

as a protease is upregulated or favorable competition via highly localized antigen concentration as compared to healthy tissues, allowing the antibody to bind to its target for tumor killing. *See*, for example, WO2019/149282. Activatable antibodies thus provide antigen-specific binding proteins that are activated predominantly in the TME while remaining largely in an inactive state in healthy tissues.

SUMMARY

[0006] The present application provides multispecific antibodies targeting CD3 and another target antigen (*e.g.*, HER2, CD20, TROP2, BCMA, or CD19), masked antibodies (including activatable antibodies such as activatable multispecific antibodies), isolated anti-CD3 antibodies, masked (*e.g.*, activatable) antibodies targeting HER2, and methods of treatment thereof.

[0007] One example of the present application provides a multispecific antibody (“multispecific T cell engager”) comprising: a first antigen-binding fragment that specifically binds CD3, wherein the first antigen-binding fragment is fused to a first masking moiety (MM1); and a second antigen-binding fragment that specifically binds a target antigen; wherein the MM1 competes with CD3 to specifically bind the CD3-binding moiety; and wherein the first antigen-binding fragment binds CD3 with half-maximal binding at a concentration of antibody (EC₅₀) that is at least 10 nM as determined by an enzyme-linked immunosorbent assay (ELISA). In some examples, the EC₅₀ is at least 50 nM. In some examples, the EC₅₀ is at least 100 nM (*e.g.*, about 110 nM). In some examples, the first antigen-binding fragment is a scFv, such as an isolated anti-CD3 scFv, an isolated anti-CD3 scFv-Fc fusion protein, or an anti-CD3 scFv fragment in a multispecific (*e.g.*, bispecific) antibody without the MM1, when used to determine the EC₅₀. In some examples, the EC₅₀ is determined using the ELISA assay as described in Example 5.

[0007a] In one aspect, the present disclosure refers to a multispecific antibody comprising: a) a first antigen-binding fragment that specifically binds CD3, wherein the first antigen-binding fragment is fused to a first masking moiety (MM1) via a first cleavable moiety (CM1) comprising a first cleavage site, wherein the first antigen-binding fragment comprises a first immunoglobulin light chain variable domain (VL1) and a first immunoglobulin heavy chain variable domain (VH1) of an anti-CD3 antibody, wherein the MM1 is fused to the N-terminus of the VL1 via the CM1, wherein the MM1 inhibits binding of the multispecific antibody to CD3 when the CM1 is not cleaved, and wherein the MM1 comprises an amino acid sequence selected from the group consisting of SEQ ID NOs: 35, 417, and 597-599 and the CM1 comprises an amino acid sequence selected from the group consisting of SEQ ID NOs: 77, 127-129, 418, 420, 431 and 477-490, and 516-555; and b) a second antigen-binding fragment that specifically binds

NO: 381; x. the VH1 comprises a CDR-H1 comprising the amino acid sequence of SEQ ID NO: 390, a CDR-H2 comprising the amino acid sequence of SEQ ID NO: 394, and a CDR-H3 comprising the amino acid sequence of SEQ ID NO: 395; and the VL1 comprises a CDR-L1 comprising the amino acid sequence of SEQ ID NO: 396, a CDR-L2 comprising the amino acid sequence of SEQ ID NO: 380, and a CDR-L3 comprising the amino acid sequence of SEQ ID NO: 381; xi. the VH1 comprises a CDR-H1 comprising the amino acid sequence of SEQ ID NO: 376, a CDR-H2 comprising the amino acid sequence of SEQ ID NO: 391, and a CDR-H3 comprising the amino acid sequence of SEQ ID NO: 378; and the VL1 comprises a CDR-L1 comprising the amino acid sequence of SEQ ID NO: 397, a CDR-L2 comprising the amino acid sequence of SEQ ID NO: 380, and a CDR-L3 comprising the amino acid sequence of SEQ ID NO: 400; xii. the VH1 comprises a CDR-H1 comprising the amino acid sequence of SEQ ID NO: 390, a CDR-H2 comprising the amino acid sequence of SEQ ID NO: 393, and a CDR-H3 comprising the amino acid sequence of SEQ ID NO: 378; and the VL1 comprises a CDR-L1 comprising the amino acid sequence of SEQ ID NO: 396, a CDR-L2 comprising the amino acid sequence of SEQ ID NO: 380, and a CDR-L3 comprising the amino acid sequence of SEQ ID NO: 381; xiii. the VH1 comprises a CDR-H1 comprising the amino acid sequence of SEQ ID NO: 390, a CDR-H2 comprising the amino acid sequence of SEQ ID NO: 391, and a CDR-H3 comprising the amino acid sequence of SEQ ID NO: 378; and the VL1 comprises a CDR-L1 comprising the amino acid sequence of SEQ ID NO: 396, a CDR-L2 comprising the amino acid sequence of SEQ ID NO: 380, and a CDR-L3 comprising the amino acid sequence of SEQ ID NO: 381; or xiv. the VH1 comprises a CDR-H1 comprising the amino acid sequence of SEQ ID NO: 390, a CDR-H2 comprising the amino acid sequence of SEQ ID NO: 391, and a CDR-H3 comprising the amino acid sequence of SEQ ID NO: 378; and the VL1 comprises a CDR-L1 comprising the amino acid sequence of SEQ ID NO: 397, a CDR-L2 comprising the amino acid sequence of SEQ ID NO: 380, and a CDR-L3 comprising the amino acid sequence of SEQ ID NO: 381.

[0008] In some examples according to any one of the multispecific antibodies described above, the multispecific antibody is a not an activatable multispecific antibody. In some examples, the first antigen-binding fragment comprises a first immunoglobulin light chain variable domain (VL1) and a first immunoglobulin heavy chain variable domain (VH1) of an anti-CD3 antibody, and wherein the MM1 is fused to the N-terminus of the VL1 via a first non-cleavable linker (NCL1).

[0009] One example of the present application provides an activatable multispecific antibody (“activatable multispecific T cell engager”) comprising: a) a first antigen-binding fragment that

specifically binds CD3, wherein the first antigen-binding fragment is fused to a first masking moiety (MM1) via a first cleavable moiety (CM1); and b) a second antigen-binding fragment that specifically binds a target antigen; wherein the CM1 comprises a first cleavage site; wherein the MM1 competes with CD3 to specifically bind the CD3-binding moiety; and wherein the first antigen-binding fragment binds CD3 with half-maximal binding at a concentration of antibody (EC_{50}) that is at least 10 nM as determined by an enzyme-linked immunosorbent assay (ELISA). In some examples, the MM1 inhibits binding of the activatable antibody to CD3 when the CM1 is not cleaved; and the activatable multispecific antibody binds to CD3 via the first antigen-binding fragment when the CM1 is cleaved. In some examples, the first antigen-binding fragment is fused to the MM1 via a first cleavable moiety (CM1), the CM1 comprises a first cleavage site, the MM1 inhibits binding of the multispecific antibody to CD3 when the CM1 is not cleaved, and the multispecific antibody binds CD3 via the first antigen-binding fragment with higher affinity when the CM1 is cleaved, *e.g.*, as compared to affinity of multispecific antibody binding to CD3 via the first antigen-binding fragment when the CM1 is not cleaved. In some examples, the EC_{50} is at least 50 nM. In some examples, the EC_{50} is at least 100 nM (*e.g.*, about 110 nM). In some examples, the first antigen-binding fragment is a scFv, such as an isolated anti-CD3 scFv, an isolated anti-CD3 scFv-Fc fusion protein, or an anti-CD3 scFv fragment in a multispecific (*e.g.*, bispecific) antibody or an activatable multispecific antibody in an activated form (*i.e.*, with CM1 cleaved or effective binding by highly localized antigen concentration in the TME vs normal tissues), when used to determine the EC_{50} . In some examples, the EC_{50} is determined using the ELISA assay as described in Example 5.

[0010] In some examples according to any one of the multispecific or activatable multispecific antibodies described above, the first antigen-binding fragment binds CD3 with a dissociation constant (K_d) of at least 50 nM or at least 100 nM. In some examples, the first antigen-binding fragment is a scFv, such as an isolated anti-CD3 scFv, an isolated anti-CD3 scFv-Fc fusion protein, or an anti-CD3 scFv fragment in a multispecific (*e.g.*, bispecific) antibody or an activatable multispecific antibody in an activated form (*e.g.*, with CM1 of the multispecific antibody cleaved or effective binding by highly localized antigen concentration in the TME vs normal tissues), when used to determine the K_d . In some examples, binding of an antigen-binding fragment to CD3 is measured when the antigen-binding fragment is unmasked.

[0011] In some examples according to any one of the multispecific or activatable multispecific antibodies described above, the MM1 has a masking efficiency of at least 250 (*e.g.*, at least 500, 1000, 2000, 3000, 5000, 10000 or higher) as determined by an ELISA assay, *e.g.*, the ELISA assay in Example 3. In some examples, the MM1 has a masking efficiency of at least 50 (*e.g.*, at least 100, 200, 300, 400, 500, 600, 800, 1000 or higher) as determined by a Jurkat NFAT reporter assay, *e.g.*, the Jurkat NFAT assay for the antigen concentration used in Example 3.

[0012] In some examples according to any one of the multispecific or activatable multispecific antibodies described above, the first antigen-binding fragment comprises a first immunoglobulin light chain variable domain (VL1) and a first immunoglobulin heavy chain variable domain (VH1) of an anti-CD3 antibody. In some examples, the first antigen-binding fragment is selected from the group consisting of a Fab, a Fv, a scFab and a scFv. In some examples, the first antigen-binding fragment is a scFv. In some examples, the scFv comprises from N-terminus to C-terminus, VL1, a linker, and VH1. In some examples, the scFv comprises from N-terminus to C-terminus, VH1, a linker, and VL1. In some examples in which the antibody is an activatable multispecific antibody, the MM1 is fused to the N-terminus of the VL1 via the CM1. In some examples in which the antibody is not an activatable multispecific antibody, the MM1 is fused to the N-terminus of the VL1 via the NCL1. In some examples, the multispecific antibody is not an activatable multispecific antibody. In some examples, the multispecific antibody does not comprise a cleavable linker. In some examples, the masking moiety is not fused with a sequence comprising a cleavage site.

[0013] In some examples according to any one of the multispecific or activatable multispecific antibodies described above, the second antigen-binding fragment comprises a second immunoglobulin light chain variable domain (VL2) and a second immunoglobulin heavy chain variable domain (VH2) of an antibody that specifically binds the target antigen. In some examples, the second antigen-binding fragment is selected from the group consisting of a Fab, a Fv, a scFab and a scFv. In some examples, the second antigen-binding fragment is a Fv. In some examples, the second antigen-binding fragment is a Fab. In some examples in which the antibody is not an activatable multispecific antibody, the multispecific antibody comprises a first polypeptide, a second polypeptide, and a third polypeptide, wherein:

- (i) the first polypeptide comprises a structure represented by the formula:

VH2-CH1-hinge-CH2-first CH3 (1a);

(ii) the second polypeptide comprises a structure represented by the formula:

MM1-NCL1-VL1-VH1-hinge-CH2-second CH3 (1b); and

(iii) the third polypeptide comprises a structure represented by the formula:

VL2-CL (1c);

wherein:

CL is an immunoglobulin light chain constant domain;

CH1 is an immunoglobulin heavy chain constant domain 1;

CH2 is an immunoglobulin heavy chain constant domain 2;

first CH3 is a first immunoglobulin heavy chain constant domain 3;

second CH3 is a second immunoglobulin heavy chain constant domain 3;

hinge is an immunoglobulin hinge region connecting the CH1 and CH2 domains;

wherein the VL1 and the VH1 associate to form a scFv that specifically binds CD3; and wherein the VL2 and the VH2 associate to form a Fv that specifically binds the target antigen. In some examples in which the antibody is an activatable multispecific antibody, the activatable multispecific antibody comprises a first polypeptide, a second polypeptide, and a third polypeptide, wherein:

(i) the first polypeptide comprises a structure represented by the formula:

VH2-CH1-hinge-CH2-first CH3 (1a);

(ii) the second polypeptide comprises a structure represented by the formula:

MM1-CM1-VL1-VH1-hinge-CH2-second CH3 (1b); and

(iii) the third polypeptide comprises a structure represented by the formula:

VL2-CL (1c);

wherein:

CL is an immunoglobulin light chain constant domain;

CH1 is an immunoglobulin heavy chain constant domain 1;

CH2 is an immunoglobulin heavy chain constant domain 2;

first CH3 is a first immunoglobulin heavy chain constant domain 3;

second CH3 is a second immunoglobulin heavy chain constant domain 3;

hinge is an immunoglobulin hinge region connecting the CH1 and CH2 domains;

wherein the VL1 and the VH1 associate to form a scFv that specifically binds CD3; and wherein the VL2 and the VH2 associate to form a Fv that specifically binds the target antigen. In some examples, the multispecific antibody (*e.g.*, the second polypeptide thereof) comprising an amino acid linker between VL1 and VH1.

[0014] In some examples according to any one of the multispecific or activatable multispecific antibodies described above, the second antigen-binding fragment is fused to a second masking moiety (MM2), wherein the MM2 competes with the target antigen to specifically bind the second antigen-binding fragment. In some examples, the second antigen-binding fragment is fused to the MM2 via a second non-cleavable linker (NCL2). In some examples, the second antigen-binding fragment is fused to the MM2 via a second cleavable moiety (CM2), wherein the CM2 comprises a second cleavage site, wherein the MM2 inhibits binding of the multispecific antibody to the target antigen when the CM2 is not cleaved, and wherein the multispecific antibody binds the target antigen via the second antigen-binding fragment when the CM2 is cleaved. In some examples, wherein the second antigen-binding fragment comprises a VH2 and a VL2 of an antibody that specifically binds the target antigen, the MM2 is fused to the N-terminus of the VL2 via the CM2. In some examples, the multispecific or activatable multispecific antibody comprises a first polypeptide, a second polypeptide, and a third polypeptide, wherein:

(i) the first polypeptide comprises a structure represented by the formula:

VH2-CH1-hinge-CH2-first CH3 (2a);

(ii) the second polypeptide comprises a structure represented by the formula:

MM1-CM1-VL1-VH1-hinge-CH2-second CH3 (2b); and

(iii) the third polypeptide comprises a structure represented by the formula:

MM2-CM2-VL2-CL (2c);

wherein:

CL is an immunoglobulin light chain constant domain;

CH1 is an immunoglobulin heavy chain constant domain 1;

CH2 is an immunoglobulin heavy chain constant domain 2;

first CH3 is a first immunoglobulin heavy chain constant domain 3;

second CH3 is a second immunoglobulin heavy chain constant domain 3;

hinge is an immunoglobulin hinge region connecting the CH1 and CH2 domains;

wherein the VL1 and the VH1 associate to form a scFv that specifically binds CD3; and wherein the VL2 and the VH2 associate to form a Fv that specifically binds the target antigen. In some examples, the multispecific antibody (*e.g.*, the second polypeptide thereof) comprises an amino acid linker between VL1 and VH1.

[0015] In some examples according to any one of the multispecific or activatable multispecific antibodies described above, the CD3 is human CD3. In some examples, the first antigen-binding fragment is cross-reactive with a CD3 polypeptide from at least one non-human species selected from the group consisting of cynomolgus monkey, mouse, rat and dog.

[0016] In some examples according to any one of the multispecific or activatable multispecific antibodies described above, wherein the first antigen-binding fragment comprises a VH1 and a VL1 of an anti-CD3 antibody, the VH1 comprises a heavy chain complementarity determining region (CDR-H) 1 comprising the amino acid sequence according to Formula (I): $X_1YAX_2X_3$ (SEQ ID NO: 382), wherein X_1 is D, S, or T, X_2 is I, L, or M, and X_3 is N or T, a CDR-H2 comprising the amino acid sequence according to Formula (II):

RIRSKYNNYATYYAX₁X₂VKX₃ (SEQ ID NO: 383), wherein X_1 is D or E, X_2 is S or T, and X_3 is D, G, or S, and a CDR-H3 comprising the amino acid sequence according to Formula (III): HGNX₁GX₂SYVSX₃X₄AY (SEQ ID NO: 384), wherein X_1 is F or Y, X_2 is N or T, X_3 is W or Y, and X_4 is F or W. In some examples, the VL1 comprises a CDR-L1 comprising the amino acid sequence according to Formula (IV): $X_1SSTGAVTX_2X_3NYX_4N$ (SEQ ID NO: 385), wherein X_1 is A, G, or R, X_2 is S or T, X_3 is G or S, and X_4 is A, P, or V, a CDR-L2 comprising the amino acid sequence according to Formula (V): GTX₁X₂RAP (SEQ ID NO: 386), wherein X_1 is K or N, and X_2 is F or K, and a CDR-L3 comprising the amino acid sequence according to Formula (VI): ALWYSX₁X₂WV (SEQ ID NO: 387), wherein X_1 is D, N, or T, and X_2 is L or R.

