CLAIMS

1. A multi-specific polypeptide molecule, wherein the multi-specific polypeptide molecule comprises a first binding region that binds a first antigen and a second binding region that binds a second antigen,

wherein the first binding region comprises an α chain variable region (TRAV1) and a β chain variable region (TRBV1) derived from a T cell receptor (TCR) that binds the first antigen-MHC complex;

wherein the second binding region comprises a heavy chain variable region VH1 and a light chain variable region VL1 derived from an antibody that binds the second antigen, or a single heavy chain variable region (VHH1) derived from a single-domain antibody or a nanobody that binds the second antigen;

wherein the first antigen is selected from the group consisting of tumor-associated antigens (TAA), tumor-specific antigens (TSA), viral antigens, and self-antigens, and the second antigen is an immune cell surface molecule;

wherein the N-terminus of the antibody is connected to the C-terminus of the TCR;

wherein the multi-specific polypeptide molecule further comprises at least one of the following functional domains:

1) a hinge region derived from an antibody;

2) an Fc domain derived from an antibody or a dimerization portion thereof;

3) a CH3 domain derived from an antibody;

4) albumin (Alb) or a binding domain thereof;

5) a constant region derived from a TCR or a fragment thereof;

preferably, wherein TRAV1 and TRBV1 are distributed on different polypeptide chains; and/or

VH1 and VL1 are distributed on one polypeptide chain (e.g., forming scFv1); and TRAV1 and TRBV1 are distributed on different polypeptide chains.

2. The multi-specific polypeptide molecule according to claim 1, wherein the multi-specific polypeptide molecule comprises, on two polypeptide chains, the first binding region that binds the first antigen and the second binding region that binds the second antigen;

preferably, TRAV1 and TRBV1 are distributed on different polypeptide chains, the VHH1 or the VH1 and VL1 are distributed on one polypeptide chain (e.g., forming scFv1) and connected to the C-terminus of the TCR; or TRAV1 and TRBV1 are distributed on different polypeptide chains, the VH1 and VL1 are distributed on different polypeptide chains, and the polypeptide chain on which one of the VH1 and VL1 is located is connected to the C-terminus of the polypeptide chain on which the TRAV1 is located, and the polypeptide chain on which the other is located is connected to the C-terminus of the polypeptide chain on which the TRBV1 is located;

or,

the multi-specific polypeptide molecule comprises, on three polypeptide chains, a first binding region that binds a first antigen and a second binding region that binds a second antigen;

preferably, TRAV1 and TRBV1 are distributed on different polypeptide chains, the VH1 and VL1 are distributed on different polypeptide chains, and the polypeptide chain on which VH1 or VL1 is located is connected to the C-terminus of the polypeptide chain on which TRBV1 or TRAV1 is located;

preferably, wherein the polypeptide chains of the multi-specific polypeptide molecule respectively comprise:

TRBV1-scFv1 and TRAV1; or
TRAV1-scFv1 and TRBV1; or
TRBV1-VHH1 and TRAV1; or
TRAV1-VHH1 and TRBV1; or
TRBV1-VL1 and TRAV1-VH1; or
TRAV1-VL1 and TRBV1-VH1; or
TRBV1-VL1-VH1 and TRAV1; or
TRBV1-VH1-VL1 and TRAV1; or
TRAV1-VL1-VH1 and TRBV1; or
TRAV1-VH1-VL1 and TRBV1; or
TRBV1-VL1, VH1, and TRAV1; or
TRBV1-VH1, VL1, and TRAV1; or
TRAV1-VL1, VH1, and TRBV1; or

TRAV1-VH1, VL1, and TRBV1.

3. The multi-specific polypeptide molecule according to claim 1 or 2, wherein the multi-specific polypeptide molecule further comprises a third binding region that binds a third antigen, and the third binding region comprises an α chain variable region (TRAV2) and a β chain variable region (TRBV2) derived from a TCR that binds the third antigen-MHC complex; wherein the third antigen is selected from the group consisting of tumor-associated antigens (TAA), tumor-specific antigens (TSA), viral antigens and self-antigens, and the third antigen is the same as or different from the first antigen;

preferably, wherein the TRAV2 is the same as the TRAV1, and/or the TRBV2 is the same as the TRBV1; and/or

wherein the TRAV2 and the TRBV2 are linked by a linker on the same polypeptide chain, and are preferably linked between VH1 and VL1;

more preferably, wherein the multi-specific polypeptide molecule comprises two polypeptide chains, and the two polypeptide chains respectively comprise:

TRBV1-VL1-TRAV2-TRBV2-VH1 and TRAV1; or
TRBV1-VL1-TRBV2-TRAV2-VH1 and TRAV1; or
TRBV1-VH1-TRBV2-TRAV2-VL1 and TRAV1; or
TRBV1-VH1-TRAV2-TRBV2-VL1 and TRAV1; or
TRAV1-VL1-TRBV2-TRAV2-VH1 and TRBV1; or
TRAV1-VL1-TRAV2-TRBV2-VH1 and TRBV1; or
TRAV1-VH1-TRAV2-TRBV2-VL1 and TRBV1; or
TRAV1-VH1-TRBV2-TRAV2-VL1 and TRBV1.

