CLAIMS

1. A GPC3/TRGV9-binding protein, comprising:
a first antigen-binding domain that specifically binds to GPC3, and
a second antigen-binding domain that specifically binds to TRGV9,
wherein:
the first antigen-binding domain comprises a heavy chain variable region (VH1) and a light chain variable region (VL1);
the VH1 comprises a HCDR1, a HCDR2, and a HCDR3 from the amino acid sequence set forth in SEQ ID NO: 39;
the VL1 comprises a LCDR1, a LCDR2, and a LCDR3 from the amino acid sequence set forth in SEQ ID NO: 40;
the CDRs are defined according to the Kabat, IMGT, Chothia, AbM, or Contact numbering scheme;
preferably, the CDRs are defined according to the Kabat numbering scheme;
preferably, the amino acid sequences of the HCDR1, the HCDR2, and the HCDR3 are set forth in SEQ ID NOs: 41-43, respectively, and the amino acid sequences of the LCDR1, the LCDR2, and the LCDR3 are set forth in SEQ ID NOs: 44-46, respectively.

2. The GPC3/TRGV9-binding protein according to claim 1, wherein the second antigen-binding domain comprises an immunoglobulin single variable domain, or comprises a heavy chain variable region (VH2) and a light chain variable region (VL2), wherein:
the immunoglobulin single variable domain comprises a CDR1, a CDR2, and a CDR3 from the amino acid sequence set forth in any one of SEQ ID NOs: 29, 26-28, and 5;
the VH2 comprises a HCDR1, a HCDR2, and a HCDR3 from the amino acid sequence set forth in any one of SEQ ID NOs: 31, 9, 30, and 32, and the VL2 comprises a LCDR1, a LCDR2, and a LCDR3 from the amino acid sequence set forth in any one of SEQ ID NOs: 34, 10, 33, and 35;
the CDRs are defined according to the Kabat, IMGT, Chothia, AbM, or Contact numbering scheme;
preferably, the CDRs are defined according to the Kabat numbering scheme;
preferably, the immunoglobulin single variable domain comprises a CDR1, a CDR2, and a CDR3 whose amino acid sequences are set forth in SEQ ID NOs: 6-8, respectively;
the amino acid sequences of the HCDR1, the HCDR2, and the HCDR3 of the VH2 are set forth in SEQ ID NOs: 11-13, respectively, and the amino acid sequences of the LCDR1, the LCDR2, and the LCDR3 of the VL2 are set forth in SEQ ID NOs: 14-16, respectively.

3. The GPC3/TRGV9-binding protein according to claim 1 or 2, wherein:
the immunoglobulin single variable domain, the heavy chain variable region, and/or the light chain variable region are/is engineered by humanization, back mutation, affinity maturation, T cell epitope removal, reduction of antibody deamidation, and/or reduction

of antibody isomerization;
preferably, framework regions of a human germline template used for humanization engineering of the immunoglobulin single variable domain are derived from IGHV3-64;
preferably, framework regions of a human germline template used for humanization engineering of the VH2 are derived from IGHV3-21, and/or framework regions of a human germline template used for humanization engineering of the VL2 are derived from IGKV1-12.

4. The GPC3/TRGV9-binding protein according to any one of claims 1 to 3, wherein:
the VH1 in the first antigen-binding domain comprises the amino acid sequence set forth in SEQ ID NO: 39 or an amino acid sequence having at least 80% or at least 90% identity thereto, and the VL1 comprises the amino acid sequence set forth in SEQ ID NO: 40 or an amino acid sequence having at least 80% or at least 90% identity thereto.

5. The GPC3/TRGV9-binding protein according to any one of claims 1 to 4, wherein:
the immunoglobulin single variable domain comprises the amino acid sequence set forth in any one of SEQ ID NOs: 29, 26-28, and 5 or an amino acid sequence having at least 80% or at least 90% identity thereto;
the VH2 in the second antigen-binding domain comprises the amino acid sequence set forth in any one of SEQ ID NOs: 9 and 30-32 or an amino acid sequence having at least 80% or at least 90% identity thereto, and the VL2 comprises the amino acid sequence set forth in any one of SEQ ID NOs: 10 and 33-35 or an amino acid sequence having at least 80% or at least 90% identity thereto.