[0017] In some examples according to any one of the multispecific or activatable multispecific antibodies described above, wherein the first antigen-binding fragment comprises a VH1 and a VL1 of an anti-CD3 antibody, the VH1 comprises a heavy chain complementarity determining region (CDR-H) 1 comprising an amino acid sequence selected from the group consisting of SEQ ID NOs: 376, 390, 601, and 602, or a variant thereof comprising up to about 3 amino acid substitutions, a CDR-H2 comprising an amino acid sequence selected from the group consisting of SEQ ID NOs: 377, 391-394, and 603, or a variant thereof comprising up to about 3 amino acid substitutions, and a CDR-H3 comprising an amino acid sequence selected from the

group consisting of SEQ ID NOs: 378, 395, 604, and 605, or a variant thereof comprising up to about 3 amino acid substitutions; and the VL1 comprises a CDR-L1 comprising an amino acid sequence selected from the group consisting of SEQ ID NOs: 396-398, and 606-609, or a variant thereof comprising up to about 3 amino acid substitutions, a CDR-L2 comprising an amino acid sequence selected from the group consisting of SEQ ID NOs: 380 and 399, or a variant thereof comprising up to about 3 amino acid substitutions, and a CDR-L3 comprising an amino acid sequence selected from the group consisting of SEQ ID NOs: 381, 400-401, and 610, or a variant thereof comprising up to about 3 amino acid substitutions.

[0018] In some examples according to any one of the multispecific or activatable multispecific antibodies described above, wherein the first antigen-binding fragment comprises a VH1 and a VL1 of an anti-CD3 antibody, the VH1 comprises a CDR-H1 comprising an amino acid sequence selected from the group consisting of SEQ ID NOs: 376 and 390, or a variant thereof comprising up to about 3 amino acid substitutions, a CDR-H2 comprising an amino acid sequence selected from the group consisting of SEQ ID NOs: 391-394, or a variant thereof comprising up to about 3 amino acid substitutions, and a CDR-H3 comprising an amino acid sequence selected from the group consisting of SEQ ID NOs: 378 and 395, or a variant thereof comprising up to about 3 amino acid substitutions; and the VL1 comprises a CDR-L1 comprising an amino acid sequence selected from the group consisting of SEQ ID NOs: 396-398, or a variant thereof comprising up to about 3 amino acid substitutions, a CDR-L2 comprising an amino acid sequence selected from the group consisting of SEQ ID NOs: 380 and 399, or a variant thereof comprising up to about 3 amino acid substitutions, and a CDR-L3 comprising an amino acid sequence selected from the group consisting of SEQ ID NOs: 381, and 400-401, or a variant thereof comprising up to about 3 amino acid substitutions.

[0019] In some examples according to any one of the multispecific or activatable multispecific antibodies described above, wherein the first antigen-binding fragment comprises a VH1 and a VL1 of an anti-CD3 antibody, the VH1 comprises a heavy chain complementarity determining region (CDR-H) 1 comprising the amino acid sequence of SEQ ID NO: 382, a CDR-H2 comprising the amino acid sequence of SEQ ID NO: 383, and a CDR-H3 comprising the amino acid sequence of SEQ ID NO: 384; and the VL1 comprises a light chain complementarity determining region (CDR-L) 1 comprising the amino acid sequence of SEQ ID NO: 385, a CDR-L2 comprising the amino acid sequence of SEQ ID NO: 386, and a CDR-L3

comprising the amino acid sequence of SEQ ID NO: 387. In some examples, the VH1 comprises a CDR-H1 comprising an amino acid sequence selected from the group consisting of SEQ ID NOs: 376 and 390, a CDR-H2 comprising an amino acid sequence selected from the group consisting of SEQ ID NOs: 391-394, and a CDR-H3 comprising an amino acid sequence selected from the group consisting of SEQ ID NOs: 378 and 395; and the VL1 comprises a CDR-L1 comprising an amino acid sequence selected from the group consisting of SEQ ID NOs: 396-398, a CDR-L2 comprising an amino acid sequence selected from the group consisting of SEQ ID NOs: 380 and 399, and a CDR-L3 comprising an amino acid sequence selected from the group consisting of SEQ ID NOs: 381, and 400-401. In some examples, the VH1 comprises a CDR-H1 comprising the amino acid sequence of SEQ ID NO: 376, a CDR-H2 comprising the amino acid sequence of SEQ ID NO: 391, and a CDR-H3 comprising the amino acid sequence of SEQ ID NO: 378; and the VL1 comprises a CDR-L1 comprising the amino acid sequence of SEQ ID NO: 396, a CDR-L2 comprising the amino acid sequence of SEQ ID NO: 380, and a CDR-L3 comprising the amino acid sequence of SEQ ID NO: 381. In some examples, the VH1 comprises a CDR-H1 comprising the amino acid sequence of SEQ ID NO: 390, a CDR-H2 comprising the amino acid sequence of SEQ ID NO: 392, and a CDR-H3 comprising the amino acid sequence of SEQ ID NO: 395; and the VL1 comprises a CDR-L1 comprising the amino acid sequence of SEQ ID NO: 397, a CDR-L2 comprising the amino acid sequence of SEQ ID NO: 380, and a CDR-L3 comprising the amino acid sequence of SEQ ID NO: 400. In some examples, the VH1 comprises a CDR-H1 comprising the amino acid sequence of SEQ ID NO: 390, a CDR-H2 comprising the amino acid sequence of SEQ ID NO: 392, and a CDR-H3 comprising the amino acid sequence of SEQ ID NO: 395; and the VL1 comprises a CDR-L1 comprising the amino acid sequence of SEQ ID NO: 396, a CDR-L2 comprising the amino acid sequence of SEQ ID NO: 380, and a CDR-L3 comprising the amino acid sequence of SEQ ID NO: 401. In some examples, the VH1 comprises a CDR-H1 comprising the amino acid sequence of SEQ ID NO: 390, a CDR-H2 comprising the amino acid sequence of SEQ ID NO: 393, and a CDR-H3 comprising the amino acid sequence of SEQ ID NO: 395; and the VL1 comprises a CDR-L1 comprising the amino acid sequence of SEQ ID NO: 397, a CDR-L2 comprising the amino acid sequence of SEQ ID NO: 380, and a CDR-L3 comprising the amino acid sequence of SEQ ID NO: 381. In some examples, the VH1 comprises a CDR-H1 comprising the amino acid sequence of SEQ ID NO: 376, a CDR-H2 comprising the amino acid

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NO: 376, a CDR-H2 comprising the amino acid sequence of SEQ ID NO: 391, and a CDR-H3 comprising the amino acid sequence of SEQ ID NO: 378; and the VL1 comprises a CDR-L1 comprising the amino acid sequence of SEQ ID NO: 397, a CDR-L2 comprising the amino acid sequence of SEQ ID NO: 380, and a CDR-L3 comprising the amino acid sequence of SEQ ID NO: 400. In some examples, the VH1 comprises a CDR-H1 comprising the amino acid sequence of SEQ ID NO: 390, a CDR-H2 comprising the amino acid sequence of SEQ ID NO: 394, and a CDR-H3 comprising the amino acid sequence of SEQ ID NO: 395; and the VL1 comprises a CDR-L1 comprising the amino acid sequence of SEQ ID NO: 396, a CDR-L2 comprising the amino acid sequence of SEQ ID NO: 380, and a CDR-L3 comprising the amino acid sequence of SEQ ID NO: 381. In some examples, the VH1 comprises a CDR-H1 comprising the amino acid sequence of SEQ ID NO: 390, a CDR-H2 comprising the amino acid sequence of SEQ ID NO: 393, and a CDR-H3 comprising the amino acid sequence of SEQ ID NO: 378; and the VL1 comprises a CDR-L1 comprising the amino acid sequence of SEQ ID NO: 396, a CDR-L2 comprising the amino acid sequence of SEQ ID NO: 380, and a CDR-L3 comprising the amino acid sequence of SEQ ID NO: 381. In some examples, the VH1 comprises a CDR-H1 comprising the amino acid sequence of SEQ ID NO: 390, a CDR-H2 comprising the amino acid sequence of SEQ ID NO: 391, and a CDR-H3 comprising the amino acid sequence of SEQ ID NO: 378; and the VL1 comprises a CDR-L1 comprising the amino acid sequence of SEQ ID NO: 396, a CDR-L2 comprising the amino acid sequence of SEQ ID NO: 380, and a CDR-L3 comprising the amino acid sequence of SEQ ID NO: 381. In some examples, the VH1 comprises a CDR-H1 comprising the amino acid sequence of SEQ ID NO: 390, a CDR-H2 comprising the amino acid sequence of SEQ ID NO: 391, and a CDR-H3 comprising the amino acid sequence of SEQ ID NO: 378; and the VL1 comprises a CDR-L1 comprising the amino acid sequence of SEQ ID NO: 397, a CDR-L2 comprising the amino acid sequence of SEQ ID NO: 380, and a CDR-L3 comprising the amino acid sequence of SEQ ID NO: 381. In some examples, the VH1 comprises the amino acid sequence according to Formula (VII):

EVQLVESGGGLVX₁PGGSLRLSCAASGFTFX₂X₃YAIX₄WVRQAPGKGLEWVX₅RIRSKY
 NNYATYYAX₆SVKX₇RFTISRDX₈SKNTLYLQX₉NSLRAEDTAVYYCX₁₀RHGNX₁₁GX₁₂S
 YVSWFAYWGQGTLVTVSS (SEQ ID NO: 388), wherein X₁ is K or Q, X₂ is N or S, X₃ is S or T, X₄ is H or N, X₅ is G or S, X₆ is D or E, X₇ is D or G, X₈ is D or N, X₉ is I or L, X₁₀ is A or V,

X₁₁ is F or Y, X₁₂ is N or T; and the VL1 comprises the amino acid sequence according to Formula (VIII):

X₁AVVTQEPSLTVSPGGTVTLTCX₂SSTGAVTTSNYX₃NWX₄QKPGQAPRGLIGGTX₅X₆RAPGX₇PARFSGSLLGGKAALTLSGAQPEDEAEYYCALWYSX₈X₉WVFGGGTKLTVL (SEQ ID NO: 389), wherein X₁ is E or Q, X₂ is A, G, P, or R, X₃ is A or P, X₄ is F or V, X₅ is K or N, X₆ is F or K, X₇ is A, I, T, or V, X₈ is A, D, N, or T, and X₉ is H or L. In some examples, the VH1 comprises an amino acid sequence selected from the group consisting of SEQ ID NOs: 67, 402, 405, 407, 409, 410, 412, 414-416, and 611-640, or a variant thereof having at least about 80% sequence identity to an amino acid sequence selected from the group consisting of SEQ ID NOs: 67, 402, 405, 407, 409, 410, 412, 414-416, and 611-640; and the VL1 comprises an amino acid sequence selected from the group consisting of SEQ ID NOs: 68, 403, 404, 406, 408, 411, 413, and 641-666, or a variant thereof having at least about 80% sequence identity to an amino acid sequence selected from the group consisting of SEQ ID NOs: 68, 403, 404, 406, 408, 411, 413, and 641-666. In some examples, the VH1 comprises an amino acid sequence selected from the group consisting of SEQ ID NOs: 67, 402, 405, 407, 409, 410, 412, 414, 415, and 416; and the VL1 comprises an amino acid sequence selected from the group consisting of SEQ ID NOs: 68, 403, 404, 406, 408, 411, and 413. In some examples, the VH1 comprises the amino acid sequence of SEQ ID NO: 388, and the VL1 comprises the amino acid sequence of SEQ ID NO: 389. In some examples, the VH1 comprises an amino acid sequence selected from the group consisting of SEQ ID NOs: 402, 405, 407, 409, 410, 412, 414, 415, and 416; and the VL1 comprises an amino acid sequence selected from the group consisting of SEQ ID NOs: 403, 404, 406, 408, 411, and 413. In some examples, the VH1 comprises the amino acid sequence of SEQ ID NO: 402, and the VL1 comprises the amino acid sequence of SEQ ID NO: 403. In some examples, the VH1 comprises the amino acid sequence of SEQ ID NO: 402, and the VL1 comprises the amino acid sequence of SEQ ID NO: 404. In some examples, the VH1 comprises the amino acid sequence of SEQ ID NO: 405, and the VL1 comprises the amino acid sequence of SEQ ID NO: 406. In some examples, the VH1 comprises the amino acid sequence of SEQ ID NO: 407, and the VL1 comprises the amino acid sequence of SEQ ID NO: 404. In some examples, the VH1 comprises the amino acid sequence of SEQ ID NO: 407, and the VL1 comprises the amino acid sequence of SEQ ID NO: 403. In some examples, the VH1 comprises the amino acid sequence of SEQ ID NO: 407,

and the VL1 comprises the amino acid sequence of SEQ ID NO: 408. In some examples, the VH1 comprises the amino acid sequence of SEQ ID NO: 409, and the VL1 comprises the amino acid sequence of SEQ ID NO: 408. In some examples, the VH1 comprises the amino acid sequence of SEQ ID NO: 410, and the VL1 comprises the amino acid sequence of SEQ ID NO: 411. In some examples, the VH1 comprises the amino acid sequence of SEQ ID NO: 412, and the VL1 comprises the amino acid sequence of SEQ ID NO: 413. In some examples, the VH1 comprises the amino acid sequence of SEQ ID NO: 410, and the VL1 comprises the amino acid sequence of SEQ ID NO: 413. In some examples, the VH1 comprises the amino acid sequence of SEQ ID NO: 414, and the VL1 comprises the amino acid sequence of SEQ ID NO: 403. In some examples, the VH1 comprises the amino acid sequence of SEQ ID NO: 415, and the VL1 comprises the amino acid sequence of SEQ ID NO: 413. In some examples, the VH1 comprises the amino acid sequence of SEQ ID NO: 416, and the VL1 comprises the amino acid sequence of SEQ ID NO: 413. In some examples, the VH1 comprises the amino acid sequence of SEQ ID NO: 416, and the VL1 comprises the amino acid sequence of SEQ ID NO: 411. In some examples, the first antigen-binding fragment comprises the amino acid sequence of SEQ ID NO: 421. In some examples, the first antigen-binding fragment comprises the amino acid sequence of SEQ ID NO: 422.

[0020] In some examples according to any one of the multispecific or activatable multispecific antibodies described above, the MM1 comprises the amino acid sequence of EVGSY (SEQ ID NO: 667) at the N-terminus of the MM1. In some examples, the MM1 comprises an amino acid sequence according to Formula (IX): PYDDPDCPSHX₁SDCDX₂ (SEQ ID NO: 668), wherein X₁ is D or E, and X₂ is N or Q. In some examples, the MM1 comprises an amino acid sequence according to Formula (X). In some examples, the MM1 comprises the amino acid sequence of SEQ ID NO: 417. In some examples, the MM1 comprises the amino acid sequence of SEQ ID NO: 35. In some examples, the MM1 comprises an amino acid sequence selected from the group consisting of SEQ ID NOs: 597-599.

[0021] In some examples according to any one of the activatable multispecific antibodies described above, the CM1 comprises an amino acid sequence selected from the group consisting of SEQ ID NOs: 77, 127-129, 418, 420, 431 and 477-490, and 516-555. In some examples, the CM1 comprises the amino acid sequence of SEQ ID NO: 77 or 418.

[0022] In some examples according to any one of the multispecific or activatable multispecific antibodies described above, the target antigen is a tumor antigen. In some examples, the tumor antigen is selected from the group consisting of CD19, CD20, EpCAM, CEA, PSMA, CD33, EGFR, HER2, EphA2, MCSP, ADAM17, PSCA, 17-A1, NKG2D, TROP2, CD79B, Nectin-4, BCMA, CD22, CD38, EGFR, GD2, SLAMF7, CD30, EpCAM, MUC1, MUC16, CD123, CD37, FOLR1, MET, FLT3, GPC3, CEACAM5, CLDN18, CSF1, Integrin alpha 5, NCAM1, PTPRC, CD138, NaPi2b, MSLN, DLL3, GPRC5D, GPNMB, ICAM1, SSTR2, carcinoma associated antigen CTAA16, CA9, ENG, ACVRL1, CD80, CSPG4, EGFL7, FLT1, HAVCR1, HGF, HLA-DRB, IGF1R, TPBG, ERBB3, and STEAP2. In some examples, the tumor antigen is HER2. In some examples, the tumor antigen is CD20. In some examples, the tumor antigen is TROP2. In some examples, the tumor antigen is BCMA. In some examples, the tumor antigen is CD19.