4. The multi-specific polypeptide molecule according to any one of claims 1-3, wherein the multi-specific polypeptide molecule further comprises a fourth binding region that binds a fourth antigen, the fourth binding region comprises a heavy chain variable region VH2 and a light chain variable region VL2 derived from an antibody that binds the fourth antigen, or a single heavy chain variable region (VHH2) derived from a single-domain antibody or nanobody that binds the fourth antigen; wherein the fourth antigen is an immune cell surface molecule, and the fourth antigen is the same as or different from the second antigen;

preferably, wherein the VH2 is the same as the VH1, and/or the VL2 is the same as the VL1, and/or the VHH2 is the same as the VHH1; and/or

wherein the VHH2 or the VH2 and VL2 are arranged in tandem on one polypeptide chain (e.g., forming scFv2 or Fab2) and connected to the N-terminus of the TRAV1 or the TRBV1, or one of the VH2 and VL2 is connected to the N-terminus of the TRAV1 and the other is connected to the N-terminus of the TRBV1;

more preferably, wherein the multi-specific polypeptide molecule comprises two polypeptide chains, and the two polypeptide chains respectively comprise:

scFv2-TRBV1-scFv1 and TRAV1; or
scFv2-TRAV1-scFv1 and TRBV1; or
scFv2-TRBV1-VHH1 and TRAV1; or
scFv2-TRAV1-VHH1 and TRBV1; or
scFv2-TRBV1-VL1 and TRAV1-VH1; or
scFv2-TRAV1-VL1 and TRBV1-VH1; or
TRBV1-scFv1 and scFv2-TRAV1; or
TRAV1-scFv1 and scFv2-TRBV1; or
TRBV1-VHH1 and scFv2-TRAV1; or
TRAV1-VHH1 and scFv2-TRBV1; or
TRBV1-VL1 and scFv2-TRAV1-VH1; or
TRAV1-VL1 and scFv2-TRBV1-VH1; or
VHH2-TRBV1-scFv1 and TRAV1; or
VHH2-TRAV1-scFv1 and TRBV1; or
VHH2-TRBV1-VHH1 and TRAV1; or
VHH2-TRAV1-VHH1 and TRBV1; or
VHH2-TRBV1-VL1 and TRAV1-VH1; or
VHH2-TRAV1-VL1 and TRBV1-VH1; or
TRBV1-scFv1 and VHH2-TRAV1; or

TRAV1-scFv1 and VHH2-TRBV1; or
TRBV1-VHH1 and VHH2-TRAV1; or
TRAV1-VHH1 and VHH2-TRBV1; or
TRBV1-VL1 and VHH2-TRAV1-VH1; or
TRAV1-VL1 and VHH2-TRBV1-VH1; or
VL2-TRBV1-scFv1 and VH2-TRAV1; or
VH2-TRBV1-scFv1 and VL2-TRAV1; or
VL2-TRAV1-scFv1 and VH2-TRBV1; or
VH2-TRAV1-scFv1 and VL2-TRBV1; or
VL2-TRBV1-VHH1 and VH2-TRAV1; or
VH2-TRBV1-VHH1 and VL2-TRAV1; or
VL2-TRAV1-VHH1 and VH2-TRBV1; or
VH2-TRAV1-VHH1 and VL2-TRBV1; or
VL2-TRBV1-VL1 and VH2-TRAV1-VH1; or
VH2-TRBV1-VL1 and VL2-TRAV1-VH1; or
VL2-TRAV1-VL1 and VH2-TRBV1-VH1; or
VH2-TRAV1-VL1 and VL2-TRBV1-VH1;

further preferably, wherein the multi-specific polypeptide molecule comprises a first binding region, a second binding region, a third binding region and a fourth binding region, and the multi-specific polypeptide molecule comprises two polypeptide chains, and the two polypeptide chains respectively comprise:

