 4 or 5 amino acid changes from the parental CDRs, as long as the CD3 ABD is still able to bind to the target antigen, as measured by at least one of a Biacore, surface plasmon resonance (SPR) and/or BLI (biolayer interferometry, e.g. Octet assay) assay, with the latter finding particular use in many embodiments.

[00385] In addition to the parental variable heavy and variable light domains disclosed herein that form an ABD to CD3, the disclosure provides variant VH and VL domains. In one embodiment, the variant VH and VL domains each can have from 1, 2, 3, 4, 5, 6, 7, 8, 9 or 10 amino acid changes from the parental VH and VL domain, as long as the ABD is still able to bind to the target antigen, as measured at least one of a Biacore, surface plasmon resonance (SPR) and/or BLI (biolayer interferometry, e.g. Octet assay) assay, with the latter finding particular use in many embodiments. In another embodiment, the variant VH and VL are at least 90, 95, 97, 98 or 99% identical to the respective parental VH or VL, as long as the ABD is still able to bind to the target antigen, as measured by at least one of a Biacore, surface plasmon resonance (SPR) and/or BLI (biolayer interferometry, e.g. Octet assay) assay, with the latter finding particular use in many embodiments.

VI. SSTR2 Binding Domains

[00386] In one aspect, provided herein are Somatostatin Receptor 2 (SSTR2) antigen binding domains (ABDs) and compositions that include such SSTR2 antigen binding domains (ABDs), including anti-SSTR2 antibodies.

[00387] Somatostatin receptors (SSTRs) belong to a superfamily of G protein-coupled receptors (GPCRs) that each contain a single polypeptide chain consisting of extracellular/intracellular domains, and seven transmembrane domains. SSTRs are highly

expressed in various cultured tumor cells and primary tumor tissues, including NETs (lung, GI, pancreatic, pituitary, medullary cancers, prostate, pancreatic lungcarcinoids, osteosarcoma, etc.) as well as non-NETs (breast, lung, colorectal, ovarian, cervical cancers, etc.) (Reubi., 2003, *Endocr. Rev.* 24: 389-427; Volante et al., 2008, *Mol. Cell. Endocrinol.* 286: 219-229; and Schulz et al., 2003, *Gynecol. Oncol.* 89: 385-390). To date, five SSTR receptor subtypes have been identified (Patel et al., 1997, *Trends Endocrinol. Metab.* 8: 398-405). SSTR2 in particular is expressed at a high concentration on many tumor cells (Volante et al., 2008, *Mol. Cell. Endocrinol.* 286: 219-229; and Reubi et al., 2003, *Eur. J. Nucl. Med. Mol. Imaging* 30: 781-793), thus making it a candidate target antigen for bispecific antibody cancer target therapeutics. In view of the high concentration of SSTR2 expressed on various tumors, it is believed that anti-SSTR2 antibodies are useful, for example, for localizing anti-tumor therapeutics (e.g., chemotherapeutic agents and T cells) to such SSTR2 expressing tumors.

[00388] Subject antibodies that include the SSTR2 antigen binding domains provided herein (e.g., anti-SSTR2 x anti-CD3 bispecific antibodies) advantageously elicit a range of different immune responses. Such SSTR2 binding domains and related antibodies find use, for example, in the treatment of SSTR2 associated cancers.

[00389] As will be appreciated by those in the art, suitable SSTR2 binding domains can comprise a set of 6 CDRs as depicted in the Figure 63, either as they are underlined or, in the case where a different numbering scheme is used as described herein and as shown in Table 2, as the CDRs that are identified using other alignments within the VH and VL sequences of those depicted in Figure 63. Suitable ABDs can also include the entire VH and VL sequences as depicted in these sequences and Figures, used as scFvs or as Fabs. In many of the embodiments herein that contain an Fv to SSTR2, it is the Fab monomer that binds SSTR2. In one embodiment, the SSTR2 antigen binding domain includes the 6 CDRs (i.e., vhCDR1-3 and vlCDR1-3) of [α SSTR2] H1.24_L1.30 (Figure 63).

[00390] In addition to the parental CDR sets disclosed in the figures and sequence listing that form an ABD to SSTR2, provided herein are variant SSTR2 ABDs having CDRs that include at least one modification of the SSTR2 ABD CDRs disclosed herein. In one embodiment, the SSTR2 ABD includes a set of 6 CDRs with 1, 2, 3, 4, 5, 6, 7, 8, 9, 10 amino acid modifications as compared to the 6 CDRs of a SSTR2 ABD described herein, including the figures and sequence listing. In exemplary embodiments, the SSTR2 ABD includes a set

of 6 CDRs with 1, 2, 3, 4, 5, 6, 7, 8, 9, 10 amino acid modifications as compared to the 6 CDRs of [α SSTR2] H1.24_L1.30 (Figure 63). In certain embodiments, the variant SSTR2 ABD is capable of binding SSTR2 antigen, as measured by at least one of a Biacore, surface plasmon resonance (SPR) and/or BLI (biolayer interferometry, e.g., Octet assay) assay, with the latter finding particular use in many embodiments. In particular embodiments, the SSTR2 ABD is capable of binding human SSTR2 antigen.

[00391] In one embodiment, the SSTR2 ABD includes 6 CDRs that are at least 90, 95, 97, 98 or 99% identical to the 6 CDRs of a SSTR2 ABD as described herein, including the figures and sequence listing. In exemplary embodiments, the SSTR2 ABD includes 6 CDRs that are at least 90, 95, 97, 98 or 99% identical to the 6 CDRs of [α SSTR2] H1.24_L1.30 (Figure 63). In certain embodiments, the SSTR2 ABD is capable of binding to SSTR2 antigen, as measured by at least one of a Biacore, surface plasmon resonance (SPR) and/or BLI (biolayer interferometry, e.g., Octet assay) assay, with the latter finding particular use in many embodiments. In particular embodiments, the SSTR2 ABD is capable of binding human SSTR2 antigen.

[00392] In another exemplary embodiment, the SSTR2 ABD include the variable heavy (VH) domain and variable light (VL) domain of any one of the SSTR2 ABDs described herein, including the figures and sequence listing. In exemplary embodiments, the SSTR2 ABD is [α SSTR2] H1.24_L1.30 (Figure 63). .

[00393] In addition to the parental SSTR2 variable heavy and variable light domains disclosed herein, provided herein are SSTR2 ABDs that include a variable heavy domain and/or a variable light domain that are variants of a SSTR2 ABD VH and VL domain disclosed herein. In one embodiment, the variant VH domain and/or VL domain has from 1, 2, 3, 4, 5, 6, 7, 8, 9 or 10 amino acid changes from a VH and/or VL domain of a SSTR2 ABD described herein, including the figures and sequence listing. In exemplary embodiments, the variant VH domain and/or VL domain has from 1, 2, 3, 4, 5, 6, 7, 8, 9 or 10 amino acid changes from a VH and/or VL domain of [α SSTR2] H1.24_L1.30 (Figure 63). In certain embodiments, the SSTR2 ABD is capable of binding to SSTR2, as measured at least one of a Biacore, surface plasmon resonance (SPR) and/or BLI (biolayer interferometry, e.g., Octet assay) assay, with the latter finding particular use in many embodiments. In particular embodiments, the SSTR2 ABD is capable of binding human SSTR2 antigen.

[00394] In one embodiment, the variant VH and/or VL domain is at least 90, 95, 97, 98 or 99% identical to the VH and/or VL of a SSTR2 ABD as described herein, including the figures and sequence listing. In exemplary embodiments, the variant VH and/or VL domain is at least 90, 95, 97, 98 or 99% identical to the VH and/or VL of [α SSTR2] H1.24_L1.30 (Figure 63). In certain embodiments, the SSTR2 ABD is capable of binding to the SSTR2, as measured by at least one of a Biacore, surface plasmon resonance (SPR) and/or BLI (biolayer interferometry, e.g., Octet assay) assay, with the latter finding particular use in many embodiments. In particular embodiments, the SSTR2 ABD is capable of binding human SSTR2 antigen.

[00395] In some embodiments, the subject antibodies described herein include at least one SSTR2 binding domain. In certain embodiments, the antibody is a heterodimeric antibody. In some embodiments, the heterodimeric antibody is a 1 + 1 Fab-scFv-Fc or 2 + 1 Fab2-scFv-Fv format antibody (see, e.g., Figures 15A and 15B). Such heterodimeric antibodies can include any of Fc variant amino acid substitutions, independently or in combination, provided herein (e.g., skew, pI and ablation variants, including those depicted in Figures 1-4). Particularly useful skew variants being selected from the group consisting of S364K/E357Q : L368D/K370S; L368D/K370S : S364K; L368E/K370S : S364K; T411T/E360E/Q362E : D401K; L368D/K370S : S364K/E357L, K370S : S364K/E357Q, T366S/L368A/Y407V : T366W and T366S/L368A/Y407V/Y349C : T366W/S354C), optionally ablation variants (including those shown in Figure 3), optionally charged scFv linkers (including those shown in Figure 5) and the heavy chain comprises pI variants (including those shown in Figure 2).

A. Useful Embodiments

[00396] Useful embodiments include 1 + 1 Fab-scFv-Fc formats that comprise: a) a first monomer (the “scFv monomer”) that comprises a charged scFv linker (with the +H sequence of Figure 5 being preferred in some embodiments), the skew variants S364K/E357Q, the ablation variants E233P/L234V/L235A/G236del/S267K, and an scFv that binds to CD3 as outlined herein; b) a second monomer (the “Fab monomer”) that comprises the skew variants L368D/K370S, the pI variants N208D/Q295E/N384D/Q418E/N421D, the ablation variants E233P/L234V/L235A/ G236del/S267K, and a variable heavy domain; and c) a light chain that includes a variable light domain light domain (VL) and a constant light

domain (CL), wherein numbering is according to EU numbering.. In some embodiments, the variable heavy domain and variable light domain make up an ENPP3 binding moiety.

[00397] Other useful embodiments include 1 + 1 Fab-scFv-Fc formats that comprise: a) a first monomer (the “scFv monomer”) that comprises a charged scFv linker (with the +H sequence of Figure 5 being preferred in some embodiments), the skew variants S364K/E357Q, the ablation variants E233P/L234V/L235A/G236del/S267K, and an scFv that binds to CD3 as outlined herein; b) a second monomer (the “Fab monomer”) that comprises the skew variants L368D/K370S, the pI variants N208D/Q295E/N384D/Q418E/N421D, the ablation variants E233P/L234V/L235A/ G236del/S267K, and a variable heavy domain; and c) a light chain that includes a variable light domain light domain (VL) and a constant light domain (CL), wherein numbering is according to EU numbering.. In some embodiments, the variable heavy domain and variable light domain make up an SSTR2 binding moiety.

[00398] Other useful embodiments include 2 + 1 Fab₂-scFv-Fc formats that comprise: a) a first monomer (the Fab-scFv-Fc side) that comprises the skew variants S364K/E357Q, the ablation variants E233P/L234V/L235A/G236del/S267K, and a variable heavy domain that, with the variable light domain of the common light chain, makes up an Fv that binds to ENPP3 as outlined herein, and an scFv domain that binds to CD3; b) a second monomer (the Fab-Fc side) that comprises the skew variants L368D/K370S, the pI variants N208D/Q295E/N384D/ Q418E/N421D, the ablation variants E233P/L234V/L235A/G236del/S267K, and a variable heavy domain that, with variable light domain of the common light chain, makes up an Fv that binds to ENPP3 as outlined herein; and c) a common light chain comprising the variable light domain and a constant light domain, where numbering is according to EU numbering. In some embodiments, the common light chain and variable heavy domains on each monomer form ENPP3 binding domains.