6. The GPC3/TRGV9-binding protein according to any one of claims 1 to 5, further comprising an immunoglobulin Fc region, wherein:
preferably, the Fc region is a human IgG1, human IgG2, human IgG3, or human IgG4 Fc region;
more preferably, the Fc region is a human IgG1 Fc region;
most preferably, the Fc region is an IgG1 Fc region comprising mutation L234F and/or mutation L235E.

7. The GPC3/TRGV9-binding protein according to claim 6, wherein the Fc region comprises a first subunit and a second subunit;
preferably, the first subunit and the second subunit of the Fc region comprise knob-into-hole mutations;
preferably, the first subunit of the Fc region comprises a mutation at position 366, and the second subunit comprises a mutation selected from the group consisting of positions 366, 368, and 407 or any combination thereof; the first subunit of the Fc region comprises a mutation at position 354 or 356, and the second subunit comprises a mutation at position 349; or
the first subunit of the Fc region comprises a mutation at position 354 or 356, and the

second subunit comprises a mutation at position 349, 366, 368, or 407 or any combination thereof;
more preferably, the first subunit of the Fc region comprises mutation 366W, and the second subunit comprises a mutation selected from the group consisting of 366S, 368A, and 407V or any combination thereof; the first subunit of the Fc region comprises mutation 354C or 356C, and the second subunit comprises mutation 349C; or the first subunit of the Fc region comprises mutations 354C/366W, and the second subunit comprises mutations 349C/366S/368A/407V;
mutation sites are numbered according to the Eu numbering scheme.

8. The GPC3/TRGV9-binding protein according to any one of claims 1 to 7, further comprising a linker, wherein:
preferably, the linker is represented by $(G_mS_n)_h$ or $(G_mQ_n)_h$ or $(GGNGT)_h$ or $(YGNGT)_h$ or $(EPKSS)_h$ or $(A_mS_n)_h$, wherein m and n are each independently selected from the group consisting of integers of 1-8, and h is independently selected from the group consisting of integers of 1-20;
more preferably, the linker is A_3S or G_4S.

9. The GPC3/TRGV9-binding protein according to any one of claims 1 to 8, comprising any one combination of polypeptide chains selected from the group consisting of (1)-(3):
(1) a first heavy chain, a second heavy chain, and a light chain, wherein:
the first heavy chain, from the N-terminus to the C-terminus, is [immunoglobulin single variable domain]-[linker 1]-[Fc1],
the second heavy chain, from the N-terminus to the C-terminus, is [VH1]-[linker 2]-[CH1]-[linker 3]-[Fc2], and
the light chain, from the N-terminus to the C-terminus, is [VL1]-[linker 4]-[CL];
wherein the linker 1, the linker 2, the linker 3, and the linker 4 may be identical or different, may independently be present or absent, and may be independently selected from the group consisting of the linker defined in claim 8;
preferably, the linker 1 is AAAS, and the linker 2, the linker 3, and the linker 4 are absent;
(2) a first heavy chain, a first light chain, a second heavy chain, and a second light chain, wherein:
the first heavy chain, from the N-terminus to the C-terminus, is [VH2]-[linker 1]-[Obscurin-O chain]-[linker 2]-[Fc1],
the first light chain, from the N-terminus to the C-terminus, is [VL2]-[linker 3]-[Titin-T chain],
the second heavy chain, from the N-terminus to the C-terminus, is [VH1]-[linker 4]-[CH1]-[linker 5]-[Fc2], and
the second light chain, from the N-terminus to the C-terminus, is [VL1]-[linker 6]-[CL];
or
the first heavy chain, from the N-terminus to the C-terminus, is [VH1]-[linker 1]-[Obscurin-O chain]-[linker 2]-[Fc1],

the first light chain, from the N-terminus to the C-terminus, is [VL1]-[linker 3]-[Titin-T chain],
the second heavy chain, from the N-terminus to the C-terminus, is [VH2]-[linker 4]-[CH1]-[linker 5]-[Fc2], and
the second light chain, from the N-terminus to the C-terminus, is [VL2]-[linker 6]-[CL];
wherein the linker 1, the linker 2, the linker 3, the linker 4, the linker 5, and the linker 6 may be identical or different, may independently be present or absent, and may be independently selected from the group consisting of the linker defined in claim 8;
preferably, the linker 1 and the linker 3 are G_4S, and the linker 2, the linker 4, the linker 5, and the linker 6 are absent;

