CLAIMS:

1. A CD3-binding domain that binds specifically to human CD3 and that comprises an immunoglobulin light chain variable region and an immunoglobulin heavy chain variable region;

wherein the immunoglobulin light chain variable region comprises an amino acid sequence that is

(a) at least about 93% identical, at least about 95% identical, at least about 97% identical, at least about 98% identical or at least about 99% identical to the amino acid sequence in SEQ ID NO:88; or

(b) at least about 94% identical, at least about 95% identical, at least about 97% identical, at least about 98% identical or at least about 99% identical to the amino acid sequence in SEQ ID NO:89; and

wherein the immunoglobulin heavy chain variable region comprises an amino acid sequence that is at least about 82% identical, at least about 85% identical, at least about 87% identical, at least about 90% identical, at least about 92% identical, at least about 95% identical, at least about 97% identical, at least about 98% identical or at least about 99% identical to the amino acid sequence in SEQ ID NO:86.

2. The CD3-binding domain of claim 1, wherein the CD3-binding domain comprises an amino acid sequence that is at least about 87% identical, at least about 90% identical, at least about 92% identical, at least about 95% identical, at least about 97% identical, at least about 98% identical or at least about 99% identical to the amino acid sequence in SEQ ID NO:83 or SEQ ID NO:84.

3. The CD3-binding domain of claim 1, wherein the immunoglobulin light chain variable region comprises

(a) an LCDR1 amino acid sequence of SEQ ID NO:94, an LCDR2 amino acid sequence of SEQ ID NO:95, and an LCDR3 amino acid sequence of SEQ ID NO:96 and wherein the immunoglobulin heavy chain variable region comprises an HCDR1 amino acid sequence of SEQ ID NO:91, an HCDR2 amino acid sequence of SEQ ID NO:92, and an HCDR3 amino acid sequence of SEQ ID NO:93; or

(b) an LCDR1 amino acid sequence of SEQ ID NO:202, an LCDR2 amino acid sequence of SEQ ID NO:203, and an LCDR3 amino acid sequence of SEQ ID NO:204 and

wherein the immunoglobulin heavy chain variable region comprises an HCDR1 amino acid sequence of SEQ ID NO:199, an HCDR2 amino acid sequence of SEQ ID NO:200, and an HCDR3 amino acid sequence of SEQ ID NO:201.

4. A CD3-binding domain that binds specifically to human CD3 and that comprises an immunoglobulin light chain variable region and an immunoglobulin heavy chain variable region, wherein

(a) the immunoglobulin light chain variable region comprises an LCDR1 amino acid sequence of SEQ ID NO:94, an LCDR2 amino acid sequence of SEQ ID NO:95, and an LCDR3 amino acid sequence of SEQ ID NO:96 and wherein the immunoglobulin heavy chain variable region comprises an HCDR1 amino acid sequence of SEQ ID NO:91, an HCDR2 amino acid sequence of SEQ ID NO:92, and an HCDR3 amino acid sequence of SEQ ID NO:93; or

(b) the immunoglobulin light chain variable region comprises an LCDR1 amino acid sequence of SEQ ID NO:202, an LCDR2 amino acid sequence of SEQ ID NO:203, and an LCDR3 amino acid sequence of SEQ ID NO:204 and wherein the immunoglobulin heavy chain variable region comprises an HCDR1 amino acid sequence of SEQ ID NO:199, an HCDR2 amino acid sequence of SEQ ID NO:200, and an HCDR3 amino acid sequence of SEQ ID NO:201; and

wherein the CD3-binding domain has any one or more of the following properties:

(i) the thermal transition midpoint of the CD3-binding domain is at least about 54ºC, at least about 55ºC, at least about 56ºC, or at least about 57ºC and up to about 72ºC;

(ii) the CD3-binding domain is stable in storage in PBS at about 25ºC for at least about 6 days, at least about 10 days, or at least about 13 days and up to about 90 days;

(iii) the CD3-binding domain binds to human CD3 with an EC50 of about 10 nM or lower; and

(iv) the CD3-binding domain binds to cynomolgus CD3 with an EC50 of about 30 nM or lower.

