[0014] The solution for this problem that has been provided by the present invention, i.e. CD3-binding molecules, in particular anti-CD3 antibodies obtained by genetic immunization of rabbits and screening of affinity matured memory B-cells, and in particular CD3-binding molecules, in particular anti-CD3 antibodies, with specificity for a novel agonistic epitope, has so far not been achieved or suggested by the prior art.

SUMMARY OF THE INVENTION

[0015] The present invention relates to novel isolated CD3-binding molecules, in particular isolated antibodies or functional fragments thereof, each comprising a binding region, particularly an antigen-binding region, wherein said binding molecules, in particular said antibodies or functional fragments thereof, are specific for an epitope of human CD3, particularly for a novel agonistic epitope of CD3ε, wherein said binding molecules, in particular said isolated antibodies or functional fragments thereof, have a higher affinity than the prior art antibodies, particularly OKT-3 and/or TR66, while simultaneously exhibiting a higher potency.

[0015a] In one aspect, there is provided an isolated antibody or functional fragment thereof comprising an antigen-binding region that is specific for an epitope of human CD3, wherein said antibody or functional fragment thereof, when tested in an IgG format, (i) is binding to human CD3 with a dissociation constant for monovalent binding of less than 3.0×10^{-8} M, particularly less than 1.5×10^{-8} M, more particularly less than 1.2×10^{-8} M, and most particularly less than 1.0×10^{-8} M; and (iia), upon cross-linking, is inducing T-cell activation at least 1.5-fold stronger than antibodies OKT-3 (BioLegend, Cat. No. 317302) or TR66 (Novus Biologicals, Cat. No. NBP1-97446) after 24 h of stimulation at an IgG concentration of 1.25 µg/ml; (iib) is resulting in T-cell activation, which lasts longer than with antibodies OKT-3 (BioLegend, Cat. No. 317302) or TR66 (Novus Biologicals, Cat. No. NBP1-97446) as indicated by at least 1.5-fold

greater increase in CD69 expression after 72 hours of stimulation at an IgG concentration of 1.25 µg/ml; (iic) is resulting in a dose-dependent homogeneous activation state of T-cells; and (iid) is specific for an epitope of human CD3ε, wherein said epitope comprises amino acid residue N4 as residue that is critical for binding, and wherein said epitope further comprises amino acid residue E6 as residue that is involved in binding.

[0015b] In one aspect, there is provided an isolated antibody or functional fragment thereof binding to essentially the same epitope as the isolated antibody or functional fragment thereof as described herein.

[0015c] In one aspect, there is provided a pharmaceutical composition comprising the isolated antibody or functional fragment thereof as described herein, and optionally a pharmaceutically acceptable carrier and/or excipient.

[0015d] In one aspect, there is provided a nucleic acid sequence or a collection of nucleic acid sequences encoding the isolated antibody or functional fragment thereof as described herein.

[0015e] In one aspect, there is provided a vector or a collection of vectors comprising the nucleic acid sequence or a collection of nucleic acid sequences as described herein.

[0015f] In one aspect, there is provided a host cell, particularly an expression host cell, comprising the nucleic acid sequence or the collection of nucleic acid sequences as described herein, or the vector or collection of vectors as described herein.

[0015g] In one aspect, there is provided a method for producing the isolated antibody or functional fragment thereof as described herein, comprising the step of expressing the nucleic acid sequence or the collection of nucleic acid sequences as described herein, or the vector or collection of vectors as described herein, or the host cell, particularly an expression host cell, as described herein.

[0016] Thus, in ~~a first~~one aspect, the present invention relates to an isolated binding molecule comprising a binding region that is specific for an epitope of human CD3ε, in particular to an isolated antibody or functional fragment thereof comprising an antigen-binding region, wherein said epitope comprises amino acid residue N4 as residue that is critical for binding.

[0017] In ~~a second~~one aspect, the present invention relates to a novel isolated CD3-binding molecule that is specific for an epitope of human CD3, wherein said isolated CD3-binding molecule is binding to human CD3 with a dissociation constant for monovalent binding of less than 3.0×10^{-8} M, particularly less than 1.5×10^{-8} M, more particularly less than 1.2×10^{-8} M, and most particularly less than 1.0×10^{-8} M, in particular to an isolated antibody or functional fragment

thereof comprising an antigen-binding region that is specific for an epitope of human CD3, wherein said antibody or functional fragment thereof, is binding to human CD3 with a dissociation constant for monovalent binding of less than 3.0 x 10^{-8} M, particularly less than 1.5 x 10^{-8} M, more particularly less than 1.2 x 10^{-8} M, and most particularly less than 1.0 x 10^{-8} M.

