

NOVEL ANTIBODIES THAT BIND TO CD3 AND LILRB4, AND USES THEREOF

FIELD OF THE INVENTION

[001] The present disclosure generally relates to novel antibodies or antigen-binding fragments thereof that bind to CD3 and LILRB4, and uses of the same.

BACKGROUND

[002] Leukocyte immunoglobulin-like receptor subfamily B member 4 (LILRB4) is a protein that in humans is encoded by the *LILRB4* gene. The encoded protein belongs to the subfamily B class of LILRs, which contains two or four extracellular immunoglobulin domains, a transmembrane domain, and two to four cytoplasmic immunoreceptor tyrosine-based inhibitory motifs (ITIMs). The LILRB4 is expressed on monocytic cells and transduces a negative signal that inhibits stimulation of an immune response. The LILRB4 can also function in antigen capture and presentation. It is thought to control inflammatory responses and cytotoxicity to help focus the immune response and limit autoreactivity. LILRB4 has also been proposed to be a potential target for tumor immunotherapy. It has been shown to express on tumor-associated macrophages and negatively regulate immune response in tumor. The expression of LILRB4 on monocytic myeloid leukemia cells supports infiltration and inhibits T cell proliferation.

[003] The CD3 (cluster of differentiation 3) T-cell co-receptor is a protein complex and is composed of four distinct chains, a CD3gamma chain, a CD3delta chain, and two CD3epsilon chains. These chains associate with a molecule known as the T-cell receptor (TCR) and the zeta-chain to generate activation signal in T lymphocytes. The TCR, zeta-chain, and CD3 molecules together form the TCR-CD3 complex, in which TCR as a subunit recognizes and binds to antigen, and CD3 as a subunit transfers and conveys the antigen-stimulation to signaling pathway, and ultimately

regulates T-cell activity. The CD3 protein is virtually present in all T cells. A more recent application of CD3 antibodies is in the form of multi-specific antibodies, binding CD3 on the one hand and a tumor cell antigen on the other hand. The simultaneous binding of such an antibody to both of its targets will force a temporary interaction between target cell and T cell, causing activation of any cytotoxic T cell and subsequent lysis of the target cell.

[004] Needs remain for novel multi-specific, especially bispecific, antibodies that bind to CD3 and LILRB4.

SUMMARY OF THE INVENTION

[005] In one respect, the present disclosure provides an antibody or antigen-binding fragment thereof that binds to CD3 and LILRB4, wherein the antibody or antigen-binding fragment thereof comprises a first binding moiety that binds to CD3 and a second binding moiety that binds to LILRB4, wherein

the first binding moiety comprises one or two or three heavy chain complementarity determining regions (HCDR1, HCDR2 and/or HCDR3) contained within the heavy chain variable (VH) region sequence of SEQ ID NOs: 7, 9, 10, 47 or 11; and/or one or two or three light chain complementarity determining regions (LCDR1, LCDR2 and/or LCDR3) contained within the light chain variable (VL) region sequence of SEQ ID NOs: 8, 12 or 13; and, wherein the second binding moiety comprises one or two or three heavy chain complementarity determining regions (HCDR1, HCDR2 and/or HCDR3) contained within the heavy chain variable (VH) region sequence of SEQ ID NOs: 20, 22, 30, or 33; and/or one or two or three light chain complementarity determining regions (LCDR1, LCDR2 and/or LCDR3) contained within the light chain variable (VL) region sequence of SEQ ID NOs: 21, 23, 31 or 34.

[006] In some embodiments, the first binding moiety comprises at least one heavy or light chain complementarity determining region (CDR) comprising an amino acid sequence selected from the group consisting of SEQ ID NOs: 1, 2, 42, 3, 4, 5 and 6.

[007] In some embodiments, the first binding moiety comprises one or two or three of HCDR1, HCDR2 and HCDR3, wherein the HCDR1 comprises an amino acid sequence as set forth in SEQ ID NO: 1, the HCDR2 comprises an amino acid sequence as set forth in SEQ ID NO: 2 or SEQ ID NO: 42, and the HCDR3 comprising an amino acid sequence as set forth in SEQ ID NO: 3.

[008] In some embodiments, the first binding moiety comprises a HCDR1 comprising an amino acid sequence as set forth in SEQ ID NO: 1, a HCDR2 comprising an amino acid sequence as set forth in SEQ ID NO: 2, and a HCDR3 comprising an amino acid sequence as set forth in SEQ ID NO: 3.

[009] In some embodiments, the first binding moiety comprises a HCDR1 comprising an amino acid sequence as set forth in SEQ ID NO: 1, a HCDR2 comprising an amino acid sequence as set forth in SEQ ID NO: 42, and a HCDR3 comprising an amino acid sequence as set forth in SEQ ID NO: 3.

[0010] In some embodiments, the first binding moiety comprises one or two or three of LCDR1, LCDR2 and LCDR3, wherein the LCDR1 comprises an amino acid sequence as set forth in SEQ ID NO: 4, the LCDR2 comprises an amino acid sequence as set forth in SEQ ID NO: 5, and the LCDR3 comprises an amino acid sequence as set forth in SEQ ID NO: 6.

[0011] In some embodiments, the first binding moiety comprises a LCDR1 comprising an amino acid sequence as set forth in SEQ ID NO: 4, a LCDR2 comprising an amino acid sequence as set forth in SEQ ID NO: 5, and a LCDR3 comprising an amino acid sequence as set forth in SEQ ID NO: 6.

[0012] In some embodiments, the first binding moiety comprises a HCDR1 comprising an amino acid sequence as set forth in SEQ ID NO: 1, a HCDR2 comprising an amino acid sequence as set forth in SEQ ID NO: 2, a HCDR3 comprising an amino acid sequence as set forth in SEQ ID NO: 3, a LCDR1 comprising an amino acid sequence as set forth in SEQ ID NO: 4, a LCDR2

comprising an amino acid sequence as set forth in SEQ ID NO: 5, and a LCDR3 comprising an amino acid sequence as set forth in SEQ ID NO: 6.

[0013] In some embodiments, the first binding moiety comprises a HCDR1 comprising an amino acid sequence as set forth in SEQ ID NO: 1, a HCDR2 comprising an amino acid sequence as set forth in SEQ ID NO: 42, a HCDR3 comprising an amino acid sequence as set forth in SEQ ID NO: 3, a LCDR1 comprising an amino acid sequence as set forth in SEQ ID NO: 4, a LCDR2 comprising an amino acid sequence as set forth in SEQ ID NO: 5, and a LCDR3 comprising an amino acid sequence as set forth in SEQ ID NO: 6.

[0014] In some embodiments, the first binding moiety comprise a VH region having an amino acid sequence as set forth in SEQ ID NOs: 7, 9, 10, 47 or 11, or a homologous sequence thereof having at least 80% sequence identity to SEQ ID NOs: 7, 9, 10, 47 or 11.

[0015] In some embodiments, the first binding moiety comprises a VL region having an amino acid sequence as set forth in SEQ ID NOs: 8, 12 or 13, or a homologous sequence thereof having at least 80% sequence identity to SEQ ID NOs: 8, 12 or 13.

[0016] In some embodiments, the first binding moiety comprises a VH/VL amino acid sequence pair of SEQ ID NOs: 7/8, 7/12, 7/13, 9/8, 9/12, 9/13, 10/8, 10/12, 10/13, 47/8, 47/12, 47/13, 11/8, 11/12 or 11/13.

[0017] In some embodiments, the second binding moiety comprises at least one heavy or light chain complementarity determining region (CDR) comprising an amino acid sequence selected from the group consisting of SEQ ID NOs: 14, 15, 16, 17, 18, 19, 24, 25, 26, 27, 28, 29, and 32.

[0018] In some embodiments, the second binding moiety comprises one or two or three of HCDR1, HCDR2 and HCDR3 comprising an amino acid sequence selected from the group consisting of SEQ ID NOs: 14, 15, 16, 24, 25, 26 and 32.

[0019] In some embodiments, the second binding moiety comprises a VL region comprising one or two or three of LCDR1, LCDR2 and LCDR3 comprising an amino acid sequence selected from the group consisting of SEQ ID NOs: 17, 18, 19, 27, 28 and 29.

[0020] In some embodiments, the second binding moiety comprises:

- i. a HCDR1 comprising an amino acid sequence selected from the group consisting of SEQ ID NOs: 14 and 24,
- ii. a HCDR2 comprising an amino acid sequence selected from the group consisting of SEQ ID NOs: 15, 25, and 32, and
- iii. a HCDR3 comprising an amino acid sequence selected from the group consisting of SEQ ID NOs: 16 and 26.

[0021] In some embodiments, the second binding moiety comprises:

- i. a LCDR1 comprising an amino acid sequence selected from the group consisting of SEQ ID NOs: 17 and 27,
- ii. a LCDR2 comprising an amino acid sequence selected from the group consisting of SEQ ID NOs: 18 and 28, and
- iii. a LCDR3 comprising an amino acid sequence selected from the group consisting of SEQ ID NOs: 19 and 29.

[0022] In some embodiments, the second binding moiety comprises:

- i. a HCDR1 comprising an amino acid sequence as set forth in SEQ ID NO: 14, a HCDR2 comprising an amino acid sequence as set forth in SEQ ID NO: 15, and a HCDR3 comprising an amino acid sequence as set forth in SEQ ID NO: 16;
- ii. a HCDR1 comprising an amino acid sequence as set forth in SEQ ID NO: 24, a HCDR2 comprising an amino acid sequence as set forth in SEQ ID NO: 25,

and a HCDR3 comprising an amino acid sequence as set forth in SEQ ID NO: 26; or

- iii. a HCDR1 comprising an amino acid sequence as set forth in SEQ ID NO: 24, a HCDR2 comprising an amino acid sequence as set forth in SEQ ID NO: 32, and a HCDR3 comprising an amino acid sequence as set forth in SEQ ID NO: 26.

[0023] In some embodiments, the second binding moiety comprises:

- i. a LCDR1 comprising an amino acid sequence as set forth in SEQ ID NO: 17, a LCDR2 comprising an amino acid sequence as set forth in SEQ ID NO: 18, and a LCDR3 comprising an amino acid sequence as set forth in SEQ ID NO: 19; or
- ii. a LCDR1 comprising an amino acid sequence as set forth in SEQ ID NO: 27, a LCDR2 comprising an amino acid sequence as set forth in SEQ ID NO: 28, and a LCDR3 comprising an amino acid sequence as set forth in SEQ ID NO: 29.

[0024] In some embodiments, the second binding moiety comprises:

- i. a HCDR1 comprising an amino acid sequence as set forth in SEQ ID NO: 14, a HCDR2 comprising an amino acid sequence as set forth in SEQ ID NO: 15, a HCDR3 comprising an amino acid sequence as set forth in SEQ ID NO: 16, a LCDR1 comprising an amino acid sequence as set forth in SEQ ID NO: 17, a LCDR2 comprising an amino acid sequence as set forth in SEQ ID NO: 18, and a LCDR3 comprising an amino acid sequence as set forth in SEQ ID NO: 19;
- ii. a HCDR1 comprising an amino acid sequence as set forth in SEQ ID NO: 24, a HCDR2 comprising an amino acid sequence as set forth in SEQ ID NO: 25, a HCDR3 comprising an amino acid sequence as set forth in SEQ ID NO: 26, a LCDR1 comprising an amino acid sequence as set forth in SEQ ID NO: 27,

a LCDR2 comprising an amino acid sequence as set forth in SEQ ID NO: 28,
and a LCDR3 comprising an amino acid sequence as set forth in SEQ ID
NO: 29; or

- iii. a HCDR1 comprising an amino acid sequence as set forth in SEQ ID NO: 24,
a HCDR2 comprising an amino acid sequence as set forth in SEQ ID NO: 32,
a HCDR3 comprising an amino acid sequence as set forth in SEQ ID NO: 26,
a LCDR1 comprising an amino acid sequence as set forth in SEQ ID NO: 27,
a LCDR2 comprising an amino acid sequence as set forth in SEQ ID NO: 28,
and a LCDR3 comprising an amino acid sequence as set forth in SEQ ID
NO: 29.

[0025] In some embodiments, the second binding moiety comprises a VH region having an amino acid sequence as set forth in SEQ ID NOs: 20, 22, 30 or 33, or a homologous sequence thereof having at least 80% sequence identity to SEQ ID NOs: 20, 22, 30 or 33.

[0026] In some embodiments, the second binding moiety comprises a VL region having an amino acid sequence as set forth in SEQ ID NOs: 21, 23, 31 or 34, or a homologous sequence thereof having at least 80% sequence identity to SEQ ID NOs: 21, 23, 31 or 34.

[0027] In some embodiments, the second binding moiety comprises a VH/VL amino acid sequence pair of SEQ ID NOs: 20/21, 22/23, 30/31, or 33/34.

[0028] In some embodiments, the first binding moiety and the second binding moiety are independently a single-chain Fv (scFv) or a Fab domain.

[0029] In some embodiments, the first binding moiety is an anti-CD3 scFv, and the second binding moiety is an anti-LILRB4 Fab domain; or the first binding moiety is an anti-CD3 Fab domain, and the second binding moiety is an anti-LILRB4 scFv.

[0030] In some embodiments, the first binding moiety is linked to the second binding moiety via a linker.

[0031] In some embodiments, the N-terminus of the first binding moiety is linked to the C-terminus of the second binding moiety via the linker.

[0032] In some embodiments, the antibody or antigen-binding fragment thereof comprises, from N-terminus to C-terminus, a first chain comprising a VH region from the second binding moiety, a CH1 region from the second binding moiety, a VH region from the first binding moiety and a VL region from the first binding moiety.

[0033] In some embodiments, the CH1 region from the second binding moiety and the VH region from the first binding moiety are directly linked or linked via a linker. In some embodiments, the VH region from the first binding moiety and the VL region from the first binding moiety are directly linked or linked via a linker. In some embodiments, the linker is a linker comprising glycine and serine, *e.g.*, a (GGGS)₂ linker or a GSSS linker.

[0034] In some embodiments, the antibody or antigen-binding fragment thereof further comprises a second chain comprising a VL region from the second binding moiety and a CL from the second binding moiety.

[0035] In some embodiments, the antibody or antigen-binding fragment thereof further comprises a third binding moiety that binds to LILRB4. In some embodiments, the third binding moiety and the second binding moiety bind to the same epitope or different epitopes on LILRB4. In some embodiments, the third binding moiety is a Fab domain.

[0036] In some embodiments, the first binding moiety is an anti-CD3 scFv, the second binding moiety is an anti-LILRB4 Fab domain, and the third binding moiety is an anti-LILRB4 Fab domain.

[0037] In some embodiments, the sequence of the second binding moiety is the same as the sequence of the third binding moiety.

[0038] In some embodiments, the first binding moiety, the second binding moiety and/or the third binding moiety further comprises one or more amino acid residue

substitutions or modifications yet retains specific binding affinity to CD3 or LILRB4. In some embodiments, at least one of the substitutions or modifications is in one or more of the CDR sequences of the VH region or VL region of the first binding moiety, the second binding moiety, or the third binding moiety. In some embodiments, at least one of the substitutions or modifications is in one or more of the non-CDR sequences of the VH region or VL region of the first binding moiety, the second binding moiety, or the third binding moiety.

[0039] In some embodiments, the antibody or antigen-binding fragment thereof further comprises one or more non-natural amino acid (NNAA) substitution. In some embodiments, the NNAA is capable of being conjugated.

[0040] In some embodiments, the antibody or antigen-binding fragment thereof is a chimeric, a humanized or a human antibody or an antigen-binding fragment thereof.

[0041] In some embodiments, the antibody or antigen-binding fragment thereof is a labeled antibody, a bivalent antibody, an anti-idiotypic antibody or a fusion protein.

[0042] In some embodiments, the antibody or antigen-binding fragment thereof further comprises an Fc region, optionally an Fc region of human immunoglobulin (Ig), or optionally an Fc region of human IgG. In some embodiments, the Fc region is derived from human IgG1, IgG2, IgG3, or IgG4.

[0043] In some embodiments, the Fc region comprises one or more amino acid substitutions selected from the group consisting of: L234A, L235A, S354C, T366W, Y349C, T366S, L368A, and Y407V (according to EU numbering).

[0044] In some embodiments, the Fc region comprises L234A and L235A (according to EU numbering) amino acid substitutions.

[0045] In some embodiments, both of the first binding moiety and the third binding moiety are linked to the Fc region.

[0046] In some embodiments, both of the second binding moiety and the third binding moiety are linked to the Fc region.

[0047] In some embodiments, the antibody or antigen-binding fragment thereof comprises, from N-terminus to C-terminus, a first chain comprising a VH region from the second binding moiety, a CH1 region from the second binding moiety, a VH region from the first binding moiety, a VL region from the first binding moiety, a CH2 from the Fc region, and a CH3 from the Fc region.

[0048] In some embodiments, the antibody or antigen-binding fragment thereof further comprises a second chain comprising a VL region from the second binding moiety, and a CL from the second binding moiety.

[0049] In some embodiments, the light chain is a λ light chain or a κ light chain.

[0050] In some embodiments, the antibody or antigen-binding fragment thereof is linked to one or more conjugate moieties. In some embodiments, the conjugate moiety comprises an antigen-binding fragment of an antibody, clearance-modifying agent, a chemotherapeutic agent, a toxin, a radioactive isotope, a lanthanide, a detectable label, a DNA-alkylator, a topoisomerase inhibitor, a tubulin-binder, a purification moiety or other anticancer drugs.

[0051] In some embodiments, the conjugate moiety is a scFv. In some embodiments, the scFv is a fourth binding moiety that binds to LILRB4.

[0052] In some embodiments, the second and third binding moieties bind to a first epitope on LILRB4, while the fourth binding moiety binds to a second epitope on LILRB4.

[0053] In some embodiments, the amino acid sequence of the second binding moiety is the same as the amino acid sequence of the third binding moiety, while the amino acid sequence of the fourth binding moiety is different from the amino acid sequence of the second and third binding moieties.

[0054] In some embodiments, the conjugate moiety is covalently attached either directly or via a linker.

[0055] In some embodiments, the linker is a linker comprising glycine and serine, *e.g.*, a (GGGGS)₂ linker or a GSSS linker.

[0056] In some embodiments, the antibody or antigen-binding fragment thereof from N-terminus to C-terminus, a first chain comprising a VH region from the second binding moiety, a CH1 region from the second binding moiety, a VH region from the first binding moiety, a VL region from the first binding moiety, a CH2 from the Fc region, a CH3 from the Fc region, a VH region from the fourth binding moiety, and a VL region from the fourth binding moiety.

[0057] In another aspect, the present disclosure provides an antibody or antigen-binding fragment thereof, which competes for binding to CD3 or LILRB4 with the antibody or antigen-binding fragment thereof provided herein.

[0058] In some embodiments, the antibody or antigen-binding fragment thereof is a multi-specific (*e.g.*, bispecific, tri-specific) antibody or an antigen-binding fragment thereof.

[0059] In some embodiments, the antibody or antigen-binding fragment thereof binds to CD3 and two different epitopes on LILRB4.

[0060] In some embodiments, the first binding moiety comprises HCDR1, HCDR2 and HCDR3 contained within the VH region sequence of SEQ ID NOs: 7, 9, 10, 47 or 11; and LCDR1, LCDR2 and LCDR3 contained within the VL region sequence of SEQ ID NOs: 8, 12 or 13; and each of the second and third binding moieties comprises HCDR1, HCDR2 and HCDR3 contained within the VH region sequence of SEQ ID NOs: 20, 22, 30, or 33; and LCDR1, LCDR2 and LCDR3 contained within the VL region sequence of SEQ ID NOs: 21, 23, 31 or 34.

[0061] In some embodiments, the first binding moiety comprises HCDR1, HCDR2, HCDR3, LCDR1, LCDR2 and LCDR3 contained within a VH/VL amino acid sequence pair of SEQ ID NOs: 7/8, 7/12, 7/13, 9/8, 9/12, 9/13, 10/8, 10/12, 10/13, 47/8, 47/12, 47/13, 11/8, 11/12 or 11/13; and each of the second and third binding moieties comprises HCDR1, HCDR2, HCDR3, LCDR1, LCDR2 and LCDR3

contained within a VH/VL amino acid sequence pair of SEQ ID NOs: 20/21, 22/23, 30/31, or 33/34.

[0062] In some embodiments, the first binding moiety comprises HCDR1, HCDR2, HCDR3, LCDR1, LCDR2 and LCDR3 contained within a VH/VL amino acid sequence pair of SEQ ID NOs: 7/8, 7/12, 7/13, 9/8, 9/12, 9/13, 10/8, 10/12, 10/13, 47/8, 47/12, 47/13, 11/8, 11/12 or 11/13; each of the second and third binding moieties comprises HCDR1, HCDR2, HCDR3, LCDR1, LCDR2 and LCDR3 contained within a VH/VL amino acid sequence pair of SEQ ID NOs: 30/31 or 33/34; and the fourth binding moiety comprises HCDR1, HCDR2, HCDR3, LCDR1, LCDR2 and LCDR3 contained within a VH/VL amino acid sequence pair of SEQ ID NOs: 20/21 or 22/23.

[0063] In some embodiments, the first binding moiety comprises HCDR1, HCDR2, HCDR3, LCDR1, LCDR2 and LCDR3 contained within a VH/VL amino acid sequence pair of SEQ ID NOs: 7/8, 7/12, 7/13, 9/8, 9/12, 9/13, 10/8, 10/12, 10/13, 47/8, 47/12, 47/13, 11/8, 11/12 or 11/13; each of the second and third binding moieties comprises HCDR1, HCDR2, HCDR3, LCDR1, LCDR2 and LCDR3 contained within a VH/VL amino acid sequence pair of SEQ ID NOs: 20/21 or 22/23; and the fourth binding moiety comprises HCDR1, HCDR2, HCDR3, LCDR1, LCDR2 and LCDR3 contained within a VH/VL amino acid sequence pair of SEQ ID NOs: 30/31 or 33/34.

[0064] In another aspect, the present disclosure provides a chimeric antigen receptor comprising the antibody or antigen-binding fragment thereof provided herein, a transmembrane region and an intracellular signal region. In some embodiments, the transmembrane region comprises a transmembrane region of CD3, CD4, CD8 or CD28. In some embodiments, the intracellular signal region is selected from the group consisting of: an intracellular signal regions sequence of CD3 (e.g. CD3ζ), FcγRI, CD27, CD28, CD137, CD134, MyD88, CD40, CD278, TLRs, or a combination thereof. In some embodiments, the antigen-binding fragment of the chimeric antigen receptor is a scFv. In some embodiments, the chimeric antigen

receptor is grafted onto an allogeneic cell, an autologous cell or a xenogeneic cell.

In some embodiments, the chimeric antigen receptor is grafted onto an immune effector cell. In some embodiments, the chimeric antigen receptor is grafted onto a T cell, a natural killer cell, a macrophage cell, or a tumor-infiltrating lymphocyte.

[0065] In another aspect, the present disclosure provides a pharmaceutical composition comprising the antibody or antigen-binding fragment thereof, and/or the chimeric antigen receptor of the present disclosure, and one or more pharmaceutically acceptable carriers.

[0066] In another aspect, the present disclosure provides an isolated polynucleotide encoding the antibody or antigen-binding fragment thereof and/or the chimeric antigen receptor of the present disclosure.

[0067] In another aspect, the present disclosure provides a vector comprising the isolated polynucleotide of the present disclosure.

[0068] In another aspect, the present disclosure provides a host expression system comprising the vector of the present disclosure or having the polynucleotide of the present disclosure integrated into genome thereof. In some embodiments, the host expression system of the present disclosure is a microorganism, a yeast, or a mammalian cell. In some embodiments, the microorganism is selected from the group consisting of *E. coli* and *B. subtilis*. In some embodiments, the yeast is *Saccharomyces*. In some embodiments, the mammalian cell is selected from the group consisting of COS, CHO-S, CHO-K1, HEK-293, and 3T3 cells.

[0069] In another aspect, the present disclosure provides a virus comprising the vector of the present disclosure.

[0070] In another aspect, the present disclosure provides a kit comprising the antibody or antigen-binding fragment thereof of the present disclosure and/or the chimeric antigen receptor of the present disclosure and/or the pharmaceutical composition of the present disclosure, and a second therapeutic agent.

[0071] In another aspect, the present disclosure provides a method of expressing the antibody or antigen-binding fragment thereof of the present disclosure and/or the chimeric antigen receptor of the present disclosure, comprising culturing the host expression system of the present disclosure under the condition at which the antibody or antigen-binding fragment of the present disclosure is expressed.

[0072] In another aspect, the present disclosure provides a method of treating, preventing or alleviating a disease, disorder or condition in a subject, comprising administering to the subject a therapeutically effective amount of the antibody or antigen-binding fragment thereof and/or the chimeric antigen receptor and/or the pharmaceutical composition of the present disclosure.

[0073] In some embodiments, the disease, disorder or condition is an immune disease, an autoimmune disease, an inflammatory disease, a cancer or a neurological disease. In some embodiments, the cancer is a solid tumor or hematologic tumor. In some embodiments, the disease, disorder or condition is a CD3 and/or LILRB4-expressing B cell cancer. In some embodiments, the disease, disorder or condition is selected from the group consisting of Kawasaki disease, T. gondii, multiple sclerosis, systematic Lupus erythematosus, lung cancer (*e.g.*, non-small-cell lung cancer (NSCLC), small cell lung cancer (SCLC), adenocarcinoma of the lung, squamous cell carcinoma of the lung, Lewis lung carcinoma, or radiation therapy resistant Lewis lung carcinoma), peritoneal cancer, carcinoid cancer, bone cancer, pancreatic cancer, primitive neuroectodermal tumor, skin cancer, gallbladder cancer, cancer of the head or neck, squamous cell cancer, uterine cancer, ovarian cancer, rectal cancer, prostate cancer, bladder cancer (*e.g.*, urothelial cancer), cancer of the anal region (*e.g.*, anal squamous cell carcinoma), gastric or stomach cancer (*e.g.*, gastrointestinal cancer), esophageal cancer, colon cancer, breast cancer, uterine cancer, liver cancer (*e.g.*, hepatoblastoma, hepatocellular carcinoma/hepatoma, or hepatic carcinoma), cholangiocarcinoma, sarcoma, colorectal cancer, carcinoma of the fallopian tubes, salivary gland carcinoma, carcinoma of the cervix, endometrial or uterine carcinoma, osteosarcoma, carcinoma of the vagina, carcinoma of the vulva, cancer of the

esophagus, cancer of the small intestine, cancer of the endocrine system, cancer of the thyroid gland, cancer of the parathyroid gland, cancer of the adrenal gland, cancer of the nasopharynx, sarcoma of soft tissue, polycythemia vera, cancer of the urethra, cancer of the penis, cancer of the kidney or ureter (*e.g.*, rhabdoid tumor of the kidney), cutaneous T-cell lymphoma, medulloblastoma, nephroblastoma, myelodysplastic syndrome, chronic and non-chronic myeloproliferative disorder, choroid plexus papilloma, renal cell carcinoma, carcinoma of the renal pelvis, neoplasms of the central nervous system (CNS), soft tissue sarcoma (*e.g.*, rhabdomyosarcoma, fibrosarcoma, Kaposi's sarcoma), spinal axis tumors, glioma (*e.g.*, ependymoma, astrocytoma, anaplastic astrocytoma, oligodendroglioma, eye cancer (*e.g.*, retinoblastoma), brain stem glioma, or mixed glioma such as oligoastrocytoma), brain tumor (*e.g.*, glioblastoma/glioblastoma multiforme (GBM), non-glioblastoma brain tumor, or meningioma), melanoma (*e.g.*, cutaneous or intraocular melanoma), thrombocythemia, mesothelioma, mycosis fungoides, Sezary syndrome, idiopathic myelofibrosis, solitary plasmacytoma, vestibular schwannoma, Ewing's sarcoma, chondrosarcoma, MYH associated polyposis, pituitary adenoma, pediatric cancers such as pediatric sarcomas (*e.g.*, neuroblastoma, rhabdomyosarcoma, and osteosarcoma), hematological cancer, lymphoma, Hodgkin's lymphoma, non-Hodgkin's lymphoma, leukemia (*e.g.*, lymphocytic/lymphoblastic leukemia), chronic or acute leukemia, mast cell leukemia, lymphocytic lymphomas, primary CNS lymphoma, chronic lymphocytic leukemia (CLL), acute lymphocytic leukemia (ALL), chronic myeloid leukemia (CML), acute myeloid leukemia (AML), chronic myelomonocytic leukemia (CMML), chronic lymphoblastic leukemia, acute lymphoblastic leukemia, hairy cell leukemia (HCL), Burkitt's lymphoma (BL), multiple myeloma (*e.g.*, relapsed or refractory multiple myeloma), T or B cell lymphoma, mantle cell lymphoma (MCL) (*e.g.*, relapsed or refractory mantle cell lymphoma), malignant melanoma, diffuse large B cell lymphoma (DLBCL), DLBCL that results from follicular lymphoma, high-grade B-cell lymphoma, primary mediastinal large B-cell lymphoma, follicular lymphoma (FL), and primary

mediastinal B-cell lymphoma. In some embodiments, the disease, disorder or condition is acute myeloid leukemia.

[0074] In some embodiments, the subject is human.

[0075] In some embodiments, the administration is through a parenteral route comprising subcutaneous, intraperitoneal, intravenous, intramuscular, or intradermal injection; or a non-parenteral route comprising transdermal, oral, intranasal, intraocular, sublingual, rectal, or topical.

[0076] In some embodiments, the method of treating, preventing or alleviating a disease, disorder or condition in a subject further includes administering to the subject in need thereof an additional therapeutic agent. In some embodiments, the additional therapeutic agent is selected from the group consisting of: an active agent, an imaging agent, a cytotoxic agent, and angiogenesis inhibitor, a kinase inhibitor, a co-stimulation molecule agonist, a co-inhibition molecule blocker, an adhesion molecule blocker, an anti-cytokine antibody or functional fragment thereof, a detectable label or reporter, an antimicrobial, a gene editing agent, a beta agonist, an viral RNA inhibitor, a polymerase inhibitor, an interferon, and a microRNA. In some embodiments, the additional therapeutic agent is administered to the subject in need before, after or simultaneously with the antibody or antigen-binding fragment thereof and/or the pharmaceutical composition of the present disclosure.

[0077] In another aspect, the present disclosure provides a method of activating a T cell expressing CD3 or LILRB4 *in vivo* or *in vitro*, comprising contacting the CD3 and/or LILRB4-expressing cell with the antibody or antigen-binding fragment thereof and/or the chimeric antigen receptor and/or the pharmaceutical composition of the present disclosure.

[0078] In another aspect, the present disclosure provides a method of modulating CD3 and/or LILRB4 activity in a CD3 and/or LILRB4-expressing cell, comprising exposing the CD3 and/or LILRB4-expressing cell to the antibody or antigen-binding

fragment thereof and/or the chimeric antigen receptor and/or the pharmaceutical composition of the present disclosure.

[0079] In another aspect, the present disclosure provides a method of promoting *in vivo* or *in vitro* processing of LILRB4 by a CD3-expressing T cell, comprising contacting the CD3-expressing T cell with the antibody or antigen-binding fragment thereof and/or the chimeric antigen receptor and/or the pharmaceutical composition, wherein the antibody or antigen-binding fragment thereof, the chimeric antigen receptor or the pharmaceutical composition is capable of binding to both the CD3-expressing T cell and LILRB4 thereby bringing both in close proximity.

[0080] In another aspect, the present disclosure provides a method of detecting presence or amount of CD3 and/or LILRB4 in a sample, comprising contacting the sample with the antibody or antigen-binding fragment thereof and/or the chimeric antigen receptor and/or the pharmaceutical composition of the present disclosure, and determining the presence or the amount of CD3 and/or LILRB4 in the sample.

[0081] In another aspect, the present disclosure provides a method of diagnosing a CD3 and/or LILRB4-related disease, disorder or condition in a subject, comprising: a) obtaining a sample from the subject; b) contacting the sample obtained from the subject with the antibody or antigen-binding fragment thereof and/or the chimeric antigen receptor and/or the pharmaceutical composition of the present disclosure; c) determining presence or amount of CD3 and/or LILRB4 in the sample; and d) correlating the presence or the amount of CD3 and/or LILRB4 to existence or status of the CD3 and/or LILRB4-related disease, disorder or condition in the subject.

[0082] In another aspect, the present disclosure provides use of the bispecific antibody or antigen-binding fragment thereof and/or the chimeric antigen receptor and/or the pharmaceutical composition of the present disclosure in the manufacture of a medicament for treating a disease, disorder or condition related to CD3 and/or LILRB4 in a subject.

[0083] In another aspect, the present disclosure provides use of the bispecific antibody or antigen-binding fragment thereof and/or the chimeric antigen receptor and/or the pharmaceutical composition of the present disclosure in the manufacture of a diagnostic reagent for diagnosing a disease, disorder or condition related to CD3 and/or LILRB4 in a subject.

[0084] In another aspect, the present disclosure provides a kit comprising the antibody or antigen-binding fragment thereof and/or the chimeric antigen receptor and/or the pharmaceutical composition of the present disclosure, useful in detecting CD3 and/or LILRB4, optionally recombinant CD3 and/or LILRB4, CD3 and/or LILRB4 expressed on cell surface, or CD3 and/or LILRB4-expressing cells.

BRIEF DESCRIPTION OF THE DRAWINGS

[0085] Figure 1 shows the constructs of two exemplary CD3/LILRB4 multi-specific antibodies BIS-PTMS16-22+40E9 (Figure 1A) and BIS-S3+S16+40E9-F3 (Figure 1B).

[0086] Figure 2 shows FACS analysis suggesting the binding affinities of BIS-PTMS16-22+40E9 and BIS-S3+S16+40E9-F3 antibodies on 293F-hLILRB4 cell (Figure 2A) and Jurkat T cells (Figure 2B).

[0087] Figure 3 shows multi-specific antibodies BIS-PTMS16-22+40E9 and BIS-S3+S16+40E9-F3 antibodies induced strong T cell activation.

[0088] Figure 4 shows LILRB4 expression on THP-1 and MOLM-13 AML cell lines.

[0089] Figure 5 shows multi-specific antibodies BIS-PTMS16-22+40E9 and BIS-S3+S16+40E9-F3 induced potent TDCC effect against LILRB4-high expressing THP-1 (Figure 5A) and LILRB4-low expressing MOLM-13 AML cells (Figure 5B).

[0090] Figure 6 shows multi-specific antibodies BIS-PTMS16-22+40E9 and BIS-S3+S16+40E9-F3 induced more potent TDCC effect against THP-1 AML cells than benchmark antibody IO-312.

[0091] Figure 7 shows the potent *in vivo* efficacy of multi-specific antibodies BIS-S3+S16+40E9-F3 and BIS-PTMS16-22+40E9 in terms of tumor growth (Figure 7A, 7C) and body change (Figure 7B).

[0092] Figure 8 shows FACS analysis suggesting the binding affinities of IO-312, BIS-PTMS16-22+40E9-L2H3, BIS-PTMS16-22+40E9-L2H3-N55S.H, BIS-F3+40E9-L2H3 and BIS-F3+40E9-L2H3-N55S.H antibodies on 293F-hLILRB4 cells (Figure 8A) and Jurkat T cells (Figure 8B).

[0093] Figure 9 shows multi-specific antibodies BIS-PTMS16-22+40E9-L2H3, BIS-PTMS16-22+40E9-L2H3-N55S.H, BIS-F3+40E9-L2H3 and BIS-F3+40E9-L2H3-N55S.H induced potent TDCC effect against LILRB4-high expressing THP-1 (Figure 9A) and LILRB4-low expressing MOLM-13 AML cells (Figure 9B).

[0094] Figure 10 shows the study design (Figure 10A) of testing *in vivo* efficacy of the humanized LILRB4 x CD3 multi-specific antibodies in mouse model, as well as tumor growth (Figures 10B, 10C, 10D) and body weight of the mouse model.

DETAILED DESCRIPTION OF THE INVENTION

[0095] The following description of the disclosure is merely intended to illustrate various embodiments of the disclosure. As such, the specific modifications discussed are not to be construed as limitations on the scope of the disclosure. It will be apparent to a person skilled in the art that various equivalents, changes, and modifications may be made without departing from the scope of the disclosure, and it is understood that such equivalent embodiments are to be included herein. All references cited herein, including publications, patents and patent applications are incorporated herein by reference in their entireties.

Definitions

[0096] Throughout the present disclosure, the articles “a”, “an”, and “the” are used herein to refer to one or to more than one (*i.e.*, to at least one) of the grammatical

object of the article. By way of example, “an antibody” means one antibody or more than one antibody.

[0097] The term “antibody” as used herein includes any immunoglobulin, monoclonal antibody, polyclonal antibody, multivalent antibody, bivalent antibody, monovalent antibody, multi-specific antibody, or bispecific antibody that binds to a specific antigen. A native intact antibody comprises two heavy (H) chains and two light (L) chains. Mammalian heavy chains are classified as alpha, delta, epsilon, gamma, and mu, each heavy chain comprises a variable region (VH) and a first, second, third, and optionally fourth constant region (CH1, CH2, CH3, CH4 respectively); mammalian light chains are classified as λ or κ , while each light chain comprises a variable region (VL) and a constant region. The antibody has a “Y” shape, with the stem of the Y comprising the second and third constant regions of two heavy chains bound together via disulfide bonding. Each arm of the Y includes the variable region and first constant region of a single heavy chain bound to the variable and constant regions of a single light chain. The variable regions of the light and heavy chains are responsible for antigen binding. The variable regions in both chains generally contain three highly variable loops called the complementarity determining regions (CDRs) (light chain CDRs including LCDR1, LCDR2, and LCDR3, heavy chain CDRs including HCDR1, HCDR2, HCDR3). The three CDRs are interposed between flanking stretches known as framework regions (FRs) (light chain FRs including LFR1, LFR2, LFR3, and LFR4, heavy chain FRs including HFR1, HFR2, HFR3, and HFR4), which are more highly conserved than the CDRs and form a scaffold to support the highly variable loops. The constant regions of the heavy and light chains are not involved in antigen-binding, but exhibit various effector functions. Antibodies are assigned to classes based on the amino acid sequences of the constant regions of their heavy chains. The five major classes or isotypes of antibodies are IgA, IgD, IgE, IgG, and IgM, which are characterized by the presence of alpha, delta, epsilon, gamma, and mu heavy chains, respectively. Several of the major antibody classes are divided into subclasses such as IgG1

(gamma1 heavy chain), IgG2 (gamma2 heavy chain), IgG3 (gamma3 heavy chain), IgG4 (gamma4 heavy chain), IgA1 (alpha1 heavy chain), or IgA2 (alpha2 heavy chain).

[0098] In certain embodiments, the antibody provided herein encompasses any antigen-binding fragments thereof. The term “antigen-binding fragment” as used herein refers to an antibody fragment formed from a portion of an antibody comprising one or more (*e.g.*, 1, 2, 3, 4, 5, or 6) CDRs, or any other antibody fragment that binds to an antigen but does not comprise an intact native antibody structure. Examples of antigen-binding fragments include, without limitation, a diabody, a Fab, a Fab', a F(ab')₂, a Fd, an Fv fragment, a disulfide stabilized Fv fragment (dsFv), a (dsFv)₂, a bispecific dsFv (dsFv-dsFv'), a disulfide stabilized diabody (ds diabody), a single-chain antibody molecule (scFv), an scFv dimer (bivalent diabody), a bispecific antibody, a multi-specific antibody, a camelized single domain antibody, a nanobody, a domain antibody, or a bivalent domain antibody. An antigen-binding fragment is capable of binding to the same antigen or epitope to which the parent antibody binds.

[0099] A multi-specific (*e.g.*, bispecific) antibody or antigen-binding fragment thereof that “specifically binds” or “specific binding” to a target (*e.g.*, an epitope) is a term well understood in the art, and methods to determine such specific binding are also well known in the art. A molecule is said to exhibit “specific binding” if it reacts or associates more frequently, more rapidly, with greater duration and/or with greater affinity with a particular cell or substance than it does with alternative cells or substances. An antibody “specifically binds” to a target if it binds with greater affinity, avidity, more readily, and/or with greater duration than it binds to other substances. For example, an antibody that specifically binds to a CD3 epitope and LILRB4 epitope is a multi-specific (*e.g.*, bispecific) antibody that binds this CD3 epitope and LILRB4 epitope with greater affinity, avidity, more readily, and/or with greater duration than it binds to other CD3 epitopes and LILRB4 epitopes or non-CD3/LILRB4 epitopes. It is also understood by reading this definition that, for

example, an antibody (or moiety or epitope) that specifically binds to a first target may or may not specifically bind to a second target. As such, “specific binding” or “specifically bind” does not necessarily require (although it can include) exclusive binding. Generally, but not necessarily, reference to binding means specific binding.

[00100] “Fab” with regard to an antibody refers to that portion of the antibody consisting of a single light chain (both variable and constant regions) bound to the variable region and first constant region of a single heavy chain by a disulfide bond.

[00101] “Fab’” refers to a Fab fragment that includes a portion of the hinge region.

[00102] “F(ab’)₂” refers to a dimer of Fab’.

[00103] “Fc” with regard to an antibody (*e.g.*, of IgG, IgA, or IgD isotype) refers to that portion of the antibody consisting of the second and third constant domains of a first heavy chain bound to the second and third constant domains of a second heavy chain via disulfide bonding. Fc with regard to antibody of IgM and IgE isotype further comprises a fourth constant domain. The Fc portion of the antibody is responsible for various effector functions such as antibody-dependent cell-mediated cytotoxicity (ADCC), and complement dependent cytotoxicity (CDC), but does not function in antigen binding.

[00104] “Fv” with regard to an antibody refers to the smallest fragment of the antibody to bear the complete antigen binding site. An Fv fragment consists of the variable region of a single light chain bound to the variable region of a single heavy chain.

[00105] “Single-chain Fv antibody” or “scFv” refers to an engineered antibody consisting of a light chain variable region and a heavy chain variable region connected to one another directly or via a linker (*e.g.*, a peptide sequence) (Huston JS *et al.*, *Proc Natl Acad Sci USA*, 85:5879(1988)).

[00106] “Single-chain Fv-Fc antibody” or “scFv-Fc” refers to an engineered antibody consisting of a scFv connected to the Fc region of an antibody directly or via a linker (*e.g.*, a peptide sequence).

[00107] “Camelized single domain antibody”, “heavy chain antibody”, or “HCAb” refers to an antibody that contains two VH domains and no light chains (Riechmann L. and Muyldermans S., *J Immunol Methods*. Dec 10; 231(1-2):25-38 (1999); Muyldermans S., *J Biotechnol*. Jun; 74(4):277-302 (2001); WO94/04678; WO94/25591; U.S. Patent No. 6,005,079). Heavy chain antibodies were originally derived from *Camelidae* (camels, dromedaries, and llamas). Although devoid of light chains, camelized antibodies have an authentic antigen-binding repertoire (Hamers-Casterman C. *et al.*, *Nature*. Jun 3; 363(6428):446-8 (1993); Nguyen VK. *et al.*, *Immunogenetics*. Apr; 54(1):39-47 (2002); Nguyen VK. *et al.*, *Immunology*. May; 109(1):93-101 (2003)). The variable domain of a heavy chain antibody (VHH domain) represents the smallest known antigen-binding unit generated by adaptive immune responses (Koch-Nolte F. *et al.*, *FASEB J*. Nov; 21(13):3490-8. Epub 2007 Jun 15 (2007)).

[00108] A “nanobody” refers to an antibody fragment that consists of a VHH domain from a heavy chain antibody and two constant domains, CH2 and CH3.

[00109] A “diabody” or “dAb” includes small antibody fragments with two antigen-binding sites, wherein the fragments comprise a VH domain connected to a VL domain in the same polypeptide chain (VH-VL or VL-VH) (see, *e.g.*, Holliger P. *et al.*, *Proc Natl Acad Sci USA*. Jul 15; 90(14):6444-8 (1993); EP404097; WO93/11161). By using a linker that is too short to allow pairing between the two domains on the same chain, the domains are forced to pair with the complementary domains of another chain, thereby creating two antigen-binding sites. The antigen-binding sites may target the same or different antigens (or epitopes). In certain embodiments, a “bispecific ds diabody” is a diabody target two different antigens (or epitopes).

