

ANTI-CD3 ANTIBODY AND USE THEREOF

FIELD

[0001] The present disclosure relates to the field of biomedicine, and more particularly, to an anti-CD3 antibody or antigen binding fragment and use thereof.

BACKGROUND

[0002] Cancer is a major disease that affects human survival and development. According to the latest data, there are about 19 million new cancer cases and around 10 million cancer-related deaths worldwide each year, with the incidence and mortality rates showing an upward trend. In addition to surgical resection, traditional cancer treatment methods such as chemotherapy and radiotherapy have serious side effects and are prone to recurrence. In recent years, immunotherapy, including tumor-targeted antibodies, immune checkpoint antibodies, and bispecific antibodies, etc., has become a new hotspot and new hope in cancer treatment. Immunotherapy medicaments, including monoclonal antibodies and bispecific antibodies, mainly achieve anti-cancer effects by promoting the function of autoimmune cells. They have the characteristics of mild side effects and long survival for benefited patients. The immune checkpoint antibodies that have been approved for marketing include PD1-1/L1, CTLA-4, and LAG-3, while the bispecific anti-cancer antibodies include Blinatumomab (CD3×CD19), Kimtrak (CD3×GP100), Mosunetuzumab (CD3×CD20), Tecvayli (CD3×BCMA), etc. The bispecific antibodies have made great progress in the treatment of hematological malignancies. They target CD19, CD20, and BCMA, and can promote T cells to eliminate tumor cells, bringing benefits to patients.

[0003] CD3-based bispecific antibodies (hereinafter referred to as "CD3 bispecific antibodies") can promote T cell activation, and kill tumors by recruiting T cells to reach the tumor site, and bridging T cells with tumors. This type of bispecific antibody does not require neoantigens and can guide T cells to kill "cold tumors". After binding to T cells and tumor cells,

the CD3 bispecific antibodies trigger strong activation signals. Therefore, the CD3 bispecific antibodies can "ignore" the inhibitory signals of immune checkpoint molecules to a certain extent. However, the CD3 bispecific antibodies can also promote the production of a large number of pro-inflammatory cytokines, such as TNF α , and IL-6, triggering a strong cytokine storm and excessive immune response, which cause damage to the body, and in severe cases, may be life-threatening. Therefore, the CD3-based bispecific antibodies have a good clinical application prospect, but their safety needs to be further improved.

[0004] One way to address the safety issues of the CD3 bispecific antibodies is to increase the affinity of the CD3 bispecific antibodies for binding to tumor targets while reducing their affinity for CD3. This enables the CD3 bispecific antibody drugs to be preferentially accumulated at the tumor site, increasing local drug concentrations at the tumor site, reducing peripheral drug concentration, reducing off-target toxicity, lowering the production level of pro-inflammatory cytokines, and reducing on-target toxicity. Therefore, there is an urgent need to develop anti-CD3 antibodies that can bind to human CD3 protein with low affinity, so as to obtain CD3 antibody drugs with better safety and high value in clinical application and drug development.

SUMMARY

[0005] The present disclosure aims to solve at least one of the technical problems existing in the prior art at least to some extent. To this end, one object of the present disclosure is to provide an anti-CD3 antibody or antigen binding fragment thereof. Another object of the present disclosure is to provide a multi-specific antibody constructed using the anti-CD3 antibody or the antigen binding fragment thereof. The anti-CD3 antibody or antigen binding fragment thereof of the present disclosure can bind to human CD3 protein and reduce the production of pro-inflammatory cytokines. The bispecific antibody constructed using the anti-CD3 antibody or antigen binding fragment thereof has high PBMC-mediated tumor-killing activity and weak ability of promoting the secretion of pro-inflammatory cytokines. Therefore, the pharmaceutical composition prepared using the anti-CD3 antibody or antigen binding fragment thereof or multi-specific antibody thereof has better safety and high value in clinical

application and drug development.

[0006] The present disclosure is made based on the inventor's discoveries and understandings of the following facts and problems.

[0007] Currently, the most commonly used CD3 antibodies among bispecific antibodies are TR66 and UCHT1. On the one hand, neither of these two antibodies binds to monkey CD3, which increases the difficulty of non-clinical evaluation of the medicament; on the other hand, these two antibodies bind very strongly to T cells, which increases the safety risk of CD3 bispecific antibody drugs constructed based thereon. The inventors performed affinity engineering on Cross3, an anti-CD3 antibody with high affinity for CD3 (KD=0.165nM) and cross-reactivity to humans and monkeys. Through extensive screening, a series of anti-CD3 antibodies that can bind to human CD3 protein with low affinity have been obtained. Furthermore, based on this series of anti-CD3 antibodies, CD3 bispecific antibodies have been constructed, and 19 anti-CD3 antibodies have been screened and obtained. Compared with Cross3, the specific recognition and binding of bispecific antibodies to tumor antigens are not affected by the CD3 antigen binding region. Further experimental results demonstrated that: 1) the CD3 bispecific antibodies of the present disclosure bind to CD3E&D proteins and specifically bind to tumor antigens (such as PDL1 and Trop2), and their tumor antigen binding activity is substantially equivalent to that of a bispecific antibody constructed based on Cross3; 2) cellular level experimental results showed that the CD3 bispecific antibodies of the present disclosure can bind to T cells and specifically bind to tumor cells (such as A-375-Trop2 cells, A-375 melanoma cells, HCT-15 colorectal cancer cells, and A549 lung cancer cells), and its tumor cell binding ability is substantially equivalent to that of a bispecific antibody constructed based on Cross3 antibody; 3) experimental results of promoting the secretion of cytokines by PBMCs showed that the CD3 bispecific antibodies of the present disclosure has reduced ability of promoting the secretion of pro-inflammatory cytokines.

[0008] The inventors further verified the tumor cell killing activity of the above-described CD3 bispecific antibodies through PBMCs tumor cell killing assays. The results showed that the bispecific antibodies (such as Cross313×Trop2, Cross316×Trop2, Cross325×Trop2, Cross313×PDL1, Cross316×PDL1, and Cross325×PDL1) constructed based on the anti-CD3 antibodies Cross313, Cross316, and Cross325 of the present disclosure can achieve the highest

killing effect or can promote 100% tumor cell killing by PBMCs, and have comparable tumor cell killing effects to the bispecific antibodies (such as Cross3×Trop2 and Cross3×PDL1) constructed based on the anti-CD3 antibody Cross3.

[0009] The above experimental results showed that the CD3 bispecific antibody developed based on the anti-CD3 antibody or antigen binding fragment thereof of the present disclosure has high PBMC-mediated tumor-killing activity and weak ability of promoting the secretion of pro-inflammatory cytokines, better safety, and high value in clinical application and drug development.

[0010] Therefore, in a first aspect of the present disclosure, the present disclosure provides an anti-CD3 antibody or antigen binding fragment thereof. According to an embodiment of the present disclosure, the anti-CD3 antibody or antigen binding fragment thereof includes heavy chain variable region CDRs and light chain variable region CDRs, the heavy chain variable region CDRs have amino acid sequences as set forth in any one of SEQ ID NOs: 1 to 3, 7 to 8, and 74 to 86, or a conservative modification form thereof; and/or the light chain variable region CDRs have amino acid sequences as set forth in any one of SEQ ID NOs: 4 to 6, 9, and 87 to 92, or a conservative modification form thereof.

[0011] The anti-CD3 antibody or antigen binding fragment thereof according to the embodiments of the present disclosure can bind to human CD3 protein and reduce the production of pro-inflammatory cytokines. The CD3 bispecific antibody developed based on this CD3 antibody or the antigen binding fragment thereof has high PBMC-mediated tumor-killing activity and weak ability of promoting the secretion of pro-inflammatory cytokines. After being prepared into a medicament, on the one hand, the anti-CD3 antibody or antigen binding fragment thereof can be preferentially accumulated at the tumor site, increasing the local drug concentration at the tumor site and reducing the peripheral drug concentration, thereby reducing the risk of off-target toxicity. On the other hand, this bispecific antibody medicament can reduce the production of pro-inflammatory cytokines and reduce the risk of on-target toxicity. Furthermore, the drugs prepared using the anti-CD3 antibody or antigen binding fragment thereof can be used to prevent and/or treat a CD3-related disease. CD3 bispecific antibody drugs developed based on the anti-CD3 antibody or antigen binding fragment thereof are safer and can be used to prevent and/or treat a cancer or tumor or infection

or autoimmune disease. Therefore, the anti-CD3 antibody or antigen binding fragment thereof of the present disclosure has good value in clinical application and drug development.

[0012] In a second aspect, the present disclosure provides a multi-specific antibody. According to an embodiment of the present disclosure, the multi-specific antibody includes a first antigen binding region and a second antigen binding region. The first antigen binding region includes the anti-CD3 antibody or antigen binding fragment thereof according to the first aspect of the present disclosure; and the second antigen binding region has a biomolecule binding activity, the biomolecule not being CD3.

[0013] As described above, the multi-specific antibody (such as bispecific antibody) developed based on the anti-CD3 antibody or antigen binding fragment thereof according to the first aspect of the present disclosure can be preferentially accumulated at the tumor site after being prepared into medicaments, increasing the local drug concentration at the tumor site and reducing the peripheral drug concentration, thereby reducing the risk of off-target toxicity. At the same time, the production of pro-inflammatory cytokines can be reduced, and the risk of on-target toxicity of multi-specific antibodies (such as bispecific antibodies) can be reduced. Therefore, the multi-specific antibody based on the anti-CD3 antibody or antigen binding fragment thereof of the first aspect of the present disclosure has better safety and high value in clinical application and drug development.

[0014] In a third aspect of the present disclosure, the present disclosure provides a conjugate. According to an embodiment of the present disclosure, the conjugate includes: the antibody or antigen binding fragment thereof according to the first aspect of the present disclosure or the multi-specific antibody according to the second aspect of the present disclosure.

[0015] As described above, the anti-CD3 antibody or antigen binding fragment thereof according to the embodiments of the present disclosure can be used to prepare bispecific antibody drugs. On the one hand, the prepared bispecific antibody drugs can be preferentially accumulated at the tumor site, increasing the local drug concentration at the tumor site and reducing the peripheral drug concentration, thereby reducing the risk of off-target toxicity. On the other hand, the bispecific antibody can reduce the production of pro-inflammatory cytokines, reducing the risk of on-target toxicity of multi-specific antibodies (such as bispecific antibodies).

Furthermore, the drugs prepared using the above-described anti-CD3 antibody or antigen

binding fragment thereof can be used to prevent and/or treat a CD3-related disease. The CD3 bispecific antibody drugs developed based on the anti-CD3 antibody or antigen binding fragment thereof are safer and can be used to prevent and/or treat a cancer or tumor or infection or autoimmune disease. Therefore, the conjugate including the anti-CD3 antibody or antigen binding fragment thereof according to the first aspect of the present disclosure and the multi-specific antibody according to the second aspect of the present disclosure has good clinical application value and drug development value.

[0016] In a fourth aspect, the present disclosure provides a nucleic acid. According to an embodiment of the present disclosure, the nucleic acid encodes the anti-CD3 antibody or antigen binding fragment thereof according to the first aspect of the present disclosure or the multi-specific antibody according to the second aspect of the present disclosure. According to an embodiment of the present disclosure, the antibody or antigen binding fragment thereof of the first aspect of the present disclosure or the multi-specific antibody of the second aspect of the present disclosure encoded by the nucleic acid is suitable for developing monoclonal antibody or bispecific antibody drugs with higher safety. Furthermore, the protein encoded by the nucleic acid can be used to prevent and/or treat a CD3-related disease or prevent and/or treat a cancer or tumor or infection or autoimmune disease.

[0017] In a fifth aspect of the present disclosure, the present disclosure provides a vector or transformant. According to an embodiment of the present disclosure, the vector or transformant contains the nucleic acid according to the fourth aspect of the present disclosure. Thus, with the constructed vector or transformant, it is feasible to effectively express the antibody or antigen binding fragment thereof of the first aspect of the present disclosure or the multi-specific antibody of the second aspect of the present disclosure, and to obtain the conjugate of the third aspect of the present disclosure.

[0018] In a sixth aspect, the present disclosure provides a cell. According to an embodiment of the present disclosure, the cell carries the nucleic acid according to the fourth aspect of the present disclosure or the vector or transformant according to the fifth aspect of the present disclosure, or expresses the antibody or antigen binding fragment thereof according to the first aspect of the present disclosure or the multi-specific antibody of the second aspect of the present disclosure. According to an embodiment of the present disclosure, the cells can efficiently

express the antibody or antigen binding fragment thereof of the first aspect of the present disclosure or the multi-specific antibody of the second aspect of the present disclosure under suitable conditions. Furthermore, an antibody or antigen binding fragment thereof or multi-specific antibody thereof with improved safety that can bind to human CD3 protein can be
5 obtained.

[0019] In a seventh aspect of the present disclosure, the present disclosure provides a pharmaceutical composition. According to an embodiment of the present disclosure, the pharmaceutical composition includes: the antibody or antigen binding fragment thereof of the first aspect of the present disclosure, the multi-specific antibody of the second aspect of the
10 present disclosure, the conjugate of the third aspect of the present disclosure, the nucleic acid of the fourth aspect of the present disclosure, or the vector or transformant of the fifth aspect of the present disclosure. Thus, the obtained medicament can be further used for preventing and/or treating a CD3-related disease, or further used for preventing and/or treating a CD3-related disease and/or cancer or tumor or infection or autoimmune disease.

[0020] In an eighth aspect of the present disclosure, the present disclosure provides a kit. According to an embodiment of the present disclosure, the kit includes the antibody or antigen binding fragment thereof according to the first aspect of the present disclosure or the multi-specific antibody according to the second aspect of the present disclosure. Thus, the obtained
15 kit can be used for a CD3-related research.

[0021] In a ninth aspect of the present disclosure, the present disclosure provides a use of the antibody or antigen binding fragment thereof according to the first aspect of the present disclosure or the multi-specific antibody according to the second aspect of the present disclosure in the manufacture of a kit for detecting CD3. Those skilled in the art will appreciate that the above-described features and advantages for the antibody or antigen binding fragment
20 thereof are also applicable to this use and will not be reiterated here.

[0022] In a tenth aspect of the present disclosure, the present disclosure provides a use of the antibody or antigen binding fragment thereof of the first aspect of the present disclosure, the multi-specific antibody of the second aspect of the present disclosure, the conjugate of the third aspect of the present disclosure, the nucleic acid of the fourth aspect of the present
25 disclosure, the vector or transformant of the fifth aspect of the present disclosure, or the
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pharmaceutical composition of the seventh aspect of the present disclosure in the manufacture of a medicament for preventing and/or treating a CD3-related disease and/or cancer or tumor or infection or autoimmune disease. The antibody or antigen binding fragment thereof, the multi-specific antibody, the conjugate, the nucleic acid, the vector or transformant, or the pharmaceutical composition of the present disclosure can be further prepared into medicaments, which can be used clinically to prevent or treat a disease.

[0023] Those skilled in the art will appreciate that the features and advantages described above for the antibody or antigen binding fragment thereof, the conjugate, the nucleic acid, the vector or transformant, or the pharmaceutical composition are also applicable to this use and will not be reiterated here.

Beneficial effects:

[0024] (1) The anti-CD3 antibodies Cross303, Cross307, Cross308, Cross309, Cross310, Cross311, Cross312, Cross313, Cross314, Cross316, Cross317, Cross318, Cross323, Cross325, Cross326, Cross3ZH07, Cross3ZH09, Cross3ZH16, and Cross3ZH23 obtained in the present disclosure can reduce the production of pro-inflammatory cytokines caused by the CD3 antigen binding moiety compared with the parental antibody Cross3.

[0025] (2) The bispecific antibody constructed from the antibodies Cross313, Cross316, and Cross325 obtained in the present disclosure have weaker T cell binding ability compared with the bispecific antibody constructed from the parental antibody Cross3.

[0026] (3) The bispecific antibody constructed from the antibodies Cross313, Cross316, and Cross325 obtained in the present disclosure can also promote PBMC to kill tumor cells to the greatest extent and has weaker performance in promoting the production of pro-inflammatory cytokines and better safety compared with the bispecific antibody constructed from the parental antibody Cross3,

[0027] Additional aspects and advantages of the present disclosure will be provided at least in part in the following description, or will become apparent at least in part from the following description, or can be learned from practicing of the present disclosure.

BRIEF DESCRIPTION OF THE DRAWINGS

[0028] The above and/or additional aspects and advantages of the present disclosure will become apparent and easily understandable from the following description of embodiments in conjunction with the accompanying drawings, in which:

5 **[0029]** FIG. 1 is a comparison of amino acid mutations between an affinity-reduced antibody and a parental antibody Cross3 according to an embodiment of the present disclosure.

[0030] FIG. 2 is a graph showing the SPR results of affinity assay between a parental antibody Cross3 as well as an affinity-reduced antibody and CD3E&D protein according to an embodiment of the present disclosure.

10 **[0031]** FIG. 3 is a graph showing the ELISA results of the binding of a parental antibody Cross3 as well as an affinity-reduced antibody to CD3E&D protein according to an embodiment of the present disclosure.

[0032] FIG. 4 is a graph showing the flow cytometry results of the binding of a parental antibody Cross3 as well as an affinity-reduced antibody to CD8 T cells according to an
15 embodiment of the present disclosure.

[0033] FIG. 5 is a schematic diagram of the formats of CD3×Trop2 and CD3×PDL1 bispecific antibody constructed from a parental antibody Cross3 and an affinity-reduced antibody according to an embodiment of the present disclosure;

[0034] FIG. 6 is a graph showing the ELISA results of the binding of a CD3×Trop2 bispecific antibody constructed from a parental antibody Cross3 and an affinity-reduced
20 antibody to CD3E&D protein according to an embodiment of the present disclosure.

[0035] FIG. 7 is a graph showing the ELISA results of the binding of a CD3×Trop2 bispecific antibody constructed from a parental antibody Cross3 and an affinity-reduced antibody to Trop2 protein according to an embodiment of the present disclosure.

25 **[0036]** FIG. 8 is a graph showing the ELISA results of a CD3×Trop2 bispecific antibody constructed from a parental antibody Cross3 and an affinity-reduced antibody bridging CD3E&D and Trop2 proteins according to an embodiment of the present disclosure.

[0037] FIG. 9 is a graph showing the flow cytometry results of the binding of a CD3×Trop2 bispecific antibody constructed from a parental antibody Cross3 and an affinity-reduced

antibody to CD8 T cells according to an embodiment of the present disclosure.

[0038] FIG. 10 is a graph showing the flow cytometry results of the binding of a CD3×Trop2 bispecific antibody constructed from a parental antibody Cross3 and an affinity-reduced antibody to A-375-Trop2 melanoma cells according to an embodiment of the present disclosure.

[0039] FIG. 11 is a graph showing the results of a CD3×Trop2 bispecific antibody constructed from a parental antibody Cross3 and an affinity-reduced antibody promoting PBMC to kill A-375-Trop2 melanoma cells according to an embodiment of the present disclosure.

[0040] FIG. 12 is a graph showing the results of Cross3×Trop2, Cross313×Trop2, Cross316×Trop2, and Cross325×Trop2 bispecific antibodies promoting PBMC to kill A-375-Trop2 melanoma cells according to an embodiment of the present disclosure.

[0041] FIG. 13 is a graph showing the results of Cross3×Trop2, Cross313×Trop2, Cross316×Trop2, and Cross325×Trop2 bispecific antibodies promoting the secretion of pro-inflammatory cytokines by PBMC according to an embodiment of the present disclosure.

[0042] FIG. 14 is a graph showing the ELISA results of the binding of a CD3×PDL1 bispecific antibody constructed from a parental antibody Cross3 and an affinity-reduced antibody to CD3E&D protein according to an embodiment of the present disclosure.

[0043] FIG. 15 is a graph showing the ELISA results of the binding of a CD3×PDL1 bispecific antibody constructed from a parental antibody Cross3 and an affinity-reduced antibody to PDL1 protein according to an embodiment of the present disclosure.

[0044] FIG. 16 is a graph showing the flow cytometry results of the binding of a CD3×PDL1 bispecific antibody constructed from a parental antibody Cross3 and an affinity-reduced antibody to CD8 T cells according to an embodiment of the present disclosure.

[0045] FIG. 17 is a graph showing the flow cytometry results of the binding of a CD3×PDL1 bispecific antibody constructed from a parental antibody Cross3 and an affinity-reduced antibody to A-375 melanoma cells according to an embodiment of the present disclosure.

[0046] FIG. 18 is a graph showing the flow cytometry results of the binding of a CD3×PDL1 bispecific antibody constructed from a parental antibody Cross3 and an affinity-

reduced antibody to HCT-15 colorectal cancer cells according to an embodiment of the present disclosure.

[0047] FIG. 19 is a graph showing the flow cytometry results of the binding of a CD3×PDL1 bispecific antibody constructed from a parental antibody Cross3 and an affinity-reduced antibody to A-549 lung cancer cells according to an embodiment of the present disclosure.

[0048] FIG. 20 is a graph showing the results of a CD3×PDL1 bispecific antibody constructed from a parental antibody Cross3 and an affinity-reduced antibody promoting PBMC to kill A-375 melanoma cells according to an embodiment of the present disclosure.

[0049] FIG. 21 is a graph showing the results of Cross3×PDL1, Cross313×PDL1, Cross316×PDL1, and Cross325×PDL1 promoting PBMC to kill A-375 melanoma cells according to an embodiment of the present disclosure.

[0050] FIG. 22 is a graph showing the results of a CD3×PDL1 bispecific antibody constructed from a parental antibody Cross3 and an affinity-reduced antibody promoting PBMC to kill HCT-15 colorectal cancer cells according to an embodiment of the present disclosure.

[0051] FIG. 23 is a graph showing the results of a CD3×PDL1 bispecific antibody constructed from a parental antibody Cross3 and an affinity-reduced antibody promoting the secretion of pro-inflammatory cytokines by PBMC after co-incubation with A-375 according to an embodiment of the present disclosure; and

[0052] FIG. 24 is a graph showing the results of a CD3×PDL1 bispecific antibody constructed from a parental antibody Cross3 and an affinity-reduced antibody promoting the secretion of pro-inflammatory cytokines by PBMC after co-incubation with HCT-15 according to an embodiment of the present disclosure.

DETAILED DESCRIPTION

[0053] Embodiments of the present disclosure will now be described in detail, examples of which are illustrated in the accompanying drawings. The embodiments described below with reference to the accompanying drawings are exemplary and are intended to explain the present

disclosure, but should not be construed as limiting the present disclosure.

[0054] In addition, terms “first” and “second” are only used for descriptive purposes, and cannot be understood as indicating or implying relative importance, or implicitly indicating the number of indicated technical features. Therefore, the features defined with “first” and “second” may explicitly or implicitly include at least one of the features. In the description of the present disclosure, “plurality/more” means at least two, such as two or three, unless otherwise explicitly specified.

[0055] The endpoint values or any values of the ranges disclosed herein are not limited to the precise ranges or values. These ranges or values should be understood to include values approximating such ranges or values. For numerical ranges, one or more new numerical ranges can be obtained by combining the endpoint values of the respective ranges, an endpoint value and an individual point value within the respective ranges, and individual point values within the respective ranges with each other, and these numerical ranges should be regarded as specifically disclosed herein.

Terms and definitions

[0056] In order to make the present disclosure more easily understood, certain technical and scientific terms are specifically defined below. Unless obviously and clearly defined elsewhere in this document, all other technical and scientific terms used herein have the meanings commonly understood by one of ordinary skill in the art to which the present disclosure belongs. Abbreviations for amino acid residues are standard 3-letter and/or 1-letter codes used in the art referring to one of the 20 commonly used L-amino acids.

[0057] As used herein, the term "antibody" generally refers to an antibody that can recognize one or more antigenic epitopes, including but not limited to monoclonal antibodies, polyclonal antibodies, dimers, multimers, multi-specific antibodies (e.g., bispecific antibodies), heavy-chain-only antibodies, triple-chain antibodies, single-chain Fv (scFv), nanobodies, etc., and also includes antibody fragments, as long as they exhibit the desired biological activity. The antibody can be murine, human, humanized, chimeric, or from other species. The antibody can refer to a full-length heavy chain, a full-length light chain, an intact immunoglobulin molecule; or an immunologically active portion of any one of these polypeptides, i.e., a molecule or portion thereof that contains an antigen-binding site that immunospecifically binds to a target

antigen of interest, and such targets include but are not limited to cancer cells or cells that produce autoimmune antibodies associated with an autoimmune disease.

[0058] As used herein, in the variable region, the amino acid composition and arrangement order of some regions have a higher degree of variation, which is called the hypervariable region (HVR). The hypervariable region is the location where the antigen and antibody bind, so it is also called the complementarity-determining region (CDR). There are three CDR regions in both the heavy chain variable region and the light chain variable region. For example, it generally includes: amino acid residues near 23-34 (L1), 50-56 (L2), and 89-97 (L3) in the light chain variable region, and near 31-35B (H1), 50-65 (H2), and 95-102 (H3) in the heavy chain variable region; and/or amino acid residues from a "hypervariable loop", such as amino acid residues near 26-32 (L1), 50-52 (L2), and 91-96 (L3) in the light chain variable region and near 26-32 (H1), 53-55 (H2), and 96-101 (H3) in the heavy chain variable region.

[0059] As used herein, the term "anti-CD3 antibody" refers to an antibody capable of binding to CD3. Such antibodies are also referred to herein as "CD3-binding antibody".

[0060] As used herein, the term "Fab-Linker" refers to a polypeptide segment obtained by linking Fab VH-CH1 and Fab VL-CL of an antibody Fab fragment via a linking peptide, including but not limited to: scFab, scFab Δ (the linking peptide contains no disulfide bond).

[0061] As used herein, the term "antigen binding fragment" is equivalent to "antibody fragment" or "antigen binding antibody fragment", and may include a portion, generally the binding region or variable region, of an intact antibody, including but is not limited to, Fv, scFv, Fab, Fab', Fab'-SH, F(ab')₂, scFv-Fc fragments, or bispecific antibodies (BsAbs), linear antibodies, or any fragment that can increase half-life by chemical modification, such as the addition of poly(alkylene) glycols, such as polyethylene glycol ("PEGylation") (PEGylated fragments referred to as Fv-PEG, scFv-PEG, Fab-PEG, F(ab')₂-PEG, or Fab'-PEG) ("PEG" is polyethylene glycol) or by incorporation into liposomes.

[0062] As used herein, the term "chimeric antibody" refers to a recombinant antibody obtained by replacing the amino acid sequence of the constant region of a monoclonal antibody from one species (e.g. mouse) with the constant region of an antibody from another species (e.g. human) using recombinant DNA technology.

[0063] As used herein, the term "humanized antibody" refers to a recombinant antibody

obtained by completely replacing the amino acid sequences of the constant region and the non-CDR (Fv framework region (FR)) in the variable region of a monoclonal antibody from one species (e.g. mouse) with the amino acid sequences of the constant region and the non-CDR in the variable region of an antibody from another species (e.g. human) using recombinant DNA technology. That is, when the constant region of an antibody is humanized, it is referred to as a chimeric antibody, and when the amino acid sequences of the constant region and the non-CDR in variable region are all humanized, it is referred to as a humanized antibody. Methods of humanization may be carried out with reference to conventional antibody engineering techniques and will not be reiterated herein.

10 **[0064]** With respect to nucleotides, the terms "homology," "identity," or "similarity" are used to describe or compare the degree of nucleotide similarity between two or more nucleotide sequences. The percentage of "sequence homology" between a first sequence and a second sequence can be calculated by dividing [the number of identical nucleotides in the first sequence that are identical to the nucleotides at corresponding positions]. [nucleotides in the second sequence] minus [the total number of nucleotides in the first sequence] and then multiplied by [100%], where each deletion, insertion, substitution, or addition of a nucleotide relative to the first nucleotide sequence in the second nucleotide sequence is considered a difference at a single nucleotide (position). Alternatively, the degree of sequence identity between two or more nucleotide sequences can be calculated using known computer algorithms for sequence alignment, such as NCBI Blast v2.0, using standard settings. Some other techniques, computer algorithms, and setups for determining the degree of sequence identity are described, for example, in WO 04/037999, EP 0 967 284, EP 1 085 089, WO 00/55318, WO 00/78972, WO 98/49185, and GB 2357768-A.

25 **[0065]** With respect to polypeptides, the terms "(substantial) homology", "identity", or "similarity" are used to describe or compare the degree of amino acid similarity between two or more polypeptides or designated sequences thereof at optimal alignment and comparison (with appropriate insertions or deletions of nucleotides). The % homology between two sequences varies with the number of identical positions shared by the sequences at optimal alignment (i.e., % homology = number of identical positions/total number of positions × 100), where optimal alignment is determined by taking into account the number of gaps that need to

be introduced, and the length of each gap, to achieve an optimal alignment of the two sequences. The comparison of two sequences and determination of identity percentage between two sequences can be accomplished using a mathematical algorithm, as described in the non-limiting examples below.

5 **[0066]** As used herein, the term “an amino acid sequence in conservative modification form” refers to an amino acid modification that does not significantly affect or alter the binding characteristics of the antibody including the amino acid sequence, and the modification includes the substitutions, additions and deletions of the amino acids. Modifications can be introduced into the antibodies of the present disclosure by standard techniques such as site-directed
10 mutagenesis and PCR-mediated mutagenesis. Conservative amino acid substitution refers to the substitution in which the amino acid residues are replaced by amino acid residues having similar side chains. Families of amino acid residues having similar side chains have been identified in the art. These families include amino acids with basic side chains, e.g. lysine, arginine, and histidine; amino acids with acidic side chains, e.g. aspartic acid, and glutamic acid;
15 amino acids with uncharged polar side chains, e.g. glycine, asparagine, glutamine, serine, threonine, tyrosine, cysteine, and tryptophan; amino acids with nonpolar side chains, e.g. alanine, valine, leucine, isoleucine, proline, phenylalanine, and methionine; amino acids with β -branched side chains, e.g. threonine, valine, and isoleucine, and amino acids with aromatic side chains, e.g. tyrosine, phenylalanine, tryptophan, and histidine.

20 **[0067]** As used herein, the term "conjugate" is understood in context to refer to an antibody or antigen binding fragment thereof conjugated to a coupling moiety such as a vector substance, medicament, toxin, cytokine, protein tag, modifier, therapeutic agent, chemotherapeutic agent, etc., using any covalent or non-covalent bioconjugation strategy.

25 **[0068]** As used herein, the term "vector" generally refers to a nucleic acid molecule capable of being inserted into a suitable host for self-replication, which transfers the inserted nucleic acid molecule into and/or between host cells. The vector may include a vector primarily used to insert DNA or RNA into cells, a vector primarily used to replicate DNA or RNA, and a vector primarily used for expression by transcription and/or translation of DNA or RNA. The vector also includes a vector having multiple functions as described above. The vector may be a
30 polynucleotide that can be transcribed and translated into a polypeptide when introduced into a

suitable host cell. Typically, by culturing appropriate host cells containing the vector, the vector can produce the desired expression product.

