

CLAIMS

What is claimed is:

1. ~~— A library comprising polynucleotides, wherein at least one of the polynucleotides encodes a polypeptide comprising, from N-terminus to C-terminus, a first peptide (FP), a cleavable moiety (CM), and a target binding moiety (TBM),~~

~~wherein the FP comprises an amino acid sequence according to Formula (XIII):~~

~~$X_mCX_nCX_o$ (SEQ ID NO: 86), wherein m is from 2-10, n is from 3-10, and o is from 1-10, and wherein each X is independently an amino acid selected from the group consisting of A, C, D, E, F, G, H, I, K, L, M, N, P, Q, R, S, T, V, W, and Y;~~

~~wherein the CM comprises at least a first cleavage site; and~~

~~wherein the TBM comprises an antibody light chain variable region and/or an antibody heavy chain variable region.~~

2. ~~— The library of claim 1, wherein the polynucleotides in the library encode at least two, at least three, at least four, at least five, or at least ten unique polypeptides and each unique polypeptide comprise, from N-terminus to C-terminus, a first peptide (FP), a cleavable moiety (CM), and a target binding moiety (TBM),~~

~~wherein the FP comprises an amino acid sequence according to Formula (XIII):~~

~~$X_mCX_nCX_o$ (SEQ ID NO: 86), wherein m is from 2-10, n is from 3-10, and o is from 1-10, and wherein each X is independently an amino acid selected from the group consisting of A, C, D, E, F, G, H, I, K, L, M, N, P, Q, R, S, T, V, W, and Y;~~

~~wherein the CM comprises at least a first cleavage site; and~~

~~wherein the TBM comprises an antibody light chain variable region and/or an antibody heavy chain variable region.~~

3. — The library of claim 1 or claim 2, wherein each of the polynucleotides in the library encodes a polypeptide comprising, from N-terminus to C-terminus, a first peptide (FP), a cleavable moiety (CM), and a target binding moiety (TBM),

wherein the FP comprises an amino acid sequence according to Formula (XIII):

$X_mCX_nCX_o$ (SEQ ID NO: 86), wherein m is from 2-10, n is from 3-10, and o is from 1-10, and wherein each X is independently an amino acid selected from the group consisting of A, C, D, E, F, G, H, I, K, L, M, N, P, Q, R, S, T, V, W, and Y;

wherein the CM comprises at least a first cleavage site; and

wherein the TBM comprises an antibody light chain variable region and/or an antibody heavy chain variable region.

4. — The library of any one of claims 1-3, wherein the FP is encoded by a polynucleotide sequence comprising a nucleic acid sequence according to Formula (XIV): $(NNK)_mTGY(NNK)_nTGY(NNK)_o$ (SEQ ID NO: 87), wherein each N is independently A, G, T, or C, wherein each K is independently T or C, and wherein each Y is independently T or C.

5. — The library of any one of claims 1-4, wherein each X is not M, W, or C.

6. — The library of any one of claims 1-5, wherein each X in X_m of Formula (XIII) is independently an amino acid selected from the group consisting of D, A, Y, S, T, N, I, L, F, V, H, and P.

7. — The library of any one of claims 1-6, wherein each X in X_n of Formula (XIII) is independently an amino acid selected from the group consisting of D, A, Y, S, T, N, I, L, F, V, H, and P.

8. — The library of any one of claims 1-7, wherein each X in X_o of Formula (XIII) is independently an amino acid selected from the group consisting of D, A, Y, S, T, N, I, L, F, V, H, and P.

9. — The library of any one of claims 1-8, wherein m is 2 or 3-6.

- ~~10. The library of any one of claims 1-9, wherein n is from 6-8.~~
- ~~11. The library of any one of claims 1-9, wherein n is 6.~~
- ~~12. The library of any one of claims 1-11, wherein o is from 1-2.~~
- ~~13. The library of any one of claims 1-11, wherein o is 2.~~
- ~~14. The library of any one of claims 1-13 wherein the FP further comprises, at its N-terminus, an additional amino acid sequence.~~
- ~~15. The library of claim 14, wherein the additional amino acid sequence comprises the amino acid sequence of SEQ ID NO: 16.~~
- ~~16. The library of any one of claims 1-15, 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.~~
- ~~17. The library of any one of claims 1-16, wherein the CM further comprises a first linker (L₁) C-terminal to the first cleavage site.~~
- ~~18. The library of claim 17, wherein the L₁ comprises an amino acid sequence selected from the group consisting of SEQ ID NOS: 17-24.~~
- ~~19. The library of any one of claims 1-18, wherein the CM further comprises a second cleavage site.~~
- ~~20. The library of claim 19, wherein the second cleavage site is C-terminal to the L₁.~~
- ~~21. The library of claim 19 or claim 20, 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.

