

CLAIMS

1. An isolated antibody or an antigen binding fragment thereof (AB) that specifically binds to mammalian CD166, wherein the AB specifically binds human CD166 and cynomolgus monkey CD166, wherein the AB comprises the VH CDR1 amino acid sequence GFSLSTYGMGVG (SEQ ID NO: 127); the VH CDR2 amino acid sequence NIWWSEDKH (SEQ ID NO: 128); the VH CDR3 amino acid sequence IDYGNDYAFTY (SEQ ID NO: 129); the VL CDR1 amino acid sequence RSSKSLLSNGITYLY (SEQ ID NO: 130) or RSSQSLLSNGITYLY (SEQ ID NO: 131); the VL CDR2 amino acid sequence QMSNLAS (SEQ ID NO: 132) or QMSNRAS (SEQ ID NO: 133); and the VL CDR3 amino acid sequence AQNLELPYT (SEQ ID NO: 134).
2. The AB of claim 1, wherein the AB comprises a heavy chain variable region comprising the amino acid sequence of SEQ ID NO: 121 or SEQ ID NO: 122, and a light chain variable region comprising an amino acid sequence selected from the group consisting of SEQ ID NOs: 123-126.
3. An activatable antibody that, in an activated state, binds CD166 comprising:
an antibody or an antigen binding fragment thereof (AB) that specifically binds to mammalian CD166, wherein the AB specifically binds human CD166 and cynomolgus monkey CD166, wherein the AB comprises the VH CDR1 amino acid sequence GFSLSTYGMGVG (SEQ ID NO: 127); the VH CDR2 amino acid sequence NIWWSEDKH (SEQ ID NO: 128); the VH CDR3 amino acid sequence IDYGNDYAFTY (SEQ ID NO: 129); the VL CDR1 amino acid sequence RSSKSLLSNGITYLY (SEQ ID NO: 130) or RSSQSLLSNGITYLY (SEQ ID NO: 131); the VL CDR2 amino acid sequence QMSNLAS (SEQ ID NO: 132) or QMSNRAS (SEQ ID NO: 133); and the VL CDR3 amino acid sequence AQNLELPYT (SEQ ID NO: 134);

a masking moiety (MM) coupled to the AB that inhibits the binding of the AB to CD166 when the activatable antibody is in an uncleaved state; and

a cleavable moiety (CM) coupled to the AB, wherein the CM is a polypeptide that functions as a substrate for a protease.

4. The activatable antibody of claim 3, wherein the MM has one or more of the characteristics selected from the group consisting of:

(i) the MM has a dissociation constant for binding to the AB that is greater than the dissociation constant of the AB to CD166;

(ii) the MM does not interfere or compete with the AB for binding to CD166 when the activatable antibody is in a cleaved state;

(iii) the MM is a polypeptide of no more than 40 amino acids in length;

(iv) the MM polypeptide sequence is different from that of human CD166;

(v) the MM polypeptide sequence is no more than 50% identical to any natural binding partner of the AB; and

(vi) the MM comprises an amino acid sequence selected from the group consisting of SEQ ID NO: 135-238.

5. The activatable antibody of claim 3, wherein the CM has one or more of the characteristics selected from the group consisting of:

(i) the CM is a substrate for a protease that is active in diseased tissue; and

(ii) the CM comprises an amino acid sequence selected from the group consisting of SEQ ID NOs: 18-87 and 318-358.

6. The activatable antibody of claim 3, wherein the antigen binding fragment thereof is selected from the group consisting of a Fab fragment, a F(ab')₂ fragment, a scFv, a scAb, a dAb, a single domain heavy chain antibody, and a single domain light chain antibody.
7. The activatable antibody of claim 3, wherein the AB comprises a heavy chain variable region comprising the amino acid sequence of SEQ ID NO: 121 or SEQ ID NO: 122, and a light chain variable region comprising an amino acid sequence selected from the group consisting of SEQ ID NOs: 123-126.
8. The activatable antibody of claim 3, wherein the AB is linked to the CM.
9. The activatable antibody of claim 3, wherein the AB is linked directly to the CM.

