

CLAIMS

What is claimed is:

1. An activatable antibody comprising:

a first polypeptide comprising, from N-terminus to C-terminus, a masking moiety (MM), a cleavable moiety (CM), and a target binding moiety (TBM),

wherein the MM comprises an amino acid sequence selected from the group consisting of SEQ ID NO 79-85;

wherein the CM comprises at least a first cleavage site and a first flexible linker (L1); and

wherein:

a) the TBM comprises an antibody light chain variable region (VL), and the activatable antibody further comprises a second polypeptide comprising an antibody heavy chain variable region (VH);

b) the TBM comprises an antibody heavy chain variable region (VH), and the activatable antibody further comprises a second polypeptide comprising an antibody light chain variable region (VL);

c) the TBM comprises from the N-terminus to the C-terminus, an antibody light chain variable region (VL) and an antibody heavy chain variable region (VH); or

d) the TBM comprises from the N-terminus to the C-terminus, an antibody heavy chain variable region (VH) and an antibody light chain variable region (VL);

wherein:

(i) the VH comprises an HVR-H1 comprising the amino acid sequence of SEQ ID NO: 65, an HVR-H2 comprising the amino acid sequence of SEQ ID NO: 66, and an HVR-H3 comprising the amino acid sequence of SEQ ID NO: 67; and

(ii) the VL comprises an HVR-L1 comprising the amino acid sequence of SEQ ID NO: 68, an HVR-L2 comprising the amino acid sequence of SEQ ID NO: 69, and an HVR-L3 comprising the amino acid sequence of SEQ ID NO: 70; and

wherein the activatable antibody binds to human CD137 via the VH and VL when the CM is cleaved.

2. The activatable antibody of claim 1, wherein the TBM comprises an antibody light chain variable region (VL), and the activatable antibody further comprises a second polypeptide comprising an antibody heavy chain variable region (VH).
3. The activatable antibody of claim 1, wherein the MM further comprises, at its N-terminus, an additional amino acid sequence.
4. The activatable antibody of claim 1, wherein the first cleavage site is a protease cleavage site for a protease selected from the group consisting of urokinase-type plasminogen activator (uPA), matrix metalloproteinase-1 (MMP-1), MMP-2, MMP-3, MMP-8, MMP-9, MMP-14, Tobacco Etch Virus (TEV) protease, plasmin, Thrombin, Factor X, PSA, PSMA, Cathepsin D, Cathepsin K, Cathepsin S, ADAM10, ADAM12, ADAMTS, Caspase-1, Caspase-2, Caspase-3, Caspase-4, Caspase-5, Caspase-6, Caspase-7, Caspase-8, Caspase-9, Caspase-10, Caspase-11, Caspase-12, Caspase-13, Caspase-14, and TACE.
5. The activatable antibody of claim 1, wherein the L₁ comprises an amino acid sequence selected from the group consisting of SEQ ID NOS: 17-24.
6. The activatable antibody of claim 1, wherein the CM further comprises a second cleavage site.
7. The activatable antibody of claim 6, wherein the second cleavage site is a protease cleavage site for a protease selected from the group consisting of urokinase-type plasminogen activator (uPA), matrix metalloproteinase-1 (MMP-1), MMP-2, MMP-3, MMP-8, MMP-9, MMP-14, Tobacco Etch Virus (TEV) protease, plasmin, Thrombin, Factor X, PSA, PSMA, Cathepsin D, Cathepsin K, Cathepsin S, ADAM10, ADAM12, ADAMTS, Caspase-1, Caspase-

2, Caspase-3, Caspase-4, Caspase-5, Caspase-6, Caspase-7, Caspase-8, Caspase-9, Caspase-10, Caspase-11, Caspase-12, Caspase-13, Caspase-14, and TACE.

8. The activatable antibody of claim 6, wherein the first and second cleavage sites are different.

9. The activatable antibody of claim 6, wherein the CM further comprises a second flexible linker (L₂).

10. The activatable antibody of claim 9, wherein the L₂ comprises an amino acid sequence selected from the group consisting of SEQ ID NOS: 17-24.

11. The activatable antibody of claim 1, wherein the first polypeptide comprises, from N-terminus to C-terminus, an amino acid sequence selected from the group consisting of SEQ ID NOS: 40-46 and the TBM, wherein the amino acid sequence selected from the group consisting of SEQ ID NOS: 40-46 comprises the MM and the CM.

12. The activatable antibody of claim 3, wherein the additional amino acid sequence comprises the amino acid sequence of SEQ ID NO: 16.

13. The activatable antibody of claim 6, wherein the second cleavage site is C-terminal to the L₁.

14. The activatable antibody of claim 1, wherein:

(a) the VH comprises the amino acid sequence of SEQ ID NO: 49; and

(b) the VL comprises the amino acid sequence of SEQ ID NO: 50.