22. — The library of any one of claims 19-21, wherein the first and second cleavage sites are different.

23. — The library of any one of claims 19-22, wherein the CM further comprises a second linker (L₂) C-terminal to the second cleavage site.

24. — The library of claim 23, wherein the L₂ comprises an amino acid sequence selected from the group consisting of SEQ ID NOS: 17-24.

25. — The library of any one of claims 1-24, wherein the polypeptide comprises an amino acid sequence according to Formula (III):

EVGSYX₁X₂X₃X₄X₅X₆CX₇X₈X₉X₁₀X₁₁X₁₂CX₁₃X₁₄SGRSAGGGGTENLYFQSGGCS (SEQ ID NO: 3), wherein X₁ is A, D, I, N, P, or Y, X₂ is A, F, N, S, or V, X₃ is A, H, L, P, S, V, or Y, X₄ is A, H, S, or Y, X₅ is A, D, P, S, V, or Y, X₆ is A, D, L, S, or Y, X₇ is D, P, or V, X₈ is A, D, H, P, S, or T, X₉ is A, D, F, H, P, or Y, X₁₀ is L, P, or Y, X₁₁ is F, P, or Y, X₁₂ is A, P, S, or Y, X₁₃ is A, D, N, S, T, or Y, and X₁₄ is A, S, or Y.

26. — The library of claim 25, wherein each of the polynucleotides in the library encodes a polypeptide comprising an amino acid sequence according to Formula (III).

27. — The library of any one of claims 1-26, wherein the polypeptide comprises an amino acid sequence selected from the group consisting of SEQ ID NOS: 25-46.

28. — The library of any one of claims 1-27, wherein the TBM comprises an antibody light chain variable region.

29. — The library of claim 28, wherein the polypeptide further comprises a heavy chain variable region C-terminal to the light chain variable region.

~~30. — The library of claim 28, further comprising polynucleotides that encode one or more antibody heavy chain variable regions.~~

~~31. — The library of any one of claims 1–27, wherein the TBM comprises an antibody heavy chain variable region.~~

~~32. — The library of claim 31, wherein the polypeptide further comprises a light chain variable region C-terminal to the heavy chain variable region.~~

~~33. — The library of claim 31, further comprising polynucleotides that encode one or more antibody light chain variable regions.~~

~~34. — The library of any one of claims 1–33, wherein at least one of the polynucleotides encoding the polypeptide is in a vector.~~

~~35. — The library of claim 34, wherein the vector is an expression vector or a display vector.~~

~~36. — The library of any one of claims 1–35, wherein at least one of the polynucleotides encoding the polypeptide is in a cell.~~

~~37. — The library of claim 36, wherein the cell is a bacterial cell, a yeast cell, an insect cell, or a mammalian cell.~~

~~38. — A method of producing an activatable antibody comprising culturing the cell of claim 36 or claim 37 under conditions suitable for producing the activatable antibody.~~

~~39. — The method of claim 38, further comprising recovering the activatable antibody produced by the cell.~~

~~40. — The method of claim 39, further comprising testing the activatable antibody for the ability to maintain an activatable phenotype while soluble.~~

~~41. — A method of using the library of any one of claims 1–37 to screen for a activatable antibody that binds to a target, the method comprising:~~

~~a) contacting the expression products of the library with the target before the CM is cleaved;~~

b) contacting the expression products of the library with the target after the CM is cleaved; and

c) isolating one or more of the expression products that binds to the target after the CM is cleaved, but has reduced binding to the target before the CM is cleaved.

42. — The method of claim 41, wherein an expression product is isolated if the binding affinity of the expression product after the CM is cleaved is at least 2 fold of the binding affinity of the expression product before the CM is cleaved.

43. — The method of claim 41 or 42, wherein the CM comprises at least a first 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.

44. — The method of any one of claims 41–43, wherein the target is CTLA4 or CD137.

45. — A polypeptide encoded by one or more polynucleotides from the library of any one of claims 1–37.

46. — A kit comprising the library of any one of claims 1–37.

