

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.

SUMMARY

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

[0004a] In one aspect, the present disclosure refers to 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) selected from the group consisting of: a. the vhCDR1-3 from SEQ ID NO:252 and the vlCDR1-3 from SEQ ID

NO:256; b. the vhCDR1-3 from SEQ ID NO:218 and the vlCDR1-3 from SEQ ID NO:222; c. the vhCDR1-3 from SEQ ID NO:228 and the vlCDR1-3 from SEQ ID NO:232; d. the vhCDR1-3 from SEQ ID NO:236 and the vlCDR1-3 from SEQ ID NO:240; e. the vhCDR1-3 from SEQ ID NO:244 and the vlCDR1-3 from SEQ ID NO:248; and f. the vhCDR1-3 from SEQ ID NO:260 and the vlCDR1-3 from SEQ ID NO:264.

[0004b] In another aspect, the present disclosure refers to a composition comprising an anti-ENPP3 binding domain comprising a variable heavy domain comprising SEQ ID NO:252 and a variable light domain comprising SEQ ID NO:256.

[0004c] In another aspect, the present disclosure refers to an anti-ENPP3 antibody comprising: a. a heavy chain comprising VH-CH1-hinge-CH2-CH3; and b. a light chain comprising VL-CL; wherein said VH and VL are selected from the group consisting: c. SEQ ID NO:252 and SEQ ID NO:256; d. SEQ ID NO:218 and SEQ ID NO:222; e. SEQ ID NO:228 and SEQ ID NO:232; f. SEQ ID NO:236 and SEQ ID NO:240; g. SEQ ID NO:244 and SEQ ID NO:248; and h. SEQ ID NO:260 and SEQ ID NO:264.

[0004d] In another aspect, the present disclosure refers to a nucleic acid composition comprising: a first nucleic acid encoding a variable heavy domain as disclosed herein; and a second nucleic acid encoding a variable light domain as disclosed herein, respectively.

[0004e] In another aspect, the present disclosure refers to an expression vector composition comprising: a. a first expression vector comprising the first nucleic acid as disclosed herein; and b. a second expression vector comprising the second nucleic acid as disclosed herein.

[0004f] In another aspect, the present disclosure refers to a host cell comprising the expression vector composition as disclosed herein.

[0004g] In another aspect, the present disclosure refers to a method of making an ENPP3 binding domain comprising culturing the host cell as disclosed herein under conditions wherein said ENPP3 binding domain is expressed, and recovering said ENPP3 binding domain.

[0004h] In another aspect, the present disclosure refers to a heterodimeric antibody comprising: a) a first monomer comprising from N-terminal to C-terminal, VH1-CH1-first domain linker-scFv-second domain linker-CH2-CH3, wherein VH1 is a first variable heavy domain, 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 VL1-CL, where VL1 is a first variable light domain and CL is a constant light domain; wherein said VH1 and said VL1 form an ENPP3 binding domain, said scFv comprises a second VH domain (VH2), a scFv linker, and a second VL domain (VL2), wherein said VH2 and said VL2 form a CD3 binding domain, and wherein the variable heavy complementary determining regions 1-3 (vhCDR1-3) of said VH1 and the variable light complementary determining regions (vlCDR1-3) of said VL1 are selected from the group consisting of: a. the vhCDR1-3 from SEQ ID NO:252 and the vlCDR1-3 from SEQ ID NO:256; b. the vhCDR1-3 from SEQ ID NO:218 and the vlCDR1-3 from SEQ ID NO:222; c. the vhCDR1-3 from SEQ ID NO:228 and the vlCDR1-3 from SEQ ID NO:232; d. the vhCDR1-3 from SEQ ID NO:236 and the vlCDR1-3 from SEQ

ID NO:240; e. the vhCDR1-3 from SEQ ID NO:244 and the vlCDR1-3 from SEQ ID NO:248; and f. the vhCDR1-3 from SEQ ID NO:260 and the vlCDR1-3 from SEQ ID NO:264.