10. The activatable antibody of claim 3, wherein the AB is linked to the CM via a linking peptide.
11. The activatable antibody of claim 3, wherein the MM is linked to the CM such that the activatable antibody in an uncleaved state comprises the structural arrangement from N-terminus to C-terminus as follows: MM-CM-AB or AB-CM-MM.
12. The activatable antibody of claim 3, wherein the activatable antibody comprises a linking peptide between the MM and the CM, a linking peptide between the CM and the AB, or both a linking peptide between the MM and the CM and a linking peptide between the CM and the AB.
13. The activatable antibody of claim 3, wherein the activatable antibody comprises a first linking peptide (LP1) and a second linking peptide (LP2), and wherein the activatable antibody in the uncleaved state has the structural arrangement from N-terminus to C-terminus as follows: MM-LP1-CM-LP2-AB or AB-LP2-CM-LP1-MM.
14. The activatable antibody of claim 13, wherein the two linking peptides are not identical to each other.
15. The activatable antibody of claim 13, wherein each of LP1 and LP2 is a peptide of about 1 to 20 amino acids in length.
16. The activatable antibody of claim 3, wherein the activatable antibody has one or more of the characteristics selected from the group consisting of:
 - (a) the activatable antibody comprises the heavy chain comprising an amino acid sequence of SEQ ID NO: 239 and a light chain comprising an amino acid sequence selected from the group consisting of SEQ ID NOs: 240, 242, 244, 246, 248, 303, 310, 312, 314, 316, 371, 372, 375, 376, 379, 380, 383, 384, 387, 388, 391, 392, 395, 396, 399, 400, 403, 404, 407, 408, 411, 412, 415, 416, 419, 420, 423, 424, 427, 428, 431, 432, 435, 436, 439, 440, 443, 444, 447, 448, 451, 452, 455, 456, 459, 460, 463, 464, 467, 468, 471, and 472;

(b) the activatable antibody comprises a combination of amino acid sequences, wherein the combination of amino acid sequences is selected from a single row in Table A, wherein for a given combination,

(i) the heavy chain of the AB comprises the amino acid sequences of the VH CDR sequences corresponding to the given combination in the single row listed in Table A,

(ii) the light chain of the AB comprises the amino acid sequences of the VL CDR sequences corresponding to the given combination in the single row listed in Table A,

(iii) the MM comprises the amino acid sequence of the mask sequence (MM) corresponding to the given combination in the single row listed in Table A, and

(iv) the CM comprises the amino acid sequence of the substrate sequence (CM) corresponding to the given combination in the single row listed in Table A;

(c) the activatable antibody comprises a combination of amino acid sequences, wherein for a given combination of amino acid sequences,

(i) the heavy chain of the AB comprises the amino acid sequences of the VH sequence or VH CDR sequences selected from the group consisting of the VH sequences or VH CDR sequences listed in the corresponding column of Table B,

(ii) the light chain of the AB comprises the amino acid sequences of the VL sequence or VL CDR sequences selected from the group consisting of: the VL sequences or VL CDR sequences listed in the corresponding column of Table B,

(iii) the MM comprises the amino acid sequence of the mask sequence (MM) selected from the group consisting of: the MM sequences listed in the corresponding column of Table B, and

(iv) the CM comprises the amino acid sequence of the substrate sequence (CM) selected from the group consisting of: the CM sequences listed in the corresponding column of Table B; and

(d) the activatable antibody comprises

a heavy chain variable region comprising an amino acid sequence selected from the group consisting of SEQ ID NOs: 121 and 122, and a light chain variable region comprising an amino acid sequence selected from the group consisting of SEQ ID NOs: 123-126, 363-370, 373, 374, 377, 378, 381, 382, 385, 386, 389, 390, 393, 394, 397, 398, 401, 402, 405, 406, 409, 410, 413, 414, 417, 418, 421, 422, 425, 426, 429, 430, 433, 434, 437, 438, 441, 442, 445, 446, 449, 450, 453, 454, 457, 458, 461, 462, 465, 466, 469, 470, 473, and 474.

17. An activatable antibody comprising an antibody or an antigen binding fragment thereof (AB) that specifically binds to mammalian CD166, a masking moiety (MM), and a cleavable moiety (CM),

wherein the AB comprises the VH CDR1 amino acid sequence GFSLSTYGMGVG (SEQ ID NO: 127); the VH CDR2 amino acid sequence NIWWSEDKH (SEQ ID NO: 128); the VH CDR3 amino acid sequence IDYGNDYAFTY (SEQ ID NO: 129); the VL CDR1 amino acid sequence RSSKSLHLSNGITYLY (SEQ ID NO: 130) or RSSQSLHLSNGITYLY (SEQ ID NO: 131); the VL CDR2 amino acid sequence QMSNLAS (SEQ ID NO: 132) or QMSNRAS (SEQ ID NO: 133); and the VL CDR3 amino acid sequence AQNLELPYT (SEQ ID NO: 134); and

wherein the activatable antibody has one or more of the following characteristics selected from the group consisting of:

(i) the activatable antibody comprises a heavy chain comprising the amino acid sequence of SEQ ID NO: 239, and a light chain sequence comprising an amino acid sequence selected from the group consisting of SEQ ID NOs: 240, 242, 244, 246, 248, 303, 310, 312, 314, 316, 371, 372, 375, 376, 379, 380, 383, 384, 387, 388, 391, 392, 395, 396, 399, 400, 403, 404, 407, 408, 411, 412, 415, 416, 419, 420, 423, 424, 427, 428, 431, 432, 435, 436, 439, 440, 443, 444, 447, 448, 451, 452, 455, 456, 459, 460, 463, 464, 467, 468, 471, and 472;