[00399] Other useful embodiments include 2 + 1 Fab₂-scFv-Fc formats that comprise: a) a first monomer (the Fab-scFv-Fc side) that comprises the skew variants S364K/E357Q, the ablation variants E233P/L234V/L235A/G236del/S267K, and a variable heavy domain that, with the variable light domain of the common light chain, makes up an Fv that binds to SSTR2 as outlined herein, and an scFv domain that binds to CD3; b) a second monomer (the Fab-Fc side) that comprises the skew variants L368D/K370S, the pI variants N208D/Q295E/N384D/ Q418E/N421D, the ablation variants

E233P/L234V/L235A/G236del/S267K, and a variable heavy domain that, with variable light domain of the common light chain, makes up an Fv that binds to SSTR2 as outlined herein; and c) a common light chain comprising the variable light domain and a constant light domain, where numbering is according to EU numbering. In some embodiments, the common light chain and variable heavy domains on each monomer form SSTR2 binding domains (e.g., [α SSTR2] H1.24_L1.30 (Figure 63)).

[00400] Some useful embodiments include: XENP24804, XENP26820, XENP28287, XENP28925, XENP29516, XENP30262, XENP26821, XENP29436, XENP28390, XENP29463, and XENP30263.

[00401] Other useful embodiments include: XENP29437, XENP29520, XENP30264, XENP26822, XENP28438, XENP29438, XENP29467, XENP30469, XENP30470, XENP30819, XENP30821, XENP31148, XENP31149, XENP31150, XENP31419, and XENP31471.

[00402] Another useful embodiment is XENP30458.

VII. Nucleic Acids of the Invention

[00403] The disclosure further provides nucleic acid compositions encoding the anti-ENPP3 antibodies provided herein, including, but not limited to, anti-ENPP3 x anti-CD3 bispecific antibodies and ENPP3 monospecific antibodies.

[00404] As will be appreciated by those in the art, the nucleic acid compositions will depend on the format and scaffold of the heterodimeric protein. Thus, for example, when the format requires three amino acid sequences, such as for the 1+1 Fab-scFv-Fc format (e.g. a first amino acid monomer comprising an Fc domain and a scFv, a second amino acid monomer comprising a heavy chain and a light chain), three nucleic acid sequences can be incorporated into one or more expression vectors for expression. Similarly, some formats (e.g. dual scFv formats such as disclosed in Figure 1) only two nucleic acids are needed; again, they can be put into one or two expression vectors.

[00405] As is known in the art, the nucleic acids encoding the components of the antibodies described herein can be incorporated into expression vectors as is known in the art, and depending on the host cells used to produce the heterodimeric antibodies described herein. Generally the nucleic acids are operably linked to any number of regulatory elements

(promoters, origin of replication, selectable markers, ribosomal binding sites, inducers, etc.). The expression vectors can be extra-chromosomal or integrating vectors.

[00406] The nucleic acids and/or expression vectors of the antibodies described herein are then transformed into any number of different types of host cells as is well known in the art, including mammalian, bacterial, yeast, insect and/or fungal cells, with mammalian cells (e.g. CHO cells), finding use in many embodiments.

[00407] In some embodiments, nucleic acids encoding each monomer and the optional nucleic acid encoding a light chain, as applicable depending on the format, are each contained within a single expression vector, generally under different or the same promoter controls. In embodiments of particular use in the antibodies described herein, each of these two or three nucleic acids are contained on a different expression vector. As shown herein and in 62/025,931, hereby incorporated by reference, different vector ratios can be used to drive heterodimer formation. That is, surprisingly, while the proteins comprise first monomer:second monomer:light chains (in the case of many of the embodiments herein that have three polypeptides comprising the heterodimeric antibody) in a 1:1:2 ratio, these are not the ratios that give the best results.

[00408] The heterodimeric antibodies described herein are made by culturing host cells comprising the expression vector(s) as is well known in the art. Once produced, traditional antibody purification steps are done, including an ion exchange chromatography step. As discussed herein, having the pIs of the two monomers differ by at least 0.5 can allow separation by ion exchange chromatography or isoelectric focusing, or other methods sensitive to isoelectric point. That is, the inclusion of pI substitutions that alter the isoelectric point (pI) of each monomer so that each monomer has a different pI and the heterodimer also has a distinct pI, thus facilitating isoelectric purification of the "1+1 Fab-scFv-Fc" and "2+1" heterodimers (e.g., anionic exchange columns, cationic exchange columns). These substitutions also aid in the determination and monitoring of any contaminating dual scFv-Fc and mAb homodimers post-purification (e.g., IEF gels, cIEF, and analytical IEX columns).

VIII. Biological and Biochemical Functionality of the Heterodimeric Bispecific Antibodies

[00409] Generally the bispecific ENPP3 x CD3 antibodies described herein are administered to patients with cancer, and efficacy is assessed, in a number of ways as described herein. Thus, while standard assays of efficacy can be run, such as cancer load, size of tumor, evaluation of presence or extent of metastasis, etc., immuno-oncology treatments can be assessed on the basis of immune status evaluations as well. This can be done in a number of ways, including both in vitro and in vivo assays.

IX. Treatments

Once made, the compositions of the antibodies described herein find use in a number of applications. ENPP3 is highly expressed in renal cell carcinoma, accordingly, the heterodimeric compositions of the antibodies described herein find use in the treatment of such ENPP3 positive cancers.

X. Antibody Compositions for In Vivo Administration

[00410] Formulations of the antibodies used in accordance with the antibodies described herein are prepared for storage by mixing an antibody having the desired degree of purity with optional pharmaceutically acceptable carriers, excipients or stabilizers (Remington's Pharmaceutical Sciences 16th edition, Osol, A. Ed. [1980]), in the form of lyophilized formulations or aqueous solutions. Acceptable carriers, excipients, or stabilizers are nontoxic to recipients at the dosages and concentrations employed, and include buffers such as phosphate, citrate, and other organic acids; antioxidants including ascorbic acid and methionine; preservatives (such as octadecyldimethylbenzyl ammonium chloride; hexamethonium chloride; benzalkonium chloride, benzethonium chloride; phenol, butyl or benzyl alcohol; alkyl parabens such as methyl or propyl paraben; catechol; resorcinol; cyclohexanol; 3-pentanol; and m-cresol); low molecular weight (less than about 10 residues) polypeptides; proteins, such as serum albumin, gelatin, or immunoglobulins; hydrophilic polymers such as polyvinylpyrrolidone; amino acids such as glycine, glutamine, asparagine, histidine, arginine, or lysine; monosaccharides, disaccharides, and other carbohydrates including glucose, mannose, or dextrins; chelating agents such as EDTA; sugars such as sucrose, mannitol, trehalose or sorbitol; salt-forming counter-ions such as sodium; metal complexes (e.g. Zn-protein complexes); and/or non-ionic surfactants such as TWEEN™, PLURONICS™ or polyethylene glycol (PEG).

XI. Administrative modalities

[00411] The antibodies and chemotherapeutic agents described herein are administered to a subject, in accord with known methods, such as intravenous administration as a bolus or by continuous infusion over a period of time.

XII. Treatment modalities

[00412] In the methods described herein, therapy is used to provide a positive therapeutic response with respect to a disease or condition. By “positive therapeutic response” is intended an improvement in the disease or condition, and/or an improvement in the symptoms associated with the disease or condition. For example, a positive therapeutic response would refer to one or more of the following improvements in the disease: (1) a reduction in the number of neoplastic cells; (2) an increase in neoplastic cell death; (3) inhibition of neoplastic cell survival; (5) inhibition (i.e., slowing to some extent, preferably halting) of tumor growth; (6) an increased patient survival rate; and (7) some relief from one or more symptoms associated with the disease or condition.

[00413] Positive therapeutic responses in any given disease or condition can be determined by standardized response criteria specific to that disease or condition. Tumor response can be assessed for changes in tumor morphology (i.e., overall tumor burden, tumor size, and the like) using screening techniques such as magnetic resonance imaging (MRI) scan, x-radiographic imaging, computed tomographic (CT) scan, bone scan imaging, endoscopy, and tumor biopsy sampling including bone marrow aspiration (BMA) and counting of tumor cells in the circulation.

[00414] In addition to these positive therapeutic responses, the subject undergoing therapy may experience the beneficial effect of an improvement in the symptoms associated with the disease.

[00415] Treatment according to the disclosure includes a “therapeutically effective amount” of the medicaments used. A “therapeutically effective amount” refers to an amount effective, at dosages and for periods of time necessary, to achieve a desired therapeutic result.

[00416] A therapeutically effective amount may vary according to factors such as the disease state, age, sex, and weight of the individual, and the ability of the medicaments to

elicit a desired response in the individual. A therapeutically effective amount is also one in which any toxic or detrimental effects of the antibody or antibody portion are outweighed by the therapeutically beneficial effects.

[00417] A “therapeutically effective amount” for tumor therapy may also be measured by its ability to stabilize the progression of disease. The ability of a compound to inhibit cancer may be evaluated in an animal model system predictive of efficacy in human tumors.

[00418] Alternatively, this property of a composition may be evaluated by examining the ability of the compound to inhibit cell growth or to induce apoptosis by in vitro assays known to the skilled practitioner. A therapeutically effective amount of a therapeutic compound may decrease tumor size, or otherwise ameliorate symptoms in a subject. One of ordinary skill in the art would be able to determine such amounts based on such factors as the subject’s size, the severity of the subject’s symptoms, and the particular composition or route of administration selected.

[00419] Dosage regimens are adjusted to provide the optimum desired response (e.g., a therapeutic response). For example, a single bolus may be administered, several divided doses may be administered over time or the dose may be proportionally reduced or increased as indicated by the exigencies of the therapeutic situation. Parenteral compositions may be formulated in dosage unit form for ease of administration and uniformity of dosage. Dosage unit form as used herein refers to physically discrete units suited as unitary dosages for the subjects to be treated; each unit contains a predetermined quantity of active compound calculated to produce the desired therapeutic effect in association with the required pharmaceutical carrier.

[00420] The specification for the dosage unit forms of the disclosure are dictated by and directly dependent on (a) the unique characteristics of the active compound and the particular therapeutic effect to be achieved, and (b) the limitations inherent in the art of compounding such an active compound for the treatment of sensitivity in individuals.

[00421] The efficient dosages and the dosage regimens for the bispecific antibodies described herein depend on the disease or condition to be treated and may be determined by the persons skilled in the art.

[00422] An exemplary, non-limiting range for a therapeutically effective amount of an bispecific antibody used in the antibodies described herein is about 0.1-100 mg/kg.

[00423] All cited references are herein expressly incorporated by reference in their entirety.

[00424] Whereas particular embodiments of the disclosure have been described above for purposes of illustration, it will be appreciated by those skilled in the art that numerous variations of the details may be made without departing from the invention as described in the appended claims.

EXAMPLES

[00425] Examples are provided below to illustrate the antibodies described herein. These examples are not meant to constrain the antibodies described herein to any particular application or theory of operation. For all constant region positions discussed in the antibodies described herein, numbering is according to the EU index as in Kabat (Kabat et al., 1991, Sequences of Proteins of Immunological Interest, 5th Ed., United States Public Health Service, National Institutes of Health, Bethesda, entirely incorporated by reference). Those skilled in the art of antibodies will appreciate that this convention consists of nonsequential numbering in specific regions of an immunoglobulin sequence, enabling a normalized reference to conserved positions in immunoglobulin families. Accordingly, the positions of any given immunoglobulin as defined by the EU index will not necessarily correspond to its sequential sequence.

[00426] General and specific scientific techniques are outlined in US Publications 2015/0307629, 2014/0288275 and WO2014/145806, all of which are expressly incorporated by reference in their entirety and particularly for the techniques outlined therein.

Example 1: Binding Domains

1A: CD3 BINDING DOMAINS

[00427] Sequences for CD3 binding domains having different CD3 binding affinities are depicted in Figure 10.