[00110] A “domain antibody” refers to an antibody fragment containing only the variable region of a heavy chain or the variable region of a light chain. In certain instances, two or more VH domains are covalently joined with a peptide linker to create a bivalent or multivalent domain antibody. The two VH domains of a bivalent domain antibody may target the same or different antigens.

[00111] The term “valent” as used herein refers to the presence of a specified number of antigen binding sites in a given molecule. The term “monovalent” refers to an antibody or an antigen-binding fragment having only one single antigen-binding site; and the term “multivalent” refers to an antibody or antigen-binding fragment having multiple antigen-binding sites. As such, the terms “bivalent”, “tetravalent”, and “hexavalent” denote the presence of two antigen-binding sites, four antigen-binding sites, and six antigen-binding sites, respectively, in an antigen-binding molecule. In some embodiments, the antibody or antigen-binding fragment thereof is bivalent.

[00112] As used herein, a “multi-specific” antibody refers to an antibody that specifically binds to at least two distinct antigens or at least two distinct epitopes within the same antigen. Multi-specific antibody may bind for example two, three, four, five or more distinct antigens or distinct epitopes within the same antigen. Accordingly, a “bispecific” antibody refers to an artificial antibody which has fragments derived from two different monoclonal antibodies and is capable of binding to two different epitopes. The two epitopes may present on the same antigen, or they may present on two different antigens.

[00113] The term “chimeric” as used herein, means an antibody or antigen-binding fragment, having a portion of heavy and/or light chain derived from one species, and the rest of the heavy and/or light chain derived from a different species. In an illustrative example, a chimeric antibody may comprise a constant region derived from human and a variable region from a non-human animal, such as from mouse. In some embodiments, the non-human animal is a mammal, for example, a mouse, a rat, a rabbit, a goat, a sheep, a guinea pig, or a hamster.

[00114] The term “humanized” as used herein means that the antibody or antigen-binding fragment comprises CDRs derived from non-human animals, FR regions derived from human, and when applicable, the constant regions derived from human. The CDRs of humanized antibodies provided in the present disclosure may contain mutation(s) compared to the CDRs of their parent antibodies.

[00115] The term “affinity” as used herein refers to the strength of non-covalent interaction between an immunoglobulin molecule (*i.e.*, antibody) or antigen-binding fragment thereof and an antigen.

[00116] The term “epitope” as used herein refers to the specific group of atoms or amino acids on an antigen to which an antibody binds. Two antibodies may bind the same or a closely related epitope within an antigen if they exhibit competitive binding for the antigen. An epitope can be linear or conformational (*i.e.*, including amino acid residues spaced apart). For example, if an antibody or antigen-binding fragment blocks binding of a reference antibody to the antigen by at least 85%, or at least 90%, or at least 95%, then the antibody or antigen-binding fragment may be considered to bind the same/closely related epitope as the reference antibody.

[00117] The term “amino acid” as used herein refers to an organic compound containing amine (-NH₂) and carboxyl (-COOH) functional groups, along with a side chain specific to each amino acid. The names of amino acids are also represented as standard single letter or three-letter codes in the present disclosure, which are summarized as follows.

Names	Three-letter Code	Single-letter Code
Alanine	Ala	A
Arginine	Arg	R
Asparagine	Asn	N
Aspartic acid	Asp	D
Cysteine	Cys	C
Glutamic acid	Glu	E
Glutamine	Gln	Q
Glycine	Gly	G
Histidine	His	H

Names	Three-letter Code	Single-letter Code
Isoleucine	Ile	I
Leucine	Leu	L
Lysine	Lys	K
Methionine	Met	M
Phenylalanine	Phe	F
Proline	Pro	P
Serine	Ser	S
Threonine	Thr	T
Tryptophan	Trp	W
Tyrosine	Tyr	Y
Valine	Val	V

[00118] A “conservative substitution” with reference to amino acid sequence refers to replacing an amino acid residue with a different amino acid residue having a side chain with similar physiochemical properties. For example, conservative substitutions can be made among amino acid residues with hydrophobic side chains (*e.g.*, Met, Ala, Val, Leu, and Ile), among amino acid residues with neutral hydrophilic side chains (*e.g.*, Cys, Ser, Thr, Asn and Gln), among amino acid residues with acidic side chains (*e.g.*, Asp, Glu), among amino acid residues with basic side chains (*e.g.*, His, Lys, and Arg), or among amino acid residues with aromatic side chains (*e.g.*, Trp, Tyr, and Phe). As known in the art, conservative substitution usually does not cause significant change in the protein conformational structure, and therefore could retain the biological activity of a protein.

[00119] The term “homologous” as used herein refers to a nucleic acid sequence (or its complementary strand) or amino acid sequence that has sequence identity of at least 60% (*e.g.*, at least 65%, 70%, 75%, 80%, 85%, 88%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99%) to another sequence when optimally aligned.

[00120] “Percent (%) sequence identity” with respect to amino acid sequence (or nucleic acid sequence) is defined as the percentage of amino acid (or nucleic acid) residues in a candidate sequence that are identical to the amino acid (or nucleic acid) residues in a reference sequence, after aligning the sequences and, if necessary, introducing gaps, to achieve the maximum number of identical amino acids (or

nucleic acids). In other words, percent (%) sequence identity of an amino acid sequence (or nucleic acid sequence) can be calculated by dividing the number of amino acid residues (or bases) that are identical relative to the reference sequence to which it is being compared by the total number of the amino acid residues (or bases) in the candidate sequence or in the reference sequence, whichever is shorter.

Conservative substitution of the amino acid residues may or may not be considered as identical residues. Alignment for purposes of determining percent amino acid (or nucleic acid) sequence identity can be achieved, for example, using publicly available tools such as BLASTN, BLASTp (available on the website of U.S. National Center for Biotechnology Information (NCBI), see also, Altschul S.F. *et al.*, *J. Mol. Biol.*, 215:403–410 (1990); Stephen F. *et al.*, *Nucleic Acids Res.*, 25:3389–3402 (1997)), ClustalW2 (available on the website of European Bioinformatics Institute, see also, Higgins D.G. *et al.*, *Methods in Enzymology*, 266:383–402 (1996); Larkin M.A. *et al.*, *Bioinformatics* (Oxford, England), 23(21): 2947–8 (2007)), and ALIGN or Megalign (DNASTAR) software. A person skilled in the art may use the default parameters provided by the tool, or may customize the parameters as appropriate for the alignment, such as for example, by selecting a suitable algorithm.

[00121] “Effector functions” as used herein refer to biological activities attributable to the binding of Fc region of an antibody to its effectors such as C1 complex and Fc receptor. Exemplary effector functions include: complement dependent cytotoxicity (CDC) mediated by interaction of antibodies and C1q on the C1 complex; antibody-dependent cell-mediated cytotoxicity (ADCC) mediated by binding of Fc region of an antibody to Fc receptor on an effector cell; and phagocytosis. Effector functions can be evaluated using various assays such as Fc receptor binding assay, C1q binding assay, and cell lysis assay.

[00122] “Antibody-dependent cell-mediated cytotoxicity” or “ADCC” as used herein refers to a cell-mediated reaction in which effector cells that express Fc receptors (FcRs) recognize bound antibody or antigen-binding fragment on a target cell and subsequently cause lysis of the target cell. “ADCC activity” or “ADCC effect”

refers to the ability of the antibody or antigen-binding fragment which is bound on the target cell to elicit an ADCC reaction as described above.

[00123] “Complement dependent cytotoxicity” or “CDC” as used herein refers to a mechanism by which antibodies can mediate specific target cell lysis through activation of an organism’s complement system. In CDC, the C1q binds the antibody and this binding triggers the complement cascade which leads to the formation of the membrane attack complex (MAC) (C5b to C9) at the surface of the target cell, as a result of the classical pathway complement activation. “CDC activity” or “CDC effect” refers to the ability of the antibody or antigen-binding fragment which is bound on the target cell to elicit a CDC reaction as described above.

[00124] “Target cells” as used herein refer to cells to which antibodies comprising an Fc region specifically bind, generally via the protein part that is C-terminal to the Fc region. “Effector cells” are leukocytes which express one or more Fc receptors and perform effector functions. Examples of human leukocytes which mediate ADCC include peripheral blood mononuclear cells (PBMCs), natural killer (NK) cells, monocytes, cytotoxic T cells and neutrophils; with PBMCs and NK cells being preferred. The effector cells may be isolated from a native source thereof, *e.g.*, from blood or PBMCs as is known in the art.

[00125] An “isolated” substance has been altered by the hand of man from the natural state. If an “isolated” composition or substance occurs in nature, it has been changed or removed from its original environment, or both. For example, a polynucleotide or a polypeptide naturally present in a living animal is not “isolated”, but the same polynucleotide or polypeptide is “isolated” if it has been sufficiently separated from the coexisting materials of its natural state so as to exist in a substantially pure state. An “isolated nucleic acid sequence” refers to the sequence of an isolated nucleic acid molecule. In certain embodiments, an “isolated antibody or an antigen-binding fragment thereof” refers to the antibody or antigen-binding fragments thereof having a purity of at least 60%, 70%, 75%, 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%,

89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% as determined by electrophoretic methods (such as SDS-PAGE, isoelectric focusing, capillary electrophoresis), or chromatographic methods (such as ion exchange chromatography or reverse phase HPLC).

[00126] The term “vector” as used herein refers to a vehicle into which a polynucleotide encoding a protein may be operably inserted so as to bring about the expression of that protein. A vector may be used to transform, transduce, or transfect a host cell so as to bring about expression of the genetic element it carries within the host cell. Examples of vectors include plasmids, phagemids, cosmids, artificial chromosomes such as yeast artificial chromosome (YAC), bacterial artificial chromosome (BAC), or P1-derived artificial chromosome (PAC), bacteriophages such as lambda phage or M13 phage, and animal viruses. Categories of animal viruses used as vectors include retrovirus (including lentivirus), adenovirus, adeno-associated virus, herpesvirus (*e.g.*, herpes simplex virus), poxvirus, baculovirus, papillomavirus, and papovavirus (*e.g.*, SV40). A vector may contain a variety of elements for controlling expression, including promoter sequences, transcription initiation sequences, enhancer sequences, selectable elements, and reporter genes. In addition, the vector may contain an origin of replication. A vector may also include materials to aid in its entry into the cell, including but not limited to a viral particle, a liposome, or a protein coating. A vector can be an expression vector or a cloning vector. The present disclosure provides vectors (*e.g.*, expression vectors) containing the nucleic acid sequence provided herein encoding the antibody or antigen-binding fragment thereof, at least one promoter (*e.g.*, SV40, CMV, EF-1 α) operably linked to the nucleic acid sequence, and at least one selection marker. Examples of vectors include, but are not limited to, retrovirus (including lentivirus), adenovirus, adeno-associated virus, herpesvirus (*e.g.*, herpes simplex virus), poxvirus, baculovirus, papillomavirus, papovavirus (*e.g.*, SV40), lambda phage, and M13 phage, plasmid pcDNA3.3, pMD18-T, pOptivec, pCMV, pEGFP, pIRES, pQD-Hyg-GSeu, pALTER, pBAD, pcDNA, pCal, pL, pET, pGEMEX, pGEX, pCI, pEGFT, pSV2, pFUSE,

pVITRO, pVIVO, pMAL, pMONO, pSELECT, pUNO, pDUO, Psg5L, pBABE, pWPXL, pBI, p15TV-L, pPro18, pTD, pRS10, pLexA, pACT2.2, pCMV-SCRIPT.RTM., pCDM8, pCDNA1.1/amp, pCDNA3.1, pRc/RSV, PCR 2.1, pEF-1, pFB, pSG5, pXT1, pCDEF3, pSVSPORT, pEF-Bos etc.

[00127] The phrase “host cell” as used herein refers to a cell into which an exogenous polynucleotide and/or a vector can be or has been introduced.

[00128] The term “subject” includes human and non-human animals. Non-human animals include all vertebrates, *e.g.*, mammals and non-mammals, such as non-human primates, mice, rats, cats, rabbits, sheep, dogs, cows, chickens, amphibians, and reptiles. Except when noted, the terms “patient”, “subject” or “individual” are used herein interchangeably.

[00129] “Treating” or “treatment” of a disease, disorder or condition as used herein includes preventing or alleviating a disease, disorder or condition, slowing the onset or rate of development of a disease, disorder or condition, reducing the risk of developing a disease, disorder or condition, preventing or delaying the development of symptoms associated with a disease, disorder or condition, reducing or ending symptoms associated with a disease, disorder or condition, generating a complete or partial regression of a disease, disorder or condition, curing a disease, disorder or condition, or some combination thereof.

[00130] The term “diagnosis”, “diagnose” or “diagnosing” refers to the identification of a pathological state, disease or condition, such as identification of a CD3 and/or LILRB4 related disease, or refer to identification of a subject with a CD3 and/or LILRB4 related disease who may benefit from a particular treatment regimen. In some embodiments, diagnosis contains the identification of abnormal amount or activity of CD3 and/or LILRB4. In some embodiments, diagnosis refers to the identification of a cancer in a subject.

[00131] As used herein, the term “biological sample” or “sample” refers to a biological composition that is obtained or derived from a subject of interest that

contains a cellular and/or other molecular entity that is to be characterized and/or identified, for example based on physical, biochemical, chemical and/or physiological characteristics. A biological sample includes, but is not limited to, cells, tissues, organs and/or biological fluids of a subject, obtained by any method known by those of skill in the art. In some embodiments, the biological sample is a fluid sample. In some embodiments, the fluid sample is whole blood, plasma, blood serum, mucus (including nasal drainage and phlegm), peritoneal fluid, pleural fluid, chest fluid, saliva, urine, synovial fluid, cerebrospinal fluid (CSF), thoracentesis fluid, abdominal fluid, ascites or pericardial fluid. In some embodiments, the biological sample is a tissue or cell obtained from stomach, heart, liver, spleen, lung, kidney, skin or blood vessels of the subject.

[00132] “CD3” as used herein, refers to the Cluster of Differentiation 3 protein and includes any variants, conformations, isoforms and species homologs of CD3 which are naturally expressed by cells or are expressed by cells transfected with the CD3 gene. For example, CD3 described herein may refer to the Cluster of Differentiation 3 protein derived from any vertebrate source, including mammals such as primates (*e.g.*, humans, monkeys) and rodents (*e.g.*, mice and rats). In mammals, the CD3 molecule is a multi-protein complex of six chains, including: a CD3gamma chain, a CD3delta chain, two CD3epsilon chains, and a homodimer of CD3zeta chains, wherein the CD3zeta chain is the intracellular tail of CD3 molecule, and the CD3gamma, CD3delta and CD3epsilon chains all contain extracellular domain (ECD) expressed on surface of T cells. Exemplary sequence of human CD3 includes human CD3epsilon protein (NCBI Ref Seq No. NP_000724), human CD3 delta protein (NCBI Ref Seq No. NP_000723), and human CD3gamma protein (NCBI Ref Seq No. NP_000064). Exemplary sequence of non-human CD3 includes *Macaca fascicularis* (monkey) CD3epsilon protein (NCBI Ref Seq No. NP_001270544), *Macaca fascicularis* (monkey) CD 3delta protein (NCBI Ref Seq No. NP_001274617), *Macaca fascicularis* (monkey) CD3gamma protein (NCBI Ref Seq No. NP_001270839); *Mus musculus* (mouse) CD3epsilon protein (NCBI Ref Seq No.

NP_031674), *Mus musculus* (mouse) CD3delta protein (NCBI Ref Seq No. NP_038515), *Mus musculus domesticus* (mouse) CD3gamma protein (NCBI Ref Seq No. AAA37400); *Rattus norvegicus* (Rat) CD3epsilon protein (NCBI Ref Seq No. NP_001101610), *Rattus norvegicus* (Rat) CD3delta protein (NCBI Ref Seq No. NP_037301), *Rattus norvegicus* (Rat) CD3gamma protein (NCBI Ref Seq No. NP_001071114). In certain embodiments, CD3 used herein can also be recombinant CD3, for example, including recombinant CD3epsilon protein, recombinant CD3delta protein, and recombinant CD3gamma protein, which may optionally be expressed as a recombinant CD3 complex. The recombinant CD3 complex may be expressed on a cell surface, or alternatively may be expressed as a soluble form which is not associated on a cell surface. In certain embodiments, the CD3 is human CD3. The terms “CD3”, “CD-3”, “CD 3”, “cluster of differentiation 3” may be used interchangeably in the present disclosure.

[00133] The term “CD3gamma” as used herein is intended to encompass any form of CD3gamma, for example, 1) native unprocessed CD3gamma molecule, “full-length” CD3gamma chain or naturally occurring variants of CD3gamma, including, for example, splice variants or allelic variants; 2) any form of CD3gamma that results from processing in the cell; or 3) full length, a fragment (*e.g.*, a truncated form, an extracellular/transmembrane domain) or a modified form (*e.g.*, a mutated form, a glycosylated/PEGylated, a His-tag/immunofluorescence fused form) of CD3gamma subunit generated through recombinant method.

[00134] The term “CD3delta” as used herein is intended to encompass any form of CD3delta, for example, 1) native unprocessed CD3delta molecule, “full-length” CD3delta chain or naturally occurring variants of CD3delta, including, for example, splice variants or allelic variants; 2) any form of CD3delta that results from processing in the cell; or 3) full length, a fragment (*e.g.*, a truncated form, an extracellular/transmembrane domain) or a modified form (*e.g.*, a mutated form, a glycosylated/PEGylated, a His-tag/immunofluorescence fused form) of CD3delta subunit generated through recombinant method.

[00135] The term “CD3epsilon” as used herein is intended to encompass any form of CD3epsilon, for example, 1) native unprocessed CD3epsilon molecule, “full-length” CD3epsilon chain or naturally occurring variants of CD3epsilon, including, for example, splice variants or allelic variants; 2) any form of CD3epsilon that results from processing in the cell; or 3) full length, a fragment (*e.g.*, a truncated form, an extracellular/transmembrane domain) or a modified form (*e.g.*, a mutated form, a glycosylated/PEGylated, a His-tag/immunofluorescence fused form) of CD3epsilon subunit generated through recombinant method.

[00136] The term “LILRB4” as used herein, refers to the leukocyte immunoglobulin-like receptor subfamily B member 4, includes any variants, conformations, isoforms and species homologs of LILRB4 which are naturally expressed by cells or are expressed by cells transfected with the LILRB4 gene. For example, LILRB4 described herein may refer to the leukocyte immunoglobulin-like receptor subfamily B member 4 protein derived from any vertebrate source, including mammals such as primates (*e.g.*, humans, monkeys) and rodents (*e.g.*, mice and rats). Exemplary sequence of human LILRB4 protein is, for example as described in UniProtKB Entry No.: Q8NHJ6 or GenBank Accession No. AAB68665.1. The term “LILRB4” as used herein is intended to encompass any form of LILRB4, for example, 1) native unprocessed LILRB4 molecule, “full-length” LILRB4 chain or naturally occurring variants of LILRB4, including, for example, splice variants or allelic variants; 2) any form of LILRB4 that results from processing in the cell; or 3) full length, a fragment (*e.g.*, a truncated form, an extracellular/transmembrane domain) or a modified form (*e.g.*, a mutated form, a glycosylated/PEGylated, a His-tag/immunofluorescence fused form) of LILRB4 subunit generated through recombinant method.

[00137] A “CD3 and/or LILRB4 related” or “CD3 and/or LILRB4-related” disease, disorder or condition, or a disease, disorder or condition “related to CD3 and/or LILRB4” as used herein refers to any disease, disorder or condition caused by, exacerbated by, or otherwise linked to increased or decreased expression or activities of CD3 and/or LILRB4. In some embodiments, the CD3 and/or LILRB4-related

disease, disorder or condition is a disorder related to excessive cell proliferation, such as, for example, cancer. In certain embodiments, the CD3 and/or LILRB4-related disease or condition is characterized in expressing or over-expressing of CD3 and/or LILRB4, and/or CD3 and/or LILRB4 related genes.

[00138] The term “pharmaceutically acceptable” indicates that the designated carrier, vehicle, diluent, excipient(s), and/or salt is generally chemically and/or physically compatible with the other ingredients comprising the formulation, and physiologically compatible with the recipient thereof.

[00139] The term “CD3 and/or LILRB4-expressing cell” as used herein refer to a cell that expresses CD3 and/or LILRB4 on the surface of the cell.

Multi-specific Antibodies

[00140] In one aspect, the present disclosure provides multi-specific (*e.g.*, bispecific) antibodies and antigen-binding fragments thereof. In particular, the multi-specific antibodies and antigen-binding fragments thereof provided herein are capable of binding (*e.g.*, specifically binding) to CD3 (*e.g.*, human CD3) and LILRB4 (*e.g.*, human LILRB4). In some embodiments, the present disclosure provides antibodies or antigen-binding fragments thereof that bind to CD3 and LILRB4, which comprise a first binding moiety that binds to CD3 and a second binding moiety that binds to LILRB4.

[00141] Binding affinity of the antibody or antigen-binding fragment thereof provided herein can be represented by K_D value, which represents the ratio of dissociation rate to association rate (k_{off}/k_{on}) when the binding between the antigen and antigen-binding molecule reaches equilibrium. The antigen-binding affinity (*e.g.*, K_D) can be appropriately determined using suitable methods known in the art, including, for example, flow cytometry assay. In some embodiments, binding of the multi-specific antibody or antigen-binding fragment thereof to the antigen at different concentrations can be determined by flow cytometry, the determined mean fluorescence intensity (MFI) can be firstly plotted against antibody concentration, K_D value can then be

calculated by fitting the dependence of specific binding fluorescence intensity (Y) and the concentration of antibodies (X) into the one site saturation equation:

$Y = B_{\max} * X / (K_D + X)$ using Prism version 5 (GraphPad Software, San Diego, CA), wherein $B_{\max}$ refers to the maximum specific binding of the tested antibody to the antigen.

[00142] Binding of the antibodies or the antigen-binding fragments thereof provided herein to CD3 and LILRB4 can also be represented by “half maximal effective concentration” (EC_{50}) value, which refers to the concentration of an antibody where 50% of its maximal binding is observed. The EC_{50} value can be measured by binding assays known in the art, for example, direct or indirect binding assay such as enzyme-linked immunosorbent assay (ELISA), FACS assay, and other binding assays.

[00143] In certain embodiments, the antibodies or antigen-binding fragments thereof provided herein are capable of binding to human CD3, cynomolgus CD3 or mouse CD3 (*e.g.*, as measured by FACS assay). For example, the antibodies or antigen-binding fragments thereof provided herein bind to human CD3 at an EC_{50} of between 100 to 200 nM (*e.g.*, 110nM, 120nM, 130nM, 140nM, 150nM, 160nM, 170nM, 180nM, 190nM, etc.). In certain embodiments, the antibodies or antigen-binding fragments thereof provided herein are capable of binding to human LILRB4 (*e.g.*, as measured by FACS assay). For example, the antibodies or antigen-binding fragments thereof provided herein bind to human LILRB4 at an EC_{50} of no more than 10 nM (*e.g.*, no more than 9 nM, no more than 8 nM, no more than 7 nM, no more than 6 nM, no more than 5 nM, no more than 4 nM, no more than 3 nM, no more than 2 nM, or no more than 1 nM) as measured by FACS assay.

[00144] In certain embodiments, the antibodies or antigen-binding fragments thereof provided herein are with a T cell activation capability. In certain embodiments, the antibodies or antigen-binding fragments thereof provided herein show potent T cell activation on CD8⁺ T cells. The T cell activation capability of multi-specific antibodies can be measured by well-known methods in the art, for example, can be

measured by determining percentage of CD69 and CD25 on CD8⁺ T cells. In certain embodiments, the T cell activation capability is measured by the method as described in Example 3 of the present disclosure.

[00145] In certain embodiments, the antibodies or antigen-binding fragments thereof provided herein show potent T-cell dependent cytotoxicity effect (*e.g.*, as measured by FACS assay) on different acute myeloid leukemia (AML) cells, including both LILRB4-high expressing AML cells (*e.g.*, THP-1 cells) and LILRB4-low expressing AML cells (*e.g.*, MOLM-13 cells). In certain embodiments, the antibodies or antigen-binding fragments thereof provided herein show more potent T-cell dependent cytotoxicity effect compared with benchmark antibody IO-312. The information of benchmark antibody IO-312 is described in U.S. patent application US2023340114A1. In certain embodiments, the T-cell dependent cytotoxicity effect is measured by the method as described in Example 4 of the present disclosure.

[00146] In certain embodiments, the antibodies or antigen-binding fragments thereof provided herein are with a PBMC-mediated cellular cytotoxicity. In certain embodiments, the antibodies or antigen-binding fragments thereof provided herein show potent PBMC-mediated cellular cytotoxicity (*e.g.*, against THP-1 AML cells) compared with benchmark antibody IO-312. The PBMC-mediated cellular cytotoxicity of multi-specific antibodies can be determined by well-known methods in the art, for example, can be determined by ELISA assay. In certain embodiments, the PBMC-mediated cellular cytotoxicity is measured by the method as described in Example 5 of the present disclosure.

[00147] In certain embodiments, the antibodies or antigen-binding fragments thereof provided herein are with an anti-tumor efficacy *in vivo*. In certain embodiments, the antibodies or antigen-binding fragments thereof provided herein show potent *in vivo* anti-tumor efficacy compared with benchmark antibody IO-312. The anti-tumor efficacy *in vivo* can be determined by well-known methods in the art, for example, can be determined by the method as described in Example 6 of the present disclosure.

[00148] In some embodiments, the antibodies or antigen-binding fragments of the present disclosure are provided in a Fab-scFv-Fab format, which is also referred as “chimeric 2+1 bispecific” or “Fab2-scFv” format in the present disclosure. An antibody in the Fab-scFv-Fab format comprises two Fab domains targeting one or two targets (*e.g.*, LILRB4 or CD3), and one scFv targeting another target (*e.g.*, CD3 or LILRB4). An illustrative example of the Fab-scFv-Fab format is shown in Figure 1A of the present disclosure.

[00149] In some embodiments, the antibodies or antigen-binding fragments of the present disclosure are provided in a Fab-scFv-Fab-Fc-scFv format. An antibody in the Fab-scFv-Fab-Fc-scFv format comprises two Fab domains targeting one or two targets (*e.g.*, LILRB4 or CD3), and two scFvs targeting another target (*e.g.*, CD3 or LILRB4). An illustrative example of the Fab-scFv-Fab-Fc-scFv format is shown in Figure 1B of the present disclosure.

Illustrative Multi-specific Antibodies

[00150] In one aspect, the present disclosure provides antibodies or antigen-binding fragments thereof that bind to CD3 and LILRB4, comprising a first binding moiety that binds to CD3 and a second binding moiety that binds to LILRB4.

[00151] In certain embodiments, the antibodies or antigen-binding fragments thereof that binds to CD3 and LILRB4, comprising a first binding moiety that binds to CD3 and a second binding moiety that binds to LILRB4,

wherein the first binding moiety comprises

one or two or three heavy chain complementarity determining regions (HCDR1, HCDR2 and/or HCDR3) contained within the heavy chain variable (VH) region sequence of SEQ ID NOS: 7, 9, 10, 47 or 11; and/or

one or two or three light chain complementarity determining regions (LCDR1, LCDR2 and/or LCDR3) contained within the light chain variable (VL) region sequence of SEQ ID NOS: 8, 12 or 13;

and,

wherein the second binding moiety comprises

one or two or three heavy chain complementarity determining regions (HCDR1, HCDR2 and/or HCDR3) contained within the heavy chain variable (VH) region sequence of SEQ ID NOs: 20, 22, 30, or 33; and/or

one or two or three light chain complementarity determining regions (LCDR1, LCDR2 and/or LCDR3) contained within the light chain variable (VL) region sequence of SEQ ID NOs: 21, 23, 31 or 34.

[00152] The first binding moiety and the second binding moiety contained in the antibodies or antigen-binding fragments thereof provided herein will be described in detailed below.

First binding moiety

[00153] As used herein, the term “first binding moiety” when referring to an antibody or antigen-binding fragment thereof provided herein, refers to a moiety that is capable of binding to CD3 (*e.g.*, human CD3, mouse CD3, and cynomolgus CD3). The first binding moiety can take any form that allows specific recognition of the target CD3. For example, the first binding moiety may be an antibody or an antigen-binding fragment thereof, *e.g.*, an IgG (such as IgG1, IgG2, IgG3, and IgG4) antibody, IgA antibody, or IgM antibody. In some embodiments, the first binding moiety of the antibody or antigen-binding fragment thereof provided herein is derived from an anti-CD3 antibody that binds and activates primary T cells. The first binding moiety of the multi-specific antibodies or antigen-binding fragments thereof provided herein may be derived from any of the anti-CD3 antibodies known in the art, *e.g.*, the anti-CD3 antibodies described in US20200048348A1, WO2013186613A1, WO2015001085A1, etc. In some embodiments, the first binding moiety is a Fab domain. In some embodiments, the first binding moiety is an anti-CD3 Fab domain. In some embodiments, the first binding moiety is a single-chain Fv (scFv). In some embodiments, the first binding moiety is an anti-CD3 scFv.

[00154] In certain embodiments, the present disclosure provides antibodies or antigen-binding fragments thereof, wherein the first binding moiety binding to CD3 comprises one or more (*e.g.*, 1, 2, 3, 4, 5, or 6) CDR sequences of an anti-CD3 antibody 40-C12-C10-E9, hu40E9-L2H1, hu40E9-L2H3, hu40E9-L2H4, hu40E9-L3H1, or hu40E9-L2H3-N55S.H.

[00155] Antibody “40-C12-C10-E9” as used herein refers to an anti-CD3 antibody or an antigen-binding fragment thereof comprising a heavy chain variable region having the sequence of SEQ ID NO: 7, and a light chain variable region having the sequence of SEQ ID NO: 8.

[00156] Antibody “hu40E9-L2H1” as used herein refers to a humanized anti-CD3 antibody or an antigen-binding fragment thereof comprising a heavy chain variable region having the sequence of SEQ ID NO: 9, and a light chain variable region having the sequence of SEQ ID NO: 12.

[00157] Antibody “hu40E9-L2H3” as used herein refers to a humanized anti-CD3 antibody or an antigen-binding fragment thereof comprising a heavy chain variable region having the sequence of SEQ ID NO: 10, and a light chain variable region having the sequence of SEQ ID NO: 12.

[00158] Antibody “hu40E9-L2H4” as used herein refers to a humanized anti-CD3 antibody or an antigen-binding fragment thereof comprising a heavy chain variable region having the sequence of SEQ ID NO: 11, and a light chain variable region having the sequence of SEQ ID NO: 12.

[00159] Antibody “hu40E9-L3H1” as used herein refers to a humanized anti-CD3 antibody or an antigen-binding fragment thereof comprising a heavy chain variable region having the sequence of SEQ ID NO: 9, and a light chain variable region having the sequence of SEQ ID NO: 13.

[00160] Antibody “hu40E9-L2H3-N55S.H” as used herein refers to a humanized anti-CD3 antibody or an antigen-binding fragment thereof comprising a heavy chain

variable region having the sequence of SEQ ID NO: 47, and a light chain variable region having the sequence of SEQ ID NO: 12.

[00161] In certain embodiments, the first binding moiety of the antibodies or antigen-binding fragments thereof provided herein comprises one or two or three heavy chain complementarity determining regions (HCDR1, HCDR2 and/or HCDR3) contained within the heavy chain variable (VH) region sequence of SEQ ID NOs: 7, 9, 10, 47 or 11; and/or one or two or three light chain complementarity determining regions (LCDR1, LCDR2 and/or LCDR3) contained within the light chain variable (VL) region sequence of SEQ ID NOs: 8, 12 or 13.

[00162] A person skilled in the art can define or identify CDR boundaries of a VH or VL region by well-known methods in the art as long as the amino acid sequence of the VH or VL region is known. For example, CDR boundaries for an antibody or antigen-binding fragment thereof may be defined or identified by the conventions of Kabat, IMGT, Chothia, or Al-Lazikani (Al-Lazikani, B., Chothia, C., Lesk, A. M., *J. Mol. Biol.*, 273(4), 927 (1997); Chothia, C. *et al.*, *J Mol Biol.* Dec 5;186(3):651-63 (1985); Chothia, C. and Lesk, A.M., *J.Mol.Biol.*, 196,901 (1987); Chothia, C. *et al.*, *Nature*. Dec 21-28;342(6252):877-83 (1989); Kabat E.A. *et al.*, Sequences of Proteins of immunological Interest, 5th Ed. Public Health Service, National Institutes of Health, Bethesda, Md. (1991); Marie-Paule Lefranc *et al.*, *Developmental and Comparative Immunology*, 27: 55-77 (2003); Marie-Paule Lefranc *et al.*, *Immunome Research*, 1(3), (2005); Marie-Paule Lefranc, *Molecular Biology of B cells* (second edition), chapter 26, 481-514, (2015)). In some embodiments, the CDR boundaries of the antibodies or antigen-binding fragments thereof provided herein are identified by the convention of Kabat. In some embodiments, the CDR boundaries of the antibodies or antigen-binding fragments thereof provided herein are identified by the convention of IMGT. In some embodiments, the CDR boundaries of the antibodies or antigen-binding fragments thereof provided herein are identified by the convention of Chothia. In some embodiments, the CDR boundaries of the antibodies or antigen-

binding fragments thereof provided herein are identified by the convention of Al-Lazikani.

[00163] In some embodiments, the first binding moiety of the antibodies or antigen-binding fragments thereof provided herein comprises HCDR1, HCDR2 and/or HCDR3 contained within the VH region sequence of SEQ ID NO: 7. In some embodiments, the first binding moiety of the antibodies or antigen-binding fragments thereof provided herein comprises HCDR1, HCDR2 and/or HCDR3 contained within the VH region sequence of SEQ ID NO: 9. In some embodiments, the first binding moiety of the antibodies or antigen-binding fragments thereof provided herein comprises HCDR1, HCDR2 and/or HCDR3 contained within the VH region sequence of SEQ ID NO: 10. In some embodiments, the first binding moiety of the antibodies or antigen-binding fragments thereof provided herein comprises HCDR1, HCDR2 and/or HCDR3 contained within the VH region sequence of SEQ ID NO: 47. In some embodiments, the first binding moiety of the antibodies or antigen-binding fragments thereof provided herein comprises HCDR1, HCDR2 and/or HCDR3 contained within the VH region sequence of SEQ ID NO: 11.

[00164] In some embodiments, the first binding moiety of the antibodies or antigen-binding fragments thereof provided herein comprises LCDR1, LCDR2 and/or LCDR3 contained within the VL region sequence of SEQ ID NO: 8. In some embodiments, the first binding moiety of the antibodies or antigen-binding fragments thereof provided herein comprises LCDR1, LCDR2 and/or LCDR3 contained within the VL region sequence of SEQ ID NO: 12. In some embodiments, the first binding moiety of the antibodies or antigen-binding fragments thereof provided herein comprises LCDR1, LCDR2 and/or LCDR3 contained within the VL region sequence of SEQ ID NO: 13.

[00165] In some embodiments, the first binding moiety of the antibodies or antigen-binding fragments thereof provided herein comprises HCDR1, HCDR2, HCDR3, LCDR1, LCDR2 and LCDR3 contained within a VH/VL amino acid sequence pair of SEQ ID NOs: 7/8, 7/12, 7/13, 9/8, 9/12, 9/13, 10/8, 10/12, 10/13, 47/8, 47/12, 47/13,

11/8, 11/12 or 11/13. In some embodiments, the first binding moiety of the antibodies or antigen-binding fragments thereof provided herein comprises HCDR1, HCDR2, HCDR3, LCDR1, LCDR2 and LCDR3 contained within a VH/VL amino acid sequence pair of SEQ ID NOs: 7/8, 10/12 or 47/12. In some embodiments, the first binding moiety of the antibodies or antigen-binding fragments thereof provided herein comprises HCDR1, HCDR2, HCDR3, LCDR1, LCDR2 and LCDR3 contained within a VH/VL amino acid sequence pair of SEQ ID NO: 7/8. In some embodiments, the first binding moiety of the antibodies or antigen-binding fragments thereof provided herein comprises HCDR1, HCDR2, HCDR3, LCDR1, LCDR2 and LCDR3 contained within a VH/VL amino acid sequence pair of SEQ ID NO: 10/12. In some embodiments, the first binding moiety of the antibodies or antigen-binding fragments thereof provided herein comprises HCDR1, HCDR2, HCDR3, LCDR1, LCDR2 and LCDR3 contained within a VH/VL amino acid sequence pair of SEQ ID NOs: 47/12.

[00166] In some embodiments, the first binding moiety is an anti-CD3 scFv, and comprises HCDR1, HCDR2, HCDR3, LCDR1, LCDR2 and LCDR3 contained within a VH/VL amino acid sequence pair of SEQ ID NOs: 7/8, 10/12 or 47/12. In some embodiments, the first binding moiety is an anti-CD3 scFv, and comprises HCDR1, HCDR2, HCDR3, LCDR1, LCDR2 and LCDR3 contained within a VH/VL amino acid sequence pair of SEQ ID NO: 7/8. In some embodiments, the first binding moiety is an anti-CD3 scFv, and comprises HCDR1, HCDR2, HCDR3, LCDR1, LCDR2 and LCDR3 contained within a VH/VL amino acid sequence pair of SEQ ID NO: 10/12. In some embodiments, the first binding moiety is an anti-CD3 scFv, and comprises HCDR1, HCDR2, HCDR3, LCDR1, LCDR2 and LCDR3 contained within a VH/VL amino acid sequence pair of SEQ ID NOs: 47/12.

[00167] In some embodiments, the CDR boundaries of the antibodies or antigen-binding fragments thereof provided herein are identified by the convention of Kabat. In some embodiments, the CDR boundaries of the antibodies or antigen-binding fragments thereof provided herein are identified by the convention of IMGT. In

some embodiments, the CDR boundaries of the antibodies or antigen-binding fragments thereof provided herein are identified by the convention of Chothia. In some embodiments, the CDR boundaries of the antibodies or antigen-binding fragments thereof provided herein are identified by the convention of Al-Lazikani.

[00168] In some embodiments, the first binding moiety of the antibodies or antigen-binding fragments thereof provided herein comprises at least one heavy or light chain CDR comprising an amino acid sequence selected from the group consisting of SEQ ID NOs: 1, 2, 42, 3, 4, 5 and 6.

[00169] In some embodiments, the first binding moiety of the antibodies or antigen-binding fragments thereof provided herein comprises one or two or three of HCDR1, HCDR2 and HCDR3, wherein the HCDR1 comprises an amino acid sequence as set forth in SEQ ID NO: 1, the HCDR2 comprises an amino acid sequence as set forth in SEQ ID NO: 2 or SEQ ID NO: 42, and the HCDR3 comprising an amino acid sequence as set forth in SEQ ID NO: 3. In some embodiments, the first binding moiety of the antibodies or antigen-binding fragments thereof provided herein comprises a HCDR1 comprising an amino acid sequence as set forth in SEQ ID NO: 1, a HCDR2 comprising an amino acid sequence as set forth in SEQ ID NO: 2, and a HCDR3 comprising an amino acid sequence as set forth in SEQ ID NO: 3. In some embodiments, the first binding moiety of the antibodies or antigen-binding fragments thereof provided herein comprises a HCDR1 comprising an amino acid sequence as set forth in SEQ ID NO: 1, a HCDR2 comprising an amino acid sequence as set forth in SEQ ID NO: 42, and a HCDR3 comprising an amino acid sequence as set forth in SEQ ID NO: 3.

[00170] In some embodiments, the first binding moiety of the antibodies or antigen-binding fragments thereof provided herein comprises one or two or three of LCDR1, LCDR2 and LCDR3, wherein the LCDR1 comprises an amino acid sequence as set forth in SEQ ID NO: 4, the LCDR2 comprises an amino acid sequence as set forth in SEQ ID NO: 5, and the LCDR3 comprises an amino acid sequence as set forth in SEQ ID NO: 6. In some embodiments, the first binding moiety of the antibodies or

antigen-binding fragments thereof provided herein comprises a LCDR1 comprising an amino acid sequence as set forth in SEQ ID NO: 4, a LCDR2 comprising an amino acid sequence as set forth in SEQ ID NO: 5, and a LCDR3 comprising an amino acid sequence as set forth in SEQ ID NO: 6.

[00171] In some embodiments, the first binding moiety of the antibodies or antigen-binding fragments thereof provided herein comprises a HCDR1 comprising an amino acid sequence as set forth in SEQ ID NO: 1, a HCDR2 comprising an amino acid sequence as set forth in SEQ ID NO: 2, a HCDR3 comprising an amino acid sequence as set forth in SEQ ID NO: 3; a LCDR1 comprising an amino acid sequence as set forth in SEQ ID NO: 4, a LCDR2 comprising an amino acid sequence as set forth in SEQ ID NO: 5, and a LCDR3 comprising an amino acid sequence as set forth in SEQ ID NO: 6.

[00172] In some embodiments, the first binding moiety of the antibodies or antigen-binding fragments thereof provided herein comprises a HCDR1 comprising an amino acid sequence as set forth in SEQ ID NO: 1, a HCDR2 comprising an amino acid sequence as set forth in SEQ ID NO: 42, a HCDR3 comprising an amino acid sequence as set forth in SEQ ID NO: 3; a LCDR1 comprising an amino acid sequence as set forth in SEQ ID NO: 4, a LCDR2 comprising an amino acid sequence as set forth in SEQ ID NO: 5, and a LCDR3 comprising an amino acid sequence as set forth in SEQ ID NO: 6.

[00173] In certain embodiments, the first binding moiety of the antibodies or antigen-binding fragments thereof provided herein comprises a VH region comprising one or two or three of HCDR1, HCDR2 and HCDR3 comprising an amino acid sequence as set forth in SEQ ID NO: 1, SEQ ID NO: 2, and SEQ ID NO: 3, respectively. In certain embodiments, the first binding moiety of the antibodies or antigen-binding fragments thereof provided herein comprises a VH region comprising HCDR1, HCDR2 and HCDR3 comprising an amino acid sequence as set forth in SEQ ID NO: 1, SEQ ID NO: 2, and SEQ ID NO: 3, respectively.

[00174] In certain embodiments, the first binding moiety of the antibodies or antigen-binding fragments thereof provided herein comprises a VH region comprising one or two or three of HCDR1, HCDR2 and HCDR3 comprising an amino acid sequence as set forth in SEQ ID NO: 1, SEQ ID NO: 42, and SEQ ID NO: 3, respectively. In certain embodiments, the first binding moiety of the antibodies or antigen-binding fragments thereof provided herein comprises a VH region comprising HCDR1, HCDR2 and HCDR3 comprising an amino acid sequence as set forth in SEQ ID NO: 1, SEQ ID NO: 42, and SEQ ID NO: 3, respectively.

[00175] In certain embodiments, the first binding moiety of the antibodies or antigen-binding fragments thereof provided herein comprises a VL region comprising one or two or three of LCDR1, LCDR2 and LCDR3 comprising an amino acid sequence as set forth in SEQ ID NO: 4, SEQ ID NO: 5, and SEQ ID NO: 6, respectively. In certain embodiments, the first binding moiety of the antibodies or antigen-binding fragments thereof provided herein comprises a VL region comprising LCDR1, LCDR2 and LCDR3 comprising an amino acid sequence as set forth in SEQ ID NO: 4, SEQ ID NO: 5, and SEQ ID NO: 6, respectively.

[00176] In certain embodiments, the first binding moiety of the antibodies or antigen-binding fragments thereof provided herein comprises a VH region having an amino acid sequence as set forth in SEQ ID NOs: 7, 9, 10, 47 or 11 below, or a homologous sequence having at least 80% (*e.g.*, at least 85%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, and at least 99%) sequence identity to SEQ ID NOs: 7, 9, 10, 47 or 11. In certain embodiments, the first binding moiety of the antibodies or antigen-binding fragments thereof provided herein comprises a VH region having a homologous sequence that has at least 80% (*e.g.*, at least 85%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, and at least 99%) sequence identity to SEQ ID NOs: 7, 9, 10, 47 or 11, and wherein the VH region comprises HCDR1, HCDR2 and HCDR3 comprising an amino acid sequence as set forth in SEQ ID NO: 1, SEQ ID NO: 2 and SEQ ID NO: 3, respectively. In

certain embodiments, the first binding moiety of the antibodies or antigen-binding fragments thereof provided herein comprises a VH region having a homologous sequence that has at least 80% (*e.g.*, at least 85%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, and at least 99%) sequence identity to SEQ ID NOs: 7, 9, 10, 47 or 11, and wherein the VH region comprises HCDR1, HCDR2 and HCDR3 comprising an amino acid sequence as set forth in SEQ ID NO: 1, SEQ ID NO: 42 and SEQ ID NO: 3, respectively.

