

Claims

- [Claim 1] A multispecific antigen-binding molecule comprising:
- (i) a first antigen-binding moiety that is capable of binding to CD3 and CD137, but does not bind to CD3 and CD137 at the same time; and
 - (ii) a second antigen-binding moiety that is capable of binding to claudin-6 (CLDN6).
- [Claim 2] A multispecific antigen-binding molecule comprising:
- (i) a first antigen-binding moiety that is capable of binding to CD3 and CD137, but does not bind to CD3 and CD137 at the same time; and
 - (ii) a second antigen-binding moiety that is capable of binding to claudin-6 (CLDN6);
- wherein the first antigen-binding moiety comprises any one of (a1) to (a4) below:
- (a1) a first antibody variable region comprising the complementarity determining region (CDR) 1 of SEQ ID NO: 9, the CDR 2 of SEQ ID NO: 15, and the CDR 3 of SEQ ID NO: 21, and a second antibody variable region comprising the CDR 1 of SEQ ID NO: 31, the CDR 2 of SEQ ID NO: 35, and the CDR 3 of SEQ ID NO: 39;
 - (a2) a first antibody variable region comprising the complementarity determining region (CDR) 1 of SEQ ID NO: 10, the CDR 2 of SEQ ID NO: 16, and the CDR 3 of SEQ ID NO: 22, and a second antibody variable region comprising the CDR 1 of SEQ ID NO: 31, the CDR 2 of SEQ ID NO: 35, and the CDR 3 of SEQ ID NO: 39;
 - (a3) a first antibody variable region comprising the complementarity determining region (CDR) 1 of SEQ ID NO: 11, the CDR 2 of SEQ ID NO: 17, and the CDR 3 of SEQ ID NO: 23, and a second antibody variable region comprising the CDR 1 of SEQ ID NO: 32, the CDR 2 of SEQ ID NO: 36, and the CDR 3 of SEQ ID NO: 40;
 - (a4) a first antibody variable region comprising the complementarity determining region (CDR) 1 of SEQ ID NO: 12, the CDR 2 of SEQ ID NO: 18, and the CDR 3 of SEQ ID NO: 24, and a second antibody variable region comprising the CDR 1 of SEQ ID NO: 32, the CDR 2 of SEQ ID NO: 36, and the CDR 3 of SEQ ID NO: 40.
- [Claim 3] The multispecific antigen-binding molecule of claim 2, wherein the second antigen-binding moiety comprises any one of (b1) to (b3) below:
- (b1) a third antibody variable region comprising the complementarity

determining region (CDR) 1 of SEQ ID NO: 8, the CDR 2 of SEQ ID NO: 14, and the CDR 3 of SEQ ID NO: 20, and a fourth antibody variable region comprising the CDR 1 of SEQ ID NO: 30, the CDR 2 of SEQ ID NO: 34, and the CDR 3 of SEQ ID NO: 38;

(b2) a third antibody variable region comprising the complementarity determining region (CDR) 1 of SEQ ID NO: 7, the CDR 2 of SEQ ID NO: 13, and the CDR 3 of SEQ ID NO: 19, and a fourth antibody variable region comprising the CDR 1 of SEQ ID NO: 29, the CDR 2 of SEQ ID NO: 33, and the CDR 3 of SEQ ID NO: 37;

(b3) a third antibody variable region comprising the complementarity determining region (CDR) 1 of SEQ ID NO: 29, the CDR 2 of SEQ ID NO: 33, and the CDR 3 of SEQ ID NO: 37, and a fourth antibody variable region comprising the CDR 1 of SEQ ID NO: 7, the CDR 2 of SEQ ID NO: 13, and the CDR 3 of SEQ ID NO: 19.

[Claim 4] The multispecific antigen-binding molecule of claim 2 or 3, wherein the antibody variable regions comprised in the first and/or second antigen-binding moiety comprise human antibody frameworks or humanized antibody frameworks.