(3) a first heavy chain, a first light chain, a second heavy chain, and a second light chain, wherein:
the first heavy chain, from the N-terminus to the C-terminus, is [VH2]-[linker 1]-[Obscurin-O chain]-[linker 2]-[Fc1],
the first light chain, from the N-terminus to the C-terminus, is [VL2]-[linker 3]-[Titin-T chain],
the second heavy chain, from the N-terminus to the C-terminus, is [VH1]-[linker 4]-[CH1]-[linker 5]-[VH1]-[linker 6]-[CH1]-[linker 7]-[Fc2], and
the second light chain, from the N-terminus to the C-terminus, is [VL1]-[linker 8]-[CL];
or
the first heavy chain, from the N-terminus to the C-terminus, is [VH1]-[linker 1]-[Obscurin-O chain]-[linker 2]-[Fc1],
the first light chain, from the N-terminus to the C-terminus, is [VL1]-[linker 3]-[Titin-T chain],
the second heavy chain, from the N-terminus to the C-terminus, is [VH2]-[linker 4]-[CH1]-[linker 5]-[VH2]-[linker 6]-[CH1]-[linker 7]-[Fc2], and
the second light chain, from the N-terminus to the C-terminus, is [VL2]-[linker 8]-[CL];
wherein the linker 1, the linker 2, the linker 3, the linker 4, the linker 5, the linker 6, the linker 7, the linker 8, the linker 9, and the linker 10 may be identical or different, may independently be present or absent, and may be independently selected from the group consisting of the linker described in claim 8;
preferably, the linker 1 and the linker 3 are G_4S, the linker 5 is $(G_4S)_2$, and the linker 2, the linker 4, the linker 6, the linker 7, and the linker 8 are absent;
wherein in the above (1)-(3), - represents a peptide bond;
preferably, the amino acid sequences of the Obscurin-O chain and the Titin-T chain are set forth in SEQ ID NOs: 47 and 48, respectively;
preferably, the CL is a Cκ, and the amino acid sequences of the CH1 and the Cκ are set forth in SEQ ID NOs: 49 and 50, respectively;
preferably, the amino acid sequences of the Fc1 and the Fc2 are set forth in SEQ ID NOs: 52 and 53, respectively.

10. The GPC3/TRGV9-binding protein according to any one of claims 1 to 9, comprising any one combination of polypeptide chains selected from the group consisting of (1)-(3):

(1) a first heavy chain whose amino acid sequence is set forth in SEQ ID NO: 54 or has at least 80% or at least 90% identity thereto, a second heavy chain whose amino acid sequence is set forth in SEQ ID NO: 55 or has at least 80% or at least 90% identity thereto, and a light chain whose amino acid sequence is set forth in SEQ ID NO: 56 or has at least 80% or at least 90% identity thereto;

(2) a first heavy chain whose amino acid sequence is set forth in SEQ ID NO: 57 or has at least 80% or at least 90% identity thereto, a first light chain whose amino acid sequence is set forth in SEQ ID NO: 58 or has at least 80% or at least 90% identity thereto, a second heavy chain whose amino acid sequence is set forth in SEQ ID NO: 59 or has at least 80% or at least 90% identity thereto, and a second light chain whose amino acid sequence is set forth in SEQ ID NO: 56 or has at least 80% or at least 90% identity thereto; or