1B: ENPP3 BINDING DOMAINS

1B(a): ENPP3 Binding Domain AN1

[00428] The variable regions of a murine ENPP3 binding domain were humanized using string content optimization (see, e.g., U.S. Patent No. 7,657,380, issued February 2,

2010). Sequences for the humanized ENPP3 binding domain, hereon referred to as AN1, are depicted in Figure 10A-10F.

[00429] AN1 variants were engineered for improved purification (in the context of α ENPP3 x α CD3 bispecific antibodies) and for modulated ENPP3 binding affinity/potency. Sequences for illustrative such variants are depicted in Figure 13.

1B(b): Additional ENPP3 Binding Domains

[00430] Sequences for additional ENPP3 binding domains which may find use in the α ENPP3 x α CD3 bispecific antibodies described herein are depicted in Figure 14.

Example 2: Engineering and Producing α ENPP3 x α CD3 bispecific antibodies

[00431] A number of formats for α ENPP3 x α CD3 bispecific antibodies (bsAbs) were conceived, illustrative formats for which are outlined below and in Figure 15.

[00432] One such format is the 1 + 1 Fab-scFv-Fc format which comprises a single-chain Fv ("scFv") covalently attached to a first heterodimeric Fc domain, a heavy chain variable region (VH) covalently attached to a complementary second heterodimeric Fc domain, and a light chain (LC) transfected separately so that a Fab domain is formed with the variable heavy domain.

[00433] Another format is the 2 + 1 Fab2-scFv-Fc format which comprises a VH domain covalently attached to a CH1 domain covalently attached to an scFv covalently attached to a first heterodimeric Fc domain (VH-CH1-scFv-Fc), a VH domain covalently attached to a complementary second heterodimeric Fc domain, and a LC transfected separately so that Fab domains are formed with the VH domains.

[00434] DNA encoding chains of the α ENPP3 x α CD3 bsAbs were generated by standard gene synthesis followed by isothermal cloning (Gibson assembly) or subcloning into a pTT5 expression vector containing fusion partners (e.g. domain linkers as depicted in Figure 6 and/or backbones as depicted in Figures 7-9). DNA was transfected into HEK293E cells for expression. Sequences for illustrative α ENPP3 x α CD3 bsAbs (based on binding domains as described in Example 1) in the 1 + 1 Fab-scFv-Fc format and in the 2 + 1 Fab2-scFv-Fc format are depicted respectively in Figures 17-23.

Example 3: α ENPP3 x α CD3 bsAbs redirect T cells to destroy ENPP3-expressing cells

[00435] Prototypic α ENPP3 x α CD3 bsAbs in the 1 + 1 Fab-scFv-Fc format were engineered using the binding domains described in Example 1. In particular, XENP26820 (comprising ENPP3 binding domain clone H16-7.8 and CD3 High scFv), XENP26821 (comprising ENPP3 binding domain clone H16-7.8 and CD3 High-Int #1 scFv), XENP28287 (comprising ENPP3 binding domain clone AN1 and CD3 High scFv), and XENP28390 (comprising ENPP3 binding domain clone AN1 and CD3 High Int #1 scFv), sequences for which are depicted in Figures 17 and 18. XENP13245 (comprising an RSV binding domain based on motavizumab and anti-CD3-High; sequences depicted in Figure 16) was used as a control.

[00436] The potential of the prototypic α ENPP3 x α CD3 bispecific antibodies (bsAbs) to redirect CD3⁺ effector T cells to destroy ENPP3-expressing cell lines was investigated. In a first experiment, KU812 (an ENPP3^{high} basophilic leukemia cell line) cells were incubated with human PBMCs (10:1 effector to target cell ratio) and indicated concentrations of the test articles described above for 24 hours at 37°C. After incubation, cells were stained with Aqua Zombie stain for 15 minutes at room temperature. Cells were then washed and stained with antibodies for cell surface markers and analyzed by flow cytometry. Two different approaches were used for investigating induction of redirected T-cell cytotoxicity (RTCC): a) decrease in the number of CSFE⁺ target cells (data for which are depicted in Figure 24A), and b) Zombie Aqua staining on CSFE⁺ target cells (data for which are depicted in Figure 24B). Activation and degranulation of CD4⁺ and CD8⁺ T cells were also determined based on CD107a, CD25, and CD69 expression (data for which are depicted in Figures 25-26).

[00437] In a second experiment, RXF393 (clinically relevant renal cell carcinoma cell line that expresses ENPP3) cells were incubated with human PBMCs (20:1 effector to target cell ratio) and indicated concentrations of the prototype test articles described above for 24 hours at 37°C. After incubation, cells were stained with Aqua Zombie stain for 15 minutes at room temperature. Cells were then washed and stained with antibodies for cell surface markers and analyzed by flow cytometry. As above, two different approaches were used for investigating induction of RTCC: a) decrease in the number of CSFE⁺ target cells (data for which are depicted in Figure 27A), and b) Zombie Aqua staining on CSFE⁺ target cells (data for which are depicted in Figure 27B). Activation and degranulation of CD4⁺ and CD8⁺ T

cells were also determined based on CD107a, CD25, and CD69 expression (data for which are depicted in Figure 28-29).

[00438] Collectively, the data show that the prototypic α ENPP3 x α CD3 bsAbs dose-dependently induced RTCC on ENPP3 cells; CD3 binding affinity correlated with RTCC potency (i.e. bsAbs with CD3 High induced RTCC more potently than bsAbs with CD3 High-Int #1); and bsAbs with AN1-based binding domain induced RTCC more potently than bsAbs with H16-7.8-based binding domain. Consistent with the RTCC data, α ENPP3 x α CD3 bsAbs dose-dependently induced activation of T cells; CD3 binding affinity correlated with activation potency (i.e. bsAbs with CD3 High induced T cell activation more potently than bsAbs with CD3 High-Int #1); and bsAbs with AN1 binding domain induced T cell activation more potently than bsAbs with H16-7.8 binding domain.

Example 4: Improving α ENPP3 x α CD3 Production

[00439] Generally, the bispecific antibodies were produced by transient transfection in HEK293E cells and were purified by a two-step purification process comprising protein A chromatography (purification part 1) followed by ion exchange chromatography (purification part 2).

4A: ENGINEERING AN1 VARIANTS TO IMPROVE PRODUCTION

4A(a): Production of XENP28287 results in a homogeneous population which includes aggregates and unpaired monomers

[00440] XENP28287 was purified from HEK293E supernatant as described above. Figure 30A depicts the chromatogram showing purification part 2 of XENP28287 (cation exchange chromatography following protein A chromatography). The chromatogram shows the isolation of two peaks (peak B and peak BC), which were further characterized by analytical size-exclusion chromatography with multi-angle light scattering (aSEC-MALS) and analytical cation-exchange chromatography (aCIEX) for identity, purity and homogeneity as generally described below.

[00441] Peaks B and BC isolated from purification part 2 for XENP28287 (as well as pre-purified material) were analyzed using aSEC-MALS to deduce their component protein species. The analysis was performed on an Agilent 1200 high-performance liquid chromatography (HPLC) system. Samples were injected onto a Superdex™ 200 10/300 GL column (GE Healthcare Life Sciences) at 1.0 mL/min using 1 x PBS, pH 7.4 as the mobile phase at 4°C for 25 minutes with UV detection wavelength at 280 nm. MALS was performed

on a miniDAWN® TREOS® with an Optilab® T-rEX Refractive Index Detector (Wyatt Technology, Santa Barbara, Cali.). Analysis was performed using Agilent OpenLab Chromatography Data System (CDS) ChemStation Edition AIC version C.01.07 and ASTRA version 6.1.7.15. Chromatograms depicting a SEC separation profiles for pre-purified material, peak B, and peak BC are depicted in Figure 30B along with approximate MW of component species as determined by MALS. The profiles show that peak B comprises a dominant species of ~126 kDa which is consistent with the calculated molecular weight of the XENP28287 heterodimer (based on amino acid sequence), but also includes a contaminating species of 75 kDa (likely to be monomers). Peak BC comprises peaks with species of 308 kDa (likely to be aggregates), 121 kDa (XENP28287), and 82 kDa (contaminating monomers). Notably, the separation profile for pre-purified material indicate that less than 85% of material was the bispecific antibody heterodimer.

[00442] The peaks from purification part 2 were also analyzed using analytical CIEC to further assess the purity and homogeneity of peaks B and BC. The analysis was performed on an Agilent 1200 high-performance liquid chromatography (HPLC) system. Samples were injected onto a Proteomix SCX-NP5 5µM non-porous column (Sepax Technologies, Inc., Newark, Del.) at 1.0 mL/min using 0-40% NaCl gradient in 20 mM MES, pH 6.0 buffer with UV detection wavelength at 280 nm. Analysis was performed using Agilent OpenLAB CDS ChemStation Edition AIC version C.01.07. Chromatogram depicting aCIEC separation of peaks B and BC are depicted in Figure 30C. Notably, the aCIEC separation show that in the peak BC material, there are many charge variants in addition to a dominant peak.

4A(b): AN1 VH variant H1.8 enabled improved separation

[00443] A number of AN1 variable heavy (VH) domains were engineered with the aim to improve bispecific antibody production. One particular VH variant (H1.8; SEQ ID NO: XXX; also depicted in Figure 13) enabled improved separation of bispecific antibody heterodimer from contaminating species. To illustrate this, XENP28925 (which comprises an ENPP3 binding domain with the AN1 H1.8 VH variant; sequences depicted in 17) was produced and purified from HEK293E supernatant as described above. Figure 31A depicts the chromatogram showing purification part 2 of XENP28925 (cation exchange chromatography following protein A chromatography). The chromatogram shows the isolation of one dominant peak (peak B), which was further characterized by aSEC-MALS and aCIEC for identity, purity and homogeneity as described above.

[00444] Chromatograms depicting aSEC separation profile (with MW of component species as determined by MALS) for pre-purified material and for peak B, and aCIEX separation profile for peak B are depicted in Figures 31B-C. The profiles show that peak B comprises a dominant species of ~128 kDa which is consistent with the calculated molecular weight of the XENP28925 heterodimer (based on amino acid sequence). Notably, the separation profile for the pre-purified material for that more than 97% of the material was the bispecific antibody heterodimer.

[00445] Collectively, this indicates that the AN1 H1.8 VH variant enables improved production of α ENPP3 x α CD3 heterodimers as well as improved separation of heterodimers from contaminating species.

4B: ENGINEERING BACKBONE OF 2 + 1 FAB₂-SCFV-FC BISPECIFIC FORMAT TO IMPROVE PRODUCTION

4B(a): Production of XENP31419 results in a protein population skewed towards VH-Fc homodimer

[00446] XENP31149 (an α ENPP3 x α CD3 bsAb in the 2 + 1 Fab₂-scFv-Fc format; sequences depicted in Figure 23) was purified from HEK293E supernatant as described above. Figure 32A depicts the chromatogram showing purification part 2 of XENP31149 (cation exchange chromatography following protein A chromatography). The chromatogram shows the isolation of two peaks (dominant peak A and minor peak B), which were further characterized by analytical size-exclusion chromatography with multi-angle light scattering (aSEC-MALS) for identity as generally described above.

[00447] Chromatograms depicting aSEC separation profiles for peaks A and B are depicted in Figure 32B along with MW of component species as determined by MALS. The profiles show that dominant peak A comprises species with molecular weight of 148.4 kDa which is consistent with the calculated molecular weight of a VH-Fc homodimer, while minor peak B comprises a species with molecular weight of 173.9 kDa which is consistent with the calculated molecular weight of XENP31149 heterodimer. As such, production yielded a very low 12.4 mg/L titre of XENP31149 heterodimer.