[00177] In certain embodiments, the first binding moiety of the antibodies or antigen-binding fragments thereof provided herein comprises a VL region having an amino acid sequence as set forth in SEQ ID NOs: 8, 12 or 13, or a homologous sequence having at least 80% (*e.g.*, at least 85%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, and at least 99%) sequence identity to SEQ ID NOs: 8, 12 or 13. In certain embodiments, the first binding moiety of the antibodies or antigen-binding fragments thereof provided herein comprises a VL region having a homologous sequence that has at least 80% (*e.g.*, at least 85%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, and at least 99%) sequence identity to SEQ ID NOs: 8, 12 or 13, and wherein the VL region comprises LCDR1, LCDR2 and LCDR3 comprising an amino acid sequence as set forth in SEQ ID NO: 4, SEQ ID NO: 5 and SEQ ID NO: 6, respectively.

[00178] In some embodiments, the first binding moiety of the antibodies or antigen-binding fragments thereof provided herein comprises a VH/VL amino acid sequence pair of SEQ ID NOs: 7/8, 7/12, 7/13, 9/8, 9/12, 9/13, 10/8, 10/12, 10/13, 47/8, 47/12, 47/13, 11/8, 11/12 or 11/13. In some embodiments, the first binding moiety of the antibodies or antigen-binding fragments thereof provided herein comprises a VH/VL amino acid sequence pair of SEQ ID NOs: 7/8, 10/12 or 47/12. In some embodiments, the first binding moiety of the antibodies or antigen-binding fragments thereof provided herein comprises a VH/VL amino acid sequence pair of SEQ ID NO:

7/8. In some embodiments, the first binding moiety of the antibodies or antigen-binding fragments thereof provided herein comprises a VH/VL amino acid sequence pair of SEQ ID NO: 10/12. In some embodiments, the first binding moiety of the antibodies or antigen-binding fragments thereof provided herein comprises a VH/VL amino acid sequence pair of SEQ ID NO: 47/12.

[00179] The SEQ ID NOs of the heavy chain (denoted as “H”) variable region, light chain (denoted as “L”) variable region, HCDRs and LCDRs of each of the exemplary first binding moieties that bind to CD3 described above are shown in Table 1 below. The amino acid sequences of each CDR of the exemplary first binding moieties that bind to CD3 are shown in Table 2 below. Unless otherwise indicated, the CDR boundaries as described in Table 2 below were defined or identified by the convention of Kabat. The amino acid sequences of each VH and VL of the exemplary first binding moieties that bind to CD3 are shown in Table 3 below.

Table 1. SEQ ID NOs of VH, VL, HCDRs and LCDRs of Exemplary First Binding Moieties that Bind to CD3

Antibody	Chain	Variable Region (SEQ ID NO)	CDR1 (SEQ ID NO)	CDR2 (SEQ ID NO)	CDR3 (SEQ ID NO)
40-C12-C10-E9	H	7	1	2	3
	L	8	4	5	6
hu40E9-L2H1	H	9	1	2	3
	L	12	4	5	6
hu40E9-L2H3	H	10	1	2	3
	L	12	4	5	6
hu40E9-L2H4	H	11	1	2	3
	L	12	4	5	6
hu40E9-L3H1	H	9	1	2	3
	L	13	4	5	6
hu40E9-L2H3-N55S.H	H	47	1	42	3
	L	12	4	5	6

Table 2. Amino Acid Sequences of CDRs of Exemplary First Binding Moieties that Bind to CD3

Sequence Description	SEQ ID NO	Amino Acid Sequence
HCDR1	1	SYVIH
HCDR2	2	YINPYNDVIKYNEKFKG
HCDR2 (N55S)	42	YINPYSDVIKYNEKFKG
HCDR3	3	DSYYSYDGFAY
LCDR1	4	SASSSVSYMH
LCDR2	5	DTSKLAS
LCDR3	6	QQWSSNPPT

Table 3. Amino Acid Sequences of Each VH and VL of Exemplary First Binding Moieties that Bind to CD3

Antibody	VH (SEQ ID NO)	VL (SEQ ID NO)
40-C12-C10-E9	EVQLQLSGPELVKPGASVKMSC KASGYTFSSYVIHWVKQKPGQG LEWIGYINPYNDVIKYNEKFKGK ATLTSDKSSSTAYMELSSLTSEDS AVYYCARDSDYYSYDGFAYWGQ GTLVTVSA (SEQ ID NO: 7)	QIVLTQSPAIMASAPGEKVTMT CSASSSVSYMHWYQQKSGTSP KRWIYDTSKLASGVPARFSGSG SGTSYSLTISSMEAEDAATYYC QQWSSNPPTFGGGTKVEIK (SEQ ID NO: 8)
hu40E9-L2H1	EVQLVQSGAEVKKPGSSVKVSC KASGGTFSSYVIHWVRQAPGQG LEWMGYINPYNDVIKYNEKFKG	EIVLTQSPDFQSVTPKEKVTITC SASSSVSYMHWYQQKPDQSPK RLIYDTSKLASGVPSRFSGSGSG

Antibody	VH (SEQ ID NO)	VL (SEQ ID NO)
	RVTITADKSTSTAYMELSSLRSED TAVYYCARDSSYYSYDGFAYWGQ GTLVTVSS (SEQ ID NO: 9)	TDFTLTINSLEAEDAATYYCQQ WSSNPPTFGGGTKVEIK (SEQ ID NO: 12)
hu40E9-L2H3	EVQLVQSGAEVKKPGSSVKVSC KASGYTFSSYVIHWVRQAPGQG LEWMGYINPYNDVIKYNEKFKG RVTLTSDKSTSTAYMELSSLRSE DTAVYYCARDSSYYSYDGFAYWG QGTLVTVSS (SEQ ID NO: 10)	EIVLTQSPDFQSVTPKEKVTITC SASSSVSYMHWYQQKPDQSPK RLIYDTSKLASGVPSRFSGSGSG TDFTLTINSLEAEDAATYYCQQ WSSNPPTFGGGTKVEIK (SEQ ID NO: 12)
hu40E9-L2H4	EVQLVQSGAEVKKPGSSVKVSC KASGYTFSSYVIHWVRQAPGQG LEWIGYINPYNDVIKYNEKFKGR ATLTSDKSTSTAYMELSSLRSED AVYYCARDSSYYSYDGFAYWGQ GTLVTVSS (SEQ ID NO: 11)	EIVLTQSPDFQSVTPKEKVTITC SASSSVSYMHWYQQKPDQSPK RLIYDTSKLASGVPSRFSGSGSG TDFTLTINSLEAEDAATYYCQQ WSSNPPTFGGGTKVEIK (SEQ ID NO: 12)
hu40E9-L3H1	EVQLVQSGAEVKKPGSSVKVSC KASGGTFSSYVIHWVRQAPGQG LEWMGYINPYNDVIKYNEKFKG RVTITADKSTSTAYMELSSLRSED TAVYYCARDSSYYSYDGFAYWGQ GTLVTVSS	EIVLTQSPDFQSVTPKEKVTITC SASSSVSYMHWYQQKPDQSPK RWIYDTSKLASGVPSRFSGSGS GTDYTLTINSLEAEDAATYYCQ QWSSNPPTFGGGTKVEIK (SEQ ID NO: 13)

Antibody	VH (SEQ ID NO)	VL (SEQ ID NO)
	(SEQ ID NO: 9)	
hu40E9- L2H3- N55S.H	EVQLVQSGAEVKKPGSSVKVSC KASGYTFSSYVIHWVRQAPGQG LEWMGYINPYSDVIKYNEKFKG RVTLTSDKSTSTAYMELSSLRSE DTAVYYCARDSEYSSYDGFAYWG QGTLVTVSS (SEQ ID NO: 47)	EIVLTQSPDFQSVTPKEKVTITC SASSSVSYMHWYQQKPDQSPK RLIYDTSKLASGVPSRFSGSGSG TDFTLTINSLEAEDAATYYCQQ WSSNPPTFGGGTKVEIK (SEQ ID NO: 12)

[00180] Given that each of the exemplary first binding moieties can bind to CD3 and that antigen-binding specificity is provided primarily by the CDR1, CDR2 and CDR3 regions, the HCDR1, HCDR2 and HCDR3 sequences and LCDR1, LCDR2 and LCDR3 sequences of each of the exemplary first binding moieties can be “mixed and matched” (*i.e.*, CDRs from different antibodies can be mixed and matched, but each antibody must contain a HCDR1, HCDR2 and HCDR3 and a LCDR1, LCDR2 and LCDR3) to create the first binding moiety of the present disclosure. CD3 binding of such “mixed and matched” binding moieties can be tested using the binding assays described above and in the Examples. Preferably, when VH CDR sequences are mixed and matched, the HCDR1, HCDR2 and/or HCDR3 sequence from a particular VH sequence is replaced with a structurally similar CDR sequence(s). Likewise, when VL CDR sequences are mixed and matched, the LCDR1, LCDR2 and/or LCDR3 sequence from a particular VL sequence preferably is replaced with a structurally similar CDR sequence(s). It will be readily apparent to a person skilled in the art that novel VH and VL sequences can be created by substituting one or more

VH and/or VL CDR sequences with structurally similar sequences from the CDR sequences disclosed herein for the exemplary first binding moieties.

[00181] CDRs are known to be responsible for antigen binding. However, it has been found that not all of the 6 CDRs are indispensable or unchangeable. In other words, it is possible to replace or change or modify one or more CDRs in each of the exemplary first binding moieties, yet substantially retain the specific binding affinity to CD3.

[00182] In certain embodiments, the first binding moieties provided herein comprise a heavy chain CDR3 sequence described herein. In certain embodiments, the first binding moieties provided herein comprise a heavy chain CDR3 sequence as set forth in SEQ ID NO: 6. Heavy chain CDR3 region is located at the center of the antigen-binding site, and therefore is believed to make the most contact with antigen and provide the most free energy to the affinity of antibody to antigen. It is also believed that the heavy chain CDR3 is by far the most diverse CDR of the antigen-binding site in terms of length, amino acid composition and conformation by multiple diversification mechanisms (Tonegawa S. *Nature*. 302:575-81). The diversity in the heavy chain CDR3 is sufficient to produce most antibody specificities (Xu JL, Davis MM. *Immunity*. 13:37-45) as well as desirable antigen-binding affinity (Schier R, *et al.*, *J Mol Biol*. 263:551-67).

[00183] In certain embodiments, the first binding moieties of the antibodies or antigen-binding fragments thereof provided herein comprise suitable framework region (FR) sequences, as long as the antibodies and antigen-binding fragments thereof can bind to CD3. The CDR sequences provided in Table 2 and Table 5 above are obtained from chimeric, humanized or human antibodies, but they can be grafted to any suitable FR sequences of any suitable species such as mouse, human, rat, rabbit, among others, using suitable methods known in the art such as recombinant techniques.

[00184] In certain embodiments, the first binding moieties of the antibodies or antigen-binding fragments thereof provided herein are humanized. A humanized first binding moiety is desirable in its reduced immunogenicity in human. A humanized antibody is chimeric in its variable regions, as non-human CDR sequences are grafted to human or substantially human FR sequences. Humanization of an antibody or antigen-binding fragment can be essentially performed by substituting the non-human (such as murine) CDR genes for the corresponding human CDR genes in a human immunoglobulin gene (see, for example, Jones *et al.*, (1986) *Nature* 321:522-525; Riechmann *et al.*, (1988) *Nature* 332:323-327; Verhoeven *et al.*, (1988) *Science* 239:1534-1536).

[00185] Suitable human heavy chain and light chain variable domains can be selected to achieve this purpose using methods known in the art. In an illustrative example, “best-fit” approach can be used, where a non-human (*e.g.*, rodent) antibody variable domain sequence is screened or BLASTed against a database of known human variable domain sequences, and the human sequence closest to the non-human query sequence is identified and used as the human scaffold for grafting the non-human CDR sequences (see, for example, Sims *et al.*, (1993) *J. Immunol.* 151:2296; Chothia *et al.*, (1987) *J. Mol. Biol.* 196:901). Alternatively, a framework derived from the consensus sequence of all human antibodies may be used for the grafting of the non-human CDRs (see, for example, Carter *et al.*, (1992) *Proc. Natl. Acad. Sci. USA*, 89:4285; Presta *et al.*, (1993) *J. Immunol.*, 151:2623).

Second binding moiety

[00186] In certain embodiments, the first binding moiety that binds to CD3 provided herein is further linked to a second binding moiety that binds to LILRB4.

[00187] As used herein, the term “second binding moiety” with regard to an antibody or antigen-binding fragment thereof provided herein, refers to a moiety that is capable of binding to LILRB4 (*e.g.*, human LILRB4, mouse LILRB4, and cynomolgus LILRB4). The second binding moiety can take any form that allows specific

recognition of the target LILRB4. For example, the second binding moiety may be an antibody or an antigen-binding fragment thereof, *e.g.*, an IgG (such as IgG1, IgG2, IgG3, and IgG4) antibody, IgA antibody, or IgM antibody. In some embodiments, the second binding moiety is a Fab domain. In some embodiments, the second binding moiety is an anti-LILRB4 Fab domain. In some embodiments, the second binding moiety is a single-chain Fv (scFv). In some embodiments, the second binding moiety is an anti-LILRB4 scFv. In some embodiments, the first binding moiety is an anti-CD3 scFv, and the second binding moiety is an anti-LILRB4 Fab domain. In some embodiments, the first binding moiety is an anti-CD3 Fab domain, and the second binding moiety is an anti-LILRB4 scFv.

[00188] In certain embodiments, the present disclosure provides antibodies or antigen-binding fragments thereof, wherein the second binding moiety binding to LILRB4 comprises one or more (*e.g.*, 1, 2, 3, 4, 5, or 6) CDR sequences of an anti-LILRB4 antibody 2-H1-D7-E5-D5, h2-H1-D7-E5-D5, 8-B3-F6-H8, or h8-B3-F6-H8.

[00189] Antibody “2-H1-D7-E5-D5” as used herein refers to a mouse monoclonal antibody or an antigen-binding fragment thereof comprising a heavy chain variable region having the sequence of SEQ ID NO: 20, and a light chain variable region having the sequence of SEQ ID NO: 21.

[00190] Antibody “h2-H1-D7-E5-D5” as used herein refers to a humanized antibody or an antigen-binding fragment thereof of antibody 2-H1-D7-E5-D5, comprising a heavy chain variable region having the sequence of SEQ ID NO: 22, and a light chain variable region having the sequence of SEQ ID NO: 23.

[00191] Antibody “8-B3-F6-H8” as used herein refers to a mouse monoclonal antibody or an antigen-binding fragment thereof comprising a heavy chain variable region having the sequence of SEQ ID NO: 30, and a light chain variable region having the sequence of SEQ ID NO: 31.

[00192] Antibody “h8-B3-F6-H8” as used herein refers to a humanized antibody or an antigen-binding fragment thereof of antibody 8-B3-F6-H8, comprising a heavy

chain variable region having the sequence of SEQ ID NO: 33, and a light chain variable region having the sequence of SEQ ID NO: 34.

[00193] In certain embodiments, the second binding moiety of the antibodies or antigen-binding fragments thereof provided herein comprises one or two or three heavy chain complementarity determining regions (HCDR1, HCDR2 and/or HCDR3) contained within the heavy chain variable (VH) region sequence of SEQ ID NOs: 20, 22, 30, or 33; and/or one or two or three light chain complementarity determining regions (LCDR1, LCDR2 and/or LCDR3) contained within the light chain variable (VL) region sequence of SEQ ID NOs: 21, 23, 31 or 34.

[00194] In some embodiments, the second binding moiety of the antibodies or antigen-binding fragments thereof provided herein comprises HCDR1, HCDR2 and/or HCDR3 contained within the VH region sequence of SEQ ID NO: 20. In some embodiments, the second binding moiety of the antibodies or antigen-binding fragments thereof provided herein comprises HCDR1, HCDR2 and/or HCDR3 contained within the VH region sequence of SEQ ID NO: 22. In some embodiments, the second binding moiety of the antibodies or antigen-binding fragments thereof provided herein comprises HCDR1, HCDR2 and/or HCDR3 contained within the VH region sequence of SEQ ID NO: 30. In some embodiments, the second binding moiety of the antibodies or antigen-binding fragments thereof provided herein comprises HCDR1, HCDR2 and/or HCDR3 contained within the VH region sequence of SEQ ID NO: 33.

[00195] In some embodiments, the second binding moiety of the antibodies or antigen-binding fragments thereof provided herein comprises LCDR1, LCDR2 and/or LCDR3 contained within the VL region sequence of SEQ ID NO: 21. In some embodiments, the second binding moiety of the antibodies or antigen-binding fragments thereof provided herein comprises LCDR1, LCDR2 and/or LCDR3 contained within the VL region sequence of SEQ ID NO: 23. In some embodiments, the second binding moiety of the antibodies or antigen-binding fragments thereof provided herein comprises LCDR1, LCDR2 and/or LCDR3 contained within the VL

region sequence of SEQ ID NO: 31. In some embodiments, the second binding moiety of the antibodies or antigen-binding fragments thereof provided herein comprises LCDR1, LCDR2 and/or LCDR3 contained within the VL region sequence of SEQ ID NO: 34.

[00196] In some embodiments, the second binding moiety of the antibodies or antigen-binding fragments thereof provided herein comprises HCDR1, HCDR2, HCDR3, LCDR1, LCDR2 and LCDR3 contained within a VH/VL amino acid sequence pair of SEQ ID NOs: 20/21, 22/23, 30/31, or 33/34. In some embodiments, the second binding moiety of the antibodies or antigen-binding fragments thereof provided herein comprises HCDR1, HCDR2, HCDR3, LCDR1, LCDR2 and LCDR3 contained within a VH/VL amino acid sequence pair of SEQ ID NO: 20/21. In some embodiments, the second binding moiety of the antibodies or antigen-binding fragments thereof provided herein comprises HCDR1, HCDR2, HCDR3, LCDR1, LCDR2 and LCDR3 contained within a VH/VL amino acid sequence pair of SEQ ID NO: 22/23. In some embodiments, the second binding moiety of the antibodies or antigen-binding fragments thereof provided herein comprises HCDR1, HCDR2, HCDR3, LCDR1, LCDR2 and LCDR3 contained within a VH/VL amino acid sequence pair of SEQ ID NO: 30/31. In some embodiments, the second binding moiety of the antibodies or antigen-binding fragments thereof provided herein comprises HCDR1, HCDR2, HCDR3, LCDR1, LCDR2 and LCDR3 contained within a VH/VL amino acid sequence pair of SEQ ID NO: 33/34.

[00197] In some embodiments, the second binding moiety of the antibodies or antigen-binding fragments thereof provided herein is an anti-LILRB4 Fab domain, and comprises HCDR1, HCDR2, HCDR3, LCDR1, LCDR2 and LCDR3 contained within a VH/VL amino acid sequence pair of SEQ ID NO: 20/21. In some embodiments, the second binding moiety of the antibodies or antigen-binding fragments thereof provided herein is an anti-LILRB4 Fab domain, and comprises HCDR1, HCDR2, HCDR3, LCDR1, LCDR2 and LCDR3 contained within a VH/VL amino acid sequence pair of SEQ ID NO: 22/23. In some embodiments, the second

binding moiety of the antibodies or antigen-binding fragments thereof provided herein is an anti-LILRB4 Fab domain, and comprises HCDR1, HCDR2, HCDR3, LCDR1, LCDR2 and LCDR3 contained within a VH/VL amino acid sequence pair of SEQ ID NO: 30/31. In some embodiments, the second binding moiety of the antibodies or antigen-binding fragments thereof provided herein is an anti-LILRB4 Fab domain, and comprises HCDR1, HCDR2, HCDR3, LCDR1, LCDR2 and LCDR3 contained within a VH/VL amino acid sequence pair of SEQ ID NO: 33/34.

[00198] In some embodiments, the second binding moiety of the antibodies or antigen-binding fragments thereof provided herein is an anti-LILRB4 scFv, and comprises HCDR1, HCDR2, HCDR3, LCDR1, LCDR2 and LCDR3 contained within a VH/VL amino acid sequence pair of SEQ ID NO: 20/21. In some embodiments, the second binding moiety of the antibodies or antigen-binding fragments thereof provided herein is an anti-LILRB4 scFv, and comprises HCDR1, HCDR2, HCDR3, LCDR1, LCDR2 and LCDR3 contained within a VH/VL amino acid sequence pair of SEQ ID NO: 22/23. In some embodiments, the second binding moiety of the antibodies or antigen-binding fragments thereof provided herein is an anti-LILRB4 scFv, and comprises HCDR1, HCDR2, HCDR3, LCDR1, LCDR2 and LCDR3 contained within a VH/VL amino acid sequence pair of SEQ ID NO: 30/31. In some embodiments, the second binding moiety of the antibodies or antigen-binding fragments thereof provided herein is an anti-LILRB4 scFv, and comprises HCDR1, HCDR2, HCDR3, LCDR1, LCDR2 and LCDR3 contained within a VH/VL amino acid sequence pair of SEQ ID NO: 33/34.

[00199] In some embodiments, the CDR boundaries of the second binding moiety are identified by the convention of Kabat. In some embodiments, the CDR boundaries of the second binding moiety are identified by the convention of IMGT. In some embodiments, the CDR boundaries of the second binding moiety are identified by the convention of Chothia. In some embodiments, the CDR boundaries of the second binding moiety are identified by the convention of Al-Lazikani.

[00200] In some embodiments, the second binding moiety of the antibodies or antigen-binding fragments thereof provided herein comprises at least one heavy or light chain complementarity determining region (CDR) comprising an amino acid sequence selected from the group consisting of SEQ ID NOs: 14, 15, 16, 17, 18, 19, 24, 25, 26, 27, 28, 29 and 32.

[00201] In some embodiments, the second binding moiety of the antibodies or antigen-binding fragments thereof provided herein comprises one or two or three of HCDR1, HCDR2 and HCDR3 comprising an amino acid sequence selected from the group consisting of SEQ ID NOs: 14, 15, 16, 24, 25, 26 and 32.

[00202] In some embodiments, the second binding moiety of the antibodies or antigen-binding fragments thereof provided herein comprises one or two or three of LCDR1, LCDR2 and LCDR3 comprising an amino acid sequence selected from the group consisting of SEQ ID NOs: 17, 18, 19, 27, 28 and 29.

[00203] In some embodiments, the second binding moiety of the antibodies or antigen-binding fragments thereof provided herein comprises:

- i. a HCDR1 comprising an amino acid sequence selected from the group consisting of SEQ ID NOs: 14 and 24,
- ii. a HCDR2 comprising an amino acid sequence selected from the group consisting of SEQ ID NOs: 15, 25, and 32, and
- iii. a HCDR3 comprising an amino acid sequence selected from the group consisting of SEQ ID NOs: 16 and 26.

[00204] In some embodiments, the second binding moiety of the antibodies or antigen-binding fragments thereof provided herein comprises:

- i. a LCDR1 comprising an amino acid sequence selected from the group consisting of SEQ ID NOs: 17 and 27,
- ii. a LCDR2 comprising an amino acid sequence selected from the group consisting of SEQ ID NOs: 18 and 28, and

- iii. a LCDR3 comprising an amino acid sequence selected from the group consisting of SEQ ID NOs: 19 and 29.

[00205] In some embodiments, the second binding moiety of the antibodies or antigen-binding fragments thereof provided herein comprises a HCDR1 comprising an amino acid sequence as set forth in SEQ ID NO: 14, a HCDR2 comprising an amino acid sequence as set forth in SEQ ID NO: 15, and a HCDR3 comprising an amino acid sequence as set forth in SEQ ID NO: 16.

[00206] In some embodiments, the second binding moiety of the antibodies or antigen-binding fragments thereof provided herein comprises a HCDR1 comprising an amino acid sequence as set forth in SEQ ID NO: 24, a HCDR2 comprising an amino acid sequence as set forth in SEQ ID NO: 25, and a HCDR3 comprising an amino acid sequence as set forth in SEQ ID NO: 26.

[00207] In some embodiments, the second binding moiety of the antibodies or antigen-binding fragments thereof provided herein comprises a HCDR1 comprising an amino acid sequence as set forth in SEQ ID NO: 24, a HCDR2 comprising an amino acid sequence as set forth in SEQ ID NO: 32, and a HCDR3 comprising an amino acid sequence as set forth in SEQ ID NO: 26.

[00208] In some embodiments, the second binding moiety of the antibodies or antigen-binding fragments thereof provided herein comprises a LCDR1 comprising an amino acid sequence as set forth in SEQ ID NO: 17, a LCDR2 comprising an amino acid sequence as set forth in SEQ ID NO: 18, and a LCDR3 comprising an amino acid sequence as set forth in SEQ ID NO: 19.

[00209] In some embodiments, the second binding moiety of the antibodies or antigen-binding fragments thereof provided herein comprises a LCDR1 comprising an amino acid sequence as set forth in SEQ ID NO: 27, a LCDR2 comprising an amino acid sequence as set forth in SEQ ID NO: 28, and a LCDR3 comprising an amino acid sequence as set forth in SEQ ID NO: 29.

[00210] In some embodiments, the second binding moiety of the antibodies or antigen-binding fragments thereof provided herein comprises: a HCDR1 comprising an amino acid sequence as set forth in SEQ ID NO: 14, a HCDR2 comprising an amino acid sequence as set forth in SEQ ID NO: 15, a HCDR3 comprising an amino acid sequence as set forth in SEQ ID NO: 16, a LCDR1 comprising an amino acid sequence as set forth in SEQ ID NO: 17, a LCDR2 comprising an amino acid sequence as set forth in SEQ ID NO: 18, and a LCDR3 comprising an amino acid sequence as set forth in SEQ ID NO: 19.

[00211] In some embodiments, the second binding moiety of the antibodies or antigen-binding fragments thereof provided herein comprises: a HCDR1 comprising an amino acid sequence as set forth in SEQ ID NO: 24, a HCDR2 comprising an amino acid sequence as set forth in SEQ ID NO: 25, a HCDR3 comprising an amino acid sequence as set forth in SEQ ID NO: 26, a LCDR1 comprising an amino acid sequence as set forth in SEQ ID NO: 27, a LCDR2 comprising an amino acid sequence as set forth in SEQ ID NO: 28, and a LCDR3 comprising an amino acid sequence as set forth in SEQ ID NO: 29.

[00212] In some embodiments, the second binding moiety of the antibodies or antigen-binding fragments thereof provided herein comprises: a HCDR1 comprising an amino acid sequence as set forth in SEQ ID NO: 24, a HCDR2 comprising an amino acid sequence as set forth in SEQ ID NO: 32, a HCDR3 comprising an amino acid sequence as set forth in SEQ ID NO: 26, a LCDR1 comprising an amino acid sequence as set forth in SEQ ID NO: 27, a LCDR2 comprising an amino acid sequence as set forth in SEQ ID NO: 28, and a LCDR3 comprising an amino acid sequence as set forth in SEQ ID NO: 29.

[00213] In certain embodiments, the second binding moiety of the antibodies or antigen-binding fragments thereof provided herein comprises a VH region comprising one or two or three of HCDR1, HCDR2 and HCDR3 comprising an amino acid sequence as set forth in SEQ ID NO: 14, SEQ ID NO: 15, and SEQ ID NO: 16, respectively. In certain embodiments, the second binding moiety of the antibodies or

antigen-binding fragments thereof provided herein comprises a VH region comprising HCDR1, HCDR2 and HCDR3 comprising an amino acid sequence as set forth in SEQ ID NO: 14, SEQ ID NO: 15, and SEQ ID NO: 16, respectively.

[00214] In certain embodiments, the second binding moiety of the antibodies or antigen-binding fragments thereof provided herein comprises a VH region comprising one or two or three of HCDR1, HCDR2 and HCDR3 comprising an amino acid sequence as set forth in SEQ ID NO: 24, SEQ ID NO: 25, and SEQ ID NO: 26, respectively. In certain embodiments, the second binding moiety of the antibodies or antigen-binding fragments thereof provided herein comprises a VH region comprising HCDR1, HCDR2 and HCDR3 comprising an amino acid sequence as set forth in SEQ ID NO: 24, SEQ ID NO: 25, and SEQ ID NO: 26, respectively.

[00215] In certain embodiments, the second binding moiety of the antibodies or antigen-binding fragments thereof provided herein comprises a VH region comprising one or two or three of HCDR1, HCDR2 and HCDR3 comprising an amino acid sequence as set forth in SEQ ID NO: 24, SEQ ID NO: 32, and SEQ ID NO: 26, respectively. In certain embodiments, the second binding moiety of the antibodies or antigen-binding fragments thereof provided herein comprises a VH region comprising HCDR1, HCDR2 and HCDR3 comprising an amino acid sequence as set forth in SEQ ID NO: 24, SEQ ID NO: 32, and SEQ ID NO: 26, respectively.

[00216] In certain embodiments, the second binding moiety of the antibodies or antigen-binding fragments thereof provided herein comprises a VL region comprising one or two or three of LCDR1, LCDR2 and LCDR3 comprising an amino acid sequence as set forth in SEQ ID NO: 17, SEQ ID NO: 18, and SEQ ID NO: 19, respectively. In certain embodiments, the second binding moiety of the antibodies or antigen-binding fragments thereof provided herein comprises a VL region comprising LCDR1, LCDR2 and LCDR3 comprising an amino acid sequence as set forth in SEQ ID NO: 17, SEQ ID NO: 18, and SEQ ID NO: 19, respectively.

[00217] In certain embodiments, the second binding moiety of the antibodies or antigen-binding fragments thereof provided herein comprises a VL region comprising one or two or three of LCDR1, LCDR2 and LCDR3 comprising an amino acid sequence as set forth in SEQ ID NO: 27, SEQ ID NO: 28, and SEQ ID NO: 29, respectively. In certain embodiments, the second binding moiety of the antibodies or antigen-binding fragments thereof provided herein comprises a VL region comprising LCDR1, LCDR2 and LCDR3 comprising an amino acid sequence as set forth in SEQ ID NO: 27, SEQ ID NO: 28, and SEQ ID NO: 29, respectively.

[00218] In certain embodiments, the second binding moiety of the antibodies or antigen-binding fragments thereof provided herein comprises a VH region having an amino acid sequence as set forth in SEQ ID NOs: 20, 22, 30 or 33 below, or a homologous sequence having at least 80% (*e.g.*, at least 85%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, and at least 99%) sequence identity to SEQ ID NOs: 20, 22, 30 or 33.

[00219] In certain embodiments, the second binding moiety of the antibodies or antigen-binding fragments thereof provided herein comprises a VH region having a homologous sequence that has at least 80% (*e.g.*, at least 85%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, and at least 99%) sequence identity to SEQ ID NOs: 20, 22, 30 or 33, and wherein the VH region comprises HCDR1, HCDR2 and HCDR3 comprising an amino acid sequence as set forth in SEQ ID NO: 14, SEQ ID NO: 15 and SEQ ID NO: 16, respectively.

[00220] In certain embodiments, the second binding moiety of the antibodies or antigen-binding fragments thereof provided herein comprises a VH region having a homologous sequence that has at least 80% (*e.g.*, at least 85%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, and at least 99%) sequence identity to SEQ ID NOs: 20, 22, 30 or 33, and wherein the VH region comprises HCDR1, HCDR2 and HCDR3 comprising an

amino acid sequence as set forth in SEQ ID NO: 24, SEQ ID NO: 25 and SEQ ID NO: 26, respectively.

[00221] In certain embodiments, the second binding moiety of the antibodies or antigen-binding fragments thereof provided herein comprises a VH region having a homologous sequence that has at least 80% (*e.g.*, at least 85%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, and at least 99%) sequence identity to SEQ ID NOs: 20, 22, 30 or 33, and wherein the VH region comprises HCDR1, HCDR2 and HCDR3 comprising an amino acid sequence as set forth in SEQ ID NO: 24, SEQ ID NO: 32 and SEQ ID NO: 26, respectively.

[00222] In certain embodiments, the second binding moiety of the antibodies or antigen-binding fragments thereof provided herein comprises a VL region having an amino acid sequence as set forth in SEQ ID NOs: 21, 23, 31 or 34, or a homologous sequence having at least 80% (*e.g.*, at least 85%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, and at least 99%) sequence identity to SEQ ID NOs: 21, 23, 31 or 34.

[00223] In certain embodiments, the second binding moiety of the antibodies or antigen-binding fragments thereof provided herein comprises a VL region having a homologous sequence that has at least 80% (*e.g.*, at least 85%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, and at least 99%) sequence identity to SEQ ID NOs: 21, 23, 31 or 34, and wherein the VL region comprises LCDR1, LCDR2 and LCDR3 comprising an amino acid sequence as set forth in SEQ ID NO: 17, SEQ ID NO: 18 and SEQ ID NO: 19, respectively.

[00224] In certain embodiments, the second binding moiety of the antibodies or antigen-binding fragments thereof provided herein comprises a VL region having a homologous sequence that has at least 80% (*e.g.*, at least 85%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%,

at least 98%, and at least 99%) sequence identity to SEQ ID NOs: 21, 23, 31 or 34, and wherein the VL region comprises LCDR1, LCDR2 and LCDR3 comprising an amino acid sequence as set forth in SEQ ID NO: 27, SEQ ID NO: 28 and SEQ ID NO: 29, respectively.

[00225] In some embodiments, the second binding moiety of the antibodies or antigen-binding fragments thereof provided herein comprises a VH/VL amino acid sequence pair of SEQ ID NOs: 20/21, 22/23, 30/31, or 33/34. In some embodiments, the second binding moiety of the antibodies or antigen-binding fragments thereof provided herein comprises a VH/VL amino acid sequence pair of SEQ ID NO: 20/21. In some embodiments, the second binding moiety of the antibodies or antigen-binding fragments thereof provided herein comprises a VH/VL amino acid sequence pair of SEQ ID NO: 22/23. In some embodiments, the second binding moiety of the antibodies or antigen-binding fragments thereof provided herein comprises a VH/VL amino acid sequence pair of SEQ ID NO: 30/31. In some embodiments, the second binding moiety of the antibodies or antigen-binding fragments thereof provided herein comprises a VH/VL amino acid sequence pair of SEQ ID NO: 33/34.

[00226] The SEQ ID NOs of the heavy chain (denoted as “H”) variable region, light chain (denoted as “L”) variable region, HCDRs and LCDRs of each of the exemplary second binding moieties that bind to LILRB4 described above are shown in Table 4 below. The amino acid sequences of each CDR of the exemplary second binding moieties that bind to LILRB4 are shown in Table 5 below. Unless otherwise indicated, the CDR boundaries as described in Table 5 below were defined or identified by the convention of IMGT. The amino acid sequences of each VH and VL of the exemplary second binding moieties that bind to LILRB4 are shown in Table 6 below.

Table 4. SEQ ID NOs of VH, VL, HCDRs and LCDRs of Exemplary Second Binding Moieties that Bind to LILRB4

Antibody	Chain	Variable Region (SEQ ID NO)	CDR1 (SEQ ID NO)	CDR2 (SEQ ID NO)	CDR3 (SEQ ID NO)
2-H1-D7-E5-D5	H	20	14	15	16
	L	21	17	18	19
h2-H1-D7-E5-D5	H	22	14	15	16
	L	23	17	18	19
8-B3-F6-H8	H	30	24	25	26
	L	31	27	28	29
h8-B3-F6-H8	H	33	24	32	26
	L	34	27	28	29

Table 5. Amino Acid Sequences of CDRs of Exemplary Second Binding Moieties that Bind to LILRB4

Antibody	Sequence Description	SEQ ID NO	Amino Acid Sequence
2-H1-D7-E5-D5 or h2-H1-D7-E5-D5	HCDR1	14	GFTFSSFG
	HCDR2	15	ISSGSSSI
	HCDR3	16	ARGTGTGAMDY
	LCDR1	17	QDISNY
	LCDR2	18	YTS
	LCDR3	19	QQGNTLPWT
8-B3-F6-H8 or h8-B3-F6-H8	HCDR1	24	GFDFSRYW
	HCDR2	25	INPDSSAI
	Humanized-HCDR2	32	INPESSAI
	HCDR3	26	ATSMTMDY
	LCDR1	27	QDIKSY
	LCDR2	28	YAT
	LCDR3	29	LQHGESPYT

Table 6. Amino Acid Sequences of Each VH and VL of Exemplary Second Binding Moieties that Bind to LILRB4

Antibody	VH (SEQ ID NO)	VL (SEQ ID NO)
2-H1-D7- E5-D5	EVHLVESGGGLVQPGGSRKLSC AASGFTFSSFGMHWRQPPEKG LEWVAYISSGSSSIYYADTVKGR FTISRDNPKNTLFLQMTSLRSED TAMYYCARGTGTGAMDYWGQ GTSVTVSS (SEQ ID NO: 20)	DIQMTQTTSSLSASLGDRVTISC RTSQDISNYLNWYQQKPDGTV KLLIYYTSKLHSGVPSRFSGSGS GTDYSLTISNLDQEDIATYFCQ QGNTLPWTFGGGKLEIK (SEQ ID NO: 21)
h2-H1-D7- E5-D5	EVQLVESGGGLVQPGGSLRLSC AASGFTFSSFGMHWRQAPGKG LEWVAYISSGSSSIYYADTVKGR FTISRDNKNSLYLQMNSLRAE DTAVYYCARGTGTGAMDYWG QGTLVTVSS (SEQ ID NO: 22)	DIQMTQSPSSLSASVGDRVTITC RTSQDISNYLNWYQQKPGKAP KLLIYYTSKLHSGVPSRFSGSGS GTDFTFTISLQPEDATYFCQQ GNTLPWTFGGGKLEIK (SEQ ID NO: 23)
8-B3-F6-H8	EVKVIESGGGLVQPGGSLKLSCA ASGFDFSRYWMSWRQAPGKG LEWIGEINPDSSAINYTPSLKDKF IISRDNKNTLYLQMSKVRSED ALYYCATSMTMDYWGQTSVT VSS (SEQ ID NO: 30)	DIVMTQSPSSMYASLGERVTIT CKASQDIKSYLSWYQQKPWKS PKTLIYYATSLADGVPSRFSGSG SGQDYSLTISLESDDTATYYCL QHGESPYTFGGGKLEK (SEQ ID NO: 31)
h8-B3-F6- H8	EVQLVESGGGLVQPGGSLRLSCA ASGFDFSRYWMSWRQAPGKG LVWIGEINPESSAINYAPSLKDRF	DIQMTQSPSSLSASVGDRVTITC KASQDIKSYLSWYQQKPGKAP KTLIYYATSLAGGVPSRFSGSGS

Antibody	VH (SEQ ID NO)	VL (SEQ ID NO)
	TISRDNAKNTLYLQMNSLRAED TAVYYCATSMTMDYWGQGTLV TVSS (SEQ ID NO: 33)	GTDYTLTISSLQPEDFATYYCLQ HGESP YTFGGG TKLEIK (SEQ ID NO: 34)

[00227] In certain embodiments, an exemplary multi-specific antibody or antigen-binding fragment thereof provided herein comprises:

- (1) a first binding moiety comprising HCDR1, HCDR2, and HCDR3 contained within VH region sequence as set forth in SEQ ID NO: 7, and LCDR1, LCDR2, and LCDR3 contained within VL region sequence as set forth in SEQ ID NO: 8; and a second binding moiety comprising HCDR1, HCDR2, and HCDR3 contained within VH region sequence as set forth in SEQ ID NO: 20, and LCDR1, LCDR2, and LCDR3 contained within VL region sequence as set forth in SEQ ID NO: 21;
- (2) a first binding moiety comprising HCDR1, HCDR2, and HCDR3 contained within VH region sequence as set forth in SEQ ID NO: 7, and LCDR1, LCDR2, and LCDR3 contained within VL region sequence as set forth in SEQ ID NO: 8; and a second binding moiety comprising HCDR1, HCDR2, and HCDR3 contained within VH region sequence as set forth in SEQ ID NO: 22, and LCDR1, LCDR2, and LCDR3 contained within VL region sequence as set forth in SEQ ID NO: 23;
- (3) a first binding moiety comprising HCDR1, HCDR2, and HCDR3 contained within VH region sequence as set forth in SEQ ID NO: 7, and LCDR1, LCDR2, and LCDR3 contained within VL region sequence as set forth in SEQ ID NO: 8; and a second binding moiety comprising HCDR1, HCDR2, and HCDR3 contained within VH region sequence as set forth in SEQ ID NO: 30, and

LCDR1, LCDR2, and LCDR3 contained within VL region sequence as set forth in SEQ ID NO: 31;

- (4) a first binding moiety comprising HCDR1, HCDR2, and HCDR3 contained within VH region sequence as set forth in SEQ ID NO: 7, and LCDR1, LCDR2, and LCDR3 contained within VL region sequence as set forth in SEQ ID NO: 8; and a second binding moiety comprising HCDR1, HCDR2, and HCDR3 contained within VH region sequence as set forth in SEQ ID NO: 33, and LCDR1, LCDR2, and LCDR3 contained within VL region sequence as set forth in SEQ ID NO: 34;
- (5) a first binding moiety comprising HCDR1, HCDR2, and HCDR3 contained within VH region sequence as set forth in SEQ ID NO: 9, and LCDR1, LCDR2, and LCDR3 contained within VL region sequence as set forth in SEQ ID NO: 12; and a second binding moiety comprising HCDR1, HCDR2, and HCDR3 contained within VH region sequence as set forth in SEQ ID NO: 20, and LCDR1, LCDR2, and LCDR3 contained within VL region sequence as set forth in SEQ ID NO: 21;
- (6) a first binding moiety comprising HCDR1, HCDR2, and HCDR3 contained within VH region sequence as set forth in SEQ ID NO: 9, and LCDR1, LCDR2, and LCDR3 contained within VL region sequence as set forth in SEQ ID NO: 12; and a second binding moiety comprising HCDR1, HCDR2, and HCDR3 contained within VH region sequence as set forth in SEQ ID NO: 22, and LCDR1, LCDR2, and LCDR3 contained within VL region sequence as set forth in SEQ ID NO: 23;
- (7) a first binding moiety comprising HCDR1, HCDR2, and HCDR3 contained within VH region sequence as set forth in SEQ ID NO: 9, and LCDR1, LCDR2, and LCDR3 contained within VL region sequence as set forth in SEQ ID NO: 12; and a second binding moiety comprising HCDR1, HCDR2, and HCDR3 contained within VH region sequence as set forth in SEQ ID NO: 30, and

LCDR1, LCDR2, and LCDR3 contained within VL region sequence as set forth in SEQ ID NO: 31;

- (8) a first binding moiety comprising HCDR1, HCDR2, and HCDR3 contained within VH region sequence as set forth in SEQ ID NO: 9, and LCDR1, LCDR2, and LCDR3 contained within VL region sequence as set forth in SEQ ID NO: 12; and a second binding moiety comprising HCDR1, HCDR2, and HCDR3 contained within VH region sequence as set forth in SEQ ID NO: 33, and LCDR1, LCDR2, and LCDR3 contained within VL region sequence as set forth in SEQ ID NO: 34;
- (9) a first binding moiety comprising HCDR1, HCDR2, and HCDR3 contained within VH region sequence as set forth in SEQ ID NO: 10, and LCDR1, LCDR2, and LCDR3 contained within VL region sequence as set forth in SEQ ID NO: 12; and a second binding moiety comprising HCDR1, HCDR2, and HCDR3 contained within VH region sequence as set forth in SEQ ID NO: 20, and LCDR1, LCDR2, and LCDR3 contained within VL region sequence as set forth in SEQ ID NO: 21;
- (10) a first binding moiety comprising HCDR1, HCDR2, and HCDR3 contained within VH region sequence as set forth in SEQ ID NO: 10, and LCDR1, LCDR2, and LCDR3 contained within VL region sequence as set forth in SEQ ID NO: 12; and a second binding moiety comprising HCDR1, HCDR2, and HCDR3 contained within VH region sequence as set forth in SEQ ID NO: 22, and LCDR1, LCDR2, and LCDR3 contained within VL region sequence as set forth in SEQ ID NO: 23;
- (11) a first binding moiety comprising HCDR1, HCDR2, and HCDR3 contained within VH region sequence as set forth in SEQ ID NO: 10, and LCDR1, LCDR2, and LCDR3 contained within VL region sequence as set forth in SEQ ID NO: 12; and a second binding moiety comprising HCDR1, HCDR2, and HCDR3 contained within VH region sequence as set forth in SEQ ID NO: 30, and

