

CLAIMS

1. ~~An isolated antigen-binding protein having one or more of the following properties:~~
 - (1) ~~capable of binding to PD-L1 protein derived from a primate with a K_D value of 1×10^9 M or less;~~
 - (2) ~~capable of blocking the binding of PD-L1 protein to PD-1 protein; and~~
 - (3) ~~capable of stimulating the secretion of cytokines in immune cells.~~antibody or antigen binding fragment binding to a human and/or a monkey PD-L1 protein, comprising HCDR1, HCDR2 and HCDR3 and LCDR1, LCDR2 and LCDR3, wherein the HCDR1 comprises an amino acid sequence as set forth in SEQ ID NO: 1, the HCDR2 comprises an amino acid sequence as set forth in SEQ ID NO: 2, and the HCDR3 comprises an amino acid sequence as set forth in SEQ ID NO: 3, the LCDR1 comprises an amino acid sequence as set forth in SEQ ID NO: 4, the LCDR2 comprises an amino acid sequence as set forth in SEQ ID NO: 5, and the LCDR3 comprises an amino acid sequence as set forth in SEQ ID NO: 6.
2. ~~The isolated antigen-binding protein according to claim 1, wherein the primate includes a human and/or a monkey.~~
3. ~~The isolated antigen-binding protein according to claim 1 or 2, including an antibody or an antigen-binding fragment thereof.~~
42. ~~The isolated antigen-binding protein~~antibody or antigen binding fragment according to claim ~~3~~1, wherein the antigen-binding fragment includes a Fab, a Fab', a F(ab)₂, a Fv fragment, a F(ab')₂, a scFv, a di-scFv, a VHH and/or a dAb.
53. ~~The isolated antigen-binding protein~~antibody or antigen binding fragment according to ~~any one of claims 3 to 4~~claim 1 or 2, wherein the antibody is selected from the group consisting of a monoclonal antibody, a chimeric antibody, a humanized antibody and a fully human antibody.
6. ~~The isolated antigen-binding protein according to any one of claims 1 to 5, wherein the isolated antigen-binding protein competes with a reference antibody for binding to PD-L1~~

protein, wherein the reference antibody comprises a heavy chain variable region VH and a light chain variable region VL, the VH of the reference antibody comprises HCDR1, HCDR2 and HCDR3, the VL of the reference antibody comprises LCDR1, LCDR2 and LCDR3, and the HCDR1 of the reference antibody comprises an amino acid sequence as set forth in SEQ ID NO: 1, the HCDR2 of the reference antibody comprises an amino acid sequence as set forth in SEQ ID NO: 2, the HCDR3 of the reference antibody comprises an amino acid sequence as set forth in SEQ ID NO: 3, the LCDR1 of the reference antibody comprises an amino acid sequence as set forth in SEQ ID NO: 4, the LCDR2 of the reference antibody comprises an amino acid sequence as set forth in SEQ ID NO: 5, and the LCDR3 of the reference antibody comprises an amino acid sequence as set forth in SEQ ID NO: 6.

7. The isolated antigen-binding protein according to any one of claims 1 to 6, wherein the isolated antigen-binding protein comprises HCDR3, and the HCDR3 comprises an amino acid sequence as set forth in SEQ ID NO: 3.
8. The isolated antigen-binding protein according to any one of claims 1 to 7, wherein the isolated antigen-binding protein comprises HCDR2, and the HCDR2 comprises an amino acid sequence as set forth in SEQ ID NO: 2.
9. The isolated antigen-binding protein according to any one of claims 1 to 8, wherein the isolated antigen-binding protein comprises HCDR1, and the HCDR1 comprises an amino acid sequence as set forth in SEQ ID NO: 1.
10. The isolated antigen-binding protein according to any one of claims 1 to 9, comprising HCDR1, HCDR2 and HCDR3, wherein the HCDR1 comprises an amino acid sequence as set forth in SEQ ID NO: 1, the HCDR2 comprises an amino acid sequence as set forth in SEQ ID NO: 2, and the HCDR3 comprises an amino acid sequence as set forth in SEQ ID NO: 3.
11. The isolated antigen-binding protein according to any one of claims 1 to 9, comprising a heavy chain variable region VH, wherein the VH comprises a framework region H-FR1, and the C-terminus of the H-FR1 is directly or indirectly linked to the N-terminus of the