(3) a first heavy chain whose amino acid sequence is set forth in SEQ ID NO: 57 or has at least 80% or at least 90% identity thereto, a first light chain whose amino acid sequence is set forth in SEQ ID NO: 58 or has at least 80% or at least 90% identity thereto, a second heavy chain whose amino acid sequence is set forth in SEQ ID NO: 60 or has at least 80% or at least 90% identity thereto, and a second light chain whose amino acid sequence is set forth in SEQ ID NO: 61 or has at least 80% or at least 90% identity thereto;

wherein preferably, the GPC3/TRGV9-binding protein comprises any one combination of polypeptide chains selected from the group consisting of (1)-(3):

(1) polypeptide chains whose amino acid sequences are set forth in SEQ ID NOs: 54-56;

(2) polypeptide chains whose amino acid sequences are set forth in SEQ ID NOs: 56-59; and

(3) polypeptide chains whose amino acid sequences are set forth in SEQ ID NOs: 57, 58, 60, and 61;

more preferably, the GPC3/TRGV9-binding protein comprises any one combination of polypeptide chains selected from the group consisting of (1)-(3):

(1) polypeptide chains whose amino acid sequences are set forth in SEQ ID NOs: 54-56 in a molar ratio of 1:1:1;

(2) polypeptide chains whose amino acid sequences are set forth in SEQ ID NOs: 56-59 in a molar ratio of 1:1:1:1; and

(3) polypeptide chains whose amino acid sequences are set forth in SEQ ID NOs: 57, 58, 60, and 61 in a molar ratio of 1:1:1:2.

11. The GPC3/TRGV9-binding protein according to any one of claims 1 to 10, being an antibody or an antigen-binding fragment thereof, preferably an anti-GPC3/TRGV9 antibody or an antigen-binding fragment thereof.

12. A TRGV9-binding protein, comprising:

(1) an immunoglobulin single variable domain, comprising a CDR1, a CDR2, and a CDR3 from the amino acid sequence set forth in any one of SEQ ID NOs: 29, 26-28, and 5, preferably a CDR1, a CDR2, and a CDR3 from the amino acid sequences set forth in SEQ ID NOs: 6-8; or

(2) a heavy chain variable region (VH) and/or a light chain variable region (VL), wherein the VH comprises a HCDR1, a HCDR2, and a HCDR3 from the amino acid sequence set forth in any one of SEQ ID NOs: 9 and 30-32, and the VL comprises a LCDR1, a LCDR2, and a LCDR3 from the amino acid sequence set forth in any one of SEQ ID NOs: 10 and 33-35;

wherein the CDRs are defined according to the Kabat, IMGT, Chothia, AbM, or Contact numbering scheme, for example, according to the Kabat numbering scheme.

13. The TRGV9-binding protein according to claim 12, wherein the immunoglobulin single variable domain, the VH, and/or the VL are/is engineered by humanization, back mutation, affinity maturation, T cell epitope removal, reduction of antibody deamidation, and/or reduction of antibody isomerization;

preferably, framework regions of a human germline template used for humanization engineering of the immunoglobulin single variable domain are derived from IGHV3-64;

preferably, framework regions of a human germline template used for humanization engineering of the VH are derived from IGHV3-21, and/or framework regions of a human germline template used for humanization engineering of the VL are derived from IGKV1-12.

14. The TRGV9-binding protein according to claim 12 or 13, wherein:

(1) the immunoglobulin single variable domain comprises the amino acid sequence set forth in any one of SEQ ID NOs: 29, 26-28, and 5 or an amino acid sequence having at least 80% or at least 90% identity thereto; or

(2) the VH comprises the amino acid sequence set forth in any one of SEQ ID NOs: 9 and 30-32 or an amino acid sequence having at least 80% or at least 90% identity thereto, and the VL comprises the amino acid sequence set forth in any one of SEQ ID NOs: 10 and 33-35 or an amino acid sequence having at least 80% or at least 90% identity thereto;

preferably, the TRGV9-binding protein is an anti-TRGV9 antibody or an antigen-binding fragment thereof;

more preferably, when the TRGV9-binding protein comprises an immunoglobulin single variable domain, the immunoglobulin single variable domain is an anti-TRGV9 single-domain antibody or VHH.