scFv2-TRBV1-VL1-TRAV2-TRBV2-VH1 and TRAV1; or
scFv2-TRBV1-VL1-TRBV2-TRAV2-VH1 and TRAV1; or
scFv2-TRBV1-VH1-TRBV2- TRAV2-VL1 and TRAV1; or
scFv2-TRBV1-VH1-TRAV2- TRBV2-VL1 and TRAV1; or
scFv2-TRAV1-VL1-TRBV2- TRAV2-VH1 and TRBV1; or
scFv2-TRAV1-VL1-TRAV2- TRBV2-VH1 and TRBV1; or
scFv2-TRAV1-VH1-TRAV2-TRBV2-VL1 and TRBV1; or
scFv2-TRAV1-VH1-TRBV2-TRAV2-VL1 and TRBV1; or
TRBV1-VL1-TRAV2-TRBV2-VH1 and scFv2-TRAV1; or
TRBV1-VL1-TRBV2-TRAV2-VH1 and scFv2-TRAV1; or
TRBV1-VH1-TRBV2- TRAV2-VL1 and scFv2-TRAV1; or
TRBV1-VH1-TRAV2- TRBV2-VL1 and scFv2-TRAV1; or
TRAV1-VL1-TRBV2- TRAV2-VH1 and scFv2-TRBV1; or
TRAV1-VL1-TRAV2- TRBV2-VH1 and scFv2-TRBV1; or
TRAV1-VH1-TRAV2-TRBV2-VL1 and scFv2-TRBV1; or
TRAV1-VH1-TRBV2-TRAV2-VL1 and scFv2-TRBV1; or
VHH2-TRBV1-VL1-TRAV2-TRBV2-VH1 and TRAV1; or
VHH2-TRBV1-VL1-TRBV2-TRAV2-VH1 and TRAV1; or
VHH2-TRBV1-VH1-TRBV2- TRAV2-VL1 and TRAV1; or
VHH2-TRBV1-VH1-TRAV2- TRBV2-VL1 and TRAV1; or
VHH2-TRAV1-VL1-TRBV2- TRAV2-VH1 and TRBV1; or
VHH2-TRAV1-VL1-TRAV2- TRBV2-VH1 and TRBV1; or
VHH2-TRAV1-VH1-TRAV2-TRBV2-VL1 and TRBV1; or
VHH2-TRAV1-VH1-TRBV2-TRAV2-VL1 and TRBV1; or
TRBV1-VL1-TRAV2-TRBV2-VH1 and VHH2-TRAV1; or
TRBV1-VL1-TRBV2-TRAV2-VH1 and VHH2-TRAV1; or
TRBV1-VH1-TRBV2- TRAV2-VL1 and VHH2-TRAV1; or
TRBV1-VH1-TRAV2- TRBV2-VL1 and VHH2-TRAV1; or
TRAV1-VL1-TRBV2- TRAV2-VH1 and VHH2-TRBV1; or
TRAV1-VL1-TRAV2- TRBV2-VH1 and VHH2-TRBV1; or
TRAV1-VH1-TRAV2-TRBV2-VL1 and VHH2-TRBV1; or
TRAV1-VH1-TRBV2-TRAV2-VL1 and VHH2-TRBV1; or
VL2-TRBV1-VL1-TRAV2-TRBV2-VH1 and VH2-TRAV1; or
VL2-TRBV1-VL1-TRBV2-TRAV2-VH1 and VH2-TRAV1; or
VH2-TRBV1-VL1-TRAV2-TRBV2-VH1 and VL2-TRAV1; or

VH2-TRBV1-VL1-TRBV2-TRAV2-VH1 and VL2-TRAV1; or
VL2-TRBV1-VH1-TRBV2- TRAV2-VL1 and VH2-TRAV1; or
VL2-TRBV1-VH1-TRAV2- TRBV2-VL1 and VH2-TRAV1; or
VH2-TRBV1-VH1-TRBV2- TRAV2-VL1 and VL2-TRAV1; or
VH2-TRBV1-VH1-TRAV2- TRBV2-VL1 and VL2-TRAV1; or
VL2-TRAV1-VL1-TRBV2- TRAV2-VH1 and VH2-TRBV1; or
VL2-TRAV1-VL1-TRAV2- TRBV2-VH1 and VH2-TRBV1; or
VH2-TRAV1-VL1-TRBV2- TRAV2-VH1 and VL2-TRBV1; or
VH2-TRAV1-VL1-TRAV2- TRBV2-VH1 and VL2-TRBV1; or
VL2-TRAV1-VH1-TRAV2-TRBV2-VL1 and VH2-TRBV1; or
VL2-TRAV1-VH1-TRBV2-TRAV2-VL1 and VH2-TRBV1; or
VH2-TRAV1-VH1-TRAV2-TRBV2-VL1 and VL2-TRBV1; or
VH2-TRAV1-VH1-TRBV2-TRAV2-VL1 and VL2-TRBV1.

5. The multi-specific polypeptide molecule according to any one of claims 1-4, wherein the functional domain is derived from albumin or a binding domain thereof and an optional antibody hinge region; and the functional domain is linked to the C-terminus or N-terminus, preferably the C-terminus, of any polypeptide chain of the multi-specific polypeptide molecule via an optional linker; and wherein the albumin is serum albumin, preferably human serum albumin; and/or the antibody hinge region is human-derived, e.g., a hinge domain derived from human IgG1, IgG2 or IgG4, or a portion thereof;

or

wherein the functional domain is a hinge region derived from an antibody, and/or an Fc domain derived from an antibody or a dimerization portion thereof, and/or a CH3 domain derived from an antibody; and any two polypeptide chains of the multi-specific polypeptide molecule are connected through covalent bonds or non-covalent bonds between the two chains of the functional domain; preferably, the two chains of the functional domain are respectively connected to the C-terminus or N-terminus, preferably the C-terminus, of any two polypeptide chains of the multi-specific polypeptide molecule, via an optional linker; or the first binding region and the second binding region are connected through covalent bonds or non-covalent bonds between the two chains of the functional domain; and preferably wherein the Antibody CH3 domain is human-derived, e.g., a CH3 domain derived from human IgG1, IgG2 or IgG4;