[0018] In ~~a third~~one aspect, the present invention relates to an isolated antibody or functional fragment thereof comprising an antigen-binding region that is specific for an epitope of human CD3, wherein said antibody or functional fragment thereof, when tested in an IgG format, upon cross-linking, is inducing T-cell activation at least 1.5-fold stronger than antibodies OKT-3 or TR66 after 24 h of stimulation at an IgG concentration of 1.25 µg/ml.

[0019] In ~~a fourth~~one aspect, the present invention relates to an isolated antibody or functional fragment thereof comprising an antigen-binding region that is specific for an epitope of human CD3, wherein said antibody or functional fragment thereof, when tested in an IgG format upon cross-linking, is resulting in T-cell activation, which lasts longer than with antibodies OKT-3 or TR66 as indicated by at least 1.5-fold greater increase in CD69 expression after 72 hours of stimulation at an IgG concentration of 1.25 µg/ml.

[0020] In ~~a fifth~~one aspect, the present invention relates to an isolated antibody or functional fragment thereof comprising an antigen-binding region that is specific for an epitope of human CD3, wherein said antibody or functional fragment thereof, when tested in an IgG format, upon cross-linking, is resulting in a dose-dependent homogeneous activation state of T-cells.

[0021] In ~~a sixth~~one aspect, the present invention relates to an isolated antibody or functional fragment thereof comprising an antigen-binding region that is specific for an epitope of human CD3, wherein said antibody or functional fragment thereof, when tested in an IgG format, (i) is binding to human CD3 with a dissociation constant for monovalent binding of less than 3.0 x 10^{-8} M, particularly less than 1.5 x 10^{-8} M, more particularly less than 1.2 x 10^{-8} M, and most particularly less than 1.0 x 10^{-8} M; and (iia), upon cross-linking, is inducing

T-cell activation at least 1.5-fold stronger than antibodies OKT-3 or TR66 after 24 h of stimulation at an IgG concentration of 1.25 µg/ml; (iib) is resulting in T-cell activation, which lasts longer than with antibodies OKT-3 or TR66 as indicated by at least 1.5-fold greater increase in CD69 expression after 72 hours of stimulation at an IgG concentration of 1.25 µg/ml; (iic) is resulting in a dose-dependent homogeneous activation state of T-cells; and/or (iid) is specific for an epitope of human CD3ε, wherein said epitope comprises amino acid residue N4 as residue that is critical for binding.

[0022] In ~~a seventh~~one aspect, the present invention relates to an isolated binding molecule, particularly an isolated antibody or functional fragment thereof, binding to essentially the same epitope as the isolated antibody or functional fragment thereof of Sections [0078] to [0080], [0083] to [0085] and [0089].

[0023] In ~~an eighth~~one aspect, the present invention relates to a pharmaceutical composition comprising a binding molecule of the present invention, in particular an isolated antibody or functional fragment thereof, and optionally a pharmaceutically acceptable carrier and/or excipient.

[0024] In ~~a ninth~~one aspect, the present invention relates to a nucleic acid sequence or a collection of nucleic acid sequences encoding a binding molecule of the present invention, in particular an isolated antibody or functional fragment thereof.

[0025] In ~~a tenth~~one aspect, the present invention relates to a vector or a collection of vectors comprising the nucleic acid sequence or a collection of nucleic acid sequences of the present invention.

[0026] In ~~an eleventh one~~ aspect, the present invention relates to a host cell, particularly an expression host cell, comprising the nucleic acid sequence or the collection of nucleic acid sequences of the present invention, or the vector or collection of vectors of the present invention.

[0027] In ~~a twelfth~~one aspect, the present invention relates to a method for producing a binding molecule of the present invention, in particular an isolated antibody or functional fragment thereof, comprising the step of expressing the nucleic acid sequence or the collection of nucleic acid sequences of the present invention, or the vector or collection of vectors of the present invention, or the host cell, particularly an expression host cell, of the present invention.

[0028] In ~~a thirteenth~~one aspect, the present invention relates to a method for generating an isolated antibody or functional antibody fragment in accordance with the present invention comprising the steps of:

a) Immunization of rabbits with a CD3ε-expressing plasmid to present the native full-length CD3ε on the surface of host cells;

b) Clonal isolation of affinity matured memory B-cells that interact with the CD3ε/γ single-chain using fluorescence activated cell-sorting;

c) Cultivation of single sorted B cells in a co-cultivation system that does not require immortalization of sorted clones;

d) Screening of B cell culture supernatants in a cell-based ELISA to identify antibodies binding to the native CD3ε embedded in the TCR complex on the surface of T cells.

