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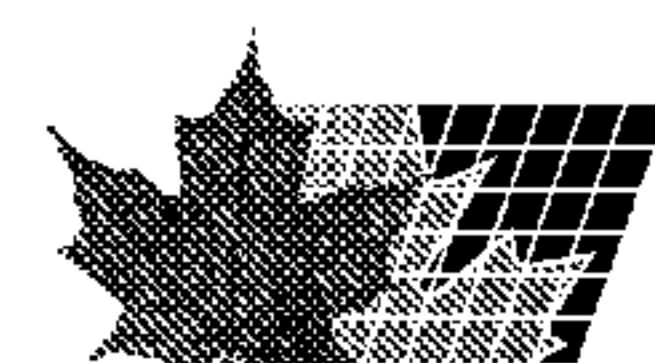
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(54) **Titre :** ANTICORPS ANTI-CD3, ANTICORPS ANTI-CD3 ACTIVABLES, ANTICORPS ANTI-CD3 MULTISPECIFIQUES,
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(54) **Title:** ANTI-CD3 ANTIBODIES, ACTIVATABLE ANTI-CD3 ANTIBODIES, MULTISPECIFIC ANTI-CD3 ANTIBODIES,
MULTISPECIFIC ACTIVATABLE ANTI-CD3 ANTIBODIES, AND METHODS OF USING THE SAME

(57) **Abrégé/Abstract:**

The invention relates generally to antibodies, activatable antibodies, multispecific antibodies, and multispecific activatable antibodies that specifically bind to at least CD3, as well as to methods of making and using these antibodies, activatable antibodies, multispecific antibodies, and/or multispecific activatable antibodies in a variety of therapeutic, diagnostic and prophylactic indications.



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- (54) Title: ANTI-CD3 ANTIBODIES, ACTIVATABLE ANTI-CD3 ANTIBODIES, MULTISPECIFIC ANTI-CD3 ANTIBODIES, MULTISPECIFIC ACTIVATABLE ANTI-CD3 ANTIBODIES, AND METHODS OF USING THE SAME
- (57) Abstract: The invention relates generally to antibodies, activatable antibodies, multispecific antibodies, and multispecific activatable antibodies that specifically bind to at least CD3, as well as to methods of making and using these antibodies, activatable antibodies, multispecific antibodies, and/or multispecific activatable antibodies in a variety of therapeutic, diagnostic and prophylactic indications.

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JUMBO APPLICATIONS/PATENTS

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**ANTI-CD3 ANTIBODIES, ACTIVATABLE ANTI-CD3 ANTIBODIES,
MULTISPECIFIC ANTI-CD3 ANTIBODIES, MULTISPECIFIC ACTIVATABLE
ANTI-CD3 ANTIBODIES, AND METHODS OF USING THE SAME**

RELATED APPLICATIONS

[0001] This application claims the benefit of U.S. Provisional Application No. 62/029,325, filed July 25, 2014, the contents of which are incorporated herein by reference in their entirety.

FIELD OF THE DISCLOSURE

[0002] The invention relates generally to antibodies, activatable antibodies, multispecific antibodies, and multispecific activatable antibodies that specifically bind to at least CD3, as well as to methods of making and using these antibodies, activatable antibodies, multispecific antibodies, and/or multispecific activatable antibodies in a variety of therapeutic, diagnostic and prophylactic indications.

BACKGROUND OF THE DISCLOSURE

[0003] CD3 (Cluster of Differentiation 3) T-cell co-receptor is a multimeric protein composed of four distinct polypeptide chains, referred to as the ϵ , γ , δ , and ζ chains. The CD3 complex serves as the signaling module of the T cell receptor that associates non-covalently with the antigen-binding α/β chains of T cell receptor (TCR).

[0004] Because direct engagement of CD3 results in T-cell activation, it is a desirable target for a variety of therapeutic and/or diagnostic indications. Accordingly, there exists a need for antibodies and therapeutics that target the CD3/TCR pathway.

SUMMARY OF THE DISCLOSURE

[0005] The present disclosure provides antibodies and antigen-binding fragments thereof that specifically bind the epsilon chain of CD3, also known as CD3 ϵ . The anti- CD3 ϵ

antibodies and antigen-binding fragments thereof of the disclosure activate T cells via engagement of CD3 ϵ on the T cells. That is, such antibodies agonize, stimulate, activate, and/or augment CD3-mediated T cell activation. These antibodies and antigen-binding fragments thereof are referred to herein as “anti-CD3 ϵ antibodies” or “anti-CD3 antibodies.” The anti-CD3 ϵ antibodies and antigen-binding fragments thereof of the disclosure include monoclonal antibodies, such as, for example, mammalian monoclonal antibodies, primate monoclonal antibodies, fully human monoclonal antibodies, as well as humanized monoclonal antibodies and chimeric antibodies, as well as antigen-binding fragments thereof. In some embodiments, the antibodies and antigen-binding fragments thereof are IgG isotype. In some embodiments, the antibodies and antigen-binding fragments thereof are IgG1 isotype. In some embodiments, the antibodies and antigen-binding fragments thereof have one of any of the isotypes disclosed herein.

[0006] In some embodiments, the anti-CD3 ϵ antibody or antigen-binding fragment thereof includes a combination of a VH CDR1 sequence, a VH CDR2 sequence, and a VH CDR3 sequence, wherein at least one of the VH CDR1 sequence, the VH CDR2 sequence, and the VH CDR3 sequence is selected from a VH CDR1 sequence that includes at least the amino acid sequence TYAMN (SEQ ID NO: 53); a VH CDR2 sequence that includes at least the amino acid sequence RIRSKYNNYATYYADSVKD (SEQ ID NO: 54); and a VH CDR3 sequence that includes at least the amino acid sequence HGNFGNSYVSWFAY (SEQ ID NO: 55).

[0007] In some embodiments, the anti-CD3 ϵ antibody or antigen-binding fragment thereof includes a combination of a VL CDR1 sequence, a VL CDR2 sequence, and a VL CDR3 sequence, wherein at least one of the VL CDR1 sequence, the VL CDR2 sequence, and the VL CDR3 sequence is selected from a VL CDR1 sequence that includes at least the amino acid sequence RSSTGAVTTSNYAN (SEQ ID NO: 56); a VL CDR2 sequence that includes at least the amino acid sequence GTNKRAP (SEQ ID NO: 57); and a VL CDR3 sequence that includes at least the amino acid sequence ALWYSNLWV (SEQ ID NO: 58).

[0008] In some embodiments, the anti-CD3 ϵ antibody or antigen-binding fragment thereof includes a combination of a VH CDR1 sequence, a VH CDR2 sequence, and a VH CDR3 sequence, wherein at least one of the VH CDR1 sequence, the VH CDR2 sequence, and the VH CDR3 sequence is selected from a VH CDR1 sequence that includes at least the amino

acid sequence TYAMN (SEQ ID NO: 53); a VH CD2 sequence that includes at least the amino acid sequence RIRSKYNNYATYYADSVKD (SEQ ID NO: 54); a VH CDR3 sequence that includes at least the amino acid sequence HGNFGNSYVSWFAY (SEQ ID NO: 55), and a combination of a VL CDR1 sequence, a VL CDR2 sequence, and a VL CDR3 sequence, wherein at least one of the VL CDR1 sequence, the VL CDR2 sequence, and the VL CDR3 sequence is selected from a VL CDR1 sequence that includes at least the amino acid sequence RSSTGAVTTSNYAN (SEQ ID NO: 56); a VL CDR2 sequence that includes at least the amino acid sequence GTNKRAP (SEQ ID NO: 57); and a VL CDR3 sequence that includes at least the amino acid sequence ALWYSNLWV (SEQ ID NO: 58).

[0009] In some embodiments, the anti-CD3 ϵ antibody or antigen-binding fragment thereof includes a VH CDR1 sequence that includes at least the amino acid sequence TYAMN (SEQ ID NO: 53); a VH CD2 sequence that includes at least the amino acid sequence RIRSKYNNYATYYADSVKD (SEQ ID NO: 54); a VH CDR3 sequence that includes at least the amino acid sequence HGNFGNSYVSWFAY (SEQ ID NO: 55), a VL CDR1 sequence that includes at least the amino acid sequence RSSTGAVTTSNYAN (SEQ ID NO: 56); a VL CDR2 sequence that includes at least the amino acid sequence GTNKRAP (SEQ ID NO: 57); and a VL CDR3 sequence that includes at least the amino acid sequence ALWYSNLWV (SEQ ID NO: 58).

[00010] In some embodiments, the anti-CD3 ϵ antibody or antigen-binding fragment thereof includes a combination of a VH CDR1 sequence, a VH CDR2 sequence, and a VH CDR3 sequence, wherein at least one of the VH CDR1 sequence, the VH CDR2 sequence, and the VH CDR3 sequence is selected from a VH CDR1 sequence that includes a sequence that is at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or more identical to the amino acid sequence TYAMN (SEQ ID NO: 53); a VH CD2 sequence that includes a sequence that is at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or more identical to the amino acid sequence RIRSKYNNYATYYADSVKD (SEQ ID NO: 54); and a VH CDR3 sequence that includes a sequence that is at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or more identical to the amino acid sequence HGNFGNSYVSWFAY (SEQ ID NO: 55).

[00011] In some embodiments, the anti-CD3 ϵ antibody or antigen-binding fragment thereof includes a combination of a VL CDR1 sequence, a VL CDR2 sequence, and a VL

CDR3 sequence, wherein at least one of the VL CDR1 sequence, the VL CDR2 sequence, and the VL CDR3 sequence is selected from a VL CDR1 sequence that includes a sequence that is at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or more identical to the amino acid sequence RSSTGAVTTSNYAN (SEQ ID NO: 56); a VL CDR2 sequence that includes a sequence that is at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or more identical to the amino acid sequence GTNKRAP (SEQ ID NO: 57); and a VL CDR3 sequence that includes a sequence that is at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or more identical to the amino acid sequence ALWYSNLWV (SEQ ID NO: 58).

[00012] In some embodiments, the anti-CD3 ϵ antibody or antigen-binding fragment thereof includes a combination of a VH CDR1 sequence, a VH CDR2 sequence, and a VH CDR3 sequence, wherein at least one of the VH CDR1 sequence, the VH CDR2 sequence, and the VH CDR3 sequence is selected from a VH CDR1 sequence that includes a sequence that is at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or more identical to the amino acid sequence TYAMN (SEQ ID NO: 53); a VH CD2 sequence that includes a sequence that is at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or more identical to the amino acid sequence RIRSKYNNYATYYADSVKD (SEQ ID NO: 54); a VH CDR3 sequence that includes a sequence that is at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or more identical to the amino acid sequence HGNFGNSYVSWFAY (SEQ ID NO: 55), and a combination of a VL CDR1 sequence, a VL CDR2 sequence, and a VL CDR3 sequence, wherein at least one of the VL CDR1 sequence, the VL CDR2 sequence, and the VL CDR3 sequence is selected from a VL CDR1 sequence that includes a sequence that is at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or more identical to the amino acid sequence RSSTGAVTTSNYAN (SEQ ID NO: 56); a VL CDR2 sequence that includes a sequence that is at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or more identical to the amino acid sequence GTNKRAP (SEQ ID NO: 57); and a VL CDR3 sequence that includes a sequence that is at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or more identical to the amino acid sequence ALWYSNLWV (SEQ ID NO: 58).

[00013] In some embodiments, the anti-CD3 ϵ antibody or antigen-binding fragment thereof includes a VH CDR1 sequence that is at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or more identical to the amino acid sequence TYAMN (SEQ ID NO: 53); a

VH CD2 sequence that is at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or more identical to the amino acid sequence RIRSKYNNYATYYADSVKD (SEQ ID NO: 54); a VH CDR3 sequence that is at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or more identical to the amino acid sequence HGNFGNSYVSWFAY (SEQ ID NO: 55), a VL CDR1 sequence that is at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or more identical to the amino acid sequence RSSTGAVTTSNYAN (SEQ ID NO: 56); a VL CDR2 sequence that is at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or more identical to the amino acid sequence GTNKRAP (SEQ ID NO: 57); and a VL CDR3 sequence that is at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or more identical to the amino acid sequence ALWYSNLWV (SEQ ID NO: 58).

[00014] In some embodiments, the anti-CD3 ϵ antibody or antigen-binding fragment thereof includes a variable heavy chain (Hv) comprising the amino acid sequence of SEQ ID NO: 4. In some embodiments, the anti-CD3 ϵ antibody or antigen-binding fragment thereof includes a variable light chain (Lv) comprising the amino acid sequence of SEQ ID NO: 2. In some embodiments, the anti-CD3 ϵ antibody or antigen-binding fragment thereof includes a variable heavy chain (Hv) comprising the amino acid sequence of SEQ ID NO: 4 and a variable light chain (Lv) comprising the amino acid sequence of SEQ ID NO: 2.

[00015] In some embodiments, the anti-CD3 ϵ antibody or antigen-binding fragment thereof includes a variable heavy chain (Hv) comprising an amino acid sequence that is at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or more identical to the amino acid sequence of SEQ ID NO: 4. In some embodiments, the anti-CD3 ϵ antibody or antigen-binding fragment thereof includes a variable light chain (Lv) comprising an amino acid sequence that is at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or more identical to the amino acid sequence of SEQ ID NO: 2. In some embodiments, the anti-CD3 ϵ antibody or antigen-binding fragment thereof includes a variable heavy chain (Hv) comprising an amino acid sequence that is at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or more identical to the amino acid sequence of SEQ ID NO: 4 and a variable light chain (Lv) comprising the amino acid sequence of SEQ ID NO: 2.

[00016] In some embodiments, the anti-CD3 ϵ antibody is an scFv antibody fragment that binds CD3 ϵ . In some embodiments, the anti-CD3 ϵ scFv antibody fragment includes the amino

acid sequence of SEQ ID NO: 6. In some embodiments, the anti-CD3 ϵ scFv antibody fragment includes the amino acid sequence of SEQ ID NO: 30. In some embodiments, the anti-CD3 ϵ scFv antibody fragment includes an amino acid sequence that is at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or more identical to the amino acid sequence of SEQ ID NO: 6. In some embodiments, the anti-CD3 ϵ scFv antibody fragment includes an amino acid sequence that is at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or more identical to the amino acid sequence of SEQ ID NO: 30.

[00017] In some embodiments, the anti-CD3 ϵ antibody also includes an agent conjugated to the antibody. In some embodiments, the agent is a therapeutic agent. In some embodiments, the agent is a detectable moiety. In some embodiments, the detectable moiety is a diagnostic agent. In some embodiments, the agent is conjugated to the anti-CD3 ϵ antibody via a linker. In some embodiments, the linker is a cleavable linker. In some embodiments, the linker is a non-cleavable linker.

[00018] In some embodiments, the anti-CD3 ϵ antibody naturally contains one or more disulfide bonds. In some embodiments, the anti-CD3 ϵ antibody can be engineered to include one or more disulfide bonds.

[00019] The disclosure also provides an isolated nucleic acid molecule encoding at least a portion of an anti-CD3 ϵ antibody described herein and/or one or more nucleic acid molecules encoding an anti-CD3 ϵ antibody described herein, such as for example, at least a first nucleic acid encoding at least a portion of the heavy chain of the antibody and a second nucleic acid encoding at least a portion of the light chain of the antibody, as well as vectors that include these isolated nucleic acid sequences. The disclosure provides methods of producing an anti-CD3 ϵ antibody by culturing a cell under conditions that lead to expression of the anti-CD3 ϵ antibody, wherein the cell comprises such a nucleic acid molecule(s). In some embodiments, the cell comprises such a vector.

[00020] The disclosure also provides activatable antibodies and activatable antibody compositions that include an antibody or antigen-binding fragment thereof (AB) that specifically binds CD3 ϵ coupled or otherwise attached to a masking moiety (MM), such that coupling of the MM reduces the ability of the AB to bind CD3 ϵ . These activatable antibodies are collectively referred to herein as activatable anti-CD3 ϵ antibodies, also referred to herein as

anti-CD3 ϵ activatable antibodies or CD3 ϵ activatable antibodies. In some embodiments, the MM is coupled via a sequence that includes a substrate for a protease. For example, the protease is produced by a tumor that is in proximity to cells that express CD3 ϵ . In some embodiments, the protease is produced by a tumor that is co-localized with cells that express CD3 ϵ . The activatable anti-CD3 ϵ antibodies provided herein are stable in circulation, activated at intended sites of therapy and/or diagnosis but not in normal, *i.e.*, healthy tissue, and, when activated, exhibit binding to CD3 ϵ that is at least comparable to the corresponding, unmodified antibody.

[00021] In some embodiments, the activatable anti-CD3 ϵ antibody or antigen-binding fragment thereof includes a combination of a VH CDR1 sequence, a VH CDR2 sequence, and a VH CDR3 sequence, wherein at least one of the VH CDR1 sequence, the VH CDR2 sequence, and the VH CDR3 sequence is selected from a VH CDR1 sequence that includes at least the amino acid sequence TYAMN (SEQ ID NO: 53); a VH CDR2 sequence that includes at least the amino acid sequence RIRSKYNNYATYYADSVKD (SEQ ID NO: 54); and a VH CDR3 sequence that includes at least the amino acid sequence HGNFGNSYVSWFAY (SEQ ID NO: 55).

[00022] In some embodiments, the activatable anti-CD3 ϵ antibody or antigen-binding fragment thereof includes a combination of a VL CDR1 sequence, a VL CDR2 sequence, and a VL CDR3 sequence, wherein at least one of the VL CDR1 sequence, the VL CDR2 sequence, and the VL CDR3 sequence is selected from a VL CDR1 sequence that includes at least the amino acid sequence RSSTGAVTTSNYAN (SEQ ID NO: 56); a VL CDR2 sequence that includes at least the amino acid sequence GTNKRAP (SEQ ID NO: 57); and a VL CDR3 sequence that includes at least the amino acid sequence ALWYSNLWV (SEQ ID NO: 58).

[00023] In some embodiments, the activatable anti-CD3 ϵ antibody or antigen-binding fragment thereof includes a combination of a VH CDR1 sequence, a VH CDR2 sequence, and a VH CDR3 sequence, wherein at least one of the VH CDR1 sequence, the VH CDR2 sequence, and the VH CDR3 sequence is selected from a VH CDR1 sequence that includes at least the amino acid sequence TYAMN (SEQ ID NO: 53); a VH CDR2 sequence that includes at least the amino acid sequence RIRSKYNNYATYYADSVKD (SEQ ID NO: 54); a VH CDR3 sequence that includes at least the amino acid sequence HGNFGNSYVSWFAY (SEQ ID

NO: 55), and a combination of a VL CDR1 sequence, a VL CDR2 sequence, and a VL CDR3 sequence, wherein at least one of the VL CDR1 sequence, the VL CDR2 sequence, and the VL CDR3 sequence is selected from a VL CDR1 sequence that includes at least the amino acid sequence RSSTGAVTTSNYAN (SEQ ID NO: 56); a VL CDR2 sequence that includes at least the amino acid sequence GTNKRAP (SEQ ID NO: 57); and a VL CDR3 sequence that includes at least the amino acid sequence ALWYSNLWV (SEQ ID NO: 58).

[00024] In some embodiments, the activatable anti-CD3 ϵ antibody or antigen-binding fragment thereof includes a VH CDR1 sequence that includes at least the amino acid sequence TYAMN (SEQ ID NO: 53); a VH CD2 sequence that includes at least the amino acid sequence RIRSKYNNYATYYADSVKD (SEQ ID NO: 54); a VH CDR3 sequence that includes at least the amino acid sequence HGNFGNSYVSWFAY (SEQ ID NO: 55), a VL CDR1 sequence that includes at least the amino acid sequence RSSTGAVTTSNYAN (SEQ ID NO: 56); a VL CDR2 sequence that includes at least the amino acid sequence GTNKRAP (SEQ ID NO: 57); and a VL CDR3 sequence that includes at least the amino acid sequence ALWYSNLWV (SEQ ID NO: 58).

[00025] In some embodiments, the activatable anti-CD3 ϵ antibody or antigen-binding fragment thereof includes a combination of a VH CDR1 sequence, a VH CDR2 sequence, and a VH CDR3 sequence, wherein at least one of the VH CDR1 sequence, the VH CDR2 sequence, and the VH CDR3 sequence is selected from a VH CDR1 sequence that includes a sequence that is at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or more identical to the amino acid sequence TYAMN (SEQ ID NO: 53); a VH CD2 sequence that includes a sequence that is at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or more identical to the amino acid sequence RIRSKYNNYATYYADSVKD (SEQ ID NO: 54); and a VH CDR3 sequence that includes a sequence that is at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or more identical to the amino acid sequence HGNFGNSYVSWFAY (SEQ ID NO: 55).

[00026] In some embodiments, the activatable anti-CD3 ϵ antibody or antigen-binding fragment thereof includes a combination of a VL CDR1 sequence, a VL CDR2 sequence, and a VL CDR3 sequence, wherein at least one of the VL CDR1 sequence, the VL CDR2 sequence, and the VL CDR3 sequence is selected from a VL CDR1 sequence that includes a sequence

that is at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or more identical to the amino acid sequence RSSTGAVTTSNYAN (SEQ ID NO: 56); a VL CDR2 sequence that includes a sequence that is at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or more identical to the amino acid sequence GTNKRAP (SEQ ID NO: 57); and a VL CDR3 sequence that includes a sequence that is at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or more identical to the amino acid sequence ALWYSNLWV (SEQ ID NO: 58).

[00027] In some embodiments, the activatable anti-CD3 ϵ antibody or antigen-binding fragment thereof includes a combination of a VH CDR1 sequence, a VH CDR2 sequence, and a VH CDR3 sequence, wherein at least one of the VH CDR1 sequence, the VH CDR2 sequence, and the VH CDR3 sequence is selected from a VH CDR1 sequence that includes a sequence that is at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or more identical to the amino acid sequence TYAMN (SEQ ID NO: 53); a VH CD2 sequence that includes a sequence that is at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or more identical to the amino acid sequence RIRSKYNNYATYYADSVKD (SEQ ID NO: 54); a VH CDR3 sequence that includes a sequence that is at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or more identical to the amino acid sequence HGNFGNSYVSWFAY (SEQ ID NO: 55), and a combination of a VL CDR1 sequence, a VL CDR2 sequence, and a VL CDR3 sequence, wherein at least one of the VL CDR1 sequence, the VL CDR2 sequence, and the VL CDR3 sequence is selected from a VL CDR1 sequence that includes a sequence that is at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or more identical to the amino acid sequence RSSTGAVTTSNYAN (SEQ ID NO: 56); a VL CDR2 sequence that includes a sequence that is at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or more identical to the amino acid sequence GTNKRAP (SEQ ID NO: 57); and a VL CDR3 sequence that includes a sequence that is at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or more identical to the amino acid sequence ALWYSNLWV (SEQ ID NO: 58).

[00028] In some embodiments, the activatable anti-CD3 ϵ antibody or antigen-binding fragment thereof includes a VH CDR1 sequence that is at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or more identical to the amino acid sequence TYAMN (SEQ ID NO: 53); a VH CD2 sequence that is at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or more identical to the amino acid sequence RIRSKYNNYATYYADSVKD (SEQ

ID NO: 54); a VH CDR3 sequence that is at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or more identical to the amino acid sequence HGNFGNSYVSWFAY (SEQ ID NO: 55), a VL CDR1 sequence that is at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or more identical to the amino acid sequence RSSTGAVTTSNYAN (SEQ ID NO: 56); a VL CDR2 sequence that is at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or more identical to the amino acid sequence GTNKRAP (SEQ ID NO: 57); and a VL CDR3 sequence that is at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or more identical to the amino acid sequence ALWYSNLWV (SEQ ID NO: 58).

[00029] In some embodiments, the activatable antibody comprises a combination of a variable heavy chain complementarity determining region 1 (VH CDR1, also referred to herein as CDRH1) sequence, a variable heavy chain complementarity determining region 2 (VH CDR2, also referred to herein as CDRH2) sequence, a variable heavy chain complementarity determining region 3 (VH CDR3, also referred to herein as CDRH3) sequence, a variable light chain complementarity determining region 1 (VL CDR1, also referred to herein as CDRL1) sequence, a variable light chain complementarity determining region 2 (VL CDR2, also referred to herein as CDRL2) sequence, and a variable light chain complementarity determining region 3 (VL CDR3, also referred to herein as CDRL3) sequence, wherein at least one CDR sequence is selected from the group consisting of a VH CDR1 sequence shown in Table 18; a VH CDR2 sequence shown in Table 18; a VH CDR3 sequence shown in Table 18; a VL CDR1 sequence shown in Table 18; a VL CDR2 sequence shown in Table 18; and a VL CDR3 sequence shown in Table 18.

[00030] In some embodiments, the activatable antibody comprises a combination of a VH CDR1 sequence, a VH CDR2 sequence, a VH CDR3 sequence, a VL CDR1 sequence, a VL CDR2 sequence, and a VL CDR3 sequence, wherein at least one CDR sequence is selected from the group consisting of a VH CDR1 sequence that includes a sequence that is at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or more identical to a VH CDR1 sequence shown in Table 18; a VH CDR2 sequence that includes a sequence that is at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or more identical to a VH CDR2 sequence shown in Table 18; a VH CDR3 sequence that includes a sequence that is at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or more identical to a VH CDR3 sequence shown in Table 18; a VL CDR1 sequence that includes a sequence that is at least 90%, 91%, 92%,

93%, 94%, 95%, 96%, 97%, 98%, 99% or more identical to a VL CDR1 sequence shown in Table 18; a VL CDR2 sequence that includes a sequence that is at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or more identical to a VL CDR2 sequence shown in Table 18; and a VL CDR3 sequence that includes a sequence that is at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or more identical to a VL CDR3 sequence shown in Table 18.

[00031] In some embodiments, the activatable antibody comprises a combination of a VH CDR1 sequence, a VH CDR2 sequence, a VH CDR3 sequence, a VL CDR1 sequence, a VL CDR2 sequence, and a VL CDR3 sequence, wherein the combination is a combination shown in Table 18.

[00032] In some embodiments, the activatable antibody comprises a combination of a VH CDR1 sequence, a VH CDR2 sequence, a VH CDR3 sequence, a VL CDR1 sequence, a VL CDR2 sequence, and a VL CDR3 sequence, wherein each CDR sequence in the combination comprises a sequence that is at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or more identical to the corresponding CDR sequence in a combination shown in Table 18.

[00033] In some embodiments, the activatable anti-CD3 ϵ antibody or antigen-binding fragment thereof includes a variable heavy chain (Hv) comprising the amino acid sequence of SEQ ID NO: 4. In some embodiments, the anti-CD3 ϵ antibody or antigen-binding fragment thereof includes a variable light chain (Lv) comprising the amino acid sequence of SEQ ID NO: 2. In some embodiments, the anti-CD3 ϵ antibody or antigen-binding fragment thereof includes a variable heavy chain (Hv) comprising the amino acid sequence of SEQ ID NO: 4 and a variable light chain (Lv) comprising the amino acid sequence of SEQ ID NO: 2.

[00034] In some embodiments, the activatable anti-CD3 ϵ antibody or antigen-binding fragment thereof includes a variable heavy chain (Hv) comprising an amino acid sequence that is at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or more identical to the amino acid sequence of SEQ ID NO: 4. In some embodiments, the anti-CD3 ϵ antibody or antigen-binding fragment thereof includes a variable light chain (Lv) comprising an amino acid sequence that is at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or more identical to the amino acid sequence of SEQ ID NO: 2. In some embodiments, the anti-CD3 ϵ

antibody or antigen-binding fragment thereof includes a variable heavy chain (Hv) comprising an amino acid sequence that is at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or more identical to the amino acid sequence of SEQ ID NO: 4 and a variable light chain (Lv) comprising the amino acid sequence of SEQ ID NO: 2.

[00035] In some embodiments, the activatable anti-CD3 ϵ antibody includes an scFv antibody fragment that binds CD3 ϵ . In some embodiments, the anti-CD3 ϵ scFv antibody fragment includes the amino acid sequence of SEQ ID NO: 6. In some embodiments, the anti-CD3 ϵ scFv antibody fragment includes the amino acid sequence of SEQ ID NO: 30. In some embodiments, the anti-CD3 ϵ scFv antibody fragment includes an amino acid sequence that is at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or more identical to the amino acid sequence of SEQ ID NO: 6. In some embodiments, the anti-CD3 ϵ scFv antibody fragment includes an amino acid sequence that is at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or more identical to the amino acid sequence of SEQ ID NO: 30.

[00036] In some embodiments, the activatable antibody comprises a heavy chain amino acid sequence selected from the group consisting of the heavy chain sequences shown in Table 17. In some embodiments, the activatable antibody comprises a light chain amino acid sequence selected from the group consisting of the light chain sequences shown in Table 17. In some embodiments, the activatable antibody comprises a heavy chain amino acid sequence selected from the group consisting of the heavy chain sequences shown in Table 17 and a light chain amino acid sequence selected from the group consisting of the light chain sequences shown in Table 17.

[00037] In some embodiments, the activatable antibody comprises a heavy chain amino acid sequence that is at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98% or 99% identical to an amino acid sequence selected from the group consisting of the heavy chain sequences shown in Table 17. In some embodiments, the activatable antibody comprises a light chain amino acid sequence that is at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98% or 99% identical to an amino acid sequence selected from the group consisting of the light chain sequences shown in Table 17. In some embodiments, the activatable antibody comprises a heavy chain amino acid sequence that is at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98% or 99% identical to an amino acid sequence selected from the group consisting of the

heavy chain sequences shown in Table 17 and a light chain amino acid sequence that is at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98% or 99% identical to an amino acid sequence selected from the group consisting of the light chain sequences shown in Table 17.

[00038] In some embodiments, the MM has a dissociation constant, i.e., dissociation constant at an equilibrium state, K_d for binding to the AB that is greater than the K_d for binding of the AB to CD3 ϵ .

[00039] In some embodiments, the MM has a K_d for binding to the AB that is no more than the K_d for binding of the AB to CD3 ϵ .

[00040] In some embodiments, the MM has a K_d for binding to the AB that is no less than the K_d for binding of the AB to CD3 ϵ .

[00041] In some embodiments, the MM has a K_d for binding to the AB that is approximately equal to the K_d for binding of the AB to CD3 ϵ .

[00042] In some embodiments, the MM has a K_d for binding to the AB that is less than the K_d for binding of the AB to CD3 ϵ .

[00043] In some embodiments, the MM has a K_d for binding to the AB that is no more than 2, 3, 4, 5, 10, 25, 50, 100, 250, 500, or 1,000 fold greater than the K_d for binding of the AB to CD3 ϵ . In some embodiments, the MM has a K_d for binding to the AB that is between 1-5, 2-5, 2-10, 5-10, 5-20, 5-50, 5-100, 10-100, 10-1,000, 20-100, 20-1000, or 100-1,000 fold greater than the K_d for binding of the AB to CD3 ϵ .

[00044] In some embodiments, the MM has an affinity for binding to the AB that is less than the affinity of binding of the AB to CD3 ϵ .

[00045] In some embodiments, the MM has an affinity for binding to the AB that is no more than the affinity of binding of the AB to CD3 ϵ .

[00046] In some embodiments, the MM has an affinity for binding to the AB that is approximately equal of the affinity of binding of the AB to CD3 ϵ .

[00047] In some embodiments, the MM has an affinity for binding to the AB that is no less than the affinity of binding of the AB to CD3 ϵ .

[00048] In some embodiments, the MM has an affinity for binding to the AB that is greater than the affinity of binding of the AB to CD3 ϵ .

[00049] In some embodiments, the MM has an affinity for binding to the AB that is 2, 3, 4, 5, 10, 25, 50, 100, 250, 500, or 1,000 less than the affinity of binding of the AB to CD3 ϵ . In some embodiments, the MM has an affinity for binding to the AB that is between 1-5, 2-5, 2-10, 5-10, 5-20, 5-50, 5-100, 10-100, 10-1,000, 20-100, 20-1000, or 100-1,000 fold less than the affinity of binding of the AB to CD3 ϵ . In some embodiments, the MM has an affinity for binding to the AB that is 2 to 20 fold less than the affinity of binding of the AB to CD3 ϵ . In some embodiments, a MM not covalently linked to the AB and at equimolar concentration to the AB does not inhibit the binding of the AB to CD3 ϵ .

[00050] In some embodiments, the MM does not interfere or compete with the AB for binding to CD3 ϵ when the activatable antibody is in a cleaved state.

[00051] In some embodiments, the MM is a polypeptide of no more than 40 amino acids in length.

[00052] In some embodiments, the MM polypeptide sequence is different from that of CD3 ϵ . In some embodiments, the MM polypeptide sequence is no more than 50% identical to any natural binding partner of the AB. In some embodiments, the MM polypeptide sequence is no more than 25% identical to any natural binding partner of the AB. In some embodiments, the MM polypeptide sequence is no more than 10% identical to any natural binding partner of the AB.

[00053] In some embodiments, the MM comprises a sequence selected from the group consisting of the sequences shown in Table 7 or Table 8.

[00054] In some embodiments, the protease is produced by a tumor that is in proximity to cells that express CD3 ϵ and/or is produced by a tumor that is co-localized with cells that express CD3 ϵ in a tissue, and wherein the protease cleaves the CM in the activatable antibody when the activatable antibody is exposed to the protease. In some embodiments, the CM is a polypeptide of up to 15 amino acids in length. In some embodiments, the CM is a substrate for a protease selected from the proteases listed in Table 3. In some embodiments, the CM is a substrate for a protease selected from the group consisting of uPA, legumain, matriptase (also referred to herein as MT-SP1 or MTSP1), ADAM17, BMP-1, TMPRSS3, TMPRSS4, MMP-9, MMP-12, MMP-13, MMP-14, and any of those shown in Table 3. In some embodiments, the

CM is a substrate for an protease selected from the group consisting of uPA, legumain, and matriptase. In some embodiments, the CM is a substrate for a matrix metalloprotease (MMP).

[00055] In some embodiments, the antigen binding fragment thereof is selected from the group consisting of a Fab fragment, a F(ab')₂ fragment, a scFv, a scab, a dAb, a single domain heavy chain antibody, and a single domain light chain antibody.

[00056] In some embodiments, the activatable antibody in the uncleaved state has the structural arrangement from N-terminus to C-terminus as follows: MM-CM-AB or AB-CM-MM. In some embodiments, the activatable antibody comprises a linking peptide between the MM and the CM. In some embodiments, the activatable antibody comprises a linking peptide between the CM and the AB. In some embodiments, the activatable antibody comprises a first linking peptide (LP1) and a second linking peptide (LP2), wherein the activatable antibody in the uncleaved state has the structural arrangement from N-terminus to C-terminus as follows: MM-LP1-CM-LP2-AB or AB-LP2-CM-LP1-MM. In some embodiments, the two linking peptides need not be identical to each other. In some embodiments, each of LP1 and LP2 is a peptide of about 1 to 20 amino acids in length.

[00057] In some embodiments, the activatable anti-CD3 ϵ antibody also includes an agent conjugated to the activatable antibody. In some embodiments, the agent is a therapeutic agent. In some embodiments, the agent is a detectable moiety. In some embodiments, the detectable moiety is a diagnostic agent. In some embodiments, the agent is conjugated to the activatable anti-CD3 ϵ antibody via a linker. In some embodiments, the linker is a cleavable linker. In some embodiments, the linker is a non-cleavable linker.

[00058] The disclosure also provides multispecific antibodies that bind the epsilon chain of CD3 (CD3 ϵ) and a second target, wherein the antibody comprises a first antibody or antigen-binding fragment thereof (AB1) that binds the epsilon chain of CD3 (CD3 ϵ) and a second antibody or antigen-binding fragment thereof (AB2) that binds a second target, and wherein AB1 comprises a VH CDR1 sequence that includes at least the amino acid sequence TYAMN (SEQ ID NO: 53); a VH CD2 sequence that includes at least the amino acid sequence RIRSKYNNYATYYADSVKD (SEQ ID NO: 54); a VH CDR3 sequence that includes at least the amino acid sequence HGNFGNSYVSWFAY (SEQ ID NO: 55), and a combination of a VL CDR1 sequence, a VL CDR2 sequence, and a VL CDR3 sequence, wherein at least one of the

VL CDR1 sequence, the VL CDR2 sequence, and the VL CDR3 sequence is selected from a VL CDR1 sequence that includes at least the amino acid sequence RSSTGAVTTSNYAN (SEQ ID NO: 56); a VL CDR2 sequence that includes at least the amino acid sequence GTNKRAP (SEQ ID NO: 57); and a VL CDR3 sequence that includes at least the amino acid sequence ALWYSNLWV (SEQ ID NO: 58).

[00059] In some embodiments, the activatable antibodies described herein in an activated state bind CD3 ϵ and include (i) an antibody or an antigen binding fragment thereof (AB) that specifically binds to CD3 ϵ ; (ii) a masking moiety (MM) that inhibits the binding of the AB to CD3 ϵ when the activatable antibody is in an uncleaved state; and (c) a cleavable moiety (CM) coupled to the AB, wherein the CM is a polypeptide that functions as a substrate for a protease. In some embodiments, the MM is coupled to the AB via the CM.

[00060] In some embodiments, the activatable antibody in the uncleaved state has the structural arrangement from N-terminus to C-terminus as follows: MM-CM-AB or AB-CM-MM.

[00061] In some embodiments, the activatable antibody comprises a linking peptide between the MM and the CM.

[00062] In some embodiments, the activatable antibody comprises a linking peptide between the CM and the AB.

[00063] In some embodiments, the activatable antibody comprises a first linking peptide (LP1) and a second linking peptide (LP2), and the activatable antibody in the uncleaved state has the structural arrangement from N-terminus to C-terminus as follows: MM-LP1-CM-LP2-AB or AB-LP2-CM-LP1-MM. In some embodiments, the two linking peptides need not be identical to each other.

[00064] In some embodiments, at least one of LP1 or LP2 includes an amino acid sequence selected from the group consisting of (GS)_n, (GGS)_n, (GSGGS)_n (SEQ ID NO: 59) and (GGGS)_n (SEQ ID NO: 60), where n is an integer of at least one. In some embodiments, at least one of LP1 or LP2 includes an amino acid sequence selected from the group consisting of GGSG (SEQ ID NO: 61), GGSGG (SEQ ID NO: 62), GSGSG (SEQ ID NO: 63), GSGGG (SEQ ID NO: 64), GGGSG (SEQ ID NO: 65), and GSSSG (SEQ ID NO: 66).

[00065] In some embodiments, the activatable antibody includes an antibody or antigen-binding fragment thereof that specifically binds CD3 ϵ . In some embodiments, the antibody or antigen-binding fragment thereof that binds CD3 ϵ is a monoclonal antibody, domain antibody, single chain, Fab fragment, a F(ab')₂ fragment, a scFv, a scAb, a dAb, a single domain heavy chain antibody, or a single domain light chain antibody. In some embodiments, such an antibody or antigen-binding fragment thereof that binds CD3 ϵ is a rodent (e.g., mouse or rat), chimeric, humanized or fully human monoclonal antibody.

[00066] In some embodiments, the AB has a dissociation constant of about 100 nM or less for binding to CD3 ϵ .

[00067] In some embodiments, the MM has a dissociation constant, i.e., dissociation constant at an equilibrium state, K_d for binding to the AB that is greater than the K_d for binding of the AB to CD3 ϵ .

[00068] In some embodiments, the MM has a K_d for binding to the AB that is no more than the K_d for binding of the AB to CD3 ϵ .

[00069] In some embodiments, the MM has a K_d for binding to the AB that is no less than the K_d for binding of the AB to CD3 ϵ .

[00070] In some embodiments, the MM has a K_d for binding to the AB that is approximately equal to the K_d for binding of the AB to CD3 ϵ .

[00071] In some embodiments, the MM has a K_d for binding to the AB that is less than the K_d for binding of the AB to CD3 ϵ .

[00072] In some embodiments, the MM has a K_d for binding to the AB that is no more than 2, 3, 4, 5, 10, 25, 50, 100, 250, 500, or 1,000 fold greater than the K_d for binding of the AB to CD3 ϵ . In some embodiments, the MM has a K_d for binding to the AB that is between 1-5, 2-5, 2-10, 5-10, 5-20, 5-50, 5-100, 10-100, 10-1,000, 20-100, 20-1000, or 100-1,000 fold greater than the K_d for binding of the AB to CD3 ϵ .

[00073] In some embodiments, the MM has an affinity for binding to the AB that is less than the affinity of binding of the AB to CD3 ϵ .

[00074] In some embodiments, the MM has an affinity for binding to the AB that is no more than the affinity of binding of the AB to CD3 ϵ .

[00075] In some embodiments, the MM has an affinity for binding to the AB that is approximately equal of the affinity of binding of the AB to CD3 ϵ .

[00076] In some embodiments, the MM has an affinity for binding to the AB that is no less than the affinity of binding of the AB to CD3 ϵ .

[00077] In some embodiments, the MM has an affinity for binding to the AB that is greater than the affinity of binding of the AB to CD3 ϵ .

[00078] In some embodiments, the MM has an affinity for binding to the AB that is 2, 3, 4, 5, 10, 25, 50, 100, 250, 500, or 1,000 less than the affinity of binding of the AB to CD3 ϵ . In some embodiments, the MM has an affinity for binding to the AB that is between 1-5, 2-5, 2-10, 5-10, 5-20, 5-50, 5-100, 10-100, 10-1,000, 20-100, 20-1000, or 100-1,000 fold less than the affinity of binding of the AB to CD3 ϵ . In some embodiments, the MM has an affinity for binding to the AB that is 2 to 20 fold less than the affinity of binding of the AB to CD3 ϵ . In some embodiments, a MM not covalently linked to the AB and at equimolar concentration to the AB does not inhibit the binding of the AB to CD3 ϵ .

[00079] In some embodiments, the MM does not interfere or compete with the AB for binding to CD3 ϵ when the activatable antibody is in a cleaved state.

[00080] In some embodiments, the MM is a polypeptide of about 2 to 40 amino acids in length. In some embodiments, the MM is a polypeptide of no more than 40 amino acids in length.

[00081] In some embodiments, the MM polypeptide sequence is different from that of CD3 ϵ . In some embodiments, the MM polypeptide sequence is no more than 50% identical to any natural binding partner of the AB. In some embodiments, the MM polypeptide sequence is different from that of CD3 ϵ and wherein the MM polypeptide sequence is no more than 25% identical to any natural binding partner of the AB. In some embodiments, the MM polypeptide sequence is different from that of CD3 ϵ and wherein the MM polypeptide sequence is no more than 10% identical to any natural binding partner of the AB.

[00082] In some embodiments, the coupling of the MM reduces the ability of the AB to bind CD3 ϵ such that the dissociation constant (K_d) of the AB when coupled to the MM towards CD3 ϵ is at least 20 times greater than the K_d of the AB when not coupled to the MM towards CD3 ϵ .

[00083] In some embodiments, the coupling of the MM reduces the ability of the AB to bind CD3 ϵ such that the dissociation constant (K_d) of the AB when coupled to the MM towards CD3 ϵ is at least 40 times greater than the K_d of the AB when not coupled to the MM towards CD3 ϵ .

[00084] In some embodiments, the coupling of the MM reduces the ability of the AB to bind CD3 ϵ such that the dissociation constant (K_d) of the AB when coupled to the MM towards CD3 ϵ is at least 100 times greater than the K_d of the AB when not coupled to the MM towards CD3 ϵ .

[00085] In some embodiments, the coupling of the MM reduces the ability of the AB to bind CD3 ϵ such that the dissociation constant (K_d) of the AB when coupled to the MM towards CD3 ϵ is at least 1000 times greater than the K_d of the AB when not coupled to the MM towards CD3 ϵ .

[00086] In some embodiments, the coupling of the MM reduces the ability of the AB to bind CD3 ϵ such that the dissociation constant (K_d) of the AB when coupled to the MM towards CD3 ϵ is at least 10,000 times greater than the K_d of the AB when not coupled to the MM towards CD3 ϵ .

[00087] In some embodiments, in the presence of CD3 ϵ , the MM reduces the ability of the AB to bind CD3 ϵ by at least 90% when the CM is uncleaved, as compared to when the CM is cleaved when assayed *in vitro* using a target displacement assay such as, for example, the assay described in PCT Publication No. WO 2010/081173, the contents of which are hereby incorporated by reference in their entirety.

[00088] In some embodiments, the MM does not interfere or compete with the AB of the activatable antibody in a cleaved state for binding to the CD3 ϵ target.

[00089] In some embodiments, the MM is an amino acid sequence selected from the group of those listed in Tables 7 or 8.

[00090] In some embodiments, the protease is produced by a tumor that is in proximity to cells that express CD3 ϵ and/or produced by a tumor that is co-localized with CD3 ϵ in a tissue, and the protease cleaves the CM in the activatable antibody when the activatable antibody is exposed to the protease.

[00091] In some embodiments, the CM is positioned in the activatable antibody such that when the activatable antibody is in the uncleaved state, binding of the activatable antibody to CD3 ϵ is reduced to occur with a dissociation constant that is at least 20-fold greater than the dissociation constant of an unmodified AB binding to CD3 ϵ , and whereas when the activatable antibody is in the cleaved state, the AB binds CD3 ϵ .

[00092] In some embodiments, the CM is positioned in the activatable antibody such that when the activatable antibody is in the uncleaved state, binding of the activatable antibody to CD3 ϵ is reduced to occur with a dissociation constant that is at least 40-fold greater than the dissociation constant of an unmodified AB binding to CD3 ϵ , and whereas when the activatable antibody is in the cleaved state, the AB binds CD3 ϵ .

[00093] In some embodiments, the CM is positioned in the activatable antibody such that when the activatable antibody is in the uncleaved state, binding of the activatable antibody to CD3 ϵ is reduced to occur with a dissociation constant that is at least 50-fold greater than the dissociation constant of an unmodified AB binding to CD3 ϵ , and whereas when the activatable antibody is in the cleaved state, the AB binds CD3 ϵ .

[00094] In some embodiments, the CM is positioned in the activatable antibody such that when the activatable antibody is in the uncleaved state, binding of the activatable antibody to CD3 ϵ is reduced to occur with a dissociation constant that is at least 100-fold greater than the dissociation constant of an unmodified AB binding to CD3 ϵ , and whereas when the activatable antibody is in the cleaved state, the AB binds CD3 ϵ .

[00095] In some embodiments, the CM is positioned in the activatable antibody such that when the activatable antibody is in the uncleaved state, binding of the activatable antibody to CD3 ϵ is reduced to occur with a dissociation constant that is at least 200-fold greater than the dissociation constant of an unmodified AB binding to CD3 ϵ , and whereas when the activatable antibody is in the cleaved state, the AB binds CD3 ϵ .

[00096] In some embodiments, the CM is a polypeptide of up to 15 amino acids in length.

[00097] In some embodiments, the CM includes the amino acid sequence LSGRSDNH (SEQ ID NO: 67). In some embodiments, the cleavable moiety is selected for use with a specific protease, for example a protease that is known to be produced by a tumor that is in

proximity to cells that express the target of the activatable antibody, e.g., CD3 ϵ , and/or produced by a tumor that is co-localized with the target of the activatable antibody. For example, suitable cleavable moieties for use in the activatable anti-CD3 ϵ antibodies of the disclosure are cleaved by at least a protease such as urokinase, legumain, and/or matriptase (also referred to herein as MT-SP1 or MTSP1). In some embodiments, a suitable cleavable moiety includes at least one of the following sequences: TGRGPSWV (SEQ ID NO: 68); SARGPSRW (SEQ ID NO: 69); TARGPSFK (SEQ ID NO: 70); LSGRSDNH (SEQ ID NO: 67); GGWHTGRN (SEQ ID NO: 71); HTGRSGAL (SEQ ID NO: 72); PLTGRSGG (SEQ ID NO: 73); AARGPAIH (SEQ ID NO: 74); RGPAFNPM (SEQ ID NO: 75); SSRGPAYL (SEQ ID NO: 76); RGPATPIM (SEQ ID NO: 77); RGPA (SEQ ID NO: 78); GGQPSGMWGW (SEQ ID NO: 79); FPRPLGITGL (SEQ ID NO: 80); VHMPLGFLGP (SEQ ID NO: 81); SPLTGRSG (SEQ ID NO: 82); SAGFSLPA (SEQ ID NO: 83); LAPLGLQRR (SEQ ID NO: 84); SGGPLGVR (SEQ ID NO: 85); and/or PLGL (SEQ ID NO: 86).

[00098] In some embodiments, the CM comprises the amino acid sequence LSGRSDNH (SEQ ID NO: 67). In some embodiments, the CM comprises the amino acid sequence TGRGPSWV (SEQ ID NO: 68). In some embodiments, the CM comprises the amino acid sequence SARGPSRW (SEQ ID NO: 69). In some embodiments, the CM comprises the amino acid sequence TARGPSFK (SEQ ID NO: 70). In some embodiments, the CM comprises the amino acid sequence LSGRSDNH (SEQ ID NO: 67). In some embodiments, the CM comprises the amino acid sequence GGWHTGRN (SEQ ID NO: 71). In some embodiments, the CM comprises the amino acid sequence HTGRSGAL (SEQ ID NO: 72). In some embodiments, the CM comprises the amino acid sequence PLTGRSGG (SEQ ID NO: 73). In some embodiments, the CM comprises the amino acid sequence AARGPAIH (SEQ ID NO: 74). In some embodiments, the CM comprises the amino acid sequence RGPAFNPM (SEQ ID NO: 75). In some embodiments, the CM comprises the amino acid sequence SSRGPAYL (SEQ ID NO: 76). In some embodiments, the CM comprises the amino acid sequence RGPATPIM (SEQ ID NO: 77). In some embodiments, the CM comprises the amino acid sequence RGPA (SEQ ID NO: 78). In some embodiments, the CM comprises the amino acid sequence GGQPSGMWGW (SEQ ID NO: 79). In some embodiments, the CM comprises the amino acid sequence FPRPLGITGL (SEQ ID NO: 80). In some embodiments, the CM comprises the

amino acid sequence VHMPLGFLGP (SEQ ID NO: 81). In some embodiments, the CM comprises the amino acid sequence SPLTGRSG (SEQ ID NO: 82). In some embodiments, the CM comprises the amino acid sequence SAGFSLPA (SEQ ID NO: 83). In some embodiments, the CM comprises the amino acid sequence LAPLGLQRR (SEQ ID NO: 84). In some embodiments, the CM comprises the amino acid sequence SGGPLGVR (SEQ ID NO: 85). In some embodiments, the CM comprises the amino acid sequence PLGL (SEQ ID NO: 86).

[00099] In some embodiments, the CM is a substrate for an MMP and includes the sequence ISSGLLSS (SEQ ID NO: 321); QNQALRMA (SEQ ID NO: 322); AQNLLGMV (SEQ ID NO: 323); STFPFGMF (SEQ ID NO: 324); PVGYTSSL (SEQ ID NO: 325); DWLYWPGI (SEQ ID NO: 326); MIAPVAYR (SEQ ID NO: 327); RPSPMWAY (SEQ ID NO: 328); WATPRPMR (SEQ ID NO: 329); FRLLDWQW (SEQ ID NO: 330); LKAAPRWA (SEQ ID NO: 331); GPSHLVLT (SEQ ID NO: 332); LPGGLSPW (SEQ ID NO: 333); MGLFSEAG (SEQ ID NO: 334); SPLPLRVP (SEQ ID NO: 335); RMHLRSLG (SEQ ID NO: 336); LAAPLGLL (SEQ ID NO: 337); AVGLLAPP (SEQ ID NO: 338); LLAPSHRA (SEQ ID NO: 339); PAGLWLDP (SEQ ID NO: 340); and/or ISSGLSS (SEQ ID NO: 341).

[000100] In some embodiments, the CM comprises the amino acid sequence ISSGLLSS (SEQ ID NO: 321). In some embodiments, the CM comprises the amino acid sequence QNQALRMA (SEQ ID NO: 322). In some embodiments, the CM comprises the amino acid sequence AQNLLGMV (SEQ ID NO: 323). In some embodiments, the CM comprises the amino acid sequence STFPFGMF (SEQ ID NO: 324). In some embodiments, the CM comprises the amino acid sequence PVGYTSSL (SEQ ID NO: 325). In some embodiments, the CM comprises the amino acid sequence DWLYWPGI (SEQ ID NO: 326). In some embodiments, the CM comprises the amino acid sequence MIAPVAYR (SEQ ID NO: 327). In some embodiments, the CM comprises the amino acid sequence RPSPMWAY (SEQ ID NO: 328). In some embodiments, the CM comprises the amino acid sequence WATPRPMR (SEQ ID NO: 329). In some embodiments, the CM comprises the amino acid sequence FRLLDWQW (SEQ ID NO: 330). In some embodiments, the CM comprises the amino acid sequence LKAAPRWA (SEQ ID NO: 331). In some embodiments, the CM comprises the amino acid sequence GPSHLVLT (SEQ ID NO: 332). In some embodiments, the CM comprises the amino acid sequence LPGGLSPW (SEQ ID NO: 333). In some embodiments, the CM comprises the amino acid sequence MGLFSEAG (SEQ ID NO: 334). In some

embodiments, the CM comprises the amino acid sequence SPLPLRVP (SEQ ID NO: 335). In some embodiments, the CM comprises the amino acid sequence RMHLRSLG (SEQ ID NO: 336). In some embodiments, the CM comprises the amino acid sequence LAAPLGLL (SEQ ID NO: 337). In some embodiments, the CM comprises the amino acid sequence AVGLLAPP (SEQ ID NO: 338). In some embodiments, the CM comprises the amino acid sequence LLAPSHRA (SEQ ID NO: 339). In some embodiments, the CM comprises the amino acid sequence PAGLWLDP (SEQ ID NO: 340). In some embodiments, the CM comprises the amino acid sequence ISSGLSS (SEQ ID NO: 341).

[000101] In some embodiments, the CM is a substrate for thrombin. In some embodiments, the CM is a substrate for thrombin and includes the sequence GPRSFGL (SEQ ID NO: 896) or GPRSFG (SEQ ID NO: 897). In some embodiments, the CM comprises the amino acid sequence GPRSFGL (SEQ ID NO: 896). In some embodiments, the CM comprises the amino acid sequence GPRSFG (SEQ ID NO: 897).

[000102] In some embodiments, the CM comprises an amino acid sequence selected from the group consisting of NTLSGRSENHSG (SEQ ID NO: 898); NTLSGRSGNHGS (SEQ ID NO: 899); TSTSGRSANPRG (SEQ ID NO: 900); TSGRSANP (SEQ ID NO: 901); VAGRSMRP (SEQ ID NO: 902); VVPEGRRS (SEQ ID NO: 903); ILPRSPAF (SEQ ID NO: 904); MVLGRSLL (SEQ ID NO: 905); QGRAITFI (SEQ ID NO: 906); SPRSIMLA (SEQ ID NO: 907); and SMLRSMPL (SEQ ID NO: 908).

[000103] In some embodiments, the CM comprises the amino acid sequence NTLSGRSENHSG (SEQ ID NO: 898). In some embodiments, the CM comprises the amino acid sequence NTLSGRSGNHGS (SEQ ID NO: 899). In some embodiments, the CM comprises the amino acid sequence TSTSGRSANPRG (SEQ ID NO: 900). In some embodiments, the CM comprises the amino acid sequence TSGRSANP (SEQ ID NO: 901). In some embodiments, the CM comprises the amino acid sequence VAGRSMRP (SEQ ID NO: 902). In some embodiments, the CM comprises the amino acid sequence VVPEGRRS (SEQ ID NO: 903). In some embodiments, the CM comprises the amino acid sequence ILPRSPAF (SEQ ID NO: 904). In some embodiments, the CM comprises the amino acid sequence MVLGRSLL (SEQ ID NO: 905). In some embodiments, the CM comprises the amino acid sequence QGRAITFI (SEQ ID NO: 906). In some embodiments, the CM comprises

the amino acid sequence SPRSIMLA (SEQ ID NO: 907). In some embodiments, the CM comprises the amino acid sequence and SMLRSMPL (SEQ ID NO: 908).

[000104] In some embodiments, the CM is a substrate for a neutrophil elastase. In some embodiments, the CM is a substrate for a serine protease. In some embodiments, the CM is a substrate for uPA. In some embodiments, the CM is a substrate for legumain. In some embodiments, the CM is a substrate for matriptase. In some embodiments, the CM is a substrate for a cysteine protease. In some embodiments, the CM is a substrate for a cysteine protease, such as a cathepsin.

[000105] In some embodiments, the CM is a CM1-CM2 substrate and includes the sequence ISSGLLSGRSDNH (SEQ ID NO: 909); ISSGLLSSGGSGGSLSGRSDNH (SEQ ID NO: 910); AVGLLAPPGGTSTSGRSANPRG (SEQ ID NO: 911); TSTSGRSANPRGGGAVGLLAPP (SEQ ID NO: 912); VHMPLGFLGPGGTSTSGRSANPRG (SEQ ID NO: 913); TSTSGRSANPRGGGVHMPLGFLGP (SEQ ID NO: 914); AVGLLAPPGGLSGRSDNH (SEQ ID NO: 915); LSGRSDNHGGAVGLLAPP (SEQ ID NO: 916); VHMPLGFLGPGGLSGRSDNH (SEQ ID NO: 917); LSGRSDNHGGGVHMPLGFLGP (SEQ ID NO: 918); LSGRSDNHGGSGGSISSGLLSS (SEQ ID NO: 919); LSGRSGNHGGSGGSISSGLLSS (SEQ ID NO: 920); ISSGLLSSGGSGGSLSGRSGNH (SEQ ID NO: 921); LSGRSDNHGGSGGGSQNQALRMA (SEQ ID NO: 922); QNQALRMAGGSGGSLSGRSDNH (SEQ ID NO: 923); LSGRSGNHGGSGGGSQNQALRMA (SEQ ID NO: 924); QNQALRMAGGSGGSLSGRSGNH (SEQ ID NO: 925) and/or ISSGLLSGRSGNH (SEQ ID NO: 926).

[000106] In some embodiments, the CM1-CM2 substrate includes the sequence ISSGLLSGRSDNH (SEQ ID NO: 909). In some embodiments, the CM1-CM2 substrate comprises the amino acid sequence ISSGLLSSGGSGGSLSGRSDNH (SEQ ID NO: 910). In some embodiments, the CM1-CM2 substrate comprises the amino acid sequence AVGLLAPPGGTSTSGRSANPRG (SEQ ID NO: 911). In some embodiments, the CM1-CM2 substrate comprises the amino acid sequence TSTSGRSANPRGGGAVGLLAPP (SEQ ID NO: 912). In some embodiments, the CM1-CM2 substrate comprises the amino acid sequence VHMPLGFLGPGGTSTSGRSANPRG (SEQ ID NO: 913). In some embodiments, the CM1-

CM2 substrate comprises the amino acid sequence TSTSGRSANPRGGGVHMPLGFLGP (SEQ ID NO: 914). In some embodiments, the CM1-CM2 substrate comprises the amino acid sequence AVGLLAPPGGLSGRSDNH (SEQ ID NO: 915). In some embodiments, the CM1-CM2 substrate comprises the amino acid sequence LSGRSDNHGGAVGLLAPP (SEQ ID NO: 916). In some embodiments, the CM1-CM2 substrate comprises the amino acid sequence VHMPLGFLGPGGLSGRSDNH (SEQ ID NO: 917). In some embodiments, the CM1-CM2 substrate comprises the amino acid sequence LSGRSDNHGGVHMPLGFLGP (SEQ ID NO: 918). In some embodiments, the CM1-CM2 substrate comprises the amino acid sequence LSGRSDNHGGSGGSISSGLLSS (SEQ ID NO: 919). In some embodiments, the CM1-CM2 substrate comprises the amino acid sequence LSGRSGNHGGSGGSISSGLLSS (SEQ ID NO: 920). In some embodiments, the CM1-CM2 substrate comprises the amino acid sequence ISSGLLSSGGSGGSLSGRSGNH (SEQ ID NO: 921). In some embodiments, the CM1-CM2 substrate comprises the amino acid sequence LSGRSDNHGGSGGSGNQALRMA (SEQ ID NO: 922). In some embodiments, the CM1-CM2 substrate comprises the amino acid sequence QNQALRMAGGSGGSLSGRSDNH (SEQ ID NO: 923). In some embodiments, the CM1-CM2 substrate comprises the amino acid sequence LSGRSGNHGGSGGSGNQALRMA (SEQ ID NO: 924). In some embodiments, the CM1-CM2 substrate comprises the amino acid sequence QNQALRMAGGSGGSLSGRSGNH (SEQ ID NO: 925). In some embodiments, the CM1-CM2 substrate comprises the amino acid sequence ISSGLLSGRSGNH (SEQ ID NO: 926).

[000107] In some embodiments, the CM is a substrate for a protease selected from the group consisting of those shown in Table 3. In some embodiments, the protease is selected from the group consisting of uPA, legumain, matriptase, ADAM17, BMP-1, TMPRSS3, TMPRSS4, MMP-9, MMP-12, MMP-13, and MMP-14. In some embodiments, the protease is a cathepsin. In some embodiments, the CM is a substrate for a protease selected from the group consisting of uPA (urokinase plasminogen activator), legumain and matriptase. In some embodiments, the protease comprises uPA. In some embodiments, the protease comprises legumain. In some embodiments, the protease comprises matriptase. In some embodiments, the protease comprises a matrix metalloproteinase (MMP).

[000108] In some embodiments, the CM is a substrate for at least two proteases. In some embodiments, each protease is selected from the group consisting of those shown in Table 3. In

some embodiments, the CM is a substrate for at least two proteases, wherein one of the proteases is selected from the group consisting of uPA, legumain and matriptase and the other protease is selected from the group consisting of those shown in Table 3. In some embodiments, the CM is a substrate for at least two proteases selected from the group consisting of uPA, legumain and matriptase.

[000109] In some embodiments, the activatable antibody includes at least a first CM and a second CM. In some embodiments, the first CM and the second CM are each polypeptides of no more than 15 amino acids long. In some embodiments, the first CM and the second CM in the activatable antibody in the uncleaved state have the structural arrangement from N-terminus to C-terminus as follows: MM-CM1-CM2-AB or AB-CM2-CM1-MM. In some embodiments, at least one of the first CM and the second CM is a polypeptide that functions as a substrate for a protease selected from the group consisting of uPA, legumain, and matriptase. In some embodiments, the first CM is cleaved by a first cleaving agent selected from the group consisting of uPA, legumain, and matriptase in a target tissue and the second CM is cleaved by a second cleaving agent in a target tissue. In some embodiments, the other protease is selected from the group consisting of those shown in Table 3. In some embodiments, the first cleaving agent and the second cleaving agent are the same protease selected from the group consisting of uPA, legumain, and matriptase, and the first CM and the second CM are different substrates for the protease. In some embodiments, the first cleaving agent and the second cleaving agent are the same protease selected from the group consisting of those shown in Table 3. In some embodiments, the first cleaving agent and the second cleaving agent are different proteases. In some embodiments, the first cleaving agent and the second cleaving agent are produced by a tumor that is in proximity to cells that express the target and/or produced by a tumor that is co-localized in the target tissue. In some embodiments, the first CM and the second CM are cleaved by at least one cleaving agent in the target tissue.

[000110] In some embodiments, the activatable antibody is exposed to and cleaved by a protease such that, when the activatable antibody is in the activated or cleaved state, the activated antibody includes a light chain amino acid sequence that includes at least a portion of LP2 and/or CM sequence after the protease has cleaved the CM.

[000111] In some embodiments, the MM and the CM include an amino acid sequence selected from the group consisting of those presented herein.

[000112] In some embodiments, the activatable antibody also includes a signal peptide. In some embodiments, the signal peptide is conjugated to the activatable antibody via a spacer. In some embodiments, the spacer is conjugated to the activatable antibody in the absence of a signal peptide. In some embodiments, the spacer is joined directly to the MM of the activatable antibody.

[000113] In some embodiments, the activatable antibody in an uncleaved state comprises a spacer that is joined directly to the MM and has the structural arrangement from N-terminus to C-terminus of spacer-MM-CM-AB. In some embodiments, the spacer includes at least the amino acid sequence QGQSGQG (SEQ ID NO: 407). In some embodiments, the spacer includes at least the amino acid sequence QGQSGQ (SEQ ID NO: 87). In some embodiments, the spacer includes at least the amino acid sequence QGQSG (SEQ ID NO: 408). In some embodiments, the spacer includes at least the amino acid sequence QGQS (SEQ ID NO: 409). In some embodiments, the spacer includes at least the amino acid sequence QGQ (SEQ ID NO: 410). In some embodiments, the spacer includes at least the amino acid sequence QG (SEQ ID NO: 411). In some embodiments, the spacer includes at least the amino acid residue Q. In some embodiments, the MM and spacer comprise an amino acid sequence selected from the sequences listed in Table 7 or Table 8.

[000114] In some embodiments, the activatable antibody includes a heavy chain sequence selected from the group consisting of SEQ ID NOs: 446, 452, 454, 456, 460, 462, 464, 466, 470, 472, 476, 478, 480, 486, 488, 490, 492, 494, 496, 498, 500, 502, 504, 510, 512, 514, 518, 524, 526, 530, 532, 534, 536, 538, 540, 542, 544, and 546.

[000115] In some embodiments, the activatable antibody includes a light chain sequence selected from the group consisting of SEQ ID NOs: 448, 450, 458, 468, 474, 482, 484, 508, 516, and 520.

[000116] In some embodiments, the activatable antibody includes a heavy chain sequence selected from the group consisting of SEQ ID NOs: 446, 452, 454, 456, 460, 462, 464, 466, 470, 472, 476, 478, 480, 486, 488, 490, 492, 494, 496, 498, 500, 502, 504, 510, 512, 514, 518, 524, 526, 530, 532, 534, 536, 538, 540, 542, 544, and 546; and a light chain sequence selected from the group consisting of SEQ ID NOs: 448, 450, 458, 468, 474, 482, 484, 508, 516, and 520.

[000117] In some embodiments, the activatable antibody includes a heavy chain sequence that is at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98% or 99% identical to an amino acid sequence selected from the group consisting of SEQ ID NOs: 446, 452, 454, 456, 460, 462, 464, 466, 470, 472, 476, 478, 480, 486, 488, 490, 492, 494, 496, 498, 500, 502, 504, 510, 512, 514, 518, 524, 526, 530, 532, 534, 536, 538, 540, 542, 544, and 546.

[000118] In some embodiments, the activatable antibody includes a light chain sequence that is at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98% or 99% identical to an amino acid sequence selected from the group consisting of SEQ ID NOs: 448, 450, 458, 468, 474, 482, 484, 508, 516, and 520.

[000119] In some embodiments, the activatable antibody includes a heavy chain sequence that is at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98% or 99% identical to an amino acid sequence selected from the group consisting of SEQ ID NOs: 446, 452, 454, 456, 460, 462, 464, 466, 470, 472, 476, 478, 480, 486, 488, 490, 492, 494, 496, 498, 500, 502, 504, 510, 512, 514, 518, 524, 526, 530, 532, 534, 536, 538, 540, 542, 544, and 546; and a light chain sequence that is at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98% or 99% identical to an amino acid sequence selected from the group consisting of SEQ ID NOs: 448, 450, 458, 468, 474, 482, 484, 508, 516, and 520.

[000120] In some embodiments, the activatable antibody includes the amino acid sequence of SEQ ID NO: 506. In some embodiments, the activatable antibody includes an amino acid sequence that is at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98% or 99% identical to the amino acid sequence of SEQ ID NO: 506.

[000121] In some embodiments, the activatable antibody includes the amino acid sequence of SEQ ID NO: 587 or SEQ ID NO: 588. In some embodiments, the activatable antibody includes an amino acid sequence that is at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98% or 99% identical to the amino acid sequence of SEQ ID NO: 587 or SEQ ID NO: 588.

[000122] In some embodiments, the activatable antibody also includes an agent conjugated to the AB. In some embodiments, the agent is a therapeutic agent. In some embodiments, the agent is a detectable moiety. In some embodiments, the detectable moiety is a diagnostic agent. In some embodiments, the agent is conjugated to the AB via a linker. In

some embodiments, the linker is a cleavable linker. In some embodiments, the linker is a non-cleavable linker.

[000123] In some embodiments, the activatable antibody also includes a detectable moiety. In some embodiments, the detectable moiety is a diagnostic agent.

[000124] In some embodiments, the AB of the activatable antibody naturally contains one or more disulfide bonds. In some embodiments, the AB can be engineered to include one or more disulfide bonds.

[000125] In some embodiments, the serum half-life of the multispecific activatable antibody is longer than that of the corresponding multispecific antibody; e.g., the pK of the multispecific activatable antibody is longer than that of the corresponding multispecific antibody. In some embodiments, the serum half-life of the multispecific activatable antibody is similar to that of the corresponding multispecific antibody. In some embodiments, the serum half-life of the multispecific activatable antibody is at least 15 days when administered to an organism. In some embodiments, the serum half-life of the multispecific activatable antibody is at least 12 days when administered to an organism. In some embodiments, the serum half-life of the multispecific activatable antibody is at least 11 days when administered to an organism. In some embodiments, the serum half-life of the multispecific activatable antibody is at least 10 days when administered to an organism. In some embodiments, the serum half-life of the multispecific activatable antibody is at least 9 days when administered to an organism. In some embodiments, the serum half-life of the multispecific activatable antibody is at least 8 days when administered to an organism. In some embodiments, the serum half-life of the multispecific activatable antibody is at least 7 days when administered to an organism. In some embodiments, the serum half-life of the multispecific activatable antibody is at least 6 days when administered to an organism. In some embodiments, the serum half-life of the activatable antibody is at least 5 days when administered to an organism. In some embodiments, the serum half-life of the activatable antibody is at least 4 days when administered to an organism. In some embodiments, the serum half-life of the activatable antibody is at least 3 days when administered to an organism. In some embodiments, the serum half-life of the activatable antibody is at least 2 days when administered to an organism. In some embodiments, the serum half-life of the activatable antibody is at least 24 hours when administered to an organism. In some embodiments, the serum half-life of the activatable antibody is at least 20 hours when

administered to an organism. In some embodiments, the serum half-life of the activatable antibody is at least 18 hours when administered to an organism. In some embodiments, the serum half-life of the activatable antibody is at least 16 hours when administered to an organism. In some embodiments, the serum half-life of the activatable antibody is at least 14 hours when administered to an organism. In some embodiments, the serum half-life of the activatable antibody is at least 12 hours when administered to an organism. In some embodiments, the serum half-life of the activatable antibody is at least 10 hours when administered to an organism. In some embodiments, the serum half-life of the activatable antibody is at least 8 hours when administered to an organism. In some embodiments, the serum half-life of the activatable antibody is at least 6 hours when administered to an organism. In some embodiments, the serum half-life of the activatable antibody is at least 4 hours when administered to an organism. In some embodiments, the serum half-life of the activatable antibody is at least 3 hours when administered to an organism.

[000126] In some embodiments, the activatable anti-CD3 ϵ antibody is monospecific. In some embodiments, the activatable anti-CD3 ϵ antibody is multispecific, *e.g.*, by way of non-limiting example, bispecific or trifunctional. In some embodiments, the activatable anti-CD3 ϵ antibody is formulated as part of a pro-Bispecific T Cell Engager (BITE) molecule. In some embodiments, the activatable anti-CD3 ϵ antibody is formulated as part of a pro-Chimeric Antigen Receptor (CAR) modified T cell or other engineered receptor.

[000127] The compositions and methods provided herein enable the attachment of one or more agents to one or more cysteine residues in the AB without compromising the activity (*e.g.*, the masking, activating or binding activity) of the activatable anti-CD3 ϵ antibody. In some embodiments, the compositions and methods provided herein enable the attachment of one or more agents to one or more cysteine residues in the AB without reducing or otherwise disturbing one or more disulfide bonds within the MM. The compositions and methods provided herein produce an activatable anti-CD3 ϵ antibody that is conjugated to one or more agents, *e.g.*, any of a variety of therapeutic, diagnostic and/or prophylactic agents, preferably without any of the agent(s) being conjugated to the MM of the activatable anti-CD3 ϵ antibody. The compositions and methods provided herein produce conjugated activatable anti-CD3 ϵ antibodies in which the MM retains the ability to effectively and efficiently mask the AB of the

activatable antibody in an uncleaved state. The compositions and methods provided herein produce conjugated activatable anti-CD3 ϵ antibodies in which the activatable antibody is still activated, *i.e.*, cleaved, in the presence of a protease that can cleave the CM.

[000128] In some embodiments, the anti-CD3 ϵ antibodies and/or the activatable anti-CD3 ϵ antibodies described herein are used in conjunction with one or more additional agents or a combination of additional agents. Suitable additional agents include current pharmaceutical and/or surgical therapies for an intended application, such as, for example, cancer. For example, the anti-CD3 ϵ antibodies and/or activatable anti-CD3 ϵ antibodies can be used in conjunction with an additional chemotherapeutic or anti-neoplastic agent.

[000129] In some embodiments, the anti-CD3 ϵ antibody and/or activatable anti-CD3 ϵ antibody and additional agent are formulated into a single therapeutic composition, and the anti-CD3 ϵ antibody and/or activatable anti-CD3 ϵ antibody and additional agent are administered simultaneously. In some embodiments, the anti-CD3 ϵ antibody and/or activatable anti-CD3 ϵ antibody and additional agent are separate from each other, *e.g.*, each is formulated into a separate therapeutic composition, and the anti-CD3 ϵ antibody and/or activatable anti-CD3 ϵ antibody and the additional agent are administered simultaneously, or the anti-CD3 ϵ antibody and/or activatable anti-CD3 ϵ antibody and the additional agent are administered at different times during a treatment regimen. For example, the anti-CD3 ϵ antibody and/or activatable anti-CD3 ϵ antibody is administered prior to the administration of the additional agent, the anti-CD3 ϵ antibody and/or activatable anti-CD3 ϵ antibody is administered subsequent to the administration of the additional agent, or the anti-CD3 ϵ antibody and/or activatable anti-CD3 ϵ antibody and the additional agent are administered in an alternating fashion. As described herein, the anti-CD3 ϵ antibody and/or activatable anti-CD3 ϵ antibody and additional agent are administered in single doses or in multiple doses.

[000130] The disclosure also provides an isolated nucleic acid molecule encoding at least a portion of an activatable anti-CD3 ϵ antibody described herein, as well as vectors that include these isolated nucleic acid sequences and/or one or more nucleic acid molecules encoding an anti-CD3 ϵ antibody described herein, such as for example, at least a first nucleic acid encoding at least a portion of the heavy chain of the activatable antibody and a second nucleic acid

encoding at least a portion of the light chain of the activatable antibody. The disclosure provides methods of producing an activatable antibody by culturing a cell under conditions that lead to expression of the activatable antibody, wherein the cell comprises such a nucleic acid molecule(s). In some embodiments, the cell comprises such a vector.

[000131] In some embodiments, the activatable antibody is encoded by a heavy chain nucleic acid sequence selected from the group consisting of SEQ ID NOs: 445, 451, 453, 455, 459, 461, 463, 465, 469, 471, 475, 477, 479, 485, 487, 489, 491, 493, 495, 497, 499, 501, 503, 511, 513, 517, 522, 525, 529, 531, 533, 535, 537, 539, 541, 543, and 545.

[000132] In some embodiments, the activatable antibody is encoded by a light chain nucleic acid selected from the group consisting of SEQ ID NOs: 447, 449, 457, 467, 473, 481, 483, 507, 515, and 519.

[000133] In some embodiments, the activatable antibody is encoded by a heavy chain nucleic acid sequence selected from the group consisting of SEQ ID NOs: 445, 451, 453, 455, 459, 461, 463, 465, 469, 471, 475, 477, 479, 485, 487, 489, 491, 493, 495, 497, 499, 501, 503, 511, 513, 517, 522, 525, 529, 531, 533, 535, 537, 539, 541, 543, and 545; and a light chain nucleic acid sequence selected from the group consisting of SEQ ID NOs: 447, 449, 457, 467, 473, 481, 483, 507, 515, and 519.

[000134] In some embodiments, the activatable antibody is encoded by a heavy chain nucleic acid sequence that is at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98% or 99% identical to a sequence selected from the group consisting of SEQ ID NOs: 445, 451, 453, 455, 459, 461, 463, 465, 469, 471, 475, 477, 479, 485, 487, 489, 491, 493, 495, 497, 499, 501, 503, 511, 513, 517, 522, 525, 529, 531, 533, 535, 537, 539, 541, 543, and 545.

[000135] In some embodiments, the activatable antibody is encoded by a light nucleic acid sequence that is at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98% or 99% identical to a sequence selected from the group consisting of SEQ ID NOs: 447, 449, 457, 467, 473, 481, 483, 507, 515, and 519.

[000136] In some embodiments, the activatable antibody is encoded by a heavy chain nucleic acid sequence that is at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98% or 99% identical to a sequence selected from the group consisting of SEQ ID NOs: 445, 451, 453, 455, 459, 461, 463, 465, 469, 471, 475, 477, 479, 485, 487, 489, 491, 493, 495, 497, 499, 501, 503, 511, 513, 517, 522, 525, 529, 531, 533, 535, 537, 539, 541, 543, and 545; and a light chain

sequence that is at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98% or 99% identical to a sequence selected from the group consisting of SEQ ID NOs: 447, 449, 457, 467, 473, 481, 483, 507, 515, and 519.

[000137] In some embodiments, the activatable antibody is encoded by a nucleic acid that includes the nucleic acid sequence of SEQ ID NO: 505. In some embodiments, the activatable antibody includes a nucleic acid sequence that is at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98% or 99% identical to the sequence of SEQ ID NO: 505.

[000138] The disclosure also provides a method of manufacturing activatable antibodies that binds CD3 ϵ in an activated state by (a) culturing a cell comprising a nucleic acid construct that encodes the activatable antibody under conditions that lead to expression of the activatable antibody, wherein the activatable antibody comprises a masking moiety (MM), a cleavable moiety (CM), and an antibody or an antigen binding fragment thereof (AB) that specifically binds CD3 ϵ , and (b) recovering the activatable antibody.

[000139] The disclosure also provides a method of manufacturing activatable antibodies that binds CD3 ϵ in an activated state by (a) culturing a cell comprising a nucleic acid construct that encodes the activatable antibody under conditions that lead to expression of the activatable antibody, wherein the activatable antibody comprises a masking moiety (MM), a cleavable moiety (CM), and an antibody or an antigen binding fragment thereof (AB) that specifically binds CD3 ϵ , (i) wherein the CM is a polypeptide that includes an amino acid sequence that functions as a substrate for a protease; and (ii) wherein the CM is positioned in the activatable antibody such that, when the activatable antibody is in an uncleaved state, the MM interferes with specific binding of the AB to CD3 and when the activatable antibody is in a cleaved state the MM does not interfere or compete with specific binding of the AB to CD3 ϵ ; and (b) recovering the activatable antibody.

[000140] The disclosure also provides multispecific antibodies that bind the epsilon chain of CD3 (CD3 ϵ) and a second target, wherein the antibody comprises a first antibody or antigen-binding fragment thereof (AB1) that binds the epsilon chain of CD3 (CD3 ϵ) and a second antibody or antigen-binding fragment thereof (AB2) that binds a second target, and wherein AB1 comprises a VH CDR1 sequence that includes an amino acid sequence that is at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or more identical to the amino acid

sequence TYAMN (SEQ ID NO: 53); a VH CD2 sequence that includes an amino acid sequence that is at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or more identical to the amino acid sequence RIRSKYNNYATYYADSVKD (SEQ ID NO: 54); a VH CDR3 sequence that includes an amino acid sequence that is at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or more identical to the amino acid sequence HGNFGNSYVSWFAY (SEQ ID NO: 55), and a combination of a VL CDR1 sequence, a VL CDR2 sequence, and a VL CDR3 sequence, wherein at least one of the VL CDR1 sequence, the VL CDR2 sequence, and the VL CDR3 sequence is selected from a VL CDR1 sequence that includes an amino acid sequence that is at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or more identical to the amino acid sequence RSSTGAVTTSNYAN (SEQ ID NO: 56); a VL CDR2 sequence that includes an amino acid sequence that is at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or more identical to the amino acid sequence GTNKRAP (SEQ ID NO: 57); and a VL CDR3 sequence that includes an amino acid sequence that is at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or more identical to the amino acid sequence ALWYSNLWV (SEQ ID NO: 58).

[000141] In some embodiments, the multispecific anti-CD3 ϵ antibody or antigen-binding fragment thereof includes a VH CDR1 sequence that includes at least the amino acid sequence TYAMN (SEQ ID NO: 53); a VH CD2 sequence that includes at least the amino acid sequence RIRSKYNNYATYYADSVKD (SEQ ID NO: 54); a VH CDR3 sequence that includes at least the amino acid sequence HGNFGNSYVSWFAY (SEQ ID NO: 55), a VL CDR1 sequence that includes at least the amino acid sequence RSSTGAVTTSNYAN (SEQ ID NO: 56); a VL CDR2 sequence that includes at least the amino acid sequence GTNKRAP (SEQ ID NO: 57); and a VL CDR3 sequence that includes at least the amino acid sequence ALWYSNLWV (SEQ ID NO: 58).

[000142] In some embodiments, AB1 comprises a variable heavy chain (Hv) comprising the amino acid sequence of SEQ ID NO: 4 and a variable light chain (Lv) comprising the amino acid sequence of SEQ ID NO: 2.

[000143] In some embodiments, AB1 comprises a variable heavy chain (Hv) comprising an amino acid sequence that is at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or more identical to the amino acid sequence of SEQ ID NO: 4 and a variable light chain (Lv)

comprising an amino acid sequence that is at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or more identical to the amino acid sequence of SEQ ID NO: 2.

[000144] In some embodiments, AB1 comprises a scFv fragment. In some embodiments, the scFv fragment comprises the amino acid sequence of SEQ ID NO: 6. In some embodiments, the scFv fragment comprises the amino acid sequence of SEQ ID NO: 30. In some embodiments, the scFv fragment comprises an amino acid sequence that is at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or more identical to the amino acid sequence of SEQ ID NO: 6. In some embodiments, the scFv fragment comprises an amino acid sequence that is at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or more identical to the amino acid sequence of SEQ ID NO: 30.

[000145] In some embodiments, the multispecific antibody includes an agent conjugated to the antibody. In some embodiments, the agent is a therapeutic agent, a detectable moiety or a diagnostic agent. In some embodiments, the agent is conjugated to the antibody via a linker. In some embodiments, the linker is a cleavable linker. In some embodiments, the linker is a non-cleavable linker. In some embodiments, the linker is a non-cleavable linker.

[000146] The present disclosure also provides multispecific antibodies and multispecific activatable antibodies that bind at least CD3ε. The multispecific antibodies provided herein are antibodies that recognize two or more different antigens or epitopes, where at least one antigen or epitope is CD3ε. The multispecific activatable antibodies provided herein are multispecific antibodies that include at least one masking moiety (MM) linked to at least one antigen- or epitope-binding domain of the multispecific antibody such that coupling of the MM reduces the ability of the antigen- or epitope-binding domain to bind its target. In some embodiments, the MM is coupled to the antigen- or epitope-binding domain of the multispecific antibody via a cleavable moiety (CM) that functions as a substrate for a protease. The activatable multispecific antibodies provided herein are stable in circulation, activated at intended sites of therapy and/or diagnosis but not in normal, *i.e.*, healthy tissue, and, when activated, exhibit binding to a target that is at least comparable to the corresponding, unmodified multispecific antibody.

[000147] In some embodiments, the multispecific antibodies and/or multispecific activatable antibodies are designed to engage immune effector cells, also referred to herein as immune effector cell engaging multispecific antibodies and/or immune effector cell engaging

multispecific activatable antibodies. In some embodiments, the multispecific antibodies and/or multispecific activatable antibodies are designed to engage leukocytes, also referred to herein as leukocyte engaging multispecific antibodies and/or leukocyte engaging multispecific activatable antibodies. In some embodiments, the multispecific antibodies and/or multispecific activatable antibodies are designed to engage T cells, also referred to herein as T-cell engaging multispecific antibodies and/or T-cell engaging multispecific activatable antibodies. In some embodiments, the multispecific antibodies and/or multispecific activatable antibodies engage a surface antigen on a leukocyte, such as on a T cell, on a natural killer (NK) cell, on a myeloid mononuclear cell, on a macrophage, and/or on another immune effector cell. In some embodiments, the immune effector cell is a leukocyte. In some embodiments, the immune effector cell is a T cell. In some embodiments, the immune effector cell is a NK cell. In some embodiments, the immune effector cell is a mononuclear cell, such as a myeloid mononuclear cell. In some embodiments, the T-cell engaging multispecific antibodies and/or T-cell engaging multispecific activatable antibodies bind CD3 ϵ .

[000148] In some embodiments, the immune effector cell engaging multispecific antibodies include a targeting antibody or antigen-binding fragment thereof and an immune effector cell engaging antibody or antigen-binding portion thereof that includes an anti-CD3 ϵ antibody or antigen-binding fragment thereof. In some embodiments, the immune effector cell engaging multispecific antibodies include a cancer targeting antibody or antigen-binding fragment thereof and an immune effector cell engaging antibody or antigen-binding portion thereof. In some embodiments, the immune effector cell engaging multispecific antibodies include a cancer targeting IgG antibody or antigen-binding fragment thereof and an immune effector cell engaging scFv. In some embodiments, the immune effector cell is a leukocyte. In some embodiments, the immune effector cell is a T cell. In some embodiments, the immune effector cell is a NK cell. In some embodiments, the immune effector cell is a myeloid mononuclear cell.

[000149] In some embodiments, the T-cell engaging multispecific antibody includes an anti-CD3 ϵ antibody or antigen-binding fragment thereof and a targeting antibody or antigen-binding fragment thereof. In some embodiments, the T-cell engaging multispecific antibody includes an anti-CD3 ϵ scFv and a targeting antibody or antigen-binding fragment thereof. In

some embodiments, the T-cell engaging multispecific antibody includes an anti-CD3 ϵ antibody or antigen-binding fragment thereof and a cancer-targeting antibody or antigen-binding fragment thereof. In some embodiments, the T-cell engaging multispecific antibody includes an anti-CD3 ϵ scFv and a cancer targeting antibody or antigen-binding fragment thereof. In some embodiments, the T-cell engaging multispecific antibody includes an anti-CD3 ϵ antibody or antigen-binding fragment thereof and a cancer-targeting IgG antibody or antigen-binding fragment thereof. In some embodiments, the T-cell engaging multispecific antibody includes an anti-CD3 ϵ scFv and a cancer targeting IgG antibody or antigen-binding fragment thereof. In some embodiments, the T-cell engaging multispecific antibody includes an anti-CD3 epsilon (CD3 ϵ) scFv that is derived from OKT3. In some embodiments, the T-cell engaging multispecific antibody includes an anti-CD3 epsilon (CD3 ϵ) scFv that is derived from SP34 (available from BD Biosciences Cat# 556610).

[000150] In some embodiments, the T-cell engaging multispecific activatable antibodies include a targeting antibody or antigen-binding fragment thereof and anti-CD3 ϵ antibody or antigen-binding fragment thereof, where at least one of the targeting antibody or antigen-binding fragment thereof and/or the anti-CD3 ϵ antibody or antigen-binding portion thereof is masked. In some embodiments, the anti-CD3 ϵ antibody or antigen binding fragment thereof includes a first antibody or antigen-binding fragment thereof (AB1) that binds CD3 ϵ , where the AB1 is attached to a masking moiety (MM1) such that coupling of the MM1 reduces the ability of the AB1 to bind CD3 ϵ . In some embodiments, the targeting antibody or antigen-binding fragment thereof includes a second antibody or fragment thereof that includes a second antibody or antigen-binding fragment thereof (AB2) that binds a second target, where the AB2 is attached to a masking moiety (MM2) such that coupling of the MM2 reduces the ability of the AB2 to bind the second target. In some embodiments, the anti-CD3 ϵ antibody or antigen binding fragment thereof includes a first antibody or antigen-binding fragment thereof (AB1) that binds CD3 ϵ , where the AB1 is attached to a masking moiety (MM1) such that coupling of the MM1 reduces the ability of the AB1 to bind CD3 ϵ , and the targeting antibody or antigen-binding fragment thereof includes a second antibody or fragment thereof that includes a second antibody or antigen-binding fragment thereof (AB2) that binds a second target, where the AB2 is attached to a masking moiety (MM2) such that coupling of the MM2 reduces the ability of the AB2 to bind the second target.

[000151] In some embodiments, the T-cell engaging multispecific activatable antibodies include a cancer targeting antibody or antigen-binding fragment thereof and an anti-CD3 ϵ antibody or antigen-binding portion thereof, where at least one of the cancer targeting antibody or antigen-binding fragment thereof and/or the anti-CD3 ϵ antibody or antigen-binding portion thereof is masked. In some embodiments, the anti-CD3 ϵ antibody or antigen binding fragment thereof includes a first antibody or antigen-binding fragment thereof (AB1) that binds CD3 ϵ , where the AB1 is attached to a masking moiety (MM1) such that coupling of the MM1 reduces the ability of the AB1 to bind CD3 ϵ . In some embodiments, the cancer targeting antibody or antigen-binding fragment thereof includes a second antibody or fragment thereof that includes a second antibody or antigen-binding fragment thereof (AB2) that binds a second, cancer-related target, where the AB2 is attached to a masking moiety (MM2) such that coupling of the MM2 reduces the ability of the AB2 to bind the second, cancer-related target. In some embodiments, the anti-CD3 ϵ antibody or antigen binding fragment thereof includes a first antibody or antigen-binding fragment thereof (AB1) that binds CD3 ϵ , where the AB1 is attached to a masking moiety (MM1) such that coupling of the MM1 reduces the ability of the AB1 to bind CD3 ϵ , and the cancer targeting antibody or antigen-binding fragment thereof includes a second antibody or fragment thereof that includes a second antibody or antigen-binding fragment thereof (AB2) that binds a second, cancer-related target, where the AB2 is attached to a masking moiety (MM2) such that coupling of the MM2 reduces the ability of the AB2 to bind the second, cancer-related target.

[000152] In some embodiments, the T-cell engaging multispecific activatable antibodies include a cancer targeting IgG antibody or antigen-binding fragment thereof and an anti-CD3 ϵ , where at least one of the cancer targeting IgG antibody or antigen-binding fragment thereof and/or the anti-CD3 ϵ antibody or antigen-binding portion thereof is masked. In some embodiments, the anti-CD3 ϵ antibody or antigen binding fragment thereof includes a first antibody or antigen-binding fragment thereof (AB1) that binds CD3 ϵ , where the AB1 is attached to a masking moiety (MM1) such that coupling of the MM1 reduces the ability of the AB1 to bind CD3 ϵ . In some embodiments, the cancer targeting IgG antibody or antigen-binding fragment thereof includes a second antibody or fragment thereof that includes a second antibody or antigen-binding fragment thereof (AB2) that binds a second, cancer-related target, where the AB2 is attached to a masking moiety (MM2) such that coupling of the MM2 reduces

the ability of the AB2 to bind the second, cancer-related target. In some embodiments, the anti-CD3 ϵ antibody or antigen binding fragment thereof includes a first antibody or antigen-binding fragment thereof (AB1) that binds CD3 ϵ , where the AB1 is attached to a masking moiety (MM1) such that coupling of the MM1 reduces the ability of the AB1 to bind CD3 ϵ , and the cancer targeting IgG antibody or antigen-binding fragment thereof includes a second antibody or fragment thereof that includes a second antibody or antigen-binding fragment thereof (AB2) that binds a second, cancer-related target, where the AB2 is attached to a masking moiety (MM2) such that coupling of the MM2 reduces the ability of the AB2 to bind the second, cancer-related target.

[000153] In some embodiments, the T-cell engaging multispecific activatable antibody includes an anti-CD3 ϵ scFv and a targeting antibody or antigen-binding fragment thereof, where at least one of the anti-CD3 ϵ scFv and/or the targeting antibody or antigen-binding portion thereof is masked. In some embodiments, the CD3 ϵ scFv includes a first antibody or antigen-binding fragment thereof (AB1) that binds CD3 ϵ , where the AB1 is attached to a masking moiety (MM1) such that coupling of the MM1 reduces the ability of the AB1 to bind CD3 ϵ . In some embodiments, the targeting antibody or antigen-binding fragment thereof includes a second antibody or fragment thereof that includes a second antibody or antigen-binding fragment thereof (AB2) that binds a second target, where the AB2 is attached to a masking moiety (MM2) such that coupling of the MM2 reduces the ability of the AB2 to bind the second target. In some embodiments, the CD3 ϵ scFv includes a first antibody or antigen-binding fragment thereof (AB1) that binds CD3 ϵ , where the AB1 is attached to a masking moiety (MM1) such that coupling of the MM1 reduces the ability of the AB1 to bind CD3 ϵ , and the targeting antibody or antigen-binding fragment thereof includes a second antibody or fragment thereof that includes a second antibody or antigen-binding fragment thereof (AB2) that binds a second target, where the AB2 is attached to a masking moiety (MM2) such that coupling of the MM2 reduces the ability of the AB2 to bind the second target.

[000154] In some embodiments, the T-cell engaging multispecific activatable antibody includes an anti-CD3 ϵ scFv and a cancer targeting antibody or antigen-binding fragment thereof, where at least one of the anti-CD3 ϵ scFv and/or the cancer targeting antibody or antigen-binding portion thereof is masked. In some embodiments, the CD3 ϵ scFv includes a first antibody or antigen-binding fragment thereof (AB1) that binds CD3 ϵ , where the AB1 is

attached to a masking moiety (MM1) such that coupling of the MM1 reduces the ability of the AB1 to bind CD3 ϵ . In some embodiments, the cancer targeting antibody or antigen-binding fragment thereof includes a second antibody or fragment thereof that includes a second antibody or antigen-binding fragment thereof (AB2) that binds a second, cancer-related target, where the AB2 is attached to a masking moiety (MM2) such that coupling of the MM2 reduces the ability of the AB2 to bind the second, cancer-related target. In some embodiments, the CD3 ϵ scFv includes a first antibody or antigen-binding fragment thereof (AB1) that binds CD3 ϵ , where the AB1 is attached to a masking moiety (MM1) such that coupling of the MM1 reduces the ability of the AB1 to bind CD3 ϵ , and the cancer targeting antibody or antigen-binding fragment thereof includes a second antibody or fragment thereof that includes a second antibody or antigen-binding fragment thereof (AB2) that binds a second, cancer-related target, where the AB2 is attached to a masking moiety (MM2) such that coupling of the MM2 reduces the ability of the AB2 to bind the second, cancer-related target.

[000155] In some embodiments, the T-cell engaging multispecific activatable antibody includes an anti-CD3 ϵ scFv and a cancer targeting IgG antibody or antigen-binding fragment thereof, where at least one of the anti-CD3 ϵ scFv and/or the cancer targeting IgG antibody or antigen-binding portion thereof is masked. In some embodiments, the CD3 ϵ scFv includes a first antibody or antigen-binding fragment thereof (AB1) that binds CD3 ϵ , where the AB1 is attached to a masking moiety (MM1) such that coupling of the MM1 reduces the ability of the AB1 to bind CD3 ϵ . In some embodiments, the cancer targeting IgG antibody or antigen-binding fragment thereof includes a second antibody or fragment thereof that includes a second antibody or antigen-binding fragment thereof (AB2) that binds a second, cancer-related target, where the AB2 is attached to a masking moiety (MM2) such that coupling of the MM2 reduces the ability of the AB2 to bind the second, cancer-related target. In some embodiments, the CD3 ϵ scFv includes a first antibody or antigen-binding fragment thereof (AB1) that binds CD3 ϵ , where the AB1 is attached to a masking moiety (MM1) such that coupling of the MM1 reduces the ability of the AB1 to bind CD3 ϵ , and the cancer targeting antibody IgG or antigen-binding fragment thereof includes a second antibody or fragment thereof that includes a second antibody or antigen-binding fragment thereof (AB2) that binds a second, cancer-related target, where the AB2 is attached to a masking moiety (MM2) such that coupling of the MM2 reduces the ability of the AB2 to bind the second, cancer-related target.

[000156] In some embodiments, the T-cell engaging multispecific activatable antibody includes an anti-CD3 epsilon (CD3 ϵ) scFv that is derived from OKT3, where at least one of the targeting antibody or antigen-binding fragment thereof and/or the OKT3 scFv or OKT3-derived scFv is masked. In some embodiments, the OKT3 scFv or OKT3-derived scFv includes a first antibody or antigen-binding fragment thereof (AB1) that binds CD3 ϵ , where the AB1 is attached to a masking moiety (MM1) such that coupling of the MM1 reduces the ability of the AB1 to bind CD3 ϵ . In some embodiments, the targeting antibody or antigen-binding fragment thereof includes a second antibody or fragment thereof that includes a second antibody or antigen-binding fragment thereof (AB2) that binds a second target, where the AB2 is attached to a masking moiety (MM2) such that coupling of the MM2 reduces the ability of the AB2 to bind the second target. In some embodiments, the OKT3 scFv or OKT3-derived scFv includes a first antibody or antigen-binding fragment thereof (AB1) that binds CD3 ϵ , where the AB1 is attached to a masking moiety (MM1) such that coupling of the MM1 reduces the ability of the AB1 to bind CD3 ϵ , and the targeting antibody or antigen-binding fragment thereof includes a second antibody or fragment thereof that includes a second antibody or antigen-binding fragment thereof (AB2) that binds a second target, where the AB2 is attached to a masking moiety (MM2) such that coupling of the MM2 reduces the ability of the AB2 to bind the second target.

[000157] In some embodiments, the T-cell engaging multispecific activatable antibody includes an OKT3 scFv or OKT3-derived scFv and a cancer targeting antibody or antigen-binding fragment thereof, where at least one of the OKT3 scFv or OKT3-derived scFv and/or the cancer targeting antibody or antigen-binding portion thereof is masked. In some embodiments, the OKT3 scFv or OKT3-derived scFv includes a first antibody or antigen-binding fragment thereof (AB1) that binds CD3 ϵ , where the AB1 is attached to a masking moiety (MM1) such that coupling of the MM1 reduces the ability of the AB1 to bind CD3 ϵ . In some embodiments, the cancer targeting antibody or antigen-binding fragment thereof includes a second antibody or fragment thereof that includes a second antibody or antigen-binding fragment thereof (AB2) that binds a second, cancer-related target, where the AB2 is attached to a masking moiety (MM2) such that coupling of the MM2 reduces the ability of the AB2 to bind the second, cancer-related target. In some embodiments, the OKT3 scFv or OKT3-derived scFv includes a first antibody or antigen-binding fragment thereof (AB1) that binds CD3 ϵ , where the

AB1 is attached to a masking moiety (MM1) such that coupling of the MM1 reduces the ability of the AB1 to bind CD3 ϵ , and the cancer targeting antibody or antigen-binding fragment thereof includes a second antibody or fragment thereof that includes a second antibody or antigen-binding fragment thereof (AB2) that binds a second, cancer-related target, where the AB2 is attached to a masking moiety (MM2) such that coupling of the MM2 reduces the ability of the AB2 to bind the second, cancer-related target.

[000158] In some embodiments, the T-cell engaging multispecific activatable antibody includes an OKT3 scFv or OKT3-derived scFv and a cancer targeting IgG antibody or antigen-binding fragment thereof, where at least one of the OKT3 scFv or OKT3-derived scFv and/or the cancer targeting IgG antibody or antigen-binding portion thereof is masked. In some embodiments, the OKT3 scFv or OKT3-derived scFv includes a first antibody or antigen-binding fragment thereof (AB1) that binds CD3 ϵ , where the AB1 is attached to a masking moiety (MM1) such that coupling of the MM1 reduces the ability of the AB1 to bind CD3 ϵ . In some embodiments, the cancer targeting IgG antibody or antigen-binding fragment thereof includes a second antibody or fragment thereof that includes a second antibody or antigen-binding fragment thereof (AB2) that binds a second, cancer-related target, where the AB2 is attached to a masking moiety (MM2) such that coupling of the MM2 reduces the ability of the AB2 to bind the second, cancer-related target. In some embodiments, the OKT3 scFv or OKT3-derived scFv includes a first antibody or antigen-binding fragment thereof (AB1) that binds CD3 ϵ , where the AB1 is attached to a masking moiety (MM1) such that coupling of the MM1 reduces the ability of the AB1 to bind CD3 ϵ , and the cancer targeting antibody IgG or antigen-binding fragment thereof includes a second antibody or fragment thereof that includes a second antibody or antigen-binding fragment thereof (AB2) that binds a second, cancer-related target, where the AB2 is attached to a masking moiety (MM2) such that coupling of the MM2 reduces the ability of the AB2 to bind the second, cancer-related target.

[000159] In some embodiments, the T-cell engaging multispecific activatable antibody includes an anti-CD3 epsilon (CD3 ϵ) scFv that is derived from SP34, where at least one of the targeting antibody or antigen-binding fragment thereof and/or the SP34 scFv or SP34-derived scFv is masked. In some embodiments, the SP34 scFv or SP34-derived scFv includes a first antibody or antigen-binding fragment thereof (AB1) that binds CD3 ϵ , where the AB1 is attached to a masking moiety (MM1) such that coupling of the MM1 reduces the ability of the

AB1 to bind CD3 ϵ . In some embodiments, the targeting antibody or antigen-binding fragment thereof includes a second antibody or fragment thereof that includes a second antibody or antigen-binding fragment thereof (AB2) that binds a second target, where the AB2 is attached to a masking moiety (MM2) such that coupling of the MM2 reduces the ability of the AB2 to bind the second target. In some embodiments, the SP34 scFv or SP34-derived scFv includes a first antibody or antigen-binding fragment thereof (AB1) that binds CD3 ϵ , where the AB1 is attached to a masking moiety (MM1) such that coupling of the MM1 reduces the ability of the AB1 to bind CD3 ϵ , and the targeting antibody or antigen-binding fragment thereof includes a second antibody or fragment thereof that includes a second antibody or antigen-binding fragment thereof (AB2) that binds a second target, where the AB2 is attached to a masking moiety (MM2) such that coupling of the MM2 reduces the ability of the AB2 to bind the second target.

[000160] In some embodiments, the T-cell engaging multispecific activatable antibody includes an SP34 scFv or SP34-derived scFv and a cancer targeting antibody or antigen-binding fragment thereof, where at least one of the SP34 scFv or SP34-derived scFv and/or the cancer targeting antibody or antigen-binding portion thereof is masked. In some embodiments, the SP34 scFv or SP34-derived scFv includes a first antibody or antigen-binding fragment thereof (AB1) that binds CD3 ϵ , where the AB1 is attached to a masking moiety (MM1) such that coupling of the MM1 reduces the ability of the AB1 to bind CD3 ϵ . In some embodiments, the cancer targeting antibody or antigen-binding fragment thereof includes a second antibody or fragment thereof that includes a second antibody or antigen-binding fragment thereof (AB2) that binds a second, cancer-related target, where the AB2 is attached to a masking moiety (MM2) such that coupling of the MM2 reduces the ability of the AB2 to bind the second, cancer-related target. In some embodiments, the SP34 scFv or SP34-derived scFv includes a first antibody or antigen-binding fragment thereof (AB1) that binds CD3 ϵ , where the AB1 is attached to a masking moiety (MM1) such that coupling of the MM1 reduces the ability of the AB1 to bind CD3 ϵ , and the cancer targeting antibody or antigen-binding fragment thereof includes a second antibody or fragment thereof that includes a second antibody or antigen-binding fragment thereof (AB2) that binds a second, cancer-related target, where the AB2 is attached to a masking moiety (MM2) such that coupling of the MM2 reduces the ability of the AB2 to bind the second, cancer-related target.

[000161] In some embodiments, the T-cell engaging multispecific activatable antibody includes an SP34 scFv or SP34-derived scFv and a cancer targeting IgG antibody or antigen-binding fragment thereof, where at least one of the SP34 scFv or SP34-derived scFv and/or the cancer targeting IgG antibody or antigen-binding portion thereof is masked. In some embodiments, the SP34 scFv or SP34-derived scFv includes a first antibody or antigen-binding fragment thereof (AB1) that binds CD3 ϵ , where the AB1 is attached to a masking moiety (MM1) such that coupling of the MM1 reduces the ability of the AB1 to bind CD3 ϵ . In some embodiments, the cancer targeting IgG antibody or antigen-binding fragment thereof includes a second antibody or fragment thereof that includes a second antibody or antigen-binding fragment thereof (AB2) that binds a second, cancer-related target, where the AB2 is attached to a masking moiety (MM2) such that coupling of the MM2 reduces the ability of the AB2 to bind the second, cancer-related target. In some embodiments, the SP34 scFv or SP34-derived scFv includes a first antibody or antigen-binding fragment thereof (AB1) that binds CD3 ϵ , where the AB1 is attached to a masking moiety (MM1) such that coupling of the MM1 reduces the ability of the AB1 to bind CD3 ϵ , and the cancer targeting antibody IgG or antigen-binding fragment thereof includes a second antibody or fragment thereof that includes a second antibody or antigen-binding fragment thereof (AB2) that binds a second, cancer-related target, where the AB2 is attached to a masking moiety (MM2) such that coupling of the MM2 reduces the ability of the AB2 to bind the second, cancer-related target.

[000162] In some embodiments, the T-cell engaging multispecific activatable antibody includes an anti-CD3 epsilon (CD3 ϵ) scFv that includes the following complementarity determining region (CDR) sequences: a VH CDR1 sequence that includes at least the amino acid sequence TYAMN (SEQ ID NO: 53); a VH CDR2 sequence that includes at least the amino acid sequence RIRSKYNNYATYYADSVKD (SEQ ID NO: 54); a VH CDR3 sequence that includes at least the amino acid sequence HGNFGNSYVSWFAY (SEQ ID NO: 55), a VL CDR1 sequence that includes at least the amino acid sequence RSSTGAVTTSNYAN (SEQ ID NO: 56); a VL CDR2 sequence that includes at least the amino acid sequence GTNKRAP (SEQ ID NO: 57); and a VL CDR3 sequence that includes at least the amino acid sequence ALWYSNLWV (SEQ ID NO: 58), where at least one of the targeting antibody or antigen-binding fragment thereof and/or the anti-CD3 ϵ scFv is masked. In some embodiments, the anti-

CD3 ϵ scFv includes a first antibody or antigen-binding fragment thereof (AB1) having the CDR sequences set for the above, i.e., SEQ ID NOs: 53, 54, 55, 56, 57, and 58, where the AB1 is attached to a masking moiety (MM1) such that coupling of the MM1 reduces the ability of the AB1 to bind CD3 ϵ . In some embodiments, the targeting antibody or antigen-binding fragment thereof includes a second antibody or fragment thereof that includes a second antibody or antigen-binding fragment thereof (AB2) that binds a second target, where the AB2 is attached to a masking moiety (MM2) such that coupling of the MM2 reduces the ability of the AB2 to bind the second target. In some embodiments, the anti-CD3 ϵ scFv includes a first antibody or antigen-binding fragment thereof (AB1) that includes the CDR sequences of SEQ ID NOs: 53, 54, 55, 56, 57, and 58, where the AB1 is attached to a masking moiety (MM1) such that coupling of the MM1 reduces the ability of the AB1 to bind CD3 ϵ , and the targeting antibody or antigen-binding fragment thereof includes a second antibody or fragment thereof that includes a second antibody or antigen-binding fragment thereof (AB2) that binds a second target, where the AB2 is attached to a masking moiety (MM2) such that coupling of the MM2 reduces the ability of the AB2 to bind the second target.

[000163] In some embodiments, the T-cell engaging multispecific activatable antibody includes an anti-CD3 ϵ scFv that includes the CDR sequences of SEQ ID NOs: 53, 54, 55, 56, 57, and 58 and a cancer targeting antibody or antigen-binding fragment thereof, where at least one of the anti-CD3 ϵ scFv and/or the cancer targeting antibody or antigen-binding portion thereof is masked. In some embodiments, the anti-CD3 ϵ scFv includes a first antibody or antigen-binding fragment thereof (AB1) that includes the CDR sequences of SEQ ID NOs: 53, 54, 55, 56, 57, and 58, where the AB1 is attached to a masking moiety (MM1) such that coupling of the MM1 reduces the ability of the AB1 to bind CD3 ϵ . In some embodiments, the cancer targeting antibody or antigen-binding fragment thereof includes a second antibody or fragment thereof that includes a second antibody or antigen-binding fragment thereof (AB2) that binds a second, cancer-related target, where the AB2 is attached to a masking moiety (MM2) such that coupling of the MM2 reduces the ability of the AB2 to bind the second, cancer-related target. In some embodiments, the anti-CD3 ϵ scFv includes a first antibody or antigen-binding fragment thereof (AB1) that includes the CDR sequences of SEQ ID NOs: 53, 54, 55, 56, 57, and 58, where the AB1 is attached to a masking moiety (MM1) such that

coupling of the MM1 reduces the ability of the AB1 to bind CD3 ϵ , and the cancer targeting antibody or antigen-binding fragment thereof includes a second antibody or fragment thereof that includes a second antibody or antigen-binding fragment thereof (AB2) that binds a second, cancer-related target, where the AB2 is attached to a masking moiety (MM2) such that coupling of the MM2 reduces the ability of the AB2 to bind the second, cancer-related target.

[000164] In some embodiments, the T-cell engaging multispecific activatable antibody includes an anti-CD3 ϵ scFv that includes the CDR sequences of SEQ ID NOs: 53, 54, 55, 56, 57, and 58 and a cancer targeting IgG antibody or antigen-binding fragment thereof, where at least one of the anti-CD3 ϵ scFv and/or the cancer targeting IgG antibody or antigen-binding portion thereof is masked. In some embodiments, the anti-CD3 ϵ scFv includes a first antibody or antigen-binding fragment thereof (AB1) that includes the CDR sequences of SEQ ID NOs: 53, 54, 55, 56, 57, and 58, where the AB1 is attached to a masking moiety (MM1) such that coupling of the MM1 reduces the ability of the AB1 to bind CD3 ϵ . In some embodiments, the cancer targeting IgG antibody or antigen-binding fragment thereof includes a second antibody or fragment thereof that includes a second antibody or antigen-binding fragment thereof (AB2) that binds a second, cancer-related target, where the AB2 is attached to a masking moiety (MM2) such that coupling of the MM2 reduces the ability of the AB2 to bind the second, cancer-related target. In some embodiments, the anti-CD3 ϵ scFv includes a first antibody or antigen-binding fragment thereof (AB1) that includes the CDR sequences of SEQ ID NOs: 53, 54, 55, 56, 57, and 58, where the AB1 is attached to a masking moiety (MM1) such that coupling of the MM1 reduces the ability of the AB1 to bind CD3 ϵ , and the cancer targeting antibody IgG or antigen-binding fragment thereof includes a second antibody or fragment thereof that includes a second antibody or antigen-binding fragment thereof (AB2) that binds a second, cancer-related target, where the AB2 is attached to a masking moiety (MM2) such that coupling of the MM2 reduces the ability of the AB2 to bind the second, cancer-related target.

[000165] In some embodiments, the multi-antigen targeting antibodies and/or multi-antigen targeting activatable antibodies include at least a first antibody or antigen-binding fragment thereof that binds a first target and/or first epitope and a second antibody or antigen-binding fragment thereof that binds a second target and/or a second epitope, where at least one of the targets and/or epitopes is CD3 ϵ . In some embodiments, the multi-antigen targeting

antibodies and/or multi-antigen targeting activatable antibodies bind two or more different targets, where at least one target is CD3 ϵ . In some embodiments, the multi-antigen targeting antibodies and/or multi-antigen targeting activatable antibodies bind two or more different epitopes on CD3 ϵ . In some embodiments, the multi-antigen targeting antibodies and/or multi-antigen targeting activatable antibodies bind a combination of two or more different targets and two or more different epitopes on the same target, where at least one of the targets and/or epitopes is CD3 ϵ .

[000166] Various embodiments of multispecific activatable antibodies of the disclosure are shown in Figures 5A-5D, 6A-6F, 7A-7J, 8A-8J, 9A-9J, 10A-10J, 11A-11J, and 12A-12D. In some embodiments, a multispecific activatable antibody comprising an IgG has the IgG variable domains masked. In some embodiments, a multispecific activatable antibody comprising a scFv has the scFv domains masked. In some embodiments, a multispecific activatable antibody has both IgG variable domains and scFv domains, where at least one of the IgG variable domains is coupled to a masking moiety. In some embodiments, a multispecific activatable antibody has both IgG variable domains and scFv domains, where at least one of the scFv domains is coupled to a masking moiety. In some embodiments, a multispecific activatable antibody has both IgG variable domains and scFv domains, where at least one of the IgG variable domains is coupled to a masking moiety and at least one of the scFv domains is coupled to a masking moiety. In some embodiments, a multispecific activatable antibody has both IgG variable domains and scFv domains, where each of the IgG variable domains and the scFv domains is coupled to its own masking moiety. In some embodiments, one antibody domain of a multispecific activatable antibody has specificity for a target antigen and another antibody domain has specificity for a T-cell surface antigen. In some embodiments, one antibody domain of a multispecific activatable antibody has specificity for a target antigen and another antibody domain has specificity for another target antigen. In some embodiments, one antibody domain of a multispecific activatable antibody has specificity for an epitope of a target antigen and another antibody domain has specificity for another epitope of the target antigen.

[000167] In a multispecific activatable antibody, a scFv can be fused to the carboxyl terminus of the heavy chain of an IgG activatable antibody, to the carboxyl terminus of the

light chain of an IgG activatable antibody, or to the carboxyl termini of both the heavy and light chains of an IgG activatable antibody. In a multispecific activatable antibody, a scFv can be fused to the amino terminus of the heavy chain of an IgG activatable antibody, to the amino terminus of the light chain of an IgG activatable antibody, or to the amino termini of both the heavy and light chains of an IgG activatable antibody. In a multispecific activatable antibody, a scFv can be fused to any combination of one or more carboxyl termini and one or more amino termini of an IgG activatable antibody. In some embodiments, a masking moiety (MM) linked to a cleavable moiety (CM) is attached to and masks an antigen binding domain of the IgG. In some embodiments, a masking moiety (MM) linked to a cleavable moiety (CM) is attached to and masks an antigen binding domain of at least one scFv. In some embodiments, a masking moiety (MM) linked to a cleavable moiety (CM) is attached to and masks an antigen binding domain of an IgG and a masking moiety (MM) linked to a cleavable moiety (CM) is attached to and masks an antigen binding domain of at least one scFv.

[000168] In some embodiments, a single chain variable domain, specific for binding CD3ε is fused to the carboxyl terminus of a fully human IgG1 antibody (targeting antibody) that binds to a cell surface antigen. Fusion of the scFv can be to the carboxyl terminus of the heavy chain, to the carboxyl terminus of the light chain or to both chains. In some embodiments, a single chain variable domain, specific for binding CD3ε is fused to the amino terminus of a fully human IgG1 antibody (targeting antibody) that binds to a cell surface antigen. Fusion of the scFv can be to the amino terminus of the heavy chain, to the amino terminus of the light chain or to both chains. The fusions are constructed as a single genetic construct and expressed in cells in culture. The targeting antibody can be specific for binding to one or more tumor surface antigens, or any cell targeted for depletion. The scFv can be specific for the same or different antigens.

[000169] Other examples of multispecific activatable antibody structures include, but are not limited to, the following: (VL-CL)₂:(VH-CH1-CH2-CH3-L4-VH*-L3-VL*-L2-CM-L1-MM)₂; (VL-CL)₂:(VH-CH1-CH2-CH3-L4-VL*-L3-VH*-L2-CM-L1-MM)₂; (MM-L1-CM-L2-VL-CL)₂:(VH-CH1-CH2-CH3-L4-VH*-L3-VL*)₂; (MM-L1-CM-L2-VL-CL)₂:(VH-CH1-CH2-CH3-L4-VL*-L3-VH*)₂; (VL-CL)₂:(MM-L1-CM-L2-VL*-L3-VH*-L4-VH-CH1-CH2-CH3)₂; (VL-CL)₂:(MM-L1-CM-L2-VH*-L3-VL*-L4-VH-CH1-CH2-CH3)₂; (MM-L1-CM-L2-

[000170] In some embodiments of a T-cell engaging multispecific activatable antibody, one antigen is typically CD3ε and the other is an antigen present on the surface of a tumor cell or other cell type associated with disease, such as, but not limited to, any target listed in Table 1, such as, but not limited to, EGFR, erbB2, EpCAM, Jagged, PD-L1, B7H3, or CD71 (transferrin receptor).

[000171] In some embodiments, the targeting antibody includes an anti-EGFR antibody. In some embodiments, the targeting antibody includes C225v5, which is specific for binding to EGFR. In some embodiments, the targeting antibody includes C225, which is specific for binding to EGFR. In some embodiments, the targeting antibody includes C225v4, which is specific for binding to EGFR. In some embodiments, the targeting antibody includes C225v6, which is specific for binding to EGFR. In some embodiments, the targeting antibody includes an anti-Jagged antibody. In some embodiments, the targeting antibody includes 4D11, which is specific for binding to human and mouse Jagged 1 and Jagged 2. In some embodiments, the targeting antibody includes 4D11v2, which is specific for binding to human and mouse Jagged 1 and Jagged 2.

[000172] In some embodiments, the targeting antibody can be in the form an activatable antibody. In some embodiments, the scFv(s) can be in the form of a Pro-scFv (see, e.g., WO 2009/025846, WO 2010/081173).

[000173] In some embodiments, the multispecific antibodies and/or multispecific activatable antibodies provided herein include at least a first antibody or antigen binding fragment thereof (AB1) that specifically binds CD3 ϵ and that contains a combination of a VH CDR1 sequence, a VH CDR2 sequence, and a VH CDR3 sequence, wherein at least one of the VH CDR1 sequence, the VH CDR2 sequence, and the VH CDR3 sequence is selected from a VH CDR1 sequence that includes at least the amino acid sequence TYAMN (SEQ ID NO: 53); a VH CDR2 sequence that includes at least the amino acid sequence RIRSKYNNYATYYADSVKD (SEQ ID NO: 54); a VH CDR3 sequence that includes at least the amino acid sequence HGNFGNSYVSWFAY (SEQ ID NO: 55), and combinations thereof.

[000174] In some embodiments, the multispecific antibodies and/or multispecific activatable antibodies provided herein include at least a first antibody or antigen binding fragment thereof (AB1) that specifically binds CD3 ϵ and that contains a combination of a VL CDR1 sequence, a VL CDR2 sequence, and a VL CDR3 sequence, wherein at least one of the VL CDR1 sequence, the VL CDR2 sequence, and the VL CDR3 sequence is selected from a VL CDR1 sequence that includes at least the amino acid sequence RSSTGAVTTSNYAN (SEQ ID NO: 56); a VL CDR2 sequence that includes at least the amino acid sequence

GTNKRAP (SEQ ID NO: 57); and a VL CDR3 sequence that includes at least the amino acid sequence ALWYSNLWV (SEQ ID NO: 58), and combinations thereof.

[000175] In some embodiments, the multispecific antibodies and/or multispecific activatable antibodies provided herein include at least a first antibody or antigen binding fragment thereof (AB1) that specifically binds CD3 ϵ and that contains a VH CDR1 sequence that includes at least the amino acid sequence TYAMN (SEQ ID NO: 53); a VH CD2 sequence that includes at least the amino acid sequence RIRSKYNNYATYYADSVKD (SEQ ID NO: 54); a VH CDR3 sequence that includes at least the amino acid sequence HGNFGNSYVSWFAY (SEQ ID NO: 55), a VL CDR1 sequence that includes at least the amino acid sequence RSSTGAVTTSNYAN (SEQ ID NO: 56); a VL CDR2 sequence that includes at least the amino acid sequence GTNKRAP (SEQ ID NO: 57); and a VL CDR3 sequence that includes at least the amino acid sequence ALWYSNLWV (SEQ ID NO: 58).

[000176] In some embodiments, the multispecific antibodies and/or multispecific activatable antibodies provided herein include at least a first antibody or antigen binding fragment thereof (AB1) that specifically binds CD3 ϵ and that contains a VH CDR1 sequence that is at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or more identical to the amino acid sequence TYAMN (SEQ ID NO: 53); a VH CD2 sequence that is at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or more identical to the amino acid sequence RIRSKYNNYATYYADSVKD (SEQ ID NO: 54); a VH CDR3 sequence that is at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or more identical to the amino acid sequence HGNFGNSYVSWFAY (SEQ ID NO: 55), a VL CDR1 sequence that is at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or more identical to the amino acid sequence RSSTGAVTTSNYAN (SEQ ID NO: 56); a VL CDR2 sequence that is at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or more identical to the amino acid sequence GTNKRAP (SEQ ID NO: 57); and a VL CDR3 sequence that is at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or more identical to the amino acid sequence ALWYSNLWV (SEQ ID NO: 58).

[000177] In some embodiments, the multispecific antibodies and/or multispecific activatable antibodies provided herein include at least a first antibody or antigen binding fragment thereof (AB1) that specifically binds a Jagged target, e.g., Jagged 1 and/or Jagged 2,

and that contains a combination of a VH CDR1 sequence, a VH CDR2 sequence, and a VH CDR3 sequence, wherein at least one of the VH CDR1 sequence, the VH CDR2 sequence, and the VH CDR3 sequence is selected from a VH CDR1 sequence that includes at least the amino acid sequence SYAMS (SEQ ID NO: 88); a VH CD2 sequence that includes at least the amino acid sequence SIDPEGRQTY YADSVKG (SEQ ID NO: 89); and a VH CDR3 sequence that includes at least the amino acid sequence DIGGRSAFDY (SEQ ID NO: 90), and combinations thereof.

[000178] In some embodiments, the multispecific antibodies and/or multispecific activatable antibodies provided herein include at least a first antibody or antigen binding fragment thereof (AB1) that specifically binds a Jagged target, e.g., Jagged 1 and/or Jagged 2, and that contains a combination of a VL CDR1 sequence, a VL CDR2 sequence, and a VL CDR3 sequence, wherein at least one of the VL CDR1 sequence, the VL CDR2 sequence, and the VL CDR3 sequence is selected from a VL CDR1 sequence that includes at least the amino acid sequence RASQSISSY (SEQ ID NO: 91); a VL CDR2 sequence that includes at least the amino acid sequence AASSLQS (SEQ ID NO: 92); and a VL CDR3 sequence that includes at least the amino acid sequence QQTVVAPPL (SEQ ID NO: 93), and combinations thereof.

[000179] In some embodiments, the multispecific antibodies and/or multispecific activatable antibodies provided herein include at least a first antibody or antigen binding fragment thereof (AB1) that specifically binds a Jagged target, e.g., Jagged 1 and/or Jagged 2, and that contains a combination of a VH CDR1 sequence, a VH CDR2 sequence, and a VH CDR3 sequence, wherein at least one of the VH CDR1 sequence, the VH CDR2 sequence, and the VH CDR3 sequence is selected from a VH CDR1 sequence that includes a sequence that is at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or more identical to the amino acid sequence SYAMS (SEQ ID NO: 88); a VH CD2 sequence that includes a sequence that is at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or more identical to the amino acid sequence SIDPEGRQTY YADSVKG (SEQ ID NO: 89); and a VH CDR3 sequence that includes a sequence that is at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or more identical to the amino acid sequence DIGGRSAFDY (SEQ ID NO: 90), and combinations thereof.

[000180] In some embodiments, the multispecific antibodies and/or multispecific activatable antibodies provided herein include at least a first antibody or antigen binding

fragment thereof (AB1) that specifically binds a Jagged target, e.g., Jagged 1 and/or Jagged 2, and that contains a combination of a VL CDR1 sequence, a VL CDR2 sequence, and a VL CDR3 sequence, wherein at least one of the VL CDR1 sequence, the VL CDR2 sequence, and the VL CDR3 sequence is selected from a VL CDR1 sequence that includes a sequence that is at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or more identical to the amino acid sequence RASQSISSY (SEQ ID NO: 91); a VL CDR2 sequence that includes a sequence that is at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or more identical to the amino acid sequence AASSLQS (SEQ ID NO: 92); and a VL CDR3 sequence that includes a sequence that is at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or more identical to the amino acid sequence QQTVVAPPL (SEQ ID NO: 93), and combinations thereof.

[000181] In some embodiments, the multispecific antibodies and/or multispecific activatable antibodies provided herein include at least a first antibody or antigen binding fragment thereof (AB1) that specifically binds a Jagged target, e.g., Jagged 1 and/or Jagged 2, and that contains a combination of a VH CDR1 sequence, a VH CDR2 sequence, a VH CDR3 sequence, a VL CDR1 sequence, a VL CDR2 sequence, and a VL CDR3 sequence, wherein the VH CDR1 sequence includes at least the amino acid sequence SYAMS (SEQ ID NO: 88); the VH CD2 sequence includes at least the amino acid sequence SIDPEGRQTTYADSVKG (SEQ ID NO: 89); the VH CDR3 sequence includes at least the amino acid sequence DIGGRSAFDY (SEQ ID NO: 90); the VL CDR1 sequence includes at least the amino acid sequence RASQSISSY (SEQ ID NO: 91); the VL CDR2 sequence includes at least the amino acid sequence AASSLQS (SEQ ID NO: 92); and the VL CDR3 sequence includes at least the amino acid sequence QQTVVAPPL (SEQ ID NO: 93).