or

wherein the functional domain is a constant region derived from a TCR or a fragment thereof and an optional hinge region derived from an antibody; and any two polypeptide chains of the multi-specific polypeptide molecule are connected through covalent bonds or non-covalent bonds between two chains of the functional domain; preferably, the two chains of the functional domain are respectively connected to the C-terminus or N-terminus of any two polypeptide chains of the multi-specific polypeptide molecule via an optional linker; or the first binding region and the second binding region are connected by covalent bonds or non-covalent bonds between the two chains of the functional domain; and preferably the constant region of the TCR or a fragment thereof is human-derived or murine-derived, and is selected from the group consisting of a TCR α chain constant region (TRAC) and a TCR β chain constant region (TRBC);

preferably, wherein the hinge region derived from an antibody comprises at least one amino acid mutation capable of reducing or eliminating binding of the multi-specific molecule to an FcR receptor and/or C1q, e.g., the mutation site is selected from the group consisting of positions 233, 234, 235 and 236; and/or

the hinge region derived from an antibody comprises at least one amino acid mutation capable of reducing or eliminating formation of a disulfide bond between the hinge region and a non-hinge region, e.g., the mutation site is at position 220; and/or

the CH2 domain in the Fc domain comprises at least one amino acid mutation capable of reducing or eliminating binding of the multi-specific molecule to an FcR receptor and/or C1q; e.g., the mutation site is selected from the group consisting of positions 237, 250, 252, 254, 256, 270, 297, 322, 327, 330 and 331; and/or

the CH3 domain in the Fc domain comprises at least one amino acid mutation capable of reducing or eliminating binding of the multi-specific molecule to an FcR receptor and/or C1q; e.g., the mutation site is selected from the group consisting of positions 428 and 434;

the CH3 domain in the Fc domain or the CH3 domain derived from an antibody comprises at least one of the following mutations capable of promoting formation of a heterodimer of the polypeptide molecule:

a) comprises at least two cysteine residue mutations to form an inter-chain disulfide bond, e.g. 354C and 349C, or 242C and 334C;

b) comprises mutations capable of enabling two CH3 to form a knob-into-hole structure, e.g., each CH3 independently comprises a mutation at one or more positions selected from the group consisting of amino acid positions 366, 368, 405 and 407; preferably, one of the CH3 domains comprises a 366W mutation and the other comprises 366S, 368A and 407V mutations;

c) comprises mutations capable of altering charge properties of amino acids at an interaction interface of the CH3 region to promote heterodimer formation, e.g., the mutation sites in the two CH3 chains are each independently selected at least one from the group consisting of Y391, K392, T393, T394, P395, P396, V397, L398, D399, S400, Q347, V348, Y349, T350, S354, R355, E356, E357, F405, Y407 and K409; and/or

the TRAC and/or TRBC comprises at least one cysteine mutation relative to a wild-type sequence, to form a disulfide bond between TRAC and TRBC, and the cysteine mutation is at position 48 of the wild-type TCR α chain constant region and position 57 of the wild-type TCR β chain constant region.

6. The multi-specific polypeptide molecule according to any one of claims 1-5, wherein the multi-specific polypeptide molecule further comprises a constant domain connected to the C-terminus of TRAV1 and/or TRBV1 via an optional linker, the constant domain is selected from the group consisting of the following (1)-(3):

(1) a TCR constant region or a fragment thereof, wherein the TCR constant region, e.g., comprises a TCR α chain constant region (TRAC) and/or a β chain constant region (TRBC);

preferably, the TRAC and/or TRBC comprises at least one cysteine mutation relative to a wild-type sequence, to form a disulfide bond between TRAC and TRBC, more preferably, the cysteine mutation is at position 48 of the wild-type TCR α chain constant region and position 57 of the wild-type TCR β chain constant region;

(2) CH3, wherein the CH3 is derived from an Fc domain of an antibody, preferably the CH3 in any one polypeptide chain or in both polypeptide chains comprises at least one mutation capable of promoting formation of a heterodimer of the polypeptide molecule;

preferably, each CH3 independently comprises a mutation at one or more positions selected from the group consisting of amino acid positions 366, 368, 405 and 407 and the two CH3 do not comprise the same mutation, more preferably, one of the CH3 comprises a T366W mutation and the other comprises T366S, L368A and Y407V mutations;

(3) CL and CH1, wherein the CL is derived from an antibody light chain CL domain, and the CH1 is derived from an antibody heavy chain CH1 domain;

preferably, wherein the multi-specific polypeptide molecule comprises TRAC and TRBC that are respectively connected to the C-terminus of TRAV1 and TRBV1 via an optional linker;

and/or

wherein the multi-specific polypeptide molecule further comprises CH1 and CL derived from an antibody that are respectively connected to the VH1 and VL1, wherein the VH1, VL1, CH1 and CL form a single-chain Fab and are connected to the C-terminus of the TRAV1 or the TRBV1 via a linker.

