

for improved methods and products useful for identifying self-blocking peptides for activatable binding polypeptides.

[0005] All references cited herein, including patent applications, patent publications, non-patent literature, and UniProtKB/Swiss-Prot/GenBank Accession numbers are herein incorporated by reference in their entirety, as if each individual reference were specifically and individually indicated to be incorporated by reference.

SUMMARY

[0006] To meet the above and other needs, disclosed herein are libraries of polynucleotides *e.g.*, that are useful for screening for and/or identifying activatable binding polypeptides (*i.e.*, activatable antibodies). The present disclosure is based, at least in part, on the finding that polypeptides described herein show a significantly improved masking efficiency before activation, allowing for better design, screening and/or identification of activatable binding polypeptides (*i.e.*, activatable antibodies) with superior therapeutic indexes and safety profiles. The present disclosure is further based, at least in part, on the surprising finding that the polynucleotide libraries described herein may be successfully constructed and screened to identify activatable binding polypeptides, (*see* Examples 1 and 2 below). Disclosed herein are precision/context-dependent activatable binding polypeptides that bind to human CTLA4 (*see* Example 3) or human CD137 (*see* Example 5) when in active form but not in inactive form, *i.e.*, they bind their target (when in active form) only after cleavage of the cleavable moiety (CM) to remove the first peptide (FP) (*i.e.*, a masking moiety (MM) or self-blocking peptide). The discovered first peptides (FPs) (*e.g.*, masking moieties) described herein are capable of efficiently masking antibody activity and/or reducing or completely inhibiting antigen binding, while in some examples being devoid of the chemically labile residues methionine and/or tryptophan. Furthermore, activatable antibodies identified using the polynucleotide libraries described herein are as efficient at treating multiple cancer types as their parental antibody, while having significantly reduced cytotoxicity even in susceptible animals (NOD mice, *see* Example 4).

[0007] Accordingly, in one example, provided herein is 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 (e.g., from 3-10), 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. In some examples, the polynucleotides in the library encode at least two, at least three, at least four, at least five, at least ten, at least 50, at least 100, at least 500, at least 1000 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 (e.g., from 3-10), 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. In some examples that may be combined with any of the preceding examples, 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 (e.g., from 3-10), 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. In some examples that may be combined with any of the preceding examples, 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 G, and wherein each Y is independently T or C.

[0008] In some examples that may be combined with any of the preceding examples, each X is not M, W, or C. In some examples that may be combined with any of the preceding examples, 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. In some examples that may be combined with any of the preceding examples, 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. In some examples that may be combined with any of the preceding examples, 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. In some examples that may be combined with any of the preceding examples, m is 6. In some examples that may be combined with any of the preceding examples, m is 2-5, *e.g.*, 2, 3, 4, or 5. In some examples that may be combined with any of the preceding examples, n is from 6-8. In some examples that may be combined with any of the preceding examples, n is 6. In some examples that may be combined with any of the preceding examples, o is from 1-2. In some examples that may be combined with any of the preceding examples, o is 2. In some examples that may be combined with any of the preceding examples, the FP further comprises, at its N-terminus, an additional amino acid sequence. In some examples, the additional amino acid sequence comprises the amino acid sequence of SEQ ID NO: 16.

[0009] In some examples that may be combined with any of the preceding examples, 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. In some examples that may be combined with any of the preceding examples, the CM further comprises a first linker (L_1) C-terminal to the first cleavage site. In some examples, the L_1 comprises an amino acid sequence selected from the group consisting of SEQ ID NOS: 17-24. In some examples that may be combined with

any of the preceding examples, the CM further comprises a second cleavage site. In some examples, the second cleavage site is C-terminal to the L₁. In some examples, 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. In some examples, the first and second cleavage sites are different. In some examples that may be combined with any of the preceding examples, the CM further comprises a second linker (L₂) C-terminal to the second cleavage site. In some examples, the L₂ comprises an amino acid sequence selected from the group consisting of SEQ ID NOS: 17-24. In some examples that may be combined with any of the preceding examples, the CM further comprises a linker N-terminal to the first cleavage site.

[0010] In some examples that may be combined with any of the preceding examples, the polypeptide encoded by one or more polynucleotides in the library comprises a first peptide (FP) and a cleavable moiety (CM) comprising an amino acid sequence according to Formula (III): EVGSYX₁X₂X₃X₄X₅X₆CX₇X₈X₉X₁₀X₁₁X₁₂CX₁₃X₁₄SGRSAGGGGTENLYFQGS 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. In some examples, each of the polynucleotides in the library encodes a polypeptide comprising an amino acid sequence according to Formula (III). In some examples that may be combined with any of the preceding examples, the polypeptide comprises an amino acid sequence selected from the group consisting of SEQ ID NOS: 25-46.

[0011] In some examples that may be combined with any of the preceding examples, the TBM comprises an antibody light chain variable region. In some examples, the TBM further comprises a heavy chain variable region C-terminal to the light

chain variable region. In some examples, the library further comprises polynucleotides that encode one or more antibody heavy chain variable regions. In some examples, the heavy chain variable region and light chain variable region forms an antigen binding site that is capable of binding to a target in the absence of a masking moiety (MM).