[000182] In some embodiments, the multispecific antibodies and/or multispecific activatable antibodies provided herein include at least a first antibody or antigen binding fragment thereof (AB1) that specifically binds a Jagged target, e.g., Jagged 1 and/or Jagged 2, and that contains a combination of a VH CDR1 sequence, a VH CDR2 sequence, a VH CDR3 sequence, a VL CDR1 sequence, a VL CDR2 sequence, and a VL CDR3 sequence, wherein the VH CDR1 sequence includes a sequence that is at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or more identical to the amino acid sequence SYAMS (SEQ ID NO: 88); the VH CD2 sequence includes a sequence that is at least 90%, 91%, 92%, 93%, 94%, 95%,

96%, 97%, 98%, 99% or more identical to the amino acid sequence SIDPEGRQTYTADSVKG (SEQ ID NO: 89); the VH CDR3 sequence includes a sequence that is at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or more identical to the amino acid sequence DIGGRSAFDY (SEQ ID NO: 90); the VL CDR1 sequence includes a sequence that is at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or more identical to the amino acid sequence RASQSISSY (SEQ ID NO: 91); the VL CDR2 sequence includes a sequence that is at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or more identical to the amino acid sequence AASSLQS (SEQ ID NO: 92); and the VL CDR3 sequence includes a sequence that is at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or more identical to the amino acid sequence QQTVVAPPL (SEQ ID NO: 93).

[000183] In some embodiments, the multispecific antibodies and/or multispecific activatable antibodies provided herein include at least a first antibody or antigen binding fragment thereof (AB1) that specifically binds Epidermal Growth Factor Receptor (EGFR) and that contains a combination of a VH CDR1 sequence, a VH CDR2 sequence, and a VH CDR3 sequence, wherein at least one of the VH CDR1 sequence, the VH CDR2 sequence, and the VH CDR3 sequence is selected from a VH CDR1 sequence that includes at least the amino acid sequence NYGVH (SEQ ID NO: 94); a VH CD2 sequence that includes at least the amino acid sequence VIWSGGNTDYNTPTS (SEQ ID NO: 95); a VH CDR3 sequence that includes at least the amino acid sequence ALTYDYEFAY (SEQ ID NO: 96); and combinations thereof.

[000184] In some embodiments, the multispecific antibodies and/or multispecific activatable antibodies provided herein include at least a first antibody or antigen binding fragment thereof (AB1) that specifically binds EGFR and that contains a combination of a VL CDR1 sequence, a VL CDR2 sequence, and a VL CDR3 sequence, wherein at least one of the VL CDR1 sequence, the VL CDR2 sequence, and the VL CDR3 sequence is selected from a VL CDR1 sequence that includes at least the amino acid sequence RASQSIGTNIH (SEQ ID NO: 97); a VL CDR2 sequence that includes at least the amino acid sequence KYASESIS (SEQ ID NO: 98); and a VL CDR3 sequence that includes at least the amino acid sequence QQNNNWPTT (SEQ ID NO: 99), and combinations thereof.

[000185] In some embodiments, the multispecific antibodies and/or multispecific activatable antibodies provided herein include at least a first antibody or antigen binding fragment thereof (AB1) that specifically binds EGFR and that contains a combination of a VH

CDR1 sequence, a VH CDR2 sequence, and a VH CDR3 sequence, wherein at least one of the VH CDR1 sequence, the VH CDR2 sequence, and the VH CDR3 sequence is selected from a VH CDR1 sequence that includes a sequence that is at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or more identical to the amino acid sequence NYGVH (SEQ ID NO: 94); a VH CD2 sequence that includes a sequence that is at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or more identical to the amino acid sequence VIWSGGNTDYNTTPFTS (SEQ ID NO: 95); a VH CDR3 sequence that includes a sequence that is at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or more identical to the amino acid sequence ALTYDYEFAY (SEQ ID NO: 96); and combinations thereof.

[000186] In some embodiments, the multispecific antibodies and/or multispecific activatable antibodies provided herein include at least a first antibody or antigen binding fragment thereof (AB1) that specifically binds EGFR and that contains a combination of a VL CDR1 sequence, a VL CDR2 sequence, and a VL CDR3 sequence, wherein at least one of the VL CDR1 sequence, the VL CDR2 sequence, and the VL CDR3 sequence is selected from a VL CDR1 sequence that includes a sequence that is at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or more identical to the amino acid sequence RASQSIGTNIH (SEQ ID NO: 97); a VL CDR2 sequence that includes a sequence that is at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or more identical to the amino acid sequence KYASESIS (SEQ ID NO: 98); and a VL CDR3 sequence that includes a sequence that is at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or more identical to the amino acid sequence QQNNNWPTT (SEQ ID NO: 99), and combinations thereof.

[000187] In some embodiments, the multispecific antibodies and/or multispecific activatable antibodies provided herein include at least a first antibody or antigen binding fragment thereof (AB1) that specifically binds EGFR and that contains a combination of a VH CDR1 sequence, a VH CDR2 sequence, a VH CDR3 sequence, a VL CDR1 sequence, a VL CDR2 sequence, and a VL CDR3 sequence, wherein the VH CDR1 sequence includes at least the amino acid sequence NYGVH (SEQ ID NO: 94); the VH CD2 sequence includes at least the amino acid sequence VIWSGGNTDYNTTPFTS (SEQ ID NO: 95); the VH CDR3 sequence includes at least the amino acid sequence ALTYDYEFAY (SEQ ID NO: 96); the VL CDR1 sequence includes at least the amino acid sequence RASQSIGTNIH (SEQ ID NO: 97); the VL CDR2 sequence includes at least the amino acid sequence KYASESIS (SEQ ID NO: 98); and

the VL CDR3 sequence includes at least the amino acid sequence QQNNNWPTT (SEQ ID NO: 99).

[000188] In some embodiments, the multispecific antibodies and/or multispecific activatable antibodies provided herein include at least a first antibody or antigen binding fragment thereof (AB1) that specifically binds EGFR and that contains a combination of a VH CDR1 sequence, a VH CDR2 sequence, a VH CDR3 sequence, a VL CDR1 sequence, a VL CDR2 sequence, and a VL CDR3 sequence, wherein the VH CDR1 sequence includes a sequence that is at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or more identical to the amino acid sequence NYGVH (SEQ ID NO: 94); the VH CD2 sequence includes a sequence that is at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or more identical to the amino acid sequence VIWSGGNTDYNTPTS (SEQ ID NO: 95); the VH CDR3 sequence includes a sequence that is at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or more identical to the amino acid sequence ALTYDYEFAY (SEQ ID NO: 96); the VL CDR1 sequence includes a sequence that is at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or more identical to the amino acid sequence RASQSIGTNIH (SEQ ID NO: 97); the VL CDR2 sequence includes a sequence that is at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or more identical to the amino acid sequence KYASESIS (SEQ ID NO: 98); and the VL CDR3 sequence includes a sequence that is at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or more identical to the amino acid sequence QQNNNWPTT (SEQ ID NO: 99).

[000189] In some embodiments, the multispecific antibodies and/or multispecific activatable antibodies provided herein include at least a heavy chain amino acid sequence selected from the group consisting of those sequences shown in the Examples provided herein. In some embodiments, the multispecific antibodies and/or multispecific activatable antibodies provided herein include at least a light chain amino acid sequence selected from the group consisting of those sequences shown in the Examples provided herein. In some embodiments, the multispecific antibodies and/or multispecific activatable antibodies provided herein include at least a heavy chain amino acid sequence selected from the group consisting of those sequences shown in the Examples provided herein, and a light chain amino acid sequence selected from the group consisting of those sequences shown in the Examples provided herein.

[000190] In some embodiments, the multispecific antibodies and/or multispecific activatable antibodies provided herein include at least a heavy chain amino acid sequence that is at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or more identical to an amino acid sequence selected from the group consisting of those sequences shown in the Examples provided herein. In some embodiments, the multispecific antibodies and/or multispecific activatable antibodies provided herein include at least a light chain amino acid sequence that is at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or more identical to an amino acid sequence selected from the group consisting of those sequences shown in the Examples provided herein. In some embodiments, the multispecific antibodies and/or multispecific activatable antibodies provided herein include at least a heavy chain amino acid sequence that is at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or more identical to an amino acid sequence selected from the group consisting of those sequences shown in the Examples provided herein, and a light chain amino acid sequence that is at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or more identical to an amino acid sequence selected from the group consisting of those sequences shown in the Examples provided herein.

[000191] In some embodiments, the multispecific antibody and/or multispecific activatable antibody also includes an agent conjugated to the AB. In some embodiments, the agent is a therapeutic agent. In some embodiments, the agent is a detectable moiety. In some embodiments, the detectable moiety is a diagnostic agent. In some embodiments, the agent is conjugated to the multispecific via a linker. In some embodiments, the linker is a cleavable linker. In some embodiments, the linker is a non-cleavable linker.

[000192] In some embodiments, the multispecific antibody and/or multispecific activatable antibody also includes a detectable moiety. In some embodiments, the detectable moiety is a diagnostic agent.

[000193] In some embodiments, the multispecific antibody and/or multispecific activatable antibody naturally contains one or more disulfide bonds. In some embodiments, the multispecific antibody and/or multispecific activatable antibody can be engineered to include one or more disulfide bonds.

[000194] The disclosure also provides an isolated nucleic acid molecule encoding at least a portion of a multispecific antibody and/or multispecific activatable antibody described herein,

such as for example, at least a first nucleic acid encoding at least a portion of the heavy chain of the multispecific antibody and/or multispecific activatable antibody and a second nucleic acid encoding at least a portion of the light chain of the multispecific antibody and/or multispecific activatable antibody, as well as vectors that include these isolated nucleic acid sequences. The disclosure provides methods of producing a multispecific antibody by culturing a cell under conditions that lead to expression of the antibody, wherein the cell comprises such a nucleic acid molecule(s). In some embodiments, the cell comprises such a vector.

[000195] The disclosure also provides multispecific activatable antibodies and/or multispecific activatable antibody compositions that include at least a first antibody or antigen-binding fragment thereof (AB1) that specifically binds a first target or first epitope and a second antibody or antigen-binding fragment thereof (AB2) that binds a second target or a second epitope, where at least AB1 is coupled or otherwise attached to a masking moiety (MM1), such that coupling of the MM1 reduces the ability of AB1 to bind its target. In some embodiments, the MM1 is coupled to AB1 via a first cleavable moiety (CM1) sequence that includes a substrate for a protease, for example, a protease that is produced by a tumor that is in proximity to cells that express the target and/or produced by a tumor that is co-localized with the target of AB1 at a treatment site or a diagnostic site in a subject. The multispecific activatable antibodies provided herein are stable in circulation, activated at intended sites of therapy and/or diagnosis but not in normal, *i.e.*, healthy tissue, and, when activated, exhibit binding to the target of AB1 that is at least comparable to the corresponding, unmodified multispecific antibody.

[000196] In some embodiments, the multispecific activatable antibody comprises a linking peptide between the MM1 and the CM1.

[000197] In some embodiments, the multispecific activatable antibody comprises a linking peptide between the CM1 and the AB1.

[000198] In some embodiments, the activatable antibody comprises a first linking peptide (LP1) and a second linking peptide (LP2), and at least a portion of the multispecific activatable antibody in the uncleaved state has the structural arrangement from N-terminus to C-terminus as follows: MM1-LP1-CM1-LP2-AB1 or AB1-LP2-CM1-LP1-MM1. In some embodiments, the two linking peptides need not be identical to each other.

[000199] In some embodiments, at least one of LP1 or LP2 includes an amino acid sequence selected from the group consisting of $(GS)_n$, $(GGS)_n$, $(GSGGS)_n$ (SEQ ID NO: 59) and $(GGGS)_n$ (SEQ ID NO: 60), where n is an integer of at least one. In some embodiments, at least one of LP1 or LP2 includes an amino acid sequence selected from the group consisting of GGSG (SEQ ID NO: 61), GGSGG (SEQ ID NO: 62), GSGSG (SEQ ID NO: 63), GSGGG (SEQ ID NO: 64), GGGSG (SEQ ID NO: 65), and GSSSG (SEQ ID NO: 66).

[000200] In some embodiments, the multispecific activatable antibody includes at least a first antibody or antigen-binding fragment thereof (AB1) that specifically binds a first target or first epitope and a second antibody or antigen-binding fragment thereof (AB2) that specifically binds a second target or second epitope. In some embodiments, each of the AB in the multispecific activatable antibody is independently selected from the group consisting of a monoclonal antibody, domain antibody, single chain, Fab fragment, a $F(ab')_2$ fragment, a scFv, a scAb, a dAb, a single domain heavy chain antibody, and a single domain light chain antibody. In some embodiments, each of the AB in the multispecific activatable antibody is a rodent (e.g., mouse or rat), chimeric, humanized or fully human monoclonal antibody.

[000201] In some embodiments, each of the AB in the multispecific activatable antibody has a dissociation constant of about 100 nM or less for binding to its corresponding target or epitope.

[000202] In some embodiments, the MM1 has a dissociation constant, i.e., dissociation constant at an equilibrium state, K_d for binding to the AB that is greater than the K_d for binding of the AB to its corresponding target or epitope.

[000203] In some embodiments, the MM1 has a K_d for binding to the AB that is no more than the K_d for binding of the AB to its corresponding target or epitope.

[000204] In some embodiments, the MM1 has a K_d for binding to the AB that is no less than the K_d for binding of the AB to its corresponding target or epitope.

[000205] In some embodiments, the MM1 has a K_d for binding to the AB that is approximately equal to the K_d for binding of the AB to its corresponding target or epitope.

[000206] In some embodiments, the MM1 has a K_d for binding to the AB that is less than the K_d for binding of the AB to its corresponding target or epitope.

[000207] In some embodiments, the MM1 has a K_d for binding to the AB that is no more than 2, 3, 4, 5, 10, 25, 50, 100, 250, 500, or 1,000 fold greater than the K_d for binding of the AB

to its corresponding target or epitope. In some embodiments, the MM1 has a K_d for binding to the AB that is between 1-5, 2-5, 2-10, 5-10, 5-20, 5-50, 5-100, 10-100, 10-1,000, 20-100, 20-1000, or 100-1,000 fold greater than the K_d for binding of the AB to its corresponding target or epitope.

[000208] In some embodiments, the MM1 has an affinity for binding to the AB that is less than the affinity of binding of the AB to its corresponding target or epitope.

[000209] In some embodiments, the MM1 has an affinity for binding to the AB that is no more than the affinity of binding of the AB to its corresponding target or epitope.

[000210] In some embodiments, the MM1 has an affinity for binding to the AB that is approximately equal of the affinity of binding of the AB to its corresponding target or epitope.

[000211] In some embodiments, the MM1 has an affinity for binding to the AB that is no less than the affinity of binding of the AB to its corresponding target or epitope.

[000212] In some embodiments, the MM1 has an affinity for binding to the AB that is greater than the affinity of binding of the AB to its corresponding target or epitope.

[000213] In some embodiments, the MM1 has an affinity for binding to the AB that is 2, 3, 4, 5, 10, 25, 50, 100, 250, 500, or 1,000 less than the affinity of binding of the AB to its corresponding target or epitope. In some embodiments, the MM1 has an affinity for binding to the AB that is between 1-5, 2-5, 2-10, 5-10, 5-20, 5-50, 5-100, 10-100, 10-1,000, 20-100, 20-1000, or 100-1,000 fold less than the affinity of binding of the AB to its corresponding target or epitope. In some embodiments, the MM1 has an affinity for binding to the AB that is 2 to 20 fold less than the affinity of binding of the AB to its corresponding target or epitope. In some embodiments, a MM not covalently linked to the AB and at equimolar concentration to the AB does not inhibit the binding of the AB to its corresponding target or epitope.

[000214] In some embodiments, MM1 does not interfere or compete with its corresponding AB for binding to the corresponding target or epitope when the multispecific activatable antibody is in a cleaved state.

[000215] In some embodiments, MM1 is a polypeptide of about 2 to 40 amino acids in length. In some embodiments, each of the MM in the multispecific activatable antibody is a polypeptide of no more than 40 amino acids in length.

[000216] In some embodiments, MM1 has a polypeptide sequence that is different from that of target of the corresponding AB.

[000217] In some embodiments, MM1 has a polypeptide sequence that is no more than 50% identical to any natural binding partner of the corresponding AB. In some embodiments, MM1 has a polypeptide sequence that is no more than 25% identical to any natural binding partner of the corresponding AB. In some embodiments, MM1 has a polypeptide sequence that is no more than 10% identical to any natural binding partner of the corresponding AB.

[000218] In some embodiments, the coupling of MM1 reduces the ability of the corresponding AB to bind its target or epitope such that the dissociation constant (K_d) of the AB when coupled to the MM1 towards its corresponding target or epitope is at least 20 times greater than the K_d of the AB when not coupled to the MM1 towards its corresponding target or epitope.

[000219] In some embodiments, the coupling of MM1 reduces the ability of the corresponding AB to bind its target or epitope such that the dissociation constant (K_d) of the AB when coupled to the MM1 towards its corresponding target or epitope is at least 40 times greater than the K_d of the AB when not coupled to the MM1 towards its corresponding target or epitope.

[000220] In some embodiments, the coupling of MM1 reduces the ability of the corresponding AB to bind its target or epitope such that the dissociation constant (K_d) of the AB when coupled to the MM1 towards its corresponding target or epitope is at least 100 times greater than the K_d of the AB when not coupled to the MM1 towards its corresponding target or epitope.

[000221] In some embodiments, the coupling of MM1 reduces the ability of the corresponding AB to bind its target or epitope such that the dissociation constant (K_d) of the AB when coupled to the MM1 towards its corresponding target or epitope is at least 1000 times greater than the K_d of the AB when not coupled to the MM1 towards its corresponding target or epitope.

[000222] In some embodiments, the coupling of MM1 reduces the ability of the corresponding AB to bind its target or epitope such that the dissociation constant (K_d) of the AB when coupled to the MM1 towards its corresponding target or epitope is at least 10,000 times greater than the K_d of the AB when not coupled to the MM1 towards its corresponding target or epitope.

[000223] In some embodiments, MM1 is an amino acid sequence selected from a MM shown in the Examples provided herein.

[000224] In some embodiments, the multispecific activatable antibody includes at least a second masking moiety (MM2) that inhibits the binding of the AB2 to its target when the multispecific activatable antibody is in an uncleaved state, and a second cleavable moiety (CM2) coupled to the AB2, wherein the CM2 is a polypeptide that functions as a substrate for a second protease. In some embodiments, CM2 is a polypeptide of no more than 15 amino acids long. In some embodiments, the second protease is produced by a tumor that is in proximity to cells that express the target and/or produced by a tumor that is co-localized with the second target or epitope in a tissue, and wherein the second protease cleaves the CM2 in the multispecific activatable antibody when the multispecific activatable antibody is exposed to the second protease. In some embodiments, the first protease and the second protease are produced by a tumor that is in proximity to cells that express the target and/or produced by a tumor that is co-localized with the first target or epitope and the second target or epitope in a tissue. In some embodiments, the first protease and the second protease are the same protease. In some embodiments, CM1 and CM2 are different substrates for the same protease. In some embodiments, the protease is selected from the group consisting of those shown in Table 3. In some embodiments, the first protease and the second protease are different proteases. In some embodiments, the first protease and the second protease are different proteases selected from the group consisting of those shown in Table 3.

[000225] In some embodiments, each of the MM in the multispecific activatable antibody has a dissociation constant, i.e., dissociation constant at an equilibrium state, K_d for binding to the AB that is greater than the K_d for binding of the AB to its corresponding target or epitope.

[000226] In some embodiments, each of the MM in the multispecific activatable antibody has a K_d for binding to the AB that is no more than the K_d for binding of the AB to its corresponding target or epitope.

[000227] In some embodiments, each of the MM in the multispecific activatable antibody has a K_d for binding to the AB that is no less than the K_d for binding of the AB to its corresponding target or epitope.

[000228] In some embodiments, each of the MM in the multispecific activatable antibody has a K_d for binding to the AB that is approximately equal to the K_d for binding of the AB to its corresponding target or epitope.

[000229] In some embodiments, each of the MM in the multispecific activatable antibody has a K_d for binding to the AB that is less than the K_d for binding of the AB to its corresponding target or epitope.

[000230] In some embodiments, each of the MM in the multispecific activatable antibody has a K_d for binding to the AB that is no more than 2, 3, 4, 5, 10, 25, 50, 100, 250, 500, or 1,000 fold greater than the K_d for binding of the AB to its corresponding target or epitope. In some embodiments, each of the MM in the multispecific activatable antibody has a K_d for binding to the AB that is between 1-5, 2-5, 2-10, 5-10, 5-20, 5-50, 5-100, 10-100, 10-1,000, 20-100, 20-1000, or 100-1,000 fold greater than the K_d for binding of the AB to its corresponding target or epitope.

[000231] In some embodiments, each of the MM in the multispecific activatable antibody has an affinity for binding to the AB that is less than the affinity of binding of the AB to its corresponding target or epitope.

[000232] In some embodiments, each of the MM in the multispecific activatable antibody has an affinity for binding to the AB that is no more than the affinity of binding of the AB to its corresponding target or epitope.

[000233] In some embodiments, each of the MM in the multispecific activatable antibody has an affinity for binding to the AB that is approximately equal of the affinity of binding of the AB to its corresponding target or epitope.

[000234] In some embodiments, each of the MM in the multispecific activatable antibody has an affinity for binding to the AB that is no less than the affinity of binding of the AB to its corresponding target or epitope.

[000235] In some embodiments, each of the MM in the multispecific activatable antibody has an affinity for binding to the AB that is greater than the affinity of binding of the AB to its corresponding target or epitope.

[000236] In some embodiments, each of the MM in the multispecific activatable antibody has an affinity for binding to the AB that is 2, 3, 4, 5, 10, 25, 50, 100, 250, 500, or 1,000 less than the affinity of binding of the AB to its corresponding target or epitope. In some

embodiments, each of the MM in the multispecific activatable antibody has an affinity for binding to the AB that is between 1-5, 2-5, 2-10, 5-10, 5-20, 5-50, 5-100, 10-100, 10-1,000, 20-100, 20-1000, or 100-1,000 fold less than the affinity of binding of the AB to its corresponding target or epitope. In some embodiments, each of the MM in the multispecific activatable antibody has an affinity for binding to the AB that is 2 to 20 fold less than the affinity of binding of the AB to its corresponding target or epitope. In some embodiments, a MM not covalently linked to the AB and at equimolar concentration to the AB does not inhibit the binding of the AB to its corresponding target or epitope.

[000237] In some embodiments, each of the MM in the multispecific activatable antibody does not interfere or compete with its corresponding AB for binding to the corresponding target or epitope when the multispecific activatable antibody is in a cleaved state.

[000238] In some embodiments, each of the MM in the multispecific activatable antibody is a polypeptide of about 2 to 40 amino acids in length. In some embodiments, each of the MM in the multispecific activatable antibody is a polypeptide of no more than 40 amino acids in length.

[000239] In some embodiments, each of the MM in the multispecific activatable antibody has a polypeptide sequence that is different from that of target of the corresponding AB.

[000240] In some embodiments, each of the MM in the multispecific activatable antibody has a polypeptide sequence that is no more than 50% identical to any natural binding partner of the corresponding AB. In some embodiments, each of the MM in the multispecific activatable antibody has a polypeptide sequence that is no more than 25% identical to any natural binding partner of the corresponding AB. In some embodiments, each of the MM in the multispecific activatable antibody has a polypeptide sequence that is no more than 10% identical to any natural binding partner of the corresponding AB.

[000241] In some embodiments, the coupling of each of the MM reduces the ability of the corresponding AB to bind its target or epitope such that the dissociation constant (K_d) of the AB when coupled to the MM towards its corresponding target or epitope is at least 20 times greater than the K_d of the AB when not coupled to the MM towards its corresponding target or epitope.

[000242] In some embodiments, the coupling of each of the MM reduces the ability of the corresponding AB to bind its target or epitope such that the dissociation constant (K_d) of the

AB when coupled to the MM towards its corresponding target or epitope is at least 40 times greater than the K_d of the AB when not coupled to the MM towards its corresponding target or epitope.

[000243] In some embodiments, the coupling of each of the MM reduces the ability of the corresponding AB to bind its target or epitope such that the dissociation constant (K_d) of the AB when coupled to the MM towards its corresponding target or epitope is at least 100 times greater than the K_d of the AB when not coupled to the MM towards its corresponding target or epitope.

[000244] In some embodiments, the coupling of each of the MM reduces the ability of the corresponding AB to bind its target or epitope such that the dissociation constant (K_d) of the AB when coupled to the MM towards its corresponding target or epitope is at least 1000 times greater than the K_d of the AB when not coupled to the MM towards its corresponding target or epitope.

[000245] In some embodiments, the coupling of each of the MM reduces the ability of the corresponding AB to bind its target or epitope such that the dissociation constant (K_d) of the AB when coupled to the MM towards its corresponding target or epitope is at least 10,000 times greater than the K_d of the AB when not coupled to the MM towards its corresponding target or epitope.

[000246] In some embodiments, the coupling of each of the MM reduces the ability of the corresponding AB to bind its target or epitope such that the dissociation constant (K_d) of the AB1 when coupled to the MM1 is at least 20 times greater than the K_d of the AB2 when coupled to the MM2. In some embodiments, the coupling of each of the MM reduces the ability of the corresponding AB to bind its target or epitope such that the dissociation constant (K_d) of the AB1 when coupled to the MM1 is at least 20 times lower than the K_d of the AB2 when coupled to the MM2.

[000247] In some embodiments, the coupling of each of the MM reduces the ability of the corresponding AB to bind its target or epitope such that the dissociation constant (K_d) of the AB1 when coupled to the MM1 is at least 40 times greater than the K_d of the AB2 when coupled to the MM2. In some embodiments, the coupling of each of the MM reduces the ability of the corresponding AB to bind its target or epitope such that the dissociation constant (K_d) of

the AB1 when coupled to the MM1 is at least 40 times lower than the K_d of the AB2 when coupled to the MM2.

[000248] In some embodiments, the coupling of each of the MM reduces the ability of the corresponding AB to bind its target or epitope such that the dissociation constant (K_d) of the AB1 when coupled to the MM1 is at least 100 times greater than the K_d of the AB2 when coupled to the MM2. In some embodiments, the coupling of each of the MM reduces the ability of the corresponding AB to bind its target or epitope such that the dissociation constant (K_d) of the AB1 when coupled to the MM1 is at least 100 times lower than the K_d of the AB2 when coupled to the MM2.

[000249] In some embodiments, the coupling of each of the MM reduces the ability of the corresponding AB to bind its target or epitope such that the dissociation constant (K_d) of the AB1 when coupled to the MM1 is at least 1,000 times greater than the K_d of the AB2 when coupled to the MM2. In some embodiments, the coupling of each of the MM reduces the ability of the corresponding AB to bind its target or epitope such that the dissociation constant (K_d) of the AB1 when coupled to the MM1 is at least 1,000 times lower than the K_d of the AB2 when coupled to the MM2.

[000250] In some embodiments, the coupling of each of the MM reduces the ability of the corresponding AB to bind its target or epitope such that the dissociation constant (K_d) of the AB1 when coupled to the MM1 is at least 10,000 times greater than the K_d of the AB2 when coupled to the MM2. In some embodiments, the coupling of each of the MM reduces the ability of the corresponding AB to bind its target or epitope such that the dissociation constant (K_d) of the AB1 when coupled to the MM1 is at least 10,000 times lower than the K_d of the AB2 when coupled to the MM2.

[000251] In some embodiments, each of the MM is an amino acid sequence selected from a MM shown in the Examples provided herein.

[000252] In some embodiments, the protease that cleaves the first cleavable moiety (CM1) sequence is produced by a tumor that is in proximity to cells that express the target and/or produced by a tumor that is co-localized with the target of the AB1 in the multispecific activatable antibody in a tissue, and the protease cleaves the CM1 in the multispecific activatable antibody when the multispecific activatable antibody is exposed to the protease.

[000253] In some embodiments, the multispecific activatable antibody includes more than one cleavable moiety sequence, and the protease that cleaves at least one cleavable moiety sequence is produced by a tumor that is in proximity to cells that express the target and/or produced by a tumor that is co-localized with the target of at least one of the AB regions in the multispecific activatable antibody in a tissue, and the protease cleaves the CM in the multispecific activatable antibody when the multispecific activatable antibody is exposed to the protease.

[000254] In some embodiments, each CM, e.g., CM1 and at least CM2, is positioned in the multispecific activatable antibody such that when the activatable antibody is in the uncleaved state, binding of the multispecific activatable antibody to a target of one of the AB regions is reduced to occur with a dissociation constant that is at least 20-fold greater than the dissociation constant of an unmodified AB binding to its target, and whereas when the activatable antibody is in the cleaved state, the AB binds its target.

[000255] In some embodiments, each CM is positioned in the multispecific activatable antibody such that when the activatable antibody is in the uncleaved state, binding of the multispecific activatable antibody to a target of one of the AB regions is reduced to occur with a dissociation constant that is at least 40-fold greater than the dissociation constant of an unmodified AB binding to its target, and whereas when the activatable antibody is in the cleaved state, the AB binds its target.

[000256] In some embodiments, each CM is positioned in the multispecific activatable antibody such when the activatable antibody is that in the uncleaved state, binding of the multispecific activatable antibody to a target of one of the AB regions is reduced to occur with a dissociation constant that is at least 50-fold greater than the dissociation constant of an unmodified AB binding to its target, and whereas when the activatable antibody is in the cleaved state, the AB binds its target.

[000257] In some embodiments, each CM is positioned in the multispecific activatable antibody such when the activatable antibody is that in the uncleaved state, binding of the multispecific activatable antibody to a target of one of the AB regions is reduced to occur with a dissociation constant that is at least 100-fold greater than the dissociation constant of an unmodified AB binding to its target, and whereas when the activatable antibody is in the cleaved state, the AB binds its target.

[000258] In some embodiments, each CM is positioned in the multispecific activatable antibody such that when the activatable antibody is in the uncleaved state, binding of the multispecific activatable antibody to a target of one of the AB regions is reduced to occur with a dissociation constant that is at least 200-fold greater than the dissociation constant of an unmodified AB binding to its target, and whereas when the activatable antibody is in the cleaved state, the AB binds its target.

[000259] In some embodiments, each CM in the multispecific activatable antibody is a polypeptide of up to 15 amino acids in length.

[000260] In some embodiments, at least one CM in the multispecific activatable antibody includes the amino acid sequence LSGRSDNH (SEQ ID NO: 67). In some embodiments, at least one cleavable moiety is selected for use with a specific protease, for example a protease that is known to be produced by a tumor that is in proximity to cells that express the target and/or produced by a tumor that is co-localized with at least one target of the multispecific activatable antibody. For example, suitable cleavable moieties for use in the multispecific activatable antibodies of the disclosure are cleaved by at least a protease such as urokinase, legumain, and/or matriptase (also referred to herein as MT-SP1 or MTSP1). In some embodiments, a suitable cleavable moiety includes at least one of the following sequences: TGRGPSWV (SEQ ID NO: 68); SARGPSRW (SEQ ID NO: 69); TARGPSFK (SEQ ID NO: 70); LSGRSDNH (SEQ ID NO: 67); GGWHTGRN (SEQ ID NO: 71); HTGRSGAL (SEQ ID NO: 72); PLTGRSGG (SEQ ID NO: 73); AARGPAIH (SEQ ID NO: 74); RGPAFNPM (SEQ ID NO: 75); SSRGPAYL (SEQ ID NO: 76); RGPATPIM (SEQ ID NO: 77); RGPA (SEQ ID NO: 78); GGQPSGMWGW (SEQ ID NO: 79); FPRPLGITGL (SEQ ID NO: 80); VHMPLGFLGP (SEQ ID NO: 81); SPLTGRSG (SEQ ID NO: 82); SAGFSLPA (SEQ ID NO: 83); LAPLGLQRR (SEQ ID NO: 84); SGGPLGVR (SEQ ID NO: 85); and/or PLGL (SEQ ID NO: 86).

[000261] In some embodiments, each CM in the multispecific activatable antibody is a substrate for a protease selected from the group consisting of those shown in Table 3. In some embodiments, the protease is selected from the group consisting of uPA, legumain, matriptase, ADAM17, BMP-1, TMPRSS3, TMPRSS4, neutrophil elastase, MMP-7, MMP-9, MMP-12, MMP-13, and MMP-14. In some embodiments, the protease is a cathepsin, such as, but not limited to, cathepsin S. In some embodiments, each CM in the multispecific activatable

antibody is a substrate for a protease selected from the group consisting of uPA (urokinase plasminogen activator), legumain and matriptase. In some embodiments, the protease comprises uPA. In some embodiments, the protease comprises legumain. In some embodiments, the protease comprises matriptase. In some embodiments, the protease comprises a matrix metalloproteinase (MMP).

[000262] In some embodiments, at least one CM in the multispecific activatable antibody is a substrate for at least two proteases. In some embodiments, each protease is selected from the group consisting of those shown in Table 3. In some embodiments, at least one CM in the multispecific activatable antibody is a substrate for at least two proteases, wherein one of the proteases is selected from the group consisting of uPA, legumain and matriptase and the other protease is selected from the group consisting of those shown in Table 3. In some embodiments, at least one CM in the multispecific activatable antibody is a substrate for at least two proteases selected from the group consisting of uPA, legumain and matriptase.

[000263] In some embodiments, at least one of the proteases that can cleave each CM in the multispecific activatable antibody is the same protease. In some embodiments, the same protease that can cleave both CMs in the multispecific activatable antibody is selected from the group consisting of those shown in Table 3. In some embodiments, the same protease that can cleave both CMs in the multispecific activatable antibody are selected from the group consisting of uPA, legumain and matriptase.

[000264] In some embodiments, at least two of the proteases that can cleave each CM in the multispecific activatable antibody are the same two proteases. In some embodiments, the same two proteases that can cleave both CMs in the multispecific activatable antibody are selected from the group consisting of those shown in Table 3. In some embodiments, at least one of the at least the two proteases that can cleave both CMs in the multispecific activatable antibody is selected from the group consisting of uPA, legumain and matriptase, and the other protease of the at least the two proteases is selected from the group consisting of those shown in Table 3. In some embodiments, the at least the two proteases that can cleave both CMs in the multispecific activatable antibody are selected from the group consisting of uPA, legumain and matriptase.

[000265] In some embodiments, at least one of the proteases that can cleave CM1 is different than at least one of the proteases that can cleave CM2 in the multispecific activatable

antibody. In some embodiments, at least one of the proteases that can cleave CM1 but cannot cleave CM2 in the multispecific activatable antibody. In some embodiments, at least one of the proteases that can cleave CM2 but cannot cleave CM1 in the multispecific activatable antibody. In some embodiments, the at least one protease that can cleave CM1 but cannot cleave CM2 in the multispecific activatable antibody is selected from the group consisting of those shown in Table 3. In some embodiments, the at least one protease that can cleave CM1 but cannot cleave CM2 in the multispecific activatable is selected from the group consisting of uPA, legumain and matriptase.

[000266] In some embodiments, the multispecific activatable antibody includes at least a first CM (CM1) and a second CM (CM2). In some embodiments, CM1 and CM2 are part of a single cleavable linker that joins an MM to an AB. In some embodiments, CM1 is part of a cleavable linker that joins MM1 to AB1, and CM2 is part of a separate cleavable linker that joins an MM2 to AB2. In some embodiments, a multispecific activatable antibody comprises more than two CMs. In some embodiments, such a multispecific activatable antibody comprises more than two CMs and more than two MMs. In some embodiments, CM1 and CM2 are each polypeptides of no more than 15 amino acids long. In some embodiments, at least one of the first CM and the second CM is a polypeptide that functions as a substrate for a protease selected from the group consisting of those listed in Table 3. In some embodiments, at least one of the first CM and the second CM is a polypeptide that functions as a substrate for a protease selected from the group consisting of uPA, legumain, and matriptase. In some embodiments, the first CM is cleaved by a first cleaving agent selected from the group consisting of uPA, legumain, and matriptase in a target tissue and the second CM is cleaved by a second cleaving agent in a target tissue. In some embodiments, the other protease is selected from the group consisting of those shown in Table 3. In some embodiments, the first cleaving agent and the second cleaving agent are the same protease selected from the group consisting of those listed in Table 3, and the first CM and the second CM are different substrates for the protease. In some embodiments, the first cleaving agent and the second cleaving agent are the same protease selected from the group consisting of uPA, legumain, and matriptase, and the first CM and the second CM are different substrates for the protease. In some embodiments, the first cleaving agent and the second cleaving agent are the same protease selected from the group listed in Table 3, and the first CM and the second CM are the same substrate. In some

embodiments, the first cleaving agent and the second cleaving agent are different proteases. In some embodiments, the first cleaving agent and the second cleaving agent are different proteases selected from the group consisting of those shown in Table 3. In some embodiments, the first cleaving agent and the second cleaving agent are produced by a tumor that is in proximity to cells that express the target and/or produced by a tumor that is co-localized in the target tissue. In some embodiments, the first CM and the second CM are cleaved by at least one cleaving agent in the target tissue.

[000267] In some embodiments, the multispecific activatable antibody is exposed to and cleaved by a protease such that, when the activatable antibody is in the activated or cleaved state, the activated multispecific activatable antibody includes a light chain amino acid sequence that includes at least a portion of LP2 and/or CM sequence after the protease has cleaved the CM.

[000268] In some embodiments, the multispecific activatable antibody also includes a signal peptide. In some embodiments, the signal peptide is conjugated to the multispecific activatable antibody via a spacer. In some embodiments, the spacer is conjugated to the multispecific activatable antibody in the absence of a signal peptide. In some embodiments, the spacer is joined directly to at least one of the MM of the multispecific activatable antibody.

[000269] In some embodiments, the multispecific activatable antibody in an uncleaved state comprises a spacer that is joined directly to a first MM and has the structural arrangement from N-terminus to C-terminus of spacer-MM1-CM-AB1. In some embodiments, the spacer includes at least the amino acid sequence QGQSGQ (SEQ ID NO: 87).

[000270] In some embodiments, the serum half-life of the multispecific activatable antibody is longer than that of the corresponding multispecific antibody; e.g., the pK of the multispecific activatable antibody is longer than that of the corresponding multispecific antibody. In some embodiments, the serum half-life of the multispecific activatable antibody is similar to that of the corresponding multispecific antibody. In some embodiments, the serum half-life of the multispecific activatable antibody is at least 15 days when administered to an organism. In some embodiments, the serum half-life of the multispecific activatable antibody is at least 12 days when administered to an organism. In some embodiments, the serum half-life of the multispecific activatable antibody is at least 11 days when administered to an organism. In some embodiments, the serum half-life of the multispecific activatable antibody is at least 10

days when administered to an organism. In some embodiments, the serum half-life of the multispecific activatable antibody is at least 9 days when administered to an organism. In some embodiments, the serum half-life of the multispecific activatable antibody is at least 8 days when administered to an organism. In some embodiments, the serum half-life of the multispecific activatable antibody is at least 7 days when administered to an organism. In some embodiments, the serum half-life of the multispecific activatable antibody is at least 6 days when administered to an organism. In some embodiments, the serum half-life of the multispecific activatable antibody is at least 5 days when administered to an organism. In some embodiments, the serum half-life of the multispecific activatable antibody is at least 4 days when administered to an organism. In some embodiments, the serum half-life of the multispecific activatable antibody is at least 3 days when administered to an organism. In some embodiments, the serum half-life of the multispecific activatable antibody is at least 2 days when administered to an organism. In some embodiments, the serum half-life of the multispecific activatable antibody is at least 24 hours when administered to an organism. In some embodiments, the serum half-life of the multispecific activatable antibody is at least 20 hours when administered to an organism. In some embodiments, the serum half-life of the multispecific activatable antibody is at least 18 hours when administered to an organism. In some embodiments, the serum half-life of the multispecific activatable antibody is at least 16 hours when administered to an organism. In some embodiments, the serum half-life of the multispecific activatable antibody is at least 14 hours when administered to an organism. In some embodiments, the serum half-life of the multispecific activatable antibody is at least 12 hours when administered to an organism. In some embodiments, the serum half-life of the multispecific activatable antibody is at least 10 hours when administered to an organism. In some embodiments, the serum half-life of the multispecific activatable antibody is at least 8 hours when administered to an organism. In some embodiments, the serum half-life of the multispecific activatable antibody is at least 6 hours when administered to an organism. In some embodiments, the serum half-life of the multispecific activatable antibody is at least 4 hours when administered to an organism. In some embodiments, the serum half-life of the multispecific activatable antibody is at least 3 hours when administered to an organism.

[000271] The disclosure also provides compositions and methods that include a multispecific activatable antibody that includes at least a first antibody or antibody fragment

(AB1) that specifically binds a target and a second antibody or antibody fragment (AB2), where at least the first AB in the multispecific activatable antibody is coupled to a masking moiety (MM1) that decreases the ability of AB1 to bind its target. In some embodiments, each AB is coupled to a MM that decreases the ability of its corresponding AB to each target. For example, in bispecific activatable antibody embodiments, AB1 is coupled to a first masking moiety (MM1) that decreases the ability of AB1 to bind its target, and AB2 is coupled to a second masking moiety (MM2) that decreases the ability of AB2 to bind its target. In some embodiments, the multispecific activatable antibody comprises more than two AB regions; in such embodiments, AB1 is coupled to a first masking moiety (MM1) that decreases the ability of AB1 to bind its target, AB2 is coupled to a second masking moiety (MM2) that decreases the ability of AB2 to bind its target, AB3 is coupled to a third masking moiety (MM3) that decreases the ability of AB3 to bind its target, and so on for each AB in the multispecific activatable antibody.

[000272] In some embodiments, the multispecific activatable antibody further includes at least one cleavable moiety (CM) that is a substrate for a protease, where the CM links a MM to an AB. For example, in some embodiments, the multispecific activatable antibody includes at least a first antibody or antibody fragment (AB1) that specifically binds a target and a second antibody or antibody fragment (AB2), where at least the first AB in the multispecific activatable antibody is coupled via a first cleavable moiety (CM1) to a masking moiety (MM1) that decreases the ability of AB1 to bind its target. In some bispecific activatable antibody embodiments, AB1 is coupled via CM1 to MM1, and AB2 is coupled via a second cleavable moiety (CM2) to a second masking moiety (MM2) that decreases the ability of AB2 to bind its target. In some embodiments, the multispecific activatable antibody comprises more than two AB regions; in some of these embodiments, AB1 is coupled via CM1 to MM1, AB2 is coupled via CM2 to MM2, and AB3 is coupled via a third cleavable moiety (CM3) to a third masking moiety (MM3) that decreases the ability of AB3 to bind its target, and so on for each AB in the multispecific activatable antibody.

[000273] In some embodiments, the multispecific antibodies and/or multispecific activatable antibodies described herein are used in conjunction with one or more additional agents or a combination of additional agents. Suitable additional agents include current pharmaceutical and/or surgical therapies for an intended application, such as, for example,

cancer. For example, the multispecific antibodies and/or multispecific activatable antibodies can be used in conjunction with an additional chemotherapeutic or anti-neoplastic agent.

[000274] In some embodiments, the multispecific antibody and/or multispecific activatable antibody and additional agent are formulated into a single therapeutic composition, and the multispecific antibody and/or multispecific activatable antibody and additional agent are administered simultaneously. In some embodiments, the multispecific antibody and/or multispecific activatable antibody and additional agent are separate from each other, *e.g.*, each is formulated into a separate therapeutic composition, and the multispecific antibody and/or multispecific activatable antibody and the additional agent are administered simultaneously, or the multispecific antibody and/or multispecific activatable antibody and the additional agent are administered at different times during a treatment regimen. For example, the multispecific antibody and/or multispecific activatable antibody is administered prior to the administration of the additional agent, the multispecific antibody and/or multispecific activatable antibody is administered subsequent to the administration of the additional agent, or the multispecific antibody and/or multispecific activatable antibody and the additional agent are administered in an alternating fashion. As described herein, the anti-multispecific antibody and/or multispecific activatable antibody and additional agent are administered in single doses or in multiple doses.

[000275] The disclosure also provides an isolated nucleic acid molecule encoding at least a portion of a multispecific antibody and/or multispecific activatable antibody described herein, such as for example, at least a first nucleic acid encoding at least a portion of the heavy chain of the multispecific antibody and/or multispecific activatable antibody and a second nucleic acid encoding at least a portion of the light chain of multispecific antibody and/or multispecific the activatable antibody, as well as vectors that include these isolated nucleic acid sequences. The disclosure provides methods of producing a multispecific antibody and/or multispecific activatable antibody by culturing a cell under conditions that lead to expression of the multispecific antibody and/or multispecific activatable antibody, wherein the cell comprises such a nucleic acid molecule(s). In some embodiments, the cell comprises such a vector.

[000276] The disclosure also provides a method of manufacturing multispecific antibodies of the disclosure and/or multispecific activatable antibodies of the disclosure by (a) culturing a cell comprising a nucleic acid construct that encodes the multispecific antibody and/or multispecific activatable antibody under conditions that lead to expression of the multispecific

antibody and/or multispecific activatable, and (b) recovering the multispecific antibody and/or multispecific activatable antibody.

[000277] The present disclosure also provides methods of treating, preventing, delaying the progression of or otherwise ameliorating a symptom of one or more pathologies or alleviating a symptom associated with such pathologies, by administering an anti-CD3 ϵ antibody, an activatable anti-CD3 ϵ antibody, a multispecific antibody that specifically binds CD3 ϵ and/or a multispecific activatable antibody of the disclosure that specifically binds CD3 ϵ to a subject in which such treatment or prevention is desired. The subject to be treated is, *e.g.*, human or other mammal. In some embodiments, the subject is a non-human mammal, such as a non-human primate, companion animal (*e.g.*, cat, dog, horse), farm animal, work animal, or zoo animal. In some embodiments, the subject is a rodent.

[000278] The present disclosure also provides methods to induce target-dependent T-cell activation and killing of a target cell by administering a multispecific activatable antibody of the disclosure to a subject in which such induction is desired, wherein when the multispecific activatable antibody is in the cleaved state, *e.g.*, each masking moiety in the multispecific activatable antibody is no longer attached or otherwise associated with the corresponding AB domain, target-dependent T-cell activation and killing of the target cell occurs, and when the multispecific activatable antibody is in the uncleaved state, *e.g.* at least one masking moiety of the multispecific activatable antibody is attached or otherwise associated with the corresponding AB domain, target-dependent T-cell activation and killing of the target cell is reduced or otherwise inhibited. Any of the multispecific activatable antibodies described herein are suitable for use in such methods. The subject to be treated is, *e.g.*, human or other mammal. In some embodiments, the subject is a non-human mammal, such as a non-human primate, companion animal (*e.g.*, cat, dog, horse), farm animal, work animal, or zoo animal. In some embodiments, the subject is a rodent.

[000279] An anti-CD3 ϵ antibody, an activatable anti-CD3 ϵ antibody, a multispecific antibody that specifically binds CD3 ϵ and/or a multispecific activatable antibody of the disclosure that specifically binds CD3 ϵ used in any of the embodiments of these methods and uses can be administered at any stage of the disease. For example, such an antibody, an activatable antibody, a multispecific antibody and/or a multispecific activatable antibody can be

administered to a patient suffering cancer of any stage, from early to metastatic. The terms subject and patient are used interchangeably herein.

[000280] An anti-CD3 ϵ antibody, an activatable anti-CD3 ϵ antibody, a multispecific antibody that specifically binds CD3 ϵ and/or a multispecific activatable antibody of the disclosure that specifically binds CD3 ϵ used in any of the embodiments of these methods and uses can be used in a treatment regimen comprising neoadjuvant therapy.

[000281] An anti-CD3 ϵ antibody, an activatable anti-CD3 ϵ antibody, a multispecific antibody that specifically binds CD3 ϵ and/or a multispecific activatable antibody of the disclosure that specifically binds CD3 ϵ used in any of the embodiments of these methods and uses can be administered either alone or in combination with one or more additional agents, including small molecule inhibitors, other antibody-based therapies, polypeptide or peptide-based therapies, nucleic acid-based therapies and/or other biologics. In some embodiments, an antibody, an activatable antibody, a multispecific antibody, and/or a multispecific activatable antibody is administered in combination with one or more additional agents such as, by way of non-limiting example, a chemotherapeutic agent, such as an alkylating agent, an anti-metabolite, an anti-microtubule agent, a topoisomerase inhibitor, a cytotoxic antibiotic, and any other nucleic acid damaging agent. In some embodiments, the additional agent is a taxane, such as paclitaxel (e.g., Abraxane®). In some embodiments, the additional agent is an anti-metabolite, such as gemcitabine. In some embodiments, the additional agent is an alkylating agent, such as platinum-based chemotherapy, such as carboplatin or cisplatin. In some embodiments, the additional agent is a targeted agent, such as a kinase inhibitor, e.g., sorafenib or erlotinib. In some embodiments, the additional agent is a targeted agent, such as another antibody, e.g., a monoclonal antibody (e.g., bevacizumab), a bispecific antibody, or a multispecific antibody. In some embodiments, the additional agent is a proteasome inhibitor, such as bortezomib or carfilzomib. In some embodiments, the additional agent is an immune modulating agent, such as lenolidomide or IL-2. In some embodiments, the additional agent is radiation. In some embodiments, the additional agent is an agent considered standard of care by those skilled in the art. In some embodiments, the additional agent is a chemotherapeutic agent well known to those skilled in the art. In some embodiments, the antibody, activatable antibody, multispecific antibody, and/or multispecific activatable antibody, and the additional

agent(s) are formulated in a single composition. In some embodiments, the antibody, activatable antibody, multispecific antibody, and/or multispecific activatable antibody, and the additional agent(s) are administered as two or more separate compositions. In some embodiments, the antibody, activatable antibody, multispecific antibody, and/or multispecific activatable antibody, and the additional agent(s) are administered simultaneously. In some embodiments, the antibody, activatable antibody, multispecific antibody, and/or multispecific activatable antibody, and the additional agent(s) are administered sequentially.

[000282] In some embodiments, the additional agent(s) is a chemotherapeutic agent, such as a chemotherapeutic agent selected from the group consisting of docetaxel, paclitaxel, abraxane (i.e., albumin-conjugated paclitaxel), doxorubicin, oxaliplatin, carboplatin, cisplatin, irinotecan, and gemcitabine.

[000283] In some embodiments, the additional agent(s) is a checkpoint inhibitor, a kinase inhibitor, an agent targeting inhibitors in the tumor microenvironment, and/or a T cell or NK agonist. In some embodiments, the additional agent(s) is radiation therapy, alone or in combination with another additional agent(s) such as a chemotherapeutic or anti-neoplastic agent. In some embodiments, the additional agent(s) is a vaccine, an oncovirus, and/or a DC-activating agent such as, by way of non-limiting example, a toll-like receptor (TLR) agonist and/or α -CD40. In some embodiments, the additional agent(s) is a tumor-targeted antibody designed to kill the tumor via ADCC or via direct conjugation to a toxin (e.g., an antibody drug conjugate (ADC)).

[000284] In some embodiments, the checkpoint inhibitor is an inhibitor of a target selected from the group consisting of CTLA-4, LAG-3, PD-1, PDL1, TIGIT, TIM-3, B7H4, and Vista. In some embodiments, the kinase inhibitor is selected from the group consisting of B-RAFi, MEKi, and Btk inhibitors, such as ibrutinib. In some embodiments, the kinase inhibitor is crizotinib. In some embodiments, the tumor microenvironment inhibitor is selected from the group consisting of an IDO inhibitor, an α -CSF1R inhibitor, an α -CCR4 inhibitor, a TGF-beta, a myeloid-derived suppressor cell, or a T-regulatory cell. In some embodiments, the agonist is selected from the group consisting of Ox40, GITR, CD137, ICOS, CD27, and HVEM. In some embodiments, the checkpoint inhibitor is an antibody that binds a target selected from CTLA-4, PD-1, and/or PD-L1. In some embodiments, the checkpoint inhibitor is an anti-CTLA4 antibody, an anti-PD-1 antibody, and an anti-PD-L1 antibody, and/or

combinations thereof. In some embodiments, the checkpoint inhibitor is an anti-CTLA4 antibody such as, e.g., Yervoy™. In some embodiments, the checkpoint inhibitor is an anti-PD-1 antibody such as, e.g., Opdivo™ and/or Keytruda™.

[000285] In some embodiments, the inhibitor is a CTLA-4 inhibitor. In some embodiments, the inhibitor is a LAG-3 inhibitor. In some embodiments, the inhibitor is a PD-1 inhibitor. In some embodiments, the inhibitor is a PDL1 inhibitor. In some embodiments, the inhibitor is a TIGIT inhibitor. In some embodiments, the inhibitor is a TIM-3 inhibitor. In some embodiments, the inhibitor is a B7H4 inhibitor. In some embodiments, the inhibitor is a Vista inhibitor. In some embodiments, the inhibitor is a B-RAFi inhibitor. In some embodiments, the inhibitor is a MEKi inhibitor. In some embodiments, the inhibitor is a Btk inhibitor. In some embodiments, the inhibitor is ibrutinib. In some embodiments, the inhibitor is crizotinib. In some embodiments, the inhibitor is an IDO inhibitor. In some embodiments, the inhibitor is an α -CSF1R inhibitor. In some embodiments, the inhibitor is an α -CCR4 inhibitor. In some embodiments, the inhibitor is a TGF-beta. In some embodiments, the inhibitor is a myeloid-derived suppressor cell. In some embodiments, the inhibitor is a T-regulatory cell.

[000286] In some embodiments, the agonist is Ox40. In some embodiments, the agonist is GITR. In some embodiments, the agonist is CD137. In some embodiments, the agonist is ICOS. In some embodiments, the agonist is CD27. In some embodiments, the agonist is HVEM.

[000287] In some embodiments, the additional agent is another antibody or antigen-binding fragment thereof, another conjugated antibody or antigen-binding fragment thereof, another activatable antibody or antigen-binding fragment thereof and/or another conjugated activatable antibody or antigen-binding fragment thereof. In some embodiments the additional agent is another antibody or antigen-binding fragment thereof, another conjugated antibody or antigen-binding fragment thereof, another activatable antibody or antigen-binding fragment thereof and/or another conjugated activatable antibody or antigen-binding fragment thereof against the same target as the first antibody or antigen-binding fragment thereof, the first conjugated antibody or antigen-binding fragment thereof, activatable antibody or antigen-binding fragment thereof and/or a conjugated activatable antibody or antigen-binding fragment thereof, e.g., against PDL1. In some embodiments the additional agent is another antibody or antigen-binding fragment thereof, another conjugated antibody or antigen-binding fragment

thereof, another activatable antibody or antigen-binding fragment thereof and/or another conjugated activatable antibody or antigen-binding fragment thereof against a target different than the target of the first antibody or antigen-binding fragment thereof, the first conjugated antibody or antigen-binding fragment thereof, activatable antibody or antigen-binding fragment thereof and/or a conjugated activatable antibody or antigen-binding fragment thereof.

[000288] In some embodiments, the anti-CD3 ϵ antibody and/or activatable antibody is administered during and/or after treatment in combination with one or more additional agents such as, for example, a chemotherapeutic agent, an anti-inflammatory agent, and/or an immunosuppressive agent. In some embodiments, the anti-CD3 ϵ antibody and/or activatable antibody and the additional agent are formulated into a single therapeutic composition, and the anti-CD3 ϵ antibody and/or activatable antibody and additional agent are administered simultaneously. Alternatively, the anti-CD3 ϵ antibody and/or activatable antibody and additional agent are separate from each other, *e.g.*, each is formulated into a separate therapeutic composition, and the anti-CD3 ϵ antibody and/or activatable antibody and the additional agent are administered simultaneously, or the anti-CD3 ϵ antibody and/or activatable antibody and the additional agent are administered at different times during a treatment regimen. For example, the anti-CD3 ϵ antibody and/or activatable antibody is administered prior to the administration of the additional agent, the anti-CD3 ϵ antibody and/or activatable antibody is administered subsequent to the administration of the additional agent, or the anti-CD3 ϵ antibody and/or activatable antibody and the additional agent are administered in an alternating fashion. As described herein, the anti-CD3 ϵ antibody and/or activatable antibody and additional agent are administered in single doses or in multiple doses.

[000289] In some embodiments, the anti-CD3 ϵ antibody and/or activatable antibody and the additional agent(s) are administered simultaneously. For example, the anti-CD3 ϵ antibody and/or activatable antibody and the additional agent(s) can be formulated in a single composition or administered as two or more separate compositions. In some embodiments, the anti-CD3 ϵ antibody and/or activatable antibody and the additional agent(s) are administered sequentially, or the anti-CD3 ϵ antibody and/or activatable antibody and the additional agent are administered at different times during a treatment regimen.

[000290] The disclosure also provides methods and kits for using the anti-CD3 ϵ antibody, activatable anti-CD3 ϵ antibody, multispecific antibody that specifically binds CD3 ϵ and/or multispecific activatable antibody of the disclosure that specifically binds CD3 ϵ in a variety of diagnostic and/or prophylactic indications. For example, the disclosure provides methods and kits for detecting presence or absence of a cleaving agent and a target of interest in a subject or a sample by (i) contacting a subject or sample with an activatable antibody or a multispecific activatable antibody that includes at least a first masking moiety (MM1), a first cleavable moiety (CM1) that is cleaved by the cleaving agent, and at least a first antigen binding domain or fragment thereof (AB1) that specifically binds the target of interest, (a) wherein the MM1 is a peptide that inhibits binding of the AB1 to the target, and wherein the MM1 does not have an amino acid sequence of a naturally occurring binding partner of the AB1 and is not a modified form of a natural binding partner of the AB1; and (b) wherein, in an uncleaved, non-activated state, the MM1 interferes with specific binding of the AB1 to the target, and in a cleaved, activated state the MM1 does not interfere or compete with specific binding of the AB1 to the target; and (ii) measuring a level of activated antibody or activated multispecific activatable antibody in the subject or sample, wherein a detectable level of activated antibody or activated multispecific activatable antibody in the subject or sample indicates that the cleaving agent and the target are present in the subject or sample and wherein no detectable level of activated antibody or activated multispecific activatable antibody in the subject or sample indicates that the cleaving agent, the target or both the cleaving agent and the target are absent and/or not sufficiently present in the subject or sample.

[000291] In some embodiments, the activatable antibody or activatable multispecific activatable antibody is an activatable antibody or an activatable multispecific activatable antibody to which a therapeutic agent is conjugated. In some embodiments, the activatable antibody or activatable multispecific activatable antibody is not conjugated to an agent. In some embodiments, the activatable antibody or activatable multispecific activatable antibody comprises a detectable label. In some embodiments, the detectable label is positioned on the AB1. In some embodiments, measuring the level of activatable antibody or multispecific activatable antibody in the subject or sample is accomplished using a secondary reagent that specifically binds to the activatable antibody or activatable multispecific activatable antibody,

wherein the reagent comprises a detectable label. In some embodiments, the secondary reagent is an antibody comprising a detectable label.

[000292] In some embodiments of these methods and kits, the activatable antibody or multispecific activatable antibody includes a detectable label. In some embodiments of these methods and kits, the detectable label includes an imaging agent, a contrasting agent, an enzyme, a fluorescent label, a chromophore, a dye, one or more metal ions, or a ligand-based label. In some embodiments of these methods and kits, the imaging agent comprises a radioisotope. In some embodiments of these methods and kits, the radioisotope is indium or technetium. In some embodiments of these methods and kits, the contrasting agent comprises iodine, gadolinium or iron oxide. In some embodiments of these methods and kits, the enzyme comprises horseradish peroxidase, alkaline phosphatase, or β -galactosidase. In some embodiments of these methods and kits, the fluorescent label comprises yellow fluorescent protein (YFP), cyan fluorescent protein (CFP), green fluorescent protein (GFP), modified red fluorescent protein (mRFP), red fluorescent protein tdimer2 (RFP tdimer2), HCRED, or a europium derivative. In some embodiments of these methods and kits, the luminescent label comprises an N- methylacrydium derivative. In some embodiments of these methods, the label comprises an Alexa Fluor[®] label, such as Alex Fluor[®] 680 or Alexa Fluor[®] 750. In some embodiments of these methods and kits, the ligand-based label comprises biotin, avidin, streptavidin or one or more haptens.

[000293] In some embodiments of these methods and kits, the subject is a mammal. In some embodiments of these methods and kits, the subject is a human. In some embodiments, the subject is a non-human mammal, such as a non-human primate, companion animal (e.g., cat, dog, horse), farm animal, work animal, or zoo animal. In some embodiments, the subject is a rodent.

[000294] In some embodiments of these methods, the method is an *in vivo* method. In some embodiments of these methods, the method is an *in situ* method. In some embodiments of these methods, the method is an *ex vivo* method. In some embodiments of these methods, the method is an *in vitro* method.

[000295] In some embodiments of the methods and kits, the method or kit is used to identify or otherwise refine a patient population suitable for treatment with a multispecific activatable antibody of the disclosure. For example, patients that test positive for both the target

and a protease that cleaves the substrate in the first cleavable moiety (CM1) of the multispecific activatable antibody being tested in these methods are identified as suitable candidates for treatment with such an activatable antibody or a multispecific activatable antibody comprising such a CM1. Likewise, patients that test negative for both the target and the protease that cleaves the substrate in the CM1 in the activatable antibody or multispecific activatable antibody being tested using these methods might be identified as suitable candidates for another form of therapy.

[000296] In some embodiments, a method or kit is used to identify or otherwise refine a patient population suitable for treatment with an activatable antibody and/or a multispecific activatable antibody of the disclosure, followed by treatment by administering that activatable antibody and/or multispecific activatable antibody to a subject in need thereof. For example, patients that test positive for both the target and a protease that cleaves the substrate in the first cleavable moiety (CM1) of the activatable antibody and/or multispecific activatable antibody being tested in these methods are identified as suitable candidates for treatment with an activatable antibody and/or a multispecific activatable antibody comprising such a CM1, and the patient is then administered a therapeutically effective amount of the activatable antibody and/or the multispecific activatable antibody that was tested. Likewise, patients that test negative for either or both of the target and the protease that cleaves the substrate in the CM1 in the activatable antibody and/or multispecific activatable antibody being tested using these methods might be identified as suitable candidates for another form of therapy.

[000297] In some embodiments, such patients can be tested with other activatable antibodies, conjugated activatable antibodies or multispecific antibodies and/or multispecific activatable antibodies and/or conjugated multispecific activatable antibodies until a suitable activatable antibody or conjugated activatable antibody or multispecific activatable antibody and/or conjugated multispecific activatable antibody for treatment is identified, *e.g.*, an activatable antibody or a conjugated activatable antibody or a multispecific activatable antibody and/or conjugated multispecific activatable antibody comprising a CM that is cleaved by the patient at the site of disease. In some embodiments, the patient is then administered a therapeutically effective amount of the activatable antibody or conjugated activatable antibody or multispecific activatable antibody and/or conjugated multispecific activatable antibody for which the patient tested positive.

[000298] Pharmaceutical compositions according to the disclosure can include a multispecific antibody and/or a multispecific activatable antibody of the disclosure and a carrier. These pharmaceutical compositions can be included in kits, such as, for example, diagnostic kits.

[000299] One skilled in the art will appreciate that the antibodies of the disclosure have a variety of uses. For example, the proteins of the disclosure are used as therapeutic agents for a variety of disorders. The antibodies of the disclosure are also used as reagents in diagnostic kits or as diagnostic tools, or these antibodies can be used in competition assays to generate therapeutic reagents.

BRIEF DESCRIPTION OF DRAWINGS

[000300] Figure 1 is an illustration depicting generic mask selection population nomenclature used herein.

[000301] Figures 2A and 2B are a series of graphs depicting the ability of scFv formats of SP34 to bind to cynomolgus CD3 ϵ . Figure 1A depicts SP34scFv(LvHv)-Fc and SP34scFv(HvLv)-Fc binding as a function of Fc fusion protein concentration. Figure 2B depicts mouse SP34-2 IgG1 binding as a function of concentration.

[000302] Figures 3A-3D are a series of graphs depicting the ability of masking moieties of the disclosure to reduce the ability of activatable anti-CD3 ϵ antibodies comprising such masking moieties to bind to CD3 ϵ on Jurkat T cells. Each activatable antibody is labeled by its masking moiety name. Antibody SP34scFv(LvHv)-Fc is identified by LvHv.

[000303] Figures 4A, 4B, and 4C are a series of illustrations depicting various antibodies and activatable antibodies of the disclosure. Figure 4A depicts component pieces for scFv-Fc antibodies. Figure 4B depicts scFv(LvHv)-Fc and scFv(HvLv)-Fc antibodies of the disclosure. Figure 4C depicts activatable antibodies comprising scFv(LvHv)-Fc or scFv(HvLv)-Fc antibodies of the disclosure.

[000304] Figures 5A, 5B, 5C, and 5D are a series of illustrations depicting multispecific antibodies and multispecific activatable antibodies of the disclosure. Figure 5A depicts a multispecific antibody of the disclosure that comprises an Ig antibody and a scFv. Figure 5B depicts a multispecific activatable antibody of the disclosure in which the primary antibody site is masked. Figure 5C depicts a multispecific activatable antibody of the disclosure in which the

secondary scFv site is masked. Figure 5D depicts a multispecific activatable antibody of the disclosure in which the with primary antibody and secondary scFv sites are masked.

[000305] Figures 6A, 6B, 6C, 6D, 6E, and 6F are a series of illustrations depicting various embodiments of multispecific activatable antibodies of the disclosure.

[000306] Figures 7A, 7B, 7C, 7D, 7E, 7F, 7G, 7H, 7I, and 7J are a series of schematic diagrams of a selected set of the possible permutations of multispecific antibodies of the disclosure.

[000307] Figures 8A, 8B, 8C, 8D, 8E, 8F, 8G, 8H, 8I, and 8J are a series of schematic diagrams of a selected set of the possible permutations of multispecific activatable antibodies of the disclosure. In particular, this figure shows multispecific activatable antibodies in which the primary antigen binding site is masked (i.e., activatable) and the additional antigen-binding domain(s) is not masked.

[000308] Figures 9A, 9B, 9C, 9D, 9E, 9F, 9G, 9H, and 9J are a series of schematic diagrams of an array of multispecific activatable antibodies in which all antigen-binding domains are masked.

[000309] Figures 10A, 10B, 10C, 10D, 10E, 10F, 10G, 10H, 10I, and 10J are a series of schematic diagrams of an array of multispecific activatable antibodies in which the secondary antigen-binding domain is masked and the additional antigen-binding domain(s) is not masked.

[000310] Figures 11A, 11B, 11C, 11D, 11E, 11F, 11G, 11H, 11I, and 11J are a series of schematic diagrams of an array of multispecific activatable antibodies in which the majority, but not all of the antigen-binding domains, are masked and at least one additional antigen-binding domain(s) is not masked.

[000311] Figures 12A, 12B, 12C, and 12D are a series of schematic diagrams of an array of multispecific activatable antibodies in which the primary antigen-binding domain and another antigen-binding domain are masked, and the remaining antigen-binding domain(s) is not masked.

[000312] Figures 13A and 13B are a series of graphs depicting SP34 scFv-Fc binding to CD3 ϵ positive Jurkat cells. Figure 13A is a graph depicting the ability of both orientations of the SP34scFv-Fc to bind to CD3 ϵ Jurkat cells. Figure 13B is a graph depicting the binding of SP34-2 IgG. All formats of SP34 bind with similar EC₅₀ values.

[000313] Figures 14A and 14B are a series of graphs depicting the ability of masking moieties of the disclosure to reduce the ability of activatable anti-CD3 ϵ antibodies comprising such masking moieties to bind to CD3 ϵ on Jurkat T cells.

[000314] Figures 15A, 15B, 15C, and 15D are a series of graphs depicting that the CD3 ϵ masking peptides shift the EC₅₀ of CD3 ϵ binding in an EGFR masked, multispecific activatable antibody that utilize two versions of an SP34 scFv while retaining effective EGFR masking.

[000315] Figures 16A and 16B are a series of graphs depicting that the cytotoxicity EC₅₀ of CD3 ϵ and EGFR masked multispecific, activatable antibodies are shifted relative to an unmasked control. This was evident with two versions of the SP34 scFv, namely the scFv in CI023 and CI024 versus the scFv in CI011 and CI010.

[000316] Figures 17A and 17B are a series of graphs depicting that in the context of a multi-specific, activatable antibody, the EGFR binding EC₅₀ is unaffected by the presence or absence of both CD3 ϵ masks; likewise, CD3 ϵ EC₅₀ is unaffected by the presence or absence of the EGFR mask.

[000317] Figure 18A and 18B are a series of graphs depicting that the largest shift in cytotoxicity EC₅₀ is obtained by masking both EGFR and CD3 binding. In addition, the cytotoxicity of the multispecific antibody and multispecific activatable antibodies is EGFR dependent.

[000318] Figure 19 is a graph depicting that the largest shift in activation EC₅₀ is obtained by masking both EGFR and CD3 binding.

[000319] Figures 20A and 20B are a series of graphs depicting that CD3 masked multispecific antibodies show negligible target independent activation as evidenced by CD69+ frequency analysis shown in Figure 20A and CD69 Mean Fluorescence Intensity analysis shown in Figure 20B.

[000320] Figures 21A, 21B, 21C, and 21D are a series of graphs depicting that uPA activation restores binding of the multispecific, activatable CI011 (M-EGFR/M-hCD3) antibody. Cell killing of all multispecific, activatable antibodies is restored by uPA activation.

[000321] Figure 22 is a graph depicting the ability of dually masked multispecific, activatable antibodies that contain different substrates to shift EC₅₀ cytotoxicity.

[000322] Figure 23 is a graph depicting that bispecific antibody CI005 at 0.1 mg/kg dosed twice over 7 days and CI059 (BiTE) at 0.5 mg/kg/day for 8 days prevented the growth of HT-29Luc2 xenograft tumors.

[000323] Figure 24 is a graph depicting that CI048 antibody limited establishment of HT-29Luc2 xenograft tumors and CI011 inhibited the growth of HT-29Luc2 xenograft tumors. The difference in effect between PBS and CI011 is significant ($p < 0.05$).

[000324] Figure 25 is a graph depicting that a dose-response for the CI048 bispecific antibody for elimination of HT-29Luc2 xenograft tumors.

[000325] Figure 26 is a graph depicting that the CI048 bispecific antibody at 0.3 mg/kg eliminated HT-29Luc2 xenograft tumors and the CI040 bispecific activatable antibody at 0.1 mg/kg limited the growth of HT-29Luc2 xenograft tumors.

[000326] Figure 27 is a graph depicting that the CI040 bispecific activatable antibody at 1.0 mg/kg and the CI048 bispecific antibody at 0.3 mg/kg eliminated HT-29Luc2 xenograft tumors.

[000327] Figure 28 is a graph depicting that the CI011 bispecific activatable antibody at 1.0 mg/kg significantly inhibited the HT-29Luc2 xenograft tumor growth ($p < 0.05$).

[000328] Figure 29 is a graph depicting dose-response plots for CI048, CI011 and CI048 for serum alanine aminotransferase (ALT) concentration 48 hours post-dose.

[000329] Figure 30 shows that mouse CD3 ϵ masks incorporated into an EGFR masked, multispecific, activatable antibody shift the EC50 of binding to mouse CD3 while retaining effective EGFR masking.

[000330] Figure 31 demonstrate that killing of IL6R+ Molp-8 cells is maximally attenuated by masking both IL6R and CD3 binding.

DETAILED DESCRIPTION

[000331] The present disclosure provides antibodies, activatable antibodies, multispecific antibodies and/or multispecific activatable antibodies that bind at least the epsilon chain of CD3 (CD3 ϵ). As used herein, an activatable antibody is an antibody that includes a masking moiety (MM) linked to the antigen- or epitope-binding domain of the antibody such that coupling of the MM reduces the ability of the antigen- or epitope-binding domain to bind its target. As used herein, a multispecific antibody is an antibody that recognizes two or more

different antigens or epitopes, and a multispecific activatable antibody is a multispecific antibody that includes at least one masking moiety (MM) linked to at least one antigen- or epitope-binding domain of the multispecific antibody such that coupling of the MM reduces the ability of the antigen- or epitope-binding domain to bind its target. The activatable antibodies and/or activatable multispecific antibodies provided herein are stable in circulation, activated at intended sites of therapy and/or diagnosis but not in normal, *i.e.*, healthy tissue, and, when activated, exhibit binding to a target that is at least comparable to the corresponding, unmodified antibody or multispecific antibody.

[000332] The antibodies, activated activatable antibodies, multispecific antibodies and/or activated multispecific activatable antibodies of the present disclosure bind to CD3 ϵ with a binding constant (K_d) of $\leq 1 \mu\text{M}$, for example, in some embodiments, $\leq 100 \text{ nM}$, $\leq 10 \text{ nM}$, or $\leq 1 \text{ nM}$.

[000333] The anti-CD3 ϵ antibodies, activatable antibodies, multispecific antibodies and/or multispecific activatable antibodies of the disclosure serve to activate T cells via engagement of CD3 ϵ on the T cells. That is, such antibodies agonize, stimulate, activate, and/or augment CD3-mediated T cell activation. Biological activities of CD3 include, for example, T cell activation and other signaling through interaction between CD3 and the antigen-binding subunits of the T-Cell Receptor (TCR). For example, the anti-CD3 ϵ antibodies, activated activatable antibodies, multispecific antibodies and/or activated multispecific activatable antibodies completely or partially activate T cells via engagement of CD3 ϵ on T cells by partially or completely modulating, *e.g.*, agonizing, stimulating, activating or otherwise augmenting CD3-mediated T cell activation.

[000334] Non-limiting examples of multispecific antibodies include bispecific antibodies, trispecific antibodies, tetraspecific antibodies, and other multispecific antibodies. Multispecific antibodies provided herein are also multivalent; as used herein, multivalency refers to the total number of binding sites on the antibody, regardless of whether the binding sites recognize the same or different antigens or epitopes. Non-limiting examples of multispecific activatable antibodies include bispecific activatable antibodies, trispecific activatable antibodies, tetraspecific activatable antibodies, and other multispecific activatable antibodies. Multispecific activatable antibodies provided herein are also multivalent.

[000335] In some embodiments, the multispecific antibodies or fragments thereof and/or multispecific activatable antibodies or fragments thereof are designed to engage T cells and/or other immune effector cells. Multispecific activatable antibodies or fragments thereof that engage T cells are also referred to herein as T-cell engaging multispecific antibodies or fragments thereof and/or T-cell engaging multispecific activatable antibodies or fragments thereof. Multispecific activatable antibodies or fragments thereof that engage immune effector cells are also referred to herein as immune effector cell engaging multispecific antibodies or fragments thereof and/or immune effector cell engaging multispecific activatable antibodies or fragments thereof. In some embodiments, the multispecific antibodies or fragments thereof and/or multispecific activatable antibodies or fragments thereof are designed to bind or otherwise interact with more than one target and/or more than one epitope, also referred to herein as multi-antigen targeting antibodies or fragments thereof and/or multi-antigen targeting activatable antibodies or fragments thereof.

[000336] In some embodiments, a multispecific antibody or fragment thereof includes an IgG domain and a scFv domain. In some embodiments, a multispecific antibody or fragment thereof includes an IgG variable domain and a scFv domain. In some embodiments, one antibody domain of a multispecific antibody or fragment thereof has specificity for a target antigen and another antibody domain has specificity for a T-cell surface antigen. In some embodiments, one antibody domain of a multispecific antibody or fragment thereof has specificity for a target antigen and another antibody domain has specificity for another target antigen. In some embodiments, one antibody domain of a multispecific antibody or fragment thereof has specificity for an epitope of a target antigen and another antibody domain has specificity for another epitope of the same target antigen.

[000337] In some embodiments, the multispecific activatable antibodies are engineered to include a masking moiety (MM) that is coupled to an antibody or antigen-binding fragment thereof (AB) via a non-cleavable linker. For example, in some embodiments, the multispecific activatable antibody is a T-cell engaging multispecific activatable antibody that includes a targeting antibody or antigen-binding fragment thereof and a T-cell engaging antibody or antigen-binding portion thereof, where the T-cell engaging antibody or antigen-binding fragment thereof includes a first antibody or antigen-binding fragment thereof (AB1) that binds a first, T-cell engaging target, where the AB1 is attached via non-cleavable linker to a masking

moiety (MM1) such that coupling of the MM reduces the ability of the AB1 to bind the first target, and the targeting antibody or antigen-binding fragment thereof includes a second antibody or antigen-binding fragment thereof (AB2) that binds a second target, where the AB2 is attached via a cleavable linker to a masking moiety (MM2) such that coupling of the MM reduces the ability of the AB2 to bind the second target.

[000338] In some embodiments, the multispecific activatable antibody is a T-cell engaging multispecific activatable antibody that includes a targeting antibody or antigen-binding fragment thereof and a T-cell engaging antibody or antigen-binding portion thereof, where the T-cell engaging antibody or antigen-binding fragment thereof includes a first antibody or antigen-binding fragment thereof (AB1) that binds a first, T-cell engaging target, where the AB1 is attached via non-cleavable linker to a masking moiety (MM1) such that coupling of the MM reduces the ability of the AB1 to bind the first target, and the targeting antibody or antigen-binding fragment thereof is unmasked.

Definitions

[000339] Unless otherwise defined, scientific and technical terms used in connection with the present disclosure shall have the meanings that are commonly understood by those of ordinary skill in the art. The term “a” entity or “an” entity refers to one or more of that entity. For example, a compound refers to one or more compounds. As such, the terms “a”, “an”, “one or more” and “at least one” can be used interchangeably. Further, unless otherwise required by context, singular terms shall include pluralities and plural terms shall include the singular. Generally, nomenclatures utilized in connection with, and techniques of, cell and tissue culture, molecular biology, and protein and oligo- or polynucleotide chemistry and hybridization described herein are those well-known and commonly used in the art. Standard techniques are used for recombinant DNA, oligonucleotide synthesis, and tissue culture and transformation (*e.g.*, electroporation, lipofection). Enzymatic reactions and purification techniques are performed according to manufacturer's specifications or as commonly accomplished in the art or as described herein. The foregoing techniques and procedures are generally performed according to conventional methods well known in the art and as described in various general and more specific references that are cited and discussed throughout the present specification. *See e.g.*, Sambrook *et al.* Molecular Cloning: A Laboratory Manual (2d ed., Cold Spring

Harbor Laboratory Press, Cold Spring Harbor, N.Y. (1989)). The nomenclatures utilized in connection with, and the laboratory procedures and techniques of, analytical chemistry, synthetic organic chemistry, and medicinal and pharmaceutical chemistry described herein are those well-known and commonly used in the art. Standard techniques are used for chemical syntheses, chemical analyses, pharmaceutical preparation, formulation, and delivery, and treatment of patients.

[000340] As utilized in accordance with the present disclosure, the following terms, unless otherwise indicated, shall be understood to have the following meanings:

[000341] As used herein, the term “antibody” refers to immunoglobulin molecules and antigen-binding portions of immunoglobulin (Ig) molecules, *i.e.*, molecules that contain an antigen binding site that specifically binds (immunoreacts with) an antigen. By “specifically bind” or “immunoreacts with” or “immunospecifically bind” is meant that the antibody reacts with one or more antigenic determinants of the desired antigen and does not react with other polypeptides or binds at much lower affinity ($K_d > 10^{-6}$). Antibodies include, but are not limited to, polyclonal, monoclonal, chimeric, fully human, domain antibody, single chain, Fab, and F(ab')₂ fragments, scFvs, and an Fab expression library.

[000342] The basic antibody structural unit is known to comprise a tetramer. Each tetramer is composed of two identical pairs of polypeptide chains, each pair having one “light” (about 25 kDa) and one “heavy” chain (about 50-70 kDa). The amino-terminal portion of each chain includes a variable region of about 100 to 110 or more amino acids primarily responsible for antigen recognition. The carboxy-terminal portion of each chain defines a constant region primarily responsible for effector function. In general, antibody molecules obtained from humans relate to any of the classes IgG, IgM, IgA, IgE and IgD, which differ from one another by the nature of the heavy chain present in the molecule. Certain classes have subclasses as well, such as IgG₁, IgG₂, IgG₃, IgG₄, and others. Furthermore, in humans, the light chain may be a kappa chain or a lambda chain.

[000343] The term “monoclonal antibody” (mAb) or “monoclonal antibody composition”, as used herein, refers to a population of antibody molecules that contain only one molecular species of antibody molecule consisting of a unique light chain gene product and a unique heavy chain gene product. In particular, the complementarity determining regions (CDRs) of the monoclonal antibody are identical in all the molecules of the population. MAbs contain an

antigen binding site capable of immunoreacting with a particular epitope of the antigen characterized by a unique binding affinity for it.

[000344] The term “antigen-binding site” or “binding portion” refers to the part of the immunoglobulin molecule that participates in antigen binding. The antigen binding site is formed by amino acid residues of the N-terminal variable (“V”) regions of the heavy (“H”) and light (“L”) chains. Three highly divergent stretches within the V regions of the heavy and light chains, referred to as “hypervariable regions,” are interposed between more conserved flanking stretches known as “framework regions,” or “FRs”. Thus, the term “FR” refers to amino acid sequences that are naturally found between, and adjacent to, hypervariable regions in immunoglobulins. In an antibody molecule, the three hypervariable regions of a light chain and the three hypervariable regions of a heavy chain are disposed relative to each other in three dimensional space to form an antigen-binding surface. The antigen-binding surface is complementary to the three-dimensional surface of a bound antigen, and the three hypervariable regions of each of the heavy and light chains are referred to as “complementarity-determining regions,” or “CDRs.” The assignment of amino acids to each domain is in accordance with the definitions of Kabat Sequences of Proteins of Immunological Interest (National Institutes of Health, Bethesda, Md. (1987 and 1991)), or Chothia & Lesk J. Mol. Biol. 196:901-917 (1987), Chothia *et al.* Nature 342:878-883 (1989).

[000345] As used herein, the term “epitope” includes any protein determinant capable of specific binding to an immunoglobulin, an scFv, or a T-cell receptor. The term “epitope” includes any protein determinant capable of specific binding to an immunoglobulin or T-cell receptor. Epitopic determinants usually consist of chemically active surface groupings of molecules such as amino acids or sugar side chains and usually have specific three dimensional structural characteristics, as well as specific charge characteristics. For example, antibodies may be raised against N-terminal or C-terminal peptides of a polypeptide. An antibody is said to specifically bind an antigen when the dissociation constant is $\leq 1 \mu\text{M}$; for example, in some embodiments $\leq 100 \text{ nM}$ and in some embodiments, $\leq 10 \text{ nM}$.

[000346] As used herein, the terms “specific binding,” “immunological binding,” and “immunological binding properties” refer to the non-covalent interactions of the type that occur between an immunoglobulin molecule and an antigen for which the immunoglobulin is specific. The strength, or affinity of immunological binding interactions can be expressed in

terms of the dissociation constant (K_d) of the interaction, wherein a smaller K_d represents a greater affinity. Immunological binding properties of selected polypeptides can be quantified using methods well known in the art. One such method entails measuring the rates of antigen-binding site/antigen complex formation and dissociation, wherein those rates depend on the concentrations of the complex partners, the affinity of the interaction, and geometric parameters that equally influence the rate in both directions. Thus, both the “on rate constant” (K_{on}) and the “off rate constant” (K_{off}) can be determined by calculation of the concentrations and the actual rates of association and dissociation. (*See* Nature 361:186-87 (1993)). The ratio of K_{off}/K_{on} enables the cancellation of all parameters not related to affinity, and is equal to the dissociation constant K_d . (*See, generally*, Davies et al. (1990) Annual Rev Biochem 59:439-473). An antibody of the present disclosure is said to specifically bind to EGFR, when the binding constant (K_d) is $\leq 1 \mu\text{M}$, for example in some embodiments $\leq 100 \text{ nM}$, in some embodiments $\leq 10 \text{ nM}$, and in some embodiments $\leq 100 \text{ pM}$ to about 1 pM , as measured by assays such as radioligand binding assays or similar assays known to those skilled in the art.

[000347] The term “isolated polynucleotide” as used herein shall mean a polynucleotide of genomic, cDNA, or synthetic origin or some combination thereof, which by virtue of its origin the “isolated polynucleotide” (1) is not associated with all or a portion of a polynucleotide in which the “isolated polynucleotide” is found in nature, (2) is operably linked to a polynucleotide that it is not linked to in nature, or (3) does not occur in nature as part of a larger sequence. Polynucleotides in accordance with the disclosure include the nucleic acid molecules encoding the heavy chain immunoglobulin molecules shown herein, and nucleic acid molecules encoding the light chain immunoglobulin molecules shown herein.

[000348] The term “isolated protein” referred to herein means a protein of cDNA, recombinant RNA, or synthetic origin or some combination thereof, which by virtue of its origin, or source of derivation, the “isolated protein” (1) is not associated with proteins found in nature, (2) is free of other proteins from the same source, *e.g.*, free of murine proteins, (3) is expressed by a cell from a different species, or (4) does not occur in nature.

[000349] The term “polypeptide” is used herein as a generic term to refer to native protein, fragments, or analogs of a polypeptide sequence. Hence, native protein fragments, and analogs are species of the polypeptide genus. Polypeptides in accordance with the disclosure

comprise the heavy chain immunoglobulin molecules shown herein, and the light chain immunoglobulin molecules shown herein, as well as antibody molecules formed by combinations comprising the heavy chain immunoglobulin molecules with light chain immunoglobulin molecules, such as kappa light chain immunoglobulin molecules, and vice versa, as well as fragments and analogs thereof.

[000350] The term “naturally-occurring” as used herein as applied to an object refers to the fact that an object can be found in nature. For example, a polypeptide or polynucleotide sequence that is present in an organism (including viruses) that can be isolated from a source in nature and that has not been intentionally modified by man in the laboratory or otherwise is naturally-occurring.

[000351] The term “operably linked” as used herein refers to positions of components so described are in a relationship permitting them to function in their intended manner. A control sequence “operably linked” to a coding sequence is ligated in such a way that expression of the coding sequence is achieved under conditions compatible with the control sequences.

[000352] The term “control sequence” as used herein refers to polynucleotide sequences that are necessary to effect the expression and processing of coding sequences to which they are ligated. The nature of such control sequences differs depending upon the host organism in prokaryotes, such control sequences generally include promoter, ribosomal binding site, and transcription termination sequence in eukaryotes, generally, such control sequences include promoters and transcription termination sequence. The term “control sequences” is intended to include, at a minimum, all components whose presence is essential for expression and processing, and can also include additional components whose presence is advantageous, for example, leader sequences and fusion partner sequences. The term “polynucleotide” as referred to herein means nucleotides of at least 10 bases in length, either ribonucleotides or deoxynucleotides or a modified form of either type of nucleotide. The term includes single and double stranded forms of DNA.

[000353] The term oligonucleotide referred to herein includes naturally occurring, and modified nucleotides linked together by naturally occurring, and non-naturally occurring oligonucleotide linkages. Oligonucleotides are a polynucleotide subset generally comprising a length of 200 bases or fewer. In some embodiments, oligonucleotides are 10 to 60 bases in length, for example in some embodiments, 12, 13, 14, 15, 16, 17, 18, 19, or 20 to 40 bases in

length. Oligonucleotides are usually single stranded, *e.g.*, for probes, although oligonucleotides may be double stranded, *e.g.*, for use in the construction of a gene mutant. Oligonucleotides of the disclosure are either sense or antisense oligonucleotides.

[000354] The term “naturally occurring nucleotides” referred to herein includes deoxyribonucleotides and ribonucleotides. The term “modified nucleotides” referred to herein includes nucleotides with modified or substituted sugar groups and the like. The term “oligonucleotide linkages” referred to herein includes oligonucleotide linkages such as phosphorothioate, phosphorodithioate, phosphoroselenoate, phosphorodiselenoate, phosphoroanilothioate, phosphoraniladate, phosphoronmidate, and the like. *See e.g.*, LaPlanche *et al.* Nucl. Acids Res. 14:9081 (1986); Stec *et al.* J. Am. Chem. Soc. 106:6077 (1984), Stein *et al.* Nucl. Acids Res. 16:3209 (1988), Zon *et al.* Anti Cancer Drug Design 6:539 (1991); Zon *et al.* Oligonucleotides and Analogues: A Practical Approach, pp. 87-108 (F. Eckstein, Ed., Oxford University Press, Oxford England (1991)); Stec *et al.* U.S. Patent No. 5,151,510; Uhlmann and Peyman Chemical Reviews 90:543 (1990). An oligonucleotide can include a label for detection, if desired.

[000355] As used herein, the twenty conventional amino acids and their abbreviations follow conventional usage. *See Immunology - A Synthesis* (2nd Edition, E.S. Golub and D.R. Gren, Eds., Sinauer Associates, Sunderland7 Mass. (1991)). Stereoisomers (*e.g.*, D- amino acids) of the twenty conventional amino acids, unnatural amino acids such as α -, α -disubstituted amino acids, N-alkyl amino acids, lactic acid, and other unconventional amino acids may also be suitable components for polypeptides of the present disclosure. Examples of unconventional amino acids include: 4 hydroxyproline, γ -carboxyglutamate, ϵ -N,N,N-trimethyllysine, ϵ -N-acetyllysine, O-phosphoserine, N- acetylserine, N-formylmethionine, 3-methylhistidine, 5-hydroxylysine, σ -N-methylarginine, and other similar amino acids and imino acids (*e.g.*, 4- hydroxyproline). In the polypeptide notation used herein, the left-hand direction is the amino terminal direction and the right-hand direction is the carboxy-terminal direction, in accordance with standard usage and convention.

[000356] Similarly, unless specified otherwise, the left-hand end of single- stranded polynucleotide sequences is the 5' end the left-hand direction of double-stranded polynucleotide sequences is referred to as the 5' direction. The direction of 5' to 3' addition of nascent RNA

transcripts is referred to as the transcription direction sequence regions on the DNA strand having the same sequence as the RNA and that are 5' to the 5' end of the RNA transcript are referred to as “upstream sequences”, sequence regions on the DNA strand having the same sequence as the RNA and that are 3' to the 3' end of the RNA transcript are referred to as “downstream sequences”.

[000357] As applied to polypeptides, the term “substantial identity” means that two peptide sequences, when optimally aligned, such as by the programs GAP or BESTFIT using default gap weights, share at least 80 percent sequence identity, for example in some embodiments, at least 90 percent sequence identity, in some embodiments at least 95 percent sequence identity, and in some embodiments at least 99 percent sequence identity.

[000358] In some embodiments, residue positions that are not identical differ by conservative amino acid substitutions.

[000359] As discussed herein, minor variations in the amino acid sequences of antibodies or immunoglobulin molecules are contemplated as being encompassed by the present disclosure, providing that the variations in the amino acid sequence maintain at least 75%, for example in some embodiments at least 80%, 90%, 95%, and in some embodiments 99%. In particular, conservative amino acid replacements are contemplated. Conservative replacements are those that take place within a family of amino acids that are related in their side chains. Genetically encoded amino acids are generally divided into families: (1) acidic amino acids are aspartate, glutamate; (2) basic amino acids are lysine, arginine, histidine; (3) non-polar amino acids are alanine, valine, leucine, isoleucine, proline, phenylalanine, methionine, tryptophan, and (4) uncharged polar amino acids are glycine, asparagine, glutamine, cysteine, serine, threonine, tyrosine. The hydrophilic amino acids include arginine, asparagine, aspartate, glutamine, glutamate, histidine, lysine, serine, and threonine. The hydrophobic amino acids include alanine, cysteine, isoleucine, leucine, methionine, phenylalanine, proline, tryptophan, tyrosine and valine. Other families of amino acids include (i) serine and threonine, which are the aliphatic-hydroxy family; (ii) asparagine and glutamine, which are the amide containing family; (iii) alanine, valine, leucine and isoleucine, which are the aliphatic family; and (iv) phenylalanine, tryptophan, and tyrosine, which are the aromatic family. For example, it is reasonable to expect that an isolated replacement of a leucine with an isoleucine or valine, an aspartate with a glutamate, a threonine with a serine, or a similar replacement of an amino acid

with a structurally related amino acid will not have a major effect on the binding or properties of the resulting molecule, especially if the replacement does not involve an amino acid within a framework site. Whether an amino acid change results in a functional peptide can readily be determined by assaying the specific activity of the polypeptide derivative. Assays are described in detail herein. Fragments or analogs of antibodies or immunoglobulin molecules can be readily prepared by those of ordinary skill in the art. In some embodiments, amino- and carboxy-termini of fragments or analogs occur near boundaries of functional domains. Structural and functional domains can be identified by comparison of the nucleotide and/or amino acid sequence data to public or proprietary sequence databases. Computerized comparison methods are used to identify sequence motifs or predicted protein conformation domains that occur in other proteins of known structure and/or function. Methods to identify protein sequences that fold into a known three-dimensional structure are known. Bowie *et al.* Science 253:164 (1991). Thus, the foregoing examples demonstrate that those of skill in the art can recognize sequence motifs and structural conformations that may be used to define structural and functional domains in accordance with the disclosure.

[000360] In some embodiments, amino acid substitutions are those that: (1) reduce susceptibility to proteolysis, (2) reduce susceptibility to oxidation, (3) alter binding affinity for forming protein complexes, (4) alter binding affinities, and (4) confer or modify other physicochemical or functional properties of such analogs. Analogs can include various muteins of a sequence other than the naturally-occurring peptide sequence. For example, single or multiple amino acid substitutions (for example, conservative amino acid substitutions) may be made in the naturally- occurring sequence (for example, in the portion of the polypeptide outside the domain(s) forming intermolecular contacts. A conservative amino acid substitution should not substantially change the structural characteristics of the parent sequence (*e.g.*, a replacement amino acid should not tend to break a helix that occurs in the parent sequence, or disrupt other types of secondary structure that characterizes the parent sequence). Examples of art-recognized polypeptide secondary and tertiary structures are described in Proteins, Structures and Molecular Principles (Creighton, Ed., W. H. Freeman and Company, New York (1984)); Introduction to Protein Structure (C. Branden and J. Tooze, eds., Garland Publishing, New York, N.Y. (1991)); and Thornton et al. Nature 354:105 (1991).

[000361] The term “polypeptide fragment” as used herein refers to a polypeptide that has an amino terminal and/or carboxy-terminal deletion and/or one or more internal deletion(s), but where the remaining amino acid sequence is identical to the corresponding positions in the naturally-occurring sequence deduced, for example, from a full length cDNA sequence. Fragments typically are at least 5, 6, 8 or 10 amino acids long, for example in some embodiments at least 14 amino acids long, in some embodiments at least 20 amino acids long, usually at least 50 amino acids long, and in some embodiments at least 70 amino acids long. The term “analog” as used herein refers to polypeptides that are comprised of a segment of at least 25 amino acids that has substantial identity to a portion of a deduced amino acid sequence and that has specific binding to EGFR, under suitable binding conditions. Typically, polypeptide analogs comprise a conservative amino acid substitution (or addition or deletion) with respect to the naturally- occurring sequence. Analogs typically are at least 20 amino acids long, for example in some embodiments at least 50 amino acids long or longer, and can often be as long as a full-length naturally-occurring polypeptide.

[000362] The term “agent” is used herein to denote a chemical compound, a mixture of chemical compounds, a biological macromolecule, or an extract made from biological materials.

[000363] As used herein, the terms “label” or “labeled” refers to incorporation of a detectable marker, *e.g.*, by incorporation of a radiolabeled amino acid or attachment to a polypeptide of biotinyl moieties that can be detected by marked avidin (*e.g.*, streptavidin containing a fluorescent marker or enzymatic activity that can be detected by optical or calorimetric methods). In certain situations, the label or marker can also be therapeutic. Various methods of labeling polypeptides and glycoproteins are known in the art and may be used. Examples of labels for polypeptides include, but are not limited to, the following: radioisotopes or radionuclides (*e.g.*, ^3H , ^{14}C , ^{15}N , ^{35}S , ^{90}Y , ^{99}Tc , ^{111}In , ^{125}I , ^{131}I), fluorescent labels (*e.g.*, a fluorophore, rhodamine, lanthanide phosphors), enzymatic labels (*e.g.*, horseradish peroxidase, p-galactosidase, luciferase, alkaline phosphatase), chemiluminescent, biotinyl groups, predetermined polypeptide epitopes recognized by a secondary reporter (*e.g.*, leucine zipper pair sequences, binding sites for secondary antibodies, metal binding domains, epitope tags). In some embodiments, labels are attached by spacer arms of various lengths to reduce potential steric hindrance. The term “pharmaceutical agent or drug” as used herein refers to a chemical

compound or composition capable of inducing a desired therapeutic effect when properly administered to a patient.

[000364] As used herein, “substantially pure” means an object species is the predominant species present (*i.e.*, on a molar basis it is more abundant than any other individual species in the composition), and a substantially purified fraction is a composition wherein the object species comprises at least about 50 percent (on a molar basis) of all macromolecular species present.

[000365] Generally, a substantially pure composition will comprise more than about 80 percent of all macromolecular species present in the composition, for example, in some embodiments, more than about 85%, 90%, 95%, and 99%. In some embodiments, the object species is purified to essential homogeneity (contaminant species cannot be detected in the composition by conventional detection methods) wherein the composition consists essentially of a single macromolecular species.

[000366] The term patient includes human and veterinary subjects.

[000367] Other chemistry terms herein are used according to conventional usage in the art, as exemplified by The McGraw-Hill Dictionary of Chemical Terms (Parker, S., Ed., McGraw-Hill, San Francisco (1985)).

Multispecific Antibodies and Multispecific Activatable Antibodies

[000368] Exemplary multispecific antibodies and/or multispecific activatable antibodies of the disclosure include, for example, those shown in the Examples provided herein, and variants thereof.

[000369] In some non-limiting embodiments, at least one of the AB in the multispecific antibody is specific for CD3 ϵ and at least one other AB is a binding partner for any target listed in Table 1.

Table 1: Exemplary Targets

1-92-LFA-3	CD52	DL44	HVEM	LAG-3	STEAP1
Alpha-4 integrin	CD56	DLK1	Hyaluronidase	LIF-R	STEAP2
Alpha-V integrin	CD64	DLL4	ICOS	Lewis X	TAG-72
alpha4beta1 integrin	CD70	DPP-4	IFNalpha	LIGHT	TAPA1

alpha4beta7 integrin	CD71	DSG1	IFNbeta	LRP4	TGFbeta
AGR2	CD74	EGFR	IFNgamma	LRRC26	TIGIT
Anti-Lewis-Y		EGFRviii	IgE	MCSP	TIM-3
Apelin J receptor	CD80	Endothelin B receptor (ETBR)	IgE Receptor (FcεRI)	Mesothelin	TLR2
APRIL	CD81	ENPP3	IGF	MRP4	TLR4
B7-H4	CD86	EpCAM	IGF1R	MUC1	TLR6
BAFF	CD95	EPHA2	IL1B	Mucin-16 (MUC16, CA-125)	TLR7
BTLA	CD117	EPHB2	IL1R	Na/K ATPase	TLR8
C5 complement	CD125	ERBB3	IL2	Neutrophil elastase	TLR9
C-242	CD132 (IL-2RG)	F protein of RSV	IL11	NGF	TMEM31
CA9	CD133	FAP	IL12	Nicastrin	TNFAalpha
CA19-9 (Lewis a)	CD137	FGF-2	IL12p40	Notch Receptors	TNFR
Carbonic anhydrase 9	CD138	FGF8	IL-12R, IL-12Rbeta1	Notch 1	TNFRS12 A
CD2	CD166	FGFR1	IL13	Notch 2	TRAIL-R1
CD3	CD172A	FGFR2	IL13R	Notch 3	TRAIL-R2
CD6	CD248	FGFR3	IL15	Notch 4	Transferrin
CD9	CDH6	FGFR4	IL17	NOV	Transferrin receptor
CD11a	CEACAM5 (CEA)	Folate receptor	IL18	OSM-R	TRK-A
CD19	CEACAM6 (NCA-90)	GAL3ST1	IL21	OX-40	TRK-B
CD20	CLAUDIN-3	G-CSF	IL23	PAR2	uPAR
CD22	CLAUDIN-4	G-CSFR	IL23R	PDGF-AA	VAP1
CD24	cMet	GD2	IL27/IL27R (wsx1)	PDGF-BB	VCAM-1
CD25	Collagen	GITR	IL29	PDGFRalpha	VEGF
CD27	Cripto	GLUT1	IL-31R	PDGFRbeta	VEGF-A
CD28	CSFR	GLUT4	IL31/IL31R	PD-1	VEGF-B
CD30	CSFR-1	GM-CSF	IL2R	PD-L1	VEGF-C
CD33	CTLA-4	GM-CSFR	IL4	PD-L2	VEGF-D
CD38	CTGF	GP IIb/IIIa receptors	IL4R	Phosphatidyl-serine	VEGFR1
CD40	CXCL10	Gp130	IL6, IL6R	P1GF	VEGFR2

CD40L	CXCL13	GPIIB/IIIA	Insulin Receptor	PSCA	VEGFR3
CD41	CXCR1	GPNMB	Jagged Ligands	PSMA	VISTA
CD44	CXCR2	GRP78	Jagged 1	RAAG12	WISP-1
CD44v6		HER2/neu	Jagged 2	RAGE	WISP-2
CD47	CXCR4	HGF		SLC44A4	WISP-3
CD51	CYR61	hGH		Sphingosine 1 Phosphate	

[000370] In some non-limiting embodiments, at least one of the AB of the multispecific antibody is or is derived from a sequence set forth in the Examples provided herein.

[000371] In some non-limiting embodiments, at least one of the AB of the multispecific antibody is or is derived from an antibody listed in Table 2.

Table 2: Exemplary sources for ABs

Antibody Trade Name (antibody name)	Target
Avastin™ (bevacizumab)	VEGF
Lucentis™ (ranibizumab)	VEGF
Erbitux™ (cetuximab)	EGFR
Vectibix™ (panitumumab)	EGFR
Remicade™ (infliximab)	TNF α
Humira™ (adalimumab)	TNF α
Tysabri™ (natalizumab)	Integrin α 4
Simulect™ (basiliximab)	IL2R
Soliris™ (eculizumab)	Complement C5
Raptiva™ (efalizumab)	CD11a
Bexxar™ (tositumomab)	CD20
Zevalin™ (ibritumomab tiuxetan)	CD20
Rituxan™ (rituximab)	CD20
Ocrelizumab	CD20
Arzerra™ (ofatumumab)	CD20
Obinutuzumab	CD20
Zenapax™ (daclizumab)	CD25
Adcetris™ (brentuximab vedotin)	CD30
Myelotarg™ (gemtuzumab)	CD33
Mylotarg™ (gemtuzumab ozogamicin)	CD33
Campath™ (alemtuzumab)	CD52
ReoPro™ (abciximab)	Glycoprotein receptor IIb/IIIa
Xolair™ (omalizumab)	IgE

Herceptin™ (trastuzumab)	Her2
Kadcyla™ (trastuzumab emtansine)	Her2
Synagis™ (palivizumab)	F protein of RSV
Yervoy™ (ipilimumab)	CTLA-4
(tremelimumab)	CTLA-4
Hu5c8	CD40L
(pertuzumab)	Her2-neu
(ertumaxomab)	CD3/Her2-neu
Orencia™ (abatacept)	CTLA-4
(tanezumab)	NGF
(bavituximab)	Phosphatidylserine
(zalutumumab)	EGFR
(mapatumumab)	EGFR
(matuzumab)	EGFR
(nimotuzumab)	EGFR
ICR62	EGFR
mAb 528	EGFR
CH806	EGFR
MDX-447	EGFR/CD64
(edrecolomab)	EpCAM
RAV12	RAAG12
huJ591	PSMA
Enbrel™ (etanercept)	TNF-R
Amevive™ (alefacept)	1-92-LFA-3
Anril™, Kineret™ (ankinra)	IL-1Ra
GC1008	TGFbeta
	Notch, e.g., Notch 1
	Jagged 1 or Jagged 2
(adecatumumab)	EpCAM
(figitumumab)	IGF1R
(tocilizumab)	IL-6 receptor
Stelara™ (ustekinumab)	IL-12/IL-23
Prolia™ (denosumab)	RANKL
Keytruda (Pembrolizumab)	PD-1
Opdivo (Nivolumab)	PD-1
Atezolizumab (MPDL3280A)	PD-L1

[000372] Exemplary antibodies, activatable antibodies and antigen-binding fragments thereof that bind CD3ε of the disclosure include the CD3ε binding sequences described in the Examples, for example in Examples 2 and 4. Exemplary multispecific antibodies and multispecific activatable antibodies include the multispecific antibodies and multispecific activatable antibodies described in the Examples, for example in Example 4.

[000373] In some embodiments, the multispecific activatable antibody includes a heavy chain sequence selected from the group consisting of SEQ ID NOs: 446, 452, 454, 456, 460, 462, 464, 466, 470, 472, 476, 478, 480, 486, 488, 490, 492, 494, 496, 498, 500, 502, 504, 510, 512, 514, 518, 524, 526, 530, 532, 534, 536, 538, 540, 542, 544, and 546.

[000374] In some embodiments, the multispecific activatable antibody includes a light chain sequence selected from the group consisting of SEQ ID NOs: 448, 450, 458, 468, 474, 482, 484, 508, 516, and 520.

[000375] In some embodiments, the multispecific activatable antibody includes a heavy chain sequence selected from the group consisting of SEQ ID NOs: 446, 452, 454, 456, 460, 462, 464, 466, 470, 472, 476, 478, 480, 486, 488, 490, 492, 494, 496, 498, 500, 502, 504, 510, 512, 514, 518, 524, 526, 530, 532, 534, 536, 538, 540, 542, 544, and 546; and a light chain sequence selected from the group consisting of SEQ ID NOs: 448, 450, 458, 468, 474, 482, 484, 508, 516, and 520.

[000376] In some embodiments, the multispecific activatable antibody includes a heavy chain sequence that is at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98% or 99% identical to an amino acid sequence selected from the group consisting of SEQ ID NOs: 446, 452, 454, 456, 460, 462, 464, 466, 470, 472, 476, 478, 480, 486, 488, 490, 492, 494, 496, 498, 500, 502, 504, 510, 512, 514, 518, 524, 526, 530, 532, 534, 536, 538, 540, 542, 544, and 546.

[000377] In some embodiments, the multispecific activatable antibody includes a light chain sequence that is at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98% or 99% identical to an amino acid sequence selected from the group consisting of SEQ ID NOs: 448, 450, 458, 468, 474, 482, 484, 508, 516, and 520.

[000378] In some embodiments, the multispecific activatable antibody includes a heavy chain sequence that is at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98% or 99% identical to an amino acid sequence selected from the group consisting of SEQ ID NOs: 446, 452, 454, 456, 460, 462, 464, 466, 470, 472, 476, 478, 480, 486, 488, 490, 492, 494, 496, 498, 500, 502, 504, 510, 512, 514, 518, 524, 526, 530, 532, 534, 536, 538, 540, 542, 544, and 546; and a light chain sequence that is at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98% or 99% identical to an amino acid sequence selected from the group consisting of SEQ ID NOs: 448, 450, 458, 468, 474, 482, 484, 508, 516, and 520.

[000379] In some embodiments, the multispecific activatable antibody includes the amino acid sequence of SEQ ID NO: 506. In some embodiments, the multispecific activatable antibody includes an amino acid sequence that is at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98% or 99% identical to the amino acid sequence of SEQ ID NO: 506.

[000380] Exemplary ABs that bind CD3 ϵ of the disclosure include the CD3 ϵ binding sequences shown below:

CD3H_vL_v

EVQLVESGGGLVQPGGSLKLSCAASGFTFNKYAMNWVRQAPGKGLEWVARIRSKYNNYATYYADSVKDRFTISRDD
SKNTAYLQMNNLKTEDTAVYYCVRHGNFGNSYISYWAYWGQGLTVTVSSGGGGSGGGGSGGGGSQTVVTQEPSLTV
SPGGTVTLTCSSTGAVTSGYYPNWVQQKPGQAPRGLIGGTKFLAPGTPARFSGSLLGGKAALTLSGVQPEDEAEY
YCALWYSNRWVFGGGTKLTVL (SEQ ID NO: 587)

CD3L_vH_v

QTVVTQEPSLTVSPGGTVTLTCRSSTGAVTTSNYANWVQQKPGQAPRGLIGGTNKRAPGTPARFSGSLLGGKAALT
LSGVQPEDEAEYYCALWYSNLWVFGGGTKLTVLGGGGSGGGGSGGGGSEVQLVESGGGLVQPGGSLKLSCAASGFT
FNTYAMNWVRQAPGKGLEWVARIRSKYNNYATYYADSVKDRFTISRDDSKNTAYLQMNNLKTEDTAVYYCVRHGNF
GNSYVSWFAYWGQGLTVTVSS (SEQ ID NO: 588)

[000381] In some embodiments, the activatable anti-CD3 ϵ antibody includes a heavy chain that comprises or is derived from a heavy chain amino acid sequence shown in Table 17. In some embodiments, the activatable anti-CD3 ϵ antibody includes a light chain that comprises or is derived from a light chain amino acid sequence shown in Table 17. In some embodiments, the activatable anti-CD3 ϵ antibody includes a heavy chain that comprises or is derived from a heavy chain amino acid sequence shown in Table 17, and a light chain that comprises or is derived from a light chain amino acid sequence shown in Table 17. In some embodiments, the activatable anti-CD3 ϵ antibody includes a combination of a heavy chain variable region sequence and a light chain variable region sequence from the combinations shown in Group A in Table 17. In some embodiments, the activatable anti-CD3 ϵ antibody includes a combination of a heavy chain variable region sequence and a light chain variable region sequence from the combinations shown in Group B in Table 17. In some embodiments, the activatable anti-CD3 ϵ antibody includes a combination of a heavy chain variable region sequence and a light chain variable region sequence from the combinations shown in Group C in Table 17. In some

embodiments, the activatable anti-CD3 ϵ antibody includes a combination of a heavy chain variable region sequence and a light chain variable region sequence from the combinations shown in Group D in Table 17. In some embodiments, the activatable anti-CD3 ϵ antibody includes a combination of a heavy chain variable region sequence and a light chain variable region sequence from the combinations shown in Group E in Table 17. In some embodiments, the activatable anti-CD3 ϵ antibody includes a combination of a heavy chain variable region sequence and a light chain variable region sequence from the combinations shown in Group F in Table 17. In some embodiments, the activatable anti-CD3 ϵ antibody includes a combination of a heavy chain variable region sequence and a light chain variable region sequence from the combinations shown in Group G in Table 17. In some embodiments, the activatable anti-CD3 ϵ antibody includes a combination of a heavy chain variable region sequence and a light chain variable region sequence from the combinations shown in Group H in Table 17. In some embodiments, the activatable anti-CD3 ϵ antibody includes a combination of a heavy chain variable region sequence and a light chain variable region sequence from the combinations shown in Group I in Table 17. In some embodiments, the activatable anti-CD3 ϵ antibody includes a combination of a heavy chain variable region sequence and a light chain variable region sequence from the combinations shown in Group J in Table 17. In some embodiments, the activatable anti-CD3 ϵ antibody includes a combination of a heavy chain variable region sequence and a light chain variable region sequence from the combinations shown in Group K in Table 17. In some embodiments, the activatable anti-CD3 ϵ antibody includes a combination of a heavy chain variable region sequence and a light chain variable region sequence from the combinations shown in Group L in Table 17. In some embodiments, the activatable anti-CD3 ϵ antibody includes a combination of a heavy chain variable region sequence and a light chain variable region sequence from the combinations shown in Group M in Table 17. In some embodiments, the activatable anti-CD3 ϵ antibody includes a combination of a heavy chain variable region sequence and a light chain variable region sequence from the combinations shown in Group N in Table 17. In some embodiments, the activatable anti-CD3 ϵ antibody includes a combination of a heavy chain variable region sequence and a light chain variable region sequence from the combinations shown in Group O in Table 17. In some embodiments, the activatable anti-CD3 ϵ antibody includes a combination of a heavy chain variable region

sequence and a light chain variable region sequence from the combinations shown in Group P in Table 17. In some embodiments, the activatable anti-CD3 ϵ antibody includes a combination of a heavy chain variable region sequence and a light chain variable region sequence from the combinations shown in Group Q in Table 17.