5. The CD3-binding domain of any one of the preceding claims, wherein the immunoglobulin light chain variable region and the immunoglobulin heavy chain variable region comprise framework regions and wherein at least one of the immunoglobulin light chain variable region and the immunoglobulin heavy chain variable region is humanized.

6. The CD3-binding domain of any one of claims 3-5, wherein the immunoglobulin light chain variable region comprises framework regions based on the human IGKV3D-20*1 germline amino acid sequence.

7. The CD3-binding domain of any one of claims 3-6, wherein the immunoglobulin heavy chain variable region comprises framework regions based on the human IGHV1-69*02 germline amino acid sequence.

8. The CD3-binding domain of any one of the preceding claims, wherein the amino acid residue at position 52 according to the IMGT numbering system of the immunoglobulin light chain variable region is arginine and/or the amino acid residue at position 53 according to the IMGT numbering system of the immunoglobulin light chain variable region is tryptophan.

9. The CD3-binding domain of claim 1, wherein the amino acid residue at position 27 according to the IMGT numbering system of the immunoglobulin heavy chain variable region is tyrosine.

10. The CD3-binding domain of claim 1, wherein the CD3-binding domain comprises one or more of the following:

(a) the amino acid residue at position 9 according to the IMGT numbering system of the immunoglobulin heavy chain variable region is proline;

(b) the amino acid residue at position 53 according to the IMGT numbering system of the immunoglobulin heavy chain variable region is isoleucine; and

(b) the amino acid residue at position 21 according to the IMGT numbering system of the immunoglobulin light chain variable region is methionine.

11. The CD3-binding domain of claim 1, wherein the amino acid residue at position 87 according to the IMGT numbering system of the immunoglobulin heavy chain variable region is tyrosine.

12. The CD3-binding domain of claim 1, wherein the amino acid residue at position 86 according to the IMGT numbering system of the immunoglobulin heavy chain variable region is aspartic acid.

13. The CD3-binding domain of claim 1, wherein the amino acid residue at position 86 according to the IMGT numbering system of the immunoglobulin heavy chain variable region is aspartic acid and the amino acid residue at position 87 according to the IMGT numbering system of the immunoglobulin heavy chain variable region is tyrosine.

14. The CD3-binding domain of any one of the preceding claims, wherein the CD3-binding domain comprises SEQ ID NO:83 or SEQ ID NO:84.

15. The CD3-binding domain of any one of the preceding claims, wherein the CD3-binding domain is a single chain variable fragment (scFv).

16. The CD3-binding domain of claim 15, wherein said scFv comprises a linker between the heavy chain variable region and the light chain variable region of said scFv and wherein said linker comprises the amino acid sequence QRHNNSSLNTGTQMAGHSPNS (SEQ ID NO:148).

17. The CD3-binding domain of claim 15, wherein the heavy chain variable region of said scFv is amino-terminal to the light chain variable region of said scFv.

18. The CD3-binding domain of claim 1, wherein the CD3-binding domain has a thermal stability that is increased at least about 10% when compared to the thermal stability of a CD3-binding domain comprising a light chain variable region comprising SEQ ID NO:90 and a heavy chain variable region comprising SEQ ID NO:87.

19. The CD3-binding domain of claim 1, wherein the thermal transition midpoint (Tm) of the CD3-binding domain is increased at least about 3ºC, at least about 4ºC, at least about 5ºC, or at least about 6ºC increased and up to about 20ºC when compared to the Tm of a CD3-binding domain comprising a light chain variable region comprising SEQ ID NO:90 and a heavy chain variable region comprising SEQ ID NO:87.

20. The CD3-binding domain of claim 1, wherein the thermal transition midpoint of the CD3-binding domain is at least about 54ºC, at least about 55ºC, at least about 56ºC, or at least about 57ºC and up to about 72ºC.

21. The CD3-binding domain of any one of claims 18-20, wherein the thermal stability or the thermal transition midpoint is measured by differential scanning calorimetry or differential scanning fluorimetry.

