

WHAT IS CLAIMED:

1. An isolated protein comprising an antigen binding domain that binds to cluster of differentiation 3 ϵ (CD3 ϵ), wherein the antigen binding domain that binds CD3 ϵ comprises:
 - a. a heavy chain complementarity determining region (HCDR) 1, a HCDR2 and a HCDR3 of a heavy chain variable region (VH) of SEQ ID NO: 23 and a light chain complementarity determining region (LCDR) 1, a LCDR2 and a LCDR3 of a light chain variable region (VL) of SEQ ID NO: 24;
 - b. the HCDR1, the HCDR2 and the HCDR3 of the VH of SEQ ID NO: 23 and the LCDR1, the LCDR2 and the LCDR3 of the VL of SEQ ID NO: 27;
 - c. the HCDR1, the HCDR2 and the HCDR3 of the VH of SEQ ID NO: 23 and the LCDR1, the LCDR2 and the LCDR3 of the VL of SEQ ID NO: 28;
 - d. the HCDR1, the HCDR2 and the HCDR3 of the VH of SEQ ID NO: 23 and the LCDR1, the LCDR2 and the LCDR3 of the VL of SEQ ID NO: 29; or
 - e. the HCDR1, the HCDR2 and the HCDR3 of the VH of SEQ ID NO: 23 and the LCDR1, the LCDR2 and the LCDR3 of the VL of SEQ ID NO: 30.
2. The isolated protein of claim 1, comprising the HCDR1, the HCDR2, the HCDR3, the LCDR1, the LCDR2 and the LCDR3 of
 - a. SEQ ID NOs: 6, 7, 8, 9, 10, and 11, respectively;
 - b. SEQ ID NOs: 12, 13, 14, 15, 16, and 17, respectively; or
 - c. SEQ ID NOs: 18, 19, 20, 21, 16, and 22, respectively.
3. The isolated protein of claim 1 or 2, wherein the antigen binding domain that binds CD3 ϵ is a scFv, a (scFv)₂, a Fv, a Fab, a F(ab')₂, a Fd, a dAb or a VHH.
4. The isolated protein of claim 3, wherein the antigen binding domain that binds CD3 ϵ is the Fab.
5. The isolated protein of claim 3, wherein the antigen binding domain that binds CD3 ϵ is the scFv.
6. The isolated protein of claim 5, wherein the scFv comprises, from the N- to C-terminus, a VH, a first linker (L1) and a VL (VH-L1-VL) or the VL, the L1 and the VH (VL-L1-VH).
7. The isolated protein of claim 6, wherein the L1 comprises
 - a. about 5-50 amino acids;
 - b. about 5-40 amino acids;
 - c. about 10-30 amino acids; or
 - d. about 10-20 amino acids.

8. The isolated protein of claim 6, wherein the L1 comprises an amino acid sequence of SEQ ID NOs: 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, 47, 48, 49, 50, 51, 52, 53, 54, 55, 56, 57, 58, 59, 60, 61, 62, 63, or 64.
9. The isolated protein of claim 8 wherein the L1 comprises the amino acid sequence of SEQ ID NO: 31, 37, or 64.
10. The isolated protein of any one of claims 1-9, wherein the antigen binding domain that binds CD3 ϵ comprises the VH of SEQ ID NOs: 23 and the VL of SEQ ID NOs: 24, 27, 28, 29 or 30.
11. The isolated protein of claim 10, wherein the antigen binding domain that binds CD3 ϵ comprises:
- the VH of SEQ ID NO: 23 and the VL of SEQ ID NO: 24;
 - the VH of SEQ ID NO: 23 and the VL of SEQ ID NO: 27;
 - the VH of SEQ ID NO: 23 and the VL of SEQ ID NO: 28;
 - the VH of SEQ ID NO: 23 and the VL of SEQ ID NO: 29; or
 - the VH of SEQ ID NO: 23 and the VL of SEQ ID NO: 30.
12. The isolated protein of any one of claims 1-11, wherein the antigen binding domain that binds CD3 ϵ comprises the amino acid sequence of SEQ ID NOs: 65, 66, 67, 68, 69, 70, 71, 72, 73, or 74.
13. An isolated protein comprising an antigen binding domain that binds CD3 ϵ , wherein the antigen binding domain that binds CD3 ϵ comprises a heavy chain variable region (VH) of SEQ ID NO: 23 and a light chain variable region (VL) of SEQ ID NO: 103.
14. The isolated protein of claim 13, wherein the antigen binding domain that binds CD3 ϵ is a scFv, a (scFv)₂, a Fv, a Fab, a F(ab')₂, a Fd, a dAb or a VHH.
15. The isolated protein of claim 14, wherein the antigen binding domain that binds CD3 ϵ is the Fab.
16. The isolated protein of claim 14, wherein the antigen binding domain that binds CD3 ϵ is the scFv.
17. The isolated protein of claim 16, wherein the scFv comprises, from the N- to C-terminus, a VH, a first linker (L1) and a VL (VH-L1-VL) or the VL, the L1 and the VH (VL-L1-VH).
18. The isolated protein of claim 17, wherein the L1 comprises
- about 5-50 amino acids;
 - about 5-40 amino acids;
 - about 10-30 amino acids; or
 - about 10-20 amino acids.
19. The isolated protein of claim 18, wherein the L1 comprises an amino acid sequence of SEQ ID NOs: 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, 47, 48, 49, 50, 51, 52, 53, 54, 55, 56, 57, 58, 59, 60, 61, 62, 63, or 64.