[0069] As used herein, the term "pharmaceutical composition" generally refers to unit dosage form, and may be prepared by any of the methods well known in the art of pharmacy.

5 All methods include the step of combining the active ingredient with the carrier which constitutes one or more accessory ingredients. In general, the compositions are prepared by uniformly and thoroughly combining the active compound with liquid carriers, finely divided solid carriers, or both.

[0070] As used herein, the term "pharmaceutically acceptable" refers to substances that are
10 suitable for use in humans and/or mammals without excessive adverse side effects (such as toxicity, irritation, and allergic response), i.e., a substance with at a reasonable benefit/risk ratio.

[0071] As used herein, the term "pharmaceutically acceptable adjuvant" may include any solvent, solid excipient, diluent, or other liquid excipient, etc., suitable for a particular target dosage form. Except for any conventional adjuvants that are incompatible with the compounds
15 of the present disclosure, for example which produce any adverse biological effects or interact in a deleterious manner with any other component of the pharmaceutically acceptable composition, their use is contemplated by the present disclosure.

[0072] As used herein, the term "administering/administration" refers to introducing a predetermined amount of a substance into a patient in a suitable manner. The antibody or
20 antigen binding fragment, recombinant protein, multi-specific antibody, conjugate, or pharmaceutical composition of the present disclosure can be administered via any common route as long as it can reach the intended tissue. Various routes of administration are contemplated, including peritoneal, intravenous, intramuscular, subcutaneous injection, and the like, but the present disclosure is not limited to these exemplified routes of administration.
25 Preferably, the composition of the present disclosure is administered by intravenous injection or subcutaneous injection.

[0073] As used herein, the term "treat/treatment" refers to the treatment intended to achieve a desired pharmacological and/or physiological effect. The effect may be prophylactic in terms of complete or partial prevention of the disease or its symptoms, and/or therapeutic in terms of
30 partial or complete cure of the disease and/or its adverse effects. As used herein,

"treat/treatment" encompasses diseases in mammals, particularly humans, and includes: (a) preventing the occurrence of a disease or condition in an individual who is susceptible to the disease but has not yet been diagnosed with the disease; (b) inhibiting the disease, such as arresting the progression of the disease; or (c) alleviating the disease, such as alleviating the symptoms associated with the disease. As used herein, "treat/treatment" encompasses any administration of a medicament or compound to a subject to treat, cure, alleviate, ameliorate, lessen, or inhibit a disease in the subject, including but not limited to administering a medicament containing the compound according to the present disclosure to a subject in need thereof.

10 **[0074]** As used herein, the term "effective amount" or "effective dose" refers to an amount that is capable of producing a function or activity in humans and/or animals and is acceptable to humans and/or animals.

[0075] The present disclosure provides an anti-CD3 antibody or antigen binding fragment thereof, a multi-specific antibody, a conjugate, a nucleic acid molecule, a vector or transformant, a cell, a pharmaceutical composition, a kit, and uses thereof, which are described in detail below.

Anti-CD3 antibody or antigen binding fragment thereof

[0076] The present disclosure provides an anti-CD3 antibody or antigen binding fragment thereof. The anti-CD3 antibody or antigen binding fragment thereof includes heavy chain variable region CDRs and light chain variable region CDRs. The heavy chain variable region CDRs have each an amino acid sequence as set forth in one of SEQ ID NOs: 1 to 3, 7 to 8, and 74 to 86, or a conservative modification form thereof; and/or the light chain variable region CDRs have each an amino acid sequences as set forth in one of SEQ ID NOs: 4 to 6, 9, and 87 to 92, or a conservative modification form thereof.

[0077] The anti-CD3 antibody or antigen binding fragment thereof according to the present disclosure can bind to human CD3 protein and reduce the production of pro-inflammatory cytokines. The CD3 bispecific antibody developed based on this CD3 antibody or its antigen binding fragment has high PBMC-mediated tumor-killing activity and weak ability of promoting the secretion of pro-inflammatory cytokines. After being prepared into a medicament, on the one hand, the anti-CD3 antibody or antigen binding fragment thereof can be preferentially accumulated at the tumor site, increasing the local drug concentration at the tumor

site and reducing the peripheral drug concentration, thereby reducing the risk of off-target toxicity. On the other hand, this bispecific antibody medicament can reduce the production of pro-inflammatory cytokines and reduce the risk of on-target toxicity. Furthermore, the drugs prepared using the anti-CD3 antibody or antigen binding fragment thereof can be used to prevent and/or treat a CD3-related disease. CD3 bispecific antibody drugs developed based on the anti-CD3 antibody or antigen binding fragment thereof are safer and can be used to prevent and/or treat a cancer or tumor or infection or autoimmune disease. Therefore, the anti-CD3 antibody or antigen binding fragment thereof of the present disclosure has good value in clinical application and drug development.

10 **[0078]** It should be noted that one or more amino acid residues in the CDR region of the antibody or antigen binding fragment thereof of the present disclosure can be replaced with other amino acid residues from the same side chain family, and the retained function of the modified antibody can be tested using the functional assay method described herein. Preferably, the number of conservative modifications does not exceed one or two.

15 **[0079]** According to an embodiment of the present disclosure, the heavy chain variable region CDRs have amino acid sequences as set forth in SEQ ID NOs: 1 to 2 or a conservative modification form thereof, and an amino acid sequence as set forth in one of SEQ ID NOs: 3, 7 to 8, and 74 to 86, or a conservative modification form thereof; and the light chain variable region CDRs have amino acid sequences as set forth in SEQ ID NOs: 4 to 5 or a conservative modification form thereof, and an amino acid sequence as set forth in one of SEQ ID NOs: 6, 9, and 87 to 92, or a conservative modification form thereof.

20 **[0080]** According to an embodiment of the present disclosure, the antibody or antigen binding fragment thereof includes: heavy chain variable region CDR1 having an amino acid sequence as set forth in SEQ ID NO: 1, heavy chain variable region CDR2 having an amino acid sequence as set forth in SEQ ID NO: 2, heavy chain variable region CDR3 having an amino acid sequence as set forth in one of SEQ ID NO: 3, SEQ ID NOs: 7 to 8, and SEQ ID NOs: 74 to 86; light chain variable region CDR1 having an amino acid sequence as set forth in SEQ ID NO: 4, light chain variable region CDR2 having an amino acid sequence as set forth in SEQ ID NO: 5, light chain variable region CDR3 having an amino acid sequence as set forth in one of SEQ ID NOs: 6, 9, and 87 to 92.

[0081] According to an embodiment of the present disclosure, the heavy chain variable region CDR3 has an amino acid sequence as set forth in one of SEQ ID NOs: 3 and 7 to 8; and/or the light chain variable region CDR3 has an amino acid sequence as set forth in SEQ ID NO: 6 or 9.

5 **[0082]** According to an embodiment of the present disclosure, the antibody or antigen binding fragment thereof includes any one of the following groups: 1) the heavy chain variable region CDR1, the heavy chain variable region CDR2, and the heavy chain variable region CDR3 as set forth in SEQ ID NOs: 1, 2, and 74, respectively, and the light chain variable region CDR1, the light chain variable region CDR2, and the light chain variable region CDR3 as set
10 forth in SEQ ID NOs: 4, 5, and 6, respectively; 2) the heavy chain variable region CDR1, the heavy chain variable region CDR2, and the heavy chain variable region CDR3 as set forth in SEQ ID NOs: 1, 2, and 75, respectively, and the light chain variable region CDR1, the light chain variable region CDR2, and the light chain variable region CDR3 as set forth in SEQ ID NOs: 4, 5, and 6, respectively; 3) the heavy chain variable region CDR1, the heavy chain
15 variable region CDR2, and the heavy chain variable region CDR3 as set forth in SEQ ID NOs: 1, 2, and 76, respectively, and the light chain variable region CDR1, the light chain variable region CDR2, and the light chain variable region CDR3 as set forth in SEQ ID NOs: 4, 5, and 6, respectively; 4) the heavy chain variable region CDR1, the heavy chain variable region CDR2, and the heavy chain variable region CDR3 as set forth in SEQ ID NOs: 1, 2, and 77, respectively,
20 and the light chain variable region CDR1, the light chain variable region CDR2, and the light chain variable region CDR3 as set forth in SEQ ID NOs: 4, 5, and 6, respectively; 5) the heavy chain variable region CDR1, the heavy chain variable region CDR2, and the heavy chain variable region CDR3 as set forth in SEQ ID NOs: 1, 2, and 78, respectively, and the light chain variable region CDR1, the light chain variable region CDR2, and the light chain variable region
25 CDR3 as set forth in SEQ ID NOs: 4, 5, and 6, respectively; 6) the heavy chain variable region CDR1, the heavy chain variable region CDR2, and the heavy chain variable region CDR3 as set forth in SEQ ID NOs: 1, 2, and 79, respectively, and the light chain variable region CDR1, the light chain variable region CDR2, and the light chain variable region CDR3 as set forth in
30 SEQ ID NOs: 4, 5, and 6, respectively; 7) the heavy chain variable region CDR1, the heavy chain variable region CDR2, and the heavy chain variable region CDR3 as set forth in SEQ ID

[illegible]

CDR3 as set forth in SEQ ID NOs: 1, 2, and 8, respectively, and the light chain variable region CDR1, the light chain variable region CDR2, and the light chain variable region CDR3 as set forth in SEQ ID NOs: 4, 5, and 9, respectively; 16) the heavy chain variable region CDR1, the heavy chain variable region CDR2, and the heavy chain variable region CDR3 as set forth in SEQ ID NOs: 1, 2, and 84, respectively, and the light chain variable region CDR1, the light chain variable region CDR2, and the light chain variable region CDR3 as set forth in SEQ ID NOs: 4, 5, and 89, respectively; 17) the heavy chain variable region CDR1, the heavy chain variable region CDR2, and the heavy chain variable region CDR3 as set forth in SEQ ID NOs: 1, 2, and 85, respectively, and the light chain variable region CDR1, the light chain variable region CDR2, and the light chain variable region CDR3 as set forth in SEQ ID NOs: 4, 5, and 90, respectively; 18) the heavy chain variable region CDR1, the heavy chain variable region CDR2, and the heavy chain variable region CDR3 as set forth in SEQ ID NOs: 1, 2, and 86, respectively, and the light chain variable region CDR1, the light chain variable region CDR2, and the light chain variable region CDR3 as set forth in SEQ ID NOs: 4, 5, and 92, respectively; or 19) the heavy chain variable region CDR1, the heavy chain variable region CDR2, and the heavy chain variable region CDR3 as set forth in SEQ ID NOs: 1, 2 and 8, respectively, and the light chain variable region CDR1, the light chain variable region CDR2, and the light chain variable region CDR3 as set forth in SEQ ID NOs: 4, 5 and 91, respectively.

[0083] It should be noted that the antigen binding fragment will be composed of a partial sequence of the heavy chain variable region or light chain variable region of the antibody from which it is derived, or contain a partial sequence of the light or heavy chain variable region, and the partial sequence is sufficient to retain the same binding specificity and sufficient affinity as the antibody from which it is derived.

[0084] According to an embodiment of the present disclosure, the antibody or antigen binding fragment thereof includes a heavy chain framework region and/or a light chain framework region.

[0085] According to an embodiment of the present disclosure, at least a portion of the heavy chain framework region and/or the light chain framework region is from at least one of a murine antibody, a human antibody, a primate antibody, a bovine antibody, an equine antibody, a dairy bovine antibody, a porcine antibody, an ovine antibody, a caprine antibody, a canine antibody,

a feline antibody, a rabbit-derived antibody, a camelid antibody, donkey-derived antibody, a deer-derived antibody, a mink-derived antibody, a chicken-derived antibody, a duck-derived antibody, a goose-derived antibody, a turkey-derived antibody, gamecock-derived antibody, or a mutant thereof, and preferably, at least one of a murine antibody, a human antibody, or a primate antibody.

[0086] It should be noted that, in order to further improve the biological acceptability of the antibody, the antibody may be humanized, that is, the antibody is a chimeric antibody or a humanized antibody.

[0087] As above described, one or more amino acid residues in the heavy or light chain variable region of the antibody or antigen binding fragment thereof of the present disclosure can be replaced with other amino acid residues from the same side chain family, and the retained function of the modified antibodies can be tested using the functional assay method described herein.

[0088] According to an embodiment of the present disclosure, the antibody or antigen binding fragment thereof includes: a heavy chain variable region having an amino acid sequence as set forth in one of SEQ ID NOs: 10, 12 to 13, 15 to 24, 27, and 31, or a conservative modification form thereof; and/or a light chain variable region having an amino acid sequence as set forth in one of SEQ ID NOs: 11, 14, 25 to 26, 28, and 30, or a conservative modification form thereof.

[0089] It should be noted that, without substantially affecting the CD3 binding activity of the antibody or antigen binding fragment thereof (retaining at least 95% of the activity), those skilled in the art may substitute, add, and/or delete one or more (e.g., 1, 2, 3, 4, 5, 6, 7, 8, 9, or 10 or more) amino acids in the amino acid sequence of the heavy chain or light chain variable region of the antibody or antigen binding fragment thereof of the present disclosure, to obtain sequence variants of the antibody or antigen binding fragment thereof, which are all considered to be included in the protection scope of the present disclosure. For example, amino acids with similar properties in the heavy chain variable region or light chain variable region are substituted. The above sequence variants of the present disclosure may be at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% identical (or homologous) to the reference sequence.

[0090] It should be noted that the sequence identity described in the present disclosure can be measured using sequence analysis software. For example, the computer program with default parameters BLAST, in particular BLASTP or TBLASTN is used.

[0091] According to an embodiment of the present disclosure, the heavy chain variable region has an amino acid sequence with at least 90% sequence identity to an amino acid sequence as set forth in one of SEQ ID NOs: 10, 12 to 13, 15 to 24, 27, and 31; and/or the light chain variable region has an amino acid sequence with at least 90% sequence identity to an amino acid sequence as set forth in one of SEQ ID NOs: 11, 14, 25 to 26, 28, and 30.

[0092] According to an embodiment of the present disclosure, the antibody or antigen binding fragment thereof includes an amino acid sequence as set forth in a combination of the heavy chain variable region and the light chain variable region in any one of the following groups, or an amino acid sequence having at least 90% sequence identity thereto:

Group number of the antibody or antigen binding fragment	Heavy chain variable region	Light chain variable region
1	SEQ ID NO: 15	SEQ ID NO: 11
2	SEQ ID NO: 16	SEQ ID NO: 11
3	SEQ ID NO: 17	SEQ ID NO: 11
4	SEQ ID NO: 17	SEQ ID NO: 11
5	SEQ ID NO: 19	SEQ ID NO: 11
6	SEQ ID NO: 20	SEQ ID NO: 11
7	SEQ ID NO: 21	SEQ ID NO: 11
8	SEQ ID NO: 22	SEQ ID NO: 11
9	SEQ ID NO: 23	SEQ ID NO: 11
10	SEQ ID NO: 24	SEQ ID NO: 11
11	SEQ ID NO: 10	SEQ ID NO: 11
12	SEQ ID NO: 12	SEQ ID NO: 11
13	SEQ ID NO: 13	SEQ ID NO: 25
14	SEQ ID NO: 13	SEQ ID NO: 26
15	SEQ ID NO: 13	SEQ ID NO: 14
16	SEQ ID NO: 27	SEQ ID NO: 28
17	SEQ ID NO: 29	SEQ ID NO: 28
18	SEQ ID NO: 13	SEQ ID NO: 30

19	SEQ ID NO: 31	SEQ ID NO: 26
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[0093] According to an embodiment of the present disclosure, the antibody or antigen binding fragment thereof further includes a constant region. The constant region including at least one of a heavy chain constant region or a light chain constant region.

5 **[0094]** According to an embodiment of the present disclosure, at least a portion of at least one of the heavy chain constant region or the light chain constant region is from at least one of a murine antibody, human antibody, primate antibody, bovine antibody, equine antibody, dairy bovine antibody, porcine antibody, ovine antibody, caprine antibody, canine antibody, feline antibody, rabbit-derived antibody, camelid antibody, donkey-derived antibody, deer-derived
10 antibody, mink-derived antibody, chicken-derived antibody, duck-derived antibody, goose-derived antibody, turkey-derived antibody, gamecock-derived antibody, or a mutant thereof.

[0095] It should be noted that the immunoglobulins described herein can be immunoglobulin molecules of any type (e.g., IgG, IgE, IgM, IgD, and IgA), class (e.g., IgG1, IgG2, IgG3, IgG4, IgA1, and IgA2), or subclass, including engineered subclasses with altered
15 Fc portions that provide reduced or enhanced effector cell activity. The immunoglobulins may be derived from any species.

[0096] According to an embodiment of the present disclosure, the heavy chain constant region includes a heavy chain constant region selected from IgG1, IgG2, IgG3, IgG4, IgA, IgM, IgE, or IgD; and/or the light chain constant region includes a light chain constant region selected
20 from a κ -type light chain constant region or a λ -type light chain constant region.

[0097] In some specific embodiments, the heavy chain constant region includes a heavy chain constant region selected from human IgG, IgA, IgM, IgE, or IgD, such as human IgG1, human IgG2, human IgG3, human IgG4, human IgA, human IgM, human IgE, or human IgD.

[0098] In some specific embodiments, the heavy chain constant region includes a heavy
25 chain constant region selected from murine IgG, IgA, IgM, IgE, or IgD, such as murine IgG1, murine IgG2a, murine IgG2b, murine IgG2c, murine IgG3, murine IgA, murine IgM, murine IgE, or murine IgD.

[0099] According to an embodiment of the present disclosure, the light chain constant region and the heavy chain constant region are both derived from a murine antibody or a mutant

thereof, or a human antibody or a mutant thereof.

[00100] It should be noted that the amino acid sequences involved in the present disclosure are all shown from the N-terminus to the C-terminus.

[00101] According to an embodiment of the present disclosure, the N-terminus of the heavy chain constant region is linked to the C-terminus of the heavy chain variable region; and/or the N-terminus of the light chain constant region is linked to the N-terminus of the light chain variable region.

[00102] As described above, without substantially affecting the CD3 binding activity of the antibody or antigen binding fragment thereof (retaining at least 95% of the activity), those skilled in the art can substitute, add, and/or delete one or more (e.g., 1, 2, 3, 4, 5, 6, 7, 8, 9, 10 or more) amino acids in the amino acid sequence of the heavy chain constant region or light chain constant region of the antibody or antigen binding fragment thereof of the present disclosure, to obtain sequence variants of the antibody or antigen binding fragment thereof, which are all considered to be included in the protection scope of the present disclosure. For example, amino acids with similar properties in the heavy chain constant region or light chain constant region are substituted. The sequence variants of the present disclosure may be at least 80%, 85%, 90%, 95%, 96%, 97%, 98%, or 99% identical (or homologous) to the reference sequence.

[00103] According to an embodiment of the present disclosure, the heavy chain constant region has an amino acid sequence as set forth in SEQ ID NO: 93 or an amino acid sequence having at least 80% identity thereto; and/or the light chain constant region has an amino acid sequence as set forth in SEQ ID NO: 94 or an amino acid sequence having at least 80% identity thereto.

[00104] According to an embodiment of the present disclosure, the heavy chain constant region and the light chain constant region have amino acid sequences as set forth in SEQ ID NO: 93 and SEQ ID NO: 94, respectively.

[00105] According to an embodiment of the present disclosure, the C-terminus of the heavy chain variable region is linked to the N-terminus of the light chain variable region, or the N-terminus of the heavy chain variable region is linked to the C-terminus of the light chain variable region.

[00106] According to an embodiment of the present disclosure, the antibody or antigen binding fragment thereof further includes a first linking peptide. The C-terminus of the heavy chain variable region is linked to the N-terminus of the first linking peptide, and the C-terminus of the first linking peptide is linked to the N-terminus of the light chain variable region, or the C-terminus of the light chain variable region is linked to the N-terminus of the first linking peptide, and the C-terminus of the first linking peptide is linked to the N-terminus of the heavy chain variable region.

[00107] In some specific embodiments, the first linking peptide has an amino acid sequence as set forth in (GGGGS)_n, wherein n is an integer greater than or equal to 1, and preferably n is 1, 2, 3, 4, 5, 6, 7, 8, 9, or 10.

[00108] As described above, the antibody of the present disclosure may be full-length (e.g., IgG1 or IgG4 antibody) or include only an antigen-binding portion (e.g., Fab, Fab', Fab'-SH, F(ab')₂, or Fv, scFv fragments), or may be modified to affect its function. The present disclosure includes anti-CD3 antibodies with modified glycosylation patterns. In some embodiments, it may be useful to perform modifications to remove undesirable glycosylation sites, or to produce antibodies lacking fucose moieties on the oligosaccharide chains, for example, to enhance antibody-dependent cellular cytotoxicity (ADCC) function. In other embodiments, galactosylation modification can be performed to alter complement dependent cytotoxicity (CDC).

[00109] According to an embodiment of the present disclosure, the antibody includes at least one of a full-length monoclonal antibody, chimeric antibody, humanized antibody, Fv, scFv, Fab, Fab', Fab'-SH, or F(ab')₂; and the antigen binding fragment of the antibody includes at least one of a F(ab')₂ fragment, Fab' fragment, Fab fragment, F(ab)₂ fragment, Fv fragment, scFv fragment, scFv-Fc fusion protein, scFv-Fv fusion protein, or a minimum recognition unit.

[00110] As described above, without substantially affecting the CD3 binding activity of the antibody or antigen binding fragment thereof (retaining at least 95% of the activity), those skilled in the art can substitute, add, and/or delete one or more (e.g., 1, 2, 3, 4, 5, 6, 7, 8, 9, 10 or more) amino acids in the amino acid sequence of the heavy chain or light chain, scFv fragment, Fab fragment, or scFab fragment of the antibody or antigen binding fragment thereof of the present disclosure, to obtain sequence variants of the antibody or antigen binding

fragment thereof, which are all considered to be included in the protection scope of the present disclosure. For example, amino acids with similar properties in the heavy chain or light chain are substituted. The sequence variants of the present disclosure may be at least 80%, 85%, 90%, 95%, 96%, 97%, 98%, or 99% identical (or homologous) to the reference sequence.

- 5 **[00111]** According to an embodiment of the present disclosure, the antibody or antigen binding fragment includes: a heavy chain having an amino acid sequence as set forth in one of SEQ ID NOs: 32, 34, 35, and 37 to 46, or an amino acid sequence having at least 80% identity thereto; and/or a light chain having an amino acid sequence as set forth in one of SEQ ID NOs: 33, 36, and 47 to 48, or an amino acid sequence having at least 80% identity thereto.
- 10 **[00112]** According to an embodiment of the present disclosure, the antibody or antigen binding fragment thereof has an amino acid sequence as set forth in a combination of a heavy chain and a light chain in any one of the following groups, or an amino acid sequence having at least 80% sequence identity thereto:

Group number of the antibody or antigen binding fragment thereof	Heavy chain	Light chain
1	SEQ ID NO: 37	SEQ ID NO: 33
2	SEQ ID NO: 38	SEQ ID NO: 33
3	SEQ ID NO: 39	SEQ ID NO: 33
4	SEQ ID NO: 40	SEQ ID NO: 33
5	SEQ ID NO: 41	SEQ ID NO: 33
6	SEQ ID NO: 42	SEQ ID NO: 33
7	SEQ ID NO: 43	SEQ ID NO: 33
8	SEQ ID NO: 44	SEQ ID NO: 33
9	SEQ ID NO: 45	SEQ ID NO: 33
10	SEQ ID NO: 46	SEQ ID NO: 33
11	SEQ ID NO: 32	SEQ ID NO: 33
12	SEQ ID NO: 34	SEQ ID NO: 33
13	SEQ ID NO: 35	SEQ ID NO: 47
14	SEQ ID NO: 35	SEQ ID NO: 48
15	SEQ ID NO: 35	SEQ ID NO: 36
16	SEQ ID NO: 95	SEQ ID NO: 119
17	SEQ ID NO: 96	SEQ ID NO: 120

18	SEQ ID NO: 97	SEQ ID NO: 121
19	SEQ ID NO: 98	SEQ ID NO: 122

[00113] According to an embodiment of the present disclosure, the antibody or antigen binding fragment thereof has an amino acid sequence as set forth in any one of SEQ ID NOs: 99 to 117, or an amino acid sequence having at least 80% sequence identity thereto.

- 5 **[00114]** According to an embodiment of the present disclosure, the antibody or antigen binding fragment thereof has an amino acid sequence as set forth in a combination of Fab VL-CL and Fab VH-CH1 in any one of the following groups , or an amino acid sequence having at least 80% sequence identity thereto:

Group number of antibody or antigen binding fragment	Fab VH-CH1	Fab VL-CL
1	SEQ ID NO: 123	SEQ ID NO: 33
2	SEQ ID NO: 124	SEQ ID NO: 33
3	SEQ ID NO: 125	SEQ ID NO: 33
4	SEQ ID NO: 126	SEQ ID NO: 33
5	SEQ ID NO: 127	SEQ ID NO: 33
6	SEQ ID NO: 128	SEQ ID NO: 33
7	SEQ ID NO: 129	SEQ ID NO: 33
8	SEQ ID NO: 130	SEQ ID NO: 33
9	SEQ ID NO: 131	SEQ ID NO: 33
10	SEQ ID NO: 132	SEQ ID NO: 33
11	SEQ ID NO: 133	SEQ ID NO: 33
12	SEQ ID NO: 134	SEQ ID NO: 33
13	SEQ ID NO: 135	SEQ ID NO: 47
14	SEQ ID NO: 136	SEQ ID NO: 36
15	SEQ ID NO: 137	SEQ ID NO: 48
16	SEQ ID NO: 138	SEQ ID NO: 119
17	SEQ ID NO: 139	SEQ ID NO: 120
18	SEQ ID NO: 140	SEQ ID NO: 121
19	SEQ ID NO: 141	SEQ ID NO: 122

- 10 **[00115]** According to an embodiment of the present disclosure, the antibody or antigen

binding fragment thereof has an amino acid sequence as set forth in any one of SEQ ID NOs: 142 to 160, or an amino acid sequence having at least 80% sequence identity thereto.

Multi-specific antibody

5 **[00116]** The present disclosure provides a multi-specific antibody. The multi-specific antibody includes: a first antigen binding region and a second antigen binding region. The first antigen binding region includes the above-described anti-CD3 antibody or antigen binding fragment thereof; and the second antigen binding region has a biomolecule binding activity, the biomolecule not being CD3.

10 **[00117]** As described above, the multi-specific antibodies (such as bispecific antibodies) developed based on the anti-CD3 antibody or antigen binding fragment thereof according to the first aspect of the present disclosure can be preferentially accumulated at the tumor site after being prepared into medicaments, increasing the local drug concentration at the tumor site and reducing the peripheral drug concentration, thereby reducing the risk of off-target toxicity. At the same time, the production of pro-inflammatory cytokines can be reduced and the risk of on-
15 target toxicity of the multi-specific antibodies (such as bispecific antibodies) can be reduced. Therefore, the multi-specific antibody based on the anti-CD3 antibody or antigen binding fragment thereof of the first aspect of the present disclosure has better safety and high value in clinical application and drug development.

[00118] According to an embodiment of the present disclosure, the multi-specific antibody
20 is selected from one of a bispecific antibody, a trispecific antibody, or a tetraspecific antibody.

[00119] The bispecific antibody is preferred.

[00120] According to an embodiment of the present disclosure, the bispecific antibody includes a symmetric bispecific antibody or an asymmetric bispecific antibody.

[00121] According to an embodiment of the present disclosure, the second antigen binding
25 region is a binding protein of the biomolecule or a fragment thereof.

[00122] According to an embodiment of the present disclosure, the biomolecule is selected from at least one of the following: PDL1, CD47, TIGIT, CD73, CD33, CEACAM1, CEACAM5, CEACAM6, STING, WNT, Beta catenin, B7H3, VISITA, CD19, BCMA, CD22, CD20, CD123, CD38, CEA, CD25, CD46, CD138, PSCA, PSMA, PSA, MUC1, MUC16, NY-ESO-1, GD2,
30 WT1, Mesothelin, MAGE-A3, GPC3, PRAME, Globo H, AFP, Trop2, FOLR1, SP, Sca-1,

CD133, or EPCAM.

[00123] According to an embodiment of the present disclosure, the binding protein or fragment thereof is selected from at least one of an antibody or antigen binding fragment thereof, a receptor, or a ligand.

5 **[00124]** According to an embodiment of the present disclosure, the bispecific antibody is an asymmetric bispecific antibody.

[00125] According to an embodiment of the present disclosure, the first antigen binding region includes a first scFv fragment, first Fv fragment, first Fab fragment, or first Fab-Linker fragment.

10 **[00126]** According to an embodiment of the present disclosure, the first antigen binding region further includes a first Fc peptide segment.

[00127] According to an embodiment of the present disclosure, the N-terminus of the first Fc peptide segment is linked to the C-terminus of the scFv fragment or the C-terminus of the Fv fragment; or the N-terminus of the first Fc peptide segment is linked to the C-terminus of CH1 of the Fab fragment or the C-terminus of the Fab-Linker fragment.

[00128] According to an embodiment of the present disclosure, the first antigen binding region further includes a second linking peptide. The N-terminus of the first Fc peptide segment is linked to the C-terminus of the second linking peptide, and the N-terminus of the second linking peptide is linked to the C-terminus of the scFv fragment or the C-terminus of the Fv fragment; or the N-terminus of the first Fc peptide segment is linked to the C-terminus of the second linking peptide, and the N-terminus of the second linking peptide is linked to the C-terminus of CH1 of the Fab fragment or the C-terminus of Fab-Linker fragment.

25 **[00129]** In some specific embodiments, the second linking peptide has an amino acid sequence as set forth in (GGGGS)_n, wherein n is an integer greater than or equal to 1, and preferably n is 1, 2, 3, 4, 5, 6, 7, 8, 9, or 10.

[00130] According to an embodiment of the present disclosure, the first Fc peptide segment is selected from a human Fc peptide segment, and preferably a human IgG1 Fc peptide.

[00131] According to an embodiment of the present disclosure, the first Fc peptide segment has an amino acid sequence as set forth in SEQ ID NO: 118; or the first Fc peptide segment has an amino acid sequence as set forth in SEQ ID NO: 166.

[00132] According to an embodiment of the present disclosure, the first scFv fragment has an amino acid sequence as set forth in any one of SEQ ID NOs: 95 to 117.