Table 17. Variable Heavy Chain Region (VH) and Variable Light Chain Region (VL)
Sequences for Activatable Antibodies that Bind CD3 ϵ

Group A	
VH	QIVLTQSPAIMASAPGEKVTMTCSASSSVSYMNWYQQKSGTSPKRWIYDTSKLGASGVPAAHFRGSGSGTSSYSL TISGMEAEDAATYYCQQWSSNPFT (SEQ ID NO: 589)
VH	QVQLQQSGAELARPGASVKMSCKASGYTFTRYTMHWVKQRPGQGLEWIGYINPSRGYTNYNQKFKDKATLT DKSSSTAYMQLSSLTSEDSAVYYCARYYDDHYCLDYWGQG (SEQ ID NO: 590)
VH	QVQLVQSGGGVVQPGRLRLSCKASGYTFTRYTMHWVRQAPGKGLEWIGYINPSRGYTNYNQKFKDRFTIST DKSKSTAFLLQMDSLRPEDTAVYYCARYYDDHYCLDYWGQGPVTVSS (SEQ ID NO: 591)
VH	QVQLVESGGGVVQPGRLRLSCSSSGYTFTRYTMHWVRQAPGKGLEWVAYINPSRGYTNYNQKFKDRFTISR DNSKNTLFLQMDSLRPEDTGVYFCARYYDDHYCLDYWGQGPVTVSS (SEQ ID NO: 592)
VL	DIQMTQSPSSLSASVGDRVTITCSASSSVSYMNWYQQTPGKAPKRWIYDTSKLGASGVPSRFSGSGSGTDYTF TISSLQPEDIAATYYCQQWSSNPFT (SEQ ID NO: 593)
VL	QIVMTQSPSSLSASVGDRVTITCSASSSVSYMNWYQQTPGKAPKRWIYDTSKLGASGVPSRFSGSGSGTDYTF TISSLQPEDIAATYYCQQWSSNPFTFGQGTLQITR (SEQ ID NO: 594)
VL	DIQMTQSPSSLSASVGDRVTITCSASSSVSYMNWYQQTPGKAPKLLIYDTSKLGASGVPSRFSGSGSGTDYTF TISSLQPEDIAATYYCQQWSSNPFTFGQGTLQITR (SEQ ID NO: 595)
Group B	
VH	QVQLQQSGAELARPGASVKMSCKASGYTFTRYTMHWVKQRPGQGLEWIGYINPSRGYTNYNQKFKDKATLT DKSSSTAYMQLSSLTSEDSAVYYCARYYDDHYCLDYWGQGTTLTVSS (SEQ ID NO: 596)
VH	EVQLVESGGGLVQPGKSLKLSCEASGFTFSGYGMHWVRQAPGRGLESVAYITSSSINIKYADAVKGRFTVSR DNAKNLLFLQMNILKSEDTAMYYCARFDWDKNYWGQGTMTVTVSS (SEQ ID NO: 597)
VL	QIVLTQSPAIMASAPGEKVTMTCRASSSVSYMNWYQQKSGTSPKRWIYDTSKVASGVPYRFSGSGSGTSSYSL TISSMEAEDAATYYCQQWSSNPFTFGSGTKLEINR (SEQ ID NO: 598)
VL	QIVLTQSPAIMASAPGEKVTMTCSASSSVSYMNWYQQKSGTSPKRWIYDTSKLGASGVPAAHFRGSGSGTSSYSL TISGMEAEDAATYYCQQWSSNPFTFGSGTKLEINR (SEQ ID NO: 599)
VL	DIQMTQSPSSLPASLGDRVTINCQASQDISNYLNWYQQKPGKAPKLLIYYTNKLADGVPSRFSGSGSGGRDSS FTISSELEDIGSYCYCQQYYNYPWTFGPGTKLEIKR (SEQ ID NO: 600)

Group C	
VH	MGWSWIFLFLLSGTAGVHSQVQLVQSGAEVKKPGASVKVSCKASGYTFISYTMHWVRQAPGQGLEWMGYINP RSGYTHYNQKLKDKATLTADKSASTAYMELSSLRSED TAVYYCARSAYYDYDGFAYWGQGT LVT VSS (SEQ ID NO: 601)
VL	METDTLLLWVLLLWVPGSTGDIQMTQSPSSLSASVGDRVTITCSASSSVSYMNWYQQKPGKAPKRLIYDTSK LASGVPSRFSGSGSGTDFTLTITSSSLQPEDFATYYCQQWSSNPPTFGGGTKVEIK (SEQ ID NO: 602)
Group D	
VH	EVQLVESGGGLVQPGGSLRLSCAASGYSFTGYTMNWVRQAPGKGLEWVALINPYKGVSTYNQKFKDRFTISV DKSKNTAYLQMNSLRAEDTAVYYCARSGYYGDS DWYFDVWGQGT LVT VSS (SEQ ID NO: 603)
VL	DIQMTQSPSSLSASVGDRVTITCRASQDIRNYLNWYQQKPGKAPKLLIYYTSRLESGVPSRFSGSGSGTDYT LTISLQPEDFATYYCQQGNTLPWTFGQGTKVEIK (SEQ ID NO: 604)
HC	MGWSCIIILFLVATATGVHSEVQLVESGGGLVQPGGSLRLSCATSGYTFTEYTMHWMRQAPGKGLEWVAGINP KNGGTSHNQRFMDRFTISVDKSTSTAYMQMNSLRAEDTAVYYCARWRGLNYGFDVRYFDVWGQGT LVT VSSA STKGPSVFPLAPCSRSTSESTAALGCLVKDYFPEPVT VSWNSGALTSGVHTFPAVLQSSGLYSLSSVVT VTS SNFGTQTYTCNV DHKPSNTKVDKTV ERKCCVECP PCPAPPVAGPSVFLFPKPKDTLMISRTPEVTCVVDV SHEDPEVQFNWYVDGMEVHNAKTKPREEQFNSTFRVSVLT VVH QDWLNGKEYKCKVSNKGLPAPIEKTISK TKGQPREPQVYTLPPSREEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPMLDSDGSFFLYSKL TVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGK (SEQ ID NO: 605)
HC	EVQLVESGGGLVQPGGSLRLSCATSGYTFTEYTMHWMRQAPGKGLEWVAGINPKNGGTSYADSVKGRFTISV DKSKNTLYLQMNSLRAEDTAVYYCARWRGLNYGFDVRYFDVWGQGT LVT VSSASTKGPSVFPLAPSSKSTSG GTAALGCLVKDYFPEPVT VSWNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSSLGTQTYICNVNHKPSNT KVDKKVEPKSCDKTHT (SEQ ID NO: 606)
HC	EVQLVESGGGLVQPGGSLRLSCATSGYTFTEYTMHWMRQAPGKGLEWVAGINPKNGGTSHNQRFMDRFTISV DKSKNTLYLQMNSLRAEDTAVYYCARWRGLNYGFDVRYFDVWGQGT LVT VSSASTKGPSVFPLAPSSKSTSG GTAALGCLVKDYFPEPVT VSWNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSSLGTQTYICNVNHKPSNT KVDKKVEPKSCDKTHT (SEQ ID NO: 607)
HC	EVQLVESGGGLVQPGGSLRLSCATSGYTFTEYTMHWMRQAPGKGLEWVAGINPKNGGTSHNQRFMDRFTLAV DKSKNTLYLQMNSLRAEDTAVYYCARWRGLNYGFDVRYFDVWGQGT LVT VSSASTKGPSVFPLAPSSKSTSG GTAALGCLVKDYFPEPVT VSWNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSSLGTQTYICNVNHKPSNT KVDKKVEPKSCDKTHT (SEQ ID NO: 608)
HC	EVQLVESGGGLVQPGGSLRLSCATSGYTFTEYTMHWMRQAPGKGLEWVAGINPKNGGTSHNQRFMDRFTISV DKSTSTAYMQMNSLRAEDTAVYYCARWRGLNYGFDVRYFDVWGQGT LVT VSSASTKGPSVFPLAPSSKSTSG GTAALGCLVKDYFPEPVT VSWNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSSLGTQTYICNVNHKPSNT KVDKKVEPKSCDKTHT (SEQ ID NO: 609)
HC	EVQLVESGGGLVQPGGSLRLSCATSGYTFTEYTMHWMRQAPGKGLEWVAGINPKNGGTSHNQRFMDRFTISV DKSTSTAYMQMNSLRAEDTAVYYCARWRGLNYGFDVRYFDVWGQGT LVT VSSASTKGPSVFPLAPSSKSTSG

	GTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSSLGTQTYICNVNHKPSNT KVDKKVEPKSCDKTHTCPP (SEQ ID NO: 610)
HC	EVQLVESGGGLVQPGGSLRLSCATSGYTFTEYTMHWMRQAPGKGLEWVAGINPKNGGTSHNQRFMDFRTISV DKSKNTLYMELRSLRAEDTAVYYCARWRGLNYGFDVRYFDVWGQGTLVTVSSASTKGPSVFPLAPSSKSTSG GTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSSLGTQTYICNVNHKPSNT KVDKKVEPKSCDKTHT (SEQ ID NO: 611)
HC	EVQLVESGGGLVQPGGSLRLSCATSGYTFTEYTMHWMRQAPGKGLEWVAGINPKNGGTSHNQRFMDFRTISV DKSKNTLYLQMNSLTSEDGIYYCARWRGLNYGFDVRYFDVWGQGTLVTVSSASTKGPSVFPLAPSSKSTSG GTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSSLGTQTYICNVNHKPSNT KVDKKVEPKSCDKTHT (SEQ ID NO: 612)
HC	EVQLVESGGGLVQPGGSLRLSCATSGYTFTEYTMHWMRQAPGKGLEWVAGINPKNGGTSHNQRFMDFRTISV DKSKNTLYLQMNSLRAEDTAVYYCARWRGLNYGFDVRYFDVWGAGTTVTVSSASTKGPSVFPLAPSSKSTSG GTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSSLGTQTYICNVNHKPSNT KVDKKVEPKSCDKTHT (SEQ ID NO: 613)
HC	EVQLQQSGPELVQPGGSLRLSCATSGYTFTEYTMHWMRQAPGKGLEWVAGINPKNGGTSHNQRFMDFRTISV DKSKNTLYLQMNSLRAEDTAVYYCARWRGLNYGFDVRYFDVWGQGTLVTVSSASTKGPSVFPLAPSSKSTSG GTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSSLGTQTYICNVNHKPSNT KVDKKVEPKSCDKTHT (SEQ ID NO: 614)
HC	EVQLVESGGGLVKPGASLRLSCATSGYTFTEYTMHWMRQAPGKGLEWVAGINPKNGGTSHNQRFMDFRTISV DKSKNTLYLQMNSLRAEDTAVYYCARWRGLNYGFDVRYFDVWGQGTLVTVSSASTKGPSVFPLAPSSKSTSG GTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSSLGTQTYICNVNHKPSNT KVDKKVEPKSCDKTHT (SEQ ID NO: 615)
HC	EVQLVESGGGLVQPGGSLKISCKTSGYTFTEYTMHWMRQAPGKGLEWVAGINPKNGGTSHNQRFMDFRTISV DKSKNTLYLQMNSLRAEDTAVYYCARWRGLNYGFDVRYFDVWGQGTLVTVSSASTKGPSVFPLAPSSKSTSG GTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSSLGTQTYICNVNHKPSNT KVDKKVEPKSCDKTHT (SEQ ID NO: 616)
HC	EVQLVESGGGLVQPGGSLRLSCATSGYTFTEYTMHWMKQSHGKSLEWVAGINPKNGGTSHNQRFMDFRTISV DKSKNTLYLQMNSLRAEDTAVYYCARWRGLNYGFDVRYFDVWGQGTLVTVSSASTKGPSVFPLAPSSKSTSG GTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSSLGTQTYICNVNHKPSNT KVDKKVEPKSCDKTHT (SEQ ID NO: 617)
HC	EVQLVESGGGLVQPGGSLRLSCATSGYTFTEYTMHWMRQAPGKGLEWIGGFNPKNGGTSHNQRFMDFRTISV DKSKNTLYLQMNSLRAEDTAVYYCARWRGLNYGFDVRYFDVWGQGTLVTVSSASTKGPSVFPLAPSSKSTSG GTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSSLGTQTYICNVNHKPSNT KVDKKVEPKSCDKTHT (SEQ ID NO: 618)
LC	MGWSCIIILFLVATATGVHSDIQMTQSPSSLSASVGRVTITCRASQDINNYLNWYQQKPGKAPKLLIYYTST LHSGVPSRFSGSGSGTDYTLTISSQLPEDFATYYCQQGNTLPPTFGQGTKVEIKRTVAAPSVFIFPPSDEQL KSGTASVVCLLNNFYPREAKVQWKVDNALQSGNSQESVTEQDSKDESTYSLSSSTLTLSKADYEEKHKVYACEVT

	HQGLSSPVTKSFNRGEC (SEQ ID NO: 619)
LC	DIQMTQSPSSLSASVGDRVTITCRASQDINNYLNWYQQKPGKAPKLLIYYTSTLESGVPSRFSGSGSGTDYT LTISSLQPEDFATYYCQQGNTLPPTFGQGTKVEIKRTVAAPSVFIFPPSDEQLKSGTASVVCLLNNFYPREA KVQWKVDNALQSGNSQESVTEQDSKDYSLSTLTLSKADYEEKHKVYACEVTHQGLSSPVTKSFNRGEC (SEQ ID NO: 620)
LC	DIQMTQSPSSLSASVGDRVTITCRASQDINNYLNWYQQKPGKAPKLLIYYTSTLHSGVPSRFSGSGSGTDYT LTISSLQPEDFATYYCQQGNTLPPTFGQGTKVEIKRTVAAPSVFIFPPSDEQLKSGTASVVCLLNNFYPREA KVQWKVDNALQSGNSQESVTEQDSKDYSLSTLTLSKADYEEKHKVYACEVTHQGLSSPVTKSFNRGEC (SEQ ID NO: 621)
Group E	
HC	MERHWIFLLLLSVTAGVHSQVQLQQSGAELARPGASVKMSCKASGYTFTTRYTMHWVKQRPGQGLEWIGYINP SRGYTNYNQKFKDKATLTDDKSSSTAYMQLSSLTSEDSAVYYCARYYDDHYCLDYWGQGTTTLTVSSAKTTAP SVYPLAPVCGDTTGSSVTLGCLVKGYFPEPVTLTWNSGSLSSGVHTFPAVLQSDLYTLSSSVTVTSSTWPSQ SITCNVAHPASSTKVDKKIEPRGPTIKPCPPCKCPAPNLLGGPSVFIFPPKIKDVLMISSLPIVTCVVVDVS EDDPDVQISWVFNNEVHTAQTQTHREDYNSTLRVVSALPIQHQQDWMMSGKEFKCKVNNKDLPAPIERTISKP KGSVRAPQVYVLPPEEEMTKKQVTLTCMVTDFMPEDIYVEWTNNGKTELNYKNTEPVLDSGYSYFMYSKLR VEKKNWVERNNSYSCSVVHEGLHNHHTTKSFSRTPGK (SEQ ID NO: 622)
LC	MDFQVQIFSFLLISASVIIISRGQIVLTQSPAISASPGKVTMTCSASSSVSYMNWYQQKSGTSPKRWIYDT SKLASGVPAHFRGSGSGTSYSLTISGMEAEDAATYYCQQWSSNPFTFGSGTKLEINRADTAPTVSIFPPSSE QLTSGGASVVCFLNNFYPKDINVKKIDGSERQNGVLNSWTDQDSKDYSLSTLTLSKADYEEKHKVYACEVTHQGLSSPVTKSFNRGEC ATHKTSTSPIVKSFNRECE (SEQ ID NO: 623)
Group F	
VH	EVQLLESGGGLVQPGGSLRLSCAASGFTFSSFPMAWVRQAPGKGLEWVSTISTSGGRTYYRDSVKGRFTISR DNSKNTLYLQMNSLRAEDTAVYYCAKFRQYSGGFDYWGQGTLLTVSS (SEQ ID NO: 624)
VL	DFMLTQPHSVSESPGKTVIIISCTLSSGNIENNYVHWYQQRPGRAPTTVIFDDDKRPDGVDPDRFSGSIDRSSN SASLTISGLQTEDEADYYCHSYVSSFNVFGGGTKLTVLGQPKAAPSVTLFPPSSEELQ (SEQ ID NO: 625)
Group G	
HC	EVQLLESGGGLVQPGGSLRLSCAASGFTFSSFPMAWVRQAPGKGLEWVSTISTSGGRTYYRDSVKGRFTISR DNSKNTLYLQMNSLRAEDTAVYYCAKFRQYSGGFDYWGQGTLLTVSSASTKGPSVFPLAPSSKSTSGGTAAL GCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVTVTPSSSLGTQTYICNVNHKPSNTKVDKK VEPKSCDKHTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHN AKTKPREEQYASTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSRDEL TKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVVFSCSVMHE ALHNHYTQKSLSLSPGK (SEQ ID NO: 626)

VL	QAVVTQANSVSTSLGSTVKLSCTLSSGNIENNYVHWYQLYEGRSPTTMIYDDDKRPDGVDPDRFSGSIDRSSN SAFLTIHNVAIEDEAIYFCHSYVSSFNVFGGGTKLTVLR (SEQ ID NO: 627)
LC	QAVVTQANSVSTSLGSTVKLSCTLSSGNIENNYVHWYQLYEGRSPTTMIYDDDKRPDGVDPDRFSGSIDRSSN SAFLTIHNVAIEDEAIYFCHSYVSSFNVFGGGTKLTVLRQPKAAPSVTLFPSPSEELQANKATLVCLISDFY PGAVTVAWKADSSPVKAGVETTTTPSKQSNNKYAASSYLSLTPEQWKSHRSYSCQVTHEGSTVEKTVAPTECS (SEQ ID NO: 628)
Group H	
VH	EVKLVESGGGLVQPGGSLRLSCAASGFTFNTYAMNWVRQAPGKGLEWVARIRSKYNNYATYYADSVKDRFTI SRDDSKSSLYLQMNNLKTEDTAMYYCVRHGNFGNSYVSWFAYWGQGTlVTVSS (SEQ ID NO: 629)
VH	EVKLVESGGGLVKPGRSLRLSCAASGFTFNTYAMNWVRQAPGKGLEWVARIRSKYNNYATYYADSVKDRFTI SRDDSKSILYLQMNNLKTEDTAMYYCVRHGNFGNSYVSWFAYWGQGTlVTVSS (SEQ ID NO: 630)
VH	EVKLVESGGGLVKPGRSLRLSCAASGFTFNTYAMNWVRQAPGKGLEWVARIRSKYNNYATYYADSVKDRFTI SRDDSKSILYLQMNSLKTEDTAMYYCVRHGNFGNSYVSWFAYWGQGTlVTVSS (SEQ ID NO: 631)
VH	EVKLVESGGGLVKPGRSLRLSCAASGFTFNTYAMNWVRQAPGKGLEWVARIRSKYNNYATYYADSVKDRFTI SRDDSKSILYLQMNSLKTEDTAMYYCVRHGNFGNSYVSWFAYWGQGTMTVTVSS (SEQ ID NO: 632)
VL	QAVVTOEPSFSVSPGGTVTLTCRSSTGAVTTSNYANWVQQTPGQAFRGLIGGTNKRAPGVPARFSGSLIGDK AALTITGAQADDESIYFCALWYSNLWVFGGGTKLTVL (SEQ ID NO: 633)
VL	QAVVTOEPSFSVSPGGTVTLTCRSSTGAVTTSNYANWVQQTPGQAFRGLIGGTNKRAPGVPARFSGSILGNK AALTITGAQADDESIYFCALWYSNLWVFGGGTKLTVL (SEQ ID NO: 634)
VL	QAVVTOEPSFSVSPGGTVTLTCRSSTGAVTTSNYANWVQQTPGQAFRGLIGGTNKRAPGVPARFSGSILGNK AALTITGAQADDES DYFCALWYSNLWVFGGGTKLTVL (SEQ ID NO: 635)
Group I	
VH	EVQLVESGGGLVQPGGSLRLSCAASGFTFDDYTMHWVRQAPGKGLEWVSGISWNSSGSIGYADSVKGRFTISR DNAKKSLLYLQMNSLRAEDTALYYCAKDNSGYGHYYYGMDVWGQGTlVTVAS (SEQ ID NO: 636)
VH	EVQLVESGGGLVQPGGSLRLSCAATGFTFDDFTMHWRQAPGKGLEWVSGISWNSSGSIGYVDSVKGRFTISR DNAKNSLLYLQMNSLRAEDTALYYCAKDNSGYGYYYYGMDVWGQGTlVTVSS (SEQ ID NO: 637)
VH	QVQLVESGGGVVQPGGSLRLSCAASGFTFRSYAMHWVRQAPGKGLEWVAMVYYDGNNQYYADSVRGRFTISR DNSKNTLYLQMNSLRADDTAVYFCARGPGYNWLDPWGQGTlVTVSS (SEQ ID NO: 638)
VH	QVQLVESGGGVVQPGGSLRLACVASGFTFRSYGMHWVRQAPGKGLQWVAMIYYDGKNKYYADSVRGRFTISR DNSKNTLYLQMNNLRVEDTAMYFCARGPGYNWLDPWGQGTlVTVSS (SEQ ID NO: 639)
VH	DVQLVQSGAEVKKPGASVKVSCKASGYTFTRYTMHWVRQAPGQGLEWIGYINPSRGYTNYADSVKGRFTITT DKSTSTAYMELSSLRSEDATYYCARYYDDHYCLDYWGQGTlVTVSS (SEQ ID NO: 640)
VL	AEIVMTQSPATLSVSPGERATLSCRASQSVSSNLAWYQQKPGQAPRLLIYGASTRATGIPARFSGSGSGTEF TLTISSLQSEDFAVYYCQHYINWPLTFGGGTKVEIK (SEQ ID NO: 641)
VL	EIVMTQSPATLSVSPGERATLSCRASHSVSRNSAWYQQKPGQAPRLLIYGASTRATGIPARFSGSGSGTEFT LTISSLQSEDFAIYYCQQYNNWPLTFGGGTKVEIK (SEQ ID NO: 642)

VL	EIVMTQSPATLSVSPGERATLSCRASQSVSSNLAWYQQKPGQAPRLLIYGASTRATGIPARFSGSGSGTEFT LTISSLQSEDFAVYYCQHYINWPLTFGGGKTKVEIK (SEQ ID NO: 643)
VL	EIVMTQSPATLSVSPGERATLSCRASQSVSRNLAWYQQKPGQAPRLLIYGASTRATGIPARFSGSGSGTDFT LTISSLQSEDFAVYYCQQYNNWPLTFGGGKTKVVIK (SEQ ID NO: 644)
VL	EIVMTQSPATLSVSPGERATLSCRASQRISSNLAWYQQKPGQAPRLLIYGASTRATGSPARFSGSGSGTDFT LTISSLQSEDVAVYYCQQHHNWPLTFGGGKTKVEIK (SEQ ID NO: 645)
VL	DIVLTQSPATLSLSPGERATLSCRASQSVSYMNWYQQKPGKAPKRWIYDTSKVASGVPARFSGSGSGTDYSL TINSLEAEDAATYYCQQWSSNPLTFGGGKTKVEIK (SEQ ID NO: 646)
Group J	
VH	QVQLVQSGAEVKKPGASVKVSCKASGYTFTRYTMHWVRQAPGQGLEWMGYINPSRGYTNYNQKFKDRVMTT DTSISTAYMELSRRLRSDDTAVYYCARYYDDHYCLDYWGQGTLVTVSS (SEQ ID NO: 647)
VH	QVQLVQSGAEVKKPGASVKVSCKASGYTFTRYTMHWVRQAPGQGLEWMGYINPSRGYTNYNQKFKDRVMTT DTSISTAYMELSRRLRSDDTAVYYCARYYDDHYSLDYWGQGTLVTVSS (SEQ ID NO: 648)
VL	EIVLTQSPATLSLSPGERATLSCSASSSVSYMNWYQQKPGQAPRLLIYDTSKLASGVPAAHFRGSGSGTDYTL TISSLEPEDFAVYYCQQWSSNPFTFGQGTKVEIK (SEQ ID NO: 649)
Group K	
VH	QVQLQQSGAELARPGASVKMSCKASGYTFTRYTMHWVKQRPGQGLEWIGYINPSRGYTNYNQKFKDKATLTT DKSSSTAYMQLSSLTSEDSAVYYCARYYDDHYCLDYWGQGTTLTVSS (SEQ ID NO: 596)
VL	QIVLTQSPAISASPGKVTMTCSASSSVSYMNWYQQKSGTSPKRWIYDTSKLASGVPAAHFRGSGSGTSYSL TISGMEAEDAATYYCQQWSSNPFTFGSGTKLEIN (SEQ ID NO: 650)
Group L	
VH	QVQLVQSGSELKKPGASVKMSCKASGYTFTRYTMHWVRQAPGKGLEWIGYINPSRGYTNYNQKFKDRATLTT DKSTSTAYMQLSSLRSEDVAVYYCARYYDDHYSLDYWGQGTLVTVSS (SEQ ID NO: 651)
VL	QIVLTQSPATLSLSPGERATMSCSASSSVSYMNWYQQKPGKAPKRWIYDTSKLASGVPSRFRGSGSGTDYTL TISSLQPEDFATYYCQQWSSNPFTFGGGKTKVEIK (SEQ ID NO: 652)
Group M	
VH	EVQLVESGGGLVQPGGSLRLSCAASGFTFSTYAMNWVRQAPGKGLEWVGRIRSKYNNYATYYADSVKDRFTI SRDDSKNSLYLQMNSLKTEDTAVYYCARHGNFGNSYVSWFAYWGQGTLVTVSS (SEQ ID NO: 653)
VH	EVQLVESGGGLVQPGGSLRLSCAASGFTFNTYAMNWVRQAPGKGLEWVGRIRSKYNNYATYYADSVKDRFTI SRDDSKNSLYLQMNSLKTEDTAVYYCARHGNFGNSYVSWFAYWGQGTLVTVSS (SEQ ID NO: 654)
VH	EVQLVESGGGLVQPGGSLRLSCAASGFTFSTYAMNWVRQAPGKGLEWVARIRSKYNNYATYYADSVKDRFTI SRDDSKNSLYLQMNSLKTEDTAVYYCARHGNFGNSYVSWFAYWGQGTLVTVSS (SEQ ID NO: 655)
VH	EVQLVESGGGLVQPGGSLRLSCAASGFTFSTYAMNWVRQAPGKGLEWVGRIRSKYNNYATYYADSVKDRFTI SRDDSKNSLYLQMNSLKTEDTAVYYCVRHGNFGNSYVSWFAYWGQGTLVTVSS (SEQ ID NO: 656)
VH	EVQLVESGGGLVQPGGSLRLSCAASGFTFNTYAMNWVRQAPGKGLEWVARIRSKYNNYATYYADSVKDRFTI

	SRDDSKNSLYLQMNSLKTEDTAVYYCARHGNFGNSYVSWFAYWGQGTTLVTVSS (SEQ ID NO: 657)
VH	EVQLVESGGGLVQPGGSLRLSCAASGFTFNTYAMNWVRQAPGKGLEWVGRIRSKYNNYATYYADSVKDRFTI SRDDSKNSLYLQMNSLKTEDTAVYYCVRHGNFGNSYVSWFAYWGQGTTLVTVSS (SEQ ID NO: 658)
VH	EVQLVESGGGLVQPGGSLRLSCAASGFTFNTYAMNWVRQAPGKGLEWVGRIRNKYNNYATEYADSVKDRFTI SRDDSKNSLYLQMNSLKTEDTAVYYCVRHGNFGNSYVSWFAYWGQGTTLVTVSS (SEQ ID NO: 659)
VH	EVQLVESGGGLVQPGGSLRLSCAASGFTFSTYAMNWVRQAPGKGLEWVARIRSKYNNYATYYADSVKDRFTI SRDDSKNSLYLQMNSLKTEDTAVYYCVRHGNFGNSYVSWFAYWGQGTTLVTVSS (SEQ ID NO: 660)
VH	EVQLVESGGGLVQPGGSLRLSCAASGFTFNTYAMNWVRQAPGKGLEWVARIRSKYNNYATYYADSVKDRFTI SRDDSKNSLYLQMNSLKTEDTAVYYCVRHGNFGNSYVSWFAYWGQGTTLVTVSS (SEQ ID NO: 661)
VH	EVQLVESGGGLVQPGGSLRLSCAASGFTFNTYAMNWVRQAPGKGLEWVARIRNKYNNYATEYADSVKDRFTI SRDDSKNSLYLQMNSLKTEDTAVYYCVRHGNFGNSYVSWFAYWGQGTTLVTVSS (SEQ ID NO: 662)
VH	EVQLVESGGGLVQPGGSLRLSCAASGFTFNTYAMNWVRQAPGKGLEWVARTRSKANSYTTYAASVKGRFTI SRDDSKNSLYLQMNSLKTEDTAVYYCARHGNFGNSYVSWFAYWGQGTTLVTVSS (SEQ ID NO: 663)
VH	EVQLVESGGGLVQPGGSLRLSCAASGFTFNTYAMNWVRQAPGKGLEWVGRTRSKANSYTTYAASVKGRFTI SRDDSKNSLYLQMNSLKTEDTAVYYCARHGNFGNSYVSWFAYWGQGTTLVTVSS (SEQ ID NO: 664)
VH	EVQLVESGGGLVQPGGSLRLSCAASGFTFNTYAMNWVRQAPGKGLEWVGRIRSKYNNYATEYAASVKDRFTI SRDDSKNSLYLQMNSLKTEDTAVYYCVRHGNFGNSYVSWFAYWGQGTTLVTVSS (SEQ ID NO: 665)
VH	EVQLVESGGGLVQPGGSLRLSCAASGFTFNTYAMNWVRQAPGKGLEWVARIRNKYNNYATEYAASVKDRFTI SRDDSKNSLYLQMNSLKTEDTAVYYCVRHGNFGNSYVSWFAYWGQGTTLVTVSS (SEQ ID NO: 666)
VH	EVQLVESGGGLVQPGGSLRLSCAASGFTFNTYAMNWVRQAPGKGLEWVGRTRSKANSYTTYAASVKGRFTI SRDDSKNSLYLQMNSLKTEDTAVYYCARHGNFGNSYVSWFAYWGQGTTLVTVSS (SEQ ID NO: 667)
VH	EVQLVESGGGLVQPGGSLRLSCAASGFTFNTYAMNWVRQAPGKGLEWVARTRSKANSYTTYAASVKGRFTI SRDDSKNSLYLQMNSLKTEDTAVYYCARHGNFGNSYVSWFAYWGQGTTLVTVSS (SEQ ID NO: 668)
VH	QVQLVQSGAEVKKPGASVKVSCKASGYTFTRSTMHWVRQAPGQGLEWIGYINPSSAYTNYNQKFKDRVITITA DKSTSTAYMELSSLRSED TAVYYCASPQVHYDNGFPYWGQGTTLVTVSS (SEQ ID NO: 669)
VL	QAVVTQEPSLTVSPGGTVTLTCRSSTGAVTTSNYANWFQQKPGQAPRTLIGGTNKRAPWTPARFSGSLLGKK AALTITGAQAEDEADYYCALWYSNLWVFGGGTKLTVLG (SEQ ID NO: 670)
VL	QAVVTQEPSLTVSPGGTVTLTCRSSTGAVTTSNYANWVQQKPGQAPRTLIGGTNKRAPWTPARFSGSLLGKK AALTITGAQAEDEADYYCALWYSNLWVFGGGTKLTVLG (SEQ ID NO: 671)
VL	QAVVTQEPSLTVSPGGTVTLTCRSSTGAVTTSNYANWFQEKPGQAPRTLIGGTNKRAPWTPARFSGSLLGKK AALTITGAQAEDEADYYCALWYSNLWVFGGGTKLTVLG (SEQ ID NO: 672)
VL	QAVVTQEPSLTVSPGGTVTLTCRSSTGAVTTSNYANWFQQKPGQAPRGLIGGTNKRAPWTPARFSGSLLGKK AALTITGAQAEDEADYYCALWYSNLWVFGGGTKLTVLG (SEQ ID NO: 673)
VL	QAVVTQEPSLTVSPGGTVTLTCRSSTGAVTTSNYANWVQEKPGQAPRTLIGGTNKRAPWTPARFSGSLLGKK AALTITGAQAEDEADYYCALWYSNLWVFGGGTKLTVLG (SEQ ID NO: 674)
VL	QAVVTQEPSLTVSPGGTVTLTCRSSTGAVTTSNYANWVQQKPGQAPRGLIGGTNKRAPWTPARFSGSLLGKK

	AALTITGAQAEDEADYYCALWYSNLWVFGGGTKLTVLG (SEQ ID NO: 675)
VL	QAVVTQEPSLTVSPGGTVTLTCRSSTGAVTTSNYANWFQEKPGQAPRGLIGGTNKRAPWTPARFSGSLLGGK AALTITGAQAEDEADYYCALWYSNLWVFGGGTKLTVLG (SEQ ID NO: 676)
VL	QAVVTQEPSLTVSPGGTVTLTCRSSTGAVTTSNYANWVQEKPGQAPRGLIGGTNKRAPWTPARFSGSLLGGK AALTITGAQAEDEADYYCALWYSNLWVFGGGTKLTVLG (SEQ ID NO: 677)
VL	QAVVTQEPSLTVSPGGTVTLTCRSSTGAVTTSNYANWVQEKPGQAFRGLIGGTNKRAPWTPARFSGSLLGGK AALTITGAQAEDEADYYCALWYSNLWVFGGGTKLTVLG (SEQ ID NO: 678)
VL	DIQMTQSPSSLSASVGDRVTITCSASSSVSYMNWYQQKPGKAPKRLIYDSSKSLASGVPSRFSGSGSGTEFTL TISSLQPEDFATYYCQQWSRNPPTFGGGTKVEIK (SEQ ID NO: 679)
VL	DVVMTQSPAIMSAFPGEKVTITCSASSSVSYMNWYQQKPGKAPKRWIYDSSKSLASGVPSRFSGSGSGTEFTL TISSLQPEDFATYYCQQWSRNPPTFGGGTKVEIK (SEQ ID NO: 680)
Group N	
VH	QVQLVQSGAEVKKPGASVKVSCKASGYTFTRSTMHWVKQAPGQGLEWIGYINPSSAYTNYNQKFKDKATLTA DKSSSTAYMQLSSLRSED TAVYYCARPQVHYDYN G F P Y W G Q G T L V T V S S (SEQ ID NO: 681)
VH	QVQLVQSGGGVVQPG RSLRLSCKASGYTFTRSTMHWVRQAPGKGLEWIGYINPSSAYTNYNQKFKDKATLTA DKSKNTAYMELSSLRSED TAVYYCARPQVHYDYN G F P Y W G Q G T L V T V S S (SEQ ID NO: 682)
VH	QVQLVQSGGGVVQPG RSLRLSCKASGYTFTRSTMHWVRQAPGKGLEWIGYINPSSAYTNYNQKFKDRFTISA DKSKSTAFLQMDSLRPED TGVYFCARPQVHYDYN G F P Y W G Q G T P V T V S S (SEQ ID NO: 683)
VH	QVQLVESGGGVVQPG RSLRLSCKASGYTFTRSTMHWVRQAPGQGLEWIGYINPSSAYTNYNQKFKDRFTISA DKSKSTAFLQMDSLRPED TGVYFCARPQVHYDYN G F P Y W G Q G T P V T V S S (SEQ ID NO: 684)
VH	QVQLVQSGAEVKKPGASVKVSCKASGYTFTRSTMHWVRQAPGQRLWVMGYINPSSAYTNYNQKFKDRVTITR DTSASTAYMELSSLRSED TAVYYCARPQVHYDYN G F P Y W G Q G T L V T V S S (SEQ ID NO: 685)
VH	EVQLVESGGGLVKPGGSLRLSCAASGFTFSRSTMHWVRQAPGKGLEWVSYINPSSAYTNYNQKFKDRFTISR DNAKNSLYLQMNSLRAED TAVYYCARPQVHYDYN G F P Y W G Q G T L V T V S S (SEQ ID NO: 686)
VL	AQDIQMTQSPSSLSASVGDRVTMTCSASSSVSYMNWYQQKPGKAPKLLIYDSSKSLASGVPARFSGSGSGTDY TLTISSLQPEDFATYYCQQWSRNPPTFGGGTKVEIK (SEQ ID NO: 687)
VL	EIVLTQSPATLSLSPGERATLSCSASSSVSYMNWYQQKPGQAPRLLIYDSSKSLASGIPARFSGSGSGTDFTL TISSLEPEDFAVYYCQQWSRNPPTFGGGTKVEIK (SEQ ID NO: 688)
VL	AQDIQMTQSPSSLSASVGDRVTMTCSASSSVSYMNWYQQKPGKAPKRWIYDSSKSLASGVPARFSGSGSGTDY TLTISSLQPEDFATYYCQQWSRNPPTFGGGTKLQIT (SEQ ID NO: 689)
VL	AQDIQMTQSPSSLSASVGDRVTITCSASSSVSYMNWYQQTPGKAPKRWIYDSSKSLASGVPSRFSGSGSGTDF TLTISSLQPEDIATYYCQQWSRNPPTFGQGTKLQIT (SEQ ID NO: 690)
Group O	
VH	QVQLVESGGGVVQPG RSLRLSCAASGFKFSGYGMHWVRQAPGKGLEWVAVIWDGSKKYYVDSVKGRFTISR DNSKNTLYLQMNSLRAED TAVYYCARQMGYWHFDLWGRGTLVTVSS (SEQ ID NO: 691)
VH	QVQLVQSGGGVVQSG RSLRLSCAASGFKFSGYGMHWVRQAPGKGLEWVAVIWDGSKKYYVDSVKGRFTISR

	DNSKNTLYLQMNSLRGEDTAVYYCARQMGYWHFDLWGRGTLVTVSS (SEQ ID NO: 692)
VH	QVQLVESGGGVVQPGRSLRLSCAASGFTFRSYGMHWVRQAPGKGLEWVAIIWYDGSKKNYADSVKGRFTISR DNSKNTLYLQMNSLRAEDTAVYYCARGTGYNWFDPWGQGTLVTVSS (SEQ ID NO: 693)
VH	QVQLVQSGGGVVQPGRSLRLSCVASGFTFSSYGMHWVRQAPGKGLEWVAIIWYNGRQDYADSVKGRFTISR DNSKNTLYLQMNSLRAEDTAVYYCTRGTGYNWFDPWGQGTLVTVSS (SEQ ID NO: 694)
VL	EIVLTQSPATLSLSPGERATLSCRASQSVSSYLAWYQQKPGQAPRLLIYDASNRATGIPARFSGSGSGTDFT LTISSELPEDFAVYYCQQRSNWPPLTFGGGTKVEIK (SEQ ID NO: 695)
VL	EIVLTQSPRTLSLSPGERATLSCRASQSVSSSYLAWYQQKPGQAPRLLIYGASSRATGIPDRFSGSGSGTDFT TLTISRDPEDFAVYYCQQYGSSPITFGQGTRLEIK (SEQ ID NO: 696)
VL	DILMTQSPSSLSASVGDRVITITCRASQGISSALAWYQQKPGKAPKLLIYYASSLQSGVPSRFSGSGSGTDYT LTISSELPEDFATYYCQQYYSTLTFGGGTKVEIK (SEQ ID NO: 697)
VL	DIVMTQSPSSLSASVGDRVITITCRASQGISSALAWYQQKPGKAPKLLIYDASSLGSGVPSRFSGSGSGTDFT LTISSELPEDFATYYCQQYYSTLTFGGGTKVEIK (SEQ ID NO: 698)
VL	DIQMTQSPFSLASVGDRVITITCWASQGISSYLAWYQQKPAKAPKLFIIYYASSLQSGVPSRFSGSGSGTDYT LTISSELPEDFATYYCQQYYSTLTFGGGTKVEIK (SEQ ID NO: 699)
VL	DIEMTQSPFSLASVGDRVITITCWASQGISSYLAWYQQKPAKAPKLFIIYYASSLQSGVPSRFSGSGSGTDYT LTISSELPEDFATYYCQQYYSTLTFGGGTKVEIK (SEQ ID NO: 700)
VL	EIVLTQSPATLSLSPGERATLSCRASQSVSSYLAWYQQKPGQAPRLLIYDASNRATGIPARFSGSGSGTDFT LTISSELPEDFAVYYCQQRSNWPWTFGQGTKVEIK (SEQ ID NO: 701)
VL	AIQLTQSPSSLSASVGDRVITITCRASQGISSALAWYQQKPGKAPKLLIYDASSLESGVPSRFSGSGSGTDFT LTISSELPEDFATYYCQQFNSYPITFGQGTRLEIK (SEQ ID NO: 702)
Group P	
VH	QVQLVQSGAEVKKPGASVKVSCKASGYTFISYTMHWVRQAPGQGLEWMGYINPRSGYTHYNQKLKDKATLTA DKSASTAYMELSSLRSEDVAVYYCARSAYDYDGFAYWGQGTLVTVSS (SEQ ID NO: 703)
VL	DIQMTQSPSSLSASVGDRVITITCSASSSVSYMNWYQQKPGKAPKRLIYDTSKLASGVPSRFSGSGSGTDFTL TISSELPEDFATYYCQQWSSNPPTFGGGGTKVEIK (SEQ ID NO: 704)
Group Q	
VH	EVQLVESGGGLVQPGGSLRLSCAASGFTFNTYAMNWVRQAPGKGLEWVGRIRSKYNNYATYYADSVKGRFTI SRDDSKNTLYLQMNSLRAEDTAVYYCVRHGNFGNSYVSWFAYWGQGTLVTVSS (SEQ ID NO: 705)
VH	EVQLVESGGGDVQPGGSLRLSCAASGFTFNTYAMNWVRQAPGKGLEWVGRIRSKYNNYATYYADSVKGRFTI SRDDSKNTLYLQMNSLRAEDTAVYYCVRHGNFGNSYVSWFAYWGQGTLVTVSS (SEQ ID NO: 706)
VH	EVQLVESGGGKVQPGGSLRLSCAASGFTFNTYAMNWVRQAPGKGLEWVGRIRSKYNNYATYYADSVKGRFTI SRDDSKNTLYLQMNSLRAEDTAVYYCVRHGNFGNSYVSWFAYWGQGTLVTVSS (SEQ ID NO: 707)
VH	EVQLVESGGGNVQPGGSLRLSCAASGFTFNTYAMNWVRQAPGKGLEWVGRIRSKYNNYATYYADSVKGRFTI SRDDSKNTLYLQMNSLRAEDTAVYYCVRHGNFGNSYVSWFAYWGQGTLVTVSS (SEQ ID NO: 708)
VH	EVQLVESGGGSVQPGGSLRLSCAASGFTFNTYAMNWVRQAPGKGLEWVGRIRSKYNNYATYYADSVKGRFTI

	SRDDSKNTLYLQMNSLRAEDTAVYYCVRHGNFGNSYVSWFAYWGQGTTLVTVSS (SEQ ID NO: 709)
VH	EVQLVESGGGLVQPGGSLKLSCAASGFTFNTYAMNWVRQAPGKGLEWVGRIRSKYNNYATYYADSVKGRFTI SRDDSKNTLYLQMNSLRAEDTAVYYCVRHGNFGNSYVSWFAYWGQGTTLVTVSS (SEQ ID NO: 710)
VH	EVQLVESGGGLVQPGGSLRLSCAASGFTFSTYAMNWVRQAPGKGLEWVGRIRSKYNNYATYYADSVKGRFTI SRDDSKNTLYLQMNSLRAEDTAVYYCVRHGNFGNSYVSWFAYWGQGTTLVTVSS (SEQ ID NO: 711)
VH	EVQLVESGGGLVQPGGSLRLSCAASGFTFNTYAMHWVRQAPGKGLEWVGRIRSKYNNYATYYADSVKGRFTI SRDDSKNTLYLQMNSLRAEDTAVYYCVRHGNFGNSYVSWFAYWGQGTTLVTVSS (SEQ ID NO: 712)
VH	EVQLVESGGGLVQPGGSLRLSCAASGFTFNTYAMSWVRQAPGKGLEWVGRIRSKYNNYATYYADSVKGRFTI SRDDSKNTLYLQMNSLRAEDTAVYYCVRHGNFGNSYVSWFAYWGQGTTLVTVSS (SEQ ID NO: 713)
VH	EVQLVESGGGLVQPGGSLRLSCAASGFTFNTYAMNWVRQASGKGLEWVGRIRSKYNNYATYYADSVKGRFTI SRDDSKNTLYLQMNSLRAEDTAVYYCVRHGNFGNSYVSWFAYWGQGTTLVTVSS (SEQ ID NO: 714)
VH	EVQLVESGGGLVQPGGSLRLSCAASGFTFNTYAMNWVRQAPGKGLEWVGRIRSKANNYATYYADSVKGRFTI SRDDSKNTLYLQMNSLRAEDTAVYYCVRHGNFGNSYVSWFAYWGQGTTLVTVSS (SEQ ID NO: 715)
VH	EVQLVESGGGLVQPGGSLRLSCAASGFTFNTYAMNWVRQAPGKGLEWVGRIRSKANSYATYYADSVKGRFTI SRDDSKNTLYLQMNSLRAEDTAVYYCVRHGNFGNSYVSWFAYWGQGTTLVTVSS (SEQ ID NO: 716)
VH	EVQLVESGGGLVQPGGSLRLSCAASGFTFNTYAMNWVRQAPGKGLEWVGRIRSKYNNYATAYADSVKGRFTI SRDDSKNTLYLQMNSLRAEDTAVYYCVRHGNFGNSYVSWFAYWGQGTTLVTVSS (SEQ ID NO: 717)
VH	EVQLVESGGGLVQPGGSLRLSCAASGFTFNTYAMNWVRQAPGKGLEWVGRIRSKYNNYATYYAASVKGRFTI SRDDSKNTLYLQMNSLRAEDTAVYYCVRHGNFGNSYVSWFAYWGQGTTLVTVSS (SEQ ID NO: 718)
VH	EVQLVESGGGLVQPGGSLRLSCAASGFTFNTYAMNWVRQAPGKGLEWVGRIRSKYNNYATYYADSVKGRFTI SRDNSKNTLYLQMNSLRAEDTAVYYCVRHGNFGNSYVSWFAYWGQGTTLVTVSS (SEQ ID NO: 719)
VH	EVQLVESGGGLVQPGGSLRLSCAASGFTFNTYAMNWVRQAPGKGLEWVGRIRSKYNNYATYYADSVKGRFTI SRDTSKNTLYLQMNSLRAEDTAVYYCVRHGNFGNSYVSWFAYWGQGTTLVTV (SEQ ID NO: 720)
VH	EVQLVESGGGLVQPGGSLRLSCAASGFTFNTYAMNWVRQAPGKGLEWVGRIRSKYNNYATYYADSVKGRFTI SRDDSKNTAYLQMNSLRAEDTAVYYCVRHGNFGNSYVSWFAYWGQGTTLVTVSS (SEQ ID NO: 721)
VH	EVQLVESGGGLVQPGGSLRLSCAASGFTFNTYAMNWVRQAPGKGLEWVGRIRSKYNNYATYYADSVKGRFTI SRDDSKNTLYLQMNSLRDEDTAVYYCVRHGNFGNSYVSWFAYWGQGTTLVTVSS (SEQ ID NO: 722)
VH	EVQLVESGGGLVQPGGSLRLSCAASGFTFNTYAMNWVRQAPGKGLEWVGRIRSKYNNYATYYADSVKGRFTI SRDDSKNTLYLQMNSLRKEDTAVYYCVRHGNFGNSYVSWFAYWGQGTTLVTVSS (SEQ ID NO: 723)
VH	EVQLVESGGGLVQPGGSLRLSCAASGFTFNTYAMNWVRQAPGKGLEWVGRIRSKYNNYATYYADSVKGRFTI SRDDSKNTLYLQMNSLRSED TAVYYCVRHGNFGNSYVSWFAYWGQGTTLVTVSS (SEQ ID NO: 724)
VH	EVQLVESGGGLVQPGGSLRLSCAASGFTFNTYAMNWVRQAPGKGLEWVGRIRSKYNNYATYYADSVKGRFTI SRDDSKNTLYLQMNSLRAEDTAVYYCARHGNFGNSYVSWFAYWGQGTTLVTVSS (SEQ ID NO: 725)
VH	EVQLVESGGGLVQPGGSLRLSCAASGFTFNTYAMNWVRQAPGKGLEWVGRIRSKYNNYATYYADSVKGRFTI SRDDSKNTLYLQMNSLRAEDTAVYYCTR HGNFGNSYVSWFAYWGQGTTLVTVSS (SEQ ID NO: 726)
VH	EVQLVESGGGLVQPGGSLRLSCAASGFTFNTYAMNWVRQAPGKGLEWVGRIRSKYNNYATYYADSVKGRFTI

	SRDDSKNTLYLQMNSLRAEDTAVYYCVRHGNFGDSYVSWFAYWGQGTTLVTVSS (SEQ ID NO: 727)
VH	EVQLVESGGGLVQPGGSLRLSCAASGFTFNTYAMNWVRQAPGKGLEWVGRIRSKYNNYATYYADSVKGRFTI SRDDSKNTLYLQMNSLRAEDTAVYYCVRHGNFGQSYVSWFAYWGQGTTLVTVSS (SEQ ID NO: 728)
VH	EVQLVESGGGLVQPGGSLRLSCAASGFTFNTYAMNWVRQAPGKGLEWVGRIRSKYNNYATYYADSVKGRFTI SRDDSKNTLYLQMNSLRAEDTAVYYCVRHGNFGNTYVSWFAYWGQGTTLVTVSS (SEQ ID NO: 729)
VH	EVQLVESGGGLVQPGGSLRLSCAASGFTFNTYAMNWVRQAPGKGLEWVGRIRSKYNNYATYYADSVKGRFTI SRDDSKNTLYLQMNSLRAEDTAVYYCVRHGNFGNSYVSWFDYWGQGTTLVTVSS (SEQ ID NO: 730)
VH	EVQLVESGGGLVQPGGSLRLSCAASGFTFNTYAMNWVRQAPGKGLEWVGRIRSKYNNYATYYADSVKGRFTI SRDDSKNTLYLQMNSLRAEDTAVYYCVRHGNFGNSYVSWFAYWGQGTTVTVTVSS (SEQ ID NO: 731)
VH	EVQLVESGGGLVQPGGSLRLSCAASGFTFNTYAMNWVRQAPGKSLEWVGRIRSKYNNYATYYADSVKGRFTI SRDDSKNTLYLQMNSLRAEDTAVYYCVRHGNFGNSYVSWFAYWGQGTTLVTVSS (SEQ ID NO: 732)
VH	EVQLVESGGGLVQPGGSLRLSCAASGFTFNTYAMNWVRQAPGKGLEWVGRIRSKYNNYATYYADSVKGRFTI SRDDSKNTLYLQMNSLRAEDTATYYCVRHGNFGNSYVSWFAYWGQGTTLVTVSS (SEQ ID NO: 733)
VH	EVQLVESGGGLVQPGGSLRLSCAASGFTFSTYAMNWVRQAPGKGLEWVGRIRSKYNNYATYYADSVKGRFTI SRDDSKNTLYLQMNSLRAEDTAVYYCVRHGNFGDSYVSWFAYWGQGTTLVTVSS (SEQ ID NO: 734)
VH	EVQLVESGGGLVQPGGSLRLSCAASGFTFSTYAMSWVRQAPGKGLEWVGRIRSKYNNYATYYADSVKGRFTI SRDDSKNTLYLQMNSLRAEDTAVYYCVRHGNFGDSYVSWFAYWGQGTTLVTVSS (SEQ ID NO: 735)
VH	EVQLVESGGGLVQPGGSLRLSCAASGFTFSTYAMNWVRQAPGKGLEWVGRIRSKANNYATYYADSVKGRFTI SRDDSKNTLYLQMNSLRAEDTAVYYCVRHGNFGDSYVSWFAYWGQGTTLVTVSS (SEQ ID NO: 736)
VH	EVQLVESGGGLVQPGGSLRLSCAASGFTFSTYAMNWVRQAPGKGLEWVGRIRSKYNNYATYYADSVKGRFTI SRDDSKNTLYLQMNSLRAEDTAVYYCVRHGNFGDSYVSWFDYWGQGTTLVTVSS (SEQ ID NO: 737)
VH	EVQLVESGGGLVQPGGSLRLSCAASGFTFSTYAMSWVRQAPGKGLEWVGRIRSKANNYATYYADSVKGRFTI SRDDSKNTLYLQMNSLRAEDTAVYYCVRHGNFGDSYVSWFDYWGQGTTLVTVSS (SEQ ID NO: 738)
VH	EVQLVESGGEVKKPGESLRLSCAASGFTFNTYAMNWVRQAPGKGLEWVGRIRSKYNNYATYYADSVKGRFTI SRDDSKNTLYLQMNSLRAEDTAVYYCVRHGNFGNSYVSWFAYWGQGTTLVTVSS (SEQ ID NO: 739)
VH	EVQLVESGGGLVQPGGSLRLSCAASGFTFNTYAMNWVRQAPGKGLEWVGRIRSNGGSTYYADSVKGRFTISR DDSKNTLYLQMNSLRAEDTAVYYCVRHGNFGNSYVSWFAYWGQGTTLVTVSS (SEQ ID NO: 740)
VH	EVQLVESGGGLVQPGGSLRLSCAASGFTFNTYAMNWVRKAPGKGLEWVGRIRSKYNNYATYYADSVKGRFTI SRDDSKNTLYLQMNSLRAEDTAVYYCVRHGNFGNSYVSWFAYWGQGTTLVTVSS (SEQ ID NO: 741)
VH	EVQLVESGGGLVQPGGSLRLSCAASGFTFSTYAMNWVRKAPGKGLEWVGRIRSKYNNYATYYADSVKGRFTI SRDDSKNTLYLQMNSLRAEDTAVYYCVRHGNFGNSYVSWFAYWGQGTTLVTVSS (SEQ ID NO: 742)
VH	EVQLVESGGGLVQPGGSLRLSCAASGFTFSTYAMNWVRKAPGKGLEWVGRIRSKYNNYATYYADSVKGRFTI SRDDSKNTLYLQMNSLRAEDTAVYYCVRHGNFGDSYVSWFAYWGQGTTLVTVSS (SEQ ID NO: 743)
VL	QAVVTQEPSLTVSPGGTVTLTCGSSTGAVTTSNYANWVQQKPGQAPRGLIGGTNKRAPGVPARFSGSLLGKK AALTLSGAQPEDEAEYYCALWYSNLWVFGGGTKLTVL (SEQ ID NO: 744)
VL	QAVVTQPPSLTVSPGGTVTLTCGSSTGAVTTSNYANWVQQKPGQAPRGLIGGTNKRAPGVPARFSGSLLGKK

	AALTLSGAQPEDEAEYYCALWYSNLWVFGGGTKLTVL (SEQ ID NO: 745)
VL	QAVVTQEPSLSVSPGGTVTLTCGSSTGAVTTSNYANWVQQKPGQAPRGLIGGTNKRAPGVPARFSGSLLGGK AALTLSGAQPEDEAEYYCALWYSNLWVFGGGTKLTVL (SEQ ID NO: 746)
VL	QAVVTQEPSLTVSPGATVTLTCGSSTGAVTTSNYANWVQQKPGQAPRGLIGGTNKRAPGVPARFSGSLLGGK AALTLSGAQPEDEAEYYCALWYSNLWVFGGGTKLTVL (SEQ ID NO: 747)
VL	QAVVTQEPSLTVSPGQTVTLTCGSSTGAVTTSNYANWVQQKPGQAPRGLIGGTNKRAPGVPARFSGSLLGGK AALTLSGAQPEDEAEYYCALWYSNLWVFGGGTKLTVL (SEQ ID NO: 748)
VL	QAVVTQEPSLTVSPGGTVTLTCGSSTGAVTSSNYANWVQQKPGQAPRGLIGGTNKRAPGVPARFSGSLLGGK AALTLSGAQPEDEAEYYCALWYSNLWVFGGGTKLTVL (SEQ ID NO: 749)
VL	QAVVTQEPSLTVSPGGTVTLTCGSSTGAVTSGHYANWVQQKPGQAPRGLIGGTNKRAPGVPARFSGSLLGGK AALTLSGAQPEDEAEYYCALWYSNLWVFGGGTKLTVL (SEQ ID NO: 750)
VL	QAVVTQEPSLTVSPGGTVTLTCGSSTGAVTTSNYANWVQQKPGQAPRGLIYDTNKRAPGVPARFSGSLLGGK AALTLSGAQPEDEAEYYCALWYSNLWVFGGGTKLTVL (SEQ ID NO: 751)
VL	QAVVTQEPSLTVSPGGTVTLTCGSSTGAVTTSNYANWVQQKPGQAPRGLIGGTNNRAPGVPARFSGSLLGGK AALTLSGAQPEDEAEYYCALWYSNLWVFGGGTKLTVL (SEQ ID NO: 752)
VL	QAVVTQEPSLTVSPGGTVTLTCGSSTGAVTTSNYANWVQQKPGQAPRGLIGGTNKRASGVPARFSGSLLGGK AALTLSGAQPEDEAEYYCALWYSNLWVFGGGTKLTVL (SEQ ID NO: 753)
VL	QAVVTQEPSLTVSPGGTVTLTCGSSTGAVTTSNYANWVQQKPGQAPRGLIGGTSNKHSWTPARFSGSLLGGK AALTLSGAQPEDEAEYYCALWYSNLWVFGGGTKLTVL (SEQ ID NO: 754)
VL	QAVVTQEPSLTVSPGGTVTLTCGSSTGAVTTSNYANWVQQKPGQAPRGLIGGTNKRAPGVDPDRFSGSLLGGK AALTLSGAQPEDEAEYYCALWYSNLWVFGGGTKLTVL (SEQ ID NO: 755)
VL	QAVVTQEPSLTVSPGGTVTLTCGSSTGAVTTSNYANWVQQKPGQAPRGLIGGTNKRAPGVPARFSGSKSGGK AALTLSGAQPEDEAEYYCALWYSNLWVFGGGTKLTVL (SEQ ID NO: 756)
VL	QAVVTQEPSLTVSPGGTVTLTCGSSTGAVTTSNYANWVQQKPGQAPRGLIGGTNKRAPGVPARFSGSSSGGK AALTLSGAQPEDEAEYYCALWYSNLWVFGGGTKLTVL (SEQ ID NO: 757)
VL	QAVVTQEPSLTVSPGGTVTLTCGSSTGAVTTSNYANWVQQKPGQAPRGLIGGTNKRAPGVPARFSGSLLGGK AALTISGAQPEDEAEYYCALWYSNLWVFGGGTKLTVL (SEQ ID NO: 758)
VL	QAVVTQEPSLTVSPGGTVTLTCGSSTGAVTTSNYANWVQQKPGQAPRGLIGGTNKRAPGVPARFSGSLLGGK AALTLSGAQAEDEAEYYCALWYSNLWVFGGGTKLTVL (SEQ ID NO: 759)
VL	QAVVTQEPSLTVSPGGTVTLTCGSSTGAVTTSNYANWVQQKPGQAPRGLIGGTNKRAPGVPARFSGSLLGGK AALTLSGAQSEDEAEYYCALWYSNLWVFGGGTKLTVL (SEQ ID NO: 760)
VL	QAVVTQEPSLTVSPGGTVTLTCGSSTGAVTTSNYANWVQQKPGQAPRGLIGGTNKRAPGVPARFSGSLLGGK AALTLSGAQPEDEADYYCALWYSNLWVFGGGTKLTVL (SEQ ID NO: 761)
VL	QAVVTQEPSLTVSPGGTVTLTCGSSTGAVTTSNYANWVQQKPGQAPRGLIGGTNKRAPGVPARFSGSLLGGK AALTLSGAQPEDEAEYYCLLWYSNLWVFGGGTKLTVL (SEQ ID NO: 762)
VL	QAVVTQEPSLTVSPGGTVTLTCGSSTGAVTTSNYANWVQQKPGQAPRGLIGGTNKRAPGVPARFSGSLLGGK

	AALTLSGAQPEDEAEYYCALWYSNHWVFEGGGTKLTVL (SEQ ID NO: 763)
VL	QAVVTQEPSLTVSPGGTVTLTCGSSTGAVTTSNYANWVQQKPGQAPRGLIGGTNKRAPGVPARFSGSLLGGK AALTLSGAQPEDEAEYYCALWYSNLWVFEGGGTKLEIKGS (SEQ ID NO: 764)
VL	EIVMTQSPATLSLSPGERATLSCRSTGAVTTSNYANWVQQKPGQAPRGLIGGTNKRAPGVPARFSGSGSGT DFTLTISSLQPEDFAVYYCALWYSNLWVFEGGGTKVEIKGS (SEQ ID NO: 765)
VL	DIVMTQSPDSLAVSLGERATINCKSSTGAVTTSNYANWVQQKPGQPPKGLIGGTNKRAPGVDPDRFSGSGSGT DFTLTISSLQAEDVAVYYCALWYSNLWVFEGGGTKVEIKGS (SEQ ID NO: 766)
VL	QAVVTQEPSLTVSPGGTVTLTCGSSTGAVTTSNYANWVQQKPGQAPRGLIGGTNKRAPGVPARFSGSLLGGK AALTDSGAQPEDEADYYCALWYSNHWVFEGGGTKLTVL (SEQ ID NO: 767)
VL	QAVVTQEPSLTVSPGGTVTLTCGSSTGAVTTSNYANWVQEKPGQAPRGLIGGTNKRAPGVPARFSGSLLGGK AALTLSGAQPEDEAEYYCALWYSNLWVFEGGGTKLTVL (SEQ ID NO: 768)
VL	QAVVTQEPSLTVSPGGTVTLTCGSSTGAVTTSNYANWVQQKPGKAPRGLIGGTNKRAPGVPARFSGSLLGGK AALTLSGAQPEDEAEYYCALWYSNLWVFEGGGTKLT (SEQ ID NO: 769)
VL	QAVVTQEPSLTVSPGGTVTLTCGSSTGAVTTSNYANWVQQKPGTAPRGLIGGTNKRAPGVPARFSGSLLGGK AALTLSGAQPEDEAEYYCALWYSNLWVFEGGGTKLTVL (SEQ ID NO: 770)
VL	QAVVTQEPSLTVSPGGTVTLTCGSSTGAVTTSNYANWVQQKPGSPRGLIGGTNKRAPGVPARFSGSLLGGK AALTLSGAQPEDEAEYYCALWYSNLWVFEGGGTKLTVL (SEQ ID NO: 771)
VL	QAVVTQEPSLTVSPGGTVTLTCGSSTGAVTTSNYANWVQQKPGQPPRGLIGGTNKRAPGVPARFSGSLLGGK AALTLSGAQPEDEAEYYCALWYSNLWVFEGGGTKLTVL (SEQ ID NO: 772)
VL	QAVVTQEPSLTVSPGGTVTLTCGSSTGAVTTSNYANWVQQKPGQGPRGLIGGTNKRAPGVPARFSGSLLGGK AALTLSGAQPEDEAEYYCALWYSNLWVFEGGGTKLTVL (SEQ ID NO: 773)
VL	QAVVTQEPSLTVSPGGTVTLTCGSSTGAVTTSNYANWVQQKPGQSPRGLIGGTNKRAPGVPARFSGSLLGGK AALTLSGAQPEDEAEYYCALWYSNLWVFEGGGTKLTVL (SEQ ID NO: 774)
VL	QAVVTQEPSLTVSPGGTVTLTCGSSTGAVTTSNYANWVQQKPGQDPRGLIGGTNKRAPGVPARFSGSLLGGK AALTLSGAQPEDEAEYYCALWYSNLWVFEGGGTKLTVL (SEQ ID NO: 775)
VL	QAVVTQEPSLTVSPGGTVTLTCGSSTGAVTTSNYANWVQQKPGQAPRGLIGGTNKRAPGVPARFSGSLLGGK AALTISGAQPEDEADYYCALWYSNHWVFEGGGTKLTVL (SEQ ID NO: 776)
VL	QAVVTQEPSLTVSPGGTVTLTCGSSTGAVTTSNYANWVQQKPGQAPRGLIGGTNKRAPGVPARFSGSLLGGK AALTISGAQPEDEAEYYCALWYSNHWVFEGGGTKLTVL (SEQ ID NO: 777)
VL	QAVVTQEPSLTVSPGGTVTLTCGSSTGAVTTSNYANWVQQKPGKAPRGLIGGTNKRAPGVPARFSGSLLGGK AALTISGAQPEDEAEYYCALWYSNHWVFEGGGTKLTVL (SEQ ID NO: 778)
VL	QAVVTQEPSLTVSPGGTVTLTCGSSTGAVTTSNYANWVQQKPGQSPRGLIGGTNKRAPGVPARFSGSLLGGK AALTISGAQPEDEAEYYCALWYSNHWVFEGGGTKLTVL (SEQ ID NO: 779)
VL	QAVVTQEPSLTVSPGGTVTLTCGSSTGAVTTSNYANWVQQKPGKSPRGLIGGTNKRAPGVPARFSGSLLGGK AALTISGAQPEDEAEYYCALWYSNHWVFEGGGTKLTVL (SEQ ID NO: 780)
VL	QAVVTQEPSLTVSPGGTVTLTCGSSTGAVTTSNYANWVQQKPGKAPRGLIGGTNKRAPGVPARFSGSLLGGK

	AALTISGAQPEDEADYYCALWYSNHWVFEGGGTKLTVL (SEQ ID NO: 781)
VL	QAVVTQEPSLTVSPGGTVTLTCGSSTGAVTTSNYANWVQQKPGQSPRGLIGGTNKRAPGVPARFSGSLLGGK AALTISGAQPEDEADYYCALWYSNHWVFEGGGTKLTVL (SEQ ID NO: 782)
VL	QAVVTQEPSLTVSPGGTVTLTCGSSTGAVTTSNYANWVQQKPGKSPRGLIGGTNKRAPGVPARFSGSLLGGK AALTISGAQPEDEADYYCALWYSNHWVFEGGGTKLTVL (SEQ ID NO: 783)

[000382] In some embodiments, the activatable anti-CD3 ϵ antibody comprises or is derived from a commercially available antibody, such as, for example, an anti-CD3 antibody from Boehringer Mannheim Corp. (Indianapolis, Ind.; Cat. No. 1273 485).

[000383] In some embodiments, the activatable anti-CD3 ϵ antibody comprises or is derived from an antibody that is manufactured, secreted or otherwise produced by a hybridoma, such as, for example, the hybridoma(s) disclosed in US Patent No. 4,361,549 and/or 4,658,019, and deposited at the American Type Culture Collection under Accession Number ATCC CRL 8001 OKT-3.

[000384] In some embodiments, the activatable anti-CD3 ϵ antibody comprises or is derived from an antibody that is manufactured, secreted or otherwise produced by a hybridoma, such as, for example, the hybridoma(s) disclosed in PCT Publication No. WO 1995/16037, and deposited at the Leibniz-Institut DSMZ - Deutsche Sammlung von Mikroorganismen und Zellkulturen GmbH under Accession Number DSM ACC2152.

[000385] In some embodiments, the activatable anti-CD3 ϵ antibody comprises or is derived from an antibody that is manufactured, secreted or otherwise produced by a hybridoma, such as, for example, the hybridoma(s) disclosed in PCT Publication No. WO 1991/01752, and deposited at the American Type Culture Collection under Accession Number HB 10166.

[000386] In some embodiments, the activatable anti-CD3 ϵ antibody includes a CDR sequence shown in Table 18, a combination of VL CDR sequences selected from the group consisting of those combinations shown in Table 18, and/or a combination of VH CDR sequences selected from the group consisting of those combinations shown in Table 18. In some embodiments, the activatable anti-CD3 ϵ antibody includes a combination of heavy chain CDR sequences selected from the group consisting of the combinations shown in Group R in Table 18. In some embodiments, the activatable anti-CD3 ϵ antibody includes a combination of light chain CDR sequences selected from the group consisting of the combinations shown in

Group R in Table 18. In some embodiments, the activatable anti-CD3 ϵ antibody includes a combination of heavy chain CDR sequences selected from the group consisting of the combinations shown in Group S in Table 18. In some embodiments, the activatable anti-CD3 ϵ antibody includes a combination of light chain CDR sequences selected from the group consisting of the combinations shown in Group S in Table 18. In some embodiments, the activatable anti-CD3 ϵ antibody includes a combination of heavy chain CDR sequences selected from the group consisting of the combinations shown in Group T in Table 18. In some embodiments, the activatable anti-CD3 ϵ antibody includes a combination of light chain CDR sequences selected from the group consisting of the combinations shown in Group T in Table 18. In some embodiments, the activatable anti-CD3 ϵ antibody includes a combination of heavy chain CDR sequences selected from the group consisting of the combinations shown in Group U in Table 18. In some embodiments, the activatable anti-CD3 ϵ antibody includes a combination of light chain CDR sequences selected from the group consisting of the combinations shown in Group U in Table 18. In some embodiments, the activatable anti-CD3 ϵ antibody includes a combination of heavy chain CDR sequences selected from the group consisting of the combinations shown in Group V in Table 18. In some embodiments, the activatable anti-CD3 ϵ antibody includes a combination of light chain CDR sequences selected from the group consisting of the combinations shown in Group V in Table 18. In some embodiments, the activatable anti-CD3 ϵ antibody includes a combination of heavy chain CDR sequences selected from the group consisting of the combinations shown in Group W in Table 18. In some embodiments, the activatable anti-CD3 ϵ antibody includes a combination of light chain CDR sequences selected from the group consisting of the combinations shown in Group W in Table 18. In some embodiments, the activatable anti-CD3 ϵ antibody includes a combination of heavy chain CDR sequences selected from the group consisting of the combinations shown in Group X in Table 18. In some embodiments, the activatable anti-CD3 ϵ antibody includes a combination of light chain CDR sequences selected from the group consisting of the combinations shown in Group X in Table 18.

Table 18. Additional CDR Sequences for Antibodies and Activatable Antibodies that Bind CD3 ϵ

Group R					
VH			VL		
CDR1 (SEQ ID NO:)	CDR2 (SEQ ID NO:)	CDR3 (SEQ ID NO:)	CDR1 (SEQ ID NO:)	CDR2 (SEQ ID NO:)	CDR3 (SEQ ID NO:)
TLSSGNIENYVH (784)	DDDKRPD (785)	HSYVSSFNV (786)	SFPMA (787)	TISTSGGRTYYR DSVKG (788)	FRQYSGGFDY (789)
Group S					
VH			VL		
CDR1 (SEQ ID NO:)	CDR2 (SEQ ID NO:)	CDR3 (SEQ ID NO:)	CDR1 (SEQ ID NO:)	CDR2 (SEQ ID NO:)	CDR3 (SEQ ID NO:)
TLSSGNIE NNYVH (790)	DDDKRPD (785)	HSYVSSFNV (786)	SFPMA (787)	TISTSGG RTYYRDS VKG (788)	FRQYSGGFDY (789)
Group T					
VH			VL		
CDR1 (SEQ ID NO:)	CDR2 (SEQ ID NO:)	CDR3 (SEQ ID NO:)	CDR1 (SEQ ID NO:)	CDR2 (SEQ ID NO:)	CDR3 (SEQ ID NO:)
GFTFNTYA (791)	IRSKYNNYAT (792)	VRHGNFGNSYV SWFAY (793)	TGAVTTSNY (794)	GTN (795)	ALWYSNLWV (58)
Group U					
VH			VL		
CDR1 (SEQ ID NO:)	CDR2 (SEQ ID NO:)	CDR3 (SEQ ID NO:)	CDR1 (SEQ ID NO:)	CDR2 (SEQ ID NO:)	CDR3 (SEQ ID NO:)
RYTMH (796)	YINPSRGYTNYNQKFK D (797)	YYDDHYSLDY (798)	SASSSVSYMN (799)	DTSKLAS (800)	QQWSSNPFT (801)

Group V					
VH			VL		
CDR1 (SEQ ID NO:)	CDR2 (SEQ ID NO:)	CDR3 (SEQ ID NO:)	CDR1 (SEQ ID NO:)	CDR2 (SEQ ID NO:)	CDR3 (SEQ ID NO:)
SYGMH (802)	VISYDGSNKYYADSVK G (803)	LSPYCTNGVCW DAFDI (804)	RASQTISNYLN (805)	AASTLQS (806)	QQFNSYPRT (807)
DYAMS (808)	FIRSKAYGGTTEYAAS VKG (809)	QLWLLQDAFDI (810)	RASQGISNYLA (811)	AASTLQS (806)	QQSYSTPPT (812)
SRNWS (813)	DIYHSGSTNYNPSLKS (814)	GYTSCRDAFDI (815)	RASQGIGNYLA (816)	WASIRES (817)	QQYYSNPQT (818)
GYWWS (819)	EINHSGSTNYNPSLKS (820)	GRGRFLGWLLG GSNWFD (821)	RASQGISNYLN (822)	DASNLET (823)	QQSYSTPYT (824)
GYWWS (819)	EINHSGSTNYNPSLKS (820)	GPDRMGHGF (825)	RASQSISSYLN (826)	AASSLQS (92)	QQSYSSP (827)
SNSAAWN (828)	RTYYRSKQYNDYAVSV KS (829)	DRRRIAARQYY GMDV (830)	RASQSVSSNYLA (831)	GASSRAT (832)	QKYNAP (833)
SYAMG (834)	AVSGSGGSTYYADSVK G (835)	AKFLGHYYGMD V (836)	RASQSISSYLN (826)	AASSLQS (92)	LQHNAYPYT (837)
NPRMGVS (838)	HIFPSDAKAHSASLKS (839)	ILGEYPPAWF DP (840)	KSSQSVLYSSNNKN YLA (841)	WASTRES (842)	QQYLKIPYT (843)
Group W					
VH			VL		
CDR1 (SEQ ID NO:)	CDR2 (SEQ ID NO:)	CDR3 (SEQ ID NO:)	CDR1 (SEQ ID NO:)	CDR2 (SEQ ID NO:)	CDR3 (SEQ ID NO:)
IYAMN (844)	RIRSKYNNYATYYADS VKS (845)	HGNFGNSYVSF FAY (846)	GSSTGAVTSGYYPN (847)	GTKFLAP (848)	ALWYSNRWV (849)
KYAMN (850)	RIRSKYNNYATYYADS VKD (54)	HGNFGNSYISY WAY (851)	RSSTGAVTSGYYPN (852)	ATDMRPS (853)	ALWYSNRWV (849)
SYAMN (854)	RIRSKYNNYATYYADS VKG (855)	HGNFGNSYLSF WAY (856)	GSSTGAVTSGNYPN (857)	GTKFLAP (848)	VLWYSNRWV (858)

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RYAMN (859)	RIRSKYNNYATYYADS VKG (855)	HGNFGNSYLSY FAY (860)			
VYAMN (861)	RIRSKYNNYATYYADS VKK (862)	HGNFGNSYLSW WAY (863)			
KYAMN (850)	RIRSKYNNYATYYADS VKS (845)	HGNFGNSYTSY YAY (864)			
GYAMN (865)	RIRSKYNNYATYYADS VKE (866)	HRNFGNSYLSW FAY (867)			
VYAMN (861)	RIRSKYNNYATYYADS VKK (862)	HGNFGNSYISW WAY (868)			
SYAMN (854)	RIRSKYNNYATYYADS VKG (855)	HGNFGNSYVSW WAY (869)			
KYAMN (850)	RIRSKYNNYATYYADS VKD (54)	HGNFGNSYISY WAY (851)			
IYAMN (844)	RIRSKYNNYATYYADS VKS (845)	HGNFGNSYVSF FAY (846)			
KYAMN (850)	RIRSKYNNYATYYADS VKD (54)	HGNFGNSYISY WAY (851)			
SYAMN (854)	RIRSKYNNYATYYADS VKG (855)	HGNFGNSYLSF WAY (856)			
			GSSTGAVTSGYYPN (847)	GTKFLAP (848)	ALWYSNRWV (849)
			RSSTGAVTSGYYPN (852)	ATDMRPS (853)	ALWYSNRWV (849)
			GSSTGAVTSGNYPN (857)	GTKFLAP (848)	VLWYSNRWV (858)
Group X					
VH			VL		
CDR1 (SEQ ID NO:)	CDR2 (SEQ ID NO:)	CDR3 (SEQ ID NO:)	CDR1 (SEQ ID NO:)	CDR2 (SEQ ID NO:)	CDR3 (SEQ ID NO:)
GYGMH (870)	VIWYDGSKKYYVDSVK G (871)	QMGYWHFDL (872)	RASQSVSSYLA (873)	DASNRAT (874)	QQRSNWPPLT (875)
SYGMH (802)	IIWYDGSKKNYADSVK G (872)	GTGYNWFDP (873)	RASQSVSSSYLA (874)	GASSRAT (832)	QQYGSSPIT (875)

SYGMH (802)	IIWYDGSKKNYADSVK G (872)	GTGYNWFDP (873)	RASQGISSALA (876)	YASSLQS (877)	QQYYSTLTF (888)
SYGMH (802)	IIWYDGSKKNYADSVK G (872)	GTGYNWFDP (873)	RASQGISSALA (876)	DASSLGS (889)	QQYYSTLTF (888)
SYGMH (802)	IIWYDGSKKNYADSVK G (872)	GTGYNWFDP (873)	WASQGISSYLA (890)	YASSLQS (877)	QQYYSTLTF (888)
SYGMH (802)	AIWYNGRKQDYADSVK G (891)	GTGYNWFDP (873)	RASQSVSSYLA (873)	DASNRAT (874)	QQRSNWPWT (892)
SYGMH (802)	AIWYNGRKQDYADSVK G (891)	GTGYNWFDP (873)	RASQGISSALA (876)	DASSLES (893)	QQFNSYPIT (894)

[000387] The ABs in the multispecific activatable antibodies of the disclosure specifically bind at least a mammalian target. In some embodiments, such ABs bind mammalian CD3 ϵ . In some embodiments, such ABs bind a human target. In some embodiments, such ABs bind a non-human primate target. Also included in the disclosure are ABs that bind to the same epitope as an antibody of the disclosure and/or an activated activatable antibody described herein. Also included in the disclosure are ABs that compete with an antibody and/or an activated activatable antibody described herein for binding to a target, e.g., a human target. Also included in the disclosure are ABs that cross-compete with an antibody and/or an activated activatable antibody described herein for binding to a target, e.g., a human target.

[000388] In some embodiments, the ABs in the multispecific activatable antibodies of the disclosure specifically bind at least a CD3 ϵ target, such as, for example, mammalian CD3 ϵ . In some embodiments, such ABs bind mammalian CD3 ϵ . In some embodiments, such ABs bind human CD3 ϵ . In some embodiments, such ABs bind non-human primate CD3 ϵ . Also included in the disclosure are ABs that bind to the same CD3 ϵ epitope as an antibody of the disclosure and/or an activated activatable antibody described herein. Also included in the disclosure are ABs that compete with an anti-CD3 ϵ antibody and/or an activated anti-CD3 ϵ activatable antibody described herein for binding to a CD3 ϵ target, e.g., human CD3 ϵ . Also included in the disclosure are ABs that cross-compete with an anti-CD3 ϵ antibody and/or an activated anti-CD3 ϵ activatable antibody described herein for binding to a CD3 ϵ target, e.g., human CD3 ϵ .

[000389] In some embodiments, at least one of the AB of the multispecific antibody and/or multispecific activatable antibody binds Epidermal Growth Factor Receptor (EGFR). In

some embodiments, the AB that binds EGFR includes one or more of the heavy chain and/or light chain sequences shown below.

C225v5 Antibody Heavy Chain Nucleotide Sequence:

CAGGTGCAGCTGAAACAGAGCGGCCCGGGCCTGGTGCAGCCGAGCCAGAGCCTGAGCATTACCTGCACCGTGAGCG
GCTTTAGCCTGACCAACTATGGCGTGCATTGGGTGCGCCAGAGCCCGGGCAAAGGCCTGGAATGGCTGGGCGTGAT
TTGGAGCGGCGGCAACACCGATTATAACACCCCGTTTACCAGCCGCCTGAGCATTAACAAAGATAACAGCAAAAGC
CAGGTGTTTTTTTAAATGAACAGCCTGCAAAGCCAGGATACCGCGATTTATTATTGCGCGCGCGCGCTGACCTATT
ATGATTATGAATTTGCGTATTGGGGCCAGGGCACCCCTGGTGACCGTGAGCGCGGCTAGCACCAAGGGCCCATCGGT
CTTCCCCCTGGCACCCCTCCTCCAAGAGCACCTCTGGGGGCACAGCGGCCCTGGGCTGCCTGGTCAAGGACTACTTC
CCCGAACCGGTGACGGTGTCTGTGGAACCTCAGGCGCCCTGACCAGCGGCGTGACACCTTCCCGGCTGTCCTACAGT
CCTCAGGACTCTACTCCCTCAGCAGCGTGGTGACCGTGCCCTCCAGCAGCTTGGGCACCCAGACCTACATCTGCAA
CGTGAATCACAAGCCCAGCAACACCAAGGTGGACAAGAAAGTTGAGCCCAAATCTTGTGACAAAACCTCACACATGC
CCACCGTGCCCAGCACCTGAACTCCTGGGGGGACCGTCAGTCTTCCTCTTCCCCCAAAACCCAAGGACACCCTCA
TGATCTCCCGGACCCCTGAGGTACATGCGTGGTGGTGGACGTGAGCCACGAAGACCCTGAGGTCAAGTTCAACTG
GTACGTGGACGGCGTGGAGGTGCATAATGCCAAGACAAAGCCGCGGGAGGAGCAGTACAACAGCACGTACCGTGTG
GTCAGCGTCCTCACCGTCCTGCACCAGGACTGGCTGAATGGCAAGGAGTACAAGTGCAAGGTCTCCAACAAAGCCC
TCCCAGCCCCCATCGAGAAAACCATCTCCAAAGCCAAAGGGCAGCCCCGAGAACCACAGGTGTACACCCTGCCCCC
ATCCCGGGATGAACTGACCAAGAACCAGGTCAGCCTGACCTGCCTGGTCAAAGGCTTCTATCCCAGCGACATCGCC
GTGGAGTGGGAGAGCAATGGGCAGCCGGAGAACAACCTACAAGACCACGCCTCCCGTGCTGGACTCCGACGGCTCCT
TCTTCCTCTACAGCAAGCTCACCGTGGACAAGAGCAGGTGGCAGCAGGGGAACGTCTTCTCATGCTCCGTGATGCA
TGAGGCTCTGCACAACCACTACACGCAGAAGAGCCTCTCCCTGTCTCCGGGTAAATGA (SEQ ID NO: 100)

C225v5 Antibody Heavy Chain Amino Acid Sequence

QVQLKQSGPGLVQPSQSLITCTVSGFSLTNYGVHWVRQSPGKGLEWLGVIWSGGNTDYNTPFTSRLSINKDNSKS
QVFFKMNSLQS^QDTAIYYCARALTYDYEFAYWGQGLVTVSAASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYF
PEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSSLGTQTYICNVNHKPSNTKVDKKVEPKSCDKHTC
PPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRV
VSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSRDELTKNQVSLTCLVKGFYPSDIA
VEWESNGQPENNYKTTTPVLDSDGSFFLYSKLTVDKSRWQQGNVFSQVMHEALHNHYTQKSLSLSPGK* (SEQ
ID NO: 101)

C225v5 Antibody Light Chain Nucleotide Sequence:

CAGATCTTGCTGACCCAGAGCCCGGTGATTCTGAGCGTGAGCCCGGGCGAACGTGTGAGCTTTAGCTGCCGCGCGA
GCCAGAGCATTGGCACCAACATTCATTGGTATCAGCAGCGCACCAACGGCAGCCCGCGCCTGCTGATTAAATATGC
GAGCGAAAGCATTAGCGGCATTCCGAGCCGCTTTAGCGGCAGCGGCAGCGGCACCGATTTTACCCTGAGCATTAAC

AGCGTGGAAGCGAAGATATTGCGGATTATTATTGCCAGCAGAACAACAACCTGGCCGACCACCTTTGGCGCGGGCA
 CCAAACCTGGAACCTGAAACGTACGGTGGCTGCACCATCTGTCTTCATCTTCCCGCCATCTGATGAGCAGTTGAAATC
 TGGAACCTGCCTCTGTTGTGTGCCTGCTGAATAACTTCTATCCCAGAGAGGCCAAAGTACAGTGGAAGGTGGATAAC
 GCCCTCCAATCGGGTAACTCCCAGGAGAGTGTACAGAGCAGGACAGCAAGGACAGCACCTACAGCCTCAGCAGCA
 CCCTGACGCTGAGCAAAGCAGACTACGAGAAACACAAAGTCTACGCCTGCGAAGTCACCCATCAGGGCCTGAGCTC
 GCCCGTCACAAAGAGCTTCAACAGGGGAGAGTGTTAG (SEQ ID NO: 102)

C225v5 Antibody Light Chain Amino Acid Sequence:

QILLTQSPVILSVSPGERVSFSCRASQSIGTNIHWYQORTNGSPRLLIKYASESISGIPSRFSGSGSGTDFTLIN
 SVESEDIADYYCQQNNNWPTTFGAGTKLELKRTVAAPSVFIFPPSDEQLKSGTASVVCLLNNFYPREAKVQWKVDN
 ALQSGNSQESVTEQDSKDYSLSTLTLSKADYEKHKVYACEVTHQGLSSPVTKSFNRGEC* (SEQ ID
 NO: 103)

C225v4 Antibody Heavy Chain Nucleotide Sequence:

CAGGTGCAGCTGAAACAGAGCGGCCCGGGCCTGGTGCAGCCGAGCCAGAGCCTGAGCATTACCTGCACCGTGAGCG
 GCTTTAGCCTGACCAACTATGGCGTGCATTGGGTGCGCCAGAGCCCGGGCAAAGGCCTGGAATGGCTGGGCGTGAT
 TTGGAGCGGCGGCAACACCGATTATAACACCCCGTTTACCAGCCGCCTGAGCATTAACAAAGATAACAGCAAAAGC
 CAGGTGTTTTTTAAATGAACAGCCTGCAAAGCAACGATACCGCGATTTATTATTGCGCGCGCGCGCTGACCTATT
 ATGATTATGAATTTGCGTATTGGGGCCAGGGCACCCCTGGTGACCGTGAGCGCGGCTAGCACCAAGGGCCCATCGGT
 CTCCCCCTGGCACCCCTCCTCCAAGAGCACCTCTGGGGGCACAGCGGCCCTGGGCTGCCTGGTCAAGGACTACTTC
 CCCGAACCGGTGACGGTGTCGTGGAACCTCAGGCGCCCTGACCAGCGGCGTGACACCTTCCCGGCTGTCCTACAGT
 CCTCAGGACTCTACTCCCTCAGCAGCGTGGTGACCGTGCCCTCCAGCAGCTTGGGCACCCAGACCTACATCTGCAA
 CGTGAATCACAAGCCCAGCAACACCAAGGTGGACAAGAAAGTTGAGCCCAAATCTTGTGACAAAACCTCACACATGC
 CCACCGTGCCCAGCACCTGAACTCCTGGGGGGACCGTCAGTCTTCCTCTTCCCCCAAACCCAAGGACACCCTCA
 TGATCTCCCGGACCCCTGAGGTCACATGCGTGGTGGTGGACGTGAGCCACGAAGACCCTGAGGTCAAGTTCAACTG
 GTACGTGGACGGCGTGGAGGTGCATAATGCCAAGACAAAGCCGCGGGAGGAGCAGTACAACAGCACGTACCGTGTG
 GTCAGCGTCCTCACCGTCCTGCACCAGGACTGGCTGAATGGCAAGGAGTACAAGTGCAAGGTCTCCAACAAAGCCC
 TCCCAGCCCCCATCGAGAAAACCATCTCCAAAGCCAAAGGGCAGCCCCGAGAACCACAGGTGTACACCCTGCCCCC
 ATCCCGGGATGAACTGACCAAGAACCAGGTCAGCCTGACCTGCCTGGTCAAAGGCTTCTATCCCAGCGACATCGCC
 GTGGAGTGGGAGAGCAATGGGCAGCCGGAGAACAACCTACAAGACCACGCCTCCCGTGCTGGACTCCGACGGCTCCT
 TCTTCCTCTACAGCAAGCTCACCGTGGACAAGAGCAGGTGGCAGCAGGGGAACGTCTTCTCATGCTCCGTGATGCA
 TGAGGCTCTGCACAACCACTACACGCAGAAGAGCCTCTCCCTGTCTCCGGGTAAATGA (SEQ ID NO: 104)

C225v4 Antibody Heavy Chain Amino Acid Sequence:

QVQLKQSGPGLVQPSQSLITCTVSGFSLTNYGVHWVRQSPGKGLEWLGVIWSGGNTDYNTPFTSRLSINKDNSKS
 QVFFKMNSLQSN~~N~~DTAIYYCARALTYDYEFAYWGQGLVTVSAASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYF
 PEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSSLGTQTYICNVNHKPSNTKVDKKVEPKSCDKTHTC

PPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRV
VSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSRDELTKNQVSLTCLVKGFYPSDIA
VEWESNGQPENNYKTTTPVLDSDGSFFLYSKLTVDKSRWQQGNVFSVMSHEALHNHYTQKSLSLSPGK* (SEQ
ID NO: 105)

C225v6 Antibody Heavy Chain Nucleotide Sequence:

CAGGTGCAGCTGAAACAGAGCGGCCCGGGCCTGGTGCAGCCGAGCCAGAGCCTGAGCATTACCTGCACCGTGAGCG
GCTTTAGCCTGACCAACTATGGCGTGCATTGGGTGCGCCAGAGCCCGGGCAAAGGCCTGGAATGGCTGGGCGTGAT
TTGGAGCGGCGGCAACACCGATTATAACACCCCGTTTACCAGCCGCCTGAGCATTAACAAAGATAACAGCAAAAGC
CAGGTGTTTTTTTAAATGAACAGCCTGCAAAGCCAGGATACCGCGATTTATTATTGCGCGCGCGCGCTGACCTATT
ATGATTATGAATTTGCGTATTGGGGCCAGGGCACCCCTGGTGACCGTGAGCGCGGCTAGCACCAAGGGCCCATCGGT
CTTCCCCCTGGCACCCCTCCTCCAAGAGCACCTCTGGGGGCACAGCGGCCCTGGGCTGCCTGGTCAAGGACTACTTC
CCCGAACCGGTGACGGTGTCTGTGGAACCTCAGGCGCCCTGACCAGCGGCGTGACACCTTCCCGGCTGTCCTACAGT
CCTCAGGACTCTACTCCCTCAGCAGCGTGGTGACCGTGCCCTCCAGCAGCTTGGGCACCCAGACCTACATCTGCAA
CGTGAATCACAAGCCCAGCAACACCAAGGTGGACAAGAAAGTTGAGCCCAAATCTTGTGACAAAACCTCACACATGC
CCACCGTGCCCAGCACCTGAACTCCTGGGGGGACCGTCAGTCTTCCTCTTCCCCCCTTCCCAAGGACACCCCTCA
TGATCTCCCGGACCCCTGAGGTACATGCGTGGTGGTGGACGTGAGCCACGAAGACCCTGAGGTCAAGTTCAACTG
GTACGTGGACGGCGTGGAGGTGCATAATGCCAAGACAAAGCCGCGGGAGGAGCAGTACGCCAGCACGTACCGTGTG
GTCAGCGTCCTCACCGTCCTGCACCAGGACTGGCTGAATGGCAAGGAGTACAAGTGCAAGGTCTCCAACAAAGCCC
TCCCAGCCCCCATCGAGAAAACCATCTCCAAAGCCAAAGGGCAGCCCCGAGAACCACAGGTGTACACCCTGCCCCC
ATCCCGGGATGAACTGACCAAGAACCAGGTCAGCCTGACCTGCCTGGTCAAAGGCTTCTATCCCAGCGACATCGCC
GTGGAGTGGGAGAGCAATGGGCAGCCGGAGAACAACCTACAAGACCACGCCTCCCGTGCTGGACTCCGACGGCTCCT
TCTTCCTCTACAGCAAGCTCACCGTGGACAAGAGCAGGTGGCAGCAGGGGAACGTCTTCTCATGCTCCGTGATGCA
TGAGGCTCTGCACAACCACTACACGCAGAAGAGCCTCTCCCTGTCTCCGGGTAAATGA] (SEQ ID NO: 106)

C225v6 Antibody Heavy Chain Amino Acid Sequence

QVQLKQSGPGLVQPSQSLITCTVSGFSLTNYGVHWVRQSPGKGLEWLGVIWSGGNTDYNTPTTSRLSINKDNSKS
QVFFKMNSLQSQDTAIYYCARALTYDYEFAYWGQGTLLVTVSAASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYF
PEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSSLGTQTYICNVNHKPSNTKVDKKVEPKSCDKHTC
PPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYASTYRV
VSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSRDELTKNQVSLTCLVKGFYPSDIA
VEWESNGQPENNYKTTTPVLDSDGSFFLYSKLTVDKSRWQQGNVFSVMSHEALHNHYTQKSLSLSPGK* (SEQ
ID NO: 107)

C225Antibody Heavy Chain Amino Acid Sequence

QVQLKQSGPGLVQPSQSLITCTVSGFSLTNYGVHWVRQSPGKGLEWLGVIWSGGNTDYNTPTTSRLSINKDNSKS
QVFFKMNSLQSQDTAIYYCARALTYDYEFAYWGQGTLLVTVSAASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYF

PEPVTVSWNSGALTSGVHTFFPAVLQSSGLYSLSSVVTVPSSSLGTQTYICNVNHKPSNTKVDKKVEPKSCDKTHTC
 PPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYQSTYRV
 VSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSREEMTKNQVSLTCLVKGFYPSDIA
 VEWESNGQPENNYKTTTPVLDSDGSFFLYSKLTVDKSRWQQGNVVFSCSVMHEALHNHYTQKSLSLSPGK* (SEQ
 ID NO: 523)

[000390] In some embodiments, at least one of the AB of the multispecific antibody and/or multispecific activatable antibody binds interleukin 6 receptor (IL-6R). In some embodiments, the AB that binds IL-6R includes one or more of the heavy chain and/or light chain sequences shown below.

Av1 Antibody Heavy Chain Amino Acid Sequence:

QVQLQESGPGLVLRPSQTLSTCTVSGYSITSDHAWSWVRQPPGRGLEWIGYISYSGITTYNPSLKSRVTISRDNK
 NTLYLQMNSLRAEDTAVYYCARSLARTTAMDYWGQGLVTVSSASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYF
 PEPVTVSWNSGALTSGVHTFFPAVLQSSGLYSLSSVVTVPSSSLGTQTYICNVNHKPSNTKVDKKVEPKSCDKTHTC
 PPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRV
 VSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSREEMTKNQVSLTCLVKGFYPSDIA
 VEWESNGQPENNYKTTTPVLDSDGSFFLYSKLTVDKSRWQQGNVVFSCSVMHEALHNHYTQKSLSLSPGK (SEQ
 ID NO: 108)

Av1 Antibody Light Chain Amino Acid Sequence:

DIQMTQSPSSLSASVGDRVTITCRASQDISSYLNWYQQKPGKAPKLLIYYTSRLHSGVPSRFSGSGSGTDFTFTIS
 SLQPEDATYYCQQGNTLPYTFGQGTKVEIKRTVAAPSVFIFPPSDEQLKSGTASVVCLLNNFYPREAKVQWKVDN
 ALQSGNSQESVTEQDSKDYSLSTLTLSKADYEKHKVYACEVTHQGLSSPVTKSFNRGEC (SEQ ID
 NO: 109)

[000391] In some embodiments, the AB binds a Jagged target, e.g., Jagged 1, Jagged 2 or both Jagged 1 and Jagged 2. In some embodiments, the AB that binds a Jagged target includes one or more of the heavy chain and/or light chain sequences shown below.

4D11 Light Chain sequence:

DIQMTQSPSSLSASVGDRVTITCRASQSISSYLNWYQQKPGKAPKLLIYAASSLQSGVPSRFSGSGSGTDFTLTIS
 SLQPEDFATYYCQQTIVAPPLFGQGTKVEIKRTVAAPSVFIFPPSDEQLKSGTASVVCLLNNFYPREAKVQWKVDN
 ALQSGNSQESVTEQDSKDYSLSTLTLSKADYEKHKVYACEVTHQGLSSPVTKSFNRGEC (SEQ ID
 NO: 110)

4D11 Heavy Chain sequence:

EVQLLES GGGGLVQPGGSLRLSCAASGFTFSSYAMSWVRQAPGKGLEWVSSIDPEGRQTYADSVKGRFTISRDN SK
NTLYLQMNSLRAEDTAVYYCAKDIGGRSAFDYWGQGT LVT VSSASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYF
PEPVT VSWNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSSLGTQTYICNVNHKPSNTKVDDKKVEPKSCDKTHTC
PPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRV
VSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSREEMTKNQVSLTCLVKGFYPSDIA
VEWESNGQPENNYKTTTPVLDSDGSFFLYSKLTVDKSRWQQGNV FSCSV MHEALHNHYTQKSLSLSPGK (SEQ
ID NO: 111)

4D11v2 Heavy Chain sequence

EVHLLLES GGGGLVQPGGSLRLSCAASGFTFSSYAMSWVRQAPGKGLEWVSSIDPEGRQTYADSVKGRFTISRDN SK
NTLYLQMNSLRAEDTAVYYCAKDIGGRSAFDYWGQGT LVT VSSASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYF
PEPVT VSWNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSSLGTQTYICNVNHKPSNTKVDDKKVEPKSCDKTHTC
PPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRV
VSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSREEMTKNQVSLTCLVKGFYPSDIA
VEWESNGQPENNYKTTTPVLDSDGSFFLYSKLTVDKSRWQQGNV FSCSV MHEALHNHYTQKSLSLSPGK (SEQ
ID NO: 112)

4D11v2 Light Chain Sequence

DIQMTQSPSSLSASVGDRVTITCRASQSISSYLNWYQQKPGKAPKLLIYAASSLQSGVPSRFS GSGSGTDFTLTIS
SLQPEDFATYYCQQTVVAPPLFGQG TKVEIKRTVAAPSVFIFPPSDEQLKSGTASVVCLLNNFYPREAKVQWKVDN
ALQSGNSQESVTEQDSKSTYSLSTLT LTKADYEKHKVYACEVTHQGLSSPVTKSFNRGEC (SEQ ID
NO: 113)

[000392] In some embodiments, at least one of the AB of the multispecific antibody and/or multispecific activatable antibody a Jagged target and includes one or more of the variable heavy chain and/or variable light chain sequences shown below.

Variable Light Chain Amino Sequence Lc4

DIQMTQSPSSLSASVGDRVTITCRASQSISSYLNWYQQKPGKAPKLLIYAASSLQSGVPSRFS GSGSGTDFTLTIS
SLQPEDFATYYCQQSVVAPLTFGQG TKVEIKR (SEQ ID NO: 114)

Variable Heavy Chain Amino Sequence Hc4

EVQLLES GGGGLVQPGGSLRLSCAASGFTFSSYAMSWVRQAPGKGLEWVSSIEQMGWQTYADSVKGRFTISRDN SK
NTLYLQMNSLRAEDTAVYYCAKDIGGRSAFDYWGQGT LVT VSS (SEQ ID NO: 115)

Variable Light Chain Amino Sequence Lc5

DIQMTQSPSSLSASVGDRVTITCRASQSISSYLNWYQQKPGKAPKLLIYAASSLQSGVPSRFSGSGSGTDFTLTIS
SLQPEDFATYYCQQSVVAPLTFGQGTKVEIKR (SEQ ID NO: 116)

Variable Heavy Chain Amino Sequence Hc5

EVQLLESGGGLVQPGGSLRLSCAASGFTFSSYAMSWVRQAPGKGLEWVSSIEQMGWQTYYADSVKGRFTISRDNK
NTLYLQMNSLRAEDTAVYYCAKSPPYHGQFDYWGQGTLLTVSS (SEQ ID NO: 117)

Variable Light Chain Amino Sequence Lc7

DIQMTQSPSSLSASVGDRVTITCRASQSISSYLNWYQQKPGKAPKLLIYAASSLQSGVPSRFSGSGSGTDFTLTIS
SLQPEDFATYYCQQSVVAPLTFGQGTKVEIKR (SEQ ID NO: 118)

Variable Heavy Chain Amino Sequence Hc7

EVQLLESGGGLVQPGGSLRLSCAASGFTFSSYAMSWVRQAPGKGLEWVSSIEQMGWQTYYADSVKGRFTISRDNK
NTLYLQMNSLRAEDTAVYYCAKSPFFGQFDYWGQGTLLTVSS (SEQ ID NO: 119)

Variable Light Chain Amino Sequence Lc8

DIQMTQSPSSLSASVGDRVTITCRASQSISSYLNWYQQKPGKAPKLLIYAASSLQSGVPSRFSGSGSGTDFTLTIS
SLQPEDFATYYCQQSVVAPLTFGQGTKVEIKR (SEQ ID NO: 120)

Variable Heavy Chain Amino Sequence Hc8

EVQLLESGGGLVQPGGSLRLSCAASGFTFSSYAMSWVRQAPGKGLEWVSSIEQMGWQTYYADSVKGRFTISRDNK
NTLYLQMNSLRAEDTAVYYCAKHIGRTNPFQFDYWGQGTLLTVSS (SEQ ID NO: 121)

Variable Light Chain Amino Sequence Lc13

DIQMTQSPSSLSASVGDRVTITCRASQSISSYLNWYQQKPGKAPKLLIYAASSLQSGVPSRFSGSGSGTDFTLTIS
SLQPEDFATYYCQQSVVAPLTFGQGTKVEIKR (SEQ ID NO: 122)

Variable Heavy Chain Amino Sequence Hc13

EVQLLESGGGLVQPGGSLRLSCAASGFTFSSYAMSWVRQAPGKGLEWVSSIEQMGWQTEYADSVKGRFTISRDNK
NTLYLQMNSLRAEDTAVYYCAKSAAAFDYWGQGTLLTVSS (SEQ ID NO: 123)

Variable Light Chain Amino Sequence Lc16

DIQMTQSPSSLSASVGDRVTITCRASQSISSYLNWYQQKPGKAPKLLIYAASSLQSGVPSRFSGSGSGTDFTLTIS
SLQPEDFATYYCQQSVVAPLTFGQGTKVEIKR (SEQ ID NO: 124)

Variable Heavy Chain Amino Sequence Hc16

EVQLLES GGGGLVQP GGS LRLSCAASGFTFSSYAMSWVRQAPGKGLEWVSSIEQMGWQTYYADSVKGRFTISRDN SK
NTLYLQMNSLRAEDTAVYYCAKSPPYYGQFDYWGQGT LVT VSS (SEQ ID NO: 125)

Variable Light Chain Amino Sequence Lc19

DIQMTQSPSSLSASVGDRVTITCRASQSISSYLNWYQQKPGKAPKLLIYAASSLQSGVPSRFSGSGSGTDFTLTIS
SLQPEDFATYYCQQSVVAPLTFGQGTKVEIKR (SEQ ID NO: 126)

Variable Heavy Chain Amino Sequence Hc19

EVQLLES GGGGLVQP GGS LRLSCAASGFTFSSYAMSWVRQAPGKGLEWVSSIEQMGWQTYYADSVKGRFTISRDN SK
NTLYLQMNSLRAEDTAVYYCAKSPFFGQFDYWGQGT LVT VSS (SEQ ID NO: 127)

Variable Light Chain Amino Sequence Lc21

DIQMTQSPSSLSASVGDRVTITCRASQSISSYLNWYQQKPGKAPKLLIYAASSLQSGVPSRFSGSGSGTDFTLTIS
SLQPEDFATYYCQQSVVAPLTFGQGTKVEIKR (SEQ ID NO: 128)

Variable Heavy Chain Amino Sequence Hc21

EVQLLES GGGGLVQP GGS LRLSCAASGFTFSSYAMSWVRQAPGKGLEWVSSIEQMGWQTYYADSVKGRFTISRDN SK
NTLYLQMNSLRAEDTAVYYCAKDIGGRSAFDYWGQGT LVT VSS (SEQ ID NO: 129)

Variable Light Chain Amino Sequence Lc24

DIQMTQSPSSLSASVGDRVTITCRASQSISSYLNWYQQKPGKAPKLLIYAASSLQSGVPSRFSGSGSGTDFTLTIS
SLQPEDFATYYCQQSVVAPLTFGQGTKVEIKR (SEQ ID NO: 130)

Variable Heavy Chain Amino Sequence Hc24

EVQLLES GGGGLVQP GGS LRLSCAASGFTFSSYAMSWVRQAPGKGLEWVSSIEEMGWQTLYADSVKGRFTISRDN SK
NTLYLQMNSLRAEDTAVYYCAKSAAAFDYWGQGT LVT VSS (SEQ ID NO: 131)

Variable Light Chain Amino Sequence Lc26

DIQMTQSPSSLSASVGDRVTITCRASQSISSYLNWYQQKPGKAPKLLIYAASSLQSGVPSRFSGSGSGTDFTLTIS
SLQPEDFATYYCQQSVVAPLTFGQGTKVEIKR (SEQ ID NO: 132)

Variable Heavy Chain Amino Sequence Hc26

EVQLLES GGGGLVQP GGS LRLSCAASGFTFSSYAMSWVRQAPGKGLEWVSSIEQMGWQTYYADSVKGRFTISRDN SK
NTLYLQMNSLRAEDTAVYYCAKDIGGRSAFDYWGQGT LVT VSS (SEQ ID NO: 133)

Variable Light Chain Amino Sequence Lc27

DIQMTQSPSSLSASVGDRVTITCRASQSISSYLNWYQQKPGKAPKLLIYAASSLQSGVPSRFSGSGSGTDFTLTIS
SLQPEDFATYYCQQSVVAPLTFGQGTKVEIKR (SEQ ID NO: 134)

Variable Heavy Chain Amino Sequence Hc27

EVQLLESGGGLVQPGGSLRLSCAASGFTFSSYAMSWVRQAPGKGLEWVSSIEQMGWQTYYADSVKGRFTISRDNK
NTLYLQMNSLRAEDTAVYYCAKSPFFYGQFDYWGQGTLLTVSS (SEQ ID NO: 135)

Variable Light Chain Amino Sequence Lc28

DIQMTQSPSSLSASVGDRVTITCRASQSISSYLNWYQQKPGKAPKLLIYAASSLQSGVPSRFSGSGSGTDFTLTIS
SLQPEDFATYYCQQSVVAPLTFGQGTKVEIKR (SEQ ID NO: 136)

Variable Heavy Chain Amino Sequence Hc28

EVQLLESGGGLVQPGGSLRLSCAASGFTFSSYAMSWVRQAPGKGLEWVSSIEQMGWQTYYADSVKGRFTISRDNK
NTLYLQMNSLRAEDTAVYYCAKSPFFYGQFDYWGQGTLLTVSS (SEQ ID NO: 137)

Variable Light Chain Amino Sequence Lc30

DIQMTQSPSSLSASVGDRVTITCRASQSISSYLNWYQQKPGKAPKLLIYAASSLQSGVPSRFSGSGSGTDFTLTIS
SLQPEDFATYYCQQSVVAPLTFGQGTKVEIKR (SEQ ID NO: 138)

Variable Heavy Chain Amino Sequence Hc30

EVQLLESGGGLVQPGGSLRLSCAASGFTFSSYAMSWVRQAPGKGLEWVSSIEEMGWQTLYADSVKGRFTISRDNK
NTLYLQMNSLRAEDTAVYYAKSAAAFDYWGQGTLLTVSS (SEQ ID NO: 139)

Variable Light Chain Amino Sequence Lc31

DIQMTQSPSSLSASVGDRVTITCRASQSISSYLNWYQQKPGKAPKLLIYAASSLQSGVPSRFSGSGSGTDFTLTIS
SLQPEDFATYYCQQSVVAPLTFGQGTKVEIKR (SEQ ID NO: 140)

Variable Heavy Chain Amino Sequence Hc31

EVQLLESGGGLVQPGGSLRLSCAASGFTFSSYAMSWVRQAPGKGLEWVSSIEQMGWQTYYADSVKGRFTISRDNK
NTLYLQMNSLRAEDTAVYYCAKDIGGRSAFDYWGQGTLLTVSS (SEQ ID NO: 141)

Variable Light Chain Amino Sequence Lc32

DIQMTQSPSSLSASVGDRVTITCRASQSISSYLNWYQQKPGKAPKLLIYAASSLQSGVPSRFSGSGSGTDFTLTIS
SLQPEDFATYYCQQSVVAPLTFGQGTKVEIKR (SEQ ID NO: 142)

Variable Heavy Chain Amino Sequence Hc32

EVQLLES GGGGLVQPGGSLRLSCAASGFTFSSYAMSWVRQAPGKGLEWVSSIDPEGWQTYYADSVKGRFTISRDN SK
NTLYLQMNSLRAEDTAVYYCAKSAAAFDYWGQGT LVTVSS (SEQ ID NO: 143)

Variable Light Chain Amino Sequence Lc37

DIQMTQSPSSLSASVGDRVTITCRASQSISSYLNWYQQKPGKAPKLLIYAASSLQSGVPSRFSGSGSGTDFTLTIS
SLQPEDFATYYCQQSVVAPLTFGQGTKVEIKR (SEQ ID NO: 144)

Variable Heavy Chain Amino Sequence Hc37

EVQLLES GGGGLVQPGGSLRLSCAASGFTFSSYAMSWVRQAPGKGLEWVSSIEQMGWQTYYADSVKGRFTISRDN SK
NTLYLQMNSLRAEDTAVYYCAKSPPHNGQFDYWGQGT LVTVSS (SEQ ID NO: 145)

Variable Light Chain Amino Sequence Lc39

DIQMTQSPSSLSASVGDRVTITCRASQSISSYLNWYQQKPGKAPKLLIYAASSLQSGVPSRFSGSGSGTDFTLTIS
SLQPEDFATYYCQQSVVAPLTFGQGTKVEIKR (SEQ ID NO: 146)

Variable Heavy Chain Amino Sequence Hc39

EVQLLES GGGGLVQPGGSLRLSCAASGFTFSSYAMSWVRQAPGKGLEWVSSIEQMGWQTEYADSVKGRFTISRDN SK
NTLYLQMNSLRAEDTAVYYCAKSAAAFDYWGQGT LVTVSS (SEQ ID NO: 147)

Variable Light Chain Amino Sequence Lc40

DIQMTQSPSSLSASVGDRVTITCRASQSISSYLNWYQQKPGKAPKLLIYAASSLQSGVPSRFSGSGSGTDFTLTIS
SLQPEDFATYYCQQSVVAPLTFGQGTKVEIKR (SEQ ID NO: 148)

Heavy Chain Amino Sequence Hc40

EVQLLES GGGGLVQPGGSLRLSCAASGFTFSSYAMSWVRQAPGKGLEWVSSIEQMGWQTYYADSVKGRFTISRDN SK
NTLYLQMNSLRAEDTAVYYCAKSPFFFGQFDYWGQGT LVTVSS (SEQ ID NO: 149)

Variable Light Chain Amino Sequence Lc47

DIQMTQSPSSLSASVGDRVTITCRASQSISSYLNWYQQKPGKAPKLLIYAASSLQSGVPSRFSGSGSGTDFTLTIS
SLQPEDFATYYCQQSVVAPLTFGQGTKVEIKR (SEQ ID NO: 150)

Variable Heavy Chain Amino Sequence Hc47

EVQLLES GGGGLVQPGGSLRLSCAASGFTFSSYAMSWVRQAPGKGLEWVSSIDEMGWQTEYADSVKGRFTISRDN SK
NTLYLQMNSLRAEDTAVYYCAKSAAAFDYWGQGT LVTVSS (SEQ ID NO: 151)

Variable 4B2 Light Chain

DIQMTQSPSSLSASVGDRVTITCRASQSISSYLNWYQQKPGKAPKLLIYAASSLQSGVPSRFSGSGSGTDFTLTIS
SLQPEDFATYYCQQTLDAPPQFGQGTKVEIKR (SEQ ID NO: 152)

Variable 4B2 Heavy Chain

EVQLLESGGGLVQPGGSLRLSCAASGFTFSSYAMSWVRQAPGKGLEWVSSIEQMGWQTYADSVKGRFTISRDNK
NTLYLQMNSLRAEDTAVYYCAKDIGGRSAFDYWGQGTLLTVSS (SEQ ID NO: 153)

Variable 4D11 Light Chain

DIQMTQSPSSLSASVGDRVTITCRASQSISSYLNWYQQKPGKAPKLLIYAASSLQSGVPSRFSGSGSGTDFTLTIS
SLQPEDFATYYCQQTVVAPPLFGQGTKVEIKR (SEQ ID NO: 154)

Variable 4D11 Heavy Chain

EVQLLESGGGLVQPGGSLRLSCAASGFTFSSYAMSWVRQAPGKGLEWVSSIDPEGRQTYADSVKGRFTISRDNK
NTLYLQMNSLRAEDTAVYYCAKDIGGRSAFDYWGQGTLLTVSS (SEQ ID NO: 155)

Variable 4E7 Light Chain

DIQMTQSPSSLSASVGDRVTITCRASQSISSYLNWYQQKPGKAPKLLIYAASSLQSGVPSRFSGSGSGTDFTLTIS
SLQPEDFATYYCQQSLVAPLTFGQGTKVEIKR (SEQ ID NO: 156)

Variable 4E7 Heavy Chain

EVQLLESGGGLVQPGGSLRLSCAASGFTFSSYAMSWVRQAPGKGLEWVSSIEEMGWQTKYADSVKGRFTISRDNK
NTLYLQMNSLRAEDTAVYYCAKSAAAFDYWGQGTLLTVSS (SEQ ID NO: 157)

Variable 4E11 Light Chain

DIQMTQSPSSLSASVGDRVTITCRASQSISSYLNWYQQKPGKAPKLLIYAASSLQSGVPSRFSGSGSGTDFTLTIS
SLQPEDFATYYCQQALDAPLMFGQGTKVEIKR (SEQ ID NO: 158)

Variable 4E11 Heavy Chain

EVQLLESGGGLVQPGGSLRLSCAASGFTFSSYAMSWVRQAPGKGLEWVSSIEPMGQLTEYADSVKGRFTISRDNK
NTLYLQMNSLRAEDTAVYYCAKDIGGRSAFDYWGQGTLLTVSS (SEQ ID NO: 159)

Variable 6B7 Light Chain

DIQMTQSPSSLSASVGDRVTITCRASQSISSYLNWYQQKPGKAPKLLIYAASSLQSGVPSRFSGSGSGTDFTLTIS
SLQPEDFATYYCQQALVAPLTFGQGTKVEIKR (SEQ ID NO: 160)

Variable 6B7 Heavy Chain

EVQLLES GGGGLVQP GGS LRLSCAASGFTFSSYAMSWVRQAPGKGLEWVSSIDEMGWQTYADSVKGRFTISRDN SK
NTLYLQMNSLRAEDTAVYYCAKSAAAFDYWGQGT LVT VSS (SEQ ID NO: 161)

Variable 6F8 Light Chain

DIQMTQSPSSLSASVGDRVTITCRASQSISSYLNWYQQKPGKAPKLLIYAASSLQSGVPSRFS GSGSGTDFTLTIS
SLQPEDFATYYCQQALVAPLTFGQGTKVEIKR (SEQ ID NO: 162)

Variable 6F8 Heavy Chain

EVQLLES GGGGLVQP GGS LRLSCAASGFTFSSYAMSWVRQAPGKGLEWVSSIDEMGWQTYADSVKGRFTISRDN SK
NTLYLQMNSLRAEDTAVYYCAKSAAAFDYWGQGT LVT VSS (SEQ ID NO: 163)

[000393] Antibodies and/or activatable antibodies of the disclosure specifically bind a given target, e.g., a human target protein such as human CD3 ϵ . Also included in the disclosure are anti-CD3 ϵ antibodies, activatable antibodies, multispecific antibodies and/or multispecific activatable antibodies that bind to the same epitope as the anti-CD3 ϵ antibodies, activatable antibodies, multispecific antibodies and/or multispecific activatable antibodies described herein. Also included in the disclosure are anti-CD3 ϵ antibodies, activatable antibodies, multispecific antibodies and/or multispecific activatable antibodies that compete with an anti-CD3 ϵ antibody, activatable antibody, multispecific antibody and/or multispecific activatable antibody described herein for binding to CD3 ϵ , e.g., human CD3 ϵ . Also included in the disclosure are anti-CD3 ϵ antibodies, activatable antibodies, multispecific antibodies and/or multispecific activatable antibodies that cross-compete with an anti-CD3 ϵ antibody, activatable antibody, multispecific antibody and/or multispecific activatable antibody described herein for binding to CD3 ϵ , e.g., human CD3 ϵ .

[000394] Those skilled in the art will recognize that it is possible to determine, without undue experimentation, if an anti-CD3 ϵ antibody, an anti-CD3 ϵ activatable antibody, a multispecific antibody and/or a multispecific activatable antibody has the same or similar specificity as an anti-CD3 ϵ antibody, an anti-CD3 ϵ activatable antibody, a multispecific antibody and/or multispecific activatable antibody of the disclosure by ascertaining whether the former prevents the latter from binding to a target. If the anti-CD3 ϵ antibody, the anti-CD3 ϵ activatable antibody, multispecific antibody and/or a multispecific activatable antibody being

tested competes with the anti-CD3 ϵ antibody, the anti-CD3 ϵ activatable antibody, the multispecific antibody and/or a multispecific activatable antibody of the disclosure, as shown by a decrease in binding by the anti-CD3 ϵ antibody, the anti-CD3 ϵ activatable antibody, the multispecific antibody and/or a multispecific activatable antibody of the disclosure, then the anti-CD3 ϵ antibody, the anti-CD3 ϵ activatable antibody, the two multispecific antibodies and/or multispecific activatable antibodies bind to the same, or a closely related, epitope.

[000395] One embodiment for determining whether an anti-CD3 ϵ antibody, an anti-CD3 ϵ activatable antibody, a multispecific antibody and/or a multispecific activatable antibody has the same or similar specificity as an anti-CD3 ϵ antibody, an anti-CD3 ϵ activatable antibody, a multispecific antibody and/or a multispecific activatable antibody of the disclosure is to pre-incubate the anti-CD3 ϵ antibody, the anti-CD3 ϵ activatable antibody, the multispecific antibody and/or a multispecific activatable antibody of the disclosure with soluble target with which it is normally reactive, and then add the anti-CD3 ϵ antibody, the anti-CD3 ϵ activatable antibody, the multispecific antibody and/or a multispecific activatable antibody being tested to determine if the anti-CD3 ϵ antibody, the anti-CD3 ϵ activatable antibody, the multispecific antibody and/or a multispecific activatable antibody being tested is inhibited in its ability to bind the target. If the anti-CD3 ϵ antibody, the anti-CD3 ϵ activatable antibody, the multispecific antibody and/or a multispecific activatable antibody being tested is inhibited then, in all likelihood, it has the same, or functionally equivalent, epitopic specificity as the anti-CD3 ϵ antibody, the anti-CD3 ϵ activatable antibody, the multispecific antibody and/or a multispecific activatable antibody of the disclosure.

[000396] An anti-CD3 ϵ antibody, an anti-CD3 ϵ activatable antibody, multispecific antibody and/or a multispecific activatable antibody is generated, for example, using the procedures described in the Examples provided below. An anti-CD3 ϵ antibody, an anti-CD3 ϵ activatable antibody, multispecific antibody and/or a multispecific activatable antibody can also be generated using any of a number of art-recognized techniques for antibody production and/or purification.

[000397] Antibody fragments, such as Fv, F(ab')₂ and Fab, for use in an anti-CD3 ϵ antibody, an anti-CD3 ϵ activatable antibody, a multispecific antibody and/or a multispecific activatable antibody may be prepared by cleavage of the intact protein, *e.g.*, by protease or

chemical cleavage. In some embodiments, a truncated gene is designed. For example, a chimeric gene encoding a portion of the F(ab')₂ fragment would include DNA sequences encoding the CH1 domain and hinge region of the H chain, followed by a translational stop codon to yield the truncated molecule.

[000398] Expression vectors include plasmids, retroviruses, YACs, EBV derived episomes, and the like. A convenient vector is one that encodes a functionally complete human CH or CL immunoglobulin sequence, with appropriate restriction sites engineered so that any VH or VL sequence can be easily inserted and expressed. In such vectors, splicing usually occurs between the splice donor site in the inserted J region and the splice acceptor site preceding the human C region, and also at the splice regions that occur within the human CH exons. Polyadenylation and transcription termination occur at native chromosomal sites downstream of the coding regions. The resulting antibody may be joined to any strong promoter, including retroviral LTRs, *e.g.*, SV-40 early promoter, (Okayama *et al.* Mol. Cell. Bio. 3:280 (1983)), Rous sarcoma virus LTR (Gorman *et al.* P.N.A.S. 79:6777 (1982)), and moloney murine leukemia virus LTR (Grosschedl *et al.* Cell 41:885 (1985)). Also, as will be appreciated, native Ig promoters and the like may be used.

[000399] Further, anti-CD3 ϵ antibodies, anti-CD3 ϵ activatable antibodies, multispecific antibodies and/or multispecific activatable antibodies can be generated through display type technologies, including, without limitation, phage display, retroviral display, ribosomal display, and other techniques, using techniques well known in the art and the resulting molecules can be subjected to additional maturation, such as affinity maturation, as such techniques are well known in the art. Wright *et al.* Crit. Reviews in Immunol. 12:125-168 (1992), Hanes and Plückthun PNAS USA 94:4937-4942 (1997) (ribosomal display), Parmley and Smith Gene 73:305-318 (1988) (phage display), Scott, TIBS, vol. 17:241-245 (1992), Cwirla *et al.* PNAS USA 87:6378-6382 (1990), Russel *et al.* Nucl. Acids Research 21:1081-1085 (1993), Hoganboom *et al.* Immunol. Reviews 130:43-68 (1992), Chiswell and McCafferty TIBTECH; 10:80-8A (1992), and U.S. Patent No. 5,733,743.

[000400] It can be desirable to modify the anti-CD3 ϵ antibody, the anti-CD3 ϵ activatable antibody, the multispecific antibody and/or multispecific activatable antibody of the disclosure with respect to effector function, so as to enhance or reduce such function to improve the

effectiveness of the antibody in recruiting CD3⁺ cells to one or more targets that are associated with a disease to be treated, such as, e.g. targets that are expressed on tumor cells. For example, cysteine residue(s) can be introduced into the Fc region, thereby allowing interchain disulfide bond formation in this region. The homodimeric antibody thus generated can have improved internalization capability and/or increased complement-mediated cell killing and antibody-dependent cellular cytotoxicity (ADCC). (See Caron et al., J. Exp Med., 176: 1191-1195 (1992) and Shopes, J. Immunol., 148: 2918-2922 (1992)). In some embodiments, an antibody can be engineered that has dual Fc regions and can thereby have enhanced complement lysis and ADCC capabilities. (See Stevenson et al., Anti-Cancer Drug Design, 3: 219-230 (1989)). In some embodiments, Fc mutations are made to remove glycosylation sites, thereby reducing Fc function.

Activatable Antibodies and Multispecific Activatable Antibodies

[000401] The activatable antibodies provided herein contain an antibody or antibody fragment thereof (collectively referred to as AB throughout the disclosure) that specifically binds a target and/or an epitope, wherein the AB is modified by a masking moiety (MM). The multispecific activatable antibodies provided herein contain at least a first antibody or antibody fragment thereof (collectively referred to as AB1 throughout the disclosure) that specifically binds a first target and/or a first epitope and a second antibody or antibody fragment thereof (collectively referred to as AB2 throughout the disclosure) that specifically binds a second target and/or a second epitope, wherein at least one of the AB is modified by a masking moiety (MM). In some embodiments, each AB in a multispecific activatable antibody is modified by its own masking moiety.

[000402] When the AB of an activatable antibody or at least one of the AB in a multispecific activatable antibody is modified with a MM and is in the presence of its target, specific binding of the AB to its target is reduced or inhibited, as compared to the specific binding of the AB not modified with an MM or the specific binding of the parental AB to the target.

[000403] The K_d of the AB modified with a MM towards the target is at least 5, 10, 20, 25, 40, 50, 100, 250, 500, 1,000, 2,500, 5,000, 10,000, 50,000, 100,000, 500,000, 1,000,000, 5,000,000, 10,000,000, 50,000,000 or greater, or between 5-10, 10-100, 10-1,000, 10-10,000,

10-100,000, 10-1,000,000, 10-10,000,000, 100-1,000, 100-10,000, 100-100,000, 100-1,000,000, 100-10,000,000, 1,000-10,000, 1,000-100,000, 1,000-1,000,000, 1000-10,000,000, 10,000-100,000, 10,000-1,000,000, 10,000-10,000,000, 100,000-1,000,000, or 100,000-10,000,000 times greater than the K_d of the AB not modified with an MM or of the parental AB towards the target. Conversely, the binding affinity of the AB modified with a MM towards the target is at least 5, 10, 20, 25, 40, 50, 100, 250, 500, 1,000, 2,500, 5,000, 10,000, 50,000, 100,000, 500,000, 1,000,000, 5,000,000, 10,000,000, 50,000,000 or greater, or between 5-10, 10-100, 10-1,000, 10-10,000, 10-100,000, 10-1,000,000, 10-10,000,000, 100-1,000, 100-10,000, 100-100,000, 100-1,000,000, 100-10,000,000, 1,000-10,000, 1,000-100,000, 1,000-1,000,000, 1000-10,000,000, 10,000-100,000, 10,000-1,000,000, 10,000-10,000,000, 100,000-1,000,000, or 100,000-10,000,000 times lower than the binding affinity of the AB not modified with an MM or of the parental AB towards the target.

[000404] The dissociation constant (K_d) of the MM towards the AB in the activatable antibody is generally greater than the K_d of the AB towards the target. The dissociation constant (K_d) of the MM towards at least one of the AB in the multispecific activatable antibody is generally greater than the K_d of the AB towards the target. The K_d of the MM towards the AB can be at least 5, 10, 25, 50, 100, 250, 500, 1,000, 2,500, 5,000, 10,000, 100,000, 1,000,000 or even 10,000,000 times greater than the K_d of the AB towards the target. Conversely, the binding affinity of the MM towards the AB is generally lower than the binding affinity of the AB towards the target. The binding affinity of MM towards the AB can be at least 5, 10, 25, 50, 100, 250, 500, 1,000, 2,500, 5,000, 10,000, 100,000, 1,000,000 or even 10,000,000 times lower than the binding affinity of the AB towards the target.

[000405] When the AB in the activatable antibody is modified with a MM and is in the presence of the target, specific binding of the AB to its target is reduced or inhibited, as compared to the specific binding of the AB not modified with an MM or the specific binding of the parental AB to the target. When at least one of the AB in the multispecific activatable antibody is modified with a MM and is in the presence of the target, specific binding of the AB to its target is reduced or inhibited, as compared to the specific binding of the AB not modified with an MM or the specific binding of the parental AB to the target. When compared to the binding of the AB not modified with an MM or the binding of the parental AB to the target, the AB's ability to bind the target when modified with an MM can be reduced by at least 50%,

60%, 70%, 80%, 90%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% and even 100% for at least 2, 4, 6, 8, 12, 28, 24, 30, 36, 48, 60, 72, 84, or 96 hours, or 5, 10, 15, 30, 45, 60, 90, 120, 150, or 180 days, or 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, or 12 months or more when measured *in vivo* or in an *in vitro* assay.

[000406] The MM inhibits the binding of the AB in the activatable antibody to its target. The MM inhibits the binding of at least one of the AB in the multispecific activatable antibody to its target. The MM binds the antigen binding domain of the AB and inhibits binding of the AB to its target. The MM can sterically inhibit the binding of the AB to the target. The MM can allosterically inhibit the binding of the AB to its target. In these embodiments when the AB is modified or coupled to a MM and in the presence of target, there is no binding or substantially no binding of the AB to the target, or no more than 0.001%, 0.01%, 0.1%, 1%, 2%, 3%, 4%, 5%, 6%, 7%, 8%, 9%, 10%, 15%, 20%, 25%, 30%, 35%, 40%, or 50% binding of the AB to the target, as compared to the binding of the AB not modified with an MM, the parental AB, or the AB not coupled to an MM to the target, for at least 2, 4, 6, 8, 12, 28, 24, 30, 36, 48, 60, 72, 84, or 96 hours, or 5, 10, 15, 30, 45, 60, 90, 120, 150, or 180 days, or 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, or 12 months or longer when measured *in vivo* or in an *in vitro* assay.

[000407] When the AB in an activatable antibody is coupled to or modified by a MM, the MM ‘masks’ or reduces or otherwise inhibits the specific binding of the AB to its target. When the AB in an activatable antibody is coupled to or modified by a MM, such coupling or modification can effect a structural change that reduces or inhibits the ability of the AB to specifically bind its target.

[000408] When at least one of the AB in a multispecific activatable antibody is coupled to or modified by a MM, the MM ‘masks’ or reduces or otherwise inhibits the specific binding of the AB to its target. When at least one of the AB in a multispecific activatable antibody is coupled to or modified by a MM, such coupling or modification can effect a structural change that reduces or inhibits the ability of the AB to specifically bind its target.

[000409] In an activatable antibody, when the AB is coupled to or modified with an MM, at least a portion of the activatable antibody can be represented by the following formulae (in order from an amino (N) terminal region to carboxyl (C) terminal region:

(MM)-(AB)

(AB)-(MM)

(MM)-L-(AB)

(AB)-L-(MM)

where MM is a masking moiety, the AB is an antibody or antibody fragment thereof, and the L is a linker. In many embodiments, it may be desirable to insert one or more linkers, *e.g.*, flexible linkers, into the composition so as to provide for flexibility.

[000410] In a multispecific activatable antibody, when at least one AB is coupled to or modified with an MM, at least a portion of the multispecific activatable antibody can be represented by the following formulae (in order from an amino (N) terminal region to carboxyl (C) terminal region:

(MM)-(AB)

(AB)-(MM)

(MM)-L-(AB)

(AB)-L-(MM)

where MM is a masking moiety, the AB is an antibody or antibody fragment thereof, and the L is a linker. In many embodiments, it may be desirable to insert one or more linkers, *e.g.*, flexible linkers, into the composition so as to provide for flexibility.

[000411] In certain embodiments, the MM is not a natural binding partner of the AB. In some embodiments, the MM contains no or substantially no homology to any natural binding partner of the AB. In other embodiments the MM is no more than 5%, 10%, 15%, 20%, 25%, 30%, 35%, 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, or 80% similar to any natural binding partner of the AB. In some embodiments, the MM is no more than 5%, 10%, 15%, 20%, 25%, 30%, 35%, 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, or 80% identical to any natural binding partner of the AB. In some embodiments, the MM is no more than 25% identical to any natural binding partner of the AB. In some embodiments, the MM is no more than 50% identical to any natural binding partner of the AB. In some embodiments, the MM is no more than 20% identical to any natural binding partner of the AB. In some embodiments, the MM is no more than 10% identical to any natural binding partner of the AB.

[000412] In some embodiments, the activatable antibodies and/or the multispecific activatable antibodies include an AB that is modified by an MM and also includes one or more cleavable moieties (CM). Such activatable antibodies and/or multispecific activatable antibodies exhibit activatable/switchable binding, to the AB's target. Activatable antibodies

and/or multispecific activatable antibodies generally include at least one antibody or antibody fragment (AB), modified by or coupled to a masking moiety (MM) and a modifiable or cleavable moiety (CM). In some embodiments, the CM contains an amino acid sequence that serves as a substrate for a protease of interest.

[000413] The elements of the activatable antibodies and/or multispecific activatable antibodies are arranged so that each MM and CM are positioned such that in a cleaved (or relatively active) state and in the presence of a target, the corresponding AB binds a target, while in an uncleaved (or relatively inactive) state in the presence of the target, specific binding of the AB to its target, is reduced or inhibited. The specific binding of the AB to its target can be reduced due to the inhibition or masking of the AB's ability to specifically bind its target by the MM.

[000414] The K_d of each AB modified with a MM and a CM towards the target, is at least 5, 10, 20, 25, 40, 50, 100, 250, 500, 1,000, 2,500, 5,000, 10,000, 50,000, 100,000, 500,000, 1,000,000, 5,000,000, 10,000,000, 50,000,000 or greater, or between 5-10, 10-100, 10-1,000, 10-10,000, 10-100,000, 10-1,000,000, 10-10,000,000, 100-1,000, 100-10,000, 100-100,000, 100-1,000,000, 100-10,000,000, 1,000-10,000, 1,000-100,000, 1,000-1,000,000, 1000-10,000,000, 10,000-100,000, 10,000-1,000,000, 10,000-10,000,000, 100,000-1,000,000, or 100,000-10,000,000 times greater than the K_d of the AB not modified with an MM and a CM or of the parental AB towards the target. Conversely, the binding affinity of each AB modified with a MM and a CM towards the target, is at least 5, 10, 20, 25, 40, 50, 100, 250, 500, 1,000, 2,500, 5,000, 10,000, 50,000, 100,000, 500,000, 1,000,000, 5,000,000, 10,000,000, 50,000,000 or greater, or between 5-10, 10-100, 10-1,000, 10-10,000, 10-100,000, 10-1,000,000, 10-10,000,000, 100-1,000, 100-10,000, 100-100,000, 100-1,000,000, 100-10,000,000, 1,000-10,000, 1,000-100,000, 1,000-1,000,000, 1000-10,000,000, 10,000-100,000, 10,000-1,000,000, 10,000-10,000,000, 100,000-1,000,000, or 100,000-10,000,000 times lower than the binding affinity of the AB not modified with an MM and a CM or of the parental AB towards the target.

[000415] When at least one AB is modified with a MM and a CM and is in the presence of the target but not in the presence of a modifying agent (for example a protease), specific binding of that AB to its target, is reduced or inhibited, as compared to the specific binding of the AB not modified with an MM and a CM or of the parental AB to the target. When compared to the binding of the parental AB or the binding of an AB not modified with an MM

and a CM to its target, the AB's ability to bind the target when modified with an MM and a CM can be reduced by at least 50%, 60%, 70%, 80%, 90%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% and even 100% for at least 2, 4, 6, 8, 12, 28, 24, 30, 36, 48, 60, 72, 84, or 96 hours or 5, 10, 15, 30, 45, 60, 90, 120, 150, or 180 days, or 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, or 12 months or longer when measured *in vivo* or in an *in vitro* assay.

[000416] As used herein, the term cleaved state refers to the condition of the activatable antibodies and/or multispecific activatable antibodies following modification of the CM by a protease. The term uncleaved state, as used herein, refers to the condition of the activatable antibodies and/or multispecific activatable antibodies in the absence of cleavage of the CM by a protease. As discussed above, the term “activatable antibodies” is used herein to refer to an activatable antibody in both its uncleaved (native) state, as well as in its cleaved state. It will be apparent to the ordinarily skilled artisan that in some embodiments, a cleaved activatable antibody may lack an MM due to cleavage of the CM by protease, resulting in release of at least the MM (*e.g.*, where the MM is not joined to the activatable antibodies by a covalent bond (*e.g.*, a disulfide bond between cysteine residues)). As discussed above, the term “multispecific activatable antibodies” is used herein to refer to a multispecific activatable antibody in both its uncleaved (native) state, as well as in its cleaved state. It will be apparent to the ordinarily skilled artisan that in some embodiments, a cleaved multispecific activatable antibody may lack an MM due to cleavage of the CM by protease, resulting in release of at least the MM (*e.g.*, where the MM is not joined to the multispecific activatable antibodies by a covalent bond (*e.g.*, a disulfide bond between cysteine residues)).

[000417] By activatable or switchable is meant that the activatable antibody and/or multispecific activatable antibody exhibits a first level of binding to a target when the activatable antibody is in a inhibited, masked or uncleaved state (*i.e.*, a first conformation), and a second level of binding to the target, when the activatable antibody is in the uninhibited, unmasked and/or cleaved state (*i.e.*, a second conformation), where the second level of target binding is greater than the first level of binding. In general, the access of target to the corresponding AB of the activatable antibody and/or multispecific activatable antibody is greater in the presence of a cleaving agent capable of cleaving the CM than in the absence of such a cleaving agent. Thus, when the activatable antibody and/or multispecific activatable antibody is in the uncleaved state, at least one AB is inhibited from target binding and can be

masked from target binding (*i.e.*, the first conformation is such the AB cannot bind the target), and when the activatable antibody is in the cleaved state the AB is not inhibited or is unmasked to target binding.

[000418] The CM and AB of the activatable antibodies are selected so that the first AB represents a binding moiety for CD3 ϵ , and the CM represents a substrate for a protease that is produced by a tumor that is in proximity to cells that express CD3 ϵ and/or produced by a tumor that is co-localized with CD3 ϵ at a treatment site or diagnostic site in a subject. The activatable antibodies disclosed herein find particular use where, for example, a protease capable of cleaving a site in the CM is present at relatively higher levels in CD3 ϵ -containing tissue of a treatment site or diagnostic site than in tissue of non-treatment sites (for example in healthy tissue).

[000419] The CM and AB of the activatable antibodies and/or multispecific activatable antibodies are selected so that the first AB represents a binding moiety for a first target and/or epitope, and the CM represents a substrate for a protease that is produced by a tumor that is in proximity to cells that express the target and/or produced by a tumor that is co-localized with the target at a treatment site or diagnostic site in a subject. The activatable antibodies and/or multispecific activatable antibodies disclosed herein find particular use where, for example, a protease capable of cleaving a site in the CM is present at relatively higher levels in target-containing tissue of a treatment site or diagnostic site than in tissue of non-treatment sites (for example in healthy tissue).

[000420] In some embodiments, activatable antibodies provide for reduced toxicity and/or adverse side effects that could otherwise result from binding of the AB at non-treatment sites if the AB were not masked or otherwise inhibited from binding CD3 ϵ .

