

CLAIMS

What Is Claimed Is:

1. A heavy chain constant region 1 (“CH1”) domain variant polypeptide comprising an amino acid substitution at one or more of the following positions: 118, 119, 124, 126-134, 136, 138-143, 145, 147-154, 163, 168, 170-172, 175, 176, 181, 183-185, 187, 190, 191, 197, 201, 203-206, 208, 210-214, 216, and 218, according to EU numbering,

optionally such that the CH1 domain variant polypeptide preferentially pairs:

- (i) with a kappa light chain constant region (“CL”) domain as compared to a lambda CL domain and/or with a kappa light chain polypeptide as compared to a lambda light chain polypeptide; or
- (ii) with a lambda CL domain as compared to a kappa CL domain and/or with a lambda light chain polypeptide as compared to a kappa light chain polypeptide;

with the proviso that optionally one or more of the following substitution combinations are excluded:

- (a) if residue 141 on CH1 is substituted to C or L, residue 166 is substituted with D or K, residue 128, 129, 162, or 171 on CH1 is substituted to C, and/or residue 147 is substituted to D, said CL does not comprise amino acid substitution;
- (b) if position 126 or 220 on CH1 is substituted with valine or alanine, non-cysteine at position 128, 141, or 168 is substituted with cysteine, or CH1 substitutions is L145F, K147A, F170V, S183F, or V185W/F, said CL does not comprise an amino acid substitution
- (c) if residue 172 on CH1 is substituted to 172R, residue 174 is mutated to 174G, or residue 190 is substituted to 190M or 190I, that these are not the only CH1 substitution(s);
- (d) if the CH1 substitutions consist of L128F, A141I/M/T/L, F170S/A/Y/M, S181M/I/T, S183A/E/K/V and/or V185A/L then CL is not modified;
- (e) if the CH1 substitutions consist of 131C/S, 133R/K, 137E/G, 138S/G, 178S/Y, 192N/S, and/or 193F/L, these are not the only CH1 substitutions and/or in a bispecific antibody containing the CH1 domains are of the same human immunoglobulin subtype or allotype;

- (f) if the CH1 substitutions consist of 145D/E/R/H/K (IMGT position 26) then there is not a corresponding LC substitution at 129D/E/R/H/K (IMGT position 18);
- (g) if the CH1 substitutions consist of 124K/E/R/D there is not a corresponding LC substitution at 176;
- (h) if the CH1 substitutions consist of 133V, 150A, 150D, 152D, 173D, and/or 188W, there are not corresponding LC substitutions;
- (i) if the CH1 substitutions consist of 133S/W/A, 139W/V/G/I, 143K/E/A, 145E/T/L/Y, 146G, 147T/E, 174V, 175D/R/S, 179K/D/R, 181R, 186R, 188F/L, and/or 190S/A/G/Y there are not corresponding LC substitutions;
- (j) if the CH1 substitutions consist of 143A/E/R/K/D and 145T/L there are not corresponding LC substitutions;
- (k) if the CH1 substitutions consist of 124A/R/E/W, 145M/T, 143E/R/D/F, 172R/T and 139W/G/C, 179E, and/or 186R, there are not corresponding LC substitutions;
- (l) if the CH1 substitutions consist of substituting with cysteine at position 126 127, 128, 134, 141, 171, or 173 then the corresponding LC positions are not modified to form a disulfide bond;
- (m) if the CH1 substitutions consist of L145Q, H168A, F170G, S183V, and/or T187E then there are not corresponding kappa or lambda LC substitutions;
- (n) if the CH1 substitutions consist of 143D/E, 145T, 190E/D, and/or 124R there are no corresponding CL substitutions; or
- (o) CH1 substitutions consisting of A140C, K147C, and/or S183C there are corresponding CL substitutions;

optionally further comprising one or more amino acid substitutions that increase pairing of a CH1 domain with:

- (i) a kappa CL domain as compared to a lambda CL domain; and/or
- (ii) a kappa light chain polypeptide as compared to a lambda light chain polypeptide.

2. The CH1 domain variant polypeptide of claim 1, comprising an amino acid substitution at one or more of the following positions: 118, 124, 126-129, 131, 132, 134, 136, 139, 143, 145, 147-151, 153, 154, 170, 172, 175, 176, 181, 183, 185, 190, 191, 197, 201, 203-206, 210, 212-214, and 218, according to EU numbering,

optionally such that the CH1 domain variant polypeptide preferentially pairs with:

(i) a kappa CL domain as compared to a lambda CL domain; and/or

(ii) a kappa light chain polypeptide as compared to a lambda light chain polypeptide;

3. ~~The~~(iii) the CH1 domain variant polypeptide ~~of claim 2, comprising~~comprises an amino acid substitution at position 147, position 183, or positions 147 and 183;

4. ~~The~~(iv) the CH1 domain variant polypeptide ~~of any of claim 2 or 3, comprising~~the foregoing comprises one or more of the following amino acid substitutions:

- a. position 118 is substituted with G;
- b. position 124 is substituted with H, R, E, L, or V;
- c. position 126 is substituted with A, T, or L;
- d. position 127 is substituted with V or L;
- e. position 128 is substituted with H;
- f. position 129 is substituted with P;
- g. position 131 is substituted with A;
- h. position 132 is substituted with P;
- i. position 134 is substituted with G;
- j. position 136 is substituted with E;
- k. position 139 is substituted with I;
- l. position 143 is substituted with V or S;
- m. position 145 is substituted with F, I, N, or T;
- n. position 147 is substituted with F, I, L, R, T, S, M, V, N, E, H, Y, Q, A or G;
- o. position 148 is substituted with I, Q, Y, or G;
- p. position 149 is substituted with C, S, or H;
- q. position 150 is substituted with L or S;
- r. position 151 is substituted with A or L;
- s. position 153 is substituted with S;
- t. position 154 is substituted with M or G;
- u. position 170 is substituted with G or L;
- v. position 172 is substituted with V;
- w. position 175 is substituted with G, L, E, A;
- x. position 176 is substituted with P;

- y. position 181 is substituted with Y, Q, or G;
- z. position 183 is substituted with I, W, F, E, Y, L, K, Q, N, R, or H;
- aa. position 185 is substituted with W;
- bb. position 190 is substituted with P;
- cc. position 191 is substituted with I;
- dd. position 197 is substituted with A;
- ee. position 201 is substituted with S;
- ff. position 203 is substituted with S;
- gg. position 204 is substituted with Y;
- hh. position 205 is substituted with Q;
- ii. position 206 is substituted with S;
- jj. position 210 is substituted with R;
- kk. position 212 is substituted with G;
- ll. position 213 is substituted with E or R;
- mm. position 214 is substituted with R; and
- ~~nn.~~ position 218 is substituted with Q;

5. ~~The~~(v) the CH1 domain variant polypeptide of any ~~one of claims 2-4, comprising:~~
~~(i) the foregoing comprises an~~ amino acid residue F, I, L, R, T, S, M, V, N, E, H, Y,
or Q at position 147; and/or
~~(ii) amino acid residue I, W, F, E, Y, L, K, Q, N, or R at position 183;~~