20. The isolated protein of claim 19, wherein the L1 comprises the amino acid sequence of SEQ ID NO: 31, 37, or 64.
21. The isolated protein of claim 13-20, wherein the antigen binding domain that binds CD3ε comprises the VH of SEQ ID NO: 23 and the VL of SEQ ID NO: 24, 27, 28, 29, or 30.
- 5 22. The isolated protein of claim 21, wherein the antigen binding domain that binds CD3ε comprises:
- a. the VH of SEQ ID NO: 23 and the VL of SEQ ID NO: 24;
 - b. the VH of SEQ ID NO: 23 and the VL of SEQ ID NO: 27;
 - c. the VH of SEQ ID NO: 23 and the VL of SEQ ID NO: 28;
 - d. the VH of SEQ ID NO: 23 and the VL of SEQ ID NO: 29; or
 - 10 e. the VH of SEQ ID NO: 23 and the VL of SEQ ID NO: 30.
23. The isolated protein of any one of claims 1-22, wherein the isolated protein is a multispecific protein.
24. The isolated protein of claim 23, wherein the multispecific protein is a bispecific protein.
- 15 25. The isolated protein of claim 23, wherein the multispecific protein is a trispecific protein.
26. The isolated protein of any one of claims 1-25, further comprising an immunoglobulin (Ig) constant region or a fragment of the Ig constant region thereof.
27. The isolated protein of claim 26, wherein the fragment of the Ig constant region comprises a Fc region.
- 20 28. The isolated protein of claim 26, wherein the fragment of the Ig constant region comprises a CH2 domain.
29. The isolated protein of claim 26, wherein the fragment of the Ig constant region comprises a CH3 domain.
30. The isolated protein of claim 26, wherein the fragment of the Ig constant region comprises a CH2 domain and a CH3 domain.
- 25 31. The isolated protein of claim 26, wherein the fragment of the Ig constant region comprises at least portion of a hinge, a CH2 domain and a CH3 domain.
32. The isolated protein of claim 26, wherein the fragment of the Ig constant region comprises a hinge, a CH2 domain and a CH3 domain.
- 30 33. The isolated protein of any one of claims 26-32, wherein the antigen binding domain that binds CD3ε is conjugated to the N-terminus of the Ig constant region or the fragment of the Ig constant region.