[000421] In general, an activatable antibody can be designed by selecting at first AB of interest and constructing the remainder of the activatable antibody so that, when conformationally constrained, the MM provides for masking of the AB or reduction of binding of the AB to CD3 ϵ . Structural design criteria can be taken into account to provide for this functional feature.

[000422] In some embodiments, multispecific activatable antibodies provide for reduced toxicity and/or adverse side effects that could otherwise result from binding of the first AB at non-treatment sites if the AB were not masked or otherwise inhibited from binding its target.

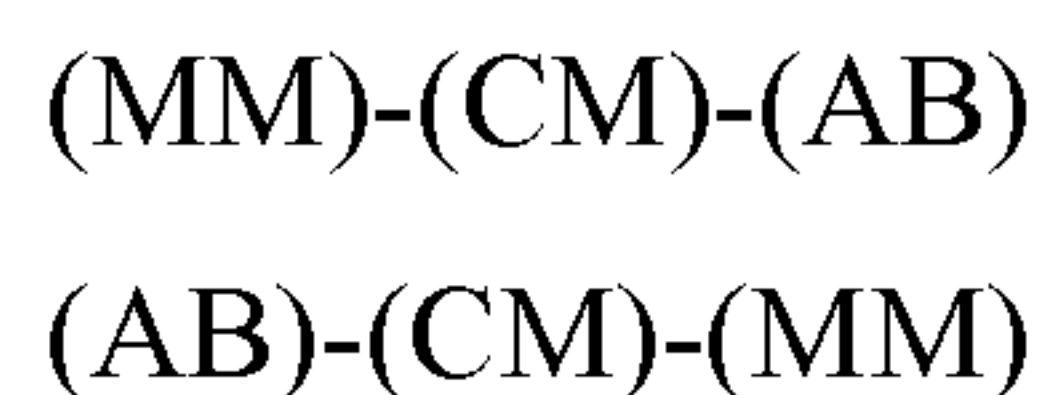
[000423] In general, a multispecific activatable antibody can be designed by selecting at first AB of interest and constructing the remainder of the activatable antibody so that, when conformationally constrained, the MM provides for masking of the AB or reduction of binding of the AB to its target. Structural design criteria can be taken into account to provide for this functional feature.

[000424] Activatable antibodies and/or multispecific activatable antibodies exhibiting a switchable phenotype of a desired dynamic range for target binding in an inhibited versus an uninhibited conformation are provided. Dynamic range generally refers to a ratio of (a) a maximum detected level of a parameter under a first set of conditions to (b) a minimum detected value of that parameter under a second set of conditions. For example, in the context of an activatable antibody and/or a multispecific activatable antibody, the dynamic range refers to the ratio of (a) a maximum detected level of target protein, binding to an activatable antibody and/or a multispecific activatable antibody in the presence of protease capable of cleaving the CM of the activatable antibodies to (b) a minimum detected level of target protein, binding to an activatable antibody and/or a multispecific activatable antibody in the absence of the protease. The dynamic range of an activatable antibody and/or a multispecific activatable antibody can be calculated as the ratio of the dissociation constant of an activatable antibody and/or a multispecific activatable antibody cleaving agent (*e.g.*, enzyme) treatment to the dissociation constant of the activatable antibodies cleaving agent treatment. The greater the dynamic range of an activatable antibody and/or multispecific activatable antibody, the better the switchable phenotype of the activatable antibody. Activatable antibodies having relatively higher dynamic range values (*e.g.*, greater than 1) exhibit more desirable switching phenotypes such that target protein binding by the activatable antibodies occurs to a greater extent (*e.g.*, predominantly occurs) in the presence of a cleaving agent (*e.g.*, enzyme) capable of cleaving the CM of the activatable antibodies than in the absence of a cleaving agent.

[000425] Activatable antibodies and/or multispecific activatable antibodies can be provided in a variety of structural configurations. Exemplary formulae for at least a portion of an activatable antibody and/or a multispecific activatable antibody are provided below. It is

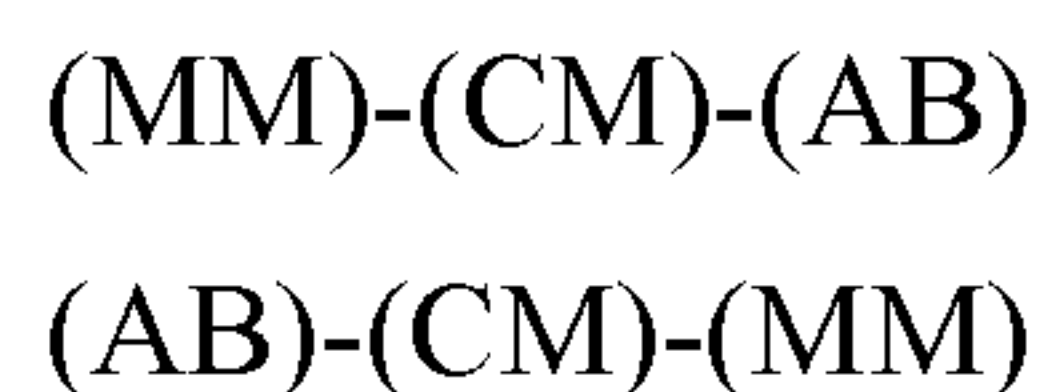
specifically contemplated that the N- to C-terminal order of the AB, the corresponding MM and CM may be reversed within an activatable antibody. It is also specifically contemplated that the CM and MM may overlap in amino acid sequence, *e.g.*, such that the CM is contained within the MM.

[000426] For example, at least a portion of the activatable antibody activatable antibodies can be represented by the following formula (in order from an amino (N) terminal region to carboxyl (C) terminal region:



where MM is a masking moiety, CM is a cleavable moiety, and AB is an antibody or fragment thereof. It should be noted that although MM and CM are indicated as distinct components in the formulae above, in all exemplary embodiments (including formulae) disclosed herein it is contemplated that the amino acid sequences of the MM and the CM could overlap, *e.g.*, such that the CM is completely or partially contained within the MM. In addition, the formulae above provide for additional amino acid sequences that may be positioned N-terminal or C-terminal to the activatable antibodies elements.

[000427] For example, at least a portion of the multispecific activatable antibodies can be represented by the following formula (in order from an amino (N) terminal region to carboxyl (C) terminal region:



where MM is a masking moiety, CM is a cleavable moiety, and AB is a first antibody or fragment thereof. It should be noted that although MM and CM are indicated as distinct components in the formulae above, in all exemplary embodiments (including formulae) disclosed herein it is contemplated that the amino acid sequences of the MM and the CM could overlap, *e.g.*, such that the CM is completely or partially contained within the MM. In addition, the formulae above provide for additional amino acid sequences that may be positioned N-terminal or C-terminal to the activatable antibodies elements.

[000428] In certain embodiments, the MM is not a natural binding partner of the AB. In some embodiments, the MM contains no or substantially no homology to any natural binding partner of the AB. In other embodiments the MM is no more than 5%, 10%, 15%, 20%, 25%,

30%, 35%, 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, or 80% similar to any natural binding partner of the AB. In some embodiments, the MM is no more than 5%, 10%, 15%, 20%, 25%, 30%, 35%, 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, or 80% identical to any natural binding partner of the AB. In some embodiments, the MM is no more than 50% identical to any natural binding partner of the AB. In some embodiments, the MM is no more than 25% identical to any natural binding partner of the AB. In some embodiments, the MM is no more than 20% identical to any natural binding partner of the AB. In some embodiments, the MM is no more than 10% identical to any natural binding partner of the AB.

[000429] In many embodiments it may be desirable to insert one or more linkers, *e.g.*, flexible linkers, into the activatable antibody and/or the multispecific activatable antibody construct so as to provide for flexibility at one or more of the MM-CM junction, the CM-AB junction, or both. For example, the AB, MM, and/or CM may not contain a sufficient number of residues (*e.g.*, Gly, Ser, Asp, Asn, especially Gly and Ser, particularly Gly) to provide the desired flexibility. As such, the switchable phenotype of such activatable antibody and/or multispecific activatable antibody constructs may benefit from introduction of one or more amino acids to provide for a flexible linker. In addition, as described below, where the activatable antibody and/or the multispecific activatable antibody is provided as a conformationally constrained construct, a flexible linker can be operably inserted to facilitate formation and maintenance of a cyclic structure in the uncleaved activatable antibody and/or the uncleaved multispecific activatable antibody.

[000430] For example, in certain embodiments an activatable antibody and/or a multispecific activatable antibody comprises one of the following formulae (where the formula below represent an amino acid sequence in either N- to C-terminal direction or C- to N-terminal direction):

(MM)-L1-(CM)-(AB)

(MM)-(CM)-L2-(AB)

(MM)-L1-(CM)-L2-(AB)

wherein MM, CM, and AB are as defined above; wherein L1 and L2 are each independently and optionally present or absent, are the same or different flexible linkers that include at least 1 flexible amino acid (*e.g.*, Gly). In addition, the formulae above provide for additional amino acid sequences that may be positioned N-terminal or C-terminal to the activatable antibody

and/or the multispecific activatable antibodies elements. Examples include, but are not limited to, targeting moieties (*e.g.*, a ligand for a receptor of a cell present in a target tissue) and serum half-life extending moieties (*e.g.*, polypeptides that bind serum proteins, such as immunoglobulin (*e.g.*, IgG) or serum albumin (*e.g.*, human serum albumin (HAS))).

[000431] In some non-limiting embodiments, at least one of the AB in the multispecific activatable antibody is a binding partner for any target listed in Table 1.

[000432] In some non-limiting embodiments, at least one of the AB in the multispecific activatable antibody comprises, is or is derived from a sequence set forth in the Examples provided herein.

[000433] In some non-limiting embodiments, at least one of the AB in the multispecific activatable antibody comprises, is or is derived from a sequence set forth in Example 2 and/or Example 4 in the Examples provided herein.

[000434] In some non-limiting embodiments, at least one of the AB in the multispecific activatable antibody is or is derived from an antibody listed in Table 2.

[000435] In some embodiments, the masking moiety is selected for use with a specific antibody or antibody fragment. For example, suitable masking moieties for use with antibodies that bind EGFR include MMs that include the sequence CISPRG (SEQ ID NO: 164). By way of non-limiting examples, the MM can include a sequence such as CISPRGC (SEQ ID NO: 165); CISPRGCG (SEQ ID NO: 166); CISPRGCPDGPYVMY (SEQ ID NO: 167); CISPRGCPDGPYVM (SEQ ID NO: 168), CISPRGCEPGTYVPT (SEQ ID NO: 169) and CISPRGCPGQIWHPP (SEQ ID NO: 170). Other suitable masking moieties include any of the EGFR-specific masks disclosed in PCT Publication No. WO 2010/081173, such as, by way of non-limiting example, GSHCLIPINMGAPSC (SEQ ID NO: 171); CISPRGCGGSSASQSGQGSHCLIPINMGAPSC (SEQ ID NO: 172); CNHHYFYTCGCISPRGCPG (SEQ ID NO: 173); ADHVFWGSYGCISPRGCPG (SEQ ID NO: 174); CHHVYWGHCISPRGCPG (SEQ ID NO: 175); CPHFTTTSCGCISPRGCPG (SEQ ID NO: 176); CNHHYHYTCGCISPRGCPG (SEQ ID NO: 177); CPHVSFGSCGCISPRGCPG (SEQ ID NO: 178); CPYYTSLSYGCISPRGCPG (SEQ ID NO: 179); CNHVYFGTCGCISPRGCPG (SEQ ID NO: 180); CNHFTLTTCGCISPRGCPG (SEQ ID NO: 181); CHHFTLTTCGCISPRGCPG (SEQ ID NO: 182); YNPCATPMCCISPRGCPG (SEQ ID NO: 183); CNHHYFYTCGCISPRGCG (SEQ ID

NO: 184); CNHHYHYCGCISPRGCG (SEQ ID NO: 185); CNHVYFGTCGCISPRGCG (SEQ ID NO: 186); CHHVYWGHC GCISPRGCG (SEQ ID NO: 187); CPHFTTTSCGCISPRGCG (SEQ ID NO: 188); CNHFTLTTCGCISPRGCG (SEQ ID NO: 189); CHHFTLTTCGCISPRGCG (SEQ ID NO: 190); CPYYTLSYCGCISPRGCG (SEQ ID NO: 191); CPHVSFGSCGCISPRGCG (SEQ ID NO: 192); ADHVFWGSYGCISPRGCG (SEQ ID NO: 193); YNPCATPMCCISPRGCG (SEQ ID NO: 194); CHHVYWGHC GCISPRGCG (SEQ ID NO: 195); C(N/P)H(H/V/F)(Y/T)(F/W/T/L)(Y/G/T/S)(T/S/Y/H)CGCISPRGCG (SEQ ID NO: 196); CISPRGCGQPIPSVK (SEQ ID NO: 197); CISPRGCTQPYHVSR (SEQ ID NO: 198); and/or CISPRGCNAVSGGLGS (SEQ ID NO: 199).

[000436] Suitable masking moieties for use with antibodies that bind a Jagged target, e.g., Jagged 1 and/or Jagged 2, include, by way of non-limiting example, masking moieties that include a sequence such as QGQSGQCNIWL VGGDCRGWQG (SEQ ID NO: 200); QGQSGQGQQWCNIWINGGDCRGWNG (SEQ ID NO: 201); PWCMQRQDFLRCPQP (SEQ ID NO: 202); QLGLPAYMCTFECLR (SEQ ID NO: 203); CNLWVSGGDCGGLQG (SEQ ID NO: 204); SCSLWTSGSCLPHSP (SEQ ID NO: 205); YCLQLPHYMQAMCGR (SEQ ID NO: 206); CFLYSCTDVSYWNNT (SEQ ID NO: 207); PWCMQRQDYLRCPP (SEQ ID NO: 208); CNLWISGGDCRGLAG (SEQ ID NO: 209); CNLWVSGGDCRGVQG (SEQ ID NO: 210); CNLWVSGGDCRGLRG (SEQ ID NO: 211); CNLWISGGDCRGLPG (SEQ ID NO: 212); CNLWVSGGDCRDAPW (SEQ ID NO: 213); CNLWVSGGDCRDLLG (SEQ ID NO: 214); CNLWVSGGDCRGLQG (SEQ ID NO: 215); CNLWLHGGDCRGWQG (SEQ ID NO: 216); CNIWL VGGDCRGWQG (SEQ ID NO: 217); CTTWFCGGDCGVMRG (SEQ ID NO: 218); CNIWGPSVDCGALLG (SEQ ID NO: 219); CNIWVNGGDCRSFEG (SEQ ID NO: 220); YCLNLPRYMQDMCWA (SEQ ID NO: 221); YCLALPHYMQADCAR (SEQ ID NO: 222); CFLYSCGDVSYWGSA (SEQ ID NO: 223); CYLYSCTDSAFWNNR (SEQ ID NO: 224); CYLYSCNDVSYWSNT (SEQ ID NO: 225); CFLYSCTDVSYW (SEQ ID NO: 226); CFLYSCTDVAYWNSA (SEQ ID NO: 227); CFLYSCTDVSYWGDT (SEQ ID NO: 228); CFLYSCTDVSYWGNS (SEQ ID NO: 229); CFLYSCTDVAYWNNT (SEQ ID NO: 230); CFLYSCGDVSYWGNPGLS (SEQ ID NO: 231); CFLYSCTDVAYWSGL (SEQ ID NO: 232); CYLYSCTDGSYWNST (SEQ ID NO: 233); CFLYSCSDVSYWGNI (SEQ ID NO: 234); CFLYSCTDVAYW (SEQ ID NO: 235); CFLYSCTDVSYWGST (SEQ ID

NO: 236); CFLYSCTDVAYWGDT (SEQ ID NO: 237); GCNIWLNGGDCRGWVDPLQG (SEQ ID NO: 238); GCNIWLVG GDCRGWIGDTNG (SEQ ID NO: 239); GCNIWLVG GDCRGWIEDSNG (SEQ ID NO: 240); GCNIWANGGDCRGWIDNIDG (SEQ ID NO: 241); GCNIWLVG GDCRGWLGEAVG (SEQ ID NO: 242); GCNIWLVG GDCRGWLEEAVG (SEQ ID NO: 243); GGPALCNIWLNGGDCRGWSG (SEQ ID NO: 244); GAPVFCNIWLNGGDCRGWMG (SEQ ID NO: 245); GQQQWCNIWINGGDCRGWNG (SEQ ID NO: 246); GKSEFCNIWLNGGDCRGWIG (SEQ ID NO: 247); GTPGGCNIWANGGDCRGWEG (SEQ ID NO: 248); GASQYCNLWINGGDCRGWRG (SEQ ID NO: 249); GCNIWLVG GDCRPFVVEGG (SEQ ID NO: 250); GCNIWAVGGDCRPFVDGG (SEQ ID NO: 251); GCNIWLNGGDCRAWVDTG (SEQ ID NO: 252); GCNIWIVGGDCRPFINDG (SEQ ID NO: 253); GCNIWLNGGDCRPVVF GG (SEQ ID NO: 254); GCNIWLSGGDCRMFMNEG (SEQ ID NO: 255); GCNIWVNGGDCRSFVYSG (SEQ ID NO: 256); GCNIWLNGGDCRGWEASG (SEQ ID NO: 257); GCNIWAHGGDCRGFIEPG (SEQ ID NO: 258); GCNIWLNGGDCRFTFVASG (SEQ ID NO: 259); GCNIWAHGGDCRGFIEPG (SEQ ID NO: 260); GFLENCNIWLNGGDCRTG (SEQ ID NO: 261); GIYENCNIWLNGGDCRMG (SEQ ID NO: 262); and/or GIPDNCNIWINGGDCRYG (SEQ ID NO: 263).

[000437] Suitable masking moieties for use with antibodies that bind an interleukin 6 target, e.g., interleukin 6 receptor (IL-6R), include, by way of non-limiting example, masking moieties that include a sequence such as QQQSGQYGSCSWNYVHIFMDC (SEQ ID NO: 264); QQQSGQGDFDIPFPAHWVPIT (SEQ ID NO: 265); QQQSGQMGPAGCVWNYAHIFMDC (SEQ ID NO: 266); YRSCNWNYSIFLDC (SEQ ID NO: 267); PGAFDIPFPAHWVPNT (SEQ ID NO: 268); ESSCVWNYVHIYMDC (SEQ ID NO: 269); YPGCKWNYDRIFLDC (SEQ ID NO: 270); YRTCSWNYVGIFLDC (SEQ ID NO: 271); YGSCSWNYVHIFMDC (SEQ ID NO: 272); YGSCSWNYVHIFLDC (SEQ ID NO: 273); YGSCNWNYSIFLDC (SEQ ID NO: 274); YTSCNWNYSIFMDC (SEQ ID NO: 275); YPGCKWNYDRIFLDC (SEQ ID NO: 276); WRSCNWNYSIFLDC (SEQ ID NO: 277); WSNCHWNYVHIFLDC (SEQ ID NO: 278); DRSCTWNYVRISYDC (SEQ ID NO: 279); SGSCKWDYVHIFLDC (SEQ ID NO: 280); SRSCIWNYAHIHLDC (SEQ ID NO: 281); SMSCYWQYERIFLDC (SEQ ID NO: 282); YRSCNWNYSIFLDC (SEQ ID

NO: 283); YGSCSWNYVHIFMDC (SEQ ID NO: 284); SGSCKWDYVHIFLDC (SEQ ID NO: 285); YKSCHWDYVHIFLDC (SEQ ID NO: 286); YGSCTWNYVHIFMEC (SEQ ID NO: 287); FSSCNWNYVHIFLDC (SEQ ID NO: 288); WRSCNWNVAHIFLDC (SEQ ID NO: 289); YGSCQWNYVHIFLDC (SEQ ID NO: 290); YRSCNWNVHIFLDC (SEQ ID NO: 291); NMSCHWDYVHIFLDC (SEQ ID NO: 292); FGPCTWNYARISWDC (SEQ ID NO: 293); XXsCXWXYvhIfXdC (SEQ ID NO: 294); MGVPAGCVWNYAHIFMDC (SEQ ID NO: 295); RDTGGQCRWDYVHIFMDC (SEQ ID NO: 296); AGVPAGCTWNYVHIFMEC (SEQ ID NO: 297); VGVPNGCVWNYAHIFMEC (SEQ ID NO: 298); DGGPAGCSWNYVHIFMEC (SEQ ID NO: 299); AVGPAGCWWNYVHIFMEC (SEQ ID NO: 300); CTWNYVHIFMDCGEGEGP (SEQ ID NO: 301); GGVPEGCTWNYAHIFMEC (SEQ ID NO: 302); AEVPAGCWWNYVHIFMEC (SEQ ID NO: 303); AGVPAGCTWNYVHIFMEC (SEQ ID NO: 304); SGASGGCKWNYVHIFMDC (SEQ ID NO: 305); MGVPAGCVWNYAHIFMDC (SEQ ID NO: 306); TPGCRWNYVHIFMECEAL (SEQ ID NO: 307); VGVPNGCVWNYAHIFMEC (SEQ ID NO: 308); PGAFDIPFPAHWVPNT (SEQ ID NO: 309); RGACDIPFPAHWIPNT (SEQ ID NO: 310); QGDFDIPFPAHWVPIT (SEQ ID NO: 311); XGafDIPFPAHWvPnT (SEQ ID NO: 312); RGDGNDSDIPFPAHWVPRT (SEQ ID NO: 313); SGVGRDRDIPFPAHWVPRT (SEQ ID NO: 314); WAGGNDSDIPFPAHWIPNT (SEQ ID NO: 315); WGDGMDVDIPFPAHWVPVT (SEQ ID NO: 316); AGSGNDSDIPFPAHWVPRT (SEQ ID NO: 317); ESRSGYADIPFPAHWVPRT (SEQ ID NO: 318); and/or RECGRCGDIPFPAHWVPRT (SEQ ID NO: 319).

[000438] In some embodiments, the cleavable moiety (CM) of the activatable antibody and/or the multispecific activatable antibody includes an amino acid sequence that can serve as a substrate for a protease, usually an extracellular protease. The CM may be selected based on a protease that is produced by a tumor that is in proximity to cells that express the target and/or produced by a tumor that is co-localized in tissue with the desired target of at least one AB of the multispecific activatable antibody. A variety of different conditions are known in which a target of interest is produced by a tumor that is in proximity to cells that express the target and/or produced by a tumor that is co-localized with a protease, where the substrate of the protease is known in the art. In the example of cancer, the target tissue can be a cancerous tissue, particularly cancerous tissue of a solid tumor. There are reports in the literature of

increased levels of proteases having known substrates in a number of cancers, *e.g.*, solid tumors. See, *e.g.*, La Rocca et al, (2004) British J. of Cancer 90(7): 1414-1421. Non-limiting examples of disease include: all types of cancers (breast, lung, colorectal, prostate, melanomas, head and neck, pancreatic, etc.), rheumatoid arthritis, Crohn's disease, SLE, cardiovascular damage, ischemia, etc. For example, indications would include leukemias, including T-cell acute lymphoblastic leukemia (T-ALL), lymphoblastic diseases including multiple myeloma, and solid tumors, including lung, colorectal, prostate, pancreatic and breast, including triple negative breast cancer. For example, indications include bone disease or metastasis in cancer, regardless of primary tumor origin; breast cancer, including by way of non-limiting example, ER/PR+ breast cancer, Her2+ breast cancer, triple-negative breast cancer; colorectal cancer; endometrial cancer; gastric cancer; glioblastoma; head and neck cancer, such as esophageal cancer; lung cancer, such as by way of non-limiting example, non-small cell lung cancer; multiple myeloma ovarian cancer; pancreatic cancer; prostate cancer; sarcoma, such as osteosarcoma; renal cancer, such as by way of nonlimiting example, renal cell carcinoma; and/or skin cancer, such as by way of nonlimiting example, squamous cell cancer, basal cell carcinoma, or melanoma. In some embodiments, the cancer is a squamous cell cancer. In some embodiments, the cancer is a skin squamous cell carcinoma. In some embodiments, the cancer is an esophageal squamous cell carcinoma. In some embodiments, the cancer is a head and neck squamous cell carcinoma. In some embodiments, the cancer is a lung squamous cell carcinoma.

[000439] The CM is specifically cleaved by an enzyme at a rate of about $0.001\text{--}1500 \times 10^4 \text{ M}^{-1}\text{S}^{-1}$ or at least 0.001, 0.005, 0.01, 0.05, 0.1, 0.5, 1, 2.5, 5, 7.5, 10, 15, 20, 25, 50, 75, 100, 125, 150, 200, 250, 500, 750, 1000, 1250, or $1500 \times 10^4 \text{ M}^{-1}\text{S}^{-1}$.

[000440] For specific cleavage by an enzyme, contact between the enzyme and CM is made. When the activatable antibody comprising an AB coupled to a MM and a CM is in the presence of target and sufficient enzyme activity, the CM can be cleaved. When the multispecific activatable antibody comprising at least a first AB coupled to a MM and a CM is in the presence of target and sufficient enzyme activity, the CM can be cleaved. Sufficient enzyme activity can refer to the ability of the enzyme to make contact with the CM and effect cleavage. It can readily be envisioned that an enzyme may be in the vicinity of the CM but unable to cleave because of other cellular factors or protein modification of the enzyme.

[000441] Exemplary substrates include but are not limited to substrates cleavable by one or more of the following enzymes or proteases in Table 3:

Table 3: Exemplary Proteases and/or Enzymes

ADAMS, ADAMTS, <i>e.g.</i> ADAM8 ADAM9 ADAM10 ADAM12 ADAM15 ADAM17/TACE ADAMDEC1 ADAMTS1 ADAMTS4 ADAMTS5	Cysteine proteinases, <i>e.g.</i> , Cruzipain Legumain Otubain-2	Serine proteases, <i>e.g.</i> , activated protein C Cathepsin A Cathepsin G Chymase coagulation factor proteases (<i>e.g.</i> , FVIIa, FIXa, FXa, FXIa, FXIIa)
		Elastase
		Granzyme B
		Guanidinobenzoatase
		HtrA1
Aspartate proteases, <i>e.g.</i> , BACE Renin	KLK10 KLK11 KLK13 KLK14	Human Neutrophil Elastase
		Lactoferrin
		Marapsin
		NS3/4A
Aspartic cathepsins, <i>e.g.</i> , Cathepsin D Cathepsin E	Metallo proteinases, <i>e.g.</i> , Meprin Neprilysin PSMA BMP-1	PACE4 Plasmin PSA tPA Thrombin
		Tryptase
Caspases, <i>e.g.</i> , Caspase 1 Caspase 2 Caspase 3 Caspase 4 Caspase 5 Caspase 6 Caspase 7 Caspase 8 Caspase 9 Caspase 10 Caspase 14	MMPs, <i>e.g.</i> , MMP1 MMP2 MMP3 MMP7 MMP8 MMP9 MMP10 MMP11 MMP12 MMP13 MMP14 MMP15 MMP16 MMP17 MMP19 MMP20	uPA Type II Transmembrane Serine Proteases (TTSPs), <i>e.g.</i> , DESC1 DPP-4 FAP Hepsin Matriptase-2 Matriptase TMPRSS2 TMPRSS3 TMPRSS4
Cysteine cathepsins, <i>e.g.</i> , Cathepsin B Cathepsin C Cathepsin K Cathepsin L Cathepsin S		

Cathepsin V/L2	MMP23
Cathepsin X/Z/P	MMP24
	MMP26
	MMP27

[000442] For example, in some embodiments, the substrate is cleavable by one or more of the following enzymes or proteases: uPA, legumain, matriptase, ADAM17, BMP-1, TMPRSS3, TMPRSS4, MMP-9, MMP-12, MMP-13, and/or MMP-14. In some embodiments, the protease is selected from the group of uPA, legumain, and matriptase. In some embodiments, the protease is a matrix metalloproteinase. In some embodiments, the protease comprises uPA. In some embodiments, the protease comprises legumain. In some embodiments, the protease comprises matriptase.

[000443] In some embodiments, the CM is selected for use with a specific protease. In some embodiments, the CM is a substrate for at least one protease selected from the group consisting of an ADAM 17, a BMP-1, a cysteine protease such as a cathepsin, a HtrA1, a legumain, a matriptase (MT-SP1), a matrix metalloprotease (MMP), a neutrophil elastase, a TMPRSS, such as TMPRSS3 or TMPRSS4, a thrombin, and a u-type plasminogen activator (uPA, also referred to as urokinase).

[000444] In some embodiments, the CM is a substrate for an ADAM17. In some embodiments, the CM is a substrate for a BMP-1. In some embodiments, the CM is a substrate for a cathepsin. In some embodiments, the CM is a substrate for a cysteine protease. In some embodiments, the CM is a substrate for a HtrA1. In some embodiments, the CM is a substrate for a legumain. In some embodiments, the CM is a substrate for matriptase. In some embodiments, the CM is a substrate for a MMP. In some embodiments, the CM is a substrate for a neutrophil elastase. In some embodiments, the CM is a substrate for a thrombin. In some embodiments, the CM is a substrate for a TMPRSS. In some embodiments, the CM is a substrate for TMPRSS3. In some embodiments, the CM is a substrate for TMPRSS4. In some embodiments, the CM is a substrate for uPA.

[000445] In some embodiments, the cleavable moiety is selected for use with a specific protease, for example a protease that is known to be produced by a tumor that is in proximity to cells that express the target and/or produced by a tumor that is co-localized with the target of the activatable antibody. For example, suitable cleavable moieties for use in the activatable

antibodies of the disclosure include the sequence TGRGPSWV (SEQ ID NO: 68); SARGPSRW (SEQ ID NO: 69); TARGPSFK (SEQ ID NO: 70); LSGRSDNH (SEQ ID NO: 67); GGWHTGRN (SEQ ID NO: 71); HTGRSGAL (SEQ ID NO: 72); PLTGRSGG (SEQ ID NO: 73); AARGPAIH (SEQ ID NO: 74); RGPAFNPM (SEQ ID NO: 75); SSRGPAYL (SEQ ID NO: 76); RGPATPIM (SEQ ID NO: 77); RGPA (SEQ ID NO: 78); GGQPSGMWGW (SEQ ID NO: 79); FPRPLGITGL (SEQ ID NO: 80); VHMPLGFLGP (SEQ ID NO: 81); SPLTGRSG (SEQ ID NO: 82); SAGFSLPA (SEQ ID NO: 83); LAPLGLQRR (SEQ ID NO: 84); SGGPLGVR (SEQ ID NO: 85); and/or PLGL (SEQ ID NO: 86).

[000446] In some embodiments, the CM is a substrate for at least one matrix metalloprotease (MMP). Examples of MMPs include MMP1; MMP2; MMP3; MMP7; MMP8; MMP9; MMP10; MMP11; MMP12; MMP13; MMP14; MMP15; MMP16; MMP17; MMP19; MMP20; MMP23; MMP24; MMP26; and MMP27. In some embodiments, the CM is a substrate for MMP9, MMP14, MMP1, MMP3, MMP13, MMP17, MMP11, and MMP19. In some embodiments, the CM is a substrate for MMP7. In some embodiments, the CM is a substrate for MMP9. In some embodiments, the CM is a substrate for MMP14. In some embodiments, the CM is a substrate for two or more MMPs. In some embodiments, the CM is a substrate for at least MMP9 and MMP14. In some embodiments, the CM comprises two or more substrates for the same MMP. In some embodiments, the CM comprises at least two or more MMP9 substrates. In some embodiments, the CM comprises at least two or more MMP14 substrates.

[000447] In some embodiments, the CM is a substrate for an MMP and includes the sequence ISSGLLSS (SEQ ID NO: 321); QNQALRMA (SEQ ID NO: 322); AQNLLGMV (SEQ ID NO: 323); STFPFGMF (SEQ ID NO: 324); PVGYTSSL (SEQ ID NO: 325); DWLYWPGI (SEQ ID NO: 326); MIAPVAYR (SEQ ID NO: 327); RPSPMWAY (SEQ ID NO: 328); WATPRPMR (SEQ ID NO: 329); FRLLDWQW (SEQ ID NO: 330); LKAAPRWA (SEQ ID NO: 331); GPSHLVLT (SEQ ID NO: 332); LPGGLSPW (SEQ ID NO: 333); MGLFSEAG (SEQ ID NO: 334); SPLPLRVP (SEQ ID NO: 335); RMHLRSLG (SEQ ID NO: 336); LAAPLGLL (SEQ ID NO: 337); AVGLLAPP (SEQ ID NO: 338); LLAPSHRA (SEQ ID NO: 339); PAGLWLDP (SEQ ID NO: 340); and/or ISSGLSS (SEQ ID NO: 341).

[000448] In some embodiments, the CM is a substrate for thrombin. In some embodiments, the CM is a substrate for thrombin and includes the sequence GPRSFG (SEQ ID NO: 896) or GPRSFG (SEQ ID NO: 897).

[000449] In some embodiments, the CM comprises an amino acid sequence selected from the group consisting of NTLSEGRSENHSG (SEQ ID NO: 898); NTLSEGRSGNHGS (SEQ ID NO: 899); TSTSEGRSANPRG (SEQ ID NO: 900); TSEGRSANP (SEQ ID NO: 901); VAGRSMRP (SEQ ID NO: 902); VVPEGRRS (SEQ ID NO: 903); ILPRSPAF (SEQ ID NO: 904); MVLGRSLL (SEQ ID NO: 905); QGRAITFI (SEQ ID NO: 906); SPRSIMLA (SEQ ID NO: 907); and SMLRSMPL (SEQ ID NO: 908).

[000450] In some embodiments, the CM is a substrate for a neutrophil elastase. In some embodiments, the CM is a substrate for a serine protease. In some embodiments, the CM is a substrate for uPA. In some embodiments, the CM is a substrate for legumain. In some embodiments, the CM is a substrate for matriptase. In some embodiments, the CM is a substrate for a cysteine protease. In some embodiments, the CM is a substrate for a cysteine protease, such as a cathepsin.

[000451] In some embodiments, the CM is a CM1-CM2 substrate and includes the sequence ISSGLLSGRSDNH (SEQ ID NO: 909); ISSGLLSGGSGGSLSGRSDNH (SEQ ID NO: 910); AVGLLAPPGGTSTSEGRSANPRG (SEQ ID NO: 911); TSTSEGRSANPRGGGAVGLLAPP (SEQ ID NO: 912); VHMPLGFLGPGGTSTSEGRSANPRG (SEQ ID NO: 913); TSTSEGRSANPRGGGVHMPLGFLGP (SEQ ID NO: 914); AVGLLAPPGGLSGRSDNH (SEQ ID NO: 915); LSGRSDNHGGAVGLLAPP (SEQ ID NO: 916); VHMPLGFLGPGGLSGRSDNH (SEQ ID NO: 917); LSGRSDNHGGGVHMPLGFLGP (SEQ ID NO: 918); LSGRSDNHGGSGGSISSGLLSS (SEQ ID NO: 919); LSGRSGNHGGSGGSISSGLLSS (SEQ ID NO: 920); ISSGLLSGGSGGSLSGRSGNH (SEQ ID NO: 921); LSGRSDNHGGSGGSGNQALRMA (SEQ ID NO: 922); QNQALRMAGGSGGSLSGRSDNH (SEQ ID NO: 923); LSGRSGNHGGSGGSGNQALRMA (SEQ ID NO: 924); QNQALRMAGGSGGSLSGRSGNH (SEQ ID NO: 925) and/or ISSGLLSGRSGNH (SEQ ID NO: 926).

[000452] In some embodiments, anti-CD3ε antibodies, activatable antibodies and/or multispecific antibodies and/or multispecific activatable antibodies of the disclosure may be made biosynthetically using recombinant DNA technology and expression in eukaryotic or prokaryotic species. For the activatable antibodies and/or multispecific activatable antibodies, the cDNAs encoding the masking moiety, linker sequence (that may include a cleavable moiety (CM), and antibody chain (heavy or light)) can be linked in an 5' to 3' (N- to C-terminal in the translated product) sequence to create the nucleic acid construct, which is expressed as the activatable antibody protein and/or multispecific activatable antibody protein following a conventional antibody expression process. In some embodiments, the activatable antibody and/or multispecific activatable antibody could be semi-synthetically produced by expressing a CM-antibody and then coupling the mask chemically at or near the N-terminus of the protein. In some embodiments, the activatable antibody and/or the multispecific activatable antibody could be produced by expressing an antibody and then coupling the mask and the CM chemically at or near the N-terminus of the protein such that the activatable antibody and/or at least a portion of the multispecific activatable antibody in the uncleaved state has the structural arrangement from N-terminus to C-terminus as follows: MM-CM-AB or AB-CM-MM.

[000453] Linkers suitable for use in compositions described herein are generally ones that provide flexibility of the modified AB or the multispecific activatable antibodies to facilitate the inhibition of the binding of at least the first AB to the target. Such linkers are generally referred to as flexible linkers. Suitable linkers can be readily selected and can be of any of a suitable of different lengths, such as from 1 amino acid (*e.g.*, Gly) to 20 amino acids, from 2 amino acids to 15 amino acids, from 3 amino acids to 12 amino acids, including 4 amino acids to 10 amino acids, 5 amino acids to 9 amino acids, 6 amino acids to 8 amino acids, or 7 amino acids to 8 amino acids, and may be 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, or 20 amino acids in length.

[000454] Exemplary flexible linkers include glycine polymers (G)_n, glycine-serine polymers (including, for example, (GS)_n, (GSGGS)_n (SEQ ID NO: 59) and (GGGS)_n (SEQ ID NO: 60), where n is an integer of at least one), glycine-alanine polymers, alanine-serine polymers, and other flexible linkers known in the art. Glycine and glycine-serine polymers are relatively unstructured, and therefore may be able to serve as a neutral tether between

components. Glycine accesses significantly more phi-psi space than even alanine, and is much less restricted than residues with longer side chains (see Scheraga, Rev. Computational Chem. 11173-142 (1992)). Exemplary flexible linkers include, but are not limited to GGSG (SEQ ID NO: 61), GGSGG (SEQ ID NO: 62), GSGSG (SEQ ID NO: 63), GSGGG (SEQ ID NO: 64), GGGSG (SEQ ID NO: 65), GSSSG (SEQ ID NO: 66), and the like. The ordinarily skilled artisan will recognize that design of an activatable antibodies can include linkers that are all or partially flexible, such that the linker can include a flexible linker as well as one or more portions that confer less flexible structure to provide for a desired activatable antibodies and/or multispecific activatable antibodies structure.

[000455] In addition to the elements described above, the activatable antibodies and/or multispecific activatable antibodies can contain additional elements such as, for example, amino acid sequence N- or C-terminal of the activatable antibodies and/or multispecific activatable antibodies. For example, activatable antibodies and/or multispecific activatable antibodies can include a targeting moiety to facilitate delivery to a cell or tissue of interest. Activatable antibodies and/or multispecific activatable antibodies can be conjugated to an agent, such as a therapeutic agent, a detectable moiety or a diagnostic agent. Examples of agents are disclosed herein.

[000456] The activatable antibodies and/or multispecific activatable antibodies can also include any of the conjugated agents, linkers and other components described herein in conjunction with an activatable antibody and/or a multispecific antibody of the disclosure, including by way of non-limiting example, any of the agents listed in Table 4 and/or any of the linkers listed in Table 5 and/or Table 6.

Conjugated Antibodies, Conjugated Activatable Antibodies, Conjugated Multispecific Antibodies and Conjugated Multispecific Activatable Antibodies

[000457] In some embodiments, the antibodies and/or activatable antibodies of the disclosure can be conjugated to an agent, such as for example, a therapeutic agent, a detectable moiety or a diagnostic agent. Suitable therapeutic agents include, by way of non-limiting example, a therapeutic agent for use in targeting a T cell-derived lymphoma. Suitable detectable agents include, by way of non-limiting example, a fluorescent dye, (e.g. a fluorophore, Fluorescein Isothiocyanate (FITC), Rhodamine Isothiocyanate (TRITC), an Alexa

Fluor® label), a near infrared (NIR) dye (e.g., Qdot® nanocrystals), a colloidal metal, a hapten, a radioactive marker, biotin and an amplification reagent such as streptavidin, or an enzyme (e.g. horseradish peroxidase or alkaline phosphatase).

[000458] The activatable anti-CD3ε antibodies have at least one point of conjugation for an agent, but in the methods and compositions provided herein less than all possible points of conjugation are available for conjugation to an agent. In some embodiments, the one or more points of conjugation are sulfur atoms involved in disulfide bonds. In some embodiments, the one or more points of conjugation are sulfur atoms involved in interchain disulfide bonds. In some embodiments, the one or more points of conjugation are sulfur atoms involved in interchain sulfide bonds, but not sulfur atoms involved in intrachain disulfide bonds. In some embodiments, the one or more points of conjugation are sulfur atoms of cysteine or other amino acid residues containing a sulfur atom. Such residues may occur naturally in the antibody structure or may be incorporated into the antibody by site-directed mutagenesis, chemical conversion, or mis-incorporation of non-natural amino acids.

[000459] Also provided are methods of preparing a conjugate of an activatable anti-CD3ε antibody having one or more interchain disulfide bonds in the AB and one or more intrachain disulfide bonds in the MM, and a drug reactive with free thiols is provided. The method generally includes partially reducing interchain disulfide bonds in the activatable antibody with a reducing agent, such as, for example, TCEP; and conjugating the drug reactive with free thiols to the partially reduced activatable antibody. As used herein, the term partial reduction refers to situations where an activatable anti-CD3ε antibody is contacted with a reducing agent and less than all disulfide bonds, *e.g.*, less than all possible sites of conjugation are reduced. In some embodiments, less than 99%, 98%, 97%, 96%, 95%, 90%, 85%, 80%, 75%, 70%, 65%, 60%, 55%, 50%, 45%, 40%, 35%, 30%, 25%, 20%, 15%, 10% or less than 5% of all possible sites of conjugation are reduced.

[000460] In yet other embodiments, a method of reducing and conjugating an agent, *e.g.*, a drug, to an activatable anti-CD3ε antibody resulting in selectivity in the placement of the agent is provided. The method generally includes partially reducing the activatable anti-CD3ε antibody with a reducing agent such that any conjugation sites in the masking moiety or other non-AB portion of the activatable antibody are not reduced, and conjugating the agent to

interchain thiols in the AB. The conjugation site(s) are selected so as to allow desired placement of an agent to allow conjugation to occur at a desired site. The reducing agent is, for example, TCEP. The reduction reaction conditions such as, for example, the ratio of reducing agent to activatable antibody, the length of incubation, the temperature during the incubation, the pH of the reducing reaction solution, etc., are determined by identifying the conditions that produce a conjugated activatable antibody in which the MM retains the ability to effectively and efficiently mask the AB of the activatable antibody in an uncleaved state. The ratio of reduction agent to activatable anti-CD3 ϵ antibody will vary depending on the activatable antibody. In some embodiments, the ratio of reducing agent to activatable anti-CD3 ϵ antibody will be in a range from about 20:1 to 1:1, from about 10:1 to 1:1, from about 9:1 to 1:1, from about 8:1 to 1:1, from about 7:1 to 1:1, from about 6:1 to 1:1, from about 5:1 to 1:1, from about 4:1 to 1:1, from about 3:1 to 1:1, from about 2:1 to 1:1, from about 20:1 to 1:1.5, from about 10:1 to 1:1.5, from about 9:1 to 1:1.5, from about 8:1 to 1:1.5, from about 7:1 to 1:1.5, from about 6:1 to 1:1.5, from about 5:1 to 1:1.5, from about 4:1 to 1:1.5, from about 3:1 to 1:1.5, from about 2:1 to 1:1.5, from about 1.5:1 to 1:1.5, or from about 1:1 to 1:1.5. In some embodiments, the ratio is in a range of from about 5:1 to 1:1. In some embodiments, the ratio is in a range of from about 5:1 to 1.5:1. In some embodiments, the ratio is in a range of from about 4:1 to 1:1. In some embodiments, the ratio is in a range from about 4:1 to 1.5:1. In some embodiments, the ratio is in a range from about 8:1 to about 1:1. In some embodiments, the ratio is in a range of from about 2.5:1 to 1:1.

[000461] In some embodiments, a method of reducing interchain disulfide bonds in the AB of an activatable anti-CD3 ϵ antibody and conjugating an agent, e.g., a thiol-containing agent such as a drug, to the resulting interchain thiols to selectively locate agent(s) on the AB is provided. The method generally includes partially reducing the AB with a reducing agent to form at least two interchain thiols without forming all possible interchain thiols in the activatable antibody; and conjugating the agent to the interchain thiols of the partially reduced AB. For example, the AB of the activatable antibody is partially reduced for about 1 hour at about 37°C at a desired ratio of reducing agent:activatable antibody. In some embodiments, the ratio of reducing agent to activatable antibody will be in a range from about 20:1 to 1:1, from about 10:1 to 1:1, from about 9:1 to 1:1, from about 8:1 to 1:1, from about 7:1 to 1:1, from

about 6:1 to 1:1, from about 5:1 to 1:1, from about 4:1 to 1:1, from about 3:1 to 1:1, from about 2:1 to 1:1, from about 20:1 to 1:1.5, from about 10:1 to 1:1.5, from about 9:1 to 1:1.5, from about 8:1 to 1:1.5, from about 7:1 to 1:1.5, from about 6:1 to 1:1.5, from about 5:1 to 1:1.5, from about 4:1 to 1:1.5, from about 3:1 to 1:1.5, from about 2:1 to 1:1.5, from about 1.5:1 to 1:1.5, or from about 1:1 to 1:1.5. In some embodiments, the ratio is in a range of from about 5:1 to 1:1. In some embodiments, the ratio is in a range of from about 5:1 to 1.5:1. In some embodiments, the ratio is in a range of from about 4:1 to 1:1. In some embodiments, the ratio is in a range from about 4:1 to 1.5:1. In some embodiments, the ratio is in a range from about 8:1 to about 1:1. In some embodiments, the ratio is in a range of from about 2.5:1 to 1:1.

[000462] The thiol-containing reagent can be, for example, cysteine or N-acetyl cysteine. The reducing agent can be, for example, TCEP. In some embodiments, the reduced activatable antibody can be purified prior to conjugation, using for example, column chromatography, dialysis, or diafiltration. In some embodiments, the reduced antibody is not purified after partial reduction and prior to conjugation.

[000463] The disclosure also provides partially reduced activatable anti-CD3 ϵ antibodies in which at least one interchain disulfide bond in the activatable antibody has been reduced with a reducing agent without disturbing any intrachain disulfide bonds in the activatable antibody, wherein the activatable antibody includes an antibody or an antigen binding fragment thereof (AB) that specifically binds to a CD3 ϵ target, a masking moiety (MM) that inhibits the binding of the AB of the activatable antibody in an uncleaved state to the CD3 ϵ target, and a cleavable moiety (CM) coupled to the AB, wherein the CM is a polypeptide that functions as a substrate for a protease. In some embodiments the MM is coupled to the AB via the CM. In some embodiments, one or more intrachain disulfide bond(s) of the activatable antibody is not disturbed by the reducing agent. In some embodiments, one or more intrachain disulfide bond(s) of the MM within the activatable antibody is not disturbed by the reducing agent. In some embodiments, the activatable antibody in the uncleaved state has the structural arrangement from N-terminus to C-terminus as follows: MM-CM-AB or AB-CM-MM. In some embodiments, reducing agent is TCEP.

[000464] The compositions and methods provided herein enable the attachment of one or more agents to one or more cysteine residues in any of the AB regions without compromising

the activity (e.g., the masking, activating or binding activity) of the multispecific activatable antibody. In some embodiments, the compositions and methods provided herein enable the attachment of one or more agents to one or more cysteine residues in any of the AB regions without reducing or otherwise disturbing one or more disulfide bonds within any of the MM. The compositions and methods provided herein produce a multispecific activatable antibody that is conjugated to one or more agents, *e.g.*, any of a variety of therapeutic, diagnostic and/or prophylactic agents, preferably without any of the agent(s) being conjugated to any of the MM of the multispecific activatable antibody. The compositions and methods provided herein produce conjugated multispecific activatable antibodies in which each of the MM retains the ability to effectively and efficiently mask its corresponding AB of the multispecific activatable antibody in an uncleaved state. The compositions and methods provided herein produce conjugated multispecific activatable antibodies in which the activatable antibody is still activated, *i.e.*, cleaved, in the presence of a protease that can cleave the CM.

[000465] The multispecific activatable antibodies have at least one point of conjugation for an agent, but in the methods and compositions provided herein less than all possible points of conjugation are available for conjugation to an agent. In some embodiments, the one or more points of conjugation are sulfur atoms involved in disulfide bonds. In some embodiments, the one or more points of conjugation are sulfur atoms involved in interchain disulfide bonds. In some embodiments, the one or more points of conjugation are sulfur atoms involved in interchain sulfide bonds, but not sulfur atoms involved in intrachain disulfide bonds. In some embodiments, the one or more points of conjugation are sulfur atoms of cysteine or other amino acid residues containing a sulfur atom. Such residues may occur naturally in the antibody structure or may be incorporated into the antibody by site-directed mutagenesis, chemical conversion, or mis-incorporation of non-natural amino acids.

[000466] Also provided are methods of preparing a conjugate of a multispecific activatable antibody having one or more interchain disulfide bonds in one or more of the AB and one or more intrachain disulfide bonds in the corresponding MM, and a drug reactive with free thiols is provided. The method generally includes partially reducing interchain disulfide bonds in the activatable antibody with a reducing agent, such as, for example, TCEP; and conjugating the drug reactive with free thiols to the partially reduced activatable antibody. As used herein, the term partial reduction refers to situations where a multispecific activatable

antibody is contacted with a reducing agent and less than all disulfide bonds, *e.g.*, less than all possible sites of conjugation are reduced. In some embodiments, less than 99%, 98%, 97%, 96%, 95%, 90%, 85%, 80%, 75%, 70%, 65%, 60%, 55%, 50%, 45%, 40%, 35%, 30%, 25%, 20%, 15%, 10% or less than 5% of all possible sites of conjugation are reduced.

[000467] In yet other embodiments, a method of reducing and conjugating an agent, *e.g.*, a drug, to a multispecific activatable antibody resulting in selectivity in the placement of the agent is provided. The method generally includes partially reducing the multispecific activatable antibody with a reducing agent such that any conjugation sites in any of the masking moieties or other non-AB portion of the activatable antibody are not reduced, and conjugating the agent to interchain thiols in one or more of the AB regions of the multispecific activatable antibody. The conjugation site(s) are selected so as to allow desired placement of an agent to allow conjugation to occur at a desired site. The reducing agent is, for example, TCEP. The reduction reaction conditions such as, for example, the ratio of reducing agent to activatable antibody, the length of incubation, the temperature during the incubation, the pH of the reducing reaction solution, etc., are determined by identifying the conditions that produce a conjugated activatable antibody in which the MM retains the ability to effectively and efficiently mask the AB of the activatable antibody in an uncleaved state. The ratio of reduction agent to multispecific activatable antibody will vary depending on the activatable antibody. In some embodiments, the ratio of reducing agent to multispecific activatable antibody will be in a range from about 20:1 to 1:1, from about 10:1 to 1:1, from about 9:1 to 1:1, from about 8:1 to 1:1, from about 7:1 to 1:1, from about 6:1 to 1:1, from about 5:1 to 1:1, from about 4:1 to 1:1, from about 3:1 to 1:1, from about 2:1 to 1:1, from about 20:1 to 1:1.5, from about 10:1 to 1:1.5, from about 9:1 to 1:1.5, from about 8:1 to 1:1.5, from about 7:1 to 1:1.5, from about 6:1 to 1:1.5, from about 5:1 to 1:1.5, from about 4:1 to 1:1.5, from about 3:1 to 1:1.5, from about 2:1 to 1:1.5, from about 1.5:1 to 1:1.5, or from about 1:1 to 1:1.5. In some embodiments, the ratio is in a range of from about 5:1 to 1:1. In some embodiments, the ratio is in a range of from about 5:1 to 1.5:1. In some embodiments, the ratio is in a range of from about 4:1 to 1:1. In some embodiments, the ratio is in a range from about 4:1 to 1.5:1. In some embodiments, the ratio is in a range from about 8:1 to about 1:1. In some embodiments, the ratio is in a range of from about 2.5:1 to 1:1.

[000468] In some embodiments, a method of reducing interchain disulfide bonds in one or more of the AB regions of a multispecific activatable antibody and conjugating an agent, e.g., a thiol-containing agent such as a drug, to the resulting interchain thiols to selectively locate agent(s) on the AB is provided. The method generally includes partially reducing one or more of the AB regions with a reducing agent to form at least two interchain thiols without forming all possible interchain thiols in the activatable antibody; and conjugating the agent to the interchain thiols of the partially reduced AB. For example, one or more of the AB regions of the multispecific activatable antibody is partially reduced for about 1 hour at about 37°C at a desired ratio of reducing agent:activatable antibody. In some embodiments, the ratio of reducing agent to activatable antibody will be in a range from about 20:1 to 1:1, from about 10:1 to 1:1, from about 9:1 to 1:1, from about 8:1 to 1:1, from about 7:1 to 1:1, from about 6:1 to 1:1, from about 5:1 to 1:1, from about 4:1 to 1:1, from about 3:1 to 1:1, from about 2:1 to 1:1, from about 20:1 to 1:1.5, from about 10:1 to 1:1.5, from about 9:1 to 1:1.5, from about 8:1 to 1:1.5, from about 7:1 to 1:1.5, from about 6:1 to 1:1.5, from about 5:1 to 1:1.5, from about 4:1 to 1:1.5, from about 3:1 to 1:1.5, from about 2:1 to 1:1.5, from about 1.5:1 to 1:1.5, or from about 1:1 to 1:1.5. In some embodiments, the ratio is in a range of from about 5:1 to 1:1. In some embodiments, the ratio is in a range of from about 5:1 to 1.5:1. In some embodiments, the ratio is in a range of from about 4:1 to 1:1. In some embodiments, the ratio is in a range from about 4:1 to 1.5:1. In some embodiments, the ratio is in a range from about 8:1 to about 1:1. In some embodiments, the ratio is in a range of from about 2.5:1 to 1:1.

[000469] The thiol-containing reagent can be, for example, cysteine or N-acetyl cysteine. The reducing agent can be, for example, TCEP. In some embodiments, the reduced activatable antibody can be purified prior to conjugation, using for example, column chromatography, dialysis, or diafiltration. In some embodiments, the reduced antibody is not purified after partial reduction and prior to conjugation.

[000470] The disclosure also provides partially reduced multispecific activatable antibodies in which at least one interchain disulfide bond in the multispecific activatable antibody has been reduced with a reducing agent without disturbing any intrachain disulfide bonds in the multispecific activatable antibody, wherein the multispecific activatable antibody includes at least a first antibody or an antigen binding fragment thereof (AB1) that specifically binds to a target, a first masking moiety (MM1) that inhibits the binding of the AB1 of the

multispecific activatable antibody in an uncleaved state to the target, a first cleavable moiety (CM1) coupled to the AB1, wherein the CM1 is a polypeptide that functions as a substrate for a protease, and a second antibody or an antigen binding fragment thereof (AB2) that specifically binds to a second target. In some embodiments, the MM1 is coupled to the AB1 via the CM1. In some embodiments, one or more intrachain disulfide bond(s) of the multispecific activatable antibody is not disturbed by the reducing agent. In some embodiments, one or more intrachain disulfide bond(s) of the MM1 within the multispecific activatable antibody is not disturbed by the reducing agent. In some embodiments, reducing agent is TCEP.

[000471] The disclosure also pertains to immunoconjugates comprising an anti-CD3 ϵ antibody, an activatable antibody, a multispecific antibody and/or a multispecific activatable antibody conjugated to a cytotoxic agent such as a toxin (*e.g.*, an enzymatically active toxin of bacterial, fungal, plant, or animal origin, or fragments thereof), or a radioactive isotope (*i.e.*, a radioconjugate). Suitable cytotoxic agents for use in targeting diseased T cells such as in a T cell-derived lymphoma include, for example, dolastatins and derivatives thereof (*e.g.* auristatin E, AFP, MMAD, MMAF, MMAE). For example, the cytotoxic agent is monomethyl auristatin E (MMAE). In some embodiments, the agent is monomethyl auristatin E (MMAD). In some embodiments, the agent is an agent selected from the group listed in Table 4. In some embodiments, the agent is a dolastatin. In some embodiments, the agent is an auristatin or derivative thereof. In some embodiments, the agent is auristatin E or a derivative thereof. In some embodiments, the agent is monomethyl auristatin E (MMAE). In some embodiments, the agent is monomethyl auristatin D (MMAD). In some embodiments, the agent is a maytansinoid or maytansinoid derivative. In some embodiments, the agent is DM1 or DM4. In some embodiments, the agent is a duocarmycin or derivative thereof. In some embodiments, the agent is a calicheamicin or derivative thereof. In some embodiments, the agent is a pyrrolobenzodiazepine.

[000472] Enzymatically active toxins and fragments thereof that can be used include diphtheria A chain, nonbinding active fragments of diphtheria toxin, exotoxin A chain (from *Pseudomonas aeruginosa*), ricin A chain, abrin A chain, modeccin A chain, alpha-sarcin, Aleurites fordii proteins, dianthin proteins, Phytolaca americana proteins (PAPI, PAPII, and PAP-S), momordica charantia inhibitor, curcin, crotin, sapaonaria officinalis inhibitor, gelonin,

mitogellin, restrictocin, phenomycin, enomycin, and the tricothecenes. A variety of radionuclides are available for the production of radioconjugated antibodies. Examples include ^{212}Bi , ^{64}Cu , ^{125}I , ^{131}I , ^{131}In , $^{99\text{m}}\text{Tc}$, ^{90}Y , ^{186}Re , and ^{89}Zr .

[000473] Conjugates of the antibody and cytotoxic agent are made using a variety of bifunctional protein-coupling agents such as N-succinimidyl-3-(2-pyridyldithiol) propionate (SPDP), iminothiolane (IT), bifunctional derivatives of imidoesters (such as dimethyl adipimidate HCL), active esters (such as disuccinimidyl suberate), aldehydes (such as glutaraldehyde), bis-azido compounds (such as bis (p-azidobenzoyl) hexanediamine), bis-diazonium derivatives (such as bis-(p-diazoniumbenzoyl)-ethylenediamine), diisocyanates (such as tolyene 2,6-diisocyanate), and bis-active fluorine compounds (such as 1,5-difluoro-2,4-dinitrobenzene). For example, a ricin immunotoxin can be prepared as described in Vitetta et al., Science 238: 1098 (1987). Carbon-14-labeled 1-isothiocyanatobenzyl-3-methyldiethylene triaminepentaacetic acid (MX-DTPA) is an exemplary chelating agent for conjugation of radionucleotide to the antibody. (See WO94/11026).

[000474] Table 4 lists some of the exemplary pharmaceutical agents that may be employed in the herein described disclosure but in no way is meant to be an exhaustive list.

Table 4: Exemplary Pharmaceutical Agents for Conjugation

CYTOTOXIC AGENTS

Auristatins	Turbostatin
Auristatin E	Phenstatins
Monomethyl auristatin D (MMAD)	Hydroxyphenstatin
Monomethyl auristatin E (MMAE)	Spongistatin 5
Desmethyl auristatin E (DMAE)	Spongistatin 7
Auristatin F	Halistatin 1
Monomethyl auristatin F (MMAF)	Halistatin 2
Desmethyl auristatin F (DMAF)	Halistatin 3
Auristatin derivatives, e.g., amides thereof	Modified Bryostatins
Auristatin tyramine	Halocomstatins
Auristatin quinolone	Pyrrolobenzimidazoles (PBI)
Dolastatins	Cibrostatin6
Dolastatin derivatives	Doxaliform
Dolastatin 16 DmJ	Anthracyclins analogues
Dolastatin 16 Dpv	
Maytansinoids, e.g. DM-1; DM-4	
Maytansinoid derivatives	Cemadotin analogue (CemCH2-SH)
Duocarmycin	Pseudomonas toxin A (PE38) variant
Duocarmycin derivatives	Pseudomonas toxin A (ZZ-PE38) variant

Alpha-amanitin
 Anthracyclines
 Doxorubicin

Daunorubicin
 Bryostatins
 Camptothecin
 Camptothecin derivatives
 7-substituted Camptothecin
 10, 11-
 Difluoromethylenedioxcamptothecin
 Combretastatins
 Debromoaplysiatoxin
 Kahalalide-F
 Discodermolide
 Ecteinasidins

ANTIVIRALS

Acyclovir
 Vira A
 Symmetrel

ANTIFUNGALS

Nystatin

ADDITIONAL ANTI-NEOPLASTICS

Adriamycin
 Cerubidine
 Bleomycin
 Alkeran
 Velban
 Oncovin
 Fluorouracil
 Methotrexate
 Thiotepa
 Bisantrone
 Novantrone
 Thioguanine
 Procarabazine
 Cytarabine

ANTI-BACTERIALS

Aminoglycosides
 Streptomycin
 Neomycin

ZJ-101
 OSW-1
 4-Nitrobenzyloxycarbonyl Derivatives of
 O6-Benzylguanine
 Topoisomerase inhibitors
 Hemiasterlin
 Cephalotaxine
 Homoharringtonine
 Pyrrolobenzodiazepine dimers (PBDs)
 Functionalized pyrrolobenzodiazepenes

Calicheamicins
 Podophyllotoxins
 Taxanes
 Vinca alkaloids

CONJUGATABLE DETECTION REAGENTS

Fluorescein and derivatives thereof
 Fluorescein isothiocyanate (FITC)

RADIOPHARMACEUTICALS

¹²⁵I
¹³¹I
⁸⁹Zr
¹¹¹In
¹²³I
¹³¹I
^{99m}Tc
²⁰¹Tl
¹³³Xe
¹¹C
⁶²Cu
¹⁸F
⁶⁸Ga
¹³N
¹⁵O
³⁸K
⁸²Rb
^{99m}Tc (Technetium)

HEAVY METALS

Barium
 Gold
 Platinum

Kanamycin
Amikacin
Gentamicin
Tobramycin
Streptomycin B
Spectinomycin
Ampicillin
Sulfanilamide
Polymyxin
Chloramphenicol

ANTI-MYCOPLASMALS

Tylosine
Spectinomycin

[000475] Those of ordinary skill in the art will recognize that a large variety of possible moieties can be coupled to the resultant anti-CD3 ϵ antibodies, activatable antibodies, multispecific antibodies and/or multispecific activatable antibodies of the disclosure. (*See, for example*, “Conjugate Vaccines”, Contributions to Microbiology and Immunology, J. M. Cruse and R. E. Lewis, Jr (eds), Carger Press, New York, (1989), the entire contents of which are incorporated herein by reference).

[000476] Coupling may be accomplished by any chemical reaction that will bind the two molecules so long as the antibody and the other moiety retain their respective activities. This linkage can include many chemical mechanisms, for instance covalent binding, affinity binding, intercalation, coordinate binding and complexation. In some embodiments, the preferred binding is, however, covalent binding. Covalent binding can be achieved either by direct condensation of existing side chains or by the incorporation of external bridging molecules. Many bivalent or polyvalent linking agents are useful in coupling protein molecules, such as the antibodies of the present disclosure, to other molecules. For example, representative coupling agents can include organic compounds such as thioesters, carbodiimides, succinimide esters, diisocyanates, glutaraldehyde, diazobenzenes and hexamethylene diamines. This listing is not intended to be exhaustive of the various classes of coupling agents known in the art but, rather, is exemplary of the more common coupling agents. (*See* Killen and Lindstrom, Jour. Immun. 133:1335-2549 (1984); Jansen et al., Immunological Reviews 62:185-216 (1982); and Vitetta et al., Science 238:1098 (1987)).

[000477] In some embodiments, in addition to the compositions and methods provided herein, the conjugated activatable antibody can also be modified for site-specific conjugation

through modified amino acid sequences inserted or otherwise included in the activatable antibody sequence. These modified amino acid sequences are designed to allow for controlled placement and/or dosage of the conjugated agent within a conjugated activatable antibody. For example, the activatable antibody can be engineered to include cysteine substitutions at positions on light and heavy chains that provide reactive thiol groups and do not negatively impact protein folding and assembly, nor alter antigen binding. In some embodiments, the activatable antibody can be engineered to include or otherwise introduce one or more non-natural amino acid residues within the activatable antibody to provide suitable sites for conjugation. In some embodiments, the activatable antibody can be engineered to include or otherwise introduce enzymatically activatable peptide sequences within the activatable antibody sequence.

[000478] Suitable linkers are described in the literature. (*See, for example*, Ramakrishnan, S. et al., Cancer Res. 44:201-208 (1984) describing use of MBS (M-maleimidobenzoyl-N-hydroxysuccinimide ester). *See also*, U.S. Patent No. 5,030,719, describing use of halogenated acetyl hydrazide derivative coupled to an antibody by way of an oligopeptide linker.

Particularly suitable linkers include: (i) SMPT (4-succinimidylloxycarbonyl- α -methyl- α -(2-pyridyl-dithio)-toluene (Pierce Chem. Co., Cat. (21558G); (ii) SPDP (succinimidyl-6 [3-(2-pyridyldithio) propionamido]hexanoate (Pierce Chem. Co., Cat #21651G); and (iii) Sulfo-LC-SPDP (sulfosuccinimidyl 6 [3-(2-pyridyldithio)-propionamide] hexanoate (Pierce Chem. Co. Cat. #2165-G. Additional linkers include, but are not limited to, SMCC, sulfo-SMCC, SPDB, or sulfo-SPDB.

[000479] The linkers described above contain components that have different attributes, thus leading to conjugates with differing physio-chemical properties. For example, the linker SMPT contains a sterically hindered disulfide bond, and can form conjugates with increased stability. Disulfide linkages, are in general, less stable than other linkages because the disulfide linkage is cleaved *in vitro*, resulting in less conjugate available.

[000480] The reagent EDC (1-ethyl-3-(3-dimethylaminopropyl)carbodiimide hydrochloride is useful to create a carboxamide starting with a carboxylic acid and a primary or secondary amine. Thus, EDC may be used to link lysine residues in an antibody with a carboxylic acid in a linker or toxin, or to link aspartate or glutamate residues in an antibody with an amine in a linker or toxin. Such conjugation reactions utilizing EDC may be enhanced

by addition of NHS (N-hydroxysuccinimide) or sulfo-NHS (N-hydroxy-3-oxysulfonylsuccinimide). Addition of NHS or sulfo-NHS to such conjugation reactions may enhance the rate, completeness, selectivity, and/or reproducibility of the conjugation reactions.

[000481] In some embodiments, the linkers are cleavable. In some embodiments, the linkers are non-cleavable. In some embodiments, two or more linkers are present. The two or more linkers are all the same, *e.g.*, cleavable or non-cleavable, or the two or more linkers are different, *e.g.*, at least one cleavable and at least one non-cleavable.

[000482] The present disclosure utilizes several methods for attaching agents to Abs of the anti-CD3 ϵ antibodies, activatable antibodies, multispecific antibodies and/or multispecific activatable antibodies: (a) attachment to the carbohydrate moieties of the AB, or (b) attachment to sulfhydryl groups of the AB, or (c) attachment to amino groups of the AB, or (d) attachment to carboxylate groups of the AB. According to the disclosure, ABs may be covalently attached to an agent through an intermediate linker having at least two reactive groups, one to react with AB and one to react with the agent. The linker, which may include any compatible organic compound, can be chosen such that the reaction with AB (or agent) does not adversely affect AB reactivity and selectivity. Furthermore, the attachment of linker to agent might not destroy the activity of the agent. Suitable linkers for reaction with oxidized antibodies or oxidized antibody fragments include those containing an amine selected from the group consisting of primary amine, secondary amine, hydrazine, hydrazide, hydroxylamine, phenylhydrazine, semicarbazide and thiosemicarbazide groups. Such reactive functional groups may exist as part of the structure of the linker, or may be introduced by suitable chemical modification of linkers not containing such groups.

[000483] According to the present disclosure, suitable linkers for attachment to reduced ABs of the anti-CD3 ϵ antibodies, activatable antibodies, multispecific antibodies and/or multispecific activatable antibodies include those having certain reactive groups capable of reaction with a sulfhydryl group of a reduced antibody or fragment. Such reactive groups include, but are not limited to: reactive haloalkyl groups (including, for example, haloacetyl groups), p-mercuribenzoate groups and groups capable of Michael-type addition reactions (including, for example, maleimides and groups of the type described by Mitra and Lawton, 1979, J. Amer. Chem. Soc. 101: 3097-3110).

[000484] According to the present disclosure, suitable linkers for attachment to neither oxidized nor reduced ABs of the anti-CD3 ϵ antibodies, activatable antibodies, multispecific antibodies and/or multispecific activatable antibodies include those having certain functional groups capable of reaction with the primary amino groups present in unmodified lysine residues in the AB. Such reactive groups include, but are not limited to, NHS carboxylic or carbonic esters, sulfo-NHS carboxylic or carbonic esters, 4-nitrophenyl carboxylic or carbonic esters, pentafluorophenyl carboxylic or carbonic esters, acyl imidazoles, isocyanates, and isothiocyanates.

[000485] According to the present disclosure, suitable linkers for attachment to neither oxidized nor reduced ABs include those having certain functional groups capable of reaction with the carboxylic acid groups present in aspartate or glutamate residues in the AB, which have been activated with suitable reagents. Suitable activating reagents include EDC, with or without added NHS or sulfo-NHS, and other dehydrating agents utilized for carboxamide formation. In these instances, the functional groups present in the suitable linkers would include primary and secondary amines, hydrazines, hydroxylamines, and hydrazides.

[000486] The agent may be attached to the linker before or after the linker is attached to the AB. In certain applications it may be desirable to first produce an AB-linker intermediate in which the linker is free of an associated agent. Depending upon the particular application, a specific agent may then be covalently attached to the linker. In other embodiments the AB is first attached to the MM, CM and associated linkers and then attached to the linker for conjugation purposes.

[000487] *Branched Linkers:* In specific embodiments, branched linkers that have multiple sites for attachment of agents are utilized. For multiple site linkers, a single covalent attachment to an AB would result in an AB-linker intermediate capable of binding an agent at a number of sites. The sites may be aldehyde or sulfhydryl groups or any chemical site to which agents can be attached.

[000488] In some embodiments, higher specific activity (or higher ratio of agents to AB) can be achieved by attachment of a single site linker at a plurality of sites on the AB. This plurality of sites may be introduced into the AB by either of two methods. First, one may generate multiple aldehyde groups and/or sulfhydryl groups in the same AB. Second, one may

attach to an aldehyde or sulfhydryl of the AB a "branched linker" having multiple functional sites for subsequent attachment to linkers. The functional sites of the branched linker or multiple site linker may be aldehyde or sulfhydryl groups, or may be any chemical site to which linkers may be attached. Still higher specific activities may be obtained by combining these two approaches; that is, attaching multiple site linkers at several sites on the AB.

[000489] *Cleavable Linkers:* Peptide linkers that are susceptible to cleavage by enzymes of the complement system, such as but not limited to urokinase, tissue plasminogen activator, trypsin, plasmin, or another enzyme having proteolytic activity may be used in one embodiment of the present disclosure. According to one method of the present disclosure, an agent is attached via a linker susceptible to cleavage by complement. The antibody is selected from a class that can activate complement. The antibody-agent conjugate, thus, activates the complement cascade and releases the agent at the target site. According to another method of the present disclosure, an agent is attached via a linker susceptible to cleavage by enzymes having a proteolytic activity such as a urokinase, a tissue plasminogen activator, plasmin, or trypsin. These cleavable linkers are useful in conjugated activatable antibodies that include an extracellular toxin, *e.g.*, by way of non-limiting example, any of the extracellular toxins shown in Table 4.

[000490] Non-limiting examples of cleavable linker sequences are provided in Table 5.

Table 5: Exemplary Linker Sequences for Conjugation

Types of Cleavable Sequences	Amino Acid Sequence
<u>Plasmin cleavable sequences</u>	
Pro-urokinase	PRFKIIGG (SEQ ID NO: 342) PRFRIIGG (SEQ ID NO: 343)
TGFβ	SSRHRRALD (SEQ ID NO: 344)
Plasminogen	RKSSIIIRMRDVVL (SEQ ID NO: 345)
Staphylokinase	SSSFDKGKYKKGDDA (SEQ ID NO: 346) SSSFDKGKYKRGDDA (SEQ ID NO: 347)
<u>Factor Xa cleavable sequences</u>	
	IEGR (SEQ ID NO: 348) IDGR (SEQ ID NO: 349) GGSIDGR (SEQ ID NO: 350)
<u>MMP cleavable sequences</u>	
Gelatinase A	PLGLWA (SEQ ID NO: 351)

Collagenase cleavable sequences

Calf skin collagen (α 1(I) chain)	GPQGIAGQ (SEQ ID NO: 352)
Calf skin collagen (α 2(I) chain)	GPQGLLGA (SEQ ID NO: 353)
Bovine cartilage collagen (α 1(II) chain)	GIAGQ (SEQ ID NO: 354)
Human liver collagen (α 1(III) chain)	GPLGIAGI (SEQ ID NO: 355)
Human α ₂ M	GPEGLRVG (SEQ ID NO: 356)
Human PZP	YGAGLGVV (SEQ ID NO: 357)
	AGLGVER (SEQ ID NO: 358)
	AGLGISST (SEQ ID NO: 359)
Rat α ₁ M	EPQALAMS (SEQ ID NO: 360)
	QALAMSAI (SEQ ID NO: 361)
Rat α ₂ M	AAVHLVSQ (SEQ ID NO: 362)
	MDAFLESS (SEQ ID NO: 363)
Rat α ₁ I ₃ (2J)	ESLPVVAV (SEQ ID NO: 364)
Rat α ₁ I ₃ (27J)	SAPAVESE (SEQ ID NO: 365)
Human fibroblast collagenase	DVAQFVLT (SEQ ID NO: 366)
<u>(autolytic cleavages)</u>	VAQFVLTE (SEQ ID NO: 367)
	AQFVLTEG (SEQ ID NO: 368)
	PVQPIGPQ (SEQ ID NO: 369)

[000491] In addition, agents may be attached via disulfide bonds (for example, the disulfide bonds on a cysteine molecule) to the AB. Since many tumors naturally release high levels of glutathione (a reducing agent) this can reduce the disulfide bonds with subsequent release of the agent at the site of delivery. In certain specific embodiments the reducing agent that would modify a CM would also modify the linker of the conjugated activatable antibody.

[000492] *Spacers and Cleavable Elements:* In still another embodiment, it may be necessary to construct the linker in such a way as to optimize the spacing between the agent and the AB of the activatable antibody. This may be accomplished by use of a linker of the general structure:



wherein

W is either --NH--CH₂-- or --CH₂--;

Q is an amino acid, peptide; and

n is an integer from 0 to 20.

[000493] In still other embodiments, the linker may comprise a spacer element and a cleavable element. The spacer element serves to position the cleavable element away from the

core of the AB such that the cleavable element is more accessible to the enzyme responsible for cleavage. Certain of the branched linkers described above may serve as spacer elements.

[000494] Throughout this discussion, it should be understood that the attachment of linker to agent (or of spacer element to cleavable element, or cleavable element to agent) need not be particular mode of attachment or reaction. Any reaction providing a product of suitable stability and biological compatibility is acceptable.

[000495] *Serum Complement and Selection of Linkers:* According to one method of the present disclosure, when release of an agent is desired, an AB that is an antibody of a class that can activate complement is used. The resulting conjugate retains both the ability to bind antigen and activate the complement cascade. Thus, according to this embodiment of the present disclosure, an agent is joined to one end of the cleavable linker or cleavable element and the other end of the linker group is attached to a specific site on the AB. For example, if the agent has an hydroxy group or an amino group, it may be attached to the carboxy terminus of a peptide, amino acid or other suitably chosen linker via an ester or amide bond, respectively. For example, such agents may be attached to the linker peptide via a carbodimide reaction. If the agent contains functional groups that would interfere with attachment to the linker, these interfering functional groups can be blocked before attachment and deblocked once the product conjugate or intermediate is made. The opposite or amino terminus of the linker is then used either directly or after further modification for binding to an AB that is capable of activating complement.

[000496] Linkers (or spacer elements of linkers) may be of any desired length, one end of which can be covalently attached to specific sites on the AB of the activatable antibody. The other end of the linker or spacer element may be attached to an amino acid or peptide linker.

[000497] Thus when these conjugates bind to antigen in the presence of complement the amide or ester bond that attaches the agent to the linker will be cleaved, resulting in release of the agent in its active form. These conjugates, when administered to a subject, will accomplish delivery and release of the agent at the target site, and are particularly effective for the in vivo delivery of pharmaceutical agents, antibiotics, antimetabolites, antiproliferative agents and the like as presented in but not limited to those in Table 4.

[000498] *Linkers for Release without Complement Activation:* In yet another application of targeted delivery, release of the agent without complement activation is desired since

activation of the complement cascade will ultimately lyse the target cell. Hence, this approach is useful when delivery and release of the agent should be accomplished without killing the target cell. Such is the goal when delivery of cell mediators such as hormones, enzymes, corticosteroids, neurotransmitters, genes or enzymes to target cells is desired. These conjugates may be prepared by attaching the agent to an AB that is not capable of activating complement via a linker that is mildly susceptible to cleavage by serum proteases. When this conjugate is administered to an individual, antigen-antibody complexes will form quickly whereas cleavage of the agent will occur slowly, thus resulting in release of the compound at the target site.

[000499] *Biochemical Cross Linkers:* In other embodiments, the activatable antibody may be conjugated to one or more therapeutic agents using certain biochemical cross-linkers. Cross-linking reagents form molecular bridges that tie together functional groups of two different molecules. To link two different proteins in a step-wise manner, hetero-bifunctional cross-linkers can be used that eliminate unwanted homopolymer formation.

[000500] Peptidyl linkers cleavable by lysosomal proteases are also useful, for example, Val-Cit, Val-Ala or other dipeptides. In addition, acid-labile linkers cleavable in the low-pH environment of the lysosome may be used, for example: bis-sialyl ether. Other suitable linkers include cathepsin-labile substrates, particularly those that show optimal function at an acidic pH.

[000501] Exemplary hetero-bifunctional cross-linkers are referenced in Table 6.

Table 6: Exemplary Hetero-Bifunctional Cross Linkers

<u>HETERO-BIFUNCTIONAL CROSS-LINKERS</u>			
Linker	Reactive Toward	Advantages and Applications	Spacer Arm Length after cross-linking (Angstroms)
SMPT	Primary amines Sulfhydryls	Greater stability	11.2 Å
SPDP	Primary amines Sulfhydryls	Thiolation Cleavable cross-linking	6.8 Å
LC-SPDP	Primary amines Sulfhydryls	Extended spacer arm	15.6 Å
Sulfo-LC-SPDP	Primary amines Sulfhydryls	Extender spacer arm Water-soluble	15.6 Å

SMCC	Primary amines	Stable maleimide reactive group	11.6 Å
	Sulfhydryls	Enzyme-antibody conjugation Hapten-carrier protein conjugation	
Sulfo-SMCC	Primary amines	Stable maleimide reactive group	11.6 Å
	Sulfhydryls	Water-soluble Enzyme-antibody conjugation	
MBS	Primary amines	Enzyme-antibody conjugation	9.9 Å
	Sulfhydryls	Hapten-carrier protein conjugation	
Sulfo-MBS	Primary amines	Water-soluble	9.9 Å
	Sulfhydryls		
SIAB	Primary amines	Enzyme-antibody conjugation	10.6 Å
	Sulfhydryls		
Sulfo-SIAB	Primary amines	Water-soluble	10.6 Å
	Sulfhydryls		
SMPB	Primary amines	Extended spacer arm	14.5 Å
	Sulfhydryls	Enzyme-antibody conjugation	
Sulfo-SMPB	Primary amines	Extended spacer arm	14.5 Å
	Sulfhydryls	Water-soluble	
EDE/Sulfo-NHS	Primary amines	Hapten-Carrier conjugation	0
	Carboxyl groups		
ABH	Carbohydrates	Reacts with sugar groups	11.9 Å
	Nonselective		

[000502] *Non-Cleavable Linkers or Direct Attachment:* In still other embodiments of the disclosure, the conjugate may be designed so that the agent is delivered to the target but not released. This may be accomplished by attaching an agent to an AB either directly or via a non-cleavable linker.

[000503] These non-cleavable linkers may include amino acids, peptides, D-amino acids or other organic compounds that may be modified to include functional groups that can subsequently be utilized in attachment to ABs by the methods described herein. A-general formula for such an organic linker could be



wherein

W is either --NH--CH₂-- or --CH₂--;

Q is an amino acid, peptide; and

n is an integer from 0 to 20.

[000504] *Non-Cleavable Conjugates:* In some embodiments, a compound may be attached to ABs that do not activate complement. When using ABs that are incapable of complement activation, this attachment may be accomplished using linkers that are susceptible to cleavage by activated complement or using linkers that are not susceptible to cleavage by activated complement.

[000505] The antibodies disclosed herein can also be formulated as immunoliposomes. Liposomes containing the antibody are prepared by methods known in the art, such as described in Epstein et al., Proc. Natl. Acad. Sci. USA, 82: 3688 (1985); Hwang et al., Proc. Natl Acad. Sci. USA, 77: 4030 (1980); and U.S. Pat. Nos. 4,485,045 and 4,544,545. Liposomes with enhanced circulation time are disclosed in U.S. Patent No. 5,013,556.

[000506] Particularly useful liposomes can be generated by the reverse-phase evaporation method with a lipid composition comprising phosphatidylcholine, cholesterol, and PEG-derivatized phosphatidylethanolamine (PEG-PE). Liposomes are extruded through filters of defined pore size to yield liposomes with the desired diameter. Fab' fragments of the antibody of the present disclosure can be conjugated to the liposomes as described in Martin et al., J. Biol. Chem., 257: 286-288 (1982) via a disulfide-interchange reaction.

Activatable Antibodies and Multispecific Activatable Antibodies Having Non-Binding Steric Moieties or Binding Partners for Non-Binding Steric Moieties

[000507] The disclosure also provides activatable antibodies and/or multispecific activatable antibodies that include non-binding steric moieties (NB) or binding partners (BP) for non-binding steric moieties, where the BP recruits or otherwise attracts the NB to the activatable antibody and/or multispecific activatable antibody. The activatable antibodies provided herein include, for example, an activatable antibody that includes a non-binding steric moiety (NB), a cleavable linker (CL) and an antibody or antibody fragment (AB) that binds a first target or epitope; a multispecific activatable antibody that includes a binding partner for a non-binding steric moiety (BP), a CL and an AB; and a multispecific activatable antibody that includes a BP to which an NB has been recruited, a CL and AB that binds a first target or epitope. The multispecific activatable antibodies provided herein include, for example, a multispecific activatable antibody that includes a non-binding steric moiety (NB), a cleavable linker (CL) and at least a first antibody or antibody fragment (AB1) that binds a first target or

epitope; a multispecific activatable antibody that includes a binding partner for a non-binding steric moiety (BP), a CL and an AB1; and a multispecific activatable antibody that includes a BP to which an NB has been recruited, a CL and AB1 that binds a first target or epitope. Activatable antibodies in which the NB is covalently linked to the CL and AB or is associated by interaction with a BP that is covalently linked to the CL and AB are referred to herein as “NB-containing activatable antibodies.” Multispecific activatable antibodies in which the NB is covalently linked to the CL and AB1 or is associated by interaction with a BP that is covalently linked to the CL and AB1 are referred to herein as “NB-containing multispecific activatable antibodies.” By activatable or switchable is meant that the activatable antibody exhibits a first level of binding to a target when the activatable antibody is in an inhibited, masked or uncleaved state (*i.e.*, a first conformation), and a second level of binding to the target when the activatable antibody is in an uninhibited, unmasked and/or cleaved state (*i.e.*, a second conformation, *i.e.*, activated antibody), where the second level of target binding is greater than the first level of target binding. The activatable antibody and/or multispecific activatable antibody compositions can exhibit increased bioavailability and more favorable biodistribution compared to conventional antibody therapeutics.

[000508] In some embodiments, activatable antibodies and/or multispecific activatable antibodies provide for reduced toxicity and/or adverse side effects that could otherwise result from binding of the activatable antibody and/or multispecific activatable antibody at non-treatment sites and/or non-diagnostic sites if the activatable antibody and/or multispecific activatable antibody were not masked or otherwise inhibited from binding to such a site.

[000509] Anti-CD3 ϵ activatable antibodies that include a non-binding steric moiety (NB) can be made using the methods set forth in PCT Publication No. WO 2013/192546, the contents of which are hereby incorporated by reference in their entirety.

Use of Anti-CD3 ϵ , Activatable Antibodies, Multispecific Antibodies and Multispecific Activatable Antibodies

[000510] It will be appreciated that administration of therapeutic entities in accordance with the disclosure will be administered with suitable carriers, excipients, and other agents that are incorporated into formulations to provide improved transfer, delivery, tolerance, and the like. A multitude of appropriate formulations can be found in the formulary known to all

pharmaceutical chemists: Remington's Pharmaceutical Sciences (15th ed., Mack Publishing Company, Easton, PA (1975)), particularly Chapter 87 by Blaug, Seymour, therein. These formulations include, for example, powders, pastes, ointments, jellies, waxes, oils, lipids, lipid (cationic or anionic) containing vesicles (such as Lipofectin™), DNA conjugates, anhydrous absorption pastes, oil-in-water and water-in-oil emulsions, emulsions carbowax (polyethylene glycols of various molecular weights), semi-solid gels, and semi-solid mixtures containing carbowax. Any of the foregoing mixtures may be appropriate in treatments and therapies in accordance with the present disclosure, provided that the active ingredient in the formulation is not inactivated by the formulation and the formulation is physiologically compatible and tolerable with the route of administration. *See also* Baldrick P. "Pharmaceutical excipient development: the need for preclinical guidance." Regul. Toxicol Pharmacol. 32(2):210-8 (2000), Wang W. "Lyophilization and development of solid protein pharmaceuticals." Int. J. Pharm. 203(1-2):1-60 (2000), Charman WN "Lipids, lipophilic drugs, and oral drug delivery-some emerging concepts." J Pharm Sci. 89(8):967-78 (2000), Powell *et al.* "Compendium of excipients for parenteral formulations" PDA J Pharm Sci Technol. 52:238-311 (1998) and the citations therein for additional information related to formulations, excipients and carriers well known to pharmaceutical chemists.

[000511] In one embodiment, an anti-CD3ε antibody, an activatable antibody, a multispecific antibody and/or a multispecific activatable antibody of the disclosure may be used as therapeutic agents. Such agents will generally be employed to diagnose, prognose, monitor, treat, alleviate, and/or prevent a disease or pathology in a subject. A therapeutic regimen is carried out by identifying a subject, *e.g.*, a human patient or other mammal suffering from (or at risk of developing) a disorder using standard methods. An anti-CD3ε antibody and/or an activatable antibody preparation is administered to the subject and will generally have an effect due to its binding with the CD3ε. A multispecific antibody and/or a multispecific activatable antibody preparation, for example in some embodiments, one having high specificity and high affinity for its two or more target antigens, is administered to the subject and will generally have an effect due to its binding with the targets. Administration of anti-CD3ε antibody, an activatable antibody, a multispecific antibody and/or a multispecific activatable antibody may activate T cells via engagement of CD3ε on the T cells. Administration of anti-CD3ε antibody,

an activatable antibody, a multispecific antibody and/or a multispecific activatable antibody may agonize, stimulate, activate, and/or augment CD3-mediated T cell activation.

[000512] Generally, alleviation or treatment of a disease or disorder involves the lessening of one or more symptoms or medical problems associated with the disease or disorder. For example, in the case of cancer, the therapeutically effective amount of the drug can accomplish one or a combination of the following: reduce the number of cancer cells; reduce the tumor size; inhibit (*i.e.*, to decrease to some extent and/or stop) cancer cell infiltration into peripheral organs; inhibit tumor metastasis; inhibit, to some extent, tumor growth; and/or relieve to some extent one or more of the symptoms associated with the cancer. In some embodiments, a composition of this disclosure can be used to prevent the onset or reoccurrence of the disease or disorder in a subject, *e.g.*, a human or other mammal, such as a non-human primate, companion animal (*e.g.*, cat, dog, horse), farm animal, work animal, or zoo animal. The terms subject and patient are used interchangeably herein.

[000513] A therapeutically effective amount of an anti-CD3 ϵ antibody, an activatable antibody, a multispecific antibody and/or a multispecific activatable antibody of the disclosure relates generally to the amount needed to achieve a therapeutic objective. As noted above, this may be a binding interaction between the anti-CD3 ϵ antibody, the activatable antibody, the multispecific antibody and/or a multispecific activatable antibody and its target antigens that, in certain cases, agonize, stimulate, activate, and/or augment CD3-mediated T cell activation. The amount required to be administered will furthermore depend on the binding affinity of the anti-CD3 ϵ antibody, the activatable antibody, the multispecific antibody and/or a multispecific activatable antibody for its specific antigen, and will also depend on the rate at which an administered anti-CD3 ϵ antibody, activatable antibody, multispecific antibody and/or multispecific activatable antibody is depleted from the free volume other subject to which it is administered. Common ranges for therapeutically effective dosing of an anti-CD3 ϵ antibody, an activatable antibody, a multispecific antibody and/or antibody fragment and/or a multispecific activatable antibody of the disclosure may be, by way of nonlimiting example, from about 0.01 μ g/kg body weight to about 10 mg/kg body weight. In some embodiments, the therapeutically effective dosing of an anti-CD3 ϵ antibody, an activatable antibody, a multispecific antibody and/or antibody fragment and/or a multispecific activatable antibody of the disclosure may be,

by way of nonlimiting example, from about 0.01 mg/kg body weight to about 5-10 mg/kg body weight. Common dosing frequencies may range, for example, from twice daily to once a week.

[000514] Efficaciousness of treatment is determined in association with any known method for diagnosing or treating the particular disorder. Methods for the screening of anti-CD3 ϵ antibodies, activatable antibodies, multispecific antibodies and/or multispecific activatable antibodies that possess the desired specificity include, but are not limited to, enzyme linked immunosorbent assay (ELISA) and other immunologically mediated techniques known within the art.

[000515] In another embodiment, an anti-CD3 ϵ antibody, an activatable antibody, a multispecific antibody and/or a multispecific activatable antibody directed two or more targets are used in methods known within the art relating to the localization and/or quantitation of the targets (*e.g.*, for use in measuring levels of one or more of the targets within appropriate physiological samples, for use in diagnostic methods, for use in imaging the protein, and the like). In a given embodiment, an anti-CD3 ϵ antibody, an activatable antibody, a multispecific antibody and/or a multispecific activatable antibody directed two or more targets, or a derivative, fragment, analog or homolog thereof, that contain the antibody derived antigen binding domain, are utilized as pharmacologically active compounds (referred to hereinafter as “Therapeutics”).

[000516] In another embodiment, an anti-CD3 ϵ antibody, an activatable antibody, a multispecific antibody and/or a multispecific activatable antibody directed two or more targets is used to isolate one or more of the targets by standard techniques, such as immunoaffinity, chromatography or immunoprecipitation. An anti-CD3 ϵ antibody, an activatable antibody, a multispecific antibody and/or a multispecific activatable antibody directed two or more targets (or a fragment thereof) are used diagnostically to monitor protein levels in tissue as part of a clinical testing procedure, *e.g.*, to determine the efficacy of a given treatment regimen. Detection can be facilitated by coupling (*i.e.*, physically linking) the antibody to a detectable substance. Examples of detectable substances include various enzymes, prosthetic groups, fluorescent materials, luminescent materials, bioluminescent materials, and radioactive materials. Examples of suitable enzymes include horseradish peroxidase, alkaline phosphatase, β -galactosidase, or acetylcholinesterase; examples of suitable prosthetic group complexes

include streptavidin/biotin and avidin/biotin; examples of suitable fluorescent materials include umbelliferone, fluorescein, fluorescein isothiocyanate, rhodamine, dichlorotriazinylamine fluorescein, dansyl chloride or phycoerythrin; an example of a luminescent material includes luminol; examples of bioluminescent materials include luciferase, luciferin, and aequorin, and examples of suitable radioactive material include ^{125}I , ^{131}I , ^{35}S or ^3H .

[000517] In yet another embodiment, an anti-CD3 ϵ antibody, an activatable antibody, a multispecific antibody and/or a multispecific activatable antibody directed two or more targets can be used as an agent for detecting the presence of one or more of the targets (or a fragment thereof) in a sample. In some embodiments, the antibody contains a detectable label.

Antibodies are polyclonal, or in some embodiments, monoclonal. An intact antibody, or a fragment thereof (*e.g.*, F_{ab} , scFv, or $F_{(ab)2}$) is used. The term “labeled”, with regard to the probe or antibody, is intended to encompass direct labeling of the probe or antibody by coupling (*i.e.*, physically linking) a detectable substance to the probe or antibody, as well as indirect labeling of the probe or antibody by reactivity with another reagent that is directly labeled. Examples of indirect labeling include detection of a primary antibody using a fluorescently-labeled secondary antibody and end-labeling of an antibody with biotin such that it can be detected with fluorescently-labeled streptavidin. The term “biological sample” is intended to include tissues, cells and biological fluids isolated from a subject, as well as tissues, cells and fluids present within a subject. Included within the usage of the term “biological sample”, therefore, is blood and a fraction or component of blood including blood serum, blood plasma, or lymph. That is, the detection method of the disclosure can be used to detect a protein in a biological sample *in vitro* as well as *in vivo*. For example, *in vitro* techniques for detection of an analyte protein include enzyme linked immunosorbent assays (ELISAs), Western blots, immunoprecipitations, and immunofluorescence. Procedures for conducting immunoassays are described, for example in “ELISA: Theory and Practice: Methods in Molecular Biology”, Vol. 42, J. R. Crowther (Ed.) Human Press, Totowa, NJ, 1995; “Immunoassay”, E. Diamandis and T. Christopoulos, Academic Press, Inc., San Diego, CA, 1996; and “Practice and Theory of Enzyme Immunoassays”, P. Tijssen, Elsevier Science Publishers, Amsterdam, 1985. Furthermore, *in vivo* techniques for detection of an analyte protein include introducing into a subject a labeled anti-analyte protein antibody. For example, the antibody can be labeled with a

radioactive marker whose presence and location in a subject can be detected by standard imaging techniques.

[000518] The anti-CD3 ϵ antibody, the activatable antibody, the multispecific antibodies and/or multispecific activatable antibodies of the disclosure are also useful in a variety of diagnostic and prophylactic formulations. In one embodiment, an anti-CD3 ϵ antibody, an activatable antibody, a multispecific antibody and/or multispecific activatable antibody is administered to patients that are at risk of developing one or more of the aforementioned disorders. A patient's or organ's predisposition to one or more of the disorders can be determined using genotypic, serological or biochemical markers.

[000519] In another embodiment of the disclosure, an anti-CD3 ϵ antibody, an activatable antibody, a multispecific antibody and/or multispecific activatable antibody is administered to human individuals diagnosed with a clinical indication associated with one or more of the aforementioned disorders. Upon diagnosis, an anti-CD3 ϵ antibody, an activatable antibody, a multispecific antibody and/or multispecific activatable antibody is administered to mitigate or reverse the effects of the clinical indication.

[000520] Anti-CD3 ϵ antibodies, activatable antibodies, multispecific antibodies and/or multispecific activatable antibodies are also useful in the detection of one or more targets in patient samples and accordingly are useful as diagnostics. For example, the anti-CD3 ϵ antibodies, the activatable antibodies, the multispecific antibodies and/or multispecific activatable antibodies of the disclosure are used in *in vitro* assays, *e.g.*, ELISA, to detect one or more target levels in a patient sample.

[000521] In one embodiment, an anti-CD3 ϵ antibody, an activatable antibody, a multispecific antibody and/or multispecific activatable antibody is immobilized on a solid support (*e.g.*, the well(s) of a microtiter plate). The immobilized antibody and/or activatable antibody serves as a capture antibody for any target(s) that may be present in a test sample. Prior to contacting the immobilized anti-CD3 ϵ antibody, activatable antibody, multispecific antibody and/or immobilized multispecific activatable antibody with a patient sample, the solid support is rinsed and treated with a blocking agent such as milk protein or albumin to prevent nonspecific adsorption of the analyte.

[000522] Subsequently the wells are treated with a test sample suspected of containing the antigen, or with a solution containing a standard amount of the antigen. Such a sample is, *e.g.*, a serum sample from a subject suspected of having levels of circulating antigen considered to be diagnostic of a pathology. After rinsing away the test sample or standard, the solid support is treated with a second antibody that is detectably labeled. The labeled second antibody serves as a detecting antibody. The level of detectable label is measured, and the concentration of target antigen(s) in the test sample is determined by comparison with a standard curve developed from the standard samples.

[000523] It will be appreciated that based on the results obtained using the anti-CD3 ϵ antibody, the activatable antibody, the multispecific antibody and/or multispecific activatable antibody in an *in vitro* diagnostic assay, it is possible to stage a disease in a subject based on expression levels of the target antigen(s). For a given disease, samples of blood are taken from subjects diagnosed as being at various stages in the progression of the disease, and/or at various points in the therapeutic treatment of the disease. Using a population of samples that provides statistically significant results for each stage of progression or therapy, a range of concentrations of the antigen that may be considered characteristic of each stage is designated.

[000524] Anti-CD3 ϵ antibodies, activatable antibodies, multispecific antibodies and/or multispecific activatable antibodies can also be used in diagnostic and/or imaging methods. In some embodiments, such methods are *in vitro* methods. In some embodiments, such methods are *in vivo* methods. In some embodiments, such methods are *in situ* methods. In some embodiments, such methods are *ex vivo* methods. For example, activatable antibodies and/or multispecific activatable antibodies having an enzymatically cleavable CM can be used to detect the presence or absence of an enzyme that is capable of cleaving the CM. Such activatable antibodies and/or multispecific activatable antibodies can be used in diagnostics, which can include *in vivo* detection (*e.g.*, qualitative or quantitative) of enzyme activity (or, in some embodiments, an environment of increased reduction potential such as that which can provide for reduction of a disulfide bond) through measured accumulation of multispecific activated antibodies (i.e., antibodies resulting from cleavage of an activatable antibody and/or a multispecific activatable antibody) in a given cell or tissue of a given host organism. Such accumulation of activated multispecific antibodies indicates not only that the tissue expresses

enzymatic activity (or an increased reduction potential depending on the nature of the CM) but also that the tissue expresses at least one target to which the activated antibody binds.

[000525] For example, the CM can be selected to be a protease substrate for a protease found at the site of a tumor, at the site of a viral or bacterial infection at a biologically confined site (*e.g.*, such as in an abscess, in an organ, and the like), and the like. At least one of the AB can be one that binds a target antigen. Using methods familiar to one skilled in the art, a detectable label (*e.g.*, a fluorescent label or radioactive label or radiotracer) can be conjugated to an AB or other region of a multispecific antibody and/or multispecific activatable antibody. Suitable detectable labels are discussed in the context of the above screening methods and additional specific examples are provided below. Using at least one AB specific to a protein or peptide of the disease state, along with a protease whose activity is elevated in the disease tissue of interest, activatable antibodies will exhibit an increased rate of binding to disease tissue relative to tissues where the CM specific enzyme is not present at a detectable level or is present at a lower level than in disease tissue or is inactive (*e.g.*, in zymogen form or in complex with an inhibitor). Since small proteins and peptides are rapidly cleared from the blood by the renal filtration system, and because the enzyme specific for the CM is not present at a detectable level (or is present at lower levels in non-disease tissues or is present in inactive conformation), accumulation of activated multispecific antibodies in the disease tissue is enhanced relative to non-disease tissues.

[000526] In another example, activatable antibodies and/or activatable multispecific antibodies can be used to detect the presence or absence of a cleaving agent in a sample. For example, where the activatable antibodies and/or multispecific activatable antibodies contain a CM susceptible to cleavage by an enzyme, the multispecific activatable antibodies can be used to detect (either qualitatively or quantitatively) the presence of an enzyme in the sample. In another example, where the activatable antibodies and/or multispecific activatable antibodies contain a CM susceptible to cleavage by reducing agent, the activatable antibodies and/or multispecific activatable antibodies can be used to detect (either qualitatively or quantitatively) the presence of reducing conditions in a sample. To facilitate analysis in these methods, the activatable antibodies and/or multispecific activatable antibodies can be detectably labeled, and can be bound to a support (*e.g.*, a solid support, such as a slide or bead). The detectable label can be positioned on a portion of the activatable antibody and/or multispecific activatable

antibody that is not released following cleavage, for example, the detectable label can be a quenched fluorescent label or other label that is not detectable until cleavage has occurred. The assay can be conducted by, for example, contacting the immobilized, detectably labeled activatable antibodies and/or multispecific activatable antibodies with a sample suspected of containing an enzyme and/or reducing agent for a time sufficient for cleavage to occur, then washing to remove excess sample and contaminants. The presence or absence of the cleaving agent (*e.g.*, enzyme or reducing agent) in the sample is then assessed by a change in detectable signal of the activatable antibodies and/or multispecific activatable antibodies prior to contacting with the sample *e.g.*, the presence of and/or an increase in detectable signal due to cleavage of the activatable antibody and/or multispecific activatable antibody by the cleaving agent in the sample.

[000527] Such detection methods can be adapted to also provide for detection of the presence or absence of a target that is capable of binding at least one AB of the multispecific activatable antibodies when cleaved. Thus, the assays can be adapted to assess the presence or absence of a cleaving agent and the presence or absence of a target of interest. The presence or absence of the cleaving agent can be detected by the presence of and/or an increase in detectable label of the multispecific activatable antibodies as described above, and the presence or absence of the target can be detected by detection of a target-AB complex *e.g.*, by use of a detectably labeled anti-target antibody.

[000528] Activatable antibodies and/or multispecific activatable antibodies are also useful in *in situ* imaging for the validation of activatable antibody activation, *e.g.*, by protease cleavage, and binding to a particular target. *In situ* imaging is a technique that enables localization of proteolytic activity and target in biological samples such as cell cultures or tissue sections. Using this technique, it is possible to confirm both binding to a given target and proteolytic activity based on the presence of a detectable label (*e.g.*, a fluorescent label).

[000529] These techniques are useful with any frozen cells or tissue derived from a disease site (*e.g.* tumor tissue) or healthy tissues. These techniques are also useful with fresh cell or tissue samples.

[000530] In these techniques, an activatable antibody and/or multispecific activatable antibody is labeled with a detectable label. The detectable label may be a fluorescent dye, (*e.g.* a fluorophore, Fluorescein Isothiocyanate (FITC), Rhodamine Isothiocyanate (TRITC), an

Alexa Fluor® label), a near infrared (NIR) dye (e.g., Qdot® nanocrystals), a colloidal metal, a hapten, a radioactive marker, biotin and an amplification reagent such as streptavidin, or an enzyme (e.g. horseradish peroxidase or alkaline phosphatase).

[000531] Detection of the label in a sample that has been incubated with the labeled, multispecific activatable antibody indicates that the sample contains the target and contains a protease that is specific for the CM of the activatable antibody and/or multispecific activatable antibody. In some embodiments, the presence of the protease can be confirmed using broad spectrum protease inhibitors such as those described herein, and/or by using an agent that is specific for the protease, for example, an antibody such as A11, which is specific for the protease matriptase (MT-SP1) and inhibits the proteolytic activity of matriptase; see e.g., International Publication Number WO 2010/129609, published 11 November 2010. The same approach of using broad spectrum protease inhibitors such as those described herein, and/or by using a more selective inhibitory agent can be used to identify a protease or class of proteases specific for the CM of the activatable antibody and/or multispecific activatable antibody. In some embodiments, the presence of the target can be confirmed using an agent that is specific for the target or the detectable label can be competed with unlabeled target. In some embodiments, unlabeled activatable antibody and/or multispecific activatable antibody could be used, with detection by a labeled secondary antibody or more complex detection system.

[000532] Similar techniques are also useful for *in vivo* imaging where detection of the fluorescent signal in a subject, e.g., a mammal, including a human, indicates that the disease site contains the target and contains a protease that is specific for the CM of the activatable antibody and/or multispecific activatable antibody.

[000533] These techniques are also useful in kits and/or as reagents for the detection, identification or characterization of protease activity in a variety of cells, tissues, and organisms based on the protease-specific CM in the multispecific activatable antibody.

Therapeutic Administration and Formulations of Anti-CD3ε Antibodies, Activatable Antibodies, Multispecific Antibodies and/or Multispecific Activatable Antibodies

[000534] It will be appreciated that administration of therapeutic entities in accordance with the disclosure will be administered with suitable carriers, excipients, and other agents that are incorporated into formulations to provide improved transfer, delivery, tolerance, and the

like. A multitude of appropriate formulations can be found in the formulary known to all pharmaceutical chemists: Remington's Pharmaceutical Sciences (15th ed., Mack Publishing Company, Easton, PA (1975)), particularly Chapter 87 by Blaug, Seymour, therein. These formulations include, for example, powders, pastes, ointments, jellies, waxes, oils, lipids, lipid (cationic or anionic) containing vesicles (such as Lipofectin™), DNA conjugates, anhydrous absorption pastes, oil-in-water and water-in-oil emulsions, emulsions carbowax (polyethylene glycols of various molecular weights), semi-solid gels, and semi-solid mixtures containing carbowax. Any of the foregoing mixtures may be appropriate in treatments and therapies in accordance with the present disclosure, provided that the active ingredient in the formulation is not inactivated by the formulation and the formulation is physiologically compatible and tolerable with the route of administration. *See also* Baldrick P. "Pharmaceutical excipient development: the need for preclinical guidance." Regul. Toxicol Pharmacol. 32(2):210-8 (2000), Wang W. "Lyophilization and development of solid protein pharmaceuticals." Int. J. Pharm. 203(1-2):1-60 (2000), Charman WN "Lipids, lipophilic drugs, and oral drug delivery-some emerging concepts." J Pharm Sci. 89(8):967-78 (2000), Powell *et al.* "Compendium of excipients for parenteral formulations" PDA J Pharm Sci Technol. 52:238-311 (1998) and the citations therein for additional information related to formulations, excipients and carriers well known to pharmaceutical chemists.

[000535] In some embodiments, the anti-CD3 ϵ antibodies, the conjugated anti-CD3 ϵ antibodies, the activatable antibodies, the conjugated activatable antibodies, the multispecific antibodies, the multispecific activatable antibodies and/or the conjugated multispecific activatable antibody compositions – referred to collectively herein as the Therapeutic(s) – are administered in conjunction with one or more additional agents, or a combination of additional agents. Suitable additional agents include current pharmaceutical and/or surgical therapies for an intended application. For example, the Therapeutic(s) can be used in conjunction with an additional chemotherapeutic or anti-neoplastic agent. For example, the Therapeutic(s) and additional agent are formulated into a single therapeutic composition, and the Therapeutic(s) and additional agent are administered simultaneously. In some embodiments, the Therapeutic(s) and additional agent are separate from each other, *e.g.*, each is formulated into a separate therapeutic composition, and the Therapeutic(s) and the additional agent are

administered simultaneously, or the Therapeutic(s) and the additional agent are administered at different times during a treatment regimen. For example, the Therapeutic(s) is administered prior to the administration of the additional agent, the Therapeutic(s) is administered subsequent to the administration of the additional agent, or the Therapeutic(s) and the additional agent are administered in an alternating fashion. As described herein, the Therapeutic(s) and additional agent are administered in single doses or in multiple doses.

[000536] In some embodiments, the additional agent is coupled or otherwise attached to the Therapeutic(s).

[000537] Suitable additional agents are selected according to the purpose of the intended application (*i.e.*, killing, prevention of cell proliferation, hormone therapy or gene therapy). Such agents may include but is not limited to, for example, pharmaceutical agents, toxins, fragments of toxins, alkylating agents, enzymes, antibiotics, antimetabolites, antiproliferative agents, hormones, neurotransmitters, DNA, RNA, siRNA, oligonucleotides, antisense RNA, aptamers, diagnostics, radiopaque dyes, radioactive isotopes, fluorogenic compounds, magnetic labels, nanoparticles, marker compounds, lectins, compounds that alter cell membrane permeability, photochemical compounds, small molecules, liposomes, micelles, gene therapy vectors, viral vectors, and the like. Finally, combinations of agents or combinations of different classes of agents may be used.

[000538] The anti-CD3 ϵ antibodies, the conjugated anti-CD3 ϵ antibodies, the activatable antibodies, the conjugated activatable antibodies, the multispecific antibodies, the multispecific activatable antibodies and/or the conjugated multispecific activatable antibody compositions of the disclosure (also referred to herein as “Therapeutic(s)” or “active compounds”), and derivatives, fragments, analogs and homologs thereof, can be incorporated into pharmaceutical compositions suitable for administration. Principles and considerations involved in preparing such compositions, as well as guidance in the choice of components are provided, for example, in Remington’s Pharmaceutical Sciences: The Science And Practice Of Pharmacy 19th ed. (Alfonso R. Gennaro, et al., editors) Mack Pub. Co., Easton, Pa. : 1995; Drug Absorption Enhancement : Concepts, Possibilities, Limitations, And Trends, Harwood Academic Publishers, Langhorne, Pa., 1994; and Peptide And Protein Drug Delivery (Advances In Parenteral Sciences, Vol. 4), 1991, M. Dekker, New York.

[000539] Such compositions typically comprise the anti-CD3 ϵ antibodies, the conjugated anti-CD3 ϵ antibodies, the activatable antibodies, the conjugated activatable antibodies, the multispecific antibodies, the multispecific activatable antibodies and/or the conjugated multispecific activatable antibody compositions and a pharmaceutically acceptable carrier. Where an antibody, activatable antibody, multispecific antibody and/or a multispecific activatable antibody includes a fragment of the AB domain, the smallest fragment of the AB that specifically binds to the binding domain of the target protein can be used. For example, based upon the variable-region sequences of an antibody, peptide molecules can be designed that retain the ability of the AB to bind the target protein sequence. Such peptides can be synthesized chemically and/or produced by recombinant DNA technology. (*See, e.g.,* Marasco et al., Proc. Natl. Acad. Sci. USA, 90: 7889-7893 (1993)).

[000540] As used herein, the term “pharmaceutically acceptable carrier” is intended to include any and all solvents, dispersion media, coatings, antibacterial and antifungal agents, isotonic and absorption delaying agents, and the like, compatible with pharmaceutical administration. Suitable carriers are described in the most recent edition of Remington’s Pharmaceutical Sciences, a standard reference text in the field, which is incorporated herein by reference. Suitable examples of such carriers or diluents include, but are not limited to, water, saline, ringer’s solutions, dextrose solution, and 5% human serum albumin. Liposomes and non-aqueous vehicles such as fixed oils may also be used. The use of such media and agents for pharmaceutically active substances is well known in the art. Except insofar as any conventional media or agent is incompatible with the active compound, use thereof in the compositions is contemplated.

[000541] The formulations to be used for *in vivo* administration must be sterile. This is readily accomplished by filtration through sterile filtration membranes.

[000542] A pharmaceutical composition of the disclosure is formulated to be compatible with its intended route of administration. Examples of routes of administration include parenteral, *e.g.*, intravenous, intradermal, subcutaneous, oral (*e.g.*, inhalation), transdermal (*i.e.*, topical), transmucosal, and rectal administration. Solutions or suspensions used for parenteral, intradermal, or subcutaneous application can include the following components: a sterile diluent such as water for injection, saline solution, fixed oils, polyethylene glycols, glycerine,

propylene glycol or other synthetic solvents; antibacterial agents such as benzyl alcohol or methyl parabens; antioxidants such as ascorbic acid or sodium bisulfite; chelating agents such as ethylenediaminetetraacetic acid (EDTA); buffers such as acetates, citrates or phosphates, and agents for the adjustment of tonicity such as sodium chloride or dextrose. The pH can be adjusted with acids or bases, such as hydrochloric acid or sodium hydroxide. The parenteral preparation can be enclosed in ampoules, disposable syringes or multiple dose vials made of glass or plastic.

[000543] Pharmaceutical compositions suitable for injectable use include sterile aqueous solutions (where water soluble) or dispersions and sterile powders for the extemporaneous preparation of sterile injectable solutions or dispersion. For intravenous administration, suitable carriers include physiological saline, bacteriostatic water, Cremophor ELTM (BASF, Parsippany, N.J.) or phosphate buffered saline (PBS). In all cases, the composition must be sterile and should be fluid to the extent that easy syringeability exists. It must be stable under the conditions of manufacture and storage and must be preserved against the contaminating action of microorganisms such as bacteria and fungi. The carrier can be a solvent or dispersion medium containing, for example, water, ethanol, polyol (for example, glycerol, propylene glycol, and liquid polyethylene glycol, and the like), and suitable mixtures thereof. The proper fluidity can be maintained, for example, by the use of a coating such as lecithin, by the maintenance of the required particle size in the case of dispersion and by the use of surfactants. Prevention of the action of microorganisms can be achieved by various antibacterial and antifungal agents, for example, parabens, chlorobutanol, phenol, ascorbic acid, thimerosal, and the like. In many cases, it will be suitable to include isotonic agents, for example, sugars, polyalcohols such as manitol, sorbitol, sodium chloride in the composition. Prolonged absorption of the injectable compositions can be brought about by including in the composition an agent that delays absorption, for example, aluminum monostearate and gelatin.

[000544] Sterile injectable solutions can be prepared by incorporating the active compound in the required amount in an appropriate solvent with one or a combination of ingredients enumerated above, as required, followed by filtered sterilization. Generally, dispersions are prepared by incorporating the active compound into a sterile vehicle that contains a basic dispersion medium and the required other ingredients from those enumerated

above. In the case of sterile powders for the preparation of sterile injectable solutions, methods of preparation are vacuum drying and freeze-drying that yields a powder of the active ingredient plus any additional desired ingredient from a previously sterile-filtered solution thereof.

[000545] Oral compositions generally include an inert diluent or an edible carrier. They can be enclosed in gelatin capsules or compressed into tablets. For the purpose of oral therapeutic administration, the active compound can be incorporated with excipients and used in the form of tablets, troches, or capsules. Oral compositions can also be prepared using a fluid carrier for use as a mouthwash, wherein the compound in the fluid carrier is applied orally and swished and expectorated or swallowed. Pharmaceutically compatible binding agents, and/or adjuvant materials can be included as part of the composition. The tablets, pills, capsules, troches and the like can contain any of the following ingredients, or compounds of a similar nature: a binder such as microcrystalline cellulose, gum tragacanth or gelatin; an excipient such as starch or lactose, a disintegrating agent such as alginic acid, Primogel, or corn starch; a lubricant such as magnesium stearate or Sterotes; a glidant such as colloidal silicon dioxide; a sweetening agent such as sucrose or saccharin; or a flavoring agent such as peppermint, methyl salicylate, or orange flavoring.

[000546] For administration by inhalation, the compounds are delivered in the form of an aerosol spray from pressured container or dispenser that contains a suitable propellant, *e.g.*, a gas such as carbon dioxide, or a nebulizer.

[000547] Systemic administration can also be by transmucosal or transdermal means. For transmucosal or transdermal administration, penetrants appropriate to the barrier to be permeated are used in the formulation. Such penetrants are generally known in the art, and include, for example, for transmucosal administration, detergents, bile salts, and fusidic acid derivatives. Transmucosal administration can be accomplished through the use of nasal sprays or suppositories. For transdermal administration, the active compounds are formulated into ointments, salves, gels, or creams as generally known in the art.

[000548] The compounds can also be prepared in the form of suppositories (*e.g.*, with conventional suppository bases such as cocoa butter and other glycerides) or retention enemas for rectal delivery.

[000549] In one embodiment, the active compounds are prepared with carriers that will protect the compound against rapid elimination from the body, such as sustained/controlled release formulations, including implants and microencapsulated delivery systems.

Biodegradable, biocompatible polymers can be used, such as ethylene vinyl acetate, polyanhydrides, polyglycolic acid, collagen, polyorthoesters, and polylactic acid. Methods for preparation of such formulations will be apparent to those skilled in the art.

[000550] For example, the active ingredients can be entrapped in microcapsules prepared, for example, by coacervation techniques or by interfacial polymerization, for example, hydroxymethylcellulose or gelatin-microcapsules and poly-(methylmethacrylate) microcapsules, respectively, in colloidal drug delivery systems (for example, liposomes, albumin microspheres, microemulsions, nano-particles, and nanocapsules) or in macroemulsions.

[000551] Sustained-release preparations can be prepared. Suitable examples of sustained-release preparations include semipermeable matrices of solid hydrophobic polymers containing the antibody, which matrices are in the form of shaped articles, *e.g.*, films, or microcapsules. Examples of sustained-release matrices include polyesters, hydrogels (for example, poly(2-hydroxyethyl-methacrylate), or poly(vinylalcohol)), polylactides (U.S. Pat. No. 3,773,919), copolymers of L-glutamic acid and γ ethyl-L-glutamate, non-degradable ethylene-vinyl acetate, degradable lactic acid-glycolic acid copolymers such as the LUPRON DEPOTTM (injectable microspheres composed of lactic acid-glycolic acid copolymer and leuprolide acetate), and poly-D-(-)-3-hydroxybutyric acid. While polymers such as ethylene-vinyl acetate and lactic acid-glycolic acid enable release of molecules for over 100 days, certain hydrogels release proteins for shorter time periods.

[000552] The materials can also be obtained commercially from Alza Corporation and Nova Pharmaceuticals, Inc. Liposomal suspensions (including liposomes targeted to infected cells with monoclonal antibodies to viral antigens) and can also be used as pharmaceutically acceptable carriers. These can be prepared according to methods known to those skilled in the art, for example, as described in U.S. Patent No. 4,522,811.

[000553] It is especially advantageous to formulate oral or parenteral compositions in dosage unit form for ease of administration and uniformity of dosage. Dosage unit form as used herein refers to physically discrete units suited as unitary dosages for the subject to be treated;

each unit containing a predetermined quantity of active compound calculated to produce the desired therapeutic effect in association with the required pharmaceutical carrier. The specification for the dosage unit forms of the disclosure are dictated by and directly dependent on the unique characteristics of the active compound and the particular therapeutic effect to be achieved, and the limitations inherent in the art of compounding such an active compound for the treatment of individuals.

[000554] The pharmaceutical compositions can be included in a container, pack, or dispenser together with instructions for administration.

[000555] The formulation can also contain more than one active compound as necessary for the particular indication being treated, for example, those with complementary activities that do not adversely affect each other. In some embodiments, or in addition, the composition can comprise an agent that enhances its function, such as, for example, a cytotoxic agent, cytokine, chemotherapeutic agent, or growth-inhibitory agent. Such molecules are suitably present in combination in amounts that are effective for the purpose intended.

[000556] In one embodiment, the active compounds are administered in combination therapy, *i.e.*, combined with other agents, *e.g.*, therapeutic agents, that are useful for treating pathological conditions or disorders, such as autoimmune disorders and inflammatory diseases. The term “in combination” in this context means that the agents are given substantially contemporaneously, either simultaneously or sequentially. If given sequentially, at the onset of administration of the second compound, the first of the two compounds is still detectable at effective concentrations at the site of treatment.

[000557] For example, the combination therapy can include one or more antibodies of the disclosure coformulated with, and/or coadministered with, one or more additional therapeutic agents, *e.g.*, one or more cytokine and growth factor inhibitors, immunosuppressants, anti-inflammatory agents, metabolic inhibitors, enzyme inhibitors, and/or cytotoxic or cytostatic agents, as described in more detail below. Furthermore, one or more antibodies described herein may be used in combination with two or more of the therapeutic agents described herein. Such combination therapies may advantageously utilize lower dosages of the administered therapeutic agents, thus avoiding possible toxicities or complications associated with the various monotherapies.

[000558] In other embodiments, one or more antibodies of the disclosure can be coformulated with, and/or coadministered with, one or more anti-inflammatory drugs, immunosuppressants, or metabolic or enzymatic inhibitors. Nonlimiting examples of the drugs or inhibitors that can be used in combination with the antibodies described herein, include, but are not limited to, one or more of: nonsteroidal anti-inflammatory drug(s) (NSAIDs), *e.g.*, ibuprofen, tenidap, naproxen, meloxicam, piroxicam, diclofenac, and indomethacin; sulfasalazine; corticosteroids such as prednisolone; cytokine suppressive anti-inflammatory drug(s) (CSAIDs); inhibitors of nucleotide biosynthesis, *e.g.*, inhibitors of purine biosynthesis, folate antagonists (*e.g.*, methotrexate (N-[4-[(2,4-diamino-6-pteridiny)methyl] methylamino] benzoyl]-L-glutamic acid); and inhibitors of pyrimidine biosynthesis, *e.g.*, dihydroorotate dehydrogenase (DHODH) inhibitors. Suitable therapeutic agents for use in combination with the antibodies of the disclosure include NSAIDs, CSAIDs, (DHODH) inhibitors (*e.g.*, leflunomide), and folate antagonists (*e.g.*, methotrexate).

[000559] Examples of additional inhibitors include one or more of: corticosteroids (oral, inhaled and local injection); immunosuppressants, *e.g.*, cyclosporin, tacrolimus (FK-506); and mTOR inhibitors, *e.g.*, sirolimus (rapamycin - RAPAMUNETM or rapamycin derivatives, *e.g.*, soluble rapamycin derivatives (*e.g.*, ester rapamycin derivatives, *e.g.*, CCI-779); agents that interfere with signaling by proinflammatory cytokines such as TNF α or IL-1 (*e.g.* IRAK, NIK, IKK, p38 or MAP kinase inhibitors); COX2 inhibitors, *e.g.*, celecoxib, rofecoxib, and variants thereof; phosphodiesterase inhibitors, *e.g.*, R973401 (phosphodiesterase Type IV inhibitor); phospholipase inhibitors, *e.g.*, inhibitors of cytosolic phospholipase 2 (cPLA2) (*e.g.*, trifluoromethyl ketone analogs); inhibitors of vascular endothelial cell growth factor or growth factor receptor, *e.g.*, VEGF inhibitor and/or VEGF-R inhibitor; and inhibitors of angiogenesis. Suitable therapeutic agents for use in combination with the antibodies of the disclosure are immunosuppressants, *e.g.*, cyclosporin, tacrolimus (FK-506); mTOR inhibitors, *e.g.*, sirolimus (rapamycin) or rapamycin derivatives, *e.g.*, soluble rapamycin derivatives (*e.g.*, ester rapamycin derivatives, *e.g.*, CCI-779); COX2 inhibitors, *e.g.*, celecoxib and variants thereof; and phospholipase inhibitors, *e.g.*, inhibitors of cytosolic phospholipase 2 (cPLA2), *e.g.*, trifluoromethyl ketone analogs.

[000560] Additional examples of therapeutic agents that can be combined with an antibody of the disclosure include one or more of: 6-mercaptopurines (6-MP); azathioprine

sulphasalazine; mesalazine; olsalazine; chloroquine/ hydroxychloroquine (PLAQUENIL[®]); pencillamine; aurothiornalate (intramuscular and oral); azathioprine; coichicine; beta-2 adrenoreceptor agonists (salbutamol, terbutaline, salmeteral); xanthines (theophylline, aminophylline); cromoglycate; nedocromil; ketotifen; ipratropium and oxitropium; mycophenolate mofetil; adenosine agonists; antithrombotic agents; complement inhibitors; and adrenergic agents.

[000561] All publications and patent documents cited herein are incorporated herein by reference as if each such publication or document was specifically and individually indicated to be incorporated herein by reference. Citation of publications and patent documents is not intended as an admission that any is pertinent prior art, nor does it constitute any admission as to the contents or date of the same. The invention having now been described by way of written description, those of skill in the art will recognize that the invention can be practiced in a variety of embodiments and that the foregoing description and examples below are for purposes of illustration and not limitation of the claims that follow.

EXAMPLES

EXAMPLE 1. Activatable anti-CD3 ϵ antibody masking moieties

[000562] This Example describes identification of masking moieties (MM) to reduce binding of activatable anti-CD3 ϵ antibodies to their target.

MACS1 and MACS2 selections

[000563] Anti-CD3 ϵ antibody SP34 (also referred to herein as SP34 antibody) was used to screen a peptide library inserted into a circularly permuted outer membrane protein OmpX in *E. coli* with 6×10^{10} members, using a method similar to the bacterial display technology described in PCT International Publication Numbers WO 2005/047461, published May 26, 2005, and WO 2009/014726, published Jan. 29, 2009. The selection involved two rounds of magnetic activated cell separation (MACS) followed by two different fluorescent activated cell sorting (FACS) based selection strategies. Figure 1 shows the generic naming scheme for MACS and FACS populations during a selection process: Magnetic activated cell separations (MACS) are denoted with an “M” and fluorescent activated cell sorting (FACS) selections are denoted with an “F”. The order of the abbreviation indicates the order of selections/sorts and the number

indicates the number of rounds of that type of selection/sort to which that the population has been subjected. For example, an M2F3 population has gone through 2 rounds of MACS selections followed by 3 rounds of FACS selections. The starting population that undergoes selection to yield a given population is assumed to be N-1 of the right most sort indicator in the nomenclature. For example, the population that was sorted to produce the M2F3 population is assumed to have been the M2F2 population.

