

CLAIMS

1. An isolated effector-competent polypeptide, comprising:
a glycosylated Fc domain comprising a first heavy chain and a second heavy chain, wherein at least one heavy chain comprises an engineered intrachain disulfide bond mediated by a pair of cysteines (C) that substitute for:
a leucine (L) at amino acid position 242 and a lysine (K) at amino acid position 334;
wherein the Fc domain further comprises a substitution at amino acid position 332, according to EU numbering, wherein the substitution at amino acid position 332 is a glutamic acid (E) and the Fc domain further comprises one or more substitutions at amino acid positions 236, 239, or 330, according to EU numbering;
wherein the Fc domain further comprises a substitution at amino acid position 256 and/or 307, according to EU numbering;
wherein the glycosylated Fc domain is capable of interacting with an antibody effector molecule; and
wherein the effector-competent polypeptide has enhanced thermal stability compared to an effector-competent polypeptide having a glycosylated Fc domain capable of interacting with an antibody effector molecule that does not comprise the engineered intrachain disulfide bond.
2. The isolated effector-competent polypeptide of claim 1, wherein the glycosylated Fc domain comprises a native glycan at amino acid position 297, according to EU numbering.
3. The isolated effector-competent polypeptide of claim 1, wherein the glycosylated Fc domain comprises an engineered or non-native glycan.
4. The isolated effector-competent polypeptide of claim 3, wherein the engineered or non-native glycan is a modified glycan that can be conjugated to a therapeutic molecule.
5. The isolated effector-competent polypeptide of claim 1, wherein the isolated effector-competent polypeptide is N-glycosylated.
6. The isolated effector-competent polypeptide of any one of claims 1-4, wherein the first heavy chain comprises the pair of cysteines.

7. The isolated effector-competent polypeptide of any one of claims 1-4, wherein the first and the second heavy chain each comprise the pair of cysteines.
8. The isolated effector-competent polypeptide of any one of claims 1-7, wherein the Fc domain is an IgG1 Fc domain.
9. The isolated effector-competent polypeptide of claim 8, wherein the IgG1 Fc domain is a human IgG1 Fc domain.
10. The isolated effector-competent polypeptide of any one of claims 1-9, wherein the antibody effector molecule is a FcRn.
11. The isolated effector-competent polypeptide of claim 10, wherein the isolated effector-competent polypeptide has enhanced binding affinity to the FcRn compared to a wild-type Fc domain.
12. The isolated effector-competent polypeptide of any one of claims 1-9, wherein the antibody effector molecule is a FcγRIIIa.
13. The isolated effector-competent polypeptide of claim 12, wherein the isolated effector-competent polypeptide has enhanced binding affinity to the FcγRIIIa compared to a polypeptide comprising a wild-type Fc domain.
14. The isolated effector-competent polypeptide of any one of claims 1-13, wherein the isolated effector-competent polypeptide has altered serum half-life compared to a wild-type Fc domain.
15. The isolated effector-competent polypeptide of claim 14, wherein the isolated effector-competent polypeptide has enhanced serum half-life compared to a wild-type Fc domain.
16. The isolated effector-competent polypeptide of any one of claims 1-15, wherein the substitution at amino acid position 236 is an alanine (A).

17. The isolated effector-competent polypeptide of any one of claims 1-15, wherein the substitution at amino acid position 239 is an aspartic acid (D).
18. The isolated effector-competent polypeptide of any one of claims 1-15, wherein the substitution at amino acid position 330 is a leucine (L).
19. The isolated effector-competent polypeptide of any one of claims 1-15, wherein the Fc domain further comprises an alanine (A) at amino acid position 236, and an aspartic acid (D) at amino acid position 239, according to EU numbering.
20. The isolated effector-competent polypeptide of any one of claims 1-15, wherein the Fc domain further comprises an alanine (A) at amino acid position 236, an aspartic acid (D) at amino acid position 239, and a leucine (L) at amino acid position 330, according to EU numbering.
21. The isolated effector-competent polypeptide of claim 1, wherein the substitution at amino acid position 256 is an aspartic acid (D).
22. The isolated effector-competent polypeptide of claim 1, wherein the substitution at amino acid position 307 is a glutamine (Q).
23. The isolated effector-competent polypeptide of claim 1, wherein the Fc domain further comprises an aspartic acid (D) at amino acid position 256 and a glutamine (Q) at amino acid position 307, according to EU numbering.
24. The isolated effector-competent polypeptide of claim 21, wherein the Fc domain further comprises an aspartic acid (D) at amino acid position 239, a glutamic acid (E) at amino acid position 332, an aspartic acid (D) at amino acid position 256, and a glutamine (Q) at amino acid position 307, according to EU numbering.
25. The isolated effector-competent polypeptide of claim 21, wherein the Fc domain further comprises an alanine (A) at amino acid position 236, an aspartic acid (D) at amino acid position 239, and a glutamic acid (E) at amino acid position 332, an aspartic acid (D) at amino acid position 256, and a glutamine (Q) at amino acid position 307, according to EU numbering.