(ii) the MM comprises an amino acid sequence selected from the group consisting of SEQ ID NOs: 135-238, and the CM comprises an amino acid sequence selected from the group consisting of SEQ ID NOs: 18-87 and 318-358; and

(iii) the activatable antibody comprises a heavy chain variable region comprising an amino acid sequence selected from the group consisting of SEQ ID NOs: 121 and 122, and a light chain variable region comprising an amino acid sequence selected from the group

consisting of SEQ ID NOs: 123-126, 363-370, 373, 374, 377, 378, 381, 382, 385, 386, 389, 390, 393, 394, 397, 398, 401, 402, 405, 406, 409, 410, 413, 414,

417, 418, 421, 422, 425, 426, 429, 430, 433, 434, 437, 438, 441, 442, 445, 446, 449, 450, 453, 454, 457, 458, 461, 462, 465, 466, 469, 470, 473, and 474.

18. A conjugated activatable antibody comprising the activatable antibody of claim 3 conjugated to an agent.

19. The conjugated activatable antibody of claim 18, wherein the agent has one or more of the characteristics selected from the group consisting of:

- (a) the agent is a toxin or fragment thereof;
- (b) the agent is a microtubule inhibitor;

- (c) the agent is a nucleic acid damaging agent;
- (d) the agent is a dolastatin;
- (e) the agent is an auristatin;
- (f) the agent is a maytansinoid;
- (g) the agent is a duocarmycin;
- (h) the agent is a calicheamicin;
- (i) the agent is a pyrrolobenzodiazepine;
- (j) the agent is auristatin E;
- (k) the agent is monomethyl auristatin E (MMAE);
- (l) the agent is monomethyl auristatin D (MMAD);
- (m) the agent is the maytansinoid DM1;
- (n) the agent is the maytansinoid DM4;
- (o) the agent is a detectable moiety; and
- (p) the agent is a diagnostic agent.

20. The conjugated activatable antibody of claim 18, wherein the agent is conjugated to the AB via a linker.

21. The conjugated activatable antibody of claim 20, wherein the linker with which the agent is conjugated to the AB comprises an SPDB moiety, a vc moiety, or a PEG2-vc moiety.
22. The conjugated activatable antibody of claim 18, wherein the agent is a toxin conjugated to the AB via a linker, and wherein the linker and the toxin conjugated to the AB comprise a moiety selected from the group consisting of: an SPDB-DM4 moiety, a vc-MMAD moiety, a vc-MMAE moiety, a vc-duocarmycin moiety, or a PEG2-vc-MMAD moiety.
23. The conjugated activatable antibody of claim 20, wherein the linker is a cleavable linker.
24. The conjugated activatable antibody of claim 20, wherein the linker is a non-cleavable linker.
25. A conjugated activatable antibody that, in an activated state, specifically binds to a mammalian CD166 comprising:
- (a) an activable antibody comprising:
 - (i) an antibody or an antigen binding fragment thereof (AB) that specifically binds to mammalian CD166, wherein the AB specifically binds human CD166 and cynomolgus monkey CD166, wherein the AB comprises the VH CDR1 amino acid sequence GFSLSTYGMGVG (SEQ ID NO: 127); the VH CDR2 amino acid sequence NIWSEDKH (SEQ ID NO: 128); the VH CDR3 amino acid sequence IDYGNDYAFTY (SEQ ID NO: 129); the VL CDR1 amino acid sequence RSSKSLLSNGITYLY (SEQ ID NO: 130) or RSSQSLLSNGITYLY (SEQ ID NO: 131); the VL CDR2 amino acid sequence QMSNLAS (SEQ ID NO: 132) or QMSNRAS (SEQ ID NO: 133); and the VL CDR3 amino acid sequence AQNLELPYT (SEQ ID NO: 134);
 - (ii) a masking moiety (MM) coupled to the AB that inhibits the binding of the AB to CD166 when the activatable antibody is in an uncleaved state;
 - (iii) a cleavable moiety (CM) coupled to the AB, wherein the CM is a polypeptide that functions as a substrate for a protease; and

(b) an agent conjugated to the AB.

26. The conjugated activatable antibody of claim 25, wherein the agent is selected from the group consisting of a dolastatin, an auristatin, a maytansinoid, a duocarmycin, a calicheamicin, a pyrrolobenzodiazepine, auristatin E, monomethyl auristatin F (MMAF), monomethyl auristatin E (MMAE), monomethyl auristatin D (MMAD), maytansinoid DM4, maytansinoid DM1, a duocarmycin, a calicheamicin, a pyrrolobenzodiazepine, and a pyrrolobenzodiazepine dimer.
27. The conjugated activatable antibody of claim 25, wherein the agent is conjugated to the AB via a linker.
28. The conjugated activatable antibody of claim 27, wherein the linker with which the agent is conjugated to the AB comprises an SPDB moiety, a vc moiety, or a PEG2-vc moiety.
29. The conjugated activatable antibody of claim 25, wherein the agent is a toxin conjugated to the AB via a linker, wherein the linker and the toxin conjugated to the AB comprise a moiety selected from the group consisting of: an SPDB-DM4 moiety, a vc-MMAD moiety, a vc-MMAE moiety, a vc-duocarmycin moiety, or a PEG2-vc-MMAD moiety.