[000564] The initial MACS (M1) was done using protein-G Dynabeads (Invitrogen Cat#: 10003D) and the SP34 antibody (available from BD Biosciences Cat# 556610) at a concentration of 100 nM. For the M1, 6×10^{11} cells were screened for binding and 6.6×10^7 cells were collected. The second round of MACS sorting (M2) was done in a manner similar to the first round with the following differences. The SP34 antibody was added to a final concentration of 50 nM, and a total of 4.5×10^{11} cells were screened for binding and 3.1×10^5 cells were collected.

FACS selections

Selection #1

[000565] For all FACS selections, 50 μ l of induced culture were spun down (5 min @ 3000xg) and labeled with the indicated concentration of antibody. For the M2, MACS2FACS1 (M2F1), and MACS2FACS2 (M2F2) selections cells were labeled with 1 nM of SP34-dylight-488 (ThermoScientific Cat#: 53025) in 500 μ l PBS, 0.5% BSA, and the top 0.5% of positive cells were sorted by fluorescent activated cell sorting (FACS) using a FACSARIAI instrument (BD Biosciences). For the M2 sort, 3×10^6 cells were sorted and 5000 cells were collected. For subsequent sorts, at least 1×10^6 cells were sorted. The MACS2FACS3 (M2F3) population was sorted as described herein, but with the addition of 100 nM unlabeled isotype control antibody (BD Biosciences Cat#: 556657). The final MACS2FACS4 (M2F4) round of FACS selection was sorted as before but was labeled using unlabeled SP34 antibody labeled with an anti-mouse IgG-488 secondary 1:100 (Jackson ImmunoResearch Cat#: 315-486-045), resulting in the MACS2FACS5 (M2F5) population. The final M2F5 population was verified to specifically bind SP34 by demonstrating inhibition of 1 nM SP34-dylight-488 binding in the presence of 100 nM soluble human CD3 ϵ protein (Sino Biologics Cat#: 10977-H08H). One hundred

individual clones were chosen for sequence analysis, and 91 gave interpretable data. The results are summarized in Table 7.

Table 7. Selection #1 M2F5 sequences

Peptide name	Peptide sequence (occurrence)
14995	YSLWGCEWGCDRGLY(73) (SEQ ID NO: 371)
15003	GYRWGCEWNCGGITT(8) (SEQ ID NO: 372)
15253	YSACEMFGEVECCFC(7) (SEQ ID NO: 373)
15263	WYSGGCEAFCGILSS(2) (SEQ ID NO: 374)
15286	GYSGGCEFRCYQLYS(1) (SEQ ID NO: 375)

Selection #2

[000566] For all FACS selections, 50 μ L of induced culture were spun down (5 min @ 3000xg) and labeled with the indicated concentration of antibody. The M2, M2F1, and M2F2 sorts, cells were labeled with 10 nM SP34-dylight-488 in 500 μ L PBS, 0.5% BSA, 100 nM unlabeled isotype control antibody as described herein, and the top 10%, 38%, and 60% of binders were sorted, respectively. In each case, approximately 3×10^6 cells were sorted. Twenty individual clones were chosen for sequence analysis, and 19 gave interpretable data. The results are summarized in Table 8.

Table 8. Selection #2 M2F2 sequences

Sequence name	Peptide Sequence
15855	KFCHCGYYCRVCTLK(1) (SEQ ID NO: 376)
15856	LGCNNLWGNEFCHPV(1) (SEQ ID NO: 377)
15858	GHPCWGNESYCHTHS(1) (SEQ ID NO: 378)
15859	GNNKWCNKPCKCWNK(1) (SEQ ID NO: 379)
15860	VYYCGGNESLCGERR(1) (SEQ ID NO: 380)
15861	WMTFGCEFSCGTDEW(1) (SEQ ID NO: 381)
15862	VYHWGCEYDCFFNDM(1) (SEQ ID NO: 382)
15863	FSGCCRGWYNCCHRG(1) (SEQ ID NO: 383)
15864	FMCQQRMWGNEFCHQ(1) (SEQ ID NO: 384)
15865	MMYCGGNEVLCGPRV(1) (SEQ ID NO: 385)

15866	TYSKCRYTVKCTKHN(1) (SEQ ID NO: 386)
15867	WYSGGCEAFCGILSS(2) (SEQ ID NO: 387)
15868	KYHCTRTSACCTKHH(1) (SEQ ID NO: 388)
15869	NCFDPYMLLTYS CNS(1) (SEQ ID NO: 389)
15870	GMCGNLWGDESRCWW(1) (SEQ ID NO: 390)
15857	SCWDPYMMMNYICNI(3) (SEQ ID NO: 391)

On Cell Peptide Characterization

[000567] Eight peptides, 5 from the selection #1 M2F5 and 3 from the selection #2 M2F2 populations, were selected for further characterization on cell. The individual clones were labeled with 100, 10, 1, and 0.1 nM SP34-dylight-488 in the presence of 100 nM unlabeled isotype control antibody as described herein. Each clone was separately labeled with yPet-Mona to quantify the cell surface expression level of the peptides, allowing the calculation of expression normalized binding.

Expression normalized binding = $\frac{\text{Mean Fab fluorescence}}{\text{Mean YpetMona fluorescence}}$

And it's assumed that:

Mean YpetMona fluorescence = Peptide expression level

[000568] To verify the ability of the binding peptides to inhibit SP34's interaction with human CD3ε, each clone was labeled with 1 nM SP34-dylight-488 in the presence of 100 nM human CD3ε. The results are summarized in Table 9. All of the peptides evaluated specifically bound to SP34-dylight-488 and were inhibited from binding by excess free human CD3ε.

Table 9. Expression normalized binding of individual SP34 binding clones

Label	14995	15003	15253	15263	15286	15860	15864	15865
100 nM SP34	0.51	0.69	2.53	2.28	1.72	4.55	1.88	2.24
10 nM SP34	0.11	0.39	0.98	0.69	0.66	1.72	0.63	1.08

1 nM SP34	0.13	0.20	0.68	0.35	0.42	0.52	0.36	0.79
0.1 nM SP34	0.08	0.11	0.55	0.24	0.28	0.48	0.15	0.21
1 nM SP34 + 100 nM hCD3ε	0.08	0.12	0.53	0.34	0.34	0.51	0.30	0.26

EXAMPLE 2. Activatable anti-CD3ε antibodies

[000569] This Example describes the production of activatable anti-CD3ε antibodies of the disclosure.

[000570] The expression vector for the SP34scFv(LvHv)-Fc fusion construct was assembled using standard molecular biology techniques. Briefly, a DNA fragment encoding the SP34 scFv (LvHv) region was amplified with primers CX2005 and CX2008 using a synthesized SP34scFv(LvHv) sequence as template. The DNA encoding the Fc domain was amplified from the pFIL-CHlg-hG1 vector (Invitrogen) using primers CX2007 and CX2006. The overlapping fragments were combined and amplified with primers CX2005 and CX2006 and subsequently cloned into the pOP Hyg expression vector (a modified pCDNA3.1 expression vector (Invitrogen)) using the EcoRI and NotI restriction sites to form antibody SP34scFv(LvHv)-Fc. A DNA fragment encoding antibody SP34scFv(HvLv)-Fc was produced in a similar manner with the following differences. The DNA fragment encoding the PSP34scFv(HvLv) was amplified using the primers CX2001 and CX200,4 and the DNA encoding the Fc domain was amplified using the primers CX2002 and CX2003. The overlapping fragments were combined and amplified using primers CX2001 and C2002. All primer sequences are shown in Table 10. Antibodies SP34scFv(LvHv)-Fc and SP34scFv(HvLv)-Fc were found to bind to Jurkat T cells with affinities similar to the ability of SP34 IgG to bind to Jurkat T cells. Figures 4A-4C depicts scFv(LvHv)-Fc and scFv(HvLv)-Fc antibodies and activatable antibodies of the disclosure.

[000571] Antibodies SP34scFv(LvHv)-Fc and SP34scFv(HvLv)-Fc were also tested for their ability to bind to cynomolgus macaque (also referred to herein as cyno) CD3 ϵ . To determine if antibodies SP34scFv(LvHv)-Fc and SP34scFv(HvLv)-Fc could bind to cynomolgus CD3 ϵ , an ELISA-based binding assay was performed. Recombinant cynomolgus (*Macaca fascicularis*) CD3 ϵ with a polyhistidine affinity tag at the C-terminus (Sino Biological, Catalog 90047-C08H) was coated in 50 μ l PBS (PBS, Gibco Life Technologies, Catalog 20012-043) onto 96-well, flat bottom plates (Maxisorb Nunc, Thermo Scientific, Catalog 12-565-226) overnight at a concentration of 0.1 μ g/ml at 4°C. The plates were washed 3 times with 250 μ l/well PBS-T (Tween 20 at 0.05%), blocked with 200 μ l PBS-T 2% BSA (BSA Fraction V, Fisher Scientific, product BP1600-1) for 1 hour at ambient temperature and washed 3 times with 250 μ l/well PBS-T. Using 50 μ l/well, either antibody SP34scFv(LvHv)-Fc or SP34scFv(HvLv)-Fc (purified to >95% monomer) was incubated in a concentration range from 37 nM to 0.017 nM diluted into PBS-T 2% BSA for 1 hour at ambient temperature. The plates were washed three times with 250 μ l/well PBS-T, incubated with the secondary anti-human Fc-HRP 1/5000 dilution into PBS-T 2% BSA (Peroxidase AffiniPure Mouse Anti-Human IgG, Fc γ Fragment Specific, Jackson ImmunoResearch, Catalog 209-035-098) for 30 minutes at ambient temperature and washed 3 times with 250 μ l/well PBS-T. HRP substrate (TMB-ELISA, Thermo Scientific, Product 34029) was added to the plate at 50 μ l/well for 2 minutes and then quenched with 50 μ l/well 1 M HCl before reading absorbance at 450 nM on the Tecan infinite 200Pro and analyzed with Prism 6 (GraphPad) by curve fitting the data to log(agonist) vs. response (three parameters). On a separate cyno CD3 ϵ coated ELISA plate, SP34 mouse antibody (SP34-2, BD Biosciences, Catalog 551916) was coated and analyzed in the same manner and using the secondary anti-mouse IgG-HRP conjugate (Jackson ImmunoResearch, Catalog 715-035-150). Figures 2A and 2B demonstrate that scFv antibodies SP34scFv(LvHv)-Fc and SP34scFv(HvLv)-Fc as well as antibody SP34-2 bound to cynomolgus CD3 ϵ HIS within a similar range of EC₅₀ between 0.1 and 0.3 nM.

[000572] The vectors encoding activatable antibody SP34scFv(LvHv)-Fc were constructed as follows. The overlapping forward primers CX2013 and CX2014 and reverse primer CX2014 were used to amplify the activatable scFv antibody using the SP34scFv(LvHv)-Fc construct as template. The DNA fragment was then cloned into the SP34scFv(LvHv)-Fc

vector using the EcoR1 and BstXI restriction sites. Using the SP34scFv(LvHv)-Fc fusion vector as template, the masking peptide sequences were added using the forward primers CX2041-CX2045 for peptides 15003, 15263, 15860, 15864, and 15865, respectively, and the reverse primer CX2002 and subsequently cloned using the SfiI and NotI restriction sites to produce nucleic acid molecules encoding activatable anti-CD3 ϵ antibodies.

Table 10. Primer sequences

PRIMER NAME	PRIMER SEQUENCE
CX2001	ACTTGTCACGAATTCGGAAGTGCAGCTGGTGGGAATCTGGGGGGCGGAC TGGTGCAGCCT (SEQ ID NO: 392)
CX2002	TAGACTCGAGCGGCCGCTCATTTACCCGGAGACAGGGAGAGGCTCTT C (SEQ ID NO: 393)
CX2003	TCGGCGGAGGCACCAAGCTGACCGTGCTGGGCGGCTCCCTGGACCCT AAGTCATCTGACAAAACCTCACACATGCCCA (SEQ ID NO: 394)
CX2004	TGGGCATGTGTGAGTTTTGTCAGATGACTTAGGGTCCAGGGAGCCGCC CAGCACGGTCAGCTTGGTGCCTCCGCCGA (SEQ ID NO: 395)
CX2005	TGCACTTGTCACGAATTCGCAGGCTGTCGTGACACAGGAAAGCGCCCT GA (SEQ ID NO: 396)
CX2006	TCTAGACTCGAGCGGCCGCTCATTTACCCGGAGACAGGGAGAGGCTC TTCT (SEQ ID NO: 397)
CX2007	TGGGGCCAGGGCACCCCTCGTGACAGTGTCTGCTGGCGGCTCCCTGGAC CCTAAGTCATCTGACAAAACCTCACACATGCCCA (SEQ ID NO: 398)
CX2008	TGGGCATGTGTGAGTTTTGTCAGATGACTTAGGGTCCAGGGAGCCGCC AGCAGACACTGTCACGAGGGTGCCCTGGCCCCA (SEQ ID NO: 399)
CX2012	AGCGGTGGCTCTGGTGGTCTGAGCGGCCGTTCCGATAATCATGGCGGC GGTTCTCAGGCTGTCGTGACACAGGAAAGCGCCCTGACCACCA (SEQ ID NO: 400)
CX2013	CTAAGTCTTGCACTTGTCACGAATTCGCAAGGCCAGTCTGGCCAAGGG TACCAAGGCTCGAGCGGTGGCAGCGGTGGCT (SEQ ID NO: 401)

CX2014	TCCGGGGCCACCCATTCCAGTCCCTTGCCAGGGGGCCTGGCGCACCCAAT TCATGGCGTAGGTGTT (SEQ ID NO: 402)
CX2041	CAAGGCCAGTCTGGCCAAGGTTATCGGTGGGGTTGCGAGTGGAATTG CGGTGGGATTACTACTGGCTCGAGCGGTGGCAGCGGTGGC (SEQ ID NO: 403)
CX2042	CAAGGCCAGTCTGGCCAATGGTATTCGGGTGGGTGCGAGGCTTTTTGC GGTATTTTGTCGTCGGGCTCGAGCGGTGGCAGCGGTGGC (SEQ ID NO: 404)
CX2043	CAAGGCCAGTCTGGCCAAGTTTATTATTGCGGTGGGAATGAGAGTCTG TGCGGTGAGAGGAGGGGCTCGAGCGGTGGCAGCGGTGGC (SEQ ID NO: 405)
CX2044	CAAGGCCAGTCTGGCCAATTTATGTGCCAGCAGCGGATGTGGGGGAA TGAGTTTTGCCATCAGGGCTCGAGCGGTGGCAGCGGTGGC (SEQ ID NO: 406)
CX2045	CAAGGCCAGTCTGGCCAAATGATGTATTGCGGTGGGAATGAGGTGTT GTGCGGGCCGCGGGTTGGCTCGAGCGGTGGCAGCGGTGGC (SEQ ID NO: 370)

SP34 Lv

Nucleotide sequence

CAGGCTGTCGTGACACAGGAAAGCGCCCTGACCACCAGCCCTGGCGAGACAGTGACCCTGACCTGCAGATCTAGCA
CAGGCGCCGTGACCACAAGCAACTACGCCAACTGGGTGCAGGAAAAGCCCGACCACCTGTTACCGGCCTGATCGG
CGGCACCAACAAAAGGGCTCCAGGCGTGCCAGCCAGATTCAGCGGCAGCCTGATTGGCGATAAGGCCGCCCTGACA
ATCACTGGCGCCCAGACCGAGGACGAGGCCATCTACTTTTGCGCCCTGTGGTACAGCAACCTGTGGGTGTTCTGGCG
GAGGCACCAAGCTGACAGTGCTG (SEQ ID NO: 1)

Amino Acid sequence

QAVVTQESALTTSPGETVTLTCRSSTGAVTTSNYANWVQEKPDHLFTGLIGGTNKRAPGVPARFSGSLIGDKAALT
ITGAQTEDEAIYFCALWYSNLWVFGGGTKLTVL (SEQ ID NO: 2)

SP34 Hv

Nucleotide sequence

GAAGTGCAGCTGGTGGGAATCTGGCGGCGGACTGGTGCAGCCTAAGGGCTCTCTGAAGCTGAGCTGTGCCGCCAGCG
 GCTTCACCTTCAACACCTACGCCATGAATTGGGTGCGCCAGGCCCTGGCAAGGGACTGGAATGGGTGGCCCGGAT
 CAGAAGCAAGTACAACAATTACGCCACCTACTACGCCGACAGCGTGAAGGACCGGTTACCATCAGCCGGGACGAC
 AGCCAGAGCATCCTGTATCTGCAGATGAACAACCTGAAAACCGAGGACACCGCCATGTACTACTGCGTGCGGCACG
 GCAACTTCGGCAACAGCTATGTGTCTTGGTTTGCCTACTGGGGCCAGGGCACCCCTCGTGACAGTGTCTGCT (SEQ
 ID NO: 3)

Amino Acid sequence

EVQLVESGGGLVQPKGSLKLSCAASGFTFNTYAMNWVRQAPGKGLEWVARIRSKYNNYATYYADSVKDRFTISRDD
 SQSILYLQMNNLKTEDTAMYYCVRHGNFGNSYVSWFAYWGQGLVTVSA (SEQ ID NO: 4)

SP34scFv(LvHv)

Nucleotide sequence

CAGGCTGTCGTGACACAGGAAAGCGCCCTGACCACCAGCCCTGGCGAGACAGTGACCCTGACCTGCAGATCTAGCA
 CAGGCGCCGTGACCACAAGCAACTACGCCAACTGGGTGCAGGAAAAGCCCGACCACCTGTTACCGGCCTGATCGG
 CGGCACCAACAAAAGGGCTCCAGGCGTGCCAGCCAGATTCAGCGGCAGCCTGATTGGCGATAAGGCCGCCCTGACA
 ATCACTGGCGCCCAGACCGAGGACGAGGCCATCTACTTTTTCGCGCCCTGTGGTACAGCAACCTGTGGGTGTTTCGGCG
 GAGGCACCAAGCTGACAGTGCTGGGAGGCGGAGGATCTGGCGGAGGCGGAAGTGGCGGAGGGGGATCTGAAGTGCA
 GCTGGTGGAATCTGGCGGCGGACTGGTGCAGCCTAAGGGCTCTCTGAAGCTGAGCTGTGCCGCCAGCGGCTTCACC
 TTCAACACCTACGCCATGAATTGGGTGCGCCAGGCCCTGGCAAGGGACTGGAATGGGTGGCCCGGATCAGAAGCA
 AGTACAACAATTACGCCACCTACTACGCCGACAGCGTGAAGGACCGGTTACCATCAGCCGGGACGACAGCCAGAG
 CATCCTGTATCTGCAGATGAACAACCTGAAAACCGAGGACACCGCCATGTACTACTGCGTGCGGCACGGCAACTTC
 GGCAACAGCTATGTGTCTTGGTTTGCCTACTGGGGCCAGGGCACCCCTCGTGACAGTGTCTGCT (SEQ ID
 NO: 5)

Amino Acid sequence

QAVVTQESALTTSPGETVTLTCRSSTGAVTTSNYANWVQEKPDHLFTGLIGGTNKRAPGVPARFSGSLIGDKAALT
 ITGAQTEDEAIYFCALWYSNLWVFGGGTKLTVLGGGSGGGGSGGGGSEVQLVESGGGLVQPKGSLKLSCAASGFT
 FNTYAMNWVRQAPGKGLEWVARIRSKYNNYATYYADSVKDRFTISRDDSQSILYLQMNNLKTEDTAMYYCVRHGNF
 GNSYVSWFAYWGQGLVTVSA (SEQ ID NO: 6)

Antibody SP34scFv(LvHv)-Fc fusion

Nucleotide sequence

CAGGCTGTCGTGACACAGGAAAGCGCCCTGACCACCAGCCCTGGCGAGACAGTGACCCTGACCTGCAGATCTAGCA
 CAGGCGCCGTGACCACAAGCAACTACGCCAACTGGGTGCAGGAAAAGCCCGACCACCTGTTACCGGCCTGATCGG
 CGGCACCAACAAAAGGGCTCCAGGCGTGCCAGCCAGATTTCAGCGGCAGCCTGATTGGCGATAAGGCCGCCCTGACA
 ATCACTGGCGCCCAGACCGAGGACGAGGCCATCTACTTTTTCGCGCCCTGTGGTACAGCAACCTGTGGGTGTTTCGGCG
 GAGGCACCAAGCTGACAGTGCTGGGAGGCGGAGGATCTGGCGGAGGCGGAAGTGGCGGAGGGGGATCTGAAGTGCA
 GCTGGTGAATCTGGCGGCGGACTGGTGCAGCCTAAGGGCTCTCTGAAGCTGAGCTGTGCCGCCAGCGGCTTCACC
 TTCAACACCTACGCCATGAATTGGGTGCGCCAGGCCCTGGCAAGGGACTGGAATGGGTGGCCCGGATCAGAAGCA
 AGTACAACAATTACGCCACCTACTACGCCGACAGCGTGAAGGACCGGTTTACCATCAGCCGGGACGACAGCCAGAG
 CATCCTGTATCTGCAGATGAACAACCTGAAAACCGAGGACACCGCCATGTACTACTGCGTGCGGCACGGCAACTTC
 GGCAACAGCTATGTGTCTTGGTTTGCCTACTGGGGCCAGGGCACCTCGTGACAGTGTCTGCTGGCGGCTCCCTGG
 ACCCTAAGTCATCTGACAAAACCTCACACATGCCCACCGTGCCCAGCACCTGAACTCCTGGGGGGACCGTCAGTCTT
 CCTCTTCCCCCCTAAACCAAGGACACCTCATGATCTCCCGGACCCCTGAGGTCACATGCGTGGTGGTGGACGTG
 AGCCACGAAGACCCTGAGGTCAAGTTCAACTGGTACGTGGACGGCGTGGAGGTGCATAATGCCAAGACAAAGCCGC
 GGGAGGAGCAGTACAACAGCACGTACCGTGTGGTCAGCGTCCTCACCGTCCTGCACCAGGACTGGCTGAATGGCAA
 GGAGTACAAGTGCAAGGTCTCCAACAAAGCCCTCCCAGCCCCCATCGAGAAAACCATCTCCAAAGCCAAAGGGCAG
 CCCCAGAAACACAGGTGTACACCCTGCCCCCATCCCGGGAGGAGATGACCAAGAACCAGGTCAGCCTGACCTGCC
 TGGTCAAAGGCTTCTATCCCAGCGACATCGCCGTGGAGTGGGAGAGCAATGGGCAGCCGGAGAACAACCTACAAGAC
 CACGCCTCCCGTGCTGGACTCCGACGGCTCCTTCTTCTCTACAGCAAGCTCACCGTGGACAAGAGCAGGTGGCAG
 CAGGGGAACGTCTTCTCATGCTCCGTGATGCATGAGGCTCTGCACAACCACTACACGCAGAAGAGCCTCTCCCTGT
 CTCCGGGTAAA (SEQ ID NO: 7)

Amino Acid sequence

QAVVTQESALTTSPGETVTLTCSRSTGAVTTSNYANWVQEKPDHLFTGLIGGTNKRAPGVPARFSGSLIGDKAALT
 ITGAQTEDEAIYFCALWYSNLWVFGGGTKLTVLGGGGSGGGGSGGGGSEVQLVESGGGLVQPKGSLKLSCAASGFT
 FNTYAMNWVRQAPGKGLEWVARIRSKYNNYATYYADSVKDRFTISRDDQSILYLQMNNLKTEDTAMYYCVRHGNF
 GNSYVSWFAYWGQGLVTVSAGGSLDPKSSDKHTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDV
 SHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQ
 PREPQVYITLPPSREEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTTPVLDSDGSFFLYSKLTVDKSRWQ
 QGNVFSCSVMHEALHNHYTQKSLSLSPGK (SEQ ID NO: 8)

15003-1204-SP34scFv(LvHv)

Nucleotide sequence

[spacer (SEQ ID NO: 507)][Antibody 15003-1204-SP34scFv(LvHv)without spacer
 (SEQ ID NO: 547)]

[CAAGGCCAGTCTGGCCAA] [GGTTATCGGTGGGGTTGCGAGTGGAATTGCGGTGGGATTACTACTGGCTCGAGCG GTGGCAGCGGTGGCTCTGGTGGTCTGAGCGGCCGTTCCGATAATCATGGCGGCGGTTCTCAGGCTGTCTGTGACACA GGAAAGCGCCCTGACCACCAGCCCTGGCGAGACAGTGACCCTGACCTGCAGATCTAGCACAGGCGCCGTGACCACA AGCAACTACGCCAACTGGGTGCAGGAAAAGCCCGACCACCTGTTTACCGGCCTGATCGGCGGCACCAACAAAAGGG CTCCAGGCGTGCCAGCCAGATTTCAGCGGCAGCCTGATTGGCGATAAGGCCGCCCTGACAATCACTGGCGCCCAGAC CGAGGACGAGGCCATCTACTTTTTCGCGCCCTGTGGTACAGCAACCTGTGGGTGTTTCGGCGGAGGCACCAAGCTGACA GTGCTGGGAGGCGGAGGATCTGGCGGAGGCGGAAGTGGCGGAGGGGGATCTGAAGTGCAGCTGGTGGAATCTGGCG GCGGACTGGTGCAGCCTAAGGGCTCTCTGAAGCTGAGCTGTGCCGCCAGCGGCTTCACCTTCAACACCTACGCCAT GAATTGGGTGCGCCAGGCCCTGGCAAGGGACTGGAATGGGTGGCCCGGATCAGAAGCAAGTACAACAATTACGCC ACCTACTACGCCGACAGCGTGAAGGACCGGTTTACCATCAGCCGGGACGACAGCCAGAGCATCCTGTATCTGCAGA TGAACAACCTGAAAACCGAGGACACCGCCATGTACTACTGCGTGCGGCACGGCAACTTCGGCAACAGCTATGTGTC TTGGTTTGCCTACTGGGGCCAGGGCACCCCTCGTGACAGTGTCTGCT] (SEQ ID NO: 9)

Amino Acid sequence

[spacer (SEQ ID NO: 87)] [Antibody 15003-1204-SP34scFv(LvHv) without spacer (SEQ ID NO: 548)]
 [QGQSGQ] [GYRWGCEWNCGGITTGSSGSGSGGLSGRSDNHGGGSQAVVTQESALTTSPGETVTLTCRSSTGAV TTSNYANWVQEKPDHLFTGLIGGTNKRAPGVPARFSGSLIGDKAALTITGAQTEDEAIYFCALWYSNLWVFGGGTK LTVLGGGGSGGGSGGGGSEVQLVESGGGLVQPKGSLKLSCAASGFTFNTYAMNWVRQAPGKGLEWVARIRSKYNN YATYYADSVKDRFTISRDDSQSILYLQMNLLKTEDTAMYCYVRHGNFNGNSYVSWFAYWGQGLVTVSA] (SEQ ID NO: 10)

Activatable antibody 15003-1204-SP34scFv(LvHv)-Fc fusion

Nucleotide sequence

[spacer (SEQ ID NO: 507)] [Activatable Antibody 15003-1204-SP34scFv(LvHv)-Fc fusion without spacer (SEQ ID NO: 549)]
 [CAAGGCCAGTCTGGCCAA] [GGTTATCGGTGGGGTTGCGAGTGGAATTGCGGTGGGATTACTACTGGCTCGAGCG GTGGCAGCGGTGGCTCTGGTGGTCTGAGCGGCCGTTCCGATAATCATGGCGGCGGTTCTCAGGCTGTCTGTGACACA GGAAAGCGCCCTGACCACCAGCCCTGGCGAGACAGTGACCCTGACCTGCAGATCTAGCACAGGCGCCGTGACCACA AGCAACTACGCCAACTGGGTGCAGGAAAAGCCCGACCACCTGTTTACCGGCCTGATCGGCGGCACCAACAAAAGGG CTCCAGGCGTGCCAGCCAGATTTCAGCGGCAGCCTGATTGGCGATAAGGCCGCCCTGACAATCACTGGCGCCCAGAC CGAGGACGAGGCCATCTACTTTTTCGCGCCCTGTGGTACAGCAACCTGTGGGTGTTTCGGCGGAGGCACCAAGCTGACA GTGCTGGGAGGCGGAGGATCTGGCGGAGGCGGAAGTGGCGGAGGGGGATCTGAAGTGCAGCTGGTGGAATCTGGCG GCGGACTGGTGCAGCCTAAGGGCTCTCTGAAGCTGAGCTGTGCCGCCAGCGGCTTCACCTTCAACACCTACGCCAT GAATTGGGTGCGCCAGGCCCTGGCAAGGGACTGGAATGGGTGGCCCGGATCAGAAGCAAGTACAACAATTACGCC ACCTACTACGCCGACAGCGTGAAGGACCGGTTTACCATCAGCCGGGACGACAGCCAGAGCATCCTGTATCTGCAGA

TGAACAACCTGAAAACCGAGGACACCGCCATGTACTACTGCGTGCGGCACGGCAACTTCGGCAACAGCTATGTGTC
 TTGGTTTGCCTACTGGGGCCAGGGCACCCCTCGTGACAGTGTCTGCTGGCGGCTCCCTGGACCCTAAGTCATCTGAC
 AAAACTCACACATGCCCACCGTGCCCAGCACCTGAACTCCTGGGGGGACCGTCAGTCTTCCTCTTCCCCC AAAAC
 CCAAGGACACCCTCATGATCTCCCGGACCCCTGAGGTCACATGCGTGGTGGTGGACGTGAGCCACGAAGACCCTGA
 GGTC AAGTTCAACTGGTACGTGGACGGCGTGGAGGTGCATAATGCCAAGACAAAGCCGCGGGAGGAGCAGTACAAC
 AGCACGTACCGTGTGGTCAGCGTCCTCACCGTCCTGCACCAGGACTGGCTGAATGGCAAGGAGTACAAGTGCAAGG
 TCTCCAACAAAGCCCTCCCAGCCCCCATCGAGAAAACCATCTCCAAAGCCAAAGGGCAGCCCCGAGAACCACAGGT
 GTACACCCTGCCCCCATCCCGGGAGGAGATGACCAAGAACCAGGTCAGCCTGACCTGCCTGGTCAAAGGCTTCTAT
 CCCAGCGACATCGCCGTGGAGTGGGAGAGCAATGGGCAGCCGGAGAACA ACTACAAGACCACGCCTCCCGTGCTGG
 ACTCCGACGGCTCCTTCTTCCTCTACAGCAAGCTCACCGTGGACAAGAGCAGGTGGCAGCAGGGGAACGTCTTCTC
 ATGCTCCGTGATGCATGAGGCTCTGCACAACCACTACACGCAGAAGAGCCTCTCCCTGTCTCCGGGTAAA] (SEQ
 ID NO: 11)

Amino Acid Sequence

[spacer (SEQ ID NO: 407)][Activatable Antibody 15003-1204-SP34scFv(LvHv)-Fc
 fusion without spacer (SEQ ID NO: 550)]
 [QGQSGQ][GYRWGCEWNCGGITTGSSGSGSGGLSGRSDNHGGGSQAVVTQESALTTSPGETVTLTCRSSTGAV
 TTSNYANWVQEKPDHLFTGLIGGTNKRAPGVPARFSGSLIGDKAALTITGAQTEDEAIYFCALWYSNLWVFGGGTK
 LTVLGGGGSGGGSGGGGSEVQLVESGGGLVQPKGSLKLSCAASGFTFNTYAMNWVRQAPKGLEWVARIRSKYNN
 YATYYADSVKDRFTISRDDSQSILYLQMNNLKTEDTAMYCYVRHGNFGNSYVSWFAYWGQGLVTVSAGGSLDPKS
 SDKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQ
 YNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSREEMTKNQVSLTCLVKG
 FYPSDIAVEWESNGQPENNYKTTTPVLDSDGSFFLYSKLTVDKSRWQQGNV FSCSVMHEALHNHYTQKSLSLSPGK
] (SEQ ID NO: 12)

15263-1204-SP34scFv(LvHv)

Nucleotide sequence

[spacer (SEQ ID NO: 507)][15263-1204-SP34scFv(LvHv) without spacer (SEQ ID
 NO: 551)]
 [CAAGGCCAGTCTGGCCAA][TGGTATTTCGGGTGGGTGCGAGGCTTTTTGCGGTATTTTGTCTCGTGGGCTCGAGCG
 GTGGCAGCGGTGGCTCTGGTGGTCTGAGCGGCCGTTCCGATAATCATGGCGGCGGTTCTCAGGCTGTCTGTGACACA
 GGAAAGCGCCCTGACCACCAGCCCTGGCGAGACAGTGACCCTGACCTGCAGATCTAGCACAGGCGCCGTGACCACA
 AGCAACTACGCCAACTGGGTGCAGGAAAAGCCCGACCACCTGTTTACCGGCCTGATCGGCGGCACCAACAAAAGGG
 CTCCAGGCGTGCCAGCCAGATTGAGCGGCAGCCTGATTGGCGATAAGGCCGCCCTGACAATCACTGGCGCCCAGAC
 CGAGGACGAGGCCATCTACTTTTGCGCCCTGTGGTACAGCAACCTGTGGGTGTTTCGGCGGAGGCACCAAGCTGACA
 GTGCTGGGAGGCGGAGGATCTGGCGGAGGCGGAAGTGGCGGAGGGGGATCTGAAGTGCAGCTGGTGGAATCTGGCG

GCGGACTGGTGCAGCCTAAGGGCTCTCTGAAGCTGAGCTGTGCCGCCAGCGGCTTCACCTTCAACACCTACGCCAT
GAATTGGGTGCGCCAGGCCCCTGGCAAGGGACTGGAATGGGTGGCCCGGATCAGAAGCAAGTACAACAATTACGCC
ACCTACTACGCCGACAGCGTGAAGGACCGGTTACCATCAGCCGGGACGACAGCCAGAGCATCCTGTATCTGCAGA
TGAACAACCTGAAAACCGAGGACACCGCCATGTACTACTGCGTGCGGCACGGCAACTTCGGCAACAGCTATGTGTC
TTGGTTTGCCTACTGGGGCCAGGGCACCCCTCGTGACAGTGTCTGCT] (SEQ ID NO: 13)

Amino Acid sequence

[spacer (SEQ ID NO: 87)][15263-1204-SP34scFv(LvHv)without spacer (SEQ ID
NO: 552)]
[QGQSGQ][WYSGGCEAFCGILSSGSSGGSGGSLSGRSDNHGGGSQAVVTQESALTTSPGETVTLTCRSSTGAV
TTSNYANWVQEKPDHLFTGLIGGTNKRAPGVPARFSGSLIGDKAALTITGAQTEDEAIYFCALWYSNLWVFGGGTK
LTVLGGGGSGGGSGGGGSEVQLVESGGGLVQPKGSLKLSCAASGFTFNTYAMNWVRQAPGKGLEWVARIRSKYNN
YATYYADSVKDRFTISRDDSQSILYLQMNNLKTEDTAMYYCVRHGNFGNSYVSWFAYWGQGLTVTVSA] (SEQ
ID NO: 14)

Activatable antibody 15263-1204-SP34scFv(LvHv)-Fc fusion

Nucleotide sequence

[spacer (SEQ ID NO: 507)][Activatable antibody 15263-1204-SP34scFv(LvHv)-Fc
fusion without spacer (SEQ ID NO: 553)]
[CAAGGCCAGTCTGGCCAA][TGGTATTCGGGTGGGTGCGAGGCTTTTTGCGGTATTTTGTCTCGGGCTCGAGCG
GTGGCAGCGGTGGCTCTGGTGGTCTGAGCGGCCGTTCCGATAATCATGGCGGCGGTTCTCAGGCTGTCTGTGACACA
GGAAAGCGCCCTGACCACCAGCCCTGGCGAGACAGTGACCCTGACCTGCAGATCTAGCACAGGCGCCGTGACCACA
AGCAACTACGCCAACTGGGTGCAGGAAAAGCCCGACCACCTGTTACCGGCCTGATCGGCGGCACCAACAAAAGGG
CTCCAGGCGTGCCAGCCAGATTGAGCGGCAGCCTGATTGGCGATAAGGCCGCCCTGACAATCACTGGCGCCCAGAC
CGAGGACGAGGCCATCTACTTTTGCGCCCTGTGGTACAGCAACCTGTGGGTGTTTCGGCGGAGGCACCAAGCTGACA
GTGCTGGGAGGCGGAGGATCTGGCGGAGGCGGAAGTGGCGGAGGGGGATCTGAAGTGCAGCTGGTGGAACTCTGGCG
GCGGACTGGTGCAGCCTAAGGGCTCTCTGAAGCTGAGCTGTGCCGCCAGCGGCTTCACCTTCAACACCTACGCCAT
GAATTGGGTGCGCCAGGCCCCTGGCAAGGGACTGGAATGGGTGGCCCGGATCAGAAGCAAGTACAACAATTACGCC
ACCTACTACGCCGACAGCGTGAAGGACCGGTTACCATCAGCCGGGACGACAGCCAGAGCATCCTGTATCTGCAGA
TGAACAACCTGAAAACCGAGGACACCGCCATGTACTACTGCGTGCGGCACGGCAACTTCGGCAACAGCTATGTGTC
TTGGTTTGCCTACTGGGGCCAGGGCACCCCTCGTGACAGTGTCTGCTGGCGGCTCCCTGGACCCTAAGTCATCTGAC
AAAACCTCACACATGCCACCGTGCCAGCACCTGAACTCCTGGGGGGACCGTCAGTCTTCCTCTTCCCCCAAAC
CCAAGGACACCCTCATGATCTCCCGGACCCCTGAGGTCACATGCGTGGTGGTGGACGTGAGCCACGAAGACCCTGA
GGTCAAGTTCAACTGGTACGTGGACGGCGTGGAGGTGCATAATGCCAAGACAAAGCCGCGGGAGGAGCAGTACAAC
AGCACGTACCGTGTGGTCAGCGTCCTACCGTCCTGCACCAGGACTGGCTGAATGGCAAGGAGTACAAGTGCAAGG
TCTCCAACAAAGCCCTCCCAGCCCCCATCGAGAAAACCATCTCCAAGCCAAAGGGCAGCCCCGAGAACCACAGGT

GTACACCCTGCCCCCATCCCGGGAGGAGATGACCAAGAACCAGGTCAGCCTGACCTGCCTGGTCAAAGGCTTCTAT
 CCCAGCGACATCGCCGTGGAGTGGGAGAGCAATGGGCAGCCGGAGAACAACACTACAAGACCACGCCTCCCGTGCTGG
 ACTCCGACGGCTCCTTCTTCTCTACAGCAAGCTCACCGTGGACAAGAGCAGGTGGCAGCAGGGGAACGTCTTCTC
 ATGCTCCGTGATGCATGAGGCTCTGCACAACCACTACACGCAGAAGAGCCTCTCCCTGTCTCCGGGTAAA] (SEQ
 ID NO: 15)

Amino acid sequence

[spacer (SEQ ID NO: 87)][Activatable antibody 15263-1204-SP34scFv(LvHv)-Fc
 fusion without spacer (SEQ ID NO: 554)]
 [QGQSGQ][WYSGGCEAFCGILSSGSSGGSGGSLGRSDNHGGGSQAVVTQESALTTSPGETVTLTCRSSTGAV
 TTSNYANWVQEKPDHLFTGLIGGTNKRAPGVPARFSGSLIGDKAALTITGAQTEDEAIYFCALWYSNLWVFGGGTK
 LTVLGGGGSGGGSGGGGSEVQLVESGGGLVQPKGSLKLSCAASGFTFNTYAMNWVRQAPGKGLEWVARIRSKYNN
 YATYYADSVKDRFTISRDDSQSILYLQMNNLKTEDTAMYYCVRHGNFGNSYVSWFAYWGQGLTVTVSAGGSLDPKS
 SDKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQ
 YNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSREEMTKNQVSLTCLVKG
 FYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSQSVMEALHNHYTQKSLSLSPGK
] (SEQ ID NO: 16)

15860-1204-SP34scFv(LvHv)

Nucleotide sequence

[spacer (SEQ ID NO: 507)][15860-1204-SP34scFv(LvHv) without spacer (SEQ ID
 NO: 555)]
 [CAAGGCCAGTCTGGCCAA][GTTTATTATTGCGGTGGGAATGAGAGTCTGTGCGGTGAGAGGAGGGGCTCGAGCG
 GTGGCAGCGGTGGCTCTGGTGGTCTGAGCGGCCGTTCCGATAATCATGGCGGCGGTTCTCAGGCTGTCTGTGACACA
 GGAAAGCGCCCTGACCACCAGCCCTGGCGAGACAGTGACCCTGACCTGCAGATCTAGCACAGGCGCCGTGACCACA
 AGCAACTACGCCAACTGGGTGCAGGAAAAGCCCGACCACCTGTTTACCGGCCTGATCGGCGGCACCAACAAAAGGG
 CTCCAGGCGTGCCAGCCAGATTTCAGCGGCAGCCTGATTGGCGATAAGGCCGCCCTGACAATCACTGGCGCCCAGAC
 CGAGGACGAGGCCATCTACTTTTGCGCCCTGTGGTACAGCAACCTGTGGGTGTTTCGGCGGAGGCACCAAGCTGACA
 GTGCTGGGAGGCGGAGGATCTGGCGGAGGCGGAAGTGGCGGAGGGGGATCTGAAGTGCAGCTGGTGGGAATCTGGCG
 GCGGACTGGTGCAGCCTAAGGGCTCTCTGAAGCTGAGCTGTGCCGCCAGCGGCTTCACCTTCAACACCTACGCCAT
 GAATTGGGTGCGCCAGGCCCTGGCAAGGGACTGGAATGGGTGGCCCGGATCAGAAGCAAGTACAACAATTACGCC
 ACCTACTACGCCGACAGCGTGAAGGACCGGTTACCATCAGCCGGGACGACAGCCAGAGCATCCTGTATCTGCAGA
 TGAACAACCTGAAAACCGAGGACACCGCCATGTACTACTGCGTGCGGCACGGCAACTTCGGCAACAGCTATGTGTC
 TTGGTTTGCCTACTGGGGCCAGGGCACCCCTCGTGACAGTGTCTGCT] (SEQ ID NO: 17)

Amino Acid sequence

[spacer (SEQ ID NO: 87)][15263-1204-SP34scFv(LvHv)without spacer (SEQ ID NO: 556)]

[QGQSGQ][VYYCGGNESLCGERRGSSGGSGGSLSGRSDNHGGGSQAVVTQESALTTSPGETVTLTCRSSTGAV
TTSNYANWVQEKPDHLFTGLIGGTNKRAPGVPARFSGSLIGDKAALTITGAQTEDEAIYFCALWYSNLWVFGGGTK
LTVLGGGGSGGGSGGGGSEVQLVESGGGLVQPKGSLKLSCAASGFTFNTYAMNWVRQAPGKGLEWVARIRSKYNN
YATYYADSVKDRFTISRDDSQSILYLQMNNLKTEDTAMYCYVRHGNFGNSYVSWFAYWGQGLVTVSA] (SEQ
ID NO: 18)

Activatable antibody 15860-1204-SP34scFv(LvHv)-Fc fusion**Nucleotide sequence**

[spacer (SEQ ID NO: 507)][Activatable antibody 15860-1204-SP34scFv(LvHv)-Fc
fusion without spacer (SEQ ID NO: 557)]

[CAAGGCCAGTCTGGCCAA][GTTTATTATTGCGGTGGGAATGAGAGTCTGTGCGGTGAGAGGAGGGGCTCGAGCG
GTGGCAGCGGTGGCTCTGGTGGTCTGAGCGGCCGTTCCGATAATCATGGCGGCGGTTCTCAGGCTGTCTGTGACACA
GGAAAGCGCCCTGACCACCAGCCCTGGCGAGACAGTGACCCTGACCTGCAGATCTAGCACAGGCGCCGTGACCACA
AGCAACTACGCCAACTGGGTGCAGGAAAAGCCCGACCACCTGTTACCGGCCTGATCGGCGGCACCAACAAAAGGG
CTCCAGGCGTGCCAGCCAGATTGAGCGGCAGCCTGATTGGCGATAAGGCCGCCCTGACAATCACTGGCGCCCAGAC
CGAGGACGAGGCCATCTACTTTTGCGCCCTGTGGTACAGCAACCTGTGGGTGTTTCGGCGGAGGCACCAAGCTGACA
GTGCTGGGAGGCGGAGGATCTGGCGGAGGCGGAAGTGGCGGAGGGGGATCTGAAGTGCAGCTGGTGAATCTGGCG
GCGGACTGGTGCAGCCTAAGGGCTCTCTGAAGCTGAGCTGTGCCGCCAGCGGCTTCACCTTCAACACCTACGCCAT
GAATTGGGTGCGCCAGGCCCTGGCAAGGGACTGGAATGGGTGGCCCGGATCAGAAGCAAGTACAACAATTACGCC
ACCTACTACGCCGACAGCGTGAAGGACCGGTTACCATCAGCCGGGACGACAGCCAGAGCATCCTGTATCTGCAGA
TGAACAACCTGAAAACCGAGGACACCGCCATGTACTACTGCGTGCGGCACGGCAACTTCGGCAACAGCTATGTGTC
TTGGTTTGCCTACTGGGGCCAGGGCACCCCTCGTGACAGTGTCTGCTGGCGGCTCCCTGGACCCTAAGTCATCTGAC
AAAACCTCACACATGCCCACCGTGCCCAGCACCTGAACTCCTGGGGGGACCGTCAGTCTTCCTCTTCCCCCCTAAAAC
CCAAGGACACCCTCATGATCTCCCGGACCCCTGAGGTCACATGCGTGGTGGTGGACGTGAGCCACGAAGACCCTGA
GGTCAAGTTCAACTGGTACGTGGACGGCGTGGAGGTGCATAATGCCAAGACAAAGCCGCGGGAGGAGCAGTACAAC
AGCACGTACCGTGTGGTCAGCGTCCTCACCGTCCTGCACCAGGACTGGCTGAATGGCAAGGAGTACAAGTGCAAGG
TCTCCAACAAAGCCCTCCCAGCCCCCATCGAGAAAACCATCTCCAAGCCAAAGGGCAGCCCCGAGAACCACAGGT
GTACACCCTGCCCCCATCCCGGGAGGAGATGACCAAGAACCAGGTCAGCCTGACCTGCCTGGTCAAAGGCTTCTAT
CCCAGCGACATCGCCGTGGAGTGGGAGAGCAATGGGCAGCCGGAGAACAACCTACAAGACCACGCCTCCCGTGCTGG
ACTCCGACGGCTCCTTCTTCTCTACAGCAAGCTCACCGTGGACAAGAGCAGGTGGCAGCAGGGGAACGTCTTCTC
ATGCTCCGTGATGCATGAGGCTCTGCACAACCACTACACGCAGAAGAGCCTCTCCCTGTCTCCGGGTAAA] (SEQ
ID NO: 19)

Amino Acid sequence

[spacer (SEQ ID NO: 87)][Activatable antibody 15860-1204-SP34scFv(LvHv)-Fc fusion without spacer (SEQ ID NO: 558)]

[QGQSGQ][VYYCGGNESLCGERRGSSGGSGGSLSGRSDNHGGGSQAVVTQESALTTSPGETVTLTCRSSTGAV TTSNYANWVQEKPDHLFTGLIGGTNKRAPGVPARFSGSLIGDKAALTITGAQTEDEAIYFCALWYSNLWVFGGGTK LTVLGGGGSGGGSGGGGSEVQLVESGGGLVQPKGSLKLSCAASGFTFNTYAMNWVRQAPGKGLEWVARIRSKYNN YATYYADSVKDRFTISRDDSQSILYLQMNNLKTEDTAMYYCVRHGNFGNSYVSWFAYWGQGLVTVSAGGSLDPKS SDKTHTCPCPCAPELLGGPSVFLFPKPKDITLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQ YNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSREEMTKNQVSLTCLVKG FYPSDIAVEWESNGQPENNYKTTTPVLDSDGSFFLYSKLTVDKSRWQQGNVFSQSVMHREALHNHYTQKSLSLSPGK] (SEQ ID NO: 20)

15864-1204-SP34scFv(LvHv)**Nucleotide sequence**

[spacer (SEQ ID NO: 507)][15864-1204-SP34scFv(LvHv) without spacer (SEQ ID NO: 559)]

[CAAGGCCAGTCTGGCCAA][TTTATGTGCCAGCAGCGGATGTGGGGGAATGAGTTTTGCCATCAGGGCTCGAGCG GTGGCAGCGGTGGCTCTGGTGGTCTGAGCGGCCGTTCCGATAATCATGGCGGCGGTTCTCAGGCTGTCTGTGACACA GGAAAGCGCCCTGACCACCAGCCCTGGCGAGACAGTGACCCTGACCTGCAGATCTAGCACAGGCGCCGTGACCACA AGCAACTACGCCAACTGGGTGCAGGAAAAGCCCGACCACCTGTTACCGGCCTGATCGGCGGCACCAACAAAAGGG CTCCAGGCGTGCCAGCCAGATTCAGCGGCAGCCTGATTGGCGATAAGGCCGCCCTGACAATCACTGGCGCCCAGAC CGAGGACGAGGCCATCTACTTTTGCGCCCTGTGGTACAGCAACCTGTGGGTGTTTCGGCGGAGGCACCAAGCTGACA GTGCTGGGAGGCGGAGGATCTGGCGGAGGCGGAAGTGGCGGAGGGGGATCTGAAGTGCAGCTGGTGGAACTCTGGCG GCGGACTGGTGCAGCCTAAGGGCTCTCTGAAGCTGAGCTGTGCCGCCAGCGGCTTCACCTTCAACACCTACGCCAT GAATTGGGTGCGCCAGGCCCTGGCAAGGGACTGGAATGGGTGGCCCGGATCAGAAGCAAGTACAACAATTACGCC ACCTACTACGCCGACAGCGTGAAGGACCGGTTACCATCAGCCGGGACGACAGCCAGAGCATCCTGTATCTGCAGA TGAACAACCTGAAAACCGAGGACACCGCCATGTACTACTGCGTGCGGCACGGCAACTTCGGCAACAGCTATGTGTC TTGGTTTGCCTACTGGGGCCAGGGCACCCCTCGTGACAGTGTCTGCT] (SEQ ID NO: 21)

Amino Acid

[spacer (SEQ ID NO: 87)][15864-1204-SP34scFv(LvHv) without spacer (SEQ ID NO: 560)]

[QGQSGQ][FMCQORMWGNEFCHQGSSGGSGGSLSGRSDNHGGGSQAVVTQESALTTSPGETVTLTCRSSTGAV TTSNYANWVQEKPDHLFTGLIGGTNKRAPGVPARFSGSLIGDKAALTITGAQTEDEAIYFCALWYSNLWVFGGGTK LTVLGGGGSGGGSGGGGSEVQLVESGGGLVQPKGSLKLSCAASGFTFNTYAMNWVRQAPGKGLEWVARIRSKYNN

YATYYADSVKDRFTISRDDSQSILYLQMNNLKTEDTAMYVCVRHGNFGNSYVSWFAYWGQGTILVTVSA] (SEQ ID NO: 22)

Activatable antibody 15864-1204-SP34scFv(LvHv)-Fc fusion

Nucleotide sequence

[spacer (SEQ ID NO: 507)][Activatable antibody 15864-1204-SP34scFv(LvHv)-Fc fusion without spacer (SEQ ID NO: 561)]

[CAAGGCCAGTCTGGCCAA][TTTATGTGCCAGCAGCGGATGTGGGGGAATGAGTTTGGCCATCAGGGCTCGAGCG GTGGCAGCGGTGGCTCTGGTGGTCTGAGCGGCCGTTCCGATAATCATGGCGGCGGTTCTCAGGCTGTCTGTGACACA GGAAAGCGCCCTGACCACCAGCCCTGGCGAGACAGTGACCCTGACCTGCAGATCTAGCACAGGCGCCGTGACCACA AGCAACTACGCCAACTGGGTGCAGGAAAAGCCCGACCACCTGTTTACCGGCCTGATCGGCGGCACCAACAAAAGGG CTCCAGGCGTGCCAGCCAGATTGAGCGGCAGCCTGATTGGCGATAAGGCCGCCCTGACAATCACTGGCGCCCAGAC CGAGGACGAGGCCATCTACTTTTGCGCCCTGTGGTACAGCAACCTGTGGGTGTTTCGGCGGAGGCACCAAGCTGACA GTGCTGGGAGGCGGAGGATCTGGCGGAGGCGGAAGTGGCGGAGGGGGATCTGAAGTGCAGCTGGTGGAAATCTGGCG GCGGACTGGTGCAGCCTAAGGGCTCTCTGAAGCTGAGCTGTGCCGCCAGCGGCTTCACCTTCAACACCTACGCCAT GAATTGGGTGCGCCAGGCCCTTGGCAAGGGACTGGAATGGGTGGCCCGGATCAGAAGCAAGTACAACAATTACGCC ACCTACTACGCCGACAGCGTGAAGGACCGGTTTACCATCAGCCGGGACGACAGCCAGAGCATCCTGTATCTGCAGA TGAACAACCTGAAAACCGAGGACACCGCCATGTACTACTGCGTGCGGCACGGCAACTTCGGCAACAGCTATGTGTC TTGGTTTGCCTACTGGGGCCAGGGCACCCCTCGTGACAGTGTCTGCTGGCGGCTCCCTGGACCCTAAGTCATCTGAC AAAACTCACACATGCCCACCGTGCCCAGCACCTGAACTCCTGGGGGGACCGTCAGTCTTCTTCCCCCCTTCCCTGA CCAAGGACACCCTCATGATCTCCCGGACCCCTGAGGTCACATGCGTGGTGGTGGACGTGAGCCACGAAGACCCTGA GGTCAAGTTCAACTGGTACGTGGACGGCGTGGAGGTGCATAATGCCAAGACAAAGCCGCGGGAGGAGCAGTACAAC AGCACGTACCGTGTGGTCAGCGTCCTCACCGTCCTGCACCAGGACTGGCTGAATGGCAAGGAGTACAAGTGCAAGG TCTCCAACAAAGCCCTCCCAGCCCCCATCGAGAAAACCATCTCCAAGCCAAAGGGCAGCCCCGAGAACCACAGGT GTACACCCTGCCCCCATCCCGGGAGGAGATGACCAAGAACCAGGTCAGCCTGACCTGCCTGGTCAAAGGCTTCTAT CCCAGCGACATCGCCGTGGAGTGGGAGAGCAATGGGCAGCCGGAGAACAACACTACAAGACCACGCCTCCCGTGCTGG ACTCCGACGGCTCCTTCTTCTTCTTCTACAGCAAGCTCACCGTGGACAAGAGCAGGTGGCAGCAGGGGAACGTCTTCTC ATGCTCCGTGATGCATGAGGCTCTGCACAACCACTACACGCAGAAGAGCCTCTCCCTGTCTCCGGGTAAA] (SEQ ID NO: 23)

Amino acid sequence

[spacer (SEQ ID NO: 87)][Activatable antibody 15864-1204-SP34scFv(LvHv)-Fc fusion without spacer (SEQ ID NO: 562)]

[QGQSGQ][FMCQQRMWGNFCHQGSSGSGSGGLSGRSDNHGGGSQAVVTQESALTTSPGETVTLTCRSSTGAV TTSNYANWVQEKPDHLFTGLIGGTNKRAPGVPARFSGSLIGDKAALTITGAQTEDEAIYFCALWYSNLWVFGGGTK LTVLGGGGSGGGSGGGGSEVQLVESGGGLVQPKGSLKLSCAASGFTFNTYAMNWVRQAPKGLEWVARIRSKYNN

YATYYADSVKDRFTISRDDSQSILYLQMNNLKTEDTAMYYCVRHGNFGNSYVSWFAYWGQGTTLVTVSAGGSLDPKS
 SDKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMI SRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQ
 YNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSREEMTKNQVSLTCLVKG
 FYPSDIAVEWESNGQPENNYKTTPVLDSGDSFFLYSKLTVDKSRWQQGNVFSQSVSMHEALHNHYTQKSLSLSPGK
] (SEQ ID NO: 24)

15865-1204-SP34scFv(LvHv)

Nucleotide sequence

[spacer (SEQ ID NO: 507)][15865-1204-SP34scFv(LvHv) without spacer (SEQ ID
 NO: 563)]
 [CAAGGCCAGTCTGGCCAA][ATGATGTATTGCGGTGGGAATGAGGTGTTGTGCGGGCCGCGGGTTGGCTCGAGCG
 GTGGCAGCGGTGGCTCTGGTGGTCTGAGCGGCCGTTCCGATAATCATGGCGGCGGTTCTCAGGCTGTCTGACACA
 GGAAAGCGCCCTGACCACCAGCCCTGGCGAGACAGTGACCCTGACCTGCAGATCTAGCACAGGCGCCGTGACCACA
 AGCAACTACGCCAACTGGGTGCAGGAAAAGCCCGACCACCTGTTTACCGGCCTGATCGGCGGCACCAACAAAAGGG
 CTCCAGGCGTGCCAGCCAGATTGAGCGGCAGCCTGATTGGCGATAAGGCCGCCCTGACAATCACTGGCGCCCAGAC
 CGAGGACGAGGCCATCTACTTTTTCGCGCCCTGTGGTACAGCAACCTGTGGGTGTTTCGGCGGAGGCACCAAGCTGACA
 GTGCTGGGAGGCGGAGGATCTGGCGGAGGCGGAAGTGGCGGAGGGGGATCTGAAGTGCAGCTGGTGGAATCTGGCG
 GCGGACTGGTGCAGCCTAAGGGCTCTCTGAAGCTGAGCTGTGCCGCCAGCGGCTTCACCTTCAACACCTACGCCAT
 GAATTGGGTGCGCCAGGCCCTGGCAAGGGACTGGAATGGGTGGCCCGGATCAGAAGCAAGTACAACAATTACGCC
 ACCTACTACGCCGACAGCGTGAAGGACCGGTTTACCATCAGCCGGGACGACAGCCAGAGCATCCTGTATCTGCAGA
 TGAACAACCTGAAAACCGAGGACACCGCCATGTACTACTGCGTGCGGCACGGCAACTTCGGCAACAGCTATGTGTC
 TTGGTTTGCCTACTGGGGCCAGGGCACCCCTCGTGACAGTGTCTGCT] (SEQ ID NO: 25)

Amino Acid sequence

[spacer (SEQ ID NO: 87)][15865-1204-SP34scFv(LvHv) without spacer (SEQ ID
 NO: 564)]
 [QGQSGQ][MMYCGGNEVLCGPRVGSSGGSGGSGGLSGRSDNHGGGSQAVVTQESALTTSPGETVTLTCRSSTGAV
 TTSNYANWVQEKPDHLFTGLIGGTNKRAPGVPARFSGSLIGDKAALTITGAQTEDEAIYFCALWYSNLWVFGGGTK
 LTVLGGGGSGGGSGGGGSEVQLVESGGGLVQPKGSLKLSCAASGFTFNTYAMNWVRQAPGKGLEWVARIRSKYNN
 YATYYADSVKDRFTISRDDSQSILYLQMNNLKTEDTAMYYCVRHGNFGNSYVSWFAYWGQGTTLVTVSA] (SEQ
 ID NO: 26)

Activatable antibody 15865-1204-SP34scFv(LvHv)-Fc fusion

Nucleotide sequence

[spacer (SEQ ID NO: 507)][Activatable antibody 15865-1204-SP34scFv(LvHv)-Fc fusion without spacer (SEQ ID NO: 565)]

[CAAGGCCAGTCTGGCCAA][ATGATGTATTGCGGTGGGAATGAGGTGTTGTGCGGGCCGCGGGTTGGCTCGAGCG GTGGCAGCGGTGGCTCTGGTGGTCTGAGCGGCCGTTCCGATAATCATGGCGGCGGTTCTCAGGCTGTCTGTGACACA GGAAAGCGCCCTGACCACCAGCCCTGGCGAGACAGTGACCCTGACCTGCAGATCTAGCACAGGCGCCGTGACCACA AGCAACTACGCCAACTGGGTGCAGGAAAAGCCCGACCACCTGTTTACCGGCCTGATCGGCGGCACCAACAAAAGGG CTCCAGGCGTGCCAGCCAGATTTCAGCGGCAGCCTGATTGGCGATAAGGCCGCCCTGACAATCACTGGCGCCCAGAC CGAGGACGAGGCCATCTACTTTTTCGCCCCCTGTGGTACAGCAACCTGTGGGTGTTTCGGCGGAGGCACCAAGCTGACA GTGCTGGGAGGCGGAGGATCTGGCGGAGGCGGAAGTGGCGGAGGGGGATCTGAAGTGCAGCTGGTGGGAATCTGGCG GCGGACTGGTGCAGCCTAAGGGCTCTCTGAAGCTGAGCTGTGCCGCCAGCGGCTTCACCTTCAACACCTACGCCAT GAATTGGGTGCGCCAGGCCCCCTGGCAAGGGACTGGAATGGGTGGCCCCGGATCAGAAGCAAGTACAACAATTACGCC ACCTACTACGCCGACAGCGTGAAGGACCGGTTTACCATCAGCCGGGACGACAGCCAGAGCATCCTGTATCTGCAGA TGAACAACCTGAAAACCGAGGACACCGCCATGTACTACTGCGTGCGGCACGGCAACTTCGGCAACAGCTATGTGTC TTGGTTTGCCTACTGGGGCCAGGGCACCCCTCGTGACAGTGTCTGCTGGCGGCTCCCTGGACCCTAAGTCATCTGAC AAAACTCACACATGCCCACCGTGCCCAGCACCTGAACTCCTGGGGGGACCGTCAGTCTTCCTCTTCCCCCCTTCC CCAAGGACACCCTCATGATCTCCCGGACCCCTGAGGTCACATGCGTGGTGGTGGACGTGAGCCACGAAGACCCTGA GGTCAAGTTCAACTGGTACGTGGACGGCGTGGAGGTGCATAATGCCAAGACAAAGCCGCGGGAGGAGCAGTACAAC AGCACGTACCGTGTGGTTCAGCGTCTCACCCTGTCACCAGGACTGGCTGAATGGCAAGGAGTACAAGTGCAAGG TCTCCAACAAAGCCCTCCCAGCCCCCATCGAGAAAACCATCTCCAAGCCAAAGGGCAGCCCCGAGAACCACAGGT GTACACCCTGCCCCCATCCCGGGAGGAGATGACCAAGAACCAGGTCAGCCTGACCTGCCTGGTCAAAGGCTTCTAT CCCAGCGACATCGCCGTGGAGTGGGAGAGCAATGGGCAGCCGGAGAACAACCTACAAGACCACGCCTCCCGTGCTGG ACTCCGACGGCTCCTTCTTCTCTACAGCAAGCTCACCGTGGACAAGAGCAGGTGGCAGCAGGGGAACGTCTTCTC ATGCTCCGTGATGCATGAGGCTCTGCACAACCACTACACGCAGAAGAGCCTCTCCCTGTCTCCGGGTAAA] (SEQ ID NO: 27)

Amino Acid sequence

[spacer (SEQ ID NO: 87)][Activatable antibody 15865-1204-SP34scFv(LvHv)-Fc fusion without spacer (SEQ ID NO: 566)]

[QGQSGQ][MMYCGGNEVLCGPRVGSSGSGSGGLSGRSDNHGGGSQAVVTQESALTTSPPGETVTLTCRSSTGAV TTSNYANWVQEKPDHLFTGLIGGTNKRAPGVPARFSGSLIGDKAALTITGAQTEDEAIYFCALWYSNLWVFGGGTK LTVLGGGGSGGGSGGGGSEVQLVESGGGLVQPKGSLKLSCAASGFTFNTYAMNWVRQAPGKGLEWVARIRSKYNN YATYYADSVKDRFTISRDDSQSILYLQMNNLKTEDTAMYYCVRHGNFGNSYVSWFAYWGQGLVTVSAGGSLDPKS SDKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQ YNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSREEMTKNQVSLTCLVKG

FYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNV FSCSVMHEALHNHYTQKSLSLSPGK
] (SEQ ID NO: 28)

SP34scFv(HvLv)

Nucleotide sequence

GAAGTGCAGCTGGTGAATCTGGGGGCGGACTGGTGCAGCCTAAGGGCAGCCTGAAGCTGAGCTGTGCCGCCAGCG
 GCTTCACCTTCAACACCTACGCCATGAACTGGGTGCGCCAGGCCCCTGGCAAAGGCCTGGAATGGGTGGCCCGGAT
 CAGAAGCAAGTACAACAATTACGCCACCTACTACGCCGACAGCGTGAAGGACAGATTCACCATCAGCCGGGACGAC
 AGCCAGAGCATCCTGTACCTGCAGATGAACAACCTGAAAACCGAGGACACCGCCATGTACTACTGCGTGCGGCACG
 GCAACTTCGGCAACAGCTATGTGTCTTGGTTTGCCTACTGGGGCCAGGGCACCCCTCGTGACAGTGTCTGCCGGTGG
 TGGTGGTAGTGGTGGCGGTGGTTCAGGCGGTGGCGGTAGCCAGGCTGTCGTGACACAGGAAAGCGCCCTGACCACC
 AGCCCTGGCGAGACAGTGACCCTGACCTGTAGAAGCAGCACAGGCGCCGTGACCACAAGCAACTACGCCAATTGGG
 TGCAGGAAAAGCCCGACCACCTGTTACCGGCCTGATCGGCGGCACCAACAAAAGGGCTCCAGGCGTGCCAGCCAG
 ATTCAGCGGCTCCCTGATCGGAGATAAGGCCGCCCTGACAATCACTGGCGCCCAGACCGAGGACGAGGCCATCTAC
 TTTTGCGCCCTGTGGTACAGCAACCTGTGGGTGTTTCGGCGGAGGCACCAAGCTGACCGTGCTG (SEQ ID
 NO: 29)

Amino Acid sequence

EVQLVESGGGLVQPKGSLKLSCAASGFTFNTYAMNWVRQAPGKGLEWVARIRSKYNNYATYYADSVKDRFTISRDD
 SQSILYLQMNNLKTEDTAMYYCVRHGNFGNSYVSWFAYWGQGLVTVSAGGGGSGGGGSGGGGSQAVVTQESALTT
 SPGETVTLTCRSSTGAVTTSNYANWVQEKPDHLFTGLIGGTNKRAPGVPARFSGSLIGDKAALTITGAQTEDEAIY
 FCALWYSNLWVFGGGTKLTVL (SEQ ID NO: 30)

Antibody SP34scFv(HvLv)-Fc fusion

Nucleotide sequence

GAAGTGCAGCTGGTGAATCTGGGGGCGGACTGGTGCAGCCTAAGGGCAGCCTGAAGCTGAGCTGTGCCGCCAGCG
 GCTTCACCTTCAACACCTACGCCATGAACTGGGTGCGCCAGGCCCCTGGCAAAGGCCTGGAATGGGTGGCCCGGAT
 CAGAAGCAAGTACAACAATTACGCCACCTACTACGCCGACAGCGTGAAGGACAGATTCACCATCAGCCGGGACGAC
 AGCCAGAGCATCCTGTACCTGCAGATGAACAACCTGAAAACCGAGGACACCGCCATGTACTACTGCGTGCGGCACG
 GCAACTTCGGCAACAGCTATGTGTCTTGGTTTGCCTACTGGGGCCAGGGCACCCCTCGTGACAGTGTCTGCCGGTGG
 TGGTGGTAGTGGTGGCGGTGGTTCAGGCGGTGGCGGTAGCCAGGCTGTCGTGACACAGGAAAGCGCCCTGACCACC
 AGCCCTGGCGAGACAGTGACCCTGACCTGTAGAAGCAGCACAGGCGCCGTGACCACAAGCAACTACGCCAATTGGG
 TGCAGGAAAAGCCCGACCACCTGTTACCGGCCTGATCGGCGGCACCAACAAAAGGGCTCCAGGCGTGCCAGCCAG
 ATTCAGCGGCTCCCTGATCGGAGATAAGGCCGCCCTGACAATCACTGGCGCCCAGACCGAGGACGAGGCCATCTAC
 TTTTGCGCCCTGTGGTACAGCAACCTGTGGGTGTTTCGGCGGAGGCACCAAGCTGACCGTGCTGGGCGGCTCCCTGG
 ACCCTAAGTCATCTGACAAAACCTCACACATGCCCACCGTGCCCAGCACCTGAACTCCTGGGGGGACCGTCAGTCTT

CCTCTTCCCCC AAAACCCAAGGACACCCTCATGATCTCCCGGACCCCTGAGGTCACATGCGTGGTGGTGGACGTG
 AGCCACGAAGACCCTGAGGTCAAGTTCAACTGGTACGTGGACGGCGTGGAGGTGCATAATGCCAAGACAAAGCCGC
 GGGAGGAGCAGTACAACAGCACGTACCGTGTGGTCAGCGTCCTCACCGTCCTGCACCAGGACTGGCTGAATGGCAA
 GGAGTACAAGTGCAAGGTCTCCAACAAAGCCCTCCCAGCCCCCATCGAGAAAACCATCTCCAAAGCCAAAGGGCAG
 CCCCAGAAACCACAGGTGTACACCCTGCCCCCATCCCGGGAGGAGATGACCAAGAACCAGGTCAGCCTGACCTGCC
 TGGTCAAAGGCTTCTATCCCAGCGACATCGCCGTGGAGTGGGAGAGCAATGGGCAGCCGGAGAACAACACTACAAGAC
 CACGCCTCCCGTGCTGGACTCCGACGGCTCCTTCTTCTCTACAGCAAGCTCACCGTGGACAAGAGCAGGTGGCAG
 CAGGGGAACGTCTTCTCATGCTCCGTGATGCATGAGGCTCTGCACAACCACTACACGCAGAAGAGCCTCTCCCTGT
 CTCCGGGTAAA (SEQ ID NO: 31)

Amino Acid sequence

EVQLVESGGGLVQPKGSLKLSCAASGFTFNTYAMNWVRQAPGKGLEWVARIRSKYNNYATYYADSVKDRFTISRDD
 SQSILYLQMNNLKTEDTAMYYCVRHGNFNGNSYVSWFAYWGQGLVTVSAGGGGSGGGGSGGGGSQAVVTQESALTT
 SPGETVTLTCSRSTGAVTTSNYANWVQEKPDHLFTGLIGGTNKRAPGVPARFSGSLIGDKAALTITGAQTEDEAIY
 FCALWYSNLWVFGGGTKLTVLGGSLDPKSSDKHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDV
 SHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQ
 PREPQVYITLPPSREEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTTPVLDSDGSFFLYSKLTVDKSRWQ
 QGNVFSCSVMHEALHNHYTQKSLSLSPGK (SEQ ID NO: 32)

15003-1204-SP34scFv(HvLv)

Nucleotide sequence

[spacer (SEQ ID NO: 507)] [15003-1204-SP34scFv(HvLv) without spacer (SEQ ID
 NO: 567)]
 [CAAGGCCAGTCTGGCCAA] [GGTTATCGGTGGGGTTGCGAGTGGAATTGCGGTGGGATTACTACTGGCTCGAGCG
 GTGGCAGCGGTGGCTCTGGTGGTCTGAGCGGCCGTTCCGATAATCATGGCGGCGGTTCTGAAGTGCAGCTGGTGGAA
 ATCTGGGGGCGGACTGGTGCAGCCTAAGGGCAGCCTGAAGCTGAGCTGTGCCGCCAGCGGCTTCACCTTCAACACC
 TACGCCATGAACTGGGTGCGCCAGGCCCCCTGGCAAAGGCCTGGAATGGGTGGCCCGGATCAGAAGCAAGTACAACA
 ATTACGCCACCTACTACGCCGACAGCGTGAAGGACAGATTACCATCAGCCGGGACGACAGCCAGAGCATCCTGTA
 CCTGCAGATGAACAACCTGAAAACCGAGGACACCGCCATGTACTACTGCGTGCGGCACGGCAACTTCGGCAACAGC
 TATGTGTCTTGGTTTGCCTACTGGGGCCAGGGCACCTCGTGACAGTGTCTGCCGGTGGTGGTGGTAGTGGTGGCG
 GTGGTTCAGGCGGTGGCGGTAGCCAGGCTGTCGTGACACAGGAAAGCGCCCTGACCACCAGCCCTGGCGAGACAGT
 GACCCTGACCTGTAGAAGCAGCACAGGCGCCGTGACCACAAGCAACTACGCCAATTGGGTGCAGGAAAAGCCCGAC
 CACCTGTTTACCGGCCTGATCGGCGGCACCAACAAAAGGGCTCCAGGCGTGCCAGCCAGATTCAGCGGCTCCCTGA
 TCGGAGATAAGGCCGCCCTGACAATCACTGGCGCCCAGACCGAGGACGAGGCCATCTACTTTTGGCGCCCTGTGGTA
 CAGCAACCTGTGGGTGTTTCGGCGGAGGCACCAAGCTGACCGTGCTG (SEQ ID NO: 33)

Amino Acid sequence

[spacer (SEQ ID NO: 87)][15003-1204-SP34scFv(HvLv)without spacer (SEQ ID NO: 568)]

[QGQSGQ][GYRWGCEWNCGGITTGSSGGSGGSGGLSGRSDNHGGGSEVQLVESGGGLVQPKGSLKLSCAASGFTF
NTYAMNWVRQAPGKGLEWVARIRSKYNNYATYYADSVKDRFTISRDDSQSILYLQMNNLKTEDTAMYVCVRHGNFG
NSYVSWFAYWGQGLTVTSAGGGGSGGGGSGGGGSQAVVTQESALTTSPGETVTLTCRSSTGAVTTSNYANWVQEK
PDHLFTGLIGGTNKRAPGVPARFSGSLIGDKAALTITGAQTEDEAIYFCALWYSNLWVFGGGTKLTVL] (SEQ
ID NO: 34)

Activatable antibody 15003-1204-SP34scFv(HvLv)-Fc fusion**Nucleotide sequence**

[spacer (SEQ ID NO: 507)][Activatable antibody 15003-1204-SP34scFv(HvLv)-Fc
fusion without spacer (SEQ ID NO: 569)]

[CAAGGCCAGTCTGGCCAA][GGTTATCGGTGGGGTTGCGAGTGGAATTGCGGTGGGATTACTACTGGCTCGAGCG
GTGGCAGCGGTGGCTCTGGTGGTCTGAGCGGCCGTTCCGATAATCATGGCGGCGGTTCTGAAGTGCAGCTGGTGGA
ATCTGGGGGCGGACTGGTGCAGCCTAAGGGCAGCCTGAAGCTGAGCTGTGCCGCCAGCGGCTTCACCTTCAACACC
TACGCCATGAACTGGGTGCGCCAGGCCCTGGCAAAGGCCTGGAATGGGTGGCCCGGATCAGAAGCAAGTACAACA
ATTACGCCACCTACTACGCCGACAGCGTGAAGGACAGATTCACCATCAGCCGGGACGACAGCCAGAGCATCCTGTA
CCTGCAGATGAACAACCTGAAAACCGAGGACACCGCCATGTACTACTGCGTGCGGCACGGCAACTTCGGCAACAGC
TATGTGTCTTGGTTTGCCTACTGGGGCCAGGGCACCCCTCGTGACAGTGTCTGCCGGTGGTGGTGGTAGTGGTGGCG
GTGGTTCAGGCGGTGGCGGTAGCCAGGCTGTCTGTGACACAGGAAAGCGCCCTGACCACCAGCCCTGGCGAGACAGT
GACCCTGACCTGTAGAAGCAGCACAGGCGCCGTGACCACAAGCAACTACGCCAATTGGGTGCAGGAAAAGCCCGAC
CACCTGTTTACCGGCCTGATCGGCGGCACCAACAAAAGGGCTCCAGGCGTGCCAGCCAGATTCAGCGGCTCCCTGA
TCGGAGATAAGGCCGCCCTGACAATCACTGGCGCCCAGACCGAGGACGAGGCCATCTACTTTTGCGCCCTGTGGTA
CAGCAACCTGTGGGTGTTTCGGCGGAGGCACCAAGCTGACCGTGCTGGGCGGCTCCCTGGACCCTAAGTCATCTGAC
AAAACCTCACACATGCCCACCGTGCCCAGCACCTGAACTCCTGGGGGGACCGTCAGTCTTCCTCTTCCCCCCTAAAC
CCAAGGACACCCTCATGATCTCCCGGACCCCTGAGGTCACATGCGTGGTGGTGGACGTGAGCCACGAAGACCCTGA
GGTCAAGTTCAACTGGTACGTGGACGGCGTGGAGGTGCATAATGCCAAGACAAAGCCGCGGGAGGAGCAGTACAAC
AGCACGTACCGTGTGGTCAGCGTCCTCACCGTCCTGCACCAGGACTGGCTGAATGGCAAGGAGTACAAGTGCAAGG
TCTCCAACAAAGCCCTCCCAGCCCCCATCGAGAAAACCATCTCCAAGCCAAAGGGCAGCCCCGAGAACCACAGGT
GTACACCCTGCCCCCATCCCGGGAGGAGATGACCAAGAACCAGGTCAGCCTGACCTGCCTGGTCAAAGGCTTCTAT
CCCAGCGACATCGCCGTGGAGTGGGAGAGCAATGGGCAGCCGGAGAACAACACTACAAGACCACGCCTCCCGTGCTGG
ACTCCGACGGCTCCTTCTTCTCTACAGCAAGCTCACCGTGGACAAGAGCAGGTGGCAGCAGGGGAACGTCTTCTC
ATGCTCCGTGATGCATGAGGCTCTGCACAACCACTACACGCAGAAGAGCCTCTCCCTGTCTCCGGGTAAA (SEQ
ID NO: 35)

Amino Acid sequence

[spacer (SEQ ID NO: 87)][Activatable antibody 15003-1204-SP34scFv(HvLv)-Fc fusion without spacer (SEQ ID NO: 570)]

[QGQSGQ][GYRWGCEWNCGGITTGSSGGSGGSGGLSGRSDNHGGGSEVQLVESGGGLVQPKGSLKLSCAASGFTFNTYAMNWVRQAPGKGLEWVARIRSKYNNYATYYADSVKDRFTISRDDSQSILYLQMNNLKTEDTAMYVCVRHGNFGNSYVSWFAYWGQGLVTVSAGGGGSGGGGSGGGGSQAVVTQESALTTSPGETVTLTCRSSTGAVTTSNYANWVQEKPDHLFTGLIGGTNKRAPGVPARFSGSLIGDKAALTITGAQTEDEAIYFCALWYSNLWVFGGGTKLTVLGGSLDPKSSDKTHTCPCPCAPELLGGPSVFLFPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSREEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSQSVMHREALHNHYTQKSLSLSPGK]

(SEQ ID NO: 36)

15263-1204-SP34scFv(HvLv)**Nucleotide sequence**

[spacer (SEQ ID NO: 507)][15263-1204-SP34scFv(HvLv) without spacer (SEQ ID NO: 571)]

[CAAGGCCAGTCTGGCCAA][TGGTATTCGGGTGGGTGCGAGGCTTTTTGCGGTATTTTGTCTCGGGCTCGAGCGGTGGCAGCGGTGGCTCTGGTGGTCTGAGCGGCCGTTCCGATAATCATGGCGGCGGTTCTGAAGTGCAGCTGGTGGGATCTGGGGGCGGACTGGTGCAGCCTAAGGGCAGCCTGAAGCTGAGCTGTGCCGCCAGCGGCTTCACCTTCAACACCTACGCCATGAACTGGGTGCGCCAGGCCCTGGCAAAGGCCTGGAATGGGTGGCCCGGATCAGAAGCAAGTACAACAATTACGCCACCTACTACGCCGACAGCGTGAAGGACAGATTCACCATCAGCCGGGACGACAGCCAGAGCATCCTGTACCTGCAGATGAACAACCTGAAAACCGAGGACACCGCCATGTACTACTGCGTGCGGCACGGCAACTTCGGCAACAGCTATGTGTCTTGGTTTGCCTACTGGGGCCAGGGCACCTCGTGACAGTGTCTGCCGGTGGTGGTAGTGGTGGCGGTGGTTCAGGCGGTGGCGGTAGCCAGGCTGTCTGTGACACAGGAAAGCGCCCTGACCACCAGCCCTGGCGAGACAGTGACCCTGACCTGTAGAAGCAGCACAGGCGCCGTGACCACAAGCAACTACGCCAATTGGGTGCAGGAAAAGCCCGACACCTGTTTACCGGCCTGATCGGCGGCACCAACAAAAGGGCTCCAGGCGTGCCAGCCAGATTCAGCGGCTCCCTGATCGGAGATAAGGCCGCCCTGACAATCACTGGCGCCCAGACCGAGGACGAGGCCATCTACTTTTGCGCCCTGTGGTACAGCAACCTGTGGGTGTTTCGGCGGAGGCACCAAGCTGACCGTGCTG]

(SEQ ID NO: 37)

Amino Acid sequence

[spacer (SEQ ID NO: 87)][15263-1204-SP34scFv(HvLv) without spacer (SEQ ID NO: 572)]

[QGQSGQ][WYSGGCEAFCGILSSGSSGGSGGSGGLSGRSDNHGGGSEVQLVESGGGLVQPKGSLKLSCAASGFTFNTYAMNWVRQAPGKGLEWVARIRSKYNNYATYYADSVKDRFTISRDDSQSILYLQMNNLKTEDTAMYVCVRHGNFGNSYVSWFAYWGQGLVTVSAGGGGSGGGGSGGGGSQAVVTQESALTTSPGETVTLTCRSSTGAVTTSNYANWVQEK

PDHLFTGLIGGTNKRAPGVPARFSGSLIGDKAALTITGAQTEDEAIYFCALWYSNLWVFGGGTKLTVL] (SEQ ID NO: 38)

Activatable antibody 15263-1204-SP34scFv(HvLv)-Fc fusion

Nucleotide sequence

[spacer (SEQ ID NO: 507)][Activatable antibody 15263-1204-SP34scFv(HvLv)-Fc fusion without spacer (SEQ ID NO: 573)]

[CAAGGCCAGTCTGGCCAA][TGGTATTCGGGTGGGTGCGAGGCTTTTTGCGGTATTTTGTCTCGTCTGGGCTCGAGCG GTGGCAGCGGTGGCTCTGGTGGTCTGAGCGGCCGTTCCGATAATCATGGCGGCGGTTCTGAAGTGCAGCTGGTGGGA ATCTGGGGGCGGACTGGTGCAGCCTAAGGGCAGCCTGAAGCTGAGCTGTGCCGCCAGCGGCTTCACCTTCAACACC TACGCCATGAACTGGGTGCGCCAGGCCCTGGCAAAGGCCTGGAATGGGTGGCCCGGATCAGAAGCAAGTACAACA ATTACGCCACCTACTACGCCGACAGCGTGAAGGACAGATTCACCATCAGCCGGGACGACAGCCAGAGCATCCTGTA CCTGCAGATGAACAACCTGAAAACCGAGGACACCGCCATGTACTACTGCGTGCGGCACGGCAACTTCGGCAACAGC TATGTGTCTTGGTTTGCCTACTGGGGCCAGGGCACCCCTCGTGACAGTGTCTGCCGGTGGTGGTGGTAGTGGTGGCG GTGGTTCAGGCGGTGGCGGTAGCCAGGCTGTCTGTGACACAGGAAAGCGCCCTGACCACCAGCCCTGGCGAGACAGT GACCCTGACCTGTAGAAGCAGCACAGGCGCCGTGACCACAAGCAACTACGCCAATTGGGTGCAGGAAAAGCCCGAC CACCTGTTCACCGGCCTGATCGGCGGCACCAACAAAAGGGCTCCAGGCGTGCCAGCCAGATTCAGCGGCTCCCTGA TCGGAGATAAGGCCGCCCTGACAATCACTGGCGCCCAGACCGAGGACGAGGCCATCTACTTTTGCGCCCTGTGGTA CAGCAACCTGTGGGTGTTTCGGCGGAGGCACCAAGCTGACCGTGCTGGGCGGCTCCCTGGACCCTAAGTCATCTGAC AAAACTCACACATGCCCACCGTGCCCAGCACCTGAACTCCTGGGGGGACCGTCAGTCTTCTTCCCCCCTTCTTCTT CCAAGGACACCCTCATGATCTCCCGGACCCCTGAGGTCACATGCGTGGTGGTGGACGTGAGCCACGAAGACCCTGA GGTCAAGTTCAACTGGTACGTGGACGGCGTGGAGGTGCATAATGCCAAGACAAAGCCGCGGGAGGAGCAGTACAAC AGCACGTACCGTGTTGGTTCAGCGTCTCCTACCGTCTCTGACCAGGACTGGCTGAATGGCAAGGAGTACAAGTGCAAGG TCTCCAACAAAGCCCTCCCAGCCCCCATCGAGAAAACCATCTCCAAAGCCAAAGGGCAGCCCCGAGAACCACAGGT GTACACCCTGCCCCCATCCCGGGAGGAGATGACCAAGAACCAGGTCAGCCTGACCTGCCTGGTCAAAGGCTTCTAT CCCAGCGACATCGCCGTGGAGTGGGAGAGCAATGGGCAGCCGGAGAACAACACTACAAGACCACGCCTCCCGTGCTGG ACTCCGACGGCTCCTTCTTCTTCTTCTTCTTCTTCTTCTTCTTCTTCTTCTTCTTCTTCTTCTTCTTCTTCTTCTT ATGCTCCGTGATGCATGAGGCTCTGCACAACCACTACACGCAGAAGAGCCTCTCCCTGTCTCCGGGTAAA] (SEQ ID NO: 39)

Amino Acid sequence

[spacer (SEQ ID NO: 87)][Activatable antibody 15263-1204-SP34scFv(HvLv)-Fc fusion without spacer (SEQ ID NO: 574)]

[QGQSGQ][WYSGGCEAFCGILSSGSSGGSGGSGGLSGRSDNHGGGSEVQLVESGGGLVQPKGSLKLSCAASGFTF NTYAMNWVRQAPGKGLEWVARIRSKYNNYATYYADSVKDRFTISRDDSQSILYLQMNNLKTEDTAMYVCVRHGNFG NSYVSWFAYWGQGLVTVSAGGGGSGGGGSGGGGSQAVVTQESALTTSPGETVTLTCRSSTGAVTTSNYANWVQEK

PDHLFTGLIGGTNKRAPGVPARFSGSLIGDKAALTITGAQTEDEAIYFCALWYSNLWVFGGGTKLTVLGGSLDPKS
 SDKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMI SRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQ
 YNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSREEMTKNQVSLTCLVKG
 FYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSQSVMEALHNHYTQKSLSLSPGK
] (SEQ ID NO: 40)

15860-1204-SP34scFv(HvLv)

Nucleotide sequence

[spacer (SEQ ID NO: 507)][15860-1204-SP34scFv(HvLv) without spacer (SEQ ID
 NO: 575)]
 [CAAGGCCAGTCTGGCCAA][GTTTATTATTGCGGTGGGAATGAGAGTCTGTGCGGTGAGAGGAGGGGCTCGAGCG
 GTGGCAGCGGTGGCTCTGGTGGTCTGAGCGGCCGTTCCGATAATCATGGCGGCGGTTCTGAAGTGCAGCTGGTGGGA
 ATCTGGGGGCGGACTGGTGCAGCCTAAGGGCAGCCTGAAGCTGAGCTGTGCCGCCAGCGGCTTCACCTTCAACACC
 TACGCCATGAACTGGGTGCGCCAGGCCCTGGCAAAGGCCTGGAATGGGTGGCCCGGATCAGAAGCAAGTACAACA
 ATTACGCCACCTACTACGCCGACAGCGTGAAGGACAGATTCACCATCAGCCGGGACGACAGCCAGAGCATCCTGTA
 CCTGCAGATGAACAACCTGAAAACCGAGGACACCGCCATGTACTACTGCGTGCGGCACGGCAACTTCGGCAACAGC
 TATGTGTCTTGGTTTGCCTACTGGGGCCAGGGCACCCCTCGTGACAGTGTCTGCCGGTGGTGGTGGTAGTGGTGGCG
 GTGGTTCAGGCGGTGGCGGTAGCCAGGCTGTCTGTGACACAGGAAAGCGCCCTGACCACCAGCCCTGGCGAGACAGT
 GACCCTGACCTGTAGAAGCAGCACAGGCGCCGTGACCACAAGCAACTACGCCAATTGGGTGCAGGAAAAGCCCGAC
 CACCTGTTCACCGGCCTGATCGGCGGCACCAACAAAAGGGCTCCAGGCGTGCCAGCCAGATTCAGCGGCTCCCTGA
 TCGGAGATAAGGCCGCCCTGACAATCACTGGCGCCCAGACCGAGGACGAGGCCATCTACTTTTGCGCCCTGTGGTA
 CAGCAACCTGTGGGTGTTTCGGCGGAGGCACCAAGCTGACCGTGCTG] (SEQ ID NO: 41)

Amino Acid sequence

[spacer (SEQ ID NO: 87)][15860-1204-SP34scFv(HvLv) without spacer (SEQ ID
 NO: 576)]
 [QGQSGQ][VYYCGGNESLCGERRGSSGGSGGSGGLSGRSDNHGGGSEVQLVESGGGLVQPKGSLKLSCAASGFTF
 NTYAMNWVRQAPGKGLEWVARIRSKYNNYATYYADSVKDRFTISRDDSQSILYLQMNNLKTEDTAMYVCVRHGNFG
 NSYVSWFAYWGQGLVTVSAGGGGSGGGGSGGGGSQAVVTQESALTTSPGETVTLTCRSSTGAVTTSNYANWVQEK
 PDHLFTGLIGGTNKRAPGVPARFSGSLIGDKAALTITGAQTEDEAIYFCALWYSNLWVFGGGTKLTVL] (SEQ
 ID NO: 42)

Activatable antibody 15860-1204-SP34scFv(HvLv)-Fc fusion

Nucleotide sequence

[spacer (SEQ ID NO: 507)][Activatable antibody 15860-1204-SP34scFv(HvLv)-Fc
 fusion without spacer (SEQ ID NO: 577)]

[CAAGGCCAGTCTGGCCAA] [GTTTATTATTGCGGTGGGAATGAGAGTCTGTGCGGTGAGAGGAGGGGCTCGAGCG GTGGCAGCGGTGGCTCTGGTGGTCTGAGCGGCCGTTCCGATAATCATGGCGGCGGTTCTGAAGTGCAGCTGGTGGA ATCTGGGGGCGGACTGGTGCAGCCTAAGGGCAGCCTGAAGCTGAGCTGTGCCGCCAGCGGCTTCACCTTCAACACC TACGCCATGAACTGGGTGCGCCAGGCCCCCTGGCAAAGGCCTGGAATGGGTGGCCCCGGATCAGAAGCAAGTACAACA ATTACGCCACCTACTACGCCGACAGCGTGAAGGACAGATTACCATCAGCCGGGACGACAGCCAGAGCATCCTGTA CCTGCAGATGAACAACCTGAAAACCGAGGACACCGCCATGTACTACTGCGTGCGGCACGGCAACTTCGGCAACAGC TATGTGTCTTGGTTTGCCTACTGGGGCCAGGGCACCCCTCGTGACAGTGTCTGCCGGTGGTGGTGGTAGTGGTGGCG GTGGTTCAGGCGGTGGCGGTAGCCAGGCTGTCTGTGACACAGGAAAGCGCCCTGACCACCAGCCCTGGCGAGACAGT GACCCTGACCTGTAGAAGCAGCACAGGCGCCGTGACCACAAGCAACTACGCCAATTGGGTGCAGGAAAAGCCCGAC CACCTGTTTACCGGCCTGATCGGCGGCACCAACAAAAGGGCTCCAGGCGTGCCAGCCAGATTACAGCGGCTCCCTGA TCGGAGATAAGGCCGCCCTGACAATCACTGGCGCCCAGACCGAGGACGAGGCCATCTACTTTTGCGCCCTGTGGTA CAGCAACCTGTGGGTGTTTCGGCGGAGGCACCAAGCTGACCGTGCTGGGCGGCTCCCTGGACCCTAAGTCATCTGAC AAAACTCACACATGCCCACCGTGCCCAGCACCTGAACTCCTGGGGGGACCGTCAGTCTTCCTCTTCCCCC AAAAC CCAAGGACACCCTCATGATCTCCCGGACCCCTGAGGTCACATGCGTGGTGGTGGACGTGAGCCACGAAGACCCTGA GGTCAAGTTCAACTGGTACGTGGACGGCGTGGAGGTGCATAATGCCAAGACAAAGCCGCGGGAGGAGCAGTACAAC AGCACGTACCGTGTGGTCAGCGTCCTCACCGTCCTGCACCAGGACTGGCTGAATGGCAAGGAGTACAAGTGCAAGG TCTCCAACAAAGCCCTCCCAGCCCCCATCGAGAAAACCATCTCCAAGCCAAAGGGCAGCCCCGAGAACCACAGGT GTACACCCTGCCCCCATCCCGGGAGGAGATGACCAAGAACCAGGTCAGCCTGACCTGCCTGGTCAAAGGCTTCTAT CCCAGCGACATCGCCGTGGAGTGGGAGAGCAATGGGCAGCCGGAGAACAACACTACAAGACCACGCCTCCCGTGCTGG ACTCCGACGGCTCCTTCTTCCTCTACAGCAAGCTCACCGTGGACAAGAGCAGGTGGCAGCAGGGGAACGTCTTCTC ATGCTCCGTGATGCATGAGGCTCTGCACAACCACTACACGCAGAAGAGCCTCTCCCTGTCTCCGGGTAAA] (SEQ ID NO: 43)

Amino Acid sequence

[spacer (SEQ ID NO: 87)][Activatable antibody 15860-1204-SP34scFv(HvLv)-Fc fusion without spacer (SEQ ID NO: 578)]
 [QGQSGQ][VYYCGGNESLCGERRGSSGGSGGSGGLSGRSDNHGGGSEVQLVESGGGLVQPKGSLKLSCAASGFTF NTYAMNWVRQAPGKGLEWVARIRSKYNNYATYYADSVKDRFTISRDDSQSILYLQMNNLKTEDTAMYYCVRHGNFG NSYVSWFAYWGQGLVTVSAGGGGSGGGGSGGGGSQAVVTQESALTTSPGETVTLTCRSSTGAVTTSNYANWVQEK PDHLFTGLIGGTNKRAPGVPARFSGSLIGDKAALTITGAQTEDEAIYFCALWYSNLWVFGGGTKLTVLGGSLDPKS SDKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQ YNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSREEMTKNQVSLTCLVKG FYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSQSVMEALHNHYTQKSLSLSPGK] (SEQ ID NO: 44)

15864-1204-SP34scFv(HvLv)**Nucleotide sequence**

[spacer (SEQ ID NO: 507)][15864-1204-SP34scFv(HvLv) without spacer (SEQ ID NO: 579)]

[CAAGGCCAGTCTGGCCAA][TTTATGTGCCAGCAGCGGATGTGGGGGAATGAGTTTGGCCATCAGGGCTCGAGCG GTGGCAGCGGTGGCTCTGGTGGTCTGAGCGGCCGTTCCGATAATCATGGCGGCGGTTCTGAAGTGCAGCTGGTGG AATCTGGGGGCGGACTGGTGCAGCCTAAGGGCAGCCTGAAGCTGAGCTGTGCCGCCAGCGGCTTCACCTTCAACACC TACGCCATGAACTGGGTGCGCCAGGCCCCCTGGCAAAGGCCTGGAATGGGTGGCCCGGATCAGAAGCAAGTACAACA ATTACGCCACCTACTACGCCGACAGCGTGAAGGACAGATTCACCATCAGCCGGGACGACAGCCAGAGCATCCTGTA CCTGCAGATGAACAACCTGAAAACCGAGGACACCGCCATGTACTACTGCGTGCGGCACGGCAACTTCGGCAACAGC TATGTGTCTTGGTTTGCCTACTGGGGCCAGGGCACCCCTCGTGACAGTGTCTGCCGGTGGTGGTGGTAGTGGTGGCG GTGGTTCAGGCGGTGGCGGTAGCCAGGCTGTCTGTGACACAGGAAAGCGCCCTGACCACCAGCCCTGGCGAGACAGT GACCCTGACCTGTAGAAGCAGCACAGGCGCCGTGACCACAAGCAACTACGCCAATTGGGTGCAGGAAAAGCCCGAC CACCTGTTTACCGGCCTGATCGGCGGCACCAACAAAAGGGCTCCAGGCGTGCCAGCCAGATTCAGCGGCTCCCTGA TCGGAGATAAGGCCGCCCTGACAATCACTGGCGCCCAGACCGAGGACGAGGCCATCTACTTTTGCGCCCTGTGGTA CAGCAACCTGTGGGTGTTTCGGCGGAGGCACCAAGCTGACCGTGCTG] (SEQ ID NO: 45)

Amino Acid sequence

[spacer (SEQ ID NO: 87)][15864-1204-SP34scFv(HvLv) without spacer (SEQ ID NO: 580)]

[QGQSGQ][FMCQQRMWGNFCHQGSSGSGSGGLSGRSDNHGGGSEVQLVESGGGLVQPKGSLKLSCAASGFTF NTYAMNWVRQAPGKGLEWVARIRSKYNNYATYYADSVKDRFTISRDDSQSILYLQMNNLKTEDTAMYVCVRHGNFG NSYVSWFAYWGQGLVTVSAGGGGSGGGGSGGGGSQAVVTQESALTTSPGETVTLTCRSSTGAVTTSNYANWVQEK PDHLFTGLIGGTNKRAPGVPARFSGSLIGDKAALTITGAQTEDEAIYFCALWYSNLWVFGGGTKLTVL] (SEQ ID NO: 46)

Activatable antibody 15864-1204-SP34scFv(HvLv)-Fc fusion

[spacer (SEQ ID NO: 507)][Activatable antibody 15864-1204-SP34scFv(HvLv)-Fc fusion without spacer (SEQ ID NO: 581)]

[CAAGGCCAGTCTGGCCAA][TTTATGTGCCAGCAGCGGATGTGGGGGAATGAGTTTGGCCATCAGGGCTCGAGCG GTGGCAGCGGTGGCTCTGGTGGTCTGAGCGGCCGTTCCGATAATCATGGCGGCGGTTCTGAAGTGCAGCTGGTGG AATCTGGGGGCGGACTGGTGCAGCCTAAGGGCAGCCTGAAGCTGAGCTGTGCCGCCAGCGGCTTCACCTTCAACACC TACGCCATGAACTGGGTGCGCCAGGCCCCCTGGCAAAGGCCTGGAATGGGTGGCCCGGATCAGAAGCAAGTACAACA ATTACGCCACCTACTACGCCGACAGCGTGAAGGACAGATTCACCATCAGCCGGGACGACAGCCAGAGCATCCTGTA CCTGCAGATGAACAACCTGAAAACCGAGGACACCGCCATGTACTACTGCGTGCGGCACGGCAACTTCGGCAACAGC TATGTGTCTTGGTTTGCCTACTGGGGCCAGGGCACCCCTCGTGACAGTGTCTGCCGGTGGTGGTGGTAGTGGTGGCG

GTGGTTCAGGCGGTGGCGGTAGCCAGGCTGTCGTGACACAGGAAAGCGCCCTGACCACCAGCCCTGGCGAGACAGT
 GACCCTGACCTGTAGAAGCAGCACAGGCGCCGTGACCACAAGCAACTACGCCAATTGGGTGCAGGAAAAGCCCGAC
 CACCTGTTTACCGGCCTGATCGGCGGCACCAACAAAAGGGCTCCAGGCGTGCCAGCCAGATTCAGCGGCTCCCTGA
 TCGGAGATAAGGCCGCCCTGACAATCACTGGCGCCCAGACCGAGGACGAGGCCATCTACTTTTGCGCCCTGTGGTA
 CAGCAACCTGTGGGTGTTTCGGCGGAGGCACCAAGCTGACCGTGCTGGGCGGCTCCCTGGACCCTAAGTCATCTGAC
 AAAACTCACACATGCCACCGTGCCCAGCACCTGAACTCCTGGGGGGACCGTCAGTCTTCCTCTTCCCCCAAAC
 CCAAGGACACCCTCATGATCTCCCGGACCCCTGAGGTCACATGCGTGGTGGTGGACGTGAGCCACGAAGACCCTGA
 GGTCAAGTTCAACTGGTACGTGGACGGCGTGGAGGTGCATAATGCCAAGACAAAGCCGCGGGAGGAGCAGTACAAC
 AGCACGTACCGTGTGGTCAGCGTCCTACCGTCCTGCACCAGGACTGGCTGAATGGCAAGGAGTACAAGTGCAAGG
 TCTCCAACAAAGCCCTCCCAGCCCCCATCGAGAAAACCATCTCCAAAGCCAAAGGGCAGCCCCGAGAACCACAGGT
 GTACACCCTGCCCCCATCCCGGGAGGAGATGACCAAGAACCAGGTCAGCCTGACCTGCCTGGTCAAAGGCTTCTAT
 CCCAGCGACATCGCCGTGGAGTGGGAGAGCAATGGGCAGCCGGAGAACAACACTACAAGACCACGCCTCCCGTGCTGG
 ACTCCGACGGCTCCTTCTTCTCTACAGCAAGCTCACCGTGGACAAGAGCAGGTGGCAGCAGGGGAACGTCTTCTC
 ATGCTCCGTGATGCATGAGGCTCTGCACAACCACTACACGCAGAAGAGCCTCTCCCTGTCTCCGGGTAAA] (SEQ
 ID NO: 47)

Amino Acid sequence

[spacer (SEQ ID NO: 87)][Activatable antibody 15864-1204-SP34scFv(HvLv)-Fc
 fusion without spacer (SEQ ID NO: 582)]
 [QGQSGQ][FMCQQRMWGNFCHQSSGGSGGSGGLSGRSDNHGGGSEVQLVESGGGLVQPKGSLKLSCAASGFTF
 NTYAMNWVRQAPGKGLEWVARIRSKYNNYATYYADSVKDRFTISRDDSQSILYLQMNNLKTEDTAMYVCVRHGNFG
 NSYVSWFAYWGQGLVTVSAGGGSGGGSGGGGSQAVVTQESALTTSPGETVTLTCRSSTGAVTTSNYANWVQEK
 PDHLFTGLIGGTNKRAPGVPARFSGSLIGDKAALTITGAQTEDEAIYFCALWYSNLWVFGGGTKLTVLGGSLDPKS
 SDKTHTCPPCPAPELLGGPSVFLFPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQ
 YNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSREEMTKNQVSLTCLVKG
 FYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSQSVMEALHNHYTQKSLSLSPGK
] (SEQ ID NO: 48)

15865-1204-SP34scFv(HvLv)

Nucleotide sequence

[spacer (SEQ ID NO: 507)][15865-1204-SP34scFv(HvLv) without spacer (SEQ ID
 NO: 583)]
 [CAAGGCCAGTCTGGCCAA][ATGATGTATTGCGGTGGGAATGAGGTGTTGTGCGGGCCGCGGGTTGGCTCGAGCG
 GTGGCAGCGGTGGCTCTGGTGGTCTGAGCGGCCGTTCCGATAATCATGGCGGCGGTTCTGAAGTGCAGCTGGTGGGA
 ATCTGGGGGCGGACTGGTGCAGCCTAAGGGCAGCCTGAAGCTGAGCTGTGCCGCCAGCGGCTTCACCTTCAACACC
 TACGCCATGAACTGGGTGCGCCAGGCCCCCTGGCAAAGGCCTGGAATGGGTGGCCCGGATCAGAAGCAAGTACAACA

ATTACGCCACCTACTACGCCGACAGCGTGAAGGACAGATTCACCATCAGCCGGGACGACAGCCAGAGCATCCTGTA
CCTGCAGATGAACAACCTGAAAACCGAGGACACCGCCATGTACTACTGCGTGCGGCACGGCAACTTCGGCAACAGC
TATGTGTCTTGGTTTGCCTACTGGGGCCAGGGCACCCCTCGTGACAGTGTCTGCCGGTGGTGGTGGTAGTGGTGGCG
GTGGTTCAGGCGGTGGCGGTAGCCAGGCTGTCTGTGACACAGGAAAGCGCCCTGACCACCAGCCCTGGCGAGACAGT
GACCCTGACCTGTAGAAGCAGCACAGGCGCCGTGACCACAAGCAACTACGCCAATTGGGTGCAGGAAAAGCCCGAC
CACCTGTTACCGGCCTGATCGGCGGCACCAACAAAAGGGCTCCAGGCGTGCCAGCCAGATTCAGCGGCTCCCTGA
TCGGAGATAAGGCCGCCCTGACAATCACTGGCGCCCAGACCGAGGACGAGGCCATCTACTTTTGCGCCCTGTGGTA
CAGCAACCTGTGGGTGTTTCGGCGGAGGCACCAAGCTGACCGTGCTG] (SEQ ID NO: 49)

Amino Acid sequence

[spacer (SEQ ID NO: 87)][15865-1204-SP34scFv(HvLv) without spacer (SEQ ID
NO: 584)]
[QGQSGQ][MMYCGGNEVLCGPRVGSSGGSGGSGGLSGRSDNHGGGSEVQLVESGGGLVQPKGSLKLSCAASGFTF
NTYAMNWVRQAPGKGLEWVARIRSKYNNYATYYADSVKDRFTISRDDSQSILYLQMNNLKTEDTAMYVCVRHGNFG
NSYVSWFAYWGQGLVTVSAGGGGSGGGGSGGGGSQAVVTQESALTTSPGETVTLTCRSSTGAVTTSNYANWVQEK
PDHLFTGLIGGTNKRAPGVPARFSGSLIGDKAALTITGAQTEDEAIYFCALWYSNLWVFGGGTKLTVL] (SEQ
ID NO: 50)

Activatable antibody 15865-1204-SP34scFv(HvLv)-Fc fusion

Nucleotide sequence

[spacer (SEQ ID NO: 507)][Activatable antibody 15865-1204-SP34scFv(HvLv)-Fc
fusion without spacer (SEQ ID NO: 585)]
[CAAGGCCAGTCTGGCCAA][ATGATGTATTGCGGTGGGAATGAGGTGTTGTGCGGGCCGCGGGTTGGCTCGAGCG
GTGGCAGCGGTGGCTCTGGTGGTCTGAGCGGCCGTTCCGATAATCATGGCGGCGGTTCTGAAGTGCAGCTGGTGGGA
ATCTGGGGGCGGACTGGTGCAGCCTAAGGGCAGCCTGAAGCTGAGCTGTGCCGCCAGCGGCTTCACCTTCAACACC
TACGCCATGAACTGGGTGCGCCAGGCCCCCTGGCAAAGGCCTGGAATGGGTGGCCCGGATCAGAAGCAAGTACAACA
ATTACGCCACCTACTACGCCGACAGCGTGAAGGACAGATTCACCATCAGCCGGGACGACAGCCAGAGCATCCTGTA
CCTGCAGATGAACAACCTGAAAACCGAGGACACCGCCATGTACTACTGCGTGCGGCACGGCAACTTCGGCAACAGC
TATGTGTCTTGGTTTGCCTACTGGGGCCAGGGCACCCCTCGTGACAGTGTCTGCCGGTGGTGGTGGTAGTGGTGGCG
GTGGTTCAGGCGGTGGCGGTAGCCAGGCTGTCTGTGACACAGGAAAGCGCCCTGACCACCAGCCCTGGCGAGACAGT
GACCCTGACCTGTAGAAGCAGCACAGGCGCCGTGACCACAAGCAACTACGCCAATTGGGTGCAGGAAAAGCCCGAC
CACCTGTTACCGGCCTGATCGGCGGCACCAACAAAAGGGCTCCAGGCGTGCCAGCCAGATTCAGCGGCTCCCTGA
TCGGAGATAAGGCCGCCCTGACAATCACTGGCGCCCAGACCGAGGACGAGGCCATCTACTTTTGCGCCCTGTGGTA
CAGCAACCTGTGGGTGTTTCGGCGGAGGCACCAAGCTGACCGTGCTGGGCGGCTCCCTGGACCCTAAGTCATCTGAC
AAAACTCACACATGCCACCGTGCCAGCACCTGAACTCCTGGGGGGACCGTCAGTCTTCTCTTCCCCCAAAC
CCAAGGACACCCTCATGATCTCCCGGACCCCTGAGGTCACATGCGTGGTGGTGGACGTGAGCCACGAAGACCCTGA

GGTCAAGTTCAACTGGTACGTGGACGGCGTGGAGGTGCATAATGCCAAGACAAAGCCGCGGGAGGAGCAGTACAAC
 AGCACGTACCGTGTGGTCAGCGTCCTCACCGTCCTGCACCAGGACTGGCTGAATGGCAAGGAGTACAAGTGCAAGG
 TCTCCAACAAAGCCCTCCCAGCCCCCATCGAGAAAACCATCTCCAAAGCCAAAGGGCAGCCCCGAGAACCACAGGT
 GTACACCCTGCCCCCATCCCGGGAGGAGATGACCAAGAACCAGGTCAGCCTGACCTGCCTGGTCAAAGGCTTCTAT
 CCCAGCGACATCGCCGTGGAGTGGGAGAGCAATGGGCAGCCGGAGAACAACACTACAAGACCACGCCTCCCGTGCTGG
 ACTCCGACGGCTCCTTCTTCTCTACAGCAAGCTCACCGTGGACAAGAGCAGGTGGCAGCAGGGGAACGTCTTCTC
 ATGCTCCGTGATGCATGAGGCTCTGCACAACCACTACACGCAGAAGAGCCTCTCCCTGTCTCCGGGTAAA] (SEQ
 ID NO: 51)

Amino Acid sequence

[spacer (SEQ ID NO: 87)][Activatable antibody 15865-1204-SP34scFv(HvLv)-Fc
 fusion without spacer (SEQ ID NO: 586)]
 [QGQSGQ][MMYCGGNEVLCGPRVGSSGGSGGSGGLSGRSDNHGGGSEVQLVESGGGLVQPKGSLKLSCAASGFTF
 NTYAMNWVRQAPGKGLEWVARIRSKYNNYATYYADSVKDRFTISRDDSQSILYLQMNNLKTEDTAMYCYVRHGNFG
 NSYVSWFAYWGQGLVTVSAGGGGSGGGGSGGGGSQAVVTQESALTTSPGETVTLTCRSSTGAVTTSNYANWVQEK
 PDHLFTGLIGGTNKRAPGVPARFSGSLIGDKAALTITGAQTEDEAIYFCALWYSNLWVFGGGTKLTVLGGSLDPKS
 SDKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQ
 YNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSREEMTKNQVSLTCLVKG
 FYPSDIAVEWESNGQPENNYKTTPVLDSDGSFFLYSKLTVDKSRWQQGNVFSQSVSMHEALHNHYTQKSLSLSPGK
] (SEQ ID NO: 52)

EXAMPLE 3. *In vitro* characterization of activatable anti-CD3 ϵ antibodies

[000573] This Example describes the ability of a masking moiety of the disclosure to reduce the ability of activatable anti-CD3 ϵ antibodies of the disclosure comprising such a masking moiety to bind to CD3 ϵ .

[000574] To determine if the masking peptides described in the previous examples could inhibit binding in the SP34 scFv-Fc context, a flow cytometry-based binding assay was performed. Jurkat T cells (Clone E6-1, ATCC, TIB-152) were cultured in RPMI-1640+glutamax (Life Technologies, Catalog 72400-120), 10% Heat Inactivated-Fetal Bovine Serum (HI-FBS, Life Technologies, Catalog 10438-026), 100 U/ml penicillin, and 100 μ g/ml streptomycin (Life Technologies, Catalog 15140-122) according to ATCC guidelines. Cells were harvested by centrifugation (200xg, 4°C, 5 min) and re-suspended in PBS supplemented with 2% HI-FBS (FACS Buffer). About 180,000 Jurkats per well were transferred to a 96-well U-bottom plate, harvested, and re-suspended in 50 μ L of primary antibody. The starting

concentration of activatable antibody was 1 μ M followed by 3-fold serial dilutions for a total of 12 concentrations. That following activatable antibodies were tested; 15003-1204-SP34scFv(LvHv)-Fc fusion; 15860-1204-SP34scFv(LvHv)-Fc fusion; 15864-1204-SP34scFv(LvHv)-Fc fusion; and 15865-1204-SP34scFv(LvHv)-Fc fusion; as was antibody SP34scFv(LvHv)-Fc.

[000575] Cells were incubated at 4°C with shaking for about 1 hour, harvested, and washed with 3x200 μ L of FACS Buffer. Jurkats were resuspended in 50 μ l Alexa Fluor 488 conjugated anti-Human IgG Fc (1:100 dilution, Jackson ImmunoResearch, Product 109-546-098) and incubated at 4°C with shaking for about 30 min. Cells were harvested, washed with 3x200 μ L of FACS Buffer, and resuspended in a final volume of 120 μ L of FACS Buffer. Samples were collected on a BD Accuri C6 (BD Biosciences) and the median fluorescence intensity (MFI) of viable cells was calculated using FlowJo V10 (Treestar). EC₅₀ values were calculated in GraphPad Prism 6 by curve fitting the data to log(agonist) vs. response (three parameters).

[000576] Figures 3A-3D demonstrate that incorporation of the masking peptides into the SP34 scFv-Fc context to produce activatable antibodies shifted the EC₅₀ values for CD3 ϵ binding from single digit to triple digit nM.

EXAMPLE 4. Bispecific activatable antibodies

[000577] This Example describes the production, expression and characterization of bispecific activatable antibodies of the disclosure.

[000578] *Vector Construction:* The heavy chain and light chain were cloned separately into a mammalian expression vector using standard molecular biology techniques. Briefly, DNA fragments encoding the region of interest were amplified with primers binding to the terminal ends. Overlapping fragments were combined and amplified with flanking primers as needed to build the entire desired region. DNA fragments were subsequently cloned into expression vector using a commercially available homologous recombination kit (MCLabs, South San Francisco, CA). The in-house mammalian expression vector is a modified version of cDNA3.1(+) from Invitrogen with selection marker of G418 or hygromycin. Mutations were introduced using the QuikChange Kit (Agilent, Santa Clara, CA).

[000579] Bispecific antibodies and bispecific activatable antibodies were expressed in mammalian cells using standard transfection kit (Life Technologies, Grand Island, NY). Briefly, 293 cells were transfected with nucleic acids using a lipid based system, following manufactures recommended protocol. Bispecific activatable antibodies and bispecific antibodies were purified from cell-free supernatant using Protein A beads (GE, Piscataway, NJ) and concentrated using standard buffer exchange columns (Millipore, Temecula, CA).

[000580] Table 11 below describes the heavy chains and light chains for various bispecific antibodies and bispecific activatable antibodies of the disclosure.

Table 11. Bispecific antibodies and bispecific activatable antibodies

Molecule Name	Molecule	Heavy Chain	Light Chain
CI005	C225v5N297Q-CD3-H-N	pLW019: HC C225v5N297Q-CD3HvLv-H-N	OPP007: LC C225
CI007	3954-1204-C225v5N297Q-CD3-H-N	pLW019: HC C225v5N297Q-CD3HvLv-H-N	OPP022: LC C225 3954-1204
CI009	C225v5N297Q-15865-CD3LvHv-H-N	pLW023: HC C225v5N297Q-15865-CD3LvHv-H-N	OPP007: LC C225
CI010	3954-1204-C225v5N297Q-15003-CD3LvHv-H-N	pLW022: HC C225v5N297Q-15003-CD3LvHv-H-N	OPP022: LC C225 3954-1204
CI011	3954-1204-C225v5N297Q-15865-CD3LvHv-H-N	pLW023: HC C225v5N297Q-15865-CD3LvHv-H-N	OPP022: LC C225 3954-1204
CI012	C225v5N297Q	pLW006: HC C225v5N297Q	OPP021: LC C225
CI015	C225v5N297Q-CD3LvHv-H-N	pLW057: HC C225v5N297Q-CD3LvHv-H-N	OPP021: LC C225
CI016	3954-1204-C225v5N297Q-CD3LvHv-H-N	pLW057: HC C225v5N297Q-CD3LvHv-H-N	OPP022: LC C225 3954-1204
CI021	C225v5N297Q-15003-CD3HvLv-H-N	pLW047: HC C225v5N297Q-15003-CD3HvLv-H-N	OPP021: LC C225

CI022	C225v5N297Q-15865- CD3HvLv-H-N	pLW048: HC C225v5N297Q-15865- CD3HvLv-H-N	OPP021: LC C225
CI023	3954-1204- C225v5N297Q-15003- CD3HvLv-H-N	pLW047: HC C225v5N297Q-15003- CD3HvLv-H-N	OPP022: LC C225 3954-1204
CI024	3954-1204- C225v5N297Q-15865- CD3HvLv-H-N	pLW048: HC C225v5N297Q-15865- CD3HvLv-H-N	OPP022: LC C225 3954-1204
CI025	IL6RN297Q	pLW078: HC IL6RN297Q	pLW077: LC IL6R
CI026	IL6RN297Q- CD3LvHv-H-N	pLW083: HC IL6RN297Q-CD3LvHv- H-N	pLW077: LC IL6R
CI027	IL6RN297Q- 15865_1204- CD3LvHv-H-N	pLW085: HC IL6RN297Q-15865_1204- CD3LvHv-H-N	pLW077: LC IL6R
CI028	IL6R 4792 Nsub N297Q-15865_1204- CD3LvHv-H-N	pLW085: HC IL6RN297Q-15865_1204- CD3LvHv-H-N	pLW079: LC IL6R 4792 Nsub
CI029	4792-1204- IL6RN297Q- 15865_1204- CD3LvHv-H-N	pLW085: HC IL6RN297Q-15865_1204- CD3LvHv-H-N	pLW080: LC IL6R 4792 1204 (C040)
CI030	IL6RN297Q- 15865_Nsub- CD3LvHv-H-N	pLW087: HC IL6RN297Q- 15865_Nsub-CD3LvHv- H-N	pLW077: LC IL6R
CI031	IL6R 4792 NsubN297Q- 15865_Nsub- CD3LvHv-H-N	pLW087: HC IL6RN297Q- 15865_Nsub-CD3LvHv- H-N	pLW079: LC IL6R 4792 Nsub
CI032	4792-1204- IL6RN297Q- 15865_Nsub- CD3LvHv-H-N	pLW087: HC IL6RN297Q- 15865_Nsub-CD3LvHv- H-N	pLW080: LC IL6R 4792 1204
CI036	4792-1204- IL6RN297Q- CD3LvHv-H-N	pLW083: HC IL6RN297Q-CD3LvHv- H-N	pLW080: LC IL6R 4792 1204
CI039	3954-2001-C225v5 N297Q-15865-2001- CD3LvHv-H-N	pLW101: HC C225v5N297Q-15865- 2001-CD3LvHv-H-N	LC C225-3954-2001

CI040	3954- 2001C225v5N297Q- 15865-2001- CD3LvHv-H-N	pLW101: HC C225v5N297Q-15865- 2001-CD3LvHv-H-N	LC C225-3954-2001
CI048	activated bispecific activatable antibody C225N297Q- *CD3LvHv-H-N	pLW023: HC C225v5N297Q-15865- CD3LvHv-H-N	OPP022: LC C225 3954-1204
CI049	3954-1204- C225v5N297Q-MC05- 2001-mCD3-H-N	pLW121: HC C225v5N297Q-MC05- 2001-mCD3-H-N	OPP022: LC C225 3954-1204
CI050	3954-1204- C225v5N297Q-MC06- 2001-mCD3-H-N	pLW122: HC C225v5N297Q-MC06- 2001-mCD3-H-N	OPP022: LC C225 3954-1204
CI051	3954-1204- C225v5N297Q-MC07- 2001-mCD3-H-N	pLW123: HC C225v5N297Q-MC07- 2001-mCD3-H-N	OPP022: LC C225 3954-1204
CI052	C225v5N297Q-mCD3- H-N	pLW100: HC C225v5N297Q-mCD3-H- N	OPP021: LC C225
CI053	3954-1204- C225v5N297Q-MC01- 2001-mCD3-H-N	pLW117: HC C225v5N297Q-MC01- 2001-mCD3-H-N	OPP022: LC C225 3954-1204
CI054	3954-1204- C225v5N297Q-MC02- 2001-mCD3-H-N	pLW118: HC C225v5N297Q-MC02- 2001-mCD3-H-N	OPP022: LC C225 3954-1204
CI055	3954-1204- C225v5N297Q-MC03- 2001-mCD3-H-N	pLW119: HC C225v5N297Q-MC03- 2001-mCD3-H-N	OPP022: LC C225 3954-1204
CI056	3954-1204- C225v5N297Q-MC04- 2001-mCD3-H-N	pLW120: HC C225v5N297Q-MC04- 2001-mCD3-H-N	OPP022: LC C225 3954-1204
CI057	3954-1204- C225v5N297Q-MC08- 2001-mCD3-H-N	pLW124: HC C225v5N297Q-MC08- 2001-mCD3-H-N	OPP022: LC C225 3954-1204
CI058	3954-1204- C225v5N297Q-MC09- 2001-mCD3-H-N	pLW125: HC C225v5N297Q-MC09- 2001-mCD3-H-N	OPP022: LC C225 3954-1204
CI059	BiTE	BiTE	

[000581] Table 12A provide the primers used for each construct, and Table 12B provides the sequences of the primers used.

Table 12A. Primers

Construct Name	Primers Used in Construction
BiTE	oLW017, oLW018
pLW006	oLW006, oLW007
pLW019	oLW048, oLW049, oLW050, oLW051
pLW022	oLW048, oLW061, oLW062, oLW063
pLW023	oLW048, oLW061, oLW062, oLW063
pLW047	oLW048, oLW049, oLW051, oLW098, oLW101, oLW102
pLW048	oLW048, oLW049, oLW051, oLW098, oLW101, oLW103
pLW057	oLW048, oLW056, oLW063, oLW109
pLW077	oLW048, oLW056, oLW115, oLW116
pLW078	oLW048, oLW049, oLW117, oLW118, oLW119, oLW120
pLW080	oLW048, oLW121
pLW083	oLW048, oLW123, oLW124, oLW125
pLW085	oLW048, oLW123, oLW124, oLW125
pLW101	oLW048, oLW151, oLW062, oLW063
pLW117	oLW048, oLW049, oLW178
pLW118	oLW048, oLW049, oLW178
pLW119	oLW048, oLW049, oLW178
pLW120	oLW048, oLW049, oLW178
pLW121	oLW048, oLW049, oLW178
pLW122	oLW048, oLW049, oLW178
pLW123	oLW048, oLW049, oLW178
pLW124	oLW048, oLW049, oLW178
pLW125	oLW048, oLW049, oLW178

Table 12B. Primer Sequences

Primer Name	Primer Sequence	SEQ ID NO:
oLW001	CATACACTGGTATCAGCAAAGAACACAGGGTTCTCCAAGGC	412
oLW002	GCCTTGGAGAACCCTGTGTTCTTTGCTGATACCAGTGTATG	413
oLW003	TTTAAAATGAACAGTCTGCAATCTCAGGACACAGCCATATAT TACTGTGCC	414

oLW004	GGCACAGTAATATATGGCTGTGTCCTGAGATTGCAGACTGTT CATTTTAAA	415
oLW006	CGGGAGGAGCAGTACCAGAGCACGTACCGTGTG	416
oLW007	CACACGGTACGTGCTCTGGTACTGCTCCTCCCG	417
oLW017	CACCCCATTGACGTCAATGGGAG	418
oLW018	GAGGGGGCAAACAACAGATGGCTG	419
oLW048	TGAACCGTCAGATCACTAGAAGCTTTATTGC	420
oLW049	TGGATGTGCACCAGGTGTGACAGC	421
oLW050	CTGGCTGTCACACCTGGTGCACATCCAGAGGTGCAGCTGGTC GAGTCTGG	422
oLW051	AGCTGCACCTGGGATCCACCACCTCCTAGGACAGTCAGTTTG GTTCTCCACC	423
oLW056	ACATCTAGCACCCACGCAGCCATAGTAGC	424
oLW061	GTGACCACGGTCTGAGAACCGCCGCCATGATTATCGG	425
oLW062	GGCGGCGGTTCTCAGACCGTGGTCACACAGGAGCC	426
oLW063	CTCTGTTTCAGCTGCACCTGGGATCCACCACCTCCTGAGGAG ACGGTGACCAGTGTCCC	427
oLW098	AGAACCGCCGCCATGATTATCG	428
oLW101	CGTTCCGATAATCATGGCGGCGGTTCTGAGGTGCAGCTGGTC GAGTCTGG	429
oLW102	CTGGCTGTCACACCTGGTGCACATCCACAAGGCCAGTCTGGC CAAGGTTATC	430
oLW103	CTGGCTGTCACACCTGGTGCACATCCACAAGGCCAGTCTGGC CAAATGATG	431
oLW109	CTCCTGCTACTATGGCTGCGTGGTGCTAGATGTCAGACCGTG GTCACACAGGAGCC	432
oLW115	CTCCTGCTACTATGGCTGCGTGGTGCTAGATGTGACATCCAG ATGACTCAGTCTCCTAGCTCC	433
oLW116	GAAGACAGATGGTGCAGCCACCG	434

oLW117	CTGGCTGTCACACCTGGTGCACATCCACAGGTGCAGCTGCAG GAGTCCG	435
oLW118	GCTAGCACCAAGGGCCCATCG	436
oLW119	GGAAGACCGATGGGCCCCTTGG	437
oLW120	GCCCTCTAGACTCGAGCGGCCGCTCATTTACCCGGAGACAGG GAGAG	438
oLW121	GCAGGACCCATACTGGCCAGACTGGCCTTGACATCTAGCACC ACGCAGCCATAGTAGC	439
oLW123	GGAGGTGGTGGATCCCAGGTGCAGCTGCAGGAGTCCG	440
oLW124	CAGCTGCACCTGGGATCCACCACCTCCTGAGGAGACG	441
oLW125	ACCGATGGGCCCCTTGGTGCTAG	442
oLW151	GACCACGGTCTGAGAACCGCCG	443
oLW178	CACCTGGTGCACATCCACAAGGC	444

[000582] The sequences for the bispecific antibodies and bispecific activatable antibodies are shown below. In some embodiments, the activatable antibody also includes a spacer sequence. In some embodiments, the spacer is joined directly to the MM of the activatable antibody. In some embodiments, the spacer is joined directly to the MM of the activatable antibody in the structural arrangement from N-terminus to C-terminus of spacer-MM-CM-AB. In some embodiments, the spacer joined directly to the N-terminus of MM of the activatable antibody is selected from the group consisting of QGQSGQG (SEQ ID NO: 407), QGQSGQ (SEQ ID NO: 87), QGQSG (SEQ ID NO: 408), QGQS (SEQ ID NO: 409), QGQ (SEQ ID NO: 410), QG (SEQ ID NO: 411), and Q. In some embodiments, the spacer includes at least the amino acid sequence QGQSGQG (SEQ ID NO: 412). In some embodiments, the spacer includes at least the amino acid sequence QGQSGQG (SEQ ID NO: 407). In some embodiments, the spacer includes at least the amino acid sequence QGQSGQ (SEQ ID NO: 87). In some embodiments, the spacer includes at least the amino acid sequence QGQSG (SEQ ID NO: 408). In some embodiments, the spacer includes at least the amino acid sequence QGQS (SEQ ID NO: 409). In some embodiments, the spacer includes at least the amino acid sequence QGQ (SEQ ID NO: 410). In some embodiments, the spacer includes at least the

amino acid sequence QG (SEQ ID NO: 411). In some embodiments, the spacer includes at least the amino acid residue Q.