7. The multi-specific polypeptide molecule according to any one of claims 1-2 and 4-6, wherein the polypeptide chains of the polypeptide molecule comprise, from the N-terminus to the C-terminus, respectively:

a first polypeptide chain: TRBV1- optional linker -TRBC- linker -VL1- linker -VH1- optional linker - hinge region -CH2-CH3, and a second polypeptide chain: TRAV1- optional linker -TRAC- optional linker - hinge region -CH2-CH3; or

a first polypeptide chain: TRAV1- optional linker -TRAC- linker -VL1- linker -VH1- optional linker - hinge region -CH2-CH3, and a second polypeptide chain: TRBV1- optional linker -TRBC- optional linker - hinge region -CH2-CH3; or

a first polypeptide chain: TRBV1- optional linker -TRBC- linker -VH1- linker -VL1- optional linker - hinge region -CH2-CH3, and a second polypeptide chain: TRAV1- optional linker -TRAC- optional linker - hinge region -CH2-CH3; or

a first polypeptide chain: TRAV1- optional linker -TRBC- linker -VH1- linker -VL1- optional linker - hinge region -CH2-CH3, and a second polypeptide chain: TRBV1- optional linker -TRBC- optional linker - hinge region -CH2-CH3; or

a first polypeptide chain: TRBV1- optional linker -TRBC- linker -VL1- linker -VH1- linker -CH2-CH3,

and a second polypeptide chain: TRAV1- optional linker -TRAC- linker -CH2-CH3; or

a first polypeptide chain: TRBV1- optional linker -TRBC- linker -VL1- linker -VH1- optional linker -hinge region -CH3, and a second polypeptide chain: TRAV1- optional linker -TRAC- optional linker -hinge region -CH3; or

a first polypeptide chain: TRBV1- optional linker -TRBC- linker -VL1- linker -VH1- optional linker -hinge region -TRBC, and a second polypeptide chain: TRAV1- optional linker -TRAC- optional linker -hinge region -TRAC; or

a first polypeptide chain: TRBV1- optional linker -TRBC- linker -VL1- linker -VH1- optional linker -hinge region, and a second polypeptide chain: TRAV1- optional linker -TRAC- optional linker -hinge region -Alb; or

a first polypeptide chain: TRBV1- optional linker -TRBC- linker -VL1- linker -VH1- linker -Alb, and a second polypeptide chain: TRAV1- optional linker -TRAC; or

a first polypeptide chain: TRAV1- optional linker -CH3- linker -VL1- linker -VH1- optional linker - optional hinge region -CH2-CH3, and a second polypeptide chain: TRBV1- optional linker -CH3- optional linker - optional hinge region -CH2-CH3; or

a first polypeptide chain: TRAV1- optional linker -CH1- linker -VL1- linker -VH1- optional linker - optional hinge region -CH2-CH3, and a second polypeptide chain: TRBV1- optional linker -CL- optional linker - optional hinge region -CH2-CH3; or

a first polypeptide chain: TRBV1- optional linker -TRBC- linker -VHH1- optional linker -hinge region -CH2-CH3, and a second polypeptide chain: TRAV1- optional linker -TRAC- optional linker -hinge region -CH2-CH3; or

the second binding region comprises an antibody-derived Fab, and wherein a first polypeptide chain: TRBV1- optional linker -TRBC- linker -VL1-CL- linker -VH1-CH1- optional linker -hinge region -CH2-CH3, and a second polypeptide chain: TRAV1- optional linker -TRAC- optional linker -hinge region -CH2-CH3; or

a first polypeptide chain: TRBV1- optional linker -TRBC- linker -VL1, a second polypeptide chain: VH1- optional linker -hinge region -CH2-CH3, and a third polypeptide chain: TRAV1- optional linker -TRAC- optional linker -hinge region -CH2-CH3; or

a first polypeptide chain: TRBV1- optional linker -TRBC- linker -VH1, a second polypeptide chain: VL1- optional linker -hinge region -CH2-CH3, and a third polypeptide chain: TRAV1- optional linker -TRAC- optional linker -hinge region -CH2-CH3; or

a first polypeptide chain: TRAV1- optional linker -TRAC- linker -VL1, a second polypeptide chain: VH1- optional linker -hinge region -CH2-CH3, and a third polypeptide chain: TRBV1- optional linker -TRBC- optional linker -hinge region -CH2-CH3; or

a first polypeptide chain: TRAV1- optional linker -TRAC- linker -VH1, a second polypeptide chain: VL1- optional linker -hinge region -CH2-CH3, and a third polypeptide chain: TRBV1- optional linker -TRBC- optional linker -hinge region -CH2-CH3; or

a first polypeptide chain: TRBV1- optional linker -TRBC- linker -VL1- optional linker -hinge region -CH2-CH3, a second polypeptide chain: VH1- optional linker -hinge region -CH2-CH3, and a third polypeptide chain: TRAV1- optional linker -TRAC; or