[Claim 5] A multispecific antigen-binding molecule comprising:
 (i) a first antigen-binding moiety that is capable of binding to CD3 and CD137, but does not bind to CD3 and CD137 at the same time; and
 (ii) a second antigen-binding moiety that is capable of binding to claudin-6 (CLDN6);
 wherein the first antigen-binding moiety comprises any one of (c1) to (c4) below:
 (c1) a first antibody variable region comprising the amino acid sequence of SEQ ID NO: 3, and a second antibody variable region comprising the amino acid sequence of SEQ ID NO: 27;
 (c2) a first antibody variable region comprising the amino acid sequence of SEQ ID NO: 4, and a second antibody variable region comprising the amino acid sequence of SEQ ID NO: 27;
 (c3) a first antibody variable region comprising the amino acid sequence of SEQ ID NO: 5, and a second antibody variable region comprising the amino acid sequence of SEQ ID NO: 28;
 (c4) a first antibody variable region comprising the amino acid sequence of SEQ ID NO: 6, and a second antibody variable region comprising the amino acid sequence of SEQ ID NO: 28.

[Claim 6] The multispecific antigen-binding molecule of claim 5, wherein the

second antigen-binding moiety comprises any one of (d1) to (d3) below:

(d1) a third antibody variable region comprising the amino acid sequence of SEQ ID NO: 2, and a fourth antibody variable region comprising the amino acid sequence of SEQ ID NO: 26;

(d2) a third antibody variable region comprising the amino acid sequence of SEQ ID NO: 1, and a fourth antibody variable region comprising the amino acid sequence of SEQ ID NO: 25;

(d3) a third antibody variable region comprising the amino acid sequence of SEQ ID NO: 25, and a fourth antibody variable region comprising the amino acid sequence of SEQ ID NO: 1.

[Claim 7] The multispecific antigen-binding molecule of any one of claims 1 to 6, which further comprises:

(iii) an Fc domain which exhibits reduced binding affinity to human Fc gamma receptor, as compared to a native human IgG1 Fc domain.

[Claim 8] The multispecific antigen-binding molecule of any one of claims 2 to 6, wherein the first antibody variable region of the first antigen-binding moiety is fused with a first heavy chain constant region, the second antibody variable region of the first antigen-binding moiety is fused with a first light chain constant region, the third antibody variable region of the second antigen-binding moiety is fused with a second heavy chain constant region, the fourth antibody variable region of the second antigen-binding moiety is fused with a second light chain constant region,

wherein the constant regions are any one of (g1) to (g7) below:

(g1) a first heavy chain constant region comprising the amino acid sequence of SEQ ID NO: 74, a first light chain constant region comprising the amino acid sequence of SEQ ID NO: 87, a second heavy chain constant region comprising the amino acid sequence of SEQ ID NO: 73, and a second light chain constant region comprising the amino acid sequence of SEQ ID NO: 88;

(g2) a first heavy chain constant region comprising the amino acid sequence of SEQ ID NO: 74, a first light chain constant region comprising the amino acid sequence of SEQ ID NO: 85, a second heavy chain constant region comprising the amino acid sequence of SEQ ID NO: 81, and a second light chain constant region comprising the amino acid sequence of SEQ ID NO: 86;

(g3) a first heavy chain constant region comprising the amino acid

sequence of SEQ ID NO: 79, a first light chain constant region comprising the amino acid sequence of SEQ ID NO: 72, a second heavy chain constant region comprising the amino acid sequence of SEQ ID NO: 80, and a second light chain constant region comprising the amino acid sequence of SEQ ID NO: 89;

(g4) a first heavy chain constant region comprising the amino acid sequence of SEQ ID NO: 83, a first light chain constant region comprising the amino acid sequence of SEQ ID NO: 87, a second heavy chain constant region comprising the amino acid sequence of SEQ ID NO: 82, and a second light chain constant region comprising the amino acid sequence of SEQ ID NO: 88;

(g5) a first heavy chain constant region comprising the amino acid sequence of SEQ ID NO: 83, a first light chain constant region comprising the amino acid sequence of SEQ ID NO: 85, a second heavy chain constant region comprising the amino acid sequence of SEQ ID NO: 84, and a second light chain constant region comprising the amino acid sequence of SEQ ID NO: 86;

(g6) a first heavy chain constant region comprising the amino acid sequence of SEQ ID NO: 77, a first light chain constant region comprising the amino acid sequence of SEQ ID NO: 72, a second heavy chain constant region comprising the amino acid sequence of SEQ ID NO: 78, and a second light chain constant region comprising the amino acid sequence of SEQ ID NO: 89;

(g7) a first heavy chain constant region comprising the amino acid sequence of SEQ ID NO: 75, a first light chain constant region comprising the amino acid sequence of SEQ ID NO: 72, a second heavy chain constant region comprising the amino acid sequence of SEQ ID NO: 76, and a second light chain constant region comprising the amino acid sequence of SEQ ID NO: 89.