[000583] While some of the sequences shown below include the spacer sequence of SEQ ID NO: 362, those of ordinary skill in the art appreciate that the multispecific activatable antibodies of the disclosure can include any suitable spacer sequence, such as, for example, a spacer sequence selected from the group consisting of QGQSGQG (SEQ ID NO: 407), QGQSGQ (SEQ ID NO: 87), QGQSG (SEQ ID NO: 408), QGQS (SEQ ID NO: 409), QGQ (SEQ ID NO: 410), QG (SEQ ID NO: 411), and Q.

CI005: C225v5N297Q-CD3-H-N

pLW019: HC C225v5N297Q-CD3HvLv-H-N

Nucleotide Sequence

GAGGTGCAGCTGGTTCGAGTCTGGAGGAGGATTGGTGCAGCCTGGAGGGTCATTGAAACTCTCATGTGCAGCCTCTG
GATTCACCTTCAATAAGTACGCCATGAACTGGGTCCGCCAGGCTCCAGGAAAGGGTTTGGAATGGGTTGCTCGCAT
AAGAAGTAAATATAATAATTATGCAACATATTATGCCGATTCAGTGAAAGACAGGTTACCATCTCCAGAGATGAT
TCAAAAAACACTGCCTATCTACAAATGAACAACTTGAAACTGAGGACACTGCCGTGTACTACTGTGTGAGACATG
GGAACCTTCGGTAATAGCTACATATCCTACTGGGCTTACTGGGGCCAAGGGACTCTGGTCACCGTCTCCTCAGGTGG
TGGTGGTTCTGGCGGCGGCGGCTCCGGTGGTGGTGGTTCTCAGACTGTTGTGACTCAGGAACCTTCACTCACCGTA
TCACCTGGTGGAACAGTCACACTCACTTGTGGCTCCTCGACTGGGGCTGTTACATCTGGCTACTACCCAAACTGGG
TCCAACAAAAACCAGGTCAGGCACCCCGTGGTCTAATAGGTGGGACTAAGTTCCTCGCCCCCGGTACTCCTGCCAG
ATTCTCAGGCTCCCTGCTTGGAGGCAAGGCTGCCCTCACCTCTCAGGGGTACAGCCAGAGGATGAGGCAGAATAT
TACTGTGCTCTATGGTACAGCAACCGCTGGGTGTTCCGGTGGAGGAACCAAACCTGACTGTCCTAGGAGGTGGTGGAT
CCCAGGTGCAGCTGAAACAGAGCGGCCCCGGGCCTGGTGCAGCCGAGCCAGAGCCTGAGCATTACCTGCACCGTGAG
CGGCTTTAGCCTGACCAACTATGGCGTGCATTGGGTGCGCCAGAGCCCCGGGCAAAGGCCTGGAATGGCTGGGCGTG
ATTTGGAGCGGCGGCAACACCGATTATAACACCCCGTTTACCAGCCGCCTGAGCATTAAACAAAGATAACAGCAAAA
GCCAGGTGTTTTTTTAAATGAACAGCCTGCAAAGCCAGGATACCGCGATTTATTATTGCGCGCGCGCGCTGACCTA
TTATGATTATGAATTTGCGTATTGGGGCCAGGGCACCCCTGGTGACCGTGAGCGCGGCTAGCACCAAGGGCCCATCG
GTCTTCCCCCTGGCACCCCTCCTCCAAGAGCACCTCTGGGGGCACAGCGGCCCTGGGCTGCCTGGTCAAGGACTACT
TCCCCGAACCGGTGACGGTGTCTGGAACCTCAGGCGCCCTGACCAGCGGCGTGCACACCTTCCCGGCTGTCCTACA
GTCCTCAGGACTCTACTCCCTCAGCAGCGTGGTGACCGTGCCCTCCAGCAGCTTGGGCACCCAGACCTACATCTGC
AACGTGAATCACAAGCCCAGCAACACCAAGGTGGACAAGAAAGTTGAGCCCAAATCTTGTGACAAAACCTCACACAT
GCCCACCGTGCCCAGCACCTGAACTCCTGGGGGGACCGTCAGTCTTCCTCTTCCCCCAAACCCAAGGACACCCT
CATGATCTCCCGGACCCCTGAGGTCACATGCGTGGTGGTGGACGTGAGCCACGAAGACCCTGAGGTCAAGTTCAAC
TGGTACGTGGACGGCGTGGAGGTGCATAATGCCAAGACAAAGCCGCGGGAGGAGCAGTACCAGAGCACGTACCGTG

TGGTCAGCGTCCTCACCGTCCTGCACCAGGACTGGCTGAATGGCAAGGAGTACAAGTGCAAGGTCTCCAACAAAGC
 CCTCCCAGCCCCCATCGAGAAAACCATCTCCAAAGCCAAAGGGCAGCCCCGAGAACCACAGGTGTACACCCTGCCC
 CCATCCCGGGAGGAGATGACCAAGAACCAGGTCAGCCTGACCTGCCTGGTCAAAGGCTTCTATCCCAGCGACATCG
 CCGTGGAGTGGGAGAGCAATGGGCAGCCGGAGAACAACACTACAAGACCACGCCTCCCGTGCTGGACTCCGACGGCTC
 CTTCTTCTCTACAGCAAGCTCACCGTGGACAAGAGCAGGTGGCAGCAGGGGAACGTCTTCTCATGCTCCGTGATG
 CATGAGGCTCTGCACAACCACTACACGCAGAAGAGCCTCTCCCTGTCTCCGGGTAAATGA (SEQ ID
 NO: 445)

Amino Acid Sequence

EVQLVESGGGLVQPGGSLKLSCAASGFTFNKYAMNWVRQAPGKGLEWVARIRSKYNNYATYYADSVKDRFTISRDD
 SKNTAYLQMNNLKTEDTAVYYCVRHGNFGNSYISYWAYWGQGLTVTVSSGGGGSGGGGSGGGGSQTVVTQEPSLTV
 SPGGTVTLTCSSTGAVTSGYYPNWVQQKPGQAPRGLIGGTKFLAPGTPARFSGSLLGGKAALTLSGVQPEDEAEY
 YCALWYSNRWVFGGGTKLTVLGGGGSQVQLKQSGPGLVQPSQSL SITCTVSGFSLTNYGVHWVRQSPGKGLEWLGV
 IWSGGNTDYNTPFTSRLSINKDNSKSQVFFKMNSLQSQDTAIYYCARALTYDYEFAYWGQGLTVTVSAASTKGPS
 VFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVTVTPSSSLGTQTYIC
 NVNHKPSNTKVDKKVEPKSCDKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVDVSHEDPEVKFN
 WYVDGVEVHNAKTKPREEQYQSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLF
 PSREEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFCFSVM
 HEALHNHYTQKSLSLSPGK* (SEQ ID NO: 446)

OPP007: LC C225 IL2ss

Nucleotide Sequence

GATATCTTGCTGACCCAGAGCCCGGTGATTCTGAGCGTGAGCCCGGGCGAACGTGTGAGCTTTAGCTGCCGCGCGA
 GCCAGAGCATTGGCACCAACATTCATTGGTATCAGCAGCGCACCAACGGCAGCCCGCGCCTGCTGATTAAATATGC
 GAGCGAAAGCATTAGCGGCATTCCGAGCCGCTTTAGCGGCAGCGGCAGCGGCACCGATTTTACCCTGAGCATTAAC
 AGCGTGGAAGCGAAGATATTGCGGATTATTATTGCCAGCAGAACAACTGGCCGACCACCTTTGGCGCGGGCA
 CCAAAGTGGAACTGAAACGTACGGTGGCTGCACCATCTGTCTTCATCTTCCCGCCATCTGATGAGCAGTTGAAATC
 TGGAAGTGCCTCTGTTGTGTGCCTGCTGAATAACTTCTATCCCAGAGAGGCCAAAGTACAGTGGAAGGTGGATAAC
 GCCCTCCAATCGGGTAACTCCCAGGAGAGTGTACAGAGCAGGACAGCAAGGACAGCACCTACAGCCTCAGCAGCA
 CCCTGACGCTGAGCAAAGCAGACTACGAGAAACACAAAGTCTACGCCTGCGAAGTCACCCATCAGGGCCTGAGCTC
 GCCCGTCACAAAGAGCTTCAACAGGGGAGAGTGTTAG (SEQ ID NO: 447)

Amino Acid Sequence

DILLTQSPVILSVSPGERVSFSCRASQSIGTNIHWYQQRNGSPRLLIKYASESISGIPSRFSGSGSGTDFTLIN
 SVESEDIADYYCQQNNNWPTTFGAGTKLELKRVAAPSVFIFPPSDEQLKSGTASVVCLLNNFYPREAKVQWKVDN
 ALQSGNSQESVTEQDSKDYSLSTLTLSKADYEKHKVYACEVTHQGLSSPVTKSFNRGEC* (SEQ ID
 NO: 448)

CI007: 3954-1204-C225v5N297Q-CD3-H-N

pLW019: HC C225v5N297Q-CD3HvLv-H-N

Nucleotide Sequence

GAGGTGCAGCTGGTCGAGTCTGGAGGAGGATTGGTGCAGCCTGGAGGGTCATTGAAACTCTCATGTGCAGCCTCTG
GATTCACCTTCAATAAGTACGCCATGAACTGGGTCCGCCAGGCTCCAGGAAAGGGTTTGGAATGGGTGCTCGCAT
AAGAAGTAAATATAATAATTATGCAACATATTATGCCGATTCAGTGAAAGACAGGTTTACCATCTCCAGAGATGAT
TCAAAAAACACTGCCTATCTACAAATGAACAACTTGAAAACAGAGGACACTGCCGTGTACTACTGTGTGAGACATG
GGAACCTTCGGTAATAGCTACATATCCTACTGGGCTTACTGGGGCCAAGGGACTCTGGTACCGTCTCCTCAGGTGG
TGGTGGTTCTGGCGGCGGCGGCTCCGGTGGTGGTGGTTCTCAGACTGTTGTGACTCAGGAACCTTCACTCACCGTA
TCACCTGGTGGAACAGTCACACTCACTTGTGGCTCCTCGACTGGGGCTGTTACATCTGGCTACTACCCAAACTGGG
TCCAACAAAAACCAGGTCAGGCACCCCGTGGTCTAATAGGTGGGACTAAGTTCCTCGCCCCCGGTACTCCTGCCAG
ATTCTCAGGCTCCCTGCTTGGAGGCAAGGCTGCCCTCACCTCTCAGGGGTACAGCCAGAGGATGAGGCAGAATAT
TACTGTGCTCTATGGTACAGCAACCGCTGGGTGTTTCGGTGGAGGAACCAAACTGACTGTCCTAGGAGGTGGTGGAT
CCCAGGTGCAGCTGAAACAGAGCGGCCCCGGGCCTGGTGCAGCCGAGCCAGAGCCTGAGCATTACCTGCACCGTGAG
CGGCTTTAGCCTGACCAACTATGGCGTGCATTGGGTGCGCCAGAGCCCCGGGCAAAGGCCTGGAATGGCTGGGCGTG
ATTTGGAGCGGCGGCAACACCGATTATAACACCCCGTTTACCAGCCGCCTGAGCATTAAACAAAGATAACAGCAAAA
GCCAGGTGTTTTTTTAAATGAACAGCCTGCAAAGCCAGGATACCGCGATTTATTATTGCGCGCGCGCGCTGACCTA
TTATGATTATGAATTTGCGTATTGGGGCCAGGGCACCTGGTGACCGTGAGCGCGGCTAGCACCAAGGGCCCATCG
GTCTTCCCCCTGGCACCCCTCCTCCAAGAGCACCTCTGGGGGCACAGCGGCCCTGGGCTGCCTGGTCAAGGACTACT
TCCCCGAACCGGTGACGGTGTCTGTGGAACCTCAGGCGCCCTGACCAGCGGCGTGACACCTTCCCGGCTGTCCTACA
GTCCTCAGGACTCTACTCCCTCAGCAGCGTGGTGACCGTGCCCTCCAGCAGCTTGGGCACCCAGACCTACATCTGC
AACGTGAATCACAAGCCCAGCAACACCAAGGTGGACAAGAAAGTTGAGCCCAAATCTTGTGACAAAACCTCACACAT
GCCCACCGTGCCCAGCACCTGAACTCCTGGGGGGACCGTCAGTCTTCCTCTTCCCCCCCCAAAACCCAAGGACACCCCT
CATGATCTCCCGGACCCCTGAGGTCACATGCGTGGTGGTGGACGTGAGCCACGAAGACCCTGAGGTCAAGTTCAAC
TGGTACGTGGACGGCGTGGAGGTGCATAATGCCAAGACAAAGCCGCGGGAGGAGCAGTACCAGAGCACGTACCGTG
TGGTCAGCGTCCTCACCGTCCTGCACCAGGACTGGCTGAATGGCAAGGAGTACAAGTGCAAGGTCTCCAACAAAGC
CCTCCCAGCCCCCATCGAGAAAACCATCTCCAAAGCCAAAGGGCAGCCCCGAGAACCACAGGTGTACACCCTGCCC
CCATCCCGGGAGGAGATGACCAAGAACCAGGTCAGCCTGACCTGCCTGGTCAAAGGCTTCTATCCCAGCGACATCG
CCGTGGAGTGGGAGAGCAATGGGCAGCCGGAGAACAACCTACAAGACCACGCCTCCCGTGCTGGACTCCGACGGCTC
CTTCTTCCTCTACAGCAAGCTCACCGTGGACAAGAGCAGGTGGCAGCAGGGGAACGTCTTCTCATGCTCCGTGATG
CATGAGGCTCTGCACAACCACTACACGCAGAAGAGCCTCTCCCTGTCTCCGGGTAAATGA (SEQ ID

NO: 445)

Amino Acid Sequence

EVQLVESGGGLVQPGGSLKLSCAASGFTFNKYAMNWVRQAPGKGLEWVARIRSKYNNYATYYADSVKDRFTISRDD
 SKNTAYLQMNNLKTEDTAVYYCVRHGNFGNSYISYWAYWGQGLTVTVSSGGGGSGGGGSGGGGSQTVVTQEPSLTV
 SPGGTVTLTCGSSTGAVTSGYYPNWVQQKPGQAPRGLIGGTKFLAPGTPARFSGSLLGGKAALTLSGVQPEDEAEY
 YCALWYSNRWVFGGGTKLTVLGGGGSQVQLKQSGPGLVQPSQSLSITCTVSGFSLTNYGVHWVRQSPGKGLEWLGV
 IWSGGNTDYNTPFTSRLSINKDNSKSQVFFKMNSLQSQDTAIYYCARALTYDYEFAYWGQGLTVTVSAASTKGPS
 VFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSSLGTQTYIC
 NVNHKPSNTKVDKKVEPKSCDKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVDVSHEDPEVKFN
 WYVDGVEVHNAKTKPREEQYQSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLF
 PSREEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTTPVLDSDGSFFLYSKLTVDKSRWQQGNVFSQSV
 HEALHNHYTQKSLSLSPGK* (SEQ ID NO: 446)

OPP022: LC C225 3954-1204

Nucleotide Sequence

[spacer (SEQ ID NO: 507)][OPP022 without spacer (SEQ ID NO: 509)]
 [CAAGGCCAGTCTGGCCAG][TGCATCTCACCTCGTGGTTGTCCGGACGGCCCATACGTCATGTACGGCTCGAGCG
 GTGGCAGCGGTGGCTCTGGTGGATCCGGTCTGAGCGGCCGTTCCGATAATCATGGCAGTAGCGGTACCCAGATCTT
 GCTGACCCAGAGCCCGGTGATTCTGAGCGTGAGCCCGGGCGAACGTGTGAGCTTTAGCTGCCGCGCGAGCCAGAGC
 ATTGGCACCAACATTTCATTGGTATCAGCAGCGCACCAACGGCAGCCCGCGCCTGCTGATTAAATATGCGAGCGAAA
 GCATTAGCGGCATTCCGAGCCGCTTTAGCGGCAGCGGCAGCGGCACCGATTTTACCCTGAGCATTAAACAGCGTGGA
 AAGCGAAGATATTGCGGATTATTATTGCCAGCAGAACAACAACACTGGCCGACCACCTTTGGCGCGGGCACCAAACTG
 GAACTGAAACGTACGGTGGCTGCACCATCTGTCTTCATCTTCCCGCCATCTGATGAGCAGTTGAAATCTGGAACCTG
 CCTCTGTTGTGTGCCTGCTGAATAACTTCTATCCCAGAGAGGCCAAAGTACAGTGGAAGGTGGATAACGCCCTCCA
 ATCGGGTAACTCCCAGGAGAGTGTACAGAGCAGGACAGCAAGGACAGCACCTACAGCCTCAGCAGCACCCCTGACG
 CTGAGCAAAGCAGACTACGAGAAACACAAAGTCTACGCCTGCGAAGTCACCCATCAGGGCCTGAGCTCGCCCGTCA
 CAAAGAGCTTCAACAGGGGAGAGTGTTAG] (SEQ ID NO: 449)

Amino Acid Sequence

[spacer (SEQ ID NO: 87)][OPP022 without spacer (SEQ ID NO: 508)]
 [QGQSGQ][CISPRGCPDGPYVMYGSSGGSGGSGGSLSGRSDNHGSSGTQILLTQSPVILSVSPGERVSFSCRAS
 QSIGTNIHWYQQRRTNGSPRLLIKYASESISGIPSRFSGSGSGTDFTLINSVESEDIADYYCQQNNNWPTTFGAGT
 KLELKRTVAAPSVFIFPPSDEQLKSGTASVVCLLNNFYPREAKVQWKVDNALQSGNSQESVTEQDSKDSTYSLSST
 LTLSKADYEKHKVYACEVTHQGLSSPVTKSFNRGEC]* (SEQ ID NO: 450)

CI009: C225v5N297Q-15865-CD3LvHv-H-N

pLW023: C225v5N297Q-15865-CD3LvHv-H-N

Nucleotide Sequence

[spacer (SEQ ID NO: 507)][pLW023 without spacer (SEQ ID NO: 511)]
 [CAAGGCCAGTCTGGCCAA][ATGATGTATTGCGGTGGGAATGAGGTGTTGTGCGGGCCGCGGGTTGGCTCGAGCG
 GTGGCAGCGGTGGCTCTGGTGGTCTGAGCGGCCGTTCCGATAATCATGGCGGCGGTTCTCAGACCGTGGTCACACA
 GGAGCCCTCACTGACAGTGAGCCCTGGCGGGACCGTCACACTGACTTGTGCGCAGTTCAACTGGCGCCGTGACTACC
 AGCAATTACGCTAACTGGGTCCAGCAGAAACCAGGACAGGCACCACGAGGACTGATCGGAGGAACTAATAAGAGAG
 CACCAGGAACCCCTGCAAGGTTCTCCGGATCTCTGCTGGGGGGAAAAGCCGCTCTGACACTGAGCGGCGTGCAGCC
 TGAGGACGAAGCTGAGTACTATTGCGCACTGTGGTACTCCAACCTGTGGGTGTTTGGCGGGGGAACTAAGCTGACC
 GTCCTGGGAGGAGGAGGAAGCGGAGGAGGAGGGAGCGGAGGAGGAGGATCCGAAGTGCAGCTGGTCGAGAGCGGAG
 GAGGACTGGTGCAGCCAGGAGGATCCCTGAAGCTGTCTTGTGCAGCCAGTGGCTTCACCTTCAACACTTACGCAAT
 GAACTGGGTGCGGCAGGCACCTGGGAAGGGACTGGAATGGGTGCGCCGGATCAGATCTAAATACAATAACTATGCC
 ACCTACTATGCTGACAGTGTGAAGGATAGGTTACCATTTTACGCGACGATAGCAAAAACACAGCTTATCTGCAGA
 TGAATAACCTGAAGACCGAGGATACAGCAGTGTACTATTGCGTCAGACACGGCAATTTTCGGGAACCTTTACGTGAG
 TTGGTTTGCCTATTGGGGACAGGGGACACTGGTCACCGTCTCCTCAGGAGGTGGTGGATCCCAGGTGCAGCTGAAA
 CAGAGCGGCCCGGGCCTGGTGCAGCCGAGCCAGAGCCTGAGCATTACCTGCACCGTGAGCGGCTTTAGCCTGACCA
 ACTATGGCGTGCATTGGGTGCGCCAGAGCCCGGGCAAAGGCCTGGAATGGCTGGGCGTGATTTGGAGCGGCGGCAA
 CACCGATTATAACACCCCGTTTACCAGCCGCTGAGCATTAACAAAGATAACAGCAAAAGCCAGGTGTTTTTTAAA
 ATGAACAGCCTGCAAAGCCAGGATACCGCGATTTATTATTGCGCGCGCGCGCTGACCTATTATGATTATGAATTTG
 CGTATTGGGGCCAGGGCACCCCTGGTGACCGTGAGCGCGGCTAGCACCAAGGGCCCATCGGTCTTCCCCCTGGCACC
 CTCCTCCAAGAGCACCTCTGGGGGCACAGCGGCCCTGGGCTGCCTGGTCAAGGACTACTTCCCCGAACCGGTGACG
 GTGTCGTGGAACCTCAGGCGCCCTGACCAGCGGCGTGACACCTTCCCGGCTGTCCTACAGTCCTCAGGACTCTACT
 CCCTCAGCAGCGTGGTGACCGTGCCCTCCAGCAGCTTGGGCACCCAGACCTACATCTGCAACGTGAATCACAAGCC
 CAGCAACACCAAGGTGGACAAGAAAGTTGAGCCCAAATCTTGTGACAAAACCTCACACATGCCCACCGTGCCCAGCA
 CCTGAACTCCTGGGGGGACCGTCAGTCTTCCTCTTCCCCCAAACCCAAGGACACCCTCATGATCTCCCGGACCC
 CTGAGGTACATGCGTGGTGGTGGACGTGAGCCACGAAGACCCTGAGGTCAAGTTCAACTGGTACGTGGACGGCGT
 GGAGGTGCATAATGCCAAGACAAAGCCGCGGGAGGAGCAGTACCAGAGCACGTACCGTGTGGTCAGCGTCCTCACC
 GTCCTGCACCAGGACTGGCTGAATGGCAAGGAGTACAAGTGCAAGGTCTCCAACAAAGCCCTCCCAGCCCCCATCG
 AGAAAACCATCTCCAAAGCCAAAGGGCAGCCCCGAGAACCACAGGTGTACACCCTGCCCCCATCCCGGGAGGAGAT
 GACCAAGAACCAGGTGAGCCTGACCTGCCTGGTCAAAGGCTTCTATCCCAGCGACATCGCCGTGGAGTGGGAGAGC
 AATGGGCAGCCGGAGAACAACCTACAAGACCACGCCTCCCGTGCTGGACTCCGACGGCTCCTTCTTCTCTACAGCA
 AGCTCACCGTGGACAAGAGCAGGTGGCAGCAGGGGAACGTCTTCTCATGCTCCGTGATGCATGAGGCTCTGCACAA
 CCACTACACGCAGAAGAGCCTCTCCCTGTCTCCGGGTAAATGA] (SEQ ID NO: 451)

Amino Acid Sequence

[spacer (SEQ ID NO: 87)][pLW023 without spacer (SEQ ID NO: 512)]
 [QGQSGQ][MMYCGGNEVLCGPRVGSSGGSGGSGGLSGRSDNHGGGSQTVVTQEPSLTVSPGGTVTLTCRSSTGAV
 TTSNYANWVQQKPGQAPRGLIGGTNKRAPGTPARFSGSLLGGKAALTLSGVQPEDEAEYYCALWYSNLWVFGGGTK
 LTVLGGGGSGGGGSGGGGSEVQLVESGGGLVQPGGSLKLSCAASGFTFNTYAMNWVRQAPGKGLEWVARIRSKYNN
 YATYYADSVKDRFTISRDDSKNTAYLQMNNLKTEDTAVYYCVRHGNFGNSYVSWFAYWGQGTLLTVTVSSGGGGSQVQ
 LKQSGPGLVQPSQSL SITCTVSGFSLTNYGVHWVRQSPGKGLEWLGVWSGGNTDYNT PFTSRLSINKDNSKSQVF
 FKMNSLQSQDTAIYYCARALTYDYEFAYWGQGTLLTVVSAASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEP
 VTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSSLGTQTYICNVNHKPSNTKVDKKVEPKSCDKTHTCPPC
 PAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYQSTYRVVSV
 LTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSREEMTKNQVSLTCLVKGFYPSDIAVEW
 ESNGQPENNYKTTTPVLDSDGSFFLYSKLTVDKSRWQQGNVVFSCSVMHEALHNHYTQKSLSLSPGK]* (SEQ ID
 NO: 452)

OPP007: LC C225 IL2ss

Nucleotide Sequence

GATATCTTGCTGACCCAGAGCCCGGTGATTCTGAGCGTGAGCCCGGGCGAACGTGTGAGCTTTAGCTGCCGCGCGA
 GCCAGAGCATTGGCACCAACATTCATTGGTATCAGCAGCGCACCAACGGCAGCCCGCGCCTGCTGATTAAATATGC
 GAGCGAAAGCATTAGCGGCATTCCGAGCCGCTTTAGCGGCAGCGGCAGCGGCACCGATTTTACCCTGAGCATTAAAC
 AGCGTGGAAGCGAAGATATTGCGGATTATTATTGCCAGCAGAACAACAACACTGGCCGACCACCTTTGGCGCGGGCA
 CCAAACCTGGAACCTGAAACGTACGGTGGCTGCACCATCTGTCTTCATCTTCCCGCCATCTGATGAGCAGTTGAAATC
 TGGAACCTGCCTCTGTTGTGTGCCTGCTGAATAACTTCTATCCCAGAGAGGCCAAAGTACAGTGGAAGGTGGATAAC
 GCCCTCCAATCGGGTAACTCCCAGGAGAGTGTACAGAGCAGGACAGCAAGGACAGCACCTACAGCCTCAGCAGCA
 CCCTGACGCTGAGCAAAGCAGACTACGAGAAACACAAAGTCTACGCCTGCGAAGTCACCCATCAGGGCCTGAGCTC
 GCCCGTCACAAAGAGCTTCAACAGGGGAGAGTGTTAG (SEQ ID NO: 447)

Amino Acid Sequence

DILLTQSPVILSVSPGERVSFSCRASQSIGTNIHWYQQRTNGSPRLLIKYASESISGIPSRFSGSGSGTDFTLSIN
 SVESEDIADYYCQQNNNWPTTFGAGTKLELKRVAAPSVFIFPPSDEQLKSGTASVVCLLNNFYPREAKVQWKVDN
 ALQSGNSQESVTEQDSKDYSLSTLTLSKADYEKHKVYACEVTHQGLSSPVTKSFNRGEC* (SEQ ID
 NO: 448)

CI010: 3954-1204-C225v5N297Q-15003-CD3LvHv-H-N

pLW022: C225v5N297Q-15865-CD3LvHv-H-N

Nucleotide Sequence

[spacer (SEQ ID NO: 507)][pLW022 without spacer (SEQ ID NO: 895)]

[CAAGGCCAGTCTGGCCAA] [GGTTATCGGTGGGGTTGCGAGTGGAATTGCGGTGGGATTACTACTGGCTCGAGCG
 GTGGCAGCGGTGGCTCTGGTGGTCTGAGCGGCCGTTCCGATAATCATGGCGGCGGTCTCAGACCGTGGTCACACA
 GGAGCCCTCACTGACAGTGAGCCCTGGCGGGACCGTCACACTGACTTGTGCGCAGTTCAACTGGCGCCGTGACTACC
 AGCAATTACGCTAACTGGGTCCAGCAGAAACCAGGACAGGCACCACGAGGACTGATCGGAGGAACATAAAGAGAG
 CACCAGGAACCCCTGCAAGGTTCTCCGGATCTCTGCTGGGGGGAAAAGCCGCTCTGACACTGAGCGGCGTGCAGCC
 TGAGGACGAAGCTGAGTACTATTGCGCACTGTGGTACTCCAACCTGTGGGTGTTTGGCGGGGGAACATAAGCTGACC
 GTCCTGGGAGGAGGAGGAAGCGGAGGAGGAGGGAGCGGAGGAGGAGGATCCGAAGTGCAGCTGGTTCGAGAGCGGAG
 GAGGACTGGTGCAGCCAGGAGGATCCCTGAAGCTGTCTTGTGCAGCCAGTGGCTTCACCTTCAACACTTACGCAAT
 GAACTGGGTGCGGCAGGCACCTGGGAAGGGACTGGAATGGGTGCGCCCGGATCAGATCTAAATACAATAACTATGCC
 ACCTACTATGCTGACAGTGTGAAGGATAGGTTTACCATTTCACGCGACGATAGCAAAAACACAGCTTATCTGCAGA
 TGAATAACCTGAAGACCGAGGATACAGCAGTGTACTATTGCGTCAGACACGGCAATTTCCGGAACTCTTACGTGAG
 TTGGTTTGCCTATTGGGGACAGGGGACACTGGTCAACGCTCTCCTCAGGAGGTGGTGGATCCCAGGTGCAGCTGAAA
 CAGAGCGGCCCGGGCCTGGTGCAGCCGAGCCAGAGCCTGAGCATTACCTGCACCGTGAGCGGCTTTAGCCTGACCA
 ACTATGGCGTGCATTGGGTGCGCCAGAGCCCGGGCAAAGGCCTGGAATGGCTGGGCGTGATTGGAGCGGCGGCAA
 CACCGATTATAACACCCCGTTTACCAGCCGCCTGAGCATTAAACAAAGATAACAGCAAAAGCCAGGTGTTTTTTAA
 ATGAACAGCCTGCAAAGCCAGGATACCGCGATTTATTATTGCGCGCGCGCGCTGACCTATTATGATTATGAATTTG
 CGTATTGGGGCCAGGGCACCCCTGGTGACCGTGAGCGCGGCTAGCACCAAGGGCCCATCGGTCTTCCCCCTGGCACC
 CTCCTCCAAGAGCACCTCTGGGGGCACAGCGGCCCTGGGCTGCCTGGTCAAGGACTACTTCCCCGAACCGGTGACG
 GTGTCGTGGAACCTCAGGCGCCCTGACCAGCGGCGTGACACCTTCCCGGCTGTCTTACAGTCCTCAGGACTCTACT
 CCCTCAGCAGCGTGGTGACCGTGCCCTCCAGCAGCTTGGGCACCCAGACCTACATCTGCAACGTGAATCACAAGCC
 CAGCAACACCAAGGTGGACAAGAAAGTTGAGCCCAAATCTTGTGACAAAACCTCACACATGCCACCGTGCCAGCA
 CCTGAACTCCTGGGGGGACCGTCAGTCTTCTCTTCCCCCAGAAACCAAGGACACCCCTCATGATCTCCCGGACCC
 CTGAGGTACATGCGTGGTGGTGGACGTGAGCCACGAAGACCCTGAGGTCAAGTTCAACTGGTACGTGGACGGCGT
 GGAGGTGCATAATGCCAAGACAAAGCCGCGGGAGGAGCAGTACCAGAGCACGTACCGTGTGGTCAGCGTCCTCACC
 GTCCTGCACCAGGACTGGCTGAATGGCAAGGAGTACAAGTGCAAGGTCTCCAACAAAGCCCTCCAGCCCCCATCG
 AGAAAACCATCTCCAAAGCCAAAGGGCAGCCCCGAGAACCACAGGTGTACACCCTGCCCCCATCCCGGGAGGAGAT
 GACCAAGAACCAGGTCAGCCTGACCTGCCTGGTCAAAGGCTTCTATCCCAGCGACATCGCCGTGGAGTGGGAGAGC
 AATGGGCAGCCGGAGAACAATAAGACCACGCCTCCCGTGCTGGACTCCGACGGCTCCTTCTTCTCTACAGCA
 AGCTCACCGTGGACAAGAGCAGGTGGCAGCAGGGGAACGTCTTCTCATGCTCCGTGATGCATGAGGCTCTGCACAA
 CCACTACACGCAGAAGAGCCTCTCCCTGTCTCCGGGTAAATGA] (SEQ ID NO: 453)

Amino Acid Sequence

[spacer (SEQ ID NO: 87)] [pLW022 without spacer (SEQ ID NO: 510)]
 [QGQSGQ] [GYRWGCEWNCGGITTGSSGGSGGSLSGRSDNHGGGSQTVVTQEPSLTVSPGGTVTLTCRSSTGAV
 TTSNYANWVQOKPGQAPRGLIGGTNKRAPGTPARFSGSLLGGKAALTLSGVQPEDEAEYYCALWYSNLWVFGGGTK
 LTVLGGGGSGGGSGGGGSEVQLVESGGGLVQPGSLKLSAASGFTFNTYAMNWVRQAPGKLEWVARIRSKYNN
 YATYYADSVKDRFTISRDDSKNTAYLQMNNLKTEDTAVYYCVRHGNFGNSYVSWFAYWGQGLTVTVSSGGGGSQVQ

WO 2016/014974

PCT/US2015/042053

LKQSGPGLVQPSQSL SITCTVSGFSLTNYGVHWVRQSPGKGLEWLGV IWSGGNTDYNTPFTSRLSINKDNSKSQVF
 FKMNSLQSQDTAIYYCARALTYDYEFAYWGQGT LVTVSAASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEP
 VTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSSLGTQTYICNVNHKPSNTKVDKKVEPKSCDKTHTCPPC
 PAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYQSTYRVVSV
 LTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSREEMTKNQVSLTCLVKGFYPSDIAVEW
 ESNGQPENNYKTTTPVLDS DGSFFLYSKLTVDKSRWQQGNV FSCSV MHEALHNHYTQKSLSLSPGK]* (SEQ ID
 NO: 454)

OPP022: LC C225 3954-1204

Nucleotide Sequence

[spacer (SEQ ID NO: 507)][OPP022 without spacer (SEQ ID NO: 509)]
 [CAAGGCCAGTCTGGCCAG][TGCATCTCACCTCGTGGTTGTCCGGACGGCCCATACGTCATGTACGGCTCGAGCG
 GTGGCAGCGGTGGCTCTGGTGGATCCGGTCTGAGCGGCCGTTCCGATAATCATGGCAGTAGCGGTACCCAGATCTT
 GCTGACCCAGAGCCCGGTGATTCTGAGCGTGAGCCCGGGCGAACGTGTGAGCTTTAGCTGCCGCGCAGCCAGAGC
 ATTGGCACCAACATTCATTGGTATCAGCAGCGCACCAACGGCAGCCCGCGCCTGCTGATTAAATATGCGAGCGAAA
 GCATTAGCGGCATTCCGAGCCGCTTTAGCGGCAGCGGCAGCGGCACCGATTTTACCCTGAGCATTAAACAGCGTGGA
 AAGCGAAGATATTGCGGATTATTATTGCCAGCAGAACAACA ACTGGCCGACCACCTTTGGCGCGGGCACCAA ACTG
 GAACTGAAACGTACGGTGGCTGCACCATCTGTCTTCATCTTCCCGCCATCTGATGAGCAGTTGAAATCTGGA ACTG
 CCTCTGTTGTGTGCCTGCTGAATAACTTCTATCCCAGAGAGGCCAAAGTACAGTGGAAGGTGGATAACGCCCTCCA
 ATCGGGTAACTCCCAGGAGAGTGTCACAGAGCAGGACAGCAAGGACAGCACCTACAGCCTCAGCAGCACCCCTGACG
 CTGAGCAAAGCAGACTACGAGAAACACAAAGTCTACGCCTGCGAAGTCACCCATCAGGGCCTGAGCTCGCCCGTCA
 CAAAGAGCTTCAACAGGGGAGAGTGTTAG] (SEQ ID NO: 449)

Amino Acid Sequence

[spacer (SEQ ID NO: 87)][OPP022 without spacer (SEQ ID NO: 508)]
 [QGQSGQ][CISPRGCPDGPYVMYGSSGGSGGSGGSLSGRSDNHGSSGTQILLTQSPVILSVSPGERVSFSCRAS
 QSIGTNIHWYQQR TNGSPRL LIKYASESISGIPSRFSGSGSGTDFTLSINSVESEDIADY YCQQNNNWPTTFGAGT
 KLELKRTVAAPSVFIFPPSDEQLKSGTASVVCLLNNFYPREAKVQWKVDNALQSGNSQESVTEQDSKDSTYSLSST
 LTL SKADYEKHKVYACEVTHQGLSSPVTKSFNRGEC*] (SEQ ID NO: 450)

CI011: 3954-1204-C225v5N297Q-15865-CD3LvHv-H-N

pLW023: HC C225v5N297Q-15865-CD3LvHv-H-N

Nucleotide Sequence

[spacer (SEQ ID NO: 507)][pLW023 without spacer (SEQ ID NO: 511)]
 [CAAGGCCAGTCTGGCCAA][ATGATGTATTGCGGTGGGAATGAGGTGTTGTGCGGGCCGCGGGTTGGCTCGAGCG
 GTGGCAGCGGTGGCTCTGGTGGTCTGAGCGGCCGTTCCGATAATCATGGCGGCGGTTCTCAGACCGTGGTCACACA

GGAGCCCTCACTGACAGTGAGCCCTGGCGGGACCGTCACACTGACTTGTCGCAGTTCAACTGGCGCCGTGACTACC
AGCAATTACGCTAACTGGGTCCAGCAGAAACCAGGACAGGCACCACGAGGACTGATCGGAGGAACTAATAAGAGAG
CACCAGGAACCCCTGCAAGGTTCTCCGGATCTCTGCTGGGGGGAAAAGCCGCTCTGACACTGAGCGGCGTGCAGCC
TGAGGACGAAGCTGAGTACTATTGCGCACTGTGGTACTCCAACCTGTGGGTGTTTGGCGGGGGAACCTAAGCTGACC
GTCCTGGGAGGAGGAGGAAGCGGAGGAGGAGGGAGCGGAGGAGGAGGATCCGAAGTGCAGCTGGTCGAGAGCGGAG
GAGGACTGGTGCAGCCAGGAGGATCCCTGAAGCTGTCTTGTGCAGCCAGTGGCTTCACCTTCAACACTTACGCAAT
GAACTGGGTGCGGCAGGCACCTGGGAAGGGACTGGAATGGGTGCGCCCGGATCAGATCTAAATACAATAACTATGCC
ACCTACTATGCTGACAGTGTGAAGGATAGGTTTACCATTTCACGCGACGATAGCAAAAACACAGCTTATCTGCAGA
TGAATAACCTGAAGACCGAGGATACAGCAGTGTACTATTGCGTCAGACACGGCAATTTTCGGGAACCTTTACGTGAG
TTGGTTTGCCTATTGGGGACAGGGGACACTGGTCACCGTCTCCTCAGGAGGTGGTGGATCCCAGGTGCAGCTGAAA
CAGAGCGGCCCCGGGCCTGGTGCAGCCGAGCCAGAGCCTGAGCATTACCTGCACCGTGAGCGGCTTTAGCCTGACCA
ACTATGGCGTGCATTGGGTGCGCCAGAGCCCCGGGCAAAGGCCTGGAATGGCTGGGCGTGATTTGGAGCGGCGGCAA
CACCGATTATAACACCCCGTTTACCAGCCGCCTGAGCATTAACAAAGATAACAGCAAAAAGCCAGGTGTTTTTTAAA
ATGAACAGCCTGCAAAGCCAGGATACCGCGATTTATTATTGCGCGCGCGCGCTGACCTATTATGATTATGAATTTG
CGTATTGGGGCCAGGGCACCCCTGGTGACCGTGAGCGCGGCTAGCACCAAGGGCCCATCGGTCTTCCCCCTGGCACC
CTCCTCCAAGAGCACCTCTGGGGGCACAGCGGCCCTGGGCTGCCTGGTCAAGGACTACTTCCCCGAACCGGTGACG
GTGTCGTGGAACCTCAGGCGCCCTGACCAGCGGCGTGCACACCTTCCCGGCTGTCCTACAGTCCTCAGGACTCTACT
CCCTCAGCAGCGTGGTGACCGTGCCCTCCAGCAGCTTGGGCACCCAGACCTACATCTGCAACGTGAATCACAAGCC
CAGCAACACCAAGGTGGACAAGAAAGTTGAGCCCAAATCTTGTGACAAAACCTCACACATGCCCACCGTGCCCAGCA
CCTGAACTCCTGGGGGGACCGTCAGTCTTCCTCTTCCCCCAAAAACCCAAGGACACCCTCATGATCTCCCGGACCC
CTGAGGTACATGCGTGGTGGTGGACGTGAGCCACGAAGACCCTGAGGTCAAGTTCAACTGGTACGTGGACGGCGT
GGAGGTGCATAATGCCAAGACAAAGCCGCGGGAGGAGCAGTACCAGAGCACGTACCGTGTGGTCAGCGTCCTCACC
GTCCTGCACCAGGACTGGCTGAATGGCAAGGAGTACAAGTGCAAGGTCTCCAACAAAGCCCTCCCAGCCCCCATCG
AGAAAACCATCTCCAAAGCCAAAGGGCAGCCCCGAGAACCACAGGTGTACACCCTGCCCCCATCCCGGGAGGAGAT
GACCAAGAACCAGGTCAGCCTGACCTGCCTGGTCAAAGGCTTCTATCCCAGCGACATCGCCGTGGAGTGGGAGAGC
AATGGGCAGCCGGAGAACAACCTACAAGACCACGCCTCCCGTGCTGGACTCCGACGGCTCCTTCTTCTCTACAGCA
AGCTCACCGTGGACAAGAGCAGGTGGCAGCAGGGGAACGTCTTCTCATGCTCCGTGATGCATGAGGCTCTGCACAA
CCACTACACGCAGAAGAGCCTCTCCCTGTCTCCGGGTAAATGA] (SEQ ID NO: 451)

Amino Acid Sequence

[spacer (SEQ ID NO: 87)][pLW023 without spacer (SEQ ID NO: 512)]
[QGQSGQ][MMYCGGNEVLCGPRVGSSGGSGGSLSGRSDNHGGGSQTVVTQEPSLTVSPGGTVTLTCRSSTGAV
TTSNYANWVQQKPGQAPRGLIGGTNKRAPGTPARFSGSLLGGKAALTLSGVQPEDEAEYYCALWYSNLWVFGGGTK
LTVLGGGGSGGGSGGGGSEVQLVESGGGLVQPGGSLKLSCAASGFTFNTYAMNWVRQAPGKGLEWVARIRSKYNN
YATYYADSVKDRFTISRDDSKNTAYLQMNNLKTEDTAVYYCVRHGNFGNSYVSWFAYWGQGLVTVSSGGGGSQVQ
LKQSGPGLVQPSQSL SITCTVSGFSLTNYGVHWVRQSPGKGLEWLGVWSGGNTDYNT PFTSRLSINKDNSKSQVF
FKMNSLQSQDTAIYYCARALTYDYEFAYWGQGLVTVSAASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEP

VTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSSLGTQTYICNVNHKPSNTKVDDKKVEPKSCDKTHTCPPC
 PAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYQSTYRVVSV
 LTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSREEMTKNQVSLTCLVKGFYPSDIAVEW
 ESNGQPENNYKTTTPVLDSDGSFFLYSKLTVDKSRWQQGNVVFSCSVMHEALHNHYTQKSLSLSPGK] (SEQ ID
 NO: 452)

OPP022: LC C225 3954-1204

Nucleotide Sequence

[spacer (SEQ ID NO: 507)][OPP022 without spacer (SEQ ID NO: 509)]
 [CAAGGCCAGTCTGGCCAG][TGCATCTCACCTCGTGGTTGTCCGGACGGCCCATACGTCATGTACGGCTCGAGCG
 GTGGCAGCGGTGGCTCTGGTGGATCCGGTCTGAGCGGCCGTTCCGATAATCATGGCAGTAGCGGTACCCAGATCTT
 GCTGACCCAGAGCCCGGTGATTCTGAGCGTGAGCCCGGGCGAACGTGTGAGCTTTAGCTGCCGCGCGAGCCAGAGC
 ATTGGCACCAACATTTCATTGGTATCAGCAGCGCACCAACGGCAGCCCGCGCCTGCTGATTAAATATGCGAGCGAAA
 GCATTAGCGGCATTCCGAGCCGCTTTAGCGGCAGCGGCAGCGGCACCGATTTTACCCTGAGCATTAAACAGCGTGGA
 AAGCGAAGATATTGCGGATTATTATTGCCAGCAGAACAACAACCTGGCCGACCACCTTTGGCGCGGGCACCAAACCTG
 GAACTGAAACGTACGGTGGCTGCACCATCTGTCTTCATCTTCCCGCCATCTGATGAGCAGTTGAAATCTGGAACCTG
 CCTCTGTTGTGTGCCTGCTGAATAACTTCTATCCCAGAGAGGCCAAAGTACAGTGGAAGGTGGATAACGCCCTCCA
 ATCGGGTAACTCCCAGGAGAGTGTACAGAGCAGGACAGCAAGGACAGCACCTACAGCCTCAGCAGCACCTTGACG
 CTGAGCAAAGCAGACTACGAGAAACACAAAGTCTACGCCTGCGAAGTCACCCATCAGGGCCTGAGCTCGCCCGTCA
 CAAAGAGCTTCAACAGGGGAGAGTGTTAG] (SEQ ID NO: 449)

Amino Acid Sequence

[spacer (SEQ ID NO: 87)][OPP022 without spacer (SEQ ID NO: 508)]
 [QGQSGQ][CISPRGCPDGPYVMYGSSGGSGGSGGSLSGRSDNHGSSGTQILLTQSPVILSVSPGERVSFSCRAS
 QSIGTNIHWYQQRRTNGSPRLLIKYASESISGIPSRFSGSGSGTDFTLINSVESEDIADYYCQQNNNWPTTFGAGT
 KLELKRTVAAPSVFIFPPSDEQLKSGTASVVCLLNFPYPREAKVQWKVDNALQSGNSQESVTEQDSKDSTYSLSST
 LTLSKADYEKHKVYACEVTHQGLSSPVTKSFNRGEC] (SEQ ID NO: 450)

CI012: C225v5N297Q

pLW006: C225v5N297Q

Nucleotide Sequence

CAGGTGCAGCTGAAACAGAGCGGCCCGGGCCTGGTGCAGCCGAGCCAGAGCCTGAGCATTACCTGCACCGTGAGCG
 GCTTTAGCCTGACCAACTATGGCGTGCATTGGGTGCGCCAGAGCCCGGGCAAAGGCCTGGAATGGCTGGGCGTGAT
 TTGGAGCGGCGGCAACACCGATTATAACACCCCGTTTACCAGCCGCTGAGCATTAAACAAAGATAACAGCAAAAGC
 CAGGTGTTTTTTAAATGAACAGCCTGCAAAGCCAGGATACCGCGATTTATTATTGCGCGCGCGCGCTGACCTATT
 ATGATTATGAATTTGCGTATTGGGGCCAGGGCACCTGGTGACCGTGAGCGCGGCTAGCACCAAGGGCCCATCGGT

CTTCCCCCTGGCACCCCTCCTCCAAGAGCACCTCTGGGGGCACAGCGGCCCTGGGCTGCCTGGTCAAGGACTACTTC
 CCCGAACCGGTGACGGTGTCTGTGGAACCTCAGGCGCCCTGACCAGCGGCGTGCACACCTTCCCGGCTGTCCTACAGT
 CCTCAGGACTCTACTCCCTCAGCAGCGTGGTGACCGTGCCCTCCAGCAGCTTGGGCACCCAGACCTACATCTGCAA
 CGTGAATCACAAGCCCAGCAACACCAAGGTGGACAAGAAAGTTGAGCCCCAAATCTTGTGACAAAACCTCACACATGC
 CCACCGTGCCCAGCACCTGAACTCCTGGGGGGACCGTCAGTCTTCCTCTTCCCCC AAAACCCAAGGACACCCTCA
 TGATCTCCCGGACCCCTGAGGTCACATGCGTGGTGGTGGACGTGAGCCACGAAGACCCTGAGGTCAAGTTCAACTG
 GTACGTGGACGGCGTGGAGGTGCATAATGCCAAGACAAAGCCGCGGGAGGAGCAGTACCAGAGCACGTACCGTGTG
 GTCAGCGTCCTCACCGTCCTGCACCAGGACTGGCTGAATGGCAAGGAGTACAAGTGCAAGGTCTCCAACAAAGCCC
 TCCCAGCCCCCATCGAGAAAACCATCTCCAAAGCCAAAGGGCAGCCCCGAGAACCACAGGTGTACACCCTGCCCCC
 ATCCCGGGAGGAGATGACCAAGAACCAGGTCAGCCTGACCTGCCTGGTCAAAGGCTTCTATCCCAGCGACATCGCC
 GTGGAGTGGGAGAGCAATGGGCAGCCGGAGAACAACCTACAAGACCACGCCTCCCGTGCTGGACTCCGACGGCTCCT
 TCTTCCTCTACAGCAAGCTCACCGTGGACAAGAGCAGGTGGCAGCAGGGGAACGTCTTCTCATGCTCCGTGATGCA
 TGAGGCTCTGCACAACCACTACACGCAGAAGAGCCTCTCCCTGTCTCCGGGTAAATGA (SEQ ID NO: 455)

Amino Acid Sequence

QVQLKQSGPGLVQPSQSL SITCTVSGFSLTNYGVHWVRQSPGKGLEWLGVIWSGGNTDYNT PFTSRLSINKDNSKS
 QVFFKMNSLQSQDTAIYYCARALTYDYEFAYWGQGLVTVSAASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYF
 PEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSSLGTQTYICNVNHKPSNTKVDKKVEPKSCDKHTC
 PPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYQSTYRV
 VSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSREEMTKNQVSLTCLVKGFYPSDIA
 VEWESNGQPENNYKTTTPVLDSDGSFFLYSKLTVDKSRWQQGNV FSCSV MHEALHNHYTQKSLSLSPGK* (SEQ
 ID NO: 456)

OPP021: LC C225

Nucleotide Sequence

GATATCTTGCTGACCCAGAGCCCGGTGATTCTGAGCGTGAGCCCGGGCGAACGTGTGAGCTTTAGCTGCCGCGCGA
 GCCAGAGCATTGGCACCAACATTCATTGGTATCAGCAGCGCACCAACGGCAGCCCGCGCCTGCTGATTAAATATGC
 GAGCGAAAGCATTAGCGGCATTCCGAGCCGCTTTAGCGGCAGCGGCAGCGGCACCGATTTTACCCTGAGCATTAAC
 AGCGTGGAAGCGAAGATATTGCGGATTATTATTGCCAGCAGAACAACAACCTGGCCGACCACCTTTGGCGCGGGCA
 CCAAACCTGGAAATCAAACGTACGGTGGCTGCACCATCTGTCTTCATCTTCCCGCCATCTGATGAGCAGTTGAAATC
 TGGAACCTGCCTCTGTTGTGTGCCTGCTGAATAACTTCTATCCCAGAGAGGCCAAAGTACAGTGGAAGGTGGATAAC
 GCCCTCCAATCGGGTAACTCCCAGGAGAGTGTACAGAGCAGGACAGCAAGGACAGCACCTACAGCCTCAGCAGCA
 CCCTGACGCTGAGCAAAGCAGACTACGAGAAACACAAAGTCTACGCCTGCGAAGTCACCCATCAGGGCCTGAGCTC
 GCCCGTCACAAAGAGCTTCAACAGGGGAGAGTGTTAG (SEQ ID NO: 457)

Amino Acid Sequence

DILLTQSPVILSVSPGERVVSFSCRASQSIGTNIHWYQQRTNGSPRLLIKYASESISGIPSRFSGSGSGTDFTLSIN
SVESEDIADYYCQQNNNWPTTFGAGTKLEIKRTVAAPSVFIFPPSDEQLKSGTASVVCLLNNFYPREAKVQWKVDN
ALQSGNSQESVTEQDSKDYSLSTLTLSKADYEKHKVYACEVTHQGLSSPVTKSFNRGEC* (SEQ ID
NO: 458)

CI015: C225v5N297Q-CD3LvHv-H-N

pLW057: HC C225v5N297Q-CD3LvHv-H-N

Nucleotide Sequence

CAGACCGTGGTCACACAGGAGCCCTCACTGACAGTGAGCCCTGGCGGGACCGTCACACTGACTTGTCGCAGTTCAA
CTGGCGCCGTGACTACCAGCAATTACGCTAACTGGGTCCAGCAGAAACCAGGACAGGCACCACGAGGACTGATCGG
AGGAACTAATAAGAGAGCACCAGGAACCCCTGCAAGGTTCTCCGGATCTCTGCTGGGGGGAAAAGCCGCTCTGACA
CTGAGCGGCGTGCAGCCTGAGGACGAAGCTGAGTACTATTGCGCACTGTGGTACTCCAACCTGTGGGTGTTTGGCG
GGGGAACCTAAGCTGACCGTCCTGGGAGGAGGAGGAAGCGGAGGAGGAGGGAGCGGAGGAGGAGGATCCGAAGTGCA
GCTGGTCGAGAGCGGAGGAGGACTGGTGCAGCCAGGAGGATCCCTGAAGCTGTCTTGTGCAGCCAGTGGCTTCACC
TTCAACACTTACGCAATGAACTGGGTGCGGCAGGCACCTGGGAAGGGACTGGAATGGGTGCGCCGGATCAGATCTA
AATACAATAACTATGCCACCTACTATGCTGACAGTGTGAAGGATAGGTTACCATTTCACGCGACGATAGCAAAAA
CACAGCTTATCTGCAGATGAATAACCTGAAGACCGAGGATACAGCAGTGTACTATTGCGTCAGACACGGCAATTTTC
GGGAACCTCTTACGTGAGTTGGTTTGCCTATTGGGGACAGGGGACACTGGTCACCGTCTCCTCAGGAGGTGGTGGAT
CCCAGGTGCAGCTGAAACAGAGCGGCCCCGGGCCTGGTGCAGCCGAGCCAGAGCCTGAGCATTACCTGCACCGTGAG
CGGCTTTAGCCTGACCAACTATGGCGTGCATTGGGTGCGCCAGAGCCCCGGGCAAAGGCCTGGAATGGCTGGGCGTG
ATTTGGAGCGGCGGCAACACCGATTATAACACCCCGTTTACCAGCCGCCTGAGCATTAAACAAAGATAACAGCAAAA
GCCAGGTGTTTTTTTAAAATGAACAGCCTGCAAAGCCAGGATACCGCGATTTATTATTGCGCGCGCGCGCTGACCTA
TTATGATTATGAATTTGCGTATTGGGGCCAGGGCACCCCTGGTGACCGTGAGCGCGGCTAGCACCAAGGGCCCATCG
GTCTTCCCCCTGGCACCCCTCCTCCAAGAGCACCTCTGGGGGCACAGCGGCCCTGGGCTGCCTGGTCAAGGACTACT
TCCCCGAACCGGTGACGGTGTCTGTGGAACCTCAGGCGCCCTGACCAGCGGCGTGACACCTTCCCGGCTGTCCTACA
GTCCTCAGGACTCTACTCCCTCAGCAGCGTGGTGACCGTGCCCTCCAGCAGCTTGGGCACCCAGACCTACATCTGC
AACGTGAATCACAAAGCCCAGCAACACCAAGGTGGACAAGAAAGTTGAGCCCAAATCTTGTGACAAAACCTCACACAT
GCCACCGTGCCAGCACCTGAACTCCTGGGGGGACCGTCAGTCTTCTCTTCCCCCAAAACCCAAGGACACCCCT
CATGATCTCCCGGACCCCTGAGGTCACATGCGTGGTGGTGGACGTGAGCCACGAAGACCCTGAGGTCAAGTTCAAC
TGGTACGTGGACGGCGTGGAGGTGCATAATGCCAAGACAAAGCCGCGGGAGGAGCAGTACCAGAGCACGTACCGTG
TGGTCAGCGTCCTCACCGTCCTGCACCAGGACTGGCTGAATGGCAAGGAGTACAAGTGCAAGGTCTCCAACAAAGC
CCTCCCAGCCCCCATCGAGAAAACCATCTCCAAAGCCAAAGGGCAGCCCCGAGAACCACAGGTGTACACCCTGCCC
CCATCCCGGGAGGAGATGACCAAGAACCAGGTCAGCCTGACCTGCCTGGTCAAAGGCTTCTATCCCAGCGACATCG
CCGTGGAGTGGGAGAGCAATGGGCAGCCGGAGAACAACCTACAAGACCACGCCTCCCGTGCTGGACTCCGACGGCTC
CTTCTTCTCTACAGCAAGCTCACCGTGGACAAGAGCAGGTGGCAGCAGGGGAACGTCTTCTCATGCTCCGTGATG

CATGAGGCTCTGCACAACCACTACACGCAGAAGAGCCTCTCCCTGTCTCCGGGTAAATGA (SEQ ID NO: 459)

Amino Acid Sequence

QTVVTQEPSLTVSPGGTVTLTCRSSTGAVTTSNYANWVQQKPGQAPRGLIGGTNKRAPGTPARFSGSLLGGKAALT
LSGVQPEDEAEYYCALWYSNLWVFGGGTKLTVLGGGGSGGGGSGGGGSEVQLVESGGGLVQPGGSLKLSCAASGFT
FNTYAMNWVRQAPGKGLEWVARIRSKYNNYATYYADSVKDRFTISRDDSKNTAYLQMNNLKTEDTAVYYCVRHGNF
GNSYVSWFAYWGQGLTVTVSSGGGGSQVQLKQSGPGLVQPSQSLSITCTVSGFSLTNYGVHWVRQSPGKGLEWLGV
IWSGGNTDYNTPFTSRLSINKDNSKSQVFFKMNSLQSQDTAIYYCARALTYDYEFAYWGQGLTVTVSAASTKGPS
VFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVTVTPSSSLGTQTYIC
NVNHKPSNTKVDKKVEPKSCDKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVDVSHEDPEVKFN
WYVDGVEVHNAKTKPREEQYQSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLF
PSREEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSQSV
HEALHNHYTQKSLSLSPGK* (SEQ ID NO: 460)

OPP021: LC C225

Nucleotide Sequence

GATATCTTGCTGACCCAGAGCCCGGTGATTCTGAGCGTGAGCCCGGGCGAACGTGTGAGCTTTAGCTGCCGCGCGA
GCCAGAGCATTGGCACCAACATTCATTGGTATCAGCAGCGCACCAACGGCAGCCCGCGCCTGCTGATTAAATATGC
GAGCGAAAGCATTAGCGGCATTCCGAGCCGCTTTAGCGGCAGCGGCAGCGGCACCGATTTTACCCTGAGCATTAAAC
AGCGTGGAAGCGAAGATATTGCGGATTATTATTGCCAGCAGAACAACAACACTGGCCGACCACCTTTGGCGCGGGCA
CCAAACTGGAAATCAAACGTACGGTGGCTGCACCATCTGTCTTCATCTTCCCGCCATCTGATGAGCAGTTGAAATC
TGGAAGTGCCTCTGTTGTGTGCCTGCTGAATAACTTCTATCCCAGAGAGGCCAAAGTACAGTGGAAGGTGGATAAC
GCCCTCCAATCGGGTAACTCCCAGGAGAGTGTCACAGAGCAGGACAGCAAGGACAGCACCTACAGCCTCAGCAGCA
CCCTGACGCTGAGCAAAGCAGACTACGAGAAACACAAAGTCTACGCCTGCGAAGTCACCCATCAGGGCCTGAGCTC
GCCCGTCACAAAGAGCTTCAACAGGGGAGAGTGTTAG (SEQ ID NO: 457)

Amino Acid Sequence

DILLTQSPVILSVSPGERVSFSCRASQSIGTNIHWYQORTNGSPRLLIKYASESISGIPSRFSGSGSGTDFTLIN
SVESEDIADYYCQNNNNWPTTFGAGTKLEIKRTVAAPSVFIFPPSDEQLKSGTASVVCLLNNFYPREAKVQWKVDN
ALQSGNSQESVTEQDSKDYSLSSLTLSKADYEKHKVYACEVTHQGLSSPVTKSFNRGEC* (SEQ ID NO: 458)

CI016: 3954-1204-C225v5N297Q-CD3LvHv-H-N

pLW057: HC C225v5N297Q-CD3LvHv-H-N

Nucleotide Sequence

CAGACCGTGGTCACACAGGAGCCCTCACTGACAGTGAGCCCTGGCGGGACCGTCACACTGACTTGTCGCAGTTCAA
CTGGCGCCGTGACTACCAGCAATTACGCTAACTGGGTCCAGCAGAAACCAGGACAGGCACCACGAGGACTGATCGG
AGGAACTAATAAGAGAGCACACAGGAACCCCTGCAAGGTTCTCCGGATCTCTGCTGGGGGGAAAAGCCGCTCTGACA
CTGAGCGGCGTGCAGCCTGAGGACGAAGCTGAGTACTATTGCGCACTGTGGTACTCCAACCTGTGGGTGTTTGGCG
GGGGAACCTAAGCTGACCGTCCTGGGAGGAGGAGGAAGCGGAGGAGGAGGGAGCGGAGGAGGAGGATCCGAAGTGCA
GCTGGTCGAGAGCGGAGGAGGACTGGTGCAGCCAGGAGGATCCCTGAAGCTGTCTTGTGCAGCCAGTGGCTTCACC
TTCAACACTTACGCAATGAACTGGGTGCGGCAGGCACCTGGGAAGGGACTGGAATGGGTGCGCCGGATCAGATCTA
AATACAATAACTATGCCACCTACTATGCTGACAGTGTGAAGGATAGGTTTACCATTTCACGCGACGATAGCAAAAA
CACAGCTTATCTGCAGATGAATAACCTGAAGACCGAGGATACAGCAGTGTACTATTGCGTCAGACACGGCAATTTT
GGGAACTCTTACGTGAGTTGGTTTGCCTATTGGGGACAGGGGACACTGGTCACCGTCTCCTCAGGAGGTGGTGGAT
CCCAGGTGCAGCTGAAACAGAGCGGCCCGGGCCTGGTGCAGCCGAGCCAGAGCCTGAGCATTACCTGCACCGTGAG
CGGCTTTAGCCTGACCAACTATGGCGTGCATTGGGTGCGCCAGAGCCCGGGCAAAGGCCTGGAATGGCTGGGCGTG
ATTTGGAGCGGCGGCAACACCGATTATAACACCCCGTTTACCAGCCGCCTGAGCATTAAACAAAGATAACAGCAAAA
GCCAGGTGTTTTTTTAAATGAACAGCCTGCAAAGCCAGGATACCGCGATTTATTATTGCGCGCGCGCGCTGACCTA
TTATGATTATGAATTTGCGTATTGGGGCCAGGGCACCCCTGGTGACCGTGAGCGCGGCTAGCACCAAGGGCCCATCG
GTCTTCCCCCTGGCACCCCTCCTCCAAGAGCACCTCTGGGGGCACAGCGGCCCTGGGCTGCCTGGTCAAGGACTACT
TCCCCGAACCGGTGACGGTGTCTGTGGAACCTCAGGCGCCCTGACCAGCGGCGTGCACACCTTCCCGGCTGTCTTACA
GTCCTCAGGACTCTACTCCCTCAGCAGCGTGGTGACCGTGCCCTCCAGCAGCTTGGGCACCCAGACCTACATCTGC
AACGTGAATCACAAGCCCAGCAACACCAAGGTGGACAAGAAAGTTGAGCCCAAATCTTGTGACAAAACCTCACACAT
GCCCACCGTGCCCAGCACCTGAACTCCTGGGGGGACCGTCAGTCTTCTCTTCCCCCCTTCCCAAGGACACCCCT
CATGATCTCCCGGACCCCTGAGGTCACATGCGTGGTGGTGGACGTGAGCCACGAAGACCCCTGAGGTCAAGTTCAAC
TGGTACGTGGACGGCGTGGAGGTGCATAATGCCAAGACAAAGCCGCGGGAGGAGCAGTACCAGAGCACGTACCGTG
TGGTCAGCGTCCTCACCGTCCTGCACCAGGACTGGCTGAATGGCAAGGAGTACAAGTGCAAGGTCTCCAACAAAGC
CCTCCCAGCCCCCATCGAGAAAACCATCTCCAAAGCCAAAGGGCAGCCCCGAGAACCACAGGTGTACACCCTGCCC
CCATCCCGGGAGGAGATGACCAAGAACCAGGTGAGCCTGACCTGCCTGGTCAAAGGCTTCTATCCCAGCGACATCG
CCGTGGAGTGGGAGAGCAATGGGCAGCCGGAGAACAATAAGACCACGCCTCCCGTGCTGGACTCCGACGGCTC
CTTCTTCTCTACAGCAAGCTCACCGTGGACAAGAGCAGGTGGCAGCAGGGGAACGTCTTCTCATGCTCCGTGATG
CATGAGGCTCTGCACAACCACTACACGCAGAAGAGCCTCTCCCTGTCTCCGGGTAAATGA (SEQ ID
NO: 459)

Amino Acid Sequence

QTVVTQEPSLTVSPGGTVTLTCRSSTGAVTTSNYANWVQQKPGQAPRGLIGGTNKRAPGTPARFSGSLLGGKAALT
LSGVQPEDEAEYYCALWYSNLWVFGGGTKLTVLGGGSGGGGSGGGGSEVQLVESGGGLVQPGGSLKLSCAASGFT

WO 2016/014974

PCT/US2015/042053

FNTYAMNWVRQAPGKGLEWVARIRSKYNNYATYYADSVKDRFTISRDDSKNTAYLQMNNLKTEDTAVYYCVRHGNF
 GNSYVSWFAYWGQGLVTVSSGGGGSQVQLKQSGPGLVQPSQSL SITCTVSGFSLTNYGVHWVRQSPGKGLEWLGV
 IWSGGNTDYNTPTFTSRLSINKDNSKSQVFFKMNSLQSQDTAIYYCARALTYDYEFAYWGQGLVTVSAASTKGPS
 VFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSSLGTQTYIC
 NVNHKPSNTKVDKKVEPKSCDKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFN
 WYVDGVEVHNAKTKPREEQYQSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLTP
 PSREEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVVFSCSVM
 HEALHNHYTQKSLSLSPGK* (SEQ ID NO: 460)

OPP022: LC C225 3954-1204

Nucleotide Sequence

[spacer (SEQ ID NO: 507)][OPP022 without spacer (SEQ ID NO: 509)]
 [CAAGGCCAGTCTGGCCAG][TGCATCTCACCTCGTGGTTGTCCGGACGGCCCATACGTCATGTACGGCTCGAGCG
 GTGGCAGCGGTGGCTCTGGTGGATCCGGTCTGAGCGGCCGTTCCGATAATCATGGCAGTAGCGGTACCCAGATCTT
 GCTGACCCAGAGCCCGGTGATTCTGAGCGTGAGCCCGGGCGAACGTGTGAGCTTTAGCTGCCGCGCGAGCCAGAGC
 ATTGGCACCAACATTCATTGGTATCAGCAGCGCACCAACGGCAGCCCGCGCCTGCTGATTAAATATGCGAGCGAAA
 GCATTAGCGGCATTCCGAGCCGCTTTAGCGGCAGCGGCAGCGGCACCGATTTTACCCTGAGCATTAACAGCGTGGA
 AAGCGAAGATATTGCGGATTATTATTGCCAGCAGAACAACAACCTGGCCGACCACCTTTGGCGCGGGCACCAAACCTG
 GAACTGAAACGTACGGTGGCTGCACCATCTGTCTTCATCTTCCCGCCATCTGATGAGCAGTTGAAATCTGGAACCTG
 CCTCTGTTGTGTGCCTGCTGAATAACTTCTATCCCAGAGAGGCCAAAGTACAGTGGAAGGTGGATAACGCCCTCCA
 ATCGGGTAACTCCCAGGAGAGTGTACAGAGCAGGACAGCAAGGACAGCACCTACAGCCTCAGCAGCACCCCTGACG
 CTGAGCAAAGCAGACTACGAGAAACACAAAGTCTACGCCTGCGAAGTCACCCATCAGGGCCTGAGCTCGCCCGTCA
 CAAAGAGCTTCAACAGGGGAGAGTGTTAG] (SEQ ID NO: 449)

Amino Acid Sequence

[spacer (SEQ ID NO: 87)][OPP022 without spacer (SEQ ID NO: 508)]
 [QGQSGQ][CISPRGCPDGPYVMYGSSGGSGGSGGSGLSGRSDNHGSSGTQILLTQSPVILSVSPGERVSFSCRAS
 QSIGTNIHWYQQRNNGSPRLLIKYASESISGIPSRFSGSGSGTDFTLSINSVESEDIADYYCQQNNNWPTTFGAGT
 KLELKRTVAAPSVFIFPPSDEQLKSGTASVVCLLNNFYPREAKVQWKVDNALQSGNSQESVTEQDSKDSTYSLSST
 LTLSKADYEKHKVYACEVTHQGLSSPVTKSFNRGEC]* (SEQ ID NO: 450)

CI025: IL6RN297Q

pLW078: HC IL6RN297Q

Nucleotide Sequence

CAGGTGCAGCTGCAGGAGTCCGGACCAGGACTGGTCCGGCCCTCACAGACTCTGAGCCTGACATGCACTGTGTCAG
 GCTACAGCATCACCTCCGATCACGCCTGGAGCTGGGTCAGGCAGCCACCTGGACGCGGCCTGGAATGGATCGGCTA

CATTTCTTATAGTGGGATCACCACATACAACCCCTCTCTGAAGAGTCGAGTGACCATTTCCAGAGACAACTCTAAA
 AATACACTGTATCTGCAGATGAATAGTCTGCGGGCCGAGGATACAGCTGTGTACTATTGTGCACGGTCTCTGGCCA
 GAACTACCGCTATGGACTATTGGGGGCAGGGAAGCCTGGTGACCGTCAGCTCCGCTAGCACCAAGGGCCCATCGGT
 CTTCCCCCTGGCACCCCTCCTCCAAGAGCACCTCTGGGGGCACAGCGGCCCTGGGCTGCCTGGTCAAGGACTACTTC
 CCCGAACCGGTGACGGTGTCTGTGGAACCTCAGGCGCCCTGACCAGCGGCGTGCACACCTTCCCGGTGTCTTACAGT
 CCTCAGGACTCTACTCCCTCAGCAGCGTGGTGACCGTGCCCTCCAGCAGCTTGGGCACCCAGACCTACATCTGCAA
 CGTGAATCACAAGCCCAGCAACACCAAGGTGGACAAGAAAGTTGAGCCCAAATCTTGTGACAAAACCTCACACATGC
 CCACCGTGCCCAGCACCTGAACTCCTGGGGGGACCGTCAGTCTTCCTCTTCCCCCCTTCCCAAGGACACCCTCA
 TGATCTCCCGGACCCCTGAGGTCACATGCGTGGTGGTGGACGTGAGCCACGAAGACCCTGAGGTCAAGTTCAACTG
 GTACGTGGACGGCGTGGAGGTGCATAATGCCAAGACAAAGCCGCGGGAGGAGCAGTACCAGAGCACGTACCGTGTG
 GTCAGCGTCCTCACCGTCCTGCACCAGGACTGGCTGAATGGCAAGGAGTACAAGTGCAAGGTCTCCAACAAAGCCC
 TCCCAGCCCCCATCGAGAAAACCATCTCCAAAGCCAAAGGGCAGCCCCGAGAACCACAGGTGTACACCCTGCCCCC
 ATCCCGGGAGGAGATGACCAAGAACCAGGTCAGCCTGACCTGCCTGGTCAAAGGCTTCTATCCCAGCGACATCGCC
 GTGGAGTGGGAGAGCAATGGGCAGCCGGAGAACAACCTACAAGACCACGCCTCCCGTGCTGGACTCCGACGGCTCCT
 TCTTCCTCTACAGCAAGCTCACCGTGGACAAGAGCAGGTGGCAGCAGGGGAACGTCTTCTCATGCTCCGTGATGCA
 TGAGGCTCTGCACAACCACTACACGCAGAAGAGCCTCTCCCTGTCTCCGGGTAAATGA (SEQ ID NO: 465)

Amino Acid Sequence

QVQLQESGPGLVLRPSQTLSTCTVSGYSITSDHAWSWVRQPPGRGLEWIGYISYSGITTYNPSLKSRVTISRDN
 SKNTLYLQMNSLRAEDTAVYYCARSLARTTAMDYWGQSLVTVSSASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYF
 PEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSSLGTQTYICNVNHKPSNTKVDKKVEPKSCDKHTC
 PPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYQSTYRV
 VSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSREEMTKNQVSLTCLVKGFYPSDIA
 VEWESNGQPENNYKTTTPVLDSDGSFFLYSKLTVDKSRWQQGNVFSVSMHEALHNHYTQKSLSLSPGK* (SEQ
 ID NO: 466)

pLW077: LC IL6R

Nucleotide Sequence

GACATCCAGATGACTCAGTCTCCTAGCTCCCTGTCCGCCTCTGTGGGGGACCGAGTCACCATCACATGCAGAGCCA
 GCCAGGATATTTCTAGTTACCTGAACTGGTATCAGCAGAAGCCCGGAAAAGCACCTAAGCTGCTGATCTACTATAC
 CTCCAGGCTGCACTCTGGCGTGCCCAGTCGGTTCAGTGGCTCAGGGAGCGGAACCGACTTCACTTTTACCATCTCA
 AGCCTGCAGCCAGAGGATATTGCCACATACTATTGTCAGCAGGGCAATACACTGCCCTACACTTTTGGCCAGGGGA
 CCAAGGTGGAAATCAAACGTACGGTGGCTGCACCATCTGTCTTCATCTTCCCGCCATCTGATGAGCAGTTGAAATC
 TGGAAGTGCCTCTGTTGTGTGCCTGCTGAATAACTTCTATCCCAGAGAGGCCAAAGTACAGTGGAAGGTGGATAAC
 GCCCTCCAATCGGGTAACTCCCAGGAGAGTGTACAGAGCAGGACAGCAAGGACAGCACCTACAGCCTCAGCAGCA
 CCCTGACGCTGAGCAAAGCAGACTACGAGAAACACAAAGTCTACGCCTGCGAAGTCACCCATCAGGGCCTGAGCTC
 GCCCGTCACAAAGAGCTTCAACAGGGGAGAGTGTTAG (SEQ ID NO: 467)

Amino Acid Sequence

DIQMTQSPSSLSASVGDRVTITCRASQDISSYLNWYQQKPGKAPKLLIYYTSRLHSGVPSRFSGSGSGTDFTFTIS
 SLQPEDIATYYCQQGNTLPYTFGQGTKVEIKRTVAAPSVFIFPPSDEQLKSGTASVVCLLNNFYPREAKVQWKVDN
 ALQSGNSQESVTEQDSKDYSLSTLTLSKADYEKHKVYACEVTHQGLSSPVTKSFNRGEC* (SEQ ID
 NO: 468)

CI026: IL6RN297Q-CD3LvHv-H-N

pLW083: HC IL6RN297Q-CD3LvHv-H-N

Nucleotide Sequence

CAGACCGTGGTCACACAGGAGCCCTCACTGACAGTGAGCCCTGGCGGGACCGTCACACTGACTTGTCGCAGTTCAA
 CTGGCGCCGTGACTACCAGCAATTACGCTAACTGGGTCCAGCAGAAACCAGGACAGGCACCACGAGGACTGATCGG
 AGGAACTAATAAGAGAGCACCAGGAACCCCTGCAAGGTTCTCCGGATCTCTGCTGGGGGGAAAAGCCGCTCTGACA
 CTGAGCGGCGTGCAGCCTGAGGACGAAGCTGAGTACTATTGCGCACTGTGGTACTCCAACCTGTGGGTGTTTGGCG
 GGGGAACTAAGCTGACCGTCCTGGGAGGAGGAGGAAGCGGAGGAGGAGGGAGCGGAGGAGGAGGATCCGAAGTGCA
 GCTGGTCGAGAGCGGAGGAGGACTGGTGCAGCCAGGAGGATCCCTGAAGCTGTCTTGTGCAGCCAGTGGCTTCACC
 TTCAACACTTACGCAATGAACTGGGTGCGGCAGGCACCTGGGAAGGGACTGGAATGGGTGCGCCGGATCAGATCTA
 AATACAATAACTATGCCACCTACTATGCTGACAGTGTGAAGGATAGGTTTACCATTTCACGCGACGATAGCAAAAA
 CACAGCTTATCTGCAGATGAATAACCTGAAGACCGAGGATACAGCAGTGTACTATTGCGTCAGACACGGCAATTTT
 GGGAACCTTACGTGAGTTGGTTTGCCTATTGGGGACAGGGGACACTGGTCACCGTCTCCTCAGGAGGTGGTGGAT
 CCCAGGTGCAGCTGCAGGAGTCCGGACCAGGACTGGTCCGGCCCTCACAGACTCTGAGCCTGACATGCACTGTGTC
 AGGCTACAGCATCACCTCCGATCACGCCTGGAGCTGGGTGAGGCAGCCACCTGGACGCGGCCTGGAATGGATCGGC
 TACATTTCTTATAGTGGGATCACACATACAACCCCTCTCTGAAGAGTCGAGTGACCATTTCAGAGACAACCTCTA
 AAAATACACTGTATCTGCAGATGAATAGTCTGCGGGCCGAGGATACAGCTGTGTACTATTGTGCACGGTCTCTGGC
 CAGAACTACCGCTATGGACTATTGGGGGCAGGGAAGCCTGGTGACCGTCAGCTCCGCTAGCACCAAGGGCCCATCG
 GTCTTCCCCCTGGCACCCCTCCTCCAAGAGCACCTCTGGGGGCACAGCGGCCCTGGGCTGCCTGGTCAAGGACTACT
 TCCCCGAACCGGTGACGGTGTCTGTGGAACCTCAGGCGCCCTGACCAGCGGCGTGCACACCTTCCCGGCTGTCCTACA
 GTCCTCAGGACTCTACTCCCTCAGCAGCGTGGTGACCGTGCCCTCCAGCAGCTTGGGCACCCAGACCTACATCTGC
 AACGTGAATCACAAGCCCAGCAACACCAAGGTGGACAAGAAAGTTGAGCCCAAATCTTGTGACAAAACCTCACACAT
 GCCCACCGTGCCCAGCACCTGAACTCCTGGGGGGACCGTCAGTCTTCTCTTCCCCCAGGACACCCCT
 CATGATCTCCCGGACCCCTGAGGTCACATGCGTGGTGGTGGACGTGAGCCACGAAGACCCTGAGGTCAAGTTCAAC
 TGGTACGTGGACGGCGTGGAGGTGCATAATGCCAAGACAAAGCCGCGGGAGGAGCAGTACCAGAGCACGTACCGTG
 TGGTCAGCGTCCTCACCGTCCTGCACCAGGACTGGCTGAATGGCAAGGAGTACAAGTGCAAGGTCTCCAACAAAGC
 CCTCCCAGCCCCCATCGAGAAAACCATCTCCAAAGCCAAAGGGCAGCCCCGAGAACCACAGGTGTACACCCTGCCC
 CCATCCCGGGAGGAGATGACCAAGAACCAGGTCAGCCTGACCTGCCTGGTCAAAGGCTTCTATCCCAGCGACATCG
 CCGTGGAGTGGGAGAGCAATGGGCAGCCGGAGAACAACCTACAAGACCACGCCTCCCGTGCTGGACTCCGACGGCTC

CTTCTTCCTCTACAGCAAGCTCACCGTGGACAAGAGCAGGTGGCAGCAGGGGAACGTCTTCTCATGCTCCGTGATG
CATGAGGCTCTGCACAACCACTACACGCAGAAGAGCCTCTCCCTGTCTCCGGGTAAATGA (SEQ ID
NO: 469)

Amino Acid Sequence

QTVVTQEPSLTVSPGGTVTLTCRSSTGAVTTSNYANWVQQKPGQAPRGLIGGTNKRAPGTPARFSGSLLGGKAALT
LSGVQPEDEAEYYCALWYSNLWVFGGGTKLTVLGGGGSGGGGSGGGGSEVQLVESGGGLVQPGGSLKLSCAASGFT
FNTYAMNWVRQAPGKGLEWVARIRSKYNNYATYYADSVKDRFTISRDDSKNTAYLQMNNLKTEDTAVYYCVRHGNF
GNSYVSWFAYWGQGLTVTVSSGGGGSQVQLQESGPGLVRPSQTLSTCTVSGYSITSDHAWSWVRQPPGRGLEWIG
YISYSGITTYNPSLKSRTISRDN SKNTLYLQMNSLRAEDTAVYYCARSLARTTAMDYWGQGS LTVTVSSASTKGPS
VFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVTVTPSSSLGTQTYIC
NVNHKPSNTKVDKKVEPKSCDKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVDVSHEDPEVKFN
WYVDGVEVHNAKTKPREEQYQSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLF
PSREEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVM
HEALHNHYTQKSLSLSPGK* (SEQ ID NO: 470)

pLW077: LC IL6R

Nucleotide Sequence

GACATCCAGATGACTCAGTCTCCTAGCTCCCTGTCCGCCTCTGTGGGGGACCGAGTCACCATCACATGCAGAGCCA
GCCAGGATATTTCTAGTTACCTGAACTGGTATCAGCAGAAGCCCGGAAAAGCACCTAAGCTGCTGATCTACTATAC
CTCCAGGCTGCACTCTGGCGTGCCAGTCGGTTCAGTGGCTCAGGGAGCGGAACCGACTTCACTTTTACCATCTCA
AGCCTGCAGCCAGAGGATATTGCCACATACTATTGTCAGCAGGGCAATACTGCCCCTACACTTTTGGCCAGGGGA
CCAAGGTGGAAATCAAACGTACGGTGGCTGCACCATCTGTCTTCATCTTCCCGCCATCTGATGAGCAGTTGAAATC
TGGAAGTGCCTCTGTTGTGTGCCTGCTGAATAACTTCTATCCCAGAGAGGCCAAAGTACAGTGGAAGGTGGATAAC
GCCCTCCAATCGGGTAACTCCCAGGAGAGTGTACAGAGCAGGACAGCAAGGACAGCACCTACAGCCTCAGCAGCA
CCCTGACGCTGAGCAAAGCAGACTACGAGAAACACAAAGTCTACGCCTGCGAAGTCACCCATCAGGGCCTGAGCTC
GCCCGTCACAAAGAGCTTCAACAGGGGAGAGTGTTAG (SEQ ID NO: 467)

Amino Acid Sequence

DIQMTQSPSSLSASVGDRVITITCRASQDISSYLNWYQQKPGKAPKLLIYYTSRLHSGVPSRFSGSGSGTDFTFTIS
SLQPEDIAITYYCQQGNTLPYTFGQGTKVEIKRTVAAPSVFIFPPSDEQLKSGTASVVCLLNNFYPREAKVQWKVDN
ALQSGNSQESVTEQDSKSTYSLSSTLTLSKADYEKHKVYACEVTHQGLSSPVTKSFNRGEC* (SEQ ID
NO: 468)

CI027: IL6RN297Q-15865_1204-CD3LvHv-H-N

pLW085: HC IL6RN297Q-15865_1204-CD3LvHv-H-N

Nucleotide Sequence

[spacer (SEQ ID NO: 507)][pLW085 without spacer (SEQ ID NO: 513)]
 [CAAGGCCAGTCTGGCCAA][ATGATGTATTGCGGTGGGAATGAGGTGTTGTGCGGGCCGCGGGTTGGCTCGAGCG
 GTGGCAGCGGTGGCTCTGGTGGTCTGAGCGGCCGTTCCGATAATCATGGCGGCGGTTCTCAGACCGTGGTCACACA
 GGAGCCCTCACTGACAGTGAGCCCTGGCGGGACCGTCACACTGACTTGTGCGCAGTTCAACTGGCGCCGTGACTACC
 AGCAATTACGCTAACTGGGTCCAGCAGAAACCAGGACAGGCACCACGAGGACTGATCGGAGGAACTAATAAGAGAG
 CACCAGGAACCCCTGCAAGGTTCTCCGGATCTCTGCTGGGGGGAAAAGCCGCTCTGACACTGAGCGGCGTGCAGCC
 TGAGGACGAAGCTGAGTACTATTGCGCACTGTGGTACTCCAACCTGTGGGTGTTTGGCGGGGGAACTAAGCTGACC
 GTCCTGGGAGGAGGAGGAAGCGGAGGAGGAGGGAGCGGAGGAGGAGGATCCGAAGTGCAGCTGGTCGAGAGCGGAG
 GAGGACTGGTGCAGCCAGGAGGATCCCTGAAGCTGTCTTGTGCAGCCAGTGGCTTCACCTTCAACACTTACGCAAT
 GAACTGGGTGCGGCAGGCACCTGGGAAGGGACTGGAATGGGTGCGCCGGATCAGATCTAAATACAATAACTATGCC
 ACCTACTATGCTGACAGTGTGAAGGATAGGTTACCATTTTACGCGACGATAGCAAAAACACAGCTTATCTGCAGA
 TGAATAACCTGAAGACCGAGGATACAGCAGTGTACTATTGCGTCAGACACGGCAATTTTCGGGAACCTTTACGTGAG
 TTGGTTTGCCTATTGGGGACAGGGGACACTGGTCACCGTCTCCTCAGGAGGTGGTGGATCCCAGGTGCAGCTGCAG
 GAGTCCGGACCAGGACTGGTCCGGCCCTCACAGACTCTGAGCCTGACATGCACTGTGTCAGGCTACAGCATCACCT
 CCGATCACGCCTGGAGCTGGGTGAGGCAGCCACCTGGACGCGGCCTGGAATGGATCGGCTACATTTCTTATAGTGG
 GATCACACATACAACCCCTCTCTGAAGAGTCGAGTGACCATTTCCAGAGACAACCTCTAAAAATACACTGTATCTG
 CAGATGAATAGTCTGCGGGCCGAGGATACAGCTGTGTACTATTGTGCACGGTCTCTGGCCAGAACTACCGCTATGG
 ACTATTGGGGGCAGGGAAGCCTGGTGACCGTCAGCTCCGCTAGCACCAAGGGCCCATCGGTCTTCCCCCTGGCACC
 CTCCTCCAAGAGCACCTCTGGGGGCACAGCGGCCCTGGGCTGCCTGGTCAAGGACTACTTCCCCGAACCGGTGACG
 GTGTCGTGGAACCTCAGGCGCCCTGACCAGCGGCGTGCACACCTTCCCGGCTGTCCTACAGTCCTCAGGACTCTACT
 CCCTCAGCAGCGTGGTGACCGTGCCCTCCAGCAGCTTGGGCACCCAGACCTACATCTGCAACGTGAATCACAAGCC
 CAGCAACACCAAGGTGGACAAGAAAGTTGAGCCCAAATCTTGTGACAAAACCTCACACATGCCCACCGTGCCCAGCA
 CCTGAACTCCTGGGGGGACCGTCAGTCTTCCTCTTCCCCC AAAACCCAAGGACACCCTCATGATCTCCCGGACCC
 CTGAGGTACATGCGTGGTGGTGGACGTGAGCCACGAAGACCCTGAGGTCAAGTTCAACTGGTACGTGGACGGCGT
 GGAGGTGCATAATGCCAAGACAAAGCCGCGGGAGGAGCAGTACCAGAGCACGTACCGTGTGGTCAGCGTCCTCACC
 GTCCTGCACCAGGACTGGCTGAATGGCAAGGAGTACAAGTGCAAGGTCTCCAACAAAGCCCTCCCAGCCCCCATCG
 AGAAAACCATCTCCAAAGCCAAAGGGCAGCCCCGAGAACCACAGGTGTACACCCTGCCCCCATCCCGGGAGGAGAT
 GACCAAGAACCAGGTGAGCCTGACCTGCCTGGTCAAAGGCTTCTATCCCAGCGACATCGCCGTGGAGTGGGAGAGC
 AATGGGCAGCCGGAGAACAACCTACAAGACCACGCCTCCCGTGCTGGACTCCGACGGCTCCTTCTTCCTCTACAGCA
 AGCTCACCGTGGACAAGAGCAGGTGGCAGCAGGGGAACGTCTTCTCATGCTCCGTGATGCATGAGGCTCTGCACAA
 CCACTACACGCAGAAGAGCCTCTCCCTGTCTCCGGGTAAATGA] (SEQ ID NO: 471)

Amino Acid Sequence

[spacer (SEQ ID NO: 87)][pLW085 without spacer (SEQ ID NO: 514)]
 [QGQSGQ][MMYCGGNEVLCGPRVGSSGGSGGSLSGRSDNHGGGSQTVVTQEPSLTVSPGGTVTLTCRSSTGAV
 TTSNYANWVQQKPGQAPRGLIGGTNKRAPGTPARFSGSLLGGKAALTLSGVQPEDEAEYYCALWYSNLWVFGGGTK
 LTVLGGGGSGGGSGGGGSEVQLVESGGGLVQPGGSLKLSCAASGFTFNTYAMNWVRQAPGKGLEWVARIRSKYNN
 YATYYADSVKDRFTISRDDSKNTAYLQMNNLKTEDTAVYYCVRHGNFGNSYVSWFAYWGQGLTVTVSSGGGGSQVQ
 LQESGPGLVRPSQTLSTCTVSGYSITSDHAWSWVRQPPGRGLEWIGYISYSGITTYNPSLKSRTVISRDNSKNTL
 YLQMNSLRAEDTAVYYCARSLARTTAMDYWGQGLVTVSSASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEP
 VTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSSLGTQTYICNVNHKPSNTKVDKKVEPKSCDKTHTCPPC
 PAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYQSTYRVVSV
 LTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSREEMTKNQVSLTCLVKGFYPSDIAVEW
 ESNGQPENNYKTTTPVLDSDGSFFLYSKLTVDKSRWQQGNVVFSCSVMHEALHNHYTQKSLSLSPGK]* (SEQ ID
 NO: 472)

pLW077: LC IL6R

Nucleotide Sequence

GACATCCAGATGACTCAGTCTCCTAGCTCCCTGTCCGCCTCTGTGGGGGACCGAGTCACCATCACATGCAGAGCCA
 GCCAGGATATTTCTAGTTACCTGAACTGGTATCAGCAGAAGCCCGGAAAAGCACCTAAGCTGCTGATCTACTATAC
 CTCCAGGCTGCACTCTGGCGTGCCAGTCGGTTCAGTGGCTCAGGGAGCGGAACCGACTTCACTTTTACCATCTCA
 AGCCTGCAGCCAGAGGATATTGCCACATACTATTGTCAGCAGGGCAATACACTGCCCTACACTTTTGGCCAGGGGA
 CCAAGGTGGAAATCAAACGTACGGTGGCTGCACCATCTGTCTTCATCTTCCCGCCATCTGATGAGCAGTTGAAATC
 TGGAAGTGCCTCTGTTGTGTGCCTGCTGAATAACTTCTATCCCAGAGAGGCCAAAGTACAGTGGAAGGTGGATAAC
 GCCCTCCAATCGGGTAACTCCCAGGAGAGTGTACAGAGCAGGACAGCAAGGACAGCACCTACAGCCTCAGCAGCA
 CCCTGACGCTGAGCAAAGCAGACTACGAGAAACACAAAGTCTACGCCTGCGAAGTCACCCATCAGGGCCTGAGCTC
 GCCCGTCACAAAGAGCTTCAACAGGGGAGAGTGTTAG (SEQ ID NO: 467)

Amino Acid Sequence

DIQMTQSPSSLSASVGDRVTITCRASQDISSYLNWYQQKPGKAPKLLIYYTSRLHSGVPSRFSGSGSGTDFTFTIS
 SLQPEDATYYCQQGNTLPYTFGQGTKVEIKRTVAAPSVFIFPPSDEQLKSGTASVVCLLNNFYPREAKVQWKVDN
 ALQSGNSQESVTEQDSKSTYSLSTLTLSKADYEKHKVYACEVTHQGLSSPVTKSFNRGEC* (SEQ ID
 NO: 468)

CI029: 4792-1204-IL6RN297Q-15865 1204-CD3LvHv-H-N

pLW085: HC IL6RN297Q-15865_1204-CD3LvHv-H-N

Nucleotide Sequence

[spacer (SEQ ID NO: 507)][pLW085 without spacer (SEQ ID NO: 513)]

[CAAGGCCAGTCTGGCCAA] [ATGATGTATTGCGGTGGGAATGAGGTGTTGTGCGGGCCGCGGGTTGGCTCGAGCG
 GTGGCAGCGGTGGCTCTGGTGGTCTGAGCGGCCGTTCCGATAATCATGGCGGCGGTTCTCAGACCGTGGTCACACA
 GGAGCCCTCACTGACAGTGAGCCCTGGCGGGACCGTCACACTGACTTGTGCGCAGTTCAACTGGCGCCGTGACTACC
 AGCAATTACGCTAACTGGGTCCAGCAGAAACCAGGACAGGCACCACGAGGACTGATCGGAGGAACATAAAGAGAG
 CACCAGGAACCCCTGCAAGGTTCTCCGGATCTCTGCTGGGGGGAAAAGCCGCTCTGACACTGAGCGGCGTGCAGCC
 TGAGGACGAAGCTGAGTACTATTGCGCACTGTGGTACTCCAACCTGTGGGTGTTTGGCGGGGGAACTAAGCTGACC
 GTCCTGGGAGGAGGAGGAAGCGGAGGAGGAGGGAGCGGAGGAGGAGGATCCGAAGTGCAGCTGGTCGAGAGCGGAG
 GAGGACTGGTGCAGCCAGGAGGATCCCTGAAGCTGTCTTGTGCAGCCAGTGGCTTCACCTTCAACACTTACGCAAT
 GAACTGGGTGCGGCAGGCACCTGGGAAGGGACTGGAATGGGTGCGCCCGGATCAGATCTAAATACAATAACTATGCC
 ACCTACTATGCTGACAGTGTGAAGGATAGGTTACCATTTACGCGACGATAGCAAAAACACAGCTTATCTGCAGA
 TGAATAACCTGAAGACCGAGGATACAGCAGTGTACTATTGCGTCAGACACGGCAATTTCCGGGAACCTTTACGTGAG
 TTGGTTTGCCTATTGGGGACAGGGGACACTGGTCACCGTCTCCTCAGGAGGTGGTGGATCCCAGGTGCAGCTGCAG
 GAGTCCGGACCAGGACTGGTCCGGCCCTCACAGACTCTGAGCCTGACATGCACTGTGTCAGGCTACAGCATCACCT
 CCGATCACGCCTGGAGCTGGGTGAGGCAGCCACCTGGACGCGGCCTGGAATGGATCGGCTACATTTCTTATAGTGG
 GATCACACATAACAACCCCTCTCTGAAGAGTCGAGTGACCATTTCCAGAGACAACCTCTAAAAATACACTGTATCTG
 CAGATGAATAGTCTGCGGGCCGAGGATACAGCTGTGTACTATTGTGCACGGTCTCTGGCCAGAACTACCGCTATGG
 ACTATTGGGGGCAGGGAAGCCTGGTGACCGTCAGCTCCGCTAGCACCAAGGGCCCATCGGTCTTCCCCCTGGCACC
 CTCCTCCAAGAGCACCTCTGGGGGCACAGCGGCCCTGGGCTGCCTGGTCAAGGACTACTTCCCCGAACCGGTGACG
 GTGTCGTGGAACCTCAGGCGCCCTGACCAGCGGCGTGCACACCTTCCCGGCTGTCCTACAGTCCTCAGGACTCTACT
 CCCTCAGCAGCGTGGTGACCGTGCCCTCCAGCAGCTTGGGCACCCAGACCTACATCTGCAACGTGAATCACAAGCC
 CAGCAACACCAAGGTGGACAAGAAAGTTGAGCCCAAATCTTGTGACAAAACCTCACACATGCCCACCGTGCCCAGCA
 CCTGAACTCCTGGGGGGACCGTCAGTCTTCCTCTTCCCCCAAAACCCAAGGACACCCTCATGATCTCCCGGACCC
 CTGAGGTCACATGCGTGGTGGTGGACGTGAGCCACGAAGACCCTGAGGTCAAGTTCAACTGGTACGTGGACGGCGT
 GGAGGTGCATAATGCCAAGACAAAGCCGCGGGAGGAGCAGTACCAGAGCACGTACCGTGTGGTCAGCGTCCTCACC
 GTCCTGCACCAGGACTGGCTGAATGGCAAGGAGTACAAGTGCAAGGTCTCCAACAAAGCCCTCCCAGCCCCCATCG
 AGAAAACCATCTCCAAGCCAAAGGGCAGCCCCGAGAACCACAGGTGTACACCCTGCCCCCATCCCGGGAGGAGAT
 GACCAAGAACCAGGTCAGCCTGACCTGCCTGGTCAAAGGCTTCTATCCCAGCGACATCGCCGTGGAGTGGGAGAGC
 AATGGGCAGCCGGAGAACAACCTACAAGACCACGCCTCCCGTGCTGGACTCCGACGGCTCCTTCTTCCTCTACAGCA
 AGCTCACCGTGGACAAGAGCAGGTGGCAGCAGGGGAACGTCTTCTCATGCTCCGTGATGCATGAGGCTCTGCACAA
 CCACTACACGCAGAAGAGCCTCTCCCTGTCTCCGGGTAAATGA] (SEQ ID NO: 471)

Amino Acid Sequence

[spacer (SEQ ID NO: 87)][pLW085 without spacer (SEQ ID NO: 514)]
 [QGQSGQ] [MMYCGGNEVLCGPRVGSSGSGSGGLSGRSDNHGGGSQTVVTQEPSLTVSPGGTVTLTCRSSTGAV
 TTSNYANWVQQKPGQAPRGLIGGTNKRAPGTPARFSGSLLGGKAALTLSGVQPEDEAEYYCALWYSNLWVFGGGTK
 LTVLGGGGSGGGSGGGGSEVQLVESGGGLVQPGGSLKLSCAASGFTFNTYAMNWVRQAPGKGLEWVARIRSKYNN
 YATYYADSVKDRFTISRDDSKNTAYLQMNNLKTEDTAVYYCVRHGNFNGNSYVSWFAYWGQGLVTVSSGGGGGSQVQ

LQESGPGLVRPSQTLSTCTVSGYSITSDHAWSWVRQPPGRGLEWIGYISYSGITTYNPSLKSRVTISRDN SKNTL
 YLQMNSLRAEDTAVYYCARSLARTTAMDYWGQGS LVTVSSASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEP
 VTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSSLGTQTYICNVNHKPSNTKVDKKVEPKSCDKTHTCPPC
 PAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYQSTYRVVSV
 LTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSREEMTKNQVSLTCLVKGFYPSDIAVEW
 ESNGQPENNYKTTTPVLDSDGSFFLYSKLTVDKSRWQQGNV FSCSV MHEALHNHYTQKSLSLSPGK]* (SEQ ID
 NO: 472)

pLW080: LC IL6R 4792 1204

Nucleotide Sequence

[spacer (SEQ ID NO: 507)][pLW080 without spacer (SEQ ID NO: 515)]
 [CAAGGCCAGTCTGGCCAG][TATGGGTCCTGCAGTTGGA ACTATGTACACATATTCATGGATTGCGGCTCGAGCG
 GTGGCAGCGGTGGCTCTGGTGGTCTGAGCGGCCGTTCCGATAATCATGGCGGCGGTTCTGACATCCAGATGACTCA
 GTCTCCTAGCTCCCTGTCCGCCTCTGTGGGGGACCGAGTCACCATCACATGCAGAGCCAGCCAGGATATTTCTAGT
 TACCTGAACTGGTATCAGCAGAAGCCCGGAAAAGCACCTAAGCTGCTGATCTACTATACTCCAGGCTGCACTCTG
 GCGTGCC CAGTCGGTTCAGTGGCTCAGGGAGCGGAACCGACTTCACTTTTACCATCTCAAGCCTGCAGCCAGAGGA
 TATTGCCACATACTATTGTCAGCAGGGCAATACACTGCCCTACACTTTTGGCCAGGGGACCAAGGTGGAAATCAAA
 CGTACGGTGGCTGCACCATCTGTCTTCATCTTCCCGCCATCTGATGAGCAGTTGAAATCTGGAAGTGCCTCTGTTG
 TGTGCCTGCTGAATAACTTCTATCCCAGAGAGGCCAAAGTACAGTGGAAGGTGGATAACGCCCTCCAATCGGGTAA
 CTCCCAGGAGAGTGTACAGAGCAGGACAGCAAGGACAGCACCTACAGCCTCAGCAGCACCCCTGACGCTGAGCAAA
 GCAGACTACGAGAAACACAAAGTCTACGCCTGCGAAGTCACCCATCAGGGCCTGAGCTCGCCCGTCACAAAGAGCT
 TCAACAGGGGAGAGTGTTAG] (SEQ ID NO: 473)

Amino Acid Sequence

[spacer (SEQ ID NO: 87)][pLW080 without spacer (SEQ ID NO: 516)]
 [QGQSGQ][YGSCSWNYVHIFMDCGSSGGSGGSGGLSGRSDNHGGGSDIQMTQSPSSLSASVGDRVTITCRASQDI
 SSYLNWYQQKPGKAPKLLIYYTSRLHSGVPSRFSGSGSGTDFTFTISSLPEDIATYYCQQGNTLPYTFGQGTKVE
 IKRTVAAPSVFIFPPSDEQLKSGTASVVCLLNNFYPREAKVQWKVDNALQSGNSQESVTEQDSKDSTYSLSSTLTL
 SKADYEKHKVYACEVTHQGLSSPVTKSFNRGEC]* (SEQ ID NO: 474)

CI036: 4792-1204-IL6RN297Q-CD3LvHv-H-N

pLW083: HC AV1N297Q-CD3LvHv-H-N

Nucleotide Sequence

CAGACCGTGGTCACACAGGAGCCCTCACTGACAGTGAGCCCTGGCGGGACCGTCACACTGACTTGTCGCAGTTCAA
 CTGGCGCCGTGACTACCAGCAATTACGCTAACTGGGTCCAGCAGAAACCAGGACAGGCACCACGAGGACTGATCGG
 AGGAACTAATAAGAGAGCACCAGGAACCCCTGCAAGGTTCTCCGGATCTCTGCTGGGGGGAAAAGCCGCTCTGACA

CTGAGCGGCGTGCAGCCTGAGGACGAAGCTGAGTACTATTGCGCACTGTGGTACTCCAACCTGTGGGTGTTTGGCG
 GGGGAACCTAAGCTGACCGTCCTGGGAGGAGGAGGAAGCGGAGGAGGAGGGAGCGGAGGAGGAGGATCCGAAGTGCA
 GCTGGTCGAGAGCGGAGGAGGACTGGTGCAGCCAGGAGGATCCCTGAAGCTGTCTTGTGCAGCCAGTGGCTTCACC
 TTCAACACTTACGCAATGAACTGGGTGCGGCAGGCACCTGGGAAGGGACTGGAATGGGTGCGCCGGATCAGATCTA
 AATACAATAACTATGCCACCTACTATGCTGACAGTGTGAAGGATAGGTTTACCATTTCACGCGACGATAGCAAAAA
 CACAGCTTATCTGCAGATGAATAACCTGAAGACCGAGGATACAGCAGTGTACTATTGCGTCAGACACGGCAATTTTC
 GGGAACTCTTACGTGAGTTGGTTTGCCTATTGGGGACAGGGGACACTGGTCACCGTCTCCTCAGGAGGTGGTGGAT
 CCCAGGTGCAGCTGCAGGAGTCCGGACCAGGACTGGTCCGGCCCTCACAGACTCTGAGCCTGACATGCACTGTGTC
 AGGCTACAGCATCACCTCCGATCACGCCTGGAGCTGGGTGAGGCAGCCACCTGGACGCGGCCTGGAATGGATCGGC
 TACATTTCTTATAGTGGGATCACACATAACAACCCCTCTCTGAAGAGTCGAGTGACCATTTCCAGAGACAACCTCTA
 AAAATACACTGTATCTGCAGATGAATAGTCTGCGGGCCGAGGATACAGCTGTGTACTATTGTGCACGGTCTCTGGC
 CAGAACTACCGCTATGGACTATTGGGGGCAGGGAAGCCTGGTGACCGTCAGCTCCGCTAGCACCAAGGGCCCATCG
 GTCTTCCCCCTGGCACCCCTCCTCCAAGAGCACCTCTGGGGGCACAGCGGCCCTGGGCTGCCTGGTCAAGGACTACT
 TCCCCGAACCGGTGACGGTGTCTGTGGAACCTCAGGCGCCCTGACCAGCGGCGTGCACACCTTCCCGGCTGTCTTACA
 GTCCTCAGGACTCTACTCCCTCAGCAGCGTGGTGACCGTGCCCTCCAGCAGCTTGGGCACCCAGACCTACATCTGC
 AACGTGAATCACAAGCCCAGCAACACCAAGGTGGACAAGAAAGTTGAGCCCAAATCTTGTGACAAAACCTCACACAT
 GCCCACCGTGCCCAGCACCTGAACTCCTGGGGGGACCGTCAGTCTTCTCTTCCCCCCTTCCCAAGGACACCCCT
 CATGATCTCCCGGACCCCTGAGGTCACATGCGTGGTGGTGGACGTGAGCCACGAAGACCCTGAGGTCAAGTTCAAC
 TGGTACGTGGACGGCGTGGAGGTGCATAATGCCAAGACAAAGCCGCGGGAGGAGCAGTACCAGAGCACGTACCGTG
 TGGTCAGCGTCCTCACCGTCCTGCACCAGGACTGGCTGAATGGCAAGGAGTACAAGTGCAAGGTCTCCAACAAAGC
 CCTCCCAGCCCCCATCGAGAAAACCATCTCCAAAGCCAAAGGGCAGCCCCGAGAACCACAGGTGTACACCCTGCCC
 CCATCCCGGGAGGAGATGACCAAGAACCAGGTCAGCCTGACCTGCCTGGTCAAAGGCTTCTATCCCAGCGACATCG
 CCGTGGAGTGGGAGAGCAATGGGCAGCCGGAGAACAACCTACAAGACCACGCCTCCCGTGCTGGACTCCGACGGCTC
 CTTCTTCTCTACAGCAAGCTCACCGTGGACAAGAGCAGGTGGCAGCAGGGGAACGTCTTCTCATGCTCCGTGATG
 CATGAGGCTCTGCACAACCACTACACGCAGAAGAGCCTCTCCCTGTCTCCGGGTAAATGA (SEQ ID
 NO: 469)

Amino Acid Sequence

QTVVTQEPSLTVSPGGTVTLTCRSSTGAVTTSNYANWVQQKPGQAPRGLIGGTNKRAPGTPARFSGSLLGGKAALT
 LSGVQPEDEAEYYCALWYSNLWVFGGGTKLTVLGGGSGGGGSGGGGSEVQLVESGGGLVQPGGSLKLSCAASGFT
 FNTYAMNWVRQAPGKGLEWVARIRSKYNNYATYYADSVKDRFTISRDDSKNTAYLQMNNLKTEDTAVYYCVRHGNF
 GNSYVSWFAYWGQGLVTVSSGGGGSQVQLQESGPGLVRPSQTLSTCTVSGYSITSDHAWSWVRQPPGRGLEWIG
 YISYSGITTYNPSLKSRTVISRDNSKNTLYLQMNSLRAEDTAVYYCARSLARTTAMDYWGQGS�TVTVSSASTKGPS
 VFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVTVTPSSSLGTQTYIC
 NVNHKPSNTKVDKKVEPKSCDKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVDVSHEDPEVKFN
 WYVDGVEVHNAKTKPREEQYQSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLF

PSREEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPVLDSGDSFFLYSKLTVDKSRWQQGNVFSVCSVM
HEALHNHYTQKSLSLSPGK* (SEQ ID NO: 470)

pLW080: LC IL6R 4792 1204

Nucleotide Sequence

[spacer (SEQ ID NO: 507)][pLW080 without spacer (SEQ ID NO: 515)]
[CAAGGCCAGTCTGGCCAG][TATGGGTCCTGCAGTTGGAACATGTACACATATTCATGGATTGCGGCTCGAGCG
GTGGCAGCGGTGGCTCTGGTGGTCTGAGCGGCCGTTCCGATAATCATGGCGGCGGTTCTGACATCCAGATGACTCA
GTCTCCTAGCTCCCTGTCCGCCTCTGTGGGGGACCGAGTCACCATCACATGCAGAGCCAGCCAGGATATTTCTAGT
TACCTGAACTGGTATCAGCAGAAGCCCGGAAAAGCACCTAAGCTGCTGATCTACTATACCTCCAGGCTGCACTCTG
GCGTGCCCAGTCGGTTCAGTGGCTCAGGGAGCGGAACCGACTTCACTTTTACCATCTCAAGCCTGCAGCCAGAGGA
TATTGCCACATACTATTGTCAGCAGGGCAATACTGCCCCTACACTTTTGGCCAGGGGACCAAGGTGGAAATCAAA
CGTACGGTGGCTGCACCATCTGTCTTCATCTTCCCGCCATCTGATGAGCAGTTGAAATCTGGAAGTGCCTCTGTTG
TGTGCCTGCTGAATAACTTCTATCCCAGAGAGGCCAAAGTACAGTGGAAGGTGGATAACGCCCTCCAATCGGGTAA
CTCCCAGGAGAGTGTACAGAGCAGGACAGCAAGGACAGCACCTACAGCCTCAGCAGCACCCCTGACGCTGAGCAAA
GCAGACTACGAGAAACACAAAGTCTACGCCTGCGAAGTCACCCATCAGGGCCTGAGCTCGCCCGTCACAAAGAGCT
TCAACAGGGGAGAGTGTTAG] (SEQ ID NO: 473)

Amino Acid Sequence

[spacer (SEQ ID NO: 87)][pLW080 without spacer (SEQ ID NO: 516)]
[QGQSGQ][YGSCSWNYVHIFMDCGSSGGSGGSGGLSGRSDNHGGGSDIQMTQSPSSLSASVGDRVITICRASQDI
SSYLNWYQQKPGKAPKLLIYYTSRLHSGVPSRFSGSGSGTDFTFTISSQLQPEDATYYCQQGNTLPYTFGQGTKVE
IKRTVAAPSVFIFPPSDEQLKSGTASVVCLLNNFYPREAKVQWKVDNALQSGNSQESVTEQDSKDSTYSLSSTLT
LTKADYEKHKVYACEVTHQGLSSPVTKSFNRGEC]* (SEQ ID NO: 474)

CI039: 3954-2001-C225v5 N297Q-15865-2001-CD3LvHv-H-N

pLW101: HC C225v5N297Q-15865-2001-CD3LvHv-H-N

Nucleotide Sequence

[spacer (SEQ ID NO: 507)][pLW101 without spacer (SEQ ID NO: 517)]
[CAAGGCCAGTCTGGCCAA][ATGATGTATTGCGGTGGGAATGAGGTGTTGTGCGGGCCGCGGTTGGCTCGAGCG
GTGGCAGCGGTGGCTCTGGTGGTATCTCTTCCGGAAGTGTGTCGCGGCGAGATCCGACAATCACGGCGGCGGTTCTCA
GACCGTGGTCACACAGGAGCCCTCACTGACAGTGAGCCCTGGCGGGACCGTCACACTGACTTGTGCGCAGTTCAACT
GGCGCCGTGACTACCAGCAATTACGCTAACTGGGTCCAGCAGAAACCAGGACAGGCACCACGAGGACTGATCGGAG
GAACTAATAAGAGAGCACCAGGAACCCCTGCAAGGTTCTCCGGATCTCTGCTGGGGGGAAAAGCCGCTCTGACACT
GAGCGGCGTGCAGCCTGAGGACGAAGCTGAGTACTATTGCGCACTGTGGTACTCCAACCTGTGGGTGTTTGGCGGG
GGAATAAGCTGACCGTCCTGGGAGGAGGAGGAAGCGGAGGAGGAGGGAGCGGAGGAGGAGGATCCGAAGTGCAGC

TGGTCGAGAGCGGAGGAGGACTGGTGCAGCCAGGAGGATCCCTGAAGCTGTCTTGTGCAGCCAGTGGCTTCACCTT
 CAACACTTACGCAATGAACTGGGTGCGGCAGGCACCTGGGAAGGGACTGGAATGGGTGCCCCGGATCAGATCTAAA
 TACAATAACTATGCCACCTACTATGCTGACAGTGTGAAGGATAGGTTCACCATTTTCACGCGACGATAGCAAAAACA
 CAGCTTATCTGCAGATGAATAACCTGAAGACCGAGGATACAGCAGTGTACTATTGCGTCAGACACGGCAATTTTCGG
 GAACTCTTACGTGAGTTGGTTTGCCTATTGGGGACAGGGGACACTGGTCACCGTCTCCTCAGGAGGTGGTGGATCC
 CAGGTGCAGCTGAAACAGAGCGGCCCGGGCCTGGTGCAGCCGAGCCAGAGCCTGAGCATTACCTGCACCGTGAGCG
 GCTTTAGCCTGACCAACTATGGCGTGCATTGGGTGCGCCAGAGCCCCGGGCAAAGGCCTGGAATGGCTGGGCGTGAT
 TTGGAGCGGCGGCAACACCGATTATAACACCCCCGTTTACCAGCCGCTGAGCATTAACAAAGATAACAGCAAAAGC
 CAGGTGTTTTTTTAAATGAACAGCCTGCAAAGCCAGGATACCGCGATTTATTATTGCGCGCGCGCGCTGACCTATT
 ATGATTATGAATTTGCGTATTGGGGCCAGGGCACCTGGTGACCGTGAGCGCGGCTAGCACCAAGGGCCCCATCGGT
 CTTCCCCCTGGCACCCCTCCTCCAAGAGCACCTCTGGGGGCACAGCGGCCCTGGGCTGCCTGGTCAAGGACTACTTC
 CCCGAACCGGTGACGGTGTTCGTGGAACCTCAGGCGCCCTGACCAGCGGCGTGACACCTTCCCGGCTGTCCTACAGT
 CCTCAGGACTCTACTCCCTCAGCAGCGTGGTGACCGTGCCCTCCAGCAGCTTGGGCACCCAGACCTACATCTGCAA
 CGTGAATCACAAGCCCAGCAACACCAAGGTGGACAAGAAAGTTGAGCCCCAAATCTTGTGACAAAACCTCACACATGC
 CCACCGTGCCCAGCACCTGAACTCCTGGGGGGACCGTCAGTCTTCCTCTTCCCCCCTTCCCAAGGACACCCCTCA
 TGATCTCCCGGACCCCTGAGGTCACATGCGTGGTGGTGGACGTGAGCCACGAAGACCCTGAGGTCAAGTTCAACTG
 GTACGTGGACGGCGTGGAGGTGCATAATGCCAAGACAAAGCCGCGGGAGGAGCAGTACCAGAGCACGTACCGTGTG
 GTCAGCGTCCTCACCGTCCTGCACCAGGACTGGCTGAATGGCAAGGAGTACAAGTGCAAGGTCTCCAACAAAGCCC
 TCCCAGCCCCCATCGAGAAAACCATCTCCAAAGCCAAAGGGCAGCCCCGAGAACCACAGGTGTACACCCTGCCCCC
 ATCCCGGGAGGAGATGACCAAGAACCAGGTCAGCCTGACCTGCCTGGTCAAAGGCTTCTATCCCAGCGACATCGCC
 GTGGAGTGGGAGAGCAATGGGCAGCCGGAGAACAACCTACAAGACCACGCCTCCCGTGCTGGACTCCGACGGCTCCT
 TCTTCCTCTACAGCAAGCTCACCGTGGACAAGAGCAGGTGGCAGCAGGGGAACGTCTTCTCATGCTCCGTGATGCA
 TGAGGCTCTGCACAACCACTACACGCAGAAGAGCCTCTCCCTGTCTCCGGGTAAATGA] (SEQ ID NO: 479)

Amino Acid Sequence

[spacer (SEQ ID NO: 87)][pLW101 without spacer (SEQ ID NO: 518)]
 [QGQSGQ][MMYCGGNEVLCGPRVGSSGGSGGSGGISSGLLSGRSDNHGGGSQTVVTQEPSLTVSPGGTVTLTCRS
 STGAVTTSNYANWVQQKPGQAPRGLIGGTNKRAPGTPARFSGSLLGGKAALTLSGVQPEDEAEYYCALWYSNLWVF
 GGGTKLTVLGGGSGGGGSGGGGSEVQLVESGGGLVQPGGSLKLSAASGFTFNTYAMNWVRQAPGKLEWVARIR
 SKYNNYATYYADSVKDRFTISRDDSKNTAYLQMNNLKTEDTAVYYCVRHGNFGNSYVSWFAYWGQGTTLTVSSGGG
 GSQVQLKQSGPGLVQPSQSLITCTVSGFSLTNYGVHWVRQSPGKLEWLGVIWSSGNTDYNTPTFTSRLSINKDNS
 KSQVFFKMNSLQSQDTAIYYCARALTYDYEFAYWGQGTTLTVSAASTKGPSVFPLAPSSKSTSGGTAALGCLVKD
 YFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSSLGTQTYICNVNHKPSNTKVDKKVEPKSCDKTH
 TCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYQSTY
 RVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSREEMTKNQVSLTCLVKGFYPSD
 IAVEWESNGQPENNYKTTTPVLDSDGSFFLYSKLTVDKSRWQQGNVVFSCSVMHEALHNHYTQKSLSLSPGK] *
 (SEQ ID NO: 480)

LC C225-3954-2001

Nucleotide Sequence

[spacer (SEQ ID NO: 507)][3954-2001-C225v5 without spacer (SEQ ID NO: 519)]
 [CAAGGCCAGTCTGGCCAG][TGCATCTCACCTCGTGGTTGTCCGGACGGCCCATACGTCATGTACGGCTCGAGCG
 GTGGCAGCGGTGGCTCTGGTGGATCCGGTATTAGCAGTGGTCTGTAAAGCGGTCGTAGCGATAATCATGGCAGTAG
 CGGTACCCAGATCTTGCTGACCCAGAGCCCGGTGATTCTGAGCGTGAGCCCGGGCGAACGTGTGAGCTTTAGCTGC
 CGCGCGAGCCAGAGCATTGGCACCAACATTCATTGGTATCAGCAGCGCACCAACGGCAGCCCGCGCCTGCTGATTA
 AATATGCGAGCGAAAGCATTAGCGGCATTCCGAGCCGCTTTAGCGGCAGCGGCAGCGGCACCGATTTTACCCTGAG
 CATTAAACAGCGTGGAAGCGAAGATATTGCGGATTATTATTGCCAGCAGAACAACAACCTGGCCGACCACCTTTGGC
 GCGGGCACCAAACTGGAACGTAAACGTACGGTGGCTGCACCATCTGTCTTCATCTTCCCGCCATCTGATGAGCAGT
 TGAAATCTGGAACCTGCCTCTGTTGTGTGCCTGCTGAATAACTTCTATCCCAGAGAGGCCAAAGTACAGTGGAAGGT
 GGATAACGCCCTCCAATCGGGTAACTCCCAGGAGAGTGTACAGAGCAGGACAGCAAGGACAGCACCTACAGCCTC
 AGCAGCACCTTGACGCTGAGCAAAGCAGACTACGAGAAACACAAAGTCTACGCCTGCGAAGTCACCCATCAGGGCC
 TGAGCTCGCCCGTCACAAAGAGCTTCAACAGGGGAGAGTGTTAG] (SEQ ID NO: 481)

Amino Acid Sequence

[spacer (SEQ ID NO: 87)][pLW080 without spacer (SEQ ID NO: 520)]
 [QGQSGQ][CISPRGCPDGPYVMYGSSGGSGGSGSGISSGLLSGRSDNHGSSGTQILLTQSPVILSVSPGERVSF
 SCRASQSIGTNIHWYQQRNNGSPRLLIKYASESISGIPSRFSGSGSGTDFTLSINSVESEDIADYYCQQNNNWPTT
 FGAGTKLELKRVAAPSVFIFPPSDEQLKSGTASVVCLLNNFYPREAKVQWKVDNALQSGNSQESVTEQDSKDSTY
 SLSSTLTLSKADYEKHKVYACEVTHQGLSSPVTKSFNRGEC]* (SEQ ID NO: 482)

CI040: 3954-2001-C225v5 N297Q-CD3LvHv-H-N

pLW057: HC C225v5N297Q-CD3LvHv-H-N

Nucleotide Sequence

CAGACCGTGGTCACACAGGAGCCCTCACTGACAGTGAGCCCTGGCGGGACCGTCACACTGACTTGTCGCAGTTCAA
 CTGGCGCCGTGACTACCAGCAATTACGCTAACTGGGTCCAGCAGAAACCAGGACAGGCACCACGAGGACTGATCGG
 AGGAACTAATAAGAGAGCACCAGGAACCCCTGCAAGGTTCTCCGGATCTCTGCTGGGGGGAAAAGCCGCTCTGACA
 CTGAGCGGCGTGCAGCCTGAGGACGAAGCTGAGTACTATTGCGCACTGTGGTACTCCAACCTGTGGGTGTTTGGCG
 GGGGAACTAAGCTGACCGTCCTGGGAGGAGGAGGAAGCGGAGGAGGAGGGAGCGGAGGAGGAGGATCCGAAGTGCA
 GCTGGTCGAGAGCGGAGGAGGACTGGTGCAGCCAGGAGGATCCCTGAAGCTGTCTTGTGCAGCCAGTGGCTTCACC
 TTCAACACTTACGCAATGAACTGGGTGCGGCAGGCACCTGGGAAGGGACTGGAATGGGTGCGCCGGATCAGATCTA
 AATACAATAACTATGCCACCTACTATGCTGACAGTGTGAAGGATAGGTTTACCATTTTACGCGACGATAGCAAAAA
 CACAGCTTATCTGCAGATGAATAACCTGAAGACCGAGGATACAGCAGTGTACTATTGCGTCAGACACGGCAATTTT
 GGGAACCTTACGTGAGTTGGTTTGCCTATTGGGGACAGGGGACACTGGTCAACCGTCTCCTCAGGAGGTGGTGGAT

