

CLAIMS

WHAT IS CLAIMED IS:

- ~~1. An isolated antibody or an antigen-binding fragment thereof, comprising 1, 2, or 3 heavy chain complementarity determining region (CDR) sequences selected from the group consisting of: SEQ ID NOs: 1, 3, 5, 7, 9, 11, 13, 15, 17, 19, 21, 23, 25, 27, 29, 31, 33, 35, 37, 39, 41, 43, 45, and 47, and/or 1, 2, or 3 kappa light chain CDR sequences selected from the group consisting of: SEQ ID NOs: 2, 4, 6, 8, 10, 12, 14, 16, 18, 20, 22, 24, 26, 28, 30, 32, 34, 36, 38, 40, 42, 44, 46 and 48.~~
- ~~2. The antibody or an antigen-binding fragment thereof of claim 1, comprising a heavy chain variable region selected from the group consisting of:
 - ~~a) a heavy chain variable region comprising 1, 2, or 3 CDR sequences selected from SEQ ID NO: 1, SEQ ID NO: 3, and SEQ ID NO: 5;~~
 - ~~b) a heavy chain variable region comprising 1, 2, or 3 CDR sequences selected from SEQ ID NO: 7, SEQ ID NO: 9, and SEQ ID NO: 11;~~
 - ~~c) a heavy chain variable region comprising 1, 2, or 3 CDR sequences selected from SEQ ID NO: 13, SEQ ID NO: 15, and SEQ ID NO: 17;~~
 - ~~d) a heavy chain variable region comprising 1, 2, or 3 CDR sequences selected from SEQ ID NO: 19, SEQ ID NO: 21, and SEQ ID NO: 23;~~
 - ~~e) a heavy chain variable region comprising 1, 2, or 3 CDR sequences selected from SEQ ID NO: 25, SEQ ID NO: 27, and SEQ ID NO: 29;~~
 - ~~f) a heavy chain variable region comprising 1, 2, or 3 CDR sequences selected from SEQ ID NO: 31, SEQ ID NO: 33, and SEQ ID NO: 35;~~
 - ~~g) a heavy chain variable region comprising 1, 2, or 3 CDR sequences selected from SEQ ID NO: 37, SEQ ID NO: 39, and SEQ ID NO: 41; and~~
 - ~~h) a heavy chain variable region comprising 1, 2, or 3 CDR sequences selected from SEQ ID NO: 43, SEQ ID NO: 45, and SEQ ID NO: 47.~~~~
- ~~3. The antibody or an antigen-binding fragment thereof of any of the preceding claims, comprising a kappa light chain variable region selected from the group consisting of:
 - ~~a) a kappa light chain variable region comprising 1, 2, or 3 CDR sequences selected from SEQ ID NO: 2, SEQ ID NO: 4, and SEQ ID NO: 6;~~
 - ~~b) a kappa light chain variable region comprising 1, 2, or 3 CDR sequences selected from SEQ ID NO: 8, SEQ ID NO: 10, and SEQ ID NO: 12;~~
 - ~~c) a kappa light chain variable region comprising 1, 2, or 3 CDR sequences selected from~~~~

SEQ ID NO: 14, SEQ ID NO: 16 and SEQ ID NO: 18;

d) ~~a kappa light chain variable region comprising 1, 2, or 3 CDR sequences selected from~~
~~SEQ ID NO: 20, SEQ ID NO: 22, and SEQ ID NO: 24;~~

e) ~~a kappa light chain variable region comprising 1, 2, or 3 CDR sequences selected from~~
~~SEQ ID NO: 26, SEQ ID NO: 28, and SEQ ID NO: 30;~~

f) ~~a kappa light chain variable region comprising 1, 2, or 3 CDR sequences selected from~~
~~SEQ ID NO: 32, SEQ ID NO: 34, and SEQ ID NO: 36;~~

g) ~~a kappa light chain variable region comprising 1, 2, or 3 CDR sequences selected from~~
~~SEQ ID NO: 38, SEQ ID NO: 40, and SEQ ID NO: 42; and~~

h) ~~a kappa light chain variable region comprising 1, 2, or 3 CDR sequences selected from~~
~~SEQ ID NO: 44, SEQ ID NO: 46, and SEQ ID NO: 48.~~