6. ~~The~~(vi) the CH1 domain variant polypeptide of any ~~one of claims 2-5, comprising:~~
~~(i) the foregoing comprises~~ amino acid residue R, K, or Y at position 183; and/or
~~(ii) amino acid residue F at position 147;~~

7. ~~The~~(vii) the CH1 domain variant polypeptide ~~of any one of claims 2-6, comprising:~~
the foregoing comprises an
~~(ia)~~ amino acid residue F at position 147 and amino acid residue R at position 183;
~~(ib)~~ amino acid residue F at position 147 and amino acid residue K at position 183;
~~(ic)~~ amino acid residue F at position 147 and amino acid residue Y at position 183;

(ivd) amino acid residue R at position 183;

(v) amino acid residue K at position 183; or

(vie) amino acid residue Y at position 183,

optionally comprising the amino acid sequence of:

(if) SEQ ID NO: 137;

(ii)₂ SEQ ID NO: 138;

(iii)₂ SEQ ID NO: 139;

(iv)₂ SEQ ID NO: 60;

(v)₂ SEQ ID NO: 41; or

~~(vi)~~ SEQ ID NO: 136; and/or

8. ~~The~~(vii) the CH1 domain variant polypeptide ~~of any one of claims 2-7, comprising of~~
the foregoing comprises an amino acid substitution at a CH1 amino acid position within the
interface between a CH1 and a VH, optionally wherein the CH1 amino acid position is
position 151, further optionally comprising amino acid residue A or L at position 151.

93. A CH1 domain variant polypeptide according to claim 2, comprising:

(i) amino acid residue F at position 147 and amino acid residue R at position 183;

(ii) amino acid residue F at position 147 and amino acid residue K at position 183;

(iii) amino acid residue F at position 147 and amino acid residue Y at position 183;

(iv) amino acid residue R at position 183;

(v) amino acid residue K at position 183; or

(vi) amino acid residue Y at position 183;₂

10. ~~The CH1 domain variant polypeptide of claim 9, which~~optionally further comprising
one or more amino acid substitutions that increase pairing of a CH1 domain with a kappa CL

domain as compared to a lambda CL domain; and/or a kappa light chain polypeptide as compared to a lambda light chain polypeptide;

which further optionally comprises amino acid substitutions consisting of:

- (i) amino acid residue F at position 147 and amino acid residue R at position 183;
- (ii) amino acid residue F at position 147 and amino acid residue K at position 183;
- (iii) amino acid residue F at position 147 and amino acid residue Y at position 183;
- (iv) amino acid residue R at position 183;
- (v) amino acid residue K at position 183; or
- (vi) amino acid residue Y at position 183.

~~11. The CH1 domain variant polypeptide of **claim 10**, comprising; and further optionally comprises~~ the amino acid sequence of:

- ~~(i)~~ SEQ ID NO: 137;
- ~~(ii)~~ SEQ ID NO: 138;
- ~~(iii)~~ SEQ ID NO: 139;
- ~~(iv)~~ SEQ ID NO: 60₁₋₂
- ~~(v)~~ SEQ ID NO: 41₁₋₂ or
- ~~(vi)~~ SEQ ID NO: 136.

~~124. The CH1 domain variant polypeptide of **any one of claims 2-11**, claim 1, further comprising one or more amino acid substitutions that increase pairing of a CH1 domain with:~~

- ~~(i) a kappa CL domain as compared to a lambda CL domain; and/or~~
- ~~(ii) a kappa light chain polypeptide as compared to a lambda light chain polypeptide.~~

~~13. The CH1 domain variant polypeptide of **any one of claims 2-11**, which results in increased pairing with:~~

- ~~(i) a kappa CL domain as compared to a lambda CL domain; and/or a kappa light chain polypeptide as compared to a lambda light chain polypeptide,~~
- ~~(ii) a kappa light chain polypeptide as compared to a lambda light chain polypeptide,~~

optionally by at least 25%, at least 30%, at least 35%, at least 40%, at least 45%, at least 50%, at least 55%, at least 60%, at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, or 100%, optionally as measured by liquid chromatography-mass spectrometry (LCMS), or

by at least 1.2-fold, at least 1.5-fold, at least 2-fold, by at least 2.5-fold, by at least 3-fold, by at least 3.5-fold, by at least 4-fold, by at least 4.5-fold, by at least 5-fold, at least 5.5-fold, at least 6-fold, at least 6.5-fold, at least 7-fold, at least 7.5-fold, at least 8-fold, at least 8.5-fold, at least 9-fold, at least 9.5-fold, at least 10-fold, at least 11-fold, at least 12-fold, at least 13-fold, at least 14-fold, at least 15-fold, at least 16-fold, at least 17-fold, at least 18-fold, at least 19-fold, at least 20-fold, at least 21-fold, at least 22-fold, at least 23-fold, at least 24-fold, or at least 25-fold, optionally as measured by flow cytometry, optionally by comparing the mean fluorescence intensity (MFI) ratio of kappa CL staining to lambda CL staining; or

14. — ~~The~~(i) the CH1 domain variant polypeptide of ~~claim 1~~, ~~comprising~~optionally comprises an amino acid substitution at one or more of the following positions: 119, 124, 126, 127, 130, 131, 133, 134, 138-142, 152, 163, 168, 170, 171, 175, 176, 181, 183-185, 187, 197, 203, 208, 210-214, 216, and 218, according to EU numbering, further optionally such that the CH1 domain variant preferentially pairs with:
(i) a lambda CL domain as compared to a kappa CL domain; and/or
(ii) a lambda light chain polypeptide as compared to a kappa light chain polypeptide.

15. — ~~The CH1 domain variant polypeptide of claim 14;~~ optionally further comprising an amino acid substitution at one or more of positions 141, 170, 171, 175, 181, 184, 185, 187, and 218;

16. — ~~The~~(ii) the CH1 domain variant polypeptide of ~~claim 14 or 15~~, ~~comprising~~(i) comprises one or more of the following amino acid substitutions:

- a. position 119 is substituted with R;
- b. position 124 is substituted with V;
- c. position 126 is substituted with V;
- d. position 127 is substituted with G;

- e. position 130 is substituted with H or S;
- f. position 131 is substituted with Q, T, N, R, V, or D;
- g. position 133 is substituted with D, T, L, E, S, or P;
- h. position 134 is substituted with A, H, I, P, V, N, or L;
- i. position 138 is substituted with R;
- j. position 139 is substituted with A;
- k. position 140 is substituted with I, V, D, Y, K, S, W, R, L or P;
- l. position 141 is substituted with D, K, E, T, R, Q, V, or M;
- m. position 142 is substituted with M;
- n. position 152 is substituted with G;
- o. position 163 is substituted with M;
- p. position 168 is substituted with F, I, or V;
- q. position 170 is substituted with N, G, E, S, or T;
- r. position 171 is substituted with N, E, G, S, A, or D;
- s. position 175 is substituted with D or M;
- t. position 176 is substituted with R or M;
- u. position 181 is substituted with V, L, A, K, or T;
- v. position 183 is substituted with L or V;
- w. position 184 is substituted with R;
- x. position 185 is substituted with M, L, S, R, or T;
- y. position 187 is substituted with R, D, E, Y, or S;
- z. position 197 is substituted with S;
- aa. position 203 is substituted with D;
- bb. position 208 is substituted with I;
- cc. position 210 is substituted with T;
- dd. position 211 is substituted with A;
- ee. position 212 is substituted with N;
- ff. position 213 is substituted with E;
- gg. position 214 is substituted with R;
- hh. position 216 is substituted with G; and
- ii. position 218 is substituted with L, E, D, P, A, H, S, Q, N, T, I, M, G, C, K, or $W_{\frac{1}{2}}$