[0004i] In another aspect, the present disclosure refers to a heterodimeric antibody comprising: a) a first monomer comprising from N-terminal to C-terminal, VH1-CH1-a first domain linker-scFv-a second domain linker-CH2-CH3, wherein VH1 is a first variable heavy domain, scFv is an anti-CD3 scFv and CH2-CH3 is a first variant Fc domain; b) a second monomer comprising from N-terminal to C-terminal, VH1-CH1-hinge-CH2-CH3, wherein CH2-CH3 is a second variant Fc domain; and c) a common light chain comprising VL1-CL, wherein said VL1 is first variable light domain and CL is a constant light domain; 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 said second monomer comprises amino acid variants N208D/Q295E/N384D/Q418E/N421D; wherein said VH1 and said VL1 form an ENPP3 binding domain, said scFv comprises a second VH domain (VH2), a scFv linker, and a second VL domain (VL2), and said VH2 and said VL2 form a CD3 binding domain; wherein VH1 and VL1 are selected from the group consisting: a. SEQ ID NO:252 and SEQ ID NO:256; b. SEQ ID NO:218 and SEQ ID NO:222; c. SEQ ID NO:228 and SEQ ID NO:232; d. SEQ ID NO:236 and SEQ ID NO:240; e. SEQ ID NO:244 and SEQ ID NO:248; and f. SEQ ID NO:260 and SEQ ID NO:264; wherein said VH2 and said VL2 are selected from the group consisting of: a. SEQ ID NO:93 and SEQ ID NO:97; b. SEQ ID NO:103 and SEQ ID NO:107; c. SEQ ID NO:113 and SEQ ID NO:117; d. SEQ ID NO:123 and SEQ ID NO:127; e. SEQ ID NO:133 and SEQ ID NO: 137; and f. SEQ ID NO:143 and SEQ ID NO:147, and wherein numbering is according to EU numbering.

[0004j] In another aspect, the present disclosure refers to a heterodimeric antibody comprising: a. a first monomer comprising SEQ ID NO:531; b. a second monomer comprising SEQ ID NO:532; and c. a light chain comprising SEQ ID NO:533.

[0004k] In another aspect, the present disclosure refers to a heterodimeric antibody comprising: a. a first monomer encoded by a nucleic acid encoding SEQ ID NO:531; b. a second monomer encoded by a nucleic acid encoding SEQ ID NO:532; and c. a light chain encoded by a nucleic acid encoding SEQ ID NO:533.

[0004l] In another aspect, the present disclosure refers to a heterodimeric antibody comprising: a) a first monomer comprising: i) an anti-CD3 scFv comprising a first variable light domain (VL1), an scFv linker and a first variable heavy domain (VH1); 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, from N- to C-terminal, VH2-CH1-hinge-CH2-CH3 monomer, wherein VH2 is a second variable heavy domain and CH2-CH3 is a second Fc domain; and c) light chain comprising VL2-CL, where VL2 is a second variable light domain and CL is a constant light domain; wherein said VH2 and said VL2 form an ENPP3 binding domain and wherein said VH2 and said VL2 are selected from the group consisting: a. SEQ ID NO:252 and SEQ ID NO:256; b. SEQ ID NO:218 and SEQ ID NO:222; c. SEQ ID NO:228 and SEQ ID NO:232; d. SEQ ID NO:236 and SEQ ID NO:240; e. SEQ ID NO:244 and SEQ ID NO:248; and f. SEQ ID NO:260 and SEQ ID NO:264.

[0004m] In another aspect, the present disclosure refers to a heterodimeric antibody comprising: a) a first monomer comprising: i) an anti-CD3 scFv comprising a first variable light domain (VL1), an scFv linker and a first variable heavy domain (VH1); 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 VH2 is a second variable heavy domain and CH2-CH3 is a second Fc domain; and c) light chain comprising VL2-CL, where VL2 is a second variable light domain and CL is a constant light domain; 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 said second monomer comprises amino acid variants N208D/Q295E/N384D/Q418E/N421D; wherein said VH2 and said VL2 form an ENPP3 binding domain and said VH1 and said VL1 form a CD3 binding domain; wherein VH2 and VL2 are selected from the group consisting of: a. SEQ ID NO:252 and SEQ ID NO:256; b. SEQ ID NO:218 and SEQ ID NO:222; c. SEQ ID NO:228 and SEQ ID NO:232; d. SEQ ID NO:236 and SEQ ID NO:240; e. SEQ ID NO:244 and SEQ ID NO:248; and f. SEQ ID NO:260 and SEQ ID NO:264; wherein said VH1 and said VL1 are selected from the group consisting of: a. SEQ ID NO:93 and SEQ ID NO:97; b. SEQ ID NO:103 and SEQ ID NO:107; c. SEQ ID NO:113 and SEQ ID NO:117; d. SEQ ID NO:123 and SEQ ID NO:127; e. SEQ ID NO:133 and SEQ ID NO: 137; and f. SEQ ID NO:143 and SEQ ID NO:147; and wherein numbering is according to EU numbering.