26. The isolated effector-competent polypeptide of claim 21, wherein the Fc domain further comprises an alanine (A) at amino acid position 236, an aspartic acid (D) at amino acid position 239, a leucine (L) at amino acid position 330, a glutamic acid (E) at amino acid position 332, an aspartic acid (D) at amino acid position 256, and a glutamine (Q) at amino acid position 307, according to EU numbering.

27. An isolated effector-competent polypeptide, comprising:
a glycosylated Fc domain comprising a first heavy chain and a second heavy chain, wherein at least one heavy chain comprises an engineered intrachain disulfide bond mediated by a pair of cysteines (C) that substitute for:

a leucine (L) at amino acid position 242 and a lysine (K) at amino acid position 334;

wherein the Fc domain further comprises a substitution at amino acid position 332, according to EU numbering, wherein the substitution at amino acid position 332 is a glutamic acid (E) and the Fc domain further comprises one or more substitutions at amino acid positions 236, 239, or 330, according to EU numbering;

wherein the Fc domain further comprises a substitution at amino acid position 256 and/or 307, according to EU numbering;

wherein the glycosylated Fc domain is capable of interacting with an antibody effector molecule; and

wherein the effector-competent polypeptide has enhanced thermal stability compared to an effector-competent polypeptide having a glycosylated Fc domain capable of interacting with an antibody effector molecule and comprising a glutamic acid (E) at amino acid position 332, that does not comprise the engineered intrachain disulfide bond.

28. The isolated effector-competent polypeptide of claim 27, wherein the glycosylated Fc domain comprises a native glycan at amino acid position 297, according to EU numbering.

29. The isolated effector-competent polypeptide of claims 27 or 28, wherein the glycosylated Fc domain comprises an engineered or non-native glycan.

30. The isolated effector-competent polypeptide of claim 29, wherein the engineered or non-native glycan is a modified glycan that can be conjugated to a therapeutic molecule.

31. The isolated effector-competent polypeptide of claim 27, wherein the isolated effector-competent polypeptide is N-glycosylated.
32. The isolated effector-competent polypeptide of any one of claims 27-31, wherein the first heavy chain comprises the pair of cysteines.
33. The isolated effector-competent polypeptide of any one of claims 27-31, wherein the first and the second heavy chain each comprise the pair of cysteines.
34. The isolated effector-competent polypeptide of any one of claims 27-33, wherein the modified Fc domain is a modified human Fc domain.
35. The isolated effector-competent polypeptide of claim 34, wherein the modified Fc domain is a modified human IgG1 Fc domain.
36. The isolated effector-competent polypeptide of any one of claims 27-35, wherein the antibody effector molecule is a FcRn.
37. The isolated effector-competent polypeptide of claim 36, wherein the isolated effector-competent polypeptide has enhanced binding affinity to the FcRn compared to a wild-type Fc domain.
38. The isolated effector-competent polypeptide of any one of claims 27-35, wherein the antibody effector molecule is a FcγRIIIa.
39. The isolated effector-competent polypeptide of claim 38, wherein the isolated effector-competent polypeptide has enhanced binding affinity to the FcγRIIIa compared to a polypeptide comprising a wild-type Fc domain.
40. The isolated effector-competent polypeptide of any one of claims 27-39, wherein the isolated effector-competent polypeptide has altered serum half-life compared to a wild-type Fc domain.