4.1. ~~The~~An isolated antibody or an antigen-binding fragment thereof ~~of any of the preceding~~
~~claims~~, comprising:

a) ~~a heavy chain variable region comprising 1, 2, or 3 CDR sequences selected~~
~~from~~comprising SEQ ID NO: 1, SEQ ID NO: 3, and SEQ ID NO: 5; and ~~a kappa light~~
chain ~~variable region comprising 1, 2, or 3 CDR sequences selected from~~comprising
SEQ ID NO: 2, SEQ ID NO: 4, and SEQ ID NO: 6;

b) ~~a heavy chain variable region comprising 1, 2, or 3 CDR sequences selected~~
~~from~~comprising SEQ ID NO: 7, SEQ ID NO: 9, and SEQ ID NO: 11; and ~~a kappa~~
light chain ~~variable region comprising 1, 2, or 3 CDR sequences selected~~
~~from~~comprising SEQ ID NO: 8, SEQ ID NO: 10, and SEQ ID NO: 12;

c) ~~a heavy chain variable region comprising 1, 2, or 3 CDR sequences selected~~
~~from~~comprising SEQ ID NO: 13, SEQ ID NO: 15, and SEQ ID NO: 17; and ~~a kappa~~
light chain ~~variable region comprising 1, 2, or 3 CDR sequences selected~~
~~from~~comprising SEQ ID NO: 14, SEQ ID NO: 16, and SEQ ID NO: 18;

d) ~~a heavy chain variable region comprising 1, 2, or 3 CDR sequences selected~~
~~from~~comprising SEQ ID NO: 19, SEQ ID NO: 21, and SEQ ID NO: 23; and ~~a kappa~~
light chain ~~variable region comprising 1, 2, or 3 CDR sequences selected~~
~~from~~comprising SEQ ID NO: 20, SEQ ID NO: 22, and SEQ ID NO: 24;

e) ~~a heavy chain variable region comprising 1, 2, or 3 CDR sequences selected~~
~~from~~comprising SEQ ID NO: 25, SEQ ID NO: 27, and SEQ ID NO: 29; and ~~a kappa~~
light chain ~~variable region comprising 1, 2, or 3 CDR sequences selected~~
~~from~~comprising SEQ ID NO: 26, SEQ ID NO: 28, and SEQ ID NO: 30;

f) ~~a heavy chain variable region comprising 1, 2, or 3 CDR sequences selected~~