LCDR1, LCDR2, and LCDR3 contained within VL region sequence as set forth in SEQ ID NO: 31;

(12) a first binding moiety comprising HCDR1, HCDR2, and HCDR3 contained within VH region sequence as set forth in SEQ ID NO: 10, and LCDR1, LCDR2, and LCDR3 contained within VL region sequence as set forth in SEQ ID NO: 12; and a second binding moiety comprising HCDR1, HCDR2, and HCDR3 contained within VH region sequence as set forth in SEQ ID NO: 33, and LCDR1, LCDR2, and LCDR3 contained within VL region sequence as set forth in SEQ ID NO: 34;

(13) a first binding moiety comprising HCDR1, HCDR2, and HCDR3 contained within VH region sequence as set forth in SEQ ID NO: 11, and LCDR1, LCDR2, and LCDR3 contained within VL region sequence as set forth in SEQ ID NO: 12; and a second binding moiety comprising HCDR1, HCDR2, and HCDR3 contained within VH region sequence as set forth in SEQ ID NO: 20, and LCDR1, LCDR2, and LCDR3 contained within VL region sequence as set forth in SEQ ID NO: 21;

(14) a first binding moiety comprising HCDR1, HCDR2, and HCDR3 contained within VH region sequence as set forth in SEQ ID NO: 11, and LCDR1, LCDR2, and LCDR3 contained within VL region sequence as set forth in SEQ ID NO: 12; and a second binding moiety comprising HCDR1, HCDR2, and HCDR3 contained within VH region sequence as set forth in SEQ ID NO: 22, and LCDR1, LCDR2, and LCDR3 contained within VL region sequence as set forth in SEQ ID NO: 23;

(15) a first binding moiety comprising HCDR1, HCDR2, and HCDR3 contained within VH region sequence as set forth in SEQ ID NO: 11, and LCDR1, LCDR2, and LCDR3 contained within VL region sequence as set forth in SEQ ID NO: 12; and a second binding moiety comprising HCDR1, HCDR2, and HCDR3 contained within VH region sequence as set forth in SEQ ID NO: 30, and

LCDR1, LCDR2, and LCDR3 contained within VL region sequence as set forth in SEQ ID NO: 31;

(16) a first binding moiety comprising HCDR1, HCDR2, and HCDR3 contained within VH region sequence as set forth in SEQ ID NO: 11, and LCDR1, LCDR2, and LCDR3 contained within VL region sequence as set forth in SEQ ID NO: 12; and a second binding moiety comprising HCDR1, HCDR2, and HCDR3 contained within VH region sequence as set forth in SEQ ID NO: 33, and LCDR1, LCDR2, and LCDR3 contained within VL region sequence as set forth in SEQ ID NO: 34;

(17) a first binding moiety comprising HCDR1, HCDR2, and HCDR3 contained within VH region sequence as set forth in SEQ ID NO: 9, and LCDR1, LCDR2, and LCDR3 contained within VL region sequence as set forth in SEQ ID NO: 13; and a second binding moiety comprising HCDR1, HCDR2, and HCDR3 contained within VH region sequence as set forth in SEQ ID NO: 20, and LCDR1, LCDR2, and LCDR3 contained within VL region sequence as set forth in SEQ ID NO: 21;

(18) a first binding moiety comprising HCDR1, HCDR2, and HCDR3 contained within VH region sequence as set forth in SEQ ID NO: 9, and LCDR1, LCDR2, and LCDR3 contained within VL region sequence as set forth in SEQ ID NO: 13; and a second binding moiety comprising HCDR1, HCDR2, and HCDR3 contained within VH region sequence as set forth in SEQ ID NO: 22, and LCDR1, LCDR2, and LCDR3 contained within VL region sequence as set forth in SEQ ID NO: 23;

(19) a first binding moiety comprising HCDR1, HCDR2, and HCDR3 contained within VH region sequence as set forth in SEQ ID NO: 9, and LCDR1, LCDR2, and LCDR3 contained within VL region sequence as set forth in SEQ ID NO: 13; and a second binding moiety comprising HCDR1, HCDR2, and HCDR3 contained within VH region sequence as set forth in SEQ ID NO: 30, and

LCDR1, LCDR2, and LCDR3 contained within VL region sequence as set forth in SEQ ID NO: 31;

(20) a first binding moiety comprising HCDR1, HCDR2, and HCDR3 contained within VH region sequence as set forth in SEQ ID NO: 9, and LCDR1, LCDR2, and LCDR3 contained within VL region sequence as set forth in SEQ ID NO: 13; and a second binding moiety comprising HCDR1, HCDR2, and HCDR3 contained within VH region sequence as set forth in SEQ ID NO: 33, and LCDR1, LCDR2, and LCDR3 contained within VL region sequence as set forth in SEQ ID NO: 34;

(21) a first binding moiety comprising HCDR1, HCDR2, and HCDR3 contained within VH region sequence as set forth in SEQ ID NO: 47, and LCDR1, LCDR2, and LCDR3 contained within VL region sequence as set forth in SEQ ID NO: 12; and a second binding moiety comprising HCDR1, HCDR2, and HCDR3 contained within VH region sequence as set forth in SEQ ID NO: 20, and LCDR1, LCDR2, and LCDR3 contained within VL region sequence as set forth in SEQ ID NO: 21;

(22) a first binding moiety comprising HCDR1, HCDR2, and HCDR3 contained within VH region sequence as set forth in SEQ ID NO: 47, and LCDR1, LCDR2, and LCDR3 contained within VL region sequence as set forth in SEQ ID NO: 12; and a second binding moiety comprising HCDR1, HCDR2, and HCDR3 contained within VH region sequence as set forth in SEQ ID NO: 22, and LCDR1, LCDR2, and LCDR3 contained within VL region sequence as set forth in SEQ ID NO: 23;

(23) a first binding moiety comprising HCDR1, HCDR2, and HCDR3 contained within VH region sequence as set forth in SEQ ID NO: 47, and LCDR1, LCDR2, and LCDR3 contained within VL region sequence as set forth in SEQ ID NO: 12; and a second binding moiety comprising HCDR1, HCDR2, and HCDR3 contained within VH region sequence as set forth in SEQ ID NO: 30, and

LCDR1, LCDR2, and LCDR3 contained within VL region sequence as set forth in SEQ ID NO: 31; or

- (24) a first binding moiety comprising HCDR1, HCDR2, and HCDR3 contained within VH region sequence as set forth in SEQ ID NO: 47, and LCDR1, LCDR2, and LCDR3 contained within VL region sequence as set forth in SEQ ID NO: 12; and a second binding moiety comprising HCDR1, HCDR2, and HCDR3 contained within VH region sequence as set forth in SEQ ID NO: 33, and LCDR1, LCDR2, and LCDR3 contained within VL region sequence as set forth in SEQ ID NO: 34.

[00228] In certain embodiments, an exemplary multi-specific antibody or antigen-binding fragment thereof provided herein comprises:

- (1) a first binding moiety comprising HCDR1, HCDR2, HCDR3, LCDR1, LCDR2, and LCDR3 comprising an amino acid sequence as set forth in SEQ ID NOs: 1, 2, 3, 4, 5 and 6, respectively; and a second binding moiety comprising HCDR1, HCDR2, HCDR3, LCDR1, LCDR2, and LCDR3 comprising an amino acid sequence as set forth in SEQ ID NOs: 14, 15, 16, 17, 18 and 19, respectively;
- (2) a first binding moiety comprising HCDR1, HCDR2, HCDR3, LCDR1, LCDR2, and LCDR3 comprising an amino acid sequence as set forth in SEQ ID NOs: 1, 2, 3, 4, 5 and 6, respectively; and a second binding moiety comprising HCDR1, HCDR2, HCDR3, LCDR1, LCDR2, and LCDR3 comprising an amino acid sequence as set forth in SEQ ID NOs: 24, 25, 26, 27, 28 and 29, respectively;
- (3) a first binding moiety comprising HCDR1, HCDR2, HCDR3, LCDR1, LCDR2, and LCDR3 comprising an amino acid sequence as set forth in SEQ ID NOs: 1, 2, 3, 4, 5 and 6, respectively; and a second binding moiety comprising HCDR1, HCDR2, HCDR3, LCDR1, LCDR2, and LCDR3 comprising an amino acid sequence as set forth in SEQ ID NOs: 24, 32, 26, 27, 28 and 29, respectively;
- (4) a first binding moiety comprising HCDR1, HCDR2, HCDR3, LCDR1, LCDR2, and LCDR3 comprising an amino acid sequence as set forth in SEQ ID NOs: 1,

- 42, 3, 4, 5 and 6, respectively; and a second binding moiety comprising HCDR1, HCDR2, HCDR3, LCDR1, LCDR2, and LCDR3 comprising an amino acid sequence as set forth in SEQ ID NOs: 14, 15, 16, 17, 18 and 19, respectively;
- (5) a first binding moiety comprising HCDR1, HCDR2, HCDR3, LCDR1, LCDR2, and LCDR3 comprising an amino acid sequence as set forth in SEQ ID NOs: 1, 42, 3, 4, 5 and 6, respectively; and a second binding moiety comprising HCDR1, HCDR2, HCDR3, LCDR1, LCDR2, and LCDR3 comprising an amino acid sequence as set forth in SEQ ID NOs: 24, 25, 26, 27, 28 and 29, respectively; or
- (6) a first binding moiety comprising HCDR1, HCDR2, HCDR3, LCDR1, LCDR2, and LCDR3 comprising an amino acid sequence as set forth in SEQ ID NOs: 1, 42, 3, 4, 5 and 6, respectively; and a second binding moiety comprising HCDR1, HCDR2, HCDR3, LCDR1, LCDR2, and LCDR3 comprising an amino acid sequence as set forth in SEQ ID NOs: 24, 32, 26, 27, 28 and 29, respectively.

[00229] Different formats of multi-specific antibodies have been described in the art, for example, in Chames and Baty (2009) *Curr Opin Drug Disc Dev* 12: 276. The multi-specific antibodies or antigen-binding fragments thereof provide herein are not limited to any particular multi-specific format, and may have all the different formats known in the art.

[00230] For example, multi-specific antibodies or antigen-binding fragments thereof provide herein may have a typical full-length antibody structure, *i.e.*, an antibody having two different full-length antibody heavy chains and two different full length antibody light chains. A full-length antibody heavy chain includes heavy chain variable region (VH) and constant domains CH1, CH2, CH3 and optionally CH4. A full-length antibody light chain includes light chain variable region (VL) and constant domain CL. For example, the multi-specific antibodies or antigen-binding fragments thereof provide herein may comprises a first light chain, a first heavy chain, a second heavy chain, and a second light chain, wherein the first light chain and the first heavy chain are paired to form a first antigen-binding site that binds to LILRB4, and the

second light chain and the second heavy chain are paired to form a second antigen-binding site that binds to CD3. For another example, the multi-specific antibodies or antigen-binding fragments thereof provide herein may comprises a first light chain, a first heavy chain, a second heavy chain, and a second light chain, wherein the first light chain and the first heavy chain are paired to form a first antigen-binding site that binds to CD3, and the second light chain and the second heavy chain are paired to form a second antigen-binding site that binds to LILRB4.

[00231] For another example, the multi-specific antibodies or antigen-binding fragments thereof provide herein may include, but are not limited to, multi-specific antibodies with complementary CH3 domains to force heterodimerization, Knobs-into-Holes molecules (Genentech, WO9850431), CrossMAbs (Roche, WO2009080253), or electrostatically-matched molecules (Amgen, EP1870459 and WO2009089004; Chugai, US201000155133; Oncomed, WO2010129304). For example, regarding the CrossMab format, the first binding moiety of the multi-specific antibodies or antigen-binding fragments thereof provided herein further comprises a constant domain CL and a constant domain CH1, and in some embodiments, the constant domains CL and CH1 are replaced by each other; or the second binding moiety of the multi-specific antibodies or antigen-binding fragments thereof provided herein further comprises a constant domain CL and a constant domain CH1, and in some embodiments, the constant domains CL and CH1 are replaced by each other.

[00232] In certain embodiments, the first binding moiety is directly linked to the second binding moiety, i.e., without a linker. In certain embodiments, the first binding moiety is linked to the second binding moiety via a linker.

[00233] The term “linker” as used herein refers to an artificial amino acid sequence having 1, 2, 3, 4 or 5 amino acid residues, or a length of between 5 and 15, 20, 30, 50 or more amino acid residues, joined by peptide bonds and are used to link one or more polypeptides. A linker may or may not have a secondary structure. Linker

sequences are known in the art, see, for example, Holliger *et al.*, *Proc. Natl. Acad. Sci. USA* 90:6444-6448 (1993); Poljak *et al.*, *Structure* 2:1121-1123 (1994).

[00234] In certain embodiments, the linker is selected from the group consisting of a cleavable linker, a non-cleavable linker, a peptide linker, a flexible linker, a rigid linker, a helical linker, and a non-helical linker. Any suitable linkers known in the art can be used. In certain embodiments, the linker comprises a peptide linker. For example, a useful linker in the present disclosure may be rich in glycine and serine residues. Examples include linkers having a single or repeated sequences comprising threonine/serine and glycine, such as TGGGG (SEQ ID NO: 35), GGGGS (SEQ ID NO: 36) or SGGGG (SEQ ID NO: 37) or its tandem repeats (e.g., 2, 3, 4, 5, 6, 7, 8, 9, 10 or more repeats). In certain embodiments, the linker used in the present disclosure comprises GGGSGGGSGGGGS (SEQ ID NO: 38). In certain embodiments, the GS linker comprises one or more repeats of GSSS (SEQ ID NO: 39). In certain embodiments, the first linker comprises or consists of an amino acid sequence having at least 80%, at least 85%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99% sequence identity to any one of SEQ ID NOs: 35-39.

[00235] In certain embodiments, the N-terminus of the first binding moiety is linked to the C-terminus of the second binding moiety via the linker. In certain embodiments, the N-terminus of the first binding moiety is directly linked to the C-terminus of the second binding moiety, i.e., without a linker.

[00236] In certain embodiments, the antibodies or antigen-binding fragments thereof provided herein comprise, from N-terminus to C-terminus, a first chain comprising a VH region from the second binding moiety, a CH1 region from the second binding moiety, a VH region from the first binding moiety and a VL region from the first binding moiety. In certain embodiments, the CH1 region from the second binding moiety and the VH region from the first binding moiety are directly linked, i.e., without a linker. In certain embodiments, the CH1 region from the second binding moiety and the VH region from the first binding moiety are linked via a linker. In

certain embodiments, the linker is a linker comprising glycine and serine, *e.g.*, a (GGGGS)₂ linker or a GSSS linker.

[00237] In certain embodiments, the antibodies or antigen-binding fragments thereof provided herein comprise, from N-terminus to C-terminus, a first chain comprising a VH region from the second binding moiety, a CH1 region from the second binding moiety, a VL region from the first binding moiety and a VH region from the first binding moiety. In certain embodiments, the CH1 region from the second binding moiety and the VL region from the first binding moiety are directly linked, *i.e.*, without a linker. In certain embodiments, the CH1 region from the second binding moiety and the VL region from the first binding moiety are linked via a linker. In certain embodiments, the linker is a linker comprising glycine and serine, *e.g.*, a (GGGGS)₂ linker or a GSSS linker.

[00238] In certain embodiments, the VH region from the first binding moiety and the VL region from the first binding moiety are directly linked, *i.e.*, without a linker. In certain embodiments, the VH region from the first binding moiety and the VL region from the first binding moiety are linked via a linker. In certain embodiments, the linker is a linker comprising glycine and serine, *e.g.*, a (GGGGS)₂ linker or a GSSS linker.

[00239] In certain embodiments, the antibodies or antigen-binding fragments thereof provided herein further comprise a second chain comprising a VL region from the second binding moiety and a CL from the second binding moiety.

Third binding moiety

[00240] In certain embodiments, the antibodies or antigen-binding fragments thereof provided herein further comprise a third binding moiety. In certain embodiments, the antibodies or antigen-binding fragments thereof provided herein further comprise a third binding moiety that binds to LILRB4.

[00241] As used herein, the term “third binding moiety” with regard to an antibody or antigen-binding fragment thereof provided herein, refers to a moiety that is capable of

binding to LILRB4 (*e.g.*, human LILRB4, mouse LILRB4, and cynomolgus LILRB4). The third binding moiety can take any form that allows specific recognition of the target LILRB4. For example, the third binding moiety may be an antibody or an antigen-binding fragment thereof, *e.g.*, an IgG (such as IgG1, IgG2, IgG3, and IgG4) antibody, IgA antibody, or IgM antibody.

[00242] In certain embodiments, the antibodies or antigen-binding fragments thereof provided herein comprise a first binding moiety that binds to CD3, a second binding moiety that binds to LILRB4, and a third binding moiety that binds to LILRB4.

[00243] In certain embodiments, the third binding moiety and the second binding moiety of the antibodies or antigen-binding fragments thereof provided herein bind to the same epitope on LILRB4. In certain embodiments, the third binding moiety and the second binding moiety of the antibodies or antigen-binding fragments thereof provided herein bind to different epitopes on LILRB4. In certain embodiments, the antibodies or antigen-binding fragments thereof provided herein bind to CD3 and two different epitopes on LILRB4.

[00244] In some embodiments, the third binding moiety that binds to LILRB4 is an antigen-binding fragment of an anti-LILRB4 antibody (*e.g.*, antibodies 2-H1-D7-E5-D5, h2-H1-D7-E5-D5, 8-B3-F6-H8, h8-B3-F6-H8). In some embodiments, the third binding moiety that binds to LILRB4 is a Fab domain. In some embodiments, the third binding moiety that binds to LILRB4 is a scFv. In some embodiments, the first binding moiety is an anti-CD3 scFv, the second binding moiety is an anti-LILRB4 Fab domain, and the third binding moiety is an anti-LILRB4 Fab domain.

[00245] In some embodiments, the amino acid sequence of the second binding moiety is the same as the amino acid sequence of the third binding moiety. In some embodiments, the HCDR1, HCDR2 and HCDR3 sequences of the second binding moiety are the same as the HCDR1, HCDR2 and HCDR3 sequences of the third binding moiety, respectively. In some embodiments, the LCDR1, LCDR2 and LCDR3 sequences of the second binding moiety are the same as the LCDR1, LCDR2

and LCDR3 sequences of the third binding moiety, respectively. In some embodiments, the HCDR1, HCDR2, HCDR3, LCDR1, LCDR2 and LCDR3 sequences of the second binding moiety are the same as the HCDR1, HCDR2, HCDR3, LCDR1, LCDR2 and LCDR3 sequences of the third binding moiety, respectively. In some embodiments, the VH sequence of the second binding moiety is the same as the VH sequence of the third binding moiety. In some embodiments, the VL sequence of the second binding moiety is the same as the VL sequence of the third binding moiety. In some embodiments, the VH and VL sequences of the second binding moiety are the same as the VH and VL sequences of the third binding moiety, respectively.

[00246] In certain embodiments, the antibodies or antigen-binding fragments thereof provided herein further comprise a third binding moiety that binds to LILRB4, wherein the third binding moiety binding to LILRB4 comprises one or more (*e.g.*, 1, 2, 3, 4, 5, or 6) CDR sequences of an anti-LILRB4 antibody 2-H1-D7-E5-D5, h2-H1-D7-E5-D5, 8-B3-F6-H8, or h8-B3-F6-H8, as described above.

[00247] In certain embodiments, the third binding moiety of the antibodies or antigen-binding fragments thereof provided herein comprises one or two or three heavy chain complementarity determining regions (HCDR1, HCDR2 and/or HCDR3) contained within the heavy chain variable (VH) region sequence of SEQ ID NOs: 20, 22, 30, or 33; and/or one or two or three light chain complementarity determining regions (LCDR1, LCDR2 and/or LCDR3) contained within the light chain variable (VL) region sequence of SEQ ID NOs: 21, 23, 31 or 34.

[00248] In some embodiments, the third binding moiety of the antibodies or antigen-binding fragments thereof provided herein comprises HCDR1, HCDR2 and/or HCDR3 contained within the VH region sequence of SEQ ID NO: 20. In some embodiments, the third binding moiety of the antibodies or antigen-binding fragments thereof provided herein comprises HCDR1, HCDR2 and/or HCDR3 contained within the VH region sequence of SEQ ID NO: 22. In some embodiments, the third binding moiety of the antibodies or antigen-binding fragments thereof provided herein

comprises HCDR1, HCDR2 and/or HCDR3 contained within the VH region sequence of SEQ ID NO: 30. In some embodiments, the third binding moiety of the antibodies or antigen-binding fragments thereof provided herein comprises HCDR1, HCDR2 and/or HCDR3 contained within the VH region sequence of SEQ ID NO: 33.

[00249] In some embodiments, the third binding moiety of the antibodies or antigen-binding fragments thereof provided herein comprises LCDR1, LCDR2 and/or LCDR3 contained within the VL region sequence of SEQ ID NO: 21. In some embodiments, the third binding moiety of the antibodies or antigen-binding fragments thereof provided herein comprises LCDR1, LCDR2 and/or LCDR3 contained within the VL region sequence of SEQ ID NO: 23. In some embodiments, the third binding moiety of the antibodies or antigen-binding fragments thereof provided herein comprises LCDR1, LCDR2 and/or LCDR3 contained within the VL region sequence of SEQ ID NO: 31. In some embodiments, the third binding moiety of the antibodies or antigen-binding fragments thereof provided herein comprises LCDR1, LCDR2 and/or LCDR3 contained within the VL region sequence of SEQ ID NO: 34.

[00250] In some embodiments, the third binding moiety of the antibodies or antigen-binding fragments thereof provided herein comprises HCDR1, HCDR2, HCDR3, LCDR1, LCDR2 and LCDR3 contained within a VH/VL amino acid sequence pair of SEQ ID NOS: 20/21, 22/23, 30/31, or 33/34. In some embodiments, the third binding moiety of the antibodies or antigen-binding fragments thereof provided herein comprises HCDR1, HCDR2, HCDR3, LCDR1, LCDR2 and LCDR3 contained within a VH/VL amino acid sequence pair of SEQ ID NO: 20/21. In some embodiments, the third binding moiety of the antibodies or antigen-binding fragments thereof provided herein comprises HCDR1, HCDR2, HCDR3, LCDR1, LCDR2 and LCDR3 contained within a VH/VL amino acid sequence pair of SEQ ID NO: 22/23. In some embodiments, the third binding moiety of the antibodies or antigen-binding fragments thereof provided herein comprises HCDR1, HCDR2, HCDR3, LCDR1, LCDR2 and LCDR3 contained within a VH/VL amino acid sequence pair of SEQ ID NO: 30/31. In some embodiments, the third binding moiety of the antibodies or antigen-binding

fragments thereof provided herein comprises HCDR1, HCDR2, HCDR3, LCDR1, LCDR2 and LCDR3 contained within a VH/VL amino acid sequence pair of SEQ ID NO: 33/34.

[00251] In some embodiments, the third binding moiety of the antibodies or antigen-binding fragments thereof provided herein is an anti-LILRB4 Fab domain, and comprises HCDR1, HCDR2, HCDR3, LCDR1, LCDR2 and LCDR3 contained within a VH/VL amino acid sequence pair of SEQ ID NO: 20/21. In some embodiments, the third binding moiety of the antibodies or antigen-binding fragments thereof provided herein is an anti-LILRB4 Fab domain, and comprises HCDR1, HCDR2, HCDR3, LCDR1, LCDR2 and LCDR3 contained within a VH/VL amino acid sequence pair of SEQ ID NO: 22/23. In some embodiments, the third binding moiety of the antibodies or antigen-binding fragments thereof provided herein is an anti-LILRB4 Fab domain, and comprises HCDR1, HCDR2, HCDR3, LCDR1, LCDR2 and LCDR3 contained within a VH/VL amino acid sequence pair of SEQ ID NO: 30/31. In some embodiments, the third binding moiety of the antibodies or antigen-binding fragments thereof provided herein is an anti-LILRB4 Fab domain, and comprises HCDR1, HCDR2, HCDR3, LCDR1, LCDR2 and LCDR3 contained within a VH/VL amino acid sequence pair of SEQ ID NO: 33/34.

[00252] In some embodiments, the CDR boundaries of the third binding moiety are identified by the convention of Kabat. In some embodiments, the CDR boundaries of the third binding moiety are identified by the convention of IMGT. In some embodiments, the CDR boundaries of the third binding moiety are identified by the convention of Chothia. In some embodiments, the CDR boundaries of the third binding moiety are identified by the convention of Al-Lazikani.

[00253] In some embodiments, the third binding moiety of the antibodies or antigen-binding fragments thereof provided herein comprises at least one heavy or light chain complementarity determining region (CDR) comprising an amino acid sequence selected from the group consisting of SEQ ID NOs: 14, 15, 16, 17, 18, 19, 24, 25, 26, 27, 28, 29 and 32.

[00254] In some embodiments, the third binding moiety of the antibodies or antigen-binding fragments thereof provided herein comprises one or two or three of HCDR1, HCDR2 and HCDR3 comprising an amino acid sequence selected from the group consisting of SEQ ID NOs: 14, 15, 16, 24, 25, 26 and 32.

[00255] In some embodiments, the third binding moiety of the antibodies or antigen-binding fragments thereof provided herein comprises one or two or three of LCDR1, LCDR2 and LCDR3 comprising an amino acid sequence selected from the group consisting of SEQ ID NOs: 17, 18, 19, 27, 28 and 29.

[00256] In some embodiments, the third binding moiety of the antibodies or antigen-binding fragments thereof provided herein comprises:

- i. a HCDR1 comprising an amino acid sequence selected from the group consisting of SEQ ID NOs: 14 and 24,
- ii. a HCDR2 comprising an amino acid sequence selected from the group consisting of SEQ ID NOs: 15, 25, and 32, and
- iii. a HCDR3 comprising an amino acid sequence selected from the group consisting of SEQ ID NOs: 16 and 26.

[00257] In some embodiments, the third binding moiety of the antibodies or antigen-binding fragments thereof provided herein comprises:

- i. a LCDR1 comprising an amino acid sequence selected from the group consisting of SEQ ID NOs: 17 and 27,
- ii. a LCDR2 comprising an amino acid sequence selected from the group consisting of SEQ ID NOs: 18 and 28, and
- iii. a LCDR3 comprising an amino acid sequence selected from the group consisting of SEQ ID NOs: 19 and 29.

[00258] In some embodiments, the third binding moiety of the antibodies or antigen-binding fragments thereof provided herein comprises a HCDR1 comprising an amino

acid sequence as set forth in SEQ ID NO: 14, a HCDR2 comprising an amino acid sequence as set forth in SEQ ID NO: 15, and a HCDR3 comprising an amino acid sequence as set forth in SEQ ID NO: 16.

[00259] In some embodiments, the third binding moiety of the antibodies or antigen-binding fragments thereof provided herein comprises a HCDR1 comprising an amino acid sequence as set forth in SEQ ID NO: 24, a HCDR2 comprising an amino acid sequence as set forth in SEQ ID NO: 25, and a HCDR3 comprising an amino acid sequence as set forth in SEQ ID NO: 26.

[00260] In some embodiments, the third binding moiety of the antibodies or antigen-binding fragments thereof provided herein comprises a HCDR1 comprising an amino acid sequence as set forth in SEQ ID NO: 24, a HCDR2 comprising an amino acid sequence as set forth in SEQ ID NO: 32, and a HCDR3 comprising an amino acid sequence as set forth in SEQ ID NO: 26.

[00261] In some embodiments, the third binding moiety of the antibodies or antigen-binding fragments thereof provided herein comprises a LCDR1 comprising an amino acid sequence as set forth in SEQ ID NO: 17, a LCDR2 comprising an amino acid sequence as set forth in SEQ ID NO: 18, and a LCDR3 comprising an amino acid sequence as set forth in SEQ ID NO: 19.

[00262] In some embodiments, the third binding moiety of the antibodies or antigen-binding fragments thereof provided herein comprises a LCDR1 comprising an amino acid sequence as set forth in SEQ ID NO: 27, a LCDR2 comprising an amino acid sequence as set forth in SEQ ID NO: 28, and a LCDR3 comprising an amino acid sequence as set forth in SEQ ID NO: 29.

[00263] In some embodiments, the third binding moiety of the antibodies or antigen-binding fragments thereof provided herein comprises: a HCDR1 comprising an amino acid sequence as set forth in SEQ ID NO: 14, a HCDR2 comprising an amino acid sequence as set forth in SEQ ID NO: 15, a HCDR3 comprising an amino acid sequence as set forth in SEQ ID NO: 16, a LCDR1 comprising an amino acid

sequence as set forth in SEQ ID NO: 17, a LCDR2 comprising an amino acid sequence as set forth in SEQ ID NO: 18, and a LCDR3 comprising an amino acid sequence as set forth in SEQ ID NO: 19.

[00264] In some embodiments, the third binding moiety of the antibodies or antigen-binding fragments thereof provided herein comprises: a HCDR1 comprising an amino acid sequence as set forth in SEQ ID NO: 24, a HCDR2 comprising an amino acid sequence as set forth in SEQ ID NO: 25, a HCDR3 comprising an amino acid sequence as set forth in SEQ ID NO: 26, a LCDR1 comprising an amino acid sequence as set forth in SEQ ID NO: 27, a LCDR2 comprising an amino acid sequence as set forth in SEQ ID NO: 28, and a LCDR3 comprising an amino acid sequence as set forth in SEQ ID NO: 29.

[00265] In some embodiments, the third binding moiety of the antibodies or antigen-binding fragments thereof provided herein comprises: a HCDR1 comprising an amino acid sequence as set forth in SEQ ID NO: 24, a HCDR2 comprising an amino acid sequence as set forth in SEQ ID NO: 32, a HCDR3 comprising an amino acid sequence as set forth in SEQ ID NO: 26, a LCDR1 comprising an amino acid sequence as set forth in SEQ ID NO: 27, a LCDR2 comprising an amino acid sequence as set forth in SEQ ID NO: 28, and a LCDR3 comprising an amino acid sequence as set forth in SEQ ID NO: 29.

[00266] In certain embodiments, the third binding moiety of the antibodies or antigen-binding fragments thereof provided herein comprises a VH region comprising one or two or three of HCDR1, HCDR2 and HCDR3 comprising an amino acid sequence as set forth in SEQ ID NO: 14, SEQ ID NO: 15, and SEQ ID NO: 16, respectively. In certain embodiments, the third binding moiety of the antibodies or antigen-binding fragments thereof provided herein comprises a VH region comprising HCDR1, HCDR2 and HCDR3 comprising an amino acid sequence as set forth in SEQ ID NO: 14, SEQ ID NO: 15, and SEQ ID NO: 16, respectively.

[00267] In certain embodiments, the third binding moiety of the antibodies or antigen-binding fragments thereof provided herein comprises a VH region comprising one or two or three of HCDR1, HCDR2 and HCDR3 comprising an amino acid sequence as set forth in SEQ ID NO: 24, SEQ ID NO: 25, and SEQ ID NO: 26, respectively. In certain embodiments, the third binding moiety of the antibodies or antigen-binding fragments thereof provided herein comprises a VH region comprising HCDR1, HCDR2 and HCDR3 comprising an amino acid sequence as set forth in SEQ ID NO: 24, SEQ ID NO: 25, and SEQ ID NO: 26, respectively.

[00268] In certain embodiments, the third binding moiety of the antibodies or antigen-binding fragments thereof provided herein comprises a VH region comprising one or two or three of HCDR1, HCDR2 and HCDR3 comprising an amino acid sequence as set forth in SEQ ID NO: 24, SEQ ID NO: 32, and SEQ ID NO: 26, respectively. In certain embodiments, the third binding moiety of the antibodies or antigen-binding fragments thereof provided herein comprises a VH region comprising HCDR1, HCDR2 and HCDR3 comprising an amino acid sequence as set forth in SEQ ID NO: 24, SEQ ID NO: 32, and SEQ ID NO: 26, respectively.

[00269] In certain embodiments, the third binding moiety of the antibodies or antigen-binding fragments thereof provided herein comprises a VL region comprising one or two or three of LCDR1, LCDR2 and LCDR3 comprising an amino acid sequence as set forth in SEQ ID NO: 17, SEQ ID NO: 18, and SEQ ID NO: 19, respectively. In certain embodiments, the third binding moiety of the antibodies or antigen-binding fragments thereof provided herein comprises a VL region comprising LCDR1, LCDR2 and LCDR3 comprising an amino acid sequence as set forth in SEQ ID NO: 17, SEQ ID NO: 18, and SEQ ID NO: 19, respectively.

[00270] In certain embodiments, the third binding moiety of the antibodies or antigen-binding fragments thereof provided herein comprises a VL region comprising one or two or three of LCDR1, LCDR2 and LCDR3 comprising an amino acid sequence as set forth in SEQ ID NO: 27, SEQ ID NO: 28, and SEQ ID NO: 29, respectively. In certain embodiments, the third binding moiety of the antibodies or

antigen-binding fragments thereof provided herein comprises a VL region comprising LCDR1, LCDR2 and LCDR3 comprising an amino acid sequence as set forth in SEQ ID NO: 27, SEQ ID NO: 28, and SEQ ID NO: 29, respectively.

[00271] In certain embodiments, the third binding moiety of the antibodies or antigen-binding fragments thereof provided herein comprises a VH region having an amino acid sequence as set forth in SEQ ID NOs: 20, 22, 30 or 33 below, or a homologous sequence having at least 80% (*e.g.*, at least 85%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, and at least 99%) sequence identity to SEQ ID NOs: 20, 22, 30 or 33.

[00272] In certain embodiments, the third binding moiety of the antibodies or antigen-binding fragments thereof provided herein comprises a VH region having a homologous sequence that has at least 80% (*e.g.*, at least 85%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, and at least 99%) sequence identity to SEQ ID NOs: 20, 22, 30 or 33, and wherein the VH region comprises HCDR1, HCDR2 and HCDR3 comprising an amino acid sequence as set forth in SEQ ID NO: 14, SEQ ID NO: 15 and SEQ ID NO: 16, respectively.

[00273] In certain embodiments, the third binding moiety of the antibodies or antigen-binding fragments thereof provided herein comprises a VH region having a homologous sequence that has at least 80% (*e.g.*, at least 85%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, and at least 99%) sequence identity to SEQ ID NOs: 20, 22, 30 or 33, and wherein the VH region comprises HCDR1, HCDR2 and HCDR3 comprising an amino acid sequence as set forth in SEQ ID NO: 24, SEQ ID NO: 25 and SEQ ID NO: 26, respectively.

[00274] In certain embodiments, the third binding moiety of the antibodies or antigen-binding fragments thereof provided herein comprises a VH region having a homologous sequence that has at least 80% (*e.g.*, at least 85%, at least 90%, at least

91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, and at least 99%) sequence identity to SEQ ID NOs: 20, 22, 30 or 33, and wherein the VH region comprises HCDR1, HCDR2 and HCDR3 comprising an amino acid sequence as set forth in SEQ ID NO: 24, SEQ ID NO: 32 and SEQ ID NO: 26, respectively.

[00275] In certain embodiments, the third binding moiety of the antibodies or antigen-binding fragments thereof provided herein comprises a VL region having an amino acid sequence as set forth in SEQ ID NOs: 21, 23, 31 or 34, or a homologous sequence having at least 80% (*e.g.*, at least 85%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, and at least 99%) sequence identity to SEQ ID NOs: 21, 23, 31 or 34.

[00276] In certain embodiments, the third binding moiety of the antibodies or antigen-binding fragments thereof provided herein comprises a VL region having a homologous sequence that has at least 80% (*e.g.*, at least 85%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, and at least 99%) sequence identity to SEQ ID NOs: 21, 23, 31 or 34, and wherein the VL region comprises LCDR1, LCDR2 and LCDR3 comprising an amino acid sequence as set forth in SEQ ID NO: 17, SEQ ID NO: 18 and SEQ ID NO: 19, respectively.

[00277] In certain embodiments, the third binding moiety of the antibodies or antigen-binding fragments thereof provided herein comprises a VL region having a homologous sequence that has at least 80% (*e.g.*, at least 85%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, and at least 99%) sequence identity to SEQ ID NOs: 21, 23, 31 or 34, and wherein the VL region comprises LCDR1, LCDR2 and LCDR3 comprising an amino acid sequence as set forth in SEQ ID NO: 27, SEQ ID NO: 28 and SEQ ID NO: 29, respectively.

[00278] In some embodiments, the third binding moiety of the antibodies or antigen-binding fragments thereof provided herein comprises a VH/VL amino acid sequence pair of SEQ ID NOs: 20/21, 22/23, 30/31, or 33/34. In some embodiments, the third binding moiety of the antibodies or antigen-binding fragments thereof provided herein comprises a VH/VL amino acid sequence pair of SEQ ID NO: 20/21. In some embodiments, the third binding moiety of the antibodies or antigen-binding fragments thereof provided herein comprises a VH/VL amino acid sequence pair of SEQ ID NO: 22/23. In some embodiments, the third binding moiety of the antibodies or antigen-binding fragments thereof provided herein comprises a VH/VL amino acid sequence pair of SEQ ID NO: 30/31. In some embodiments, the third binding moiety of the antibodies or antigen-binding fragments thereof provided herein comprises a VH/VL amino acid sequence pair of SEQ ID NO: 33/34.

[00279] In certain embodiments, in the antibodies or antigen-binding fragments thereof provided herein, the first binding moiety comprises HCDR1, HCDR2 and HCDR3 contained within the VH region sequence of SEQ ID NOs: 7, 9, 10, 47 or 11; and LCDR1, LCDR2 and LCDR3 contained within the VL region sequence of SEQ ID NOs: 8, 12 or 13; and

each of the second and third binding moieties comprises HCDR1, HCDR2 and HCDR3 contained within the VH region sequence of SEQ ID NOs: 20, 22, 30, or 33; and LCDR1, LCDR2 and LCDR3 contained within the VL region sequence of SEQ ID NOs: 21, 23, 31 or 34.

[00280] In certain embodiments, in the antibodies or antigen-binding fragments thereof provided herein, the first binding moiety comprises HCDR1, HCDR2, HCDR3, LCDR1, LCDR2 and LCDR3 contained within a VH/VL amino acid sequence pair of SEQ ID NOs: 7/8, 7/12, 7/13, 9/8, 9/12, 9/13, 10/8, 10/12, 10/13, 47/8, 47/12, 47/13, 11/8, 11/12 or 11/13; and

each of the second and third binding moieties comprises HCDR1, HCDR2, HCDR3, LCDR1, LCDR2 and LCDR3 contained within a VH/VL amino acid sequence pair of SEQ ID NOs: 20/21, 22/23, 30/31, or 33/34.

[00281] In certain embodiments, in the antibodies or antigen-binding fragments thereof provided herein, the first binding moiety comprises HCDR1, HCDR2, HCDR3 contained within a VH amino acid sequence as set forth in SEQ ID NO: 7; and LCDR1, LCDR2 and LCDR3 contained within a VL amino acid sequence of SEQ ID NO: 8; and

each of the second and third binding moieties comprises HCDR1, HCDR2, and HCDR3 contained within a VH amino acid sequence of SEQ ID NO: 20; and LCDR1, LCDR2 and LCDR3 contained within a VL amino acid sequence of SEQ ID NO: 21.

[00282] In certain embodiments, in the antibodies or antigen-binding fragments thereof provided herein, the first binding moiety comprises HCDR1, HCDR2, HCDR3 contained within a VH amino acid sequence as set forth in SEQ ID NO: 7; and LCDR1, LCDR2 and LCDR3 contained within a VL amino acid sequence of SEQ ID NO: 8; and

each of the second and third binding moieties comprises HCDR1, HCDR2, and HCDR3 contained within a VH amino acid sequence of SEQ ID NO: 22; and LCDR1, LCDR2 and LCDR3 contained within a VL amino acid sequence of SEQ ID NO: 23.

[00283] In certain embodiments, in the antibodies or antigen-binding fragments thereof provided herein, the first binding moiety comprises HCDR1, HCDR2, HCDR3 contained within a VH amino acid sequence as set forth in SEQ ID NO: 7; and LCDR1, LCDR2 and LCDR3 contained within a VL amino acid sequence of SEQ ID NO: 8; and

each of the second and third binding moieties comprises HCDR1, HCDR2, and HCDR3 contained within a VH amino acid sequence of SEQ ID NO: 30; and LCDR1, LCDR2 and LCDR3 contained within a VL amino acid sequence of SEQ ID NO: 31.

[00284] In certain embodiments, in the antibodies or antigen-binding fragments thereof provided herein, the first binding moiety comprises HCDR1, HCDR2, HCDR3 contained within a VH amino acid sequence as set forth in SEQ ID NO: 7; and LCDR1, LCDR2 and LCDR3 contained within a VL amino acid sequence of SEQ ID NO: 8; and

each of the second and third binding moieties comprises HCDR1, HCDR2, and HCDR3 contained within a VH amino acid sequence of SEQ ID NO: 33; and LCDR1, LCDR2 and LCDR3 contained within a VL amino acid sequence of SEQ ID NO: 34.

[00285] In certain embodiments, in the antibodies or antigen-binding fragments thereof provided herein, the first binding moiety comprises HCDR1, HCDR2, HCDR3 contained within a VH amino acid sequence as set forth in SEQ ID NO: 9; and LCDR1, LCDR2 and LCDR3 contained within a VL amino acid sequence of SEQ ID NO: 12; and

each of the second and third binding moieties comprises HCDR1, HCDR2, and HCDR3 contained within a VH amino acid sequence of SEQ ID NO: 20; and LCDR1, LCDR2 and LCDR3 contained within a VL amino acid sequence of SEQ ID NO: 21.

[00286] In certain embodiments, in the antibodies or antigen-binding fragments thereof provided herein, the first binding moiety comprises HCDR1, HCDR2, HCDR3 contained within a VH amino acid sequence as set forth in SEQ ID NO: 9; and LCDR1, LCDR2 and LCDR3 contained within a VL amino acid sequence of SEQ ID NO: 12; and

each of the second and third binding moieties comprises HCDR1, HCDR2, and HCDR3 contained within a VH amino acid sequence of SEQ ID NO: 22; and LCDR1, LCDR2 and LCDR3 contained within a VL amino acid sequence of SEQ ID NO: 23.

[00287] In certain embodiments, in the antibodies or antigen-binding fragments thereof provided herein, the first binding moiety comprises HCDR1, HCDR2, HCDR3 contained within a VH amino acid sequence as set forth in SEQ ID NO: 9;

and LCDR1, LCDR2 and LCDR3 contained within a VL amino acid sequence of SEQ ID NO: 12; and

each of the second and third binding moieties comprises HCDR1, HCDR2, and HCDR3 contained within a VH amino acid sequence of SEQ ID NO: 30; and LCDR1, LCDR2 and LCDR3 contained within a VL amino acid sequence of SEQ ID NO: 31.

[00288] In certain embodiments, in the antibodies or antigen-binding fragments thereof provided herein, the first binding moiety comprises HCDR1, HCDR2, HCDR3 contained within a VH amino acid sequence as set forth in SEQ ID NO: 9; and LCDR1, LCDR2 and LCDR3 contained within a VL amino acid sequence of SEQ ID NO: 12; and

each of the second and third binding moieties comprises HCDR1, HCDR2, and HCDR3 contained within a VH amino acid sequence of SEQ ID NO: 33; and LCDR1, LCDR2 and LCDR3 contained within a VL amino acid sequence of SEQ ID NO: 34.

[00289] In certain embodiments, in the antibodies or antigen-binding fragments thereof provided herein, the first binding moiety comprises HCDR1, HCDR2, HCDR3 contained within a VH amino acid sequence as set forth in SEQ ID NO: 10; and LCDR1, LCDR2 and LCDR3 contained within a VL amino acid sequence of SEQ ID NO: 12; and

each of the second and third binding moieties comprises HCDR1, HCDR2, and HCDR3 contained within a VH amino acid sequence of SEQ ID NO: 20; and LCDR1, LCDR2 and LCDR3 contained within a VL amino acid sequence of SEQ ID NO: 21.