30. The conjugated activatable antibody of claim 25, wherein the activatable antibody has one or more of the characteristics selected from the group consisting of:

(i) the activatable antibody comprises a heavy chain variable region comprising an amino acid sequence selected from the group consisting of SEQ ID NOs: 121 and 122, and a light chain variable region comprising an amino acid sequence selected from the group consisting of SEQ ID NOs: 123-126, 363-370, 373, 374, 377, 378, 381, 382, 385, 386, 389, 390, 393, 394, 397, 398, 401, 402, 405, 406, 409, 410, 413, 414, 417, 418, 421, 422, 425, 426, 429, 430, 433, 434, 437, 438, 441, 442, 445, 446, 449, 450, 453, 454, 457, 458, 461, 462, 465, 466, 469, 470, 473, and 474; and

(ii) the activatable antibody comprises a heavy chain comprising an amino acid sequence of SEQ ID NO: 239, and a light chain sequence comprising an amino acid sequence selected from the group consisting of SEQ ID NOs: 240, 242, 244, 246, 248, 303, 310, 312, 314, 316, 371, 372, 375, 376, 379, 380, 383, 384, 387, 388, 391, 392, 395, 396, 399, 400, 403, 404, 407, 408, 411, 412, 415, 416, 419, 420, 423, 424, 427, 428, 431, 432, 435, 436, 439, 440, 443, 444, 447, 448, 451, 452, 455, 456, 459, 460, 463, 464, 467, 468, 471, and 472.

31. The conjugated activatable antibody of claim 25, wherein the MM comprises an amino acid sequence selected from the group consisting of SEQ ID NOs: 135-238.

32. The conjugated activatable antibody of claim 25, wherein the CM comprises an amino acid sequence selected from the group consisting of SEQ ID NOs: 18-87 and 318-358.

33. The conjugated activatable antibody of claim 25, wherein the activatable antibody has one or more of the following characteristics selected from the group consisting of:

(i) the activatable antibody comprises a combination of amino acid sequences, wherein the combination of amino acid sequences is selected from a single row in Table A, wherein for a given combination,

(a) the heavy chain of the AB comprises the amino acid sequences of the VH CDR sequences corresponding to the given combination in the single row listed in Table A,

(b) the light chain of the AB comprises the amino acid sequences of the VL CDR sequences corresponding to the given combination in the single row listed in Table A,

- (c) the MM comprises the amino acid sequence of the mask sequence (MM) corresponding to the given combination in the single row listed in Table A, and
- (d) the CM comprises the amino acid sequence of the substrate sequence (CM) corresponding to the given combination in the single row listed in Table A; and
- (ii) the activatable antibody comprises a combination of amino acid sequences, wherein for a given combination of amino acid sequences,
 - (a) the heavy chain of the AB comprises the amino acid sequences of the VH sequence or VH CDR sequences selected from the group consisting of: the VH sequence or VH CDR sequences listed in the corresponding column of Table B,
 - (b) the light chain of the AB comprises the amino acid sequences of the VL sequence or VL CDR sequences selected from the group consisting of: the VL sequence or VL CDR sequences listed in the corresponding column of Table B,
 - (c) the MM comprises the amino acid sequence of the mask sequence (MM) selected from the group consisting of: the MM sequences listed in the corresponding column of Table B, and
 - (d) the CM comprises the amino acid sequence of the substrate sequence (CM) selected from the group consisting of: the CM sequences listed in the corresponding column of Table B.

34. A conjugated antibody comprising:

- (a) an antibody or an antigen binding fragment thereof (AB) that specifically binds to mammalian CD166, wherein the AB comprises:
 - (i) the VH CDR1 amino acid sequence GFSLSTYGMGVG (SEQ ID NO: 127); the VH CDR2 amino acid sequence NIWWSEDKH (SEQ ID NO: 128);

the VH CDR3 amino acid sequence IDYGNDYAFTY (SEQ ID NO: 129); the VL CDR1 amino acid sequence RSSKSLHLSNGITYLY (SEQ ID NO: 130) or RSSQSLHLSNGITYLY (SEQ ID NO: 131); the VL CDR2 amino acid sequence QMSNLAS (SEQ ID NO: 132) or QMSNRAS (SEQ ID NO: 133); and the VL CDR3 amino acid sequence AQNLELPYT (SEQ ID NO: 134), or

(ii) a heavy chain variable region comprising an amino acid sequence selected from the group consisting of SEQ ID NOs: 121 and 122, and a light chain variable region comprising an amino acid sequence selected from the group consisting of SEQ ID NOs: 123-126, 363-370, 373, 374, 377, 378, 381, 382, 385, 386, 389, 390, 393, 394, 397, 398, 401, 402, 405, 406, 409, 410, 413, 414, 417, 418, 421, 422, 425, 426, 429, 430, 433, 434, 437, 438, 441, 442, 445, 446, 449, 450, 453, 454, 457, 458, 461, 462, 465, 466, 469, 470, 473, and 474, or