47. — A library comprising antigen binding domains, wherein at least one of the antigen binding domains comprises a polypeptide comprising, from N-terminus to C-terminus, a first peptide (FP), a cleavable moiety (CM), and a target binding moiety (TBM),

wherein the FP comprises an amino acid sequence according to Formula (XIII):

$X_mCX_nCX_o$ (SEQ ID NO: 86), wherein m is from 2–10, n is from 3–10, and o is from 1–10, and wherein each X is independently an amino acid selected from the group consisting of A, C, D, E, F, G, H, I, K, L, M, N, P, Q, R, S, T, V, W, and Y;

wherein the CM comprises at least a first cleavage site; and

~~wherein the TBM comprises an antibody light chain variable region and/or an antibody heavy chain variable region.~~

~~48. The library of claim 47, wherein at least two, at least three, at least four, at least five, or at least ten of the antigen binding domains comprise a unique polypeptide comprising, from N-terminus to C-terminus, a first peptide (FP), a cleavable moiety (CM), and a target binding moiety (TBM);~~

~~wherein the FP comprises an amino acid sequence according to Formula (XIII): $X_mCX_nCX_o$ (SEQ ID NO: 86), wherein m is from 2-10, n is from 3-10, and o is from 1-10, and wherein each X is independently an amino acid selected from the group consisting of A, C, D, E, F, G, H, I, K, L, M, N, P, Q, R, S, T, V, W, and Y;~~

~~wherein the CM comprises at least a first cleavage site; and~~

~~wherein the TBM comprises an antibody light chain variable region and/or an antibody heavy chain variable region.~~

~~49. The library of claim 47 or claim 48, wherein each of the antigen binding domains comprises a unique polypeptide comprising, from N-terminus to C-terminus, a first peptide (FP), a cleavable moiety (CM), and a target binding moiety (TBM);~~

~~wherein the FP comprises an amino acid sequence according to Formula (XIII): $X_mCX_nCX_o$ (SEQ ID NO: 86), wherein m is from 2-10, n is from 3-10, and o is from 1-10, and wherein each X is independently an amino acid selected from the group consisting of A, C, D, E, F, G, H, I, K, L, M, N, P, Q, R, S, T, V, W, and Y;~~

~~wherein the CM comprises at least a first cleavage site; and~~

~~wherein the TBM comprises an antibody light chain variable region and/or an antibody heavy chain variable region.~~

~~50. The library of any one of claims 47-49, wherein the TBM comprises an antibody light chain variable region and the antigen binding domain further comprises an antibody heavy chain variable region.~~

~~51. The library of any one of claims 47-49, wherein the TBM comprises an antibody heavy chain variable region and the antigen binding domain further comprises an antibody light chain variable region.~~

~~52. The library of any one of claims 47-51, wherein each X is not M, W, or C.~~

~~53. The library of any one of claims 47-52, wherein each X in X_m in formula (XIII) is independently an amino acid selected from the group consisting of D, A, Y, S, T, N, I, L, F, V, H, and P.~~

~~54. The library of any one of claims 47-53, wherein each X in X_n of Formula (XIII) is independently an amino acid selected from the group consisting of D, A, Y, S, T, N, I, L, F, V, H, and P.~~

~~55. The library of any one of claims 47-54, wherein each X in X_o of Formula (XIII) is independently an amino acid selected from the group consisting of D, A, Y, S, T, N, I, L, F, V, H, and P.~~

~~56. The library of any one of claims 47-55, wherein each of the antigen binding domains is displayed on a cell surface or on a phage.~~

~~57. An antibody light chain comprising a polypeptide comprising, from N-terminus to C-terminus, a first peptide (FP), a cleavable moiety (CM), and a target binding moiety (TBM),~~

~~wherein the FP comprises an amino acid sequence according to Formula (I): $X_mCX_nCZ_o$ (SEQ ID NO: 1), wherein m is from 2-10, n is from 3-10, and o is from 1-10, wherein each X is independently an amino acid selected from the group consisting of A, C, D, E, F, G, H, I, K, L, M, N, P, Q, R, S, T, V, W, and Y, and wherein each Z is independently an amino acid selected from the group consisting of D, A, Y, S, T, N, I, L, F, V, H, and P;~~

~~wherein the CM comprises at least a first cleavage site; and~~

~~wherein the TBM comprises an antibody light chain variable region.~~

~~58. — An antibody comprising a heavy chain and a light chain, wherein the light chain is a light chain of claim 57.~~

~~59. — An antibody heavy chain comprising a polypeptide comprising, from N terminus to C terminus, a first peptide (FP), a cleavable moiety (CM), and a target binding moiety (TBM),~~

~~wherein the FP comprises an amino acid sequence according to Formula (I): $X_mCX_nCZ_o$ (SEQ ID NO: 1), wherein m is from 2-10, n is from 3-10, and o is from 1-10, wherein each X is independently an amino acid selected from the group consisting of A, C, D, E, F, G, H, I, K, L, M, N, P, Q, R, S, T, V, W, and Y, and wherein each Z is independently an amino acid selected from the group consisting of D, A, Y, S, T, N, I, L, F, V, H, and P;~~