a first polypeptide chain: TRBV1- optional linker -TRBC- linker -VH1- optional linker -hinge region -CH2-CH3, a second polypeptide chain: VL1- optional linker -hinge region -CH2-CH3, and a third polypeptide chain:TRAV1- optional linker -TRAC; or

a first polypeptide chain: TRAV1- optional linker -TRAC- linker -VL1- optional linker -hinge region -CH2-CH3, a second polypeptide chain: VH1- optional linker -hinge region -CH2-CH3, and a third polypeptide chain: TRBV1- optional linker -TRBC; or

a first polypeptide chain: TRAV1- optional linker -TRAC- linker -VH1- optional linker -hinge region -CH2-CH3, a second polypeptide chain: VL1- optional linker -hinge region -CH2-CH3, and a third polypeptide chain: TRBV1- optional linker -TRBC.

8. The multi-specific polypeptide molecule according to any one of claims 3-6, wherein the polypeptide molecule comprises a first polypeptide chain and a second polypeptide chain; wherein:

the first polypeptide chain comprises, from the N-terminus to the C-terminus: TRBV1- optional linker -TRBC- linker -VL1- linker -TRAV2- linker -TRBV2- linker -VH1- optional linker -hinge region -CH2-CH3, and the second polypeptide chain comprises, from N-terminus to C-terminus: TRAV1- optional linker -TRAC- optional linker -hinge region -CH2-CH3; or

the first polypeptide chain comprises, from the N-terminus to the C-terminus: VL2- linker -VH2- linker -TRBV1- optional linker -TRBC- linker -VL1- linker -VH1- optional linker -hinge region -CH2-CH3, and the second polypeptide chain comprises, from the N-terminus to the C-terminus: TRAV1- optional linker -TRAC- optional linker -hinge region -CH2-CH3; or

the first polypeptide chain comprises, from the N-terminus to the C-terminus: VL2- linker -TRBV1- optional linker -TRBC- linker -VL1- linker -VH1- optional linker -hinge region -CH2-CH3, and the second polypeptide chain comprises, from the N-terminus to the C-terminus: VH2- linker -TRAV1- optional linker -TRAC- optional linker -hinge region -CH2-CH3; or

the first polypeptide chain comprises, from the N-terminus to the C-terminus: VL2- linker -TRBV1- optional linker -TRBC- linker -VL1- linker -VH1- optional linker -hinge region -CH2-CH3, and the second polypeptide chain comprises, from the N-terminus to the C-terminus: VH2- linker -TRAV1- optional linker -TRAC- optional linker -hinge region -CH2-CH3; or

the first polypeptide chain comprises, from the N-terminus to the C-terminus: TRBV1- optional linker -TRBC- linker -scFv1- optional linker -hinge region -CH2-CH3, and the second polypeptide chain comprises, from the N-terminus to the C-terminus: scFv2- linker -TRAV1- optional linker -TRAC- optional linker -hinge region -CH2-CH3; or

the first polypeptide chain comprises, from the N-terminus to the C-terminus: VL2- linker -VH2- linker -TRBV1- optional linker -TRBC- linker -VL1- linker -TRAV2- linker -TRBV2- linker -VH1- optional linker -hinge region -CH2-CH3, and the second polypeptide chain comprises, from the N-terminus to the C-terminus: TRAV1- optional linker -TRAC- optional linker -hinge region -CH2-CH3.

9. The multi-specific polypeptide molecule according to any one of claims 1-8, wherein

the VH1 and VL1 comprise at least one cysteine mutation to form a disulfide bond between VH1 and VL1, preferably, the cysteine mutations are at position 44 of VH1 and position 100 of VL1; and/or

the VH2 and VL2 comprise at least one cysteine mutation to form a disulfide bond between VH2 and VL2, preferably, the cysteine mutations are at position 44 of VH2 and position 100 of VL2; and/or

the TRAV1 and VL1 or VL2 comprise at least one cysteine mutation to form a disulfide bond between TRAV1 and VL1 or VL2, preferably, the cysteine mutations are at position 104 of TRAV1, and position 80 of VL1 or VL2; and/or

the TRAV2 and VL1 or VL2 comprise at least one cysteine mutation to form a disulfide bond between TRAV2 and VL1 or VL2, preferably, the cysteine mutations are at position 104 of TRAV2, and position 80 of VL1 or VL2; and/or

the TRBV1 and VL1 or VL2 comprise at least one cysteine mutation to form a disulfide bond between TRBV1 and VL1 or VL2, preferably, the cysteine mutations are at position 84 of TRBV1, and position 80 of VL1 or VL2; and/or

the TRBV2 and VL1 or VL2 comprise at least one cysteine mutation to form a disulfide bond between TRBV2 and VL1 or VL2, preferably, the cysteine mutations are at position 84 of TRBV2, and position 80 of VL1 or VL2; and/or

the TRAV1 and VH1 or VH2 comprise at least one cysteine mutation to form a disulfide bond between TRAV1 and VH1 or VH2, preferably, the cysteine mutations are at position 104 of TRAV1, and position 89 of VH1 or VH2; and/or