(iii) a heavy chain comprising an amino acid sequence selected from the group consisting of SEQ ID NO: 239, and a light chain comprising an amino acid sequence selected from the group consisting of SEQ ID NOS: 240, 242, 244, 246, 248, 303, 310, 312, 314, 316, 371, 372, 375, 376, 379, 380, 383, 384, 387, 388, 391, 392, 395, 396, 399, 400, 403, 404, 407, 408, 411, 412, 415, 416, 419, 420, 423, 424, 427, 428, 431, 432, 435, 436, 439, 440, 443, 444, 447, 448, 451, 452, 455, 456, 459, 460, 463, 464, 467, 468, 471, and 472; and

(b) an agent conjugated to the AB, wherein the agent is selected from the group consisting of auristatin E, monomethyl auristatin F (MMAF), monomethyl auristatin E (MMAE), monomethyl auristatin D (MMAD), maytansinoid DM4, maytansinoid DM1, a pyrrolobenzodiazepine, a pyrrolobenzodiazepine dimer, and a duocarmycin.

35. A conjugated activatable antibody that, in an activated state, binds to CD166, comprising:

(a) an activatable antibody comprising:

(i) an antibody or an antigen binding fragment thereof (AB) that specifically binds to mammalian CD166, wherein the AB specifically binds human CD166 and cynomolgus monkey CD166;

(ii) a masking moiety (MM) coupled to the AB that inhibits the binding of the AB to CD166 when the activatable antibody is in an uncleaved state;

- (iii) a cleavable moiety (CM) coupled to the AB, wherein the CM is a polypeptide that functions as a substrate for a protease; and
- (b) an agent conjugated to the AB,
 - wherein the activatable antibody comprises:

(i) the VH CDR1 amino acid sequence GFSLSTYGMGVG (SEQ ID NO: 127); the VH CDR2 amino acid sequence NIWWSEDKH (SEQ ID NO: 128); the VH CDR3 amino acid sequence IDYGNDYAFTY (SEQ ID NO: 129); the VL CDR1 amino acid sequence RSSKSLHLSNGITYLY (SEQ ID NO: 130) or RSSQSLHLSNGITYLY (SEQ ID NO: 131); the VL CDR2 amino acid sequence QMSNLAS (SEQ ID NO: 132) or QMSNRAS (SEQ ID NO: 133); and the VL CDR3 amino acid sequence AQNLELPYT (SEQ ID NO: 134), or

(ii) a heavy chain variable region comprising an amino acid sequence selected from the group consisting of SEQ ID NOs: 121 and 122, and a light chain variable region comprising an amino acid sequence selected from the group consisting of SEQ ID NOs: 123-126, 363-370, 373, 374, 377, 378, 381, 382, 385, 386, 389, 390, 393, 394, 397, 398, 401, 402, 405, 406, 409, 410, 413, 414, 417, 418, 421, 422, 425, 426, 429, 430, 433, 434, 437, 438, 441, 442, 445, 446, 449, 450, 453, 454, 457, 458, 461, 462, 465, 466, 469, 470, 473, and 474, or

(iii) a heavy chain comprising an amino acid sequence selected from the group consisting of SEQ ID NOS: 121, 122, and 239, and a light chain comprising an amino acid sequence selected from the group consisting of SEQ ID NOS: 240, 242, 244, 246, 248, 303, 310, 312, 314, 316, 371, 372, 375, 376, 379, 380, 383, 384, 387, 388, 391, 392, 395, 396, 399, 400, 403, 404, 407, 408, 411, 412, 415, 416, 419, 420, 423, 424, 427, 428, 431, 432, 435, 436, 439, 440, 443, 444, 447, 448, 451, 452, 455, 456, 459, 460, 463, 464, 467, 468, 471, and 472; and

wherein the agent is selected from the group consisting of auristatin E, monomethyl auristatin F (MMAF), monomethyl auristatin E (MMAE), monomethyl auristatin D (MMAD), maytansinoid DM4, maytansinoid DM1, a calicheamicin, a pyrrolobenzodiazepine, a pyrrolobenzodiazepine dimer, and a duocarmycin.

36. The conjugated activatable antibody of claim 35, wherein the MM comprises an amino acid sequence selected from the group consisting of SEQ ID NOs: 135-238.

37. The conjugated activatable antibody of claim 35, wherein the CM comprises an amino acid sequence selected from the group consisting of SEQ ID NOs: 18-87 and 318-358.