[00133] According to an embodiment of the present disclosure, the first Fab fragment has an amino acid sequence as set forth in any one of the following groups of combinations of Fab

5 VL-CL and Fab VH-CH1:

Group number of the first Fab fragment	Fab VH-CH1	Fab VL-CL
1	SEQ ID NO: 123	SEQ ID NO: 33
2	SEQ ID NO: 124	SEQ ID NO: 33
3	SEQ ID NO: 125	SEQ ID NO: 33
4	SEQ ID NO: 126	SEQ ID NO: 33
5	SEQ ID NO: 127	SEQ ID NO: 33
6	SEQ ID NO: 128	SEQ ID NO: 33
7	SEQ ID NO: 129	SEQ ID NO: 33
8	SEQ ID NO: 130	SEQ ID NO: 33
9	SEQ ID NO: 131	SEQ ID NO: 33
10	SEQ ID NO: 132	SEQ ID NO: 33
11	SEQ ID NO: 133	SEQ ID NO: 33
12	SEQ ID NO: 134	SEQ ID NO: 33
13	SEQ ID NO: 135	SEQ ID NO: 47
14	SEQ ID NO: 136	SEQ ID NO: 36
15	SEQ ID NO: 137	SEQ ID NO: 48
16	SEQ ID NO: 138	SEQ ID NO: 119
17	SEQ ID NO: 139	SEQ ID NO: 120
18	SEQ ID NO: 140	SEQ ID NO: 121
19	SEQ ID NO: 141	SEQ ID NO: 122

[00134] According to an embodiment of the present disclosure, the first Fab-Linker has an amino acid sequence as set forth in any one of SEQ ID NOs: 142 to 160.

[00135] According to an embodiment of the present disclosure, the first antigen binding
10 region has an amino acid sequence as set forth in any one of SEQ ID NOs: 49 to 52, and 54 to 68.

- [00136]** According to an embodiment of the present disclosure, the biomolecule is Trop2 or PDL1.
- [00137]** According to an embodiment of the present disclosure, the second antigen binding region includes a first anti-Trop2 antibody or antigen binding fragment thereof; or a first anti-PDL1 antibody or an antigen binding fragment thereof.
- [00138]** According to an embodiment of the present disclosure, the first anti-Trop2 antibody or antigen binding fragment thereof is a second scFab fragment or a second Fab fragment of anti-Trop2; or the first anti-PDL1 antibody or antigen binding fragment thereof is a third scFab fragment or a third Fab fragment of anti-PDL1.
- [00139]** According to an embodiment of the present disclosure, the second scFab fragment has an amino acid sequence as set forth in SEQ ID NO: 161; or the second Fab fragment has an amino acid sequence as set forth in SEQ ID NO: 162 and 70; or the third scFab fragment has an amino acid sequence as set forth in SEQ ID NO: 163; or the third Fab fragment has an amino acid sequence as set forth in SEQ ID NO: 164 and 72.
- [00140]** According to an embodiment of the present disclosure, the second antigen binding region further includes a second Fc fragment. The N-terminus of the second Fc fragment is linked to the C-terminus of CH1 of the second scFab fragment, the second Fab fragment, the third scFab fragment, or the third Fab fragment.
- [00141]** According to an embodiment of the present disclosure, the second Fc peptide segment is selected from a human Fc peptide segment, and preferably a human IgG1 Fc peptide segment.
- [00142]** According to an embodiment of the present disclosure, the second Fc peptide segment has an amino acid sequence as set forth in SEQ ID NO: 165.
- [00143]** According to an embodiment of the present disclosure, the first Fc peptide segment and the second Fc peptide segment are linked via a knob-into-hole structure. According to an embodiment of the present disclosure, the second antigen binding region has an amino acid sequence as set forth in SEQ ID NO: 161 or 163; or the second antigen binding region has an amino acid sequence as set forth in SEQ ID NO: 162 and 70; or the second antigen binding region has an amino acid sequence as set forth in SEQ ID NO: 164 and 72.
- [00144]** In some optional embodiments of the present disclosure, the multi-specific antibody

of the present disclosure has the structure shown in FIG. 5.

[00145] In some optional embodiments of the present disclosure, as shown in FIG. 5A, the multi-specific antibody of the present disclosure includes: a first peptide chain having an amino acid sequence as set forth in any one of SEQ ID NOs: 49 to 52 and 54 to 68, a second peptide chain having an amino acid sequence as set forth in SEQ ID NO: 69, and a third peptide chain having an amino acid sequence as set forth in SEQ ID NO: 70; or a second peptide chain having an amino acid sequence as set forth in SEQ ID NO: 71, and a third peptide chain having an amino acid sequence as set forth in SEQ ID NO: 72.

[00146] In some optional embodiments of the present disclosure, as shown in FIG. 5B, the multi-specific antibody of the present disclosure includes: a first peptide chain having an amino acid sequence as set forth in any one of SEQ ID NOs: 49 to 52 and 54 to 68, and a second peptide chain having an amino acid sequence as set forth in SEQ ID NO: 161 or 163.

[00147] In some optional embodiments of the present disclosure, as shown in FIG. 5C, the multi-specific antibody of the present disclosure includes: a first peptide chain having an amino acid sequence as set forth in any one of SEQ ID NOs: 142 to 160, a second peptide chain having an amino acid sequence as set forth in SEQ ID NO: 69, and a third peptide chain having an amino acid sequence as set forth in SEQ ID NO: 70; or a second peptide chain having an amino acid sequence as set forth in SEQ ID NO: 71, and a third peptide chain having an amino acid sequence as set forth in SEQ ID NO: 72.

[00148] In some optional embodiments of the present disclosure, as shown in FIG. 5D, the multi-specific antibody of the present disclosure includes: a first peptide chain having an amino acid sequence as set forth in any one of SEQ ID NOs: 142 to 160, and a second peptide chain having an amino acid sequence as set forth in SEQ ID NO: 161 or 163.

[00149] In some optional embodiments of the present disclosure, as shown in FIG. 5E, the multi-specific antibody of the present disclosure includes: a first peptide chain having an amino acid sequence as set forth in SEQ ID NO: 70, a third peptide chain having an amino acid sequence as set forth in SEQ ID NO: 69, and a fourth peptide chain having an amino acid sequence as set forth in SEQ ID NO: 70; or a first peptide chain having an amino acid sequence as set forth in SEQ ID NO: 72, a third peptide chain having an amino acid sequence as set forth in SEQ ID NO: 71, a fourth peptide chain having an amino acid sequence as set forth in SEQ ID

NO: 72; and a second peptide chain including a Fab VH-CH1 having an amino acid sequence as set forth in SEQ ID NO: 162 or 164 and a scFv having an amino acid sequence as set forth in SEQ ID NOs: 99 to 117, the C-terminus of the Fab VH-CH1 being linked to the N-terminus of the scFv.

5 **Conjugate**

[00150] The present disclosure provides a conjugate. The conjugate includes: the above-described antibody or antigen binding fragment thereof or the above-described multi-specific antibody; and a conjugate moiety, the conjugated moiety being linked to the antibody or antigen binding fragment thereof or the multi-specific antibody.

10 **[00151]** As described above, the anti-CD3 antibodies or antigen binding fragments thereof of the embodiments of the present disclosure can be used to prepare multi-specific antibody drug, such as bispecific antibody drug. On the one hand, the prepared bispecific antibody drug can be preferentially accumulated at the tumor site, increasing the local drug concentration at the tumor site and reducing the peripheral drug concentration, thereby reducing the risk of off-
15 target toxicity. On the other hand, the bispecific antibody can reduce the production of pro-inflammatory cytokines, reducing the risk of on-target toxicity of the bispecific antibody. Furthermore, the drugs prepared using the anti-CD3 antibodies or antigen binding fragments thereof can be used to prevent and/or a treat CD3-related disease. The CD3 bispecific antibody drugs developed based on the anti-CD3 antibodies or antigen binding fragments thereof are
20 safer and can be used to prevent and/or treat a cancer or tumor or infection or autoimmune disease. Therefore, the conjugate including the anti-CD3 antibody or antigen binding fragment thereof of the first aspect of the present disclosure and the multi-specific antibody of the second aspect of the present disclosure has good clinical application value and drug development value.

[00152] According to an embodiment of the present disclosure, the conjugated moiety
25 includes at least one selected from a carrier, a medicament, a toxin, a cytokine, a protein tag, a modifier, a therapeutic agent, or a chemotherapeutic agent.

Nucleic acid

[00153] The present disclosure provides a nucleic acid. The nucleic acid encodes the above-described antibody or antigen binding fragment thereof or the above-described multi-specific
30 antibody.

[00154] According to an embodiment of the present disclosure, the antibody or antigen binding fragment thereof of the first aspect of the present disclosure or the multi-specific antibody of the second aspect of the present disclosure encoded by the nucleic acid is suitable for developing a safer monoclonal antibody or bispecific antibody drug. Furthermore, the protein encoded by the nucleic acid can be used to prevent and/or treat a CD3-related disease or prevent and/or treat a cancer or tumor or infection or autoimmune disease.

[00155] It should be noted that, for the nucleic acid molecules described herein, those skilled in the art should understand that they actually include any one or both of the complementary double strands. For convenience, although only one chain is shown in most cases herein, the other chain complementary thereto is actually also disclosed. In addition, the molecular sequences in the present disclosure include DNA forms or RNA forms, and disclosure of one of them means that the other is also disclosed.

Vector or transformant

[00156] The present disclosure provides a vector or transformant. The vector or transformant contains the above-described nucleic acid. The vector or transformant may include optional control sequences operably linked to the nucleic acid molecule. The control sequence is one or more control sequences that can direct the expression of the nucleic acid molecule in the host. The vector or transformant thus constructed can effectively express the antibody or antigen binding fragment thereof of the first aspect of the present disclosure or the multi-specific antibody of the second aspect of the present disclosure, and can obtain the conjugate of the third aspect of the present disclosure.

[00157] When the above-described nucleic acid molecule is linked to the vector or transformant, such as an expression vector, the nucleic acid molecule can be directly or indirectly linked to the control elements on the expression vector, as long as these control elements can control the translation and expression of the nucleic acid molecule. Of course, these control elements may be derived directly from the vector itself, or they can be exogenous, that is, not from the vector itself. The nucleic acid molecule is operably linked to the control element.

[00158] According to an embodiment of the present disclosure, the vector may refer to a cloning vector or an expression vector, which may be obtained by operably linking the nucleic

acid to a commercially available vector (such as a plasmid or a viral vector). The vector in the present disclosure is not particularly limited, and commonly used plasmids can be used, such as pSeTag2, PEE14, pMH3, etc.

5 **[00159]** As used herein, the term "operably linked" means that the exogenous gene is linked to the vector. In this way, the control elements in the vector, such as transcription control sequences and translation control sequences, can play their intended function of regulating the transcription and translation of the exogenous gene. Commonly used vectors include viral vectors, plasmids, phages, and the like. After the expression vector according to some specific embodiments of the present disclosure is introduced into appropriate recipient cells, the
10 expression of the above-described nucleic acid molecules can be effectively achieved under the mediation of the regulatory system, thereby achieving large-scale *in vitro* production of the protein encoded by the nucleic acid molecules.

[00160] According to an embodiment of the present disclosure, the vector is a eukaryotic carrier or a prokaryotic carrier.

15 **[00161]** According to an embodiment of the present disclosure, the vector includes at least one selected from a plasmid vector, an adenovirus vector, a lentivirus vector, or an adeno-associated virus vector.

Cell

[00162] The present disclosure provides a cell. The cell carries the above-described nucleic acid, or the above-described vector or transformant; or expresses the above-described antibody
20 or antigen binding fragment thereof or the above-described multi-specific antibody. Thus, according to an embodiment of the present disclosure, the cells can efficiently express the antibody or antigen binding fragment thereof of the first aspect of the present disclosure or the multi-specific antibody of the second aspect of the present disclosure under suitable conditions.
25 Furthermore, an antibody or antigen binding fragment thereof or multi-specific antibody that can bind to human CD3 protein and has improved safety can be obtained.

[00163] According to an embodiment of the present disclosure, the cell is a prokaryotic cell, a eukaryotic cell, or a bacteriophage.

[00164] According to an embodiment of the present disclosure, the prokaryotic cell is
30 *Escherichia coli*, *Bacillus subtilis*, *Streptomyces*, or *Proteus mirabilis*.

[00165] According to an embodiment of the present disclosure, the eukaryotic cell is a fungus, an insect cell, a plant cell, or a mammalian cell.

[00166] According to an embodiment of the present disclosure, the fungus is *Pichia pastoris*, *Saccharomyces cerevisiae*, *Schizosaccharomyces pombe*, or *Trichoderma*.

5 [00167] According to an embodiment of the present disclosure, the insect cell is a *Spodoptera frugiperda* cell. According to an embodiment of the present disclosure, the plant cell is a tobacco plant cell. According to an embodiment of the present disclosure, the mammalian cell is a BHK cell, a CHO cell, a COS cell, a myeloma cell, or a human embryonic kidney 293 cell; and does not include animal germ cells, fertilized eggs, or embryonic stem
10 cells.

[00168] According to an embodiment of the present disclosure, the cell is a mammalian cell.

[00169] According to an embodiment of the present disclosure, the cell is a BHK cell, a CHO cell, a COS cell, or a NSO cell.

[00170] It should be noted that the "suitable conditions" described in the specification of the
15 present disclosure refer to conditions suitable for the expression of the antibody or antigen binding fragment thereof or multi-specific antibody of the present disclosure. It is readily understood by those skilled in the art that conditions suitable for the expression of antibodies or antigen binding fragments thereof or multi-specific antibodies include, but are not limited to, suitable transformation or transfection methods, suitable transformation or transfection
20 conditions, healthy host cell status, suitable host cell density, suitable cell culture environment, and suitable cell culture time. The "suitable conditions" are not particularly limited, and those skilled in the art can optimize the most suitable conditions for expressing the recombinant antibody according to the specific environment of the laboratory.

Pharmaceutical composition

25 [00171] The present disclosure provides a pharmaceutical composition. The pharmaceutical composition includes: the above-described antibody or antigen binding fragment thereof, the above-described multi-specific antibody, the above-described conjugate, the above-described nucleic acid, or the above-described vector or transformant. Thus, the obtained medicament can be further used for the prevention and/or treatment of a CD3-related disease, or further used for
30 the prevention and/or treatment of a CD3-related disease and/or cancer or tumor or infection or

autoimmune disease.

[00172] According to an embodiment of the present disclosure, the composition further includes a pharmaceutically acceptable adjuvant.

[00173] According to an embodiment of the present disclosure, the adjuvant includes: one
5 or more pharmaceutically acceptable excipients, diluents, stabilizers, or carriers.

[00174] According to an embodiment of the present disclosure, the pharmaceutical composition is an injection.

[00175] It should be noted that the pharmaceutical composition includes combinations separated in time and/or space, as long as they can work together to achieve the purpose of the
10 present disclosure. For example, the components contained in the composition can be administered to the subject as a whole, or separately. When the components contained in the composition are administered to the subject separately, the individual components can be administered to the subject simultaneously or sequentially.

[00176] The pharmaceutical composition of the present disclosure contains a safe and
15 effective amount of the active ingredient of the present disclosure and pharmaceutically acceptable adjuvants. Such adjuvants include, but are not limited to, saline, buffer, dextrose, water, glycerol, ethanol, and combinations thereof. Generally, pharmaceutical preparations should be compatible with the mode of administration. The dosage forms of the pharmaceutical composition of the present disclosure are injections, oral preparations (tablets, capsules, oral
20 liquids), transdermal preparations, and sustained-release preparations. For example, it can be prepared by conventional methods using physiological saline or an aqueous solution containing glucose and other adjuvants. The pharmaceutical composition is preferably manufactured under sterile conditions.

[00177] The effective amount of the active ingredient of the present disclosure may vary
25 depending on the mode of administration and the severity of the disease to be treated. The selection of the preferred effective amount can be determined by those skilled in the art based on various factors (e.g., through clinical trials). The factors include, but are not limited to: pharmacokinetic parameters of the active ingredient, such as bioavailability, metabolism, half-life, etc.; severity of the disease to be treated, weight of the patient, immune status of the patient,
30 route of administration, etc. For example, several divided doses may be administered daily or

the dose may be proportionally reduced depending on the exigencies of the therapeutic situation.

[00178] The pharmaceutically acceptable adjuvants of the present disclosure include (but are not limited to): water, saline, liposomes, lipids, proteins, protein-antibody conjugates, peptides, cellulose, nanogels, or combinations thereof. The selection of the carrier should be compatible with the mode of administration, which is well known to those skilled in the art.

Kit

[00179] The present disclosure provides a kit. The kit includes the above-described antibody or antigen binding fragment thereof or the above-described multi-specific antibody. As described above, the antibodies or antigen binding fragments thereof according to the embodiments of the present disclosure can effectively and specifically bind to CD3. The CD3-related kit developed by utilizing this property can be used in CD3-related research.

[00180] The kit can effectively detect, enrich, or separate and purify CD3 in biological samples, and is further used in scientific research, such as qualitatively or quantitatively detecting CD3 protein molecules in biological samples. More specifically, it can be used in kits for immunoblotting, immunoprecipitation, and the like that involve detection using the specific binding properties of CD3 and antibodies or binding fragments thereof. These kits may contain any one or more of the following: antagonists, CD3 antibodies, or medicament reference materials; protein purification columns; immunoglobulin affinity purification buffers; and cell assay diluents. CD3 antibodies can be used in various types of diagnostic tests, such as in vitro or in vivo detection of the presence of various diseases, or medicaments, toxins, or other proteins. For example, CD3-related diseases can be detected by testing the serum or blood of the subject.

Use in the manufacture of a kit

[00181] The present disclosure provides use of the above-described antibody or antigen binding fragment thereof or the above-described multi-specific antibody in the manufacture of a kit for detecting CD3.

[00182] As described above, the antibodies or antigen binding fragments thereof according to the embodiments of the present disclosure can specifically bind to CD3. Therefore, the antibodies or antigen binding fragments thereof can be used to detect CD3. Furthermore, it can be used to prepare CD3-related kits and used in scientific research, such as qualitative or

quantitative detection of CD3 protein molecules in biological samples. More specifically, it can be used in kits for immunoblotting, immunoprecipitation, and the like that involve detection using the specific binding properties of CD3 and antibodies or binding fragments thereof. These kits may contain any one or more of the following: antagonists, CD3 antibodies, or medicament
5 reference materials; protein purification columns; immunoglobulin affinity purification buffers; and cell assay diluents. CD3 antibodies or antigen fragments can be used in various types of diagnostic tests, such as in vitro or in vivo detection of the presence of various diseases or medicaments, toxins or other proteins. For example, CD3-related diseases can be detected by testing the serum or blood of the subject.

10 **Use in the manufacture of a medicament**

[00183] The present disclosure provides use of the above-described antibody or antigen binding fragment thereof, the above-described multi-specific antibody, the above-described conjugate, the above-described nucleic acid, the above-described vector or transformant, or the above-described pharmaceutical composition in the manufacture of a medicament for
15 regulating an immune response and/or inhibiting tumor growth, or for preventing and/or treating a CD3-related disease and/or cancer or tumor or infection or autoimmune disease.

[00184] According to an embodiment of the present disclosure, the cancer or tumor or infection or autoimmune disease is a Trop2- or PDL1-related disease.

[00185] According to an embodiment of the present disclosure, the cancer or tumor is at least
20 one of lung cancer, liver cancer, ovarian cancer, cervical cancer, skin cancer, bladder cancer, colon cancer, rectal cancer, breast cancer, pancreatic cancer, prostate cancer, glioma, renal cancer, gastric cancer, esophageal cancer, oral squamous cell carcinoma, or head-and-neck cancer

[00186] According to an embodiment of the present disclosure, the cancer or tumor is at least
25 one of non-small cell lung cancer, malignant melanoma, renal cell carcinoma, head-and-neck squamous cell carcinoma, urothelial carcinoma, colorectal cancer, liver cancer, or classical Hodgkin's lymphoma.

A method for treating diseases

[00187] The present disclosure provides a method for regulating an immune response and/or
30 inhibiting tumor growth or preventing and/or treating a CD3-related disease and/or cancer,

tumor, infection, or autoimmune disease.

[00188] According to an embodiment of the present disclosure, the method includes: administering to a subject a pharmaceutically acceptable amount of the above-described antibody or antigen binding fragment thereof, the above-described multi-specific antibody, the
5 above-described conjugate, the above-described nucleic acid, the above-described vector or transformant, or the above-described pharmaceutical composition.

[00189] It should be noted that the terms "subject", "individual", and "patient" may be used interchangeably herein, and refer to a mammal being evaluated for treatment and/or being treated. In one embodiment, the mammal is a human. The terms "subject", "individual", and
10 "patient" include, but are not limited to, individuals with cancer, individuals with autoimmune diseases, individuals with pathogen infection, and the like. The subject can be a human, but also includes other mammals, particularly mammals that can be used as laboratory models of human disease, such as mice, rats, etc.

[00190] The effective amount of the antibody or antigen binding fragment thereof, multi-specific antibody, conjugate, nucleic acid, vector or transformant, or pharmaceutical
15 composition of the present disclosure may vary depending on the mode of administration and the severity of the disease to be treated. The selection of the preferred effective amount can be determined by those skilled in the art based on various factors (e.g., through clinical trials). The factors include, but are not limited to: pharmacokinetic parameters of the active ingredient such
20 as bioavailability, metabolism, half-life, etc.; severity of the disease to be treated, weight of the patient, immune status of the patient, route of administration, etc. For example, several divided doses may be administered daily or the dose may be proportionally reduced depending on the exigencies of the therapeutic situation.

[00191] According to an embodiment of the present disclosure, the cancer or tumor or
25 infection or autoimmune disease is a Trop2- or PDL1-related disease.

[00192] According to an embodiment of the present disclosure, the cancer or tumor is at least one of lung cancer, liver cancer, ovarian cancer, cervical cancer, skin cancer, bladder cancer, colon cancer, rectal cancer, breast cancer, pancreatic cancer, prostate cancer, glioma, renal cancer, gastric cancer, esophageal cancer, oral squamous cell carcinoma, or head-and-neck
30 cancer.

[00193] According to an embodiment of the present disclosure, the cancer or tumor is at least one of non-small cell lung cancer, malignant melanoma, renal cell carcinoma, head-and-neck squamous cell carcinoma, urothelial carcinoma, colorectal cancer, liver cancer, or classical Hodgkin's lymphoma.

5 **[00194]** The description of the sequences involved in the present disclosure are detailed in Table 1.

[00195] [Table 1] Amino acid sequence description table

SEQ ID NO:	Sequence	Description
1	GFTFNTYA	Cross303/307/308/309/310/311/312/313/314/316/317/318/323/325/326/Cross3ZH07/Cross3ZH09/Cross3ZH16/Cross3ZH23 HCDR1
2	IRSKYNNYAT	Cross303/307/308/309/310/311/312/313/314/316/317/318/323/325/326/Cross3ZH07/Cross3ZH09/Cross3ZH16/Cross3ZH23 HCDR2
3	VRHGNFGNSYQSWFAY	Cross313 HCDR3
4	TGAVTTSNY	Cross303/307/308/309/310/311/312/313/314/316/317/318/323/325/326/Cross3ZH07/Cross3ZH09/Cross3ZH16/Cross3ZH23 LCDR1
5	GTN	Cross303/307/308/309/310/311/312/313/314/316/317/318/323/325/326/Cross3ZH07/Cross3ZH09/Cross3ZH16/Cross3ZH23 LCDR2
6	ALWYSNLWV	Cross303/307/308/309/310/311/312/313/314/316/317/318 LCDR3
7	VRHGNFGNSYVSWFLY	Cross316 HCDR3
8	VRHGNFGNSYVSWFAY	Cross325/323/326/Cross3ZH16 HCDR3
9	ALWYSNDWV	Cross325 LCDR3
10	EVQLLESGGGLVQPGGSLKLSCAASGFTFNTYAMNWVRQAPGKGLEWVARIRSKYNNYATYYADSVKDRFTISRDDSKNTAYLQMNNLKTEDTAVYYCVRHGNFGNSYQSWFAYWGQGLTVTVSS	Cross313 VH
11	ELVVVTQEPSLTVSPGGTVTLTCSRSTGAVTTSNYANWVQKPGQAPRGLIGGTNKRAPGTPARFSGSLLGGKAALTLSGVQPEDEAEYYCALWYSNLWVFGGGTKLTVL	Cross3/303/307/308/309/310/311/312/313/314//316/317/318 VL
12	EVQLLESGGGLVQPGGSLKLSCAASGFTFNTYAMNWVRQAPGKGLEWVARIRSKYNNYATYYADSVKDRFTISRDDSKNTAYLQMNNLKTEDTAVYYCVRHGNFGNSYVSWFLYWGQGLTVTVSS	Cross316 VH
13	EVQLLESGGGLVQPGGSLKLSCAASGFTFNTYAMNWVRQAPGKGLEWVARIRSKYNNYATYYADSVKDRFTISRDDSKNTAYLQMNNLKTEDTAVYYCVRHGNFGNSYVSWFAYWGQGLTVTVSS	Cross3/323/325/326/ZH16 VH

14	ELVVTQEPSLTVSPGGTVTLTCSRSTGAVTTSNYANWVQQKPGQAPRGLIGGTN KRAPGTPARFSGSLLGGKAALTLSGVQPEDEAEYYCALWYSNDWVFGGGTKL TVL	Cross325 VL
15	EVQLES GGGLVQPGGSLKLSCAASGFTFNTYAMNWVRQAPGKGLEWVARIR SKYNNYATYYADSVKDRFTISRDDSKNTAYLQMNNLKTEDTAVYYCVRHGMF GNSYVSWFAYWGQGLTVTVSS	Cross303/ZH07 VH
16	EVQLES GGGLVQPGGSLKLSCAASGFTFNTYAMNWVRQAPGKGLEWVARIR SKYNNYATYYADSVKDRFTISRDDSKNTAYLQMNNLKTEDTAVYYCVRHGMF GNEYVSWFAYWGQGLTVTVSS	Cross307 VH
17	EVQLES GGGLVQPGGSLKLSCAASGFTFNTYAMNWVRQAPGKGLEWVARIR SKYNNYATYYADSVKDRFTISRDDSKNTAYLQMNNLKTEDTAVYYCVRHGMF GNPYVSWFAYWGQGLTVTVSS	Cross308 VH
18	EVQLES GGGLVQPGGSLKLSCAASGFTFNTYAMNWVRQAPGKGLEWVARIR SKYNNYATYYADSVKDRFTISRDDSKNTAYLQMNNLKTEDTAVYYCVRHGMF GNVYVSWFAYWGQGLTVTVSS	Cross309 VH
19	EVQLES GGGLVQPGGSLKLSCAASGFTFNTYAMNWVRQAPGKGLEWVARIR SKYNNYATYYADSVKDRFTISRDDSKNTAYLQMNNLKTEDTAVYYCVRHGMF GNYYVSWFAYWGQGLTVTVSS	Cross310 VH
20	EVQLES GGGLVQPGGSLKLSCAASGFTFNTYAMNWVRQAPGKGLEWVARIR SKYNNYATYYADSVKDRFTISRDDSKNTAYLQMNNLKTEDTAVYYCVRHGMF GNSEVSWFAYWGQGLTVTVSS	Cross311 VH
21	EVQLES GGGLVQPGGSLKLSCAASGFTFNTYAMNWVRQAPGKGLEWVARIR SKYNNYATYYADSVKDRFTISRDDSKNTAYLQMNNLKTEDTAVYYCVRHGMF GNSYHSWFAYWGQGLTVTVSS	Cross312 VH
22	EVQLES GGGLVQPGGSLKLSCAASGFTFNTYAMNWVRQAPGKGLEWVARIR SKYNNYATYYADSVKDRFTISRDDSKNTAYLQMNNLKTEDTAVYYCVRHGMF GNSYSSWFAYWGQGLTVTVSS	Cross314 VH
23	EVQLES GGGLVQPGGSLKLSCAASGFTFNTYAMNWVRQAPGKGLEWVARIR SKYNNYATYYADSVKDRFTISRDDSKNTAYLQMNNLKTEDTAVYYCVRHGMF GNSYVSWFAGWGQGLTVTVSS	Cross317 VH
24	EVQLES GGGLVQPGGSLKLSCAASGFTFNTYAMNWVRQAPGKGLEWVARIR SKYNNYATYYADSVKDRFTISRDDSKNTAYLQMNNLKTEDTAVYYCVRHGMF GNSYVSWFAMWGQGLTVTVSS	Cross318 VH
25	ELVVTQEPSLTVSPGGTVTLTCSRSTGAVTTSNYANWVQQKPGQAPRGLIGGTN KRAPGTPARFSGSLLGGKAALTLSGVQPEDEAEYYCALWYPNLWVFGGGTKL TVL	Cross323 VL
26	ELVVTQEPSLTVSPGGTVTLTCSRSTGAVTTSNYANWVQQKPGQAPRGLIGGTN KRAPGTPARFSGSLLGGKAALTLSGVQPEDEAEYYCALWYSPWVFGGGTKL TVL	Cross326/ZH23 VL
27	EVQLES GGGLVQPGGSLKLSCAASGFTFNTYAMNWVRQAPGKGLEWVARIR SKYNNYATYYADSVKDRFTISRDDSKNTAYLQMNNLKTEDTAVYYCVRHGMF GNSYVSWFAYWGQGLTVTVSS	Cross3ZH07 VH
28	ELVVTQEPSLTVSPGGTVTLTCSRSTGAVTTSNYANWVQQKPGQAPRGLIGGTN KRAPGTPARFSGSLLGGKAALTLSGVQPEDEAEYYCALWYPNPWVFGGGTKL TVL	Cross3ZH07/ZH09 VL
29	EVQLES GGGLVQPGGSLKLSCAASGFTFNTYAMNWVRQAPGKGLEWVARIR SKYNNYATYYADSVKDRFTISRDDSKNTAYLQMNNLKTEDTAVYYCVRHGMF GNSYVSWFLYWGQGLTVTVSS	Cross3ZH09 VH
30	ELVVTQEPSLTVSPGGTVTLTCSRSTGAVTTSNYANWVQQKPGQAPRGLIGGTN KRAPGTPARFSGSLLGGKAALTLSGVQPEDEAEYYCALWYPNDWVFGGGTKL TVL	Cross3ZH16 VL
31	EVQLES GGGLVQPGGSLKLSCAASGFTFNTYAMNWVRQAPGKGLEWVARIR SKYNNYATYYADSVKDRFTISRDDSKNTAYLQMNNLKTEDTAVYYCVRHGMF GNDYVSWFAYWGQGLTVTVSS	Cross3ZH23 VH