the TRAV2 and VH1 or VH2 comprise at least one cysteine mutation to form a disulfide bond between TRAV2 and VH1 or VH2, preferably, the cysteine mutations are at position 104 of TRAV2, and position 89 of VH1 or VH2; and/or

wherein the linkers in the polypeptide molecule are each independently selected from linkers consisting of 1-35 amino acids; preferably, the linkers are each independently selected from the group consisting of S, GS, GGGS, GGGGS, GGGSGGGG, GGSGGS, GGSGGSGGS, GGGGSGGGGS, GGGGSGGGGSGGGGS, GGGGSGGGGSGGGGSGGGGSGGGGS, GGGGSGGGGSGGGGSGGGGSGGGGSGGGGS, GQPKAAP, TVLRT, TVSSAS, GGEGG, GSEGGGS, RTSGPGDGGKGGPGKGPGGEGTKGTGPGG, GKGPGGEGTKGTGPGG, TVLSSAS, EDLKN or a variant thereof, EDLNK or a variant thereof, and ANIQK or a variant thereof, or any combination thereof; preferably, a variant of EDLKN is an amino acid sequence formed by substitution, deletion, or addition of one or several amino acids in EDLKN; and/or a variant of EDLNK is an amino acid sequence formed by substitution, deletion, or addition of one or several amino acids in EDLNK; and/or a variant of ANIQK is an amino acid sequence formed by substitution, deletion, or addition of one or several amino acids in ANIQK; and/or

wherein the polypeptide molecule further comprises a signal peptide sequence at the N-terminus of one or more polypeptide chains, preferably comprises a signal peptide sequence at the N-terminus of each polypeptide chain, e.g. a signal peptide sequence derived from albumin or an immunoglobulin, preferably as shown in SEQ ID NO: 164;

and/or

wherein the first antigen and the third antigen are each independently a TAA selected from the group consisting of the following antigens: melanoma-associated antigens (e.g., gp100, MAGEA1, MAGEA3, MAGEA6, MAGEA4, MAGEA2, MAGEA12, MAGEA2B, MAGEA9B, MAGEA10, MAGEA11, MAGEB2, MAGEC1, MAGEC2), IGF2BP1, GNGT1, PI4K2B, CCR8, NPSR1, COX7B2, ONECUT3, SMC1B, FOXI3, GAGE2A, FBXO43, BRDT, PAGE2, GAGE13, POU5F1B, CTAG1A, and endogenous reverse transcriptase antigen; or

wherein the first antigen and the third antigen are each independently a TSA selected from the group consisting of the following antigens: KRAS antigens (e.g., a KRAS G12 mutant antigens), TP53, PIK3CA, CTNNB1, EGFR, BRAF, and GNAS; or

wherein the first antigen and the third antigen are each independently a viral antigen selected from the group consisting of the following antigens: HPV antigens (e.g., an HPV E6 or E7 antigen), CMV antigens, HBV antigens, EBV antigens, herpesvirus antigens, human immunodeficiency virus (HIV) antigens, influenza virus antigens, and coronavirus antigens; or

the first antigen and the third antigen are each independently an self-antigen selected from the group consisting of the following antigens: AFP, CEA, CD19, CD20, BCMA, CD22, CD30, SLAM, CLDN18.2, GD2, mesothelin, CD38, Her2, GPC3, MUC1, Ro52, Ro60, La, Jo-1, SRP, IFIH1, CENPA, CENPB, SNRPA1, SNRNP70, SNR-PD3, RNAP3, TOPO1, Insulin, GAD65, IA2, Znt8, PL7, TARS, ARS, MI2, topoisomerase 1, EXOSC9, EXOSC107, POLR3A, POLR3K, PTRN, GAD2, SLC30A8, AchR, MUSK, LRP4, PLA2R, THSD7A, TSHR, IFN-γ, CHRNA1, MUSK, LRP4, AQP4, MOG, GRIN1, COL4A3, PLA2R, GM-SCF, PR3 and MPO;

and/or

wherein the second antigen and the fourth antigen are each independently selected from the group consisting of CD3 (e.g., CD3γ, CD3δ and CD3ε chains), CD4, CD8, CD10, CD11b, CD11c, CD14, CD16, CD18, CD25, CD32a, CD32b, CD41, CD41b, CD42a, CD42b, CD44, CD45RA, CD49, CD61, CD64, CD68, CD94, CD90, CD117, Nkp46, NKG2D, FcεRI, TCRα/β, TCR γ/δ, HLA-DR, CD28, 4-1BB(CD137), OX40(CD134), ICOS(CD278), 2B4 (CD244), HVEM, LAG3, DAP10, DAP12, CD27, CD40, GITR, LFA-1, MyD88, CD2, CD7, LIGHT, B7-H3, CTLA-4, PD-1, PD-L1, CD80, BTLA, TIM3, TIGIT and LAG-3;

preferably, wherein the first antigen and the third antigen are each independently selected from the group consisting of melanoma-associated antigens (e.g., gp100, MAGEA1), KRAS antigens (e.g., a KRAS G12 mutant antigen), and HPV antigens (e.g., an HPV E7 antigen), and/or the second antigen and the fourth antigen are each independently selected from the group consisting of CD3, CD28, 4-1BB(CD137), PD-1, and PD-L1.