CCCAGGTGCAGCTGAAACAGAGCGGCCCGGGCCTGGTGCAGCCGAGCCAGAGCCTGAGCATTACCTGCACCGTGAG
 CGGCTTTAGCCTGACCAACTATGGCGTGCATTGGGTGCGCCAGAGCCCGGGCAAAGGCCTGGAATGGCTGGGCGTG
 ATTTGGAGCGGCGGCAACACCGATTATAACACCCCGTTTACCAGCCGCCTGAGCATTAAACAAAGATAACAGCAAAA
 GCCAGGTGTTTTTTTAAATGAACAGCCTGCAAAGCCAGGATACCGCGATTTATTATTGCGCGCGCGCTGACCTA
 TTATGATTATGAATTTGCGTATTGGGGCCAGGGCACCCCTGGTGACCGTGAGCGCGGCTAGCACCAAGGGCCCATCG
 GTCTTCCCCCTGGCACCCCTCCTCCAAGAGCACCTCTGGGGGCACAGCGGCCCTGGGCTGCCTGGTCAAGGACTACT
 TCCCCGAACCGGTGACGGTGTCTGGAACCTCAGGCGCCCTGACCAGCGGCGTGACACCTTCCCGGCTGTCTACA
 GTCCTCAGGACTCTACTCCCTCAGCAGCGTGGTGACCGTGCCCTCCAGCAGCTTGGGCACCCAGACCTACATCTGC
 AACGTGAATCACAAGCCCAGCAACACCAAGGTGGACAAGAAAGTTGAGCCCAAATCTTGTGACAAAACCTCACACAT
 GCCACCGTGCCAGCACCTGAACCTCTGGGGGGACCGTCAGTCTTCTCTTCCCCCAAACCCAAGGACACCCT
 CATGATCTCCCGGACCCCTGAGGTACATGCGTGGTGGTGACGTGAGCCACGAAGACCCTGAGGTCAAGTTCAAC
 TGGTACGTGGACGGCGTGGAGGTGCATAATGCCAAGACAAAGCCGCGGGAGGAGCAGTACCAGAGCACGTACCGTG
 TGGTCAGCGTCCTCACCGTCCTGCACCAGGACTGGCTGAATGGCAAGGAGTACAAGTGCAAGGTCTCCAACAAAGC
 CCTCCCAGCCCCCATCGAGAAAACCATCTCCAAAGCCAAAGGGCAGCCCCGAGAACCACAGGTGTACACCCTGCCC
 CCATCCCGGGAGGAGATGACCAAGAACCAGGTACGCTGACCTGCCTGGTCAAAGGCTTCTATCCCAGCGACATCG
 CCGTGGAGTGGGAGAGCAATGGGCAGCCGGAGAACAACCTACAAGACCACGCCTCCCGTGCTGGACTCCGACGGCTC
 CTTCTTCTCTACAGCAAGCTCACCGTGGACAAGAGCAGGTGGCAGCAGGGGAACGTCTTCTCATGCTCCGTGATG
 CATGAGGCTCTGCACAACCACTACACGCAGAAGAGCCTCTCCCTGTCTCCGGGTAAATGA (SEQ ID
 NO: 882)

Amino Acid Sequence

QTVVTQEPSLTVSPGGTVTLTCSRSTGAVTTSNYANWVQQKPGQAPRGLIGGTNKRAPGTPARFSGSLLGGKAALT
 LSGVQPEDEAEYYCALWYSNLWVFGGKLTVLGGGGSGGGSGGGGSEVOLVESGGGLVQPGGSLKLSAASGFT
 FNTYAMNWVRQAPGKLEWVARIRSKYNNYATYYADSVKDRFTISRDDSKNTAYLQMNNLKTEDTAVYYCVRHGNF
 GNSYVSWFAYWGQGLVTVSSGGGSQVQLKQSGPGLVQPSQSLITCTVSGFSLTNYGVHWRQSPGKLEWLGV
 IWSGGNTDYNTPFTSRLSINKDNSKSQVFFKMNSLQSQDTAIYYCARALTYDYEFAYWGQGLVTVSAASTKGPS
 VFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVTVPSSSLGTQTYIC
 NVNHKPSNTKVDKKVEPKSCDKHTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFN
 WYVDGVEVHNAKTKPREEQYQSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLP
 PSREEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTTPVLDSGDSFFLYSKLTVDKSRWQQGNVFSQSV
 HEALHNHYTQKSLSLSPGK* (SEQ ID NO: 883)

LC C225-3954-2001

Nucleotide Sequence

[spacer (SEQ ID NO: 507)][3954-2001-C225v5without spacer (SEQ ID NO: 519)]
 [CAAGGCCAGTCTGGCCAG][TGCATCTCACCTCGTGGTTGTCCGGACGGCCCATACGTCATGTACGGCTCGAGCG
 GTGGCAGCGGTGGCTCTGGTGGATCCGGTATTAGCAGTGGTCTGTTAAGCGGTCTAGCGATAATCATGGCAGTAG

CGGTACCCAGATCTTGCTGACCCAGAGCCCGGTGATTCTGAGCGTGAGCCCGGGCGAACGTGTGAGCTTTAGCTGC
 CGCGCGAGCCAGAGCATTGGCACCAACATTCATTGGTATCAGCAGCGCACCAACGGCAGCCCGCGCCTGCTGATTA
 AATATGCGAGCGAAAGCATTAGCGGCATTCCGAGCCGCTTTAGCGGCAGCGGCAGCGGCACCGATTTTACCCTGAG
 CATTAACAGCGTGGAAGCGAAGATATTGCGGATTATTATTGCCAGCAGAACAACAACCTGGCCGACCACCTTTGGC
 GCGGGCACCAAACTGGAACGTAAACGTACGGTGGCTGCACCATCTGTCTTCATCTTCCCGCCATCTGATGAGCAGT
 TGAAATCTGGAACGTGCTCTGTTGTGTGCCTGCTGAATAACTTCTATCCCAGAGAGGCCAAAGTACAGTGGAAGGT
 GGATAACGCCCTCCAATCGGGTAACTCCCAGGAGAGTGTACAGAGCAGGACAGCAAGGACAGCACCTACAGCCTC
 AGCAGCACCTTGACGCTGAGCAAAGCAGACTACGAGAAACACAAAGTCTACGCCTGCGAAGTCACCCATCAGGGCC
 TGAGCTCGCCCGTCACAAAGAGCTTCAACAGGGGAGAGTGTTAG] (SEQ ID NO: 481)

Amino Acid Sequence

[spacer (SEQ ID NO: 87)][pLW080 without spacer (SEQ ID NO: 520)]
 [QGQSGQ][CISPRGCPDGPYVMYGSSGGSGSGSGSGISSGLLSGRSDNHGSSGTQILLTQSPVILSVSPGERVSF
 SCRASQSIGTNIHWYQQRNGSPRLLIKYASESISGIPSRFSGSGSGTDFTLSINSVESEDIADYYCQQNNNWPTT
 FGAGTKLELKRTVAAPSVFIFPPSDEQLKSGTASVVCLLNNFYPREAKVQWKVDNALQSGNSQESVTEQDSKDSTY
 SLSSTLTLSKADYEEKHKVYACEVTHQGLSSPVTKSFNRGEC]* (SEQ ID NO: 482)

CI021: C225v5N297Q-15003-CD3HvLv-H-N

pLW047: HC C225v5N297Q-15003-CD3HvLv-H-N

Nucleotide Sequence

[spacer (SEQ ID NO: 507)][pLW047 without spacer (SEQ ID NO: 522)]
 [CAAGGCCAGTCTGGCCAA][GGTTATCGGTGGGGTTGCGAGTGGAATTGCGGTGGGATTACTACTGGCTCGAGCG
 GTGGCAGCGGTGGCTCTGGTGGTCTGAGCGGCCGTTCCGATAATCATGGCGGCGGTTCTGAGGTGCAGCTGGTCGA
 GTCTGGAGGAGGATTGGTGCAGCCTGGAGGGTCATTGAAACTCTCATGTGCAGCCTCTGGATTCACCTTCAATAAG
 TACGCCATGAACTGGGTCCGCCAGGCTCCAGGAAAGGGTTTGAATGGGTTGCTCGCATAAGAAGTAAATATAATA
 ATTATGCAACATATTATGCCGATTCAGTGAAAGACAGGTTACCATCTCCAGAGATGATTCAAAAAACACTGCCTA
 TCTACAAATGAACAACCTGAAAACCTGAGGACACTGCCGTGTACTACTGTGTGAGACATGGGAACCTTCGGTAATAGC
 TACATATCCTACTGGGCTTACTGGGGCCAAGGGACTCTGGTCACCGTCTCCTCAGGTGGTGGTGGTCTTGGCGGCG
 GCGGCTCCGGTGGTGGTGGTCTCAGACTGTTGTGACTCAGGAACCTTCACTCACCGTATCACCTGGTGGAACAGT
 CACACTCACTTGTGGCTCCTCGACTGGGGCTGTTACATCTGGCTACTACCCAAACTGGGTCCAACAAAAACCAGGT
 CAGGCACCCCGTGGTCTAATAGGTGGGACTAAGTTCCTCGCCCCCGGTACTCCTGCCAGATTCTCAGGCTCCCTGC
 TTGGAGGCAAGGCTGCCCTCACCTCTCAGGGGTACAGCCAGAGGATGAGGCAGAATATTACTGTGCTCTATGGTA
 CAGCAACCGCTGGGTGTTTCGGTGGAGGAACCAAACTGACTGTCCTAGGAGGTGGTGGATCCCAGGTGCAGCTGAAA
 CAGAGCGGCCCCGGGCCTGGTGCAGCCGAGCCAGAGCCTGAGCATTACCTGCACCGTGAGCGGCTTTAGCCTGACCA
 ACTATGGCGTGCATTGGGTGCGCCAGAGCCCCGGGCAAAGGCCTGGAATGGCTGGGCGTGATTTGGAGCGGCGGCAA
 CACCGATTATAACACCCCGTTTACCAGCCGCCTGAGCATTAACAAAGATAACAGCAAAAGCCAGGTGTTTTTTAAA

ATGAACAGCCTGCAAAGCCAGGATACCGCGATTTATTATTGCGCGCGCGCTGACCTATTATGATTATGAATTTG
 CGTATTGGGGCCAGGGCACCCCTGGTGACCGTGAGCGCGGCTAGCACCAAGGGCCCATCGGTCTTCCCCCTGGCACC
 CTCCTCCAAGAGCACCTCTGGGGGCACAGCGGCCCTGGGCTGCCTGGTCAAGGACTACTTCCCCGAACCGGTGACG
 GTGTCGTGGAACCTCAGGCGCCCTGACCAGCGGCGTGACACCTTCCCGGCTGTCCTACAGTCCTCAGGACTCTACT
 CCCTCAGCAGCGTGGTGACCGTGCCCTCCAGCAGCTTGGGCACCCAGACCTACATCTGCAACGTGAATCACAAGCC
 CAGCAACACCAAGGTGGACAAGAAAGTTGAGCCCAAATCTTGTGACAAAACCTCACACATGCCCACCGTGCCCAGCA
 CCTGAACTCCTGGGGGGACCGTCAGTCTTCCTCTTCCCCCAAACCCAAGGACACCCTCATGATCTCCCGGACCC
 CTGAGGTCACATGCGTGGTGGTGGACGTGAGCCACGAAGACCCTGAGGTCAAGTTCAACTGGTACGTGGACGGCGT
 GGAGGTGCATAATGCCAAGACAAAGCCGCGGGAGGAGCAGTACCAGAGCACGTACCGTGTGGTCAGCGTCCTCACC
 GTCCTGCACCAGGACTGGCTGAATGGCAAGGAGTACAAGTGCAAGGTCTCCAACAAAGCCCTCCCAGCCCCCATCG
 AGAAAACCATCTCCAAAGCCAAAGGGCAGCCCCGAGAACCACAGGTGTACACCCTGCCCCCATCCCGGGAGGAGAT
 GACCAAGAACCAGGTCAGCCTGACCTGCCTGGTCAAAGGCTTCTATCCCAGCGACATCGCCGTGGAGTGGGAGAGC
 AATGGGCAGCCGGAGAACAACCTACAAGACCACGCCTCCCGTGCTGGACTCCGACGGCTCCTTCTTCCTCTACAGCA
 AGCTCACCGTGGACAAGAGCAGGTGGCAGCAGGGGAACGTCTTCTCATGCTCCGTGATGCATGAGGCTCTGCACAA
 CCACTACACGCAGAAGAGCCTCTCCCTGTCTCCGGGTAAATGA] (SEQ ID NO: 461)

Amino Acid Sequence

[spacer (SEQ ID NO: 87)][pLW047 without spacer (SEQ ID NO: 524)]
 [QGQSGQ][GYRWGCEWNCGGITTGSSGGSGGSGGLSGRSDNHGGGSEVQLVESGGGLVQPGGSLKLSCAASGFTF
 NKYAMNWVRQAPGKGLEWVARIRSKYNNYATYYADSVKDRFTISRDDSKNTAYLQMNNLKTEDTAVYYCVRHGNFG
 NSYISYWAYWGQGLTVTVSSGGGGSGGGGSGGGGSQTVVTQEPSLTVSPGGTVTLTCGSSTGAVTSGYYPNWVQQK
 PGQAPRGLIGGTKFLAPGTPARFSGSLLGGKAALTLSGVQPEDEAEYYCALWYSNRWVFGGGTKLTVLGGGGSQVQ
 LKQSGPGLVQPSQSL SITCTVSGFSLTNYGVHWVRQSPGKGLEWLGVWISGGNTDYNTPFTSRLSINKDNSKSQVF
 FKMNSLQSQDTAIYYCARALTYDYEFAYWGQGLTVTVSAASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEP
 VTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSSLGTQTYICNVNHKPSNTKVDKKVEPKSCDKTHTCPPC
 PAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYQSTYRVVSV
 LTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSREEMTKNQVSLTCLVKGFYPSDIAVEW
 ESNGQPENNYKTTTPVLDSDGSFFLYSKLTVDKSRWQQGNVVFSCSVMH EALHNHYTQKSLSLSPGK]* (SEQ ID
 NO: 462)

OPP021: LC C225

Nucleotide Sequence

GATATCTTGCTGACCCAGAGCCCGGTGATTCTGAGCGTGAGCCCGGGCGAACGTGTGAGCTTTAGCTGCCGCGCGA
 GCCAGAGCATTGGCACCAACATTCATTGGTATCAGCAGCGCACCAACGGCAGCCCGCGCCTGCTGATTAAATATGC
 GAGCGAAAGCATTAGCGGCATTCCGAGCCGCTTTAGCGGCAGCGGCAGCGGCACCGATTTTACCCTGAGCATTAAC
 AGCGTGAAAGCGAAGATATTGCGGATTATTATTGCCAGCAGAACAACTGGCCGACCACCTTTGGCGCGGGCA
 CCAAACCTGGAAATCAAACGTACGGTGGCTGCACCATCTGTCTTCATCTTCCCGCCATCTGATGAGCAGTTGAAATC

TGGAAGTGCCTCTGTTGTGTGCCTGCTGAATAACTTCTATCCCAGAGAGGCCAAAGTACAGTGGAAGGTGGATAAC
 GCCCTCCAATCGGGTAACTCCCAGGAGAGTGTACAGAGCAGGACAGCAAGGACAGCACCTACAGCCTCAGCAGCA
 CCCTGACGCTGAGCAAAGCAGACTACGAGAAACACAAAGTCTACGCCTGCGAAGTCACCCATCAGGGCCTGAGCTC
 GCCCGTCACAAAGAGCTTCAACAGGGGAGAGTGTTAG (SEQ ID NO: 457)

Amino Acid Sequence

DILLTQSPVILSVSPGERVSFSCRASQSIGTNIHWYQORTNGSPRLLIKYASESISGIPSRFSGSGSGTDFTLIN
 SVESEDIADYYCQQNNNWPTTFGAGTKLEIKRTVAAPSVFIFPPSDEQLKSGTASVVCLLNNFYPREAKVQWKVDN
 ALQSGNSQESVTEQDSKDYSLSTLTLSKADYEKHKVYACEVTHQGLSSPVTKSFNRGEC* (SEQ ID
 NO: 458)

CI022: C225v5N297Q-15865-CD3HvLv-H-N

pLW048: HC C225v5N297Q-15865-CD3HvLv-H-N

Nucleotide Sequence

[spacer (SEQ ID NO: 507)][pLW048 without spacer (SEQ ID NO: 525)]
 [CAAGGCCAGTCTGGCCAA][ATGATGTATTGCGGTGGGAATGAGGTGTTGTGCGGGCCGCGGGTTGGCTCGAGCG
 GTGGCAGCGGTGGCTCTGGTGGTCTGAGCGGCCGTTCCGATAATCATGGCGGCGGTTCTGAGGTGCAGCTGGTCGA
 GTCTGGAGGAGGATTGGTGCAGCCTGGAGGGTCATTGAAACTCTCATGTGCAGCCTCTGGATTCACCTTCAATAAG
 TACGCCATGAACTGGGTCCGCCAGGCTCCAGGAAAGGGTTTGAATGGGTGCTCGCATAAGAAGTAAATATAATA
 ATTATGCAACATATTATGCCGATTCAGTGAAAGACAGGTTACCATCTCCAGAGATGATTCAAAAAACACTGCCTA
 TCTACAAATGAACAACCTTGAAAACCTGAGGACACTGCCGTGTACTACTGTGTGAGACATGGGAACTTCGGTAATAGC
 TACATATCCTACTGGGCTTACTGGGGCCAAGGGACTCTGGTCACCGTCTCCTCAGGTGGTGGTGGTCTGGCGGCG
 GCGGCTCCGGTGGTGGTGGTCTCAGACTGTTGTGACTCAGGAACCTTCACTCACCGTATCACCTGGTGGAACAGT
 CAACTCACTTGTGGCTCCTCGACTGGGGCTGTTACATCTGGCTACTACCCAACTGGGTCCAACAAAAACCAGGT
 CAGGCACCCCGTGGTCTAATAGGTGGGACTAAGTTCCTCGCCCCCGGTACTCCTGCCAGATTCTCAGGCTCCCTGC
 TTGGAGGCAAGGCTGCCCTCACCTCTCAGGGGTACAGCCAGAGGATGAGGCAGAATATTACTGTGCTCTATGGTA
 CAGCAACCGCTGGGTGTTCCGGTGGAGGAACCAAACCTGACTGTCCTAGGAGGTGGTGGATCCCAGGTGCAGCTGAAA
 CAGAGCGGCCCCGGGCCTGGTGCAGCCGAGCCAGAGCCTGAGCATTACCTGCACCGTGAGCGGCTTTAGCCTGACCA
 ACTATGGCGTGCAATTGGGTGCGCCAGAGCCCCGGGCAAAGGCCTGGAATGGCTGGGCGTGATTTGGAGCGGCGGCAA
 CACCGATTATAACACCCCGTTTACCAGCCGCTGAGCATTAACAAAGATAACAGCAAAAGCCAGGTGTTTTTTTAAA
 ATGAACAGCCTGCAAAGCCAGGATACCGCGATTTATTATTGCGCGCGCGCGCTGACCTATTATGATTATGAATTTG
 CGTATTGGGGCCAGGGCACCTTGGTGACCGTGAGCGCGGCTAGCACCAAGGGCCCATCGGTCTTCCCCCTGGCACC
 CTCCTCCAAGAGCACCTCTGGGGGCACAGCGGCCCTGGGCTGCCTGGTCAAGGACTACTTCCCCGAACCGGTGACG
 GTGTCGTGGAACCTCAGGCGCCCTGACCAGCGGCGTGACACCTTCCCGGCTGTCCTACAGTCCTCAGGACTCTACT
 CCCTCAGCAGCGTGGTGACCGTGCCCTCCAGCAGCTTGGGCACCCAGACCTACATCTGCAACGTGAATCACAAGCC
 CAGCAACACCAAGGTGGACAAGAAAGTTGAGCCCCAAATCTTGTGACAAAACCTCACACATGCCCACCGTGCCCAGCA

CCTGAACTCCTGGGGGGACCGTCAGTCTTCCTCTTCCCCCAAACCCAAGGACACCCTCATGATCTCCCGGACCC
 CTGAGGTCACATGCGTGGTGGTGGACGTGAGCCACGAAGACCCTGAGGTCAAGTTCAACTGGTACGTGGACGGCGT
 GGAGGTGCATAATGCCAAGACAAAGCCGCGGGAGGAGCAGTACCAGAGCACGTACCGTGTGGTCAGCGTCCTCACC
 GTCCTGCACCAGGACTGGCTGAATGGCAAGGAGTACAAGTGCAAGGTCTCCAACAAAGCCCTCCCAGCCCCCATCG
 AGAAAACCATCTCCAAAGCCAAAGGGCAGCCCCGAGAACCACAGGTGTACACCCTGCCCCCATCCCGGGAGGAGAT
 GACCAAGAACCAGGTCAGCCTGACCTGCCTGGTCAAAGGCTTCTATCCCAGCGACATCGCCGTGGAGTGGGAGAGC
 AATGGGCAGCCGGAGAACAACACTACAAGACCACGCCTCCCGTGCTGGACTCCGACGGCTCCTTCTTCCTCTACAGCA
 AGCTCACCGTGGACAAGAGCAGGTGGCAGCAGGGGAACGTCTTCTCATGCTCCGTGATGCATGAGGCTCTGCACAA
 CCACTACACGCAGAAGAGCCTCTCCCTGTCTCCGGGTAAATGA] (SEQ ID NO: 463)

Amino Acid Sequence

[spacer (SEQ ID NO: 87)][pLW048 without spacer (SEQ ID NO: 526)]
 [QGQSGQ][MMYCGGNEVLCGPRVGSSGGSGGSLSGRSDNHGGGSEVQLVESGGGLVQPGGSLKLSCAASGFTF
 NKYAMNWVRQAPGKGLEWVARIRSKYNNYATYYADSVKDRFTISRDDSKNTAYLQMNNLKTEDTAVYYCVRHGNFG
 NSYISYWAYWGQGLTVTVSSGGGGSGGGSGGGGSQTVVTQEPSLTVSPGGTVTLTCGSSTGAVTSGYYPNWWVQOK
 PGQAPRGLIGGTKFLAPGTPARFSGSLLGGKAALTLSGVQPEDEAEYYCALWYSNRWVFGGGTKLTVLGGGGSQVQ
 LKQSGPGLVQPSQSL SITCTVSGFSLTNYGVHWVRQSPGKGLEWLGVWSGGNTDYNTPFTSRLSINKDNSKSQVF
 FKMNSLQSQDTAIYYCARALTYDYEFAYWGQGLTVTVSAASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEP
 VTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSSLGTQTYICNVNHKPSNTKVDKKVEPKSCDKTHTCPPC
 PAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYQSTYRVVSV
 LTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSREEMTKNQVSLTCLVKGFYPSDIAVEW
 ESNGQPENNYKTTTPVLDSDGSFFLYSKLTVDKSRWQQGNVFSQSVMEALHNHYTQKSLSLSPGK]* (SEQ ID
 NO: 464)

OPP021: LC C225

Nucleotide Sequence

GATATCTTGCTGACCCAGAGCCCGGTGATTCTGAGCGTGAGCCCGGGCGAACGTGTGAGCTTTAGCTGCCGCGCGA
 GCCAGAGCATTGGCACCAACATTCATTGGTATCAGCAGCGCACCAACGGCAGCCCGCGCCTGCTGATTAAATATGC
 GAGCGAAAGCATTAGCGGCATTCCGAGCCGCTTTAGCGGCAGCGGCAGCGGCACCGATTTTACCCTGAGCATTAAC
 AGCGTGGAAGCGAAGATATTGCGGATTATTATTGCCAGCAGAACAACAACACTGGCCGACCACCTTTGGCGCGGGCA
 CCAAACCTGGAAATCAAACGTACGGTGGCTGCACCATCTGTCTTCATCTTCCCGCCATCTGATGAGCAGTTGAAATC
 TGGAACCTGCCTCTGTTGTGTGCCTGCTGAATAACTTCTATCCCAGAGAGGCCAAAGTACAGTGGAAGGTGGATAAC
 GCCCTCCAATCGGGTAACTCCCAGGAGAGTGTACAGAGCAGGACAGCAAGGACAGCACCTACAGCCTCAGCAGCA
 CCCTGACGCTGAGCAAAGCAGACTACGAGAAACACAAAGTCTACGCCTGCGAAGTCACCCATCAGGGCCTGAGCTC
 GCCCGTCACAAAGAGCTTCAACAGGGGAGAGTGTTAG (SEQ ID NO: 457)

Amino Acid Sequence

DILLTQSPVILSVSPGERVVSFSCRASQSIGTNIHWYQQRNNGSPRLLIKYASESISGIPSRFSGSGSGTDFTLSIN
 SVESEDIADYYCQQNNNWPTTFGAGTKLEIKRTVAAPSVFIFPPSDEQLKSGTASVVCLLNNFYPREAKVQWKVDN
 ALQSGNSQESVTEQDSKDYSLSTLTLSKADYEKHKVYACEVTHQGLSSPVTKSFNRGEC* (SEQ ID
 NO: 458)

CI023: 3954-1204-C225v5N297Q-15003-CD3HvLv-H-N

pLW047: HC C225v5N297Q-15003-CD3HvLv-H-N

Nucleotide Sequence

[spacer (SEQ ID NO: 507)][pLW047 without spacer (SEQ ID NO: 522)]
 [CAAGGCCAGTCTGGCCAA][GGTTATCGGTGGGGTTGCGAGTGGAATTGCGGTGGGATTACTACTGGCTCGAGCG
 GTGGCAGCGGTGGCTCTGGTGGTCTGAGCGGCCGTTCCGATAATCATGGCGGCGGTTCTGAGGTGCAGCTGGTCGA
 GTCTGGAGGAGGATTGGTGCAGCCTGGAGGGTCATTGAAACTCTCATGTGCAGCCTCTGGATTACCTTCAATAAG
 TACGCCATGAACTGGGTCCGCCAGGCTCCAGGAAAGGGTTTGAATGGGTTGCTCGCATAAGAAGTAAATATAATA
 ATTATGCAACATATTATGCCGATTCAGTGAAAGACAGGTTACCATCTCCAGAGATGATTCAAAAAACACTGCCTA
 TCTACAAATGAACAACCTGAAAACCTGAGGACACTGCCGTGTACTACTGTGTGAGACATGGGAACTTCGGTAATAGC
 TACATATCCTACTGGGCTTACTGGGGCCAAGGGACTCTGGTCACCGTCTCCTCAGGTGGTGGTGGTCTGGCGGCG
 GCGGCTCCGGTGGTGGTGGTCTCAGACTGTTGTGACTCAGGAACCTTCACTCACCGTATCACCTGGTGGAACAGT
 CACACTCACTTGTGGCTCCTCGACTGGGGCTGTTACATCTGGCTACTACCCAACTGGGTCCAACAAAAACCAGGT
 CAGGCACCCCGTGGTCTAATAGGTGGGACTAAGTTCCTCGCCCCCGGTACTCCTGCCAGATTCTCAGGCTCCCTGC
 TTGGAGGCAAGGCTGCCCTCACCTCTCAGGGGTACAGCCAGAGGATGAGGCAGAATATTACTGTGCTCTATGGTA
 CAGCAACCGCTGGGTGTTTCGGTGGAGGAACCAAACTGACTGTCCTAGGAGGTGGTGGATCCCAGGTGCAGCTGAAA
 CAGAGCGGCCCCGGGCCTGGTGCAGCCGAGCCAGAGCCTGAGCATTACCTGCACCGTGAGCGGCTTTAGCCTGACCA
 ACTATGGCGTGCATTGGGTGCGCCAGAGCCCCGGGCAAAGGCCTGGAATGGCTGGGCGTGATTTGGAGCGGCGGCAA
 CACCGATTATAACACCCCGTTTACCAGCCGCTGAGCATTAACAAAGATAACAGCAAAAGCCAGGTGTTTTTTAAA
 ATGAACAGCCTGCAAAGCCAGGATACCGCGATTTATTATTGCGCGCGCGCGCTGACCTATTATGATTATGAATTTG
 CGTATTGGGGCCAGGGCACCTGGTGACCGTGAGCGCGGCTAGCACCAAGGGCCCATCGGTCTTCCCCCTGGCACC
 CTCCTCCAAGAGCACCTCTGGGGGCACAGCGGCCCTGGGCTGCCTGGTCAAGGACTACTTCCCCGAACCGGTGACG
 GTGTCGTGGAACCTCAGGCGCCCTGACCAGCGGCGTGACACCTTCCCGGCTGTCCTACAGTCCTCAGGACTCTACT
 CCCTCAGCAGCGTGGTGACCGTGCCCTCCAGCAGCTTGGGCACCCAGACCTACATCTGCAACGTGAATCACAAGCC
 CAGCAACACCAAGGTGGACAAGAAAGTTGAGCCCAAATCTTGTGACAAAACCTCACACATGCCCACCGTGCCCAGCA
 CCTGAACTCCTGGGGGGACCGTCAGTCTTCCTCTTCCCCCAAAACCCAAGGACACCCTCATGATCTCCCGGACCC
 CTGAGGTACATGCGTGGTGGTGGACGTGAGCCACGAAGACCCTGAGGTCAAGTTCAACTGGTACGTGGACGGCGT
 GGAGGTGCATAATGCCAAGACAAAGCCGCGGGAGGAGCAGTACCAGAGCACGTACCGTGTGGTCAGCGTCCTCACC
 GTCCTGCACCAGGACTGGCTGAATGGCAAGGAGTACAAGTGCAAGGTCTCCAACAAAGCCCTCCCAGCCCCCATCG
 AGAAAACCATCTCCAAGCCAAAGGGCAGCCCCGAGAACCACAGGTGTACACCCTGCCCCCATCCCGGGAGGAGAT

GACCAAGAACCAGGTCAGCCTGACCTGCCTGGTCAAAGGCTTCTATCCCAGCGACATCGCCGTGGAGTGGGAGAGC
 AATGGGCAGCCGGAGAACAACACTACAAGACCACGCCTCCCGTGCTGGACTCCGACGGCTCCTTCTTCCTCTACAGCA
 AGCTCACCGTGGACAAGAGCAGGTGGCAGCAGGGGAACGTCTTCTCATGCTCCGTGATGCATGAGGCTCTGCACAA
 CCACTACACGCAGAAGAGCCTCTCCCTGTCTCCGGGTAAATGA] (SEQ ID NO: 461)

Amino Acid Sequence

[spacer (SEQ ID NO: 87)][pLW047 without spacer (SEQ ID NO: 524)]
 [QGQSGQ][GYRWGCEWNCGGITTGSSGSGSGGSLSGRSDNHGGGSEVQLVESGGGLVQPGGSLKLSCAASGFTF
 NKYAMNWVRQAPGKGLEWVARIRSKYNNYATYYADSVKDRFTISRDDSKNTAYLQMNNLKTEDTAVYYCVRHGNFG
 NSYISYWAYWGQGLTVTVSSGGGSGGGGSGGGGSQTVVTQEPSLTVSPGGTVTLTCGSSTGAVTSGYYPNWVQQK
 PGQAPRGLIGGTKFLAPGTPARFSGSLLGGKAALTLSGVQPEDEAEYYCALWYSNRWVFGGGTKLTVLGGGGSQVQ
 LKQSGPGLVQPSQSL SITCTVSGFSLTNYGVHWVRQSPGKGLEWLGVWSGGNTDYNT PFTSRLSINKDNSKSQVF
 FKMNSLQSQDTAIYYCARALTYDYEFAYWGQGLTVTVSAASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEP
 VTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSSLGTQTYICNVNHKPSNTKVDKKVEPKSCDKTHTCPPC
 PAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYQSTYRVVSV
 LTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSREEMTKNQVSLTCLVKGFYPSDIAVEW
 ESNGQPENNYKTTTPVLDSDGSFFLYSKLTVDKSRWQQGNV FSCSV MHEALHNHYTQKSLSLSPGK]* (SEQ ID
 NO: 462)

OPP022: LC C225 3954-1204

Nucleotide Sequence

[spacer (SEQ ID NO: 507)][OPP022 without spacer (SEQ ID NO: 509)]
 [CAAGGCCAGTCTGGCCAG][TGCATCTCACCTCGTGGTTGTCCGGACGGCCCATACGTCATGTACGGCTCGAGCG
 GTGGCAGCGGTGGCTCTGGTGGATCCGGTCTGAGCGGCCGTTCCGATAATCATGGCAGTAGCGGTACCCAGATCTT
 GCTGACCCAGAGCCCGGTGATTCTGAGCGTGAGCCCGGGCGAACGTGTGAGCTTTAGCTGCCGCGCGAGCCAGAGC
 ATTGGCACCAACATTCATTGGTATCAGCAGCGCACCAACGGCAGCCCGCGCCTGCTGATTAAATATGCGAGCGAAA
 GCATTAGCGGCATTCCGAGCCGCTTTAGCGGCAGCGGCAGCGGCACCGATTTTACCCTGAGCATTAAACAGCGTGGA
 AAGCGAAGATATTGCGGATTATTATTGCCAGCAGAACAACAACACTGGCCGACCACCTTTGGCGCGGGCACCAAACCTG
 GAACTGAAACGTACGGTGGCTGCACCATCTGTCTTCATCTTCCCGCCATCTGATGAGCAGTTGAAATCTGGAACCTG
 CCTCTGTTGTGTGCCTGCTGAATAACTTCTATCCCAGAGAGGCCAAAGTACAGTGGAAGGTGGATAACGCCCTCCA
 ATCGGGTAACTCCCAGGAGAGTGTACAGAGCAGGACAGCAAGGACAGCACCTACAGCCTCAGCAGCACCCCTGACG
 CTGAGCAAAGCAGACTACGAGAAACACAAAGTCTACGCCTGCGAAGTCACCCATCAGGGCCTGAGCTCGCCCGTCA
 CAAAGAGCTTCAACAGGGGAGAGTGTTAG] (SEQ ID NO: 449)

Amino Acid Sequence

[spacer (SEQ ID NO: 87)][OPP022 without spacer (SEQ ID NO: 508)]

[QGQSGQ][CISPRGCPDGPYVMYGSSGGSGGSGGSGLSGRSDNHGSSGTQILLTQSPVILSVSPGERVSFSCRAS
QSIGTNIHWYQQRRTNGSPRLLIKYASESISGIPSRFSGSGSGTDFTLINSVESEDIADYYCQQNNNWPTTFGAGT
KLELKRTVAAPSVFIFPPSDEQLKSGTASVVCLLNFPREAKVQWKVDNALQSGNSQESVTEQDSKDYSLST
LTLSKADYEKHKVYACEVTHQGLSSPVTKSFNRGEC]* (SEQ ID NO: 450)

CI024: 3954-1204-C225v5N297Q-15865-CD3HvLv-H-N

pLW048: HC C225v5N297Q-15865-CD3HvLv-H-N

Nucleotide Sequence

[spacer (SEQ ID NO: 507)][pLW048 without spacer (SEQ ID NO: 525)]

[CAAGGCCAGTCTGGCCAA][ATGATGTATTGCGGTGGGAATGAGGTGTTGTGCGGGCCGCGGGTTGGCTCGAGCG
GTGGCAGCGGTGGCTCTGGTGGTCTGAGCGGCCGTTCCGATAATCATGGCGGCGGTTCTGAGGTGCAGCTGGTCGA
GTCTGGAGGAGGATTGGTGCAGCCTGGAGGGTCATTGAAACTCTCATGTGCAGCCTCTGGATTACCTTCAATAAG
TACGCCATGAACTGGGTCCGCCAGGCTCCAGGAAAGGGTTTGAATGGGTTGCTCGCATAAGAAGTAAATATAATA
ATTATGCAACATATTATGCCGATTCAGTGAAAGACAGGTTACCATCTCCAGAGATGATTCAAAAAACACTGCCTA
TCTACAAATGAACAACTTGAAAACCTGAGGACACTGCCGTGTACTACTGTGTGAGACATGGGAACTTCGGTAATAGC
TACATATCCTACTGGGCTTACTGGGGCCAAGGGACTCTGGTCACCGTCTCCTCAGGTGGTGGTGGTTCTGGCGGCG
GCGGCTCCGGTGGTGGTGGTTCTCAGACTGTTGTGACTCAGGAACCTTCACTCACCGTATCACCTGGTGGAACAGT
CACACTCACTTGTGGCTCCTCGACTGGGGCTGTTACATCTGGCTACTACCCAACTGGGTCCAACAAAAACCAGGT
CAGGCACCCCGTGGTCTAATAGGTGGGACTAAGTTCCTCGCCCCCGGTACTCCTGCCAGATTCTCAGGCTCCCTGC
TTGGAGGCAAGGCTGCCCTCACCTCTCAGGGGTACAGCCAGAGGATGAGGCAGAATATTACTGTGCTCTATGGTA
CAGCAACCGCTGGGTGTTTCGGTGGAGGAACCAAACTGACTGTCCTAGGAGGTGGTGGATCCCAGGTGCAGCTGAAA
CAGAGCGGCCCCGGGCCTGGTGCAGCCGAGCCAGAGCCTGAGCATTACCTGCACCGTGAGCGGCTTTAGCCTGACCA
ACTATGGCGTGCAATTGGGTGCGCCAGAGCCCCGGGCAAAGGCCTGGAATGGCTGGGCGTGATTTGGAGCGGCGGCAA
CACCGATTATAACACCCCGTTTACCAGCCGCCTGAGCATTAACAAAGATAACAGCAAAAGCCAGGTGTTTTTTAAA
ATGAACAGCCTGCAAAGCCAGGATACCGCGATTTATTATTGCGCGCGCGCGCTGACCTATTATGATTATGAATTTG
CGTATTGGGGCCAGGGCACCTGGTGACCGTGAGCGCGGCTAGCACCAAGGGCCCATCGGTCTTCCCCCTGGCACC
CTCCTCCAAGAGCACCTCTGGGGGCACAGCGGCCCTGGGCTGCCTGGTCAAGGACTACTTCCCCGAACCGGTGACG
GTGTGCTGGAACCTCAGGCGCCCTGACCAGCGGCGTGACACCTTCCCGGCTGTCCTACAGTCCTCAGGACTCTACT
CCCTCAGCAGCGTGGTGACCGTGCCCTCCAGCAGCTTGGGCACCCAGACCTACATCTGCAACGTGAATCACAAGCC
CAGCAACACCAAGGTGGACAAGAAAGTTGAGCCCAAATCTTGTGACAAAACCTCACACATGCCCACCGTGCCCAGCA
CCTGAACTCCTGGGGGGACCGTCAGTCTTCCTCTTCCCCCAAAACCCAAGGACACCCTCATGATCTCCCGGACCC
CTGAGGTACATGCGTGGTGGTGGACGTGAGCCACGAAGACCCTGAGGTCAAGTTCAACTGGTACGTGGACGGCGT
GGAGGTGCATAATGCCAAGACAAAGCCGCGGGAGGAGCAGTACCAGAGCACGTACCGTGTGGTCAGCGTCCTCACC
GTCCTGCACCAGGACTGGCTGAATGGCAAGGAGTACAAGTGCAAGGTCTCCAACAAAGCCCTCCCAGCCCCCATCG
AGAAAACCATCTCCAAGCCAAAGGGCAGCCCCGAGAACCACAGGTGTACACCCTGCCCCCATCCCGGGAGGAGAT
GACCAAGAACCAGGTCAGCCTGACCTGCCTGGTCAAAGGCTTCTATCCCAGCGACATCGCCGTGGAGTGGGAGAGC

AATGGGCAGCCGGAGAACAACACTACAAGACCACGCCTCCCGTGCTGGACTCCGACGGCTCCTTCTTCCTCTACAGCA
AGCTCACCGTGGACAAGAGCAGGTGGCAGCAGGGGAACGTCTTCTCATGCTCCGTGATGCATGAGGCTCTGCACAA
CCACTACACGCAGAAGAGCCTCTCCCTGTCTCCGGGTAAATGA] (SEQ ID NO: 463)

Amino Acid Sequence

[spacer (SEQ ID NO: 87)][pLW048 without spacer (SEQ ID NO: 526)]
[QGQSGQ][MMYCGGNEVLCGPRVGSSGGSGGSGGLSGRSDNHGGGSEVQLVESGGGLVQPGGSLKLSCAASGFTF
NKYAMNWVRQAPGKGLEWVARIRSKYNNYATYYADSVKDRFTISRDDSKNTAYLQMNNLKTEDTAVYYCVRHGNFG
NSYISYWAYWGQGLTVTVSSGGGGSGGGGSGGGGSQTVVTQEPSLTVSPGGTVTLTCGSSTGAVTSGYYPNWVQQK
PGQAPRGLIGGTKFLAPGTPARFSGSLLGGKAALTLSGVQPEDEAEYYCALWYSNRWVFGGGTKLTVLGGGGSQVQ
LKQSGPGLVQPSQSL SITCTVSGFSLTNYGVHWVRQSPGKGLEWLGVWSGGNTDYNTPFTSRLSINKDNSKSQVF
FKMNSLQSQDTAIYYCARALTYDYEFAYWGQGLTVTVSAASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEP
VTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSSLGTQTYICNVNHKPSNTKVDKKVEPKSCDKTHTCPPC
PAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYQSTYRVVSV
LTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSREEMTKNQVSLTCLVKGFYPSDIAVEW
ESNGQPENNYKTTTPVLDSDGSFFLYSKLTVDKSRWQQGNVVFSCSVMEALHNHYTQKSLSLSPGK]* (SEQ ID
NO: 464)

OPP022: LC C225 3954-1204

Nucleotide Sequence

[spacer (SEQ ID NO: 507)][OPP022 without spacer (SEQ ID NO: 509)]
[CAAGGCCAGTCTGGCCAG][TGCATCTCACCTCGTGGTTGTCCGGACGGCCCATACGTCATGTACGGCTCGAGCG
GTGGCAGCGGTGGCTCTGGTGGATCCGGTCTGAGCGGCCGTTCCGATAATCATGGCAGTAGCGGTACCCAGATCTT
GCTGACCCAGAGCCCGGTGATTCTGAGCGTGAGCCCGGGCGAACGTGTGAGCTTTAGCTGCCGCGCGAGCCAGAGC
ATTGGCACCAACATTCATTGGTATCAGCAGCGCACCAACGGCAGCCCGCGCCTGCTGATTAAATATGCGAGCGAAA
GCATTAGCGGCATTCCGAGCCGCTTTAGCGGCAGCGGCAGCGGCACCGATTTTACCCTGAGCATTAACAGCGTGGA
AAGCGAAGATATTGCGGATTATTATTGCCAGCAGAACAACAACACTGGCCGACCACCTTTGGCGCGGGCACCAAACCTG
GAACTGAAACGTACGGTGGCTGCACCATCTGTCTTCATCTTCCCGCCATCTGATGAGCAGTTGAAATCTGGAACCTG
CCTCTGTTGTGTGCCTGCTGAATAACTTCTATCCCAGAGAGGCCAAAGTACAGTGGAAGGTGGATAACGCCCTCCA
ATCGGGTAACTCCCAGGAGAGTGTACAGAGCAGGACAGCAAGGACAGCACCTACAGCCTCAGCAGCACCCCTGACG
CTGAGCAAAGCAGACTACGAGAAACACAAAGTCTACGCCTGCGAAGTCACCCATCAGGGCCTGAGCTCGCCCGTCA
CAAAGAGCTTCAACAGGGGAGAGTGTTAG] (SEQ ID NO: 449)

Amino Acid Sequence

[spacer (SEQ ID NO: 87)][OPP022 without spacer (SEQ ID NO: 508)]
[QGQSGQ][CISPRGCPDGPYVMYSSGGSGGSGGSGLSGRSDNHGSSGTQILLTQSPVILSVSPGERVSFSCRAS
QSIGTNIHWYQQRTNGSPRLLIKYASESISGIPSRFSGSGSGTDFTLINSVESEDIADYYCQQNNNWPTTFGAGT

KLELKRTVAAPSVFIFPPSDEQLKSGTASVVCLLNNFYPREAKVQWKVDNALQSGNSQESVTEQDSKDSTYSLSST
LTLISKADYEEKHKVYACEVTHQGLSSPVTKSFNRGEC]* (SEQ ID NO: 450)

CI028: IL6R 4792 Nsub N297Q-15865 1204-CD3LvHv-H-N

pLW085: HC IL6RN297Q-15865_1204-CD3LvHv-H-N

Nucleotide Sequence

[spacer (SEQ ID NO: 507)][pLW085 without spacer (SEQ ID NO: 513)]
[CAAGGCCAGTCTGGCCAA][ATGATGTATTGCGGTGGGAATGAGGTGTTGTGCGGGCCGCGGGTTGGCTCGAGCG
GTGGCAGCGGTGGCTCTGGTGGTCTGAGCGGCCGTTCCGATAATCATGGCGGCGGTTCTCAGACCGTGGTCACACA
GGAGCCCTCACTGACAGTGAGCCCTGGCGGGACCGTCACACTGACTTGTGCGCAGTTCAACTGGCGCCGTGACTACC
AGCAATTACGCTAACTGGGTCCAGCAGAAACCAGGACAGGCACCACGAGGACTGATCGGAGGAACTAATAAGAGAG
CACCAGGAACCCCTGCAAGGTTCTCCGGATCTCTGCTGGGGGGAAAAGCCGCTCTGACACTGAGCGGCGTGCAGCC
TGAGGACGAAGCTGAGTACTATTGCGCACTGTGGTACTCCAACCTGTGGGTGTTTGGCGGGGGAATAAGCTGACC
GTCCTGGGAGGAGGAGGAAGCGGAGGAGGAGGGAGCGGAGGAGGAGGATCCGAAGTGCAGCTGGTCGAGAGCGGAG
GAGGACTGGTGCAGCCAGGAGGATCCCTGAAGCTGTCTTGTGCAGCCAGTGGCTTCACCTTCAACACTTACGCAAT
GAACTGGGTGCGGCAGGCACCTGGGAAGGGACTGGAATGGGTGCGCCCGGATCAGATCTAAATACAATAACTATGCC
ACCTACTATGCTGACAGTGTGAAGGATAGGTTACCATTTACGCGACGATAGCAAAAACACAGCTTATCTGCAGA
TGAATAACCTGAAGACCGAGGATACAGCAGTGTACTATTGCGTCAGACACGGCAATTTCCGGAACTCTTACGTGAG
TTGGTTTGCCTATTGGGGACAGGGGACACTGGTCACCGTCTCCTCAGGAGGTGGTGGATCCCAGGTGCAGCTGCAG
GAGTCCGGACCAGGACTGGTCCGGCCCTCACAGACTCTGAGCCTGACATGCACTGTGTCAGGCTACAGCATCACCT
CCGATCACGCCTGGAGCTGGGTGAGGCAGCCACCTGGACGCGGCCTGGAATGGATCGGCTACATTTCTTATAGTGG
GATCACACATAACAACCCCTCTCTGAAGAGTCGAGTGACCATTTCCAGAGACAACCTCTAAAAATACACTGTATCTG
CAGATGAATAGTCTGCGGGCCGAGGATACAGCTGTGTACTATTGTGCACGGTCTCTGGCCAGAACTACCGCTATGG
ACTATTGGGGGCAGGGAAGCCTGGTGACCGTCAGCTCCGCTAGCACCAAGGGCCCATCGGTCTTCCCCCTGGCACC
CTCCTCCAAGAGCACCTCTGGGGGCACAGCGGCCCTGGGCTGCCTGGTCAAGGACTACTTCCCCGAACCGGTGACG
GTGTCGTGGAACCTCAGGCGCCCTGACCAGCGGCGTGCACACCTTCCCGGCTGTCCTACAGTCCTCAGGACTCTACT
CCCTCAGCAGCGTGGTGACCGTGCCCTCCAGCAGCTTGGGCACCCAGACCTACATCTGCAACGTGAATCACAAGCC
CAGCAACACCAAGGTGGACAAGAAAGTTGAGCCCAAATCTTGTGACAAAACCTCACACATGCCCACCGTGCCCAGCA
CCTGAACTCCTGGGGGGACCGTCAGTCTTCCTCTTCCCCCAAAACCCAAGGACACCCCTCATGATCTCCCGGACCC
CTGAGGTCACATGCGTGGTGGTGGACGTGAGCCACGAAGACCCTGAGGTCAAGTTCAACTGGTACGTGGACGGCGT
GGAGGTGCATAATGCCAAGACAAAGCCGCGGGAGGAGCAGTACCAGAGCACGTACCGTGTGGTCAGCGTCCTCACC
GTCCTGCACCAGGACTGGCTGAATGGCAAGGAGTACAAGTGCAAGGTCTCCAACAAAGCCCTCCCAGCCCCCATCG
AGAAAACCATCTCCAAAGCCAAAGGGCAGCCCCGAGAACCACAGGTGTACACCTGCCCCCATCCCGGGAGGAGAT
GACCAAGAACCAGGTCAGCCTGACCTGCCTGGTCAAAGGCTTCTATCCCAGCGACATCGCCGTGGAGTGGGAGAGC
AATGGGCAGCCGGAGAACAACCTACAAGACCACGCCTCCCGTGCTGGACTCCGACGGCTCCTTCTTCCTCTACAGCA

AGCTCACCGTGGACAAGAGCAGGTGGCAGCAGGGGAACGTCTTCTCATGCTCCGTGATGCATGAGGCTCTGCACAA
CCACTACACGCAGAAGAGCCTCTCCCTGTCTCCGGGTAAATGA] (SEQ ID NO: 471)

Amino Acid Sequence

[spacer (SEQ ID NO: 87)] [pLW085 without spacer (SEQ ID NO: 514)]
[QGQSGQ] [MMYCGGNEVLCGPRVGSSGSGSGGLSGRSDNHGGGSQTVVTQEPSLTVSPGGTVTLTCRSSTGAV
TTSNYANWVQOKPGQAPRGLIGGTNKRAPGTPARFSGSLLGGKAALTLSGVQPEDEAEYYCALWYSNLWVFGGGTK
LTVLGGGSGGGGSGGGGSEVQLVESGGGLVQPGGSLKLSAASGFTFNTYAMNWVRQAPGKLEWVARIRSKYNN
YATYYADSVKDRFTISRDDSKNTAYLQMNNLKTEDTAVYYCVRHGNFGNSYVSWFAYWGQGLTVTVSSGGGGSQVQ
LQESGPGLVLRPSQTLSTCTVSGYSITSDHAWSWVRQPPGRGLEWIGYISYSGITTYNPSLKSRVTISRDN SKNTL
YLQMNSLRAEDTAVYYCARSLARTTAMDYWGQGLVTVSSASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEP
VTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVTVTPSSSLGTQTYICNVNHKPSNTKVDKKVEPKSCDKTHTCPPC
PAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYQSTYRVVSV
LTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSREEMTKNQVSLTCLVKGFYPSDIAVEW
ESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFCFSVMHEALHNHYTQKSLSLSPGK] * (SEQ ID
NO: 472)

pLW079: LC IL6R 4792 Nsub

Nucleotide Sequence

[spacer (SEQ ID NO: 507)] [pLW079 without spacer (SEQ ID NO: 527)]
[CAAGGCCAGTCTGGCCAG] [TATGGGTCCTGCAGTTGGAACATATGTACACATATTCATGGATTGCGGCTCGAGCG
GTGGCAGCGGTGGCTCTGGTGGCTCAGGTGGAGGCTCGGGCGGTGGGAGCGGCGGTCTGACATCCAGATGACTCA
GTCTCCTAGCTCCCTGTCCGCCTCTGTGGGGGACCGAGTCACCATCACATGCAGAGCCAGCCAGGATATTTCTAGT
TACCTGAACTGGTATCAGCAGAAGCCCGGAAAAGCACCTAAGCTGCTGATCTACTATACCTCCAGGCTGCACTCTG
GCGTGCCCACTCGGTTCACTGGCTCAGGGAGCGGAACCGACTTCACTTTTACCATCTCAAGCCTGCAGCCAGAGGA
TATTGCCACATACTATTGTCAGCAGGGCAATACACTGCCCTACACTTTTGGCCAGGGGACCAAGGTGGAAATCAAA
CGTACGGTGGCTGCACCATCTGTCTTCATCTTCCCGCCATCTGATGAGCAGTTGAAATCTGGAAGTGCCTCTGTTG
TGTGCCTGCTGAATAACTTCTATCCCAGAGAGGCCAAAGTACAGTGAAGGTGGATAACGCCCTCCAATCGGGTAA
CTCCCAGGAGAGTGTACAGAGCAGGACAGCAAGGACAGCACCTACAGCCTCAGCAGCACCTGACGCTGAGCAAA
GCAGACTACGAGAAACACAAAGTCTACGCCTGCGAAGTCACCCATCAGGGCCTGAGCTCGCCCGTCACAAAGAGCT
TCAACAGGGGAGAGTGTTAG] (SEQ ID NO: 880)

Amino Acid Sequence

[spacer (SEQ ID NO: 87)] [pLW079 without spacer (SEQ ID NO: 528)]
[QGQSGQ] [YGSCSWNYVHIFMDCGSSGSGSGSGSGSGSGSGSDIQMTQSPSSLSASVGDRVTITCRASQDI
SSYLNWYQOKPGKAPKLLIYYTSRLHSGVPSRFSGSGSGTDFTFITISLQPEDATYYCQQGNTLPYTFGQGTKVE

DEMANDE OU BREVET VOLUMINEUX

LA PRÉSENTE PARTIE DE CETTE DEMANDE OU CE BREVET COMPREND PLUS D'UN TOME.

CECI EST LE TOME 1 DE 2
CONTENANT LES PAGES 1 À 268

NOTE : Pour les tomes additionels, veuillez contacter le Bureau canadien des brevets

JUMBO APPLICATIONS/PATENTS

THIS SECTION OF THE APPLICATION/PATENT CONTAINS MORE THAN ONE VOLUME

THIS IS VOLUME 1 OF 2
CONTAINING PAGES 1 TO 268

NOTE: For additional volumes, please contact the Canadian Patent Office

NOM DU FICHER / FILE NAME :

NOTE POUR LE TOME / VOLUME NOTE:

What is claimed is:

1. An isolated single chain variable antibody fragment that binds the epsilon chain of CD3 (CD3 ϵ) comprising a variable heavy chain complementarity determining region 1 (VH CDR1) comprising the amino acid sequence TYAMN (SEQ ID NO: 53); a variable heavy chain complementarity determining region 2 (VH CD2) comprising the amino acid sequence RIRSKYNNYATYYADSVKD (SEQ ID NO: 54); a variable heavy chain complementarity determining region 3 (VH CDR3) comprising the amino acid sequence HGNFGNSYVSWFAY (SEQ ID NO: 55), a variable light chain complementarity determining region 1 (VL CDR1) comprising the amino acid sequence RSSTGAVTTSNYAN (SEQ ID NO: 56); a variable light chain complementarity determining region 2 (VL CDR2) comprising the amino acid sequence GTNKRAP (SEQ ID NO: 57); and a variable light chain complementarity determining region 3 (VL CDR3) comprising the amino acid sequence ALWYSNLWV (SEQ ID NO: 58).
2. The isolated single chain variable antibody fragment of claim 1, wherein the AB comprises a variable heavy chain (Hv) comprising the amino acid sequence of SEQ ID NO: 4 and a variable light chain (Lv) comprising the amino acid sequence of SEQ ID NO: 2.
3. The isolated single chain variable antibody fragment of claim 1, wherein the scFv fragment comprises the amino acid sequence of SEQ ID NO: 6 or the amino acid sequence of SEQ ID NO: 30.
4. The isolated single chain variable antibody fragment of claim 1, wherein the AB comprises the amino acid sequence of SEQ ID NO: 587.
5. The isolated single chain variable antibody fragment of claim 1, wherein the AB comprises the amino acid sequence of SEQ ID NO: 588.
6. The isolated single chain variable antibody fragment of claim 1 comprising an agent conjugated to the antibody.

7. The isolated single chain variable antibody fragment of claim 6, wherein the agent is a therapeutic agent, an antineoplastic agent, a toxin or fragment thereof, a detectable moiety or a diagnostic agent.
8. The isolated single chain variable antibody fragment of claim 6, wherein the agent is conjugated to the antibody via a linker.
9. A pharmaceutical composition comprising an antibody as in any of claims 1 to 8 and a carrier.
10. A pharmaceutical composition comprising an antibody as in any of claims 1 to 5 and a carrier.
11. An activatable antibody that in an activated state binds the epsilon chain of CD3 (CD3 ϵ), the activatable antibody comprising:
 - an antibody or an antigen binding fragment thereof (AB) that specifically binds to CD3 ϵ ;
 - a masking moiety (MM) that inhibits the binding of the AB to CD3 ϵ when the activatable antibody is in an uncleaved state; and
 - a cleavable moiety (CM) coupled to the AB, wherein the CM is a polypeptide that functions as a substrate for a protease.
12. The activatable antibody of claim 11, wherein the MM has a dissociation constant for binding to the AB that is selected from the group consisting of: greater than the dissociation constant of the AB to CD3 ϵ ; equivalent to the dissociation constant of the AB to CD3 ϵ ; and less than the dissociation constant of the AB to CD3 ϵ .
13. The activatable antibody of claim 11, wherein the MM does not interfere or compete with the AB for binding to CD3 ϵ when the activatable antibody is in a cleaved state.

14. The activatable antibody of claim 11, wherein the MM is a polypeptide of no more than 40 amino acids in length.
15. The activatable antibody of claim 11, wherein the MM polypeptide sequence is different from that of CD3 ϵ and wherein the MM polypeptide sequence is no more than 50% identical to any natural binding partner of the AB.
16. The activatable antibody of claim 11, wherein the MM comprises a sequence selected from the group consisting of the sequences shown in Table 7 or Table 8.
17. The activatable antibody of claim 11, wherein the protease is produced by a tumor that is in proximity to cells that express CD3 ϵ in a tissue and/or produced by a tumor that is co-localized with CD3 ϵ in a tissue, and wherein the protease cleaves the CM in the activatable antibody when the activatable antibody is exposed to the protease.
18. The activatable antibody of claim 11, wherein the CM is a polypeptide of up to 15 amino acids in length.
19. The activatable antibody of claim 11, wherein the CM is a substrate for a protease selected from the group consisting of the proteases shown in Table 3 or wherein the CM comprises an amino acid sequence selected from the group consisting of SEQ ID NO: 67-86, 321-341, and 896-926.
20. The activatable antibody of claim 11, wherein the antigen binding fragment thereof is selected from the group consisting of a Fab fragment, a F(ab')₂ fragment, a scFv, a scab, a dAb, a single domain heavy chain antibody, and a single domain light chain antibody.
21. The activatable antibody of claim 11, wherein the activatable antibody in the uncleaved state has the structural arrangement from N-terminus to C-terminus as follows: MM-CM-AB or AB-CM-MM.

22. The activatable antibody of claim 11, wherein the activatable antibody comprises a linking peptide between the MM and the CM.
23. The activatable antibody of claim 11, wherein the activatable antibody comprises a linking peptide between the CM and the AB.
24. The activatable antibody of claim 11, wherein the activatable antibody comprises a first linking peptide (LP1) and a second linking peptide (LP2), and wherein the activatable antibody in the uncleaved state has the structural arrangement from N-terminus to C-terminus as follows: MM-LP1-CM-LP2-AB or AB-LP2-CM-LP1-MM.
25. The activatable antibody of claim 24, wherein the two linking peptides are not identical to each other.
26. The activatable antibody of claim 24, wherein each of LP1 and LP2 is a peptide of about 1 to 20 amino acids in length.
27. The activatable antibody as in any of claims 11 to 26, wherein the AB comprises a VH CDR1 comprising the amino acid sequence TYAMN (SEQ ID NO: 53); a VH CD2 comprising the amino acid sequence RIRSKYNNYATYYADSVKD (SEQ ID NO: 54); a VH CDR3 comprising the amino acid sequence HGNFGNSYVSWFAY (SEQ ID NO: 55), a VL CDR1 comprising the amino acid sequence RSSTGAVTTSNYAN (SEQ ID NO: 56); a VL CDR2 comprising the amino acid sequence GTNKRAP (SEQ ID NO: 57); and a VL CDR3 comprising the amino acid sequence ALWYSNLWV (SEQ ID NO: 58).
28. The activatable antibody as in any of claims 11 to 26, wherein the AB comprises the amino acid sequence of SEQ ID NO: 587.
29. The activatable antibody as in any of claims 11 to 26, wherein the AB comprises the amino acid sequence of SEQ ID NO: 588.

30. The activatable antibody as in any of claims 11 to 26, wherein the activatable antibody comprises a heavy chain sequence selected from the group consisting of SEQ ID NOs: 446, 452, 454, 456, 460, 462, 464, 466, 470, 472, 476, 478, 480, 486, 488, 490, 492, 494, 496, 498, 500, 502, 504, 510, 512, 514, 518, 524, 526, 530, 532, 534, 536, 538, 540, 542, 544, and 546.

31. The activatable antibody as in any of claims 11 to 26, wherein the activatable antibody comprises a light chain sequence selected from the group consisting of SEQ ID NOs: 448, 450, 458, 468, 474, 482, 484, 508, 516, and 520.

32. The activatable antibody as in any of claims 11 to 26, wherein the activatable antibody comprises a heavy chain sequence selected from the group consisting of SEQ ID NOs: 446, 452, 454, 456, 460, 462, 464, 466, 470, 472, 476, 478, 480, 486, 488, 490, 492, 494, 496, 498, 500, 502, 504, 510, 512, 514, 518, 524, 526, 530, 532, 534, 536, 538, 540, 542, 544, and 546; and a light chain sequence selected from the group consisting of SEQ ID NOs: 448, 450, 458, 468, 474, 482, 484, 508, 516, and 520.

33. The activatable antibody as in any of claims 11 to 26, wherein the activatable antibody comprises the amino acid sequence of SEQ ID NO: 506.

34. The activatable antibody as in any of claims 11 to 33 comprising an agent conjugated to the AB.

35. The activatable antibody of claim 34, wherein the agent is a therapeutic agent, an antineoplastic agent, a toxin or fragment thereof, a detectable moiety or a diagnostic agent.

36. The activatable antibody of claim 34, wherein the agent is conjugated to the AB via a linker.

37. The activatable antibody of claim 11, wherein the antibody or antigen-binding fragment thereof comprises a variable heavy chain (Hv) comprising the amino acid sequence of SEQ ID NO: 4 and a variable light chain (Lv) comprising the amino acid sequence of SEQ ID NO: 2.

38. The activatable antibody of claim 11, wherein the antibody or antigen-binding fragment thereof comprises a scFv fragment.
39. The activatable antibody of claim 38, wherein the scFv fragment comprises the amino acid sequence of SEQ ID NO: 6 or the amino acid sequence of SEQ ID NO: 30.
40. A pharmaceutical composition comprising an activatable antibody as in any of claims 11 to 33 or 37-39 and a carrier.
41. A pharmaceutical composition comprising an activatable antibody as in any of claims 11 to 39 and a carrier.
42. An isolated nucleic acid molecule encoding at least a portion of the isolated single chain variable antibody as in any of claims 1 to 5.
43. An isolated nucleic acid molecule encoding at least a portion of the activatable antibody as in any of claims 11-33 or 37-39.
44. A vector comprising the isolated nucleic acid molecule of claim 42 or claim 43.
45. A method of producing an isolated single chain variable antibody or an activatable antibody by culturing a cell under conditions that lead to expression of the antibody or the activatable antibody, wherein the cell comprises the nucleic acid molecule of claim 42 or claim 43.
46. A method of manufacturing an activatable antibody that binds the epsilon chain of CD3 (CD3 ϵ) in an activated state, the method comprising:
- (a) culturing a cell comprising a nucleic acid construct that encodes the activatable antibody under conditions that lead to expression of the activatable antibody, wherein the activatable antibody comprises a masking moiety (MM), a cleavable moiety (CM), and an

antibody or an antigen binding fragment thereof (AB) that specifically binds the epsilon chain of CD3 (CD3 ϵ),

and

(b) recovering the activatable antibody.

47. The method of claim 46, wherein the CM is a polypeptide that functions as a substrate for a protease.

48. The method of claim 46, wherein the CM is positioned in the activatable antibody such that, when the activatable antibody is in an uncleaved state, the MM interferes with specific binding of the AB to CD3 ϵ and when the activatable antibody is in a cleaved state the MM does not interfere or compete with specific binding of the AB to CD3 ϵ .

49. An isolated multispecific antibody that binds the epsilon chain of CD3 (CD3 ϵ) and a second target, wherein the antibody comprises a first antibody or antigen-binding fragment thereof (AB1) that binds the epsilon chain of CD3 (CD3 ϵ) and a second antibody or antigen-binding fragment thereof (AB2) that binds a second target, and wherein AB1 comprises a VH CDR1 comprising the amino acid sequence TYAMN (SEQ ID NO: 53); a VH CDR2 comprising the amino acid sequence RIRSKYNNYATYYADSVKD (SEQ ID NO: 54); a VH CDR3 comprising the amino acid sequence HGNFGNSYVSWFAY (SEQ ID NO: 55), a VL CDR1 comprising the amino acid sequence RSSTGAVTTSNYAN (SEQ ID NO: 56); a VL CDR2 comprising the amino acid sequence GTNKRAP (SEQ ID NO: 57); and a VL CDR3 comprising the amino acid sequence ALWYSNLWV (SEQ ID NO: 58).

50. The multispecific antibody of claim 49, wherein AB1 comprises a variable heavy chain (Hv) comprising the amino acid sequence of SEQ ID NO: 4 and a variable light chain (Lv) comprising the amino acid sequence of SEQ ID NO: 2.

51. The multispecific antibody of claim 49, wherein the AB1 comprises a scFv fragment.

52. The multispecific antibody of claim 51, wherein the scFv fragment comprises the amino acid sequence of SEQ ID NO: 6 or the amino acid sequence of SEQ ID NO: 30.
53. The multispecific antibody of claim 49, wherein the AB comprises the amino acid sequence of SEQ ID NO: 587.
54. The multispecific antibody of claim 49, wherein the AB comprises the amino acid sequence of SEQ ID NO: 588.
55. The antibody of claim 49 comprising an agent conjugated to the antibody.
56. The multispecific antibody of claim 49, wherein the agent is a therapeutic agent, an antineoplastic agent, a toxin or fragment thereof, a detectable moiety or a diagnostic agent.
57. The multispecific antibody of claim 49, wherein the agent is conjugated to the antibody via a linker.
58. The multispecific antibody of claim 57, wherein the linker is a cleavable linker.
59. The multispecific antibody of claim 57, wherein the linker is a non-cleavable linker.
60. The multispecific antibody of claim 49, wherein the multispecific antibody is selected from:
a multispecific antibody comprising:
a cleavable moiety (CM) coupled to the AB2, wherein the CM is a polypeptide that functions as a substrate for a protease; and
a masking moiety (MM) that inhibits the binding of the AB2 to the second target when the CM is in an uncleaved state; and
a multispecific antibody comprising:
a cleavable moiety (CM) coupled to the AB1, wherein the CM is a polypeptide that functions as a substrate for a protease; and

a masking moiety (MM) that inhibits the binding of the AB1 to CD3 ϵ when the CM is in an uncleaved state.

61. An isolated multispecific activatable antibody that binds the epsilon chain of CD3 (CD3 ϵ) and a second target, the multispecific activatable antibody comprising
- a first antibody or an antigen binding fragment thereof (AB1) that specifically binds to CD3 ϵ and a second antibody or antigen-binding fragment thereof (AB2) that binds a second target, wherein AB1 comprises a VH CDR1 comprising the amino acid sequence TYAMN (SEQ ID NO: 53); a VH CDR2 comprising the amino acid sequence RIRSKYNNYATYYADSVKD (SEQ ID NO: 54); a VH CDR3 comprising the amino acid sequence HGNFGNSYVSWFAY (SEQ ID NO: 55), a VL CDR1 comprising the amino acid sequence RSSTGAVTTSNYAN (SEQ ID NO: 56); a VL CDR2 comprising the amino acid sequence GTNKRAP (SEQ ID NO: 57); and a VL CDR3 comprising the amino acid sequence ALWYSNLWV (SEQ ID NO: 58);
 - a masking moiety (MM) that inhibits the binding of the AB2 to the second target when the multispecific activatable antibody is in an uncleaved state; and
 - a cleavable moiety (CM) coupled to the AB2, wherein the CM is a polypeptide that functions as a substrate for a protease.

62. The multispecific activatable antibody of claim 61, wherein the target of AB2 is selected from the group of targets listed in Table 1.

63. The multispecific activatable antibody of claim 61, wherein AB2 is or is derived from an antibody selected from the group of antibodies listed in Table 2.

64. The multispecific activatable antibody of claim 61, wherein the antigen binding fragment thereof is selected from the group consisting of a Fab fragment, a F(ab')₂ fragment, a scFv, a scAb, a dAb, a single domain heavy chain antibody, and a single domain light chain antibody.

65. The multispecific activatable antibody of claim 61, wherein the MM has a dissociation constant for binding to the AB that is selected from the group consisting of: greater than the dissociation constant of the AB to the target; equivalent to the dissociation constant of the AB to CD3 ϵ ; and less than the dissociation constant of the AB to CD3 ϵ .
66. The multispecific activatable antibody of claim 61, wherein the MM does not interfere or compete with the AB for binding to the target when the multispecific activatable antibody is in a cleaved state.
67. The multispecific activatable antibody of claim 61, wherein the MM is a polypeptide of no more than 40 amino acids in length.
68. The multispecific activatable antibody of claim 61, wherein the MM polypeptide sequence is different from that of the target of AB2, and wherein the MM polypeptide sequence is no more than 50% identical to any natural binding partner of AB2.
69. The multispecific activatable antibody of claim 61, wherein the CM is a polypeptide of up to 15 amino acids in length.
70. The multispecific activatable antibody of claim 61, wherein the protease is produced by a tumor that is in proximity to cells that express CD3 ϵ in a tissue and/or produced by a tumor that is co-localized with the target of AB2 in a tissue, and wherein the protease cleaves the CM in the multispecific activatable antibody when the multispecific activatable antibody is exposed to the protease.
71. The multispecific activatable antibody of claim 61, wherein the multispecific activatable antibody comprises a linking peptide between the MM and the CM.
72. The multispecific activatable antibody of claim 61, wherein the multispecific activatable antibody comprises a linking peptide between the CM and the AB.

73. The multispecific activatable antibody as in any of claims 61 to 72, wherein the multispecific activatable antibody comprises a first linking peptide (LP1) and a second linking peptide (LP2), and wherein the multispecific activatable antibody in an uncleaved state has the structural arrangement from N-terminus to C-terminus as follows: MM-LP1-CM-LP2-AB or AB-LP2-CM-LP1-MM.

74. The multispecific activatable antibody of claim 73, wherein the two linking peptides are not identical to each other.

75. The multispecific activatable antibody of claim 73, wherein each of LP1 and LP2 is a peptide of about 1 to 20 amino acids in length.

76. The multispecific activatable antibody of claim 73, wherein at least one of LP1 or LP2 comprises an amino acid sequence selected from the group consisting of (GS)_n, (GGS)_n, (GSGGS)_n (SEQ ID NO: 59) and (GGGS)_n (SEQ ID NO: 60), GGSG (SEQ ID NO: 61), GGSGG (SEQ ID NO: 62), GSGSG (SEQ ID NO: 63), GSGGG (SEQ ID NO: 64), GGGSG (SEQ ID NO: 65), and GSSSG (SEQ ID NO: 66), where n is an integer of at least one.

77. The multispecific activatable antibody of claim 61, wherein the CM is a substrate for a protease selected from the group consisting of the proteases shown in Table 3 or wherein the CM comprises an amino acid sequence selected from the group consisting of SEQ ID NO: 67-86, 321-341, and 896-926.

78. A multispecific activatable antibody that in an activated state binds the epsilon chain of CD3 (CD3 ϵ) and a second target, the multispecific activatable antibody comprising:

at least a first antibody or an antigen binding fragment thereof (AB1) that specifically binds CD3 ϵ ;

a second antibody or an antigen binding fragment thereof (AB2) that specifically binds a second target or epitope;

at least a first masking moiety (MM1) that inhibits the binding of the AB1 to CD3 ϵ when the multispecific activatable antibody is in an uncleaved state; and

at least a first cleavable moiety (CM1) coupled to the AB1, wherein the CM1 is a polypeptide that functions as a substrate for a protease.

79. A multispecific activatable antibody that in an activated state binds the epsilon chain of CD3 (CD3 ϵ) and a second target, the multispecific activatable antibody comprising:

at least a first antibody or an antigen binding fragment thereof (AB1) that specifically binds CD3 ϵ ;

at least a first masking moiety (MM1) that inhibits the binding of the AB1 to CD3 ϵ when the multispecific activatable antibody is in an uncleaved state;

at least a first cleavable moiety (CM1) coupled to the AB1, wherein the CM1 is a polypeptide that functions as a substrate for a protease;

at least a second antibody or an antigen binding fragment thereof (AB2) that specifically binds a second target or epitope;

at least a second masking moiety (MM2) that inhibits the binding of the AB2 to the second target or epitope when the multispecific activatable antibody is in an uncleaved state; and

at least a second cleavable moiety (CM2) coupled to the AB2, wherein the CM2 is a polypeptide that functions as a substrate for a protease.

80. The multispecific activatable antibody of claim 61, claim 78 or claim 79, wherein the MM1 and/or the MM2 has a dissociation constant for binding to the AB1 and/or AB2 which is selected from the group consisting of: greater than the dissociation constant of the AB1 to CD3 ϵ and/or than the dissociation constant of the AB2 to the second target or epitope; equivalent to the dissociation constant of the AB1 to CD3 ϵ and/or than the dissociation constant of the AB2 to the second target or epitope; and less than the dissociation constant of the AB1 to CD3 ϵ and/or than the dissociation constant of the AB2 to the second target or epitope.

81. The multispecific activatable antibody of claim 61, claim 78 or claim 79, wherein the MM1 polypeptide sequence is different from that of CD3 ϵ , the MM2 polypeptide sequence is different from that of the second target or epitope, and wherein the MM1 polypeptide sequence and/or the MM2 polypeptide sequence is no more than 50% identical to any natural binding partner of AB1 and/or AB2.
82. The multispecific activatable antibody of claim 61, claim 78 or claim 79, wherein the MM1 and/or MM2 does not interfere or compete with the AB1 for binding to CD3 ϵ and/or with the AB2 for binding to the second target or epitope when the multispecific activatable antibody is in a cleaved state.
83. The multispecific activatable antibody of claim 61, claim 78 or claim 79, wherein the MM1 comprises a sequence selected from the group consisting of the sequences shown in Table 7 or Table 8.
84. The multispecific activatable antibody of claim 61, claim 78 or claim 79, wherein the protease that cleaves CM1 and/or CM2 is produced by a tumor that is in proximity to cells that express CD3 ϵ in a tissue and/or produced by a tumor that is co-localized with the CD3 ϵ and/or the second target or epitope in a tissue, and wherein the protease cleaves CM1 and/or CM2 in the multispecific activatable antibody when the multispecific activatable antibody is exposed to the protease.
85. The multispecific activatable antibody of claim 61, claim 78 or claim 79, wherein at least a portion of the multispecific activatable antibody in the uncleaved state has the structural arrangement from N-terminus to C-terminus as follows: MM1-CM1-AB1, AB1-CM1-MM1, MM2-CM2-AB2, or AB2-CM2-MM2.
86. The multispecific activatable antibody of claim 61, claim 78 or claim 79, wherein the multispecific activatable antibody comprises a linking peptide between the MM1 and the CM1 and/or between the MM2 and the CM2.

87. The multispecific activatable antibody of claim 61, claim 78 or claim 79, wherein the multispecific activatable antibody comprises a linking peptide between the CM1 and the AB1 and/or between the CM2 and the AB2.

88. The multispecific activatable antibody of claim 61, claim 78 or claim 79, wherein the multispecific activatable antibody comprises a first linking peptide (LP1) and a second linking peptide (LP2), and wherein the multispecific activatable antibody in the uncleaved state has the structural arrangement from N-terminus to C-terminus as follows: MM1-LP1-CM1-LP2-AB1, AB1-LP2-CM1-LP1-MM1, MM2-LP1-CM2-LP2-AB2, or AB2-LP2-CM2-LP1-MM2.

89. The multispecific activatable antibody of claim 88, wherein the two linking peptides need not be identical to each other.

90. The multispecific activatable antibody of claim 88, wherein each of LP1 and LP2 is a peptide of about 1 to 20 amino acids in length.

91. The multispecific activatable antibody of claim 61, claim 78 or claim 79, wherein the MM1 and/or the MM2 is a polypeptide of no more than 40 amino acids in length.

92. The multispecific activatable antibody of claim 61, claim 78 or claim 79, wherein the CM1 and/or CM2 is a polypeptide of up to 15 amino acids in length.

93. The multispecific activatable antibody of claim 61, claim 78 or claim 79, wherein the CM1 and/or CM2 is a substrate for a protease selected from the group consisting of the proteases listed in Table 3 or wherein the CM1 and/or CM2 comprises an amino acid sequence selected from the group consisting of SEQ ID NO: 67-86, 321-341, and 896-926.

94. The multispecific activatable antibody of claim 61, claim 78 or claim 79, wherein the antigen binding fragment thereof of AB1 and/or AB2 is selected from the group consisting of a Fab fragment, a F(ab')₂ fragment, a scFv, a scab, a dAb, a single domain heavy chain antibody, and a single domain light chain antibody.

95. The multispecific activatable antibody as in any of claims 78 to 94, wherein AB1 comprises a VH CDR1 comprising the amino acid sequence TYAMN (SEQ ID NO: 53); a VH CD2 comprising the amino acid sequence RIRSKYNNYATYYADSVKD (SEQ ID NO: 54); a VH CDR3 comprising the amino acid sequence HGNFGNSYVSWFAY (SEQ ID NO: 55), a VL CDR1 comprising the amino acid sequence RSSTGAVTTSNYAN (SEQ ID NO: 56); a VL CDR2 comprising the amino acid sequence GTNKRAP (SEQ ID NO: 57); and a VL CDR3 comprising the amino acid sequence ALWYSNLWV (SEQ ID NO: 58).
96. The multispecific activatable antibody as in any of claims 78 to 94, wherein AB1 comprises a variable heavy chain (Hv) comprising the amino acid sequence of SEQ ID NO: 4 and a variable light chain (Lv) comprising the amino acid sequence of SEQ ID NO: 2.
97. The multispecific activatable antibody as in any of claims 78 to 94, wherein the AB1 and/or AB2 comprises a scFv fragment.
98. The multispecific activatable antibody as in any of claims 78 to 94, wherein the AB1 comprises a scFv fragment, and wherein the scFv fragment comprises the amino acid sequence of SEQ ID NO: 6 or the amino acid sequence of SEQ ID NO: 30.
99. The multispecific activatable antibody as in any of claims 78 to 94, wherein the AB1 comprises the amino acid sequence of SEQ ID NO: 587.
100. The multispecific activatable antibody as in any of claims 78 to 94, wherein the AB1 comprises the amino acid sequence of SEQ ID NO: 588.
101. The multispecific activatable antibody as in any of claims 78 to 94, wherein the target of AB2 is selected from the group of targets listed in Table 1.
102. The multispecific activatable antibody as in any of claims 78 to 94, wherein AB2 is or is derived from an antibody selected from the group of antibodies listed in Table 2.

103. The multispecific activatable antibody of claim 78 or claim 79 comprising an agent conjugated to at least one of AB1 and AB2.

104. The multispecific activatable antibody of claim 103, wherein the agent is a therapeutic agent, an antineoplastic agent, a toxin or fragment thereof, a detectable moiety or a diagnostic agent.

105. The multispecific activatable antibody of claim 103, wherein the agent is conjugated to at least one of AB1 and AB2 via a linker.

106. A pharmaceutical composition comprising the multispecific antibody as in any of claims 49-55 or 60-77 or the multispecific activatable antibody as in any of claims 78-102 and a carrier.

107. A pharmaceutical composition comprising the multispecific antibody as in any of claims 49-77 or the multispecific activatable antibody as in any of claims 78-102 and a carrier.

108. An isolated nucleic acid molecule encoding at least a portion of the multispecific antibody as in any of claims 49-54.

109. An isolated nucleic acid molecule encoding at least a portion of the multispecific activatable antibody as in any of claims 95-100.

110. A vector comprising the isolated nucleic acid molecule of claim 108 or claim 109.

111. A method of producing a multispecific antibody or a multispecific activatable antibody that specifically binds at least the epsilon chain of CD3 (CD3 ϵ), the method comprising culturing a cell under conditions that lead to expression of the multispecific antibody or multispecific activatable antibody, wherein the cell comprises the nucleic acid molecule of claim 108 or claim 109.

112. A method of manufacturing a multispecific activatable antibody that in an activated state binds the epsilon chain of CD3 (CD3 ϵ) and a second target, the method comprising:

(a) culturing a cell comprising a nucleic acid construct that encodes the multispecific activatable antibody under conditions that lead to expression of the multispecific activatable antibody, wherein the activatable antibody is selected from the group consisting of:

- (i) an activatable antibody comprising at least a first antibody or an antigen binding fragment thereof (AB1) that specifically binds CD3 ϵ , a second antibody or an antigen binding fragment thereof (AB2) that specifically binds a second target or epitope, at least a first masking moiety (MM1) that inhibits the binding of the AB1 to CD3 ϵ when the multispecific activatable antibody is in an uncleaved state, and at least a first cleavable moiety (CM1) coupled to the AB1, and
- (ii) an activatable antibody comprising at least a first antibody or an antigen binding fragment thereof (AB1) that specifically binds CD3 ϵ , a second antibody or an antigen binding fragment thereof (AB2) that specifically binds a second target or epitope, a first masking moiety (MM1) that inhibits the binding of the AB1 to CD3 ϵ when the multispecific activatable antibody is in an uncleaved state, a second masking moiety (MM2) that inhibits the binding of the AB2 to its target when the multispecific activatable antibody is in an uncleaved state, a first cleavable moiety (CM1) coupled to the AB1, and a second cleavable moiety (CM2) coupled to the AB2;

and

(b) recovering the activatable antibody.

113. The method of claim 112, wherein the CM1, the CM2, or both CM1 and CM2 is a polypeptide that functions as a substrate for a protease.

114. The method of claim 112, wherein the CM1 is positioned in the activatable antibody such that, when the activatable antibody is in an uncleaved state, the MM1 interferes with specific binding of the AB1 to CD3 ϵ and when the activatable antibody is in a cleaved state the MM1 does not interfere or compete with specific binding of the AB1 to CD3 ϵ , and wherein the CM2 is positioned in the activatable antibody such that, when the activatable antibody is in an

uncleaved state, the MM2 interferes with specific binding of the AB2 to its target and when the activatable antibody is in a cleaved state the MM2 does not interfere or compete with specific binding of the AB2 to its target.

115. A method of alleviating a symptom of a clinical indication associated with a disorder in a subject, the method comprising administering the isolated single chain variable antibody as in any of claims 1-5 or the activatable antibody as in any of claims 11-33 or 37-39, or the multispecific antibody as in any of claims 49-54 or 60, or the multispecific activatable antibody as in any of claims 61-102 to a subject in need thereof in an amount sufficient to alleviate the symptom of the clinical indication associated with the disorder.

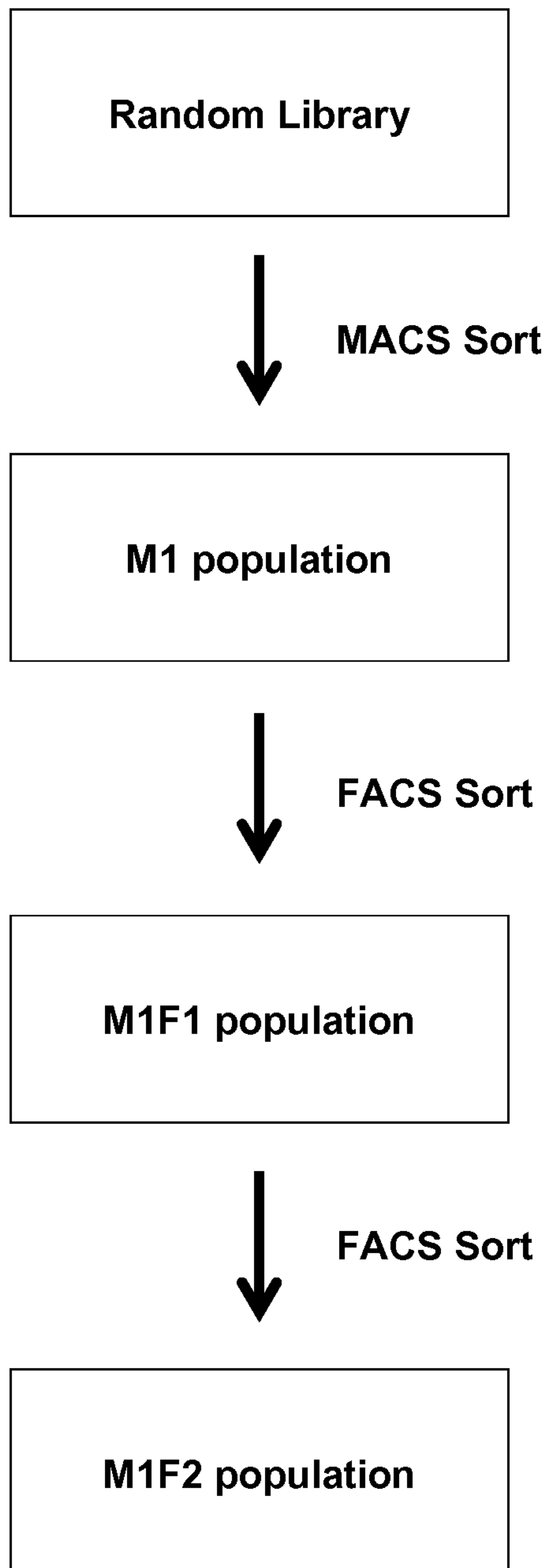
116. A method of alleviating a symptom of a clinical indication associated with a disorder in a subject, the method comprising administering the isolated single chain variable antibody as in any of claims 1-8 or the activatable antibody as in any of claims 11-39, or the multispecific antibody as in any of claims 49-60, or the multispecific activatable antibody as in any of claims 61-105 to a subject in need thereof in an amount sufficient to alleviate the symptom of the clinical indication associated with the disorder.

117. The method of claim 115 or claim 116, wherein said subject is a human.

118. The method of claim 115 or claim 116, wherein the disorder is cancer.

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FIGURE 1



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FIGURE 2A

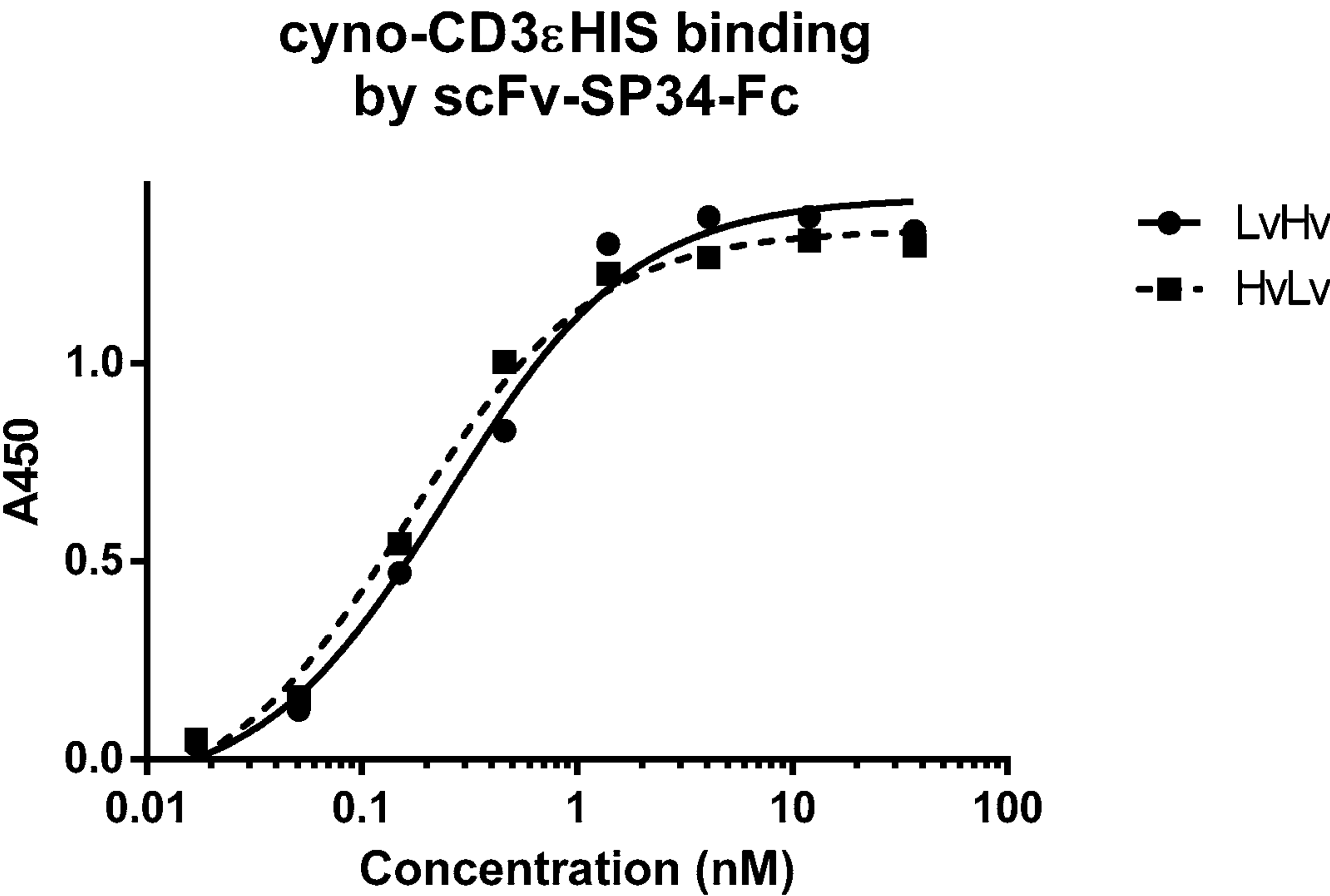


FIGURE 2B

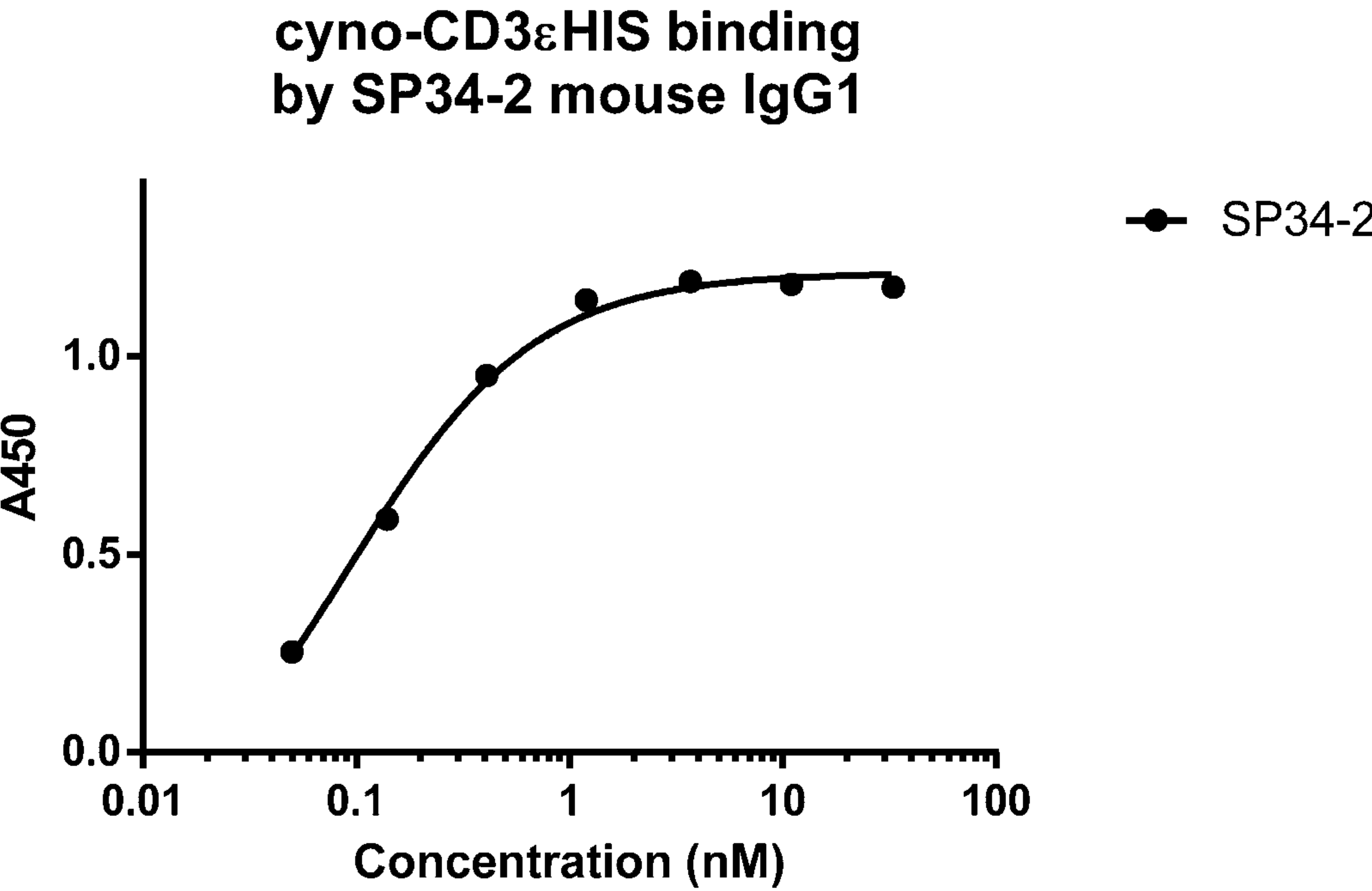
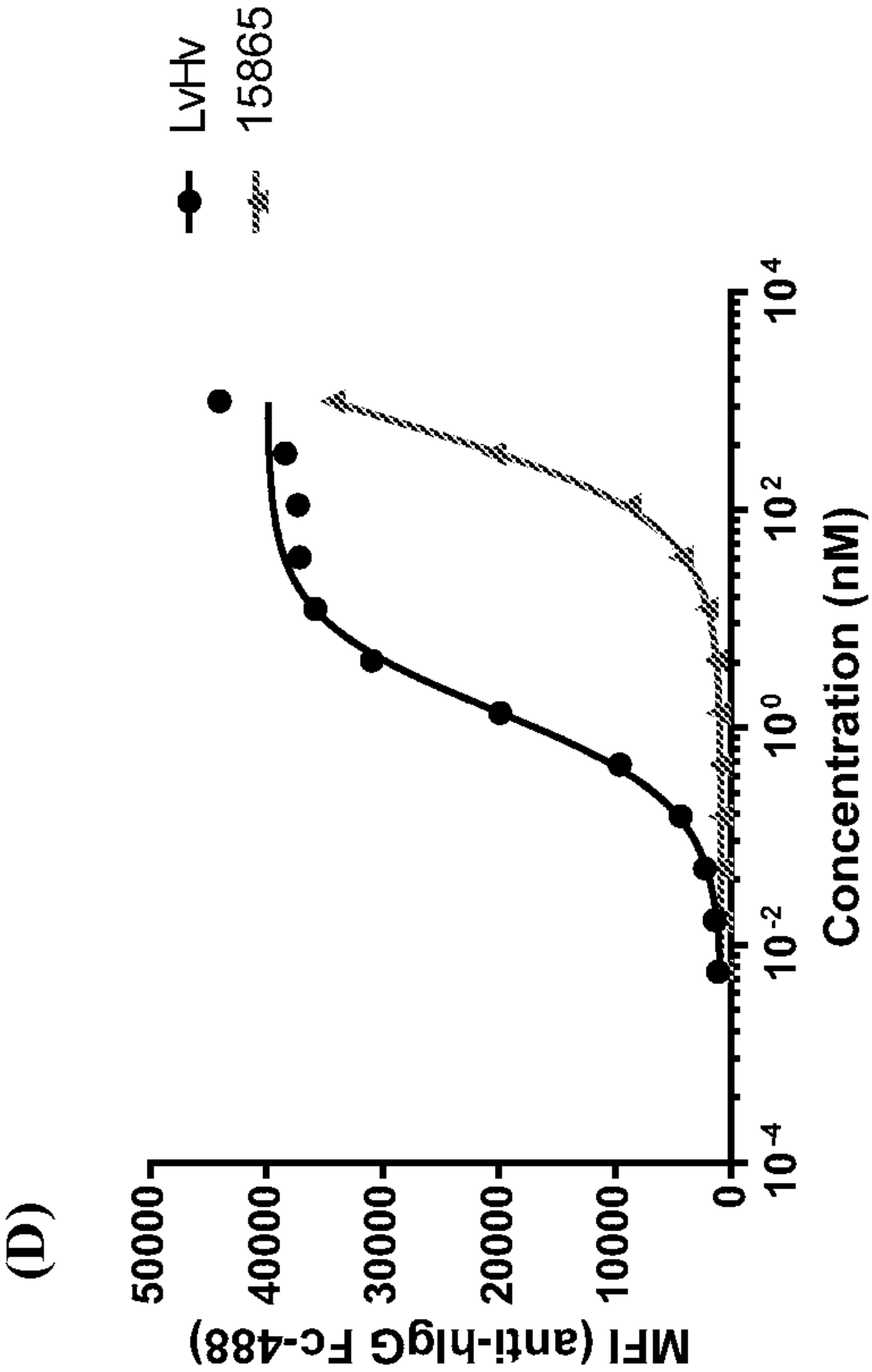
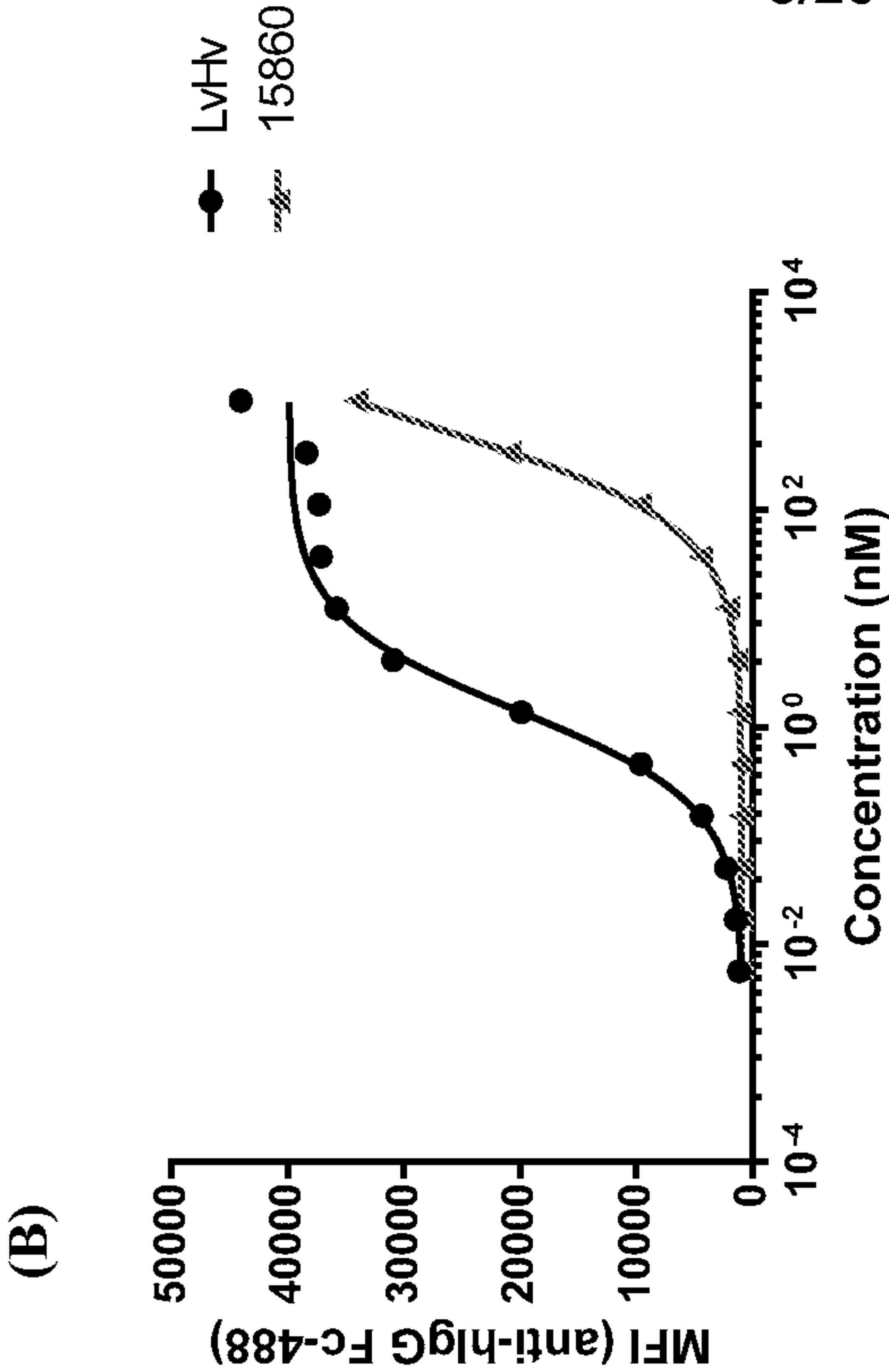
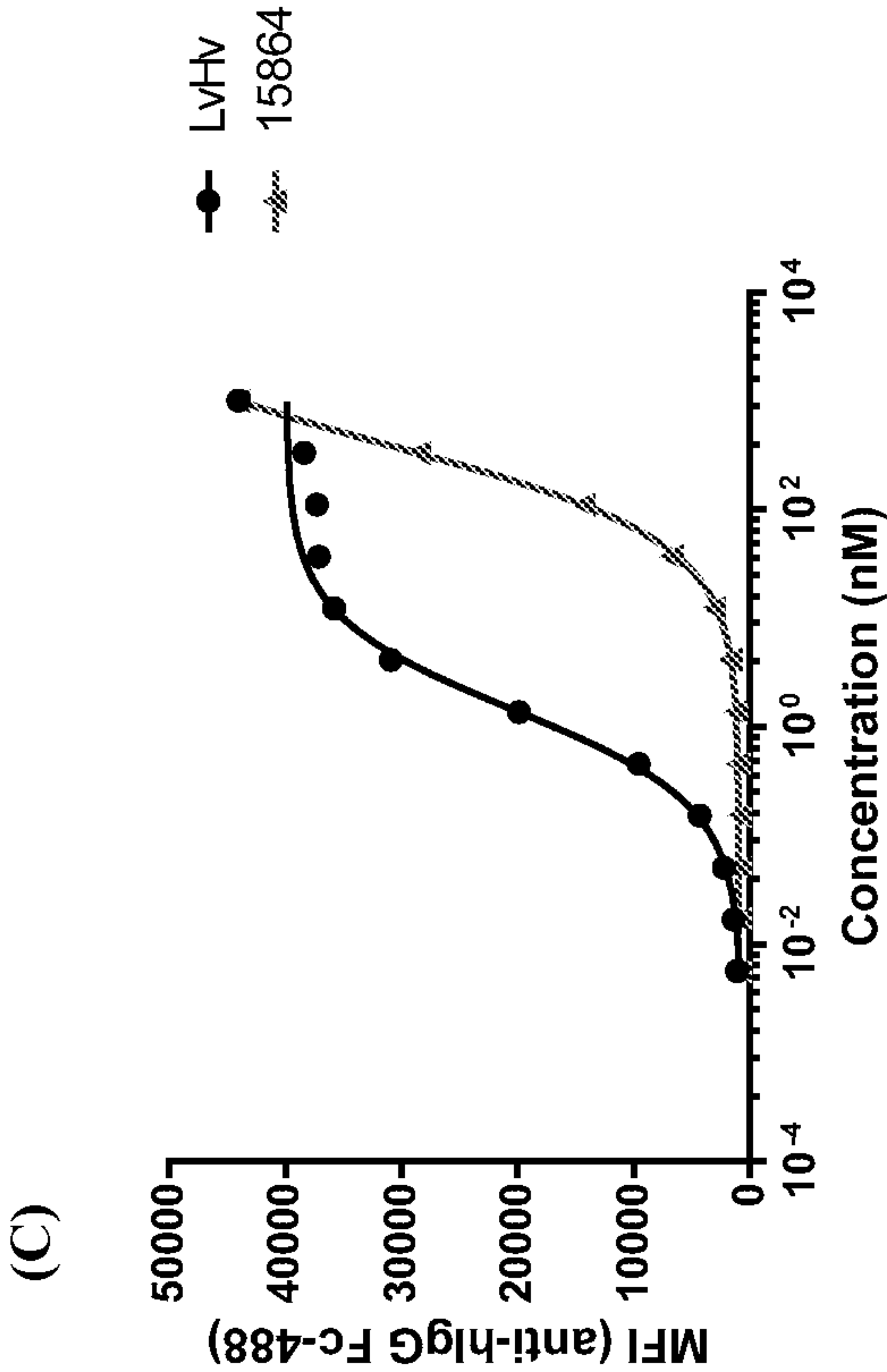
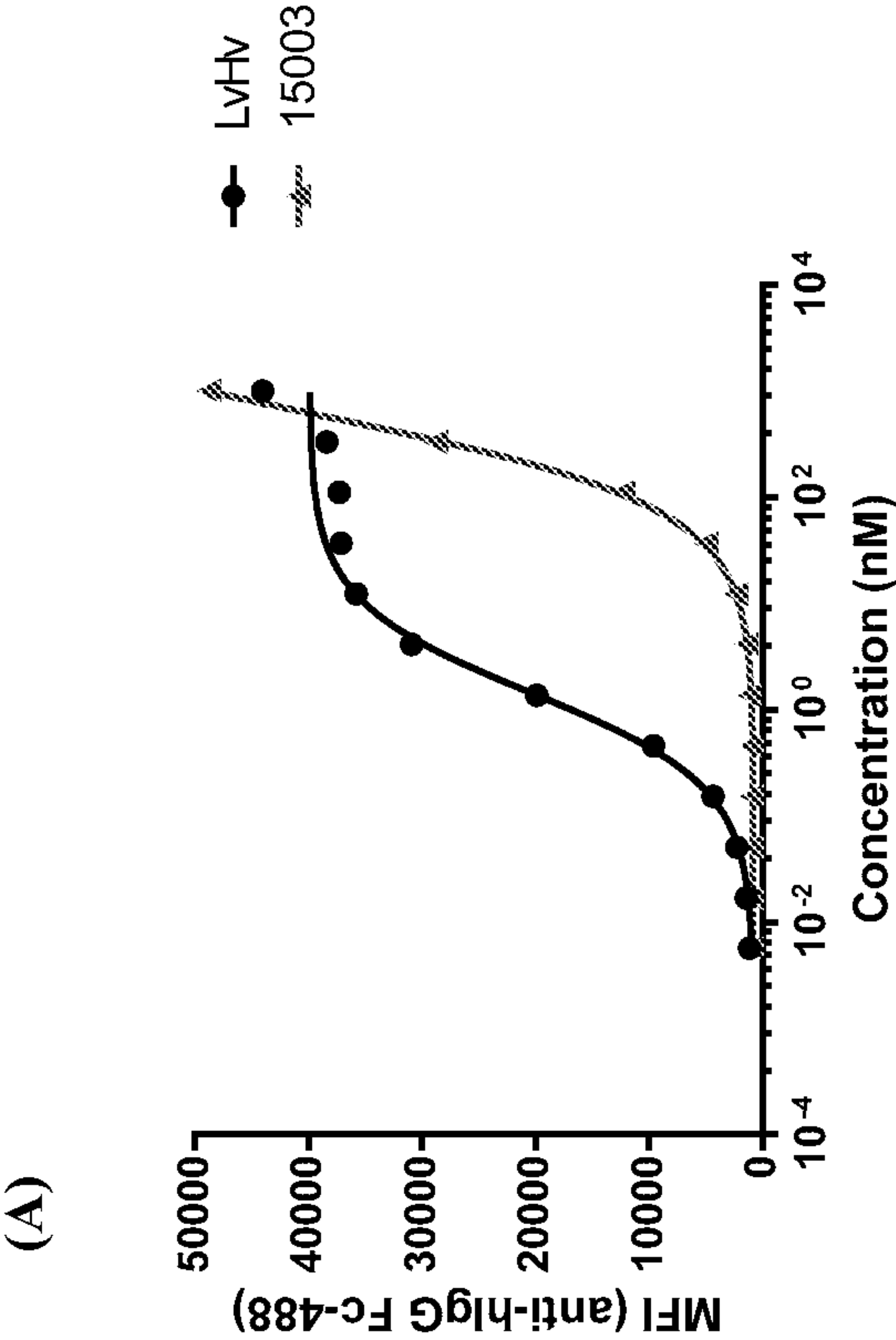


FIGURE 3



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FIGURE 4A

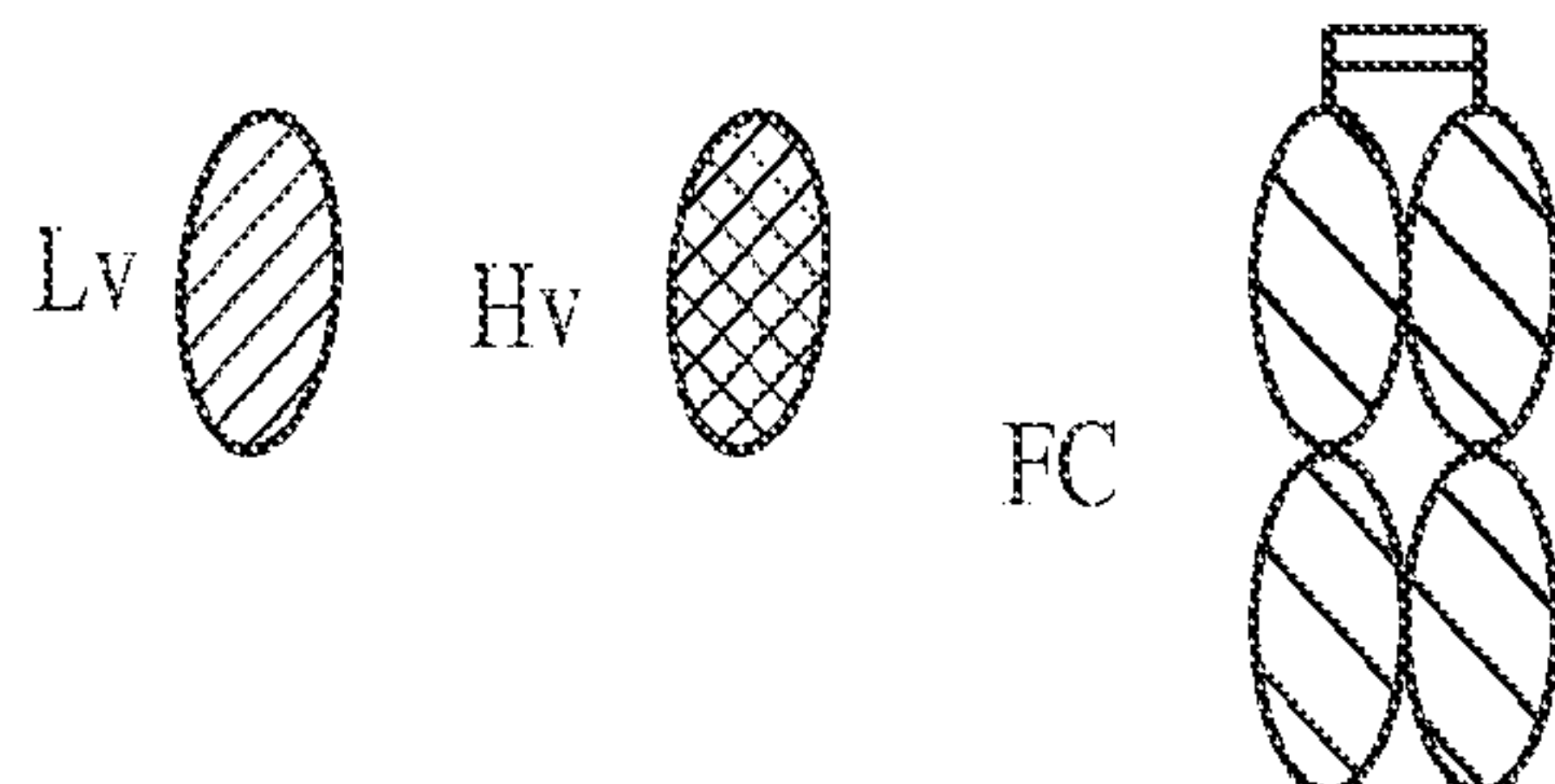


FIGURE 4B

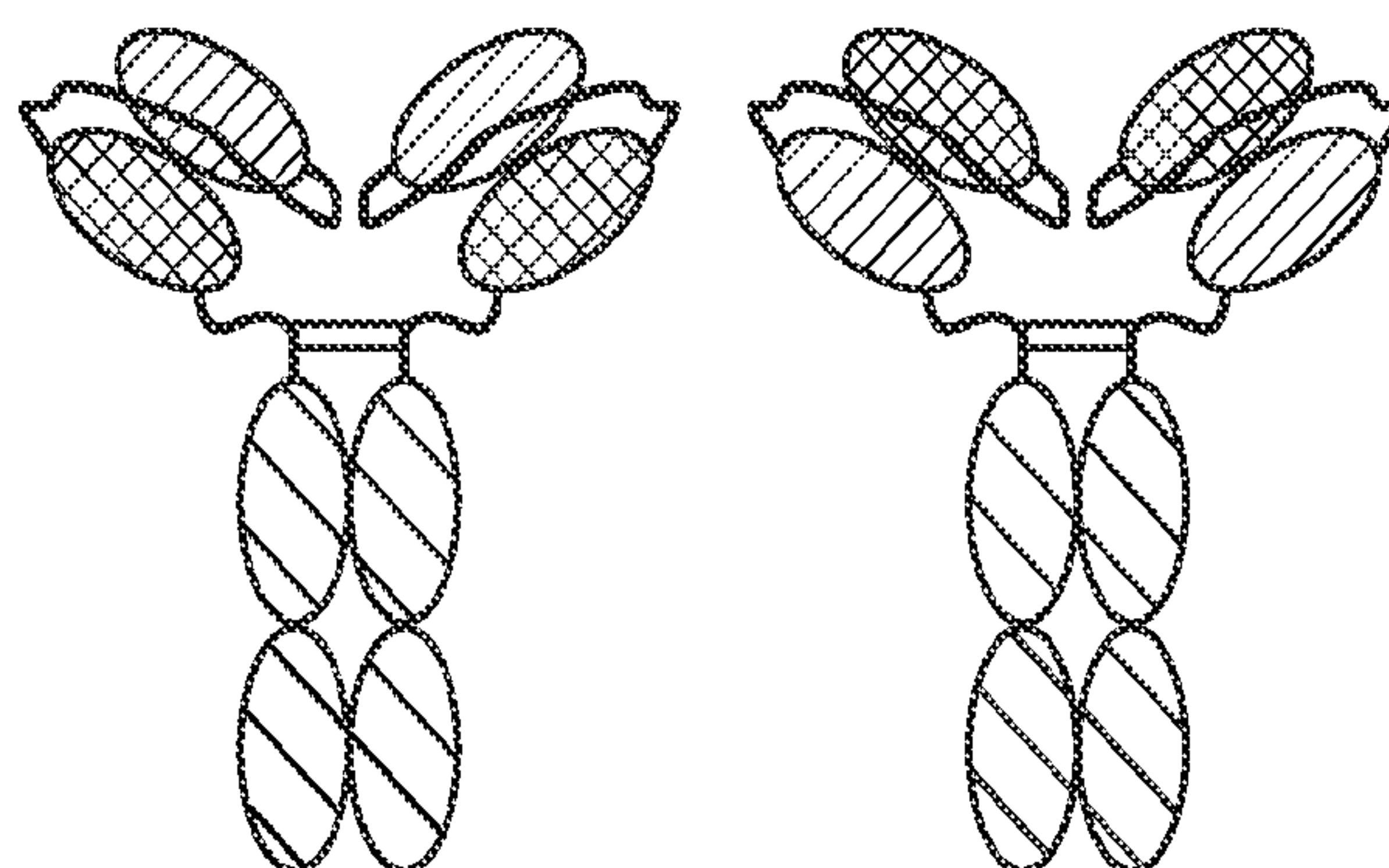
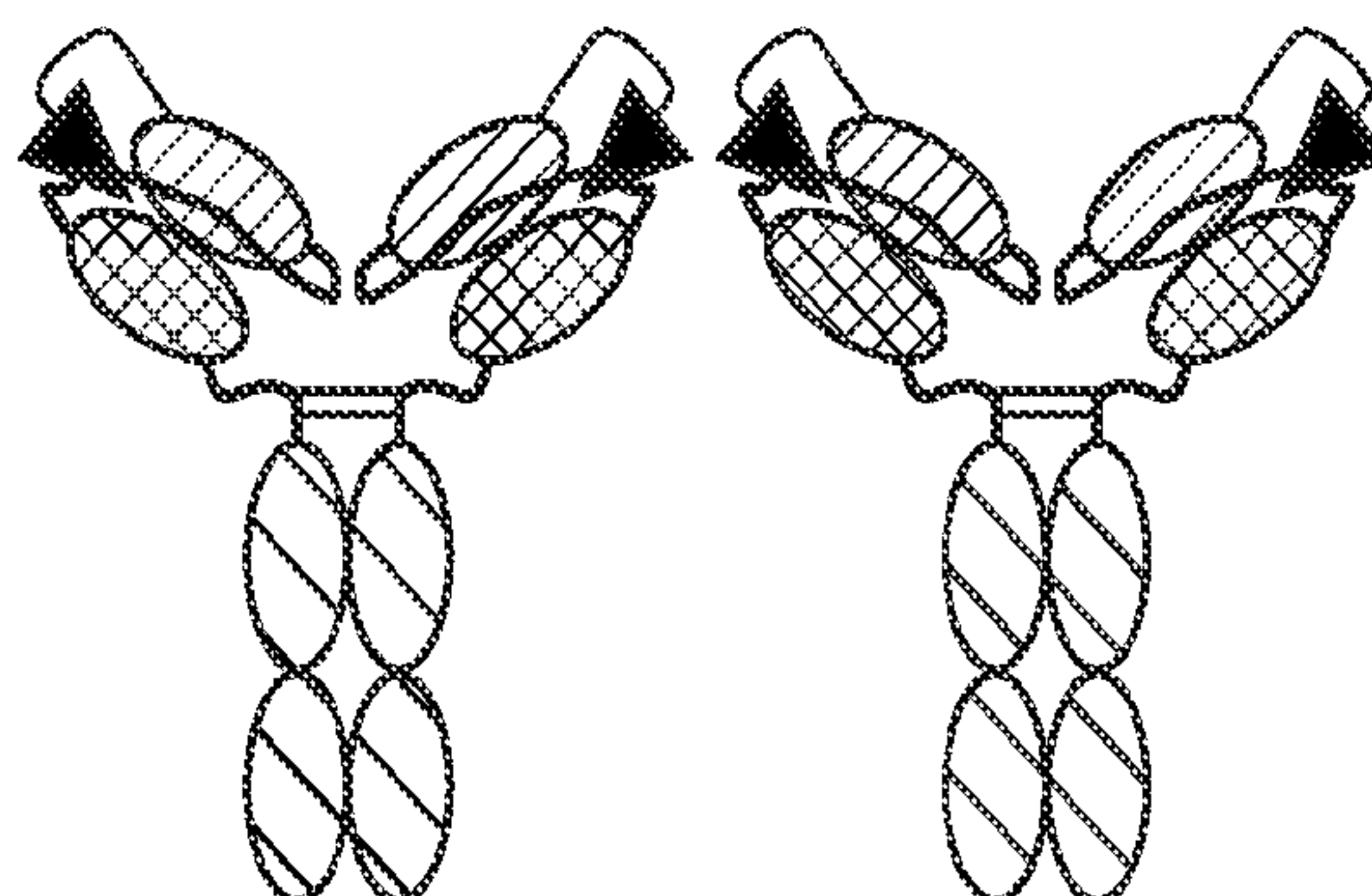