[00290] In certain embodiments, in the antibodies or antigen-binding fragments thereof provided herein, the first binding moiety comprises HCDR1, HCDR2, HCDR3 contained within a VH amino acid sequence as set forth in SEQ ID NO: 10; and LCDR1, LCDR2 and LCDR3 contained within a VL amino acid sequence of SEQ ID NO: 12; and

each of the second and third binding moieties comprises HCDR1, HCDR2, and HCDR3 contained within a VH amino acid sequence of SEQ ID NO: 22; and LCDR1, LCDR2 and LCDR3 contained within a VL amino acid sequence of SEQ ID NO: 23.

[00291] In certain embodiments, in the antibodies or antigen-binding fragments thereof provided herein, the first binding moiety comprises HCDR1, HCDR2, HCDR3 contained within a VH amino acid sequence as set forth in SEQ ID NO: 10; and LCDR1, LCDR2 and LCDR3 contained within a VL amino acid sequence of SEQ ID NO: 12; and

each of the second and third binding moieties comprises HCDR1, HCDR2, and HCDR3 contained within a VH amino acid sequence of SEQ ID NO: 30; and LCDR1, LCDR2 and LCDR3 contained within a VL amino acid sequence of SEQ ID NO: 31.

[00292] In certain embodiments, in the antibodies or antigen-binding fragments thereof provided herein, the first binding moiety comprises HCDR1, HCDR2, HCDR3 contained within a VH amino acid sequence as set forth in SEQ ID NO: 10; and LCDR1, LCDR2 and LCDR3 contained within a VL amino acid sequence of SEQ ID NO: 12; and

each of the second and third binding moieties comprises HCDR1, HCDR2, and HCDR3 contained within a VH amino acid sequence of SEQ ID NO: 33; and LCDR1, LCDR2 and LCDR3 contained within a VL amino acid sequence of SEQ ID NO: 34.

[00293] In certain embodiments, in the antibodies or antigen-binding fragments thereof provided herein, the first binding moiety comprises HCDR1, HCDR2, HCDR3 contained within a VH amino acid sequence as set forth in SEQ ID NO: 11; and LCDR1, LCDR2 and LCDR3 contained within a VL amino acid sequence of SEQ ID NO: 12; and

each of the second and third binding moieties comprises HCDR1, HCDR2, and HCDR3 contained within a VH amino acid sequence of SEQ ID NO: 20; and LCDR1, LCDR2 and LCDR3 contained within a VL amino acid sequence of SEQ ID NO: 21.

[00294] In certain embodiments, in the antibodies or antigen-binding fragments thereof provided herein, the first binding moiety comprises HCDR1, HCDR2, HCDR3 contained within a VH amino acid sequence as set forth in SEQ ID NO: 11; and LCDR1, LCDR2 and LCDR3 contained within a VL amino acid sequence of SEQ ID NO: 12; and

each of the second and third binding moieties comprises HCDR1, HCDR2, and HCDR3 contained within a VH amino acid sequence of SEQ ID NO: 22; and LCDR1, LCDR2 and LCDR3 contained within a VL amino acid sequence of SEQ ID NO: 23.

[00295] In certain embodiments, in the antibodies or antigen-binding fragments thereof provided herein, the first binding moiety comprises HCDR1, HCDR2, HCDR3 contained within a VH amino acid sequence as set forth in SEQ ID NO: 11; and LCDR1, LCDR2 and LCDR3 contained within a VL amino acid sequence of SEQ ID NO: 12; and

each of the second and third binding moieties comprises HCDR1, HCDR2, and HCDR3 contained within a VH amino acid sequence of SEQ ID NO: 30; and LCDR1, LCDR2 and LCDR3 contained within a VL amino acid sequence of SEQ ID NO: 31.

[00296] In certain embodiments, in the antibodies or antigen-binding fragments thereof provided herein, the first binding moiety comprises HCDR1, HCDR2, HCDR3 contained within a VH amino acid sequence as set forth in SEQ ID NO: 11; and LCDR1, LCDR2 and LCDR3 contained within a VL amino acid sequence of SEQ ID NO: 12; and

each of the second and third binding moieties comprises HCDR1, HCDR2, and HCDR3 contained within a VH amino acid sequence of SEQ ID NO: 33; and LCDR1, LCDR2 and LCDR3 contained within a VL amino acid sequence of SEQ ID NO: 34.

[00297] In certain embodiments, in the antibodies or antigen-binding fragments thereof provided herein, the first binding moiety comprises HCDR1, HCDR2, HCDR3 contained within a VH amino acid sequence as set forth in SEQ ID NO: 9;

and LCDR1, LCDR2 and LCDR3 contained within a VL amino acid sequence of SEQ ID NO: 13; and

each of the second and third binding moieties comprises HCDR1, HCDR2, and HCDR3 contained within a VH amino acid sequence of SEQ ID NO: 20; and LCDR1, LCDR2 and LCDR3 contained within a VL amino acid sequence of SEQ ID NO: 21.

[00298] In certain embodiments, in the antibodies or antigen-binding fragments thereof provided herein, the first binding moiety comprises HCDR1, HCDR2, HCDR3 contained within a VH amino acid sequence as set forth in SEQ ID NO: 9; and LCDR1, LCDR2 and LCDR3 contained within a VL amino acid sequence of SEQ ID NO: 13; and

each of the second and third binding moieties comprises HCDR1, HCDR2, and HCDR3 contained within a VH amino acid sequence of SEQ ID NO: 22; and LCDR1, LCDR2 and LCDR3 contained within a VL amino acid sequence of SEQ ID NO: 23.

[00299] In certain embodiments, in the antibodies or antigen-binding fragments thereof provided herein, the first binding moiety comprises HCDR1, HCDR2, HCDR3 contained within a VH amino acid sequence as set forth in SEQ ID NO: 9; and LCDR1, LCDR2 and LCDR3 contained within a VL amino acid sequence of SEQ ID NO: 13; and

each of the second and third binding moieties comprises HCDR1, HCDR2, and HCDR3 contained within a VH amino acid sequence of SEQ ID NO: 30; and LCDR1, LCDR2 and LCDR3 contained within a VL amino acid sequence of SEQ ID NO: 31.

[00300] In certain embodiments, in the antibodies or antigen-binding fragments thereof provided herein, the first binding moiety comprises HCDR1, HCDR2, HCDR3 contained within a VH amino acid sequence as set forth in SEQ ID NO: 9; and LCDR1, LCDR2 and LCDR3 contained within a VL amino acid sequence of SEQ ID NO: 13; and

each of the second and third binding moieties comprises HCDR1, HCDR2, and HCDR3 contained within a VH amino acid sequence of SEQ ID NO: 33; and LCDR1, LCDR2 and LCDR3 contained within a VL amino acid sequence of SEQ ID NO: 34.

[00301] In certain embodiments, in the antibodies or antigen-binding fragments thereof provided herein, the first binding moiety comprises HCDR1, HCDR2, HCDR3 contained within a VH amino acid sequence as set forth in SEQ ID NO: 47; and LCDR1, LCDR2 and LCDR3 contained within a VL amino acid sequence of SEQ ID NO: 12; and

each of the second and third binding moieties comprises HCDR1, HCDR2, and HCDR3 contained within a VH amino acid sequence of SEQ ID NO: 20; and LCDR1, LCDR2 and LCDR3 contained within a VL amino acid sequence of SEQ ID NO: 21.

[00302] In certain embodiments, in the antibodies or antigen-binding fragments thereof provided herein, the first binding moiety comprises HCDR1, HCDR2, HCDR3 contained within a VH amino acid sequence as set forth in SEQ ID NO: 47; and LCDR1, LCDR2 and LCDR3 contained within a VL amino acid sequence of SEQ ID NO: 12; and

each of the second and third binding moieties comprises HCDR1, HCDR2, and HCDR3 contained within a VH amino acid sequence of SEQ ID NO: 22; and LCDR1, LCDR2 and LCDR3 contained within a VL amino acid sequence of SEQ ID NO: 23.

[00303] In certain embodiments, in the antibodies or antigen-binding fragments thereof provided herein, the first binding moiety comprises HCDR1, HCDR2, HCDR3 contained within a VH amino acid sequence as set forth in SEQ ID NO: 47; and LCDR1, LCDR2 and LCDR3 contained within a VL amino acid sequence of SEQ ID NO: 12; and

each of the second and third binding moieties comprises HCDR1, HCDR2, and HCDR3 contained within a VH amino acid sequence of SEQ ID NO: 30; and LCDR1, LCDR2 and LCDR3 contained within a VL amino acid sequence of SEQ ID NO: 31.

[00304] In certain embodiments, in the antibodies or antigen-binding fragments thereof provided herein, the first binding moiety comprises HCDR1, HCDR2, HCDR3 contained within a VH amino acid sequence as set forth in SEQ ID NO: 47; and LCDR1, LCDR2 and LCDR3 contained within a VL amino acid sequence of SEQ ID NO: 12; and

each of the second and third binding moieties comprises HCDR1, HCDR2, and HCDR3 contained within a VH amino acid sequence of SEQ ID NO: 33; and LCDR1, LCDR2 and LCDR3 contained within a VL amino acid sequence of SEQ ID NO: 34.

[00305] In certain embodiments, an exemplary multi-specific antibody or antigen-binding fragment thereof provided herein comprises:

- (1) a first binding moiety comprising HCDR1, HCDR2, HCDR3, LCDR1, LCDR2, and LCDR3 comprising an amino acid sequence as set forth in SEQ ID NOS: 1, 2, 3, 4, 5 and 6, respectively; a second binding moiety comprising HCDR1, HCDR2, HCDR3, LCDR1, LCDR2, and LCDR3 comprising an amino acid sequence as set forth in SEQ ID NOS: 14, 15, 16, 17, 18 and 19, respectively, and a third binding moiety comprising HCDR1, HCDR2, HCDR3, LCDR1, LCDR2, and LCDR3 comprising an amino acid sequence as set forth in SEQ ID NOS: 14, 15, 16, 17, 18 and 19, respectively;
- (2) a first binding moiety comprising HCDR1, HCDR2, HCDR3, LCDR1, LCDR2, and LCDR3 comprising an amino acid sequence as set forth in SEQ ID NOS: 1, 2, 3, 4, 5 and 6, respectively; a second binding moiety comprising HCDR1, HCDR2, HCDR3, LCDR1, LCDR2, and LCDR3 comprising an amino acid sequence as set forth in SEQ ID NOS: 24, 25, 26, 27, 28 and 29, respectively, and a third binding moiety comprising HCDR1, HCDR2, HCDR3, LCDR1, LCDR2, and LCDR3 comprising an amino acid sequence as set forth in SEQ ID NOS: 24, 25, 26, 27, 28 and 29, respectively;
- (3) a first binding moiety comprising HCDR1, HCDR2, HCDR3, LCDR1, LCDR2, and LCDR3 comprising an amino acid sequence as set forth in SEQ ID NOS: 1, 2,

- 3, 4, 5 and 6, respectively; a second binding moiety comprising HCDR1, HCDR2, HCDR3, LCDR1, LCDR2, and LCDR3 comprising an amino acid sequence as set forth in SEQ ID NOs: 24, 32, 26, 27, 28 and 29, respectively, and a third binding moiety comprising HCDR1, HCDR2, HCDR3, LCDR1, LCDR2, and LCDR3 comprising an amino acid sequence as set forth in SEQ ID NOs: 24, 32, 26, 27, 28 and 29, respectively;
- (4) a first binding moiety comprising HCDR1, HCDR2, HCDR3, LCDR1, LCDR2, and LCDR3 comprising an amino acid sequence as set forth in SEQ ID NOs: 1, 42, 3, 4, 5 and 6, respectively; a second binding moiety comprising HCDR1, HCDR2, HCDR3, LCDR1, LCDR2, and LCDR3 comprising an amino acid sequence as set forth in SEQ ID NOs: 14, 15, 16, 17, 18 and 19, respectively, and a third binding moiety comprising HCDR1, HCDR2, HCDR3, LCDR1, LCDR2, and LCDR3 comprising an amino acid sequence as set forth in SEQ ID NOs: 14, 15, 16, 17, 18 and 19, respectively;
- (5) a first binding moiety comprising HCDR1, HCDR2, HCDR3, LCDR1, LCDR2, and LCDR3 comprising an amino acid sequence as set forth in SEQ ID NOs: 1, 42, 3, 4, 5 and 6, respectively; a second binding moiety comprising HCDR1, HCDR2, HCDR3, LCDR1, LCDR2, and LCDR3 comprising an amino acid sequence as set forth in SEQ ID NOs: 24, 25, 26, 27, 28 and 29, respectively, and a third binding moiety comprising HCDR1, HCDR2, HCDR3, LCDR1, LCDR2, and LCDR3 comprising an amino acid sequence as set forth in SEQ ID NOs: 24, 25, 26, 27, 28 and 29, respectively; or
- (6) a first binding moiety comprising HCDR1, HCDR2, HCDR3, LCDR1, LCDR2, and LCDR3 comprising an amino acid sequence as set forth in SEQ ID NOs: 1, 42, 3, 4, 5 and 6, respectively; a second binding moiety comprising HCDR1, HCDR2, HCDR3, LCDR1, LCDR2, and LCDR3 comprising an amino acid sequence as set forth in SEQ ID NOs: 24, 32, 26, 27, 28 and 29, respectively, and a third binding moiety comprising HCDR1, HCDR2, HCDR3, LCDR1, LCDR2,

and LCDR3 comprising an amino acid sequence as set forth in SEQ ID NOs: 24, 32, 26, 27, 28 and 29, respectively.

FR region

[00306] In some embodiments, the bispecific or multi-specific antibodies or antigen-binding fragments thereof provided herein are humanized. In certain embodiments, the humanized antibodies or antigen-binding fragments thereof provided herein are composed of substantially all human sequences except for the CDR sequences which are non-human. In some embodiments, the variable region FRs, and constant regions if present, are entirely or substantially from human immunoglobulin sequences. The human FR sequences and human constant region sequences may be derived from different human immunoglobulin genes, for example, FR sequences derived from one human antibody and constant region from another human antibody. In some embodiments, the humanized antibody or antigen-binding fragment thereof comprises human heavy chain HFR1, HFR2, HFR3 and HFR4, and/or light chain LFR1, LFR2, LFR3 and LFR4.

[00307] In some embodiments, the FR regions derived from human may comprise the same amino acid sequence as the human immunoglobulin from which it is derived. In some embodiments, one or more amino acid residues of the human FR are substituted with the corresponding residues from the parent non-human antibody. This may be desirable in certain embodiments to make the humanized antibody or its fragment closely approximate the non-human parent antibody structure, so as to optimize binding characteristics (for example, increase binding affinity). In certain embodiments, the humanized antibody or antigen-binding fragment thereof provided herein comprises no more than 10, 9, 8, 7, 6, 5, 4, 3, 2, or 1 amino acid residue substitutions in each of the human FR sequences, or no more than 10, 9, 8, 7, 6, 5, 4, 3, 2, or 1 amino acid residue substitutions in all the FR sequences of a heavy or a light chain variable domain. In some embodiments, such change in amino acid residue could be present in heavy chain FR regions only, in light chain FR regions only, or in both chains. In certain embodiments, one or more amino acids of the human FR

sequences are randomly mutated to increase binding affinity. In certain embodiments, one or more amino acids of the human FR sequences are back mutated to the corresponding amino acid(s) of the parent non-human antibody so as to increase binding affinity.

[00308] In some embodiments, the bispecific or multi-specific antibodies and antigen-binding fragments thereof provided herein comprise all or a portion of the heavy chain variable domain and/or all or a portion of the light chain variable domain. In one embodiment, the bispecific or multi-specific antibody or antigen-binding fragment thereof provided herein is a single domain antibody which consists of all or a portion of the heavy chain variable domain provided herein. More information of such a single domain antibody is available in the art (see, *e.g.*, U.S. Pat. No. 6,248,516).

Fc region

[00309] In certain embodiments, the antibodies or antigen-binding fragments thereof provided herein comprise an Fc region. In certain embodiments, the antibodies or antigen-binding fragments thereof provided herein further comprise an Fc region, optionally an Fc region of human immunoglobulin (Ig), or optionally an Fc region of human IgG. In certain embodiments, the bispecific or multi-specific antibodies or antigen-binding fragments thereof provided herein further comprise a constant region, which optionally further comprises a heavy chain and/or a light chain constant region. In certain embodiments, the heavy chain constant region comprises CH1, hinge, and/or CH2-CH3 regions (or optionally CH2-CH3-CH4 regions). In certain embodiments, the antibodies or antigen-binding fragments thereof provided herein comprise heavy chain constant regions of human IgG1, IgG2, IgG3 or IgG4. In certain embodiments, the first binding moiety and the third binding moiety are linked to the Fc region. In certain embodiments, the bispecific or multi-specific antibodies or antigen-binding fragments thereof provided herein comprise a lambda (λ) light chain or a kappa (κ) light chain. The constant region of the bispecific or multi-specific antibodies or antigen-binding fragments thereof provided herein may be

identical to the wild-type constant region sequence or be different in one or more mutations.

[00310] In certain embodiments, the heavy chain constant region comprises an Fc region. Fc region is known to mediate effector functions such as antibody-dependent cellular cytotoxicity (ADCC) and complement-dependent cytotoxicity (CDC) of the antibody. Fc regions of different Ig isotypes have different abilities to induce effector functions. For example, Fc regions of IgG1 and IgG3 have been recognized to induce both ADCC and CDC more effectively than those of IgG2 and IgG4. In certain embodiments, the bispecific or multi-specific antibodies and antigen-binding fragments thereof provided herein comprises an Fc region of IgG1, or IgG3 isotype, which could induce ADCC or CDC; or alternatively, a constant region of IgG4 or IgG2 isotype, which has reduced or depleted effector function. In some embodiments, the Fc region derived from human IgG1 with enhanced effector functions.

[00311] In certain embodiments, the antibodies or antigen-binding fragments thereof provided herein comprise an Fc region, and both of the first binding moiety and the third binding moiety are linked to the Fc region. In certain embodiments, the antibodies or antigen-binding fragments thereof provided herein comprise an Fc region, and both of the second binding moiety and the third binding moiety are linked to the Fc region.

[00312] In certain embodiments, the antibodies or antigen-binding fragments thereof provided herein comprise, from N-terminus to C-terminus, a first chain comprising a VH region from the second binding moiety, a CH1 from the second binding moiety, a VH region from the first binding moiety, a VL region from the first binding moiety, a CH2 from the Fc region, and a CH3 from the Fc region.

[00313] In certain embodiments, the antibodies or antigen-binding fragments thereof provided herein comprise, from N-terminus to C-terminus, a first chain comprising a VH region from the second binding moiety, a CH1 from the second binding moiety, a

VL region from the first binding moiety, a VH region from the first binding moiety, a CH2 from the Fc region, and a CH3 from the Fc region.

[00314] In certain embodiments, the antibodies or antigen-binding fragments thereof provided herein further comprise a second chain comprising a VL region from the second binding moiety, and a CL from the second binding moiety.

Competing for binding to epitopes

[00315] In certain embodiments, the present disclosure provides an antibody or antigen-binding fragment thereof, which competes for binding to CD3 and/or LILRB4 with the antibody or antigen-binding fragment thereof provided herein. In certain embodiments, the present disclosure provides an antibody or antigen-binding fragment thereof, which competes for binding to human CD3 and/or LILRB4 with any one of the anti-CD3 and/or anti-LILRB4 antibodies provided herein. In some embodiments, the present disclosure provides an antibody or antigen-binding fragment thereof, which competes for the same epitope with the antibody or antigen-binding fragment thereof provided herein.

[00316] The ability to “block binding” or “compete for the same epitope” as used herein refers to the ability of an antibody or antigen-binding fragment to inhibit the binding interaction between two molecules (*e.g.*, human CD3 and an anti-CD3 antibody) to any detectable degree. In certain embodiments, an antibody or antigen-binding fragment that blocks binding between two molecules inhibits the binding interaction between the two molecules by at least 85%, or at least 90%. In certain embodiments, this inhibition may be greater than 85%, or greater than 90%.

[00317] Those skilled in the art will recognize that it is possible to determine, without undue experimentation, if a human monoclonal antibody binds to the same epitope as the antibody of present disclosure by ascertaining whether the former prevents the latter from binding to a CD3 antigen polypeptide and/or LILRB4 antigen polypeptide. If the test antibody competes with the antibody of the present disclosure, as shown by a decrease in binding by the antibody of present disclosure to the CD3 antigen

polypeptide and/or LILRB4 antigen polypeptide, then the two antibodies bind to the same, or a closely related, epitope. Or if the binding of a test antibody to the CD3 antigen polypeptide and/or LILRB4 antigen polypeptide was inhibited by the antibody of the present disclosure, then the two antibodies bind to the same, or a closely related, epitope.

[00318] In certain embodiments, the present disclosure provides bispecific or multi-specific antibodies or antigen-binding fragments thereof which have higher or comparable binding affinity to CD3 (*e.g.*, human CD3 or cynomolgus CD3) and LILRB4 (*e.g.*, human LILRB4) compared with IO-312.

[00319] In certain embodiments, the bispecific antibody or antigen-binding fragment which competes for binding to CD3 and LILRB4 with the antibody or antigen-binding fragment thereof provided herein is not IO-312.

[00320] “IO-312” as used here refers to a bispecific antibody or antigen-binding fragment thereof that binds to CD3 and LILRB4, comprising a first binding moiety that binds to CD3 and a second binding moiety that binds to LILRB4, wherein the first binding moiety that binds to CD3 comprises a heavy chain variable region having an amino acid sequence of SEQ ID NO: 43, a light chain variable region having an amino acid sequence of SEQ ID NO: 44; the second moiety that binds to LILRB4 comprises a heavy chain variable region having an amino acid sequence of SEQ ID NO: 45, and a light chain variable region having an amino acid sequence of SEQ ID NO: 46. The CDR sequences were bold and underlined in SEQ ID NO: 43, SEQ ID NO: 44, SEQ ID NO: 45 and SEQ ID NO: 46, respectively.

QVQLVQSGAEVKKPGSSVKVSCKAS**GFAFTDY**YIHWVRQAPGQGLEWMGW
IS**PGNVN**TKYNENFKGRVTITADKSTSTAYMELSSLRSEDVAVYYCAR**DGYSL**
YYFDYWGQGLTVTVSS (SEQ ID NO: 43)

DIVMTQSPDSLAVSLGERATINC**KSSQSLLNSRTRKNYLA**WYQQKPGQPPL
LIY**WASTRQS**GVPDRFSGSGSGTDFLTISLQAEDVAVYYC**TQSHTLR**TFGG
GTKVEIK (SEQ ID NO: 44)

EVQLVESGGGLVQPGGSLRLSCAASGFSLSSYWISWVRQAPGKGLEWIGSID
SGSVGITYYATWVKGRFTISRDN SKNTLYLQMNSLRAEDTAVYYCARHGDN
WALDLWGQGTLVTVLE (SEQ ID NO: 45)

DIQMTQSPSTLSASVGDRVTITCRASQSISSWLAWYQQKPGKAPKLLIYKAST
LASGVPSRFSGSGSGTEFTLTISSLQPDDFATYYCQHGYIRGDLDNVFGGGTK
 VEIK (SEQ ID NO: 46)

Antibody Variants

[00321] The antibodies and antigen-binding fragments thereof provided herein also encompass various variants of the antibody sequences provided herein.

[00322] In certain embodiments, the antibody variants comprise one or more amino acid residue substitutions or modifications yet retains binding affinity to CD3 and/or LILRB4. In certain embodiments, the first binding moiety, the second binding moiety and/or the third binding moiety of the antibodies or antigen-binding fragments thereof provided herein further comprises one or more amino acid residue substitutions or modifications yet retains specific binding affinity to CD3 or LILRB4. In certain embodiments, at least one of the substitutions or modifications is in one or more of the CDR sequences of the VH region or VL region. In certain embodiments, at least one of the substitutions or modifications is in one or more of the non-CDR sequences of the VH region or VL region. In certain embodiments, at least one of the substitutions or modifications is in one or more of the CDR sequences of the VH region or VL region of the first binding moiety, the second binding moiety, or the third binding moiety. In certain embodiments, at least one of the substitutions or modifications is in one or more of the non-CDR sequences of the VH region or VL region of the first binding moiety, the second binding moiety, or the third binding moiety.

[00323] In certain embodiments, the antibodies or antigen-binding fragments thereof provided herein further comprise one or more non-natural amino acid (NNAA) substitution. In certain embodiments, the NNAA is capable of being conjugated.

[00324] For example, the antibody variants comprise one or more amino acid residue substitutions or modifications in one or more of the CDR sequences provided in Tables 2 and 5 above, one or more of the non-CDR sequences of the heavy chain variable region or light chain variable region provided in Tables 3 and 6 above, and/or the constant region (*e.g.*, Fc region). Such variants retain binding specificity to CD3 and/or LILRB4 of their parent antibodies, but have one or more desirable properties conferred by the modification(s) or substitution(s). For example, the antibody variants may have improved antigen-binding affinity, improved glycosylation pattern, reduced risk of glycosylation, reduced deamination, enhanced effector function(s), improved FcRn receptor binding, increased pharmacokinetic half-life, pH sensitivity, and/or compatibility to conjugation (*e.g.*, one or more introduced cysteine residues), etc.

[00325] The parent antibody sequence may be screened to identify suitable or preferred residues to be modified or substituted, using methods known in the art, for example, “alanine scanning mutagenesis” (see, for example, Cunningham and Wells (1989) *Science*, 244:1081-1085). Briefly, target residues (*e.g.*, charged residues such as Arg, Asp, His, Lys, and Glu) can be identified and replaced by a neutral or negatively charged amino acid (*e.g.*, alanine or polyalanine), and the modified antibodies are produced and screened for the interested property. If substitution at a particular amino acid location demonstrates an interested functional change, then the position can be identified as a potential residue for modification or substitution. The potential residues may be further assessed by substituting with a different type of residue (*e.g.*, cysteine residue, positively charged residue, etc.).

[00326] In certain embodiments, the antibodies or antigen-binding fragments thereof provided herein can be a monoclonal antibody, a polyclonal antibody, a humanized antibody, a human antibody, a chimeric antibody, a recombinant antibody, a bispecific antibody, a multi-specific antibody, a labeled antibody, a bivalent antibody, an anti-idiotypic antibody, or a fusion protein. A recombinant antibody is an antibody prepared *in vitro* using recombinant methods rather than in animals.

Affinity Variants

[00327] Affinity variants of antibodies may contain modifications or substitutions in one or more CDR sequences provided in Tables 2 and 5 above, one or more FR sequences, or the heavy or light chain variable region sequences provided in Tables 3 and 6 above. FR sequences can be readily identified by a person skilled in the art based on the CDR sequences and variable region sequences in Tables 2, 3, 5 and 6 above, as it is well-known in the art that a CDR region is flanked by two FR regions in the variable region. The affinity variants retain specific binding affinity to CD3 and/or LILRB4 of the parent antibody, or even have improved CD3 and/or LILRB4 binding affinity over the parent antibody. In certain embodiments, at least one (or all) of the substitution(s) in the CDR sequences, FR sequences, or variable region sequences comprises a conservative substitution.

[00328] A person skilled in the art will understand that in the CDR sequences and variable region sequences provided in Tables 2, 3, 5 and 6 above, one or more amino acid residues may be substituted yet the resulting antibody or antigen-binding fragment still retain the binding affinity or binding capacity to CD3 and/or LILRB4, or even have an improved binding affinity or capacity. Various methods known in the art can be used to achieve this purpose. For example, a library of antibody variants (such as Fab or scFv variants) can be generated and expressed with phage display technology, and then screened for the binding affinity to human CD3 and/or LILRB4. For another example, computer software can be used to virtually simulate the binding of the antibodies to human CD3 and/or LILRB4, and identify the amino acid residues on the antibodies which form the binding interface. Such residues may be either avoided in the substitution so as to prevent reduction in binding affinity, or targeted for substitution to provide for a stronger binding.

[00329] In certain embodiments, the humanized antibody or antigen-binding fragment thereof provided herein comprises one or more amino acid residue substitutions in one or more of the CDR sequences, and/or one or more of the FR sequences. In certain

embodiments, an affinity variant comprises no more than 20, 15, 10, 9, 8, 7, 6, 5, 4, 3, 2, or 1 substitutions in the CDR sequences and/or FR sequences in total.

[00330] In certain embodiments, the antibodies or antigen-binding fragments thereof provided herein comprise 1, 2, or 3 CDR sequences having at least 80% (*e.g.*, at least 85%, 88%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99%) sequence identity to that (or those) listed in Tables 2 and 5 above yet retaining the specific binding affinity to CD3 and/or LILRB4 at a level similar to or even higher than its parent antibody.

[00331] In certain embodiments, the antibodies or antigen-binding fragments thereof comprise one or more variable region sequences having at least 80% (*e.g.*, at least 85%, 88%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99%) sequence identity to that (or those) listed in Tables 3 and 6 above yet retaining the specific binding affinity to CD3 and/or LILRB4 at a level similar to or even higher than its parent antibody. In some embodiments, a total of 1 to 10 amino acids have been substituted, inserted, or deleted in a variable region sequence listed in Tables 3 and 6 above. In some embodiments, the substitutions, insertions, or deletions occur in regions outside the CDRs (*e.g.*, in the FRs).

Glycosylation Variants

[00332] The antibodies or antigen-binding fragments thereof provided herein also encompass glycosylation variants, which can be obtained to either increase or decrease the extent of glycosylation of the antibodies or antigen-binding fragments thereof.

[00333] The antibodies or antigen-binding fragments thereof provided herein may comprise one or more modifications that introduce or remove a glycosylation site. A glycosylation site is an amino acid residue with a side chain to which a carbohydrate moiety (*e.g.*, an oligosaccharide structure) can be attached. Glycosylation of antibodies is typically either N-linked or O-linked. N-linked refers to the attachment of the carbohydrate moiety to the side chain of an asparagine residue, for example, an

asparagine residue in a tripeptide sequence such as asparagine-X-serine and asparagine-X-threonine, where X is any amino acid except proline. O-linked glycosylation refers to the attachment of one of the sugars N-acylgalactosamine, galactose, or xylose to a hydroxyamino acid, most commonly to serine or threonine. Removal of a native glycosylation site can be conveniently accomplished, for example, by altering the amino acid sequence such that one of the above-described tripeptide sequences (for N-linked glycosylation sites) or serine or threonine residues (for O-linked glycosylation sites) present in the sequence is substituted. A new glycosylation site can be created in a similar way by introducing such a tripeptide sequence or serine or threonine residue.

Cysteine-engineered Variants

[00334] The antibodies or antigen-binding fragments thereof provided herein also encompass cysteine-engineered variants, which comprise one or more introduced free cysteine amino acid residues.

[00335] A free cysteine residue is one which is not part of a disulfide bridge. A cysteine-engineered variant is useful for conjugation with for example, a cytotoxic and/or imaging compound, a label, or a radioisotope among others, at the site of the engineered cysteine, through for example a maleimide or haloacetyl. Methods for engineering antibodies or antigen-binding fragments thereof to introduce free cysteine residues are known in the art, see, for example, WO2006/034488.

Fc Variants

[00336] The antibodies and antigen-binding fragments provided herein also encompass an Fc variant, which comprises one or more amino acid residue modifications or substitutions at its Fc region and/or hinge region.

[00337] In certain embodiments, the antibodies or antigen-binding fragments thereof provided herein comprise one or more amino acid substitution(s) that improves pH-dependent binding to neonatal Fc receptor (FcRn). Such a variant can have an extended pharmacokinetic half-life, as it binds to FcRn at acidic pH which allows it to

escape from degradation in the lysosome and then be translocated and released out of the cell. Methods of engineering an antibody or antigen-binding fragment thereof to improve binding affinity with FcRn are well-known in the art, see, for example, Vaughn, D. *et al.*, *Structure*, 6(1): 63-73, 1998; Kontermann, R. *et al.*, *Antibody Engineering*, Volume 1, Chapter 27: Engineering of the Fc region for improved PK, published by Springer, 2010; Yeung, Y. *et al.*, *Cancer Research*, 70: 3269-3277 (2010); and Hinton, P. *et al.*, *J. Immunology*, 176:346-356 (2006).

[00338] In certain embodiments, the antibodies or antigen-binding fragments thereof provided herein comprise one or more amino acid substitution(s) that alters ADCC. Certain amino acid residues at CH2 domain of the Fc region can be substituted to provide for enhanced ADCC activity. Alternatively or additionally, carbohydrate structures on the antibody can be changed to enhance ADCC activity. Methods of altering ADCC activity by antibody engineering have been described in the art, see for example, Shields RL. *et al.*, *J Biol Chem*. 2001. 276(9): 6591-604; Idusogie EE. *et al.*, *J Immunol*. 2000.164(8):4178-84; Steurer W. *et al.*, *J Immunol*. 1995, 155(3): 1165- 74; Idusogie EE. *et al.*, *J Immunol*. 2001, 166(4): 2571-5; Lazar GA. *et al.*, *PNAS*, 2006, 103(11): 4005-4010; Ryan MC. *et al.*, *Mol. Cancer Ther.*, 2007, 6: 3009-3018; Richards JO., *et al.*, *Mol Cancer Ther*. 2008, 7(8): 2517-27; Shields R. L. *et al.*, *J. Biol. Chem*, 2002, 277: 26733-26740; Shinkawa T. *et al.*, *J. Biol. Chem*, 2003, 278: 3466-3473.

[00339] In certain embodiments, the antibodies or antigen-binding fragments thereof provided herein comprise one or more amino acid substitution(s) that alters CDC, for example, by improving or diminishing C1q binding and/or CDC (see, for example, WO99/51642; Duncan & Winter *Nature* 322:738-40 (1988); U.S. Pat. No. 5,648,260; U.S. Pat. No. 5,624,821); and WO94/29351 concerning other examples of Fc region variants. One or more amino acids selected from amino acid residues 329, 331 and 322 of the Fc region can be replaced with a different amino acid residue to alter C1q binding and/or enhance CDC (see, U.S. Pat. No. 6,194,551 by Idusogie *et al.*). One

or more amino acid substitution(s) can also be introduced to alter the ability of the antibody to fix complement (see PCT Publication WO 94/29351 by Bodmer *et al.*).

[00340] In certain embodiments, the antibodies or antigen-binding fragments thereof provided herein comprise one or more amino acid substitution(s) in human immunoglobulin (*e.g.*, IgG1) at position 234 and/or 235 (according to EU numbering). In certain embodiments, the antibodies or antigen-binding fragments thereof provided herein comprise two amino acid substitutions in human immunoglobulin (*e.g.*, IgG1) at positions 234 and 235 (according to EU numbering). In certain embodiments, the antibodies or antigen-binding fragments thereof provided herein comprise L234A and L235A (according to EU numbering) amino acid substitutions.

[00341] In certain embodiments, the antibodies or antigen-binding fragments thereof provided herein comprise one or more amino acid substitution(s) in the interface of the Fc region to facilitate and/or promote heterodimerization. These modifications comprise introduction of a protuberance into a first Fc polypeptide and a cavity into a second Fc polypeptide, wherein the protuberance can be positioned in the cavity so as to promote interaction of the first and second Fc polypeptides to form a heterodimer or a complex. Methods of generating antibodies with these modifications are known in the art, *e.g.*, as described in U.S. Pat. No. 5,731,168.

[00342] In certain embodiments, the antibodies or antigen-binding fragments thereof provided herein comprise an amino acid substitution at position 366 (according to EU numbering) of a first Fc polypeptide, and comprises one, two or three amino acid substitutions at one, two or three positions of 366, 368, and 407 (according to EU numbering) of a second Fc polypeptide. In certain embodiments, the antibodies or antigen-binding fragments thereof provided herein comprise a T366W substitution (according to EU numbering) of a first Fc polypeptide, and comprise T366S+L368A+Y407V substitutions (according to EU numbering) of a second Fc polypeptide.

[00343] In certain embodiments, the antibodies or antigen-binding fragments thereof provided herein further comprise one or more amino acid substitution(s) of a first Fc polypeptide, and comprise one or more amino acid substitution(s) of a second Fc polypeptide, so as to introduce a non-natural disulfide bond between the two Fc polypeptides. For example, the antibodies or antigen-binding fragments thereof provided herein comprise an amino acid substitution at position 354 (according to EU numbering) of a first Fc polypeptide, and comprise an amino acid substitution at position 349 (according to EU numbering) of a second Fc polypeptide. In certain embodiments, the antibodies or antigen-binding fragments thereof provided herein comprise S354C substitution (according to EU numbering) of a first Fc polypeptide, and comprise Y349C substitution (according to EU numbering) of a second Fc polypeptide.

[00344] In certain embodiments, the antibodies or antigen-binding fragments thereof provided herein comprises one or more (*e.g.*, 2, 3, 4, 5, 6, 7 or 8) amino acid substitutions selected from the group consisting of: L234A, L235A, S354C, T366W, Y349C, T366S, L368A, and Y407V (according to EU numbering). In certain embodiments, the antibodies or antigen-binding fragments thereof provided herein comprise S354C and T366W (according to EU numbering) amino acid substitutions of a first Fc polypeptide, and comprise Y349C, T366S, L368A, and Y407V (according to EU numbering) amino acid substitutions of a second Fc polypeptide.

[00345] In certain embodiments, the antibodies or antigen-binding fragments thereof provided herein comprise a first Fc polypeptide comprising the amino acid sequence as set forth in SEQ ID NO: 40, and a second Fc polypeptide comprising the amino acid sequence as set forth in SEQ ID NO: 41.

ASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTF
PAVLQSSGLYSLSSVVTVPSSSLGTQTYICNVNHKPSNTKVDKKVEPKSCDKT
HTCPPCPAPEAAGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFN
WYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNK
ALPAPIEKTISKAKGQPREPQVYTLPPCREEMTKNQVSLWCLVKGFYPSDIAV

EWESNGQPENNYKTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHE
ALHNHYTQKSLSLSPGK (SEQ ID NO: 40)

DKTHTCPPCPAPEAAGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEV
KFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKV
SNKALPAPIEKTISKAKGQPREPQVCTLPSSREEMTKNQVSLSCAVKGFYPSDI
AVEWESNGQPENNYKTPPVLDSDGSFFLVSKLTVDKSRWQQGNVFSCSVM
HEALHNHYTQKSLSLSPGK (SEQ ID NO: 41)

Antigen-binding Fragments

[00346] Provided herein are also anti-CD3 and/or LILRB4 antigen-binding fragments. Various types of antigen-binding fragments are known in the art and can be developed based on the antibodies provided herein, including for example, the exemplary antibodies whose CDRs are shown in Tables 2 and 5 above, and their different variants (such as affinity variants, glycosylation variants, Fc variants, cysteine-engineered variants and so on).

[00347] In certain embodiments, an anti-CD3 or anti-LILRB4 antigen-binding fragment provided herein is a diabody, a Fab, a Fab', a F(ab')₂, a Fd, an Fv fragment, a disulfide stabilized Fv fragment (dsFv), a (dsFv)₂, a dsFv (dsFv-dsFv'), a disulfide stabilized diabody (ds diabody), a single-chain antibody molecule (scFv), an scFv dimer (bivalent diabody), a camelized single domain antibody, a nanobody, a domain antibody, or a bivalent domain antibody.

[00348] Various techniques can be used for the production of such antigen-binding fragments. Illustrative methods include, enzymatic digestion of intact antibodies (see, e.g., Morimoto *et al.*, *Journal of Biochemical and Biophysical Methods* 24:107-117 (1992); and Brennan *et al.*, *Science*, 229:81 (1985)), recombinant expression by host cells such as *E. Coli* (e.g., for Fab, Fv and ScFv antibody fragments), screening from a phage display library as discussed above (e.g., for ScFv), and chemical coupling of two Fab'-SH fragments to form F(ab')₂ fragments (Carter *et al.*,

Bio/Technology 10:163-167 (1992)). Other techniques for the production of antibody fragments will be apparent to a person skilled in the art.

[00349] In certain embodiments, the antigen-binding fragment is a scFv. Generation of scFv is described in, for example, WO 93/16185; U.S. Pat. Nos. 5,571,894; and 5,587,458. ScFv may be fused to an effector protein at either the amino or the carboxyl terminus to provide for a fusion protein (see, for example, *Antibody Engineering*, ed. Borrebaeck).

[00350] In certain embodiments, the antibodies or antigen-binding fragments thereof provided herein are bivalent, tetravalent, hexavalent, or multivalent. Any molecule being more than bivalent is considered multivalent, encompassing for example, trivalent, tetravalent, hexavalent, and so on. In certain embodiments, the antibodies or antigen-binding fragments thereof provided herein are multi-specific, e.g., bispecific or tri-specific.

[00351] A bivalent molecule can be monospecific if the two binding sites are both specific for binding to the same antigen or the same epitope. This, in certain embodiments, provides for stronger binding to the antigen or the epitope than a monovalent counterpart. Similar, a multivalent molecule may also be monospecific. In certain embodiments, in a bivalent or multivalent antigen-binding moiety, the first valent of binding site and the second valent of binding site are structurally identical (*i.e.*, having the same sequences), or structurally different (*i.e.*, having different sequences albeit with the same specificity).

[00352] A bivalent can be bispecific, if the two binding sites are specific for different antigens or epitopes. This also applies to a multivalent molecule. For example, a trivalent molecule can be bispecific when two binding sites are monospecific for a first antigen (or epitope) and the third binding site is specific for a second antigen (or epitope).

Conjugates

[00353] In some embodiments, the antibodies or antigen-binding fragments thereof provided herein further comprise one or more conjugate moieties. The conjugate moiety can be linked to the antibodies or antigen-binding fragments thereof. A conjugate moiety is a moiety that can be attached to the antibody or antigen-binding fragment thereof. It is contemplated that a variety of conjugate moieties may be linked to the antibodies or antigen-binding fragments thereof provided herein (see, for example, “Conjugate Vaccines”, Contributions to Microbiology and Immunology, J. M. Cruse and R. E. Lewis, Jr. (eds.), Carger Press, New York, (1989)). These conjugate moieties may be linked to the antibodies or antigen-binding fragments thereof by covalent binding (*e.g.*, disulfide bond), affinity binding, intercalation, coordinate binding, complexation, association, blending, or addition, among other methods. In some embodiments, the antibodies or antigen-binding fragments thereof can be linked to one or more conjugates via a linker or a crosslinking agent. The linker or crosslinking agent comprises a reactive chemical group that can react with the antibodies or fragments thereof. The reactive chemical groups can be N-succinimidyl esters and N-sulfosuccinimidyl esters. Additionally, the linker comprises a reactive chemical group, which can be a dithiopyridyl group that can react with the drug to form a disulfide bond. Linker molecules include, for example, N-succinimidyl 4-(maleimidomethyl) cyclohexanecarboxylate (SMCC), N-succinimidyl 3-(2-pyridyldithio) propionate (SPDP) (see, *e.g.*, Carlsson *et al.*, *Biochem. J.*, 173: 723-737 (1978)), N-succinimidyl 4-(2-pyridyldithio)butanoate (SPDB) (see, *e.g.*, U.S. Patent No. 4,563,304), N-succinimidyl 4-(2-pyridyldithio)2-sulfobutanoate (sulfo-SPDB) (see US Publication No. 20090274713), N-succinimidyl 4-(2-pyridyldithio) pentanoate (SPP) (see, *e.g.*, CAS Registry number 341498-08-6), 2-iminothiolane, or acetylsuccinic anhydride. For example, the antibody or cell binding agent can be modified with crosslinking reagents and the antibody or cell binding agent containing free or protected thiol groups thus derived is then reacted with a disulfide- or thiol-containing maytansinoid to produce conjugates. The conjugates can be purified by chromatography, including but not limited to HPLC,

size-exclusion, adsorption, ion exchange and affinity capture, dialysis or tangential flow filtration.

[00354] In certain embodiments, the antibodies or antigen-binding fragments thereof provided herein may be engineered to contain specific sites outside the epitope binding portion that may be utilized for binding to one or more conjugate moieties. For example, such a site may include one or more reactive amino acid residues, such as for example cysteine or histidine residues, to facilitate covalent linkage to a conjugate moiety.