4B(b): Engineering a full hinge in the Fab-scFv-Fc chain improved 2 + 1 Fab₂-scFv-Fc heterodimer yield

[00448] Various approaches were investigated towards enhancing 2 + 1 Fab₂-scFv-Fc heterodimer yield including varying the linker between the VH and scFv or the linker between the scFv and CH2 in the Fab-scFv-Fc chain. XENP31419 (sequence depicted in Figure 23) was engineered as a XENP31149 counterpart with a full-hinge (EPKSCDKTHTCPPCP; SEQ ID NO: XX) rather than flex half-hinge (GGGSGGGGSKTHTCPPCP; SEQ ID NO: XX) between the scFv and the CH2 region in the Fab-scFv-Fc chain. XENP31419 was produced and purified from HEK293E supernatant as described above. Figure 33A depicts the chromatogram showing purification part 2 of XENP31419 (cation exchange chromatography following protein A chromatography). The chromatogram shows the isolation of two peaks (minor peak A and dominant peak B), which were further characterized by analytical size-exclusion chromatography with multi-angle light scattering (aSEC-MALS) for identity as generally described above.

[00449] Chromatograms depicting aSEC separation profiles for peaks A and B are depicted in Figure 33B along with MW of component species as determined by MALS. The profiles show that minor peak A comprises species with molecular weight of 152.2 kDa which is consistent with the calculated molecular weight of a VH-Fc homodimer, while dominant peak B comprises a species with molecular weight of 180 kDa which is consistent with the calculated molecular weight of XENP31419 heterodimer. As such, production yielded a significantly improved 107.8 mg/L titre XENP31419 heterodimer.

Example 5: Tuning α ENPP3 x α CD3 bsAbs to Enhance Selectivity and Therapeutic Index

[00450] The following experiments were generally performed using KU812 as ENPP3^{high} target cells (as a surrogate for ENPP3⁺ tumor cells) or RCC4 as ENPP3^{low} target cells (as a surrogate for cells outside of the tumor environment). Target cells were incubated with human PBMCs and test articles at indicated effector to target cell ratios at 37°C. After incubation, cells were stained with Aqua Zombie stain for 15 minutes at room temperature. Cells were then washed and stained with antibodies for cell surface markers, and analyzed by flow cytometry. Induction of RTCC was determined using Zombie Aqua staining on CSFE+ target cells; and activation and degranulation of T cells were determined by CD107a, CD25,

and CD69 expression on lymphocytes. It should also be noted that some of the data sets are from the same experiment, as several engineering approaches were simultaneously explored.

[00451] To investigate the potential for on-target/off-tumor killing by prototypic 1 + 1 Fab-scFv-Fc bispecific antibody having high affinity CD3 binding and high affinity ENPP3 binding, KU812 and RCC4 cells were incubated with human PBMCs (10:1 effector to target cell ratio) and indicated concentrations of XENP28925 for 18 hours at 37°C. The data as depicted in Figure 34 show that XENP28925 induced RTCC on ENPP3^{high} KU812 cells; however, XENP28925 also induced RTCC on ENPP3^{low} RCC4 cells indicating that there was room for improving therapeutic index of α ENPP3 x α CD3 bispecific antibodies. Accordingly, the prototypic α ENPP3 x α CD3 bsAbs were further engineered with the aim to enhance selectivity and therapeutic index.

5A: TUNING ENPP3 BINDING AFFINITY

[00452] A first approach explored tuning ENPP3 binding affinity. Variant ENPP3 binding arms were engineered with variable light domain variants with the aim to create a ladder of ENPP3 binding affinity, illustrative sequences for which are depicted in Figure 13 (for variable regions) and Figure 16 (in the context of 1 + 1 Fab-scFv-Fc bsAbs).

[00453] Binding of the affinity-engineered α ENPP3 x α CD3 bsAbs to cell-surface ENPP3 was investigated. KU812 cells were incubated with indicated concentrations of the indicated test articles. Cells were then stained with a Fc γ fragment specific secondary antibody to detect the test articles and analyzed by flow cytometry. The data as depicted in Figure 35 show that the affinity-engineered α ENPP3 x α CD3 bsAbs demonstrated a range of binding potencies to ENPP3^{high} KU812 cells, from high (XENP28925 having L1 variable light) to intermediate (XENP29516 having L1.33 variable light) to low (XENP30262 having L1.77 variable light).

[00454] Next to investigate the effect of modulating ENPP3 binding affinity on selectivity of the bispecific antibodies, KU812 (ENPP3^{high}) and RCC4 (ENPP3^{low}) cells were incubated with human PBMCs (10:1 effector to target cell ratio) and indicated concentrations of the following bispecific antibodies having fixed CD3 potency (CD3 High): XENP28925 (WT high ENPP3 binding), XENP29516 (intermediate ENPP3 binding), or XENP30262 (low ENPP3 binding) for 42 hours at 37°C. The data as depicted in Figure 36 show that both XENP29516 and XENP30262 demonstrated substantially less potent induction of RTCC on ENPP3^{low} cells in comparison to XENP28925, with RTCC potency correlating with binding

potency as shown above. However, XENP29516 and XENP30262 also demonstrated less potent induction of RTCC on ENPP3^{high} cells.

5B: TUNING CD3 BINDING POTENCY

[00455] Reducing the affinity for CD3 was also explored towards improving pharmacokinetics and attenuating cytokine release. α ENPP3 x α CD3 1 + 1 Fab-scFv-Fc bsAbs having CD3 High-Int #1 scFv were engineered, illustrative sequences for which are depicted in Figure 18.

[00456] To investigate induction of cytokine release, an experiment was performed in which KU812 and RCC4 cells were incubated with human PBMCs (10:1 effector to target cell ratio) and indicated concentrations of XENP28925 (CD3 High) or XENP29436 (CD3 High-Int #1) for 18 hours at 37°C. Release of IFN γ , IL-6, and TNF α was determined using V-PLEX Proinflammatory Panel 1 Human Kit (according to manufacturer protocol; Meso Scale Discovery, Rockville, Md.), data for which are depicted in Figures 37 and 38.

[00457] To investigate if tuning CD3 binding potency had any impact on selectivity, another experiment was performed in which KU812 (ENPP3^{high}) and RCC4 (ENPP3^{low}) cells were incubated with human PBMCs (10:1 effector to target cell ratio) and indicated concentrations of XENP28925 (CD3 High) or XENP29436 (CD3 High-Int #1) for 42 hours at 37°C. The data as depicted in Figure 39 show that XENP29436 demonstrated substantially less potent induction of RTCC on ENPP3^{low} cells in comparison to XENP28925; however, XENP29436 also demonstrated reduced potency in induction of RTCC on ENPP3^{high} cells.

5C: TUNING BOTH ENPP3 BINDING AFFINITY AND CD3 BINDING POTENCY

[00458] Next, the effect of reducing both ENPP3 and CD3 binding potency was investigated. α ENPP3 x α CD3 1 + 1 Fab-scFv-Fc bsAbs having reduced potency ENPP3 binding domains and CD3 High-Int #1 scFv were engineered, sequences for which are depicted in Figure 18.

[00459] KU812 cells were incubated with human PBMCs (10:1 effector to target cell ratio) and indicated concentrations of XENP28925 (ENPP3 High; CD3 High), XENP29436 (ENPP3 High; CD3 High-Int #1), XENP29518 (ENPP3 Intermediate; CD3 High), XENP29463 (ENPP3 Intermediate; CD3 High-Int #1), XENP30262 (ENPP3 Low; CD3

High), or XENP30263 (ENPP3 Low; CD3 High-Int #1) for 18 hours at 37°C. Release of IFN γ was determined using V-PLEX Proinflammatory Panel 1 Human Kit. The data as depicted in Figure 40 show that reducing either CD3 or ENPP3 binding potency reduces induction of cytokine release. Notably, reducing CD3 and ENPP3 binding potency further reduces induction of cytokine release.

5D: TUNING BOTH ENPP3 BINDING VALENCY AND ENPP3 BINDING POTENCY

[00460] It was hypothesized that while reduced ENPP3 binding potency reduces binding to both ENPP3^{low} and ENPP3^{high} cells, increased binding valency may restore potency toward ENPP3^{high} cells. Accordingly, α ENPP3 x α CD3 bispecific antibodies having reduced ENPP3 binding potency were engineered in the 2 + 1 Fab₂-scFv-Fc format, sequences for which are depicted in Figure 19.

[00461] KU812 (ENPP3^{high}) and RCC4 (ENPP3^{low}) cells were incubated with human PBMCs (10:1 effector to target cell ratio) and indicated concentrations of XENP28925 (monovalent high ENPP3 binding), XENP29516 (monovalent intermediate ENPP3 binding), XENP29520 (bivalent intermediate ENPP3 binding), XENP30262 (monovalent low ENPP3 binding), or XENP30264 (bivalent low ENPP3 binding) for 42 hours at 37°C.

[00462] The data (as depicted in Figure 41) show that bivalent binding (with intermediate ENPP3 binding) maintained reduced RTCC potency on ENPP3^{low} cells, but restored RTCC potency on ENPP3^{high} cells close to that demonstrated by XENP28925. The data (as depicted in Figure 42) show that bivalent binding (with low ENPP3 binding) further reduced RTCC potency on ENPP3^{low} cells, and restored some RTCC potency on ENPP3^{high} cells. Collectively, the data validates the hypothesis that combining reduced ENPP3 binding affinity and increased ENPP3 binding valency enhances selectivity.

5E: TUNING BOTH ENPP3 BINDING VALENCY AND CD3 BINDING POTENCY

[00463] Next, the combination of increased ENPP3 binding valency with reduced CD3 binding affinity was explored. Accordingly, α ENPP3 x α CD3 bispecific antibodies having reduced CD3 binding potency were engineered in the 2 + 1 Fab₂-scFv-Fc format, sequences for which are depicted in Figure 20.

[00464] KU812 cells were incubated with human PBMCs (10:1 effector to target cell ratio) and indicated concentrations of XENP28925 (CD3 High; monovalent ENPP3 binding), XENP29437 (CD3 High; bivalent ENPP3 binding), XENP29436 (CD3 High-Int #1; monovalent ENPP3 binding), or XENP29438 (CD3 High-Int #1; bivalent ENPP3 binding) for 44 hours at 37°C. Unexpectedly, the data (as depicted in Figure 43) show that XENP29438 was unable to induce RTCC on KU812 cells.

5E(a): Repairing activity of reduced potency CD3 binding domains

[00465] One approach explored towards repairing the activity of High-Int #1 CD3 binding domain for use in 2 + 1 Fab₂-scFv-Fc bsAbs was swapping the orientation of the variable heavy and variable light domain in the αCD3 scFv. Sequences for the new scFvs are depicted in Figure 10. Hereon, αCD3 scFvs are designated as either VH/VL or VL/VH to indicate the orientation of their component variable domains. αENPP3 x αCD3 bispecific antibodies VL/VH CD3 scFvs were engineered in the 2 + 1 Fab₂-scFv-Fc format, sequences for which are depicted in Figures 21-22.

[00466] KU812 (ENPP3^{high}) and RCC4 (ENPP3^{low}) cells were incubated with human PBMCs (10:1 effector to target cell ratio) and indicated concentrations of XENP29437 (CD3 High VH/VL; bivalent ENPP3 binding), XENP30469 (CD3 High VL/VH; bivalent ENPP3 binding), XENP29428 (CD3 High-Int #1 VH/VL; bivalent ENPP3 binding), or XENP30470 (CD3 High-Int #2 VL/VH; bivalent ENPP3 binding) for 44 hours at 37°C. The data as depicted in Figure 44 showed that swapping the orientation of the variable heavy and variable light domains in the CD3 High-Int #1 scFv restored its activity in the context of 2 + 1 Fab₂-scFv-Fc bsAb format. This is surprising in view of the much more modest increase in potency when swapping the orientation of the variable heavy and variable light domains in the CD3 High scFv in the context of the 2 + 1 Fab₂-scFv-Fc bsAb format (as in XENP30469). In addition in an Octet experiment (data not shown), it was found that swapping the orientation of the variable domains did not impact the binding affinity of the molecules for CD3 antigen, further highlighting the unexpected restoration of RTCC activity by the VL/VH swap.