[0004n] In another aspect, the present disclosure refers to a nucleic acid composition comprising: a. a first nucleic acid encoding said first monomer as disclosed herein; b. a second nucleic acid encoding said second monomer as disclosed herein; and c. a third nucleic acid encoding said light chain as disclosed herein, respectively.

[0004o] In another aspect, the present disclosure refers to an expression vector composition comprising: a. a first expression vector comprising said first nucleic acid as disclosed herein; b. a second expression vector comprising said second nucleic acid as disclosed herein; and c. a third expression vector comprising said third nucleic acid as disclosed herein; respectively.

[0004p] In another aspect, the present disclosure refers to a method of making a heterodimeric antibody as disclosed herein comprising culturing said host cell as disclosed herein under conditions wherein said heterodimeric antibody is expressed and recovering said heterodimeric antibody.

[0005] In one example, 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 examples, 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 example, 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 example, the present disclosure 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 example, the present disclosure 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 examples, 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 example, the present disclosure 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-

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[0010] In some examples, the present disclosure 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 examples, the present disclosure provides a host cell that includes the expression vector composition.

[0011] In some examples, the present disclosure 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 example, the present disclosure 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 examples, 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 example, the present disclosure 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 example, 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 examples, 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 examples, first antigen binding domain and the second antigen binding domain bind different antigens. In further examples, the first antigen binding domain is the ENPP3 binding domain and the second antigen binding domain is a CD3 binding domain. In further examples, 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 examples, 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 examples, 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 examples, the CD3 binding domain is an anti-CD3 scFv.

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

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

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

[0021] In some examples, the first and second monomers include a set of heterodimerization variants are any one of the variants depicted in Figures 1A-1E. In some examples, 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 examples, the first and second monomers each further include an ablation variant. In further examples, the ablation variant is E233P/L234V/L235A/G236del/S267K.

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

[0024] In some examples, the present disclosure provides a nucleic acid composition including nucleic acids encoding the anti-ENPP3. In some examples, the composition including nucleic acids encoding first and second monomers. In some examples, the present

disclosure provides expression vectors that include the nucleic acids. In some examples, the present disclosure provides a host cell transformed with the expression vector.

[0025] In some examples, the present disclosure 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 examples, the present disclosure provides a method of treating a cancer that includes administering to a patient in need thereof the antibody.

[0026] In another example, the present disclosure 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 examples, 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 examples, 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 examples, 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 examples, 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 examples, 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 examples, 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_H1.32, L1.47_H1.89, L1.47_H1.90, L1.47_H1.33, and L1.47_H1.31 (Figures 10A-10F).

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

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

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

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

[0037] In some examples, the first and second monomers includes a set of heterodimerization variants selected from any of the heterodimerization variants in Figures 1A-1E. In some examples, 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 examples, the first and second monomers further includes an ablation variant. In some examples, the ablation variant is E233P/L234V/L235A/G236del/S267K, wherein numbering is according to EU numbering.

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

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

[0041] In some examples, 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 examples, the scFv linker is a charged scFv linker having the amino acid sequence (GKPGS)₄.

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

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

[0045] In another example, the present disclosure 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 examples, the scFv includes a charged scFv linker having the amino acid sequence (GKPGS)₄.

[0047] In some examples, 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 examples, the present disclosure 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 examples, the present disclosure provides an expression vector that includes the nucleic acids. In some examples, the present disclosure provides a host cell transformed with the expression vector.

[0050] In some examples, the present disclosure 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 example, the present disclosure 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 examples, 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 examples, 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 examples, 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 examples, 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 examples, 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 examples, 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 examples, 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 examples, 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 examples, 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 examples, the scFv linker is a charged scFv linker.