- HCDR1, and the H-FR1 comprises an amino acid sequence as set forth in SEQ ID NO: 50.
12. The isolated antigen-binding protein according to claim 11, wherein the H-FR1 comprises an amino acid sequence as set forth in any one of SEQ ID NO: 7 or SEQ ID NO: 8.
13. The isolated antigen-binding protein according to any one of claims 11 to 12, wherein the VH comprises a framework region H-FR2, the H-FR2 is located between the HCDR1 and the HCDR2, and the H-FR2 comprises an amino acid sequence as set forth in SEQ ID NO: 51.
14. The isolated antigen-binding protein according to claim 13, wherein the H-FR2 comprises an amino acid sequence as set forth in any one of SEQ ID NO: 9 to 11.
15. The isolated antigen-binding protein according to any one of claims 11 to 14, wherein the VH comprises a framework region H-FR3, the H-FR3 is located between the HCDR2 and the HCDR3, and the H-FR3 comprises an amino acid sequence as set forth in SEQ ID NO: 52.
16. The isolated antigen-binding protein according to claim 15, wherein the H-FR3 comprises an amino acid sequence as set forth in any one of SEQ ID NO: 12 to 17.
17. The isolated antigen-binding protein according to any one of claims 11 to 16, wherein the VH comprises a framework region H-FR4, the N-terminus of the H-FR4 is linked to the C-terminus of the HCDR3, and the H-FR4 comprises an amino acid sequence as set forth in SEQ ID NO: 53.
18. The isolated antigen-binding protein according to claim 17, wherein the H-FR4 comprises an amino acid sequence as set forth in SEQ ID NO: 18 or 19.
19. The isolated antigen-binding protein according to any one of claims 1 to 18, comprising H-FR1, H-FR2, H-FR3 and H-FR4, wherein the H-FR1 comprises an amino acid sequence as set forth in SEQ ID NO: 7 or 8, the H-FR2 comprises an amino acid sequence as set forth in any one of SEQ ID NO: 9 to 11, the H-FR3 comprises an amino acid sequence as set forth in any one of SEQ ID NO: 12 to 17 and the H-FR4 comprises an amino acid sequence as set forth in SEQ ID NO: 18 or 19.

~~204.~~ The ~~isolated antigen binding protein~~antibody or antigen binding fragment according to any one of claims 1 to ~~493~~, comprising H-FR1, H-FR2, H-FR3 and H-FR4, and the H-FR1, H-FR2, H-FR3 and H-FR4 are selected from any one of groups of amino acid sequences as follows:

- (1) H-FR1: SEQ ID NO: 7, H-FR2: SEQ ID NO: 9, H-FR3: SEQ ID NO: 12 and H-FR4: SEQ ID NO: 18;
- (2) H-FR1: SEQ ID NO: 8, H-FR2: SEQ ID NO: 10, H-FR3: SEQ ID NO: 13 and H-FR4: SEQ ID NO: 19;
- (3) H-FR1: SEQ ID NO: 8, H-FR2: SEQ ID NO: 11, H-FR3: SEQ ID NO: 14 and H-FR4: SEQ ID NO: 18;
- (4) H-FR1: SEQ ID NO: 8, H-FR2: SEQ ID NO: 10, H-FR3: SEQ ID NO: 15 and H-FR4: SEQ ID NO: 19;
- (5) H-FR1: SEQ ID NO: 8, H-FR2: SEQ ID NO: 10, H-FR3: SEQ ID NO: 16 and H-FR4: SEQ ID NO: 19; and
- (6) H-FR1: SEQ ID NO: 8, H-FR2: SEQ ID NO: 11, H-FR3: SEQ ID NO: 17 and H-FR4: SEQ ID NO: 19.

~~21.~~ The ~~isolated antigen binding protein~~ according to any one of claims 1 to 20, wherein the ~~isolated antigen binding protein~~ comprises a heavy chain variable region VH, and the VH comprises an amino acid sequence as set forth in SEQ ID NO: 54.

~~225.~~ The ~~isolated antigen binding protein~~antibody or antigen binding fragment according to ~~claim 21~~any one of claims 1 to 4, wherein the VH comprises an amino acid sequence as set forth in any one of SEQ ID NO: 20 to 25.

~~236.~~ The ~~isolated antigen binding protein~~antibody or antigen binding fragment according to any one of claims 1 to ~~225~~, wherein the ~~isolated antigen binding protein~~antibody or antigen binding fragment comprises a heavy chain constant region, and the heavy chain constant region is derived from a human IgG constant region, optionally.

~~24.~~ The ~~isolated antigen binding protein~~ according to ~~claim 23~~, wherein the heavy chain constant region is derived from a human IgG1 constant region, and the human IgG1

constant region comprises an amino acid sequence as set forth in SEQ ID NO: 41.