15. The TRGV9-binding protein according to any one of claims 12 to 14, further comprising an immunoglobulin Fc region, wherein:

preferably, the Fc region is a human IgG1, human IgG2, human IgG3, or human IgG4 Fc region; more preferably, the Fc region is a human IgG1 Fc region;

preferably, the Fc region is an IgG1 Fc region comprising mutation L234F and/or mutation L235E.

16. The TRGV9-binding protein according to any one of claims 12 to 15, further comprising a tumor-associated antigen (TAA)-binding domain or a tumor-specific antigen-binding

domain, preferably an antigen-binding domain that specifically binds to GPC3.

17. The GPC3/TRGV9-binding protein according to any one of claims 1 to 11 or the TRGV9-binding protein according to any one of claims 12 to 16, having any one or more properties or functions selected from the group consisting of (a)-(d):
(a) specific binding to a γ9 chain of a TCR, preferably specific binding to a γ9δ1 TCR or a γ9δ2 TCR;
(b) undetectable binding to a γ8 chain of the TCR, preferably undetectable binding to a γ8δ2 TCR;
(c) specific binding to a variable region of the γ9 chain of the TCR (TRGV9); and
(d) specific binding to a VγδCαβ chimeric TCR and undetectable binding to a VαβCγδ chimeric TCR.

18. A polynucleotide, encoding the GPC3/TRGV9-binding protein according to any one of claims 1 to 11 or the TRGV9-binding protein according to any one of claims 12 to 16, or a combination thereof, wherein:
preferably, the polynucleotide is a DNA or RNA.

19. A vector, comprising the polynucleotide according to claim 18, wherein:
preferably, the vector expresses the polynucleotide according to claim 18.

20. A host cell, comprising or expressing the polynucleotide according to claim 18 or the vector according to claim 19.

21. A method for preparing the GPC3/TRGV9-binding protein according to any one of claims 1 to 11 or the TRGV9-binding protein according to any one of claims 12 to 16, comprising:
expressing the polynucleotide according to claim 18 or the vector according to claim 19 in the host cell according to claim 20, and isolating an expressed GPC3/TRGV9-binding protein or TRGV9-binding protein from the host cell,
wherein optionally, the method further comprises a step of purifying the GPC3/TRGV9-binding protein or the TRGV9-binding protein.

22. A pharmaceutical composition, comprising the GPC3/TRGV9-binding protein according to any one of claims 1 to 11, the TRGV9-binding protein according to any one of claims 12 to 16, the polynucleotide according to claim 18, and/or the vector according to claim 19, wherein:
preferably, the pharmaceutical composition further comprises a T cell, and the T cell is preferably a γδ T cell;
preferably, the pharmaceutical composition further comprises one or more pharmaceutically acceptable excipients, diluents, or auxiliary materials.

23. A combination or kit, comprising:
(1) the GPC3/TRGV9-binding protein according to any one of claims 1 to 11 and a T cell, or
(2) the TRGV9-binding protein according to any one of claims 12 to 16 and a T cell,
wherein the T cell is preferably a γδ T cell.

24. The pharmaceutical composition of claim 22 or the combination or kit of claim 23 for use in therapy.

25. Use of the GPC3/TRGV9-binding protein according to any one of claims 1 to 11, the TRGV9-binding protein according to any one of claims 12 to 16, the polynucleotide according to claim 18, the vector according to claim 19, or the combination or kit according to claim 23 in the manufacture of a medicament for treating a disease, wherein:
the disease is cancer;
preferably, the disease is liver cancer or GPC3-positive cancer; most preferably, the disease is GPC3-positive liver cancer.

26. The use of claim 25, wherein:
the medicament is to be used in combination with a T cell;
the T cell is preferably a γδ T cell.

27. Use of a T cell for the manufacture of a medicament for treating a disease, wherein:
the medicament is to be used in combination with the GPC3/TRGV9-binding protein according to any one of claims 1 to 11, the TRGV9-binding protein according to any one of claims 12 to 16, the polynucleotide according to claim 18, or the vector according to claim 19;
the T cell is preferably a γδ T cell;
the disease is cancer; preferably, the disease is liver cancer or GPC3-positive cancer; most preferably, the disease is GPC3-positive liver cancer.