[0029] In ~~a fourteenth~~one aspect the present invention relates to a particular epitope of human CD3 epsilon comprising exclusively amino acid residues of CD3 epsilon that are not located in the interface between CD3 epsilon and CD3 gamma and that still can be bound by an antibody in the context of the native TCR expressed on T cells, binding of which by a cross-linked antibody of the invention is inducing T-cell activation at least 1.5-fold stronger than antibodies OKT-3 or TR66 after 24 h of stimulation at an IgG concentration of 1.25 µg/ml; (iib) is resulting in T-cell activation, which lasts longer than with antibodies OKT-3 or TR66 as indicated by at least 1.5-fold greater increase in CD69 expression after 72 hours of stimulation at an IgG concentration of 1.25 µg/ml; and/or (iic) is resulting in a dose-dependent homogeneous activation state of T-cells.

[0030] In ~~a fifteenth~~one aspect, the present invention relates to a method for identifying a binding molecule comprising a binding region that is specific for a novel epitope of human CD3ε, comprising the step of (a) selecting from one or more molecules binding to human CD3 at least one binding molecule, which comprises a binding region that is specific for an epitope of human CD3ε, wherein said epitope comprises amino acid residue N4 as residue that is critical for binding.

BRIEF DESCRIPTION OF THE DRAWINGS

[0031] **Figure 1** shows the phylogenetic clustering of joined VH and VL CDR Sequences from monoclonal rabbit antibodies.

[0032] **Figure 2** shows binding of purified monoclonal rabbit antibodies to Jurkat T cells.

[0033] **Figure 3** shows the stimulation of CD69 expression by cross-linked anti-CD3ε mAbs. The potential of purified monoclonal rabbit anti-CD3 antibodies and comparator antibodies TR66 and OKT-3 to induce T-cell activation was assessed by measurement of CD69 expression. Three different concentrations of cross-linked antibodies were used to stimulate Jurkat cells and CD69 expression was assessed by flow-cytometry 24 h later. Antibody concentrations were 1.25 µg/ml (a), 5.0 µg/ml (b) and 20 µg/ml (c).

[0034] **Figure 4** shows the stimulation of CD69 by cross-linked rabbit mAbs over time. The potential of purified monoclonal rabbit anti-CD3 antibodies to induce T-cell activation was assessed by measurement of CD69 expression. Cross-linked antibodies were used at a concentration of 5.0 µg/ml to stimulate Jurkat cells and CD69 expression was assessed by flow-cytometry 0, 4, 15, 24, 48 and 72 h later. For the qualitative detection of CD69 expression the mean fluorescence intensity (MFI), reflecting the signal intensity at the geometric mean, was measured for both, the negative control as well as for the test

CLAIMS

1. An isolated antibody or functional fragment thereof comprising an antigen-binding region that is specific for an epitope of human CD3ε, wherein said epitope comprises amino acid residue N4 as residue that is critical for binding.

2. The isolated antibody or functional fragment thereof of claim 2, wherein said epitope further comprises amino acid residue E6 as residue that is involved in binding.

3. The isolated antibody or functional fragment thereof of claim 1 or 2, that is cross-reactive with cynomolgus CD3, particularly cynomolgus CD3ε, particularly having an affinity to cynomolgus monkey CD3ε that is less than 100-fold, particularly less than 30-fold, even more particularly less than 15-fold and most particularly less than 5-fold different to that of human CD3ε.

4. An isolated antibody or functional fragment thereof comprising an antigen-binding region that is specific for an epitope of human CD3, wherein said antibody or functional fragment thereof, is binding to human CD3 with a dissociation constant for monovalent binding of less than 3.0×10^{-8} M, particularly less than 1.5×10^{-8} M, more particularly less than 1.2×10^{-8} M, and most particularly less than 1.0×10^{-8} M.

5. An isolated antibody or functional fragment thereof comprising an antigen-binding region that is specific for an epitope of human CD3, wherein said antibody or functional fragment thereof, when tested in an IgG format, upon cross-linking, is inducing T-cell activation at least 1.5-fold stronger than antibodies OKT-3 or TR66 after 24 h of stimulation at an IgG concentration of 1.25 µg/ml.

6. An isolated antibody or functional fragment thereof comprising an antigen-binding region that is specific for an epitope of human CD3, wherein said

antibody or functional fragment thereof, when tested in an IgG format, upon cross-linking, is resulting in T-cell activation, which lasts longer than with antibodies OKT-3 or TR66 as indicated by at least 1.5-fold greater increase in CD69 expression after 72 hours of stimulation at an IgG concentration of 1.25 µg/ml.

7. An isolated antibody or functional fragment thereof comprising an antigen-binding region that is specific for an epitope of human CD3, wherein said antibody or functional fragment thereof, when tested in an IgG format, upon cross-linking, is resulting in a dose-dependent homogeneous activation state of T-cells.