5F: FINE TUNING ENPP3 AND CD3 BINDING POTENCIES IN 2 + 1 FAB₂-SCFV-FC FORMAT

[00467] In view of the collective findings above (that is, there is a tradeoff between selectivity and potency), additional α ENPP3 x α CD3 bispecific antibodies in the 2 + 1 Fab₂-scFv-Fc format having different combinations of ENPP3 and CD3 binding potencies to provide for a range of molecules with different selectivity/potency profiles were generated, sequences for which are depicted throughout Figures 17-23.

[00468] KU812 (ENPP3^{high}) and RCC4 (ENPP3^{low}) cells were incubated with human PBMCs (10:1 effector to target cell ratio) and indicated concentrations XENP29520 (CD3 High[VH/VL]; bivalent ENPP3 intermediate binding), XENP30819 (CD3 High-Int #1[VL/VHL]; bivalent ENPP3 intermediate binding), XENP31149 (CD3 High-Int #2[VL/VHL]; bivalent ENPP3 intermediate binding), XENP30264 (CD3 High[VH/VL]; bivalent ENPP3 low binding), XENP30821 (CD3 High-Int #1[VL/VHL]; bivalent ENPP3 low binding), or XENP31150 (CD3 High-Int #2[VL/VHL]; bivalent ENPP3 low binding). The data as depicted in Figure 45 show that each of the molecules provided good separation between RTCC potency on ENPP3^{high} cells and ENPP3^{low} cells.

Example 6: α ENPP3 x α CD3 bsAbs enhance allogeneic anti-tumor effect of T cells in vivo and combine well with PD-1 blockade

6A: ANTI-ENPP3 X ANTI-CD3 BSABS ARE ACTIVE ON KU812 CELLS IN VIVO

[00469] In a first study, NOD SCID gamma (NSG) mice (n = 10) were engrafted with 5×10^6 KU812 cells in the right flank on Day -15. On Day 0, mice were engrafted intraperitoneally with 5×10^6 human PBMCs. Mice were then treated on Days 0, 7, 14, and 21 with XENP30819, XENP30821, or XENP31419 at either low or high dose and either alone or in combination with 3 mg/kg XENP16432 (a bivalent anti-PD-1 mAb, a checkpoint inhibitor which enhances anti-tumor activity by de-repressing the engrafted human T cells; sequences depicted in Figure 46). Tumor volume was measured by caliper three times per week (data for which are shown in Figure 47) and blood was drawn to investigate lymphocyte expansion (data for which are shown in Figure 48). Individual mouse plots for each treatment are shown in Figures 50A-50N.

[00470] The data show that each of the α ENPP3 x α CD3 bsAbs, at low and/or higher dose treatment, were able to enhance allogeneic anti-tumor effect of T cells on KU812 cells. Notably, treatment with 3 mg/kg XENP30819 alone significantly enhanced anti-tumor activity (as indicated by change in tumor volume) by Day 5 in comparison to PD-1 blockade (XENP16432) alone. By day 7, treatment with lower 1 mg/kg dose XENP30819 alone significantly enhanced anti-tumor activity in comparison to PD-1 blockade alone. Statistics were performed on baseline corrected data using Mann-Whitney test; significance denote $p < 0.5$. Further, by Day 7, treatment with a combination of 6 mg/kg XENP30821 (which has lower potency in vitro than XENP30819) and PD-1 blockade significantly enhanced anti-tumor activity in comparison to PD-1 blockade alone; and by Day 16, treatment with a combination of 6 mg/kg XENP31419 (which has a lower affinity for CD3 than both XENP30819 and XENP30821) and PD-1 blockade significantly enhanced anti-tumor activity in comparison to PD-1 blockade alone. Collectively, this demonstrated that α ENPP3 x α CD3 bsAbs combine productively with PD-1 blockade. Statistics were performed on baseline corrected data using Mann-Whitney test; significance denote $p < 0.5$. In addition, as depicted Figure 48, combining the α ENPP3 x α CD3 bsAbs with PD-1 blockade enhanced lymphocyte expansion.

6B: ANTI-ENPP3 X ANTI-CD3 BSABS ARE ACTIVE ON RXF-393 CELLS IN VIVO

[00471] In a second study, RXF-393 which is a more clinically relevant human kidney renal cell carcinoma cell line was used. NOD SCID gamma (NSG) mice ($n = 10$) were engrafted with 1×10^6 RXF-393 cells in the right flank on Day -8. On Day 0, mice were engrafted intraperitoneally with 5×10^6 human PBMCs. Mice were then treated on Days 0, 7, 14, 21, and 28 with XENP30819 or XENP31419 at either low, mid, or high dose and either alone or in combination with 3 mg/kg XENP16432 (PD-1 blockade). Tumor volume was measured by caliper three times per week (data for which are shown in Figure 49). Individual mouse plots for each treatment are shown in Figures 51A-51L. The data show that each of the α ENPP3 x α CD3 bsAbs, at low, intermediate and/or higher dose treatment, were able to enhance allogeneic anti-tumor effect of T cells on RXF-393 cells. Although XENP31419 (which has lower potency CD3 binding) alone is less effective than XENP30819, combining with PD-1 blockade enhances its anti-tumor effect.

Example 7: Tumor selective cytotoxicity by TAA x CD3 bispecifics utilizing a 2:1 mixed-valency format

[00472] Tumor-associated antigen (TAA) x CD3 bispecifics have been shown to recruit T cells to mediate cytotoxicity against tumor cells. The pharmacodynamics and tolerability of TAA x CD3 bispecifics are impacted by multiple aspects of TAA biology such as tumor load, cell surface antigen density, and normal tissue expression. Using a bivalent/monovalent (2:1) mixed-valency format, multiple examples of TAA x CD3 bispecifics have been engineered so that such bispecifics exhibit selective redirected T-cell cytotoxicity (RTCC) of high versus low antigen density cell lines that mimic tumor versus normal tissue, respectively. The selectivity exhibited by the 2:1 format potentially empowers TAA x CD3 bispecifics to address an expanded set of tumor antigen biologies.

[00473] Heterodimeric Fc have empowered next-generation bispecific formats with altered valencies. Such heterodimeric Fc proteins (see, e.g., Figure 53) include, but are not limited to, 2:1 Fab₂-scFv-Fc bispecific proteins (e.g., CD3 bispecifics when avidity or selectivity is required), 1:1 Fab-scFv-Fc bispecific proteins (e.g., dual checkpoint target or checkpoint target x costimulatory target), Y/Z-Fc proteins (e.g., heterocytokines), anti-X x Y/Z-Fc proteins (e.g., targeted cytokines), and one-arm Fc proteins (e.g., monovalent cytokines).

[00474] Stable and well-behaved heterodimeric Fc regions have enabled the 2:1 Fab₂-scFv-Fc bispecific format. A novel set of Fc substitutions were capable of achieving heterodimer yields over 95% with little change in thermostability (Figure 54). In addition, engineered isoelectric point differences in the Fc region allowed for straightforward purification of the heterodimers. Isosteric substitutions were used to minimize the impact to tertiary structure. Figure 55 shows the distribution after standard protein A purification as determined by analytical IEX of the pI-engineered Fc dimer and the pI-engineered Fc heterodimer. There was little difference between the thermostability of the pI-engineered Fc dimer and the pI-engineered Fc heterodimer. Hinge and CH2 substitutions abolished FcγR binding (Figure 56). The Fc-silenced construct showed substantially no FcγRI, FcγRIIa (H), FcγRIIa (R), FcγRIIb, FcγRIIIa (V), and FcγRIIIa (F) binding.

[00475] The 2:1 Fab₂-scFv-Fc format also enabled targeting of solid tumor antigens with low density on normal tissue. Tuning TAA valency and TAA/CD3 affinities enabled selective cytotoxicity of cell lines mimicking cancer tissue and normal tissue (high/low antigen density). Bispecific formats targeting TAAs such as FAP, SSTR2, and ENPP3 were tested.

The tuned 1:1 format showed broad reactivity and the tuned 2:1 format showed high selectivity (Figures 57A-57C). the tuned 2:1 bispecifics also had reduced interference from soluble antigen and reduced cytokine release.

[00476] The 2:1 Fab2-scFv-Fc CD3 bispecifics described herein are stable, well-behaved, and easily purified. In addition, production including research scale production was straightforward. The 2:1 Fab2-scFv-Fc CD3 bispecifics displayed antibody-like thermostability as determined by DSC and favorable solution properties as measured by SEC (Figure 58). The bispecifics also had high purity as determined by IEX.

[00477] Stable cell lines expressing the bispecifics described herein had a high titer and high heterodimer prevalence. For example, top clones had shake flask yields of 1-2g/L with about 90% heterodimer content (Figure 59).

[00478] The 2:1 mixed valency format of TAA x CD3 bispecifics described herein are stable and easily purified. They also exhibit tumor selective cytotoxicity.

Example 8: Tuning α SSTR2 x α CD3 Bispecific Antibodies Enabled Improved Selectivity and Attenuated Cytokine Release

[00479] Untuned XENP18087 (1 + 1 Fab-scFv-Fc bsAb) and tuned XENP30458 (2 + 1 (Fab)₂-scFv-Fc bsAb) were investigated in RTCC experiments.

[00480] In a first experiment, improvement in selectivity by tuning α SSTR2 x α CD3 bispecific antibodies was explored. A549 cells transfected with different densities (high, medium, and low) of SSTR2 were used. CFSE-labeled A549 cells were incubated with human PBMCs (effector:target ration of 20:1) for 48 hours in the presence of XENP18087 or XENP30458. Data depicting RTCC activity (as indicated by Zombie Aqua staining) are depicted in Figure 60. The data show that although XENP30458 induced RTCC less potently than XENP18087 on high- and medium-density cell lines, efficacious target cell kill was still achievable at high concentrations of XENP30458. Notably, however, XENP30458 induced very little RTCC on low-density cell lines even at very high concentrations in comparison to XENP18087 which induced efficacious target cell kill at higher concentrations.

[00481] In a second experiment, the attenuation of cytokine release by tuning α SSTR2 x α CD3 bispecific antibodies was explored. In this experiment, COR-L279 which is a more clinically relevant human lung small cell carcinoma cell line known to be SSTR2-positive

was used. CFSE-labeled COR-L279 was incubated with human PBMCs (effector:target ratio of 20:1) for 48 hours in the presence of XENP18087 or XENP30458. Data depicting target cell killing are depicted in Figure 61A, and data depicting release of cytokines by effector cells are depicted in Figures 61B-E. As shown in Figure 61A, although XENP30458 induced RTCC less potently than XENP18087, complete target cell kill was still achievable at high concentrations of XENP30458. However as shown in Figures 61B-E, XENP30458 induced substantially decreased cytokine release in comparison to XENP18087 even at high doses.

[00482] While exemplary embodiments of the present invention have been shown and described herein, it will be obvious to those skilled in the art that such embodiments are provided by way of example only. Numerous variations, changes, and substitutions will now occur to those skilled in the art without departing from the invention. It should be understood that various alternatives to the embodiments of the invention described herein may be employed in practicing the invention. It is intended that the following claims define the scope of the invention and that methods and structures within the scope of these claims and their equivalents be covered thereby.