22. The CD3-binding domain of claim 1, wherein the CD3-binding domain has storage stability that is increased at least about 5%, at least about 10%, at least about 20%, at least about 30%, at least about 40%, at least about 50%, at least about 60%, at least about 70%, at least about 80%, at least about 90% and up to about 100% when compared to the storage stability of a CD3-binding domain comprising a light chain variable region comprising SEQ ID NO:90 and a heavy chain variable region comprising SEQ ID NO:87.

23. The CD3-binding domain of claim 22, wherein the CD3-binding domain is stored in PBS at about 25ºC.

24. The CD3-binding domain of claim 1, wherein the CD3-binding domain is stable in storage in PBS at about 25ºC for at least about 6 days, at least about 10 days, or at least about 13 days and up to about 90 days.

25. The CD3-binding domain of claim 1, wherein the CD3-binding domain has a level of high molecular weight aggregates produced during recombinant expression that are at least about 5%, at least about 10%, at least about 20% decreased, at least about 30% decreased and up to about 50% decreased when compared to the level of high molecular weight aggregates produced during recombinant expression of a CD3-binding domain comprising a light chain variable region comprising SEQ ID NO:90 and a heavy chain variable region comprising SEQ ID NO:87.

26. The CD3-binding domain of claim 1, wherein the CD3-binding domain binds to human CD3 with an EC50 of about 10 nM or lower.

27. The CD3-binding domain of claim 1, wherein the CD3-binding domain binds specifically to cynomolgus CD3.

28. The CD3-binding domain of claim 27, wherein the CD3-binding domain binds to cynomolgus CD3 with an EC50 of about 30 nM or lower.

29. A CD3-binding polypeptide comprising the CD3-binding domain of any one of the preceding claims.

30. The CD3-binding polypeptide of claim 29, wherein the CD3-binding polypeptide comprises an immunoglobulin constant region.

31. The CD3-binding polypeptide of claim 29 or 30, wherein the CD3-binding polypeptide when bound to a CD3 protein on a T cell does not induce or induces a minimally detectable cytokine release from said T cell.

32. The CD3-binding polypeptide of any one of claims 29-31, wherein the CD3-binding polypeptide induces T-cell activation or T-cell proliferation.

33. The CD3-binding polypeptide of any one of claims 29-32, further comprising a second binding domain.

34. The CD3-binding polypeptide of claim 33, wherein said CD3-binding polypeptide comprises, in order from amino-terminus to carboxyl-terminus, (i) the second-binding domain, (ii) a hinge region, (iii) an immunoglobulin constant region, (iv) a carboxyl-terminus linker, and (v) the CD3-binding domain.

35. The CD3-binding polypeptide of claim 33 or 34, wherein

(i) the CD3-binding domain comprises (a) an immunoglobulin light chain variable region comprising LCDR1, LCDR2, and LCDR3, and (b) an immunoglobulin heavy chain variable region comprising HCDR1, HCDR2, and HCDR3; and

(ii) the second binding domain comprises (a) an immunoglobulin light chain variable region comprising LCDR1, LCDR2, and LCDR3, and (b) an immunoglobulin heavy chain variable region comprising HCDR1, HCDR2, and HCDR3.

36. The CD3-binding polypeptide of claim 34 or 35, wherein the hinge region is derived from an immunoglobulin hinge region.

37. The CD3-binding polypeptide of any one of claims 34-36, wherein the carboxyl-terminus linker comprises or consists of SEQ ID NO:196.

38. The CD3-binding polypeptide of any one of claims 27-37, wherein the immunoglobulin constant region comprises immunoglobulin CH2 and CH3 domains of IgG1, IgG2, IgG3, IgG4, IgA1, IgA2 or IgD.

39. The CD3-binding polypeptide of claim 38, wherein the immunoglobulin constant region comprises a human IgG1 CH2 domain comprising the substitutions L234A, L235A, G237A, and K322A, according to the EU numbering system.

40. The CD3-binding polypeptide of any one of claims 33-39, wherein the CD3-binding polypeptide induces redirected T-cell cytotoxicity (RTCC).