8. An isolated antibody or functional fragment thereof comprising an antigen-binding region that is specific for an epitope of human CD3, wherein said antibody or functional fragment thereof, when tested in an IgG format, (i) is binding to human CD3 with a dissociation constant for monovalent binding of less than 3.0×10^{-8} M, particularly less than 1.5×10^{-8} M, more particularly less than 1.2×10^{-8} M, and most particularly less than 1.0×10^{-8} M; and (iia), upon cross-linking, is inducing T-cell activation at least 1.5-fold stronger than antibodies OKT-3 or TR66 after 24 h of stimulation at an IgG concentration of 1.25 µg/ml; (iib) is resulting in T-cell activation, which lasts longer than with antibodies OKT-3 or TR66 as indicated by at least 1.5-fold greater increase in CD69 expression after 72 hours of stimulation at an IgG concentration of 1.25 µg/ml; (iic) is resulting in a dose-dependent homogeneous activation state of T-cells; and/or (iid) is specific for an epitope of human CD3ε, wherein said epitope comprises amino acid residue N4 as residue that is critical for binding.

9. The isolated antibody or functional fragment thereof of any one of claims 4 to 8, wherein said epitope is located on the epsilon chain of human CD3.

10. The isolated antibody or functional fragment thereof of any one of claims 1 to 3, and 9, wherein said binding to human CD3ε is determined by determining the affinity of said antibody or functional fragment thereof in an

IgG format to the purified extracellular domain of heterodimeric CD3εγ of human origin using a surface plasmon resonance experiment, particularly using the following conditions: MASS-1 SPR instrument (Sierra Sensors); capture antibody: antibody specific for the Fc region of said IgG immobilized on an SPR-2 Affinity Sensor chip, Amine, Sierra Sensors, using a standard amine-coupling procedure; two-fold serial dilutions of human heterodimeric single-chain CD3εγ extracellular domain ranging from 90 to 2.81 nM, injection into the flow cells for 3 min and dissociation of the protein from the IgG captured on the sensor chip for 5 min, surface regeneration after each injection cycle with two injections of 10 mM glycine-HCl, calculation of the apparent dissociation (kd) and association (ka) rate constants and the apparent dissociation equilibrium constant (K_D) with the MASS-1 analysis software (Analyzer, Sierra Sensors) using one-to-one Langmuir binding model.

11. The isolated antibody or functional fragment thereof of any one of claims 1 to 10, wherein said inducing of T-cell activation according to (iia) and/or (iic) is determined by determining the stimulation of CD69 expression by said isolated antibody or functional fragment thereof in an IgG format, particularly using the following conditions: stimulation of Jurkat cells (100,000 cells/well) for 24 h with 20 µg/ml, 5 µg/ml and 1.25 µg/ml of said isolated antibody or functional fragment thereof in an IgG format after prior cross-linking by addition of 3-fold excess of an anti-IgG antibody (control: OKT3 (BioLegend, Cat. No. 317302) or TR66 (Novus Biologicals, Cat. No. NBP1-97446), cross-linking with rabbit anti-mouse IgG antibody (JacksonImmuno Research, Cat. No. 315-005-008)); cell staining for CD69 expression after stimulation using a Phycoerithrin (PE)-labeled antibody specific for human CD69 (BioLegend, Cat. No. 310906), analysis with a flow cytometer (FACS aria III, Becton Dickinson); negative control: unstimulated Jurkat cells incubated with the cross-linking antibody stained with said anti-CD69 antibody.

12. The isolated antibody or functional fragment thereof of any one of claims 1 to 11, wherein said longer lasting T-cell activation according to (iib) is determined by determining the time course of stimulation of CD69

expression by said isolated antibody or functional fragment thereof in an IgG format, particularly using the following conditions: stimulation of 100,000 Jurkat cells/well for 0 h, 4 h, 15 h, 24 h, 48 h and 72 h with 5 µg/ml of said isolated antibody or functional fragment thereof in an IgG format anti-CD3 antibodies that have been cross-linked as in claim 10 and analysis of CD69 expression by flow cytometry as in claim 10.

13. The isolated antibody or functional fragment thereof of any one of claims 1 to 12, wherein said inducing of T-cell activation according to (iia) and/or (iic) is determined by determining the stimulation of IL-2 secretion by said isolated antibody or functional fragment thereof in an IgG format, particularly using the following conditions: stimulation of Jurkat cells (200,000 cells/well) with said isolated antibody or functional fragment thereof in an IgG format at a concentration of 5 µg/ml using 4 different assay setups: (a) stimulation of Jurkat cells with said isolated antibody or functional fragment thereof in an IgG format cross-linked by addition of 3-fold higher concentrations of an anti IgG antibody (control: OKT3 (BioLegend, Cat. No. 317302) or TR66 (Novus Biologicals, Cat. No. NBP1-97446), cross-linking with rabbit anti-mouse IgG antibody (JacksonImmuno Research, Cat. No. 315-005-008)); (b) T-cell activation in absence of cross-linking antibody; (c) immobilization of said cross-linking antibodies on the tissue culture plates by over-night incubation; (d) immobilization of said isolated antibody or functional fragment thereof in an IgG format (or of control antibodies) on the tissue culture plate by over-night incubation in absence of cross-linking antibodies; in each setup, one hour after addition, stimulation of cells with 10 ng/ml PMA and collection of supernatant after 24, 48 and 72 h to measure IL-2 release, quantified using a commercially available ELISA (BioLegend, Cat. No. 431801).