41. The isolated effector-competent polypeptide of claim 40, wherein the isolated effector-competent polypeptide has enhanced serum half-life compared to a wild-type Fc domain.
42. The isolated effector-competent polypeptide of any one of claims 27-41, further comprising:
- (i) an aspartic acid (D) at amino acid position 239;
 - (ii) an alanine (A) at amino acid position 236;
 - (iii) a leucine (L) at amino acid position 330;
 - (iv) an aspartic acid (D) at amino acid position 256; and/or
 - (v) a glutamine (Q) at amino acid position 307.
43. The isolated effector-competent polypeptide of claims 42, wherein the Fc domain further comprises an aspartic acid (D) at amino acid position 256 and a glutamine (Q) at amino acid position 307, according to EU numbering.
44. The isolated effector-competent polypeptide of claim 42 or 43, wherein the one or more substitutions are on the same heavy chain as the engineered disulfide bond.
45. The isolated effector-competent polypeptide of claim 42 or 43, wherein the one or more substitutions are on a different heavy chain as the engineered disulfide bond.
46. The isolated effector-competent polypeptide of any one of claims 27-45, wherein the isolated effector-competent polypeptide further comprises a binding domain.
47. The isolated effector-competent polypeptide of claim 46, wherein the binding domain comprises one or more antigen binding domains.
48. The isolated effector-competent polypeptide of claim 47, wherein the one or more antigen binding domains specifically bind to a tumor antigen.
49. The isolated effector-competent polypeptide of claim 47, wherein the one or more antigen binding domains specifically bind to an antigen on an immune cell.

50. The isolated effector-competent polypeptide of any one of claims 27-49, wherein the polypeptide is an antibody.
51. The isolated effector-competent polypeptide of claim 50, wherein the polypeptide is monoclonal antibody.
52. The isolated effector-competent polypeptide of claim 50 or 51, wherein the antibody is a chimeric, humanized, or human antibody.
53. The isolated effector-competent polypeptide of any one of claims 50-62, wherein the antibody is a full-length antibody.
54. The isolated effector-competent polypeptide of any one of claims 27-49, wherein the polypeptide is a single-domain antibody.
55. The isolated effector-competent polypeptide of claim 54, wherein the single-domain antibody is a VHH antibody.
56. The isolated effector-competent polypeptide of any one of claims 27-49, wherein the antibody is a multi-specific antibody.
57. The isolated effector-competent polypeptide of claim 56, wherein the multi-specific antibody is of a format selected from the group consisting of: DVD-Ig, a CODV based format that is CODV-Ig, CrossMab, CrossMab-Fab, or Tandem Fabs.
58. The isolated effector-competent polypeptide of claim 56 or 57, wherein the multi-specific antibody is a T cell engager.
59. The isolated effector-competent polypeptide of claim 56 or 57, wherein the multi-specific antibody is an NK cell engager.
60. The isolated effector-competent polypeptide of claim 46, wherein the binding polypeptide comprises a therapeutic polypeptide.

61. The isolated effector-competent polypeptide of claim 60, wherein the therapeutic polypeptide may be a receptor, a ligand, or an enzyme.
62. The isolated effector-competent polypeptide of any one of claims 46-51, wherein the binding polypeptide is linked to the N-terminus and/or the C-terminus of the Fc domain.
63. The isolated effector-competent polypeptide of any one of claims 27-62, wherein the isolated effector-competent polypeptide is capable of depleting a target cell by antibody-dependent cellular cytotoxicity (ADCC) and/or complement-dependent cytotoxicity (CDC).
64. The isolated effector-competent polypeptide of claim 63, wherein the target cell is a cancer cell.
65. The isolated effector-competent polypeptide of claim 63, wherein the target cell is an immune cell.
66. The isolated effector-competent polypeptide of any one of claims 27-49, wherein the polypeptide is an Fc-fusion polypeptide.
67. An isolated nucleic acid molecule comprising a nucleic acid encoding the isolated effector-competent polypeptide of any one of claims 1-66.
68. A vector comprising the isolated nucleic acid molecule of claim 67.
69. The vector of claim 68, wherein the vector is an expression vector.
70. A host cell comprising the vector of claim 68 or 69.
71. The host cell of claim 70, wherein the host cell is of eukaryotic or prokaryotic origin.
72. The host cell of claim 70 or 71, wherein the host cell is of mammalian origin.
73. The host cell of claim 70 or 71, wherein the host cell is of bacterial origin.