32	EVQLLESGGGLVQPGGSLKLSCAASGFTFTNTYAMNWVRQAPGKGLEWVARIR SKYNNYATYYADSVKDRFTISRDDSKNTAYLQMNNLKTEDTAVYYCVRHGNTF GNSYQSWFAYWGQGLTVTVSSASTKGPSVFPLAPSSKSTSGGTAALGCLVKDY FPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVTVPSSSLGTQTYICNVNH KPSNTKVDKKVEPKSCDKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEV TCVVDVSDHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLH QDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSREEMTKNQV SLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRW QQGNVFSCSVMHEALHNHYTQKSLSLSPGK	Cross313 heavy chain
33	ELVVTQEPSTLVSPGGTVTLTCSRSTGAVTTSNYANWVQQKPGQAPRGLIGGTN KRAPGTPARFSGSLLGGKAALTLSGVQPEDEAEYYCALWYSNLWVFGGGTKL TVLRTVAAPSVFIFPPSDEQLKSGTASVVCLLNNFYPREAKVQWKVDNALQSG NSQESVTEQDSKDYSLSSLTLSKADYEKHKVYACEVTHQGLSSPVTKSFNR GEC	Cross3/303/307/308/309/310/3 11/312/313/314//316/317/318 light chain
34	EVQLLESGGGLVQPGGSLKLSCAASGFTFTNTYAMNWVRQAPGKGLEWVARIR SKYNNYATYYADSVKDRFTISRDDSKNTAYLQMNNLKTEDTAVYYCVRHGNTF GNSYVSWFLYWGQGLTVTVSSASTKGPSVFPLAPSSKSTSGGTAALGCLVKDY FPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVTVPSSSLGTQTYICNVNH KPSNTKVDKKVEPKSCDKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEV TCVVDVSDHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLH QDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSREEMTKNQV SLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRW QQGNVFSCSVMHEALHNHYTQKSLSLSPGK	Cross316 heavy chain
35	EVQLLESGGGLVQPGGSLKLSCAASGFTFTNTYAMNWVRQAPGKGLEWVARIR SKYNNYATYYADSVKDRFTISRDDSKNTAYLQMNNLKTEDTAVYYCVRHGNTF GNSYVSWFAYWGQGLTVTVSSASTKGPSVFPLAPSSKSTSGGTAALGCLVKDY FPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVTVPSSSLGTQTYICNVNH KPSNTKVDKKVEPKSCDKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEV TCVVDVSDHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLH QDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSREEMTKNQV SLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRW QQGNVFSCSVMHEALHNHYTQKSLSLSPGK	Cross3/323/325/326 heavy chain
36	ELVVTQEPSTLVSPGGTVTLTCSRSTGAVTTSNYANWVQQKPGQAPRGLIGGTN KRAPGTPARFSGSLLGGKAALTLSGVQPEDEAEYYCALWYSNDWVFGGGTKL TVLRTVAAPSVFIFPPSDEQLKSGTASVVCLLNNFYPREAKVQWKVDNALQSG NSQESVTEQDSKDYSLSSLTLSKADYEKHKVYACEVTHQGLSSPVTKSFNR GEC	Cross325 light chain
37	EVQLLESGGGLVQPGGSLKLSCAASGFTFTNTYAMNWVRQAPGKGLEWVARIR SKYNNYATYYADSVKDRFTISRDDSKNTAYLQMNNLKTEDTAVYYCVRHGMF GNSYVSWFAYWGQGLTVTVSSASTKGPSVFPLAPSSKSTSGGTAALGCLVKDY FPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVTVPSSSLGTQTYICNVNH KPSNTKVDKKVEPKSCDKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEV TCVVDVSDHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLH QDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSREEMTKNQV SLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRW QQGNVFSCSVMHEALHNHYTQKSLSLSPGK	Cross303 heavy chain
38	EVQLLESGGGLVQPGGSLKLSCAASGFTFTNTYAMNWVRQAPGKGLEWVARIR SKYNNYATYYADSVKDRFTISRDDSKNTAYLQMNNLKTEDTAVYYCVRHGNTF GNEYVSWFAYWGQGLTVTVSSASTKGPSVFPLAPSSKSTSGGTAALGCLVKDY FPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVTVPSSSLGTQTYICNVNH KPSNTKVDKKVEPKSCDKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEV TCVVDVSDHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLH QDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSREEMTKNQV SLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRW QQGNVFSCSVMHEALHNHYTQKSLSLSPGK	Cross307 heavy chain

39	EVQLLESGGGLVQPGGSLKLSCAASGFTFNTYAMNWVRQAPGKGLEWVARIR SKYNNYATYYADSVKDRFTISRDDSKNTAYLQMNNLKTEDTAVYYCVRHGNF GNPYVSWFAYWGQGLTVTVSSASTKGPSVFPLAPSSKSTSGGTAALGCLVKDY FPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVTVPSSSLGTQTYICNVNH KPSNTKVDKKVEPKSCDKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEV TCVVDVSDHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLH QDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSREEMTKNQV SLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRW QQGNVFSCSVMHEALHNHYTQKSLSLSPGK	Cross308 heavy chain
40	EVQLLESGGGLVQPGGSLKLSCAASGFTFNTYAMNWVRQAPGKGLEWVARIR SKYNNYATYYADSVKDRFTISRDDSKNTAYLQMNNLKTEDTAVYYCVRHGNF GNVYVSWFAYWGQGLTVTVSSASTKGPSVFPLAPSSKSTSGGTAALGCLVKDY FPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVTVPSSSLGTQTYICNVNH KPSNTKVDKKVEPKSCDKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEV TCVVDVSDHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLH QDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSREEMTKNQV SLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRW QQGNVFSCSVMHEALHNHYTQKSLSLSPGK	Cross309 heavy chain
41	EVQLLESGGGLVQPGGSLKLSCAASGFTFNTYAMNWVRQAPGKGLEWVARIR SKYNNYATYYADSVKDRFTISRDDSKNTAYLQMNNLKTEDTAVYYCVRHGNF GNVYVSWFAYWGQGLTVTVSSASTKGPSVFPLAPSSKSTSGGTAALGCLVKDY FPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVTVPSSSLGTQTYICNVNH KPSNTKVDKKVEPKSCDKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEV TCVVDVSDHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLH QDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSREEMTKNQV SLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRW QQGNVFSCSVMHEALHNHYTQKSLSLSPGK	Cross310 heavy chain
42	EVQLLESGGGLVQPGGSLKLSCAASGFTFNTYAMNWVRQAPGKGLEWVARIR SKYNNYATYYADSVKDRFTISRDDSKNTAYLQMNNLKTEDTAVYYCVRHGNF GNSEVSWFAYWGQGLTVTVSSASTKGPSVFPLAPSSKSTSGGTAALGCLVKDY FPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVTVPSSSLGTQTYICNVNH KPSNTKVDKKVEPKSCDKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEV TCVVDVSDHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLH QDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSREEMTKNQV SLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRW QQGNVFSCSVMHEALHNHYTQKSLSLSPGK	Cross311 heavy chain
43	EVQLLESGGGLVQPGGSLKLSCAASGFTFNTYAMNWVRQAPGKGLEWVARIR SKYNNYATYYADSVKDRFTISRDDSKNTAYLQMNNLKTEDTAVYYCVRHGNF GNSYHSWFAYWGQGLTVTVSSASTKGPSVFPLAPSSKSTSGGTAALGCLVKDY FPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVTVPSSSLGTQTYICNVNH KPSNTKVDKKVEPKSCDKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEV TCVVDVSDHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLH QDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSREEMTKNQV SLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRW QQGNVFSCSVMHEALHNHYTQKSLSLSPGK	Cross312 heavy chain
44	EVQLLESGGGLVQPGGSLKLSCAASGFTFNTYAMNWVRQAPGKGLEWVARIR SKYNNYATYYADSVKDRFTISRDDSKNTAYLQMNNLKTEDTAVYYCVRHGNF GNSYSSWFAYWGQGLTVTVSSASTKGPSVFPLAPSSKSTSGGTAALGCLVKDY FPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVTVPSSSLGTQTYICNVNH KPSNTKVDKKVEPKSCDKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEV TCVVDVSDHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLH QDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSREEMTKNQV SLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRW QQGNVFSCSVMHEALHNHYTQKSLSLSPGK	Cross314 heavy chain

45	EVQLES GGG LVQP GGS LKLS CAAS GFT FNTYAMNWVRQAPGKGLEWVARIR SKYNNYATYYADSVKDRFTISRDDSKNTAYLQMNNLKTEDTAVYYCVRHGNTF GNSYVSWFAGWGQGLTVTVSSASTKGPSVFLAPSSKSTSGGTAALGCLVKDY FPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVTVPSSSLGTQTYICNVNH KPSNTKVDKKVEPKSCDKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEV TCVVDVSDHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLH QDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSREEMTKNQV SLTCLVKGFYPSDIAVEWESNGQPENNYKTTPVLDS DGSFFLYSKLTVDKSRW QQGNVFSCSVMHEALHNHYTQKSLSLSPGK	Cross317 heavy chain
46	EVQLES GGG LVQP GGS LKLS CAAS GFT FNTYAMNWVRQAPGKGLEWVARIR SKYNNYATYYADSVKDRFTISRDDSKNTAYLQMNNLKTEDTAVYYCVRHGNTF GNSYVSWFAMWGQGLTVTVSSASTKGPSVFLAPSSKSTSGGTAALGCLVKDY FPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVTVPSSSLGTQTYICNVNH KPSNTKVDKKVEPKSCDKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEV TCVVDVSDHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLH QDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSREEMTKNQV SLTCLVKGFYPSDIAVEWESNGQPENNYKTTPVLDS DGSFFLYSKLTVDKSRW QQGNVFSCSVMHEALHNHYTQKSLSLSPGK	Cross318 heavy chain
47	ELVVTQEP SLTVSPGGTVTLTCSRSTGAVTTSNYANWVQQKPGQAPRGLIGGTN KRAPGTPARFSGSLLGGKAALTLSGVQPEDEAEYYCALWYNLWVFGGGTKL TVLRTVAAPSVFIFPPSDEQLKSGTASVCLLNNFYPREAKVQWKVDNALQSG NSQESVTEQDSKSTYSLSTLTLSKADYEKHKVYACEVTHQGLSSPVTKSFNR GEC	Cross323 light chain
48	ELVVTQEP SLTVSPGGTVTLTCSRSTGAVTTSNYANWVQQKPGQAPRGLIGGTN KRAPGTPARFSGSLLGGKAALTLSGVQPEDEAEYYCALWYNLWVFGGGTKL TVLRTVAAPSVFIFPPSDEQLKSGTASVCLLNNFYPREAKVQWKVDNALQSG NSQESVTEQDSKSTYSLSTLTLSKADYEKHKVYACEVTHQGLSSPVTKSFNR GEC	Cross326 light chain
49	EVQLES GGG LVQP GGS LKLS CAAS GFT FNTYAMNWVRQAPGKGLEWVARIR SKYNNYATYYADSVKDRFTISRDDSKNTAYLQMNNLKTEDTAVYYCVRHGNTF GNSYQSWFAYWGQGLTVTVSSGGGGSGGGSGGGGSELVVTQEP SLTVSPGG TVTLTCSRSTGAVTTSNYANWVQQKPGQAPRGLIGGTNKRAPGTPARFSGSLL GGKAALTLSGVQPEDEAEYYCALWYNLWVFGGGTKLTVLGGGGSPKSSDKT HTCPPCPAEEAAGGPSVFLFPPKPKDTLMISRTPEVTCVVDVSDHEDPEVKFNW YVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKAL PAPIEKTISKAKGQPREPQVYTLPPCREEMTKNQVSLWCLVKGFYPSDIAVEWE SNGQPENNYKTTPVLDS DGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHN HYTQKSLSLSPGK	Cross313-kbss
50	EVQLES GGG LVQP GGS LKLS CAAS GFT FNTYAMNWVRQAPGKGLEWVARIR SKYNNYATYYADSVKDRFTISRDDSKNTAYLQMNNLKTEDTAVYYCVRHGNTF GNSYVSWFLYWGQGLTVTVSSGGGGSGGGSGGGGSELVVTQEP SLTVSPGG TVTLTCSRSTGAVTTSNYANWVQQKPGQAPRGLIGGTNKRAPGTPARFSGSLL GGKAALTLSGVQPEDEAEYYCALWYNLWVFGGGTKLTVLGGGGSPKSSDKT HTCPPCPAEEAAGGPSVFLFPPKPKDTLMISRTPEVTCVVDVSDHEDPEVKFNW YVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKAL PAPIEKTISKAKGQPREPQVYTLPPCREEMTKNQVSLWCLVKGFYPSDIAVEWE SNGQPENNYKTTPVLDS DGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHN HYTQKSLSLSPGK	Cross316-kbss
51	EVQLES GGG LVQP GGS LKLS CAAS GFT FNTYAMNWVRQAPGKGLEWVARIR SKYNNYATYYADSVKDRFTISRDDSKNTAYLQMNNLKTEDTAVYYCVRHGNTF GNSYVSWFAYWGQGLTVTVSSGGGGSGGGSGGGGSELVVTQEP SLTVSPGG TVTLTCSRSTGAVTTSNYANWVQQKPGQAPRGLIGGTNKRAPGTPARFSGSLL GGKAALTLSGVQPEDEAEYYCALWYNLWVFGGGTKLTVLGGGGSPKSSDKT HTCPPCPAEEAAGGPSVFLFPPKPKDTLMISRTPEVTCVVDVSDHEDPEVKFNW YVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKAL PAPIEKTISKAKGQPREPQVYTLPPCREEMTKNQVSLWCLVKGFYPSDIAVEWE SNGQPENNYKTTPVLDS DGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHN HYTQKSLSLSPGK	Cross325-kbss

52	EVQLES GGGGLVQPGGSLKLSCAASGFTFNTYAMNWVRQAPGKGLEWVARIR SKYNNYATYYADSVKDRFTISRDDSKNTAYLQMNNLKTEDTAVYYCVRHGNTF GNSYVSWFAYWGQGLTVTVSSGGGGSGGGGSGGGGSELVVTQEPSLTVSPGG TVTLTCRSSTGAVTTSNYANWVQQKPGQAPRGLIGGTNKRAPGTPARFSGSLL GGKAALTL SGVQPEDEAEYYCALWYSNLWVFGGGTKLTVLGGGGSPKSSDKT HTCPPCPAPEAAGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNW YVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKAL PAPIEKTISKAKGQPREPQVYTLPPCREEMTKNQVSLWCLVKGFYPSDIAVEWE SNGQPENNYKTTTPVLDSGDSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHN HYTQKSLSLSPGK	Cross3-kbss
53	EVQLES GGGGLVQPGGSLKLSCAASGFTFNTYAMNWVRQAPGKGLEWVARIR SKYNNYATYYADSVKDRFTISRDDSKNTAYLQMNNLKTEDTAVYYCVRHGNTF GNSYVSWFAYWGQGLTVTVSSGGGGSGGGGSGGGGSELVVTQEPSLTVSPGG TVTLTCRSSTGAVTTSNYANWVQQKPGQAPRGLIGGTNKRAPGTPARFSGSLL GGKAALTL SGVQPEDEAEYYCALWYSNLWVFGGGTKLTVLGGGGSPKSSDKT HTCPPCPAPEAAGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNW YVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKAL PAPIEKTISKAKGQPREPQVYTLPPCREEMTKNQVSLWCLVKGFYPSDIAVEWE SNGQPENNYKTTTPVLDSGDSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHN HYTQKSLSLSPGK	Cross303-kbss
54	EVQLES GGGGLVQPGGSLKLSCAASGFTFNTYAMNWVRQAPGKGLEWVARIR SKYNNYATYYADSVKDRFTISRDDSKNTAYLQMNNLKTEDTAVYYCVRHGNTF GNEYVSWFAYWGQGLTVTVSSGGGGSGGGGSGGGGSELVVTQEPSLTVSPGG TVTLTCRSSTGAVTTSNYANWVQQKPGQAPRGLIGGTNKRAPGTPARFSGSLL GGKAALTL SGVQPEDEAEYYCALWYSNLWVFGGGTKLTVLGGGGSPKSSDKT HTCPPCPAPEAAGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNW YVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKAL PAPIEKTISKAKGQPREPQVYTLPPCREEMTKNQVSLWCLVKGFYPSDIAVEWE SNGQPENNYKTTTPVLDSGDSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHN HYTQKSLSLSPGK	Cross307-kbss
55	EVQLES GGGGLVQPGGSLKLSCAASGFTFNTYAMNWVRQAPGKGLEWVARIR SKYNNYATYYADSVKDRFTISRDDSKNTAYLQMNNLKTEDTAVYYCVRHGNTF GNPYVSWFAYWGQGLTVTVSSGGGGSGGGGSGGGGSELVVTQEPSLTVSPGG TVTLTCRSSTGAVTTSNYANWVQQKPGQAPRGLIGGTNKRAPGTPARFSGSLL GGKAALTL SGVQPEDEAEYYCALWYSNLWVFGGGTKLTVLGGGGSPKSSDKT HTCPPCPAPEAAGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNW YVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKAL PAPIEKTISKAKGQPREPQVYTLPPCREEMTKNQVSLWCLVKGFYPSDIAVEWE SNGQPENNYKTTTPVLDSGDSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHN HYTQKSLSLSPGK	Cross308-kbss
56	EVQLES GGGGLVQPGGSLKLSCAASGFTFNTYAMNWVRQAPGKGLEWVARIR SKYNNYATYYADSVKDRFTISRDDSKNTAYLQMNNLKTEDTAVYYCVRHGNTF GNVYVSWFAYWGQGLTVTVSSGGGGSGGGGSGGGGSELVVTQEPSLTVSPGG TVTLTCRSSTGAVTTSNYANWVQQKPGQAPRGLIGGTNKRAPGTPARFSGSLL GGKAALTL SGVQPEDEAEYYCALWYSNLWVFGGGTKLTVLGGGGSPKSSDKT HTCPPCPAPEAAGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNW YVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKAL PAPIEKTISKAKGQPREPQVYTLPPCREEMTKNQVSLWCLVKGFYPSDIAVEWE SNGQPENNYKTTTPVLDSGDSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHN HYTQKSLSLSPGK	Cross309-kbss

57	EVQLES GGGGLVQPGGSLKLSCAASGFTFNTYAMNWVRQAPGKGLEWVARIR SKYNNYATYYADSVKDRFTISRDDSKNTAYLQMNNLKTEDTAVYYCVRHGNTF GNYVSWFAYWGQGLTVTVSSGGGGSGGGSGGGGSELVVTQEPSLTVSPGG TVTLTCRSSTGAVTTSNYANWVQQKPGQAPRGLIGGTNKRAPGTPARFSGSLL GGKAALTL SGVQPEDEAEYYCALWYSNLWVFGGGTKLTVLGGGGSPKSSDKT HTCPPCPAPEAAGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNW YVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKAL PAPIEKTISKAKGQPREPQVYTLPPCREEMTKNQVSLWCLVKGFYPSDIAVEWE SNGQPENNYKTTTPVLDSGDSFFLYSKLTVDKSRWQQGNVFCFSVMHEALHN HYTQKSLSLSPGK	Cross310-kbss
58	EVQLES GGGGLVQPGGSLKLSCAASGFTFNTYAMNWVRQAPGKGLEWVARIR SKYNNYATYYADSVKDRFTISRDDSKNTAYLQMNNLKTEDTAVYYCVRHGNTF GNSEVSWFAYWGQGLTVTVSSGGGGSGGGSGGGGSELVVTQEPSLTVSPGG TVTLTCRSSTGAVTTSNYANWVQQKPGQAPRGLIGGTNKRAPGTPARFSGSLL GGKAALTL SGVQPEDEAEYYCALWYSNLWVFGGGTKLTVLGGGGSPKSSDKT HTCPPCPAPEAAGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNW YVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKAL PAPIEKTISKAKGQPREPQVYTLPPCREEMTKNQVSLWCLVKGFYPSDIAVEWE SNGQPENNYKTTTPVLDSGDSFFLYSKLTVDKSRWQQGNVFCFSVMHEALHN HYTQKSLSLSPGK	Cross311-kbss
59	EVQLES GGGGLVQPGGSLKLSCAASGFTFNTYAMNWVRQAPGKGLEWVARIR SKYNNYATYYADSVKDRFTISRDDSKNTAYLQMNNLKTEDTAVYYCVRHGNTF GNSYHSWFAYWGQGLTVTVSSGGGGSGGGSGGGGSELVVTQEPSLTVSPGG TVTLTCRSSTGAVTTSNYANWVQQKPGQAPRGLIGGTNKRAPGTPARFSGSLL GGKAALTL SGVQPEDEAEYYCALWYSNLWVFGGGTKLTVLGGGGSPKSSDKT HTCPPCPAPEAAGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNW YVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKAL PAPIEKTISKAKGQPREPQVYTLPPCREEMTKNQVSLWCLVKGFYPSDIAVEWE SNGQPENNYKTTTPVLDSGDSFFLYSKLTVDKSRWQQGNVFCFSVMHEALHN HYTQKSLSLSPGK	Cross312-kbss
60	EVQLES GGGGLVQPGGSLKLSCAASGFTFNTYAMNWVRQAPGKGLEWVARIR SKYNNYATYYADSVKDRFTISRDDSKNTAYLQMNNLKTEDTAVYYCVRHGNTF GNSYSSWFAYWGQGLTVTVSSGGGGSGGGSGGGGSELVVTQEPSLTVSPGGT VTLTCRSSTGAVTTSNYANWVQQKPGQAPRGLIGGTNKRAPGTPARFSGSLLG GKAALTL SGVQPEDEAEYYCALWYSNLWVFGGGTKLTVLGGGGSPKSSDKTH TCPPCPAPEAAGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWY VDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALP APIEKTISKAKGQPREPQVYTLPPCREEMTKNQVSLWCLVKGFYPSDIAVEWES NGQPENNYKTTTPVLDSGDSFFLYSKLTVDKSRWQQGNVFCFSVMHEALHNH YTQKSLSLSPGK	Cross314-kbss
61	EVQLES GGGGLVQPGGSLKLSCAASGFTFNTYAMNWVRQAPGKGLEWVARIR SKYNNYATYYADSVKDRFTISRDDSKNTAYLQMNNLKTEDTAVYYCVRHGNTF GNSYVSWFAGWGQGLTVTVSSGGGGSGGGSGGGGSELVVTQEPSLTVSPGG TVTLTCRSSTGAVTTSNYANWVQQKPGQAPRGLIGGTNKRAPGTPARFSGSLL GGKAALTL SGVQPEDEAEYYCALWYSNLWVFGGGTKLTVLGGGGSPKSSDKT HTCPPCPAPEAAGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNW YVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKAL PAPIEKTISKAKGQPREPQVYTLPPCREEMTKNQVSLWCLVKGFYPSDIAVEWE SNGQPENNYKTTTPVLDSGDSFFLYSKLTVDKSRWQQGNVFCFSVMHEALHN HYTQKSLSLSPGK	Cross317-kbss

62	EVQLES GGGLVQPGGSLKLSCAASGFTFNTYAMNWVRQAPGKGLEWVARIR SKYNNYATYYADSVKDRFTISRDDSKNTAYLQMNNLKTEDTAVYYCVRHGNF GNSYVSWFAMWGQGLTVTVSSGGGGSGGGSGGGGSELVVTQEPSLTVSPGG TVTLTCRSSTGAVTTSNYANWVQQKPGQAPRGLIGGTNKRAPGTPARFSGSLL GGKAALTL SGVQPEDEAEYYCALWYSNLWVFGGGTKLTVLGGGGSPKSSDKT HTCPPCPAPEAAGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNW YVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKAL PAPIEKTISKAKGQPREPQVYTLPPCREEMTKNQVSLWCLVKGFYPSDIAVEWE SNGQPENNYKTTTPVLDSGDSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHN HYTQKSLSLSPGK	Cross318-kbss
63	EVQLES GGGLVQPGGSLKLSCAASGFTFNTYAMNWVRQAPGKGLEWVARIR SKYNNYATYYADSVKDRFTISRDDSKNTAYLQMNNLKTEDTAVYYCVRHGNF GNSYVSWFAYWGQGLTVTVSSGGGGSGGGSGGGGSELVVTQEPSLTVSPGG TVTLTCRSSTGAVTTSNYANWVQQKPGQAPRGLIGGTNKRAPGTPARFSGSLL GGKAALTL SGVQPEDEAEYYCALWYPNLWVFGGGTKLTVLGGGGSPKSSDKT HTCPPCPAPEAAGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNW YVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKAL PAPIEKTISKAKGQPREPQVYTLPPCREEMTKNQVSLWCLVKGFYPSDIAVEWE SNGQPENNYKTTTPVLDSGDSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHN HYTQKSLSLSPGK	Cross323-kbss
64	EVQLES GGGLVQPGGSLKLSCAASGFTFNTYAMNWVRQAPGKGLEWVARIR SKYNNYATYYADSVKDRFTISRDDSKNTAYLQMNNLKTEDTAVYYCVRHGNF GNSYVSWFAYWGQGLTVTVSSGGGGSGGGSGGGGSELVVTQEPSLTVSPGG TVTLTCRSSTGAVTTSNYANWVQQKPGQAPRGLIGGTNKRAPGTPARFSGSLL GGKAALTL SGVQPEDEAEYYCALWYSNPWFVFGGGTKLTVLGGGGSPKSSDKT HTCPPCPAPEAAGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNW YVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKAL PAPIEKTISKAKGQPREPQVYTLPPCREEMTKNQVSLWCLVKGFYPSDIAVEWE SNGQPENNYKTTTPVLDSGDSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHN HYTQKSLSLSPGK	Cross326-kbss
65	EVQLES GGGLVQPGGSLKLSCAASGFTFNTYAMNWVRQAPGKGLEWVARIR SKYNNYATYYADSVKDRFTISRDDSKNTAYLQMNNLKTEDTAVYYCVRHGMF GNSYVSWFAYWGQGLTVTVSSGGGGSGGGSGGGGSELVVTQEPSLTVSPGG TVTLTCRSSTGAVTTSNYANWVQQKPGQAPRGLIGGTNKRAPGTPARFSGSLL GGKAALTL SGVQPEDEAEYYCALWYPNPWFVFGGGTKLTVLGGGGSPKSSDKT HTCPPCPAPEAAGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNW YVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKAL PAPIEKTISKAKGQPREPQVYTLPPCREEMTKNQVSLWCLVKGFYPSDIAVEWE SNGQPENNYKTTTPVLDSGDSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHN HYTQKSLSLSPGK	Cross3ZH07-kbss
66	EVQLES GGGLVQPGGSLKLSCAASGFTFNTYAMNWVRQAPGKGLEWVARIR SKYNNYATYYADSVKDRFTISRDDSKNTAYLQMNNLKTEDTAVYYCVRHGNF GNSYVSWFLYWGQGLTVTVSSGGGGSGGGSGGGGSELVVTQEPSLTVSPGG TVTLTCRSSTGAVTTSNYANWVQQKPGQAPRGLIGGTNKRAPGTPARFSGSLL GGKAALTL SGVQPEDEAEYYCALWYPNPWFVFGGGTKLTVLGGGGSPKSSDKT HTCPPCPAPEAAGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNW YVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKAL PAPIEKTISKAKGQPREPQVYTLPPCREEMTKNQVSLWCLVKGFYPSDIAVEWE SNGQPENNYKTTTPVLDSGDSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHN HYTQKSLSLSPGK	Cross3ZH09-kbss

67	EVQLLESQGGGLVQPGGSLKLSAASGFTFTNTYAMNWVRQAPGKGLEWVARIR SKYNNYATYYADSVKDRFTISRDDSKNTAYLQMNNLKTEDTAVYYCVRHGNF GNSYVSWFAYWGQGLTVTVSSGGGGSGGGGSGGGGSELVVTQEPSTVSPGG TVTLTCRSSTGAVTTSNYANWVQKPGQAPRGLIGGTNKRAPGTPARFSGSLL GGKAALTLSGVQPEDEAEYYCALWYPNDWVFGGGTKLTVLGGGGSPKSSDKT HTCPPCPAPEAAGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNW YVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKAL PAPIEKTISKAKGQPREPQVYTLPPCREEMTKNQVSLWCLVKGFYPSDIAVEWE SNGQPENNYKTTTPVLDSDGSFFLYSKLTVDKSRWQQGNVFCFSVMHEALHN HYTQKSLSLSPGK	Cross3ZH16-kbss
68	EVQLLESQGGGLVQPGGSLKLSAASGFTFTNTYAMNWVRQAPGKGLEWVARIR SKYNNYATYYADSVKDRFTISRDDSKNTAYLQMNNLKTEDTAVYYCVRHGNF GNDYVSWFAYWGQGLTVTVSSGGGGSGGGGSGGGGSELVVTQEPSTVSPGG TVTLTCRSSTGAVTTSNYANWVQKPGQAPRGLIGGTNKRAPGTPARFSGSLL GGKAALTLSGVQPEDEAEYYCALWYSNPWFVFGGGTKLTVLGGGGSPKSSDKT HTCPPCPAPEAAGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNW YVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKAL PAPIEKTISKAKGQPREPQVYTLPPCREEMTKNQVSLWCLVKGFYPSDIAVEWE SNGQPENNYKTTTPVLDSDGSFFLYSKLTVDKSRWQQGNVFCFSVMHEALHN HYTQKSLSLSPGK	Cross3ZH23-kbss
69	QVQLQQSGSELKKPGASVKVSCASGYTFTNYGMNWVKQAPGQGLKWMG WINTYTGTEPTTDDFKGRFAFLDTSVSTAYLQISSLKADDTAVYFCARGGFGS SYWYFDVWGQGLVTVSSASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPE PVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVTVPSSSLGTQTYICNVNHKP SNTKVDKKVEPKSSDKTHTCPPCPAPEAAGGPSVFLFPPKPKDTLMISRTPEVT CVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQ DWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVCTLPSSREEMTKNQVS LSCAVKGFYPSDIAVEWESNGQPENNYKTTTPVLDSDGSFFLVSKLTVDKSRWQ QGNVFCFSVMHEALHNHYTQKSLSLSPGK	Trop2 antibody heavy chain
70	DIQLTQSPSSLSASVGDVRSITCKASQDVSAVAWYQKPGKAPKLLIYSASYRY TGVDPDRFSGSGSGTDFTLTISLQPEDFAVYYCQQHYYITPLTFGAGTKVEIKRTV AAPSVFIFPPSDEQLKSGTASVCLLNNFYPREAKVQWKVDNALQSGNSQESV TEQDSKDSSTYSLSSTLTLSKADYEKHKVYACEVTHQGLSSPVTKSFNRGEC	Trop2 antibody light chain
71	QVQLVQSGAEVKKPGASVKVSCASGYSTGYMHVVRQAPGQGLEWIGRI NPYNGATTYSQNFKDRATMTVDTSISTAYMELSRLSDDTAVYYCVPTGMGYF LMDYWGQGLTVTVSSASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVT VSWNSGALTSGVHTFPAVLQSSGLYSLSSVTVPSSSLGTQTYICNVNHKPSNT KVDKKVEPKSCDKTHTCPPCPAPEAAGGPSVFLFPPKPKDTLMISRTPEVTCVV VDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWL NGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVCTLPSSREEMTKNQVSLSCA VKGFYPSDIAVEWESNGQPENNYKTTTPVLDSDGSFFLVSKLTVDKSRWQQGN VFSCFSVMHEALHNHYTQKSLSLSPGK	PDL1 antibody heavy chain
72	DIQLTQSPSFLSASVGDVRSITCKASQDVGSAAVWYQKPGKAPKLLIYWASTR HTGVPSRFRSGSGSGTEFTLTISLQPEDFATYFCQQFTSYPTFGQGTKEIKRTVA APSVFIFPPSDEQLKSGTASVCLLNNFYPREAKVQWKVDNALQSGNSQESV TEQDSKDSSTYSLSSTLTLSKADYEKHKVYACEVTHQGLSSPVTKSFNRGEC	PDL1 antibody light chain
73	MQSGTHWRVLGCLLSVGWVGQDGNEEMGGITQTPYKVSISGTTVILTCPQYP GSEILWQHNDKNIGGDEDDKNIGSDEDHLSLKEFSELEQSGYYVCYPRGSKPE DANFYLYLRARCENCMEMDVMSVATIVDITGGLLLLWYWSKNRKAKA KPVTRGAGAGGRQGRQNKERPPVPNPDYEPKRGQRDLYSGLNQRRRI	CD3e
74	VRHGMFGNSYVSWFAY	Cross303 HCDR3
75	VRHGNFGNEYVSWFAY	Cross307 HCDR3
76	VRHGNFGNPYVSWFAY	Cross308 HCDR3
77	VRHGNFGNVYVSWFAY	Cross309 HCDR3
78	VRHGNFGNYVSWFAY	Cross310 HCDR3
79	VRHGNFGNSEVSWFAY	Cross311 HCDR3
80	VRHGNFGNSYHSWFAY	Cross312 HCDR3
81	VRHGNFGNSYSSWFAY	Cross314 HCDR3