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

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

[0064] In some examples, 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 examples, the first and second monomers further include an ablation variant.

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

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

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

[0069] In some examples, 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 examples, the scFv linker is a charged scFv linker having the amino acid sequence (GKPGS)₄.

[0071] In some examples, 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 examples, 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 example, 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 examples, the scFv includes a charged scFv linker having the amino acid sequence (GKPGS)₄.

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

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

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

[0078] In another example, the present disclosure 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 examples, the present disclosure provides nucleic acid composition that includes the nucleic acids encoding the heterodimeric antibody. In some examples, the present disclosure provides an expression vector includes the nucleic acids. In some examples, the present disclosure provides a host cell transformed with the expression vector.

[0079] In some examples, 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).

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) selected from the group consisting of:
 - a. the vhCDR1-3 from SEQ ID NO:252 and the vlCDR1-3 from SEQ ID NO:256;
 - b. the vhCDR1-3 from SEQ ID NO:218 and the vlCDR1-3 from SEQ ID NO:222;
 - c. the vhCDR1-3 from SEQ ID NO:228 and the vlCDR1-3 from SEQ ID NO:232;
 - d. the vhCDR1-3 from SEQ ID NO:236 and the vlCDR1-3 from SEQ ID NO:240;
 - e. the vhCDR1-3 from SEQ ID NO:244 and the vlCDR1-3 from SEQ ID NO:248; and
 - f. the vhCDR1-3 from SEQ ID NO:260 and the vlCDR1-3 from SEQ ID NO:264.
2. A composition according to claim 1 wherein the variable heavy domain and the variable light domain are selected from the group consisting of:
 - a. SEQ ID NO:252 and SEQ ID NO:256;
 - b. SEQ ID NO:218 and SEQ ID NO:222;
 - c. SEQ ID NO:228 and SEQ ID NO:232;
 - d. SEQ ID NO:236 and SEQ ID NO:240;
 - e. SEQ ID NO:244 and SEQ ID NO:248; and
 - f. SEQ ID NO:260 and SEQ ID NO:264.
3. A composition comprising an anti-ENPP3 binding domain comprising a variable heavy domain comprising SEQ ID NO:252 and a variable light domain comprising SEQ ID NO:256.

4. A nucleic acid composition comprising:
a first nucleic acid encoding a variable heavy domain according to claims 1, 2 or 3;
and a second nucleic acid encoding a variable light domain according to claims 1, 2 or 3,
respectively.
5. An expression vector composition comprising:
 - a. a first expression vector comprising the first nucleic acid of claim 4; and
 - b. a second expression vector comprising the second nucleic acid of claim 4.
6. A host cell comprising the expression vector composition of claim 5.
7. A method of making an ENPP3 binding domain comprising culturing the host cell according to claim 6 under conditions wherein said ENPP3 binding domain is expressed, and recovering said ENPP3 binding domain.
8. An anti-ENPP3 antibody comprising:
 - a. A heavy chain comprising VH-CH1-hinge-CH2-CH3; and
 - b. A light chain comprising VL-CL;wherein said VH and VL are selected from the group consisting:
 - c. SEQ ID NO:252 and SEQ ID NO:256;
 - d. SEQ ID NO:218 and SEQ ID NO:222;
 - e. SEQ ID NO:228 and SEQ ID NO:232;
 - f. SEQ ID NO:236 and SEQ ID NO:240;
 - g. SEQ ID NO:244 and SEQ ID NO:248; and
 - h. SEQ ID NO:260 and SEQ ID NO:264.
9. A nucleic acid composition comprising:
 - a. a first nucleic acid encoding the heavy chain according to claim 8; and
 - b. a second nucleic acid encoding the light chain according to claim 8, respectively.
10. An expression vector composition comprising:
 - a. a first expression vector comprising the first nucleic acid of claim 9; and
 - b. a second expression vector comprising the second nucleic acid of claim 9.