[0023] In some examples according to any one of the multispecific or activatable multispecific antibodies described above, the target antigen is HER2. In some examples, wherein the second antigen-binding fragment comprises a VH2 and a VL2 of an anti-HER2 antibody, the VH2 comprises a CDR-H1 comprising the amino acid sequence of SEQ ID NO: 423, a CDR-H2 comprising the amino acid sequence of SEQ ID NO: 424, and a CDR-H3 comprising the amino acid sequence of SEQ ID NO: 71; and the VL2 comprises a CDR-L1 comprising the amino acid sequence of SEQ ID NO: 72, a CDR-L2 comprising the amino acid sequence of SEQ ID NO: 73, and a CDR-L3 comprising the amino acid sequence of SEQ ID NO: 74. In some examples, the VH2 comprises the amino acid sequence of SEQ ID NO: 75, and the VL2 comprises the amino acid sequence of SEQ ID NO: 76. In some examples, the second antigen-binding fragment is fused to a second masking moiety (MM2) via a second cleavable moiety (CM2), wherein a) the MM2 comprises an amino acid sequence according to Formula (XI): $ESX_1X_2CX_3X_4DPFX_5CQX_6$ (SEQ ID NO: 670), wherein X_1 is D or E, X_2 is A, F, V, or Y, X_3 is D or E, X_4 is A or L, X_5 is D or E, and X_6 is A, F, or Y; b) the MM2 comprises an amino acid sequence according to Formula (XII): $X_1X_2X_3X_4X_5X_6CX_7X_8DPYECX_9X_{10}$ (SEQ ID NO: 671), wherein X_1 is A, H, or S, X_2 is A, D, or S, X_3 is A, T, or V, X_4 is P, S, or T, X_5 is D or E, X_6 is A or V, X_7 is D or E, X_8 is A or L, X_9 is Q, S, or T, and X_{10} is A, H, or V; or c) the MM2 comprises an amino acid sequence according to Formula (XIII): $YNSDDDCX_1SX_2YDPYTCYY$ (SEQ ID NO: 672), wherein X_1 is A, I, or V, and X_2 is H or R. In some examples, the second

antigen-binding fragment is fused to a second masking moiety (MM2) via a second cleavable moiety (CM2), wherein the MM2 comprises an amino acid sequence selected from the group consisting of SEQ ID NOs: 36, 419, 432-476, and 491-515. In some examples, the MM2 comprises the amino acid sequence of SEQ ID NO: 419. In some examples, the CM2 comprises an amino acid sequence selected from the group consisting of SEQ ID NOs: 77, 127-129, 418, 420, 431 and 477-490, and 516-555. In some examples, the CM2 comprises the amino acid sequence of SEQ ID NO: 420. In some examples, the CM2 comprises the amino acid sequence of SEQ ID NO: 77. In some examples, the multispecific or activatable multispecific antibody comprises: a first polypeptide comprising an amino acid sequence having at least 80% (*e.g.*, at least 85%, 90%, 95%, 98%, or 99%; or 100%) sequence identity with SEQ ID NO: 425, a second polypeptide comprising an amino acid sequence having at least 80% (*e.g.*, at least 85%, 90%, 95%, 98%, or 99%; or 100%) sequence identity with SEQ ID NO: 426, and a third polypeptide comprising an amino acid sequence having at least at least 80% (*e.g.*, at least 85%, 90%, 95%, 98%, or 99%; or 100%) sequence identity with SEQ ID NO: 112. In some examples, the multispecific or activatable multispecific antibody comprises: a first polypeptide comprising an amino acid sequence having at least at least 80% (*e.g.*, at least 85%, 90%, 95%, 98%, or 99%; or 100%) sequence identity with SEQ ID NO: 427, a second polypeptide comprising an amino acid sequence having at least 80% (*e.g.*, at least 85%, 90%, 95%, 98%, or 99%; or 100%) sequence identity with SEQ ID NO: 428, and a third polypeptide comprising an amino acid sequence having at least 80% (*e.g.*, at least 85%, 90%, 95%, 98%, or 99%; or 100%) sequence identity with SEQ ID NO: 112. In some examples, the multispecific or activatable multispecific antibody comprises: a first polypeptide comprising an amino acid sequence having at least at least 80% (*e.g.*, at least 85%, 90%, 95%, 98%, or 99%; or 100%) sequence identity with SEQ ID NO: 429, a second polypeptide comprising an amino acid sequence having at least at least 80% (*e.g.*, at least 85%, 90%, 95%, 98%, or 99%; or 100%) sequence identity with SEQ ID NO: 430, and a third polypeptide comprising an amino acid sequence having at least at least 80% (*e.g.*, at least 85%, 90%, 95%, 98%, or 99%; or 100%) sequence identity with SEQ ID NO: 115. In some examples, the multispecific or activatable multispecific antibody comprises: a first polypeptide comprising an amino acid sequence having at least at least 80% (*e.g.*, at least 85%, 90%, 95%, 98%, or 99%; or 100%) sequence identity with SEQ ID NO: 83, a second polypeptide comprising an amino acid sequence having at least at

least 80% (*e.g.*, at least 85%, 90%, 95%, 98%, or 99%; or 100%) sequence identity with SEQ ID NO: 84, and a third polypeptide comprising an amino acid sequence having at least at least 80% (*e.g.*, at least 85%, 90%, 95%, 98%, or 99%; or 100%) sequence identity with SEQ ID NO: 85. In some examples, the multispecific or activatable multispecific antibody comprises: a first polypeptide comprising an amino acid sequence having at least at least 80% (*e.g.*, at least 85%, 90%, 95%, 98%, or 99%; or 100%) sequence identity with SEQ ID NO: 683, a second polypeptide comprising an amino acid sequence having at least at least 80% (*e.g.*, at least 85%, 90%, 95%, 98%, or 99%; or 100%) sequence identity with SEQ ID NO: 684, and a third polypeptide comprising an amino acid sequence having at least at least 80% (*e.g.*, at least 85%, 90%, 95%, 98%, or 99%; or 100%) sequence identity with SEQ ID NO: 685. In some examples, the multispecific or activatable multispecific antibody comprises: a first polypeptide comprising an amino acid sequence having at least 80% (*e.g.*, at least 85%, 90%, 95%, 98%, or 99%; or 100%) sequence identity with SEQ ID NO: 425 optionally without the C-terminal lysine, a second polypeptide comprising an amino acid sequence having at least 80% (*e.g.*, at least 85%, 90%, 95%, 98%, or 99%; or 100%) sequence identity with SEQ ID NO: 426 optionally without the C-terminal lysine, and a third polypeptide comprising an amino acid sequence having at least at least 80% (*e.g.*, at least 85%, 90%, 95%, 98%, or 99%; or 100%) sequence identity with SEQ ID NO: 112. In some examples, the multispecific or activatable multispecific antibody comprises: a first polypeptide comprising an amino acid sequence having at least at least 80% (*e.g.*, at least 85%, 90%, 95%, 98%, or 99%; or 100%) sequence identity with SEQ ID NO: 427 optionally without the C-terminal lysine, a second polypeptide comprising an amino acid sequence having at least 80% (*e.g.*, at least 85%, 90%, 95%, 98%, or 99%; or 100%) sequence identity with SEQ ID NO: 428 optionally without the C-terminal lysine, and a third polypeptide comprising an amino acid sequence having at least 80% (*e.g.*, at least 85%, 90%, 95%, 98%, or 99%; or 100%) sequence identity with SEQ ID NO: 112. In some examples, the multispecific or activatable multispecific antibody comprises: a first polypeptide comprising an amino acid sequence having at least at least 80% (*e.g.*, at least 85%, 90%, 95%, 98%, or 99%; or 100%) sequence identity with SEQ ID NO: 429 optionally without the C-terminal lysine, a second polypeptide comprising an amino acid sequence having at least at least 80% (*e.g.*, at least 85%, 90%, 95%, 98%, or 99%; or 100%) sequence identity with SEQ ID NO: 430 optionally without the C-terminal lysine, and a third polypeptide comprising an amino

acid sequence having at least at least 80% (*e.g.*, at least 85%, 90%, 95%, 98%, or 99%; or 100%) sequence identity with SEQ ID NO: 115. In some examples, the multispecific or activatable multispecific antibody comprises: a first polypeptide comprising an amino acid sequence having at least at least 80% (*e.g.*, at least 85%, 90%, 95%, 98%, or 99%; or 100%) sequence identity with SEQ ID NO: 83, a second polypeptide comprising an amino acid sequence having at least at least 80% (*e.g.*, at least 85%, 90%, 95%, 98%, or 99%; or 100%) sequence identity with SEQ ID NO: 84 optionally without the C-terminal lysine, and a third polypeptide comprising an amino acid sequence having at least at least 80% (*e.g.*, at least 85%, 90%, 95%, 98%, or 99%; or 100%) sequence identity with SEQ ID NO: 85 optionally without the C-terminal lysine. In some examples, the multispecific or activatable multispecific antibody comprises: a first polypeptide comprising an amino acid sequence having at least at least 80% (*e.g.*, at least 85%, 90%, 95%, 98%, or 99%; or 100%) sequence identity with SEQ ID NO: 683, a second polypeptide comprising an amino acid sequence having at least at least 80% (*e.g.*, at least 85%, 90%, 95%, 98%, or 99%; or 100%) sequence identity with SEQ ID NO: 684 optionally without the C-terminal lysine, and a third polypeptide comprising an amino acid sequence having at least at least 80% (*e.g.*, at least 85%, 90%, 95%, 98%, or 99%; or 100%) sequence identity with SEQ ID NO: 685 optionally without the C-terminal lysine. In some examples according to any one of the multispecific or activatable multispecific antibodies described above, the multispecific or activatable multispecific antibody comprises a mixture of heavy chain species, wherein some species comprise the C-terminal lysine, and some species lack the C-terminal lysine.

[0024] In some examples according to any one of the multispecific or activatable multispecific antibodies described above, the target antigen is CD20. In some examples, wherein the second antigen-binding fragment comprises a VH2 and a VL2 of an anti-CD20 antibody, the VH2 comprises a CDR-H1 comprising the amino acid sequence of SEQ ID NO: 556, a CDR-H2 comprising the amino acid sequence of SEQ ID NO: 557, and a CDR-H3 comprising the amino acid sequence of SEQ ID NO: 558; and the VL2 comprises a CDR-L1 comprising the amino acid sequence of SEQ ID NO: 559, a CDR-L2 comprising the amino acid sequence of SEQ ID NO: 560, and a CDR-L3 comprising the amino acid sequence of SEQ ID NO: 561. In some examples, wherein the second antigen-binding fragment comprises a VH2 and a VL2 of an anti-CD20 antibody, the VH2 comprises a CDR-H1 comprising the amino acid sequence of SEQ ID NO: 86, a CDR-H2 comprising the amino acid sequence of SEQ ID NO:

557, and a CDR-H3 comprising the amino acid sequence of SEQ ID NO: 558; and the VL2 comprises a CDR-L1 comprising the amino acid sequence of SEQ ID NO: 559, a CDR-L2 comprising the amino acid sequence of SEQ ID NO: 560, and a CDR-L3 comprising the amino acid sequence of SEQ ID NO: 561. In some examples, the VH2 comprises the amino acid sequence of SEQ ID NO: 562, and the VL2 comprises the amino acid sequence of SEQ ID NO: 563. In some examples, the multispecific or activatable multispecific antibody comprises: a first polypeptide comprising an amino acid sequence having at least 80% (*e.g.*, at least 85%, 90%, 95%, 98%, or 99%; or 100%) sequence identity with SEQ ID NO: 564, a second polypeptide comprising an amino acid sequence having at least 80% (*e.g.*, at least 85%, 90%, 95%, 98%, or 99%; or 100%) sequence identity with SEQ ID NO: 565, and a third polypeptide comprising an amino acid sequence having at least at least 80% (*e.g.*, at least 85%, 90%, 95%, 98%, or 99%; or 100%) sequence identity with SEQ ID NO: 567. In some examples, the multispecific or activatable multispecific antibody comprises: a first polypeptide comprising an amino acid sequence having at least 80% (*e.g.*, at least 85%, 90%, 95%, 98%, or 99%; or 100%) sequence identity with SEQ ID NO: 564, a second polypeptide comprising an amino acid sequence having at least 80% (*e.g.*, at least 85%, 90%, 95%, 98%, or 99%; or 100%) sequence identity with SEQ ID NO: 565, and a third polypeptide comprising an amino acid sequence having at least at least 80% (*e.g.*, at least 85%, 90%, 95%, 98%, or 99%; or 100%) sequence identity with SEQ ID NO: 569. In some examples, the multispecific or activatable multispecific antibody comprises: a first polypeptide comprising an amino acid sequence having at least 80% (*e.g.*, at least 85%, 90%, 95%, 98%, or 99%; or 100%) sequence identity with SEQ ID NO: 564, a second polypeptide comprising an amino acid sequence having at least 80% (*e.g.*, at least 85%, 90%, 95%, 98%, or 99%; or 100%) sequence identity with SEQ ID NO: 565 optionally without the C-terminal lysine, and a third polypeptide comprising an amino acid sequence having at least at least 80% (*e.g.*, at least 85%, 90%, 95%, 98%, or 99%; or 100%) sequence identity with SEQ ID NO: 567 optionally without the C-terminal lysine. In some examples, the multispecific or activatable multispecific antibody comprises: a first polypeptide comprising an amino acid sequence having at least 80% (*e.g.*, at least 85%, 90%, 95%, 98%, or 99%; or 100%) sequence identity with SEQ ID NO: 564, a second polypeptide comprising an amino acid sequence having at least 80% (*e.g.*, at least 85%, 90%, 95%, 98%, or 99%; or 100%) sequence identity with SEQ ID NO: 565 optionally without the C-terminal

lysine, and a third polypeptide comprising an amino acid sequence having at least at least 80% (*e.g.*, at least 85%, 90%, 95%, 98%, or 99%; or 100%) sequence identity with SEQ ID NO: 569 optionally without the C-terminal lysine. In some examples according to any one of the multispecific or activatable multispecific antibodies described above, the multispecific or activatable multispecific antibody comprises a mixture of heavy chain species, wherein some species comprise the C-terminal lysine, and some species lack the C-terminal lysine.

[0025] In some examples according to any one of the multispecific or activatable multispecific antibodies described above, the multispecific or activatable multispecific antibody comprises an Fc region. In some examples, the Fc region is of the human IgG1 subclass. In some examples, the Fc region is of the human IgG2 subclass. In some examples, the Fc region is of the human IgG4 subclass. In some examples, the Fc region has enhanced ADCC and/or cross-linking efficiency. In some examples, the Fc region has reduced or no antibody-dependent cell cytotoxicity (ADCC) effect and/or reduced or no cross-linking effect. In some examples, the Fc region is of the human IgG1 subclass and has an N297A amino acid substitution.