17. — ~~The~~(iii) the CH1 domain variant polypeptide of any ~~one of claims 14-16~~, comprising ~~any~~the foregoing comprises one or more of ~~(i)-(xvii)~~the following:

- (~~ia~~) amino acid residue V at position 126;
- (~~ib~~) amino acid residue G at position 127;
- (~~ic~~) amino acid residue V at position 131;
- (~~id~~) amino acid residue S at position 133;
- (~~ie~~) amino acid residue R at position 138;
- (~~if~~) amino acid residue I or V at position 140;
- (~~ig~~) amino acid residue D, K, E, or T at position 141;
- (~~ih~~) amino acid residue M at position 142;
- (~~ii~~) amino acid residue I at position 168;
- (~~ij~~) amino acid residue E, G, or S at position 170;
- (~~ik~~) amino acid residue E, D, G, S, or A at position 171;
- (~~il~~) amino acid residue M at position 175;
- (~~im~~) amino acid residue R at position 176;
- (~~in~~) amino acid residue K, V, A, or L at position 181;
- (~~io~~) amino acid residue R at position 184;
- (~~ip~~) amino acid residue R at position 185;
- (~~iq~~) amino acid residue R at position 187; ~~and/or~~
- (~~iiir~~) amino acid residue L, E, D, P, A, H, S, Q, N, T, I, M, G, C, or W at position 218;

18. — ~~The~~(iv) the CH1 domain variant polypeptide of any ~~one of claims 14-17~~, wherein the foregoing comprises CH1 substitutions that comprise or consist of one or more of the following substitutions: 141D, 141E, 171E, 170E, 185R and 187R;

~~(v)~~19. ~~The the~~ CH1 domain variant polypeptide of any ~~one of claims 14-17~~, wherein the foregoing comprises CH1 substitutions ~~that comprise~~ comprise or consist of two or more of the following substitutions: 141D, 141E, 171E, 170E, 185R and 187R;

~~(vi)~~20. ~~The the~~ CH1 domain variant polypeptide of any ~~one of claims 14-17~~, wherein the foregoing comprises CH1 substitutions ~~that comprise~~ CH1 substitutions comprise or consist of three or more of the following substitutions: 141D, 141E, 171E, 170E, 185R and 187R.

21. —The

~~(vii)~~ the CH1 domain variant polypeptide of any ~~one of claims 14-17~~, wherein the foregoing comprises CH1 substitutions ~~that comprise~~ or consist of the following substitutions: ~~(i)~~ 141E and 185R; ~~(ii)~~ 141E and 187R; ~~(iii)~~ 141E, 170E or 171E, and 185R; ~~(iv)~~ 141E, 170E or 171E, and 187R; ~~(v)~~ 141D and 185R; (vi) 141D and 187R; (vii) 141D, 170E or 171E, and 185R; (viii) 141D, 170E or 171E, and 187R; (ix) 141E, 185R, and 187R; or (x) 141D, 185R, and 187R;

22. —The ~~(viii)~~ the CH1 domain variant polypeptide of any ~~one of claims 14-17~~, comprising the foregoing comprises a substitution at position 141 to D, K, or E optionally paired with a substitution at position 181 to K and further optionally paired with a substitution at position 218 to L, E, D, P, A, H, S, Q, N, T, I, M, G, C, or W;

23. —The ~~(ix)~~ the CH1 domain variant polypeptide of ~~any one of claims 14-17~~, comprising a substitution at position 141 to D, K, or E paired with a substitution at position 181 to K and/or a substitution at position 218 to L, E, D, P, A, H, S, Q, N, T, I, M, G, C, or W.

24. —The CH1 domain variant polypeptide of ~~any one of claims 14-17~~, comprising any comprises one or more of ~~(i)-(ix)~~ the following:

(~~i~~) amino acid residue D, E, or K at position 141;

(~~ii~~) amino acid residue E at position 170;

(~~iii~~) amino acid residue E at position 171;

- (iv~~4~~) amino acid residue M at position 175;
- (v~~5~~) amino acid residue K at position 181;
- (vi~~6~~) amino acid residue R at position 184;
- (vii~~7~~) amino acid residue R at position 185;
- (viii~~8~~) amino acid residue R at position 187; and/or
- (ix~~9~~) amino acid residue P, A, or E at position 218;

25. ~~The~~(x) the CH1 domain variant polypeptide of any of the foregoing comprises one of
~~claims 14-17, comprising or more of the following:~~

- (i~~1~~) amino acid residue D at position 141;
- (ii~~2~~) amino acid residue D at position 141 and amino acid residue K at position 181;
- (iii~~3~~) amino acid residue D at position 141, amino acid residue K at position 181, and amino acid residue A at position 218;
- (iv~~4~~) amino acid residue D at position 141, amino acid residue K at position 181, and amino acid residue P at position 218;
- (v~~5~~) amino acid residue E at position 141;
- (vi~~6~~) amino acid residue E at position 141 and amino acid residue K at position 181;
- (vii~~7~~) amino acid residue K at position 141;
- (viii~~8~~) amino acid residue K at position 141 and amino acid residue K at position 181;
- (ix~~9~~) amino acid residue K at position 141, amino acid residue K at position 181, and amino acid residue E at position 218;
- (x~~10~~) amino acid residue K at position 141, amino acid residue K at position 181, and amino acid residue P at position 218;
- (xi~~11~~) amino acid residue E at position 141, amino acid residue E at position 170, amino acid residue V at position 181, and amino acid residue R at position 187;

(~~xi~~12) amino acid residue E at position 141, amino acid residue D at position 171, and amino acid residue R at position 185;

(~~xiii~~13) amino acid residue E at position 141, amino acid residue E at position 171, and amino acid residue R at position 185;

(~~xiv~~14) amino acid residue E at position 141, amino acid residue G at position 171, amino acid residue R at position 185, and amino acid residue R at position 187;

(~~xv~~15) amino acid residue E at position 141, amino acid residue R at position 185, and amino acid residue R at position 187;

(~~xvi~~16) amino acid residue E at position 141, amino acid residue S at position 171, and amino acid residue K at position 181;

(~~xvii~~17) amino acid residue E at position 141, amino acid residue G at position 170, amino acid residue M at position 175, amino acid residue V at position 181, amino acid residue R at position 184, and amino acid residue R at position 187;

(~~xviii~~18) amino acid residue E at position 141 and amino acid residue R at position 185;

(~~xix~~19) amino acid residue E at position 141 and amino acid residue R at position 187;

(~~xx~~20) amino acid residue E at position 141, amino acid residue E at position 170, and amino acid residue R at position 185;

(~~xxi~~21) amino acid residue E at position 141, amino acid residue E at position 170, and amino acid residue R at position 187;