WHAT IS CLAIMED IS:

1. A composition comprising an Ectonucleotide pyrophosphatase/phosphodiesterase family member 3 (ENPP3) binding domain comprising the variable heavy complementary determining regions 1-3 (vhCDR1-3) and the variable light complementary determining regions (vlCDR1-3) of any of the following ENPP3 binding domains: AN1[ENPP3] H1L1, AN1[ENPP3] H1 L1.33, AN1[ENPP3] H1 L1.77, AN1[ENPP3] H1.8 L1, AN1[ENPP3] H1.8 L1.33, AN1[ENPP3] H1 L1.77, H16-7.213, H16-9.69, H16-1.52, Ha16-1(1)23, H16-9.44, H16-1.67, Ha1 6-1 (3,5)36, H16-1.86, H16-9.10, H16-9.33, H16-1.68, Ha16-1(1)1, Ha1 6-1(3,5)18, Ha16-1(2,4)4, Ha16-1(3,5)56, H16-7.8, H16-1.93, Ha1 6-1(3,5)27.1, H16-1.61, H16-1(3,5)5, H16-7.200, H16-1(3,5)42, H1 6-9.65, Ha1-1(3,5)19, and Ha16-1.80, (Figures 12, 13A-13B, and 14A-14I).
2. A composition according to claim 1, wherein the vhCDR1-3 and vlCDR1-3 are selected from the vhCDR1-3 and vlCDR1-3 sequences of an ENPP3 binding domain provided in Figures 12, 13A-13B, and 14A-14I.
3. A composition comprising an Ectonucleotide pyrophosphatase/phosphodiesterase family member 3 (ENPP3) binding domain comprising a variable heavy domain and a variable light domain of any of the following ENPP3 binding domains: AN1[ENPP3] H1L1, AN1[ENPP3] H1 L1.33, AN1[ENPP3] H1 L1.77, AN1[ENPP3] H1.8 L1, AN1[ENPP3] H1.8 L1.33, AN1[ENPP3] H1 L1.77, H16-7.213, H16-9.69, H16-1.52, Ha16-1(1)23, H16-9.44, H16-1.67, Ha1 6-1 (3,5)36, H16-1.86, H16-9.10, H16-9.33, H16-1.68, Ha16-1(1)1, Ha1 6-1(3,5)18, Ha16-1(2,4)4, Ha16-1(3,5)56, H16-7.8, H16-1.93, Ha1 6-1(3,5)27.1, H16-1.61, H16-1(3,5)5, H16-7.200, H16-1(3,5)42, H1 6-9.65, Ha1-1(3,5)19, and Ha16-1.80, (Figures 12, 13A-13B, and 14A-14I).
4. A composition comprising a Ectonucleotide pyrophosphatase/phosphodiesterase family member 3 (ENPP3) binding domain selected from the following ENPP3 binding domains: AN1[ENPP3] H1L1, AN1[ENPP3] H1 L1.33, AN1[ENPP3] H1 L1.77, AN1[ENPP3] H1.8 L1, AN1[ENPP3] H1.8 L1.33, AN1[ENPP3] H1 L1.77, H16-7.213, H16-9.69, H16-1.52, Ha16-1(1)23, H16-9.44, H16-1.67, Ha1 6-1 (3,5)36, H16-1.86, H16-9.10, H16-9.33, H16-1.68, Ha16-1(1)1, Ha1 6-1(3,5)18, Ha16-1(2,4)4, Ha16-1(3,5)56, H16-7.8,

H16-1.93, Ha1 6-1(3,5)27.1, H16-1.61, H16-1(3,5)5, H16-7.200, H16-1(3,5)42, H1 6-9.65, Ha1-1(3,5)19, and Ha16-1.80, (Figures 12, 13A-13B, and 14A-14I).

5. A nucleic acid composition comprising:

a) a first nucleic acid encoding a variable heavy domain comprising the variable heavy complementary determining regions 1-3 (vhCDR1-3) of an ENPP3 binding domain; and

b) a second nucleic acid encoding a variable light domain comprising the variable light complementary determining regions 1-3 (vlCDR1-3) of the ENPP3 binding domain, wherein the ENPP3 binding domain is one of the following ENPP3 binding domains: AN1[ENPP3] H1L1, AN1[ENPP3] H1 L1.33, AN1[ENPP3] H1 L1.77, AN1[ENPP3] H1.8 L1, AN1[ENPP3] H1.8 L1.33, AN1[ENPP3] H1 L1.77, H16-7.213, H16-9.69, H16-1.52, Ha16-1(1)23, H16-9.44, H16-1.67, Ha1 6-1 (3,5)36, H16-1.86, H16-9.10, H16-9.33, H16-1.68, Ha16-1(1)1, Ha1 6-1(3,5)18, Ha16-1(2,4)4, Ha16-1(3,5)56, H16-7.8, H16-1.93, Ha1 6-1(3,5)27.1, H16-1.61, H16-1(3,5)5, H16-7.200, H16-1(3,5)42, H1 6-9.65, Ha1-1(3,5)19, and Ha16-1.80, (Figures 12, 13A-13B, and 14A-14I).

6. A nucleic acid composition according to claim 5, wherein the vhCDR1-3 and vlCDR1-3 are selected from the vhCDR1-3 and vlCDR1-3 sequences provided in Figures 12, 13A-13B, and 14A-14I.

7. A nucleic acid composition comprising:

a) a first nucleic acid encoding a variable heavy domain comprising the variable heavy domain of an ENPP3 binding domain; and

b) a second nucleic acid encoding a variable light domain comprising the variable light domain of the ENPP3 binding domain,

wherein the ENPP3 binding domain is any one of the following ENPP3 binding domains:

AN1[ENPP3] H1L1, AN1[ENPP3] H1 L1.33, AN1[ENPP3] H1 L1.77, AN1[ENPP3] H1.8 L1, AN1[ENPP3] H1.8 L1.33, AN1[ENPP3] H1 L1.77, H16-7.213, H16-9.69, H16-1.52, Ha16-1(1)23, H16-9.44, H16-1.67, Ha1 6-1 (3,5)36, H16-1.86, H16-9.10, H16-9.33, H16-1.68, Ha16-1(1)1, Ha1 6-1(3,5)18, Ha16-1(2,4)4, Ha16-1(3,5)56, H16-7.8, H16-1.93, Ha1 6-1(3,5)27.1, H16-1.61, H16-1(3,5)5, H16-7.200, H16-1(3,5)42, H1 6-9.65, Ha1-1(3,5)19, and Ha16-1.80, (Figures 12, 13A-13B, and 14A-14I).

8. An expression vector composition comprising:
- a) a first expression vector comprising the first nucleic acid according to any one of claims 5 to 7; and
 - b) a second expression vector comprising a second nucleic acid according to any one of claims 5 to 7.
9. A host cell comprising the expression vector composition according to claim 8.
10. A method of making an Ectonucleotide pyrophosphatase/phosphodiesterase family member 3 (ENPP3) binding domain according to any one of claims 1-9 comprising culturing the host cell according to claim 9 under conditions wherein the ENPP3 binding domain is expressed, and recovering the ENPP3 binding domain.
11. An anti-ENPP3 antibody comprising an Ectonucleotide pyrophosphatase/phosphodiesterase family member 3 (ENPP3) binding domain, the ENPP3 binding domain comprising the variable heavy complementary determining regions 1-3 (vhCDR1-3) and the variable light complementary determining regions (vlCDR1-3) of any of the following ENPP3 binding domains: AN1[ENPP3] H1L1, AN1[ENPP3] H1 L1.33, AN1[ENPP3] H1 L1.77, AN1[ENPP3] H1.8 L1, AN1[ENPP3] H1.8 L1.33, AN1[ENPP3] H1 L1.77, H16-7.213, H16-9.69, H16-1.52, Ha16-1(1)23, H16-9.44, H16-1.67, Ha1 6-1 (3,5)36, H16-1.86, H16-9.10, H16-9.33, H16-1.68, Ha16-1(1)1, Ha1 6-1(3,5)18, Ha16-1(2,4)4, Ha16-1(3,5)56, H16-7.8, H16-1.93, Ha1 6-1(3,5)27.1, H16-1.61, H16-1(3,5)5, H16-7.200, H16-1(3,5)42, H1 6-9.65, Ha1-1(3,5)19, and Ha16-1.80, (Figures 12, 13A-13B, and 14A-14I).
12. An anti-ENPP3 antibody according to claim 11, wherein the vhCDR1-3 and vlCDR1-3 are selected from the vhCDR1-3 and vlCDR1-3 of any of the following ENPP3 binding domains in Figures 12, 13A-13B, and 14A-14I.
13. An anti-ENPP3 antibody comprising an Ectonucleotide pyrophosphatase/phosphodiesterase family member 3 (ENPP3) binding domain, the ENPP3 binding domain comprising a variable heavy domain and a variable light domain of any of

the following ENPP3 binding domains: AN1[ENPP3] H1L1, AN1[ENPP3] H1 L1.33, AN1[ENPP3] H1 L1.77, AN1[ENPP3] H1.8 L1, AN1[ENPP3] H1.8 L1.33, AN1[ENPP3] H1 L1.77, H16-7.213, H16-9.69, H16-1.52, Ha16-1(1)23, H16-9.44, H16-1.67, Ha1 6-1 (3,5)36, H16-1.86, H16-9.10, H16-9.33, H16-1.68, Ha16-1(1)1, Ha1 6-1(3,5)18, Ha16-1(2,4)4, Ha16-1(3,5)56, H16-7.8, H16-1.93, Ha1 6-1(3,5)27.1, H16-1.61, H16-1(3,5)5, H16-7.200, H16-1(3,5)42, H1 6-9.65, Ha1-1(3,5)19, and Ha16-1.80, (Figures 12, 13A-13B, and 14A-14I).

14. An anti-ENPP3 antibody comprising an Ectonucleotide pyrophosphatase/phosphodiesterase family member 3 (ENPP3) binding domain selected from any one of the following ENPP3 binding domains: AN1[ENPP3] H1L1, AN1[ENPP3] H1 L1.33, AN1[ENPP3] H1 L1.77, AN1[ENPP3] H1.8 L1, AN1[ENPP3] H1.8 L1.33, AN1[ENPP3] H1 L1.77, H16-7.213, H16-9.69, H16-1.52, Ha16-1(1)23, H16-9.44, H16-1.67, Ha1 6-1 (3,5)36, H16-1.86, H16-9.10, H16-9.33, H16-1.68, Ha16-1(1)1, Ha1 6-1(3,5)18, Ha16-1(2,4)4, Ha16-1(3,5)56, H16-7.8, H16-1.93, Ha1 6-1(3,5)27.1, H16-1.61, H16-1(3,5)5, H16-7.200, H16-1(3,5)42, H1 6-9.65, Ha1-1(3,5)19, and Ha16-1.80, (Figures 12, 13A-13B, and 14A-14I).

15. An antibody according to any of claims 11-14, wherein the antibody comprises:

- a) a first monomer comprising a first antigen binding domain and a first constant domain; and
- b) a second monomer comprising a second antigen binding domain and a second constant domain,

wherein either of the first antigen binding domain or second antigen binding domain is the ENPP3 binding domain.

16. An antibody according to claim 15, wherein the first antigen binding domain and the second antigen binding domain bind different antigens.

17. An antibody according to claim 16, wherein the first antigen binding domain is the ENPP3 binding domain and the second antigen binding domain is a CD3 binding domain.