38. The conjugated activatable antibody of claim 35, wherein the agent is conjugated to the AB via a linker, and wherein the linker to which the agent is conjugated to the AB comprises an SPDB moiety, a vc moiety, or a PEG2-vc moiety,

39. The conjugated activatable antibody of claim 35, wherein the agent is a toxin conjugated to the AB via a linker, and wherein the linker and toxin conjugated to the AB comprise a moiety selected from the group consisting of: an SPDB-DM4 moiety, a vc-MMAD moiety, a vc-MMAE moiety, a vc-duocarmycin moiety, or a PEG2-vc-MMAD moiety.

40. A conjugated activatable antibody or conjugated antibody comprising:
an antibody or antigen binding fragment thereof (AB) that, in an activated state, binds CD166; and
a toxin conjugated to the AB via a linker,
wherein the conjugated activatable antibody or the conjugated antibody comprises amino acid sequences, a linker, and a toxin selected from a single row in Table C, wherein for the given combination:
(a) the AB comprises a heavy chain comprising the amino acid sequence of the heavy chain sequence or heavy chain variable domain sequence corresponding to the given combination in the single row listed in Table C,
(b) the AB comprises a light chain comprising the amino acid sequence of the light chain sequence or light chain variable domain sequence corresponding to the given combination in the single row listed in Table C, and
(c) the linker and the toxin comprise the linker and the toxin corresponding to the given combination in the single row listed in Table C.

41. A pharmaceutical composition comprising the activatable antibody of claim 3 and a carrier.

42. The pharmaceutical composition of claim 41 comprising an additional agent.
43. The pharmaceutical composition of claim 42, wherein the additional agent is a therapeutic agent.
44. An isolated nucleic acid molecule encoding the activatable antibody of claim 6.
45. A vector comprising the isolated nucleic acid molecule of claim 44.
46. A method of producing an antibody or an activatable antibody by culturing a cell under conditions that lead to expression of the activatable antibody, wherein the cell comprises the nucleic acid molecule of claim 44.
47. A method of manufacturing an activatable antibody that, in an activated state, binds CD166, the method comprising:
- (a) culturing a cell comprising a nucleic acid construct that encodes the activatable antibody under conditions that lead to expression of the activatable antibody, wherein the activatable antibody comprises an activatable antibody of claim 3;
- and
- (b) recovering the activatable antibody.
48. A pharmaceutical composition comprising the conjugated activatable antibody of claim 18; and a carrier.
49. The pharmaceutical composition of claim 48 comprising an additional agent.
50. The activatable antibody of claim 3, wherein the MM is linked to the CM.
51. The activatable antibody of claim 3, wherein the MM is linked directly to the CM.
52. The activatable antibody of claim 3, wherein the MM is linked to the CM via a linking peptide.

53. A conjugated activatable antibody that, in an activated state, specifically binds to mammalian CD166 comprising:

an antibody or an antigen binding fragment thereof (AB) that specifically binds to mammalian CD166, wherein the AB specifically binds human CD166 and cynomolgus monkey CD166;

a masking moiety (MM) coupled to the AB that inhibits the binding of the AB to CD166 when the activatable antibody is in an uncleaved state;

a cleavable moiety (CM) coupled to the AB, wherein the CM is a polypeptide that functions as a substrate for a protease; and

an agent conjugated to the AB via a linker,

wherein the AB comprises the VH CDR1 amino acid sequence GFSLSTYGMGVG (SEQ ID NO: 127); the VH CDR2 amino acid sequence NIWSEDKH (SEQ ID NO: 128); the VH CDR3 amino acid sequence IDYGNDYAFTY (SEQ ID NO: 129); the VL CDR1 amino acid sequence RSSKSLHSNGITYLY (SEQ ID NO: 130) or RSSQSLHSNGITYLY (SEQ ID NO: 131); the VL CDR2 amino acid sequence QMSNLAS (SEQ ID NO: 132) or QMSNRAS (SEQ ID NO: 133); and the VL CDR3 amino acid sequence AQNLELPYT (SEQ ID NO: 134);

wherein the MM comprises the amino acid sequence of SEQ ID NO: 222,

wherein the CM comprises the amino acid sequence of SEQ ID NO: 76,

wherein the MM is linked to the CM such that the activatable antibody in an uncleaved state comprises the structural arrangement from N-terminus to C-terminus as follows: MM-CM-AB or AB-CM-MM, and

wherein the linker and the agent comprises vc-MMAE.

54. The conjugated activatable antibody of claim 53, wherein the conjugated activatable antibody comprises a first linking peptide (LP1) and a second linking peptide (LP2), and wherein the conjugated activatable antibody in the uncleaved state has the structural arrangement from N-terminus to C-terminus as follows: MM-LP1-CM-LP2-AB.

55. The conjugated activatable antibody of claim 54, wherein the first linking peptide (LP1) comprises the amino acid sequence of GGGSSGGS (positions 23-30 of SEQ ID NO: 455) and a second linking peptide (LP2) comprises the amino acid sequence of GGS.