82	VRHGNFGNSYVSWFAG	Cross317 HCDR3
83	VRHGNFGNSYVSWFAM	Cross318 HCDR3
84	VRHGMFGNSYVSWFAY	Cross3ZH07 HCDR3
85	VRHGNFGNSYVSWFLY	Cross3ZH09 HCDR3
86	VRHGNFGNDYVSWFAY	Cross3ZH23 HCDR3
87	ALWYPNLWV	Cross323 LCDR3
88	ALWYSPWV	Cross326 LCDR3
89	ALWYPPWV	Cross3ZH07 LCDR3
90	ALWYPPWV	Cross3ZH09 LCDR3
91	ALWYPNDWV	Cross3ZH16 LCDR3
92	ALWYSPWV	Cross3ZH23 LCDR3
93	ASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVTPVSSSLGTQTYICNVNHKPSNTKVDKKVEPKSCDKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSREEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGK	Heavy chain constant region of CD3 antibody
94	RTVAAPSVFIFPPSDEQLKSGTASVVCLLNNFYPREAKVQWKVDNALQSGNSQESVTEQDSKDSTYSLSSTLTLSKADYEKHKVYACEVTHQGLSSPVTKSFNRGEC	Light chain constant region of CD3 antibody
95	EVQLLESGGGLVQPGGSLKLSCAASGFTFTNYAMNWVRQAPGKGLEWVARIRSKYNNYATYYADSVKDRFTISRDDSKNTAYLQMNNLKTEDTAVYYCVRHGMFGNSYVSWFAYWGQGLTVTVSSASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVTPVSSSLGTQTYICNVNHKPSNTKVDKKVEPKSCDKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSREEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGK	Cross3ZH07 heavy chain
96	EVQLLESGGGLVQPGGSLKLSCAASGFTFTNYAMNWVRQAPGKGLEWVARIRSKYNNYATYYADSVKDRFTISRDDSKNTAYLQMNNLKTEDTAVYYCVRHGMFGNSYVSWFLYWGQGLTVTVSSASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVTPVSSSLGTQTYICNVNHKPSNTKVDKKVEPKSCDKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSREEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGK	Cross3ZH09 heavy chain
97	EVQLLESGGGLVQPGGSLKLSCAASGFTFTNYAMNWVRQAPGKGLEWVARIRSKYNNYATYYADSVKDRFTISRDDSKNTAYLQMNNLKTEDTAVYYCVRHGMFGNSYVSWFAYWGQGLTVTVSSASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVTPVSSSLGTQTYICNVNHKPSNTKVDKKVEPKSCDKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSREEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGK	Cross3ZH16 heavy chain
98	EVQLLESGGGLVQPGGSLKLSCAASGFTFTNYAMNWVRQAPGKGLEWVARIRSKYNNYATYYADSVKDRFTISRDDSKNTAYLQMNNLKTEDTAVYYCVRHGMFGNDYVSWFAYWGQGLTVTVSSASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVTPVSSSLGTQTYICNVNHKPSNTKVDKKVEPKSCDKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSREEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGK	Cross3ZH23 heavy chain

99	EVQLES GGGLVQPGGSLKLSCAASGFTFNTYAMNWVRQAPGKGLEWVARIR SKYNNYATYYADSVKDRFTISRDDSKNTAYLQMNNLKTEDTAVYYCVRHGFMF GNSYVSWFAYWGQGLTVTVSSGGGGSGGGGSGGGGSELVVTQEPSLTVSPGG TVTLTCRSSTGAVTTSNYANWVQKPGQAPRGLIGGTNKRAPGTPARFSGSLL GGKAALTLSGVQPEDEAEYYCALWYSNLWVFGGGTKLTVL	Cross303 scFv
100	EVQLES GGGLVQPGGSLKLSCAASGFTFNTYAMNWVRQAPGKGLEWVARIR SKYNNYATYYADSVKDRFTISRDDSKNTAYLQMNNLKTEDTAVYYCVRHGFMF GNEYVSWFAYWGQGLTVTVSSGGGGSGGGGSGGGGSELVVTQEPSLTVSPGG TVTLTCRSSTGAVTTSNYANWVQKPGQAPRGLIGGTNKRAPGTPARFSGSLL GGKAALTLSGVQPEDEAEYYCALWYSNLWVFGGGTKLTVL	Cross307 scFv
101	EVQLES GGGLVQPGGSLKLSCAASGFTFNTYAMNWVRQAPGKGLEWVARIR SKYNNYATYYADSVKDRFTISRDDSKNTAYLQMNNLKTEDTAVYYCVRHGFMF GNPYVSWFAYWGQGLTVTVSSGGGGSGGGGSGGGGSELVVTQEPSLTVSPGG TVTLTCRSSTGAVTTSNYANWVQKPGQAPRGLIGGTNKRAPGTPARFSGSLL GGKAALTLSGVQPEDEAEYYCALWYSNLWVFGGGTKLTVL	Cross308 scFv
102	EVQLES GGGLVQPGGSLKLSCAASGFTFNTYAMNWVRQAPGKGLEWVARIR SKYNNYATYYADSVKDRFTISRDDSKNTAYLQMNNLKTEDTAVYYCVRHGFMF GNVYVSWFAYWGQGLTVTVSSGGGGSGGGGSGGGGSELVVTQEPSLTVSPGG TVTLTCRSSTGAVTTSNYANWVQKPGQAPRGLIGGTNKRAPGTPARFSGSLL GGKAALTLSGVQPEDEAEYYCALWYSNLWVFGGGTKLTVL	Cross309 scFv
103	EVQLES GGGLVQPGGSLKLSCAASGFTFNTYAMNWVRQAPGKGLEWVARIR SKYNNYATYYADSVKDRFTISRDDSKNTAYLQMNNLKTEDTAVYYCVRHGFMF GNYVSWFAYWGQGLTVTVSSGGGGSGGGGSGGGGSELVVTQEPSLTVSPGG TVTLTCRSSTGAVTTSNYANWVQKPGQAPRGLIGGTNKRAPGTPARFSGSLL GGKAALTLSGVQPEDEAEYYCALWYSNLWVFGGGTKLTVL	Cross310 scFv
104	EVQLES GGGLVQPGGSLKLSCAASGFTFNTYAMNWVRQAPGKGLEWVARIR SKYNNYATYYADSVKDRFTISRDDSKNTAYLQMNNLKTEDTAVYYCVRHGFMF GNSEVSWFAYWGQGLTVTVSSGGGGSGGGGSGGGGSELVVTQEPSLTVSPGG TVTLTCRSSTGAVTTSNYANWVQKPGQAPRGLIGGTNKRAPGTPARFSGSLL GGKAALTLSGVQPEDEAEYYCALWYSNLWVFGGGTKLTVL	Cross311 scFv
105	EVQLES GGGLVQPGGSLKLSCAASGFTFNTYAMNWVRQAPGKGLEWVARIR SKYNNYATYYADSVKDRFTISRDDSKNTAYLQMNNLKTEDTAVYYCVRHGFMF GNSYHSWFAYWGQGLTVTVSSGGGGSGGGGSGGGGSELVVTQEPSLTVSPGG TVTLTCRSSTGAVTTSNYANWVQKPGQAPRGLIGGTNKRAPGTPARFSGSLL GGKAALTLSGVQPEDEAEYYCALWYSNLWVFGGGTKLTVL	Cross312 scFv
106	EVQLES GGGLVQPGGSLKLSCAASGFTFNTYAMNWVRQAPGKGLEWVARIR SKYNNYATYYADSVKDRFTISRDDSKNTAYLQMNNLKTEDTAVYYCVRHGFMF GNSYQSWFAYWGQGLTVTVSSGGGGSGGGGSGGGGSELVVTQEPSLTVSPGG TVTLTCRSSTGAVTTSNYANWVQKPGQAPRGLIGGTNKRAPGTPARFSGSLL GGKAALTLSGVQPEDEAEYYCALWYSNLWVFGGGTKLTVL	Cross313 scFv
107	EVQLES GGGLVQPGGSLKLSCAASGFTFNTYAMNWVRQAPGKGLEWVARIR SKYNNYATYYADSVKDRFTISRDDSKNTAYLQMNNLKTEDTAVYYCVRHGFMF GNSYSSWFAYWGQGLTVTVSSGGGGSGGGGSGGGGSELVVTQEPSLTVSPGGT VTLTCRSSTGAVTTSNYANWVQKPGQAPRGLIGGTNKRAPGTPARFSGSLLG GKAALTLSGVQPEDEAEYYCALWYSNLWVFGGGTKLTVL	Cross314 scFv
108	EVQLES GGGLVQPGGSLKLSCAASGFTFNTYAMNWVRQAPGKGLEWVARIR SKYNNYATYYADSVKDRFTISRDDSKNTAYLQMNNLKTEDTAVYYCVRHGFMF GNSYVSWFLYWGQGLTVTVSSGGGGSGGGGSGGGGSELVVTQEPSLTVSPGG TVTLTCRSSTGAVTTSNYANWVQKPGQAPRGLIGGTNKRAPGTPARFSGSLL GGKAALTLSGVQPEDEAEYYCALWYSNLWVFGGGTKLTVL	Cross316 scFv
109	EVQLES GGGLVQPGGSLKLSCAASGFTFNTYAMNWVRQAPGKGLEWVARIR SKYNNYATYYADSVKDRFTISRDDSKNTAYLQMNNLKTEDTAVYYCVRHGFMF GNSYVSWFAGWGQGLTVTVSSGGGGSGGGGSGGGGSELVVTQEPSLTVSPGG TVTLTCRSSTGAVTTSNYANWVQKPGQAPRGLIGGTNKRAPGTPARFSGSLL GGKAALTLSGVQPEDEAEYYCALWYSNLWVFGGGTKLTVL	Cross317 scFv

110	EVQLLESGGGLVQPGGSLKLSCAASGFTFTNTYAMNWVRQAPGKGLEWVARIR SKYNNYATYYADSVKDRFTISRDDSKNTAYLQMNNLKTEDTAVYYCVRHGNF GNSYVSWFAMWGQGLTVTVSSGGGGSGGGSGGGGSELVVTQEPSLTVSPGG TVTLTCRSSTGAVTTSNYANWVQKPGQAPRGLIGGTNKRAPGTPARFSGSLL GGKAALTLSGVQPEDEAEYYCALWYSNLWVFGGGTKLTVL	Cross318 scFv
111	EVQLLESGGGLVQPGGSLKLSCAASGFTFTNTYAMNWVRQAPGKGLEWVARIR SKYNNYATYYADSVKDRFTISRDDSKNTAYLQMNNLKTEDTAVYYCVRHGNF GNSYVSWFAYWGQGLTVTVSSGGGGSGGGSGGGGSELVVTQEPSLTVSPGG TVTLTCRSSTGAVTTSNYANWVQKPGQAPRGLIGGTNKRAPGTPARFSGSLL GGKAALTLSGVQPEDEAEYYCALWYPNLWVFGGGTKLTVL	Cross323 scFv
112	EVQLLESGGGLVQPGGSLKLSCAASGFTFTNTYAMNWVRQAPGKGLEWVARIR SKYNNYATYYADSVKDRFTISRDDSKNTAYLQMNNLKTEDTAVYYCVRHGNF GNSYVSWFAYWGQGLTVTVSSGGGGSGGGSGGGGSELVVTQEPSLTVSPGG TVTLTCRSSTGAVTTSNYANWVQKPGQAPRGLIGGTNKRAPGTPARFSGSLL GGKAALTLSGVQPEDEAEYYCALWYSNDWVFGGGTKLTVL	Cross325 scFv
113	EVQLLESGGGLVQPGGSLKLSCAASGFTFTNTYAMNWVRQAPGKGLEWVARIR SKYNNYATYYADSVKDRFTISRDDSKNTAYLQMNNLKTEDTAVYYCVRHGNF GNSYVSWFAYWGQGLTVTVSSGGGGSGGGSGGGGSELVVTQEPSLTVSPGG TVTLTCRSSTGAVTTSNYANWVQKPGQAPRGLIGGTNKRAPGTPARFSGSLL GGKAALTLSGVQPEDEAEYYCALWYSNPWVFGGGTKLTVL	Cross326 scFv
114	EVQLLESGGGLVQPGGSLKLSCAASGFTFTNTYAMNWVRQAPGKGLEWVARIR SKYNNYATYYADSVKDRFTISRDDSKNTAYLQMNNLKTEDTAVYYCVRHGMF GNSYVSWFAYWGQGLTVTVSSGGGGSGGGSGGGGSELVVTQEPSLTVSPGG TVTLTCRSSTGAVTTSNYANWVQKPGQAPRGLIGGTNKRAPGTPARFSGSLL GGKAALTLSGVQPEDEAEYYCALWYPNPWVFGGGTKLTVL	Cross3ZH07 scFv
115	EVQLLESGGGLVQPGGSLKLSCAASGFTFTNTYAMNWVRQAPGKGLEWVARIR SKYNNYATYYADSVKDRFTISRDDSKNTAYLQMNNLKTEDTAVYYCVRHGNF GNSYVSWFLYWGQGLTVTVSSGGGGSGGGSGGGGSELVVTQEPSLTVSPGG TVTLTCRSSTGAVTTSNYANWVQKPGQAPRGLIGGTNKRAPGTPARFSGSLL GGKAALTLSGVQPEDEAEYYCALWYPNPWVFGGGTKLTVL	Cross3ZH09 scFv
116	EVQLLESGGGLVQPGGSLKLSCAASGFTFTNTYAMNWVRQAPGKGLEWVARIR SKYNNYATYYADSVKDRFTISRDDSKNTAYLQMNNLKTEDTAVYYCVRHGNF GNSYVSWFAYWGQGLTVTVSSGGGGSGGGSGGGGSELVVTQEPSLTVSPGG TVTLTCRSSTGAVTTSNYANWVQKPGQAPRGLIGGTNKRAPGTPARFSGSLL GGKAALTLSGVQPEDEAEYYCALWYPNDWVFGGGTKLTVL	Cross3ZH16 scFv
117	EVQLLESGGGLVQPGGSLKLSCAASGFTFTNTYAMNWVRQAPGKGLEWVARIR SKYNNYATYYADSVKDRFTISRDDSKNTAYLQMNNLKTEDTAVYYCVRHGNF GNDYVSWFAYWGQGLTVTVSSGGGGSGGGSGGGGSELVVTQEPSLTVSPGG TVTLTCRSSTGAVTTSNYANWVQKPGQAPRGLIGGTNKRAPGTPARFSGSLL GGKAALTLSGVQPEDEAEYYCALWYSNPWVFGGGTKLTVL	Cross3ZH23 scFv
118	PKSSDKTHTCPPEAPEAAGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHED PEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVHLQDNLNGKEYKC KVSNAKALPAIEKTISKAKGQPREPQVYTLPPCREEMTKNQVSLWCLVKGFYPS DIAVEWESNGQPENNYKTTPVLDSGDSFFLYSKLTVDKSRWQQGNVFCFSVM HEALHNHYTQKSLSLSPGK	Corresponding first Fc peptide segment when CD3 binding region is an scFv structure
119	ELVVTTQEPSLTVSPGGTVTLTCRSSTGAVTTSNYANWVQKPGQAPRGLIGGTN KRAPGTPARFSGSLLGGKAALTLSGVQPEDEAEYYCALWYPNPWVFGGGTKL TVLRTVAAPSVFIFPPSDEQLKSGTASVVCLLNNFYPREAKVQWKVDNALQSG NSQESVTEQDSKDSTYLSSTLTLSKADYEKHKVYACEVTHQGLSSPVTKSFNR GEC	Cross3ZH07 light chain
120	ELVVTTQEPSLTVSPGGTVTLTCRSSTGAVTTSNYANWVQKPGQAPRGLIGGTN KRAPGTPARFSGSLLGGKAALTLSGVQPEDEAEYYCALWYPNPWVFGGGTKL TVLRTVAAPSVFIFPPSDEQLKSGTASVVCLLNNFYPREAKVQWKVDNALQSG NSQESVTEQDSKDSTYLSSTLTLSKADYEKHKVYACEVTHQGLSSPVTKSFNR GEC	Cross3ZH09 light chain

121	ELVVTQEPSLTVSPGGTVTLTCSRSTGAVTTSNYANWVQQKPGQAPRGLIGGTN KRAPGTPARFSGSLLGGKAALTLSGVQPEDEAEYYCALWYPNDWVFGGGTKL TVLRTVAAPSVFIFPPSDEQLKSGTASVVCLLNNFYPREAKVQWKVDNALQSG NSQESVTEQDSKDSTYLSSTLTLSKADYEKHKVYACEVTHQGLSSPVTKSFNR GEC	Cross3ZH16 light chain
122	ELVVTQEPSLTVSPGGTVTLTCSRSTGAVTTSNYANWVQQKPGQAPRGLIGGTN KRAPGTPARFSGSLLGGKAALTLSGVQPEDEAEYYCALWYNSNPWVFGGGTKL TVLRTVAAPSVFIFPPSDEQLKSGTASVVCLLNNFYPREAKVQWKVDNALQSG NSQESVTEQDSKDSTYLSSTLTLSKADYEKHKVYACEVTHQGLSSPVTKSFNR GEC	Cross3ZH23 light chain
123	EVQLLESGGGLVQPGGSLKLSCAASGFTFTNTYAMNWVRQAPGKGLEWVARIR SKYNNYATYYADSVKDRFTISRDDSKNTAYLQMNNLKTEDTAVYYCVRHGFMF GNSYVSWFAYWGQGLTVTVSSASTKGPSVFPLAPSSKSTSGGTAALGCLVKDY FPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSSLGTQTYICNVNH KPSNTKVDKKVE	Cross303 Fab VH-CH1
124	EVQLLESGGGLVQPGGSLKLSCAASGFTFTNTYAMNWVRQAPGKGLEWVARIR SKYNNYATYYADSVKDRFTISRDDSKNTAYLQMNNLKTEDTAVYYCVRHGFMF GNEYVSWFAYWGQGLTVTVSSASTKGPSVFPLAPSSKSTSGGTAALGCLVKDY FPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSSLGTQTYICNVNH KPSNTKVDKKVE	Cross307Fab VH-CH1
125	EVQLLESGGGLVQPGGSLKLSCAASGFTFTNTYAMNWVRQAPGKGLEWVARIR SKYNNYATYYADSVKDRFTISRDDSKNTAYLQMNNLKTEDTAVYYCVRHGFMF GNPYVSWFAYWGQGLTVTVSSASTKGPSVFPLAPSSKSTSGGTAALGCLVKDY FPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSSLGTQTYICNVNH KPSNTKVDKKVE	Cross308Fab VH-CH1
126	EVQLLESGGGLVQPGGSLKLSCAASGFTFTNTYAMNWVRQAPGKGLEWVARIR SKYNNYATYYADSVKDRFTISRDDSKNTAYLQMNNLKTEDTAVYYCVRHGFMF GNVYVSWFAYWGQGLTVTVSSASTKGPSVFPLAPSSKSTSGGTAALGCLVKDY FPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSSLGTQTYICNVNH KPSNTKVDKKVE	Cross309Fab VH-CH1
127	EVQLLESGGGLVQPGGSLKLSCAASGFTFTNTYAMNWVRQAPGKGLEWVARIR SKYNNYATYYADSVKDRFTISRDDSKNTAYLQMNNLKTEDTAVYYCVRHGFMF GNYVYVSWFAYWGQGLTVTVSSASTKGPSVFPLAPSSKSTSGGTAALGCLVKDY FPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSSLGTQTYICNVNH KPSNTKVDKKVE	Cross310Fab VH-CH1
128	EVQLLESGGGLVQPGGSLKLSCAASGFTFTNTYAMNWVRQAPGKGLEWVARIR SKYNNYATYYADSVKDRFTISRDDSKNTAYLQMNNLKTEDTAVYYCVRHGFMF GNSEVSWFAYWGQGLTVTVSSASTKGPSVFPLAPSSKSTSGGTAALGCLVKDY FPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSSLGTQTYICNVNH KPSNTKVDKKVE	Cross311Fab VH-CH1
129	EVQLLESGGGLVQPGGSLKLSCAASGFTFTNTYAMNWVRQAPGKGLEWVARIR SKYNNYATYYADSVKDRFTISRDDSKNTAYLQMNNLKTEDTAVYYCVRHGFMF GNSYHSWFAYWGQGLTVTVSSASTKGPSVFPLAPSSKSTSGGTAALGCLVKDY FPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSSLGTQTYICNVNH KPSNTKVDKKVE	Cross312Fab VH-CH1
130	EVQLLESGGGLVQPGGSLKLSCAASGFTFTNTYAMNWVRQAPGKGLEWVARIR SKYNNYATYYADSVKDRFTISRDDSKNTAYLQMNNLKTEDTAVYYCVRHGFMF GNSYQSWFAYWGQGLTVTVSSASTKGPSVFPLAPSSKSTSGGTAALGCLVKDY FPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSSLGTQTYICNVNH KPSNTKVDKKVE	Cross313Fab VH-CH1
131	EVQLLESGGGLVQPGGSLKLSCAASGFTFTNTYAMNWVRQAPGKGLEWVARIR SKYNNYATYYADSVKDRFTISRDDSKNTAYLQMNNLKTEDTAVYYCVRHGFMF GNSYSSWFAYWGQGLTVTVSSASTKGPSVFPLAPSSKSTSGGTAALGCLVKDY FPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSSLGTQTYICNVNH KPSNTKVDKKVE	Cross314Fab VH-CH1

132	EVQLLESGGGLVQPGGSLKLSCAASGFTFNTYAMNWVRQAPGKGLEWVARIR SKYNNYATYYADSVKDRFTISRDDSKNTAYLQMNNLKTEDTAVYYCVRHGNTF GNSYVSWFLYWGGTLTVTVSSASTKGPSVFPLAPSSKSTSGGTAALGCLVKDY FPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVTVTPSSSLGTQTYICNVNH KPSNTKVDKKVE	Cross316Fab VH-CH1
133	EVQLLESGGGLVQPGGSLKLSCAASGFTFNTYAMNWVRQAPGKGLEWVARIR SKYNNYATYYADSVKDRFTISRDDSKNTAYLQMNNLKTEDTAVYYCVRHGNTF GNSYVSWFAGWGGTLTVTVSSASTKGPSVFPLAPSSKSTSGGTAALGCLVKDY FPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVTVTPSSSLGTQTYICNVNH KPSNTKVDKKVE	Cross317Fab VH-CH1
134	EVQLLESGGGLVQPGGSLKLSCAASGFTFNTYAMNWVRQAPGKGLEWVARIR SKYNNYATYYADSVKDRFTISRDDSKNTAYLQMNNLKTEDTAVYYCVRHGNTF GNSYVSWFAMWGGTLTVTVSSASTKGPSVFPLAPSSKSTSGGTAALGCLVKDY FPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVTVTPSSSLGTQTYICNVNH KPSNTKVDKKVE	Cross318Fab VH-CH1
135	EVQLLESGGGLVQPGGSLKLSCAASGFTFNTYAMNWVRQAPGKGLEWVARIR SKYNNYATYYADSVKDRFTISRDDSKNTAYLQMNNLKTEDTAVYYCVRHGNTF GNSYVSWFAYWGGTLTVTVSSASTKGPSVFPLAPSSKSTSGGTAALGCLVKDY FPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVTVTPSSSLGTQTYICNVNH KPSNTKVDKKVE	Cross323Fab VH-CH1
136	EVQLLESGGGLVQPGGSLKLSCAASGFTFNTYAMNWVRQAPGKGLEWVARIR SKYNNYATYYADSVKDRFTISRDDSKNTAYLQMNNLKTEDTAVYYCVRHGNTF GNSYVSWFAYWGGTLTVTVSSASTKGPSVFPLAPSSKSTSGGTAALGCLVKDY FPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVTVTPSSSLGTQTYICNVNH KPSNTKVDKKVE	Cross325Fab VH-CH1
137	EVQLLESGGGLVQPGGSLKLSCAASGFTFNTYAMNWVRQAPGKGLEWVARIR SKYNNYATYYADSVKDRFTISRDDSKNTAYLQMNNLKTEDTAVYYCVRHGNTF GNSYVSWFAYWGGTLTVTVSSASTKGPSVFPLAPSSKSTSGGTAALGCLVKDY FPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVTVTPSSSLGTQTYICNVNH KPSNTKVDKKVE	Cross326Fab VH-CH1
138	EVQLLESGGGLVQPGGSLKLSCAASGFTFNTYAMNWVRQAPGKGLEWVARIR SKYNNYATYYADSVKDRFTISRDDSKNTAYLQMNNLKTEDTAVYYCVRHGMF GNSYVSWFAYWGGTLTVTVSSASTKGPSVFPLAPSSKSTSGGTAALGCLVKDY FPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVTVTPSSSLGTQTYICNVNH KPSNTKVDKKVE	Cross3ZH07Fab VH-CH1
139	EVQLLESGGGLVQPGGSLKLSCAASGFTFNTYAMNWVRQAPGKGLEWVARIR SKYNNYATYYADSVKDRFTISRDDSKNTAYLQMNNLKTEDTAVYYCVRHGNTF GNSYVSWFLYWGGTLTVTVSSASTKGPSVFPLAPSSKSTSGGTAALGCLVKDY FPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVTVTPSSSLGTQTYICNVNH KPSNTKVDKKVE	Cross3ZH09Fab VH-CH1
140	EVQLLESGGGLVQPGGSLKLSCAASGFTFNTYAMNWVRQAPGKGLEWVARIR SKYNNYATYYADSVKDRFTISRDDSKNTAYLQMNNLKTEDTAVYYCVRHGNTF GNSYVSWFAYWGGTLTVTVSSASTKGPSVFPLAPSSKSTSGGTAALGCLVKDY FPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVTVTPSSSLGTQTYICNVNH KPSNTKVDKKVE	Cross3ZH16Fab VH-CH1
141	EVQLLESGGGLVQPGGSLKLSCAASGFTFNTYAMNWVRQAPGKGLEWVARIR SKYNNYATYYADSVKDRFTISRDDSKNTAYLQMNNLKTEDTAVYYCVRHGNTF GNDYVSWFAYWGGTLTVTVSSASTKGPSVFPLAPSSKSTSGGTAALGCLVKDY FPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVTVTPSSSLGTQTYICNVNH KPSNTKVDKKVE	Cross3ZH23Fab VH-CH1