[0026] In some examples according to any one of the multispecific or activatable multispecific antibodies described above, wherein the multispecific or activatable multispecific antibody comprises a first CH3 domain and a second CH3 domain, i) the first CH3 domain comprises a cysteine (C) residue at position 390 and the second CH3 domain comprises a cysteine residue at position 400, or the first CH3 domain comprises a cysteine residue at position 400 and the second CH3 domain comprises a cysteine residue at position 390; or ii) the first CH3 domain comprises a cysteine residue at position 392 and the second CH3 domain comprises a cysteine residue at position 397, or the first CH3 domain comprises a cysteine residue at position 397 and the second CH3 domain comprises a cysteine residue at position 392; or iii) the first CH3 domain comprises a cysteine residue at position 392 and the second CH3 domain comprises a cysteine residue at position 400, or the first CH3 domain comprises a cysteine residue at position 400 and the second CH3 domain comprises a cysteine residue at position 392; and wherein the amino acid residue numbering is based on EU numbering. In some examples, i) the first CH3 domain comprises N390C substitution and the second CH3 domain comprises S400C substitution, or the first CH3 domain comprises S400C substitution and the second CH3 domain comprises N390C substitution; or ii) the first CH3 domain

comprises K392C substitution and the second CH3 domain comprises V397C substitution, or the first CH3 domain comprises V397C substitution and the second CH3 domain comprises K392C substitution; or iii) the first CH3 domain comprises K392C substitution and the second CH3 domain comprises S400C substitution, or the first CH3 domain comprises S400C substitution and the second CH3 domain comprises K392C substitution. In some examples, i) the first CH3 domain further comprises a positively charged residue at position 357 and the second CH3 domain further comprises a negatively charged residue at position 351, or the first CH3 domain further comprises a negatively charged residue at position 351 and the second CH3 domain further comprises a positively charged residue at position 357; or ii) the first CH3 domain further comprises a positively charged residue at position 411 and the second CH3 domain further comprises a negatively charged residue at position 370, or the first CH3 domain further comprises a negatively charged residue at position 370 and the second CH3 domain further comprises a positively charged residue at position 411; or iii) the first CH3 domain further comprises a positively charged residue at position 364 and the second CH3 domain further comprises a negatively charged residue at position 370, or the first CH3 domain further comprises a negatively charged residue at position 370 and the second CH3 domain further comprises a positively charged residue at position 364; or a combination of i) and ii), or a combination of i) and iii), and wherein the amino acid residue numbering is based on EU numbering. In some examples, the first CH3 domain further comprises a positively charged residue at position 356 and the second CH3 domain further comprises a negatively charged residue at position 439, or first CH3 domain further comprises a negatively charged residue at position 439 and the second CH3 domain further comprises a positively charged residue at position 356; and wherein the amino acid residue numbering is based on EU numbering. In some examples, i) the positively charged residue is a lysine (K) residue, and the negatively charged residue is an aspartic acid (D) residue; or ii) the positively charged residue is a lysine (K) residue, and the negatively charged residue is a glutamic acid (E) residue; or iii) the positively charged residue is an arginine (R) residue, and the negatively charged residue is an aspartic acid (D) residue; or iv) the positively charged residue is an arginine (R) residue, and the negatively charged residue is a glutamic acid (E) residue. In some examples, i) the first CH3 domain comprises E357K and T411K substitutions and the second CH3 domain comprises L351D and K370D substitutions, or the first CH3 domain comprises L351D and K370D substitutions and

the second CH3 domain comprises E357K and T411K substitutions; or ii) the first CH3 domain comprises E357K and S364K substitutions and the second CH3 domain comprises L351D and K370D substitutions, or the first CH3 domain comprises L351D and K370D substitutions and the second CH3 domain comprises E357K and S364K substitutions; or iii) the first CH3 domain comprises D356K, E357K and S364K substitutions and the second CH3 domain comprises L351D, K370D and K439D substitutions, or the first CH3 domain comprises L351D, K370D and K439D substitutions and the second CH3 domain comprises D356K, E357K and S364K substitutions. In some examples, i) the first CH3 domain further comprises K392D and K409D substitutions and the second CH3 domain further comprises D356K, and D399K substitutions, or the first CH3 domain further comprises D356K and D399K substitutions and the second CH3 domain further comprises K392D and K409D substitutions; or ii) the first CH3 domain further comprises L368D and K370S substitutions and the second CH3 domain further comprises E357Q and S364K substitutions, or the first CH3 domain further comprises E357Q and S364K substitutions and the second CH3 domain further comprises L368D and K370S substitutions; or iii) the first CH3 domain further comprises L351K and T366K substitutions and the second CH3 domain further comprises L351D and L368E substitutions, or the first CH3 domain further comprises L351D and L368E substitutions and the second CH3 domain further comprises L351K and T366K substitutions; or (iv) the first CH3 domain further comprises P395K, P396K and V397K substitutions and the second CH3 domain comprises T394D, P395D and P396D substitutions, or the first CH3 domain further comprises T394D, P395D and P396D substitutions and the second CH3 domain further comprises P395K, P396K and V397K substitutions; or (v) the first CH3 domain further comprises F405E, Y407E and K409E substitutions and the second CH3 domain comprises F405K and Y407K substitutions, or the first CH3 domain further comprises F405K and Y407K substitutions and the second CH3 domain further comprises F405E, Y407E and K409E substitutions.

[0027] In some examples according to any one of the multispecific or activatable multispecific antibodies described above, wherein the multispecific or activatable multispecific antibody comprises a first CH3 domain and a second CH3 domain, the first CH3 domain comprises E357K, S364K and N390C substitutions and the second CH3 domain comprises L351D, K370D, and S400C substitutions, or the first CH3 domain comprises L351D, K370D, and S400C substitutions and the second CH3 domain comprises E357K, S364K and N390C

substitutions. In some examples, the first CH3 domain comprises E357K, S364K and S400C substitutions and the second CH3 domain comprises L351D, K370D, and N390C substitutions, or the first CH3 domain comprises L351D, K370D, and N390C substitutions and the second CH3 domain comprises E357K, S364K and S400C substitutions. In some examples, the first CH3 domain comprises D356K, E357K, S364K and S400C substitutions and the second CH3 domain comprises L351D, K370D, N390C and K439D substitutions, or the first CH3 domain comprises L351D, K370D, N390C and K439D substitutions and the second CH3 domain comprises D356K, E357K, S364K and S400C substitutions. In some examples, the first CH3 domain comprises D356K, E357K, S364K and N390C substitutions and the second CH3 domain comprises L351D, K370D, K439D and S400C substitutions, or the first CH3 domain comprises L351D, K370D, K439D and S400C substitutions and the second CH3 domain comprises D356K, E357K, S364K and N390C substitutions.

[0028] In some examples according to any one of the multispecific or activatable multispecific antibodies described above, the multispecific or activatable multispecific antibody is a bispecific antibody.

[0029] One example of the present application provides an isolated antibody or antigen-binding fragment thereof that specifically binds CD3 (“anti-CD3 antibody”), comprising: a VH comprising a heavy chain complementarity determining region (CDR-H) 1 comprising the amino acid sequence according to Formula (I): $X_1YAX_2X_3$ (SEQ ID NO: 382), wherein X_1 is D, S, or T, X_2 is I, L, or M, and X_3 is N or T, a CDR-H2 comprising the amino acid sequence according to Formula (II): $RIRSKYNNYATYYAX_1X_2VKX_3$ (SEQ ID NO: 383), wherein X_1 is D or E, X_2 is S or T, and X_3 is D, G, or S, and a CDR-H3 comprising the amino acid sequence according to Formula (III): $HGNX_1GX_2SYVSX_3X_4AY$ (SEQ ID NO: 384), wherein X_1 is F or Y, X_2 is N or T, X_3 is W or Y, and X_4 is F or W; and b) a VL comprising a CDR-L1 comprising the amino acid sequence according to Formula (IV): $X_1SSTGAVTX_2X_3NYX_4N$ (SEQ ID NO: 385), wherein X_1 is A, G, or R, X_2 is S or T, X_3 is G or S, and X_4 is A, P, or V, a CDR-L2 comprising the amino acid sequence according to Formula (V): GTX_1X_2RAP (SEQ ID NO: 386), wherein X_1 is K or N, and X_2 is F or K, and a CDR-L3 comprising the amino acid sequence according to Formula (VI): $ALWYSX_1X_2WV$ (SEQ ID NO: 387), wherein X_1 is D, N, or T, and X_2 is L or R. In some examples the VH comprises a heavy chain complementarity determining region (CDR-H) 1 comprising an amino acid sequence selected from the group consisting of SEQ ID NOs: 376,

390, 601, and 602, or a variant thereof comprising up to about 3 amino acid substitutions, a CDR-H2 comprising an amino acid sequence selected from the group consisting of SEQ ID NOs: 377, 391-394, and 603, or a variant thereof comprising up to about 3 amino acid substitutions, and a CDR-H3 comprising an amino acid sequence selected from the group consisting of SEQ ID NOs: 378, 395, 604, and 605, or a variant thereof comprising up to about 3 amino acid substitutions; and the VL comprises a CDR-L1 comprising an amino acid sequence selected from the group consisting of SEQ ID NOs: 396-398, and 606-609, or a variant thereof comprising up to about 3 amino acid substitutions, a CDR-L2 comprising an amino acid sequence selected from the group consisting of SEQ ID NOs: 380 and 399, or a variant thereof comprising up to about 3 amino acid substitutions, and a CDR-L3 comprising an amino acid sequence selected from the group consisting of SEQ ID NOs: 381, 400-401, and 610, or a variant thereof comprising up to about 3 amino acid substitutions. In some examples the VH comprises a CDR-H1 comprising an amino acid sequence selected from the group consisting of SEQ ID NOs: 376 and 390, or a variant thereof comprising up to about 3 amino acid substitutions, a CDR-H2 comprising an amino acid sequence selected from the group consisting of SEQ ID NOs: 391-394, or a variant thereof comprising up to about 3 amino acid substitutions, and a CDR-H3 comprising an amino acid sequence selected from the group consisting of SEQ ID NOs: 378 and 395, or a variant thereof comprising up to about 3 amino acid substitutions; and the VL comprises a CDR-L1 comprising an amino acid sequence selected from the group consisting of SEQ ID NOs: 396-398, or a variant thereof comprising up to about 3 amino acid substitutions, a CDR-L2 comprising an amino acid sequence selected from the group consisting of SEQ ID NOs: 380 and 399, or a variant thereof comprising up to about 3 amino acid substitutions, and a CDR-L3 comprising an amino acid sequence selected from the group consisting of SEQ ID NOs: 381, and 400-401, or a variant thereof comprising up to about 3 amino acid substitutions. In some examples, the VH comprises a CDR-H1 comprising the amino acid sequence of SEQ ID NO: 382, a CDR-H2 comprising the amino acid sequence of SEQ ID NO: 383, and a CDR-H3 comprising the amino acid sequence of SEQ ID NO: 384; and a VL comprising a CDR-L1 comprising the amino acid sequence of SEQ ID NO: 385, a CDR-L2 comprising the amino acid sequence of SEQ ID NO: 386, and a CDR-L3 comprising the amino acid sequence of SEQ ID NO: 387. In some examples, the VH comprises a CDR-H1 comprising an amino acid sequence selected from the group consisting of SEQ ID NOs: 376 and

390, a CDR-H2 comprising an amino acid sequence selected from the group consisting of SEQ ID NOs: 391-394, and a CDR-H3 comprising an amino acid sequence selected from the group consisting of SEQ ID NOs: 378 and 395; and the VL comprises a CDR-L1 comprising an amino acid sequence selected from the group consisting of SEQ ID NOs: 396-398, a CDR-L2 comprising an amino acid sequence selected from the group consisting of SEQ ID NOs: 380 and 399, and a CDR-L3 comprising an amino acid sequence selected from the group consisting of SEQ ID NOs: 381, and 400-401. In some examples, the VH comprises a CDR-H1 comprising the amino acid sequence of SEQ ID NO: 376, a CDR-H2 comprising the amino acid sequence of SEQ ID NO: 391, and a CDR-H3 comprising the amino acid sequence of SEQ ID NO: 378; and the VL comprises a CDR-L1 comprising the amino acid sequence of SEQ ID NO: 396, a CDR-L2 comprising the amino acid sequence of SEQ ID NO: 380, and a CDR-L3 comprising the amino acid sequence of SEQ ID NO: 381. In some examples, the VH comprises a CDR-H1 comprising the amino acid sequence of SEQ ID NO: 390, a CDR-H2 comprising the amino acid sequence of SEQ ID NO: 392, and a CDR-H3 comprising the amino acid sequence of SEQ ID NO: 395; and the VL comprises a CDR-L1 comprising the amino acid sequence of SEQ ID NO: 397, a CDR-L2 comprising the amino acid sequence of SEQ ID NO: 380, and a CDR-L3 comprising the amino acid sequence of SEQ ID NO: 400. In some examples, the VH comprises a CDR-H1 comprising the amino acid sequence of SEQ ID NO: 390, a CDR-H2 comprising the amino acid sequence of SEQ ID NO: 392, and a CDR-H3 comprising the amino acid sequence of SEQ ID NO: 395; and the VL comprises a CDR-L1 comprising the amino acid sequence of SEQ ID NO: 396, a CDR-L2 comprising the amino acid sequence of SEQ ID NO: 380, and a CDR-L3 comprising the amino acid sequence of SEQ ID NO: 401. In some examples, the VH comprises a CDR-H1 comprising the amino acid sequence of SEQ ID NO: 390, a CDR-H2 comprising the amino acid sequence of SEQ ID NO: 393, and a CDR-H3 comprising the amino acid sequence of SEQ ID NO: 395; and the VL comprises a CDR-L1 comprising the amino acid sequence of SEQ ID NO: 397, a CDR-L2 comprising the amino acid sequence of SEQ ID NO: 380, and a CDR-L3 comprising the amino acid sequence of SEQ ID NO: 381. In some examples, the VH comprises a CDR-H1 comprising the amino acid sequence of SEQ ID NO: 376, a CDR-H2 comprising the amino acid sequence of SEQ ID NO: 393, and a CDR-H3 comprising the amino acid sequence of SEQ ID NO: 395; and the VL comprises a CDR-L1 comprising the amino acid sequence of SEQ ID NO:

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comprising the amino acid sequence of SEQ ID NO: 397, a CDR-L2 comprising the amino acid sequence of SEQ ID NO: 380, and a CDR-L3 comprising the amino acid sequence of SEQ ID NO: 400. In some examples, the VH comprises a CDR-H1 comprising the amino acid sequence of SEQ ID NO: 390, a CDR-H2 comprising the amino acid sequence of SEQ ID NO: 394, and a CDR-H3 comprising the amino acid sequence of SEQ ID NO: 395; and the VL comprises a CDR-L1 comprising the amino acid sequence of SEQ ID NO: 396, a CDR-L2 comprising the amino acid sequence of SEQ ID NO: 380, and a CDR-L3 comprising the amino acid sequence of SEQ ID NO: 381. In some examples, the VH comprises a CDR-H1 comprising the amino acid sequence of SEQ ID NO: 390, a CDR-H2 comprising the amino acid sequence of SEQ ID NO: 393, and a CDR-H3 comprising the amino acid sequence of SEQ ID NO: 378; and the VL comprises a CDR-L1 comprising the amino acid sequence of SEQ ID NO: 396, a CDR-L2 comprising the amino acid sequence of SEQ ID NO: 380, and a CDR-L3 comprising the amino acid sequence of SEQ ID NO: 381. In some examples, the VH comprises a CDR-H1 comprising the amino acid sequence of SEQ ID NO: 390, a CDR-H2 comprising the amino acid sequence of SEQ ID NO: 391, and a CDR-H3 comprising the amino acid sequence of SEQ ID NO: 378; and the VL comprises a CDR-L1 comprising the amino acid sequence of SEQ ID NO: 396, a CDR-L2 comprising the amino acid sequence of SEQ ID NO: 380, and a CDR-L3 comprising the amino acid sequence of SEQ ID NO: 381. In some examples, the VH comprises a CDR-H1 comprising the amino acid sequence of SEQ ID NO: 390, a CDR-H2 comprising the amino acid sequence of SEQ ID NO: 391, and a CDR-H3 comprising the amino acid sequence of SEQ ID NO: 378; and the VL comprises a CDR-L1 comprising the amino acid sequence of SEQ ID NO: 397, a CDR-L2 comprising the amino acid sequence of SEQ ID NO: 380, and a CDR-L3 comprising the amino acid sequence of SEQ ID NO: 381. In some examples, the VH comprises the amino acid sequence according to Formula (VII):

EVQLVESGGGLVX₁PGGSLRLSCAASGFTFX₂X₃YAIX₄WVRQAPGKGLEWVX₅RIRSKY
NNYATYYAX₆SVKX₇RFTISRDX₈SKNTLYLQX₉NSLRAEDTAVYYCX₁₀RHGNX₁₁GX₁₂S
YVSWFAYWGQGTLLTVSS (SEQ ID NO: 388), wherein X₁ is K or Q, X₂ is N or S, X₃ is S or T, X₄ is H or N, X₅ is G or S, X₆ is D or E, X₇ is D or G, X₈ is D or N, X₉ is I or L, X₁₀ is A or V, X₁₁ is F or Y, X₁₂ is N or T; and the VL comprises the amino acid sequence according to Formula (VIII):

X_1 AVVTQEPSLTVSPGGTVTLTC X_2 SSTGAVTTSNY X_3 NWX X_4 QQKPGQAPRGLIGGT X_5 X_6
 RAPGX X_7 PARFSGSLLGGKAALTLGAQPEDEAEYYCALWYS X_8 X_9 WVFGGGTKLTVL
 (SEQ ID NO: 389), wherein X_1 is E or Q, X_2 is A, G, P, or R, X_3 is A or P, X_4 is F or V, X_5 is K or N, X_6 is F or K, X_7 is A, I, T, or V, X_8 is A, D, N, or T, and X_9 is H or L. In some examples, the VH comprises an amino acid sequence selected from the group consisting of SEQ ID NOs: 67, 402, 405, 407, 409, 410, 412, 414-416, and 611-640, or a variant thereof having at least about 80% sequence identity to an amino acid sequence selected from the group consisting of SEQ ID NOs: 67, 402, 405, 407, 409, 410, 412, 414-416, and 611-640; and the VL comprises an amino acid sequence selected from the group consisting of SEQ ID NOs: 68, 403, 404, 406, 408, 411, 413, and 641-666, or a variant thereof having at least about 80% sequence identity to an amino acid sequence selected from the group consisting of SEQ ID NOs: 68, 403, 404, 406, 408, 411, 413, and 641-666. In some examples, the VH comprises an amino acid sequence selected from the group consisting of SEQ ID NOs: 67, 402, 405, 407, 409, 410, 412, 414, 415, and 416; and the VL comprises an amino acid sequence selected from the group consisting of SEQ ID NOs: 68, 403, 404, 406, 408, 411, and 413. In some examples according to any one of the isolated anti-CD3 antibodies or antigen-binding fragments thereof, the VH comprises an amino acid sequence selected from the group consisting of SEQ ID NOs: 402, 405, 407, 409, 410, 412, 414, 415, and 416; and the VL comprises an amino acid sequence selected from the group consisting of SEQ ID NOs: 403, 404, 406, 408, 411, and 413. In some examples, the VH comprises the amino acid sequence of SEQ ID NO: 388, and the VL comprises the amino acid sequence of SEQ ID NO: 389. In some examples, the VH comprises the amino acid sequence of SEQ ID NO: 402, and the VL comprises the amino acid sequence of SEQ ID NO: 403. In some examples, the VH comprises the amino acid sequence of SEQ ID NO: 402, and the VL comprises the amino acid sequence of SEQ ID NO: 404. In some examples, the VH comprises the amino acid sequence of SEQ ID NO: 405, and the VL comprises the amino acid sequence of SEQ ID NO: 406. In some examples, the VH comprises the amino acid sequence of SEQ ID NO: 407, and the VL comprises the amino acid sequence of SEQ ID NO: 404. In some examples, the VH comprises the amino acid sequence of SEQ ID NO: 407, and the VL comprises the amino acid sequence of SEQ ID NO: 403. In some examples, the VH comprises the amino acid sequence of SEQ ID NO: 407, and the VL comprises the amino acid sequence of SEQ ID NO: 408. In some examples, the VH

comprises the amino acid sequence of SEQ ID NO: 409, and the VL comprises the amino acid sequence of SEQ ID NO: 408. In some examples, the VH comprises the amino acid sequence of SEQ ID NO: 410, and the VL comprises the amino acid sequence of SEQ ID NO: 411. In some examples, the VH comprises the amino acid sequence of SEQ ID NO: 412, and the VL comprises the amino acid sequence of SEQ ID NO: 413. In some examples, the VH comprises the amino acid sequence of SEQ ID NO: 410, and the VL comprises the amino acid sequence of SEQ ID NO: 413. In some examples, the VH comprises the amino acid sequence of SEQ ID NO: 414, and the VL comprises the amino acid sequence of SEQ ID NO: 403. In some examples, the VH comprises the amino acid sequence of SEQ ID NO: 415, and the VL comprises the amino acid sequence of SEQ ID NO: 413. In some examples, the VH comprises the amino acid sequence of SEQ ID NO: 416, and the VL comprises the amino acid sequence of SEQ ID NO: 413. In some examples, the VH comprises the amino acid sequence of SEQ ID NO: 416, and the VL comprises the amino acid sequence of SEQ ID NO: 411.