56. A conjugated activatable antibody that, in an activated state, specifically binds to mammalian CD166 comprising:

- an antibody or an antigen binding fragment thereof (AB) that specifically binds mammalian CD166, wherein the AB specifically binds human CD166 and cynomolgus monkey CD166;

- a masking moiety (MM) coupled to the AB that inhibits the binding of the AB to CD166 when the activatable antibody is in an uncleaved state;

- a cleavable moiety (CM) coupled to the AB, wherein the CM is a polypeptide that functions as a substrate for a protease; and

- an agent conjugated to the AB via a linker,

- wherein the AB comprises a heavy chain variable region comprising the amino acid sequence of SEQ ID NOs: 122, and a light chain variable region comprising the amino acid sequence of SEQ ID NOs: 123,

- wherein the MM comprises an amino acid sequence of SEQ ID NO: 222,

- wherein the CM comprises an amino acid sequence of SEQ ID NO: 76,

- wherein the MM is linked to the CM such that the activatable antibody in an uncleaved state comprises the structural arrangement from N-terminus to C-terminus as follows: MM-CM-AB or AB-CM-MM, and

- wherein the linker and the agent comprises vc-MMAE.

57. The conjugated activatable antibody of claim 56, wherein the conjugated activatable antibody comprises a first linking peptide (LP1) and a second linking peptide (LP2), and wherein the conjugated activatable antibody in the uncleaved state has the structural arrangement from N-terminus to C-terminus as follows: MM-LP1-CM-LP2-AB.

58. The conjugated activatable antibody of claim 57, wherein the first linking peptide (LP1) comprises the amino acid sequence of GGGSSGGS (positions 23-30 of SEQ ID NO: 455) and a second linking peptide (LP2) comprises the amino acid sequence of GGS.

59. A conjugated activatable antibody that, in an activated state, specifically binds to mammalian CD166 comprising:

- an antibody or an antigen binding fragment thereof (AB) that specifically binds mammalian CD166, wherein the AB specifically binds human CD166 and cynomolgus monkey CD166;

- a masking moiety (MM) coupled to the AB that inhibits the binding of the AB to CD166 when the activatable antibody is in an uncleaved state;

- a cleavable moiety (CM) coupled to the AB, wherein the CM is a polypeptide that functions as a substrate for a protease; and

- an agent conjugated to the AB via a linker,

- wherein the activatable antibody comprises a heavy chain variable region comprising the amino acid sequence of SEQ ID NOs: 122, and a light chain variable region comprising the amino acid sequence of SEQ ID NOs: 368, and

- wherein the linker and the agent comprises vc-MMAE.

60. A conjugated activatable antibody that, in an activated state, specifically binds to mammalian CD166 comprising:

- an antibody or an antigen binding fragment thereof (AB) that specifically binds mammalian CD166, wherein the AB specifically binds human CD166 and cynomolgus monkey CD166;

- a masking moiety (MM) coupled to the AB that inhibits the binding of the AB to CD166 when the activatable antibody is in an uncleaved state;

- a cleavable moiety (CM) coupled to the AB, wherein the CM is a polypeptide that functions as a substrate for a protease; and

- an agent conjugated to the AB via a linker,

- wherein the activatable antibody comprises a heavy chain comprising the amino acid sequence of SEQ ID NOs: 239, and a light chain comprising the amino acid sequence of SEQ ID NOs: 240, and

- wherein the MM comprises an amino acid sequence of SEQ ID NO: 222,

- wherein the CM comprises an amino acid sequence of SEQ ID NO: 76,

wherein the linker and the agent comprises vc-MMAE.

61. The conjugated activatable antibody of claim 60, wherein the conjugated activatable antibody comprises a first linking peptide (LP1) and a second linking peptide (LP2), and wherein the conjugated activatable antibody in the uncleaved state has the structural arrangement from N-terminus to C-terminus as follows: MM-LP1-CM-LP2-AB.

62. The conjugated activatable antibody of claim 61, wherein the first linking peptide (LP1) comprises the amino acid sequence of GGGSSGGS (positions 23-30 of SEQ ID NO: 455) and a second linking peptide (LP2) comprises the amino acid sequence of GGS.

63. A conjugated activatable antibody that, in an activated state, specifically binds to mammalian CD166 comprising:

an antibody or an antigen binding fragment thereof (AB) that specifically binds mammalian CD166, wherein the AB specifically binds human CD166 and cynomolgus monkey CD166;

a masking moiety (MM) coupled to the AB that inhibits the binding of the AB to CD166 when the activatable antibody is in an uncleaved state;

a cleavable moiety (CM) coupled to the AB, wherein the CM is a polypeptide that functions as a substrate for a protease; and

an agent conjugated to the AB via a linker,

wherein the activatable antibody comprises a heavy chain comprising the amino acid sequence of SEQ ID NOs: 239, and a light chain comprising the amino acid sequence of SEQ ID NOs: 314, and

wherein the linker and the agent comprises vc-MMAE.