10. The multi-specific polypeptide molecule according to any one of claims 1-9, wherein

TRAV1 and TRBV1 is sected from one of (1) to (10): (1) SEQ ID NOs:91 and 92, (2) SEQ ID NOs:93 and 94, (3) SEQ ID NOs:41 and 42, (4) SEQ ID NOs:107 and 108, (5) SEQ ID NOs:49 and 50, (6) SEQ ID NOs:57 and 58, (7) SEQ ID NOs:65 and 66, (8) SEQ ID NOs:73 and 74, (9) SEQ ID NOs:81 and 82, (10) SEQ ID NOs:89 and 90; and/or

TRAV2 and TRBV2 is sected from one of (1) to (10): (1) SEQ ID NOs:91 and 92, (2) SEQ ID NOs:93 and 94, (3) SEQ ID NOs:41 and 42, (4) SEQ ID NOs:107 and 108, (5) SEQ ID NOs:49 and 50, (6) SEQ ID NOs:57 and 58, (7) SEQ ID NOs:65 and 66, (8) SEQ ID NOs:73 and 74, (9) SEQ ID NOs:81 and 82, (10) SEQ ID NOs:89 and 90; and/or

VH1 and VL1 is selected from one of (1) to (6): (1) SEQ ID NOs: 95 and 101, (2) SEQ ID NOs: 96 and 102, (3) SEQ ID NOs: 97 and 103, (4) SEQ ID NOs: 98 and 104, (5) SEQ ID NOs: 99 and 105, (6) SEQ ID NOs: 100 and 106; and/or

VH2 and VL2 is selected from one of (1) to (6): (1) SEQ ID NOs: 95 and 101, (2) SEQ ID NOs: 96 and

102, (3) SEQ ID NOs: 97 and 103, (4) SEQ ID NOs: 98 and 104, (5) SEQ ID NOs: 99 and 105, (6) SEQ ID NOs: 100 and 106; and/or

TRAC is selected from SEQ ID NO: 8, 9 and 10; and/or

TRBC is selected from SEQ ID NO: 11, 12, 13 and 14; and/or

the two Hinge region-CH2-CH3 in two polypeptide chains are selected from one of (1) to (6): (1) SEQ ID NOs: 109 and 110, (2) SEQ ID NOs: 111 and 112, (3) SEQ ID NOs: 113 and 114, (4) SEQ ID NOs: 115 and 116, (5) SEQ ID NOs: 117 and 118, (6) SEQ ID NO: 119 and 120; and/or

CL is selected from SEQ ID NO: 15 and 16; and/or

CH1 is selected from SEQ ID NO: 17 and 18; and/or

Alb has the amino acid sequence as showin in SEQ ID NO: 2; and/or

the hinge region comprises the amino acid sequence as showin in SEQ ID NO: 4; and/or

the CH2 domain comprises the amino acid sequence as showin in SEQ ID NO: 5 or 161; and/or

the CH3 domain in one chain of the polypeptide molecule comprises the amino acid sequence as shown in SEQ ID NO: 6, and the CH3 domain in the other chain comprises the amino acid sequence as shown in SEQ ID NO: 7.

11. A nucleic acid, comprising a nucleotide sequence encoding each chain of the multi-specific polypeptide molecule according to any one of claims 1-10.

12. A vector, comprising the nucleic acid according to claim 11.

13. A pharmaceutical composition, comprising the multi-specific polypeptide molecule according to any one of claims 1-10, the nucleic acid according to claim 11, or the vector according to claim 12, and a pharmaceutically acceptable excipient; preferably wherein the pharmaceutical composition further comprises a second therapeutic agent, preferably, the second therapeutic agent is selected from the group consisting of an antibody, a chemotherapeutic agent, and a small-molecule drug.

14. The multi-specific polypeptide molecule according to any one of claims 1-10, the nucleic acid according to claim 11, the vector according to claim 12, or the pharmaceutical composition according to claim 13 for use in preventing or treating a disease in a subject; wherein the disease is selected from the group consisting of cancer, an infectious disease, an autoimmune disease, and an inflammatory disease; preferably, the method further comprises administering a second therapeutic agent to the subject, preferably, the second therapeutic agent is selected from the group consisting of an antibody, a chemotherapeutic agent, and a small-molecule drug.

15. The multi-specific polypeptide molecule according to any one of claims 1-10 or a conjugate comprising the multi-specific polypeptide molecule for use in a method for detecting or diagnosing a disease in a subject, wherein the method comprises (i) contacting a sample obtained from the subject with the multi-specific polypeptide molecule according to any one of claims 1-10 or a conjugate comprising the multi-specific polypeptide molecule; and (ii) detecting the presence of a target antigen in the sample, wherein the presence of the target antigen indicates that the subject has the corresponding disease.