14. The isolated antibody or functional fragment thereof of any one of claims 4 to 13, wherein said isolated antibody or functional fragment thereof is cross-reactive with cynomolgus CD3.

15. The isolated antibody or functional fragment thereof of any one of claims 1 to 14, wherein said antibody or functional fragment thereof is (i) a rabbit

antibody or functional fragment thereof, or (ii) an antibody or functional fragment thereof obtained by humanizing the rabbit antibody or functional fragment thereof of (i).

16. The isolated antibody or functional fragment thereof of any one of claims 1 to 15, wherein said isolated antibody or functional fragment thereof comprises an antigen-binding region comprising a VH domain comprising a combination of one CDR1, one CDR2 and one CDR3 region present in SEQ ID NOs: 2, 4, 6, 8, 10, 12, 14, 16, 18, 20, and 22, particularly SEQ ID NOs: 4, 6, 10, and 22, more particularly SEQ ID NO: 10 and 22, particularly wherein said VH domain comprises framework domains selected from the framework domains present in SEQ ID NOs: 2, 4, 6, 8, 10, 12, 14, 16, 18, 20, and 22, particularly SEQ ID NOs: 4, 6, 10, and 22, more particularly SEQ ID NO: 10 and 22, and a VL domain comprising a combination of one CDR1, one CDR2 and one CDR3 region present in SEQ ID NOs: 1, 3, 5, 7, 9, 11, 13, 15, 17, 19, and 21, particularly SEQ ID NOs: 3, 5, 9, and 21, more particularly SEQ ID NO: 9 and 21, particularly wherein said VL domain comprises framework domains selected from the framework domains present in SEQ ID NOs: 1, 3, 5, 7, 9, 11, 13, 15, 17, 19, and 21, particularly SEQ ID NOs: 3, 5, 9, and 21, more particularly SEQ ID NO: 9 and 21.

17. The isolated antibody or functional fragment thereof of claim 16, wherein said isolated antibody or functional fragment thereof comprises an antigen-binding region comprising a VH domain comprising the combination of CDR1, CDR2 and CDR3 present in one of SEQ ID NOs: 2, 4, 6, 8, 10, 12, 14, 16, 18, 20, and 22, particularly SEQ ID NOs: 4, 6, 10, and 20, more particularly SEQ ID NO: 10 and 22, particularly wherein said VH domain comprises the combination of framework domains present in one of SEQ ID NOs: 2, 4, 6, 8, 10, 12, 14, 16, 18, and 20, and 22, particularly SEQ ID NOs: 4, 6, 10, and 22, more particularly SEQ ID NO: 10 and 22, and a VL domain comprising the combination of CDR1, CDR2 and CDR3 present in one of SEQ ID NOs: 1, 3, 5, 7, 9, 11, 13, 15, 17, 19, and 21, particularly SEQ ID NOs: 3, 5, 9, and 21, more particularly SEQ ID NO: 9 and 21, particularly wherein said VL domain comprises the combination of framework domains

present in one of SEQ ID NOs: 1, 3, 5, 7, 9, 11, 13, 15, 17, 19, and 21, particularly SEQ ID NOs: 3, 5, 9, and 21, more particularly SEQ ID NO: 9 and 21.

18. The isolated antibody or functional fragment thereof of claim 17, wherein said isolated antibody or functional fragment thereof comprises an antigen-binding region comprising a VH domain selected from SEQ ID NOs: 2, 4, 6, 8, 10, 12, 14, 16, 18, 20, and 22, particularly SEQ ID NOs: 4, 6, 10, and 22, more particularly SEQ ID NO: 10 and 22, and a VL domain selected from SEQ ID NOs: 1, 3, 5, 7, 9, 11, 13, 15, 17, 19, and 21, particularly SEQ ID NOs: 3, 5, 9, and 21, more particularly SEQ ID NO: 9 and 21.