(~~xxii~~22) amino acid residue D at position 141 and amino acid residue R at position 185;

(~~xxiii~~23) amino acid residue D at position 141 and amino acid residue R at position 187;

(~~xxiv~~24) amino acid residue D at position 141, amino acid residue R at position 185, and amino acid residue R at position 187;

(~~xxv~~25) amino acid residue D at position 141, amino acid residue E at position 170, and amino acid residue R at position 185;

(~~xxvi~~26) amino acid residue D at position 141, amino acid residue E at position 170, and amino acid residue R at position 187;

(~~xxvii~~27) amino acid residue E at position 141, amino acid residue E at position 171, and amino acid residue R at position 187;

(~~xxiii~~28) amino acid residue D at position 141, amino acid residue E at position 171, and amino acid residue R at position 185; or

(~~xxix~~29) amino acid residue D at position 141, amino acid residue E at position 171, and amino acid residue R at position 187;

and optionally comprising the CH1 variant of any of the foregoing comprises the amino acid sequence of:

(i) one of SEQ ID NO: 140;

(ii) SEQ ID NO: 141;

(iii) SEQ ID NO: 142;

(iv) SEQ ID NO: 143;

(v) SEQ ID NO: 144;

(vi) SEQ ID NO: 145;

(vii) SEQ ID NO: 146;

(viii) SEQ ID NO: 147;

(ix) SEQ ID NO: 148;

(x) SEQ ID NO: 149;

(xi) SEQ ID NO: 155;

(xii) SEQ ID NO: 157;

(xiii) SEQ ID NO: 159;

(xiv) SEQ ID NO: 162;

(xv) SEQ ID NO: 163;

(xvi) SEQ ID NO: 164;

(xvii) SEQ ID NO: 165;

(xviii) SEQ ID NO: 178;

(xix) SEQ ID NO: 179;

(xx) SEQ ID NO: 180;

(xxi) SEQ ID NO: 181;

(xxii) SEQ ID NO: 182;

~~(xxiii)~~₂ SEQ ID NO: 183;
~~(xxiv)~~₂ SEQ ID NO: 184;
~~(xxv)~~₂ SEQ ID NO: 185;
~~(xxvi)~~₂ SEQ ID NO: 186;
~~(xxvii)~~₂ SEQ ID NO: 187;
~~(xxviii)~~₂ SEQ ID NO: 188₂ or
~~(xxix)~~₂ SEQ ID NO: 189.

~~265.~~ A heavy chain CH1 domain variant polypeptide according to claim 2, comprising:

(i) amino acid residue D at position 141, amino acid residue E at position 171, and amino acid residue R at position 185;

(ii) amino acid residue D at position 141, amino acid residue E at position 170, and amino acid residue R at position 187; or

(iii) amino acid residue D at position 141, amino acid residue K at position 181, and amino acid residue P at position 218₂;

~~27.~~ ~~The~~optionally wherein the heavy chain CH1 domain variant polypeptide ~~according to claim 26, comprising:~~

~~(i)~~comprises SEQ ID NO: 188;

~~(ii)~~ SEQ ID NO: 186; or

~~(iii)~~ SEQ ID NO: 143.

~~286.~~ The CH1 domain variant polypeptide of ~~any one of claims 14-27,~~claim 4 further comprising

(i) one or more amino acid substitutions that increase pairing of a CH1 domain with:

~~(i)~~ a lambda CL domain as compared to a kappa CL domain; and/or

~~(ii)~~ a lambda light chain polypeptide as compared to a kappa light chain polypeptide₂; and/or

29. ~~The CH1 domain variant polypeptide of **any one of claims 14-28**, which (ii) it comprises one or more amino acid substitutions~~ results in increased pairing with:

- ~~(i)~~ a lambda CL domain as compared to a kappa CL domain; and/or
- ~~(ii)~~ a lambda light chain polypeptide as compared to a kappa light chain polypeptide,

by at least 25%, at least 30%, at least 35%, at least 40%, at least 45%, at least 50%, at least 55%, at least 60%, at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, or 100%, optionally as measured by liquid chromatography-mass spectrometry (LCMS), or

by at least 1.2-fold, at least 1.5-fold, at least 2-fold, by at least 2.5-fold, by at least 3-fold, by at least 3.5-fold, by at least 4-fold, by at least 4.5-fold, by at least 5-fold, at least 5.5-fold, at least 6-fold, at least 6.5-fold, at least 7-fold, at least 7.5-fold, at least 8-fold, at least 8.5-fold, at least 9-fold, at least 9.5-fold, at least 10-fold, at least 11-fold, at least 12-fold, at least 13-fold, at least 14-fold, at least 15-fold, at least 16-fold, at least 17-fold, at least 18-fold, at least 19-fold, at least 20-fold, at least 21-fold, at least 22-fold, at least 23-fold, at least 24-fold, or at least 25-fold, optionally as measured by flow cytometry, optionally by comparing the MFI value ration of lambda CL staining to kappa CL staining.

307. An antibody heavy chain polypeptide comprising a variable region and a constant region, wherein the constant region comprises ~~the~~ CH1 domain variant according to ~~any one of claims~~claim 1-29, optionally further comprising one or more amino acid substitutions outside the CH1 domain which further promotes preferential pairing of the heavy chain with:

(I) (i) a kappa CL domain as compared to a lambda CL domain, and/or

(ii) a kappa light chain polypeptide as compared to a lambda light chain polypeptide; or

(II) (i) a lambda CL domain as compared to a kappa CL domain, and/or

(ii) a lambda light chain polypeptide as compared to a kappa light chain polypeptide.

318. The antibody heavy chain polypeptide of claim ~~307~~, wherein the CH1 domain variant is according to ~~any one of claims 7-11 and 25-27~~claim 2.

329. An antibody or antibody fragment comprising a first heavy chain polypeptide and a first light chain polypeptide, wherein:

(a) the first heavy chain polypeptide and the first light chain polypeptide form a first cognate pair; and

(b) the first heavy chain polypeptide comprises a first CH1 domain variant according to claim 1 comprising an amino acid substitution at one or more of the following positions: 118, 119, 124, 126-134, 136, 138-143, 145, 147-154, 163, 168, 170-172, 175, 176, 181, 183-185, 187, 190, 191, 197, 201, 203-206, 208, 210-214, 216, and 218, according to EU numbering, such that the first CH1 domain variant preferentially binds to the first light chain;

optionally wherein the first light chain polypeptide comprises a first CL domain which is a wild-type CL domain;

with the proviso that optionally one or more of the following substitution combinations in said first CH` domain are excluded:

- (a) if residue 141 on CH1 is substituted to C or L, residue 166 is substituted with D or K, residue 128, 129, 162, or 171 on CH1 is substituted to C , and/or residue 147 is substituted to D, said CL does not comprise amino acid substitution;
- (b) if position 126 or 220 on CH1 is substituted with valine or alanine, non-cysteine at position 128, 141, or 168 is substituted with cysteine, or CH1 substitutions is L145F, K147A, F170V, S183F, or V185W/F, said CL does not comprise an amino acid substitution
- (c) if residue 172 is substituted to 172R, residue 174 is mutated to 174G, or residue 190 is substituted to 190M or 190I, that these are not the only CH1 substitution(s);
- (d) if the CH1 substitutions consist of L128F, A141I/M/T/L, F170S/A/Y/M, S181M/I/T, S183A/E/K/V and/or V185A/L, then CL is not modified;
- (e) if the CH1 substitutions consist of 131C/S, 133R/K, 137E/G, 138S/G, 178S/Y, 192N/S, and/or 193F/L, these are not the only CH1 substitutions s and/or in a

bispecific antibody containing the CH1 domains are of the same human immunoglobulin subtype or allotype;

- (f) if the CH1 substitutions consist of 145D/E/R/H/K (IMGT position 26) then there is not a corresponding LC substitution at 129D/E/R/H/K (IMGT position 18);
- (g) if the CH1 substitutions consist of 124K/E/R/D there is not a corresponding LC substitution at 176;
- (h) if the CH1 substitutions consist of 133V, 150A, 150D, 152D, 173D, and/or 188W, there are not corresponding LC substitutions;
- (i) if the CH1 substitutions consist of 133S/W/A, 139W/V/G/I, 143K/E/A, 145E/T/L/Y, 146G, 147T/E, 174V, 175D/R/S, 179K/D/R, 181R, 186R, 188F/L, and/or 190S/A/G/Y there are not corresponding LC substitutions;
- (j) if the CH1 substitutions consist of 143A/E/R/K/D and 145T/L there are not corresponding LC substitutions;
- (k) if the CH1 substitutions consist of 124A/R/E/W, 145M/T, 143E/R/D/F, 172R/T and 139W/G/C, 179E, and/or 186R, there are not corresponding LC substitutions;
- (l) if the CH1 substitutions consist of substituting with cysteine at position 126, 127, 128, 134, 141, 171, and/or 173, then the corresponding LC positions are not modified to form a disulfide bond;
- (m) if the CH1 substitutions consist of L145Q, H168A, F170G, S183V, and/or T187E then there are not corresponding kappa or lambda LC substitutions;
- (n) if the CH1 substitutions consist of 143D/E, 145T, 190E/D and/or 124R there are no corresponding CL substitutions; or
- (o) CH1 substitutions consisting of A140C, K147C and/or S183C there are corresponding CL substitutions; and

~~33. The antibody or antibody fragment of claim 32, further comprising~~ it further optionally comprises a second heavy chain polypeptide and a second light chain polypeptide, wherein:

- (a) the second heavy chain polypeptide and the second light chain polypeptide form a second cognate pair; and
- (b) the second heavy chain polypeptide comprises a second CH1 domain variant comprising an amino acid substitution at one or more of the following positions: 118,

119, 124, 126-134, 136, 138-143, 145, 147-154, 163, 168, 170-172, 175, 176, 181, 183-185, 187, 190, 191, 197, 201, 203-206, 208, 210-214, 216, and 218, according to EU numbering, such that the second CH1 domain variant preferentially binds to the second light chain polypeptide comprising a second CL domain,

with the proviso that optionally one or more of the following substitution combinations in said second CH1 domain are excluded:

- ~~(p)~~(a) _____ if residue 141 on CH1 is substituted to C or L, residue 166 is substituted with D or K, residue 128, 129, 162, or 171 on CH1 is substituted to C, and/or residue 147 is substituted to D, said CL does not comprise amino acid substitution;
- ~~(q)~~(b) _____ if position 126 or 220 on CH1 is substituted with valine or alanine, non-cysteine at position 128, 141, or 168 is substituted with cysteine, or CH1 substitutions is L145F, K147A, F170V, S183F, or V185W/F, said CL does not comprise an amino acid substitution;
- ~~(r)~~(c) _____ if residue 172 is substituted to 172R, residue 174 is mutated to 174G, or residue 190 is substituted to 190M or 190I, that these are not the only CH1 substitutions;
- ~~(s)~~(d) _____ if the CH1 substitutions consist of L128F, A141I/M/T/L, F170S/A/Y/M, S181M/I/T, S183A/E/K/V and/or V185A/L, then CL is not modified;
- (e) if the CH1 substitutions consist of 131C/S, 133R/K, 137E/G, 138S/G, 178S/Y, 192N/S, and/or 193F/L, these are not the only CH1 substitutions and/or in a bispecific antibody containing the CH1 domains are of the same human immunoglobulin subtype or allotype;
- (f) if the CH1 substitutions consist of 145D/E/R/H/K (IMGT position 26) then there is not a corresponding LC substitution at 129D/E/R/H/K (IMGT position 18);
- (g) if the CH1 substitutions consist of 124K/E/R/D there is not a corresponding LC substitution at 176;
- (h) if the CH1 substitutions consist of 133V, 150A, 150D, 152D, 173D, and/or 188W, there are not corresponding LC substitutions;
- (i) if the CH1 substitutions consist of 133S/W/A, 139W/V/G/I, 143K/E/A, 145E/T/L/Y, 146G, 147T/E, 174V, 175D/R/S, 179K/D/R, 181R, 186R, 188F/L, and/or 190S/A/G/Y there are not corresponding LC substitutions;
- (j) if the CH1 substitutions consist of 143A/E/R/K/D and 145T/L there are not corresponding LC substitutions;

- (k) if the CH1 substitutions consist of 124A/R/E/W, 145M/T, 143E/R/D/F, 172R/T and 139W/G/C, 179E, and/or 186R, there are not corresponding LC substitutions;
- (l) if the CH1 substitutions consist of substituting with cysteine at position 126 127, 128, 134, 141, 171, or 173 then the corresponding LC positions are not modified to form a disulfide bond;
- (m) if the CH1 substitutions consist of L145Q, H168A, F170G, S183V, and T187E then there are not corresponding kappa or lambda LC substitutions;
- (n) if the CH1 substitutions consist of 143D/E, 145T, 190E/D, and/or 124R there are no corresponding CL substitutions; or
- (o) CH1 substitutions consisting of A140C, K147C, and/or S183C there are corresponding CL substitutions;

further optionally wherein the antibody or antibody fragment comprises one or more of features (i)-(ix):

- (i) the first CL domain is a wild-type CL domain;
- (ii) the second CL Domain is a wild-type CL domain;
- (iii) the first CL domain is a kappa CL domain;
- (iv) the first CL domain is a lambda CL domain;
- (v) the second CL domain is a kappa CL domain;
- (vi) the second CL domain is a lambda CL domain;
- (vii) the first CH1 domain variant is the CH1 domain variant is one according to ~~any one of claims~~ claim 1-29;
- (viii) the second CH1 domain variant is the CH1 domain variant is one according to ~~any one of claims~~ claim 1-29; and/or

___(ix) the amino acid substitution(s) in the first CH1 domain variant are different from the amino acid substitution(s) in the second CH1 domain variant.