11. A host cell comprising the expression vector composition of claim 10.
12. A method of making an anti-ENPP3 antibody comprising culturing the host cell according to claim 11 under conditions wherein said anti-ENPP3 antibody is expressed, and recovering said anti-ENPP3 antibody.
13. A heterodimeric antibody comprising:
- a) a first monomer comprising from N-terminal to C-terminal, VH1-CH1-first domain linker-scFv-second domain linker-CH2-CH3,
wherein VH1 is a first variable heavy domain, 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 VL1-CL, where VL1 is a first variable light domain and CL is a constant light domain;
wherein said VH1 and said VL1 form an ENPP3 binding domain, said scFv comprises a second VH domain (VH2), a scFv linker, and a second VL domain (VL2), wherein said VH2 and said VL2 form a CD3 binding domain, and wherein the variable heavy complementary determining regions 1-3 (vhCDR1-3) of said VH1 and the variable light complementary determining regions (vlCDR1-3) of said VL1 are selected from the group consisting of:
 - a. the vhCDR1-3 from SEQ ID NO:252 and the vlCDR1-3 from SEQ ID NO:256;
 - b. the vhCDR1-3 from SEQ ID NO:218 and the vlCDR1-3 from SEQ ID NO:222;
 - c. the vhCDR1-3 from SEQ ID NO:228 and the vlCDR1-3 from SEQ ID NO:232;
 - d. the vhCDR1-3 from SEQ ID NO:236 and the vlCDR1-3 from SEQ ID NO:240;
 - e. the vhCDR1-3 from SEQ ID NO:244 and the vlCDR1-3 from SEQ ID NO:248; and
 - f. the vhCDR1-3 from SEQ ID NO:260 and the vlCDR1-3 from SEQ ID NO:264.
14. A heterodimeric antibody according to claim 13, wherein said VH1 and VL1 are selected from the group consisting:
- a. SEQ ID NO:252 and SEQ ID NO:256;
 - b. SEQ ID NO:218 and SEQ ID NO:222;
 - c. SEQ ID NO:228 and SEQ ID NO:232;
 - d. SEQ ID NO:236 and SEQ ID NO:240;

- e. SEQ ID NO:244 and SEQ ID NO:248; and
 - f. SEQ ID NO:260 and SEQ ID NO:264.
15. A heterodimeric antibody according to any of claims 13 and 14 wherein said scFv comprises, from N- to C-terminal, VL2-scFv linker-VH2.
16. A heterodimeric antibody according to any of claims 13 to 14 wherein said scFv comprises, from N- to C-terminal, VH2-scFv linker-VL2.
17. A heterodimeric antibody according to any of claims 13 to 14, wherein said first monomer comprises, from N- to C-terminal, VH1-CH1-first domain linker-VL2-scFv linker-VH2-second domain linker-CH2-CH3.
18. A heterodimeric antibody according to any of claims 13 to 14, wherein said first monomer comprises, from N- to C-terminal, VH1-CH1-first domain linker-VH2-scFv linker-VL2-second domain linker-CH2-CH3.
19. A heterodimeric antibody according to any of claims 13 to 19, wherein said VH2 and said VL2 are selected from the group consisting of:
- a. SEQ ID NO:93 and SEQ ID NO:97;
 - b. SEQ ID NO:103 and SEQ ID NO:107;
 - c. SEQ ID NO:113 and SEQ ID NO:117;
 - d. SEQ ID NO:123 and SEQ ID NO:127;
 - e. SEQ ID NO:133 and SEQ ID NO: 137; and
 - f. SEQ ID NO:143 and SEQ ID NO:147.
20. A heterodimeric antibody according to any of claims 13 to 19, wherein said scFv linker is a charged scFv linker.
21. A heterodimeric antibody according to claim 20, wherein said charged scFv linker comprises the sequence (GKPGS)₄.
22. A heterodimeric antibody according to any of claims 13 to 21, wherein said first and second Fc domains comprise a set of heterodimerization variants selected from the sets

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.

23. A heterodimeric antibody according to any of claims 13 to 22, wherein said first and second Fc domains each comprise an ablation variant.

24. A heterodimeric antibody according to claim 23, wherein said ablation variant is E233P/L234V/L235A/G236del/S267K, wherein numbering is according to EU numbering.

25. A heterodimeric antibody according to any of claims 13 to 24, wherein said second monomer comprises a pI variant.

26. A heterodimeric antibody according to claim 25, wherein said pI variant is N208D/Q295E/N384D/Q418E/N421D, wherein numbering is according to EU numbering.