19. The isolated antibody or functional fragment thereof of claim 18, wherein said isolated antibody or functional fragment thereof comprises an antigen-binding region comprising a VH/VL domain combination selected from SEQ ID NO: 1/SEQ ID NO: 2; SEQ ID NO: 3/SEQ ID NO: 4; SEQ ID NO: 5/SEQ ID NO: 6; SEQ ID NO: 7/SEQ ID NO: 8, SEQ ID NO: 9/SEQ ID NO: 10, SEQ ID NO: 11/SEQ ID NO: 12, SEQ ID NO: 13/SEQ ID NO: 14, SEQ ID NO: 15/SEQ ID NO: 16, SEQ ID NO: 17/SEQ ID NO: 18, SEQ ID NO: 19/SEQ ID NO: 20, and SEQ ID NO: 21/SEQ ID NO: 22; particularly SEQ ID NO: 3/SEQ ID NO: 4; SEQ ID NO: 5/SEQ ID NO: 6; SEQ ID NO: 9/SEQ ID NO: 10, and SEQ ID NO: 21/SEQ ID NO: 22; more particularly SEQ ID NO: 9/SEQ ID NO: 10 and SEQ ID NO: 21/SEQ ID NO: 22;.

20. An isolated antibody or functional fragment thereof binding to essentially the same epitope as the isolated antibody or functional fragment thereof of any one of claims 16 to 19.

21. The isolated antibody or functional fragment thereof of any one of claims 1 to 17, wherein said isolated antibody or functional fragment thereof comprises an antigen-binding region which is obtained by humanizing an antigen-binding region of claim 18 or 19.

22. A pharmaceutical composition comprising the isolated antibody or functional fragment thereof of any one of claims 1 to 21, and optionally a pharmaceutically acceptable carrier and/or excipient.

23. A nucleic acid sequence or a collection of nucleic acid sequences encoding the isolated antibody or functional fragment thereof of any one of claims 1 to 21.

24. A vector or a collection of vectors comprising the nucleic acid sequence or a collection of nucleic acid sequences of claim 23.

25. A host cell, particularly an expression host cell, comprising the nucleic acid sequence or the collection of nucleic acid sequences of claim 23, or the vector or collection of vectors of claim 24.

26. A method for producing the isolated antibody or functional fragment thereof of any one of claims 1 to 21, comprising the step of expressing the nucleic acid sequence or the collection of nucleic acid sequences of claim 23, or the vector or collection of vectors of claim 24, or the host cell, particularly an expression host cell, of claim 25.

CLAIMS

1. An isolated antibody or functional fragment thereof comprising an antigen-binding region that is specific for an epitope of human CD3, wherein said antibody or functional fragment thereof, when tested in an IgG format, (i) is binding to human CD3 with a dissociation constant for monovalent binding of less than 3.0 x 10^{-8} M, particularly less than 1.5 x 10^{-8} M, more particularly less than 1.2 x 10^{-8} M, and most particularly less than 1.0 x 10^{-8} M; and (iia), upon cross-linking, is inducing T-cell activation at least 1.5-fold stronger than antibodies OKT-3 (BioLegend, Cat. No. 317302) or TR66 (Novus Biologicals, Cat. No. NBP1-97446) after 24 h of stimulation at an IgG concentration of 1.25 µg/ml; (iib) is resulting in T-cell activation, which lasts longer than with antibodies OKT-3 (BioLegend, Cat. No. 317302) or TR66 (Novus Biologicals, Cat. No. NBP1-97446) as indicated by at least 1.5-fold greater increase in CD69 expression after 72 hours of stimulation at an IgG concentration of 1.25 µg/ml; (iic) is resulting in a dose-dependent homogeneous activation state of T-cells; and (iid) is specific for an epitope of human CD3ε, wherein said epitope comprises amino acid residue N4 as residue that is critical for binding, and wherein said epitope further comprises amino acid residue E6 as residue that is involved in binding.

2. The isolated antibody or functional fragment thereof of claim 1, wherein said binding to human CD3ε is determined by determining the affinity of said antibody or functional fragment thereof in an IgG format to the purified extracellular domain of heterodimeric CD3εγ of human origin using a surface plasmon resonance experiment, particularly using the following conditions: MASS-1 SPR instrument (Sierra Sensors); capture antibody: antibody specific for the Fc region of said IgG immobilized on an SPR-2 Affinity Sensor chip, Amine, Sierra Sensors, using a standard amine-coupling procedure; two-fold serial dilutions of human heterodimeric single-chain CD3εγ extracellular domain ranging from 90 to 2.81 nM, injection into the flow cells for 3 min and dissociation of the protein from the IgG captured on the sensor chip for 5 min, surface regeneration after each injection cycle with two injections of 10 mM glycine-HCl, calculation of the apparent dissociation (kd) and association (ka) rate constants and the apparent dissociation

equilibrium constant (K_D) with the MASS-1 analysis software (Analyzer, Sierra Sensors) using one-to-one Langmuir binding model.