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10. An antibody or antibody fragment comprising a first heavy chain polypeptide and a first light chain polypeptide, wherein:

(a) the first heavy chain polypeptide and the first light chain polypeptide form a first cognate pair;

(b) the first heavy chain polypeptide comprises a first CH1 domain variant according to ~~any one of claims 2-13~~claim 2; and

(c) the first light chain polypeptide comprises a kappa CL domain and optionally is a kappa light chain polypeptide,

optionally wherein:

(i) the kappa CL domain is a wild-type CL domain; and/or

(ii) the first light chain polypeptide is a wild-type light chain polypeptide,

further optionally wherein the first heavy chain polypeptide comprises one or more amino acid substitutions outside the CH1 domain which further promotes preferential pairing of the heavy chain with:

(i) a kappa CL domain as compared to a lambda CL domain, and/or

(ii) a kappa light chain polypeptide as compared to a lambda light chain polypeptide.

~~35~~11. An antibody or antibody fragment comprising a second heavy chain polypeptide and a second light chain polypeptide, wherein:

(a) the second heavy chain polypeptide and the second light chain polypeptide form a first cognate pair;

(b) the second heavy chain polypeptide comprises a second CH1 domain variant according to ~~any one of claims 14-29~~claim 13; and

(c) the second light chain polypeptide comprises a lambda CL domain and optionally is a lambda light chain polypeptide;

optionally wherein:

(i) the lambda CL domain is a wild-type CL domain; and/or

(ii) the second light chain polypeptide is a wild-type light chain polypeptide,

further optionally wherein the second heavy chain polypeptide comprises one or more amino acid substitutions outside the CH1 domain which further promotes preferential pairing of the heavy chain with:

- (i) a lambda CL domain as compared to a kappa CL domain, and/or
- (ii) a lambda light chain polypeptide as compared to a kappa light chain polypeptide.

3612. An antibody or antibody fragment comprising a first heavy chain polypeptide, a first light chain polypeptide, a second heavy chain polypeptide, and a second light chain polypeptide, wherein:

- (a) the first heavy chain polypeptide and the first light chain polypeptide form a first cognate pair;
- (b) the first heavy chain polypeptide comprises a first CH1 domain comprising the CH1 domain variant according to ~~any one of claims 2-13~~ claim 2-13;
- (c) the first light chain polypeptide comprises a kappa CL domain and optionally is a kappa light chain polypeptide;
- (d) the second heavy chain polypeptide and the second light chain polypeptide form a second cognate pair;
- (e) the second heavy chain polypeptide comprises a second CH1 domain comprising the CH1 domain variant according to ~~any one of claims 14-29~~ claim 2; and
- (f) the second light chain polypeptide comprises a lambda CL domain and optionally is a lambda light chain polypeptide,

further optionally wherein the first heavy chain polypeptide comprises one or more amino acid substitutions outside the CH1 domain which further promotes preferential pairing of the heavy chain with:

- (i) a kappa CL domain as compared to a lambda CL domain, and/or
- (ii) a kappa light chain polypeptide as compared to a lambda light chain polypeptide,

and further optionally wherein the second heavy chain polypeptide comprises one or more amino acid substitutions outside the CH1 domain which further promotes preferential pairing of the heavy chain with:

(i) a lambda CL domain as compared to a kappa CL domain, and/or

(ii) a lambda light chain polypeptide as compared to a kappa light chain polypeptide.

~~37~~13. The antibody or antibody fragment of ~~any one of claims 32-36~~claim 9, which is multispecific, optionally bispecific,

further optionally wherein

(i) the structure of said antibody or antibody fragment is as depicted in any one of FIGS. 24-29;

~~38. The antibody or antibody fragment of claim 32 or 36, which is multispecific, wherein~~(ii) the first and second CH1 domain variants:

~~(i)~~(1) reduce formation of non-cognate heavy chain-light chain pairs by at least 25%, at least 30%, at least 35%, at least 40%, at least 45%, at least 50%, at least 55%, at least 60%, at least 65%, at least 70%, at least 75%, or at least 80%, or by at least 1.2-fold, at least 1.5-fold, at least 2-fold, by at least 2.5-fold, by at least 3-fold, by at least 3.5-fold, by at least 4-fold, by at least 4.5-fold, by at least 5-fold, at least 5.5-fold, at least 6-fold, at least 6.5-fold, at least 7-fold, at least 7.5-fold, at least 8-fold, at least 8.5-fold, at least 9-fold, at least 9.5-fold, at least 10-fold, at least 11-fold, at least 12-fold, at least 13-fold, at least 14-fold, at least 15-fold, at least 16-fold, at least 17-fold, at least 18-fold, at least 19-fold, at least 20-fold, at least 21-fold, at least 22-fold, at least 23-fold, at least 24-fold, or at least 25-fold;

~~(ii)~~(2) provide at least 75%, at least 76%, at least 77%, at least 78%, at least 79%, at least 80%, at least 81%, at least 82%, at least 83%, at least 84%, at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% formation of the desired first and second cognate pairs;

(iii3) provide about 85% to about 95% formation of the desired first and second cognate pairs; and/or

(iv4) provide decreased formation of non-cognate heavy chain-light chain pairs of less than 25%, less than 20%, less than 15%, less than 14%, less than 13%, less than 12%, less than 11%, less than 10%, less than 9%, less than 8%, less than 7%, less than 6%, less than 5%, less than 4%, less than 3%, less than 2%, or less than 1%,

optionally wherein the quantity of cognate and/or non-cognate pairs is determined by LCMS or flow cytometry;

39. ~~The antibody or antibody fragment of claim 33, 36, or 38, which is multispecific and comprises one or more of features (i)-(iv):~~

~~(i)(iii)~~ wherein:

(1) the first CH1 domain variant comprises a substitution at position 147 and/or 183 and reduces formation of non-cognate heavy chain-light chain pairs by at least about 50%;

(ii2) the second CH1 domain variant comprises a substitution at one or more of positions 141, 170, 171, 175, 181, 184, 185, 187, and 218 and reduces formation of non-cognate heavy chain-light chain pairs by at least about 50%;

(iii3) the first CH1 domain variant comprises a substitution at position 147 and/or 183 and the second CH1 domain variant comprises a substitution at one or more of positions 141, 170, 171, 175, 181, 184, 185, 187, and 218 and reduces formation of non-cognate heavy chain-light chain pairs by at least about 50% to at least about 75%;
or

(iv4) the first CH1 domain variant comprises a substitution at position 147 and/or 183 and the second CH1 domain variant comprises a substitution at one or more of positions 141, 170, 171, 175, 181, 184, 185, 187, and 218 and provide about 85% to at least about 95% formation of the desired first and second cognate pairs;

40. ~~The antibody or antibody fragment of claim 33, 36, 38, or 39, which is multispecific and wherein:~~

(iv) wherein:

(a) the first CH1 domain variant comprises

- (i) amino acid residue F at position 147 and/or
- (ii) amino acid residue R, K, or Y at position 183; and

(b) the second CH1 domain variant comprises

- (~~i~~1) amino acid residue E or D at position 141;
- (~~ii~~2) amino acid residue E at position 170;
- (~~iii~~3) amino acid residue E at position 171;
- (~~iv~~4) amino acid residue K at position 181;
- (~~v~~5) amino acid residue R at position 185;
- (~~vi~~6) amino acid residue R at position 187;
- (~~vii~~7) amino acid residue P, A, E, or K at position 218;