27. A heterodimeric antibody according to any one claims 13 to 26, wherein said 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.

28. A heterodimeric antibody according to any of claims 13 to 27, wherein the first and second variant Fc domains each further comprise amino acid variants 428L/434S, wherein numbering is according to EU numbering.

29. A heterodimeric antibody comprising:

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

wherein VH1 is a first variable heavy domain, scFv is an anti-CD3 scFv and CH2-CH3 is a first variant Fc domain;

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

c) a common light chain comprising VL1-CL, wherein said VL1 is first variable light domain and CL is a constant light domain;

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 said second monomer comprises amino acid variants N208D/Q295E/N384D/Q418E/N421D;

wherein said VH1 and said VL1 form an ENPP3 binding domain, said scFv comprises a second VH domain (VH2), a scFv linker, and a second VL domain (VL2), and said VH2 and said VL2 form a CD3 binding domain; wherein VH1 and VL1 are selected from the group consisting:

- a. SEQ ID NO:252 and SEQ ID NO:256;
- b. SEQ ID NO:218 and SEQ ID NO:222;
- c. SEQ ID NO:228 and SEQ ID NO:232;
- d. SEQ ID NO:236 and SEQ ID NO:240;
- e. SEQ ID NO:244 and SEQ ID NO:248; and
- f. SEQ ID NO:260 and SEQ ID NO:264;

Wherein said VH2 and said VL2 are selected from the group consisting of:

- a. SEQ ID NO:93 and SEQ ID NO:97;
- b. SEQ ID NO:103 and SEQ ID NO:107;
- c. SEQ ID NO:113 and SEQ ID NO:117;
- d. SEQ ID NO:123 and SEQ ID NO:127;
- e. SEQ ID NO:133 and SEQ ID NO: 137; and
- f. SEQ ID NO:143 and SEQ ID NO:147, and wherein numbering is according to

EU numbering.

30. A heterodimeric antibody comprising:

- a. A first monomer comprising SEQ ID NO:531;
- b. A second monomer comprising SEQ ID NO:532; and
- c. A light chain comprising SEQ ID NO:533.

31. A heterodimeric antibody comprising:

- a. A first monomer encoded by a nucleic acid encoding SEQ ID NO:531;

- b. A second monomer encoded by a nucleic acid encoding SEQ ID NO:532; and
 - c. A light chain encoded by a nucleic acid encoding SEQ ID NO:533.
32. A heterodimeric antibody comprising:
- a) a first monomer comprising:
 - i) an anti-CD3 scFv comprising a first variable light domain (VL1), an scFv linker and a first variable heavy domain (VH1); 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, from N- to C-terminal, VH2-CH1-hinge-CH2-CH3 monomer, wherein VH2 is a second variable heavy domain and CH2-CH3 is a second Fc domain; and
 - c) light chain comprising VL2-CL, where VL2 is a second variable light domain and CL is a constant light domain;
- wherein said VH2 and said VL2 form an ENPP3 binding domain and wherein said VH2 and said VL2 are selected from the group consisting:
- a. SEQ ID NO:252 and SEQ ID NO:256;
 - b. SEQ ID NO:218 and SEQ ID NO:222;
 - c. SEQ ID NO:228 and SEQ ID NO:232;
 - d. SEQ ID NO:236 and SEQ ID NO:240;
 - e. SEQ ID NO:244 and SEQ ID NO:248; and
 - f. SEQ ID NO:260 and SEQ ID NO:264.
33. A heterodimeric antibody according to claim 32 wherein said scFv comprises, from N- to C-terminal, VL1-scFv linker-VH1.
34. A heterodimeric antibody according to claim 32 wherein said scFv comprises, from N- to C-terminal, VH1-scFv linker-VL1.
35. A heterodimeric antibody according to claim 32 wherein said first monomer comprises, from N- to C-terminal, VH2-CH1-first domain linker-VL1-scFv linker-VH1-second domain linker-CH2-CH3.