[0030] In some examples according to any one of the isolated anti-CD3 antibodies or antigen-binding fragments thereof, the anti-CD3 antibody further comprises a second antigen-binding fragment that specifically binds a target antigen. In some examples, the target antigen is a tumor antigen. In some examples, the tumor antigen is selected from the group consisting of CD19, CD20, EpCAM, CEA, PSMA, CD33, EGFR, HER2, EphA2, MCSP, ADAM17, PSCA, 17-A1, NKG2D, TROP2, CD79B, Nectin-4, BCMA, CD22, CD38, EGFR, GD2, SLAMF7, CD30, EpCAM, MUC1, MUC16, CD123, CD37, FOLR1, MET, FLT3, GPC3, CEACAM5, CLDN18, CSF1, Integrin alpha 5, NCAM1, PTPRC, CD138, NaPi2b, MSLN, DLL3, GPRC5D, GPNMB, ICAM1, SSTR2, carcinoma associated antigen CTAA16, CA9, ENG, ACVRL1, CD80, CSPG4, EGFL7, FLT1, HAVCR1, HGF, HLA-DRB, IGF1R, TPBG, ERBB3, and STEAP2. In some examples, the tumor antigen is HER2. In some examples, the tumor antigen is CD20. In some examples, the tumor antigen is TROP2. In some examples, the tumor antigen is BCMA. In some examples, the tumor antigen is CD19.

[0031] One example of the present application provides an activatable antibody (“activatable anti-CD3 antibody”), comprising, from N-terminus to C-terminus, a masking moiety (MM), a cleavable moiety (CM), and a CD3-binding moiety, wherein: a) the CD3-binding moiety comprises a VL and the activatable antibody further comprises a second polypeptide comprising a VH; b) the CD3-binding moiety comprises a VH and the activatable antibody further comprises

a second polypeptide comprising a VL; c) the CD3-binding moiety comprises from the N-terminus to the C-terminus, a VL and a VH; or d) the CD3-binding moiety comprise from the N-terminus to the C-terminus, a VH and a VL; wherein the CM comprises a cleavage site; wherein the MM inhibits binding of the activatable antibody to CD3 when the CM is not cleaved; wherein the activatable antibody binds CD3 via the VH and the VL when the CM is cleaved; and wherein the activatable antibody binds CD3 with half-maximal binding at a concentration of antibody (EC_{50}) that is at least 10 nM (*e.g.*, at least 50 nM, or at least 100 nM, or about 110 nM) as determined by an enzyme-linked immunosorbent assay (ELISA). In some examples, the first antigen-binding fragment is a scFv, such as an isolated anti-CD3 scFv, an isolated anti-CD3 scFv-Fc fusion protein, or an anti-CD3 scFv fragment in a multispecific (*e.g.*, bispecific) antibody or an activatable multispecific antibody in an activated form (*i.e.*, with CM1 cleaved), when used to determine the EC_{50} . In some examples, the EC_{50} is determined using the ELISA assay as described in Example 5.

[0032] In some examples according to any one of the activatable anti-CD3 antibodies described above, the first antigen-binding fragment binds CD3 with a dissociation constant (K_d) of at least 50 nM. In some examples, the first antigen-binding fragment is a scFv, such as an isolated anti-CD3 scFv, an isolated anti-CD3 scFv-Fc fusion protein, or an anti-CD3 scFv fragment in a multispecific (*e.g.*, bispecific) antibody or an activatable multispecific antibody in an activated form (*i.e.*, with CM1 cleaved), when used to determine the K_d .

[0033] In some examples according to any one of the activatable anti-CD3 antibodies described above, the MM comprises the amino acid sequence of EVGSY (SEQ ID NO: 667) at the N-terminus of the MM. In some examples, the MM comprises an amino acid sequence according to Formula (IX): PYDDPDCPSHX₁SDCDX₂ (SEQ ID NO: 668), wherein X₁ is D or E, and X₂ is N or Q. In some examples, the MM comprises an amino acid sequence according to Formula (X): X₁X₂X₃DX₄X₅CX₆X₇DX₈X₉X₁₀CX₁₁X₁₂ (SEQ ID NO: 669), wherein X₁ is A or D, X₂ is A, D, or P, X₃ is D, H, or P, X₄ is F or P, X₅ is D or P, X₆ is D or P, X₇ is A or P, X₈ is D, N, or P, X₉ is A, N, or P, X₁₀ is D, H, or S, X₁₁ is H, P, or Y, and X₁₂ is N, P, or Y. In some examples, the MM comprises the amino acid sequence of SEQ ID NO: 35. In some examples, the MM comprises the amino acid sequence of SEQ ID NO: 417. In some examples, the MM comprises an amino acid sequence selected from the group consisting of SEQ ID NOs: 585-588 and 597-591. In some examples, the CD3 is human CD3.

[0034] One example of the present application provides an activatable antibody (“activatable anti-CD3 antibody”), comprising, from N-terminus to C-terminus, a masking moiety (MM), a cleavable moiety (CM), and a CD3-binding moiety, wherein: a) the CD3-binding moiety comprises a VL and the activatable antibody further comprises a second polypeptide comprising a VH; b) the CD3-binding moiety comprises a VH and the activatable antibody further comprises a second polypeptide comprising a VL; c) the CD3-binding moiety comprises from the N-terminus to the C-terminus, a VL and a VH; or d) the CD3-binding moiety comprise from the N-terminus to the C-terminus, a VH and a VL; wherein the CM comprises a cleavage site; wherein the MM inhibits binding of the activatable antibody to CD3 when the CM is not cleaved; wherein the activatable antibody binds CD3 via the VH and the VL when the CM is cleaved; and wherein a) the MM comprises the amino acid sequence of EVGSY (SEQ ID NO: 667) at the N-terminus of the MM; b) the MM comprises an amino acid sequence according to Formula (IX): PYDDPDCPSHX₁SDCDX₂ (SEQ ID NO: 668), wherein X₁ is D or E, and X₂ is N or Q; or c) the MM comprises an amino acid sequence according to Formula (X): X₁X₂X₃DX₄X₅CX₆X₇DX₈X₉X₁₀CX₁₁X₁₂ (SEQ ID NO: 669), wherein X₁ is A or D, X₂ is A, D, or P, X₃ is D, H, or P, X₄ is F or P, X₅ is D or P, X₆ is D or P, X₇ is A or P, X₈ is D, N, or P, X₉ is A, N, or P, X₁₀ is D, H, or S, X₁₁ is H, P, or Y, and X₁₂ is N, P, or Y. In some examples, the MM comprises the amino acid sequence of SEQ ID NOs: 35, 417, 585-588, and 597-599. In some examples, the CD3 is human CD3.

[0035] In some examples according to any one of the activatable anti-CD3 antibodies described above, the activatable anti-CD3 antibody comprises an anti-CD3 antigen-binding fragment selected from the group consisting of a Fab, a Fv, a scFab and a scFv. In some examples, the anti-CD3 antigen-binding fragment is a scFv. In some examples, the scFv comprises from the N-terminus to the C-terminus, the VL, a linker and the VH.

[0036] In some examples according to any one of the activatable anti-CD3 antibodies described above, the VH comprising a heavy chain complementarity determining region (CDR-H) 1 comprising the amino acid sequence according to Formula (I): X₁YAX₂X₃ (SEQ ID NO: 382), wherein X₁ is D, S, or T, X₂ is I, L, or M, and X₃ is N or T, a CDR-H2 comprising the amino acid sequence according to Formula (II): RIRSKYNNYATYYAX₁X₂VKX₃ (SEQ ID NO: 383), wherein X₁ is D or E, X₂ is S or T, and X₃ is D, G, or S, and a CDR-H3 comprising the amino acid sequence according to Formula (III): HGNX₁GX₂SYVSX₃X₄AY (SEQ ID NO:

384), wherein X_1 is F or Y, X_2 is N or T, X_3 is W or Y, and X_4 is F or W; and b) the VL comprising a CDR-L1 comprising the amino acid sequence according to Formula (IV): $X_1SSTGAVTX_2X_3NYX_4N$ (SEQ ID NO: 385), wherein X_1 is A, G, or R, X_2 is S or T, X_3 is G or S, and X_4 is A, P, or V, a CDR-L2 comprising the amino acid sequence according to Formula (V): GTX_1X_2RAP (SEQ ID NO: 386), wherein X_1 is K or N, and X_2 is F or K, and a CDR-L3 comprising the amino acid sequence according to Formula (VI): $ALWYSX_1X_2WV$ (SEQ ID NO: 387), wherein X_1 is D, N, or T, and X_2 is L or R. In some examples the VH comprises a heavy chain complementarity determining region (CDR-H) 1 comprising an amino acid sequence selected from the group consisting of SEQ ID NOs: 376, 390, 601, and 602, or a variant thereof comprising up to about 3 amino acid substitutions, a CDR-H2 comprising an amino acid sequence selected from the group consisting of SEQ ID NOs: 377, 391-394, and 603, or a variant thereof comprising up to about 3 amino acid substitutions, and a CDR-H3 comprising an amino acid sequence selected from the group consisting of SEQ ID NOs: 378, 395, 604, and 605, or a variant thereof comprising up to about 3 amino acid substitutions; and the VL comprises a CDR-L1 comprising an amino acid sequence selected from the group consisting of SEQ ID NOs: 396-398, and 606-609, or a variant thereof comprising up to about 3 amino acid substitutions, a CDR-L2 comprising an amino acid sequence selected from the group consisting of SEQ ID NOs: 380 and 399, or a variant thereof comprising up to about 3 amino acid substitutions, and a CDR-L3 comprising an amino acid sequence selected from the group consisting of SEQ ID NOs: 381, 400-401, and 610, or a variant thereof comprising up to about 3 amino acid substitutions. In some examples the VH comprises a CDR-H1 comprising an amino acid sequence selected from the group consisting of SEQ ID NOs: 376 and 390, or a variant thereof comprising up to about 3 amino acid substitutions, a CDR-H2 comprising an amino acid sequence selected from the group consisting of SEQ ID NOs: 391-394, or a variant thereof comprising up to about 3 amino acid substitutions, and a CDR-H3 comprising an amino acid sequence selected from the group consisting of SEQ ID NOs: 378 and 395, or a variant thereof comprising up to about 3 amino acid substitutions; and the VL comprises a CDR-L1 comprising an amino acid sequence selected from the group consisting of SEQ ID NOs: 396-398, or a variant thereof comprising up to about 3 amino acid substitutions, a CDR-L2 comprising an amino acid sequence selected from the group consisting of SEQ ID NOs: 380 and 399, or a variant thereof comprising up to about 3 amino acid substitutions, and a CDR-L3

comprising an amino acid sequence selected from the group consisting of SEQ ID NOs: 381, and 400-401, or a variant thereof comprising up to about 3 amino acid substitutions. In some examples, the VH comprises a CDR-H1 comprising the amino acid sequence of SEQ ID NO: 382, a CDR-H2 comprising the amino acid sequence of SEQ ID NO: 383, and a CDR-H3 comprising the amino acid sequence of SEQ ID NO: 384; and the VL comprises a CDR-L1 comprising the amino acid sequence of SEQ ID NO: 385, a CDR-L2 comprising the amino acid sequence of SEQ ID NO: 386, and a CDR-L3 comprising the amino acid sequence of SEQ ID NO: 387. In some examples, the VH comprises a CDR-H1 comprising an amino acid sequence selected from the group consisting of SEQ ID NOs: 376 and 390, a CDR-H2 comprising an amino acid sequence selected from the group consisting of SEQ ID NOs: 391-394, and a CDR-H3 comprising an amino acid sequence selected from the group consisting of SEQ ID NOs: 378 and 395; and the VL comprises a CDR-L1 comprising an amino acid sequence selected from the group consisting of SEQ ID NOs: 396-398, a CDR-L2 comprising an amino acid sequence selected from the group consisting of SEQ ID NOs: 380 and 399, and a CDR-L3 comprising an amino acid sequence selected from the group consisting of SEQ ID NOs: 381, and 400-401. In some examples, the VH comprises a CDR-H1 comprising the amino acid sequence of SEQ ID NO: 376, a CDR-H2 comprising the amino acid sequence of SEQ ID NO: 391, and a CDR-H3 comprising the amino acid sequence of SEQ ID NO: 378; and the VL comprises a CDR-L1 comprising the amino acid sequence of SEQ ID NO: 396, a CDR-L2 comprising the amino acid sequence of SEQ ID NO: 380, and a CDR-L3 comprising the amino acid sequence of SEQ ID NO: 381. In some examples, the VH comprises a CDR-H1 comprising the amino acid sequence of SEQ ID NO: 390, a CDR-H2 comprising the amino acid sequence of SEQ ID NO: 392, and a CDR-H3 comprising the amino acid sequence of SEQ ID NO: 395; and the VL comprises a CDR-L1 comprising the amino acid sequence of SEQ ID NO: 397, a CDR-L2 comprising the amino acid sequence of SEQ ID NO: 380, and a CDR-L3 comprising the amino acid sequence of SEQ ID NO: 400. In some examples, the VH comprises a CDR-H1 comprising the amino acid sequence of SEQ ID NO: 390, a CDR-H2 comprising the amino acid sequence of SEQ ID NO: 392, and a CDR-H3 comprising the amino acid sequence of SEQ ID NO: 395; and the VL comprises a CDR-L1 comprising the amino acid sequence of SEQ ID NO: 396, a CDR-L2 comprising the amino acid sequence of SEQ ID NO: 380, and a CDR-L3 comprising the amino acid sequence of SEQ ID NO: 401. In some

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380, and a CDR-L3 comprising the amino acid sequence of SEQ ID NO: 381. In some examples, the VH comprises the amino acid sequence of SEQ ID NO: 388, and the VL comprises the amino acid sequence of SEQ ID NO: 389.