41. The CD3-binding polypeptide of claim 40, wherein the CD3-binding polypeptide induces RTCC with an EC50 of about 30 pM or lower.

42. The CD3-binding polypeptide of any one of claims 33-41, wherein the second binding domain is a single chain variable fragment (scFv).

43. The CD3-binding polypeptide of any one of claims 33-42, wherein the second binding domain binds or interacts with a tumor associated antigen.

44. The CD3-binding polypeptide of claim 43, wherein said CD3-binding polypeptide induces T-cell-dependent lysis of cells expressing the tumor associated antigen.

45. The CD3-binding polypeptide of claim 43, wherein the tumor associated antigen is selected from the group consisting of PSMA, CD19, CD20, CD37, CD38, CD123, Her2, ROR1, RON, glycoprotein A33 antigen (gpA33), and CEA..

46. The CD3-binding polypeptide of any one of claims 29-45, wherein said CD3-binding polypeptide further comprises an immunoglobulin heterodimerization domain.

47. The CD3-binding polypeptide of claim 46, wherein the immunoglobulin heterodimerization domain comprises an immunoglobulin CH1 domain or an immunoglobulin CL domain.

48. The CD3-binding polypeptide of any one of claims 29-47, wherein said CD3-binding polypeptide is a heterodimeric CD3-binding protein comprising (i) a first polypeptide chain comprising, in order from amino-terminus to carboxyl-terminus or from carboxyl-terminus to amino-terminus, (a) a CD3-binding domain that specifically binds human CD3, (b) a first hinge region, (c) a first immunoglobulin constant region, and (d) a first immunoglobulin heterodimerization domain; and (ii) a second polypeptide chain comprising, in order from amino-terminus to carboxyl-terminus or from carboxyl-terminus to amino-terminus, (a') a second hinge region, (b') a second immunoglobulin constant region, and (c') a second immunoglobulin heterodimerization domain that is different from the first immunoglobulin heterodimerization domain of the first single chain polypeptide, wherein the first and second immunoglobulin heterodimerization domains associate with each other to form a heterodimer.

49. The CD3-binding polypeptide of claim 48, wherein the first immunoglobulin heterodimerization domain comprises an immunoglobulin CH1 domain and the second immunoglobulin heterodimerization domain comprises an immunoglobulin CL domain, or wherein the first immunoglobulin heterodimerization domain comprises an immunoglobulin CL domain and the second immunoglobulin heterodimerization domain comprises an immunoglobulin CH1 domain.

50. The CD3-binding polypeptide of claim 49, wherein at least one of the first and second immunoglobulin constant regions comprises immunoglobulin CH2 and CH3 domains of IgG1, IgG2, IgG3, IgG4, IgA1, IgA2, IgD or any combination thereof; an immunoglobulin CH3 domain of IgG1, IgG2, IgG3, IgG4, IgA1, IgA2, IgD, IgE, IgM or any combination thereof; or immunoglobulin CH3 and CH4 domains of IgE, IgM or a combination thereof.

51. The CD3-binding polypeptide of claim 48, wherein said second polypeptide chain further comprises a second binding domain.

52. The CD3-binding polypeptide of claim 51, wherein the second binding domain is amino-terminal to the second hinge region.

53. The CD3-binding polypeptide of any one of claims 29-45, wherein said CD3-binding polypeptide is a bispecific single-chain antibody molecule comprising a CD3-binding domain and a second binding domain, wherein the binding domains are arranged in the order VH CD3-VL CD3-VH second binding domain-VL second binding domain or VL CD3-VH CD3-VH second binding domain -VL second binding domain or VH second binding domain-VL second binding domain-VH CD3-VL CD3 or VH second binding domain-VL second binding domain-VL CD3-VH CD3.

54. An isolated nucleic acid molecule encoding the CD3-binding domain of any one of claims 1-28 or the CD3-binding polypeptide of any one of claims 29-53 or a portion of said CD3-binding domain or polypeptide.