[00355] In certain embodiments, the antibodies or antigen-binding fragments thereof provided herein may be linked to a conjugate moiety indirectly, or through another conjugate moiety. For example, the antibodies or antigen-binding fragments thereof provided herein may be conjugated to biotin, then indirectly conjugated to a second conjugate that is conjugated to avidin. In some embodiments, the conjugate moiety comprises a clearance-modifying agent (*e.g.*, a polymer such as PEG which extends half-life), a chemotherapeutic agent, a toxin, a radioactive isotope, a lanthanide, a detectable label (*e.g.*, a luminescent label, a fluorescent label, an enzyme-substrate label), a DNA-alkylator, a topoisomerase inhibitor, a tubulin-binder, a purification moiety or other anticancer drugs (*e.g.*, agonist of toll-like receptor 7 (TLR-7), TLR-8 and/or TLR-9, siRNA, antibody or antigen-binding fragments thereof, a peptide (such as a short peptide), etc.).

[00356] A “toxin” can be any agent that is detrimental to cells or that can damage or kill cells. Examples of toxin include, without limitation, taxol, taxoids, CC-1065 and CC-1065 analogs, duocarmycins and duocarmycin analogs, enediynes such as calicheamicins, dolastatin and dolastatin analogs including auristatins, tomaymycin derivatives, leptomycin derivatives, cisplatin, carboplatin, daunorubicin, doxorubicin, vincristine, vinblastine, melphalan, mitomycin C, chlorambucil and morpholino doxorubicin, cytochalasin B, gramicidin D, ethidium bromide, emetine, mitomycin, etoposide, tenoposide, vincristine, MMAE, MMAF, DM1, DM4, vinblastine, colchicin, doxorubicin, daunorubicin, dihydroxy anthracin dione, mitoxantrone,

mithramycin, actinomycin D, 1-dehydrotestosterone, glucocorticoids, procaine, tetracaine, lidocaine, propranolol, puromycin and analogs thereof, antimetabolites (*e.g.*, methotrexate, 6-mercaptopurine, 6-thioguanine, cytarabine, 5-fluorouracil decarbazine), alkylating agents (*e.g.*, mechlorethamine, thioepa chlorambucil, melphalan, carmustine (BSNU) and lomustine (CCNU), cyclophosphamide, busulfan, dibromomannitol, streptozotocin, mitomycin C, and cis-dichlorodiamine platinum (II) (DDP) cisplatin), anthracyclines (*e.g.*, daunorubicin (formerly daunomycin) and doxorubicin), antibiotics (*e.g.*, dactinomycin (formerly actinomycin), bleomycin, mithramycin, and anthramycin (AMC)), anti-mitotic agents (*e.g.*, vincristine and vinblastine), a topoisomerase inhibitor, and a tubulin-binders.

[00357] Examples of detectable labels may include a fluorescent label (*e.g.*, fluorescein, rhodamine, dansyl, phycoerythrin, or Texas Red), an enzyme-substrate label (*e.g.*, horseradish peroxidase, alkaline phosphatase, luciferases, glucoamylase, lysozyme, saccharide oxidases or β -D-galactosidase), a radioisotope (*e.g.*, ^{123}I , ^{124}I , ^{125}I , ^{131}I , ^{35}S , ^3H , ^{111}In , ^{112}In , ^{14}C , ^{64}Cu , ^{67}Cu , ^{86}Y , ^{88}Y , ^{90}Y , ^{177}Lu , ^{211}At , ^{186}Re , ^{188}Re , ^{153}Sm , ^{212}Bi , and ^{32}P , other lanthanides), a luminescent label, a chromophoric moiety, digoxigenin, biotin/avidin, a DNA molecule or gold for detection.

[00358] In certain embodiments, the conjugate moiety can be a clearance-modifying agent which helps increase half-life of the antibody. Illustrative examples include water-soluble polymers, such as PEG, carboxymethylcellulose, dextran, polyvinyl alcohol, polyvinyl pyrrolidone, copolymers of ethylene glycol/propylene glycol, and the like. The polymer may be of any molecular weight, and may be branched or unbranched. The number of polymers attached to the antibody may vary, and if more than one polymers are attached, they can be the same or different molecules.

[00359] In certain embodiments, the conjugate moiety can be a purification moiety such as a magnetic bead.

[00360] In certain embodiments, the antibody or an antigen-binding fragment thereof provided herein is used as a base for a conjugate.

[00361] In certain embodiments, the antibody or an antigen-binding fragment thereof provided herein is conjugated to a signal peptide. A signal peptide (sometimes referred to as signal sequence, leader sequence or leader peptide) can be used to facilitate secretion and isolation of the antibodies or antigen-binding fragments thereof provided herein. Signal peptides are typically characterized by a core of hydrophobic amino acids which are generally cleaved from the mature protein during secretion in one or more cleavage events. Such signal peptides contain processing sites that allow cleavage of the signal sequence from the mature proteins as they pass through the secretory pathway. Thus, the invention pertains to the described polypeptides having a signal sequence, as well as to polypeptides from which the signal sequence has been proteolytically cleaved (*i.e.*, the cleavage products). In one embodiment, a nucleic acid sequence encoding a signal sequence can be operably linked in an expression vector to a protein of interest, such as a protein which is ordinarily not secreted or is otherwise difficult to isolate. The signal sequence directs secretion of the protein, such as from a eukaryotic host into which the expression vector is transformed, and the signal sequence is subsequently or concurrently cleaved. The protein can then be readily purified from the extracellular medium by art recognized methods. Alternatively, the signal sequence can be linked to the protein of interest using a sequence which facilitates purification, such as with a GST domain.

Fourth binding moiety

[00362] In some embodiments, the conjugate moiety is an antigen-binding fragment of an antibody. For example, the conjugate moiety can be a scFv. In some embodiments, the scFv is a fourth binding moiety that binds to LILRB4. The descriptions under the Section Second binding moiety and the Section Third binding moiety are also applicable to the fourth binding moiety, and would not be discussed in details herein.

[00363] In some embodiments, the second and third binding moieties of the antibodies or antigen-binding fragments thereof provided herein bind to a first epitope

on LILRB4, while the fourth binding moiety binds to a second epitope on LILRB4. In some embodiments, the first and second epitopes on LILRB4 are different from each other. In some embodiments, the first and second epitopes on LILRB4 are the same, i.e., the second, third and fourth binding moieties bind to the same epitope on LILRB4.

[00364] In some embodiments, the amino acid sequence of the second binding moiety is the same as the amino acid sequence of the third binding moiety, while the amino acid sequence of the fourth binding moiety is different from the amino acid sequence of the second and third binding moieties.

[00365] In some embodiments, the amino acid sequence of the second binding moiety is the same as the amino acid sequence of the fourth binding moiety, while the amino acid sequence of the third binding moiety is different from the amino acid sequence of the second and fourth binding moieties.

[00366] In some embodiments, the amino acid sequence of the third binding moiety is the same as the amino acid sequence of the fourth binding moiety, while the amino acid sequence of the second binding moiety is different from the amino acid sequence of the third and fourth binding moieties.

[00367] In some embodiments, the amino acid sequences of the second, third and fourth binding moieties are identical to each other.

[00368] In some embodiments, the conjugate moiety is covalently attached to the antibodies or antigen-binding fragments thereof provided herein directly (*i.e.*, without a linker). In some embodiments, the conjugate moiety is covalently attached to the antibodies or antigen-binding fragments thereof provided herein via a linker. In some embodiments, the linker is a linker comprising glycine and serine, *e.g.*, a (GGGGS)₂ linker or a GSSS linker. In some embodiments, the fourth binding moiety that binds to LILRB4 is an anti-LILRB4 scFv, which is covalently attached to the antibodies or antigen-binding fragments thereof provided herein via a linker, *e.g.*, a (GGGGS)₂ linker or a GSSS linker.

[00369] In some embodiments, the antibodies or antigen-binding fragments thereof provided herein comprise, from N-terminus to C-terminus, a first chain comprising a VH region from the second binding moiety, a CH1 from the second binding moiety, a VH region from the first binding moiety, a VL region from the first binding moiety, a CH2 from the Fc region, a CH3 from the Fc region, a VH region from the fourth binding moiety, and a VL region from the fourth binding moiety.

[00370] In some embodiments, the antibodies or antigen-binding fragments thereof provided herein comprise, from N-terminus to C-terminus, a first chain comprising a VH region from the second binding moiety, a CH1 from the second binding moiety, a VH region from the first binding moiety, a VL region from the first binding moiety, a CH2 from the Fc region, a CH3 from the Fc region, a VL region from the fourth binding moiety, and a VH region from the fourth binding moiety.

[00371] In some embodiments, the first binding moiety of the antibody or antigen-binding fragment thereof provided herein comprises HCDR1, HCDR2, HCDR3, LCDR1, LCDR2 and LCDR3 contained within a VH/VL amino acid sequence pair of SEQ ID NOs: 7/8, 7/12, 7/13, 9/8, 9/12, 9/13, 10/8, 10/12, 10/13, 47/8, 47/12, 47/13, 11/8, 11/12 or 11/13; each of the second and third binding moieties of the antibody or antigen-binding fragment thereof provided herein comprises HCDR1, HCDR2, HCDR3, LCDR1, LCDR2 and LCDR3 contained within a VH/VL amino acid sequence pair of SEQ ID NOs: 30/31 or 33/34; and the fourth binding moiety of the antibody or antigen-binding fragment thereof provided herein comprises HCDR1, HCDR2, HCDR3, LCDR1, LCDR2 and LCDR3 contained within a VH/VL amino acid sequence pair of SEQ ID NOs: 20/21 or 22/23.

[00372] In some embodiments, the first binding moiety of the antibody or antigen-binding fragment thereof provided herein comprises HCDR1, HCDR2, HCDR3, LCDR1, LCDR2 and LCDR3 contained within a VH/VL amino acid sequence pair of SEQ ID NOs: 7/8, 7/12, 7/13, 9/8, 9/12, 9/13, 10/8, 10/12, 10/13, 47/8, 47/12, 47/13, 11/8, 11/12 or 11/13; each of the second and third binding moieties of the antibody or antigen-binding fragment thereof provided herein comprises HCDR1, HCDR2,

HCDR3, LCDR1, LCDR2 and LCDR3 contained within a VH/VL amino acid sequence pair of SEQ ID NOs: 20/21 or 22/23; and the fourth binding moiety of the antibody or antigen-binding fragment thereof provided herein comprises HCDR1, HCDR2, HCDR3, LCDR1, LCDR2 and LCDR3 contained within a VH/VL amino acid sequence pair of SEQ ID NOs: 30/31 or 33/34.

[00373] In some embodiments, the first binding moiety of the antibody or antigen-binding fragment thereof provided herein comprises HCDR1, HCDR2, HCDR3, LCDR1, LCDR2 and LCDR3 contained within a VH/VL amino acid sequence pair of SEQ ID NOs: 7/8, 10/12 or 47/12; each of the second and third binding moieties of the antibody or antigen-binding fragment thereof provided herein comprises HCDR1, HCDR2, HCDR3, LCDR1, LCDR2 and LCDR3 contained within a VH/VL amino acid sequence pair of SEQ ID NOs: 30/31 or 33/34; and the fourth binding moiety of the antibody or antigen-binding fragment thereof provided herein comprises HCDR1, HCDR2, HCDR3, LCDR1, LCDR2 and LCDR3 contained within a VH/VL amino acid sequence pair of SEQ ID NOs: 20/21 or 22/23.

[00374] In some embodiments, the first binding moiety of the antibody or antigen-binding fragment thereof provided herein comprises HCDR1, HCDR2, HCDR3, LCDR1, LCDR2 and LCDR3 contained within a VH/VL amino acid sequence pair of SEQ ID NOs: 7/8, 10/12 or 47/12; each of the second and third binding moieties of the antibody or antigen-binding fragment thereof provided herein comprises HCDR1, HCDR2, HCDR3, LCDR1, LCDR2 and LCDR3 contained within a VH/VL amino acid sequence pair of SEQ ID NOs: 20/21 or 22/23; and the fourth binding moiety of the antibody or antigen-binding fragment thereof provided herein comprises HCDR1, HCDR2, HCDR3, LCDR1, LCDR2 and LCDR3 contained within a VH/VL amino acid sequence pair of SEQ ID NOs: 30/31 or 33/34.

[00375] In some embodiments, the first binding moiety of the antibody or antigen-binding fragment thereof provided herein comprises HCDR1, HCDR2, HCDR3, LCDR1, LCDR2 and LCDR3 contained within a VH/VL amino acid sequence pair of SEQ ID NO: 7/8; each of the second and third binding moieties of the antibody or

antigen-binding fragment thereof provided herein comprises HCDR1, HCDR2, HCDR3, LCDR1, LCDR2 and LCDR3 contained within a VH/VL amino acid sequence pair of SEQ ID NO: 33/34; and the fourth binding moiety of the antibody or antigen-binding fragment thereof provided herein comprises HCDR1, HCDR2, HCDR3, LCDR1, LCDR2 and LCDR3 contained within a VH/VL amino acid sequence pair of SEQ ID NO: 22/23.

[00376] In some embodiments, the first binding moiety of the antibody or antigen-binding fragment thereof provided herein comprises HCDR1, HCDR2, HCDR3, LCDR1, LCDR2 and LCDR3 contained within a VH/VL amino acid sequence pair of SEQ ID NO: 7/8; each of the second and third binding moieties of the antibody or antigen-binding fragment thereof provided herein comprises HCDR1, HCDR2, HCDR3, LCDR1, LCDR2 and LCDR3 contained within a VH/VL amino acid sequence pair of SEQ ID NO: 22/23; and the fourth binding moiety of the antibody or antigen-binding fragment thereof provided herein comprises HCDR1, HCDR2, HCDR3, LCDR1, LCDR2 and LCDR3 contained within a VH/VL amino acid sequence pair of SEQ ID NO: 33/34.

[00377] In some embodiments, the first binding moiety of the antibody or antigen-binding fragment thereof provided herein comprises HCDR1, HCDR2, HCDR3, LCDR1, LCDR2 and LCDR3 contained within a VH/VL amino acid sequence pair of SEQ ID NO: 10/12; each of the second and third binding moieties of the antibody or antigen-binding fragment thereof provided herein comprises HCDR1, HCDR2, HCDR3, LCDR1, LCDR2 and LCDR3 contained within a VH/VL amino acid sequence pair of SEQ ID NO: 33/34; and the fourth binding moiety of the antibody or antigen-binding fragment thereof provided herein comprises HCDR1, HCDR2, HCDR3, LCDR1, LCDR2 and LCDR3 contained within a VH/VL amino acid sequence pair of SEQ ID NO: 22/23.

[00378] In some embodiments, the first binding moiety of the antibody or antigen-binding fragment thereof provided herein comprises HCDR1, HCDR2, HCDR3, LCDR1, LCDR2 and LCDR3 contained within a VH/VL amino acid sequence pair of

SEQ ID NO: 10/12; each of the second and third binding moieties of the antibody or antigen-binding fragment thereof provided herein comprises HCDR1, HCDR2, HCDR3, LCDR1, LCDR2 and LCDR3 contained within a VH/VL amino acid sequence pair of SEQ ID NO: 22/23; and the fourth binding moiety of the antibody or antigen-binding fragment thereof provided herein comprises HCDR1, HCDR2, HCDR3, LCDR1, LCDR2 and LCDR3 contained within a VH/VL amino acid sequence pair of SEQ ID NO: 33/34.

[00379] In some embodiments, the first binding moiety of the antibody or antigen-binding fragment thereof provided herein comprises HCDR1, HCDR2, HCDR3, LCDR1, LCDR2 and LCDR3 contained within a VH/VL amino acid sequence pair of SEQ ID NO: 47/12; each of the second and third binding moieties of the antibody or antigen-binding fragment thereof provided herein comprises HCDR1, HCDR2, HCDR3, LCDR1, LCDR2 and LCDR3 contained within a VH/VL amino acid sequence pair of SEQ ID NO: 33/34; and the fourth binding moiety of the antibody or antigen-binding fragment thereof provided herein comprises HCDR1, HCDR2, HCDR3, LCDR1, LCDR2 and LCDR3 contained within a VH/VL amino acid sequence pair of SEQ ID NO: 22/23.

[00380] In some embodiments, the first binding moiety of the antibody or antigen-binding fragment thereof provided herein comprises HCDR1, HCDR2, HCDR3, LCDR1, LCDR2 and LCDR3 contained within a VH/VL amino acid sequence pair of SEQ ID NO: 47/12; each of the second and third binding moieties of the antibody or antigen-binding fragment thereof provided herein comprises HCDR1, HCDR2, HCDR3, LCDR1, LCDR2 and LCDR3 contained within a VH/VL amino acid sequence pair of SEQ ID NO: 22/23; and the fourth binding moiety of the antibody or antigen-binding fragment thereof provided herein comprises HCDR1, HCDR2, HCDR3, LCDR1, LCDR2 and LCDR3 contained within a VH/VL amino acid sequence pair of SEQ ID NO: 33/34.

[00381] In some embodiments, the first binding moiety of the antibody or antigen-binding fragment thereof provided herein comprises HCDR1, HCDR2, HCDR3,

LCDR1, LCDR2 and LCDR3 comprising an amino acid sequence as set forth in SEQ ID NO: 1, SEQ ID NO: 2, SEQ ID NO: 3, SEQ ID NO: 4, SEQ ID NO: 5, and SEQ ID NO: 6, respectively; each of the second and third binding moieties of the antibody or antigen-binding fragment thereof provided herein comprises HCDR1, HCDR2, HCDR3, LCDR1, LCDR2 and LCDR3 comprising an amino acid sequence as set forth in SEQ ID NO: 14, SEQ ID NO: 15, SEQ ID NO: 16, SEQ ID NO: 17, SEQ ID NO: 18, and SEQ ID NO: 19, respectively; and the fourth binding moiety of the antibody or antigen-binding fragment thereof provided herein comprises HCDR1, HCDR2, HCDR3, LCDR1, LCDR2 and LCDR3 comprising an amino acid sequence as set forth in SEQ ID NO: 24, SEQ ID NO: 25, SEQ ID NO: 26, SEQ ID NO: 27, SEQ ID NO: 28, and SEQ ID NO: 29, respectively.

[00382] In some embodiments, the first binding moiety of the antibody or antigen-binding fragment thereof provided herein comprises HCDR1, HCDR2, HCDR3, LCDR1, LCDR2 and LCDR3 comprising an amino acid sequence as set forth in SEQ ID NO: 1, SEQ ID NO: 2, SEQ ID NO: 3, SEQ ID NO: 4, SEQ ID NO: 5, and SEQ ID NO: 6, respectively; each of the second and third binding moieties of the antibody or antigen-binding fragment thereof provided herein comprises HCDR1, HCDR2, HCDR3, LCDR1, LCDR2 and LCDR3 comprising an amino acid sequence as set forth in SEQ ID NO: 14, SEQ ID NO: 15, SEQ ID NO: 16, SEQ ID NO: 17, SEQ ID NO: 18, and SEQ ID NO: 19, respectively; and the fourth binding moiety of the antibody or antigen-binding fragment thereof provided herein comprises HCDR1, HCDR2, HCDR3, LCDR1, LCDR2 and LCDR3 comprising an amino acid sequence as set forth in SEQ ID NO: 24, SEQ ID NO: 32, SEQ ID NO: 26, SEQ ID NO: 27, SEQ ID NO: 28, and SEQ ID NO: 29, respectively.

[00383] In some embodiments, the first binding moiety of the antibody or antigen-binding fragment thereof provided herein comprises HCDR1, HCDR2, HCDR3, LCDR1, LCDR2 and LCDR3 comprising an amino acid sequence as set forth in SEQ ID NO: 1, SEQ ID NO: 2, SEQ ID NO: 3, SEQ ID NO: 4, SEQ ID NO: 5, and SEQ ID NO: 6, respectively; each of the second and third binding moieties of the antibody

or antigen-binding fragment thereof provided herein comprises HCDR1, HCDR2, HCDR3, LCDR1, LCDR2 and LCDR3 comprising an amino acid sequence as set forth in SEQ ID NO: 24, SEQ ID NO: 25, SEQ ID NO: 26, SEQ ID NO: 27, SEQ ID NO: 28, and SEQ ID NO: 29, respectively; and the fourth binding moiety of the antibody or antigen-binding fragment thereof provided herein comprises HCDR1, HCDR2, HCDR3, LCDR1, LCDR2 and LCDR3 comprising an amino acid sequence as set forth in SEQ ID NO: 14, SEQ ID NO: 15, SEQ ID NO: 16, SEQ ID NO: 17, SEQ ID NO: 18, and SEQ ID NO: 19, respectively.

[00384] In some embodiments, the first binding moiety of the antibody or antigen-binding fragment thereof provided herein comprises HCDR1, HCDR2, HCDR3, LCDR1, LCDR2 and LCDR3 comprising an amino acid sequence as set forth in SEQ ID NO: 1, SEQ ID NO: 2, SEQ ID NO: 3, SEQ ID NO: 4, SEQ ID NO: 5, and SEQ ID NO: 6, respectively; each of the second and third binding moieties of the antibody or antigen-binding fragment thereof provided herein comprises HCDR1, HCDR2, HCDR3, LCDR1, LCDR2 and LCDR3 comprising an amino acid sequence as set forth in SEQ ID NO: 24, SEQ ID NO: 32, SEQ ID NO: 26, SEQ ID NO: 27, SEQ ID NO: 28, and SEQ ID NO: 29, respectively; and the fourth binding moiety of the antibody or antigen-binding fragment thereof provided herein comprises HCDR1, HCDR2, HCDR3, LCDR1, LCDR2 and LCDR3 comprising an amino acid sequence as set forth in SEQ ID NO: 14, SEQ ID NO: 15, SEQ ID NO: 16, SEQ ID NO: 17, SEQ ID NO: 18, and SEQ ID NO: 19, respectively.

[00385] In some embodiments, the first binding moiety of the antibody or antigen-binding fragment thereof provided herein comprises HCDR1, HCDR2, HCDR3, LCDR1, LCDR2 and LCDR3 comprising an amino acid sequence as set forth in SEQ ID NO: 1, SEQ ID NO: 42, SEQ ID NO: 3, SEQ ID NO: 4, SEQ ID NO: 5, and SEQ ID NO: 6, respectively; each of the second and third binding moieties of the antibody or antigen-binding fragment thereof provided herein comprises HCDR1, HCDR2, HCDR3, LCDR1, LCDR2 and LCDR3 comprising an amino acid sequence as set forth in SEQ ID NO: 14, SEQ ID NO: 15, SEQ ID NO: 16, SEQ ID NO: 17, SEQ ID

NO: 18, and SEQ ID NO: 19, respectively; and the fourth binding moiety of the antibody or antigen-binding fragment thereof provided herein comprises HCDR1, HCDR2, HCDR3, LCDR1, LCDR2 and LCDR3 comprising an amino acid sequence as set forth in SEQ ID NO: 24, SEQ ID NO: 25, SEQ ID NO: 26, SEQ ID NO: 27, SEQ ID NO: 28, and SEQ ID NO: 29, respectively.

[00386] In some embodiments, the first binding moiety of the antibody or antigen-binding fragment thereof provided herein comprises HCDR1, HCDR2, HCDR3, LCDR1, LCDR2 and LCDR3 comprising an amino acid sequence as set forth in SEQ ID NO: 1, SEQ ID NO: 42, SEQ ID NO: 3, SEQ ID NO: 4, SEQ ID NO: 5, and SEQ ID NO: 6, respectively; each of the second and third binding moieties of the antibody or antigen-binding fragment thereof provided herein comprises HCDR1, HCDR2, HCDR3, LCDR1, LCDR2 and LCDR3 comprising an amino acid sequence as set forth in SEQ ID NO: 14, SEQ ID NO: 15, SEQ ID NO: 16, SEQ ID NO: 17, SEQ ID NO: 18, and SEQ ID NO: 19, respectively; and the fourth binding moiety of the antibody or antigen-binding fragment thereof provided herein comprises HCDR1, HCDR2, HCDR3, LCDR1, LCDR2 and LCDR3 comprising an amino acid sequence as set forth in SEQ ID NO: 24, SEQ ID NO: 32, SEQ ID NO: 26, SEQ ID NO: 27, SEQ ID NO: 28, and SEQ ID NO: 29, respectively.

[00387] In some embodiments, the first binding moiety of the antibody or antigen-binding fragment thereof provided herein comprises HCDR1, HCDR2, HCDR3, LCDR1, LCDR2 and LCDR3 comprising an amino acid sequence as set forth in SEQ ID NO: 1, SEQ ID NO: 42, SEQ ID NO: 3, SEQ ID NO: 4, SEQ ID NO: 5, and SEQ ID NO: 6, respectively; each of the second and third binding moieties of the antibody or antigen-binding fragment thereof provided herein comprises HCDR1, HCDR2, HCDR3, LCDR1, LCDR2 and LCDR3 comprising an amino acid sequence as set forth in SEQ ID NO: 24, SEQ ID NO: 25, SEQ ID NO: 26, SEQ ID NO: 27, SEQ ID NO: 28, and SEQ ID NO: 29, respectively; and the fourth binding moiety of the antibody or antigen-binding fragment thereof provided herein comprises HCDR1, HCDR2, HCDR3, LCDR1, LCDR2 and LCDR3 comprising an amino acid sequence

as set forth in SEQ ID NO: 14, SEQ ID NO: 15, SEQ ID NO: 16, SEQ ID NO: 17, SEQ ID NO: 18, and SEQ ID NO: 19, respectively.

[00388] In some embodiments, the first binding moiety of the antibody or antigen-binding fragment thereof provided herein comprises HCDR1, HCDR2, HCDR3, LCDR1, LCDR2 and LCDR3 comprising an amino acid sequence as set forth in SEQ ID NO: 1, SEQ ID NO: 42, SEQ ID NO: 3, SEQ ID NO: 4, SEQ ID NO: 5, and SEQ ID NO: 6, respectively; each of the second and third binding moieties of the antibody or antigen-binding fragment thereof provided herein comprises HCDR1, HCDR2, HCDR3, LCDR1, LCDR2 and LCDR3 comprising an amino acid sequence as set forth in SEQ ID NO: 24, SEQ ID NO: 32, SEQ ID NO: 26, SEQ ID NO: 27, SEQ ID NO: 28, and SEQ ID NO: 29, respectively; and the fourth binding moiety of the antibody or antigen-binding fragment thereof provided herein comprises HCDR1, HCDR2, HCDR3, LCDR1, LCDR2 and LCDR3 comprising an amino acid sequence as set forth in SEQ ID NO: 14, SEQ ID NO: 15, SEQ ID NO: 16, SEQ ID NO: 17, SEQ ID NO: 18, and SEQ ID NO: 19, respectively.

Chimeric Antigen Receptor

[00389] In certain embodiments, the present disclosure provides a chimeric antigen receptor comprising the antibody or an antigen-binding fragment thereof provided herein, a transmembrane region and an intracellular signal region.

[00390] The term “chimeric antigen receptor” or “CAR” or “CARs” as used herein refers to engineered receptors, which graft an antigen specificity onto cells (for example, T cells such as naive T cells, central memory T cells, effector memory T cells, regulatory T cells or combination thereof). CARs are also known as artificial T-cell receptors, chimeric T-cell receptors or chimeric immunoreceptors. In some embodiments, CARs comprise an antigen-specific targeting region (for example, the antigen-binding fragments of the antibody as provided herein), an extracellular region, a transmembrane region, one or more co-stimulatory regions, and an intracellular signal region.

[00391] In some embodiments, the antigen-binding fragment is an scFv. In some embodiments, the transmembrane region comprises a transmembrane region of CD3, CD4, CD8 or CD28. In some embodiments, the co-stimulatory region comprises a co-stimulatory domain of CD28, ICOS, CD27, 4-1BB, OX40 and CD40L. In some embodiments, the intracellular signal region is selected from the group consisting of: an intracellular signal region sequence of CD3 (e.g. CD3 ζ), Fc γ RI, CD27, CD28, CD137, CD134, MyD88, CD40, CD278, TLRs, or a combination thereof.

[00392] The CARs may be grafted onto various cells, for example, allogeneic cells, autologous cells or xenogeneic cells.

[00393] The term “allogeneic cell” as used herein refers to any cells derived from a different subject of the same species.

[00394] The term “autologous cell” as used herein refers to any cells derived from the same subject into which they are later to be re-introduced.

[00395] The term “xenogeneic cell” as used herein refers to any cells derived from a different subject of a different species.

[00396] In some embodiments, the CARs are grafted on immune effector cells, for example, T cells, natural killer cells, macrophage cells, tumor-infiltrating lymphocytes, etc.

Polynucleotides and Recombinant Methods

[00397] The present disclosure provides isolated polynucleotides that encode the antibodies or antigen-binding fragments thereof, and/or the chimeric antigen receptors provided herein. The term “nucleic acid” or “polynucleotide” as used herein refers to deoxyribonucleic acids (DNA) or ribonucleic acids (RNA) and polymers thereof in either single- or double-stranded form. Unless otherwise indicated, a particular polynucleotide sequence also implicitly encompasses conservatively modified variants thereof (*e.g.*, degenerate codon substitutions), alleles, orthologs, SNPs, and complementary sequences as well as the sequence explicitly indicated. Specifically,

degenerate codon substitutions may be achieved by generating sequences in which the third position of one or more selected (or all) codons is substituted with mixed-base and/or deoxyinosine residues (see Batzer *et al.*, *Nucleic Acid Res.* 19:5081 (1991); Ohtsuka *et al.*, *J. Biol. Chem.* 260:2605-2608 (1985); and Rossolini *et al.*, *Mol. Cell. Probes* 8:91-98 (1994)).

[00398] DNA encoding the antibody or antigen-binding fragment thereof provided herein is readily isolated and sequenced using conventional procedures (*e.g.*, by using oligonucleotide probes that are capable of binding specifically to genes encoding the heavy and light chains of the antibody). The encoding DNA may also be obtained by synthetic methods.

[00399] The isolated polynucleotide that encodes the antibodies or antigen-binding fragments thereof and/or the chimeric antigen receptors provided herein can be inserted into a vector for further cloning (amplification of the DNA) or for expression, using recombinant techniques known in the art. Many vectors are available. The vector components generally include, but are not limited to, one or more of the following: a signal sequence, an origin of replication, one or more marker genes, an enhancer element, a promoter (*e.g.*, SV40, CMV, EF-1 α), and a transcription termination sequence.

[00400] The present disclosure provides vectors comprising the isolated polynucleotides provided herein. In certain embodiments, the polynucleotides provided herein encodes the antibodies or antigen-binding fragments thereof and/or the chimeric antigen receptors provided herein, at least one promoter (*e.g.*, SV40, CMV, EF-1 α) operably linked to the nucleic acid sequence, and at least one selection marker. Examples of vectors include, but are not limited to, retrovirus (including lentivirus), adenovirus, adeno-associated virus, herpesvirus (*e.g.*, herpes simplex virus), poxvirus, baculovirus, papillomavirus, papovavirus (*e.g.*, SV40), lambda phage, and M13 phage, plasmid pcDNA3.3, pMD18-T, pOptivec, pCMV, pEGFP, pIRES, pQD-Hyg-GSeu, pALTER, pBAD, pcDNA, pCal, pL, pET, pGEMEX, pGEX, pCI, pEGFT, pSV2, pFUSE, pVITRO, pVIVO, pMAL, pMONO, pSELECT,

pUNO, pDUO, Psg5L, pBABE, pWPXL, pBI, p15TV-L, pPro18, pTD, pRS10, pLexA, pACT2.2, pCMV-SCRIPT.RTM., pCDM8, pCDNA1.1/amp, pCDNA3.1, pRc/RSV, PCR 2.1, pEF-1, pFB, pSG5, pXT1, pCDEF3, pSVSPORT, pEF-Bos etc.

[00401] Vectors comprising the polynucleotide sequence encoding the antibody or antigen-binding fragment thereof and/or the chimeric antigen receptors provided herein can be introduced to a host expression system (*e.g.*, a host cell) for cloning or gene expression. In certain embodiments, the host expression system provided herein is a microorganism, a yeast, or a mammalian cell. In certain embodiments, the microorganism is selected from the group consisting of *E. coli* and *B. subtilis*. In certain embodiments, the yeast is *Saccharomyces*. In certain embodiments, the mammalian cell is selected from the group consisting of COS, CHO-S, CHO-K1, HEK-293, and 3T3 cells.

[00402] Suitable host cells for cloning or expressing the DNA in the vectors herein are the prokaryote, yeast, or higher eukaryote cells described above. Suitable prokaryotes for this purpose include eubacteria, such as Gram-negative or Gram-positive organisms, for example, *Enterobacteriaceae* such as *Escherichia*, *e.g.*, *E. coli*, *Enterobacter*, *Erwinia*, *Klebsiella*, *Proteus*, *Salmonella*, *e.g.*, *Salmonella typhimurium*, *Serratia*, *e.g.*, *Serratia marcescans*, and *Shigella*, as well as *Bacilli* such as *B. subtilis* and *B. licheniformis*, *Pseudomonas* such as *P. aeruginosa*, and *Streptomyces*.

[00403] In addition to prokaryotes, eukaryotic microbes such as filamentous fungi or yeast are suitable cloning or expression hosts for antibody-encoding vectors. *Saccharomyces cerevisiae*, or common baker's yeast, is the most commonly used among lower eukaryotic host microorganisms. However, a number of other genera, species, and strains are commonly available and useful herein, such as *Schizosaccharomyces pombe*; *Kluyveromyces* hosts such as, *e.g.*, *K. lactis*, *K. fragilis* (ATCC 12,424), *K. bulgaricus* (ATCC 16,045), *K. wickerhamii* (ATCC 24,178), *K. waltii* (ATCC 56,500), *K. drosophilarum* (ATCC 36,906), *K. thermotolerans*, and *K. marxianus*; *yarrowia* (EP 402,226); *Pichia pastoris* (EP 183,070); *Candida*;

Trichoderma reesia (EP 244,234); *Neurospora crassa*; *Schwanniomyces* such as *Schwanniomyces occidentalis*; and filamentous fungi such as, e.g., *Neurospora*, *Penicillium*, *Tolypocladium*, and *Aspergillus* hosts such as *A. nidulans* and *A. niger*.

[00404] Suitable host cells for the expression of glycosylated antibodies or antigen-fragment thereof provided herein are derived from multicellular organisms.

Examples of invertebrate cells include plant and insect cells. Numerous baculoviral strains and variants and corresponding permissive insect host cells from hosts such as *Spodoptera frugiperda* (caterpillar), *Aedes aegypti* (mosquito), *Aedes albopictus* (mosquito), *Drosophila melanogaster* (fruitfly), and *Bombyx mori* have been identified. A variety of viral strains for transfection are publicly available, e.g., the L-1 variant of *Autographa californica* NPV and the Bm-5 strain of *Bombyx mori* NPV, and such viruses may be used as the virus herein according to the present invention, particularly for transfection of *Spodoptera frugiperda* cells. Plant cell cultures of cotton, corn, potato, soybean, petunia, tomato, and tobacco can also be utilized as hosts.

[00405] However, interest has been greatest in vertebrate cells, and propagation of vertebrate cells in culture (tissue culture) has become a routine procedure. Examples of useful mammalian host cell lines are monkey kidney CV1 line transformed by SV40 (COS-7, ATCC CRL 1651); human embryonic kidney line (293 or 293 cells subcloned for growth in suspension culture, Graham *et al.*, *J. Gen Virol.* 36:59 (1977)); baby hamster kidney cells (BHK, ATCC CCL 10); Chinese hamster ovary cells/-DHFR (CHO, Urlaub *et al.*, *Proc. Natl. Acad. Sci. USA* 77:4216 (1980)); mouse sertoli cells (TM4, Mather, *Biol. Reprod.* 23:243-251 (1980)); monkey kidney cells (CV1 ATCC CCL 70); African green monkey kidney cells (VERO-76, ATCC CRL-1587); human cervical carcinoma cells (HELA, ATCC CCL 2); canine kidney cells (MDCK, ATCC CCL 34); buffalo rat liver cells (BRL 3A, ATCC CRL 1442); human lung cells (W138, ATCC CCL 75); human liver cells (Hep G2, HB 8065); mouse mammary tumor (MMT 060562, ATCC CCL51); TRI cells (Mather *et al.*, *Annals N.Y. Acad. Sci.* 383:44-68 (1982)); MRC 5 cells; FS4 cells; mouse forestomach

carcinoma cells (MFC), SNU620 cells, and a human hepatoma line (Hep G2). In some embodiments, the host cell is a mammalian cultured cell line, such as CHO, BHK, NS0, 293, MFC, SNU620 and their derivatives.

[00406] Host cells are transformed with the above-described expression or cloning vectors for antibody production and cultured in conventional nutrient media modified as appropriate for inducing promoters, selecting transformants, or amplifying the genes encoding the desired sequences. In another embodiment, the antibody may be produced by homologous recombination known in the art. In certain embodiments, the host cell is capable of producing the antibody or antigen-binding fragment thereof provided herein.

[00407] The present disclosure also provides a method of expressing the antibody or antigen-binding fragment thereof and/or the chimeric antigen receptors provided herein, comprising culturing the host expression system provided herein under the condition at which the antibody or antigen-binding fragment thereof and/or the chimeric antigen receptor is expressed. The host expression systems used to produce the antibodies or antigen-binding fragments thereof and/or the chimeric antigen receptors provided herein may be cultured in a variety of media. Commercially available media such as Ham's F10 (Sigma), Minimal Essential Medium (MEM) (Sigma), RPMI-1640 (Sigma), and Dulbecco's Modified Eagle's Medium (DMEM) (Sigma) are suitable for culturing the host cells. In addition, any of the media described in Ham *et al.*, *Meth. Enz.* 58:44 (1979), Barnes *et al.*, *Anal. Biochem.* 102:255 (1980), U.S. Pat. No. 4,767,704; 4,657,866; 4,927,762; 4,560,655; or 5,122,469; WO 90/03430; WO 87/00195; or U.S. Pat. Re. 30,985 may be used as culture media for the host cells. Any of these media may be supplemented as necessary with hormones and/or other growth factors (such as insulin, transferrin, or epidermal growth factor), salts (such as sodium chloride, calcium, magnesium, and phosphate), buffers (such as HEPES), nucleotides (such as adenosine and thymidine), antibiotics (such as GENTAMYCINTM drug), trace elements (defined as inorganic compounds usually present at final concentrations in the micromolar range), and

glucose or an equivalent energy source. Any other necessary supplements may also be included at appropriate concentrations that would be known to a person skilled in the art. The culture conditions, such as temperature, pH, and the like, are those previously used with the host cell selected for expression, and will be apparent to a person skilled in the art.

[00408] When using recombinant techniques, the antibody can be produced intracellularly, in the periplasmic space, or directly secreted into the medium. If the antibody is produced intracellularly, as a first step, the particulate debris, either host cells or lysed fragments, is removed, for example, by centrifugation or ultrafiltration. Carter *et al.*, *Bio/Technology* 10:163-167 (1992) describe a procedure for isolating antibodies which are secreted to the periplasmic space of *E. coli*. Briefly, cell paste is thawed in the presence of sodium acetate (pH 3.5), EDTA, and phenylmethylsulfonylfluoride (PMSF) over about 30 min. Cell debris can be removed by centrifugation. Where the antibody is secreted into the medium, supernatants from such expression systems are generally first concentrated using a commercially available protein concentration filter, for example, an Amicon or Millipore Pellicon ultrafiltration unit. A protease inhibitor such as PMSF may be included in any of the foregoing steps to inhibit proteolysis and antibiotics may be included to prevent the growth of adventitious contaminants.

[00409] The antibodies or antigen-binding fragments thereof and/or the chimeric antigen receptors prepared from the host expression systems can be purified using, for example, hydroxylapatite chromatography, gel electrophoresis, dialysis, DEAE-cellulose ion exchange chromatography, ammonium sulfate precipitation, salting out, and affinity chromatography, with affinity chromatography being the preferred purification technique.

[00410] In certain embodiments, Protein A immobilized on a solid phase is used for immunoaffinity purification of the antibody and antigen-binding fragment thereof and/or the chimeric antigen receptors. The suitability of protein A as an affinity ligand depends on the species and isotype of any immunoglobulin Fc domain that is

present in the antibody. Protein A can be used to purify antibodies that are based on human gamma1, gamma2, or gamma4 heavy chains (Lindmark *et al.*, *J. Immunol. Meth.* 62:1-13 (1983)). Protein G is recommended for all mouse isotypes and for human gamma3 (Guss *et al.*, *EMBO J.* 5:1567-1575 (1986)). The matrix to which the affinity ligand is attached is most often agarose, but other matrices are available. Mechanically stable matrices such as controlled pore glass or poly(styrenedivinyl)benzene allow for faster flow rates and shorter processing times than can be achieved with agarose. Where the antibody comprises a CH3 domain, the Bakerbond ABXTM resin (J. T. Baker, Phillipsburg, N.J.) is useful for purification. Other techniques for protein purification such as fractionation on an ion-exchange column, ethanol precipitation, Reverse Phase HPLC, chromatography on silica, chromatography on heparin SEPHAROSETM chromatography on an anion or cation exchange resin (such as a polyaspartic acid column), chromatofocusing, SDS-PAGE, and ammonium sulfate precipitation are also available depending on the antibody to be recovered.

[00411] Following any preliminary purification step(s), the mixture comprising the antibody of interest and contaminants may be subjected to low pH hydrophobic interaction chromatography using an elution buffer at a pH between about 2.5-4.5, preferably performed at low salt concentrations (*e.g.*, from about 0-0.25M salt).

Pharmaceutical Composition

[00412] The present disclosure further provides pharmaceutical compositions comprising the antibodies or antigen-binding fragments thereof and/or the chimeric antigen receptors provided herein, and one or more pharmaceutically acceptable carriers.

[00413] Pharmaceutical acceptable carriers for use in the pharmaceutical compositions disclosed herein may include, for example, pharmaceutically acceptable liquid, gels, or solid carriers, aqueous vehicles, nonaqueous vehicles, antimicrobial agents, isotonic agents, buffers, antioxidants, anesthetics, suspending/dispersing

agents, sequestering or chelating agents, diluents, adjuvants, excipients, or non-toxic auxiliary substances, other components known in the art, or various combinations thereof.

[00414] Suitable components may include, for example, antioxidants, fillers, binders, disintegrants, buffers, preservatives, lubricants, flavorings, thickeners, coloring agents, emulsifiers or stabilizers such as sugars and cyclodextrins. Suitable antioxidants may include, for example, methionine, ascorbic acid, EDTA, sodium thiosulfate, platinum, catalase, citric acid, cysteine, thioglycerol, thioglycolic acid, thiosorbitol, butylated hydroxanisole, butylated hydroxytoluene, and/or propyl gallate. As disclosed herein, inclusion of one or more antioxidants such as methionine in a composition comprising an antibody or antigen-binding fragment thereof and conjugates provided herein decreases oxidation of the antibody or antigen-binding fragment thereof. This reduction in oxidation prevents or reduces loss of binding affinity, thereby improving antibody stability and maximizing shelf-life. Therefore, in certain embodiments, pharmaceutical compositions are provided that comprise one or more antibodies or antigen-binding fragments thereof as disclosed herein and one or more antioxidants such as methionine. Further provided are methods for preventing oxidation of, extending the shelf-life of, and/or improving the efficacy of an antibody or antigen-binding fragment provided herein by mixing the antibody or antigen-binding fragment with one or more antioxidants such as methionine.

[00415] To further illustrate, pharmaceutical acceptable carriers may include, for example, aqueous vehicles such as sodium chloride injection, Ringer's injection, isotonic dextrose injection, sterile water injection, or dextrose and lactated Ringer's injection, nonaqueous vehicles such as fixed oils of vegetable origin, cottonseed oil, corn oil, sesame oil, or peanut oil, antimicrobial agents at bacteriostatic or fungistatic concentrations, isotonic agents such as sodium chloride or dextrose, buffers such as phosphate or citrate buffers, antioxidants such as sodium bisulfate, local anesthetics such as procaine hydrochloride, suspending and dispersing agents such as sodium carboxymethylcellulose, hydroxypropyl methylcellulose, or polyvinylpyrrolidone,

emulsifying agents such as Polysorbate 80 (TWEEN-80), sequestering or chelating agents such as EDTA (ethylenediaminetetraacetic acid) or EGTA (ethylene glycol tetraacetic acid), ethyl alcohol, polyethylene glycol, propylene glycol, sodium hydroxide, hydrochloric acid, citric acid, or lactic acid. Antimicrobial agents utilized as carriers may be added to pharmaceutical compositions in multiple-dose containers that include phenols or cresols, mercurials, benzyl alcohol, chlorobutanol, methyl and propyl p-hydroxybenzoic acid esters, thimerosal, benzalkonium chloride and benzethonium chloride. Suitable excipients may include, for example, water, saline, dextrose, glycerol, or ethanol. Suitable non-toxic auxiliary substances may include, for example, wetting or emulsifying agents, pH buffering agents, stabilizers, solubility enhancers, or agents such as sodium acetate, sorbitan monolaurate, triethanolamine oleate, or cyclodextrin.