- ~~from comprising~~ SEQ ID NO: 31, SEQ ID NO: 33, and SEQ ID NO: 35; and ~~a~~ kappa light chain ~~variable region comprising 1, 2, or 3~~ CDR sequences ~~selected from comprising~~ SEQ ID NO: 32, SEQ ID NO: 34, and SEQ ID NO: 36;
- g) ~~a~~ heavy chain ~~variable region comprising 1, 2, or 3~~ CDR sequences ~~selected from comprising~~ SEQ ID NO: 37, SEQ ID NO: 39, and SEQ ID NO: 41; and ~~a~~ kappa light chain ~~variable region comprising 1, 2, or 3~~ CDR sequences ~~selected from comprising~~ SEQ ID NO: 38, SEQ ID NO: 40, and SEQ ID NO: 42; or
- h) ~~a~~ heavy chain ~~variable region comprising 1, 2, or 3~~ CDR sequences ~~selected from comprising~~ SEQ ID NO: 43, SEQ ID NO: 45, and SEQ ID NO: 47; and ~~a~~ kappa light chain ~~variable region comprising 1, 2, or 3~~ CDR sequences ~~selected from comprising~~ SEQ ID NO: 44, SEQ ID NO: 46, and SEQ ID NO: 48.
5. ~~The antibody or an antigen-binding fragment thereof of any of the preceding claims, further comprising 1, 2, 3, or 4 heavy chain framework region (FR) sequences selected from the group consisting of: SEQ ID NO: 57, 59, 61, 63, 73, 75, 77 and 79, and/or 1, 2, 3, or 4 light chain framework region (FR) sequences selected from SEQ ID NO: 58, 60, 62, 64, 74, 76, 78 and 80.~~
6. ~~The antibody or an antigen-binding fragment thereof of any of the preceding claims, comprising a heavy chain variable region selected from the group consisting of: SEQ ID NO: 81, SEQ ID NO: 85, SEQ ID NO: 89, SEQ ID NO: 93, SEQ ID NO: 97, SEQ ID NO: 101, SEQ ID NO: 105, SEQ ID NO: 109, SEQ ID NO: 113, SEQ ID NO: 117 and the homologue sequences of at least 80% sequence identity thereof.~~
7. ~~The antibody or an antigen-binding fragment thereof of any of the preceding claims, comprising a kappa light chain variable region selected from the group consisting of: SEQ ID NO: 83, SEQ ID NO: 87, SEQ ID NO: 91, SEQ ID NO: 95, SEQ ID NO: 99, SEQ ID NO: 103, SEQ ID NO: 107, SEQ ID NO: 111, SEQ ID NO: 115, SEQ ID NO: 119 and the homologue sequences of at least 80% sequence identity thereof.~~
- 8.2. The antibody or an antigen-binding fragment thereof of ~~any of the preceding~~ claims 1, comprising:
- a) a heavy chain variable region comprising SEQ ID NO: 81 and a kappa light chain variable region comprising SEQ ID NO: 83;
- b) a heavy chain variable region comprising SEQ ID NO: 85 and a kappa light chain variable region comprising SEQ ID NO: 87;
- c) a heavy chain variable region comprising SEQ ID NO: 89 and a kappa light chain variable region comprising SEQ ID NO: 91;

- d) a heavy chain variable region comprising SEQ ID NO: 93 and a kappa light chain variable region comprising SEQ ID NO: 95;
- e) a heavy chain variable region comprising SEQ ID NO: 97 and a kappa light chain variable region comprising SEQ ID NO: 99;
- f) a heavy chain variable region comprising SEQ ID NO: 101 and a kappa light chain variable region comprising SEQ ID NO: 103;
- g) a heavy chain variable region comprising SEQ ID NO: 105 and a kappa light chain variable region comprising SEQ ID NO: 107;
- h) a heavy chain variable region comprising SEQ ID NO: 109 and a kappa light chain variable region comprising SEQ ID NO: 111;
- i) a heavy chain variable region comprising SEQ ID NO: 113 and a kappa light chain variable region comprising SEQ ID NO: 115; or
- j) a heavy chain variable region comprising SEQ ID NO: 117 and a kappa light chain variable region comprising SEQ ID NO: 119.

~~9. The antibody or antigen-binding fragment thereof of any of the preceding claims, further comprising one or more amino acid residue substitutions yet retains specific binding affinity to CD3epsilon.~~

~~10. The antibody or antigen-binding fragment thereof of claim 9, wherein the substitution is in one or more CDR sequences, and/or in one or more FR sequences, in one or both variable region sequences, and/or in Fc region.~~

~~11. The antibody or antigen-binding fragment thereof of claim 9 or 10, wherein the substitution confers one or more desirable properties selected from:~~

- ~~a) improving binding affinity to CD3epsilon,~~
- ~~b) introducing or removing a glycosylation site,~~
- ~~c) introducing a free cysteine residue,~~
- ~~d) enhancing or reducing ADCC or CDC,~~
- ~~e) increasing serum half-life; and~~
- ~~f) increasing FcRn binding.~~

12.3. The antibody or antigen-binding fragment thereof of ~~any of the preceding~~ claims 1, further comprising an immunoglobulin constant region, optionally a constant region of IgG, optionally a constant region of human IgG1.

13.4. The antibody or an antigen-binding fragment thereof ~~of any of the preceding~~ claims 1, which is a humanized antibody.