55. An expression vector comprising a nucleic acid segment encoding the CD3-binding domain of any one of claims 1-28 or the CD3-binding polypeptide of any one of claims 29-47, wherein the nucleic acid segment is operatively linked to regulatory sequences suitable for expression of the nucleic acid segment in a host cell.

56. A recombinant host cell comprising the expression vector of claim 55.

57. An expression vector comprising first and second expression units,

wherein the first and second expression units respectively comprise first and second nucleic acid segments encoding the first and second polypeptide chains of the heterodimeric CD3-binding polypeptide of any one of claims 48-52, and

wherein the first and second nucleic acid segments are operably linked to regulatory sequences suitable for expression of the nucleic acid segments in a host cell.

58. A recombinant host cell comprising the expression vector of claim 57.

59. A method for producing a CD3-binding polypeptide, the method comprising

culturing a recombinant host cell comprising the expression vector of claim 56 under conditions whereby the nucleic acid segment is expressed, thereby producing the CD3-binding polypeptide.

60. The method of claim 59, further comprising recovering the CD3-binding polypeptide.

61. A method for producing a heterodimeric CD3-binding protein, the method comprising

culturing a recombinant host cell comprising first and second expression units, wherein the first and second expression units respectively comprise first and second nucleic acid segments encoding the first and second polypeptide chains of a heterodimeric CD3-binding protein as set forth in any one of claims 48-52, wherein the first and second nucleic acid segments are operably linked to regulatory sequences suitable for expression of the nucleic acid segments in a host cell, and

wherein said culturing is under conditions whereby the first and second nucleic acid segments are expressed and the encoded polypeptide chains are produced as the heterodimeric CD3-binding protein.

62. The method of claim 61, further comprising recovering the heterodimeric CD3-binding protein.

63. A pharmaceutical composition comprising the CD3-binding polypeptide of any one of claims 29-53 and a pharmaceutically acceptable carrier, diluent, or excipient.

64. A method for inducing redirected T-cell cytotoxicity (RTCC) against a cell expressing a tumor associated antigen, the method comprising: contacting said tumor associated antigen-expressing cell with the CD3-binding polypeptide of any one of claims 44-53, wherein said contacting is under conditions whereby RTCC against the tumor associated antigen-expressing cell is induced.

65. A method for inhibiting tumor growth in a subject in need thereof, comprising administering a therapeutically effective amount of the CD3-binding polypeptide of any one of claims 29-53 or the composition of claim 63 to the subject.

66. A method for treating cancer or an autoimmune disorder in a subject in need thereof, comprising administering a therapeutically effective amount of the CD3-binding polypeptide of any one of claims 29-53 or the composition of claim 63 to the subject.

67. The method of claim 66, wherein the cancer is prostate cancer, colorectal cancer, renal cell carcinoma, bladder cancer, salivary gland cancer, pancreatic cancer, ovarian cancer, non-small cell lung cancer, breast cancer (e.g., triple negative breast cancer). melanoma, adrenal cancer, mantle cell lymphoma, acute lymphoblastic leukemia, chronic lymphocytic leukemia, Non-Hodgkin's lymphoma, acute myeloid leukemia (AML), B-lymphoid leukemia, blastic plasmocytoid dendritic neoplasm (BPDCN), or hairy cell leukemia.

68. A CD3-binding domain that binds specifically to human CD3 and that comprises SEQ ID NO: 4, 6, 8, 10, 12, 14, 16, 18, 20, 22, 24, 26, 28, 30, 32, 34, 36, 38, 40, 42, 44, 46, 48, 50, 52, 54, 56, 58, or 60.

69. A CD3-binding protein that is a dimer of two identical polypeptides, wherein each polypeptide is the CD3-binding polypeptide of any one of claims 29-45.

70. The CD3-binding polypeptide of any one of claims 29-53, wherein the CD3-binding polypeptide does not exhibit or exhibits minimal antibody-dependent cell-mediated cytotoxicity (ADCC) activity and/or complement-dependent cytotoxicity (CDC) activity.