74. A pharmaceutical composition comprising the isolated effector-competent polypeptide of any one of claims 1-66.

75. A method of increasing yield of an isolated effector-competent polypeptide, comprising: expressing a glycosylated Fc domain comprising a first heavy chain and a second heavy chain, wherein at least one heavy chain comprises an engineered intrachain disulfide bond mediated by a pair of cysteines (C) that substitute for:

a leucine (L) at amino acid position 242 and a lysine (K) at amino acid position 334;

wherein the Fc domain further comprises a substitution at amino acid position 332, according to EU numbering, wherein the substitution at amino acid position 332 is a glutamic acid (E) and the Fc domain further comprises one or more substitutions at amino acid positions 236, 239, or 330, according to EU numbering;

wherein the Fc domain further comprises a substitution at amino acid position 256 and/or 307, according to EU numbering;

wherein the glycosylated Fc domain is capable of interacting with an antibody effector molecule; and

wherein the effector-competent polypeptide has enhanced thermal stability compared to an effector-competent polypeptide having a glycosylated Fc domain capable of interacting with an antibody effector molecule that does not comprise the engineered intrachain disulfide bond; and

purifying the effector-competent polypeptide, wherein yield of said polypeptide is increased compared to a polypeptide comprising a wild-type glycosylated Fc domain.

76. The method of claim 75, wherein the glycosylated Fc domain comprises a native glycan at amino acid position 297, according to EU numbering.

77. The method of claim 75, wherein the glycosylated Fc domain comprises an engineered or non-native glycan, that is optionally a modified glycan that can be conjugated to a therapeutic molecule.

78. The method of claim 75, wherein the isolated effector-competent polypeptide is N-glycosylated.

79. The method of any one of claims 75-78, wherein the first heavy chain comprises the pair of cysteines.
80. The method of any one of claims 75-78, wherein the first and the second heavy chain each comprise the pair of cysteines.
81. The method of any one of claims 75-80, wherein the Fc domain is a human Fc domain.
82. The method of claim 81, wherein the Fc domain is a IgG1 Fc domain.
83. The method of any one of claims 75-82, wherein the antibody effector molecule is a FcRn.
84. The method of claim 83, wherein the isolated effector-competent polypeptide has enhanced binding affinity to the FcRn compared to a wild-type Fc domain.
85. The method of any one of claims 75-82, wherein the antibody effector molecule is a FcγRIIIa.
86. The method of claim 85, wherein the isolated effector-competent polypeptide has enhanced binding affinity to the FcγRIIIa compared to a polypeptide comprising a wild-type Fc domain.
87. The method of any one of claims 75-86, wherein the isolated effector-competent polypeptide has altered serum half-life compared to a wild-type Fc domain.
88. The method of claim 87, wherein the isolated effector-competent polypeptide has enhanced serum half-life compared to a wild-type Fc domain.
89. The method of any one of claims 75-88, wherein the isolated polypeptide further comprises one or more effector function-enhancing amino acid substitutions, optionally a substitution at amino acid position 332, according to EU numbering.
90. The method of claim 89, wherein the substitution at amino acid position 332 is a glutamic acid (E).

91. The method of claim 90, wherein the Fc domain further comprises one or more substitutions at amino acid positions 236, 239, or 330, according to EU numbering.
92. The method of claim 91, wherein the substitution at amino acid position 236 is an alanine (A).
93. The method of claim 91, wherein the substitution at amino acid position 239 is an aspartic acid (D).
94. The method of claim 91, wherein the substitution at amino acid position 330 is a leucine (L).
95. The method any one of claims 75-91, wherein the Fc domain further comprises an aspartic acid (D) at amino acid position 239, and a glutamic acid (E) at amino acid position 332, according to EU numbering.
96. The method of any one of claims 75-91, wherein the Fc domain further comprises an alanine (A) at amino acid position 236, an aspartic acid (D) at amino acid position 239, and a glutamic acid (E) at amino acid position 332, according to EU numbering.
97. The method of any one of claims 75-91, wherein the Fc domain further comprises an alanine (A) at amino acid position 236, an aspartic acid (D) at amino acid position 239, a leucine (L) at amino acid position 330, and a glutamic acid (E) at amino acid position 332, according to EU numbering.
98. The method of claim 75, wherein the substitution at amino acid position 256 is an aspartic acid (D).
99. The method of claim 75, wherein the substitution at amino acid position 307 is a glutamine (Q).