[0037] In some examples according to any one of the activatable anti-CD3 antibodies described above, the VH comprises the amino acid sequence according to Formula (VII):

EVQLVESGGGLVX₁PGGSLRLSCAASGFTFX₂X₃YAIX₄WVRQAPGKGLEWVX₅RIRSKY
NNYATYYAX₆SVKX₇RFTISRDX₈SKNTLYLQX₉NSLRAEDTAVYYCX₁₀RHGNX₁₁GX₁₂S
YVSWFAYWGQGTLLTVSS (SEQ ID NO: 388), wherein X₁ is K or Q, X₂ is N or S, X₃ is S or T, X₄ is H or N, X₅ is G or S, X₆ is D or E, X₇ is D or G, X₈ is D or N, X₉ is I or L, X₁₀ is A or V, X₁₁ is F or Y, X₁₂ is N or T; and the VL comprises the amino acid sequence according to Formula (VIII):

X₁AVVTQEPSLTVSPGGTVTLTCX₂SSTGAVTTSNYX₃NWX₄QKPGQAPRGLIGGTGX₅X₆
RAPGX₇PARFSGSLLGGKAALTLSGAQPEDEAEYYCALWYSX₈X₉WVFGGGTKLTVL
(SEQ ID NO: 389), wherein X₁ is E or Q, X₂ is A, G, P, or R, X₃ is A or P, X₄ is F or V, X₅ is K or N, X₆ is F or K, X₇ is A, I, T, or V, X₈ is A, D, N, or T, and X₉ is H or L. In some examples, the VH comprises an amino acid sequence selected from the group consisting of SEQ ID NOs: 67, 402, 405, 407, 409, 410, 412, 414-416, and 611-640, or a variant thereof having at least about 80% sequence identity to an amino acid sequence selected from the group consisting of SEQ ID NOs: 67, 402, 405, 407, 409, 410, 412, 414-416, and 611-640; and the VL comprises an amino acid sequence selected from the group consisting of SEQ ID NOs: 68, 403, 404, 406, 408, 411, 413, and 641-666, or a variant thereof having at least about 80% sequence identity to an amino acid sequence selected from the group consisting of SEQ ID NOs: 68, 403, 404, 406, 408, 411, 413, and 641-666. In some examples, the VH comprises an amino acid sequence selected from the group consisting of SEQ ID NOs: 67, 402, 405, 407, 409, 410, 412, 414, 415, and 416; and the VL comprises an amino acid sequence selected from the group consisting of SEQ ID NOs: 68, 403, 404, 406, 408, 411, and 413.

[0038] In some examples according to any one of the activatable anti-CD3 antibodies described above, the VH comprises an amino acid sequence selected from the group consisting of SEQ ID NOs: 402, 405, 407, 409, 410, 412, 414, 415, and 416; and the VL comprises an amino acid sequence selected from the group consisting of SEQ ID NOs: 403, 404, 406, 408, 411, and 413. In some examples, the VH comprises the amino acid sequence of SEQ ID NO:

402, and the VL comprises the amino acid sequence of SEQ ID NO: 403. In some examples, the VH comprises the amino acid sequence of SEQ ID NO: 402, and the VL comprises the amino acid sequence of SEQ ID NO: 404. In some examples, the VH comprises the amino acid sequence of SEQ ID NO: 405, and the VL comprises the amino acid sequence of SEQ ID NO: 406. In some examples, the VH comprises the amino acid sequence of SEQ ID NO: 407, and the VL comprises the amino acid sequence of SEQ ID NO: 404. In some examples, the VH comprises the amino acid sequence of SEQ ID NO: 407, and the VL comprises the amino acid sequence of SEQ ID NO: 403. In some examples, the VH comprises the amino acid sequence of SEQ ID NO: 407, and the VL comprises the amino acid sequence of SEQ ID NO: 408. In some examples, the VH comprises the amino acid sequence of SEQ ID NO: 409, and the VL comprises the amino acid sequence of SEQ ID NO: 408. In some examples, the VH comprises the amino acid sequence of SEQ ID NO: 410, and the VL comprises the amino acid sequence of SEQ ID NO: 411. In some examples, the VH comprises the amino acid sequence of SEQ ID NO: 412, and the VL comprises the amino acid sequence of SEQ ID NO: 413. In some examples, the VH comprises the amino acid sequence of SEQ ID NO: 410, and the VL comprises the amino acid sequence of SEQ ID NO: 413. In some examples, the VH comprises the amino acid sequence of SEQ ID NO: 414, and the VL comprises the amino acid sequence of SEQ ID NO: 403. In some examples, the VH comprises the amino acid sequence of SEQ ID NO: 415, and the VL comprises the amino acid sequence of SEQ ID NO: 413. In some examples, the VH comprises the amino acid sequence of SEQ ID NO: 416, and the VL comprises the amino acid sequence of SEQ ID NO: 413. In some examples, the VH comprises the amino acid sequence of SEQ ID NO: 416, and the VL comprises the amino acid sequence of SEQ ID NO: 411. In some examples, the CD3-binding moiety comprises the amino acid sequence of SEQ ID NO: 421 or SEQ ID NO: 422.

[0039] In some examples according to any one of the activatable anti-CD3 antibodies described above, the CM comprises an amino acid sequence selected from the group consisting of SEQ ID NOs: 77, 127-129, 418, 420, 431 and 477-490, and 516-555. In some examples, the CM comprises the amino acid sequence of SEQ ID NO: 77 or 418.

[0040] One example of the present application provides a masked antibody (“masked anti-CD3 antibody”), comprising, from N-terminus to C-terminus, a masking moiety (MM), and a CD3-binding moiety, wherein: a) the CD3-binding moiety comprises a VL and the activatable

antibody further comprises a second polypeptide comprising a VH; b) the CD3-binding moiety comprises a VH and the activatable antibody further comprises a second polypeptide comprising a VL; c) the CD3-binding moiety comprises from the N-terminus to the C-terminus, a VL and a VH; or d) the CD3-binding moiety comprise from the N-terminus to the C-terminus, a VH and a VL; wherein the MM competes with CD3 to specifically bind the CD3-binding moiety; wherein the activatable antibody binds CD3 via the VH and the VL; and wherein the masked antibody binds CD3 with half-maximal binding at a concentration of antibody (EC_{50}) that is at least 10 nM (*e.g.*, at least 50 nM, or at least 100 nM, or about 110 nM) as determined by an enzyme-linked immunosorbent assay (ELISA). In some examples, the masked anti-CD3 antibody is an activatable antibody. In some examples, the masked anti-CD3 antibody comprises, from N-terminus to C-terminus, the masking moiety (MM), a cleavable moiety (CM), and the CD3-binding moiety. In some examples, the masked anti-CD3 antibody is a not an activatable antibody. In some examples, the masked anti-CD3 antibody comprises, from N-terminus to C-terminus, the masking moiety (MM), a non-cleavable linker (NCL), and the CD3-binding moiety. One example of the present application provides a masked antibody (“masked anti-CD3 antibody”), comprising a masking moiety (MM) and an antibody or antigen-binding fragment that binds CD3, wherein the antibody or antigen-binding fragment comprises a VH and a VL; wherein the masked antibody comprises a single polypeptide chain and the VH and the VL of the antibody or antigen-binding fragment are part of the single polypeptide chain, or the masked antibody comprises two polypeptide chains, and the VH and the VL of the antibody or antigen-binding fragment are part of different polypeptide chains of the masked antibody; wherein the C-terminus of the MM is fused to the N-terminus of the VH or the VL of the antibody or antigen-binding fragment; wherein the MM competes with CD3 to specifically bind the antibody or antigen-binding fragment; and wherein the antibody or antigen-binding fragment binds CD3 with half-maximal binding at a concentration of antibody (EC_{50}) that is at least 10 nM (*e.g.*, at least 50 nM, or at least 100 nM, or about 110 nM) as determined by an enzyme-linked immunosorbent assay (ELISA). In some examples, the masked antibody comprises an amino acid linker between the C-terminus of the MM and the N-terminus of the VH or the VL of the antibody or antigen-binding fragment. In some examples, the masked antibody further comprises a cleavable linker, *e.g.*, between the C-terminus of the MM and the N-terminus of the VH or the VL of the antibody or antigen-binding fragment. In some examples, the masked antibody

does not comprise a cleavable linker (*e.g.*, fused to the MM, or between the C-terminus of the MM and the N-terminus of the antibody or fragment).

[0041] One example of the present application provides a masked antibody (“masked anti-CD3 antibody”), comprising, from N-terminus to C-terminus, a masking moiety (MM), a non-cleavable linker (NCL), and a CD3-binding moiety, wherein: a) the CD3-binding moiety comprises a VL and the activatable antibody further comprises a second polypeptide comprising a VH; b) the CD3-binding moiety comprises a VH and the activatable antibody further comprises a second polypeptide comprising a VL; c) the CD3-binding moiety comprises from the N-terminus to the C-terminus, a VL and a VH; or d) the CD3-binding moiety comprise from the N-terminus to the C-terminus, a VH and a VL; wherein the MM competes with CD3 to specifically bind the CD3-binding moiety; wherein the activatable antibody binds CD3 via the VH and the VL; and wherein the masked antibody binds CD3 with half-maximal binding at a concentration of antibody (EC_{50}) that is at least 10 nM (*e.g.*, at least 50 nM, or at least 100 nM, or about 110 nM) as determined by an enzyme-linked immunosorbent assay (ELISA). In some examples, the first antigen-binding fragment is a scFv, such as an isolated anti-CD3 scFv, an isolated anti-CD3 scFv-Fc fusion protein, or an anti-CD3 scFv fragment in a multispecific (*e.g.*, bispecific) antibody or multispecific antibody in an un-masked form (*i.e.*, without the MM), when used to determine the EC_{50} . In some examples, the EC_{50} is determined using the ELISA assay as described in Example 5.

[0042] In some examples according to any one of the masked anti-CD3 antibodies described above, the first antigen-binding fragment binds CD3 with a dissociation constant (K_d) of at least 50 nM. In some examples, the first antigen-binding fragment is a scFv, such as an isolated anti-CD3 scFv, an isolated anti-CD3 scFv-Fc fusion protein, or an anti-CD3 scFv fragment in a multispecific (*e.g.*, bispecific) antibody or a multispecific antibody in an un-masked form (*i.e.*, without the MM), when used to determine the K_d .

[0043] In some examples according to any one of the masked anti-CD3 antibodies described above, the MM comprises the amino acid sequence of EVGSY (SEQ ID NO: 667) at the N-terminus of the MM. In some examples, the MM comprises an amino acid sequence according to Formula (IX): PYDDPDCPSHX₁SDCDX₂ (SEQ ID NO: 668), wherein X₁ is D or E, and X₂ is N or Q. In some examples, the MM comprises an amino acid sequence according to Formula (X): X₁X₂X₃DX₄X₅CX₆X₇DX₈X₉X₁₀CX₁₁X₁₂ (SEQ ID NO: 669), wherein

X₁ is A or D, X₂ is A, D, or P, X₃ is D, H, or P, X₄ is F or P, X₅ is D or P, X₆ is D or P, X₇ is A or P, X₈ is D, N, or P, X₉ is A, N, or P, X₁₀ is D, H, or S, X₁₁ is H, P, or Y, and X₁₂ is N, P, or Y. In some examples, the MM comprises the amino acid sequence of SEQ ID NO: 35. In some examples, the MM comprises the amino acid sequence of SEQ ID NO: 417. In some examples, the MM comprises an amino acid sequence selected from the group consisting of SEQ ID NOs: 585-588 and 597-591. In some examples, the CD3 is human CD3.

[0044] One example of the present application provides a masked antibody (“masked anti-CD3 antibody”), comprising, from N-terminus to C-terminus, a masking moiety (MM), and a CD3-binding moiety, wherein: a) the CD3-binding moiety comprises a VL and the activatable antibody further comprises a second polypeptide comprising a VH; b) the CD3-binding moiety comprises a VH and the activatable antibody further comprises a second polypeptide comprising a VL; c) the CD3-binding moiety comprises from the N-terminus to the C-terminus, a VL and a VH; or d) the CD3-binding moiety comprise from the N-terminus to the C-terminus, a VH and a VL; wherein the MM competes with CD3 to specifically bind the CD3-binding moiety; wherein the activatable antibody binds CD3 via the VH and the VL; and wherein a) the MM comprises the amino acid sequence of EVGSY (SEQ ID NO: 667) at the N-terminus of the MM; b) the MM comprises an amino acid sequence according to Formula (IX): PYDDPDCPSHX₁SDCDX₂ (SEQ ID NO: 668), wherein X₁ is D or E, and X₂ is N or Q; or c) the MM comprises an amino acid sequence according to Formula (X): X₁X₂X₃DX₄X₅CX₆X₇DX₈X₉X₁₀CX₁₁X₁₂ (SEQ ID NO: 669), wherein X₁ is A or D, X₂ is A, D, or P, X₃ is D, H, or P, X₄ is F or P, X₅ is D or P, X₆ is D or P, X₇ is A or P, X₈ is D, N, or P, X₉ is A, N, or P, X₁₀ is D, H, or S, X₁₁ is H, P, or Y, and X₁₂ is N, P, or Y. In some examples, the masked anti-CD3 antibody is an activatable antibody. In some examples, the masked anti-CD3 antibody comprises, from N-terminus to C-terminus, the masking moiety (MM), a cleavable moiety (CM), and the CD3-binding moiety. In some examples, the masked anti-CD3 antibody is a not an activatable antibody. In some examples, the masked anti-CD3 antibody comprises, from N-terminus to C-terminus, the masking moiety (MM), a non-cleavable linker (NCL), and the CD3-binding moiety.

[0045] One example of the present application provides a masked antibody (“masked anti-CD3 antibody”), comprising, from N-terminus to C-terminus, a masking moiety (MM), a non-cleavable linker (NCL), and a CD3-binding moiety, wherein: a) the CD3-binding moiety comprises a VL and the activatable antibody further comprises a second polypeptide comprising

a VH; b) the CD3-binding moiety comprises a VH and the activatable antibody further comprises a second polypeptide comprising a VL; c) the CD3-binding moiety comprises from the N-terminus to the C-terminus, a VL and a VH; or d) the CD3-binding moiety comprise from the N-terminus to the C-terminus, a VH and a VL; wherein the MM competes with CD3 to specifically bind the CD3-binding moiety; wherein the activatable antibody binds CD3 via the VH and the VL; and wherein a) the MM comprises the amino acid sequence of EVGSY (SEQ ID NO: 667) at the N-terminus of the MM; b) the MM comprises an amino acid sequence according to Formula (IX): PYDDPDCPSHX₁SDCDX₂ (SEQ ID NO: 668), wherein X₁ is D or E, and X₂ is N or Q; or c) the MM comprises an amino acid sequence according to Formula (X): X₁X₂X₃DX₄X₅CX₆X₇DX₈X₉X₁₀CX₁₁X₁₂ (SEQ ID NO: 669), wherein X₁ is A or D, X₂ is A, D, or P, X₃ is D, H, or P, X₄ is F or P, X₅ is D or P, X₆ is D or P, X₇ is A or P, X₈ is D, N, or P, X₉ is A, N, or P, X₁₀ is D, H, or S, X₁₁ is H, P, or Y, and X₁₂ is N, P, or Y. In some examples, the MM comprises the amino acid sequence of SEQ ID NOs: 35, 417, 585-588, and 597-599. In some examples, the CD3 is human CD3.

[0046] In some examples according to any one of the masked anti-CD3 antibodies described above, the activatable anti-CD3 antibody comprises an anti-CD3 antigen-binding fragment selected from the group consisting of a Fab, a Fv, a scFab and a scFv. In some examples, the anti-CD3 antigen-binding fragment is a scFv. In some examples, the scFv comprises from the N-terminus to the C-terminus, the VL, a linker and the VH.