[00416] The pharmaceutical compositions can be a liquid solution, suspension, emulsion, pill, capsule, tablet, sustained release formulation, or powder. Oral formulations can include standard carriers such as pharmaceutical grades of mannitol, lactose, starch, magnesium stearate, polyvinyl pyrrolidone, sodium saccharine, cellulose, magnesium carbonate, etc.

[00417] In certain embodiments, the pharmaceutical compositions are formulated into an injectable composition. The injectable pharmaceutical compositions may be prepared in any conventional form, such as for example liquid solution, suspension, emulsion, or solid forms suitable for generating liquid solution, suspension, or emulsion. Preparations for injection may include sterile and/or non-pyretic solutions ready for injection, sterile dry soluble products, such as lyophilized powders, ready to be combined with a solvent just prior to use, including hypodermic tablets, sterile suspensions ready for injection, sterile dry insoluble products ready to be combined with a vehicle just prior to use, and sterile and/or non-pyretic emulsions. The solutions may be either aqueous or nonaqueous.

[00418] In certain embodiments, unit-dose parenteral preparations are packaged in an ampoule, a vial or a syringe with a needle. All preparations for parenteral administration should be sterile and not pyretic, as is known and practiced in the art.

[00419] In certain embodiments, a sterile, lyophilized powder is prepared by dissolving an antibody or antigen-binding fragment as disclosed herein in a suitable solvent. The solvent may contain an excipient which improves the stability or other pharmacological components of the powder or reconstituted solution, prepared from the powder. Excipients that may be used include, but are not limited to, water, dextrose, sorbitol, fructose, corn syrup, xylitol, glycerin, glucose, sucrose or other suitable agent. The solvent may contain a buffer, such as citrate, sodium or potassium phosphate or other such buffer known to a person skilled in the art at, in one embodiment, about neutral pH. Subsequent sterile filtration of the solution followed by lyophilization under standard conditions known to a person skilled in the art provides a desirable formulation. In one embodiment, the resulting solution will be apportioned into vials for lyophilization. Each vial can contain a single dosage or multiple dosages of the antibody or antigen-binding fragment thereof or composition thereof. Overfilling vials with a small amount above that needed for a dose or set of doses (*e.g.*, about 10%) is acceptable so as to facilitate accurate sample withdrawal and accurate dosing. The lyophilized powder can be stored under appropriate conditions, such as at about 4 °C to room temperature.

[00420] Reconstitution of a lyophilized powder with water for injection provides a formulation for use in parenteral administration. In one embodiment, for reconstitution the sterile and/or non-pyretic water or other liquid suitable carrier is added to lyophilized powder. The precise amount depends upon the selected therapy being given, and can be empirically determined.

Kits

[00421] In certain embodiments, the present disclosure provides a kit comprising the antibody or an antigen-binding fragment thereof provided herein and/or the chimeric

antigen receptors provided herein and/or the pharmaceutical composition provided herein. In certain embodiments, the present disclosure provides a kit comprising the antibody or an antigen-binding fragment thereof provided herein and/or the chimeric antigen receptors provided herein and/or the pharmaceutical composition provided herein, and a second therapeutic agent. In certain embodiments, the second therapeutic agent is selected from the group consisting of a chemotherapeutic agent, an anti-cancer drug, radiation therapy agent, an immunotherapy agent, an anti-angiogenesis agent, a targeted therapy agent, a cellular therapy agent, a gene therapy agent, a hormonal therapy agent, an antiviral agent, an antibiotic, an analgesics, an antioxidant, a metal chelator, and cytokines.

[00422] Such kits can further include, if desired, one or more of various conventional pharmaceutical kit components, such as, for example, containers with one or more pharmaceutically acceptable carriers, additional containers etc., as will be readily apparent to a person skilled in the art. Instructions, either as inserts or a labels, indicating quantities of the components to be administered, guidelines for administration, and/or guidelines for mixing the components, can also be included in the kit.

Methods of Use

[00423] The present disclosure also provides methods of treating, preventing or alleviating a disease, disorder or condition in a subject, comprising administering to the subject a therapeutically effective amount of the antibodies or antigen-binding fragments thereof provided herein, and/or the chimeric antigen receptors provided herein, and/or the pharmaceutical compositions provided herein. In certain embodiments, the disease, disorder or condition is a CD3 and/or LILRB4-related disease, disorder or condition. In certain embodiments, the subject is human.

[00424] In some embodiments, the CD3 and/or LILRB4-related disease, disorder or condition is characterized in expressing or over-expressing of CD3 and/or LILRB4.

[00425] In certain embodiments, the disease, disorder or condition is an immune disease, an autoimmune disease, an inflammatory disease, a cancer or a neurological disease. In certain embodiments, the disease, disorder or condition is cancer. In certain embodiments, the cancer is a solid tumor or hematologic tumor. In certain embodiments, the cancer is a CD3 and/or LILRB4-expressing cancer. “CD3 and/or LILRB4-expressing” cancer as used herein refers to a cancer characterized in expressing CD3 and/or LILRB4 protein in a cancer cell, a tumor infiltrating immune cell, or expressing CD3 and/or LILRB4 in a cancer cell, a tumor infiltrating immune cell at a level significantly higher than that would have been expected of a normal cell. Various methods can be used to determine the presence and/or amount of CD3 and/or LILRB4 in a test biological sample from the subject. For example, the test biological sample can be exposed to an antibody or antigen-binding fragment thereof, which binds to and detects the expressed CD3 and/or LILRB4 protein. Alternatively, CD3 and/or LILRB4 can also be detected at nucleic acid expression level, using methods such as qPCR, reverse transcriptase PCR, microarray, SAGE, FISH, and the like. In some embodiments, the test sample is derived from a cancer cell or tissue, or tumor infiltrating immune cells. The reference sample can be a control sample obtained from a healthy or non-diseased individual, or a healthy or non-diseased sample obtained from the same individual from whom the test sample is obtained. For example, the reference sample can be a non-diseased sample adjacent to or in the neighborhood of the test sample (*e.g.*, tumor). In certain embodiments, the cancer is a CD3 and/or LILRB4-expressing B cell cancer.

[00426] In some embodiments, the disease, disorder or condition is selected from the group consisting of Kawasaki disease, T. gondii, multiple sclerosis, systematic Lupus erythematosus, lung cancer (*e.g.*, non-small-cell lung cancer (NSCLC), small cell lung cancer (SCLC), adenocarcinoma of the lung, squamous cell carcinoma of the lung, Lewis lung carcinoma, or radiation therapy resistant Lewis lung carcinoma), peritoneal cancer, carcinoid cancer, bone cancer, pancreatic cancer, primitive neuroectodermal tumor, skin cancer, gallbladder cancer, cancer of the head or neck,

squamous cell cancer, uterine cancer, ovarian cancer, rectal cancer, prostate cancer, bladder cancer (*e.g.*, urothelial cancer), cancer of the anal region (*e.g.*, anal squamous cell carcinoma), gastric or stomach cancer (*e.g.*, gastrointestinal cancer), esophageal cancer, colon cancer, breast cancer, uterine cancer, liver cancer (*e.g.*, hepatoblastoma, hepatocellular carcinoma/hepatoma, or hepatic carcinoma), cholangiocarcinoma, sarcoma, colorectal cancer, carcinoma of the fallopian tubes, salivary gland carcinoma, carcinoma of the cervix, endometrial or uterine carcinoma, osteosarcoma, carcinoma of the vagina, carcinoma of the vulva, cancer of the esophagus, cancer of the small intestine, cancer of the endocrine system, cancer of the thyroid gland, cancer of the parathyroid gland, cancer of the adrenal gland, cancer of the nasopharynx, sarcoma of soft tissue, polycythemia vera, cancer of the urethra, cancer of the penis, cancer of the kidney or ureter (*e.g.*, rhabdoid tumor of the kidney), cutaneous T-cell lymphoma, medulloblastoma, nephroblastoma, myelodysplastic syndrome, chronic and non-chronic myeloproliferative disorder, choroid plexus papilloma, renal cell carcinoma, carcinoma of the renal pelvis, neoplasms of the central nervous system (CNS), soft tissue sarcoma (*e.g.*, rhabdomyosarcoma, fibrosarcoma, Kaposi's sarcoma), spinal axis tumors, glioma (*e.g.*, ependymoma, astrocytoma, anaplastic astrocytoma, oligodendroglioma, eye cancer (*e.g.*, retinoblastoma), brain stem glioma, or mixed glioma such as oligoastrocytoma), brain tumor (*e.g.*, glioblastoma/glioblastoma multiforme (GBM), non-glioblastoma brain tumor, or meningioma), melanoma (*e.g.*, cutaneous or intraocular melanoma), thrombocythemia, mesothelioma, mycosis fungoides, Sezary syndrome, idiopathic myelofibrosis, solitary plasmacytoma, vestibular schwannoma, Ewing's sarcoma, chondrosarcoma, MYH associated polyposis, pituitary adenoma, pediatric cancers such as pediatric sarcomas (*e.g.*, neuroblastoma, rhabdomyosarcoma, and osteosarcoma), hematological cancer, lymphoma, Hodgkin's lymphoma, non-Hodgkin's lymphoma, leukemia (*e.g.*, lymphocytic/lymphoblastic leukemia), chronic or acute leukemia, mast cell leukemia, lymphocytic lymphomas, primary CNS lymphoma, chronic lymphocytic leukemia (CLL), acute lymphocytic leukemia (ALL), chronic myeloid leukemia (CML), acute myeloid leukemia (AML), chronic

myelomonocytic leukemia (CMML), chronic lymphoblastic leukemia, acute lymphoblastic leukemia, hairy cell leukemia (HCL), Burkitt's lymphoma (BL), multiple myeloma (*e.g.*, relapsed or refractory multiple myeloma), T or B cell lymphoma, mantle cell lymphoma (MCL) (*e.g.*, relapsed or refractory mantle cell lymphoma), malignant melanoma, diffuse large B cell lymphoma (DLBCL), DLBCL that results from follicular lymphoma, high-grade B-cell lymphoma, primary mediastinal large B-cell lymphoma, follicular lymphoma (FL), and primary mediastinal B-cell lymphoma. In some embodiments, the disease, disorder or condition is acute myeloid leukemia.

[00427] In some embodiments, the subject has been identified as having a cancer cell or tumor infiltrating immune cells expressing CD3 and/or LILRB4, optionally at a level significantly higher from the level normally found on non-cancer cells.

[00428] In another aspect, methods are provided to treat, prevent or alleviate a disease, disorder or condition in a subject that would benefit from modulation of CD3 and/or LILRB4 activity, comprising administering to the subject a therapeutically effective amount of the antibody or antigen-binding fragment thereof provided herein, and/or the pharmaceutical composition provided herein, and/or the chimeric antigen receptor provided herein. In certain embodiments, the disease, disorder or condition is a CD3 and/or LILRB4-related disease, disorder or condition, which is defined above.

[00429] The therapeutically effective amount of an antibody or antigen-binding fragment provided herein will depend on various factors known in the art, such as for example body weight, age, past medical history, present medications, state of health of the subject and potential for cross-reaction, allergies, sensitivities and adverse side-effects, as well as the administration route and extent of disease development. Dosages may be proportionally reduced or increased by a person skilled in the art (*e.g.*, physician or veterinarian) as indicated by these and other circumstances or requirements.

[00430] In certain embodiments, the antibody or antigen-binding fragment provided herein and/or the chimeric antigen receptors provided herein may be administered at a therapeutically effective dosage of about 0.01 mg/kg to about 100 mg/kg. In certain embodiments, the administration dosage may change over the course of treatment. For example, in certain embodiments the initial administration dosage may be higher than subsequent administration dosages. In certain embodiments, the administration dosage may vary over the course of treatment depending on the reaction of the subject.

[00431] Dosage regimens may be adjusted to provide the optimum desired response (*e.g.*, a therapeutic response). For example, a single dose may be administered, or several divided doses may be administered over time.

[00432] The antibodies or antigen-binding fragments thereof provided herein and/or the chimeric antigen receptors provided herein and/or the pharmaceutical composition provided herein may be administered by any route known in the art, for example the administration is through a parenteral route comprising subcutaneous, intraperitoneal, intravenous, intramuscular, or intradermal injection; or a non-parenteral route comprising transdermal, oral, intranasal, intraocular, sublingual, rectal, or topical.

[00433] In some embodiments, the antibodies or antigen-binding fragments thereof provided herein and/or the chimeric antigen receptors provided herein and/or the pharmaceutical compositions provided herein may be administered alone or in combination with a therapeutically effective amount of an additional therapeutic agent. For example, the antibodies or antigen-binding fragments thereof disclosed herein and/or the chimeric antigen receptors provided herein and/or the pharmaceutical compositions provided herein may be administered in combination with an additional therapeutic agent, for example, a chemotherapeutic agent, an anti-cancer drug, a radiation therapy agent, an immunotherapy agent, a targeted therapy agent, a cellular therapy agent, a gene therapy agent, a hormonal therapy agent, an antiviral agent, an antibiotic, an analgesics, an antioxidant, a metal chelator, cytokines, an active agent, an imaging agent, a cytotoxic agent, an angiogenesis

inhibitor, a kinase inhibitor, a co-stimulation molecule agonist, a co-inhibition molecule blocker, an adhesion molecule blocker, an anti-cytokine antibody or functional fragment thereof, a detectable label or reporter, an antimicrobial, a gene editing agent, a beta agonist, an viral RNA inhibitor, a polymerase inhibitor, an interferon, or a microRNA.

[00434] The term “immunotherapy” as used herein, refers to a type of therapy that stimulates immune system to fight against disease such as cancer or that boosts immune system in a general way. Examples of immunotherapy include, without limitation, checkpoint modulators, adoptive cell transfer, cytokines, oncolytic virus and therapeutic vaccines.

[00435] “Targeted therapy” is a type of therapy that acts on specific molecules associated with cancer, such as specific proteins that are present in cancer cells but not normal cells or that are more abundant in cancer cells, or the target molecules in the cancer microenvironment that contributes to cancer growth and survival. Targeted therapy targets a therapeutic agent to a tumor, thereby sparing of normal tissue from the effects of the therapeutic agent.

[00436] In certain of these embodiments, an antibody or antigen-binding fragment thereof provided herein, and/or the chimeric antigen receptors provided herein, and/or the pharmaceutical composition provided herein that is administered in combination with one or more additional therapeutic agents may be administered simultaneously with the one or more additional therapeutic agents, and in certain of these embodiments the antibody or antigen-binding fragment thereof and/or the pharmaceutical composition provided herein, and the additional therapeutic agent(s) may be administered as part of the same pharmaceutical composition. However, an antibody or antigen-binding fragment thereof and/or the chimeric antigen receptors provided herein and/or a pharmaceutical composition provided herein administered “in combination” with another therapeutic agent does not have to be administered simultaneously with or in the same composition as the agent. An antibody or antigen-binding fragment thereof, or a chimeric antigen receptor or pharmaceutical

composition administered prior to or after another agent is considered to be administered “in combination” with that agent as the phrase is used herein, even if the antibody or antigen-binding fragment, or the pharmaceutical composition or the chimeric antigen receptor, and the second agent are administered via different routes. Where possible, additional therapeutic agents administered in combination with the antibodies or antigen-binding fragments thereof, chimeric antigen receptors or pharmaceutical compositions disclosed herein are administered according to the schedule listed in the product information sheet of the additional therapeutic agent, or according to the Physicians’ Desk Reference 2003 (Physicians’ Desk Reference, 57th Ed; Medical Economics Company; ISBN: 1563634457; 57th edition (November 2002)) or protocols well known in the art.

[00437] In another aspect, the present disclosure further provides methods of activating a T cell expressing CD3 or LILRB4 *in vivo* or *in vitro*, comprising contacting the T cell with the antibodies or antigen-binding fragments thereof provided herein, and/or the chimeric antigen receptors provided herein, and/or the pharmaceutical composition provided herein.

[00438] In another aspect, the present disclosure further provides methods of modulating CD3 and/or LILRB4 activity in CD3 and/or LILRB4-expressing cells, comprising exposing the CD3 and/or LILRB4-expressing cells to the antibodies or antigen-binding fragments thereof provided herein, and/or the chimeric antigen receptors provided herein, and/or the pharmaceutical compositions provided herein. In some embodiments, the CD3 and/or LILRB4-expressing cell is a dendritic cell, monocyte, macrophage, B cell, Treg, progenitor mast cell, endothelial cell, or osteoclast.

[00439] In another aspect, the present disclosure further provides methods of promoting *in vivo* or *in vitro* processing of LILRB4 by CD3-expressing T cells, comprising contacting the CD3-expressing T cells with the antibodies or antigen-binding fragments thereof provided herein, and/or the chimeric antigen receptors provided herein, and/or the pharmaceutical compositions provided herein, wherein the

antibodies or antigen-binding fragments thereof, and/or the chimeric antigen receptors, and/or the pharmaceutical compositions are capable of specifically binding to both the CD3-expressing T cells and LILRB4 thereby bringing both in close proximity.

[00440] In another aspect, the present disclosure provides methods of detecting the presence or amount of CD3 and/or LILRB4 in a sample, comprising contacting the sample with the antibodies or antigen-binding fragments thereof provided herein, and/or the chimeric antigen receptors provided herein, and/or the pharmaceutical compositions provided herein, and determining the presence or the amount of CD3 and/or LILRB4 in the sample.

[00441] In another aspect, the present disclosure provides a method of diagnosing a CD3 and/or LILRB4-related disease, disorder or condition in a subject, comprising: a) contacting a sample obtained from the subject with the antibodies or an antigen-binding fragments thereof provided herein and/or the chimeric antigen receptors provided herein and/or the pharmaceutical compositions provided herein; b) determining the presence or amount of CD3 and/or LILRB4 in the sample; and c) correlating the presence or the amount of CD3 and/or LILRB4 to existence or status of the CD3 and/or LILRB4-related disease, disorder or condition in the subject.

[00442] In another aspect, the present disclosure provides kits comprising the antibodies or antigen-binding fragments thereof provided herein and/or the chimeric antigen receptors provided herein and/or the pharmaceutical compositions provided herein, optionally conjugated with a detectable moiety, which is useful in detecting CD3 and/or LILRB4, optionally recombinant CD3 and/or LILRB4, CD3 and/or LILRB4 expressed on cell surface, or CD3 and/or LILRB4-expressing cells. The kits may further comprise instructions for use.

[00443] In another aspect, the present disclosure also provides uses of the antibodies or antigen-binding fragments thereof provided herein and/or the chimeric antigen receptors provided herein and/or the pharmaceutical compositions provided herein in

the manufacture of a medicament for treating, preventing or alleviating diseases, disorders or conditions related to CD3 and/or LILRB4 in a subject.

[00444] In another aspect, the present disclosure also provides uses of the antibodies or antigen-binding fragments thereof provided herein and/or the chimeric antigen receptors provided herein and/or the pharmaceutical compositions provided herein in the manufacture of diagnostic reagents for diagnosing diseases, disorders or conditions related to CD3 and/or LILRB4 in a subject.

[00445] The following examples are provided to better illustrate the claimed invention and are not to be interpreted as limiting the scope of the invention. All specific compositions, materials, and methods described below, in whole or in part, fall within the scope of the present invention. These specific compositions, materials, and methods are not intended to limit the invention, but merely to illustrate specific embodiments falling within the scope of the invention. A person skilled in the art may develop equivalent compositions, materials, and methods without the exercise of inventive capacity and without departing from the scope of the invention. It will be understood that many variations can be made in the procedures herein described while still remaining within the bounds of the present invention. It is the intention of the inventors that such variations are included within the scope of the invention.

EXAMPLES

Example 1. Antibody Generation

[00446] Multi-specific antibodies targeting CD3 and LILRB4 were generated and characterized, wherein the characterizations include binding affinity to 293F-hLILRB4 cells and Jurkat T cells, T cell activation, T-cell dependent cytotoxicity, PBMC-mediated cellular cytotoxicity, and *in vivo* anti-tumor efficacy.

[00447] One multi-specific antibody BIS-PTMS16-22+40E9 was constructed in Fab-scFv-Fab (also referred to as “chimeric 2+1 bispecific” or “Fab2-scFv”) format by introducing a high affinity anti-CD3 scFv domain between the CH1 region and hinge

region of one of the heavy chains of an anti-LILRB4 antibody h8-B3-F6-H8 (see Figure 1A). In particular, the chimeric 2+1 bispecific antibody generated herein comprises two anti-LILRB4 Fab domains and one anti-CD3 scFv domain, wherein each of the anti-LILRB4 Fab domains comprises a LILRB4 binding moiety comprising the VH and VL regions from the anti-LILRB4 antibody h8-B3-F6-H8, and the anti-CD3 scFv domain comprises the VH and VL regions from clone 40-C12-C10-E9, wherein the VH and VL regions of clone 40-C12-C10-E9 were linked by a (GGGGS)₄ linker, and the VL region of the anti-CD3 scFv domain was linked to the N-terminal of the hinge region of the chimeric antibody by a (GGGGS)₂ linker, the VH region of the anti-CD3 scFv domain was linked to CH1 region of one of the anti-LILRB4 Fab domains by a (GGGGS)₂ linker. LALA mutations (L234A, L235A, according to EU numbering) were introduced to each bispecific antibody to abolish the Fc receptor binding capability. The generated chimeric 2+1 bispecific antibody comprising two Fab domains (VL region and VH region from the anti-LILRB4 antibody h8-B3-F6-H8) targeting LILRB4 and the scFv (VL region and VH region from the clone 40-C12-C10-E9) targeting CD3 was also named as “BIS-PTMS16-22+40E9” in the present disclosure. In BIS-PTMS16-22+40E9, each of the Fab domain targeting LILRB4 comprises a VH region as set forth in SEQ ID NO: 33 and a VL region as set forth in SEQ ID NO: 34, the scFv targeting CD3 comprises a VH region as set forth in SEQ ID NO: 7 and a VL region as set forth in SEQ ID NO: 8.

[00448] Another multi-specific antibody BIS-S3+S16+40E9-F3 was constructed in Fab-scFv-Fab-Fc-scFv format by introducing a high affinity anti-CD3 scFv domain between the CH1 region and hinge region of one of the heavy chains of an anti-LILRB4 antibody h2-H1-D7-E5-D5, and another anti-LILRB4 scFv targeting another epitope of LILRB4 is linked to C terminal of the same heavy chain by a GSSS linker, enabling a trivalent, dual epitope recognition of LILRB4 (see Figure 1B). In particular, the Fab-scFv-Fab-Fc-scFv antibody generated herein comprises two anti-LILRB4 Fab domains, one anti-CD3 scFv domain, and one anti-LILRB4 scFv domain, wherein each of the anti-LILRB4 Fab domains comprises a LILRB4 binding

moiety comprising the VH and VL regions from the anti-LILRB4 antibody h2-H1-D7-E5-D5, the anti-CD3 scFv domain comprises the VH and VL regions from clone 40-C12-C10-E9, and the anti-LILRB4 scFv domain comprises a LILRB4 binding moiety comprising the VH and VL regions from the anti-LILRB4 antibody h8-B3-F6-H8, wherein the VH and VL regions of clone 40-C12-C10-E9 were linked by a (GGGGS)₄ linker, and the VL region of the anti-CD3 scFv domain was linked to the N-terminal of the hinge region of the chimeric antibody by a (GGGGS)₂ linker, the VH region of the anti-CD3 scFv domain was linked to CH1 region of one of the anti-LILRB4 Fab domains by a (GGGGS)₂ linker. LALA mutations (L234A, L235A, according to EU numbering) were introduced to each bispecific antibody to abolish the Fc receptor binding capability. The generated antibody comprising two Fab domains (VL region and VH region from the anti-LILRB4 antibody h2-H1-D7-E5-D5) targeting LILRB4, one scFv (VL region and VH region from the clone 40-C12-C10-E9) targeting CD3, and one scFv (VL region and VH region from the anti-LILRB4 antibody h8-B3-F6-H8) targeting another epitope of LILRB4 was also named as “BIS-S3+S16+40E9-F3” in the present disclosure. In BIS-S3+S16+40E9-F3, each of the Fab domain targeting LILRB4 comprises a VH region as set forth in SEQ ID NO: 22 and a VL region as set forth in SEQ ID NO: 23, the scFv targeting CD3 comprises a VH region as set forth in SEQ ID NO: 7 and a VL region as set forth in SEQ ID NO: 8, and the scFv targeting another epitope on LILRB4 comprises a VH region as set forth in SEQ ID NO: 33 and a VL region as set forth in SEQ ID NO: 34.

[00449] Humanized bispecific antibodies were then generated and characterized, wherein the characterizations include binding affinity to 293F-hLILRB4 cells and Jurkat T cells, T-cell dependent cytotoxicity, and *in vivo* anti-tumor efficacy.

[00450] A humanized multi-specific antibody BIS-PTMS16-22+40E9-L2H3 was constructed in Fab-scFv-Fab format, which has the same amino acid sequence and construct as BIS-PTMS16-22+40E9 except for the anti-CD3 scFv domains, *i.e.*, VH and VL regions of clone 40-C12-C10-E9 were used in the anti-CD3 scFv domain of BIS-PTMS16-22+40E9, while VH and VL regions of clone hu40E9-L2H3 were used

in the anti-CD3 scFv domain of BIS-PTMS16-22+40E9-L2H3. Similarly, a humanized multi-specific antibody BIS-PTMS16-22+40E9-L2H3-N55S.H was constructed in Fab-scFv-Fab format, which has the same amino acid sequence and construct as BIS-PTMS16-22+40E9 except for the anti-CD3 scFv domains, *i.e.*, VH and VL regions of clone 40-C12-C10-E9 were used in the anti-CD3 scFv domain of BIS-PTMS16-22+40E9, while VH and VL regions of clone hu40E9-L2H3-N55S.H were used in the anti-CD3 scFv domain of BIS-PTMS16-22+40E9-L2H3-N55S.H. The VH and VL amino acid sequences of the humanized multi-specific antibodies BIS-PTMS16-22+40E9-L2H3 and BIS-PTMS16-22+40E9-L2H3-N55S.H were shown in Table 7.

[00451] A humanized multi-specific antibody BIS-F3+40E9-L2H3 was constructed in Fab-scFv-Fab-Fc-scFv format, which has the same amino acid sequence and construct as BIS-S3+S16+40E9-F3 except for the anti-CD3 scFv domains, *i.e.*, VH and VL regions of clone 40-C12-C10-E9 were used in the anti-CD3 scFv domain of BIS-S3+S16+40E9-F3, while VH and VL regions of clone hu40E9-L2H3 were used in the anti-CD3 scFv domain of BIS-F3+40E9-L2H3. Similarly, a humanized multi-specific antibody BIS-F3+40E9-L2H3-N55S.H was constructed in Fab-scFv-Fab-Fc-scFv format, which has the same amino acid sequence and construct as BIS-S3+S16+40E9-F3 except for the anti-CD3 scFv domains, *i.e.*, VH and VL regions of clone 40-C12-C10-E9 were used in the anti-CD3 scFv domain of BIS-S3+S16+40E9-F3, while VH and VL regions of clone hu40E9-L2H3-N55S.H were used in the anti-CD3 scFv domain of BIS-F3+40E9-L2H3-N55S.H. The VH and VL amino acid sequences of the humanized multi-specific antibodies BIS-F3+40E9-L2H3 and BIS-F3+40E9-L2H3-N55S.H were shown in Table 7.

Table 7. Amino Acid Sequences of Humanized Multi-specific Antibodies

Sequence Description	SEQ ID NO	Amino Acid Sequence
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BIS-PTMS16-22+40E9-L2H3	Anti-CD3 scFv	VH	10	EVQLVQSGAEVKKPGSSVKVSCKA SGYTFSSYVIHWVRQAPGQGLEW MGYINPYNDVIKYNEKFKGRVTLT SDKSTSTAYMELSSLRSEDNAVYY CARDSYYSYDGFAYWGQGLVTV SS
		VL	12	EIVLTQSPDFQSVTPKEKVTITCSAS SSVSYMHWYQQKPDQSPKRLIYDT SKLASGVPSRFSGSGSGTDFTLTINS LEAEDAATYYCQQWSSNPPTFGGG TKVEIK
	Anti-LILRB4 Fab	VH	33	EVQLVESGGGLVQPGGSLRLSCAA SGFDFSRYWMSWVRQAPGKGLVW IGEINPESSAINYAPSLKDRFTISRDN AKNTLYLQMNSLRAEDNAVYYCA TSMTMDYWGQGLVTVSS
		VL	34	DIQMTQSPSSLSASVGDRVTITCKA SQDIKSYLSWYQQKPGKAPKTLIY YATSLAGGVPSRFSGSGSGTDYTLT ISSLPEDFATYYCLQHGESPYTFG GGTKLEIK
BIS-PTMS16-22+40E9-L2H3-N55S.H	Anti-CD3 scFv	VH	47	EVQLVQSGAEVKKPGSSVKVSCKA SGYTFSSYVIHWVRQAPGQGLEW MGYINPYSDVIKYNEKFKGRVTLT SDKSTSTAYMELSSLRSEDNAVYY CARDSYYSYDGFAYWGQGLVTV SS

		VL	12	EIVLTQSPDFQSVTPKEKVTITCSAS SSVSYMHWYQQKPDQSPKRLIYDT SKLASGVPSRFSGSGSGTDFTLTINS LEAEDAATYYCQQWSSNPPTFGGG TKVEIK
	Anti- LILRB4 Fab	VH	33	EVQLVESGGGLVQPGGSLRLSCAA SGFDFSRYWMSWVRQAPGKGLVW IGEINPESSAINYAPSLKDRFTISRDN AKNTLYLQMNSLRAEDTAVYYCA TSMTMDYWGQGLVTVSS
		VL	34	DIQMTQSPSSLSASVGDRVTITCKA SQDIKSYLSWYQQKPGKAPKTLIY YATSLAGGVPSRFSGSGSGTDYTLT ISSLPEDFATYYCLQHGESPYTFG GGTKLEIK
BIS- F3+40E9 -L2H3	Anti-CD3 scFv	VH	10	EVQLVQSGAEVKKPGSSVKVSCKA SGYTFSSYVIHWVRQAPGQGLEW MGYINPYNDVIKYNEKFKGRVTLT SDKSTSTAYMELSSLRSEDVAVYY CARDSEYSSYDGFAYWGQGLVTV SS
		VL	12	EIVLTQSPDFQSVTPKEKVTITCSAS SSVSYMHWYQQKPDQSPKRLIYDT SKLASGVPSRFSGSGSGTDFTLTINS LEAEDAATYYCQQWSSNPPTFGGG TKVEIK
		VH	33	EVQLVESGGGLVQPGGSLRLSCAA SGFDFSRYWMSWVRQAPGKGLVW IGEINPESSAINYAPSLKDRFTISRDN

	Anti-LILRB4 scFv			AKNTLYLQMNSLRAEDTAVYYCA TSMTMDYWGQGTLVTVSS
		VL	34	DIQMTQSPSSLSASVGDRVTITCKA SQDIKSYLSWYQQKPGKAPKTLIY YATSLAGGVPSRFSGSGSGTDYTLT ISSLPEDFATYYCLQHGESPYTFG GGTKLEIK
	Anti-LILRB4 Fab	VH	22	EVQLVESGGGLVQPGGSLRLSCAA SGFTFSSFGMHWVRQAPGKGLEW VAYISSGSSSIYYADTVKGRFTISR NAKNSLYLQMNSLRAEDTAVYYC ARGTGTGAMDYWGQGTLVTVSS
		VL	23	DIQMTQSPSSLSASVGDRVTITCRT SQDISNYLNWYQQKPGKAPKLLIY YTSKLHSGVPSRFSGSGSGTDFTFTI SSLQPEDIATYFCQQGNTLPWTFGG GTKLEIK
BIS- F3+40E9 -L2H3- N55S.H	Anti-CD3 scFv	VH	47	EVQLVQSGAEVKKPGSSVKVSCA SGYTFSSYVIHWVRQAPGQGLEW MGYINPYSDVIKYNEKFKGRVTLT SDKSTSTAYMELSSLRSEDVAVYY CARDSEYSDGFAYWGQGTLVTV SS
		VL	12	EIVLTQSPDFQSVTPKEKVTITCSAS SSVSVMHWYQQKPDQSPKRLIYDT SKLASGVPSRFSGSGSGTDFTLTINS LEAEDAATYYCQQWSSNPPTFGGG TKVEIK

	Anti-LILRB4 scFv	VH	33	EVQLVESGGGLVQPGGSLRLSCAA SGFDFSRYWMSWVRQAPGKGLVW IGEINPESSAINYAPSLKDRFTISRDN AKNTLYLQMNSLRAEDTAVYYCA TSMTMDYWGQGTLVTVSS
		VL	34	DIQMTQSPSSLSASVGDRVTITCKA SQDIKSYLSWYQQKPGKAPKTLIY YATSLAGGVPSRFSGSGSGTDYTLT ISSLPEDFATYYCLQHGESPYTFG GGTKLEIK
	Anti-LILRB4 Fab	VH	22	EVQLVESGGGLVQPGGSLRLSCAA SGFTFSSFGMHWVRQAPGKGLEW VAYISSGSSSIYYADTVKGRFTISR NAKNSLYLQMNSLRAEDTAVYYC ARGTGTGAMDYWGQGTLVTVSS
		VL	23	DIQMTQSPSSLSASVGDRVTITCRT SQDISNYLNWYQQKPGKAPKLLIY YTSKLHSGVPSRFSGSGSGTDFTFTI SSLQPEDIATYFCQQGNTLPWTFGG GTKLEIK

[00452] A bispecific antibody targeting CD3 and another antigen other than LILRB4 was also constructed in the Fab-scFv-Fab format and used a negative control, and name as “ATG-Ctrl” in the following Examples. ATG-Ctrl comprises one anti-CD3 scFv domain and two Fab domains that bind to another antigen other than LILRB4.

[00453] The benchmark antibody IO-312 was constructed in the Fab3 format (as described in WO2019154890A1), which binds to CD3 and LILRB4, and comprises one anti-CD3 Fab domain and two anti-LILRB4 Fab domains, wherein the anti-CD3

Fab domain comprises a heavy chain variable region having an amino acid sequence of SEQ ID NO: 43, and a light chain variable region having an amino acid sequence of SEQ ID NO: 44; each of the two anti-LILRB4 Fab domains comprises a heavy chain variable region having an amino acid sequence of SEQ ID NO: 45, and a light chain variable region having an amino acid sequence of SEQ ID NO: 46.

Example 2. Bispecific Antibody Characterization: Cell Based Binding Affinity to 293F-hLILRB4 cells and Jurkat T cells

[00454] The LILRB4 binding affinity of the bispecific antibodies (BIS-PTMS16-22+40E9 and BIS-S3+S16+40E9-F3) with 293F cell stably over-expressing human LILRB4 protein (293F-hLILRB4) and Jurkat cells was determined by FACS analysis. The protocol for FACS analysis was described as follows:

- (a) Harvested cells. Then centrifuged the harvested cells at 300 g for 3 min and discarded the supernatant.
- (b) Washed the cells 2 times with FACS buffer by centrifuging at 300 g for 3 min and discarded the supernatant.
- (c) Resuspended the cells, and seeded 2×10^5 cell/well into the assay plate in 50 μ l FACS buffer, then added 50 μ l primary antibody with primary antibody final concentration starting with max conc. 100 nM for LILRB4 binding, max conc. 100 nM for CD3 binding. Incubated at 4 degree for 1 h.
- (d) Cells were washed using the condition in step (b). Resuspended the cells with 100 μ l/well diluted 2nd antibody, incubated at 4 degree for 1h in the dark.
- (e) The cells were washed using condition in step (b). Resuspended the cells with 100 μ l/well FACS buffer. Kept the cells in dark for FACS analysis.

[00455] The CD3 binding affinity of the bispecific antibodies (BIS-PTMS16-22+40E9 and BIS-S3+S16+40E9-F3) with Jurkat T cells was determined by FACS analysis. The protocol for FACS analysis was described as follows:

- (a) Harvested cells. Then centrifuged the harvested cells at 300 g for 5 min and discarded the supernatant.
- (b) Resuspend the cells, and seed 1×10^5 cells/well into the assay plate.
- (c) Washed the cells once with FACS buffer.
- (d) Resuspend the cells with 100 μ L/well serially diluted primary antibodies starting with max conc. 100 nM for LILRB4 binding and max conc. 600 nM for CD3 binding. Incubate at 4 degree for 30-40 min.
- (e) Washed the cells twice with FACS buffer.
- (f) Resuspend the cells with 100 μ L/well 2nd antibodies in a dilution of 1: 500. Incubated at 4 degree for 30-40 min.
- (g) Washed the cells twice with FACS buffer.
- (h) Resuspended the cells with 200 μ L/well FACS buffer. Cells were kept in the dark were analyzed by flow cytometry.

[00456] As shown in Figure 2A, Figure 2B and Table 8 below, BIS-PTMS16-22+40E9 and BIS-S3+S16+40E9-F3 bound to LILRB4 positive cells with a single-digit nM affinity, and bound to Jurkat T cells with a quite low affinity from 100 to 200 nM.

Table 8. Binding affinity of BIS-PTMS16-22+40E9 and BIS-S3+S16+40E9-F3 on 293F-hLILRB4 cell line and Jurkat T cells by FACS.

Antibodies	293F-hLILRB4		Jurkat T cells	
	EC ₅₀ (nM)	Max MFI	EC ₅₀ (nM)	Max MFI
Isotype (hIgG1-LALA)	/	240	/	65
ATG-Ctrl	35.16	253	182	1379
BIS-PTMS16-22+40E9	1.089	40248	124.5	995
BIS-S3+S16+40E9-F3	2.139	38978	159.8	1066

Example 3. Bispecific Antibody Characterization: T Cell Activation

[00457] The T cell activation of the bispecific antibodies BIS-PTMS16-22+40E9 and BIS-S3+S16+40E9-F3 was tested by FACS analysis. The protocol for FACS analysis is described as follows:

- (a) Isolated T cells from fresh human PBMC and resuspend in complete RPMI1640 media.
- (b) Seeded 5×10^4 , 100 μL /well T cells to 96-well cell culture plate.
- (c) Seeded 1×10^4 , 50 μL /well tumor cells to the plate.
- (d) Added 50 μL /well 4 $\times$ final conc. increasing concentrations of antibodies beginning with max conc. 10 nM.
- (e) After 24 hrs and 72 hrs incubation, centrifuged at 500 g for 5 min and collected cells followed by CD69 (24 hrs) and CD25 (72 hrs) analysis gated from CD8+ T cells by FACS.

[00458] As shown in Table 9 and Figure 3, BIS-PTMS16-22+40E9 and BIS-S3+S16+40E9-F3 bispecific antibodies induced strong T cell activation accompanied by very low CD3 binding affinity via FACS analysis of early marker CD69 and latter marker CD25 of T cell activation.

Table 9. EC₅₀ and max percentage of CD69 and CD25 induced by BIS-PTMS16-22+40E9 and BIS-S3+S16+40E9-F3.

Antibodies	CD69 ⁺ of CD8 ⁺ T cells		CD25 ⁺ of CD8 ⁺ T cells	
	EC ₅₀ (pM)	Max (%)	EC ₅₀ (pM)	Max (%)
Isotype (hIgG1-LALA)	/	3.12	/	0
ATG-Ctrl	97.53	86.6	85.9	85.9
BIS-PTMS16-22+40E9	90.37	88.6	98.33	99.2
BIS-S3+S16+40E9-F3	90.67	92.5	98.46	99.3

Example 4. Bispecific Antibody Characterization: T-Cell Dependent Cytotoxicity

[00459] 1×10^4 /well CFSE labeled target cells were seeded to the non-treated 96-well U-bottom plate, then 1×10^4 (E: T=1: 1) or 5×10^4 (E: T=5: 1) freshly isolated T cells from human PBMCs were added as effector cell. After that, human T cells, CFSE

labeled target cells, and increasing concentrations of antibodies (human IgG1-LALA as a negative control) were combined in 200 μ L total in RPMI-1640 medium in U-shaped 96-well plate and incubated for 24 hours at 37 degree, 5% CO₂ for 24 hours. Finally, cells were washed and resuspended in 200 μ L of staining buffer. Cells were acquired by FACS and percentages of CFSE labeled target cells were measured. Percent of cell cytotoxicity = 100 - ([AT/NAT] $\times$ 100) (AT: antibody treated group, NAT: no antibody treated group).

[00460] As shown in Figure 4, LILRB4 is highly expressed on THP-1 AML cells (M5) and lowly expressed on MOLM-13 AML cells (M5a). The bispecific antibodies BIS-PTMS16-22+40E9 and BIS-S3+S16+40E9-F3 showed potent TDCC effect on LILRB4-high expressing THP-1 (M5) AML cells. The antibody BIS-PTMS16-22+40E9 showed lower EC₅₀ and higher max cytotoxicity compared with benchmark antibody IO-312, while BIS-S3+S16+40E9-F3 showed higher EC₅₀ and higher max cytotoxicity compared with benchmark antibody IO-312 (Figure 5A and Table 10).

[00461] The bispecific antibodies BIS-PTMS16-22+40E9 and BIS-S3+S16+40E9-F3 showed potent TDCC effect on LILRB4-low expressing MOLM-13 (a human AML cell line, M5a). The antibody BIS-S3+S16+40E9-F3 showed higher max cytotoxicity compared with benchmark antibody IO-312 (Figure 5B and Table 10), while BIS-PTMS16-22+40E9 showed comparable cytotoxicity compared with benchmark antibody IO-312.

Table 10. EC₅₀ and max cell cytotoxicity of antibodies induced TDCC effect on THP-1 cell.

Antibodies	LILRB4-high expressing THP-1		LILRB4-low expressing MOLM-13	
	EC ₅₀ (pM)	Max (%)	EC ₅₀ (pM)	Max (%)
Isotype (hIgG1-LALA)	/	0.6908	45.94	-3.714
ATG-Ctrl	/	14.06	333.7	-3.569
IO-312	0.5549	63.98	2.662	57.44
BIS-PTMS16-22+40E9	0.3216	72.57	2.426	53.07

BIS-S3+S16+40E9-F3	0.9197	64.29	10.74	67.62
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Example 5. Bispecific Antibody Characterization: PBMC-mediated cellular cytotoxicity

[00462] To further characterize the bispecific antibodies, the PBMC-mediated cellular cytotoxicity thereof was tested. The protocol is described as follows.

[00463] 1×10^4 /well CFSE labeled target cells as target cells were seeded to the non-treated 96-well U-bottom plate, then 1×10^5 freshly isolated human PBMCs were added as effector cell. After that, human PBMC cells, CFSE labeled target cells, and increasing concentrations of antibodies (human IgG1-LALA as a negative control) were combined in 200 μ L total in RPMI-1640 medium in U-shaped 96-well plate and incubated for 24 hours at 37 degree, 5% CO₂ for 24 hour. Finally, cells were washed and resuspended in 200 μ L of staining buffer. Cells were acquired by FACS and percentages of CFSE labeled target cells were measured. Percent of cell cytotoxicity = 100 - ([AT/NAT] $\times$ 100) (AT: antibody treated group, NAT: no antibody treated group).

[00464] As shown in Figure 6 and Table 11, both the antibodies BIS-PTMS16-22+40E9 and BIS-S3+S16+40E9-F3 induced more potent TDCC effect against THP-1 AML cells than benchmark antibody IO-312.

Table 11. EC₅₀ and max cell cytotoxicity of antibodies induced PBMC-mediated cellular cytotoxicity effect on THP-1 cell

Antibodies	PBMC-mediated cellular cytotoxicity	
	EC ₅₀ (pM)	Max (%)
Isotype (hIgG1-LALA)	/	0.2333
ATG-Ctrl	3167	51.25
IO-312	1.401	48.41
BIS-PTMS16-22+40E9	0.3959	67.79
BIS-S3+S16+40E9-F3	1.375	69.96

Example 6. Bispecific Antibody Characterization: *in vivo* efficacy in the treatment of PBMC-humanized NCG mice bearing THP-1-luciferase cells

[00465] The *in vivo* efficacy of the bispecific antibodies was tested. The protocol is described as follows.