CLAIMS:

1. A CD3-binding protein comprising a CD3-binding domain that binds specifically to human CD3 and that comprises a humanized immunoglobulin light chain variable region and a humanized immunoglobulin heavy chain variable region wherein the immunoglobulin light chain variable region comprises an LCDR1 amino acid sequence of SEQ ID NO:94, an LCDR2 amino acid sequence of SEQ ID NO:95, and an LCDR3 amino acid sequence of SEQ ID NO:96 and wherein the immunoglobulin heavy chain variable region comprises an HCDR1 amino acid sequence of SEQ ID NO:91, an HCDR2 amino acid sequence of SEQ ID NO:92, and an HCDR3 amino acid sequence of SEQ ID NO:93;

wherein the amino acid residue at position 9 according to the IMGT numbering system of the immunoglobulin heavy chain variable region is proline; and

wherein the amino acid residue at position 21 according to the IMGT numbering system of the immunoglobulin light chain variable region is methionine; and

wherein the amino acid residue at position 87 according to the IMGT numbering system of the immunoglobulin light chain variable region is tyrosine.

2. The CD3-binding protein of claim 1, wherein the immunoglobulin light chain variable region comprises framework regions based on the human IGKV3D-20*1 germline amino acid sequence.

3. The CD3-binding protein of claim 1, wherein the immunoglobulin heavy chain variable region comprises framework regions based on the human IGHV1-69*02 germline amino acid sequence.

4. The CD3-binding protein of claim 1, wherein the amino acid residue at position 52 according to the IMGT numbering system of the immunoglobulin light chain variable region is arginine and/or the amino acid residue at position 53 according to the IMGT numbering system of the immunoglobulin light chain variable region is tryptophan.

5. The CD3-binding protein of claim 1, wherein the amino acid residue at position 27 according to the IMGT numbering system of the immunoglobulin heavy chain variable region is tyrosine.

6. The CD3-binding protein of claim 1, wherein the amino acid residue at position 53 according to the IMGT numbering system of the immunoglobulin heavy chain variable region is isoleucine.

7. The CD3-binding protein of claim 1, wherein the amino acid residue at position 86 according to the IMGT numbering system of the immunoglobulin light chain variable region is aspartic acid.

8. The CD3-binding protein of claim 1, wherein the CD3-binding domain comprises SEQ ID NO:83 or SEQ ID NO:84.

9. The CD3-binding protein of claim 1, wherein the CD3-binding domain is a single chain variable fragment (scFv).

10. The CD3-binding protein of claim 9, wherein said scFv comprises a linker between the heavy chain variable region and the light chain variable region of said scFv and wherein said linker comprises the amino acid sequence QRHNNSSLNTGTQMAGHSPNS (SEQ ID NO:148).

11. The CD3-binding protein of claim 9, wherein the heavy chain variable region of said scFv is amino-terminal to the light chain variable region of said scFv.

12. The CD3-binding protein of claim 1, wherein the CD3-binding protein binds specifically to cynomolgus CD3.

13. The CD3-binding protein of claim 1, wherein the CD3-binding protein comprises an immunoglobulin constant region.

14. The CD3-binding protein of claim 1, further comprising a second binding domain.

15. The CD3-binding protein of claim 14, wherein said CD3-binding protein is a single chain molecule comprising, in order from amino-terminus to carboxyl-terminus, (i) the second binding domain, (ii) a hinge region, (iii) an immunoglobulin constant region, (iv) a carboxyl-terminus linker, and (v) the CD3-binding domain.

16. The CD3-binding protein of claim 14, wherein the second binding domain comprises (a) an immunoglobulin light chain variable region comprising LCDR1, LCDR2, and LCDR3, and (b) an immunoglobulin heavy chain variable region comprising HCDR1, HCDR2, and HCDR3.

17. The CD3-binding protein of claim 15, wherein the hinge region is derived from an immunoglobulin hinge region.

18. The CD3-binding protein of claim 15, wherein the carboxyl-terminus linker comprises or consists of SEQ ID NO:196.

19. The CD3-binding protein of claim 13, wherein the immunoglobulin constant region comprises immunoglobulin CH2 and CH3 domains of IgG1, IgG2, IgG3, IgG4, IgA1, IgA2 or IgD.