[0012] In some examples that may be combined with any of the preceding examples, the TBM comprises an antibody heavy chain variable region. In some examples, the TBM further comprises a light chain variable region C-terminal to the heavy chain variable region. In some examples, the library further comprises polynucleotides that encode one or more antibody light chain variable regions. In some examples, the heavy chain variable region and light chain variable region forms an antigen binding site that is capable of binding to a target in the absence of a masking moiety (MM).

[0013] In some examples that may be combined with any of the preceding examples, at least one of the polynucleotides encoding the polypeptide is in a vector. In some examples, the vector is an expression vector or a display vector. In some examples that may be combined with any of the preceding examples, at least one of the polynucleotides encoding the polypeptide is in a cell. In some examples, the cell is a bacterial cell, a yeast cell, an insect cell, or a mammalian cell.

[0014] Other examples of the present disclosure relate to a method of producing an activatable antibody comprising culturing any of the cells described herein under conditions suitable for producing the activatable antibody. In some examples, the method further comprises recovering the activatable antibody produced by the cell. In some examples, the method further comprises testing the activatable antibody for the ability to maintain an activatable phenotype while soluble.

[0015] Other examples of the present disclosure relate to a method of using any of the libraries described herein to screen for an activatable antibody that binds to a target, comprising the steps of 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 does not bind to the target before the CM is cleaved.

Also provided here is a method using any of the libraries described herein to screen for an activatable antibody that binds to a target, comprising the steps of 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 affinity to the target before the CM is cleaved as compared to the binding affinity after the CM is cleaved. In some examples, an expression product is isolated if the K_D of the expression product before the CM is cleaved is at least 2-fold (e.g., at least 5-fold, at least 10-fold, at least 15-fold, or higher) of the K_D of the expression product after the CM is cleaved. In some examples, 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. In some examples, the target is CTLA4, CD137, PD-1, PD-L1, PD-L2, LAG3, TIM3, B7-H3, OX40, CD3, CD19, CD20, CD40, CD95, CD120a, BTLA, VISTA, ICOS, BCMA, Her1, Her2, Her3, and/or B7-H4. In some examples, the target is CTLA4 or CD137.

[0016] Other examples of the present disclosure relate to a polypeptide encoded by one or more polynucleotides of any of the libraries described herein or a library of the polypeptides encoded by one or more polynucleotides of any of the libraries described herein.

[0017] Other examples of the present disclosure relate to a kit comprising any of the libraries described herein.

[0018] Other examples of the present disclosure relate to 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 (e.g., from 3-10), 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. In some examples, at least two, at least three, at least four, at least five, at least ten, at least 50, at least 100, at least 1000 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 (e.g., from 3-10), 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. In some examples that may be combined with any of the preceding examples, 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 (e.g., from 3-10), 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. In some examples that may be combined with any of the preceding examples, the TBM comprises an antibody light chain variable region and the antigen binding domain further comprises an antibody heavy chain variable region. In some examples that may be combined with any of the preceding examples, the TBM comprises an antibody heavy chain variable region and the antigen binding domain further comprises an antibody light chain variable region. In some examples that may be combined with any of the preceding examples, each X is not M, W, or C. In some examples that may be combined with any of the preceding examples, 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. In some examples that may be combined with any of the

preceding examples, 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. In some examples that may be combined with any of the preceding examples, 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. In some examples, each of the antigen binding domains in the library is displayed on a phage or a cell (e.g., a yeast cell).

[0019] Other examples of the present disclosure relate to 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 (e.g., from 3-10), 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. Other examples of the present disclosure relate to an antibody comprising a heavy chain and a light chain, wherein the light chain is any of the antibody light chains described herein. In some examples that may be combined with any of the preceding examples, each X is not M, W, or C. In some examples that may be combined with any of the preceding examples, 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.

[0020] Other examples of the present disclosure relate to 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 (e.g., from 3-10), 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. Other examples

of the present disclosure relate to an antibody comprising a heavy chain and a light chain, wherein the heavy chain is any of the antibody heavy chains described herein. In some examples that may be combined with any of the preceding examples, each X is not M, W, or C. In some examples that may be combined with any of the preceding examples, 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.

[0021] Other examples of the present disclosure relate to 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 (e.g., from 3-10), 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. In some examples, the cell is a bacterial cell, a yeast cell, an insect cell, or a mammalian cell. In some examples that may be combined with any of the preceding examples, each X is not M, W, or C. In some examples that may be combined with any of the preceding examples, 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. In some examples that may be combined with any of the preceding examples, 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.