18. An antibody according to claim 17, wherein the CD3 binding domain comprises the vhCDR1-3, and vlCDR1-3 of any of the following CD3 binding domains: H1.30_L1.47, H1.32_L1.47, H1.89_L1.47, H1.90_L1.47, H1.33_L1.47, H1.31_L1.47, L1.47_H1.30, L1.47_H1.30, L1.47_H1.32, L1.47_H1.89, L1.47_H1.90, L1.47_H1.33, and L1.47_H1.31 (Figures 10A-10F).
19. An antibody according to 17, wherein the vhCDR1-3 and vlCDR1-3 of the CD3 binding domain are selected from the vhCDR1-3 and vlCDR1-3 in Figures 10A-10F.
20. An antibody according to 17, wherein the CD3 binding domain comprises the variable heavy domain and variable light domain of any of the following CD3 binding domains: H1.30_L1.47, H1.32_L1.47, H1.89_L1.47, H1.90_L1.47, H1.33_L1.47, H1.31_L1.47, L1.47_H1.30, L1.47_H1.30, L1.47_H1.32, L1.47_H1.89, L1.47_H1.90, L1.47_H1.33, and L1.47_H1.31 (Figures 10A-10F).
21. An antibody according to any of claims 17 to 20, wherein the CD3 binding domain is an anti-CD3 scFv.
22. An antibody according to any of claims 15 to 21, wherein the first and second constant domains each comprise CH2-CH3.
23. An antibody according to any of claims 15 to 21, wherein the first and second constant domains each comprise CH1-hinge-CH2-CH3.
24. An antibody according to any of claims 16 to 23, where the first and second constant domains each are a variant constant domain.
25. An antibody according to any of claims 16 to 24, wherein the first and second monomers comprise a set of heterodimerization variants selected from the group consisting of those depicted in Figures 1A-1E.
26. An antibody according to claim 25, wherein the set of heterodimerization variants selected is from the group consisting of S364K/E357Q : L368D/K370S; S364K :

L368D/K370S; S364K : L368E/K370S; D401K: T411E/K360E/Q362E; and T366W : T366S/L368A/Y407V.

27. An antibody according to claims 2 or 26, wherein the first and second monomers each further comprise an ablation variant.

28. An antibody according to claim 27, wherein the ablation variant is E233P/L234V/L235A/G236del/S267K.

29. An antibody according to any of claims 25 to 28, wherein the at least one of the first or second monomer further comprises a pI variant.

30. An antibody according to claim 29, wherein the pI variant is N208D/Q295E/N384D/Q418E/N421D.

31. An antibody according to claim 21, wherein the scFv comprises a charged scFv linker.

32. A nucleic acid composition comprising nucleic acids encoding the anti-ENPP3 antibody according to any of claims 11 to 31.

33. A nucleic acid composition comprising nucleic acids encoding the first and second monomers according to any of claims 15 to 31.

34. An expression vector comprising the nucleic acids of claim 32 or 33.

35. A host cell transformed with an expression vector according to claim 34.

36. A method of making an anti-ENPP3 antibody comprising culturing the host cell according to claim 35 under conditions wherein the anti-ENPP3 antibody is expressed, and recovering the anti-ENPP3 antibody.

37. A method of treating a cancer comprising administering to a patient in need thereof an antibody according to any of claims 11 to 31.
38. A heterodimeric antibody comprising:
- a) a first monomer comprising:
 - i) an anti-CD3 scFv comprising a first variable light domain, an scFv linker and a first variable heavy domain; and
 - ii) a first Fc domain, wherein the scFv is covalently attached to the N-terminus of the first Fc domain using a domain linker;
 - b) a second monomer comprising a VH2-CH1-hinge-CH2-CH3 monomer, wherein VH is a second variable heavy domain and CH2-CH3 is a second Fc domain; and
 - c) a light chain comprising a second variable light domain,
- wherein the second variable heavy domain and the second variable light domain form an ENPP3 binding domain.
39. A heterodimeric antibody according to claim 38, wherein the ENPP3 binding domain comprises the vhCDR1-3 and vlCDR1-3 of any of the following ENPP3 binding domains: AN1[ENPP3] H1L1, AN1[ENPP3] H1 L1.33, AN1[ENPP3] H1 L1.77, AN1[ENPP3] H1.8 L1, AN1[ENPP3] H1.8 L1.33, AN1[ENPP3] H1 L1.77, H16-7.213, H16-9.69, H16-1.52, Ha16-1(1)23, H16-9.44, H16-1.67, Ha1 6-1 (3,5)36, H16-1.86, H16-9.10, H16-9.33, H16-1.68, Ha16-1(1)1, Ha1 6-1(3,5)18, Ha16-1(2,4)4, Ha16-1(3,5)56, H16-7.8, H16-1.93, Ha1 6-1(3,5)27.1, H16-1.61, H16-1(3,5)5, H16-7.200, H16-1(3,5)42, H1 6-9.65, Ha1-1(3,5)19, and Ha16-1.80, (Figures 12, 13A-13B, and 14A-14I).
40. A heterodimeric antibody according to claim 39, wherein the vhCDR1-3 and vlCDR1-3 of the ENPP3 binding domain are selected from the vhCDR1-3 and vlCDR1-3 sequences of the ENPP3 binding domains provided in Figures 12, 13A-13B, and 14A-14I.
41. A heterodimeric antibody according to claim 38, wherein the second heavy variable domain comprises a heavy variable domain and the second light variable domain comprises a variable light domain of any of the following ENPP3 binding domains: AN1[ENPP3] H1L1, AN1[ENPP3] H1 L1.33, AN1[ENPP3] H1 L1.77, AN1[ENPP3] H1.8 L1, AN1[ENPP3] H1.8 L1.33, AN1[ENPP3] H1 L1.77, H16-7.213, H16-9.69, H16-1.52, Ha16-1(1)23, H16-

9.44, H16-1.67, Hal 6-1 (3,5)36, H16-1.86, H16-9.10, H16-9.33, H16-1.68, Hal6-1(1)1, Hal 6-1(3,5)18, Hal6-1(2,4)4, Hal6-1(3,5)56, H16-7.8, H16-1.93, Hal 6-1(3,5)27.1, H16-1.61, H16-1(3,5)5, H16-7.200, H16-1(3,5)42, H1 6-9.65, Hal-1(3,5)19, and Hal6-1.80, (Figures 12, 13A-13B, and 14A-14I).

42. A heterodimeric antibody according to any one of claims 38 to 41, wherein the anti-CD3 scFv comprises the vhCDR1-3 and the vlCDR1-3 of any of the following CD3 binding domains: H1.30_L1.47, H1.32_L1.47, H1.89_L1.47, H1.90_L1.47, H1.33_L1.47, H1.31_L1.47, L1.47_H1.30, L1.47_H1.30, L1.47_H1.32, L1.47_H1.89, L1.47_H1.90, L1.47_H1.33, and L1.47_H1.31 (Figures 10A-10F).

43. A heterodimeric antibody according to 42, wherein the vhCDR1-3 and vlCDR1-3 of the anti-CD3 scFv are selected from the vhCDR1-3 and vlCDR1-3 in Figures 10A-10F.

44. A heterodimeric antibody according to 43, wherein the anti-CD3 scFv comprises the variable heavy domain and variable light domain of any of the following CD3 binding domains: H1.30_L1.47, H1.32_L1.47, H1.89_L1.47, H1.90_L1.47, H1.33_L1.47, H1.31_L1.47, L1.47_H1.30, L1.47_H1.30, L1.47_H1.32, L1.47_H1.89, L1.47_H1.90, L1.47_H1.33, and L1.47_H1.31 (Figures 10A-10F).

45. A heterodimeric antibody according to any one of claims 38 to 44, wherein the first variable light domain is covalently attached to the N-terminus of the first Fc domain using a domain linker.

46. A heterodimeric antibody according to any one of claims 38 to 45, wherein the first variable heavy domain is covalently attached to the N-terminus of the first Fc domain using a domain linker.

47. A heterodimeric antibody according to any one of claims 38 to 46, wherein the scFv linker is a charged scFv linker.

48. A heterodimeric antibody according to any one of claims 38 to 47, wherein the first and second Fc domains are variant Fc domains.

49. A heterodimeric antibody according to any of claims 38 to 48, wherein the first and second monomers comprise a set of heterodimerization variants selected from the group consisting of those depicted in Figures 1A-1E.
50. A heterodimeric antibody according to claim 49, wherein the set of heterodimerization variants selected is from the group consisting of S364K/E357Q : L368D/K370S; S364K : L368D/K370S; S364K : L368E/K370S; D401K: T411E/K360E/Q362E; and T366W : T366S/L368A/Y407V, wherein numbering is according to EU numbering.
51. A heterodimeric antibody according to claims 49 or 50, wherein the first and second monomers further comprise an ablation variant.
52. A heterodimeric antibody according to claim 51, wherein the ablation variant is E233P/L234V/L235A/G236del/S267K, wherein numbering is according to EU numbering.
53. A heterodimeric antibody according to any of claims 38 to 52, wherein one of the first or second monomer comprises a pI variant.
54. A heterodimeric antibody according to claim 53, wherein the pI variant is N208D/Q295E/N384D/Q418E/N421D, wherein numbering is according to EU numbering.
55. A heterodimeric antibody according to any of claims 38 to 47, wherein the first monomer comprises amino acid variants S364K/E357Q/E233P/L234V/L235A/G236del/S267K,
wherein the second monomer comprises amino acid variants
L368D/K370S/N208D/Q295E/N384D/Q418E/N421D/E233P/L234V/L235A/G236del/S267K, and
wherein numbering is according to EU numbering.
56. A heterodimeric antibody according to claim 55, wherein the scFv linker is a charged scFv linker having the amino acid sequence (GKPGS)₄.

57. A heterodimeric antibody according to claim 55, wherein the first and second monomers each further comprise amino acid variants 428/434S.

58. A heterodimeric antibody according to claim 38, selected from the following heterodimeric antibodies: XENP24804, XENP26820, XENP28287, XENP28925, XENP29516, XENP30262, XENP26821, XENP29436, XENP28390, XENP29463, and XENP30263.

59. A heterodimeric antibody comprising:

- a) a first monomer comprising from N-terminal to C-terminal, a scFv-linker-CH2-CH3,
 - wherein scFv is an anti-CD3 scFV and CH2-CH3 is a first Fc domain;
- b) a second monomer comprising from N-terminal to C-terminal a VH-CH1-hinge-CH2-CH3, wherein CH2-CH3 is a second Fc domain; and
- c) a light chain comprising VL-CL;
 - wherein the first variant Fc domain comprises amino acid variants S364K/E357Q,
 - wherein the second variant Fc domain comprises amino acid variants L368D/K370S,
 - wherein the first and second variant Fc domains each comprises amino acid variants E233P/L234V/L235A/G236del/S267K,
 - wherein the hinge-CH2-CH3 of the second monomer comprises amino acid variants N208D/Q295E/N384D/Q418E/N421D,
 - wherein the VH and VL form an ENPP3 binding domain comprising the variable heavy domain and the variable light domain, respectively, of an ENPP3 binding domain selected from AN1[ENPP3] H1L1, AN1[ENPP3] H1 L1.33, AN1[ENPP3] H1 L1.77, AN1[ENPP3] H1.8 L1, AN1[ENPP3] H1.8 L1.33, AN1[ENPP3] H1 L1.77, H16-7.213, H16-9.69, H16-1.52, Ha16-1(1)23, H16-9.44, H16-1.67, Ha1 6-1 (3,5)36, H16-1.86, H16-9.10, H16-9.33, H16-1.68, Ha16-1(1)1, Ha1 6-1(3,5)18, Ha16-1(2,4)4, Ha16-1(3,5)56, H16-7.8, H16-1.93, Ha1 6-1(3,5)27.1, H16-1.61, H16-1(3,5)5, H16-7.200, H16-1(3,5)42, H1 6-9.65, Ha1-1(3,5)19, and Ha16-1.80, (Figures 12, 13A-13B, and 14A-14I),
 - wherein the anti-CD3 scFv comprises the variable heavy domain and the variable light domain of a CD3 binding domain selected from H1.30_L1.47, H1.32_L1.47, H1.89_L1.47,

H1.90_L1.47, H1.33_L1.47, H1.31_L1.47, L1.47_H1.30, L1.47_H1.30, L1.47_H1.32, L1.47_H1.89, L1.47_H1.90, L1.47_H1.33, and L1.47_H1.31 (Figures 10A-10F), and wherein numbering is according to EU numbering.

60. A heterodimeric antibody according to claim 59, wherein the scFv comprises a charged scFv linker having the amino acid sequence (GKPGS)₄.

61. A heterodimeric antibody according to 59, wherein the first and second variant Fc domains each further comprise amino acid variants 428/434S, wherein numbering is according to EU numbering.