~~wherein the CM comprises at least a first cleavage site; and~~

~~wherein the TBM comprises an antibody heavy chain variable region.~~

~~60. — An antibody comprising a heavy chain and a light chain, wherein the heavy chain is a heavy chain of claim 59.~~

~~61. — A cell comprising at least one polypeptide displayed on its surface, wherein the at least polypeptide comprises, from N terminus to C terminus, a first peptide (FP), a cleavable moiety (CM), and a target binding moiety (TBM),~~

~~wherein the FP comprises an amino acid sequence according to Formula (I): $X_mCX_nCZ_o$ (SEQ ID NO: 1), wherein m is from 2-10, n is from 3-10, and o is from 1-10, wherein each X is independently an amino acid selected from the group consisting of A, C, D, E, F, G, H, I, K, L, M, N, P, Q, R, S, T, V, W, and Y, and wherein each Z is independently an amino acid selected from the group consisting of D, A, Y, S, T, N, I, L, F, V, H, and P;~~

~~wherein the CM comprises at least a first cleavage site; and~~

~~wherein the TBM comprises an antibody light chain variable region and/or an antibody heavy chain variable region.~~

~~62. The cell of claim 61, wherein the cell is a bacterial cell, a yeast cell, an insect cell, or a mammalian cell.~~

~~63. The cell of claim 61 or claim 62, wherein each X is not M, W, or C.~~

~~64. The cell of any one of claims 61-63, wherein each X in X_m of formula (I) is independently an amino acid selected from the group consisting of D, A, Y, S, T, N, I, L, F, V, H, and P.~~

~~65. The cell of any one of claims 61-64, wherein each X in X_n of formula (I) is independently an amino acid selected from the group consisting of D, A, Y, S, T, N, I, L, F, V, H, and P.~~

~~66~~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 according to Formula (I): $X_mCX_nCZ_o$ (SEQ ID NO: 1), wherein m is from 2-10, n is from 3-10, and o is from 1-10, wherein each X is independently an amino acid selected from the group consisting of A, C, D, E, F, G, H, I, K, L, M, N, P, Q, R, S, T, V, W, and Y, and wherein each Z is independently an amino acid selected from the group consisting of D, A, Y, S, T, N, I, L, F, V, H, and P; wherein the MM inhibits the binding of the activatable antibody to human CD137 when the CM is not cleaved;

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); ~~and~~

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.

~~672.~~ The activatable antibody of claim ~~166~~, 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).

~~68. — The activatable antibody of claim 66 or claim 67, wherein m is 2 or 3-6.~~

~~69. — The activatable antibody of any one of claims 66-68, wherein n is from 6-8.~~

~~70. — The activatable antibody of claim 69, wherein n is 6.~~

~~71. — The activatable antibody of any one of claims 66-69, wherein o is 1 or 2.~~

~~72. — The activatable antibody of any one of claims 66-71, wherein each X is not M, W, or C.~~

~~73. — The activatable antibody of any one of claims 66–72, wherein each X in X_m of formula (I) is independently an amino acid selected from the group consisting of D, A, Y, S, T, N, I, L, F, V, H, and P.~~

~~74. — The activatable antibody of any one of claims 66–73, wherein each X in X_n of formula (I) is independently an amino acid selected from the group consisting of D, A, Y, S, T, N, I, L, F, V, H, and P.~~

~~75. — The activatable antibody of any one of claims 66–74, wherein the MM comprises an amino acid sequence selected from the group consisting of SEQ ID NO: 79–85 and X_mCADAPNHCXX (SEQ ID NO: 88), X_mCHHSPANCXX (SEQ ID NO: 89), X_mCPILRHRCXX (SEQ ID NO: 90), X_mCKWRPSRCXX (SEQ ID NO: 91), X_mCRVLPTRCXX (SEQ ID NO: 92), X_mCLWRHRSCXX (SEQ ID NO: 93), and X_mCPRLRRKCXX (SEQ ID NO: 94), where m is from 2–10, and where each X is independently an amino acid selected from the group consisting of A, C, D, E, F, G, H, I, K, L, M, N, P, Q, R, S, T, V, W, and Y.~~

~~763. The activatable antibody of claim 1any one of claims 66–75, wherein the MM further comprises, at its N-terminus, an additional amino acid sequence.~~

~~77. — The activatable antibody of claim 76, wherein the additional amino acid sequence comprises the amino acid sequence of SEQ ID NO: 16.~~

~~784. The activatable antibody of claim 1any one of claims 66–77, 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.~~

~~79. — The activatable antibody of any one of claims 66–78, wherein the CM further comprises a first linker (L₁) C-terminal to the first cleavage site.~~

~~805.~~ The activatable antibody of claim ~~179~~, wherein the L₁ comprises an amino acid sequence selected from the group consisting of SEQ ID NOS: 17-24.