3. The isolated antibody or functional fragment thereof of claim 1 or 2, wherein said inducing of T-cell activation according to (iia) and/or (iic) is determined by determining the stimulation of CD69 expression by said isolated antibody or functional fragment thereof in an IgG format, particularly using the following conditions: stimulation of Jurkat cells (100,000 cells/well) for 24 h with 20 µg/ml, 5 µg/ml and 1.25 µg/ml of said isolated antibody or functional fragment thereof in an IgG format after prior cross-linking by addition of 3-fold excess of an anti-IgG antibody (control: OKT3 (BioLegend, Cat. No. 317302) or TR66 (Novus Biologicals, Cat. No. NBP1-97446), cross-linking with rabbit anti-mouse IgG antibody (JacksonImmuno Research, Cat. No. 315-005-008)); cell staining for CD69 expression after stimulation using a Phycoerithrin (PE)-labeled antibody specific for human CD69 (BioLegend, Cat. No. 310906), analysis with a flow cytometer (FACS aria III, Becton Dickinson); negative control: unstimulated Jurkat cells incubated with the cross-linking antibody stained with said anti-CD69 antibody.

4. The isolated antibody or functional fragment thereof of any one of claims 1 to 3, wherein said longer lasting T-cell activation according to (iib) is determined by determining the time course of stimulation of CD69 expression by said isolated antibody or functional fragment thereof in an IgG format, particularly using the following conditions: stimulation of 100,000 Jurkat cells/well for 0 h, 4 h, 15 h, 24 h, 48 h and 72 h with 5 µg/ml of said isolated antibody or functional fragment thereof in an IgG format anti-CD3 antibodies that have been cross-linked as in claim 10 and analysis of CD69 expression by flow cytometry as in claim 10.

5. The isolated antibody or functional fragment thereof of any one of claims 1 to 4, wherein said inducing of T-cell activation according to (iia) and/or (iic) is determined by determining the stimulation of IL-2 secretion by said isolated antibody or functional fragment thereof in an IgG format, particularly using the following conditions: stimulation of Jurkat cells (200,000 cells/well) with said isolated antibody or functional fragment thereof in an IgG format at a concentration of 5 µg/ml using 4 different assay setups: (a) stimulation of Jurkat cells with said isolated antibody or functional fragment thereof in an IgG format cross-linked by addition of 3-fold higher concentrations of an anti IgG antibody (control: OKT3 (BioLegend, Cat. No. 317302) or TR66 (Novus Biologicals, Cat. No. NBP1-97446), cross-linking with

rabbit anti-mouse IgG antibody (JacksonImmuno Research, Cat. No. 315-005-008)); (b) T-cell activation in absence of cross-linking antibody; (c) immobilization of said cross-linking antibodies on the tissue culture plates by over-night incubation; (d) immobilization of said isolated antibody or functional fragment thereof in an IgG format (or of control antibodies) on the tissue culture plate by over-night incubation in absence of cross-linking antibodies; in each setup, one hour after addition, stimulation of cells with 10 ng/ml PMA and collection of supernatant after 24, 48 and 72 h to measure IL-2 release, quantified using a commercially available ELISA (BioLegend, Cat. No. 431801).

6. The isolated antibody or functional fragment thereof of any one of claims 1 to 5, wherein said isolated antibody or functional fragment thereof is cross-reactive with cynomolgus CD3.

7. The isolated antibody or functional fragment thereof of any one of claims 1 to 6, wherein said antibody or functional fragment thereof is (i) a rabbit antibody or functional fragment thereof, or (ii) an antibody or functional fragment thereof obtained by humanizing the rabbit antibody or functional fragment thereof of (i).

8. The isolated antibody or functional fragment thereof of any one of claims 1 to 7, wherein said isolated antibody or functional fragment thereof comprises an antigen-binding region comprising a VH domain comprising a combination of one CDR1, one CDR2 and one CDR3 region present in SEQ ID NOs: 2, 4, 6, 8, 10, 12, 14, 16, 18, 20, and 22, particularly SEQ ID NOs: 4, 6, 10, and 22, more particularly SEQ ID NO: 10 and 22, particularly wherein said VH domain comprises framework domains selected from the framework domains present in SEQ ID NOs: 2, 4, 6, 8, 10, 12, 14, 16, 18, 20, and 22, particularly SEQ ID NOs: 4, 6, 10, and 22, more particularly SEQ ID NO: 10 and 22, and a VL domain comprising a combination of one CDR1, one CDR2 and one CDR3 region present in SEQ ID NOs: 1, 3, 5, 7, 9, 11, 13, 15, 17, 19, and 21, particularly SEQ ID NOs: 3, 5, 9, and 21, more particularly SEQ ID NO: 9 and 21, particularly wherein said VL domain comprises framework domains selected from the framework domains present in SEQ ID NOs: 1, 3, 5, 7, 9, 11, 13, 15, 17, 19, and 21, particularly SEQ ID NOs: 3, 5, 9, and 21, more particularly SEQ ID NO: 9 and 21, particularly wherein said isolated antibody or functional fragment thereof comprises an antigen-binding region comprising a VH domain comprising the combination of CDR1, CDR2 and CDR3 present in one of SEQ ID NOs: 2, 4, 6, 8, 10, 12, 14, 16, 18, 20, and 22, particularly SEQ ID NOs: 4, 6, 10, and 20, more