100. The method of claim 75, wherein the Fc domain further comprises an aspartic acid (D) at amino acid position 256 and a glutamine (Q) at amino acid position 307, according to EU numbering.

101. The method of claim 75, wherein the Fc domain further comprises an aspartic acid (D) at amino acid position 239, a glutamic acid (E) at amino acid position 332, an aspartic acid (D) at amino acid position 256, and a glutamine (Q) at amino acid position 307, according to EU numbering.

102. The method of claims 75, wherein the Fc domain further comprises an alanine (A) at amino acid position 236, an aspartic acid (D) at amino acid position 239, and a glutamic acid (E) at amino acid position 332, an aspartic acid (D) at amino acid position 256, and a glutamine (Q) at amino acid position 307, according to EU numbering.

103. The method of claim 75, wherein the Fc domain further comprises an alanine (A) at amino acid position 236, an aspartic acid (D) at amino acid position 239, a leucine (L) at amino acid position 330, a glutamic acid (E) at amino acid position 332, an aspartic acid (D) at amino acid position 256, and a glutamine (Q) at amino acid position 307, according to EU numbering.

104. The method of any one of claims 89-103, wherein the one or more substitutions are on the same heavy chain as the engineered disulfide bond.

105. The method of any one of claims 89-103, wherein the one or more substitutions are on a different heavy chain as the engineered disulfide bond.

106. The method of any one of claims 89-105, wherein the isolated effector-competent polypeptide further comprises a binding polypeptide.

107. The method of claim 106, wherein the binding polypeptide comprises one or more antigen binding domains.

108. The method of claim 107, wherein the one or more antigen binding domains specifically bind a tumor antigen.

109. The method of claim 108, wherein the one or more antigen binding domains specifically bind to an antigen on an immune cell.
110. The method of any one of claims 75-109, wherein the isolated effector-competent polypeptide is an antibody.
111. The method of claim 110, wherein the isolated effector-competent polypeptide is monoclonal antibody.
112. The method of claim 110 or 111, wherein the antibody is a chimeric, humanized, or human antibody.
113. The method of any one of claims 110-112, wherein the antibody is a full-length antibody.
114. The method of any one of claims 75-109, wherein the isolated effector-competent polypeptide is a single-domain antibody.
115. The method of claim 114, wherein the single-domain antibody is a VHH antibody.
116. The method of any one of claims 110-115, wherein the antibody is a multi-specific antibody.
117. The method of claim 116, wherein the multi-specific antibody is of a format selected from the group consisting of: DVD-Ig, a CODV based format that is optionally CODV-Ig, CrossMab, CrossMab-Fb, or Tandem Fabs.
118. The method of claim 116 or 117, wherein the multi-specific antibody is a T cell engager.
119. The method of claim 116 or 117, wherein the multi-specific antibody is an NK cell engager.
120. The method of claim 106, wherein the binding polypeptide comprises a therapeutic polypeptide.

121. The method of claim 120, wherein the therapeutic polypeptide may be a receptor, a ligand, or an enzyme.

122. The method of any one of claims 106-121, wherein the binding polypeptide is linked to the N-terminus and/or the C-terminus of the Fc domain.

123. The method of any one of claims 75-122, wherein the isolated effector-competent polypeptide is capable of depleting a target cell by antibody-dependent cellular cytotoxicity (ADCC) and/or complement-dependent cytotoxicity (CDC).

124. The method of claim 123, wherein the target cell is a cancer cell.

125. The method of claim 123, wherein the target cell is an immune cell.

126. The method of any one of claims 75-109, wherein the isolated effector-competent polypeptide is an Fc-fusion polypeptide.

127. Use of an effective amount of an effector-competent polypeptide of any one of claims 1-66 in the manufacture of a medicament for the treatment of a disease or disorder in a subject in need thereof, wherein the effector-competent polypeptide further comprises a binding polypeptide or wherein the effector-competent polypeptide is an Fc-fusion polypeptide.

128. The use of claim 127, wherein the disease or disorder is a cancer.

129. The use of claim 127, wherein the disease or disorder is an inflammatory disease.

130. The use of claim 127, wherein the disease or disorder is an autoimmune disease.