~~41. The antibody or antibody fragment of claim 33, 36, 38, or 39, which is multispecific; and wherein:~~or

(v) wherein:

(a) the first CH1 domain variant comprises amino acid substitution(s) consisting of:

- (~~i~~1) amino acid residue F at position 147 and/or
- (~~ii~~2) amino acid residue R, K, or Y at position 183; and

(b) the second CH1 domain variant comprises amino acid substitution(s) consisting of:

- (~~i~~1) amino acid residue D at position 141, amino acid residue E at position 171, and amino acid residue R at position 185;
- (~~ii~~2) amino acid residue D at position 141, amino acid residue E at position 170, and amino acid residue R at position 187; or
- (~~iii~~3) amino acid residue D at position 141, amino acid residue K at position 181, and amino acid residue P at position 218;

42. ~~The antibody or antibody fragment according to **claim 33** or **36**, wherein:~~

(vi) wherein:

(a) the first CH1 domain variant comprises the amino acid sequence of:

(i) SEQ ID NO: 137;

(ii) SEQ ID NO: 138;

(iii) SEQ ID NO: 139;

(iv) SEQ ID NO: 60;

(v) SEQ ID NO: 41; or

(vi) SEQ ID NO: 136; and/or

(b) the second CH1 domain variant comprises the amino acid sequence of:

(i) SEQ ID NO: 188;

(ii) SEQ ID NO: 186; or

(iii) SEQ ID NO: 143.

4314. The antibody or antibody fragment of ~~any one of claims 40-42~~claim 13, wherein the first CH1 variant and the second CH1 variant:

(i) reduces formation of non-cognate heavy chain-light chain pairs by at least 50% to at least 75%; and/or

(ii) provides about 85% to at least about 95% formation of the desired first and second cognate pairs.

4415. A pharmaceutical composition comprising:

(i) the CH1 domain variant polypeptide of ~~claims~~claim 1-29;

(ii) ~~the~~a antibody heavy chain polypeptide comprising the CH1 domain variant polypeptide of **claim 30 or 31**;(i); and/or

(iii) ~~the~~an antibody or antibody fragment comprising the CH1 domain variant polypeptide of ~~any one~~(i) or the heavy chain polypeptide of ~~claims 32-43~~(ii).

4516. A method of generating a CH1 domain variant library, the method comprising:

(a) providing (i) one or more sets of a polypeptide comprising a CH1 domain paired with a polypeptide comprising a kappa CL domain (“C_κ set”) and/or (ii) one or more sets of a polypeptide comprising a CH1 domain paired with a polypeptide comprising a lambda CL domain (“C_λ set”), optionally wherein the polypeptide comprising a CH1 domain further comprises a heavy chain variable region (VH), further optionally wherein the polypeptide comprising a kappa or a lambda CL domain further comprises a light chain variable region (VL);

(b) selecting one or more amino acid positions of the CH1 domain that are in contact with one or more amino acid positions (i) in the kappa CL domain in the C_κ set, (ii) in the lambda CL domain in the C_λ set, and/or (iii) in the VH in the C_κ set and/or in the C_λ set; and

(c) producing a library of CH1 domain variant polypeptides or a library of CH1 domain variant-encoding constructs, wherein one or more of the one or more amino acid positions selected in step (b) are substituted with any non-wild-type amino acid,

optionally wherein:

(I) in step (a), said CH1 domain, said kappa CL domain, and said lambda CL domain are wild-type and/or human;

(II) in step (a), both (i) said polypeptide comprising a CH1 domain paired with a polypeptide comprising a kappa CL domain and (ii) said polypeptide comprising a CH1 domain paired with a polypeptide comprising a lambda CL domain are an intact antibody or are an fragment antigen-binding (“Fab”);

(III) in step (b), one or more amino acid positions of the CH1 domain is selected if the amino acid residue at said one or more amino acid positions of the CH1 domain have a side-chain atom within a distance of 5 Å of (i) a side-chain atom of the amino acid residue at said one or more amino acid positions in the kappa CL domain, (ii) a side-chain atom of the amino acid residue at said one or more amino acid positions in the

lambda CL domain, and/or (iii) a side-chain atom of the amino acid residue at said one or more amino acid positions in the VH; and/or

(IV) said producing in step (c) is via a degenerate codon, optionally a degenerate RMW codon representing six naturally occurring amino acids (D, T, A, E, K, and N) or a degenerate NNK codon representing all 20 naturally occurring amino acid residues;

46. — ~~The method of claim 45,~~ wherein further optionally the one or more CH1 amino acid positions selected in step (b) are:

(i) at an interface with the kappa CL domain in at least 10% of a representative set of the C_κ set and has a fractional solvent accessible surface area greater than 10% in at least 90% of a representative set of the C_κ set;

(ii) at an interface with the lambda CL domain in at least 10% of a representative set of the C_λ set and has a fractional solvent accessible surface area greater than 10% in at least 90% of a representative set of the C_λ set; and/or

(iii) at an interface with the VH in at least 10% of a representative set of the C_κ and/or C_λ set and has a fractional solvent accessible surface area greater than 10% in at least 90% of a representative set of the C_κ and/or C_λ set.

~~47~~17. The method of claim ~~45 or 46~~16, the amino acid positions selected in step (b) comprise one or more of positions 118, 119, 124, 126-134, 136, 138-143, 145, 147-154, 163, 168, 170-172, 175, 176, 181, 183-185, 187, 190, 191, 197, 201, 203-206, 208, 210-214, 216, and 218 according to EU numbering;

with the proviso that optionally one or more of the following substitution combinations are excluded:

(a) if residue 141 on CH1 is substituted to C or L, residue 166 is substituted with D or K, residue 128, 129, 162, or 171 on CH1 is substituted to C, and/or residue 147 is substituted to D, said CL does not comprise amino acid substitution;

- (b) if position 126 or 220 on CH1 is substituted with valine or alanine, non-cysteine at position 128, 141, or 168 is substituted with cysteine, or CH1 substitutions is L145F, K147A, F170V, S183F, or V185W/F, said CL does not comprise an amino acid substitution;
- (c) if residue 172 is substituted to 172R, residue 174 is mutated to 174G, or residue 190 is substituted to 190M or 190I, that these are not the only CH1 substitutions;
- (d) if the CH1 substitutions consist of L128F, A141I/M/T/L, F170S/A/Y/M, S181M/I/T, S183A/E/K/V and/or V185A/L then CL is not modified;
- (e) if the CH1 substitutions consist of 131C/S, 133R/K, 137E/G, 138S/G, 178S/Y, 192N/S, and/or 193F/L, these are not the only CH1 substitutions and/or in a bispecific antibody containing the CH1 domains are of the same human immunoglobulin subtype or allotype;
- (f) if the CH1 substitutions consist of 145D/E/R/H/K (IMGT position 26) then there is not a corresponding LC substitution at 129D/E/R/H/K (IMGT position 18);
- (g) if the CH1 substitutions consist of 124K/E/R/D there is not a corresponding LC substitution at 176;
- (h) if the CH1 substitutions consist of 133V, 150A, 150D, 152D, 173D, and/or 188W, there are not corresponding LC substitutions;
- (i) if the CH1 substitutions consist of 133S/W/A, 139W/V/G/I, 143K/E/A, 145E/T/L/Y, 146G, 147T/E, 174V, 175D/R/S, 179K/D/R, 181R, 186R, 188F/L, and/or 190S/A/G/Y there are not corresponding LC substitutions;
- (j) if the CH1 substitutions consist of 143A/E/R/K/D and 145T/L there are not corresponding LC substitutions;
- (k) if the CH1 substitutions consist of 124A/R/E/W, 145M/T, 143E/R/D/F, 172R/T and 139W/G/C, 179E, and/or 186R, there are not corresponding LC substitutions;
- (l) if the CH1 substitutions consist of substituting with cysteine at 126, 127, 128, 134, 141, 171, or 173 then the corresponding LC positions are not modified to form a disulfide bond;
- (m) if the CH1 substitutions consist of L145Q, H168A, F170G, S183V, and T187E then there are not corresponding kappa or lambda LC substitutions;
- (n) if the CH1 substitutions consist of 143D/E, 145T, 190E/D and/or 124R there are no corresponding CL substitutions; or
- (o) CH1 substitutions consisting of A140C, K147C and/or S183C there are corresponding CL substitutions.