34. The isolated protein of any one of claims 26-32, wherein the antigen binding domain that binds CD3 ϵ is conjugated to the C-terminus of the Ig constant region or the fragment of the Ig constant region.
- 5 35. The isolated protein of any one of claims 26-32, wherein the antigen binding domain that binds CD3 ϵ is conjugated to the Ig constant region or the fragment of the Ig constant region via a second linker (L2).
36. The isolated protein of claim 35, wherein the L2 comprises the amino acid sequence of SEQ ID NOs: 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, 47, 48, 49, 50, 51, 52, 53, 54, 55, 56, 57, 58, 59, 60, 61, 62, 63, or 64.
- 10 37. The isolated protein of any one of claims 23-36, wherein the multispecific protein comprises an antigen binding domain that binds an antigen other than CD3 ϵ .
38. The multispecific antibody of claim 37, wherein the cell antigen is a tumor associated antigen.
39. The isolated protein of any one of claims 26-38, wherein the Ig constant region or the fragment of the Ig constant region is an IgG1, an IgG2, an IgG3 or an IgG4 isotype.
- 15 40. The isolated protein of any one of claims 26-39, wherein the Ig constant region or the fragment of the Ig constant region comprises at least one mutation that results in reduced binding of the protein to a Fc γ receptor (Fc γ R).
41. The isolated protein of claim 40, wherein the at least one mutation that results in reduced binding of the protein to the Fc γ R is selected from the group consisting of F234A/L235A, L234A/L235A, 20 L234A/L235A/D265S, V234A/G237A/ P238S/H268A/V309L/A330S/P331S, F234A/L235A, S228P/F234A/ L235A, N297A, V234A/G237A, K214T/E233P/ L234V/L235A/G236-deleted/A327G/P331A/D365E/L358M, H268Q/V309L/A330S/P331S, S267E/L328F, L234F/L235E/D265A, L234A/L235A/G237A/P238S/H268A/A330S/P331S, S228P/F234A/L235A/G237A/P238S and S228P/F234A/L235A/G236-deleted/G237A/P238S, 25 wherein residue numbering is according to the EU index.
42. The isolated protein of any one of claims 40-41, wherein the Fc γ R is Fc γ RI, Fc γ RIIA, Fc γ RIIB or Fc γ RIII, or any combination thereof.
43. The isolated protein of any one of the claims 26-42, wherein the protein comprises at least one mutation in a CH3 domain of the Ig constant region.
- 30 44. The isolated protein of claim 38, wherein the at least one mutation in the CH3 domain of the Ig constant region is selected from the group consisting of T350V, L351Y, F405A, Y407V, T366Y, T366W, T366L, F405W, T394W, K392L, T394S, Y407T, Y407A, T366S/L368A/Y407V, L351Y/F405A/Y407V, T366I/K392M/T394W, F405A/Y407V, T366L/K392M/T394W, T366L/K392L/T394W, L351Y/Y407A, L351Y/Y407V, T366A/K409F, T366V/K409F,

T366A/K409F, T350V/L351Y/F405A/Y407V and T350V/T366L/K392L/T394W, wherein residue numbering is according to the EU index.

45. A pharmaceutical composition comprising the isolated protein of any one of claims 1-44 and a pharmaceutically acceptable carrier.
- 5 46. A polynucleotide encoding the isolated protein of any one of claims 1-44.
47. A vector comprising the polynucleotide of claim 46.
48. A host cell comprising the vector of claim 47.
49. A method of producing the isolated protein of any one of claims 1-44, comprising culturing the host cell of claim 48 in conditions that the protein is expressed, and recovering the protein
- 10 produced by the host cell.
50. A method of treating a cancer in a subject, comprising administering a therapeutically effective amount of the isolated antibody of any one of claims 1-44 to the subject in need thereof to treat the cancer.
- 15 51. An isolated protein comprising an antigen binding domain that binds to an epitope on CD3 ϵ (SEQ ID NO: 1), wherein the epitope is a discontinuous epitope comprising the amino acid sequences of SEQ ID NO: 100, 101, and 102.
52. The isolated protein of claim 1 comprising an amino acid sequence selected from the group consisting of SEQ ID NOs: 747, 748, 77, 78, 749, 750, 751, 752, 753, and 754.
- 20 53. The isolated protein of claim 1 comprising amino acid sequences of SEQ ID NO: 747.
54. The isolated protein of claim 1 comprising amino acid sequences of SEQ ID NO: 748.
55. The isolated protein of claim 1 comprising amino acid sequences of SEQ ID NO: 77.
56. The isolated protein of claim 1 comprising amino acid sequences of SEQ ID NO: 78.
57. The isolated protein of claim 1 comprising amino acid sequences of SEQ ID NO: 749.
- 25 58. The isolated protein of claim 1 comprising amino acid sequences of SEQ ID NO: 750.
59. The isolated protein of claim 1 comprising amino acid sequences of SEQ ID NO: 751.
60. The isolated protein of claim 1 comprising amino acid sequences of SEQ ID NO: 752.
61. The isolated protein of claim 1 comprising amino acid sequences of SEQ ID NO: 753.
62. The isolated protein of claim 1 comprising amino acid sequences of SEQ ID NO: 754.
- 30 63. The isolated protein of claim 1 comprising amino acid sequences of SEQ ID NOs: 85 and 86.
64. The isolated protein of claim 1 comprising amino acid sequences of SEQ ID NOs: 85 and 88.
65. The isolated protein of claim 1 comprising amino acid sequences of SEQ ID NOs: 85 and 90.
66. The isolated protein of claim 1 comprising amino acid sequences of SEQ ID NOs: 85 and 92.
67. The isolated protein of claim 1 comprising amino acid sequences of SEQ ID NOs: 85 and 94.