[0047] In some examples according to any one of the masked anti-CD3 antibodies described above, the VH comprising a heavy chain complementarity determining region (CDR-H) 1 comprising the amino acid sequence according to Formula (I): X₁YAX₂X₃ (SEQ ID NO: 382), wherein X₁ is D, S, or T, X₂ is I, L, or M, and X₃ is N or T, a CDR-H2 comprising the amino acid sequence according to Formula (II): RIRSKYNNYATYYAX₁X₂VKX₃ (SEQ ID NO: 383), wherein X₁ is D or E, X₂ is S or T, and X₃ is D, G, or S, and a CDR-H3 comprising the amino acid sequence according to Formula (III): HGNX₁GX₂SYVSX₃X₄AY (SEQ ID NO: 384), wherein X₁ is F or Y, X₂ is N or T, X₃ is W or Y, and X₄ is F or W; and b) the VL comprising a CDR-L1 comprising the amino acid sequence according to Formula (IV): X₁SSTGAVTX₂X₃NYX₄N (SEQ ID NO: 385), wherein X₁ is A, G, or R, X₂ is S or T, X₃ is G or S, and X₄ is A, P, or V, a CDR-L2 comprising the amino acid sequence according to Formula (V): GTX₁X₂RAP (SEQ ID NO: 386), wherein X₁ is K or N, and X₂ is F or K, and a CDR-L3

comprising the amino acid sequence according to Formula (VI): $ALWYSX_1X_2WV$ (SEQ ID NO: 387), wherein X_1 is D, N, or T, and X_2 is L or R. In some examples the VH comprises a heavy chain complementarity determining region (CDR-H) 1 comprising an amino acid sequence selected from the group consisting of SEQ ID NOs: 376, 390, 601, and 602, or a variant thereof comprising up to about 3 amino acid substitutions, a CDR-H2 comprising an amino acid sequence selected from the group consisting of SEQ ID NOs: 377, 391-394, and 603, or a variant thereof comprising up to about 3 amino acid substitutions, and a CDR-H3 comprising an amino acid sequence selected from the group consisting of SEQ ID NOs: 378, 395, 604, and 605, or a variant thereof comprising up to about 3 amino acid substitutions; and the VL comprises a CDR-L1 comprising an amino acid sequence selected from the group consisting of SEQ ID NOs: 396-398, and 606-609, or a variant thereof comprising up to about 3 amino acid substitutions, a CDR-L2 comprising an amino acid sequence selected from the group consisting of SEQ ID NOs: 380 and 399, or a variant thereof comprising up to about 3 amino acid substitutions, and a CDR-L3 comprising an amino acid sequence selected from the group consisting of SEQ ID NOs: 381, 400-401, and 610, or a variant thereof comprising up to about 3 amino acid substitutions. In some examples the VH comprises a CDR-H1 comprising an amino acid sequence selected from the group consisting of SEQ ID NOs: 376 and 390, or a variant thereof comprising up to about 3 amino acid substitutions, a CDR-H2 comprising an amino acid sequence selected from the group consisting of SEQ ID NOs: 391-394, or a variant thereof comprising up to about 3 amino acid substitutions, and a CDR-H3 comprising an amino acid sequence selected from the group consisting of SEQ ID NOs: 378 and 395, or a variant thereof comprising up to about 3 amino acid substitutions; and the VL comprises a CDR-L1 comprising an amino acid sequence selected from the group consisting of SEQ ID NOs: 396-398, or a variant thereof comprising up to about 3 amino acid substitutions, a CDR-L2 comprising an amino acid sequence selected from the group consisting of SEQ ID NOs: 380 and 399, or a variant thereof comprising up to about 3 amino acid substitutions, and a CDR-L3 comprising an amino acid sequence selected from the group consisting of SEQ ID NOs: 381, and 400-401, or a variant thereof comprising up to about 3 amino acid substitutions. In some examples, the VH comprises a CDR-H1 comprising the amino acid sequence of SEQ ID NO: 382, a CDR-H2 comprising the amino acid sequence of SEQ ID NO: 383, and a CDR-H3 comprising the amino acid sequence of SEQ ID NO: 384; and the VL comprises a CDR-L1

comprising the amino acid sequence of SEQ ID NO: 385, a CDR-L2 comprising the amino acid sequence of SEQ ID NO: 386, and a CDR-L3 comprising the amino acid sequence of SEQ ID NO: 387. In some examples, the VH comprises a CDR-H1 comprising an amino acid sequence selected from the group consisting of SEQ ID NOs: 376 and 390, a CDR-H2 comprising an amino acid sequence selected from the group consisting of SEQ ID NOs: 391-394, and a CDR-H3 comprising an amino acid sequence selected from the group consisting of SEQ ID NOs: 378 and 395; and the VL comprises a CDR-L1 comprising an amino acid sequence selected from the group consisting of SEQ ID NOs: 396-398, a CDR-L2 comprising an amino acid sequence selected from the group consisting of SEQ ID NOs: 380 and 399, and a CDR-L3 comprising an amino acid sequence selected from the group consisting of SEQ ID NOs: 381, and 400-401. In some examples, the VH comprises a CDR-H1 comprising the amino acid sequence of SEQ ID NO: 376, a CDR-H2 comprising the amino acid sequence of SEQ ID NO: 391, and a CDR-H3 comprising the amino acid sequence of SEQ ID NO: 378; and the VL comprises a CDR-L1 comprising the amino acid sequence of SEQ ID NO: 396, a CDR-L2 comprising the amino acid sequence of SEQ ID NO: 380, and a CDR-L3 comprising the amino acid sequence of SEQ ID NO: 381. In some examples, the VH comprises a CDR-H1 comprising the amino acid sequence of SEQ ID NO: 390, a CDR-H2 comprising the amino acid sequence of SEQ ID NO: 392, and a CDR-H3 comprising the amino acid sequence of SEQ ID NO: 395; and the VL comprises a CDR-L1 comprising the amino acid sequence of SEQ ID NO: 397, a CDR-L2 comprising the amino acid sequence of SEQ ID NO: 380, and a CDR-L3 comprising the amino acid sequence of SEQ ID NO: 400. In some examples, the VH comprises a CDR-H1 comprising the amino acid sequence of SEQ ID NO: 390, a CDR-H2 comprising the amino acid sequence of SEQ ID NO: 392, and a CDR-H3 comprising the amino acid sequence of SEQ ID NO: 395; and the VL comprises a CDR-L1 comprising the amino acid sequence of SEQ ID NO: 396, a CDR-L2 comprising the amino acid sequence of SEQ ID NO: 380, and a CDR-L3 comprising the amino acid sequence of SEQ ID NO: 401. In some examples, the VH comprises a CDR-H1 comprising the amino acid sequence of SEQ ID NO: 390, a CDR-H2 comprising the amino acid sequence of SEQ ID NO: 393, and a CDR-H3 comprising the amino acid sequence of SEQ ID NO: 395; and the VL comprises a CDR-L1 comprising the amino acid sequence of SEQ ID NO: 397, a CDR-L2 comprising the amino acid sequence of SEQ ID NO: 380, and a CDR-L3 comprising the amino acid sequence of SEQ ID

[illegible]

comprising the amino acid sequence of SEQ ID NO: 381. In some examples, the VH comprises a CDR-H1 comprising the amino acid sequence of SEQ ID NO: 376, a CDR-H2 comprising the amino acid sequence of SEQ ID NO: 391, and a CDR-H3 comprising the amino acid sequence of SEQ ID NO: 378; and the VL comprises a CDR-L1 comprising the amino acid sequence of SEQ ID NO: 397, a CDR-L2 comprising the amino acid sequence of SEQ ID NO: 380, and a CDR-L3 comprising the amino acid sequence of SEQ ID NO: 400. In some examples, the VH comprises a CDR-H1 comprising the amino acid sequence of SEQ ID NO: 390, a CDR-H2 comprising the amino acid sequence of SEQ ID NO: 394, and a CDR-H3 comprising the amino acid sequence of SEQ ID NO: 395; and the VL comprises a CDR-L1 comprising the amino acid sequence of SEQ ID NO: 396, a CDR-L2 comprising the amino acid sequence of SEQ ID NO: 380, and a CDR-L3 comprising the amino acid sequence of SEQ ID NO: 381. In some examples, the VH comprises a CDR-H1 comprising the amino acid sequence of SEQ ID NO: 390, a CDR-H2 comprising the amino acid sequence of SEQ ID NO: 393, and a CDR-H3 comprising the amino acid sequence of SEQ ID NO: 378; and the VL comprises a CDR-L1 comprising the amino acid sequence of SEQ ID NO: 396, a CDR-L2 comprising the amino acid sequence of SEQ ID NO: 380, and a CDR-L3 comprising the amino acid sequence of SEQ ID NO: 381. In some examples, the VH comprises a CDR-H1 comprising the amino acid sequence of SEQ ID NO: 390, a CDR-H2 comprising the amino acid sequence of SEQ ID NO: 391, and a CDR-H3 comprising the amino acid sequence of SEQ ID NO: 378; and the VL comprises a CDR-L1 comprising the amino acid sequence of SEQ ID NO: 396, a CDR-L2 comprising the amino acid sequence of SEQ ID NO: 380, and a CDR-L3 comprising the amino acid sequence of SEQ ID NO: 381. In some examples, the VH comprises a CDR-H1 comprising the amino acid sequence of SEQ ID NO: 390, a CDR-H2 comprising the amino acid sequence of SEQ ID NO: 391, and a CDR-H3 comprising the amino acid sequence of SEQ ID NO: 378; and the VL comprises a CDR-L1 comprising the amino acid sequence of SEQ ID NO: 397, a CDR-L2 comprising the amino acid sequence of SEQ ID NO: 380, and a CDR-L3 comprising the amino acid sequence of SEQ ID NO: 381. In some examples, the VH comprises the amino acid sequence of SEQ ID NO: 388, and the VL comprises the amino acid sequence of SEQ ID NO: 389.

[0048] In some examples according to any one of the masked anti-CD3 antibodies described above, the VH comprises the amino acid sequence according to Formula (VII):

EVQLVESGGGLVX₁PGGSLRLSCAASGFTFX₂X₃YAIX₄WVRQAPGKGLEWVX₅RIRSKY
 NNYATYYAX₆SVKX₇RFTISRDX₈SKNTLYLQX₉NSLRAEDTAVYYCX₁₀RHGNX₁₁GX₁₂S
 YVSWFAYWGQGLTVTVSS (SEQ ID NO: 388), wherein X₁ is K or Q, X₂ is N or S, X₃ is S or
 T, X₄ is H or N, X₅ is G or S, X₆ is D or E, X₇ is D or G, X₈ is D or N, X₉ is I or L, X₁₀ is A or V,
 X₁₁ is F or Y, X₁₂ is N or T; and the VL comprises the amino acid sequence according to
 Formula (VIII):

X₁AVVTQEPSLTVSPGGTVTLTCX₂SSTGAVTTSNYX₃NWX₄QQKPGQAPRGLIGGTXX₅X₆
 RAPGX₇PARFSGSLLGGKAALTLGAQPEDEAEYYCALWYSX₈X₉WVFGGGTKLTVL
 (SEQ ID NO: 389), wherein X₁ is E or Q, X₂ is A, G, P, or R, X₃ is A or P, X₄ is F or V, X₅ is K
 or N, X₆ is F or K, X₇ is A, I, T, or V, X₈ is A, D, N, or T, and X₉ is H or L. In some examples,
 the VH comprises an amino acid sequence selected from the group consisting of SEQ ID NOs:
 67, 402, 405, 407, 409, 410, 412, 414-416, and 611-640, or a variant thereof having at least about
 80% sequence identity to an amino acid sequence selected from the group consisting of SEQ ID
 NOs: 67, 402, 405, 407, 409, 410, 412, 414-416, and 611-640; and the VL comprises an amino
 acid sequence selected from the group consisting of SEQ ID NOs: 68, 403, 404, 406, 408, 411,
 413, and 641-666, or a variant thereof having at least about 80% sequence identity to an amino
 acid sequence selected from the group consisting of SEQ ID NOs: 68, 403, 404, 406, 408, 411,
 413, and 641-666. In some examples, the VH comprises an amino acid sequence selected from
 the group consisting of SEQ ID NOs: 67, 402, 405, 407, 409, 410, 412, 414, 415, and 416; and
 the VL comprises an amino acid sequence selected from the group consisting of SEQ ID NOs:
 68, 403, 404, 406, 408, 411, and 413.

[0049] In some examples according to any one of the masked anti-CD3 antibodies described
 above, the VH comprises an amino acid sequence selected from the group consisting of SEQ ID
 NOs: 402, 405, 407, 409, 410, 412, 414, 415, and 416; and the VL comprises an amino acid
 sequence selected from the group consisting of SEQ ID NOs: 403, 404, 406, 408, 411, and 413.
 In some examples, the VH comprises the amino acid sequence of SEQ ID NO: 402, and the VL
 comprises the amino acid sequence of SEQ ID NO: 403. In some examples, the VH comprises
 the amino acid sequence of SEQ ID NO: 402, and the VL comprises the amino acid sequence of
 SEQ ID NO: 404. In some examples, the VH comprises the amino acid sequence of SEQ ID NO:
 405, and the VL comprises the amino acid sequence of SEQ ID NO: 406. In some examples, the
 VH comprises the amino acid sequence of SEQ ID NO: 407, and the VL

comprises the amino acid sequence of SEQ ID NO: 404. In some examples, the VH comprises the amino acid sequence of SEQ ID NO: 407, and the VL comprises the amino acid sequence of SEQ ID NO: 403. In some examples, the VH comprises the amino acid sequence of SEQ ID NO: 407, and the VL comprises the amino acid sequence of SEQ ID NO: 408. In some examples, the VH comprises the amino acid sequence of SEQ ID NO: 409, and the VL comprises the amino acid sequence of SEQ ID NO: 408. In some examples, the VH comprises the amino acid sequence of SEQ ID NO: 410, and the VL comprises the amino acid sequence of SEQ ID NO: 411. In some examples, the VH comprises the amino acid sequence of SEQ ID NO: 412, and the VL comprises the amino acid sequence of SEQ ID NO: 413. In some examples, the VH comprises the amino acid sequence of SEQ ID NO: 410, and the VL comprises the amino acid sequence of SEQ ID NO: 413. In some examples, the VH comprises the amino acid sequence of SEQ ID NO: 414, and the VL comprises the amino acid sequence of SEQ ID NO: 403. In some examples, the VH comprises the amino acid sequence of SEQ ID NO: 415, and the VL comprises the amino acid sequence of SEQ ID NO: 413. In some examples, the VH comprises the amino acid sequence of SEQ ID NO: 416, and the VL comprises the amino acid sequence of SEQ ID NO: 413. In some examples, the VH comprises the amino acid sequence of SEQ ID NO: 416, and the VL comprises the amino acid sequence of SEQ ID NO: 411. In some examples, the CD3-binding moiety comprises the amino acid sequence of SEQ ID NO: 421 or SEQ ID NO: 422.

[0050] One example of the present application provides an activatable antibody (“activatable anti-HER2 antibody”) comprising, from N-terminus to C-terminus, a masking moiety (MM), a cleavable moiety (CM), and a HER2-binding moiety, wherein: a) the HER2-binding moiety comprises a VL and the activatable antibody further comprises a second polypeptide comprising a VH; b) the HER2-binding moiety comprises a VH and the activatable antibody further comprises a second polypeptide comprising a VL; c) the HER2-binding moiety comprises from the N-terminus to the C-terminus, a VL and a VH; or d) the HER2-binding moiety comprise from the N-terminus to the C-terminus, a VH and a VL; and wherein the CM comprises a cleavage site; wherein the MM inhibits binding of the activatable antibody to HER2 when the CM is not cleaved; and wherein the activatable antibody binds HER2 via the VH and VL when the CM is cleaved, and wherein the MM comprises: a) an amino acid sequence according to Formula (XI): $ESX_1X_2CX_3X_4DPFX_5CQX_6$ (SEQ ID NO: 670), wherein X_1 is D or E, X_2 is A, F,

V, or Y, X₃ is D or E, X₄ is A or L, X₅ is D or E, and X₆ is A, F, or Y; b) an amino acid sequence according to Formula (XII): X₁X₂X₃X₄X₅X₆CX₇X₈DPYECX₉X₁₀ (SEQ ID NO: 671), wherein X₁ is A, H, or S, X₂ is A, D, or S, X₃ is A, T, or V, X₄ is P, S, or T, X₅ is D or E, X₆ is A or V, X₇ is D or E, X₈ is A or L, X₉ is Q, S, or T, and X₁₀ is A, H, or V; or c) an amino acid sequence according to Formula (XIII): YNSDDDCX₁SX₂YDPYTCYY (SEQ ID NO: 672), wherein X₁ is A, I, or V, and X₂ is H or R. In some examples, the MM comprises an amino acid sequence selected from the group consisting of SEQ ID NOs: 36, 419, 432-476, and 491-515. In some examples, the CM comprises an amino acid sequence selected from the group consisting of SEQ ID NOs: 77, 127-129, 418, 420, 431 and 477-490, and 516-555.

[0051] In some examples according to any one of the activatable anti-HER2 antibodies described above, the VH comprises a CDR-H1 comprising the amino acid sequence of SEQ ID NO: 423, a CDR-H2 comprising the amino acid sequence of SEQ ID NO: 424, and a CDR-H3 comprising the amino acid sequence of SEQ ID NO: 71, and the VL comprises a CDR-L1 comprising the amino acid sequence of SEQ ID NO: 72, a CDR-L2 comprising the amino acid sequence of SEQ ID NO: 73, and a CDR-L3 comprising the amino acid sequence of SEQ ID NO: 74. In some examples, the VH comprises the amino acid sequence of SEQ ID NO: 75, and the VL comprises the amino acid sequence of SEQ ID NO: 76.