particularly SEQ ID NO: 10 and 22, particularly wherein said VH domain comprises the combination of framework domains present in one of SEQ ID NOs: 2, 4, 6, 8, 10, 12, 14, 16, 18, and 20, and 22, particularly SEQ ID NOs: 4, 6, 10, and 22, more particularly SEQ ID NO: 10 and 22, and a VL domain comprising the combination of CDR1, CDR2 and CDR3 present in one of SEQ ID NOs: 1, 3, 5, 7, 9, 11, 13, 15, 17, 19, and 21, particularly SEQ ID NOs: 3, 5, 9, and 21, more particularly SEQ ID NO: 9 and 21, particularly wherein said VL domain comprises the combination of framework domains present in one of SEQ ID NOs: 1, 3, 5, 7, 9, 11, 13, 15, 17, 19, and 21, particularly SEQ ID NOs: 3, 5, 9, and 21, more particularly SEQ ID NO: 9 and 21.

9. The isolated antibody or functional fragment thereof of claim 8, wherein said isolated antibody or functional fragment thereof comprises an antigen-binding region comprising a VH domain selected from SEQ ID NOs: 2, 4, 6, 8, 10, 12, 14, 16, 18, 20, and 22, particularly SEQ ID NOs: 4, 6, 10, and 22, more particularly SEQ ID NO: 10 and 22, and a VL domain selected from SEQ ID NOs: 1, 3, 5, 7, 9, 11, 13, 15, 17, 19, and 21, particularly SEQ ID NOs: 3, 5, 9, and 21, more particularly SEQ ID NO: 9 and 21, particularly wherein said isolated antibody or functional fragment thereof comprises an antigen-binding region comprising a VH/VL domain combination selected from SEQ ID NO: 1/SEQ ID NO: 2; SEQ ID NO: 3/SEQ ID NO: 4; SEQ ID NO: 5/SEQ ID NO: 6; SEQ ID NO: 7/SEQ ID NO: 8, SEQ ID NO: 9/SEQ ID NO: 10, SEQ ID NO: 11/SEQ ID NO: 12, SEQ ID NO: 13/SEQ ID NO: 14, SEQ ID NO: 15/SEQ ID NO: 16, SEQ ID NO: 17/SEQ ID NO: 18, SEQ ID NO: 19/SEQ ID NO: 20, and SEQ ID NO: 21/SEQ ID NO: 22; particularly SEQ ID NO: 3/SEQ ID NO: 4; SEQ ID NO: 5/SEQ ID NO: 6; SEQ ID NO: 9/SEQ ID NO: 10, and SEQ ID NO: 21/SEQ ID NO: 22; more particularly SEQ ID NO: 9/SEQ ID NO: 10 and SEQ ID NO: 21/SEQ ID NO: 22;.

10. An isolated antibody or functional fragment thereof binding to essentially the same epitope as the isolated antibody or functional fragment thereof of claim 8 or 9.

11. The isolated antibody or functional fragment thereof of any one of claims 1 to 8, wherein said isolated antibody or functional fragment thereof comprises an antigen-binding region which is obtained by humanizing an antigen-binding region of claim 9.

12. A pharmaceutical composition comprising the isolated antibody or functional fragment thereof of any one of claims 1 to 11, and optionally a pharmaceutically acceptable carrier and/or excipient.

13. A nucleic acid sequence or a collection of nucleic acid sequences encoding the isolated antibody or functional fragment thereof of any one of claims 1 to 12.

14. A vector or a collection of vectors comprising the nucleic acid sequence or a collection of nucleic acid sequences of claim 13.

15. A host cell, particularly an expression host cell, comprising the nucleic acid sequence or the collection of nucleic acid sequences of claim 15, or the vector or collection of vectors of claim 16.

16. A method for producing the isolated antibody or functional fragment thereof of any one of claims 1 to 12, comprising the step of expressing the nucleic acid sequence or the collection of nucleic acid sequences of claim 13, or the vector or collection of vectors of claim 14, or the host cell, particularly an expression host cell, of claim 15.