~~846.~~ The activatable antibody of ~~claim 1~~~~any one of claims 66-80~~, wherein the CM further comprises a second cleavage site.

~~82.~~ ~~The activatable antibody of claim 81, wherein the second cleavage site is C-terminal to the L₁.~~

~~837.~~ The activatable antibody of claim ~~681 or claim 82~~, 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.

~~848.~~ The activatable antibody of ~~claim 6~~~~any one of claims 81-83~~, wherein the first and second cleavage sites are different.

~~859.~~ The activatable antibody of ~~claim 6~~~~any one of claims 81-84~~, wherein the CM further comprises a second flexible linker (L₂)~~C-terminal to the second cleavage site.~~

~~8610.~~ The activatable antibody of claim ~~985~~, wherein the L₂ comprises an amino acid sequence selected from the group consisting of SEQ ID NOS: 17-24.

~~87.~~ ~~The activatable antibody of any one of claims 66-86, wherein the CM comprises at least a first protease cleavage site and is cleaved with one or more proteases 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.~~

~~88. The activatable antibody of any one of claims 66-87, wherein the activatable antibody comprises an amino acid sequence according to Formula (III):~~

~~EVGSYX₁X₂X₃X₄X₅CX₆X₇X₈X₉X₁₀X₁₁X₁₂CX₁₃X₁₄SGRSAGGGGTENLYFQGS GGS (SEQ ID NO: 3), wherein X₁ is A, D, I, N, P, or Y, X₂ is A, F, N, S, or V, X₃ is A, H, L, P, S, V, or Y, X₄ is A, H, S, or Y, X₅ is A, D, P, S, V, or Y, X₆ is A, D, L, S, or Y, X₇ is D, P, or V, X₈ is A, D, H, P, S, or T, X₉ is A, D, F, H, P, or Y, X₁₀ is L, P, or Y, X₁₁ is F, P, or Y, X₁₂ is A, P, S, or Y, X₁₃ is A, D, N, S, T, or Y, and X₁₄ is A, S, or Y.~~

~~8911. The activatable antibody of claim 1any one of claims 66-88, wherein the activatable antibodyfirst 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.~~

~~90. The activatable antibody of any one of claims 66-89, wherein 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.~~

~~91. The activatable antibody of any one of claims 66-90, wherein the VL comprises the amino acid sequence of SEQ ID NO: 50.~~

~~92. The activatable antibody of any one of claims 66-91, wherein 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.~~

~~93. The activatable antibody of any one of claims 66-92, wherein the VH comprises the amino acid sequence of SEQ ID NO: 49.~~

~~94. A method of treating or delaying progression of cancer in a subject in need thereof, the method comprising administering to the subject an effective amount of a polypeptide encoded by~~

~~one or more polynucleotides from the library of any one of claims 1-37, or an activatable antibody of any one of claims 66-93.~~

~~95. — The method of claim 94, further comprising administering to the subject an effective amount of at least one additional therapeutic agent.~~

~~96. — The method of claim 95, wherein the at least one additional therapeutic agent is selected from the group consisting of viral gene therapy, immune checkpoint inhibitors, target therapies, radiation therapies, and chemotherapies.~~

~~97. — The method of claim 95 or claim 96, wherein the at least one additional therapeutic agent is selected from the group consisting of pomalyst, revlimid, lenalidomide, pomalidomide, thalidomide, a DNA-alkylating platinum-containing derivative, cisplatin, 5-fluorouracil, cyclophosphamide, an anti-CD137 antibody, an anti-CTLA4 antibody, an anti-PD-1 antibody, an anti-PD-L1 antibody, an anti-CD20 antibody, an anti-CD40 antibody, an anti-DR5 antibody, an anti-CD1d antibody, an anti-TIM3 antibody, an anti-SLAMF7 antibody, an anti-KIR receptor antibody, an anti-OX40 antibody, an anti-HER2 antibody, an anti-ErbB-2 antibody, an anti-EGFR antibody, cetuximab, rituximab, trastuzumab, pembrolizumab, radiotherapy, single-dose radiation, fractionated radiation, focal radiation, whole-organ radiation, IL-12, IFN α , GM-CSF, a chimeric antigen receptor, adoptively transferred T cells, an anti-cancer vaccine, and an oncolytic virus.~~

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.