64. Use of a therapeutically effective amount of (1) the conjugated activatable antibody of any one of claims 18 – 40 and 53 – 63, (2) the AB of claim 1 or claim 2, (3) the activatable antibody of any one of claims 3 – 17 and 50 – 52, or (4) the pharmaceutical composition of any one of claims 41 – 43, 48, and 49, in the manufacture of a medicament for alleviating a symptom of, or delaying the progression of a disorder or disease in which diseased cells express CD166.

65. The use of claim 64, wherein the disorder or disease is cancer.
66. Use of a therapeutically effective amount of (1) the conjugated activatable antibody of any one of claims 18 – 40 and 53 – 63, (2) the AB of claim 1 or claim 2, (3) the activatable antibody of any one of claims 3 – 17 and 50 – 52, or (4) the pharmaceutical composition of any one of claims 41 – 43, 48, and 49, in the manufacture of a medicament for alleviating a symptom of, or delaying the progression of a disorder or disease associated with cells expressing CD166.
67. The use of claim 66, wherein the disorder or disease associated with cells expressing CD166 is cancer.
68. The use of claim 67, wherein the cancer is an adenocarcinoma, a bile duct (biliary) cancer, a bladder cancer, a bone cancer, a breast cancer, a Her2-negative breast cancer, a triple-negative breast cancer, an endometrial cancer, an estrogen receptor-positive breast cancer, a carcinoid, a cervical cancer, a cholangiocarcinoma, a colorectal cancer, a colon cancer, , a glioma, a head and neck cancer, a head and neck squamous cell cancer, a leukemia, a liver cancer, a lung cancer, a non-small cell lung cancer, a small cell lung cancer, a lymphoma, a melanoma, an oropharyngeal cancer, an ovarian cancer, a pancreatic cancer, a prostate cancer, a metastatic castration-resistant prostate carcinoma, a renal cancer, a sarcoma, a skin cancer, a squamous cell cancer, a stomach cancer, a testis cancer, a thyroid cancer, a urogenital cancer, or a urothelial cancer.
69. The use of claim 67, wherein the cancer is selected from the group consisting of a cholangiocarcinoma, an endometrial cancer, triple negative breast cancer (TNBC), an estrogen receptor-positive breast cancer, non-small cell lung cancer (NSCLC), a prostate carcinoma, small cell lung cancer (SCLC), oropharyngeal cancer, cervical cancer, an ovarian cancer, head and neck squamous cell carcinoma (HNSCC), and prostate cancer.
70. Use of a therapeutically effective amount of (1) the conjugated activatable antibody of any one of claims 18 – 40 and 53 – 63, (2) the AB of claim 1 or claim 2, (3) the activatable antibody of any one of claims 3 – 17 and 50 – 52, or (4) the pharmaceutical

composition of any one of claims 41 – 43, 48, and 49, in the manufacture of a medicament for inhibiting or reducing the growth, proliferation, or metastasis of cells expressing mammalian CD166.

71. Use of a therapeutically effective amount of (1) the conjugated activatable antibody of any one of claims 18 – 40 and 53 – 63, (2) the AB of claim 1 or claim 2, (3) the activatable antibody of any one of claims 3 – 17 and 50 – 52, or (4) the pharmaceutical composition of any one of claims 41 – 43, 48, and 49, in the manufacture of a medicament for inhibiting, blocking, or preventing the binding of a natural ligand or receptor to mammalian CD166.

72. The use of claim 71, wherein the natural ligand or receptor is mammalian CD6.

73. The use of claim 70, wherein the expression and/or activity of the mammalian CD166 is aberrant.

74. The use of claim 64, wherein the medicament comprises an additional agent.

75. The use of claim 74, wherein the additional agent is a therapeutic agent.

76. The use of claim 65, wherein the cancer is an adenocarcinoma, a bile duct (biliary) cancer, a bladder cancer, a bone cancer, a breast cancer, a Her2-negative breast cancer, a triple-negative breast cancer, an endometrial cancer, an estrogen receptor-positive breast cancer, a carcinoid, a cervical cancer, a cholangiocarcinoma, a colorectal cancer, a colon cancer, a glioma, a head and neck cancer, a head and neck squamous cell cancer, a leukemia, a liver cancer, a lung cancer, a non-small cell lung cancer, a small cell lung cancer, a lymphoma, a melanoma, an oropharyngeal cancer, an ovarian cancer, a pancreatic cancer, a prostate cancer, a metastatic castration-resistant prostate carcinoma, a renal cancer, a sarcoma, a skin cancer, a squamous cell cancer, a stomach cancer, a testis cancer, a thyroid cancer, a urogenital cancer, or a urothelial cancer.

77. The use of claim 65, wherein the cancer is selected from the group consisting of a cholangiocarcinoma, an endometrial cancer, triple negative breast cancer (TNBC), an estrogen receptor-positive breast cancer, non-small cell lung cancer (NSCLC), a prostate carcinoma, small cell lung cancer (SCLC), oropharyngeal cancer, cervical cancer, an ovarian cancer, head and neck squamous cell carcinoma (HNSCC), and prostate cancer.