142	ELVVTQEPSLTVSPGGTVTLTCSRSTGAVTTSNYANWVQQKPGQAPRGLIGGTN KRAPGTPARFSGSLLGGKAALTLSGVQPEDEAEYYCALWYSNLWVFGGGTKL TVLRTVAAPSVFIFPPSDEQLKSGTASVVCLLNFPYAPREKVVQWKVDNALQSG NSQESVTEQDSKDYSLSTLTLSKADYEKHKVYACEVTHQGLSSPVTKSFNR GECGGGGSEKSSGSGSESKSTEGKSSGSGSESKSTGGGGSEVQLLESGGGLV QPGGSLKLSCAASGFTFNTYAMNWVRQAPGKGLEWVARIRSKYNNYATYYAD SVKDRFTISRDDSKNTAYLQMNNLKTEDTAVYYCVRHGMFGNSYVSWFAYW GQGTLLTVSSASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSG ALTSGVHTFPAVLQSSGLYSLSSVVTVPSSSLGTQTYICNVNHKPSNTKVDKKV E	Cross303 scFab
143	ELVVTQEPSLTVSPGGTVTLTCSRSTGAVTTSNYANWVQQKPGQAPRGLIGGTN KRAPGTPARFSGSLLGGKAALTLSGVQPEDEAEYYCALWYSNLWVFGGGTKL TVLRTVAAPSVFIFPPSDEQLKSGTASVVCLLNFPYAPREKVVQWKVDNALQSG NSQESVTEQDSKDYSLSTLTLSKADYEKHKVYACEVTHQGLSSPVTKSFNR GECGGGGSEKSSGSGSESKSTEGKSSGSGSESKSTGGGGSEVQLLESGGGLV QPGGSLKLSCAASGFTFNTYAMNWVRQAPGKGLEWVARIRSKYNNYATYYAD SVKDRFTISRDDSKNTAYLQMNNLKTEDTAVYYCVRHGNFGNEYVSWFAYWG QGTLLTVSSASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGA LTSGVHTFPAVLQSSGLYSLSSVVTVPSSSLGTQTYICNVNHKPSNTKVDKKVE	Cross307scFab
163	ELVVTQEPSLTVSPGGTVTLTCSRSTGAVTTSNYANWVQQKPGQAPRGLIGGTN KRAPGTPARFSGSLLGGKAALTLSGVQPEDEAEYYCALWYSNLWVFGGGTKL TVLRTVAAPSVFIFPPSDEQLKSGTASVVCLLNFPYAPREKVVQWKVDNALQSG NSQESVTEQDSKDYSLSTLTLSKADYEKHKVYACEVTHQGLSSPVTKSFNR GECGGGGSEKSSGSGSESKSTEGKSSGSGSESKSTGGGGSEVQLLESGGGLV QPGGSLKLSCAASGFTFNTYAMNWVRQAPGKGLEWVARIRSKYNNYATYYAD SVKDRFTISRDDSKNTAYLQMNNLKTEDTAVYYCVRHGNFGNPYVSWFAYWG QGTLLTVSSASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGA LTSGVHTFPAVLQSSGLYSLSSVVTVPSSSLGTQTYICNVNHKPSNTKVDKKVE	Cross308scFab
145	ELVVTQEPSLTVSPGGTVTLTCSRSTGAVTTSNYANWVQQKPGQAPRGLIGGTN KRAPGTPARFSGSLLGGKAALTLSGVQPEDEAEYYCALWYSNLWVFGGGTKL TVLRTVAAPSVFIFPPSDEQLKSGTASVVCLLNFPYAPREKVVQWKVDNALQSG NSQESVTEQDSKDYSLSTLTLSKADYEKHKVYACEVTHQGLSSPVTKSFNR GECGGGGSEKSSGSGSESKSTEGKSSGSGSESKSTGGGGSEVQLLESGGGLV QPGGSLKLSCAASGFTFNTYAMNWVRQAPGKGLEWVARIRSKYNNYATYYAD SVKDRFTISRDDSKNTAYLQMNNLKTEDTAVYYCVRHGNFGNVYVSWFAYW GQGTLLTVSSASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSG ALTSGVHTFPAVLQSSGLYSLSSVVTVPSSSLGTQTYICNVNHKPSNTKVDKKV E	Cross309scFab
146	ELVVTQEPSLTVSPGGTVTLTCSRSTGAVTTSNYANWVQQKPGQAPRGLIGGTN KRAPGTPARFSGSLLGGKAALTLSGVQPEDEAEYYCALWYSNLWVFGGGTKL TVLRTVAAPSVFIFPPSDEQLKSGTASVVCLLNFPYAPREKVVQWKVDNALQSG NSQESVTEQDSKDYSLSTLTLSKADYEKHKVYACEVTHQGLSSPVTKSFNR GECGGGGSEKSSGSGSESKSTEGKSSGSGSESKSTGGGGSEVQLLESGGGLV QPGGSLKLSCAASGFTFNTYAMNWVRQAPGKGLEWVARIRSKYNNYATYYAD SVKDRFTISRDDSKNTAYLQMNNLKTEDTAVYYCVRHGNFGNYVSWFAYW GQGTLLTVSSASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSG ALTSGVHTFPAVLQSSGLYSLSSVVTVPSSSLGTQTYICNVNHKPSNTKVDKKV E	Cross310scFab
147	ELVVTQEPSLTVSPGGTVTLTCSRSTGAVTTSNYANWVQQKPGQAPRGLIGGTN KRAPGTPARFSGSLLGGKAALTLSGVQPEDEAEYYCALWYSNLWVFGGGTKL TVLRTVAAPSVFIFPPSDEQLKSGTASVVCLLNFPYAPREKVVQWKVDNALQSG NSQESVTEQDSKDYSLSTLTLSKADYEKHKVYACEVTHQGLSSPVTKSFNR GECGGGGSEKSSGSGSESKSTEGKSSGSGSESKSTGGGGSEVQLLESGGGLV QPGGSLKLSCAASGFTFNTYAMNWVRQAPGKGLEWVARIRSKYNNYATYYAD SVKDRFTISRDDSKNTAYLQMNNLKTEDTAVYYCVRHGNFGNSEVSWFAYWG QGTLLTVSSASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGA LTSGVHTFPAVLQSSGLYSLSSVVTVPSSSLGTQTYICNVNHKPSNTKVDKKVE	Cross311scFab

148	ELVVTQEPSLTVSPGGTVTLTCSRSTGAVTTSNYANWVQQKPGQAPRGLIGGTN KRAPGTPARFSGSLLGGKAALTLSGVQPEDEAEYYCALWYSNLWVFGGGTKL TVLRTVAAPSVFIFPPSDEQLKSGTASVVCLLNFPYAPREKVQWKVDNALQSG NSQESVTEQDSKDYSLSSLTLSKADYEKHKVYACEVTHQGLSSPVTKSFNR GECGGGGSEKSSGSGSESKSTEGKSSGSGSESKSTGGGGSEVQLLESGGGLV QPGGSLKLSCAASGFTFNTYAMNWVRQAPGKGLEWVARIRSKYNNYATYYAD SVKDRFTISRDDSKNTAYLQMNNLKTEDTAVYYCVRHGNFGNSYHSWFAYWG QGTLVTVSSASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGA LTSGVHTFPAVLQSSGLYSLSSVTVPSSSLGTQTYICNVNHKPSNTKVDKKVE	Cross312scFab
149	ELVVTQEPSLTVSPGGTVTLTCSRSTGAVTTSNYANWVQQKPGQAPRGLIGGTN KRAPGTPARFSGSLLGGKAALTLSGVQPEDEAEYYCALWYSNLWVFGGGTKL TVLRTVAAPSVFIFPPSDEQLKSGTASVVCLLNFPYAPREKVQWKVDNALQSG NSQESVTEQDSKDYSLSSLTLSKADYEKHKVYACEVTHQGLSSPVTKSFNR GECGGGGSEKSSGSGSESKSTEGKSSGSGSESKSTGGGGSEVQLLESGGGLV QPGGSLKLSCAASGFTFNTYAMNWVRQAPGKGLEWVARIRSKYNNYATYYAD SVKDRFTISRDDSKNTAYLQMNNLKTEDTAVYYCVRHGNFGNSYQSWFAYWG QGTLVTVSSASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGA LTSGVHTFPAVLQSSGLYSLSSVTVPSSSLGTQTYICNVNHKPSNTKVDKKVE	Cross313scFab
150	ELVVTQEPSLTVSPGGTVTLTCSRSTGAVTTSNYANWVQQKPGQAPRGLIGGTN KRAPGTPARFSGSLLGGKAALTLSGVQPEDEAEYYCALWYSNLWVFGGGTKL TVLRTVAAPSVFIFPPSDEQLKSGTASVVCLLNFPYAPREKVQWKVDNALQSG NSQESVTEQDSKDYSLSSLTLSKADYEKHKVYACEVTHQGLSSPVTKSFNR GECGGGGSEKSSGSGSESKSTEGKSSGSGSESKSTGGGGSEVQLLESGGGLV QPGGSLKLSCAASGFTFNTYAMNWVRQAPGKGLEWVARIRSKYNNYATYYAD SVKDRFTISRDDSKNTAYLQMNNLKTEDTAVYYCVRHGNFGNSYSSWFAYWG QGTLVTVSSASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGA LTSGVHTFPAVLQSSGLYSLSSVTVPSSSLGTQTYICNVNHKPSNTKVDKKVE	Cross314scFab
151	ELVVTQEPSLTVSPGGTVTLTCSRSTGAVTTSNYANWVQQKPGQAPRGLIGGTN KRAPGTPARFSGSLLGGKAALTLSGVQPEDEAEYYCALWYSNLWVFGGGTKL TVLRTVAAPSVFIFPPSDEQLKSGTASVVCLLNFPYAPREKVQWKVDNALQSG NSQESVTEQDSKDYSLSSLTLSKADYEKHKVYACEVTHQGLSSPVTKSFNR GECGGGGSEKSSGSGSESKSTEGKSSGSGSESKSTGGGGSEVQLLESGGGLV QPGGSLKLSCAASGFTFNTYAMNWVRQAPGKGLEWVARIRSKYNNYATYYAD SVKDRFTISRDDSKNTAYLQMNNLKTEDTAVYYCVRHGNFGNSYVSWFLYWG QGTLVTVSSASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGA LTSGVHTFPAVLQSSGLYSLSSVTVPSSSLGTQTYICNVNHKPSNTKVDKKVE	Cross316scFab
152	ELVVTQEPSLTVSPGGTVTLTCSRSTGAVTTSNYANWVQQKPGQAPRGLIGGTN KRAPGTPARFSGSLLGGKAALTLSGVQPEDEAEYYCALWYSNLWVFGGGTKL TVLRTVAAPSVFIFPPSDEQLKSGTASVVCLLNFPYAPREKVQWKVDNALQSG NSQESVTEQDSKDYSLSSLTLSKADYEKHKVYACEVTHQGLSSPVTKSFNR GECGGGGSEKSSGSGSESKSTEGKSSGSGSESKSTGGGGSEVQLLESGGGLV QPGGSLKLSCAASGFTFNTYAMNWVRQAPGKGLEWVARIRSKYNNYATYYAD SVKDRFTISRDDSKNTAYLQMNNLKTEDTAVYYCVRHGNFGNSYVSWFAGWG QGTLVTVSSASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGA LTSGVHTFPAVLQSSGLYSLSSVTVPSSSLGTQTYICNVNHKPSNTKVDKKVE	Cross317scFab
153	ELVVTQEPSLTVSPGGTVTLTCSRSTGAVTTSNYANWVQQKPGQAPRGLIGGTN KRAPGTPARFSGSLLGGKAALTLSGVQPEDEAEYYCALWYSNLWVFGGGTKL TVLRTVAAPSVFIFPPSDEQLKSGTASVVCLLNFPYAPREKVQWKVDNALQSG NSQESVTEQDSKDYSLSSLTLSKADYEKHKVYACEVTHQGLSSPVTKSFNR GECGGGGSEKSSGSGSESKSTEGKSSGSGSESKSTGGGGSEVQLLESGGGLV QPGGSLKLSCAASGFTFNTYAMNWVRQAPGKGLEWVARIRSKYNNYATYYAD SVKDRFTISRDDSKNTAYLQMNNLKTEDTAVYYCVRHGNFGNSYVSWFAMW GGQTLVTVSSASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSG ALTSGVHTFPAVLQSSGLYSLSSVTVPSSSLGTQTYICNVNHKPSNTKVDKKV E	Cross318scFab

154	ELVVTQEPSLTVSPGGTVTLTCSRSTGAVTTSNYANWVQQKPGQAPRGLIGGTN KRAPGTPARFSGSLLGGKAALTLSGVQPEDEAEYYCALWYPNLWVFGGGTKL TVLRTVAAPSVFIFPPSDEQLKSGTASVVCLLNNFYPREAKVQWKVDNALQSG NSQESVTEQDSKDSTYLSSTLTLSKADYEKHKVYACEVTHQGLSSPVTKSFNR GECGGGGSEKSSGSGSESKSTEGKSSGSGSESKSTGGGGSEVQLLESGGGLV QPGGSLKLSCAASGFTFNTYAMNWVRQAPGKGLEWVARIRSKYNNYATYYAD SVKDRFTISRDDSKNTAYLQMNNLKTEDTAVYYCVRHGNFGNSYVSWFAYWG QGTLVTVSSASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGA LTSGVHTFPAVLQSSGLYSLSSVTVTPSSSLGTQTYICNVNHKPSNTKVDKKVE	Cross323scFab
155	ELVVTQEPSLTVSPGGTVTLTCSRSTGAVTTSNYANWVQQKPGQAPRGLIGGTN KRAPGTPARFSGSLLGGKAALTLSGVQPEDEAEYYCALWYSNDWVFGGGTKL TVLRTVAAPSVFIFPPSDEQLKSGTASVVCLLNNFYPREAKVQWKVDNALQSG NSQESVTEQDSKDSTYLSSTLTLSKADYEKHKVYACEVTHQGLSSPVTKSFNR GECGGGGSEKSSGSGSESKSTEGKSSGSGSESKSTGGGGSEVQLLESGGGLV QPGGSLKLSCAASGFTFNTYAMNWVRQAPGKGLEWVARIRSKYNNYATYYAD SVKDRFTISRDDSKNTAYLQMNNLKTEDTAVYYCVRHGNFGNSYVSWFAYWG QGTLVTVSSASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGA LTSGVHTFPAVLQSSGLYSLSSVTVTPSSSLGTQTYICNVNHKPSNTKVDKKVE	Cross325scFab
156	ELVVTQEPSLTVSPGGTVTLTCSRSTGAVTTSNYANWVQQKPGQAPRGLIGGTN KRAPGTPARFSGSLLGGKAALTLSGVQPEDEAEYYCALWYSPWVFGGGTKL TVLRTVAAPSVFIFPPSDEQLKSGTASVVCLLNNFYPREAKVQWKVDNALQSG NSQESVTEQDSKDSTYLSSTLTLSKADYEKHKVYACEVTHQGLSSPVTKSFNR GECGGGGSEKSSGSGSESKSTEGKSSGSGSESKSTGGGGSEVQLLESGGGLV QPGGSLKLSCAASGFTFNTYAMNWVRQAPGKGLEWVARIRSKYNNYATYYAD SVKDRFTISRDDSKNTAYLQMNNLKTEDTAVYYCVRHGNFGNSYVSWFAYWG QGTLVTVSSASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGA LTSGVHTFPAVLQSSGLYSLSSVTVTPSSSLGTQTYICNVNHKPSNTKVDKKVE	Cross326scFab
157	ELVVTQEPSLTVSPGGTVTLTCSRSTGAVTTSNYANWVQQKPGQAPRGLIGGTN KRAPGTPARFSGSLLGGKAALTLSGVQPEDEAEYYCALWYPNPWVFGGGTKL TVLRTVAAPSVFIFPPSDEQLKSGTASVVCLLNNFYPREAKVQWKVDNALQSG NSQESVTEQDSKDSTYLSSTLTLSKADYEKHKVYACEVTHQGLSSPVTKSFNR GECGGGGSEKSSGSGSESKSTEGKSSGSGSESKSTGGGGSEVQLLESGGGLV QPGGSLKLSCAASGFTFNTYAMNWVRQAPGKGLEWVARIRSKYNNYATYYAD SVKDRFTISRDDSKNTAYLQMNNLKTEDTAVYYCVRHGMFGNSYVSWFAYW GGTLVTVSSASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSG ALTSGVHTFPAVLQSSGLYSLSSVTVTPSSSLGTQTYICNVNHKPSNTKVDKKV E	Cross3ZH07scFab
158	ELVVTQEPSLTVSPGGTVTLTCSRSTGAVTTSNYANWVQQKPGQAPRGLIGGTN KRAPGTPARFSGSLLGGKAALTLSGVQPEDEAEYYCALWYPNPWVFGGGTKL TVLRTVAAPSVFIFPPSDEQLKSGTASVVCLLNNFYPREAKVQWKVDNALQSG NSQESVTEQDSKDSTYLSSTLTLSKADYEKHKVYACEVTHQGLSSPVTKSFNR GECGGGGSEKSSGSGSESKSTEGKSSGSGSESKSTGGGGSEVQLLESGGGLV QPGGSLKLSCAASGFTFNTYAMNWVRQAPGKGLEWVARIRSKYNNYATYYAD SVKDRFTISRDDSKNTAYLQMNNLKTEDTAVYYCVRHGNFGNSYVSWFLYWG QGTLVTVSSASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGA LTSGVHTFPAVLQSSGLYSLSSVTVTPSSSLGTQTYICNVNHKPSNTKVDKKVE	Cross3ZH09scFab
159	ELVVTQEPSLTVSPGGTVTLTCSRSTGAVTTSNYANWVQQKPGQAPRGLIGGTN KRAPGTPARFSGSLLGGKAALTLSGVQPEDEAEYYCALWYPNDWVFGGGTKL TVLRTVAAPSVFIFPPSDEQLKSGTASVVCLLNNFYPREAKVQWKVDNALQSG NSQESVTEQDSKDSTYLSSTLTLSKADYEKHKVYACEVTHQGLSSPVTKSFNR GECGGGGSEKSSGSGSESKSTEGKSSGSGSESKSTGGGGSEVQLLESGGGLV QPGGSLKLSCAASGFTFNTYAMNWVRQAPGKGLEWVARIRSKYNNYATYYAD SVKDRFTISRDDSKNTAYLQMNNLKTEDTAVYYCVRHGNFGNSYVSWFAYWG QGTLVTVSSASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGA LTSGVHTFPAVLQSSGLYSLSSVTVTPSSSLGTQTYICNVNHKPSNTKVDKKVE	Cross3ZH16scFab

160	ELVVTQEPLSTVSPGGTVTLTCSRSTGAVTTSNYANWVQQKPGQAPRGLIGGTN KRAPGTPARFSGSLLGGKAALTLSGVQPEDEAEYYCALWYSNPWFVGGGKTL TVLRTVAAPSVFIFPPSDEQLKSGTASVVCLLNNFYPREAKVQWKVDNALQSG NSQESVTEQDSKDSTYLSSTLTLSKADYEKHKVYACEVTHQGLSSPVTKSFNR GECGGGGSEGKSSGSGSESKSTEGKSSGSGSESKSTGGGGSEVQLLESGGGLV QPGGSLKLSCAASGFTFTNYAMNWRQAPGKGLEWVARIRSKYNNYATYYAD SVKDRFTISRDDSKNTAYLQMNNLKTEDTAVYYCVRHGNGNDYVSWFAYW GQGTLLTVSSASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSG ALTSGVHTFPAVLQSSGLYSLSSVVTVPSSSLGTQTYICNVNHKPSNTKVDKKV E	Cross3ZH23scFab
161	RTVAAPSVFIFPPSDEQLKSGTASVVCLLNNFYPREAKVQWKVDNALQSGNSQ ESVTEQDSKDSTYLSSTLTLSKADYEKHKVYACEVTHQGLSSPVTKSFNRGEC GGGGSEGKSSGSGSESKSTEGKSSGSGSESKSTGGGGSQVQLQSGSELKKPG ASVKVSCASGYTFTNYGMNWVKQAPGQGLKWMGWINTYTGEPTYTDDFK GRFAFSLDTSVSTAYLQISSLKADDTAVYFCARGGFGSSYWFYFDVWGQGLVT VSSASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVH TFPAVLQSSGLYSLSSVVTVPSSSLGTQTYICNVNHKPSNTKVDKKVE	Trop2 antibody scFab
162	QVQLQSGSELKKPGASVKVSCASGYTFTNYGMNWVKQAPGQGLKWMG WINTYTGEPTYTDDFKGRFAFSLDTSVSTAYLQISSLKADDTAVYFCARGGFGS SYWYFDVWGQGLVTVSSASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPE PVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSSLGTQTYICNVNHKPS NTKVDKKVE	Trop2 antibody Fab VH-CH1
163	DIQLTQSPSFLSASVGDRTITCKASQDVGSVAVVWYQQKPGKAPKLLIYWASTR HTGVPSRFSGSGSGTEFTLTISSLQPEDFATYFCQQFTSYPTFGQGTKEIKRTVA APSVFIFPPSDEQLKSGTASVVCLLNNFYPREAKVQWKVDNALQSGNSQESVT EQDSKDSTYLSSTLTLSKADYEKHKVYACEVTHQGLSSPVTKSFNRGECGGG GSEGKSSGSGSESKSTEGKSSGSGSESKSTGGGGSQVQLVQSGAEVKKPGASV KVSCASGYSFTGYMHVWRQAPGQGLEWIGRINPYNGATTYSQNFKDRAT MTVDTISISTAYMELSRLRSDDTAVYYCVPTGMGYFLMDYWGQGTLLTVSSAS TKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAV LQSSGLYSLSSVVTVPSSSLGTQTYICNVNHKPSNTKVDKKVE	PDL1 antibody scFab
164	QVQLVQSGAEVKKPGASVKVSCASGYSFTGYMHVWRQAPGQGLEWIGRI NPYNGATTYSQNFKDRATMTVDTISISTAYMELSRLRSDDTAVYYCVPTGMGYF LMDYWGQGTLLTVSSASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVT VSWNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSSLGTQTYICNVNHKPSNT KVDKKVE	PDL1 antibody Fab VH-CH1
165	PKSCDKTHTCPPCPAPEAAGGPSVFLFPPKPKDITLMISRTPEVTCVVVDVSHED PEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVHLQDWLNGKEYKC KVSNAKALPAIEKTISKAKGQPREPQVCTLPPSREEMTKNQVSLCAVKGFYPS DIAVEWESNGQPENNYKTTTPVLDSDGSFFLVSKLTVDKSRWQQGNVFSCSVM HEALHNHYTQKSLSLSPGK	Second Fc peptide segment
166	PKSCDKTHTCPPCPAPEAAGGPSVFLFPPKPKDITLMISRTPEVTCVVVDVSHED PEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVHLQDWLNGKEYKC KVSNAKALPAIEKTISKAKGQPREPQVYTLPPCREEMTKNQVSLWCLVKGFYPS DIAVEWESNGQPENNYKTTTPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVM HEALHNHYTQKSLSLSPGK	Corresponding first Fc peptide segment when CD3 binding region is a Fab or scFab structure

[00196] Hereinafter, the present disclosure will be described in detail by way of examples.

In the examples or test examples, the experimental methods without specifying the specific conditions were carried out according to conventional conditions.

[00197] Hereinafter, the scheme of the present disclosure will be explained in conjunction

5 with examples. Those skilled in the art will understand that the following examples are only used to illustrate the present disclosure and should not be considered as limiting the scope of

the present disclosure. Where specific techniques or conditions are not specified in the examples, they are performed according to techniques or conditions described in the literature in the art or according to the product specification. The reagents or instruments used without specifying the manufacturer are all conventional products that can be obtained commercially.

5 **Example 1: Acquisition and production of an affinity-reduced antibody**

[00198] During the affinity maturation process of natural antibodies, somatic hypermutations are mainly concentrated in the CDR region. Generally, single-point saturation mutagenesis is performed at each site in the CDR region through *in vitro* experiments to obtain sufficient mutation diversity without destroying the protein structure. This approach can achieve *in vitro* reproduction that is most similar to the somatic hypermutation of natural antibodies *in vivo*. The above method is generally used for affinity maturation (enhancement) of antibodies. With reference to this technical route, the present disclosure, conversely, reduces the affinity of CD3 antibodies.

[00199] The single-point saturation mutagenesis was performed on each amino acid site in the CDR region to construct an unbiased single-point saturation mutagenesis plasmid library of the parental antibody. Mutation hotspots with weakened specific binding to the antigen were screened out, and these hotspots were then combined for further screening to obtain candidate antibody mutation sequences.

[00200] Through this technical route and after a large number of screening tests, the affinity of the parental antibody Cross3 (the amino acid sequence of the heavy chain variable region is as set forth in SEQ ID NO: 15, and the amino acid sequence of the light chain variable region is as set forth in SEQ ID NO: 16) was reduced, and the following low-affinity CD3 monoclonal antibodies were obtained: Cross303, Cross307, Cross308, Cross309, Cross310, Cross311, Cross312, Cross313, Cross314, Cross316, Cross317, Cross318, Cross323, Cross325, Cross326, Cross3ZH07, Cross3ZH09, Cross3ZH16, and Cross3ZH23. The amino acid sequences of the CDR region and the heavy and light chain amino acid sequences of the above-described low-affinity CD3 monoclonal antibody were shown in Table 1. The amino acid mutations in the CDR region compared with the parental antibody Cross3 were shown in FIG. 1.

[00201] The specific experimental operations for the production of the CD3 monoclonal antibodies were as follows. (1) ExpiCHO cells (purchased from Thermo Fisher) were cultured

using ExpiCHO Expression Medium (purchased from Thermo Fisher), and the cell concentration was adjusted to $6 \times 10^6/\text{mL}$ to obtain ExpiCHO cell solution. (2) The pcDNA3.4 vectors containing the heavy chain and light chain of the CD3 antibody (for Cross3, heavy chain amino acid sequence is as set forth in SEQ ID NO: 35, and light chain amino acid sequence thereof is as set forth in SEQ ID NO: 33; for Cross303, heavy chain amino acid sequence is as set forth in SEQ ID NO: 37, and light chain amino acid sequence thereof is as set forth in SEQ ID NO: 33; for Cross307, heavy chain amino acid sequence is as set forth in SEQ ID NO: 38, and light chain amino acid sequence thereof is as set forth in SEQ ID NO: 33; for Cross308, heavy chain amino acid sequence is as set forth in SEQ ID NO: 39, and light chain amino acid sequence thereof is as set forth in SEQ ID NO: 33; for Cross309, heavy chain amino acid sequence is as set forth in SEQ ID NO: 40, and light chain amino acid sequence thereof is as set forth in SEQ ID NO: 33; for Cross310, heavy chain amino acid sequence is as set forth in SEQ ID NO: 41, and light chain amino acid sequence thereof is as set forth in SEQ ID NO: 33; for Cross311, heavy chain amino acid sequence is as set forth in SEQ ID NO: 42, and light chain amino acid sequence thereof is as set forth in SEQ ID NO: 33; for Cross312, heavy chain amino acid sequence is as set forth in SEQ ID NO: 43, and light chain amino acid sequence is as set forth in SEQ ID NO: 33; for Cross313, heavy chain amino acid sequence is as set forth in SEQ ID NO: 32, and light chain amino acid sequence is as set forth in SEQ ID NO: 33; for Cross314, heavy chain amino acid sequence is as set forth in SEQ ID NO: 44, and light chain amino acid sequence is as set forth in SEQ ID NO: 33; for Cross316, heavy chain amino acid sequence is as set forth in SEQ ID NO: 34, and light chain amino acid sequence is as set forth in SEQ ID NO: 33; for Cross317, heavy chain amino acid sequence is as set forth in SEQ ID NO: 45, and light chain amino acid sequence is as set forth in SEQ ID NO: 33; for Cross318, heavy chain amino acid sequence is as set forth in SEQ ID NO: 46, and light chain amino acid sequence is as set forth in SEQ ID NO: 33; for Cross323, heavy chain amino acid sequence is as set forth in SEQ ID NO: 45, and light chain amino acid sequence is as set forth in SEQ ID NO: 47; for Cross325, heavy chain amino acid sequence is as set forth in SEQ ID NO: 45, and light chain amino acid sequence thereof is as set forth in SEQ ID NO: 36; and for Cross326, heavy chain amino acid sequence is as set forth in SEQ ID NO: 46, and light chain amino acid sequence thereof is as set forth in SEQ ID NO: 48) (Synthesized by Nanjing GenScript) were

added to 2 mL of OptiSFM medium (purchased from Thermo Fisher) at a ratio of 1: 1 to obtain solution a. (3) 160 μ L of ExpiFectamine CHO transfection reagent (purchased from Thermo Fisher) was added to 2 mL of OptiSFM medium (purchased from Thermo Fisher) to obtain solution b. (4) The solution a and the solution b were then mixed to obtain a transfection mixture, and the entire transfection mixture was added to 50 mL of the ExpiCHO cell solution within 5 minutes. (5) After culturing at 37°C and 5% CO₂ for 1 day, 8 mL of Feed and 300 μ L of Enhancer (purchased from Thermo Fisher) were added, and the cells were transferred to 32°C and 5% CO₂ and cultured for 9 days, with 8 mL of Feed added on day 5. The culture supernatant was harvested. (6) The target antibody was obtained by affinity purification from the culture supernatant using a Protein A purification column (purchased from NanoMicro).