[0022] Other examples of the present disclosure relate to an activatable antibody comprising: a 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 according to Formula (I): $X_mCX_nCZ_o$ (SEQ ID NO: 1), wherein m is from 2-10 (e.g., from 3-10), 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 CTLA4 when the CM is not cleaved; wherein the CM comprises at least a first cleavage site; and wherein the TBM comprises an antibody light chain variable region (VL) and/or an antibody heavy chain variable region (VH); and wherein the activatable antibody binds to human CTLA4 via the VH and VL when the CM is cleaved. In some examples, the TBM comprises the VL and the activatable antibody further comprises a VH. In some examples, the TBM comprises the VH and the activatable antibody further comprises a VL. In some examples, the TBM comprises, from N-terminus to C-terminus, VH and VL or VL and VH. In some examples, 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. In some examples that may be combined with any of the preceding examples, each X is not M, W, or C. In some examples that may be combined with any of the preceding examples, 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. In some examples that may be combined with any of the preceding examples, 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. In some examples that may be combined with any of the preceding examples, the MM comprises an amino acid sequence selected from the group consisting of SEQ ID NO: 72-78. In some examples that may be combined with any of the preceding examples, the VL comprises an HVR-L1 comprising the amino acid sequence of SEQ ID NO: 62, an HVR-L2 comprising the amino acid sequence of SEQ ID NO: 63, and an HVR-L3 comprising the amino acid sequence of SEQ ID NO: 64. In some examples that may be combined with any of the preceding examples, the VL comprises the amino acid sequence of SEQ ID NO: 48. In some examples that may be combined with any of the preceding examples, the VH comprises an HVR-H1 comprising the amino acid sequence of SEQ ID NO: 59, an HVR-

H2 comprising the amino acid sequence of SEQ ID NO: 60, and an HVR-H3 comprising the amino acid sequence of SEQ ID NO: 61. In some examples that may be combined with any of the preceding examples, the VH comprises the amino acid sequence of SEQ ID NO: 47. In some examples, m is 3-10.

[0023] Other examples of the present disclosure relate to an activatable antibody comprising: a 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 according to Formula (I): $X_mCX_nCZ_o$ (SEQ ID NO: 1), wherein m is from 2-10 (e.g., from 3-10), 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 wherein the TBM comprises an antibody light chain variable region (VL) and/or an antibody heavy chain variable region (VH); and wherein the activatable antibody binds to human CD137 via the VH and VL when the CM is cleaved. In some examples, the TBM comprises the VL and the activatable antibody further comprises a VH. In some examples, the TBM comprises the VH and the activatable antibody further comprises a VL. In some examples, the TBM comprises, from N-terminus to C-terminus, VH and VL or VL and VH. In some examples, 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. In some examples that may be combined with any of the preceding examples, each X is not M, W, or C. In some examples that may be combined with any of the preceding examples, 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. In some examples that

may be combined with any of the preceding examples, 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. In some examples that may be combined with any of the preceding examples, the MM comprises an amino acid sequence selected from the group consisting of SEQ ID NO: 79-85 and 88-94. In some examples that may be combined with any of the preceding examples, 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. In some examples that may be combined with any of the preceding examples, the VL comprises the amino acid sequence of SEQ ID NO: 50. In some examples that may be combined with any of the preceding examples, 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. In some examples that may be combined with any of the preceding examples, the VH comprises the amino acid sequence of SEQ ID NO: 49. In some examples, m is 3-10.

[0023a] In one aspect, the present disclosure refers to 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.

[0023b] In one aspect, the present disclosure refers to 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.

[0024] Other examples of the present disclosure relate to a polynucleotide encoding any of the activatable antibodies described herein. In other examples, the present disclosure relates to a vector comprising any of the polynucleotides described herein (*e.g.*, a polynucleotide encoding an activatable antibody). In some examples, the vector is an expression vector and/or a display vector. In other examples, the present disclosure relates to a host cell comprising any of the polynucleotides and/or vectors described herein (*e.g.*, a polynucleotide and/or vector encoding an activatable antibody). In some examples, the host cell is a eukaryotic cell. In some examples, the host cell is a Chinese Hamster Ovary (CHO) cell. In other examples, the present disclosure relates to a method of making an activatable antibody comprising culturing any of the host cells described herein under conditions suitable for producing the antibody or activatable antibody. In some examples, the method further comprises recovering the antibody or activatable antibody produced by the cell.

[0025] Other examples of the present disclosure relate to 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 any

of the libraries described herein and/or any of the activatable antibodies (e.g., activatable antibody to human CTLA4 or activatable antibody to human CD137) described herein. In some examples, the method further comprises administering to the subject an effective amount of at least one additional therapeutic agent. In some examples, 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. In some examples, 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.

[0026] It is to be understood that one, some, or all of the properties of the various examples described above and herein may be combined to form other examples of the present disclosure. These and other examples of the present disclosure will become apparent to one of skill in the art. These and other examples of the present disclosure are further described by the detailed description that follows.

BRIEF DESCRIPTION OF THE DRAWINGS

[0027] **FIG. 1** shows a schematic of an exemplary selection process for self-blocking peptides using the Fab fragment of the target antibody displayed on yeast surface.

[0028] **FIG. 2** shows a schematic of an exemplary selection process for self-blocking peptide using the scFv fragment of the target antibody displayed on yeast surface.

[0029] **FIGS. 3A-B** show functional display of Fabs and scFvs targeting CTLA4 on yeast, as determined by flow cytometry. **FIG. 3A** shows functional display of Fabs targeting CTLA4