[Claim 9]

A multispecific antigen-binding molecule comprising four polypeptide chains, wherein the four polypeptide chains are any one of (h01) to (h18) below:

(h01) a heavy chain comprising the amino acid sequence of SEQ ID NO: 41 (chain 1) and a light chain comprising the amino acid sequence of SEQ ID NO: 50 (chain 2), and a heavy chain comprising the amino acid sequence of SEQ ID NO: 54 (chain 3) and a light chain comprising the amino acid sequence of SEQ ID NO: 68 (chain 4);

(h02) a heavy chain comprising the amino acid sequence of SEQ ID

NO: 41 (chain 1) and a light chain comprising the amino acid sequence of SEQ ID NO: 50 (chain 2), and a heavy chain comprising the amino acid sequence of SEQ ID NO: 55 (chain 3) and a light chain comprising the amino acid sequence of SEQ ID NO: 68 (chain 4);

(h03) a heavy chain comprising the amino acid sequence of SEQ ID NO: 42 (chain 1) and a light chain comprising the amino acid sequence of SEQ ID NO: 51 (chain 2), and a heavy chain comprising the amino acid sequence of SEQ ID NO: 56 (chain 3) and a light chain comprising the amino acid sequence of SEQ ID NO: 69 (chain 4);

(h04) a heavy chain comprising the amino acid sequence of SEQ ID NO: 42 (chain 1) and a light chain comprising the amino acid sequence of SEQ ID NO: 51 (chain 2), and a heavy chain comprising the amino acid sequence of SEQ ID NO: 57 (chain 3) and a light chain comprising the amino acid sequence of SEQ ID NO: 69 (chain 4);

(h05) a heavy chain comprising the amino acid sequence of SEQ ID NO: 44 (chain 1) and a light chain comprising the amino acid sequence of SEQ ID NO: 52 (chain 2), and a heavy chain comprising the amino acid sequence of SEQ ID NO: 60 (chain 3) and a light chain comprising the amino acid sequence of SEQ ID NO: 70 (chain 4);

(h06) a heavy chain comprising the amino acid sequence of SEQ ID NO: 44 (chain 1) and a light chain comprising the amino acid sequence of SEQ ID NO: 52 (chain 2), and a heavy chain comprising the amino acid sequence of SEQ ID NO: 61 (chain 3) and a light chain comprising the amino acid sequence of SEQ ID NO: 70 (chain 4);

(h07) a heavy chain comprising the amino acid sequence of SEQ ID NO: 45 (chain 1) and a light chain comprising the amino acid sequence of SEQ ID NO: 50 (chain 2), and a heavy chain comprising the amino acid sequence of SEQ ID NO: 62 (chain 3) and a light chain comprising the amino acid sequence of SEQ ID NO: 68 (chain 4);

(h08) a heavy chain comprising the amino acid sequence of SEQ ID NO: 45 (chain 1) and a light chain comprising the amino acid sequence of SEQ ID NO: 50 (chain 2), and a heavy chain comprising the amino acid sequence of SEQ ID NO: 63 (chain 3) and a light chain comprising the amino acid sequence of SEQ ID NO: 68 (chain 4);

(h09) a heavy chain comprising the amino acid sequence of SEQ ID NO: 46 (chain 1) and a light chain comprising the amino acid sequence of SEQ ID NO: 51 (chain 2), and a heavy chain comprising the amino acid sequence of SEQ ID NO: 64 (chain 3) and a light chain comprising

the amino acid sequence of SEQ ID NO: 69 (chain 4);