[00202] The specific experimental operations for the production of bispecific antibodies were as follows. (1) ExpiCHO cells (purchased from Thermo Fisher) were cultured using ExpiCHO Expression Medium (purchased from Thermo Fisher), and the cell concentration was adjusted to 6×10^6 /mL to obtain ExpiCHO cell solution. (2) The pcDNA3.4 vectors containing CD3 antibody, Trop2 antibody heavy chain, and Trop2 antibody (Cross3×Trop2 contains CD3 antibody as set forth in SEQ ID NO: 52, Trop2 antibody heavy chain having an amino acid sequence as set forth in SEQ ID NO: 69, and Trop2 antibody light chain having an amino acid sequence as set forth in SEQ ID NO: 70; Cross303×Trop2 contains CD3 antibody as set forth in SEQ ID NO: 53, Trop2 antibody heavy chain having an amino acid sequence as set forth in SEQ ID NO: 69, and Trop2 antibody light chain having an amino acid sequence as set forth in SEQ ID NO: 70; Cross307×Trop2 contains CD3 antibody set forth in SEQ ID NO: 54, Trop2 antibody heavy chain having an amino acid sequence as set forth in SEQ ID NO: 69, and Trop2 antibody light chain having an amino acid sequence set forth in SEQ ID NO: 70; Cross308×Trop2 contains CD3 antibody as set forth in SEQ ID NO: 55, Trop2 antibody heavy chain having an amino acid sequence as set forth in SEQ ID NO: 69, and Trop2 antibody having an amino acid sequence as set forth in SEQ ID NO: 70; Cross309×Trop2 contains CD3 antibody as set forth in SEQ ID NO: 56, Trop2 antibody heavy chain having an amino acid sequence as set forth in SEQ ID NO: 69, and Trop2 antibody light chain having an amino acid sequence as set forth in SEQ ID NO: 70; Cross310×Trop2 contains CD3 antibody as set forth in SEQ ID NO: 57, Trop2 antibody heavy chain having an amino acid sequence as set forth in SEQ ID NO:

69, and Trop2 antibody light chain having an amino acid sequence as set forth in SEQ ID NO: 70; Cross311×Trop2 contains CD3 antibody as set forth in SEQ ID NO: 58, Trop2 antibody heavy chain having an amino acid sequence as set forth in SEQ ID NO: 69, and Trop2 antibody light chain having an amino acid sequence as set forth in SEQ ID NO: 70; Cross312×Trop2 contains CD3 antibody as set forth in SEQ ID NO: 59, Trop2 antibody heavy chain having an amino acid sequence as set forth in SEQ ID NO: 69, and Trop2 antibody light chain having an amino acid sequence as set forth in SEQ ID NO: 70; Cross313×Trop2 contains CD3 antibody as set forth in SEQ ID NO: 49, Trop2 antibody heavy chain having an amino acid sequence as set forth in SEQ ID NO: 69, and Trop2 antibody light chain having an amino acid sequence as set forth in SEQ ID NO: 70; Cross314×Trop2 contains CD3 antibody as set forth in SEQ ID NO: 60, Trop2 antibody heavy chain having an amino acid sequence as set forth in SEQ ID NO: 69, and Trop2 antibody light chain having an amino acid sequence as set forth in SEQ ID NO: 70; Cross316×Trop2 contains CD3 antibody as set forth in SEQ ID NO: 50, Trop2 antibody heavy chain having an amino acid sequence as set forth in SEQ ID NO: 69, and Trop2 antibody light chain having an amino acid sequence as set forth in SEQ ID NO: 70; Cross317×Trop2 contains CD3 antibody as set forth in SEQ ID NO: 61, Trop2 antibody heavy chain having an amino acid sequence as set forth in SEQ ID NO: 69, and Trop2 antibody light chain having an amino acid sequence as set forth in SEQ ID NO: 70; Cross318×Trop2 contains CD3 antibody as set forth in SEQ ID NO: 62, Trop2 antibody heavy chain having an amino acid sequence as set forth in SEQ ID NO: 69, and Trop2 antibody light chain having an amino acid sequence as set forth in SEQ ID NO: 70; Cross323×Trop2 contains CD3 antibody as set forth in SEQ ID NO: 63, Trop2 antibody heavy chain having an amino acid sequence as set forth in SEQ ID NO: 69, and Trop2 antibody light chain having an amino acid sequence as set forth in SEQ ID NO: 70; Cross325×Trop2 contains CD3 antibody as set forth in SEQ ID NO: 51, Trop2 antibody heavy chain having an amino acid sequence as set forth in SEQ ID NO: 69, and Trop2 antibody light chain having an amino acid sequence as set forth in SEQ ID NO: 70; Cross326×Trop2 contains CD3 antibody as set forth in SEQ ID NO: 64, Trop2 antibody heavy chain having an amino acid sequence as set forth in SEQ ID NO: 69, and Trop2 antibody light chain having an amino acid sequence as set forth in SEQ ID NO: 70) (synthesized by Nanjing GenScript), or the pcDNA3.4 vectors containing CD3 antibody, PDL1 antibody heavy chain, and PDL1

[illegible]

acid sequence as set forth in SEQ ID NO: 72; Cross317×PDL1 contains CD3 antibody as set forth in SEQ ID NO: 61, PDL1 antibody heavy chain having an amino acid sequence as set forth in SEQ ID NO: 71, and PDL1 antibody light chain having an amino acid sequence as set forth in SEQ ID NO: 72; Cross318×PDL1 contains CD3 antibody as set forth in SEQ ID NO: 62, PDL1 antibody heavy chain having an amino acid sequence as set forth in SEQ ID NO: 71, and PDL1 antibody light chain having an amino acid sequence as set forth in SEQ ID NO: 72; Cross323×PDL1 contains CD3 antibody as set forth in SEQ ID NO: 63, PDL1 antibody heavy chain having an amino acid sequence as set forth in SEQ ID NO: 71, and PDL1 antibody light chain having an amino acid sequence as set forth in SEQ ID NO: 72; Cross325×PDL1 contains CD3 antibody as set forth in SEQ ID NO: 51, PDL1 antibody heavy chain having an amino acid sequence as set forth in SEQ ID NO: 71, and PDL1 antibody light chain having an amino acid sequence as set forth in SEQ ID NO: 72; Cross326×PDL1 contains CD3 antibody as set forth in SEQ ID NO: 64, PDL1 antibody heavy chain having an amino acid sequence as set forth in SEQ ID NO: 71, and PDL1 antibody light chain having an amino acid sequence as set forth in SEQ ID NO: 72; Cross3ZH07×PDL1 contains CD3 antibody as set forth in SEQ ID NO: 65, PDL1 antibody heavy chain having an amino acid sequence set forth in SEQ ID NO: 71, and PDL1 antibody light chain having an amino acid sequence as set forth in SEQ ID NO: 72; Cross3ZH09×PDL1 contains CD3 antibody as set forth in SEQ ID NO: 66, PDL1 antibody heavy chain having an amino acid sequence as set forth in SEQ ID NO: 71, and PDL1 antibody light chain having an amino acid sequence as set forth in SEQ ID NO: 72; Cross3ZH16×PDL1 contains CD3 antibody as set forth in SEQ ID NO: 67, PDL1 antibody heavy chain having an amino acid sequence as set forth in SEQ ID NO: 71, and PDL1 antibody light chain having an amino acid sequence as set forth in SEQ ID NO: 72; Cross3ZH23×PDL1 contains CD3 antibody as set forth in SEQ ID NO: 68, PDL1 antibody heavy chain having an amino acid sequence as set forth in SEQ ID NO: 71, and PDL1 antibody light chain having an amino acid sequence as set forth in SEQ ID NO: 72) (synthesized by Nanjing GenScript) were added to 2 mL of OptiSFM medium (purchased from Thermo Fisher) at a ratio of 1: 1: 1 to obtain solution a. (3) 160 µL of ExpiFectamine CHO transfection reagent (purchased from Thermo Fisher) was added to 2 mL of OptiSFM medium (purchased from Thermo Fisher) to obtain solution b. (4) The solution a and the solution b were then mixed to obtain a transfection mixture, and the

entire transfection mixture was added to 50 mL of the ExpiCHO cell solution within 5 minutes.

(5) After culturing at 37°C and 5% CO₂ for 1 day, 8 mL of Feed and 300 µL of Enhancer (purchased from Thermo Fisher) were added, and the cells were transferred to 32°C and 5% CO₂ and cultured for 9 days, with 8 mL of Feed added on day 5. The culture supernatant was harvested. (6) The target CD3×Trop2 or CD3×PDL1 antibodies were obtained by affinity purification from the culture supernatant using a Protein A purification column (purchased from NanoMicro). The antibody configuration was shown in FIG. 5.

Example 2: Antibody affinity assay

[00203] Biacore is a method for analyzing biomolecular interactions based on the principle of optical surface plasmon resonance (SPR). It can not only detect the specific binding between antigens and antibodies, but also obtain intermolecular binding rate constant (ka), dissociation rate constant (kd), equilibrium dissociation constant (KD), etc., which are very important data in drug development, thereby enabling calculation of antibody affinity.

[00204] In a Biacore 8K (Cytiva) system, the antibody was diluted to 10 µg/mL in HBS-EP running buffer and coupled to a CM5 chip (Cytiva) at a flow rate of 10 µL/min. At a flow rate of 30 µL/min, the kinetics and affinity data of the binding of CD3 E&D antigens to the low-affinity CD3 monoclonal antibody of Example 1 of the present disclosure were detected, with the association time set to 120 s and the dissociation time set to 800 s. The results of the antibody affinity assay were shown in FIG. 2.

Example 3: CD3 monoclonal antibody ELISA binding assay

[00205] In this example, the binding properties of the parental antibody Cross3 and the CD3 affinity-reduced antibody (the production method and the amino acid sequences of the heavy and light chains were shown in Example 1) were detected by ELISA. The CD3E&D protein was coated onto a 96-well plate by the inventors, and the strength of the signal after the addition of the antibody was used to determine the binding properties of the antibody and CD3E&D.

[00206] CD3E&D protein (purchased from Acro) was diluted to 2 µg/ml with PBS buffer, added to a 96-well plate at a volume of 100 µL/well, and placed at 4°C overnight. The PBS buffer in the 96-well plate was aspirated, and the plate was washed 6 times with PBST (pH 7.2 PBS containing 0.1% Tween 20) buffer. Then, 200 µL/well PBS/10% BSA was added and incubated at 37°C for 2 h for blocking. The blocking solution was removed, and the plate was

washed six times with PBST. Then, the to-be-tested antibodies serially diluted with PBST/0.05% BSA, Cross3, Cross303, Cross307, Cross308, Cross309, Cross310, Cross311, Cross312, Cross313, Cross314, Cross316, Cross317, Cross318, Cross323, Cross325, Cross326, and control IgG1 (purchased from Bio-Tech) were added at 100 μ L/well, and incubated at 37°C for 1 h. The reaction system was removed, and the plate was washed 6 times with PBST. Then, the cells were incubated at 37°C for 1 h with HRP (horseradish peroxidase)-labeled anti-human secondary antibody (Fab specific) (purchased from Sigma) serially diluted with PBST/0.05% BSA at 100 μ L/well. The plate was washed six times with PBST, TMB (tetramethylbenzidine) was added at 80 μ L/well and incubated at room temperature for 3 min. 4 M sulfuric acid was added at 80 μ L/well to terminate the reaction. The absorbance was read at 450 nm using a microplate reader.

[00207] The results were shown in FIG. 3, which indicate that all CD3 antibodies of the present disclosure can bind to CD3 E&D. However, due to the limited resolution of the ELISA assay, the ELISA results cannot reflect the differences in affinity among various CD3 monoclonal antibodies.

Example 4: CD3 monoclonal antibody flow cytometry binding assay

[00208] PBMCs were diluted with PBS to 2×10^6 /mL and added to a 1.5 ml EP tube at a volume of 100 μ L/tube, 10 μ L/tube of goat serum was added thereto and incubated at 4°C for 30 min for blocking. The to-be-tested antibodies serially diluted, Cross3, Cross303, Cross307, Cross308, Cross309, Cross310, Cross311, Cross312, Cross313, Cross314, Cross316, Cross317, Cross318, Cross323, Cross325, Cross326 (the production method and the amino acid sequences of the heavy and light chains of the antibody Cross3 and the CD3 affinity-reduced antibody were shown in Example 1), and control IgG1 (purchased from Bio-Tech) were added and incubate at 4°C for 30 min. 1 mL of PBS was added to the EP tube, followed by centrifugation at 3500 rpm for 5 min at 4°C. The supernatant was discarded, and the cells were washed again with PBS. After centrifugation, the supernatant was discarded, and the cells were resuspended with 100 μ L/tube PBS. 1 μ L/tube Alexa-647-labeled goat anti-human secondary antibody (purchased from Jackson lab) and 0.5 μ L/tube PerCP-Cy5.5-labeled anti-human CD8 antibody were added and incubated at 4°C in the dark for 30 min. The cells were washed twice with PBS, and the supernatant was discarded after centrifugation. The cells were resuspended with 200

μL/tube of PBS and assayed using the flow cytometry.

[00209] The results were shown in FIG. 4, which show that the CD3 antibodies, Cross309, Cross310, Cross311, Cross312, Cross313, Cross314, Cross316, Cross317, Cross318, Cross323, and Cross325 of the present disclosure can bind to CD8 T cells with reduced CD3 affinity, and the CD3 antibody binding ability is weaker than that of the parental antibody Cross3.

Example 5: CD3×Trop2 and CD3×PDL1 bispecific antibody ELISA binding assay

[00210] In this example, the binding properties of the CD3×Trop2 and CD3×PDL1 bispecific antibody (the production method and the amino acid sequences of the heavy and light chains were shown in Example 1) were detected by using ELISA. The CD3E&D, Trop2, or PDL1 protein was coated onto 96-well plates by inventors, and the strength of the signal after the addition of the antibody was used to determine the binding properties of the antibody and the corresponding protein.

[00211] (1) CD3E&D protein (purchased from Acro) was diluted to 2 μg/ml with PBS buffer, added to a 96-well plate at a volume of 100 μL/well, and placed at 4°C overnight. The PBS buffer in the 96-well plate was aspirated, and the plate was washed 6 times with PBST (pH 7.2 PBS containing 0.1% Tween 20) buffer. Then, 200 μL/well PBS/10% BSA was added and incubated at 37°C for 2 h for blocking. The blocking solution was removed, and the plate was washed 6 times with PBST. Then, the to-be-tested bispecific antibodies serially diluted with PBST/0.05% BSA, Cross3×Trop2, Cross303×Trop2, Cross307×Trop2, Cross308×Trop2, Cross309×Trop2, Cross310×Trop2, Cross311×Trop2, Cross312×Trop2, Cross313×Trop2, Cross314×Trop2, Cross316×Trop2, Cross317×Trop2, Cross318×Trop2, Cross323×Trop2, Cross325×Trop2, Cross326×Trop2, Cross3×PDL1, Cross303×PDL1, Cross307×PDL1, Cross308×PDL1, Cross309×PDL1, Cross310×PDL1, Cross311×PDL1, Cross312×PDL1, Cross313×PDL1, Cross314×PDL1, Cross316×PDL1, Cross317×PDL1, Cross318×PDL1, Cross323×PDL1, Cross325×PDL1, Cross326×PDL1, and control IgG1LALA (purchased from Bio-Tech) were added at 100 μL/well, and incubated at 37°C for 1 h. The reaction system was removed, and the plate was washed 6 times with PBST. Then, the cells were incubated at 37°C for 1 h with HRP (horseradish peroxidase)-labeled anti-human secondary antibody (Fab specific) (purchased from Sigma) diluted with PBST/0.05% BSA at 100 μL/well. After the plate was washed six times with PBST, 80 μL/well TMB (tetramethylbenzidine) was added and

incubated at room temperature for 3 min. 4 M sulfuric acid was added at 80 μ L/well to terminate the reaction. The absorbance was read at 450 nm using a microplate reader.

[00212] As shown in FIG. 6, the CD3 $\times$ Trop2 antibody of the present disclosure can bind to CD3E&D. Compared with the parental Cross3 $\times$ Trop2 bispecific antibody, the binding of the affinity-reduced CD3 bispecific antibody to CD3E&D protein is weakened, which is consistent with expectations.

[00213] As shown in FIG. 14, the CD3 $\times$ PDL1 antibody of the present disclosure can bind to CD3E&D. Compared with the parent Cross3 $\times$ PDL1 bispecific antibody, the binding of the affinity-reduced CD3 bispecific antibody to CD3E&D protein is weakened, which is consistent with expectations.

[00214] (2) Trop2 protein (purchased from Acro) was diluted to 2 μ g/ml with PBS buffer, added to a 96-well plate at a volume of 100 μ L/well, and placed at 4°C overnight. The PBS buffer in the 96-well plate was aspirated, and the plate was washed 6 times with PBST (pH 7.2 PBS containing 0.1% Tween 20) buffer. Then, 200 μ L/well PBS/10% BSA was added and incubated at 37°C for 2 h for blocking. The blocking solution was removed, and the plate was washed six times with PBST. Then, the to-be-tested bispecific antibodies, Cross3 $\times$ Trop2, Cross303 $\times$ Trop2, Cross307 $\times$ Trop2, Cross308 $\times$ Trop2, Cross309 $\times$ Trop2, Cross310 $\times$ Trop2, Cross311 $\times$ Trop2, Cross312 $\times$ Trop2, Cross313 $\times$ Trop2, Cross314 $\times$ Trop2, Cross316 $\times$ Trop2, Cross317 $\times$ Trop2, Cross318 $\times$ Trop2, Cross323 $\times$ Trop2, Cross325 $\times$ Trop2, Cross326 $\times$ Trop2, and control IgG1LALA (purchased from Bio-Tech), serially diluted with PBST/0.05% BSA, were added at 100 μ L/well and incubated at 37°C for 1 h. The reaction system was removed, and the plate was washed 6 times with PBST. Then, HRP (horseradish peroxidase)-labeled anti-human secondary antibody (Fab specific) (purchased from Sigma) diluted with PBST/0.05% BSA at 100 μ L/well. After the plate was washed six times with PBST, TMB (tetramethylbenzidine) was added at 80 μ L/well, and incubated at room temperature for 3 min. 4 M sulfuric acid was added at 80 μ L/well to terminate the reaction. The absorbance was read at 450 nm using a microplate reader.

[00215] As shown in FIG. 7, it indicates that the CD3 $\times$ Trop2 antibody of the present disclosure can bind to the Trop2 protein, and all bispecific antibodies exhibit similar binding to the Trop2 protein.

[00216] (3) PDL1 protein (purchased from Acro) was diluted to 2 µg/ml with PBS buffer, added to a 96-well plate at a volume of 100 µL/well, and placed at 4°C overnight. The PBS buffer in the 96-well plate was aspirated, and the plate was washed 6 times with PBST (pH 7.2 PBS containing 0.1% Tween 20) buffer. Then, PBS/10% BSA was added at 200 µL/well and incubated at 37°C for 2 h for blocking. The blocking solution was removed, and the plate was washed six times with PBST. Then, the to-be-tested bispecific antibodies serially diluted with PBST/0.05% BSA, Cross3×PDL1, Cross303×PDL1, Cross307×PDL1, Cross308×PDL1, Cross309×PDL1, Cross310×PDL1, Cross311×PDL1, Cross312×PDL1, Cross313×PDL1, Cross314×PDL1, Cross316×PDL1, Cross317×PDL1, Cross318×PDL1, Cross323×PDL1, Cross325×PDL1, Cross326×PDL1, and control IgG1LALA (purchased from Bio-Tech) were added at 100 µL/well, and incubated at 37°C for 1 h. The reaction system was removed, and the plate was washed 6 times with PBST. Then, the cells were incubated at 37°C for 1 h with HRP (horseradish peroxidase)-labeled anti-human secondary antibody (Fab specific) (purchased from Sigma) serially diluted with PBST/0.05% BSA at 100 µL/well. After the plate was washed six times with PBST, TMB (tetramethylbenzidine) was added at 80 µL/well and incubated at room temperature for 3 min. 4 M sulfuric acid was added at 80 µL/well to terminate the reaction. The absorbance was read at 450 nm using a microplate reader.

[00217] As shown in FIG. 15, it indicates that the CD3×PDL1 antibody of the present disclosure can bind to the PDL1 protein.

20 **Example 6: CD3×Trop2 antibody ELISA bridging assay**

[00218] ELISA assay was used to detect the bridging binding properties of the CD3×Trop2 bispecific antibody (the production method and amino acid sequences of the heavy and light chains were shown in Example 1). CD3E&D antigen protein was coated onto a 96-well plate. After the antibody was added, detection was performed by using biotin-labeled Trop2 protein. The strength of the signal was used to determine the binding properties of the bispecific antibody bridging CD3E&D and Trop2 proteins.

[00219] CD3E&D protein (purchased from Acro) was diluted to 2 µg/ml with PBS buffer, added to a 96-well plate at a volume of 100 µL/well, and placed at 4°C overnight. The PBS buffer in the 96-well plate was aspirated, and the plate was washed 6 times with PBST (pH 7.2 PBS containing 0.1% Tween 20) buffer. Then, PBS/10% BSA was added at 200 µL/well, and

incubated at 37°C for 2 h for blocking. The blocking solution was removed, and the plate was washed six times with PBST. Then, the to-be-tested bispecific antibodies serially diluted with PBST/0.05% BSA, Cross3×Trop2, Cross303×Trop2, Cross307×Trop2, Cross308×Trop2, Cross309×Trop2, Cross310×Trop2, Cross311×Trop2, Cross312×Trop2, Cross313×Trop2, Cross314×Trop2, Cross316×Trop2, Cross317×Trop2, Cross318×Trop2, Cross323×Trop2, Cross325×Trop2, Cross326×Trop2, and the control hIgG1LALA (purchased from Bio-Tech), were added at 100 µl/well, and incubated at 37°C for 1 h. The reaction system was removed, and the plate was washed 6 times with PBST. Trop2-Biotin protein diluted to an appropriate concentration was added and incubated at 37°C for 1 h. The reaction system was removed, and the plate was washed 6 times with PBST. The cells were incubated at 37°C for 1 h with HRP (horseradish peroxidase)-labeled Streptavidin secondary antibody (purchased from Southern Biotech) serially diluted with PBST/0.05% BSA at 100 µl/well. After the plate was washed six times with PBST, TMB (tetramethylbenzidine) was added at 80 µl/well, and incubated at room temperature for 3 min. 4 M sulfuric acid was added at 80 µl/well to terminate the reaction. The absorbance was read at 450 nm using a microplate reader.

[00220] The results were shown in FIG. 8, indicating that the bispecific antibodies of the present disclosure can bridge CD3E&D and Trop2 proteins, and the bridging ability of the parental Cross3×Trop2 antibody is stronger than that of the affinity-reduced CD3 bispecific antibodies, which is consistent with expectations.

Example 7: Bispecific antibody flow cytometry binding assay

[00221] The flow cytometry experiment was used to detect the binding properties of the bispecific antibodies. The bispecific antibodies (the production method and amino acid sequences of heavy and light chains were shown in Example 1) were added to cells, and the strength of the signal after the addition of the antibody was measured.

[00222] (1) PBMCs were diluted to 2×10^6 /ml with PBS and added to a 1.5 ml EP tube at 100 µl/tube, goat serum was added thereto at 10 µl/tube. The cells were blocked at 4°C for 30 min. The serially diluted bispecific antibodies, Cross3×Trop2, Cross303×Trop2, Cross307×Trop2, Cross308×Trop2, Cross309×Trop2, Cross310×Trop2, Cross311×Trop2, Cross312×Trop2, Cross313×Trop2, Cross314×Trop2, Cross316×Trop2, Cross317×Trop2, Cross318×Trop2, Cross323×Trop2, Cross325×Trop2, Cross326×Trop2, Cross3×PDL1, Cross313×PDL1,

Cross316×PDL1, Cross325×PDL1, and control hIgG1LALA (purchased from Bio-Tech) were added and incubated at 4°C for 30 min. 1 ml of PBS was added to the EP tube, followed by centrifugation at 3500 rpm for 5 min at 4°C. The supernatant was discarded, and the system was washed again with PBS. After centrifugation, the supernatant was discarded, and the cells were resuspended with PBS at 100 µl/tube. 1 µl/tube Alexa-647-labeled goat anti-human IgG secondary antibody (purchased from Jackson lab) and 0.5 µl/tube PerCP-Cy5.5-labeled anti-human CD8 antibody were added and incubated at 4°C in the dark for 30 min. The cells were washed twice with PBS. After centrifugation, the supernatant was discarded. The cells were resuspended with 200 µl/tube of PBS and assay is performed by flow cytometry.

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[00223] The results, as shown in FIG. 9, indicate that the CD3×Trop2 antibody of the present disclosure can bind to CD8 T cells, and compared with the parental CD3 antibody Cross3×Trop2, the ability of the CD3 affinity-reduced bispecific antibody to bind to T cells is weakened, which is consistent with expectations.

[00224] The results, as shown in FIG. 16, indicate that the Cross3×PDL1, Cross313×PDL1, Cross316×PDL1, and Cross325×PDL1 antibodies of the present disclosure can bind to CD8 T cells, and the binding ability of Cross313×PDL1, Cross316×PDL1, and Cross325×PDL1 to T cells is weaker than that of Cross3×PDL1, which is consistent with expectations.

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[00225] (2) The tumor cells were diluted to 2×10^6 /ml with PBS and added to a 1.5ml EP tube at a volume of 100µl/tube, and goat serum was added thereto at 10µl/tube. The cells were blocked at 4°C for 30 min. The serially diluted bispecific antibodies, Cross3×Trop2, Cross303×Trop2, Cross307×Trop2, Cross308×Trop2, Cross309×Trop2, Cross310×Trop2, Cross311×Trop2, Cross312×Trop2, Cross313×Trop2, Cross314×Trop2, Cross316×Trop2, Cross317×Trop2, Cross318×Trop2, Cross323×Trop2, Cross325×Trop2, Cross326×Trop2, and Cross3×PDL1, Cross303×PDL1, Cross307×PDL1, Cross308×PDL1, Cross309×PDL1, Cross310×PDL1, Cross311×PDL1, Cross312×PDL1, Cross313×PDL1, Cross314×PDL1, Cross316×PDL1, Cross317×PDL1, Cross318×PDL1, Cross323×PDL1, Cross325×PDL1, Cross326×PDL1, and control hIgG1LALA (purchased from Bio-Tech) were added and incubated at 4°C for 30 min. 1 ml of PBS was added to the EP tube, followed by centrifugation at 3500 rpm for 5 min at 4°C. The supernatant was discarded, and the cells were washed again with PBS. After centrifugation, the supernatant was completely discarded, and the cells were

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resuspended with PBS at 100 μ l/tube. Alexa-647-labeled goat anti-human IgG secondary antibody (purchased from Jackson lab) was added at 1 μ l/tube and incubated at 4°C in the dark for 30 min. The cells were washed twice with PBS, and the supernatant was completely discarded after centrifugation. The cells were resuspended with PBS at 200 μ l/tube and assay
5 was performed using the flow cytometry.

[00226] The results, as shown in FIG. 10, indicate that the CD3 $\times$ Trop2 antibodies of the present disclosure can bind to A-375-Trop2 melanoma cells. Based on the mean fluorescence intensity, all bispecific antibodies have similar binding abilities to A-375-Trop2 cells.

[00227] The results, as shown in FIG.S 17, 18, and 19, indicate that the CD3 $\times$ PDL1 antibody
10 of the present disclosure can bind to A-375 melanoma cells, HCT-15 colorectal cancer cells, and A549 lung cancer cells.

Example 8: Experiment on bispecific antibody promoting the killing of tumor cells by PBMCs

[00228] The ability of bispecific antibodies to promote the killing of A-375 melanoma cells
15 and HCT-15 colorectal cancer cells by PBMCs was detected.

[00229] (a) Complete RPMI-1450 medium was added to a 16-well RTCA plate at a volume of 50 μ L/well, and the instrument was calibrated after loading.

[00230] (b) The tumor cells were diluted to 2×10^5 /mL with the complete RPMI-1450 medium and added separately to the RTCA plates obtained in step (1) at a volume of 50 μ L/well,
20 respectively. The cell index was then detected for 24 h using the xCELLigence RTCA MP instrument under the conditions of 37°C and 5% CO₂.

[00231] (c) The bispecific antibodies serially diluted with the complete RPMI-1450 medium, Cross3 $\times$ Trop2, Cross303 $\times$ Trop2, Cross307 $\times$ Trop2, Cross308 $\times$ Trop2, Cross309 $\times$ Trop2, Cross310 $\times$ Trop2, Cross311 $\times$ Trop2, Cross312 $\times$ Trop2, Cross313 $\times$ Trop2, Cross314 $\times$ Trop2,
25 Cross316 $\times$ Trop2, Cross317 $\times$ Trop2, Cross318 $\times$ Trop2, Cross323 $\times$ Trop2, Cross325 $\times$ Trop2, Cross326 $\times$ Trop2, Cross3 $\times$ PDL1, and Cross303 $\times$ PDL1, Cross307 $\times$ PDL1, Cross308 $\times$ PDL1, Cross309 $\times$ PDL1, Cross310 $\times$ PDL1, Cross311 $\times$ PDL1, Cross312 $\times$ PDL1, Cross313 $\times$ PDL1, Cross314 $\times$ PDL1, Cross316 $\times$ PDL1, Cross317 $\times$ PDL1, Cross318 $\times$ PDL1, Cross323 $\times$ PDL1, Cross325 $\times$ PDL1, Cross326 $\times$ PDL1, Cross3ZH07 $\times$ PDL1, Cross3ZH09 $\times$ PDL1,
30 Cross3ZH16 $\times$ PDL1, Cross3ZH23 $\times$ PDL1, and control hIgG1LALA (purchased from Bio-Tech)

were added to the RTCA plate obtained in step (2) at a volume of 20 μ l/well.

[00232] (4) PBMCs (purchased from Miaoshun Biotechnology) were diluted to 1.25×10^6 cells/ml with the complete RPMI-1450 medium and added to the RTCA plate obtained in step (3) at a volume of 80 μ l/well.

5 [00233] (5) The reaction system obtained in step (4) was incubated at 37°C and 5% CO₂, and the cell index was detected for 24 h by using an xCELLigence RTCA MP instrument.

[00234] As shown in FIG. 11, the CD3 $\times$ Trop2 antibody of the present disclosure promotes the killing of A-375-Trop2 melanoma cells by PBMCs. The effective dose of the Cross3 $\times$ Trop2 bispecific antibody is lower than that of the affinity-reduced bispecific antibody. Although the
10 effective doses of Cross313 $\times$ Trop2, Cross316 $\times$ Trop2, and Cross325 $\times$ Trop2 are high, the maximum killing ratio is consistent with that of Cross3 $\times$ Trop2, and all can achieve the highest killing.

[00235] As shown in FIG. 12, Cross3 $\times$ Trop2, Cross313 $\times$ Trop2, Cross316 $\times$ Trop2, and Cross325 $\times$ Trop2 promote the killing of A-375-Trop2 melanoma cells by PBMCs. Although the
15 effective doses of Cross313 $\times$ Trop2 and Cross316 $\times$ Trop2 are high, the highest killing ratio is consistent with that of Cross3 $\times$ Trop2, and both can achieve the highest killing.

[00236] As shown in FIG. 20, CD3 $\times$ PDL1 of the present disclosure promotes the killing of kill A-375 melanoma cells by PBMCs, and Cross313 $\times$ PDL1, Cross316 $\times$ PDL1, and the maximum killing of Cross325 $\times$ PDL1 is superior to that of other bispecific antibodies.

20 [00237] As shown in FIG. 21, Cross3 $\times$ PDL1, Cross313 $\times$ PDL1, Cross316 $\times$ PDL1, and Cross325 $\times$ PDL1 of the present disclosure promote PBMC to kill A-375 melanoma cells, and the maximum killing of Cross316 $\times$ PDL1 is superior to that of Cross313 $\times$ PDL1 and Cross325 $\times$ PDL1.

[00238] As shown in FIG. 22, CD3 $\times$ PDL1 of the present disclosure promotes the killing of
25 HCT-15 colorectal cancer cells by PBMCs, and Cross313 $\times$ PDL1, Cross316 $\times$ PDL1, and Cross325 $\times$ PDL1 can promote 100% killing of HCT-15 colorectal cancer cells by PBMCs.

Example 9: Experiment on bispecific antibodies promoting cytokine secretion by PBMCs

[00239] The bispecific antibodies (the production method and amino acid sequences of heavy and light chains were shown in Example 1) were added to the co-incubation system of
30 PBMCs and tumor cells. After 48 h of incubation, the culture supernatant was collected, and

the cytokine content in the supernatant was detected to determine the characteristics of the bispecific antibody in inducing cytokine release.

[00240] (1) The tumor cells were diluted to $1 \times 10^5/\text{ml}$ with complete RPMI 1450 medium, added to a 96-well plate, and cultured in an incubator at 37°C and 5% CO_2 for 24 h.

5 **[00241]** (2) The bispecific antibodies Cross3×Trop2, Cross313×Trop2, Cross316×Trop2, Cross325×Trop2, Cross3×PDL1, Cross313×PDL1, Cross316×PDL1, Cross325×PDL1, and control hIgG1LALA (purchased from Bio-Bio) were serially diluted with complete RPMI 1450 medium and added to a 96-well plate at $20 \mu\text{l}/\text{well}$.

[00242] (3) PBMCs (purchased from Saili Biotechnology) were diluted to $1.25 \times 10^6/\text{ml}$ with
10 complete RPMI 1450 medium and added to a 96-well plate at $80 \mu\text{l}/\text{well}$.

[00243] (4) The 96-well plate was incubated in an incubator at 37°C and 5% CO_2 for 48 h.

[00244] (5) The cells were centrifuged at 300g for 10 min at room temperature, and the cell culture supernatant was collected.

[00245] (6) The cytokine content in the supernatant was detected using CBA kit (purchased
15 from BD).