14.5. The antibody or antigen-binding fragment thereof of ~~any of the preceding~~ claims 1,

which is a camelized single domain antibody, a diabody, a scFv, an scFv dimer, a BsFv, a dsFv, a (dsFv)₂, a dsFv-dsFv', an Fv fragment, a Fab, a Fab', a F(ab')₂, a bispecific antibody, a ds diabody, a nanobody, a domain antibody, or a bivalent domain antibody.

~~15.6.~~ The antibody or an antigen-binding fragment thereof of ~~any of the preceding~~ claims 5, wherein the antibody or an antigen-binding fragment thereof ~~which~~ is bispecific and has a first specificity for CD3epsilon, and a second specificity.

~~16.~~ The antibody or an antigen-binding fragment thereof of claim ~~15~~, wherein the antibody or an antigen-binding fragment thereof has a first specificity for CD3epsilon, and a second specificity.

~~17.7.~~ The antibody or an antigen-binding fragment thereof of claim ~~16~~, wherein the second specificity is for a second antigen different from CD3epsilon wherein presence of the second antigen in proximity to a CD3epsilon-expressing T cells is desirable for the second antigen to be recognized by immune system.

~~18.~~ The antibody or an antigen-binding fragment thereof of claim ~~16~~, wherein the first antigenic specificity is for CD3epsilon, and the second antigenic specificity is for a tumor associated antigen.

~~19.8.~~ The antibody or antigen-binding fragment thereof of ~~any of the preceding~~ claims 1 linked to one or more conjugates, wherein the conjugate comprises a chemotherapeutic agent, a toxin, a radioactive isotope, a lanthanide, a luminescent label, a fluorescent label, or an enzyme-substrate label.

~~20.~~ The antibody or antigen-binding fragment thereof of claim ~~19~~, wherein the conjugate comprises a chemotherapeutic agent, a toxin, a radioactive isotope, a lanthanide, a luminescent label, a fluorescent label, or an enzyme-substrate label.

~~21.9.~~ The antibody or an antigen-binding fragment thereof ~~any of the preceding~~ claims 1, capable of specifically binding to CD3epsilon, and optionally wherein the CD3epsilon are derived from mouse, rat, monkey or human, and optionally wherein the CD3epsilon is a recombinant CD3epsilon or a CD3epsilon expressed on a cell surface.

~~22.~~ The antibody or an antigen-binding fragment thereof of ~~any of the preceding~~ claims, capable of specifically binding to human CD3epsilon expressed on a cell surface at a K_D value of no more than $5 \times 10^{-9}M$, no more than $4 \times 10^{-9}M$, no more than $3 \times 10^{-9}M$, no more than $2 \times 10^{-9}M$, no more than $10^{-9}M$, no more than $5 \times 10^{-10}M$, no more than $4 \times 10^{-10}M$, no more than $3 \times 10^{-10}M$, no more than $2 \times 10^{-10}M$, no more than $10^{-10}M$, no more than $5 \times 10^{-11}M$, no more than $4 \times 10^{-11}M$, no more than $3 \times 10^{-11}M$, no more than $2 \times 10^{-11}M$, or no more than $10^{-11}M$ as measured by flow cytometry assay.