- (a) THP-1-luciferase cells were maintained in vitro with RPMI-1640 medium supplemented with 10% fetal bovine serum at 37 degree in an atmosphere of 5% CO₂ in air. The cells in exponential growth phase were harvested and quantitated by cell counter before tumor inoculation.
- (b) Each NCG mouse was inoculated intravenously with THP-1-luciferase cells (1 × 10⁶) in 0.2 ml of PBS for tumor development.
- (c) The randomization was start 4 hrs after tumor inoculation. 36 mice were enrolled in this study. All animals were randomly allocated to 6 study groups. Randomization were performed based on randomized block design.
- (d) 3 days after tumor inoculation, NCG mice were injected with human PBMC i.v. (10×10⁶ cells), followed by i.v. injection of 200 μL of antibodies at 0.5 hr after PBMC engraftment.
- (e) Tumor flux was measured once per week by caliper and bioluminescence imaging (BLI). Dosing as well as tumor and body weight measurements were conducted in a Laminar Flow Cabinet. (Table 12).

Table 12. Study design for *in vivo* efficacy test of PBMC-THP-1-luc-humanized NCG mice

Group	NO.	Treatment	Tumor model	Dose	ROA	Dosing Frequency & Duration
1	6	Isotype (hIgG1-LALA)	PBMC-THP-1-luc-humanized NCG mice	1 mg/kg	<i>i.p.</i>	BIW, 5 doses
2	6	IO-312	PBMC-THP-1-luc-humanized NCG mice	1 mg/kg	<i>i.p.</i>	BIW, 5 doses

3	6	BIS-PTMS16-22+40E9	PBMC-THP-1-luc-humanized NCG mice	1 mg/kg	<i>i.p.</i>	BIW, 5 doses
4	6	BIS-S3+S16+40E9-F3	PBMC-THP-1-luc-humanized NCG mice	1 mg/kg	<i>i.p.</i>	BIW, 5 doses
5	6	IO-312	PBMC-THP-1-luc-humanized NCG mice	0.1 mg/kg	<i>i.p.</i>	BIW, 5 doses
6	6	BIS-S3+S16+40E9-F3	PBMC-THP-1-luc-humanized NCG mice	0.1 mg/kg	<i>i.p.</i>	BIW, 5 doses

[00466] BIS-S3+S16+40E9-F3 showed more potent *in vivo* anti-tumor efficacy compared with the benchmark antibody IO-312 at 1 mg/kg and 0.1 mg/kg dose level in PBMC-humanized NCG mice bearing THP-1-luciferase cells (Figure 7A).

[00467] BIS-PTMS16-22+40E9 showed more potent *in vivo* anti-tumor efficacy compared with the benchmark antibody IO-312 at 1 mg/kg dose level in PBMC-humanized NCG mice bearing THP-1-luciferase cells (Figure 7A).

**Example 7. Humanized LILRB4 x CD3 Bispecific Antibody Characterization:
Cell Based Binding Affinity to 293F-hLILRB4 cells and Jurkat T cells**

[00468] The LILRB4 binding affinity of BIS-PTMS16-22+40E9-L2H3, BIS-PTMS16-22+40E9-L2H3-N55S.H, BIS-F3+40E9-L2H3 and BIS-F3+40E9-L2H3-N55S.H antibodies and benchmark antibody IO-312 with 293F cell stably over-expressing human LILRB4 protein (293F-hLILRB4) and Jurkat T cells was determined by FACS analysis. The protocol for FACS analysis was described in Example 2.

[00469] The CD3 binding affinity of BIS-PTMS16-22+40E9-L2H3, BIS-PTMS16-22+40E9-L2H3-N55S.H, BIS-F3+40E9-L2H3 and BIS-F3+40E9-L2H3-N55S.H antibodies and benchmark antibody IO-312 with Jurkat T cells was determined by FACS analysis. The protocol for FACS analysis was described in Example 2.

[00470] As shown in Figure 8A and Table 13 below, BIS-PTMS16-22+40E9-L2H3, BIS-PTMS16-22+40E9-L2H3-N55S.H, BIS-F3+40E9-L2H3, BIS-F3+40E9-L2H3-N55S.H and benchmark antibody IO-312 bound to LILRB4 positive cells with a single-digit nM affinity.

[00471] As shown in Figure 8B and Table 13 below, BIS-PTMS16-22+40E9-L2H3, BIS-PTMS16-22+40E9-L2H3-N55S.H, BIS-F3+40E9-L2H3 and BIS-F3+40E9-L2H3-N55S.H bound to Jurkat T cells with low affinity (EC_{50}) ranged from 300 to 4000 nM in the absence of LILRB4 positive cells (Figure 8B and Table 13), while IO-312 showed stronger T cell binding with an EC_{50} of 153.2nM.

Table 13. Binding affinity of IO-312, BIS-PTMS16-22+40E9-L2H3, BIS-PTMS16-22+40E9-L2H3-N55S.H, BIS-F3+40E9-L2H3 and BIS-F3+40E9-L2H3-N55S.H antibodies on 293F-hLILRB4 cells and Jurkat T cells by FACS.

Antibodies	293F-hLILRB4		Jurkat T cells	
	EC_{50} (nM)	Max MFI	EC_{50} (nM)	Max MFI
Isotype (hIgG1-LALA)	/	249.6	/	65.67
IO-312	1.035	32239	153.2	1762
BIS-PTMS16-22+40E9-L2H3	1.005	33585	578.2	597.6
BIS-PTMS16-22+40E9-L2H3-N55S.H	1.098	32800	3612	1589
BIS-F3+40E9-L2H3	1.88	31354	514.8	364.3
BIS-F3+40E9-L2H3-N55S.H	1.921	31100	323.7	128

**Example 8. Humanized LILRB4 x CD3 Bispecific Antibody Characterization:
T-Cell Dependent Cytotoxicity**

[00472] 1×10^4 /well CFSE labeled target cells were seeded to the non-treated 96-well U-bottom plate, then 1×10^4 (E: T=1: 1) or 5×10^4 (E: T=5: 1) freshly isolated T cells from human PBMCs were added as effector cell. After that, human T cells, CFSE labeled target cells, and increasing concentrations of antibodies (human IgG1-LALA as a negative control) were combined in 200 μ L total in RPMI-1640 medium in U-shaped 96-well plate and incubated for 24 hours at 37 degree, 5% CO₂ for 24 hours. Finally, cells were washed and resuspended in 200 μ L of staining buffer. Cells were acquired by FACS and percentages of CFSE labeled target cells were measured. Percent of cell cytotoxicity = $100 - ([AT/NAT] \times 100)$ (AT: antibody treated group, NAT: no antibody treated group).

[00473] LILRB4 is highly expressed on THP-1 AML cells (M5) and lowly expressed on MOLM-13 AML cells (M5a). As shown in Figure 9A and Table 14, all the generated multi-specific antibodies (*i.e.*, BIS-PTMS16-22+40E9-L2H3, BIS-PTMS16-22+40E9-L2H3-N55S.H, BIS-F3+40E9-L2H3 and BIS-F3+40E9-L2H3-N55S.H) showed potent TDCC effect on LILRB4-high expressing THP-1 (M5) AML cells. The antibody BIS-F3+40E9-L2H3-N55S.H showed higher max cytotoxicity compared with benchmark antibody IO-312.

Table 14. EC₅₀ and max cell cytotoxicity of antibodies induced TDCC effect on THP-1 AML cells and MOLM-13 AML cells.

Antibodies	LILRB4-high expressing THP-1 AML cell		LILRB4-low expressing MOLM-13 AML cell	
	EC ₅₀ (pM)	Max (%)	EC ₅₀ (pM)	Max (%)
Isotype (hIgG1-LALA)	/	1.977	/	/
IO-312	0.4513	87.92	7.575	50.59
BIS-PTMS16-22+40E9-L2H3	0.8831	89.86	7.159	43.49
BIS-PTMS16-22+40E9-L2H3-N55S.H	0.675	85.45	6.958	43.51
BIS-F3+40E9-L2H3	1.529	88.95	26.86	56.76
BIS-F3+40E9-L2H3-	0.6193	90.6	23.41	54.63

N55S.H				
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Example 9. Humanized LILRB4 x CD3 Bispecific Antibody Characterization:
***in vivo* Efficacy in the Treatment of PBMC-humanized NCG Mice Bearing THP-1-luciferase Cells**

[00474] The *in vivo* efficacies of the humanized LILRB4 x CD3 bispecific antibodies were tested. The protocol is described in Example 6. Study design was shown in Table 15.

Table 15. Study design for *in vivo* efficacy test of PBMC-THP-1-luc-humanized NCG mice

Group	NO.	Treatment	Tumor model	Dose	ROA	Dosing Frequency & Duration
1	6	Isotype (hIgG1-LALA)	PBMC-THP-1-luc-humanized B-NDG mice	1 mg/kg	<i>i.p.</i>	BIW, 5 doses
2	6	IO-312	PBMC-THP-1-luc-humanized B-NDG mice	1 mg/kg	<i>i.p.</i>	BIW, 5 doses
3	6	BIS-PTMS16-22+40E9-L2H3	PBMC-THP-1-luc-humanized B-NDG mice	1 mg/kg	<i>i.p.</i>	BIW, 5 doses
4	6	BIS-PTMS16-22+40E9-L2H3-N55S.H	PBMC-THP-1-luc-humanized B-NDG mice	1 mg/kg	<i>i.p.</i>	BIW, 5 doses
5	6	BIS-F3+40E9-L2H3	PBMC-THP-1-luc-humanized B-NDG mice	1 mg/kg	<i>i.p.</i>	BIW, 5 doses
6	6	BIS-F3+40E9-L2H3-N55S.H	PBMC-THP-1-luc-humanized B-NDG mice	1 mg/kg	<i>i.p.</i>	BIW, 5 doses

[00475] As shown in Figure 10, BIS-F3+40E9-L2H3-N55S.H (a LILRB4 x CD3 T cell engager targeting dual LILRB4 epitopes) showed more potent *in vivo* anti-tumor

efficacy compared with the benchmark antibody IO-312 and BIS-PTMS16-22+40E9-L2H3-N55S.H that are LILRB4 x CD3 T cell engagers targeting single LILRB4 epitopes at 1 mg/kg dose level in PBMC-humanized B-NDG mice bearing THP-1-luciferase cells.

WHAT CLAIMED IS:

1. An antibody or antigen-binding fragment thereof that binds to CD3 and LILRB4, comprising a first binding moiety that binds to CD3 and a second binding moiety that binds to LILRB4,

wherein the first binding moiety comprises

one or two or three heavy chain complementarity determining regions (HCDR1, HCDR2 and/or HCDR3) contained within the heavy chain variable (VH) region sequence of SEQ ID NOs: 7, 9, 10, 47 or 11; and/or

one or two or three light chain complementarity determining regions (LCDR1, LCDR2 and/or LCDR3) contained within the light chain variable (VL) region sequence of SEQ ID NOs: 8, 12 or 13;

and,

wherein the second binding moiety comprises

one or two or three heavy chain complementarity determining regions (HCDR1, HCDR2 and/or HCDR3) contained within the heavy chain variable (VH) region sequence of SEQ ID NOs: 20, 22, 30, or 33; and/or

one or two or three light chain complementarity determining regions (LCDR1, LCDR2 and/or LCDR3) contained within the light chain variable (VL) region sequence of SEQ ID NOs: 21, 23, 31 or 34.

2. The antibody or antigen-binding fragment thereof of claim 1,

wherein the first binding moiety comprises at least one heavy or light chain complementarity determining region (CDR) comprising an amino acid sequence selected from the group consisting of SEQ ID NOs: 1, 2, 42, 3, 4, 5 and 6.

3. The antibody or antigen-binding fragment thereof of claim 1 or 2, wherein the first binding moiety comprises one or two or three of HCDR1, HCDR2 and HCDR3,

wherein the HCDR1 comprises an amino acid sequence as set forth in SEQ ID NO: 1, the HCDR2 comprises an amino acid sequence as set forth in SEQ ID NO: 2 or SEQ ID NO: 42, and the HCDR3 comprising an amino acid sequence as set forth in SEQ ID NO: 3.

4. The antibody or antigen-binding fragment thereof of any one of the preceding claims, wherein the first binding moiety comprises a HCDR1 comprising an amino acid sequence as set forth in SEQ ID NO: 1, a HCDR2 comprising an amino acid sequence as set forth in SEQ ID NO: 2, and a HCDR3 comprising an amino acid sequence as set forth in SEQ ID NO: 3; or

wherein the first binding moiety comprises a HCDR1 comprising an amino acid sequence as set forth in SEQ ID NO: 1, a HCDR2 comprising an amino acid sequence as set forth in SEQ ID NO: 42, and a HCDR3 comprising an amino acid sequence as set forth in SEQ ID NO: 3.

5. The antibody or antigen-binding fragment thereof of any one of the preceding claims, wherein the first binding moiety comprises one or two or three of LCDR1, LCDR2 and LCDR3,

wherein the LCDR1 comprises an amino acid sequence as set forth in SEQ ID NO: 4, the LCDR2 comprises an amino acid sequence as set forth in SEQ ID NO: 5, and the LCDR3 comprises an amino acid sequence as set forth in SEQ ID NO: 6.

6. The antibody or antigen-binding fragment thereof of claim 5, wherein the first binding moiety comprises a LCDR1 comprising an amino acid sequence as set forth in SEQ ID NO: 4, a LCDR2 comprising an amino acid sequence as set forth in SEQ ID NO: 5, and a LCDR3 comprising an amino acid sequence as set forth in SEQ ID NO: 6.

7. The antibody or antigen-binding fragment thereof of any one of the preceding claims, wherein the first binding moiety comprises:

a HCDR1 comprising an amino acid sequence as set forth in SEQ ID NO: 1,

- a HCDR2 comprising an amino acid sequence as set forth in SEQ ID NO: 2,
- a HCDR3 comprising an amino acid sequence as set forth in SEQ ID NO: 3,
- a LCDR1 comprising an amino acid sequence as set forth in SEQ ID NO: 4,
- a LCDR2 comprising an amino acid sequence as set forth in SEQ ID NO: 5, and
- a LCDR3 comprising an amino acid sequence as set forth in SEQ ID NO: 6; or

wherein the first binding moiety comprises:

- a HCDR1 comprising an amino acid sequence as set forth in SEQ ID NO: 1,
- a HCDR2 comprising an amino acid sequence as set forth in SEQ ID NO: 42,
- a HCDR3 comprising an amino acid sequence as set forth in SEQ ID NO: 3,
- a LCDR1 comprising an amino acid sequence as set forth in SEQ ID NO: 4,
- a LCDR2 comprising an amino acid sequence as set forth in SEQ ID NO: 5, and
- a LCDR3 comprising an amino acid sequence as set forth in SEQ ID NO: 6.

8. The antibody or antigen-binding fragment thereof of any one of the preceding claims, wherein the first binding moiety comprise a VH region having an amino acid sequence as set forth in SEQ ID NOS: 7, 9, 10, 47 or 11, or a homologous sequence thereof having at least 80% sequence identity to SEQ ID NOS: 7, 9, 10, 47 or 11.

9. The antibody or antigen-binding fragment thereof of any one of the preceding claims, wherein the first binding moiety comprises a VL region having an amino acid sequence as set forth in SEQ ID NOS: 8, 12 or 13, or a homologous sequence thereof having at least 80% sequence identity to SEQ ID NOS: 8, 12 or 13.

10. The antibody or antigen-binding fragment thereof of any one of the preceding claims, wherein the first binding moiety comprises a VH/VL amino acid sequence pair of SEQ ID NOS: 7/8, 7/12, 7/13, 9/8, 9/12, 9/13, 10/8, 10/12, 10/13, 47/8, 47/12, 47/13, 11/8, 11/12 or 11/13.

11. The antibody or antigen-binding fragment thereof of any one of the preceding claims, wherein the second binding moiety comprises at least one heavy or light chain complementarity determining region (CDR) comprising an amino acid sequence selected from the group consisting of SEQ ID NOS: 14, 15, 16, 17, 18, 19, 24, 25, 26, 27, 28, 29 and 32.

12. The antibody or antigen-binding fragment thereof of any one of the preceding claims, wherein the second binding moiety comprises one or two or three of HCDR1, HCDR2 and HCDR3 comprising an amino acid sequence selected from the group consisting of SEQ ID NOs: 14, 15, 16, 24, 25, 26 and 32.

13. The antibody or antigen-binding fragment thereof of any one of the preceding claims, wherein the second binding moiety comprises one or two or three of LCDR1, LCDR2 and LCDR3 comprising an amino acid sequence selected from the group consisting of SEQ ID NOs: 17, 18, 19, 27, 28 and 29.

14. The antibody or antigen-binding fragment thereof of any one of the preceding claims, wherein the second binding moiety comprises:

- i. a HCDR1 comprising an amino acid sequence selected from the group consisting of SEQ ID NOs: 14 and 24,
- ii. a HCDR2 comprising an amino acid sequence selected from the group consisting of SEQ ID NOs: 15, 25, and 32, and
- iii. a HCDR3 comprising an amino acid sequence selected from the group consisting of SEQ ID NOs: 16 and 26.

15. The antibody or antigen-binding fragment thereof of any one of the preceding claims, wherein the second binding moiety comprises:

- i. a LCDR1 comprising an amino acid sequence selected from the group consisting of SEQ ID NOs: 17 and 27,
- ii. a LCDR2 comprising an amino acid sequence selected from the group consisting of SEQ ID NOs: 18 and 28, and
- iii. a LCDR3 comprising an amino acid sequence selected from the group consisting of SEQ ID NOs: 19 and 29.

16. The antibody or antigen-binding fragment thereof of any one of the preceding claims, wherein the second binding moiety comprises:

- i. a HCDR1 comprising an amino acid sequence as set forth in SEQ ID NO: 14, a HCDR2 comprising an amino acid sequence as set forth in SEQ ID NO: 15, and a HCDR3 comprising an amino acid sequence as set forth in SEQ ID NO: 16;
 - ii. a HCDR1 comprising an amino acid sequence as set forth in SEQ ID NO: 24, a HCDR2 comprising an amino acid sequence as set forth in SEQ ID NO: 25, and a HCDR3 comprising an amino acid sequence as set forth in SEQ ID NO: 26; or
 - iii. a HCDR1 comprising an amino acid sequence as set forth in SEQ ID NO: 24, a HCDR2 comprising an amino acid sequence as set forth in SEQ ID NO: 32, and a HCDR3 comprising an amino acid sequence as set forth in SEQ ID NO: 26.
17. The antibody or antigen-binding fragment thereof of any one of the preceding claims, wherein the second binding moiety comprises:
- i. a LCDR1 comprising an amino acid sequence as set forth in SEQ ID NO: 17, a LCDR2 comprising an amino acid sequence as set forth in SEQ ID NO: 18, and a LCDR3 comprising an amino acid sequence as set forth in SEQ ID NO: 19; or
 - ii. a LCDR1 comprising an amino acid sequence as set forth in SEQ ID NO: 27, a LCDR2 comprising an amino acid sequence as set forth in SEQ ID NO: 28, and a LCDR3 comprising an amino acid sequence as set forth in SEQ ID NO: 29.
18. The antibody or antigen-binding fragment thereof of any one of the preceding claims,
- wherein the second binding moiety comprises:
- i. a HCDR1 comprising an amino acid sequence as set forth in SEQ ID NO: 14, a HCDR2 comprising an amino acid sequence as set forth in SEQ ID NO: 15,

a HCDR3 comprising an amino acid sequence as set forth in SEQ ID NO: 16,
a LCDR1 comprising an amino acid sequence as set forth in SEQ ID NO: 17,
a LCDR2 comprising an amino acid sequence as set forth in SEQ ID NO: 18,
and a LCDR3 comprising an amino acid sequence as set forth in SEQ ID
NO: 19;

- ii. a HCDR1 comprising an amino acid sequence as set forth in SEQ ID NO: 24,
a HCDR2 comprising an amino acid sequence as set forth in SEQ ID NO: 25,
a HCDR3 comprising an amino acid sequence as set forth in SEQ ID NO: 26,
a LCDR1 comprising an amino acid sequence as set forth in SEQ ID NO: 27,
a LCDR2 comprising an amino acid sequence as set forth in SEQ ID NO: 28,
and a LCDR3 comprising an amino acid sequence as set forth in SEQ ID
NO: 29; or

- iii. a HCDR1 comprising an amino acid sequence as set forth in SEQ ID NO: 24,
a HCDR2 comprising an amino acid sequence as set forth in SEQ ID NO: 32,
a HCDR3 comprising an amino acid sequence as set forth in SEQ ID NO: 26,
a LCDR1 comprising an amino acid sequence as set forth in SEQ ID NO: 27,
a LCDR2 comprising an amino acid sequence as set forth in SEQ ID NO: 28,
and a LCDR3 comprising an amino acid sequence as set forth in SEQ ID
NO: 29.

19. The antibody or antigen-binding fragment thereof of any one of the preceding claims, wherein the second binding moiety comprises a VH region having an amino acid sequence as set forth in SEQ ID NOs: 20, 22, 30 or 33, or a homologous sequence thereof having at least 80% sequence identity to SEQ ID NOs: 20, 22, 30 or 33.

20. The antibody or antigen-binding fragment thereof of any one of the preceding claims, wherein the second binding moiety comprises a VL region having an amino acid sequence as set forth in SEQ ID NOs: 21, 23, 31 or 34, or a homologous

sequence thereof having at least 80% sequence identity to SEQ ID NOs: 21, 23, 31 or 34.

21. The antibody or antigen-binding fragment thereof of any one of the preceding claims, wherein the second binding moiety comprises a VH/VL amino acid sequence pair of SEQ ID NOs: 20/21, 22/23, 30/31, or 33/34.

22. The antibody or antigen-binding fragment thereof of any one of any one of the preceding claims, wherein the first binding moiety and the second binding moiety are independently a single-chain Fv (scFv) or a Fab domain.

23. The antibody or antigen-binding fragment thereof of claim 22, wherein the first binding moiety is an anti-CD3 scFv, and the second binding moiety is an anti-LILRB4 Fab domain; or the first binding moiety is an anti-CD3 Fab domain, and the second binding moiety is an anti-LILRB4 scFv.

24. The antibody or antigen-binding fragment thereof of claim 22 or 23, wherein the first binding moiety is linked to the second binding moiety via a linker.

25. The antibody or antigen-binding fragment thereof of claim 24, wherein the N-terminus of the first binding moiety is linked to the C-terminus of the second binding moiety via the linker.

26. The antibody or antigen-binding fragment thereof of any one of the preceding claims, comprising, from N-terminus to C-terminus, a first chain comprising a VH region from the second binding moiety, a CH1 from the second binding moiety, a VH region from the first binding moiety and a VL region from the first binding moiety.

27. The antibody or antigen-binding fragment thereof of claim 26, wherein the CH1 region from the second binding moiety and the VH region from the first binding moiety are directly linked or linked via a linker.

28. The antibody or antigen-binding fragment thereof of claim 26, wherein the VH region from the first binding moiety and the VL region from the first binding moiety are directly linked or linked via a linker.

29. The antibody or antigen-binding fragment thereof of any one of claims 24-28, wherein the linker is a linker comprising glycine and serine, *e.g.*, a (GGGGS)₂ linker or a GSSS linker.
30. The antibody or antigen-binding fragment thereof of claim 26, further comprising a second chain comprising a VL region from the second binding moiety and a CL from the second binding moiety.
31. The antibody or antigen-binding fragment thereof of any one of the preceding claims, comprising a third binding moiety that binds to LILRB4.
32. The antibody or antigen-binding fragment thereof of claim 31, wherein the third binding moiety and the second binding moiety bind to the same epitope or different epitopes on LILRB4.
33. The antibody or antigen-binding fragment thereof of claim 32, wherein the third binding moiety is a Fab domain.
34. The antibody or antigen-binding fragment thereof of any one of claims 31-33, wherein the first binding moiety is an anti-CD3 scFv, the second binding moiety is an anti-LILRB4 Fab domain, and the third binding moiety is an anti-LILRB4 Fab domain.
35. The antibody or antigen-binding fragment thereof of claim 34, wherein the amino acid sequence of the second binding moiety is the same as the amino acid sequence of the third binding moiety.
36. The antibody or antigen-binding fragment thereof of any one of the preceding claims, wherein the first binding moiety, the second binding moiety and/or the third binding moiety further comprises one or more amino acid residue substitutions or modifications yet retains specific binding affinity to CD3 or LILRB4.
37. The antibody or antigen-binding fragment thereof of claim 36, wherein at least one of the substitutions or modifications is within one or more of the CDR sequences

of the VH region or VL region of the first binding moiety, the second binding moiety, or the third binding moiety.

38. The antibody or antigen-binding fragment thereof of claim 36, wherein at least one of the substitutions or modifications is within one or more of the non-CDR sequences of the VH region or VL region of the first binding moiety, the second binding moiety, or the third binding moiety.

39. The antibody or antigen-binding fragment thereof of any one of the preceding claims, further comprising one or more non-natural amino acid (NNAA) substitution.

40. The antibody or antigen-binding fragment thereof of claim 39, wherein the NNAA is capable of being conjugated.

41. The antibody or antigen-binding fragment thereof of any one of the preceding claims, which is a chimeric, a humanized or a human antibody or an antigen-binding fragment thereof.

42. The antibody or antigen-binding fragment thereof of any one of the preceding claims, which is a labeled antibody, a bivalent antibody, an anti-idiotypic antibody or a fusion protein.

43. The antibody or antigen-binding fragment thereof of any one of the preceding claims, further comprising an Fc region, optionally an Fc region of human immunoglobulin (Ig), or optionally an Fc region of human IgG.

44. The antibody or antigen-binding fragment thereof of claim 43, wherein the Fc region is derived from human IgG1, IgG2, IgG3, or IgG4.

45. The antibody or antigen-binding fragment thereof of claim 44, wherein the Fc region comprises one or more amino acid substitutions selected from the group consisting of: L234A, L235A, S354C, T366W, Y349C, T366S, L368A, and Y407V (according to EU numbering).

46. The antibody or antigen-binding fragment thereof of claim 45, wherein the Fc region comprises L234A and L235A (according to EU numbering) amino acid substitutions.
47. The antibody or antigen-binding fragment thereof of any one of claims 31-46, wherein both of the first binding moiety and the third binding moiety are linked to the Fc region.
48. The antibody or antigen-binding fragment thereof of any one of claims 31-46, wherein both of the second binding moiety and the third binding moiety are linked to the Fc region.
49. The antibody or antigen-binding fragment thereof of claim 47, comprising, from N-terminus to C-terminus, a first chain comprising a VH region from the second binding moiety, a CH1 from the second binding moiety, a VH region from the first binding moiety, a VL region from the first binding moiety, a CH2 from the Fc region, and a CH3 from the Fc region.
50. The antibody or antigen-binding fragment thereof of claim 49, further comprising a second chain comprising a VL region from the second binding moiety, and a CL from the second binding moiety.
51. The antibody or antigen-binding fragment thereof of any one of the preceding claims, wherein the light chain is a λ light chain or a κ light chain.
52. The antibody or antigen-binding fragment thereof of any one of preceding claims, which is linked to one or more conjugate moieties.
53. The antibody or antigen-binding fragment thereof of claim 52, wherein the conjugate moiety comprises an antigen-binding fragment of an antibody, a clearance-modifying agent, a chemotherapeutic agent, a toxin, a radioactive isotope, a lanthanide, a detectable label, a DNA-alkylator, a topoisomerase inhibitor, a tubulin-binder, a purification moiety or other anticancer drugs.

54. The antibody or antigen-binding fragment thereof of claim 53, wherein the conjugate moiety is a scFv.
55. The antibody or antigen-binding fragment thereof of claim 54, wherein the scFv is a fourth binding moiety that binds to LILRB4.
56. The antibody or antigen-binding fragment thereof of claim 55, wherein the second and third binding moieties bind to a first epitope on LILRB4, while the fourth binding moiety binds to a second epitope on LILRB4.
57. The antibody or antigen-binding fragment thereof of claim 56, wherein the amino acid sequence of the second binding moiety is the same as the amino acid sequence of the third binding moiety, while the amino acid sequence of the fourth binding moiety is different from the amino acid sequence of the second and third binding moieties.
58. The antibody or antigen-binding fragment thereof of any one of claims 52-57, wherein the conjugate moiety is covalently attached either directly or via a linker.
59. The antibody or antigen-binding fragment thereof of claim 58, wherein the linker is a linker comprising glycine and serine, *e.g.*, a (GGGGS)₂ linker or a GSSS linker.
60. The antibody or antigen-binding fragment thereof of any one of claims 55-59, comprising, from N-terminus to C-terminus, a first chain comprising a VH region from the second binding moiety, a CH1 from the second binding moiety, a VH region from the first binding moiety, a VL region from the first binding moiety, a CH2 from the Fc region, a CH3 from the Fc region, a VH region from the fourth binding moiety, and a VL region from the fourth binding moiety.
61. An antibody or antigen-binding fragment thereof, which competes for binding to CD3 or LILRB4 with the antibody or antigen-binding fragment thereof of any one of the preceding claims.
62. The antibody or antigen-binding fragment thereof of any one of the preceding claims, which is a multi-specific (*e.g.*, bispecific, tri-specific) antibody or an antigen-binding fragment thereof.

63. The antibody or antigen-binding fragment thereof of claim 62, which binds to CD3 and two different epitopes on LILRB4.

64. The antibody or antigen-binding fragment thereof of any one of the preceding claims, wherein

the first binding moiety comprises HCDR1, HCDR2 and HCDR3 contained within the VH region sequence of SEQ ID NOs: 7, 9, 10, 47 or 11; and LCDR1, LCDR2 and LCDR3 contained within the VL region sequence of SEQ ID NOs: 8, 12 or 13; and

each of the second and third binding moieties comprises HCDR1, HCDR2 and HCDR3 contained within the VH region sequence of SEQ ID NOs: 20, 22, 30, or 33; and LCDR1, LCDR2 and LCDR3 contained within the VL region sequence of SEQ ID NOs: 21, 23, 31 or 34.

65. The antibody or antigen-binding fragment thereof of claim 64, wherein

the first binding moiety comprises HCDR1, HCDR2, HCDR3, LCDR1, LCDR2 and LCDR3 contained within a VH/VL amino acid sequence pair of SEQ ID NOs: 7/8, 7/12, 7/13, 9/8, 9/12, 9/13, 10/8, 10/12, 10/13, 47/8, 47/12, 47/13, 11/8, 11/12 or 11/13; and

each of the second and third binding moieties comprises HCDR1, HCDR2, HCDR3, LCDR1, LCDR2 and LCDR3 contained within a VH/VL amino acid sequence pair of SEQ ID NOs: 20/21, 22/23, 30/31, or 33/34.

66. The antibody or antigen-binding fragment thereof of claim 65, wherein

the first binding moiety comprises HCDR1, HCDR2, HCDR3, LCDR1, LCDR2 and LCDR3 contained within a VH/VL amino acid sequence pair of SEQ ID NOs: 7/8, 7/12, 7/13, 9/8, 9/12, 9/13, 10/8, 10/12, 10/13, 47/8, 47/12, 47/13, 11/8, 11/12 or 11/13;

each of the second and third binding moieties comprises HCDR1, HCDR2, HCDR3, LCDR1, LCDR2 and LCDR3 contained within a VH/VL amino acid sequence pair of SEQ ID NOs: 30/31 or 33/34; and

the fourth binding moiety comprises HCDR1, HCDR2, HCDR3, LCDR1, LCDR2 and LCDR3 contained within a VH/VL amino acid sequence pair of SEQ ID NOs: 20/21 or 22/23.

67. The antibody or antigen-binding fragment thereof of claim 65, wherein

the first binding moiety comprises HCDR1, HCDR2, HCDR3, LCDR1, LCDR2 and LCDR3 contained within a VH/VL amino acid sequence pair of SEQ ID NOs: 7/8, 7/12, 7/13, 9/8, 9/12, 9/13, 10/8, 10/12, 10/13, 47/8, 47/12, 47/13, 11/8, 11/12 or 11/13;

each of the second and third binding moieties comprises HCDR1, HCDR2, HCDR3, LCDR1, LCDR2 and LCDR3 contained within a VH/VL amino acid sequence pair of SEQ ID NOs: 20/21 or 22/23; and

the fourth binding moiety comprises HCDR1, HCDR2, HCDR3, LCDR1, LCDR2 and LCDR3 contained within a VH/VL amino acid sequence pair of SEQ ID NOs: 30/31 or 33/34.

68. A chimeric antigen receptor, comprising the antibody or antigen-binding fragment thereof of any one of claims 1-67, a transmembrane region and an intracellular signal region.

69. The chimeric antigen receptor of claim 68, wherein the transmembrane region comprises a transmembrane region of CD3, CD4, CD8 or CD28.

70. The chimeric antigen receptor of claim 68, wherein the intracellular signal region is selected from the group consisting of: an intracellular signal regions sequence of CD3 (e.g. CD3 ζ), Fc γ RI, CD27, CD28, CD137, CD134, MyD88, CD40, CD278, TLRs, or a combination thereof.

71. The chimeric antigen receptor of any one of claims 68-70, wherein the antigen-binding fragment is a scFv.
72. The chimeric antigen receptor of any one of claims 68-71, wherein the chimeric antigen receptor is grafted onto an allogeneic cell, an autologous cell or a xenogeneic cell.
73. The chimeric antigen receptor of any one of claims 68-72, wherein the chimeric antigen receptor is grafted onto an immune effector cell.
74. The chimeric antigen receptor of any one of claims 68-73, wherein the chimeric antigen receptor is grafted onto a T cell, a natural killer cell, a macrophage cell, or a tumor-infiltrating lymphocyte.
75. A pharmaceutical composition comprising the antibody or antigen-binding fragment thereof of any one of claims 1-67, and/or the chimeric antigen receptor of any one of claims 68-74, and one or more pharmaceutically acceptable carriers.
76. An isolated polynucleotide encoding the antibody or antigen-binding fragment thereof of any one of claims 1-67, and/or the chimeric antigen receptor of any one of claims 68-74.
77. A vector comprising the isolated polynucleotide of claim 76.
78. A host expression system comprising the vector of claim 77 or having the polynucleotide of claim 76 integrated into genome thereof.
79. The host expression system of claim 78, which is a microorganism, a yeast, or a mammalian cell, optionally, wherein the microorganism is selected from the group consisting of *E. coli* and *B. subtilis*, optionally wherein the yeast is *Saccharomyces*, optionally wherein the mammalian cell is selected from the group consisting of COS, CHO-S, CHO-K1, HEK-293, and 3T3 cells.
80. A virus comprising the vector of claim 77.

81. A kit comprising the antibody or antigen-binding fragment thereof of any one of claims 1-67 and/or the chimeric antigen receptor of any one of claims 68-74 and/or the pharmaceutical composition of claim 75, and a second therapeutic agent.
82. A method of expressing the antibody or antigen-binding fragment thereof of any one of claims 1-67 or the chimeric antigen receptor of any one of claims 68-74, comprising culturing the host expression system of claim 78 under the condition at which the antibody or antigen-binding fragment of any one of claims 1-67 is expressed.
83. A method of treating, preventing or alleviating a disease, disorder or condition in a subject, comprising administering to the subject a therapeutically effective amount of the antibody or antigen-binding fragment thereof of any one of claims 1-67 and/or the chimeric antigen receptor of any one of claims 68-74 and/or the pharmaceutical composition of claim 75.
84. The method of claim 83, wherein the disease, disorder or condition is an immune disease, an autoimmune disease, an inflammatory disease, a cancer or a neurological disease.
85. The method of claim 84, wherein the cancer is a solid tumor or hematologic tumor.
86. The method of claim 83, wherein the disease, disorder or condition is a CD3 and/or LILRB4-expressing cancer.
87. The method of any one of claims 83-86, wherein the disease, disorder or condition is selected from the group consisting of Kawasaki disease, T. gondii, multiple sclerosis, systematic Lupus erythematosus, lung cancer (*e.g.*, non-small-cell lung cancer (NSCLC), small cell lung cancer (SCLC), adenocarcinoma of the lung, squamous cell carcinoma of the lung, Lewis lung carcinoma, or radiation therapy resistant Lewis lung carcinoma), peritoneal cancer, carcinoid cancer, bone cancer, pancreatic cancer, primitive neuroectodermal tumor, skin cancer, gallbladder cancer, cancer of the head or neck, squamous cell cancer, uterine cancer, ovarian cancer,

rectal cancer, prostate cancer, bladder cancer (*e.g.*, urothelial cancer), cancer of the anal region (*e.g.*, anal squamous cell carcinoma), gastric or stomach cancer (*e.g.*, gastrointestinal cancer), esophageal cancer, colon cancer, breast cancer, uterine cancer, liver cancer (*e.g.*, hepatoblastoma, hepatocellular carcinoma/hepatoma, or hepatic carcinoma), cholangiocarcinoma, sarcoma, colorectal cancer, carcinoma of the fallopian tubes, salivary gland carcinoma, carcinoma of the cervix, endometrial or uterine carcinoma, osteosarcoma, carcinoma of the vagina, carcinoma of the vulva, cancer of the esophagus, cancer of the small intestine, cancer of the endocrine system, cancer of the thyroid gland, cancer of the parathyroid gland, cancer of the adrenal gland, cancer of the nasopharynx, sarcoma of soft tissue, polycythemia vera, cancer of the urethra, cancer of the penis, cancer of the kidney or ureter (*e.g.*, rhabdoid tumor of the kidney), cutaneous T-cell lymphoma, medulloblastoma, nephroblastoma, myelodysplastic syndrome, chronic and non-chronic myeloproliferative disorder, choroid plexus papilloma, renal cell carcinoma, carcinoma of the renal pelvis, neoplasms of the central nervous system (CNS), soft tissue sarcoma (*e.g.*, rhabdomyosarcoma, fibrosarcoma, Kaposi's sarcoma), spinal axis tumors, glioma (*e.g.*, ependymoma, astrocytoma, anaplastic astrocytoma, oligodendroglioma, eye cancer (*e.g.*, retinoblastoma), brain stem glioma, or mixed glioma such as oligoastrocytoma), brain tumor (*e.g.*, glioblastoma/glioblastoma multiforme (GBM), non-glioblastoma brain tumor, or meningioma), melanoma (*e.g.*, cutaneous or intraocular melanoma), thrombocytopenia, mesothelioma, mycosis fungoides, Sezary syndrome, idiopathic myelofibrosis, solitary plasmacytoma, vestibular schwannoma, Ewing's sarcoma, chondrosarcoma, MYH associated polyposis, pituitary adenoma, pediatric cancers such as pediatric sarcomas (*e.g.*, neuroblastoma, rhabdomyosarcoma, and osteosarcoma), hematological cancer, lymphoma, Hodgkin's lymphoma, non-Hodgkin's lymphoma, leukemia (*e.g.*, lymphocytic/lymphoblastic leukemia), chronic or acute leukemia, mast cell leukemia, lymphocytic lymphomas, primary CNS lymphoma, chronic lymphocytic leukemia (CLL), acute lymphocytic leukemia (ALL), chronic myeloid leukemia (CML), acute myeloid leukemia (AML), chronic myelomonocytic leukemia (CMML), chronic lymphoblastic leukemia, acute

lymphoblastic leukemia, hairy cell leukemia (HCL), Burkitt's lymphoma (BL), multiple myeloma (*e.g.*, relapsed or refractory multiple myeloma), T or B cell lymphoma, mantle cell lymphoma (MCL) (*e.g.*, relapsed or refractory mantle cell lymphoma), malignant melanoma, diffuse large B cell lymphoma (DLBCL), DLBCL that results from follicular lymphoma, high-grade B-cell lymphoma, primary mediastinal large B-cell lymphoma, follicular lymphoma (FL), and primary mediastinal B-cell lymphoma.

88. The method of claim 87, wherein the disease, disorder or condition is acute myeloid leukemia.

89. The method of any one of claims 83-88, wherein the subject is human.

90. The method of any one of claims 83-89, wherein the administration is through a parenteral route comprising subcutaneous, intraperitoneal, intravenous, intramuscular, or intradermal injection; or a non-parenteral route comprising transdermal, oral, intranasal, intraocular, sublingual, rectal, or topical.

91. The method of any one of claims 83-90, wherein the method further includes administering to the subject in need thereof an additional therapeutic agent.

92. The method of claim 91, wherein the additional therapeutic agent is selected from the group consisting of: an active agent, an imaging agent, a cytotoxic agent, and angiogenesis inhibitor, a kinase inhibitor, a co-stimulation molecule agonist, a co-inhibition molecule blocker, an adhesion molecule blocker, an anti-cytokine antibody or functional fragment thereof, a detectable label or reporter, an antimicrobial, a gene editing agent, a beta agonist, an viral RNA inhibitor, a polymerase inhibitor, an interferon, and a microRNA.

93. The method of claim 91 or 92, wherein the additional therapeutic agent is administered to the subject in need before, after or simultaneously with the antibody or antigen-binding fragment thereof of any one of claims 1-67 and/or the chimeric antigen receptor of any one of claims 68-74 and/or the pharmaceutical composition of claim 75.

94. A method of activating a T cell expressing CD3 or LILRB4 *in vivo* or *in vitro*, comprising contacting the T cell with the antibody or antigen-binding fragment thereof of any one of claims 1-67 and/or the chimeric antigen receptor of any one of claims 68-74 and/or the pharmaceutical composition of claim 75.

95. A method of modulating CD3 and/or LILRB4 activity in a CD3 and/or LILRB4-expressing cell, comprising exposing the CD3 and/or LILRB4-expressing cell to the specific antibody or antigen-binding fragment thereof of any of claims 1-67 and/or the chimeric antigen receptor of any one of claims 68-74 and/or the pharmaceutical composition of claim 75.

96. A method of promoting *in vivo* or *in vitro* processing of LILRB4 by a CD3-expressing T cell, comprising contacting the CD3-expressing T cell with the antibody or antigen-binding fragment thereof of any of claims 1-67 and/or the chimeric antigen receptor of any one of claims 68-74 and/or the pharmaceutical composition of claim 75, wherein the antibody or antigen-binding fragment thereof, the chimeric antigen receptor or the pharmaceutical composition is capable of binding to both the CD3-expressing T cell and LILRB4 thereby bringing both in close proximity.

97. A method of detecting presence or amount of CD3 and/or LILRB4 in a sample, comprising contacting the sample with the antibody or antigen-binding fragment thereof of any of claims 1-67 and/or the chimeric antigen receptor of any one of claims 68-74 and/or the pharmaceutical composition of claim 75, and determining the presence or the amount of CD3 and/or LILRB4 in the sample.

98. A method of diagnosing a CD3 and/or LILRB4-related disease, disorder or condition in a subject, comprising: a) contacting a sample obtained from the subject with the antibody or antigen-binding fragment thereof of any of claims 1-67 and/or the chimeric antigen receptor of any one of claims 68-74 and/or the pharmaceutical composition of claim 75; b) determining presence or amount of CD3 and/or LILRB4 in the sample; and c) correlating the presence or the amount of CD3 and/or LILRB4 to

existence or status of the CD3 and/or LILRB4-related disease, disorder or condition in the subject.

99. Use of the antibody or antigen-binding fragment thereof of any of claims 1-67 and/or the chimeric antigen receptor of any one of claims 68-74 and/or the pharmaceutical composition of claim 75 in the manufacture of a medicament for treating a disease, disorder or condition related to CD3 and/or LILRB4 in a subject.

100. Use of the antibody or antigen-binding fragment thereof of any of claims 1-67 and/or the chimeric antigen receptor of any one of claims 68-74 and/or the pharmaceutical composition of claim 75 in the manufacture of a diagnostic reagent for diagnosing a disease, disorder or condition related to CD3 and/or LILRB4 in a subject.

101. A kit comprising the antibody or antigen-binding fragment thereof of any of claims 1-67 and/or the chimeric antigen receptor of any one of claims 68-74 and/or the pharmaceutical composition of claim 75, useful in detecting CD3 and/or LILRB4, optionally recombinant CD3 and/or LILRB4, CD3 and/or LILRB4 expressed on cell surface, or CD3 and/or LILRB4-expressing cells.