[00246] As shown in FIG. 13, Cross3×Trop2, Cross313×Trop2, Cross316×Trop2, and Cross325×Trop2 of the present disclosure promote the secretion of pro-inflammatory cytokines $\text{TNF-}\alpha$, IL-6, $\text{IFN-}\gamma$, and IL-2 by PBMCs, and the ability of the Cross313×Trop2, Cross316×Trop2, and Cross325×Trop2 antibodies in promoting the secretion of pro-
20 inflammatory cytokines is weaker than that of Cross3×Trop2.

[00247] As shown in FIG. 23 and FIG. 24, Cross3×PDL1, Cross313×PDL1, Cross316×PDL1, and Cross325×PDL1 of the present disclosure promote the secretion of pro-inflammatory cytokines $\text{TNF-}\alpha$ and $\text{IFN-}\gamma$ by PBMCs, and the ability of the Cross313×PDL1, Cross316×PDL1, and Cross325×PDL1 antibodies in promoting the secretion of pro-
25 inflammatory cytokines is weaker than that of Cross3×PDL1.

[00248] In the description of the present specification, reference to the terms such as “one embodiment”, “some embodiments”, “an example”, “a specific example”, or “some examples” mean that the specific features, structures, materials, or characteristics described in connection with the embodiment or example are included in at least an embodiment or example of the
30 present disclosure. In the present specification, the illustrative descriptions of the above terms

do not necessarily refer to the same embodiment or example. Moreover, the specific features, structures, materials, or characteristics described may be combined in any one or more embodiments or examples in a suitable manner. Furthermore, where there is no mutual contradiction, those skilled in the art may combine and integrate different embodiments or examples and features of different embodiments or examples described in this specification.

[00249] Although embodiments of the present disclosure are illustrated and described above, it can be understood that the above embodiments are illustrative and should not be construed as limiting the present disclosure. Those skilled in the art can make changes, modifications, substitutions, and variations to the above embodiments within the scope of the present disclosure.

CLAIMS

What is claimed is:

1. An anti-CD3 antibody or antigen binding fragment thereof, comprising:

heavy chain variable region CDRs; and

5 light chain variable region CDRs, wherein:

the heavy chain variable region CDRs have each an amino acid sequence as set forth in one of SEQ ID NOs: 1 to 3, 7 to 8, and 74 to 86, or a conservative modification form thereof; and/or

the light chain variable region CDRs have each an amino acid sequence as set forth in one of SEQ ID NOs: 4 to 6, 9, and 87 to 92, or a conservative modification form thereof.

2. The antibody or antigen binding fragment thereof according to claim 1, wherein:

the heavy chain variable region CDRs have amino acid sequences as set forth in SEQ ID NOs: 1 to 2 or a conservative modification form thereof, and an amino acid sequence as set forth in one of SEQ ID NOs: 3, 7 to 8, and 74 to 86 or a conservative modification form thereof;

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the light chain variable region CDRs have amino acid sequences as set forth in SEQ ID NOs: 4 to 5 or a conservative modification form thereof, and an amino acid sequence as set forth in one of SEQ ID NOs: 6, 9, and 87 to 92 or a conservative modification form thereof.

3. The antibody or antigen binding fragment thereof according to claim 2, comprising:

20 a heavy chain variable region CDR1 having an amino acid sequence as set forth in SEQ ID NO: 1,

a heavy chain variable region CDR2 having an amino acid sequence as set forth in SEQ ID NO: 2,

25 a heavy chain variable region CDR3 having an amino acid sequence as set forth in one of SEQ ID NO: 3, SEQ ID NOs: 7 to 8, and SEQ ID NOs: 74 to 86;

a light chain variable region CDR1 having an amino acid sequence as set forth in SEQ ID NO: 4,

a light chain variable region CDR2 having an amino acid sequence as set forth in SEQ ID

NO: 5,

a light chain variable region CDR3 having an amino acid sequence as set forth in one of SEQ ID NOs: 6, 9, and 87 to 92.

4. The antibody or antigen binding fragment thereof according to claim 3, comprising any
5 one of the following groups:

1) the heavy chain variable region CDR1, the heavy chain variable region CDR2, and the heavy chain variable region CDR3 as set forth in SEQ ID NOs: 1, 2, and 74, respectively, and the light chain variable region CDR1, the light chain variable region CDR2, and the light chain variable region CDR3 as set forth in SEQ ID NOs: 4, 5, and 6, respectively;

10 2) the heavy chain variable region CDR1, the heavy chain variable region CDR2, and the heavy chain variable region CDR3 as set forth in SEQ ID NOs: 1, 2, and 75, respectively; and the light chain variable region CDR1, the light chain variable region CDR2, and the light chain variable region CDR3 as set forth in SEQ ID NOs: 4, 5, and 6, respectively;

3) the heavy chain variable region CDR1, the heavy chain variable region CDR2, and the
15 heavy chain variable region CDR3 as set forth in SEQ ID NOs: 1, 2, and 76, respectively; and the light chain variable region CDR1, the light chain variable region CDR2, and the light chain variable region CDR3 as set forth in SEQ ID NOs: 4, 5, and 6, respectively;

4) the heavy chain variable region CDR1, the heavy chain variable region CDR2, and the heavy chain variable region CDR3 as set forth in SEQ ID NOs: 1, 2, and 77, respectively; and
20 the light chain variable region CDR1, the light chain variable region CDR2, and the light chain variable region CDR3 as set forth in SEQ ID NOs: 4, 5, and 6, respectively;

5) the heavy chain variable region CDR1, the heavy chain variable region CDR2, and the heavy chain variable region CDR3 as set forth in SEQ ID NOs: 1, 2, and 78, respectively; and the light chain variable region CDR1, the light chain variable region CDR2, and the light chain
25 variable region CDR3 as set forth in SEQ ID NOs: 4, 5, and 6, respectively;

6) the heavy chain variable region CDR1, the heavy chain variable region CDR2, and the heavy chain variable region CDR3 as set forth in SEQ ID NOs: 1, 2, and 79, respectively; and the light chain variable region CDR1, the light chain variable region CDR2, and the light chain variable region CDR3 as set forth in SEQ ID NOs: 4, 5, and 6, respectively;

30 7) the heavy chain variable region CDR1, the heavy chain variable region CDR2, and the

variable region CDR3 as set forth in SEQ ID NOs: 4, 5, and 88, respectively;

15) the heavy chain variable region CDR1, the heavy chain variable region CDR2, and the heavy chain variable region CDR3 as set forth in SEQ ID NOs: 1, 2, and 8, respectively; and the light chain variable region CDR1, the light chain variable region CDR2, and the light chain variable region CDR3 as set forth in SEQ ID NOs: 4, 5, and 9, respectively;

16) the heavy chain variable region CDR1, the heavy chain variable region CDR2, and the heavy chain variable region CDR3 as set forth in SEQ ID NOs: 1, 2, and 84, respectively; and the light chain variable region CDR1, the light chain variable region CDR2, and the light chain variable region CDR3 as set forth in SEQ ID NOs: 4, 5, and 89, respectively;

17) the heavy chain variable region CDR1, the heavy chain variable region CDR2, and the heavy chain variable region CDR3 as set forth in SEQ ID NOs: 1, 2, and 85, respectively; and the light chain variable region CDR1, the light chain variable region CDR2, and the light chain variable region CDR3 as set forth in SEQ ID NOs: 4, 5, and 90, respectively;

18) the heavy chain variable region CDR1, the heavy chain variable region CDR2, and the heavy chain variable region CDR3 as set forth in SEQ ID NOs: 1, 2, and 86, respectively; and the light chain variable region CDR1, the light chain variable region CDR2, and the light chain variable region CDR3 as set forth in SEQ ID NOs: 4, 5, and 92, respectively; or

19) the heavy chain variable region CDR1, the heavy chain variable region CDR2, and the heavy chain variable region CDR3 as set forth in SEQ ID NOs: 1, 2 and 8, respectively; and the light chain variable region CDR1, the light chain variable region CDR2, and the light chain variable region CDR3 as set forth in SEQ ID NOs: 4, 5 and 91, respectively.

5. The antibody or antigen binding fragment thereof according to any one of claims 1 to 4, comprising a heavy chain framework region and/or a light chain framework region.

6. The antibody or antigen binding fragment thereof according to claim 5, wherein at least a portion of the heavy chain framework region and/or the light chain framework region is from at least one of a murine antibody, a human antibody, a primate antibody, a bovine antibody, an equine antibody, a dairy bovine antibody, a porcine antibody, an ovine antibody, a caprine antibody, a canine antibody, a feline antibody, a rabbit-derived antibody, a camelid antibody, donkey-derived antibody, a deer-derived antibody, a mink-derived antibody, a chicken-derived antibody, a duck-derived antibody, a goose-derived antibody, a turkey-derived antibody,

gamecock-derived antibody, or a mutant thereof, and preferably, at least one of a murine antibody, a human antibody, or a primate antibody.

7. The antibody or antigen binding fragment thereof according to claim 5, comprising:

a heavy chain variable region having an amino acid sequence as set forth in one of SEQ

5 ID NOs: 10, 12 to 13, 15 to 24, 27, and 31, or a conservative modification form thereof; and/or

a light chain variable region having an amino acid sequence as set forth in one of SEQ ID NOs: 11, 14, 25 to 26, 28, and 30, or a conservative modification form thereof.

8. The antibody or antigen binding fragment thereof according to claim 5, comprising:

10 a heavy chain variable region having an amino acid sequence with at least 90% sequence identity to an amino acid sequence as set forth in one of SEQ ID NOs: 10, 12 to 13, 15 to 24, 27, and 31; and/or

a light chain variable region having an amino acid sequence with at least 90% sequence identity to an amino acid sequence as set forth in one of SEQ ID NOs: 11, 14, 25 to 26, 28, and 30.

15 9. The antibody or antigen binding fragment thereof according to claim 8, comprising amino acid sequences as set forth in a combination of the heavy chain variable region and the light chain variable region in any one of the following groups, or amino acid sequences having at least 90% sequence identity thereto:

Group number of the antibody or antigen binding fragment	Heavy chain variable region	Light chain variable region
1	SEQ ID NO: 15	SEQ ID NO: 11
2	SEQ ID NO: 16	SEQ ID NO: 11
3	SEQ ID NO: 17	SEQ ID NO: 11
4	SEQ ID NO: 17	SEQ ID NO: 11
5	SEQ ID NO: 19	SEQ ID NO: 11
6	SEQ ID NO: 20	SEQ ID NO: 11
7	SEQ ID NO: 21	SEQ ID NO: 11
8	SEQ ID NO: 22	SEQ ID NO: 11
9	SEQ ID NO: 23	SEQ ID NO: 11
10	SEQ ID NO: 24	SEQ ID NO: 11
11	SEQ ID NO: 10	SEQ ID NO: 11

12	SEQ ID NO: 12	SEQ ID NO: 11
13	SEQ ID NO: 13	SEQ ID NO: 25
14	SEQ ID NO: 13	SEQ ID NO: 26
15	SEQ ID NO: 13	SEQ ID NO: 14
16	SEQ ID NO: 27	SEQ ID NO: 28
17	SEQ ID NO: 29	SEQ ID NO: 28
18	SEQ ID NO: 13	SEQ ID NO: 30
19	SEQ ID NO: 31	SEQ ID NO: 26

10. The antibody or antigen binding fragment thereof according to any one of claims 1 to 4, further comprising a constant region, wherein:

the constant region comprises at least one of a heavy chain constant region or a light chain constant region.

11. The antibody or antigen binding fragment thereof according to claim 10, wherein at least a portion of at least one of the heavy chain constant region or the light chain constant region is derived from at least one of a murine antibody, a human antibody, a primate antibody, a bovine antibody, an equine antibody, a dairy bovine antibody, a porcine antibody, an ovine antibody, a caprine antibody, a canine antibody, a feline antibody, a rabbit-derived antibody, a camelid antibody, a donkey-derived antibody, a deer-derived antibody, a mink-derived antibody, a chicken-derived antibody, a duck-derived antibody, a goose-derived antibody, a turkey-derived antibody, a gamecock-derived antibody, or a mutant thereof.

12. The antibody or antigen binding fragment thereof according to claim 10, wherein:

the heavy chain constant region comprises a heavy chain constant region selected from IgG1, IgG2, IgG3, IgG4, IgA, IgM, IgE, or IgD; and/or

the light chain constant region comprises a light chain constant region selected from a κ -type light chain constant region or a λ -type light chain constant region.

13. The antibody or antigen binding fragment thereof according to claim 11, wherein the light chain constant region and the heavy chain constant region are both derived from a murine antibody or a mutant thereof; or a human antibody or a mutant thereof.

14. The antibody or antigen binding fragment thereof according to claim 10, wherein:

the N-terminus of the heavy chain constant region is linked to the C-terminus of the heavy

chain variable region; and/or

the N-terminus of the light chain constant region is linked to the N-terminus of the light chain variable region.

15. The antibody or antigen binding fragment thereof according to claim 10, wherein:

5 the heavy chain constant region has an amino acid sequence as set forth in SEQ ID NO: 93 or an amino acid sequence having at least 80% identity thereto; and/or

the light chain constant region has an amino acid sequence as set forth in SEQ ID NO: 94 or an amino acid sequence having at least 80% identity thereto.

16. The antibody or antigen binding fragment thereof according to claim 10, wherein:

10 the C-terminus of the heavy chain variable region is linked to the N-terminus of the light chain variable region, or

the N-terminus of the heavy chain variable region is linked to the C-terminus of the light chain variable region.

17. The antibody or antigen binding fragment thereof according to claim 16, further

15 comprising a first linking peptide, wherein:

the C-terminus of the heavy chain variable region is linked to the N-terminus of the first linking peptide, and the C-terminus of the first linking peptide is linked to the N-terminus of the light chain variable region, or

20 the C-terminus of the light chain variable region is linked to the N-terminus of the first linking peptide, and the C-terminus of the first linking peptide is linked to the N-terminus of the heavy chain variable region.

18. The antibody or antigen binding fragment thereof according to any one of claims 1 to 4, wherein:

25 the antibody comprises at least one of a full-length monoclonal antibody, chimeric antibody, humanized antibody, Fv, scFv, Fab, Fab', Fab'-SH, or F(ab')₂; and

the antigen binding fragment of the antibody comprises at least one of a F(ab')₂ fragment, Fab' fragment, Fab fragment, F(ab)₂ fragment, Fv fragment, scFv fragment, scFv-Fc fusion protein, scFv-Fv fusion protein, or a minimum recognition unit.

19. The antibody or antigen binding fragment thereof according to claim 18, comprising:

30 a heavy chain having an amino acid sequence as set forth in one of SEQ ID NOs: 32, 34,

35, and 37 to 46, or an amino acid sequence having at least 80% identity thereto; and/or

a light chain having an amino acid sequence as set forth in one of SEQ ID NOs: 33, 36, and 47 to 48, or an amino acid sequence having at least 80% identity thereto.

20. The antibody or antigen binding fragment thereof according to claim 18, wherein the
5 antibody or antigen binding fragment thereof has amino acid sequences as set forth in a combination of a heavy chain and a light chain in any one of the following groups, or amino acid sequences having at least 80% sequence identity thereto:

Group number of the antibody or antigen binding fragment thereof	Heavy chain	Light chain
1	SEQ ID NO: 37	SEQ ID NO: 33
2	SEQ ID NO: 38	SEQ ID NO: 33
3	SEQ ID NO: 39	SEQ ID NO: 33
4	SEQ ID NO: 40	SEQ ID NO: 33
5	SEQ ID NO: 41	SEQ ID NO: 33
6	SEQ ID NO: 42	SEQ ID NO: 33
7	SEQ ID NO: 43	SEQ ID NO: 33
8	SEQ ID NO: 44	SEQ ID NO: 33
9	SEQ ID NO: 45	SEQ ID NO: 33
10	SEQ ID NO: 46	SEQ ID NO: 33
11	SEQ ID NO: 32	SEQ ID NO: 33
12	SEQ ID NO: 34	SEQ ID NO: 33
13	SEQ ID NO: 35	SEQ ID NO: 47
14	SEQ ID NO: 35	SEQ ID NO: 48
15	SEQ ID NO: 35	SEQ ID NO: 36
16	SEQ ID NO: 95	SEQ ID NO: 119
17	SEQ ID NO: 96	SEQ ID NO: 120
18	SEQ ID NO: 97	SEQ ID NO: 121
19	SEQ ID NO: 98	SEQ ID NO: 122

21. The antibody or antigen binding fragment thereof according to claim 18, having an
10 amino acid sequence as set forth in any one of SEQ ID NOs: 99 to 117, or an amino acid sequence having at least 80% sequence identity thereto.

22. The antibody or antigen binding fragment thereof according to claim 18, having amino acid sequences as set forth in a combination of Fab VL-CL and Fab VH-CH1 in any one of the following groups, or amino acid sequences having at least 80% sequence identity thereto:

Group number of the antibody or antigen binding fragment	Fab VH-CH1	Fab VL-CL
1	SEQ ID NO: 123	SEQ ID NO: 33
2	SEQ ID NO: 124	SEQ ID NO: 33
3	SEQ ID NO: 125	SEQ ID NO: 33
4	SEQ ID NO: 126	SEQ ID NO: 33
5	SEQ ID NO: 127	SEQ ID NO: 33
6	SEQ ID NO: 128	SEQ ID NO: 33
7	SEQ ID NO: 129	SEQ ID NO: 33
8	SEQ ID NO: 130	SEQ ID NO: 33
9	SEQ ID NO: 131	SEQ ID NO: 33
10	SEQ ID NO: 132	SEQ ID NO: 33
11	SEQ ID NO: 133	SEQ ID NO: 33
12	SEQ ID NO: 134	SEQ ID NO: 33
13	SEQ ID NO: 135	SEQ ID NO: 47
14	SEQ ID NO: 136	SEQ ID NO: 36
15	SEQ ID NO: 137	SEQ ID NO: 48
16	SEQ ID NO: 138	SEQ ID NO: 119
17	SEQ ID NO: 139	SEQ ID NO: 120
18	SEQ ID NO: 140	SEQ ID NO: 121
19	SEQ ID NO: 141	SEQ ID NO: 122

5 23. The antibody or antigen binding fragment thereof according to claim 18, wherein the antibody or antigen binding fragment thereof has an amino acid sequence as set forth in any one of SEQ ID NOs: 142 to 160, or an amino acid sequence having at least 80% sequence identity thereto.

10 24. A multi-specific antibody, comprising:
a first antigen binding region, wherein the first antigen binding region comprises the anti-CD3 antibody or antigen binding fragment thereof according to any one of claims 1 to 23; and
a second antigen binding region, wherein the second antigen binding region has

biomolecule binding activity, the biomolecule not being CD3.

25. The multi-specific antibody according to claim 24, wherein the multi-specific antibody is selected from one of a bispecific antibody, a trispecific antibody, or a tetraspecific antibody, and preferably, the multi-specific antibody is a bispecific antibody.

5 26. The multi-specific antibody according to claim 25, wherein the bispecific antibody comprises a symmetric bispecific antibody or an asymmetric bispecific antibody.

27. The multi-specific antibody according to claim 24, wherein the second antigen binding region is a binding protein of the biomolecule or a fragment thereof.

28. The multi-specific antibody according to claim 24, wherein the biomolecule is selected
10 from at least one of PDL1, CD47, TIGIT, CD73, CD33, CEACAM1, CEACAM5, CEACAM6, STING, WNT, Beta catenin, B7H3, VISITA, CD19, BCMA, CD22, CD20, CD123, CD38, CEA, CD25, CD46, CD138, PSCA, PSMA, PSA, MUC1, MUC16, NY-ESO-1, GD2, WT1, Mesothelin, MAGE-A3, GPC3, PRAME, Globo H, AFP, Trop2, FOLR1, SP, Sca-1, CD133, or EPCAM.

15 29. The multi-specific antibody according to claim 27, wherein the binding protein or fragment thereof is selected from at least one of an antibody or antigen binding fragment thereof, a receptor, or a ligand.

30. The multi-specific antibody according to claim 29, wherein the bispecific antibody is an asymmetric bispecific antibody.

20 31. The multi-specific antibody according to claim 24, wherein the first antigen binding region comprises a first scFv fragment, a first Fv fragment, a first Fab fragment, or a first Fab-Linker fragment.

32. The multi-specific antibody according to claim 31, wherein the first antigen binding region further comprises a first Fc peptide segment.

25 33. The multi-specific antibody according to claim 32, wherein:

the N-terminus of the first Fc peptide segment is linked to the C-terminus of the scFv fragment or the C-terminus of the Fv fragment; or

the N-terminus of the first Fc peptide segment is linked to the C-terminus of CH1 of the Fab fragment or the C-terminus of the Fab-Linker fragment.

30 34. The multi-specific antibody according to claim 32, wherein the first antigen binding

region further comprises a second linking peptide, wherein:

the N-terminus of the first Fc peptide segment is linked to the C-terminus of the second linking peptide, and the N-terminus of the second linking peptide is linked to the C-terminus of the scFv fragment or the C-terminus of the Fv fragment; or

5 the N-terminus of the first Fc peptide segment is linked to the C-terminus of the second linking peptide, and the N-terminus of the second linking peptide is linked to the C-terminus of CH1 of the Fab fragment or the C-terminus of the Fab-Linker fragment.

35. The multi-specific antibody according to claim 32, wherein the first Fc peptide segment is selected from a human Fc peptide segment.

10 36. The multi-specific antibody according to claim 35, wherein the first Fc peptide segment is a human IgG1 Fc peptide segment.

37. The multi-specific antibody according to claim 32, wherein:

the first Fc peptide segment has an amino acid sequence as set forth in SEQ ID NO: 118;

or

15 the first Fc peptide segment has an amino acid sequence as set forth in SEQ ID NO: 166.

38. The multi-specific antibody according to claim 31, wherein the first scFv fragment has an amino acid sequence as set forth in any one of SEQ ID NOs: 95 to 117.

39. The multi-specific antibody according to claim 31, wherein the first Fab fragment has amino acid sequences as set forth in a combination of Fab VL-CL and Fab VH-CH1 in any one
20 of the following groups:

Group number of the first Fab fragment	Fab VH-CH1	Fab VL-CL
1	SEQ ID NO: 123	SEQ ID NO: 33
2	SEQ ID NO: 124	SEQ ID NO: 33
3	SEQ ID NO: 125	SEQ ID NO: 33
4	SEQ ID NO: 126	SEQ ID NO: 33
5	SEQ ID NO: 127	SEQ ID NO: 33
6	SEQ ID NO: 128	SEQ ID NO: 33
7	SEQ ID NO: 129	SEQ ID NO: 33
8	SEQ ID NO: 130	SEQ ID NO: 33
9	SEQ ID NO: 131	SEQ ID NO: 33
10	SEQ ID NO: 132	SEQ ID NO: 33

11	SEQ ID NO: 133	SEQ ID NO: 33
12	SEQ ID NO: 134	SEQ ID NO: 33
13	SEQ ID NO: 135	SEQ ID NO: 47
14	SEQ ID NO: 136	SEQ ID NO: 36
15	SEQ ID NO: 137	SEQ ID NO: 48
16	SEQ ID NO: 138	SEQ ID NO: 119
17	SEQ ID NO: 139	SEQ ID NO: 120
18	SEQ ID NO: 140	SEQ ID NO: 121
19	SEQ ID NO: 141	SEQ ID NO: 122

40. The multi-specific antibody according to claim 31, wherein the first Fab-Linker has an amino acid sequence as set forth in any one of SEQ ID NOs: 142 to 160.

41. The multi-specific antibody according to claim 24, wherein the first antigen binding region has an amino acid sequence as set forth in any one of SEQ ID NOs: 49 to 52 and 54 to 68.

42. The multi-specific antibody according to claim 28, wherein the biomolecule is Trop2 or PDL1.

43. The multi-specific antibody according to claim 42, wherein the second antigen binding region comprises:

a first anti-Trop2 antibody or antigen binding fragment thereof; or

a first anti-PDL1 antibody or an antigen binding fragment thereof.

44. The multi-specific antibody according to claim 43, wherein:

the first anti-Trop2 antibody or antigen binding fragment thereof is a second scFab fragment or a second Fab fragment of anti-Trop2; or

the first anti-PDL1 antibody or antigen binding fragment thereof is a third scFab fragment or a third Fab fragment of anti-PDL1.

45. The multi-specific antibody according to claim 44, wherein:

the second scFab fragment has an amino acid sequence as set forth in SEQ ID NO: 161;

the second Fab fragment has an amino acid sequence as set forth in SEQ ID NO: 162 and 70;

the third scFab fragment has an amino acid sequence as set forth in SEQ ID NO: 163; or

the third Fab fragment has an amino acid sequence as set forth in SEQ ID NO: 164 and 72.

46. The multi-specific antibody according to claim 44, wherein the second antigen binding region further comprises a second Fc fragment, wherein:

5 the N-terminus of the second Fc fragment is linked to the C-terminus of CH1 of the second scFab fragment, the C-terminus of CH1 of the second Fab fragment, the C-terminus of CH1 of the third scFab fragment, or the C-terminus of CH1 of the third Fab fragment.

47. The multi-specific antibody according to claim 46, wherein the second Fc peptide segment is selected from a human Fc peptide segment.

10 48. The multi-specific antibody according to claim 47, wherein the second Fc peptide segment is a human IgG1 Fc peptide segment.

49. The multi-specific antibody according to claim 46, wherein the second Fc peptide segment has an amino acid sequence as set forth in SEQ ID NO: 165.

15 50. The multi-specific antibody according to claim 46, wherein the first Fc peptide segment and the second Fc peptide segment are linked via a knob-into-hole structure.

51. The multi-specific antibody according to claim 43, wherein:

the second antigen binding region has an amino acid sequence as set forth in SEQ ID NO: 161 or 163;

20 the second antigen binding region has an amino acid sequence as set forth in SEQ ID NO: 162 and 70; or

the second antigen binding region has an amino acid sequence as set forth in SEQ ID NO: 164 and 72.

52. A conjugate, comprising:

25 the antibody or antigen binding fragment thereof according to any one of claims 1 to 23, or the multi-specific antibody according to any one of claims 24 to 51; and

a conjugated moiety, the conjugated moiety being linked to the antibody or antigen binding fragment thereof or the multi-specific antibody.

53. The conjugate according to claim 52, wherein the conjugated moiety comprises at least one selected from a carrier, a medicament, a toxin, a cytokine, a protein tag, a modifier, a therapeutic agent, or a chemotherapeutic agent.

54. A nucleic acid, encoding the antibody or antigen binding fragment thereof according to any one of claims 1 to 23, or the multi-specific antibody according to any one of claims 24 to 51.
55. A vector or transformant, comprising the nucleic acid according to claim 54.
- 5 56. The vector or transformant according to claim 55, wherein the vector is a eukaryotic vector or prokaryotic vector.
57. The vector or transformant according to claim 55, wherein the vector comprises at least one selected from a plasmid vector, an adenovirus vector, a lentivirus vector, or an adeno-associated virus vector.
- 10 58. A cell, carrying the nucleic acid according to claim 54, or the vector or transformant according to any one of claims 55 to 57; or expressing the antibody or antigen binding fragment thereof according to any one of claims 1 to 23 or the multi-specific antibody according to any one of claims 24 to 51.
59. The cell according to claim 58, wherein the cell is a prokaryotic cell, a eukaryotic cell,
15 or a bacteriophage.
60. A pharmaceutical composition, comprising:
the antibody or antigen binding fragment thereof according to any one of claims 1 to 23;
the multi-specific antibody according to any one of claims 24 to 51;
the conjugate according to any one of claims 52 to 53;
20 the nucleic acid according to claim 54; or
the vector or transformant according to any one of claims 55 to 57.
61. The pharmaceutical composition according to claim 60, further comprising a pharmaceutically acceptable adjuvant.
62. The pharmaceutical composition according to claim 61, wherein the adjuvant
25 comprises one or more pharmaceutically acceptable excipients, diluents, stabilizers, or carriers.
63. The pharmaceutical composition according to claim 60, wherein the pharmaceutical composition is an injection.
64. A kit, comprising:
the antibody or antigen binding fragment thereof according to any one of claims 1 to 23;
30 or

the multi-specific antibody according to any one of claims 24 to 51.

65. Use of the antibody or antigen binding fragment thereof according to any one of claims 1 to 23 or the multi-specific antibody according to any one of claims 24 to 51 in the manufacture of a kit for detecting CD3.

5 66. Use of the antibody or antigen binding fragment thereof according to any one of claims 1 to 23, the multi-specific antibody according to any one of claims 24 to 51, the conjugate according to any one of claims 52 to 53, the nucleic acid according to claim 54, the vector or transformant according to any one of claims 55 to 57, or the pharmaceutical composition according to any one of claims 60 to 63 in the manufacture of a medicament for regulating an
10 immune response and/or inhibiting tumor growth, or for the prevention and/or treatment of a CD3-related disease and/or cancer or tumor or infection or autoimmune disease.

67. The use according to claim 66, wherein the cancer or tumor or infection or autoimmune disease is a Trop2- or PDL1-related disease.

68. The use according to claim 67, wherein:

15 the cancer or tumor is at least one of lung cancer, liver cancer, ovarian cancer, cervical cancer, skin cancer, bladder cancer, colon cancer, rectal cancer, breast cancer, pancreatic cancer, prostate cancer, glioma, renal cancer, gastric cancer, esophageal cancer, oral squamous cell carcinoma, or head-and-neck cancer; and/or

20 the cancer or tumor is at least one of non-small cell lung cancer, malignant melanoma, renal cell carcinoma, head-and-neck squamous cell carcinoma, urothelial carcinoma, colorectal cancer, liver cancer, or classical Hodgkin's lymphoma.

69. A method for regulating an immune response and/or inhibiting tumor growth or preventing and/or treating a CD3-related disease and/or cancer, tumor, infection, or autoimmune disease, comprising:

25 administering to a subject a pharmaceutically acceptable amount of the antibody or antigen binding fragment thereof according to any one of claims 1 to 23, the multi-specific antibody according to any one of claims 24 to 51, the conjugate according to any one of claims 52 to 53, the nucleic acid according to claim 54, the vector or transformant according to any one of claims 55 to 57, or the pharmaceutical composition according to any one of claims 60 to 63.

30 70. The method according to claim 69, wherein the cancer or tumor or infection or

autoimmune disease is a Trop2- or PDL1-related disease.

71. The method according to claim 70, wherein:

the cancer or tumor is at least one of lung cancer, liver cancer, ovarian cancer, cervical cancer, skin cancer, bladder cancer, colon cancer, rectal cancer, breast cancer, pancreatic cancer,
5 prostate cancer, glioma, renal cancer, gastric cancer, esophageal cancer, oral squamous cell carcinoma, or head-and-neck cancer; and/or

the cancer or tumor is at least one of non-small cell lung cancer, malignant melanoma, renal cell carcinoma, head-and-neck squamous cell carcinoma, urothelial carcinoma, colorectal cancer, liver cancer, or classical Hodgkin's lymphoma.

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