(h10) a heavy chain comprising the amino acid sequence of SEQ ID NO: 46 (chain 1) and a light chain comprising the amino acid sequence of SEQ ID NO: 51 (chain 2), and a heavy chain comprising the amino acid sequence of SEQ ID NO: 65 (chain 3) and a light chain comprising the amino acid sequence of SEQ ID NO: 69 (chain 4);

(h11) a heavy chain comprising the amino acid sequence of SEQ ID NO: 47 (chain 1) and a light chain comprising the amino acid sequence of SEQ ID NO: 52 (chain 2), and a heavy chain comprising the amino acid sequence of SEQ ID NO: 66 (chain 3) and a light chain comprising the amino acid sequence of SEQ ID NO: 70 (chain 4);

(h12) a heavy chain comprising the amino acid sequence of SEQ ID NO: 47 (chain 1) and a light chain comprising the amino acid sequence of SEQ ID NO: 52 (chain 2), and a heavy chain comprising the amino acid sequence of SEQ ID NO: 67 (chain 3) and a light chain comprising the amino acid sequence of SEQ ID NO: 70 (chain 4);

(h13) a heavy chain comprising the amino acid sequence of SEQ ID NO: 48 (chain 1) and a light chain comprising the amino acid sequence of SEQ ID NO: 53 (chain 2), and a heavy chain comprising the amino acid sequence of SEQ ID NO: 56 (chain 3) and a light chain comprising the amino acid sequence of SEQ ID NO: 71 (chain 4);

(h14) a heavy chain comprising the amino acid sequence of SEQ ID NO: 48 (chain 1) and a light chain comprising the amino acid sequence of SEQ ID NO: 53 (chain 2), and a heavy chain comprising the amino acid sequence of SEQ ID NO: 57 (chain 3) and a light chain comprising the amino acid sequence of SEQ ID NO: 71 (chain 4);

(h15) a heavy chain comprising the amino acid sequence of SEQ ID NO: 49 (chain 1) and a light chain comprising the amino acid sequence of SEQ ID NO: 53 (chain 2), and a heavy chain comprising the amino acid sequence of SEQ ID NO: 64 (chain 3) and a light chain comprising the amino acid sequence of SEQ ID NO: 71 (chain 4);

(h16) a heavy chain comprising the amino acid sequence of SEQ ID NO: 49 (chain 1) and a light chain comprising the amino acid sequence of SEQ ID NO: 53 (chain 2), and a heavy chain comprising the amino acid sequence of SEQ ID NO: 65 (chain 3) and a light chain comprising the amino acid sequence of SEQ ID NO: 71 (chain 4);

(h17) a heavy chain comprising the amino acid sequence of SEQ ID NO: 43 (chain 1) and a light chain comprising the amino acid sequence

- of SEQ ID NO: 52 (chain 2), and a heavy chain comprising the amino acid sequence of SEQ ID NO: 58 (chain 3) and a light chain comprising the amino acid sequence of SEQ ID NO: 70 (chain 4); and
- (h18) a heavy chain comprising the amino acid sequence of SEQ ID NO: 43 (chain 1) and a light chain comprising the amino acid sequence of SEQ ID NO: 52 (chain 2), and a heavy chain comprising the amino acid sequence of SEQ ID NO: 59 (chain 3) and a light chain comprising the amino acid sequence of SEQ ID NO: 70 (chain 4).
- [Claim 10] An isolated nucleic acid encoding the multispecific antigen-binding molecule of any one of claims 1 to 9.
- [Claim 11] A host cell comprising the nucleic acid of claim 10.
- [Claim 12] A method of producing a multispecific antigen-binding molecule comprising culturing the host cell of claim 11 so that the multispecific antigen-binding molecule is produced.
- [Claim 13] A pharmaceutical composition comprising the multispecific antigen-binding molecule of any one of claims 1 to 9, and a pharmaceutically acceptable carrier.
- [Claim 14] The pharmaceutical composition of claim 13, which is a pharmaceutical composition used for treatment and/or prevention of cancer.
- [Claim 15] The pharmaceutical composition of claim 14, wherein the cancer comprises CLDN6 expressing cells.