[0052] One example of the present application provides a masked antibody (“masked anti-HER2 antibody”) comprising, from N-terminus to C-terminus, a masking moiety (MM), and a HER2-binding moiety, wherein: a) the HER2-binding moiety comprises a VL and the activatable antibody further comprises a second polypeptide comprising a VH; b) the HER2-binding moiety comprises a VH and the activatable antibody further comprises a second polypeptide comprising a VL; c) the HER2-binding moiety comprises from the N-terminus to the C-terminus, a VL and a VH; or d) the HER2-binding moiety comprise from the N-terminus to the C-terminus, a VH and a VL; wherein the MM competes with HER2 to specifically bind the HER2-binding moiety; and wherein the activatable antibody binds HER2 via the VH and VL, and wherein the MM comprises: a) an amino acid sequence according to Formula (XI): ESX₁X₂CX₃X₄DPFX₅CQX₆ (SEQ ID NO: 670), wherein X₁ is D or E, X₂ is A, F, V, or Y, X₃ is D or E, X₄ is A or L, X₅ is D or E, and X₆ is A, F, or Y; b) an amino acid sequence according to Formula (XII): X₁X₂X₃X₄X₅X₆CX₇X₈DPYECX₉X₁₀ (SEQ ID NO: 671), wherein X₁ is A, H, or S, X₂ is A, D, or S, X₃ is A, T, or V, X₄ is P, S, or T, X₅ is D or E, X₆ is A or V, X₇ is D or E, X₈ is A or L, X₉ is

Q, S, or T, and X₁₀ is A, H, or V; or c) an amino acid sequence according to Formula (XIII): YNSDDDCX₁SX₂YDPYTCYY (SEQ ID NO: 672), wherein X₁ is A, I, or V, and X₂ is H or R. In some examples, the masked anti-HER2 antibody is an activatable antibody. In some examples, the masked anti-HER2 antibody comprises, from N-terminus to C-terminus, the masking moiety (MM), a cleavable moiety (CM), and the HER2-binding moiety. In some examples, the masked anti-HER2 antibody is a not an activatable antibody. In some examples, the masked anti-HER2 antibody comprises, from N-terminus to C-terminus, the masking moiety (MM), a non-cleavable linker (NCL), and the HER2-binding moiety. One example of the present application provides a masked antibody (“masked anti-HER2 antibody”) comprising a masking moiety (MM) and an antibody or antigen-binding fragment that binds HER2, wherein the antibody or antigen-binding fragment comprises a VH and a VL; wherein the masked antibody comprises a single polypeptide chain and the VH and the VL of the antibody or antigen-binding fragment are part of the single polypeptide chain, or the masked antibody comprises two polypeptide chains, and the VH and the VL of the antibody or antigen-binding fragment are part of different polypeptide chains of the masked antibody; wherein the C-terminus of the MM is fused to the N-terminus of the VH or the VL of the antibody or antigen-binding fragment; wherein the MM competes with HER2 to specifically bind the antibody or antigen-binding fragment; and wherein the MM comprises: a) an amino acid sequence according to Formula (XI): ESX₁X₂CX₃X₄DPFX₅CQX₆ (SEQ ID NO: 670), wherein X₁ is D or E, X₂ is A, F, V, or Y, X₃ is D or E, X₄ is A or L, X₅ is D or E, and X₆ is A, F, or Y; b) an amino acid sequence according to Formula (XII): X₁X₂X₃X₄X₅X₆CX₇X₈DPYECX₉X₁₀ (SEQ ID NO: 671), wherein X₁ is A, H, or S, X₂ is A, D, or S, X₃ is A, T, or V, X₄ is P, S, or T, X₅ is D or E, X₆ is A or V, X₇ is D or E, X₈ is A or L, X₉ is Q, S, or T, and X₁₀ is A, H, or V; or c) an amino acid sequence according to Formula (XIII): YNSDDDCX₁SX₂YDPYTCYY (SEQ ID NO: 672), wherein X₁ is A, I, or V, and X₂ is H or R. In some examples, the masked antibody comprises an amino acid linker between the C-terminus of the MM and the N-terminus of the VH or the VL of the antibody or antigen-binding fragment. In some examples, the masked antibody further comprises a cleavable linker, *e.g.*, between the C-terminus of the MM and the N-terminus of the VH or the VL of the antibody or antigen-binding fragment. In some examples, the masked antibody does not comprise a cleavable linker (*e.g.*, between the C-terminus of the MM and the N-terminus of the antibody or fragment).

[0053] One example of the present application provides a masked antibody (“masked anti-HER2 antibody”) comprising, from N-terminus to C-terminus, a masking moiety (MM), a non-cleavable linker (NCL), and a HER2-binding moiety, wherein: a) the HER2-binding moiety comprises a VL and the activatable antibody further comprises a second polypeptide comprising a VH; b) the HER2-binding moiety comprises a VH and the activatable antibody further comprises a second polypeptide comprising a VL; c) the HER2-binding moiety comprises from the N-terminus to the C-terminus, a VL and a VH; or d) the HER2-binding moiety comprise from the N-terminus to the C-terminus, a VH and a VL; wherein the MM competes with HER2 to specifically bind the HER2-binding moiety; and wherein the activatable antibody binds HER2 via the VH and VL, and wherein the MM comprises: a) an amino acid sequence according to Formula (XI): ESX₁X₂CX₃X₄DPFX₅CQX₆ (SEQ ID NO: 670), wherein X₁ is D or E, X₂ is A, F, V, or Y, X₃ is D or E, X₄ is A or L, X₅ is D or E, and X₆ is A, F, or Y; b) an amino acid sequence according to Formula (XII): X₁X₂X₃X₄X₅X₆CX₇X₈DPYECX₉X₁₀ (SEQ ID NO: 671), wherein X₁ is A, H, or S, X₂ is A, D, or S, X₃ is A, T, or V, X₄ is P, S, or T, X₅ is D or E, X₆ is A or V, X₇ is D or E, X₈ is A or L, X₉ is Q, S, or T, and X₁₀ is A, H, or V; or c) an amino acid sequence according to Formula (XIII): YNSDDDCX₁SX₂YDPYTCYY (SEQ ID NO: 672), wherein X₁ is A, I, or V, and X₂ is H or R. In some examples, the MM comprises an amino acid sequence selected from the group consisting of SEQ ID NOs: 36, 419, 432-476, and 491-515.

[0054] In some examples according to any one of the masked anti-HER2 antibodies described above, the VH comprises a CDR-H1 comprising the amino acid sequence of SEQ ID NO: 423, a CDR-H2 comprising the amino acid sequence of SEQ ID NO: 424, and a CDR-H3 comprising the amino acid sequence of SEQ ID NO: 71, and the VL comprises a CDR-L1 comprising the amino acid sequence of SEQ ID NO: 72, a CDR-L2 comprising the amino acid sequence of SEQ ID NO: 73, and a CDR-L3 comprising the amino acid sequence of SEQ ID NO: 74. In some examples, the VH comprises the amino acid sequence of SEQ ID NO: 75, and the VL comprises the amino acid sequence of SEQ ID NO: 76.

[0055] In other examples, the present disclosure provides an anti-HER2 antibody comprising the 6 CDRs and/or VH and VL sequences of any anti-HER2 binding domain provided herein. In some examples, an anti-HER2 antibody comprises a VH that comprises a CDR-H1 comprising the amino acid sequence of SEQ ID NO: 423, a CDR-H2 comprising the amino acid sequence of

SEQ ID NO: 424, and a CDR-H3 comprising the amino acid sequence of SEQ ID NO: 71, and a VL that comprises a CDR-L1 comprising the amino acid sequence of SEQ ID NO: 72, a CDR-L2 comprising the amino acid sequence of SEQ ID NO: 73, and a CDR-L3 comprising the amino acid sequence of SEQ ID NO: 74. In some examples, the VH comprises the amino acid sequence of SEQ ID NO: 75, and the VL comprises the amino acid sequence of SEQ ID NO: 76. In some examples, an anti-HER2 antibody comprises a VH that comprises a CDR-H1 comprising the amino acid sequence of SEQ ID NO: 69, a CDR-H2 comprising the amino acid sequence of SEQ ID NO: 70, and a CDR-H3 comprising the amino acid sequence of SEQ ID NO: 71, and a VL that comprises a CDR-L1 comprising the amino acid sequence of SEQ ID NO: 72, a CDR-L2 comprising the amino acid sequence of SEQ ID NO: 73, and a CDR-L3 comprising the amino acid sequence of SEQ ID NO: 74.

[0056] In other examples, the present disclosure provides an anti-CD20 antibody comprising the 6 CDRs and/or VH and VL sequences of any anti-CD20 binding domain provided herein. In some examples, an anti-CD20 antibody comprises a VH that comprises a CDR-H1 comprising the amino acid sequence of SEQ ID NO: 556, a CDR-H2 comprising the amino acid sequence of SEQ ID NO: 557, and a CDR-H3 comprising the amino acid sequence of SEQ ID NO: 558, and a VL that comprises a CDR-L1 comprising the amino acid sequence of SEQ ID NO: 559, a CDR-L2 comprising the amino acid sequence of SEQ ID NO: 560, and a CDR-L3 comprising the amino acid sequence of SEQ ID NO: 561. In some examples, an anti-CD20 antibody comprises a VH that comprises a CDR-H1 comprising the amino acid sequence of SEQ ID NO: 86, a CDR-H2 comprising the amino acid sequence of SEQ ID NO: 557, and a CDR-H3 comprising the amino acid sequence of SEQ ID NO: 558, and a VL that comprises a CDR-L1 comprising the amino acid sequence of SEQ ID NO: 559, a CDR-L2 comprising the amino acid sequence of SEQ ID NO: 560, and a CDR-L3 comprising the amino acid sequence of SEQ ID NO: 561. In some examples, the VH comprises the amino acid sequence of SEQ ID NO: 562, and the VL comprises the amino acid sequence of SEQ ID NO: 563.

[0057] One example of the present application provides one or more isolated nucleic acids encoding any one of the antibodies, multispecific antibodies, masked antibodies, activatable multispecific antibodies, isolated anti-CD3 antibodies or antigen-binding fragments thereof, masked anti-CD3 antibodies, activatable anti-CD3 antibodies, masked anti-HER2 antibodies or activatable anti-HER2 antibodies described above. In some examples, there is provided a

vector comprising the one or more nucleic acids according to any one of the nucleic acids described above. In some examples, there is provided a host cell comprising the one or more nucleic acids according to any one of the nucleic acids described above or any one of the vectors described above. In some examples, there is provided a method for preparing a masked antibody, a multispecific antibody, an activatable multispecific antibody, an isolated anti-CD3 antibody or antigen-binding fragment thereof, a masked anti-CD3 antibody, an activatable anti-CD3 antibody, a masked anti-HER2 antibody or an activatable anti-HER2 antibody, comprising: a) culturing any one of the host cells under conditions that allow expression of the one or more nucleic acids or vector; and b) recovering the multispecific antibody, the activatable multispecific antibody, the anti-CD3 antibody or antigen-binding fragment thereof, the masked anti-CD3 antibody, the activatable anti-CD3 antibody, the masked anti-Her2 antibody, or the activatable antibody from the host cell culture.

[0058] Also provided are pharmaceutical compositions comprising any one of the antibodies, multispecific antibodies, masked antibodies, activatable multispecific antibodies, isolated anti-CD3 antibodies or antigen-binding fragments thereof, masked anti-CD3 antibodies, activatable anti-CD3 antibodies, masked anti-HER2 antibodies, or activatable anti-HER2 antibodies described above, and a pharmaceutically acceptable carrier.

[0059] Another example of the present application provides a method for treating a disease or condition in a subject in need thereof, comprising administering to the subject an effective amount of any one of the pharmaceutical compositions described above. In some examples, wherein the pharmaceutical composition comprises an activatable multispecific antibody, wherein the CM1 and the CM2 are cleaved at a diseased site, thereby unblocking binding of the multispecific activatable antibody to CD3 and the target antigen at the diseased site. In some examples, the disease or condition is cancer such as liquid cancer and solid cancer. In some examples, wherein the target antigen is HER2, the cancer is selected from the group consisting of breast cancer, ovarian cancer, and lung cancer. In some examples, wherein the target antigen is CD20, the cancer is lymphoma or leukemia. In some examples, the target antigen is TROP2, and wherein the cancer is breast cancer or lymphoma. In some examples, the pharmaceutical composition is administered such that the multispecific antibody, isolated antibody or antigen-binding fragment thereof, or masked antibody is provided to the subject at a dose of 0.02mg/kg, 0.2mg/kg, 2mg/kg, 10mg/kg, 30mg/kg, or 60mg/kg. In some examples,

the multispecific antibody, isolated antibody or antigen-binding fragment thereof, or masked antibody comprises: a first polypeptide comprising an amino acid sequence having at least 90% sequence identity with SEQ ID NO: 427, a second polypeptide comprising an amino acid sequence having at least 90% sequence identity with SEQ ID NO: 428, and a third polypeptide comprising an amino acid sequence having at least 90% sequence identity with SEQ ID NO: 112; a first polypeptide comprising an amino acid sequence having at least 90% sequence identity with SEQ ID NO: 83, a second polypeptide comprising an amino acid sequence having at least 90% sequence identity with SEQ ID NO: 84, and a third polypeptide comprising an amino acid sequence having at least 90% sequence identity with SEQ ID NO: 85; a first polypeptide comprising an amino acid sequence having at least 90% sequence identity with SEQ ID NO: 683, a second polypeptide comprising an amino acid sequence having at least 90% sequence identity with SEQ ID NO: 684, and a third polypeptide comprising an amino acid sequence having at least 90% sequence identity with SEQ ID NO: 685; a first polypeptide comprising the amino acid sequence of SEQ ID NO: 427, a second polypeptide comprising the amino acid sequence of SEQ ID NO: 428, and a third polypeptide comprising the amino acid sequence of SEQ ID NO: 112; a first polypeptide comprising the amino acid sequence of SEQ ID NO: 83, a second polypeptide comprising the amino acid sequence of SEQ ID NO: 84, and a third polypeptide comprising the amino acid sequence of SEQ ID NO: 85; a first polypeptide comprising the amino acid sequence of SEQ ID NO: 683, a second polypeptide comprising the amino acid sequence of SEQ ID NO: 684, and a third polypeptide comprising the amino acid sequence of SEQ ID NO: 685; a first polypeptide comprising the amino acid sequence of SEQ ID NO: 427 without the C-terminal lysine, a second polypeptide comprising the amino acid sequence of SEQ ID NO: 428 without the C-terminal lysine, and a third polypeptide comprising the amino acid sequence of SEQ ID NO: 112; a first polypeptide comprising the amino acid sequence of SEQ ID NO: 83, a second polypeptide comprising the amino acid sequence of SEQ ID NO: 84 without the C-terminal lysine, and a third polypeptide comprising the amino acid sequence of SEQ ID NO: 85 without the C-terminal lysine; or a first polypeptide comprising the amino acid sequence of SEQ ID NO: 683, a second polypeptide comprising the amino acid sequence of SEQ ID NO: 684 without the C-terminal lysine, and a third polypeptide comprising the amino acid sequence of SEQ ID NO: 685 without the C-terminal lysine. In some examples, the methods further comprise administering to the subject an anti-PD-1 or anti-

PD-L1 antibody. In some examples, the methods further comprise administering to the subject a CD137 agonist or antibody. In some examples, the CD137 agonist or antibody comprises a heavy chain variable region and a light chain variable region, wherein the heavy chain variable region comprises a CDR-H1 comprising the amino acid sequence of TGGVGVG (SEQ ID NO:700), a CDR-H2 comprising the amino acid sequence of LIDWADDKYYSPSLKS (SEQ ID NO:701), and a CDR-H3 comprising the amino acid sequence of GGSDTVIGDWFAY (SEQ ID NO:702); and/or wherein the light chain variable region comprises a CDR-L1 comprising the amino acid sequence of RASQSIGSYLA (SEQ ID NO:703), a CDR-L2 comprising the amino acid sequence of DASNLET (SEQ ID NO:704), and a CDR-L3 comprising the amino acid sequence of QQGYLWT (SEQ ID NO:705). In some examples, the heavy chain variable region comprises the amino acid sequence of SEQ ID NO:706, and/or wherein the light chain variable region comprises the amino acid sequence of SEQ ID NO:707. In some examples, the heavy chain comprises the amino acid sequence of SEQ ID NO:710, and/or the light chain comprises the amino acid sequence of SEQ ID NO:711.

[0060] Also provided are compositions, kits and articles of manufacture comprising any one of the multispecific antibodies, masked antibodies, activatable multispecific antibodies, isolated anti-CD3 antibodies or antigen-binding fragments thereof, masked anti-CD3 antibodies, activatable anti-CD3 antibodies, masked anti-HER2 antibodies, or activatable anti-HER2 antibodies described above.

BRIEF DESCRIPTION OF THE DRAWINGS

[0061] FIGs. 1-5 provide schematic diagrams of exemplary antibody designs of the present application. The antibodies may be converted to activatable antibodies by fusing one or more antigen-binding sites to masking peptide(s).

[0062] FIG. 1 shows a Fab-Fc/Fc one-armed scaffold schematic.

[0063] FIG. 2 shows a schematic of a common light chain scaffold, in which the bispecific antibody has a first antibody heavy chain, a second antibody heavy chain and two copies of a common light chain. The first antibody heavy chain and a first common light chain form a first antigen-binding site, and the second antibody heavy chain and a second common light chain form a second antigen-binding site. The first antigen-binding site and the second antigen-binding site can bind to different targets.