FIGURE 4C



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FIGURE 5A

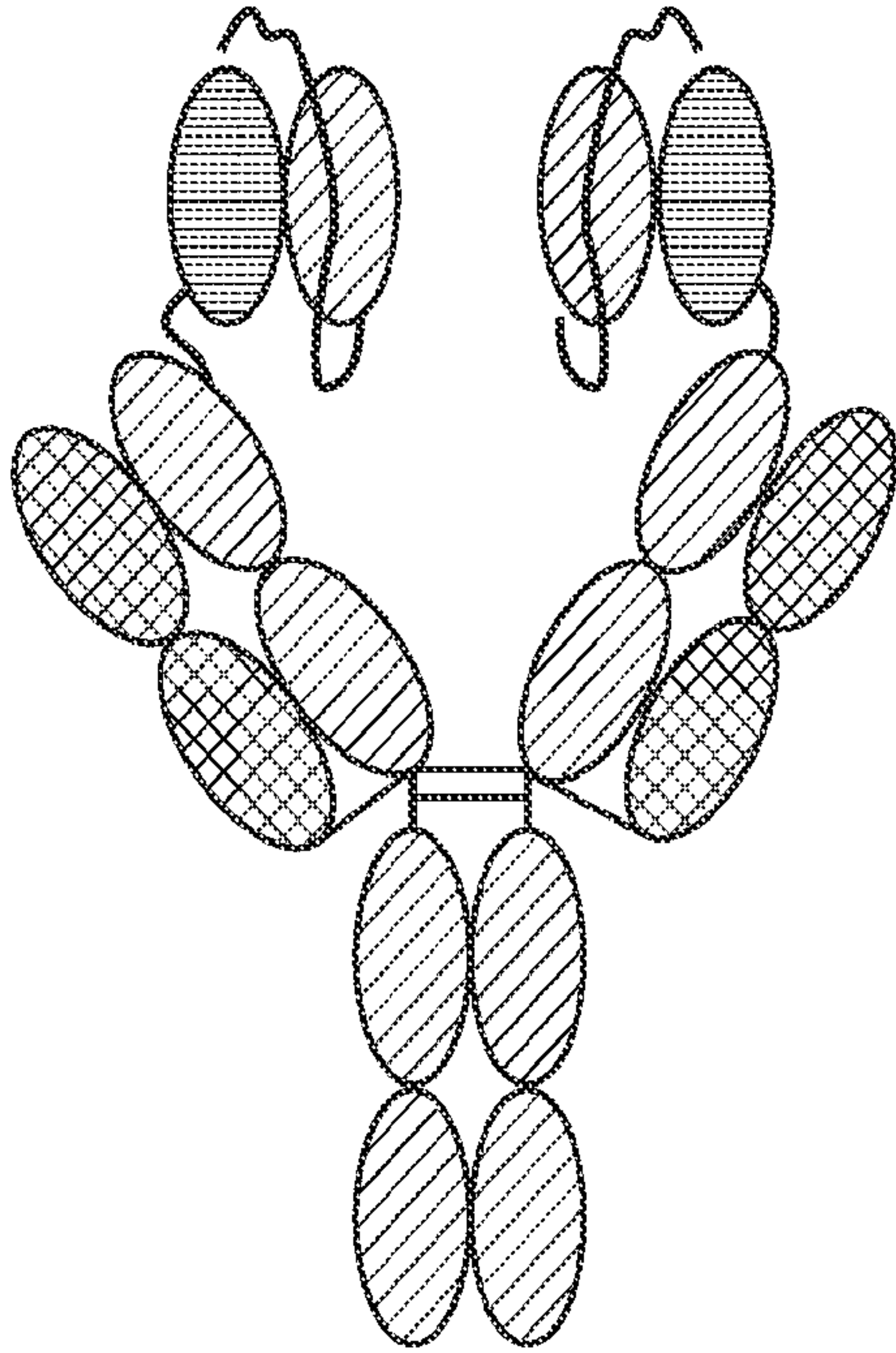


FIGURE 5B

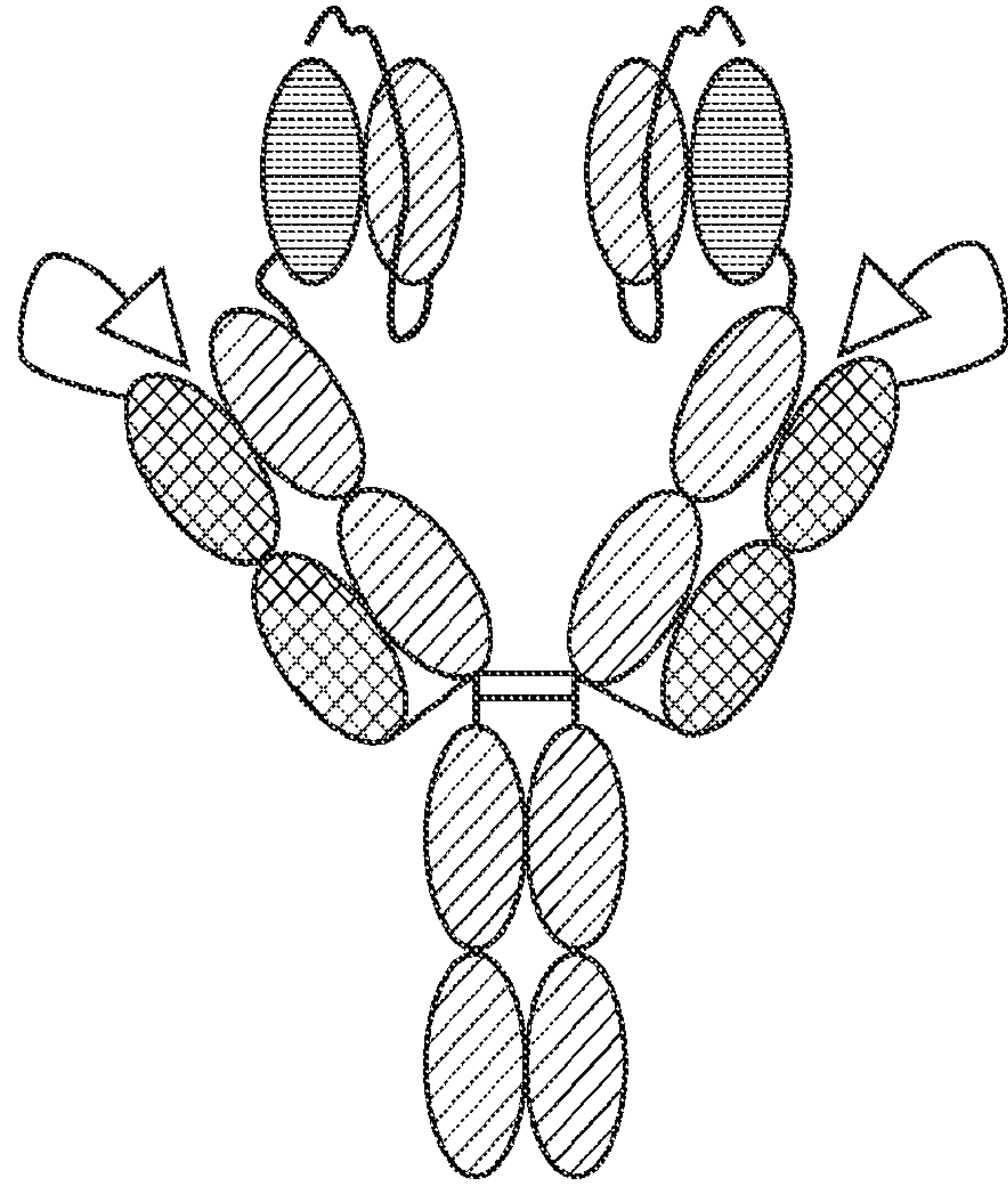


FIGURE 5C

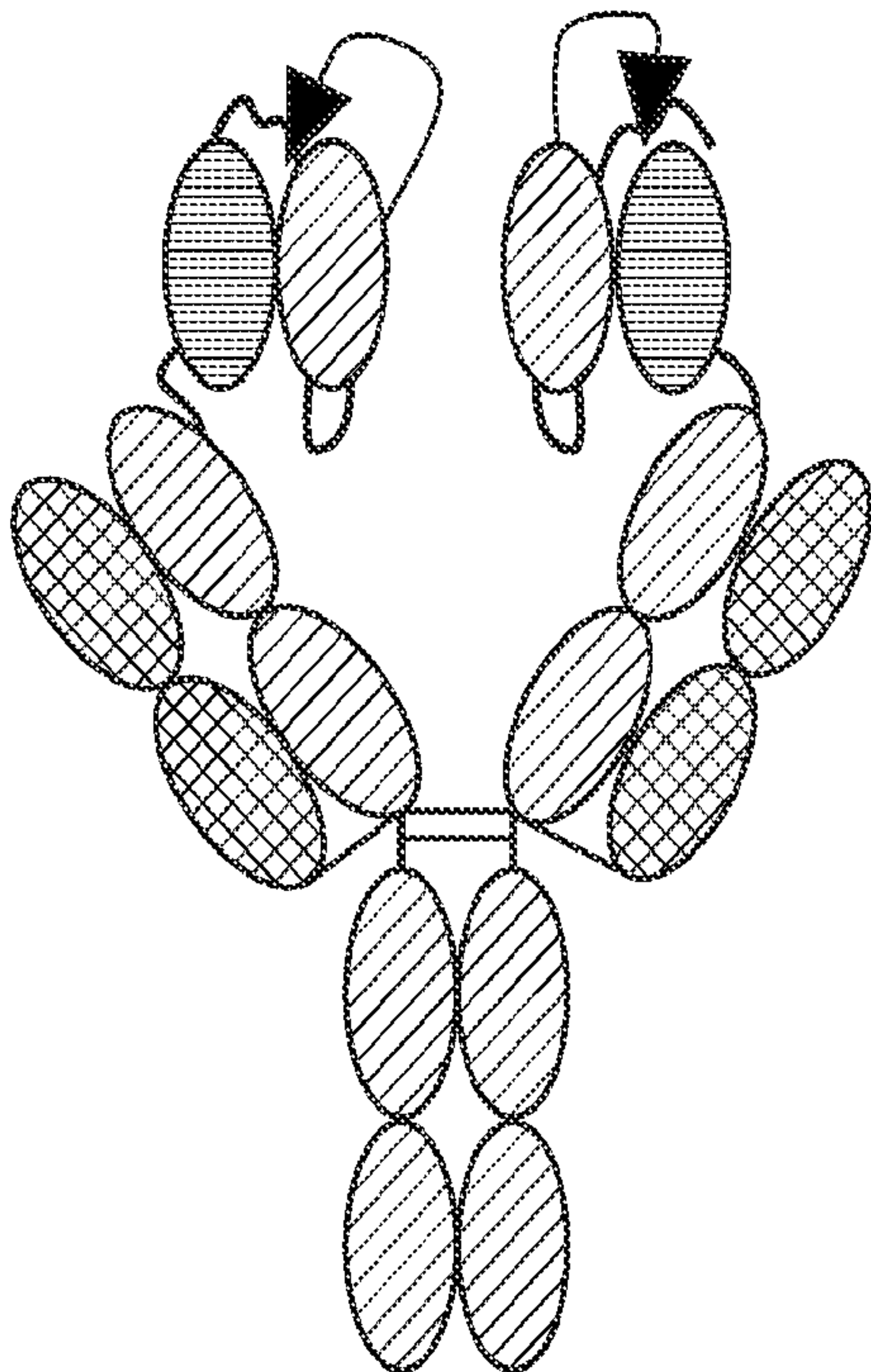
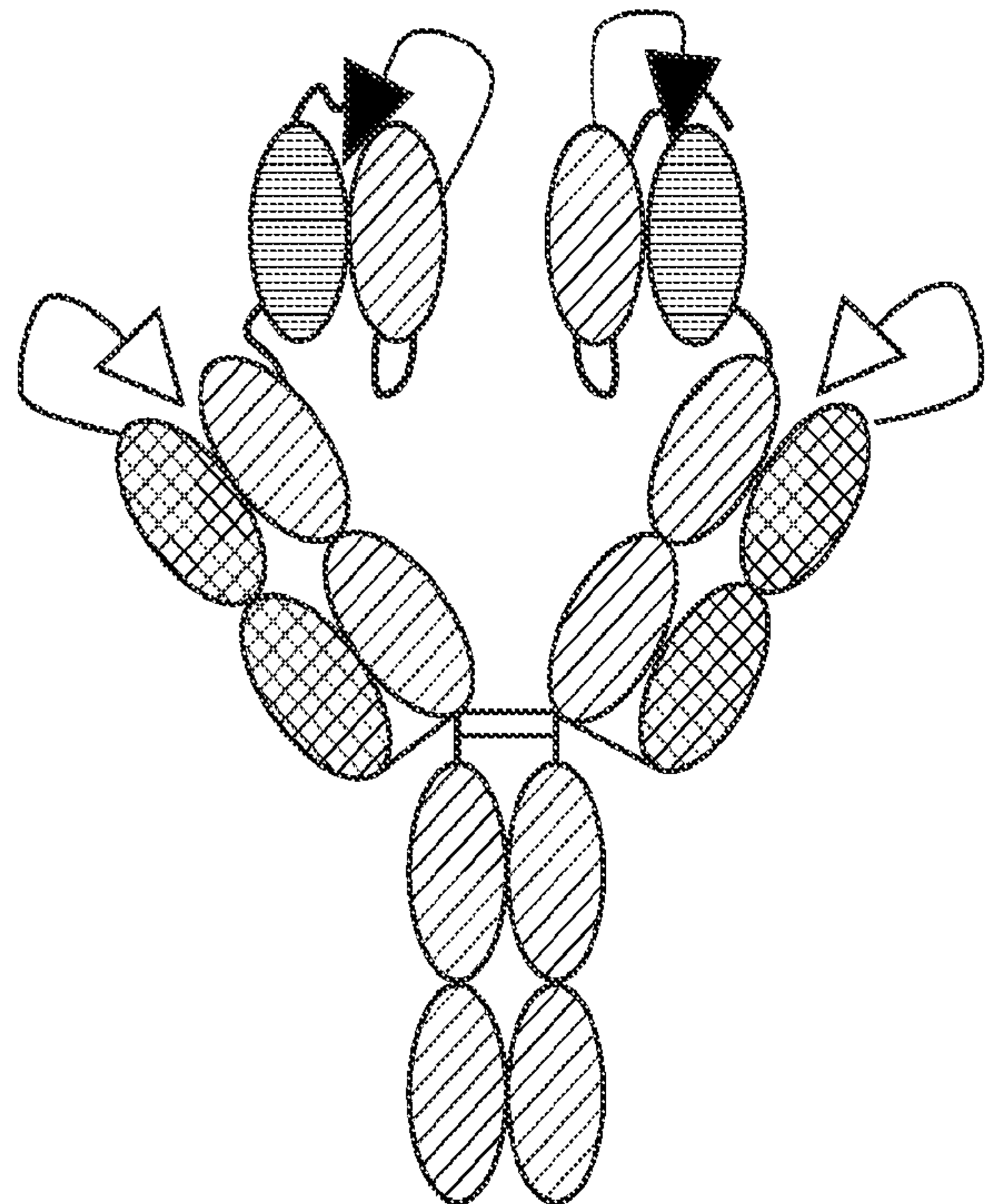


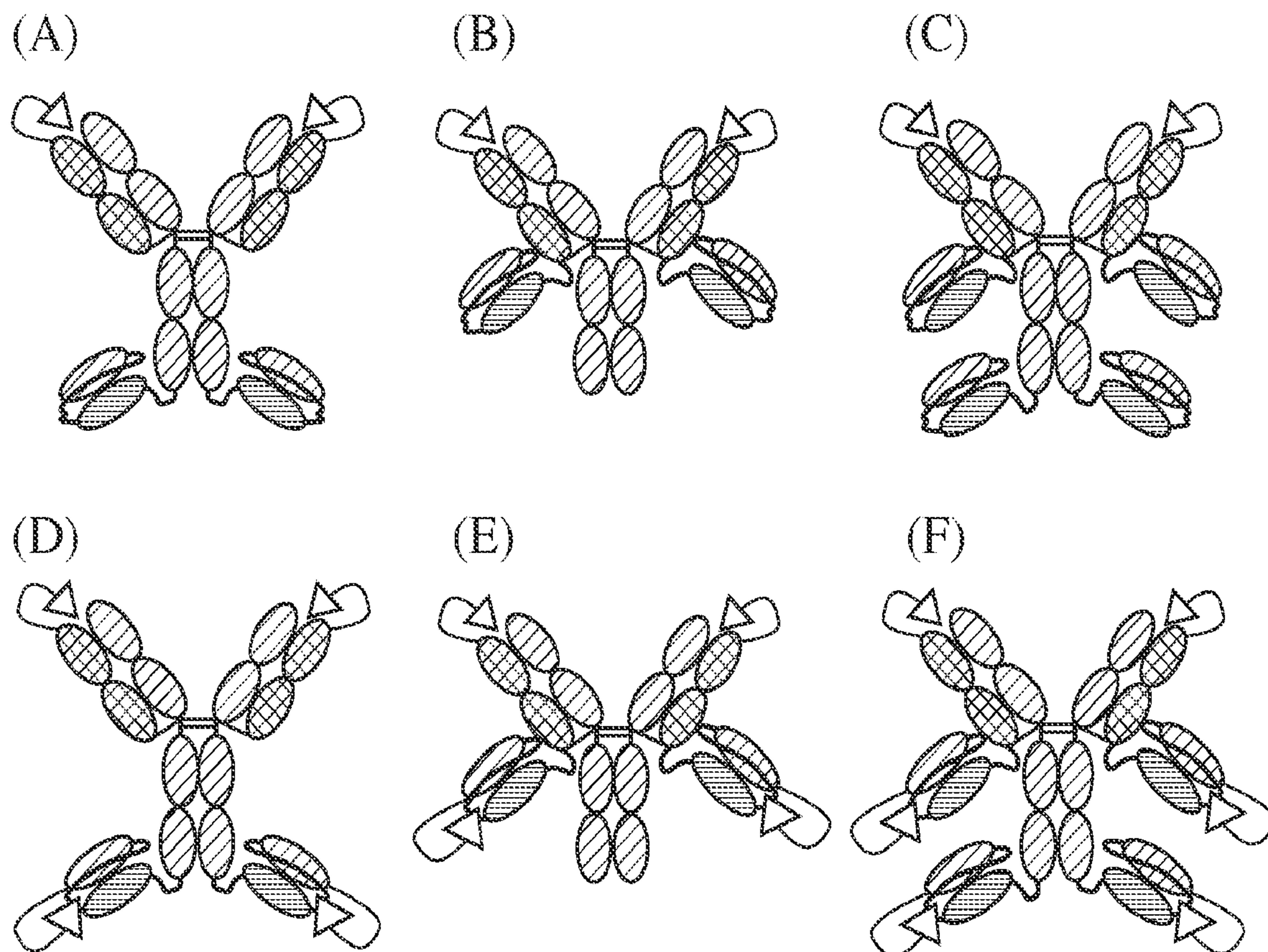
FIGURE 5D



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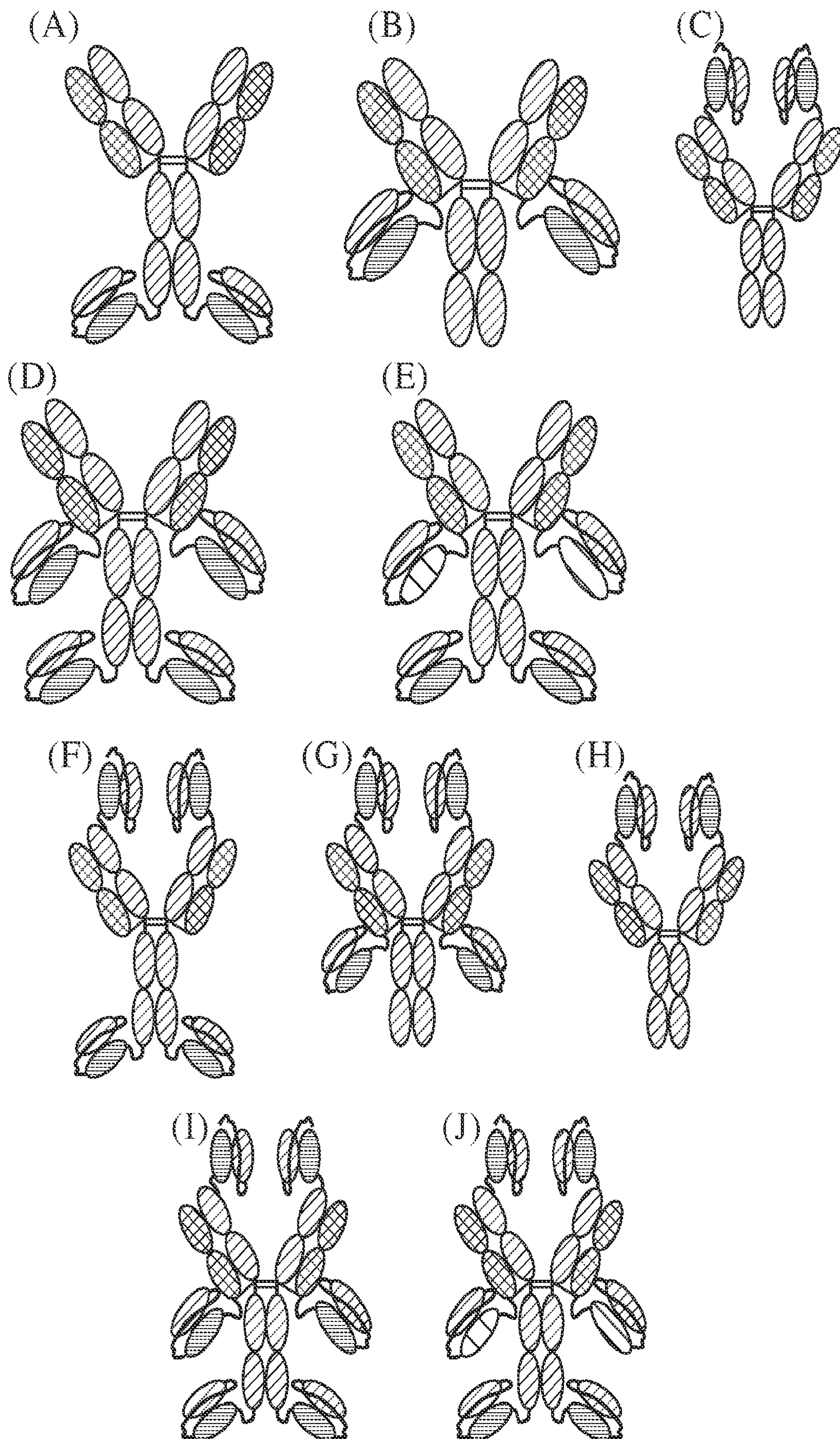
FIGURE 6

Multispecific activatable antibody arrays:



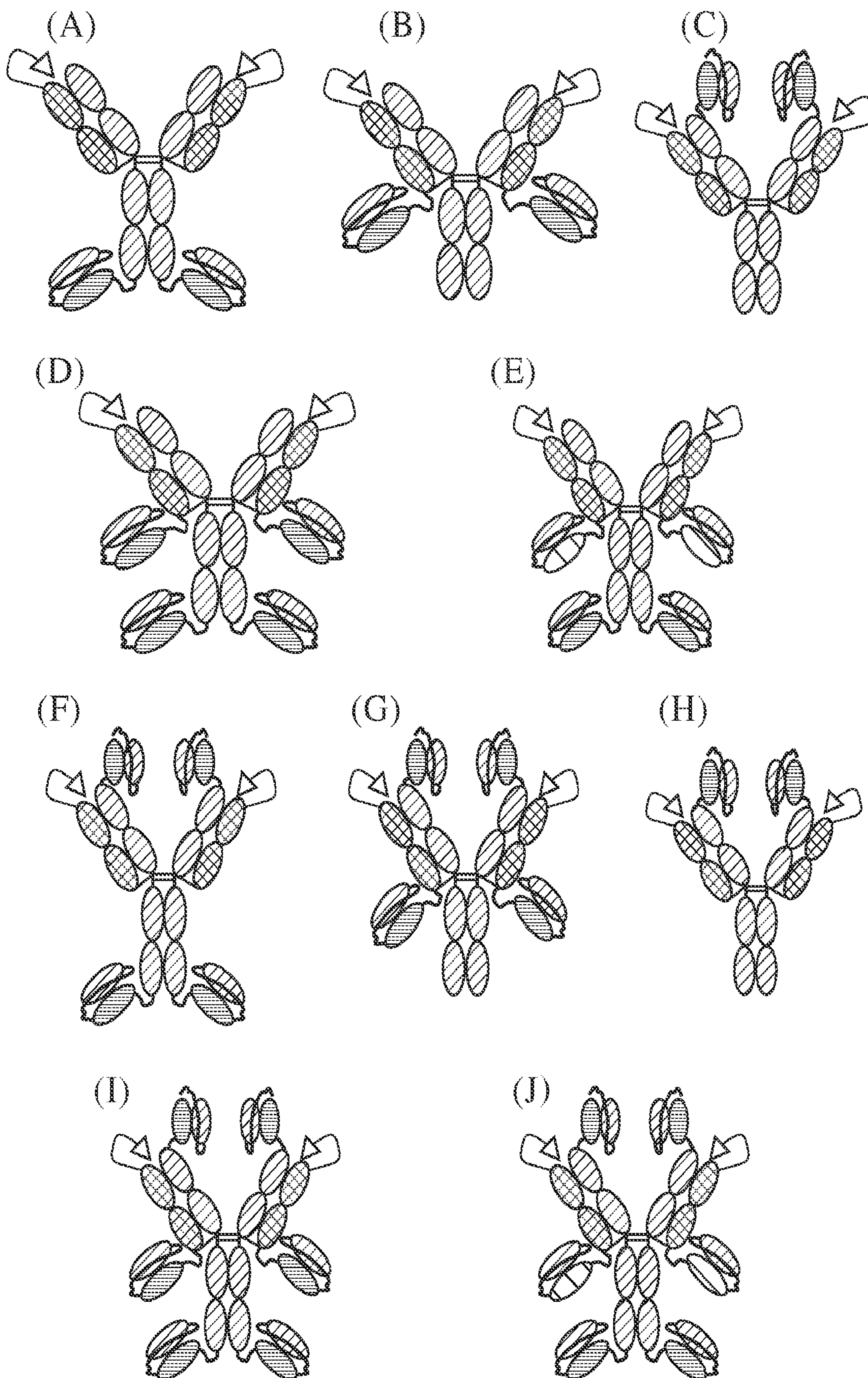
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FIGURE 7



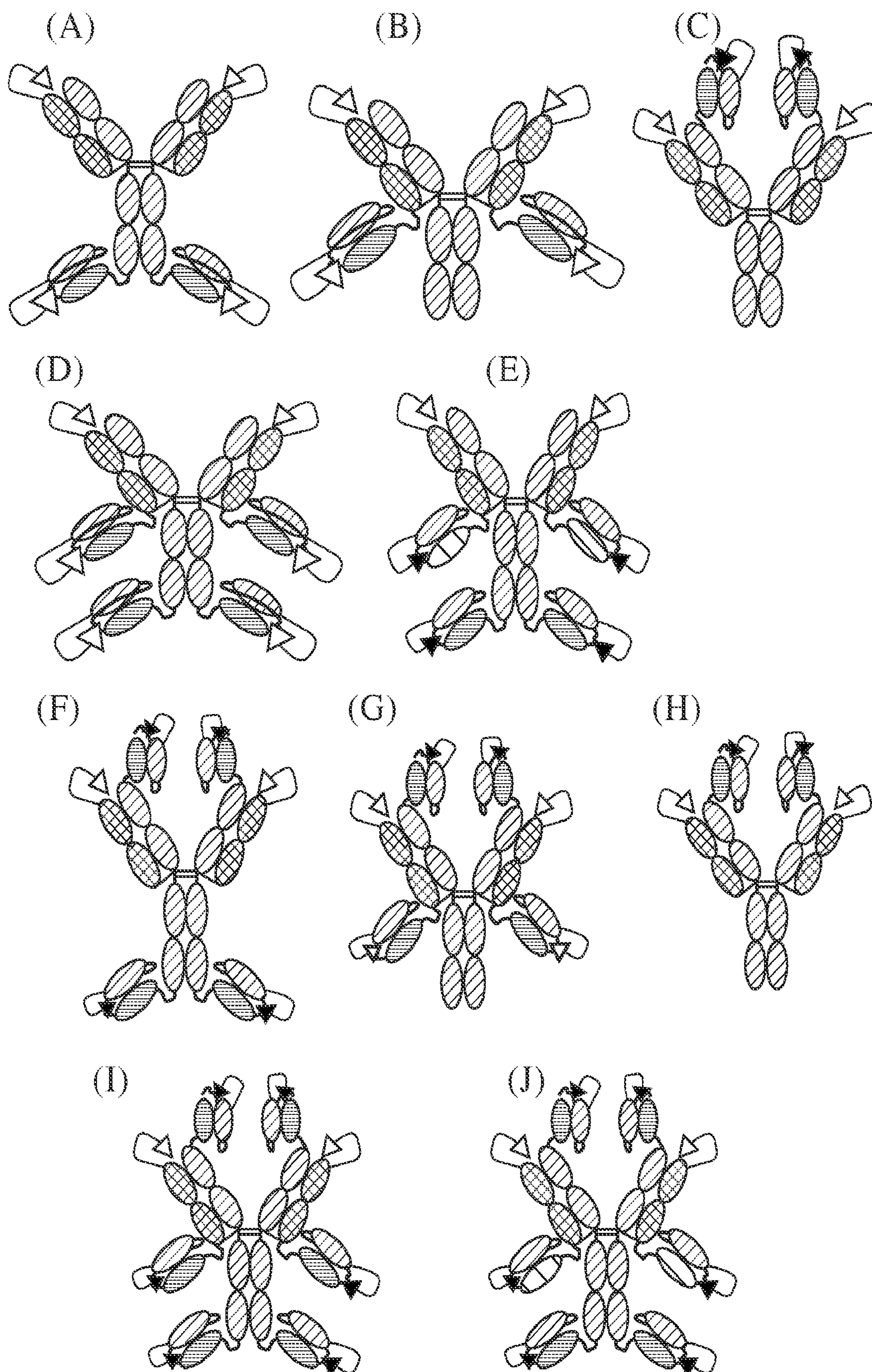
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FIGURE 8



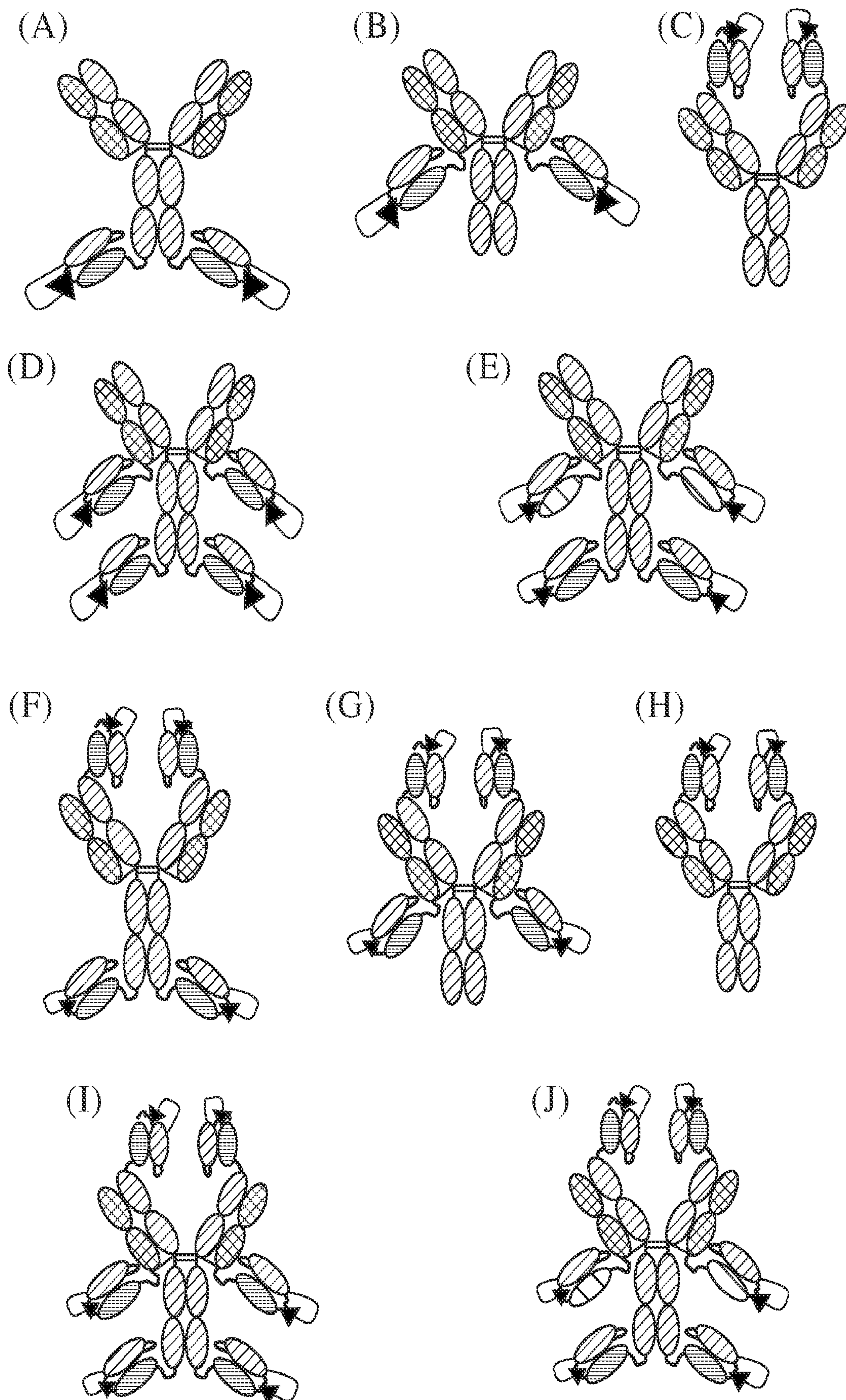
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FIGURE 9



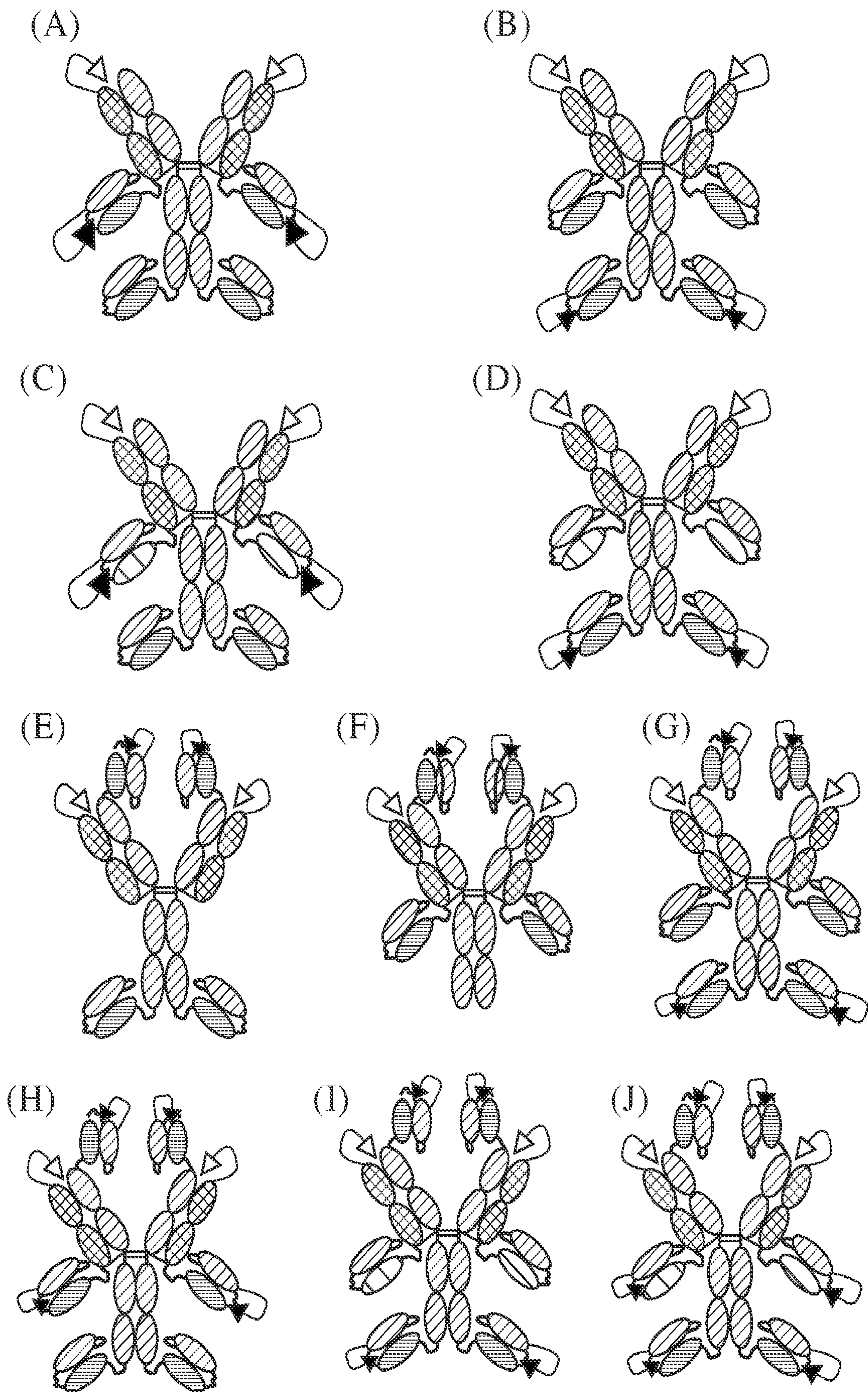
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FIGURE 10



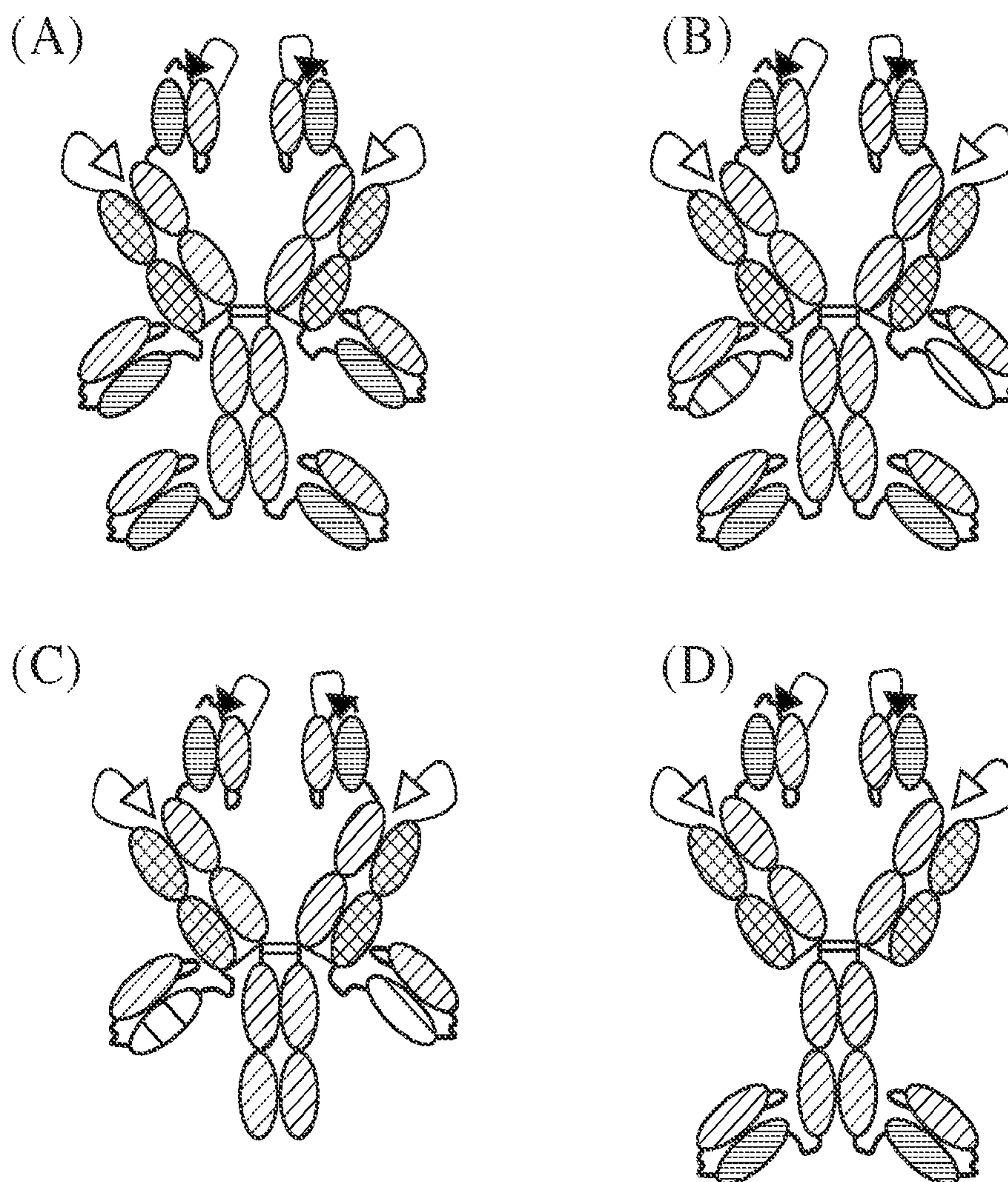
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FIGURE 11



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FIGURE 12



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FIGURE 13A

Jurkat Binding

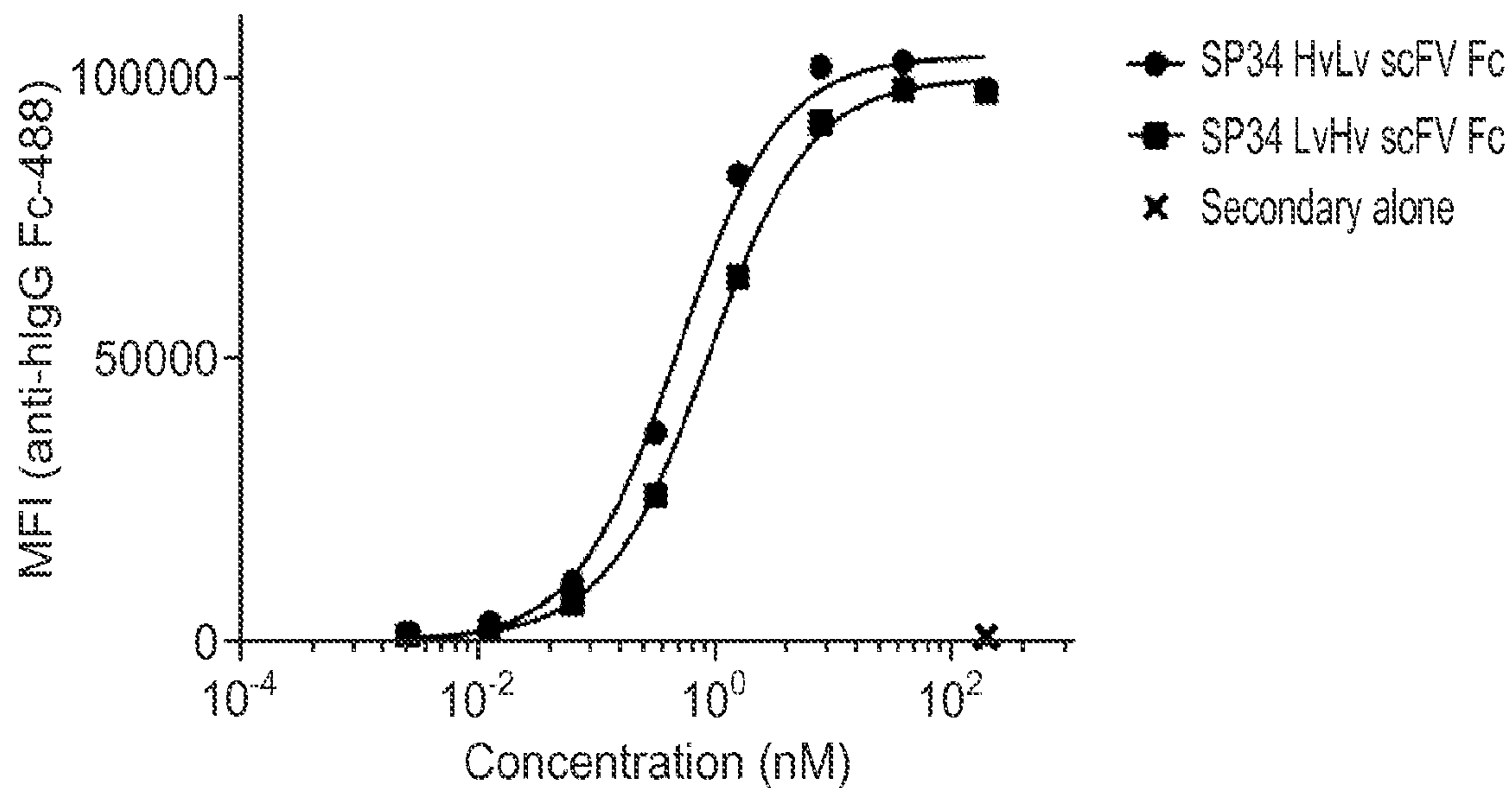
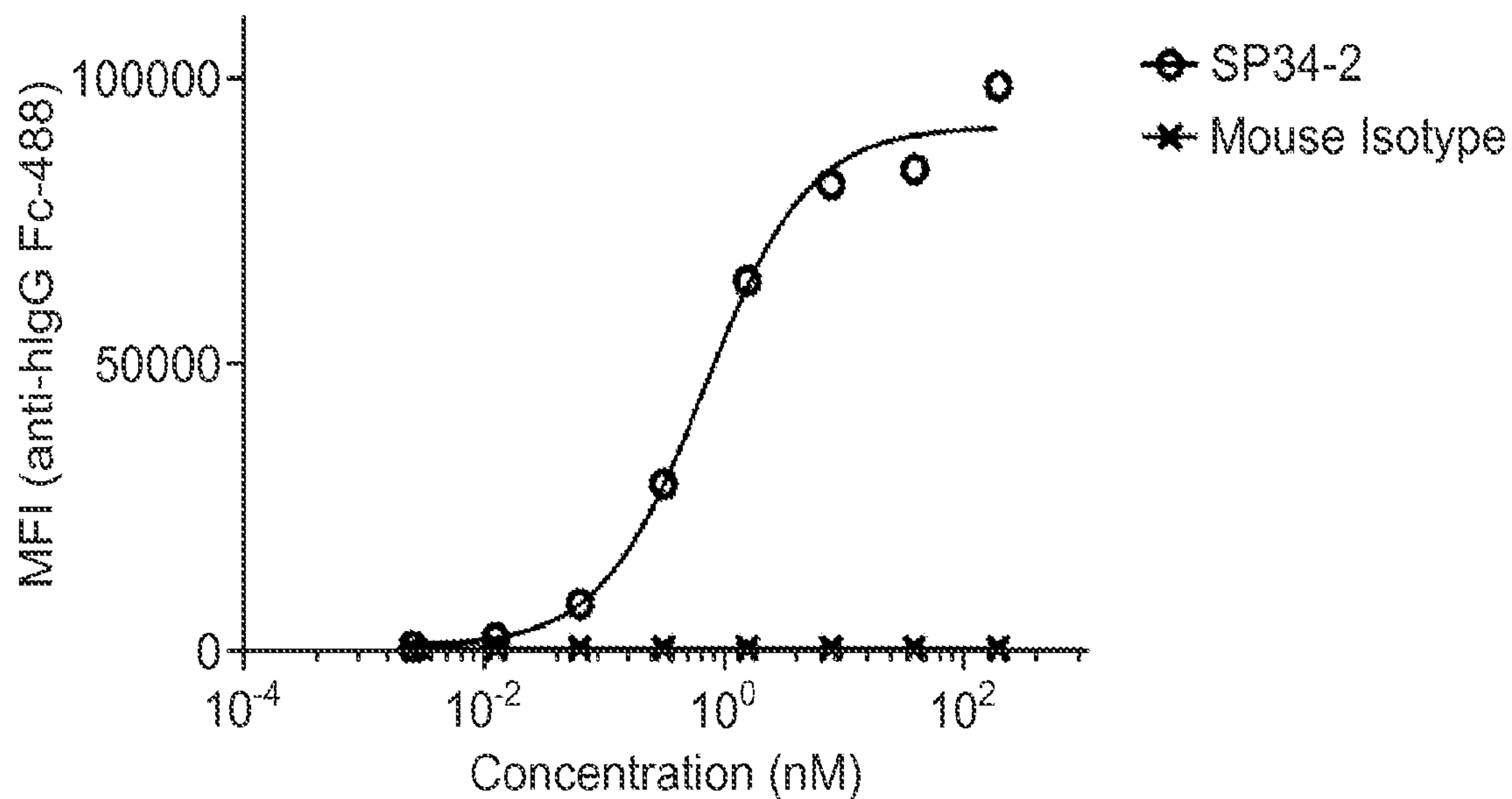


FIGURE 13B

Jurkat Binding



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FIGURE 14A

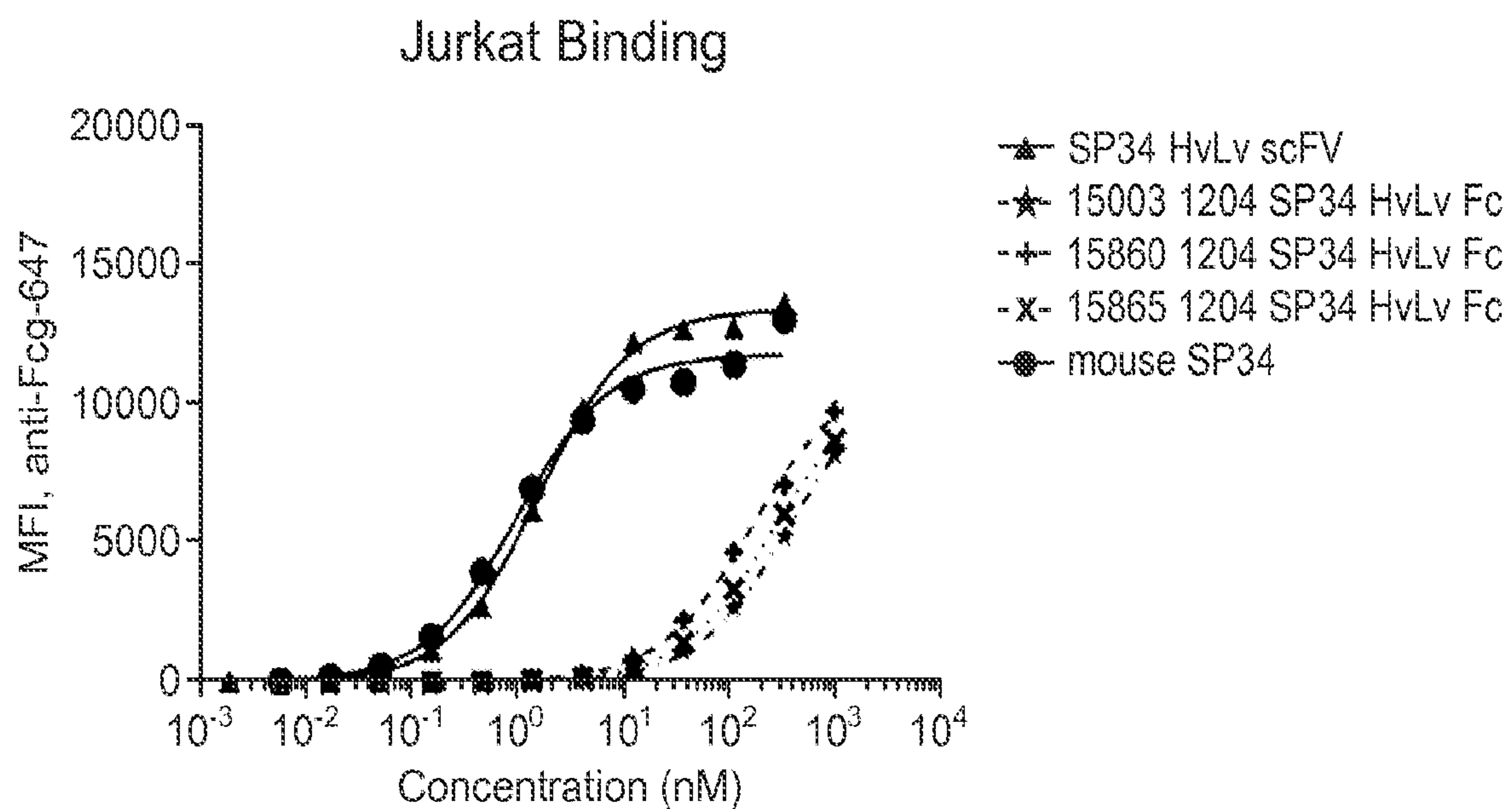


FIGURE 14B

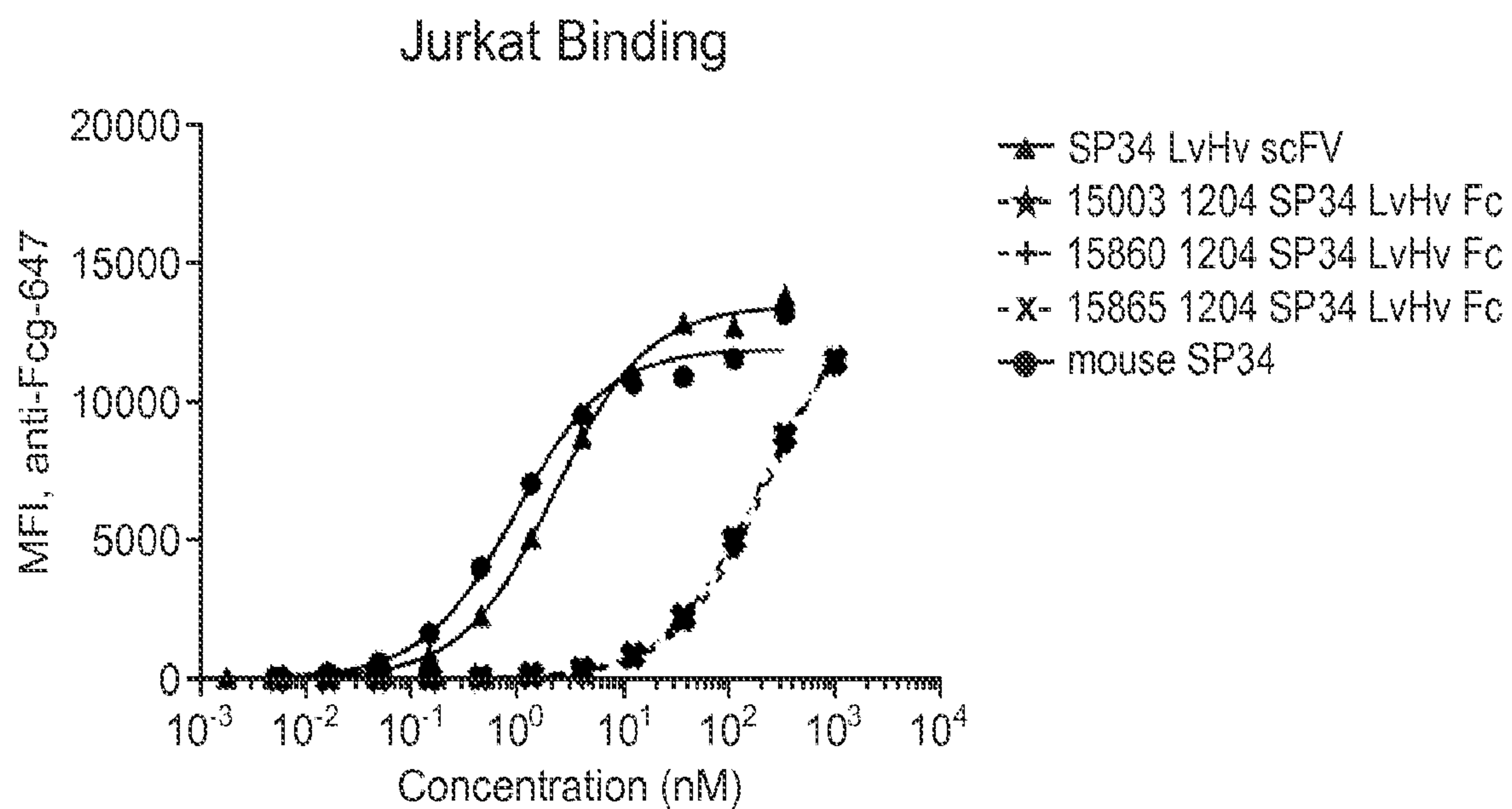
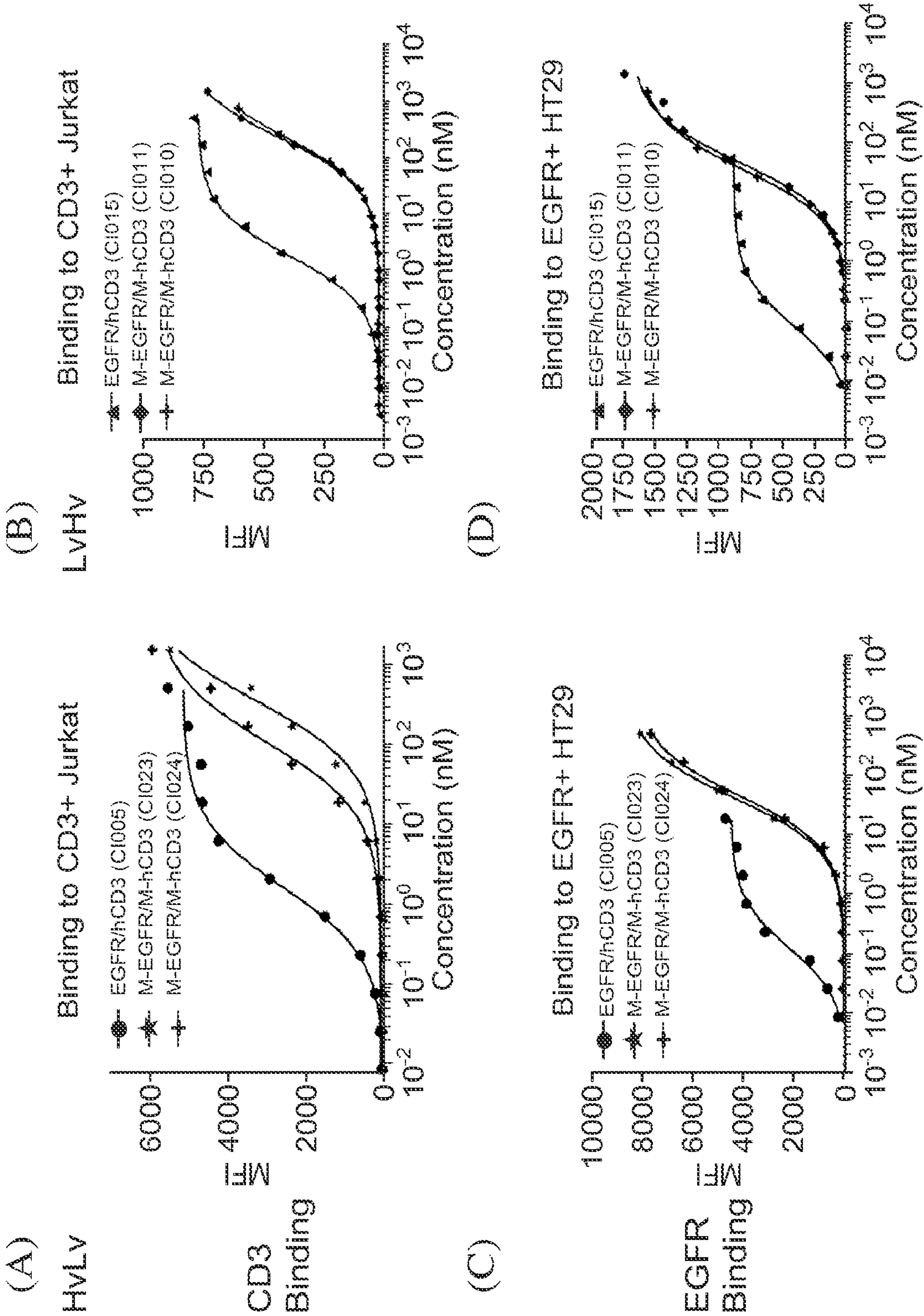


FIGURE 15



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FIGURE 16A

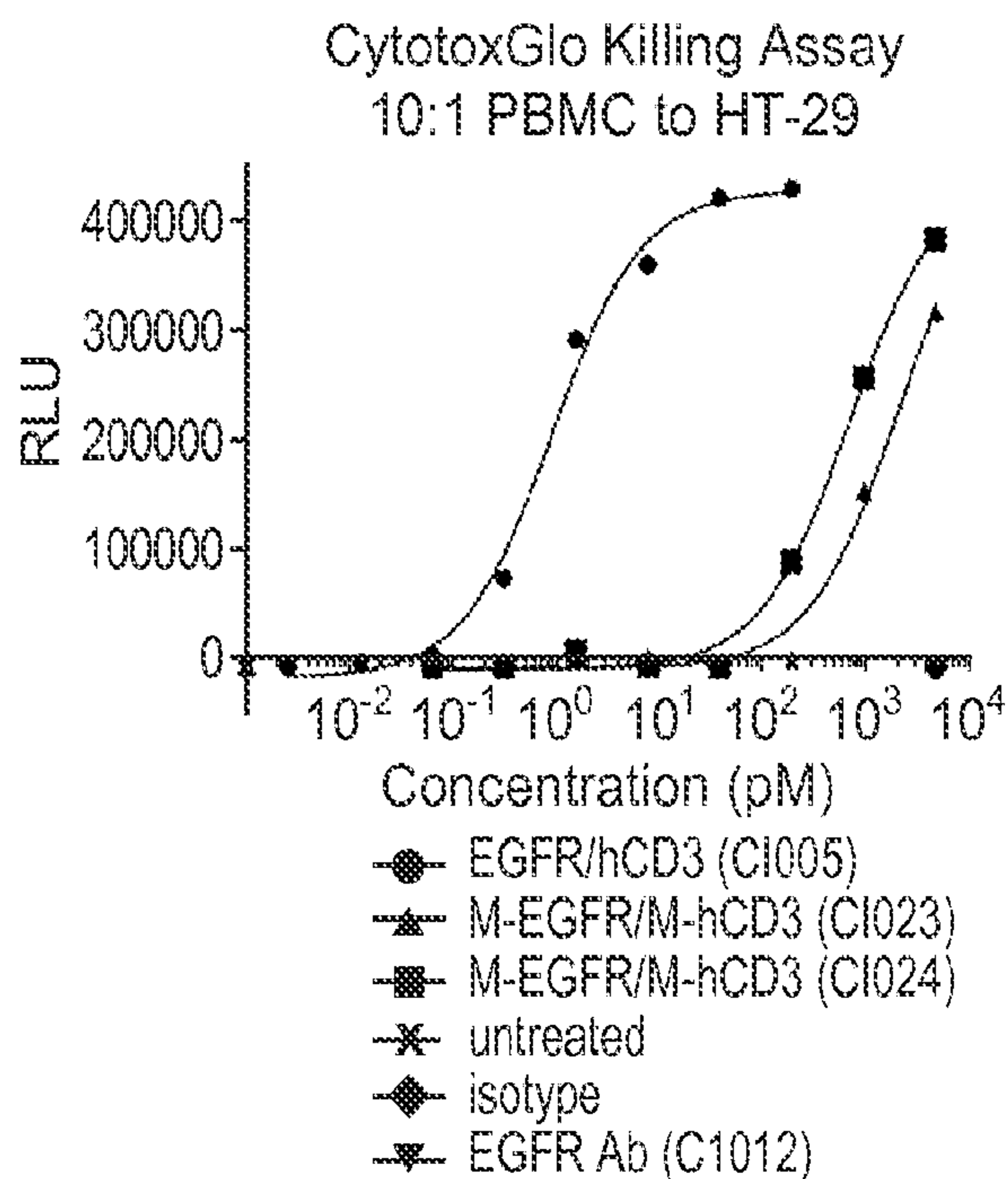


FIGURE 16B

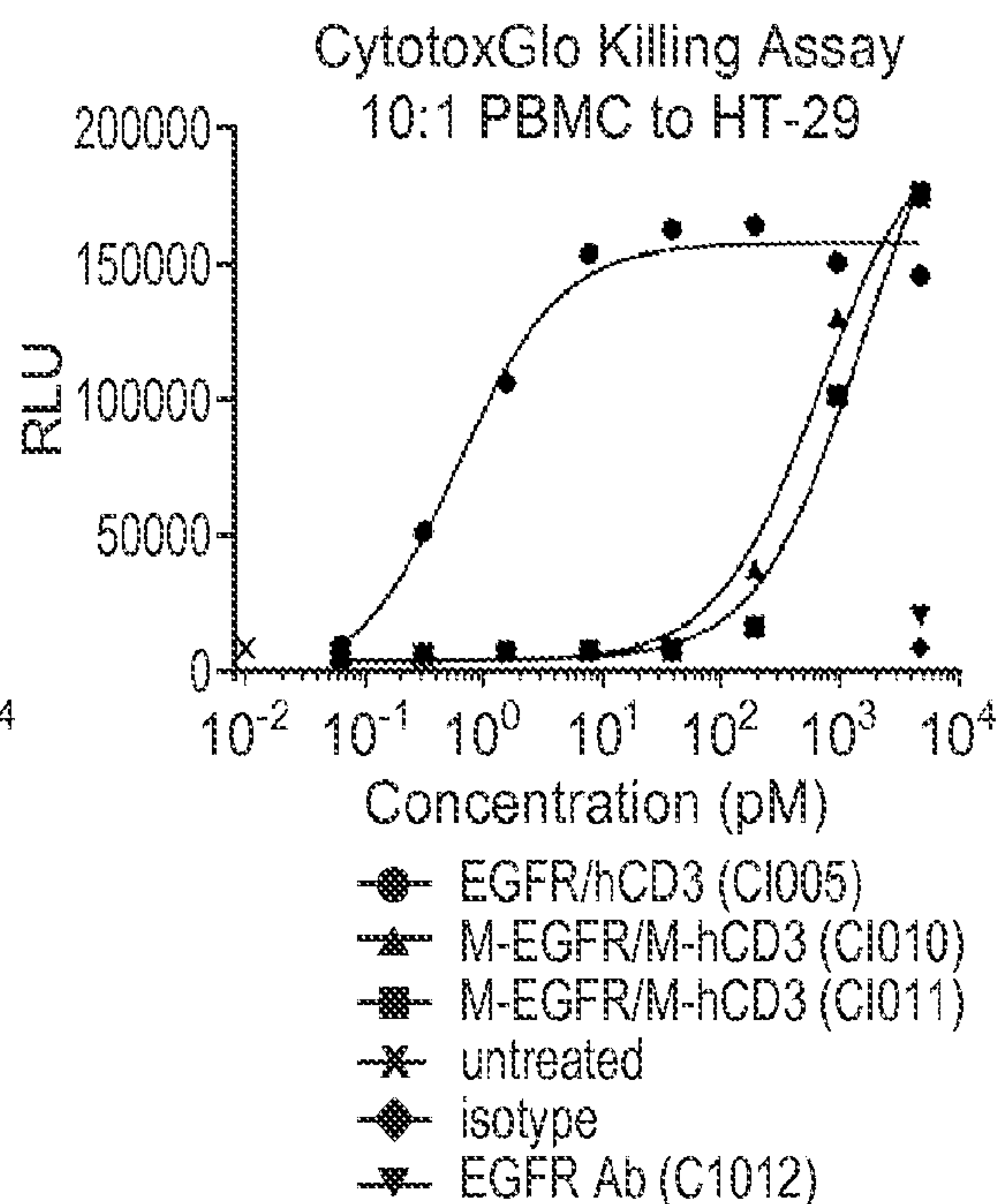


FIGURE 17A

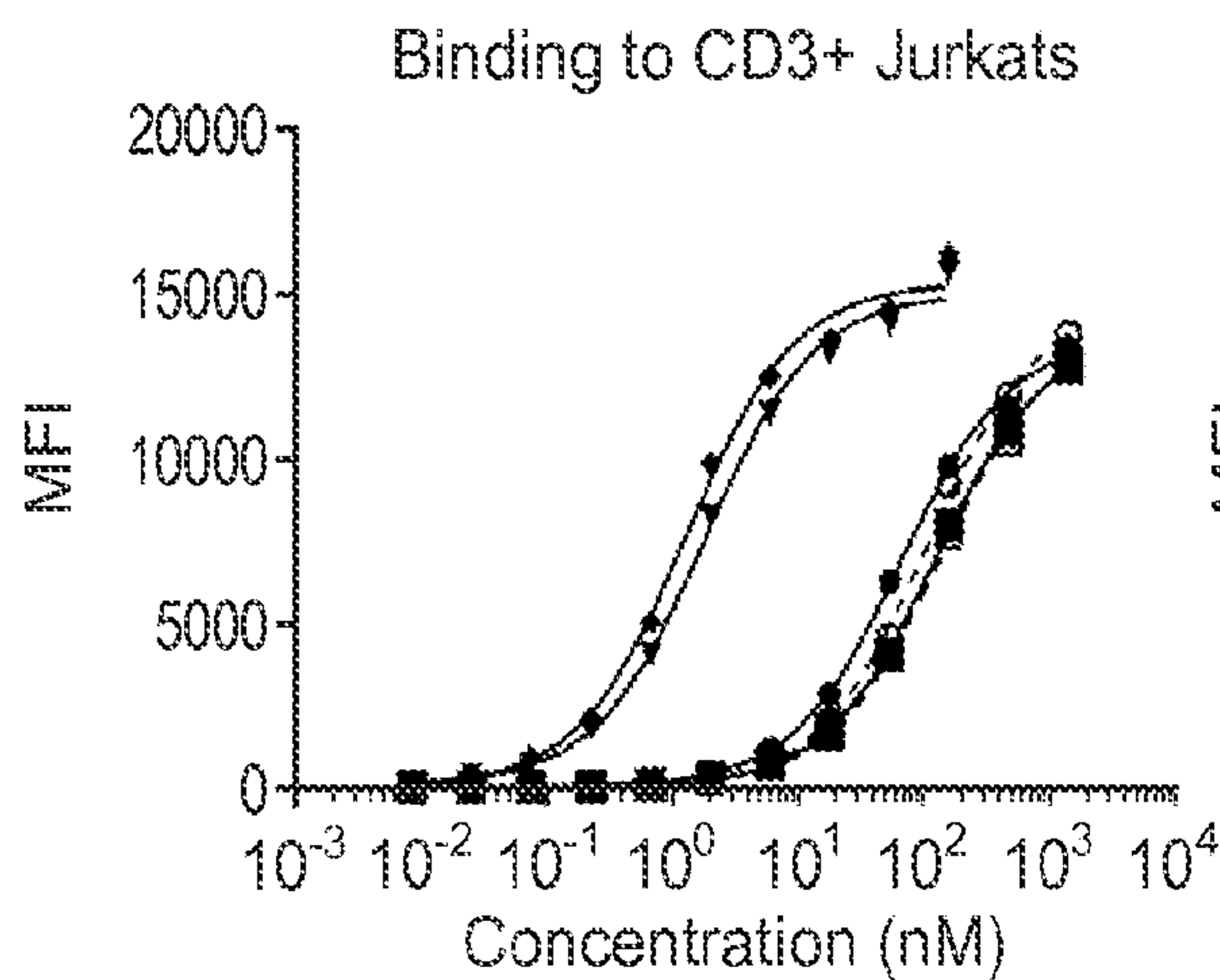
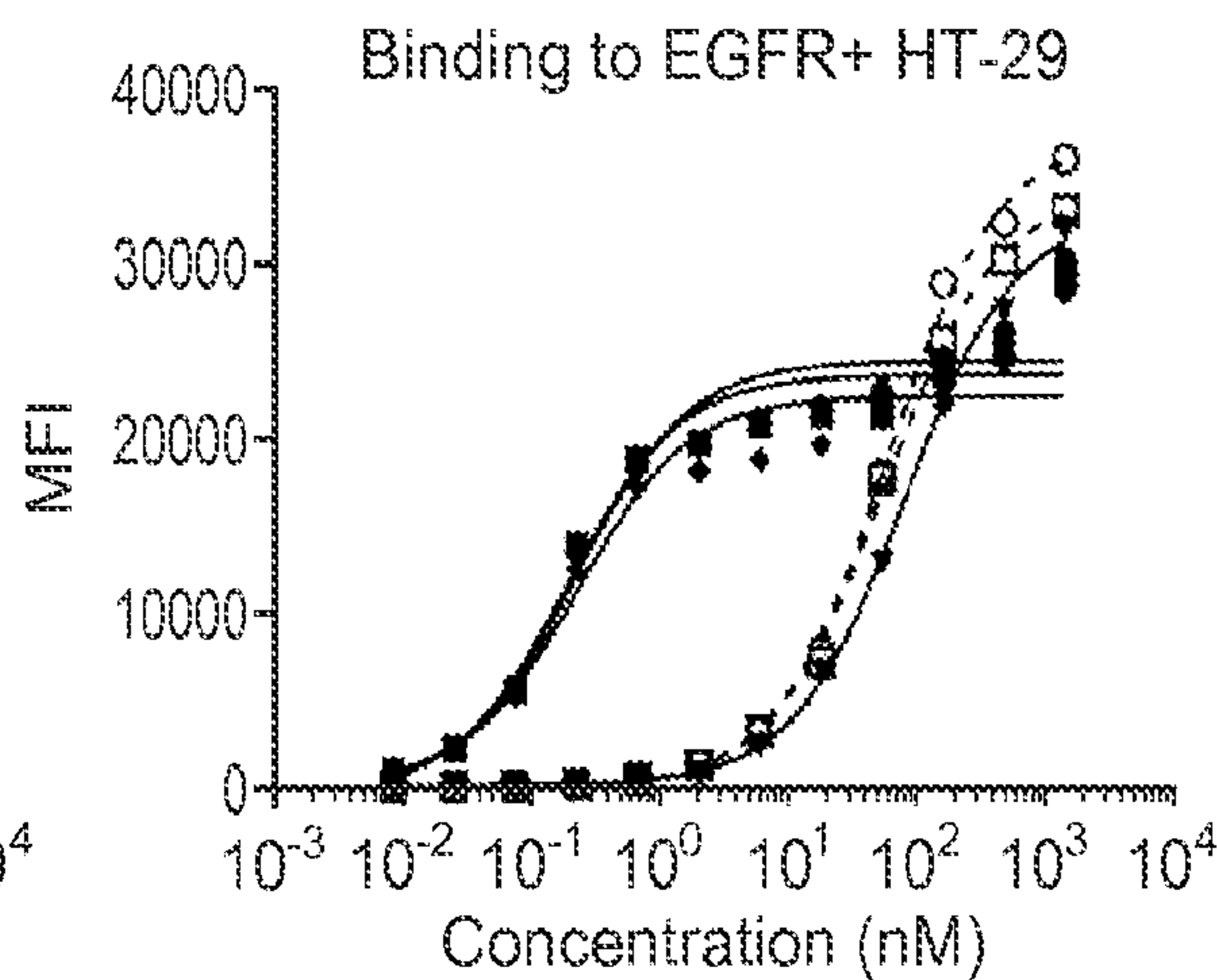


FIGURE 17B



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FIGURE 18A

CytotoxGlo Killing Assay
3:1 Purified CD8s:HT29

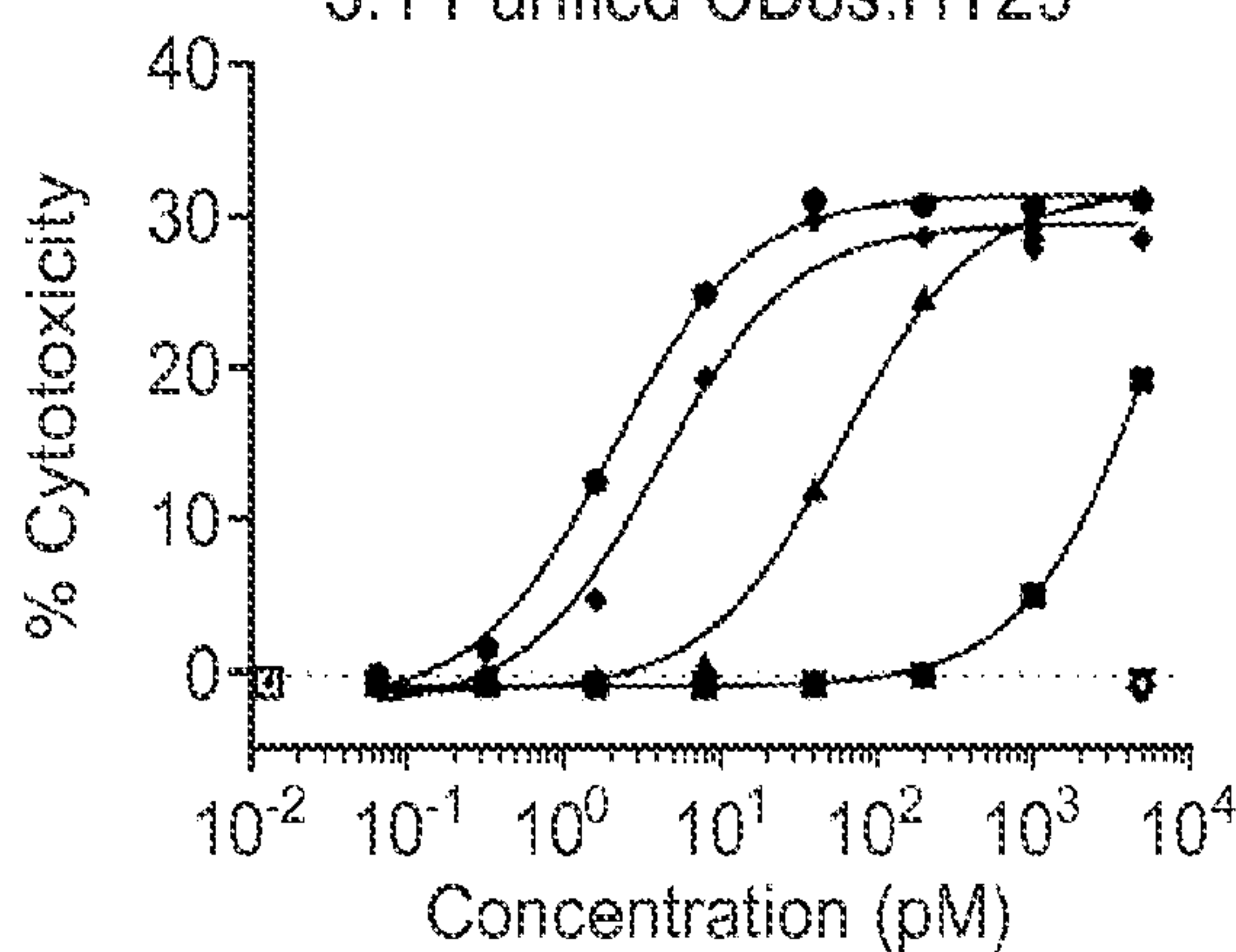
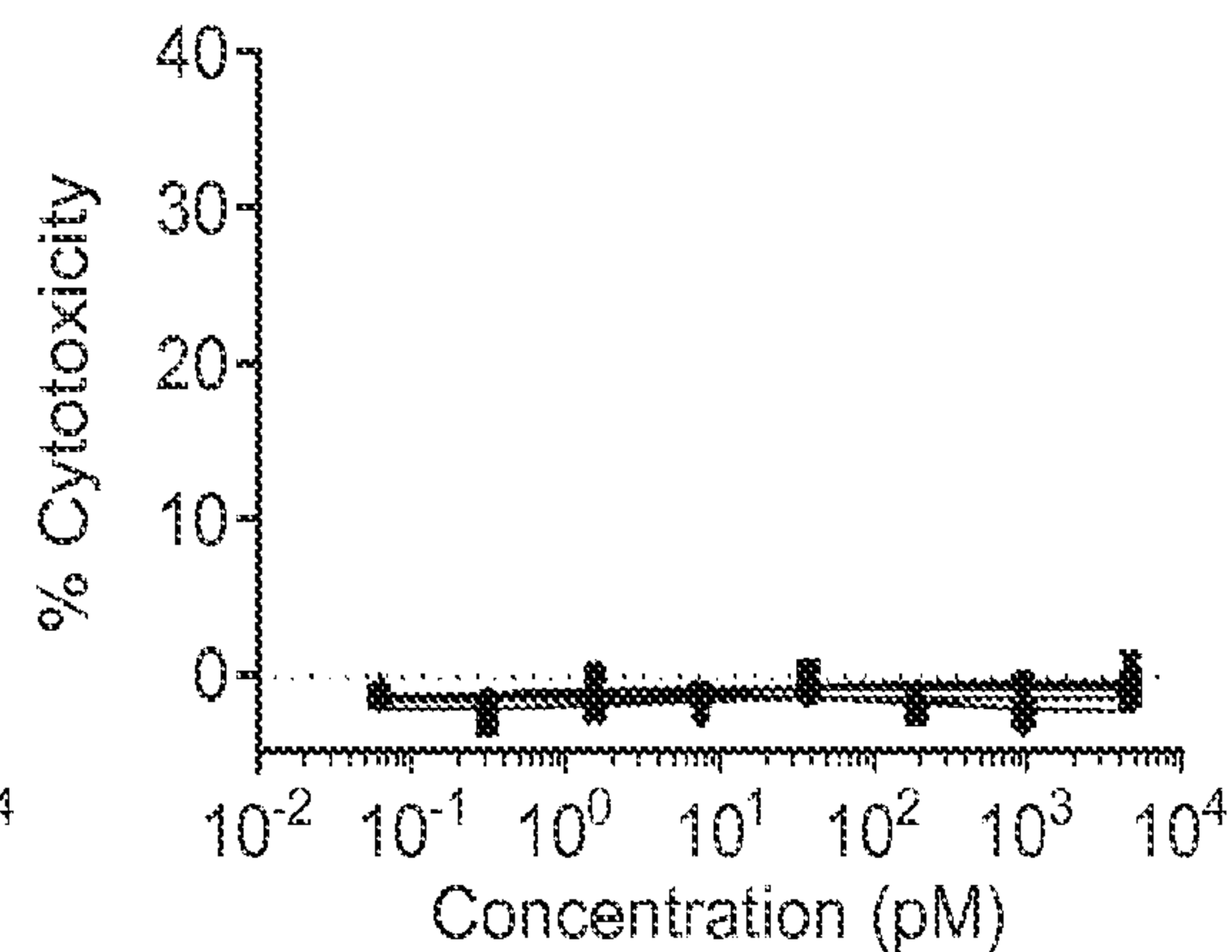


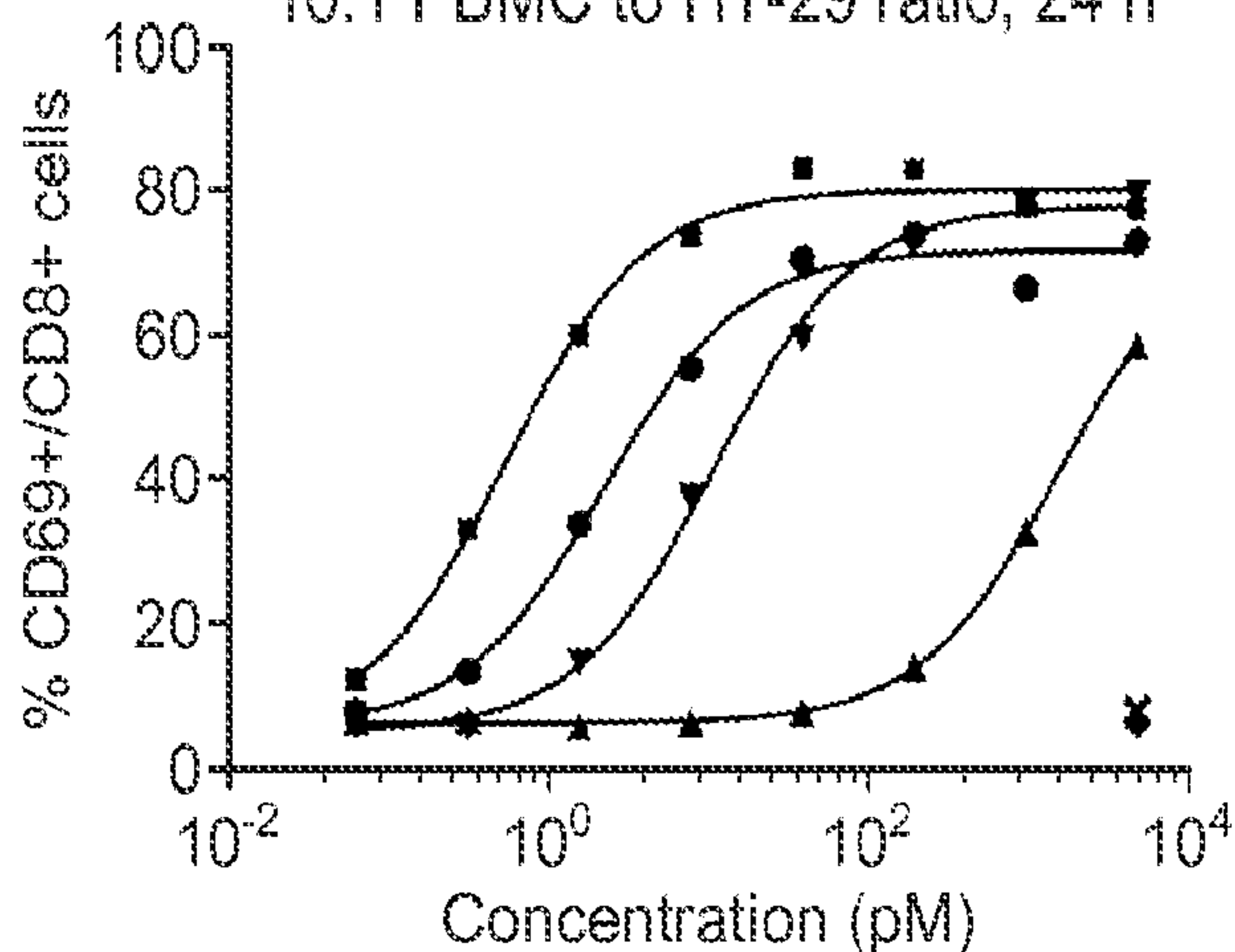
FIGURE 18B



- EGFR/CD3 (CI005)
- ▲ M-EGFR/CD3 (CI007)
- ◆ EGFR/M2-CD3 (CI009)
- M-EGFR/M2-CD3 (CI011)
- ◇ EGFR Ab (CI012)
- ▽ isotype
- untreated

FIGURE 19

Activation of CD8+ T cells
10:1 PBMC to HT-29 ratio, 24 h



- EGFR/CD3 (CI005)
- ▲ M-EGFR/CD3 (CI007)
- ◆ EGFR/M2-CD3 (CI009)
- M-EGFR/M2-CD3 (CI011)
- ◆ EGFR (C225V5)
- × ISOTYPE

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FIGURE 20A

10:1 PBMC to U266 cells (24 hours)

Percent CD69+ CD8+ T cells

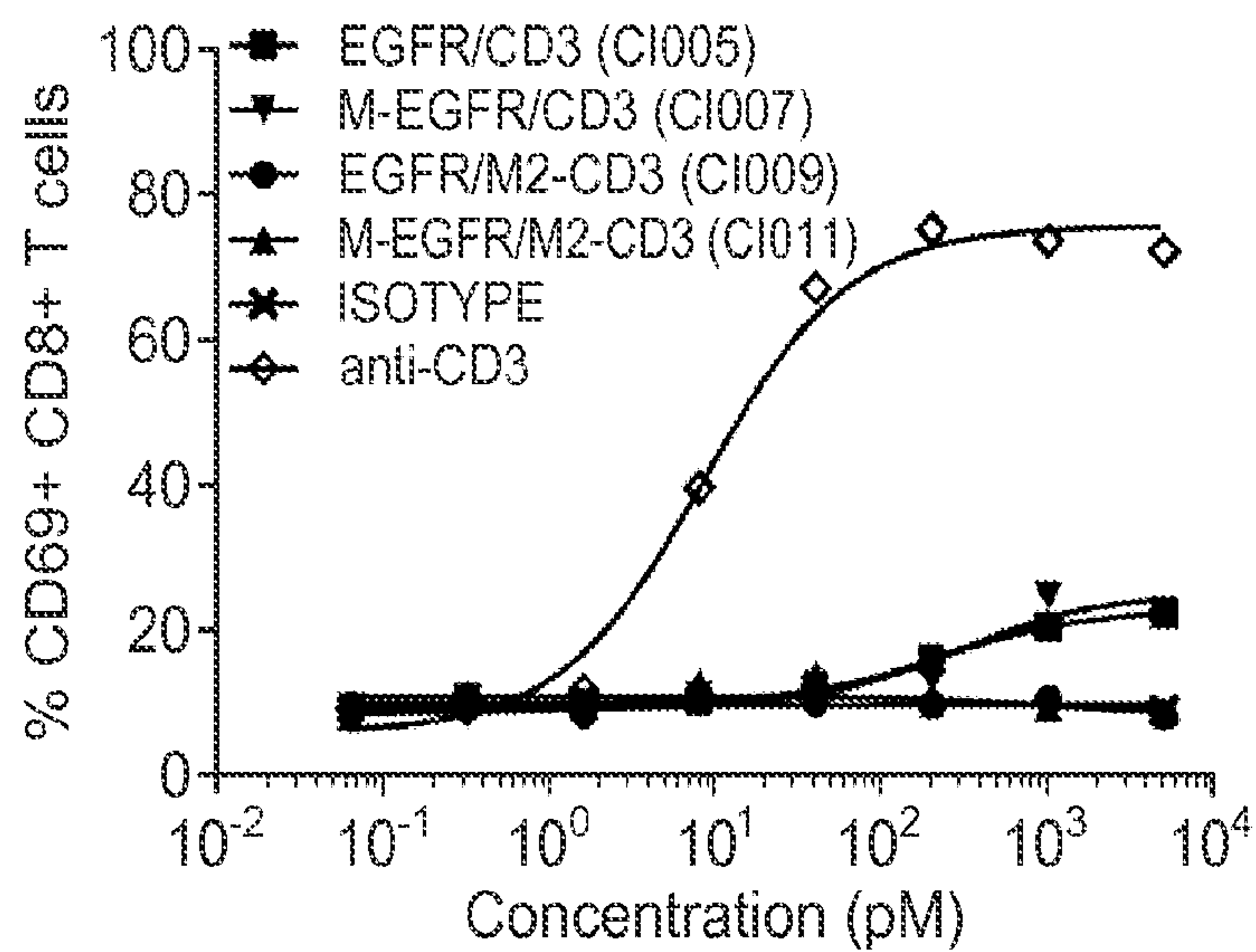
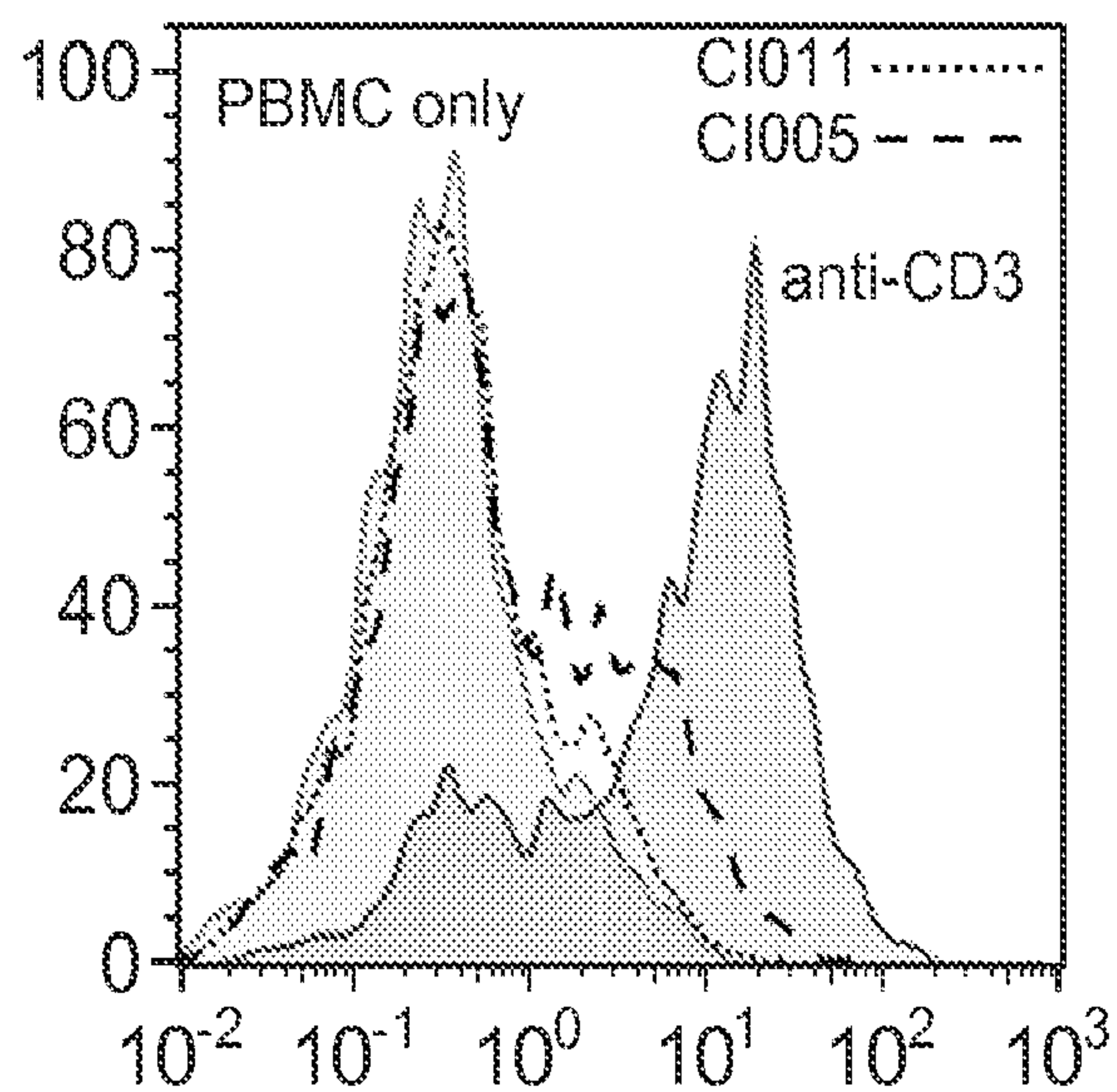


FIGURE 20B



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FIGURE 21

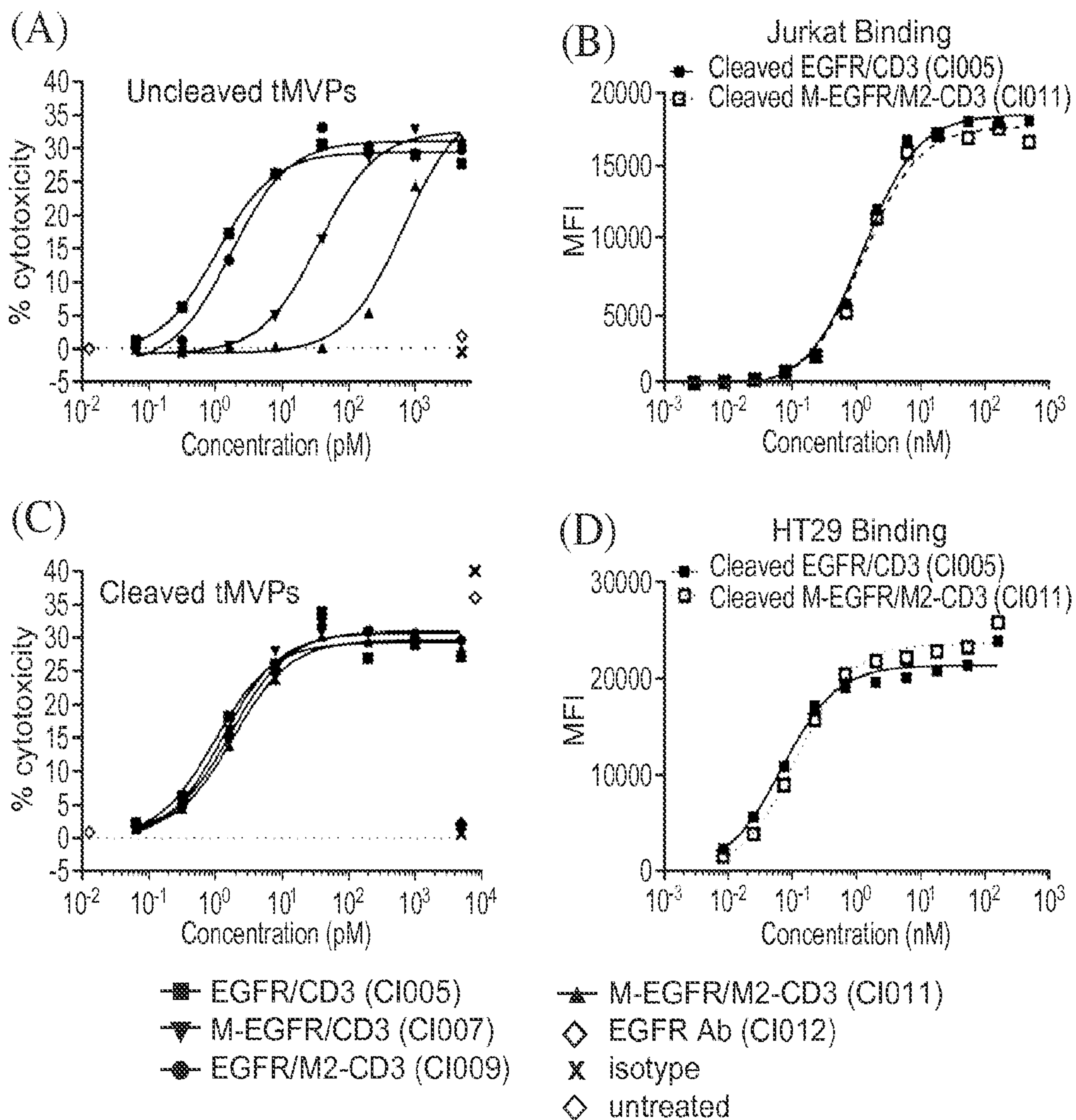
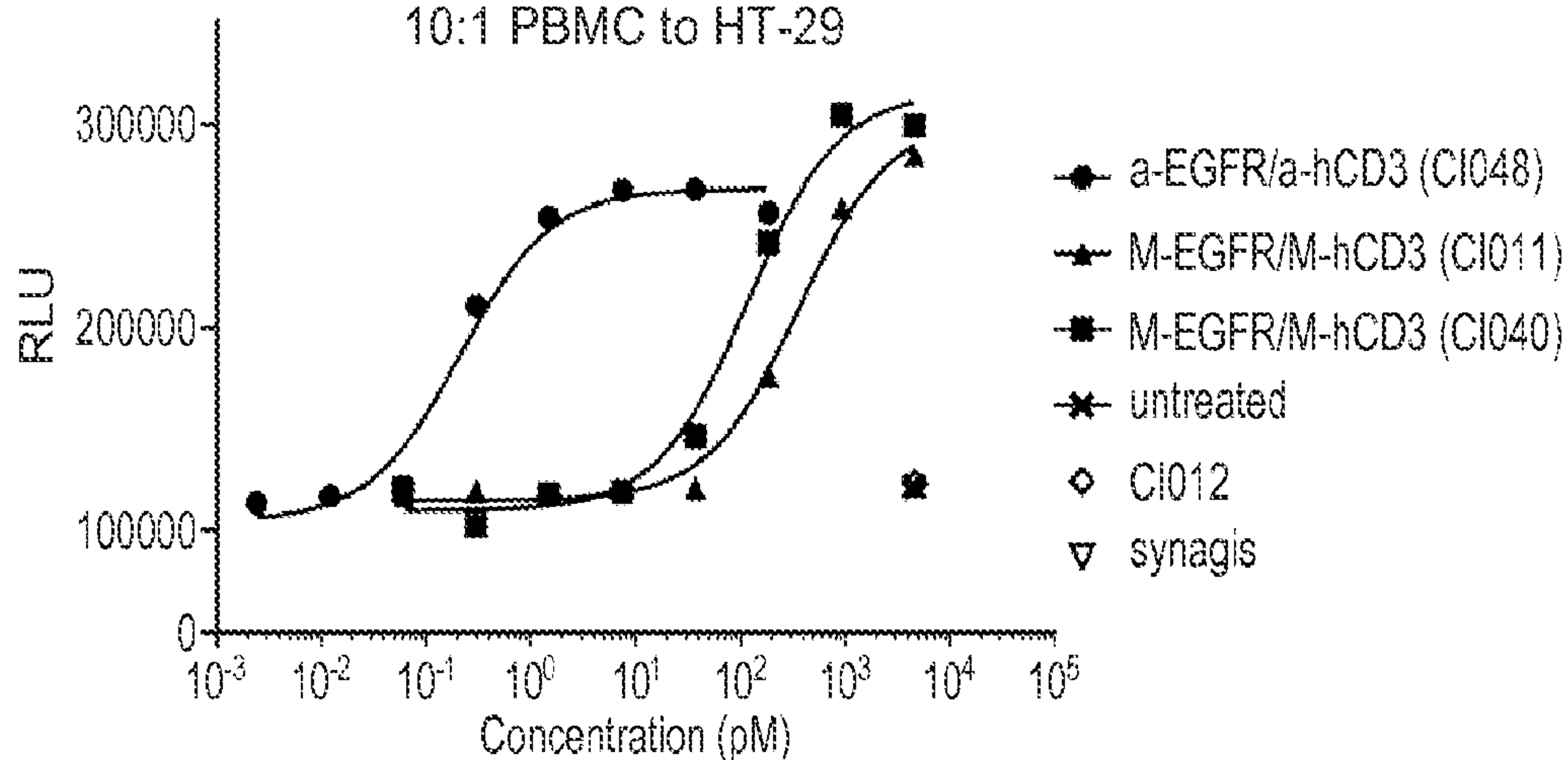
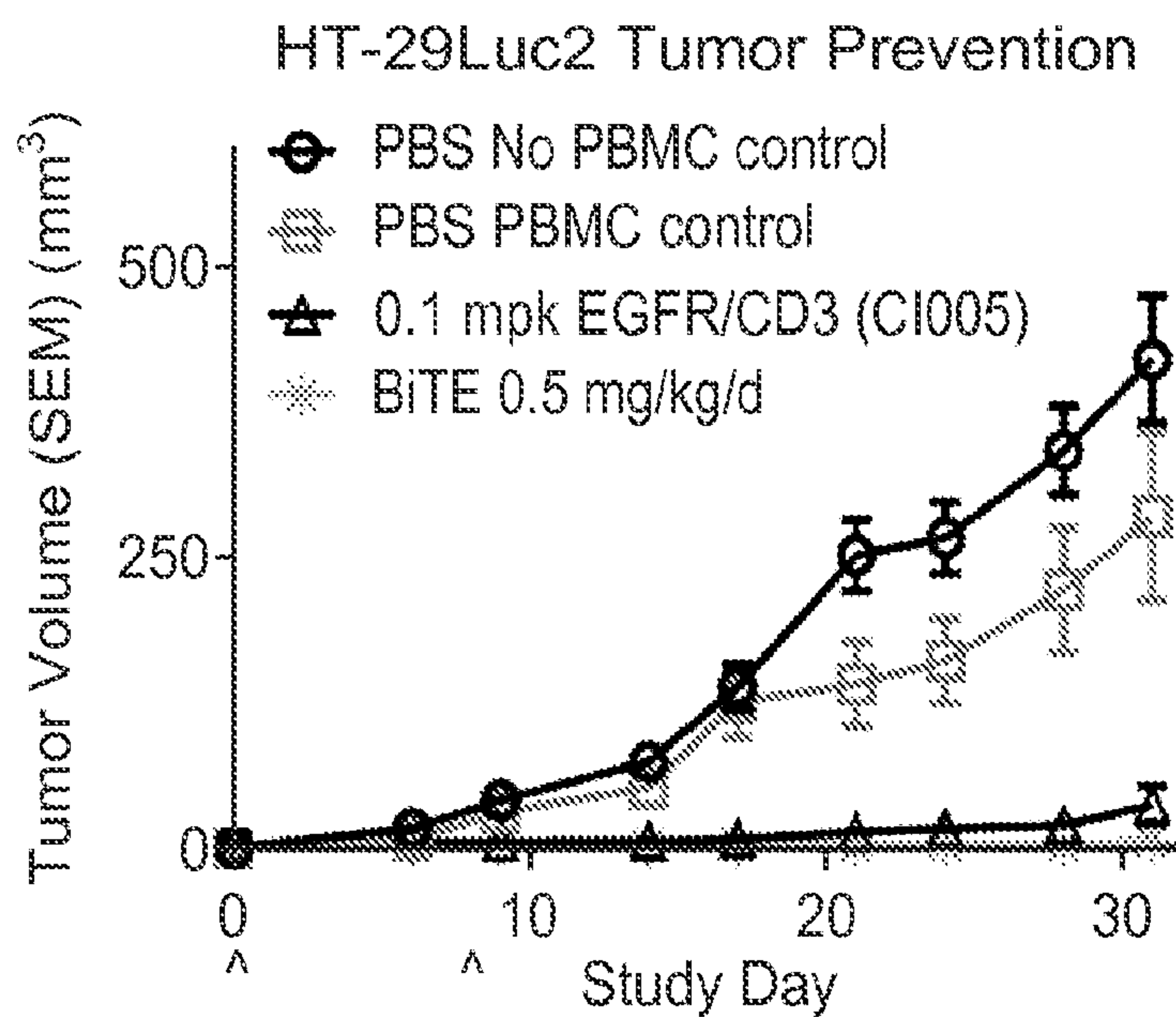


FIGURE 22
CytotoxGlo Killing Assay
10:1 PBMC to HT-29



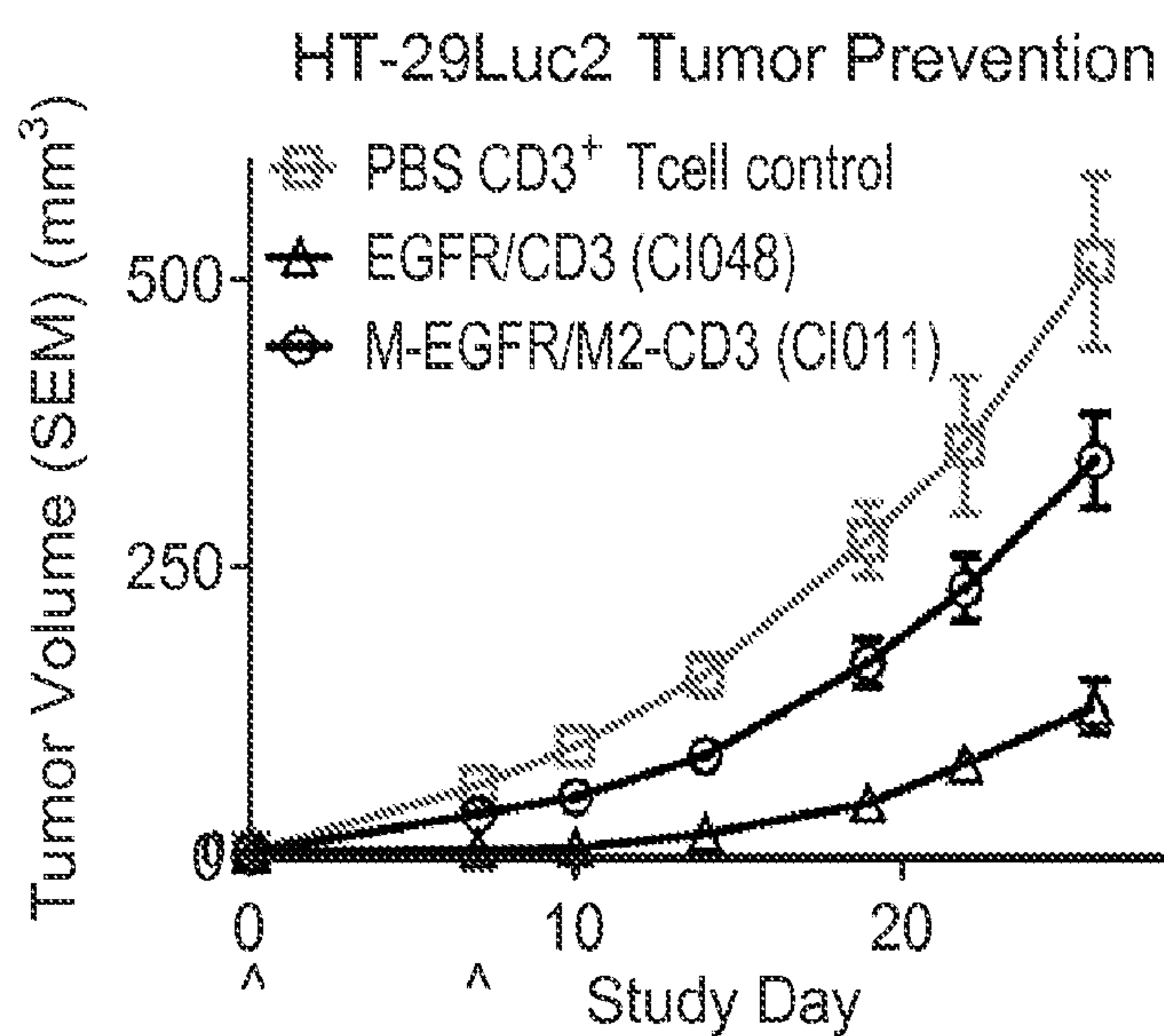
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FIGURE 23



^Dosed IV on days 0 & 7 for CI005
& dosed daily days 0-7 for BiTE

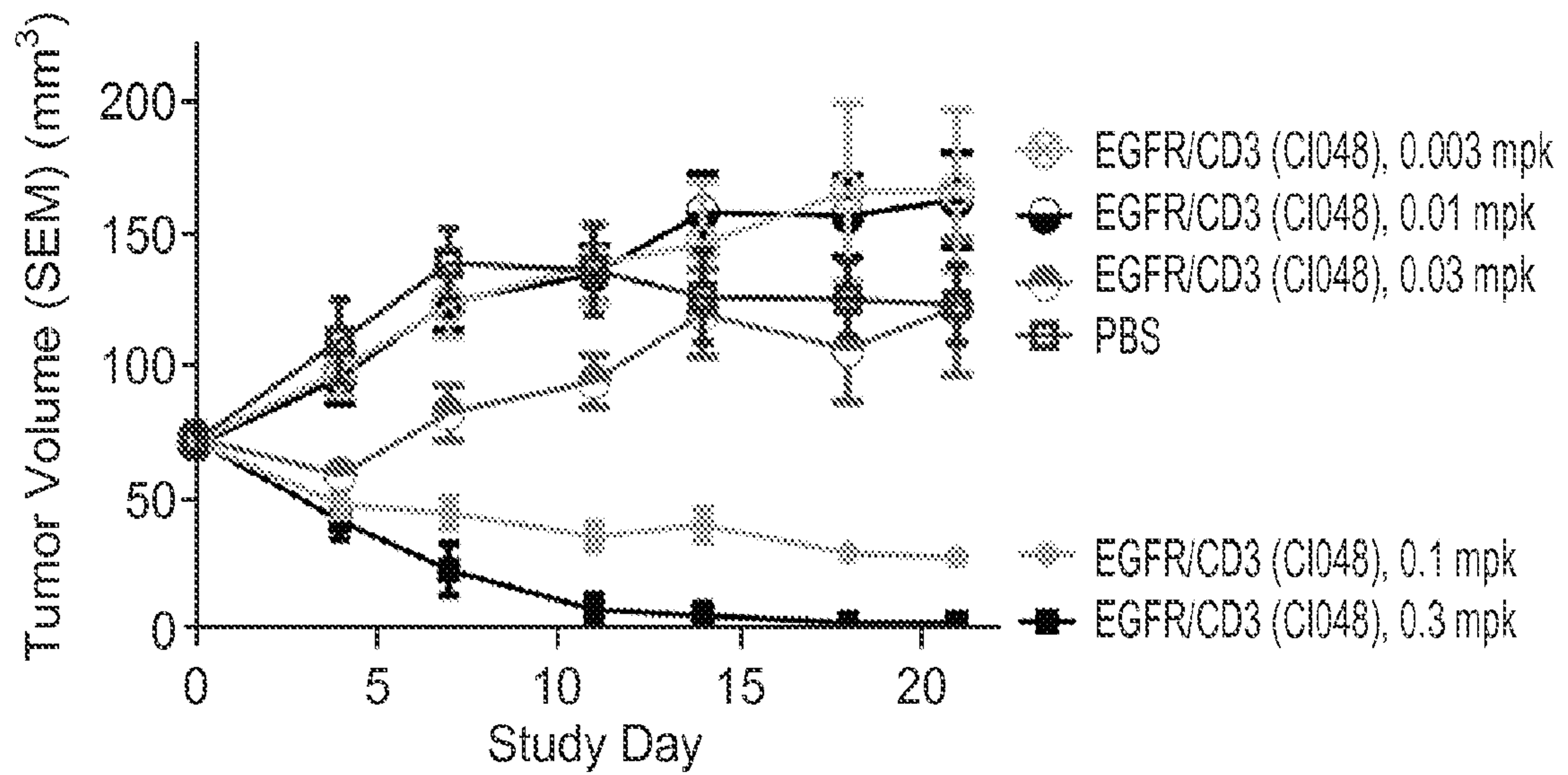
FIGURE 24



^CI048 & CI011 were dosed IV on days 0 & 7.
Dose 0.1 mg/kg

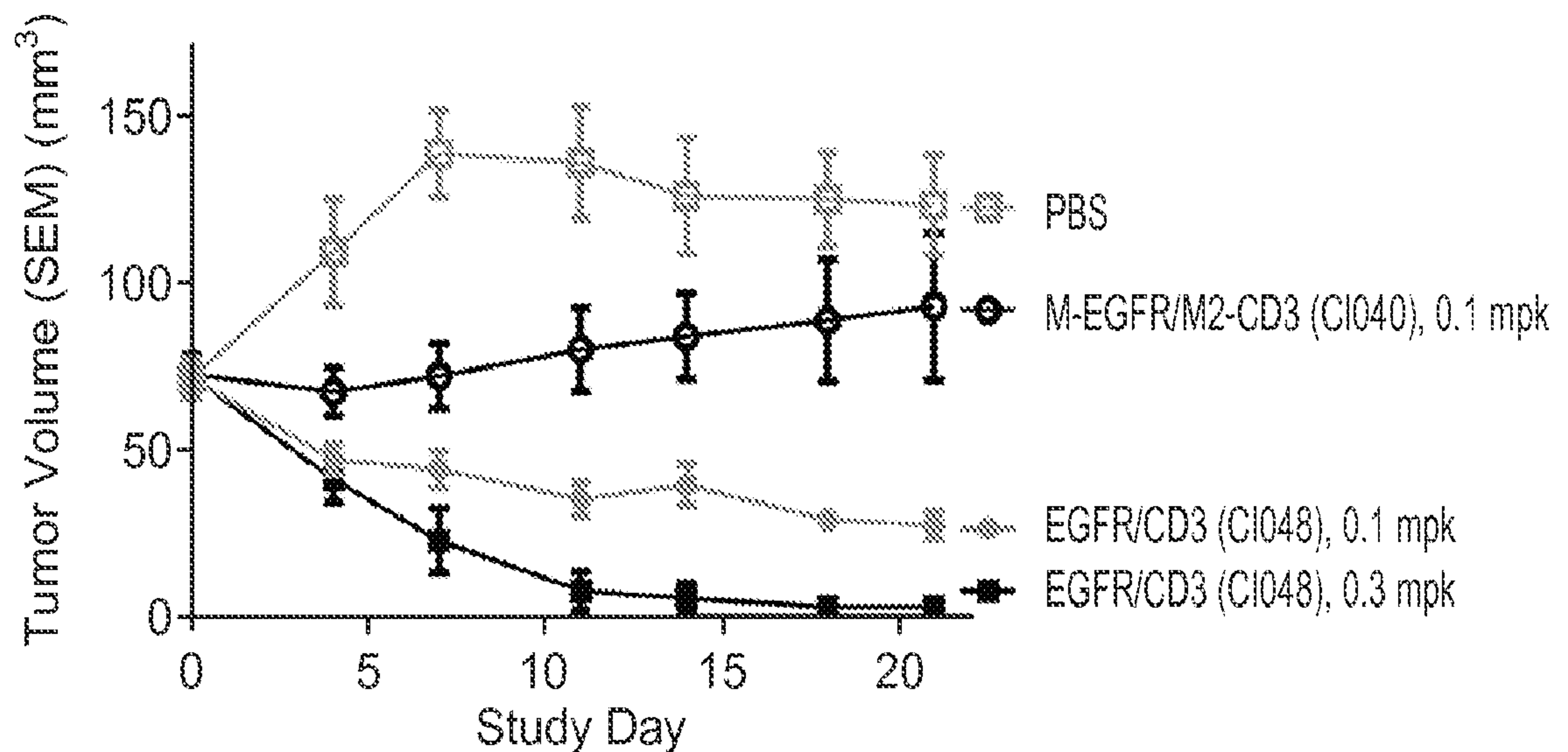
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FIGURE 25



8 mice/grp IV dosing on day 1, 8, 15 post-group assignment

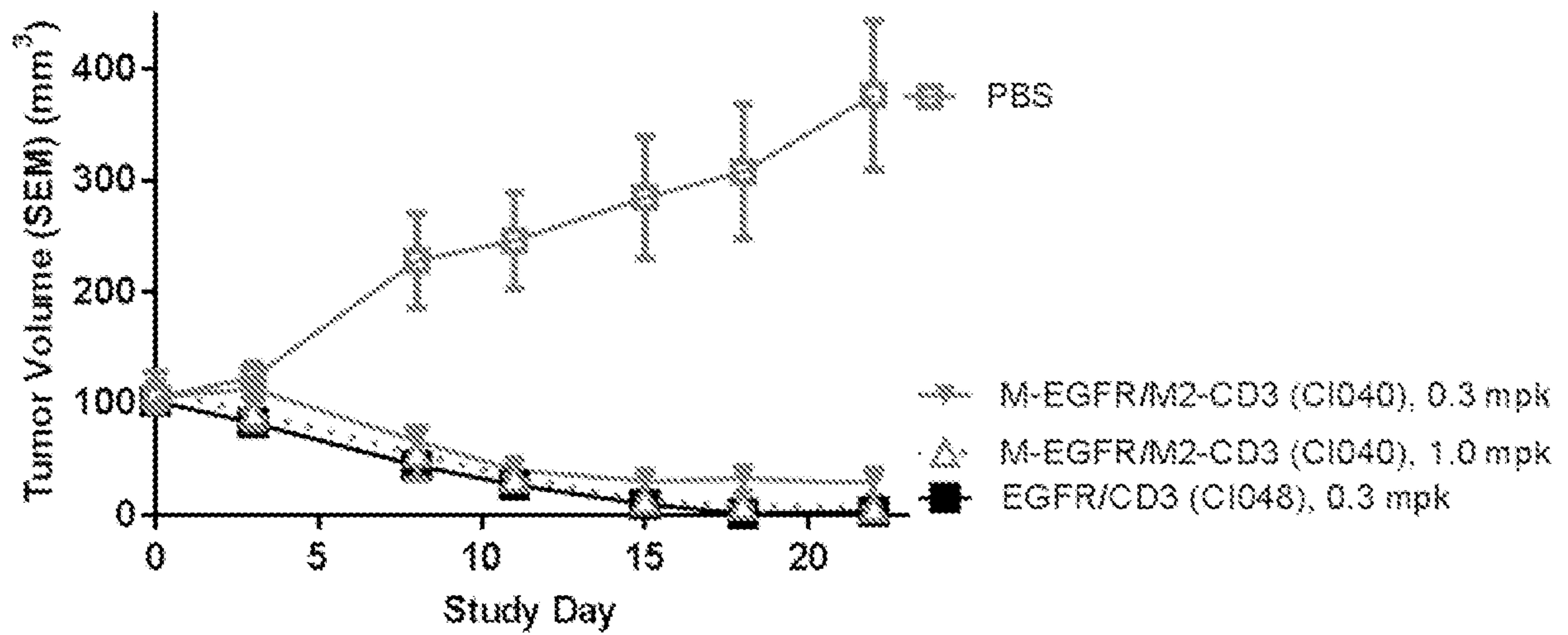
FIGURE 26



8 mice/grp IV dosing on day 1, 8, 15 post-group assignment

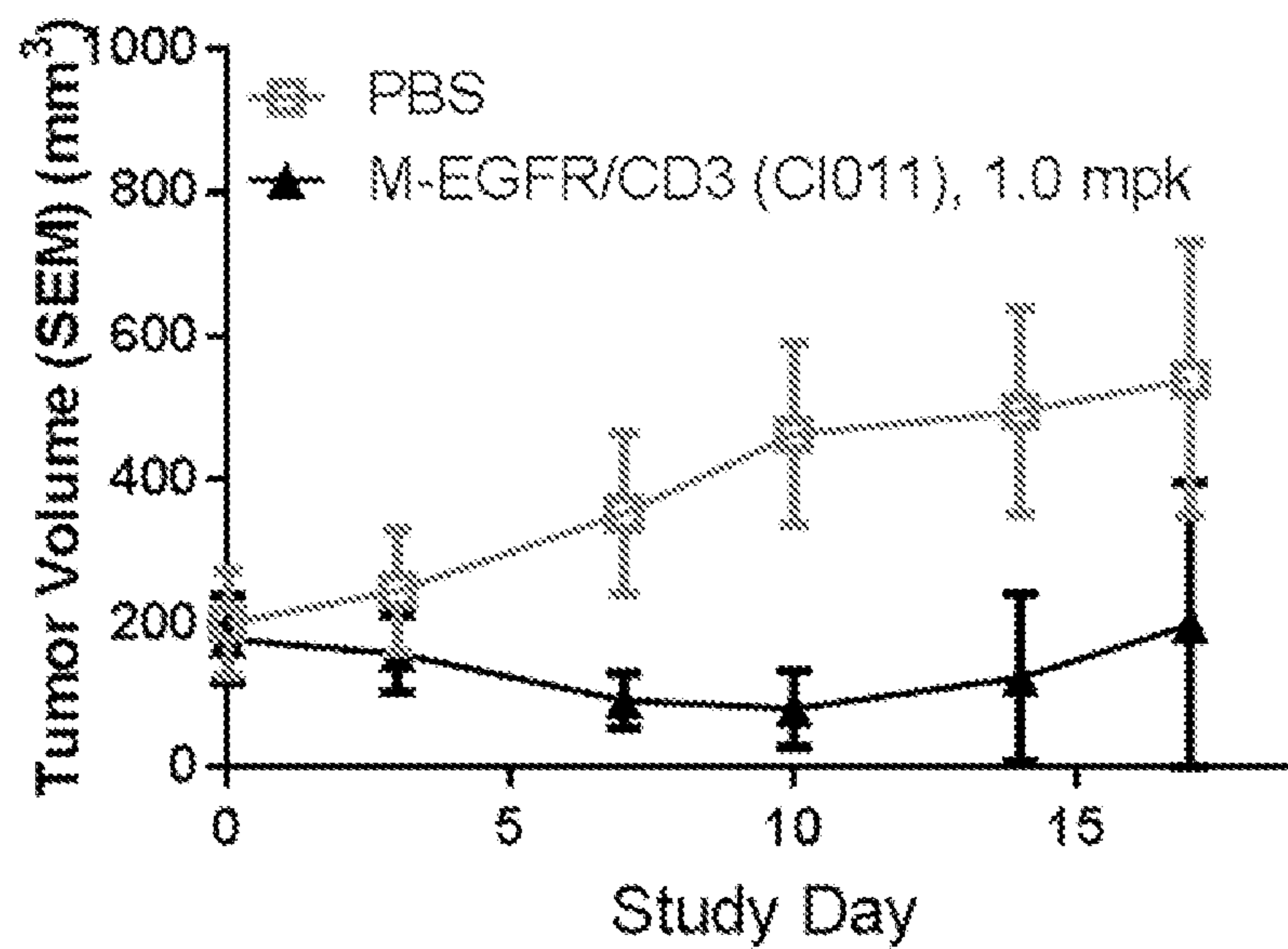
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FIGURE 27



7 mice/grp IV dosing on day 1, 8, 15, 22 post-group assignment

FIGURE 28



4 mice/grp, IV dosed day 1, 8 post-group assignment

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FIGURE 29

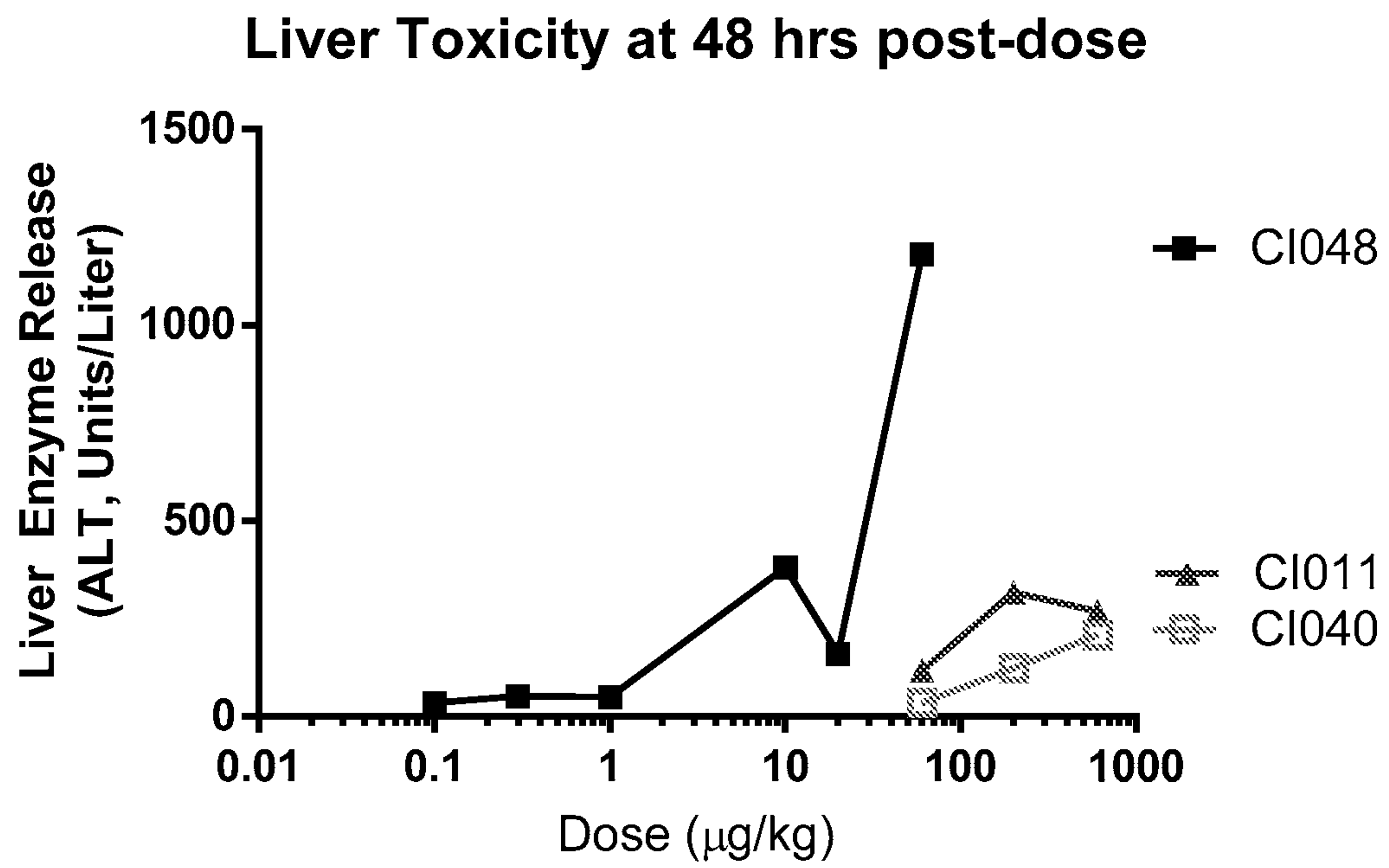
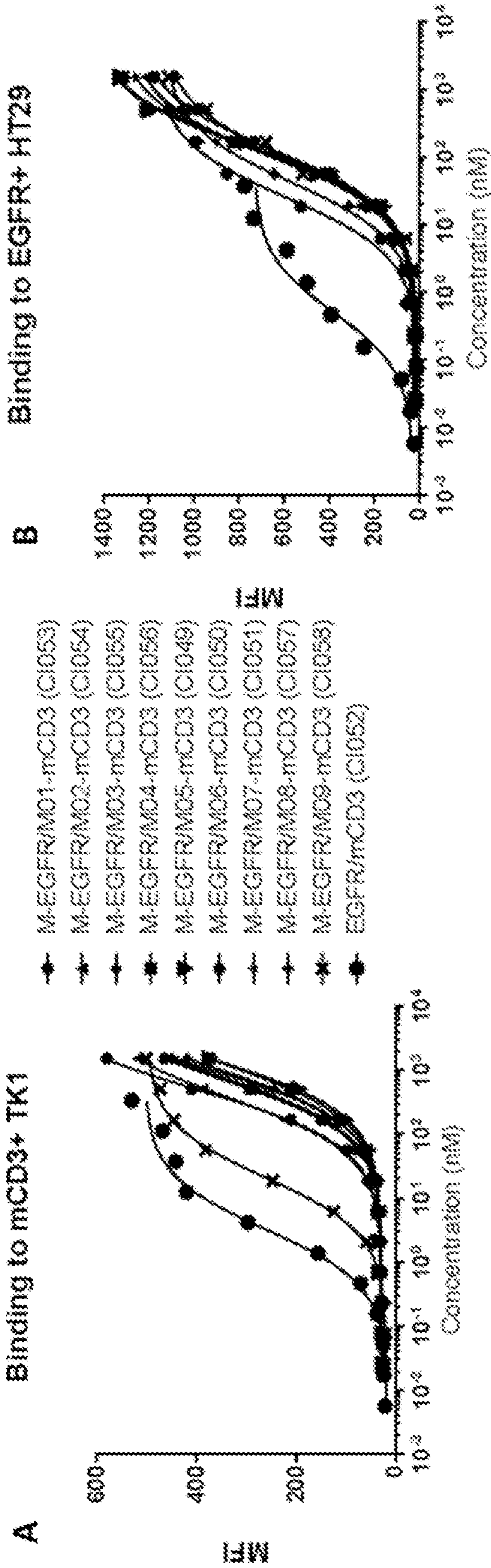


FIGURE 30



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FIGURE 31