4818. The method of ~~any one of claims 45-47~~claim 16, the library of CH1 domain variants in step (c) is expressed in:

(I) a yeast strain;

(II) *Saccharomyces cerevisiae*; and/or

(III) a cell system which co-expresses (i) one or more polypeptides comprising a kappa CL domain and (ii) one or more polypeptides comprising a lambda CL domain, optionally wherein the kappa and/or lambda CL domains are wild-type,

further optionally the kappa and/or lambda CL domains are human.

4919. A method of generating a CH1 domain variant library optionally comprising CH1 domain variants according to claim 1, the method comprising:

(a) selecting one or more of the following CH1 amino acid positions: 118, 119, 124, 126-134, 136, 138-143, 145, 147-154, 163, 168, 170-172, 175, 176, 181, 183-185, 187, 190, 191, 197, 201, 203-206, 208, 210-214, 216, and 218, according to EU numbering,

(b) selecting one or more CH1 amino acid positions of interest different from the position(s) selected in step (a); and

(c) producing a library of CH1 domain variant polypeptides or a library of CH1 domain variant-encoding constructs, wherein one or more of the one or more amino acid positions selected in step (a) and (b) are substituted with any non-wild-type amino acid,

optionally wherein:

(I) the amino acid position(s) selected in (a) comprises position 141, 147, 151, 170, 171, 181, 183, 185, 187, or 218, or any combination thereof;

(II) said producing in step (c) is via a degenerate codon, optionally a degenerate RMW codon representing six naturally occurring amino acids (D, T, A, E, K, and N)

or a degenerate NNK codon representing all 20 naturally occurring amino acid residues; and/or

(III) in step (c), the amino acid position(s) selected in step (a) is substituted to a pre-determined amino acid and the amino acid position(s) selected in (b) is substituted via a degenerate codon, optionally wherein the substitution to a pre-determined amino acid in step (a) comprises A141D, A141E, K147F, P151A, P151L, F170E, P171E, S181K, S183R, V185R, T187R, or K218P, or any combination thereof.

5020. A method of identifying one or more CH1 domain variant polypeptides that preferentially pair with:

(A) a polypeptide comprising a kappa CL domain as compared to a polypeptide comprising a lambda CL domain; or

(B) a polypeptide comprising a lambda CL domain as compared to a polypeptide comprising a kappa CL domain,

the method comprising:

(a) co-expressing one or more candidate CH1 domain variant polypeptides from a CH1 domain variant library generated via the method of ~~any one of claims 44-49~~ claim 16 with (i) one or more polypeptides comprising a kappa CL domain and (ii) one or more polypeptides comprising a lambda CL domain;

(b) comparing (i) the amount of a candidate CH1 domain variant polypeptide paired with a polypeptide comprising a kappa CL domain and (ii) the amount of a candidate CH1 domain variant polypeptide paired with a polypeptide comprising a lambda CL domain;

(c) based on the comparison in step (b), selecting one or more CH1 domain variants that provide preferential pairing with

(A) a polypeptide comprising a kappa CL domain as compared to a polypeptide comprising a lambda CL domain; or

(B) a polypeptide comprising a lambda CL domain as compared to a polypeptide comprising a kappa CL domain,

wherein in step (a), optionally the total amount of the candidate CH1 domain variant polypeptides expressed and the total amount of the polypeptides comprising a (kappa and lambda) CL domain expressed are approximately the same,

optionally wherein in step (a), the candidate CH1 domain variant polypeptides, the polypeptides comprising a kappa CL domain, and the polypeptides comprising a lambda CL domain are expressed approximately at the ratio of 2:1:1; optionally wherein;

51. ~~The method of claim 50, wherein:~~

(1) in step (a), said (i) one or more polypeptides comprising a kappa CL domain and (ii) one or more polypeptides comprising a lambda CL domain are wild-type and/or human;

52. ~~The method of claim 50 or 51, wherein;~~ (2) in step (b), the amount is determined via fluorescence-activated cell sorting or via liquid chromatography-mass spectrometry; and/or

53. ~~The method of any one of claims 50-52, wherein;~~ (3) the method further comprises (d) co-expressing one or more control CH1 domain variants with (i) one or more polypeptides comprising a kappa CL domain and (ii) one or more polypeptides comprising a lambda CL domain, optionally wherein one or more of said one or more control CH1 domain variants is according to the CH1 domain variant of any one of claims 1-29; domain variant polypeptide comprising an amino acid substitution at one or more of the following positions: 118, 119, 124, 126-134, 136, 138-143, 145, 147-154, 163, 168, 170-172, 175, 176, 181, 183-185, 187, 190, 191, 197, 201, 203-206, 208, 210-214, 216, and 218, according to EU numbering,

optionally such that the CH1 domain variant polypeptide preferentially pairs with a kappa light chain constant region ("CL") domain as compared to a lambda CL domain and/or with a kappa light chain polypeptide as compared to a lambda light chain polypeptide; or with a lambda CL domain as compared to a kappa CL domain and/or with a lambda light chain polypeptide as compared to a kappa light chain polypeptide; and

(4) optionally the method further comprises (d) co-expressing one or more control CH1 domain variants with (i) one or more polypeptides comprising a kappa CL domain and (ii) one or more polypeptides comprising a lambda CL domain, optionally wherein one or more of said one or more control CH1 domain variants is according to a CH1 domain variant polypeptide comprising an amino acid substitution at one or more of the following positions: 118, 119, 124, 126-134, 136, 138-143, 145, 147-154, 163, 168, 170-172, 175, 176, 181, 183-185, 187, 190, 191, 197, 201, 203-206, 208, 210-214, 216, and 218, according to EU numbering,

optionally such that the CH1 domain variant polypeptide preferentially pairs:

(i) with a kappa light chain constant region ("CL") domain as compared to a lambda CL domain and/or with a kappa light chain polypeptide as compared to a lambda light chain polypeptide; or

(ii) with a lambda CL domain as compared to a kappa CL domain and/or with a lambda light chain polypeptide as compared to a kappa light chain polypeptide.