62. A nucleic acid composition comprising nucleic acids encoding the first and second monomers and the light chain of the antibody according to any of claims 38 to 61.

63. An expression vector comprising the nucleic acids according to claim 62.

64. A host cell transformed with an expression vector according to claim 63.

65. A method of treating an ENPP3 associated cancer comprising administering to a patient in need thereof an antibody according to any of claims 38 to 61.

66. A heterodimeric antibody comprising:

a) a first monomer comprising from N-terminal to C-terminal, a VH1-CH1-linker 1-scFv-linker 2-CH2-CH3,

wherein VH1 is a first variable heavy domain, scFv is an anti-CD3 scFV, linker 1 and linker 2 are a first domain linker and second domain linker, respectively, and CH2-CH3 is a first Fc domain;

b) a second monomer comprising from N-terminal to C-terminal a VH2-CH1-hinge-CH2-CH3, wherein VH2 is a second variable heavy domain and CH2-CH3 is a second Fc domain; and

c) a common light chain comprising a variable light domain;

wherein the first variable heavy domain and the variable light domain form a first ENPP3 binding domain, and the second variable heavy domain and the variable light domain form a second ENPP3 binding domain.

67. A heterodimeric antibody according to claim 66, wherein the first and second ENPP3 binding domains each comprise the vhCDR1-3 and vlCDR1-3 of any of the following ENPP3 binding domains: AN1[ENPP3] H1L1, AN1[ENPP3] H1 L1.33, AN1[ENPP3] H1 L1.77, AN1[ENPP3] H1.8 L1, AN1[ENPP3] H1.8 L1.33, AN1[ENPP3] H1 L1.77, H16-7.213, H16-9.69, H16-1.52, Ha16-1(1)23, H16-9.44, H16-1.67, Ha1 6-1 (3,5)36, H16-1.86, H16-9.10, H16-9.33, H16-1.68, Ha16-1(1)1, Ha1 6-1(3,5)18, Ha16-1(2,4)4, Ha16-1(3,5)56, H16-7.8, H16-1.93, Ha1 6-1(3,5)27.1, H16-1.61, H16-1(3,5)5, H16-7.200, H16-1(3,5)42, H1 6-9.65, Ha1-1(3,5)19, and Ha16-1.80, (Figures 12, 13A-13B, and 14A-14I).,

68. A heterodimeric antibody according to claim 66, wherein the vhCDR1-3 and vlCDR1-3 of the first and second ENPP3 binding domains are selected from the vhCDR1-3 and vlCDR1-3 provided in Figures 14 and 45.

69. A heterodimeric antibody according to claim 66, wherein the first and second variable heavy domain each comprise a variable heavy domain of a ENPP3 binding domain, and the first and second variable light domain each comprise a variable light domain of the ENPP3 binding domain, wherein the ENPP3 binding domain is any of the following ENPP3 binding domains: AN1[ENPP3] H1L1, AN1[ENPP3] H1 L1.33, AN1[ENPP3] H1 L1.77, AN1[ENPP3] H1.8 L1, AN1[ENPP3] H1.8 L1.33, AN1[ENPP3] H1 L1.77, H16-7.213, H16-9.69, H16-1.52, Ha16-1(1)23, H16-9.44, H16-1.67, Ha1 6-1 (3,5)36, H16-1.86, H16-9.10, H16-9.33, H16-1.68, Ha16-1(1)1, Ha1 6-1(3,5)18, Ha16-1(2,4)4, Ha16-1(3,5)56, H16-7.8, H16-1.93, Ha1 6-1(3,5)27.1, H16-1.61, H16-1(3,5)5, H16-7.200, H16-1(3,5)42, H1 6-9.65, Ha1-1(3,5)19, and Ha16-1.80, (Figures 12, 13A-13B, and 14A-14I).

70. A heterodimeric antibody according to claim 66, wherein the scFv comprises the vhCDR1-3 and the vlCDR1-3 of any of the following CD3 binding domains: H1.30_L1.47, H1.32_L1.47, H1.89_L1.47, H1.90_L1.47, H1.33_L1.47, H1.31_L1.47, L1.47_H1.30, L1.47_H1.30, L1.47_H1.32, L1.47_H1.89, L1.47_H1.90, L1.47_H1.33, and L1.47_H1.31 (Figures 10A-10F).

71. A heterodimeric antibody according to 70, wherein the vhCDR1-3 and vlCDR1-3 of the scFv are selected from the vhCDR1-3 and vlCDR1-3 in Figures 10A-10F.

72. A heterodimeric antibody according to 71, wherein the scFv comprises the variable heavy domain and variable light domain of any of the following CD3 binding domains: H1.30_L1.47, H1.32_L1.47, H1.89_L1.47, H1.90_L1.47, H1.33_L1.47, H1.31_L1.47, L1.47_H1.30, L1.47_H1.30, L1.47_H1.32, L1.47_H1.89, L1.47_H1.90, L1.47_H1.33, and L1.47_H1.31 (Figures 10A-10F).

73. A heterodimeric antibody according to any one of 66 to 72, wherein the scFv comprises an scFv variable heavy domain, an scFv variable light domain and an scFv linker that connects the scFv variable heavy domain and the scFv variable light domain.

74. A heterodimeric antibody according to any one of claims 66 to 73, wherein the scFv variable heavy domain is attached to the C-terminus of the CH1 of the first monomer using the first domain linker and the scFv variable light domain is covalently attached to the N-terminus of the first Fc domain using the second domain linker.

75. A heterodimeric antibody according to any one of claims 66 to 73, wherein the scFv variable light domain is attached to the C-terminus of the CH1 of the first monomer using the first domain linker and the scFv variable heavy domain is covalently attached to the N-terminus of the first Fc domain using the second domain linker.

76. A heterodimeric antibody according to any one of claims 66 to 75, wherein the scFv linker is a charged scFv linker.

77. A heterodimeric antibody according to any one of claims 66 to 76, wherein the first and second Fc domains are variant Fc domains.

78. A heterodimeric antibody according to any of claims 66 to 77, wherein the first and second monomers comprise a set of heterodimerization variants selected from the group consisting of those depicted in Figures 1A-1E.

79. A heterodimeric antibody according to claim 78, wherein the set of heterodimerization variants selected is from the group consisting of S364K/E357Q : L368D/K370S; S364K : L368D/K370S; S364K : L368E/K370S; D401K: T411E/K360E/Q362E; and T366W : T366S/L368A/Y407V, wherein numbering is according to EU numbering.
80. A heterodimeric antibody according to any of claims 78 or 79, wherein the first and second monomers further comprise an ablation variant.
81. A heterodimeric antibody according to claim 80, wherein the ablation variant is E233P/L234V/L235A/G236del/S267K, wherein numbering is according to EU numbering.
82. A heterodimeric antibody according to any of claims 78 to 81, wherein one of the first or second monomer further comprises a pI variant.
83. A heterodimeric antibody according to claim 82, wherein the pI variant is N208D/Q295E/N384D/Q418E/N421D, wherein numbering is according to EU numbering.
84. A heterodimeric antibody according to any one claims 66 to 76, wherein the monomer comprises amino acid variants S364K/E357Q/ E233P/L234V/L235A/G236del/S267K, wherein the second monomer comprises amino acid variants L368D/K370S/N208D/Q295E/N384D/Q418E/N421D/E233P/L234V/L235A/G236del/S267 K, and wherein numbering is according to EU numbering.
85. A heterodimeric antibody according to claim 84, wherein the scFv linker is a charged scFv linker having the amino acid sequence (GKPGS)₄.
86. A heterodimeric antibody according to 84, wherein the first and second variant Fc domains each further comprise amino acid variants 428/434S, wherein numbering is according to EU numbering.

87. A heterodimeric antibody according to claim 66, selected from the following heterodimeric antibodies: XENP29437, XENP29520, XENP30264, XENP26822, XENP28438, XENP29438, XENP29467, XENP30469, XENP30470, XENP30819, XENP30821, XENP31148, XENP31149, XENP31150, XENP31419, and XENP31471.

88. A heterodimeric antibody comprising:

a) a first monomer comprising from N-terminal to C-terminal, a VH1-CH1-linker 1-scFv-linker 2-CH2-CH3,

wherein scFv is an anti-CD3 scFv and CH2-CH3 is a first Fc domain;

b) a second monomer comprising from N-terminal to C-terminal a VH1-CH1-hinge-CH2-CH3, wherein CH2-CH3 is a second Fc domain; and

c) a common light chain comprising VL-CL;

wherein the first variant Fc domain comprises amino acid variants S364K/E357Q,

wherein the second variant Fc domain comprises amino acid variants L368D/K370S,

wherein the first and second variant Fc domains each comprises amino acid variants E233P/L234V/L235A/G236del/S267K,

wherein the hinge-CH2-CH3 of the second monomer comprises amino acid variants N208D/Q295E/N384D/Q418E/N421D,

wherein said VH and VL comprise the variable heavy domain and the variable light domain of a ENPP3 binding domain selected from AN1[ENPP3] H1L1, AN1[ENPP3] H1 L1.33, AN1[ENPP3] H1 L1.77, AN1[ENPP3] H1.8 L1, AN1[ENPP3] H1.8 L1.33, AN1[ENPP3] H1 L1.77, H16-7.213, H16-9.69, H16-1.52, Ha16-1(1)23, H16-9.44, H16-1.67, Ha1 6-1 (3,5)36, H16-1.86, H16-9.10, H16-9.33, H16-1.68, Ha16-1(1)1, Ha1 6-1(3,5)18, Ha16-1(2,4)4, Ha16-1(3,5)56, H16-7.8, H16-1.93, Ha1 6-1(3,5)27.1, H16-1.61, H16-1(3,5)5, H16-7.200, H16-1(3,5)42, H1 6-9.65, Ha1-1(3,5)19, and Ha16-1.80, (Figures 12, 13A-13B, and 14A-14I),

wherein the anti-CD3 scFv comprises the variable heavy domain and the variable light domain of a CD3 binding domain selected from H1.30_L1.47, H1.32_L1.47, H1.89_L1.47, H1.90_L1.47, H1.33_L1.47, H1.31_L1.47, L1.47_H1.30, L1.47_H1.30, L1.47_H1.32, L1.47_H1.89, L1.47_H1.90, L1.47_H1.33, and L1.47_H1.31 (Figures 10A-10F), and wherein numbering is according to EU numbering.

89. A heterodimeric antibody according to claim 88, wherein the scFv comprises a charged scFv linker having the amino acid sequence (GKPGS)₄.
90. A heterodimeric antibody according to 88, wherein the first and second variant Fc domains each further comprise amino acid variants 428/434S.
91. A nucleic acid composition comprising nucleic acids encoding the first and second monomers and the common light chain of the antibody according to any of claims 66 to 90.
92. An expression vector comprising the nucleic acids according to claim 91.
93. A host cell transformed with an expression vector according to claim 92.
94. A method of treating an ENPP3 associated cancer comprising administering to a patient in need thereof an antibody according to any of claims 66 to 90.
95. A heterodimeric antibody selected from the following heterodimeric antibodies: XENP24804, XENP26820, XENP28287, XENP28925, XENP29516, XENP30262, XENP26821, XENP29436, XENP28390, XENP29463, and XENP30263.
96. A heterodimeric antibody selected from the following: XENP29437, XENP29520, XENP30264, XENP26822, XENP28438, XENP29438, XENP29467, XENP30469, XENP30470, XENP30819, XENP30821, XENP31148, XENP31149, XENP31150, XENP31419, and XENP31471.
97. A nucleic acid composition comprising nucleic acids encoding the heterodimeric antibody of any one of claims 95 or 96.
98. An expression vector comprising the nucleic acids according to claim 97.
99. A host cell transformed with an expression vector according to claim 98.

100. A method of treating an ENPP3 related cancer comprising administering to a patient in need thereof a heterodimeric antibody according to any one of claims 85 or 86.