- ~~23. The antibody or an antigen-binding fragment thereof of any of the preceding claims, capable of specifically binding to recombinant Cynomolgus monkey CD3epsilon with an EC₅₀ of no more than 0.001 nM, no more than 0.005 nM, no more than 0.01 nM, no more than 0.02 nM, no more than 0.03 nM, no more than 0.04 nM, or no more than 0.05 nM as measured by ELISA.~~
- ~~24. The antibody or an antigen-binding fragment thereof of any of the preceding claims, capable of specifically binding to recombinant human CD3epsilon at an EC₅₀ of no more than 0.01 nM, no more than 0.02 nM, no more than 0.03 nM, no more than 0.04 nM, no more than 0.05 nM, no more than 0.06 nM, no more than 0.07 nM or no more than 0.08 nM as measured by ELISA.~~
- ~~25. An antibody or an antigen-binding fragment thereof, which competes for the same epitope with the antibody or antigen-binding fragment thereof of any of the preceding claims.~~
- 26.10. A pharmaceutical composition comprising the antibody or antigen-binding fragment thereof of ~~any of the preceding~~ claims 1, and a pharmaceutically acceptable carrier.
- 27.11. An isolated polynucleotide encoding the antibody or an antigen-binding fragment thereof of claims ~~1-25~~.
- ~~28. The isolated polynucleotide of claim 27, which comprises a nucleotide sequence selecting from a group consisting of SEQ ID NO: 82, 84, 86, 88, 90, 92, 94, 96, 98, 100, 102, 104, 106, 108, 110, 112, 114, 116, 118 and 120.~~
- 29.12. A vector comprising the isolated polynucleotide of claim ~~27 or 28~~11.
- 30.13. A host cell comprising the vector of claim ~~29~~12.
- 31.14. A method of expressing the antibody or antigen-binding fragment thereof of ~~any of~~ claims ~~1-24~~, comprising culturing ~~the~~a host cell ~~of claim 30 under the condition at which~~ comprising a vector of claim 29 is expressed comprising an isolated polynucleotide encoding the antibody or antigen-binding fragment thereof of claim 1 under the condition at which the vector is expressed
- 32.15. ~~A method of treating a disease or condition in a subject that would benefit from modulation of CD3epsilon activity, comprising administering to the subject a therapeutically effective amount of the~~Use of an antibody or antigen-binding fragment thereof of ~~any of~~ claims ~~1-25 or the pharmaceutical composition of claim 26 in the~~ manufacture of a medicament for the treatment of CD3 related disease or condition.
- ~~33. The method of claim 32, wherein the disease or condition is a CD3-related disease or condition.~~
- ~~34. The method of claim 32, wherein the disease or condition is cancer, autoimmune disease,~~

~~inflammatory disease, or infectious disease.~~

35.16. The ~~method~~use of claim 3415, wherein the antibody or antigen-binding fragment thereof is bispecific and the disease or condition is cancer.

~~36. The method of claim 32, wherein the subject is human.~~

37.17. A method of activating CD3epsilon-expressing T cells ~~in vivo or~~ in vitro, comprising contacting the CD3epsilon-expressing T cells with the antibody or antigen-binding fragment thereof of ~~any of~~ claims 1-25.

~~38. A method of modulating CD3 activity in a CD3epsilon-expressing cell, comprising exposing the CD3epsilon-expressing cell to the antibody or antigen-binding fragment thereof of any of claims 1-25.~~

39.18. A method of promoting ~~in vivo or~~ in vitro processing of a second antigen by CD3epsilon-expressing T cell, comprising contacting the CD3epsilon-expressing T cells with the bispecific antibody or antigen-binding fragment thereof of ~~any of~~ claims ~~15-18~~ 6, wherein the bispecific antibody or antigen-binding fragment is capable of specifically binding to both the CD3epsilon-expressing T cells and a second antigen thereby bringing both in close proximity.

~~40. A method of detecting presence or amount of CD3epsilon in a sample, comprising contacting the sample with the antibody or antigen-binding fragment thereof of any of claims 1-25, and determining the presence or the amount of CD3epsilon in the sample.~~

~~41. A method of diagnosing a CD3 related disease 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 of any of claims 1-25; c) determining presence or amount of CD3epsilon in the sample; and d) correlating the presence or the amount of CD3epsilon to existence or status of the CD3 related disease or condition in the subject.~~

~~42. Use of the antibody or antigen-binding fragment thereof of any of claims 1-25 in the manufacture of a medicament for treating a CD3 related disease or condition in a subject.~~

~~43. Use of the antibody or antigen-binding fragment thereof of any of claims 1-25 in the manufacture of a diagnostic reagent for diagnosing a CD3 related disease or condition.~~

44.19. A kit comprising the antibody or antigen-binding fragment thereof ~~of any~~ of claims 1-25, useful in detecting CD3epsilon, optionally recombinant CD3epsilon, CD3epsilon expressed on cell surface, or CD3epsilon-expressing cells, or useful in diagnosing a CD3 related disease or condition in a subject.