36. A heterodimeric antibody according to claim 32 wherein said first monomer comprises, from N- to C-terminal, VH2-CH1-first domain linker-VH1-scFv linker-VL1-second domain linker-CH2-CH3.
37. A heterodimeric antibody according to any of claims 32 to 36, wherein said VH1 and said VL1 are selected from the group consisting of:
- SEQ ID NO:93 and SEQ ID NO:97;
 - SEQ ID NO:103 and SEQ ID NO:107;
 - SEQ ID NO:113 and SEQ ID NO:117;
 - SEQ ID NO:123 and SEQ ID NO:127;
 - SEQ ID NO:133 and SEQ ID NO: 137; and
 - SEQ ID NO:143 and SEQ ID NO:147.
38. A heterodimeric antibody according to any of claims 32 to 37 wherein said scFv linker is a charged scFv linker.
39. A heterodimeric antibody according to claim 38, wherein said charged scFv linker has the sequence (GKPGS)₄.
40. A heterodimeric antibody according to any of claims 32 to 39 wherein said first and second Fc domains comprise a set of heterodimerization variants selected from the sets 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.
41. A heterodimeric antibody according to any of claims 32 to 40 wherein said first and second Fc domains each comprise an ablation variant.
42. A heterodimeric antibody according to claim 41 wherein said ablation variant is E233P/L234V/L235A/G236del/S267K, wherein numbering is according to EU numbering.
43. A heterodimeric antibody according to any of claims 32 to 42 wherein said second monomer comprises a pI variant.

44. A heterodimeric antibody according to claim 43 wherein said pI variant is N208D/Q295E/N384D/Q418E/N421D, wherein numbering is according to EU numbering.
45. A heterodimeric antibody according to any of claims 32 to 44, wherein said 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.
46. A heterodimeric antibody according to any of claims 32 to 45, wherein the first and second variant Fc domains each further comprise amino acid variants 428L/434S, wherein numbering is according to EU numbering.
47. A heterodimeric antibody comprising:
- a) a first monomer comprising:
 - i) an anti-CD3 scFv comprising a first variable light domain (VL1), an scFv linker and a first variable heavy domain (VH1); 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 VH2 is a second variable heavy domain and CH2-CH3 is a second Fc domain; and
 - c) light chain comprising VL2-CL, where VL2 is a second variable light domain and CL is a constant light domain;
- 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 said second monomer comprises amino acid variants N208D/Q295E/N384D/Q418E/N421D;
- wherein said VH2 and said VL2 form an ENPP3 binding domain and said VH1 and said VL1 form a CD3 binding domain; wherein VH2 and VL2 are selected from the group consisting of:
- a. SEQ ID NO:252 and SEQ ID NO:256;
 - b. SEQ ID NO:218 and SEQ ID NO:222;

- c. SEQ ID NO:228 and SEQ ID NO:232;
- d. SEQ ID NO:236 and SEQ ID NO:240;
- e. SEQ ID NO:244 and SEQ ID NO:248; and
- f. SEQ ID NO:260 and SEQ ID NO:264;

wherein said VH1 and said VL1 are selected from the group consisting of:

- a. SEQ ID NO:93 and SEQ ID NO:97;
- b. SEQ ID NO:103 and SEQ ID NO:107;
- c. SEQ ID NO:113 and SEQ ID NO:117;
- d. SEQ ID NO:123 and SEQ ID NO:127;
- e. SEQ ID NO:133 and SEQ ID NO: 137; and
- f. SEQ ID NO:143 and SEQ ID NO:147;

and wherein numbering is according to EU numbering.

48. A nucleic acid composition comprising:

- a. a first nucleic acid encoding said first monomer of any of claims 13–47;
- b. a second nucleic acid encoding said second monomer of any of claims 13-47; and
- c. a third nucleic acid encoding said light chain of any of claims 13-47, respectively.

49. An expression vector composition comprising:

- a. a first expression vector comprising said first nucleic acid of claim 48;
- b. a second expression vector comprising said second nucleic acid of claim 48; and
- c. a third expression vector comprising said third nucleic acid of claim 48;

respectively.

50. A host cell comprising the expression vector composition of claim 49.

51. A method of making a heterodimeric antibody according to any of claims 13 to 47 comprising culturing said host cell of claim 51 under conditions wherein said heterodimeric antibody is expressed and recovering said heterodimeric antibody.