20. The CD3-binding protein of claim 19, wherein the immunoglobulin constant region comprises a human IgG1 CH2 domain comprising the substitutions L234A, L235A, G237A, and K322A, according to the EU numbering system.

21. The CD3-binding protein of claim 14, wherein the CD3-binding protein induces redirected T-cell cytotoxicity (RTCC).

22. The CD3-binding protein of claim 14, wherein the second binding domain is a single chain variable fragment (scFv).

23. The CD3-binding protein of claim 14, wherein the second binding domain binds a tumor associated antigen.

24. The CD3-binding protein of claim 23, wherein said CD3-binding protein induces T-cell-dependent lysis of cells expressing the tumor associated antigen.

25. The CD3-binding protein of claim 23, wherein the tumor associated antigen is selected from the group consisting of PSMA, CD19, CD20, CD37, CD38, CD123, Her2, ROR1, RON, glycoprotein A33 antigen (gpA33), and CEA.

26. The CD3-binding protein of claim 1, wherein said CD3-binding protein further comprises an immunoglobulin heterodimerization domain.

27. The CD3-binding protein of claim 26, wherein the immunoglobulin heterodimerization domain comprises an immunoglobulin CH1 domain or an immunoglobulin CL domain.

28. The CD3-binding protein of claim 1, wherein said CD3-binding protein is a bispecific single chain antibody molecule comprising a CD3-binding domain and a second binding domain, wherein the binding domains are arranged in the order VH CD3-VL CD3-VH second binding domain-VL second binding domain, VL CD3-VH CD3-VH second binding domain -VL second binding domain, VH second binding domain-VL second binding domain-VH CD3-VL CD3 or VH second binding domain-VL second binding domain-VL CD3-VH CD3.

29. A pharmaceutical composition comprising the CD3-binding protein of claim 1 and a pharmaceutically acceptable carrier, diluent, or excipient.

30. The CD3-binding protein of claim 23 for use in the treatment of cancer in a subject in need thereof.

31. Use of the CD3-binding protein of claim 23 for the manufacture of a medicament for treating cancer in a subject in need thereof.

32. The CD3-binding protein of claim 30 for use in the treatment of cancer, or the use of claim 31 for the manufacture of a medicament for treating cancer, wherein the cancer is prostate cancer, colorectal cancer, renal cell carcinoma, bladder cancer, salivary gland cancer, pancreatic cancer, ovarian cancer, non-small cell lung cancer, breast cancer, melanoma, adrenal cancer, mantle cell lymphoma, acute lymphoblastic leukemia, chronic lymphocytic leukemia, Non-Hodgkin's lymphoma, acute myeloid leukemia (AML), B-lymphoid leukemia, blastic plasmocytoid dendritic neoplasm (BPDCN), or hairy cell leukemia.

33. A CD3-binding protein that is a dimer of two identical proteins, wherein each protein is the CD3-binding protein of claim 15.

34. The CD3-binding protein of claim 1, wherein

(a) the immunoglobulin light chain variable region comprises SEQ ID NO:88 and the immunoglobulin heavy chain variable region comprises SEQ ID NO:86; or

(b) the immunoglobulin light chain variable region comprises SEQ ID NO:89 and the immunoglobulin heavy chain variable region comprises SEQ ID NO:86.

35. The CD3-binding protein of claim 1, wherein

(a) the immunoglobulin light chain variable region comprises an amino acid sequence that is at least 96% identical to SEQ ID NO:88 and the immunoglobulin heavy chain variable region comprises an amino acid sequence that is at least 96% identical to SEQ ID NO:86; or

(b) the immunoglobulin light chain variable region comprises an amino acid sequence that is at least 96% identical to SEQ ID NO:89 and the immunoglobulin heavy chain variable region comprises an amino acid sequence that is at least 96% identical to SEQ ID NO:86.

36. The CD3-binding protein of claim 32 for use in the treatment of cancer, or the use of claim 32 for the manufacture of a medicament for treating cancer, wherein the breast cancer is triple negative breast cancer.