15. The activatable antibody of claim 1, wherein

the MM comprises the amino acid sequence of SEQ ID NO: 79.

16. The activatable antibody of claim 15, wherein:

(a) the VH comprises the amino acid sequence of SEQ ID NO: 49; and

- (b) the VL comprises the amino acid sequence of SEQ ID NO: 50.
17. The activatable antibody of claim 15, wherein the CM comprises the amino acid sequence of SEQ ID NO: 12.
18. The activatable antibody of claim 1, wherein:
- (a) the TBM comprises an antibody light chain variable region (VL), and the activatable antibody further comprises a second polypeptide comprising an antibody heavy chain variable region (VH);
 - (b) the MM comprises the amino acid sequence of SEQ ID NO: 79; and
 - (c) the CM comprises the amino acid sequence of SEQ ID NO: 12.
19. The activatable antibody of claim 1, wherein:
- (a) the TBM comprises an antibody light chain variable region (VL), and the activatable antibody further comprises a second polypeptide comprising an antibody heavy chain variable region (VH);
 - (b) the MM comprises the amino acid sequence of SEQ ID NO: 79;
 - (c) the CM comprises the amino acid sequence of SEQ ID NO: 12;
 - (d) the VH comprises the amino acid sequence of SEQ ID NO: 49; and
 - (e) the VL comprises the amino acid sequence of SEQ ID NO: 50.
20. The activatable antibody of claim 1, wherein the first polypeptide comprises, from N-terminus to C-terminus, the amino acid sequence of SEQ ID NO: 40 and the TBM, and wherein:
- (a) the TBM comprises an antibody light chain variable region (VL), and the activatable antibody further comprises a second polypeptide comprising an antibody heavy chain variable region (VH);
 - (b) the VH comprises the amino acid sequence of SEQ ID NO: 49; and

- (c) the VL comprises the amino acid sequence of SEQ ID NO: 50.
21. The activatable antibody of claim 1, wherein
- the MM comprises the amino acid sequence of SEQ ID NO: 82.
22. The activatable antibody of claim 21, wherein:
- (a) the VH comprises the amino acid sequence of SEQ ID NO: 49; and
- (b) the VL comprises the amino acid sequence of SEQ ID NO: 50.
23. The activatable antibody of claim 21, wherein the CM comprises the amino acid sequence of SEQ ID NO: 12.
24. The activatable antibody of claim 1, wherein:
- (a) the TBM comprises an antibody light chain variable region (VL), and the activatable antibody further comprises a second polypeptide comprising an antibody heavy chain variable region (VH);
- (b) the MM comprises the amino acid sequence of SEQ ID NO: 82; and
- (c) the CM comprises the amino acid sequence of SEQ ID NO: 12.
25. The activatable antibody of claim 1, wherein:
- (a) the TBM comprises an antibody light chain variable region (VL), and the activatable antibody further comprises a second polypeptide comprising an antibody heavy chain variable region (VH);
- (b) the MM comprises the amino acid sequence of SEQ ID NO: 82;
- (c) the CM comprises the amino acid sequence of SEQ ID NO: 12;
- (d) the VH comprises the amino acid sequence of SEQ ID NO: 49; and
- (e) the VL comprises the amino acid sequence of SEQ ID NO: 50.

26. The activatable antibody of claim 1, wherein the first polypeptide comprises, from N-terminus to C-terminus, the amino acid sequence of SEQ ID NO: 43 and the TBM, and wherein:
- (a) the TBM comprises an antibody light chain variable region (VL), and the activatable antibody further comprises a second polypeptide comprising an antibody heavy chain variable region (VH);
 - (b) the VH comprises the amino acid sequence of SEQ ID NO: 49; and
 - (c) the VL comprises the amino acid sequence of SEQ ID NO: 50.
27. The activatable antibody of claim 1, wherein
- the MM comprises the amino acid sequence of SEQ ID NO: 85.
28. The activatable antibody of claim 27, wherein:
- (a) the VH comprises the amino acid sequence of SEQ ID NO: 49; and
 - (b) the VL comprises the amino acid sequence of SEQ ID NO: 50.
29. The activatable antibody of claim 27, wherein the CM comprises the amino acid sequence of SEQ ID NO: 12.
30. The activatable antibody of claim 1, wherein:
- (a) the TBM comprises an antibody light chain variable region (VL), and the activatable antibody further comprises a second polypeptide comprising an antibody heavy chain variable region (VH);
 - (b) the MM comprises the amino acid sequence of SEQ ID NO: 85; and
 - (c) the CM comprises the amino acid sequence of SEQ ID NO: 12.
31. The activatable antibody of claim 1, wherein:

- (a) the TBM comprises an antibody light chain variable region (VL), and the activatable antibody further comprises a second polypeptide comprising an antibody heavy chain variable region (VH);
 - (b) the MM comprises the amino acid sequence of SEQ ID NO: 85;
 - (c) the CM comprises the amino acid sequence of SEQ ID NO: 12;
 - (d) the VH comprises the amino acid sequence of SEQ ID NO: 49; and
 - (e) the VL comprises the amino acid sequence of SEQ ID NO: 50.
32. The activatable antibody of claim 1, wherein the first polypeptide comprises, from N-terminus to C-terminus, the amino acid sequence of SEQ ID NO: 46 and the TBM, and wherein:
- (a) the TBM comprises an antibody light chain variable region (VL), and the activatable antibody further comprises a second polypeptide comprising an antibody heavy chain variable region (VH);
 - (b) the VH comprises the amino acid sequence of SEQ ID NO: 49; and
 - (c) the VL comprises the amino acid sequence of SEQ ID NO: 50.
33. An activatable antibody comprising:
- a first polypeptide comprising, from N-terminus to C-terminus, a masking moiety (MM), a cleavable moiety (CM), and an antibody light chain variable region (VL), and
 - a second polypeptide comprising an antibody heavy chain variable region (VH);
- wherein the MM comprises an amino acid sequence selected from the group consisting of SEQ ID NO 79-85;
- wherein the CM comprises at least a first cleavage site and a first flexible linker (L1);
- wherein:
- (i) the VH comprises an HVR-H1 comprising the amino acid sequence of SEQ ID NO: 65, an HVR-H2 comprising the amino acid sequence of SEQ ID NO: 66, and an HVR-H3 comprising the amino acid sequence of SEQ ID NO: 67; and

(ii) the VL comprises an HVR-L1 comprising the amino acid sequence of SEQ ID NO: 68, an HVR-L2 comprising the amino acid sequence of SEQ ID NO: 69, and an HVR-L3 comprising the amino acid sequence of SEQ ID NO: 70; and

wherein the activatable antibody binds to human CD137 via the VH and VL when the CM is cleaved.

34. The activatable antibody of claim 33, wherein:

(a) the VH comprises the amino acid sequence of SEQ ID NO: 49; and

(b) the VL comprises the amino acid sequence of SEQ ID NO: 50.

35. The activatable antibody of claim 33, wherein the MM comprises the amino acid sequence of SEQ ID NO: 79.

36. The activatable antibody of claim 33, wherein:

(a) the MM comprises the amino acid sequence of SEQ ID NO: 79;

(b) the CM comprises the amino acid sequence of SEQ ID NO: 12;

(c) the VH comprises the amino acid sequence of SEQ ID NO: 49; and

(d) the VL comprises the amino acid sequence of SEQ ID NO: 50.

37. The activatable antibody of claim 33, wherein the first polypeptide comprises, from N-terminus to C-terminus, the amino acid sequence of SEQ ID NO: 40 and the VL, and wherein:

(a) the VH comprises the amino acid sequence of SEQ ID NO: 49; and

(b) the VL comprises the amino acid sequence of SEQ ID NO: 50.

38. The activatable antibody of claim 33, wherein the MM comprises the amino acid sequence of SEQ ID NO: 82.

39. The activatable antibody of claim 33, wherein:

- (a) the MM comprises the amino acid sequence of SEQ ID NO: 82;
 - (b) the CM comprises the amino acid sequence of SEQ ID NO: 12;
 - (c) the VH comprises the amino acid sequence of SEQ ID NO: 49; and
 - (d) the VL comprises the amino acid sequence of SEQ ID NO: 50.
40. The activatable antibody of claim 33, wherein the first polypeptide comprises, from N-terminus to C-terminus, the amino acid sequence of SEQ ID NO: 43 and the VL, and wherein:
- (a) the VH comprises the amino acid sequence of SEQ ID NO: 49; and
 - (b) the VL comprises the amino acid sequence of SEQ ID NO: 50.
41. The activatable antibody of claim 33, wherein the MM comprises the amino acid sequence of SEQ ID NO: 85.
42. The activatable antibody of claim 33, wherein:
- (a) the MM comprises the amino acid sequence of SEQ ID NO: 85;
 - (b) the CM comprises the amino acid sequence of SEQ ID NO: 12;
 - (c) the VH comprises the amino acid sequence of SEQ ID NO: 49; and
 - (d) the VL comprises the amino acid sequence of SEQ ID NO: 50.
43. The activatable antibody of claim 33, wherein the first polypeptide comprises, from N-terminus to C-terminus, the amino acid sequence of SEQ ID NO: 46 and the VL, and wherein:
- (a) the VH comprises the amino acid sequence of SEQ ID NO: 49; and
 - (b) the VL comprises the amino acid sequence of SEQ ID NO: 50.