- ~~257. The isolated antigen-binding proteinantibody or antigen binding fragment according to any one of claims 1 to 246, wherein the isolated antigen-binding proteinantibody or antigen binding fragment comprises an antibody heavy chain HC, and the HC comprises an amino acid sequence as set forth in any one of SEQ ID NO: 42 to 46.~~
- ~~26. The isolated antigen-binding protein according to any one of claims 1 to 25, wherein the isolated antigen-binding protein comprises LCDR3, and the LCDR3 comprises an amino acid sequence as set forth in SEQ ID NO: 6.~~
- ~~27. The isolated antigen-binding protein according to any one of claims 1 to 26, wherein the isolated antigen-binding protein comprises LCDR2, and the LCDR2 comprises an amino acid sequence as set forth in SEQ ID NO: 5.~~
- ~~28. The isolated antigen-binding protein according to any one of claims 1 to 27, wherein the isolated antigen-binding protein comprises LCDR1, and the LCDR1 comprises an amino acid sequence as set forth in SEQ ID NO: 4.~~
- ~~29. The isolated antigen-binding protein according to any one of claims 1 to 28, comprising LCDR1, LCDR2 and LCDR3, wherein the LCDR1 comprises an amino acid sequence as set forth in SEQ ID NO: 4, the LCDR2 comprises an amino acid sequence as set forth in SEQ ID NO: 5, and the LCDR3 comprises an amino acid sequence as set forth in SEQ ID NO: 6.~~
- ~~30. The isolated antigen-binding protein according to any one of claims 1 to 29, comprising a light chain variable region VL, wherein the VL comprises a framework region L-FR1, the C terminus of the L-FR1 is directly or indirectly linked to the N terminus of the LCDR1, and the L-FR1 comprises an amino acid sequence as set forth in SEQ ID NO: 55.~~
- ~~31. The isolated antigen-binding protein according to claim 30, wherein the L-FR1 comprises an amino acid sequence as set forth in SEQ ID NO: 26 or 27.~~
- ~~32. The isolated antigen-binding protein according to any one of claims 30 to 31, wherein the VL comprises a framework region L-FR2, the L-FR2 is located between the LCDR1 and the LCDR2, and the L-FR2 comprises an amino acid sequence as set forth in SEQ ID NO:~~

56.

~~33. The isolated antigen-binding protein according to claim 32, wherein the L-FR2 comprises an amino acid sequence as set forth in SEQ ID NO: 28 or 29.~~

~~34. The isolated antigen-binding protein according to any one of claims 30 to 33, wherein the VL comprises a framework region L-FR3, the L-FR3 is located between the LCDR2 and the LCDR3, and the L-FR3 comprises an amino acid sequence as set forth in SEQ ID NO: 57.~~

~~35. The isolated antigen-binding protein according to claim 34, wherein the L-FR3 comprises an amino acid sequence as set forth in any one of SEQ ID NO: 30 to 33.~~

~~36. The isolated antigen-binding protein according to any one of claims 30 to 34, wherein the VL comprises a framework region L-FR4, the N-terminus of the L-FR4 is linked to the C-terminus of the LCDR3, and the L-FR4 comprises an amino acid sequence as set forth in SEQ ID NO: 58.~~

~~37. The isolated antigen-binding protein according to claim 36, wherein the L-FR4 comprises an amino acid sequence as set forth in SEQ ID NO: 34 or 35.~~

~~38. The isolated antigen-binding protein of any one of claims 1 to 37, comprising L-FR1, L-FR2, L-FR3 and L-FR4, wherein the L-FR1 comprises an amino acid sequence as set forth in SEQ ID NO: 26 or 27, the L-FR2 comprises an amino acid sequence as set forth in SEQ ID NO: 28 or 29, the L-FR3 comprises an amino acid sequence as set forth in any one of SEQ ID NO: 30 to 33, and the L-FR4 comprises an amino acid sequence as set forth in SEQ ID NO: 34 or 35.~~

~~39~~8. The isolated antigen-binding protein~~antibody or antigen binding fragment~~ according to any one of claims 1 to ~~38~~7, comprising L-FR1, L-FR2, L-FR3 and L-FR4, and the L-FR1, L-FR2, L-FR3 and L-FR4 are selected from any one of groups of amino acid sequences as follows:

(1) L-FR1: SEQ ID NO: 26, L-FR2: SEQ ID NO: 28, L-FR3: SEQ ID NO: 30 and L-FR4: SEQ ID NO: 34;

(2) L-FR1: SEQ ID NO: 27, L-FR2: SEQ ID NO: 29, L-FR3: SEQ ID NO: 31 and

L-FR4: SEQ ID NO: 35;

(3) L-FR1: SEQ ID NO: 27, L-FR2: SEQ ID NO: 29, L-FR3: SEQ ID NO: 32 and
L-FR4: SEQ ID NO: 35; and

(4) L-FR1: SEQ ID NO: 27, L-FR2: SEQ ID NO: 29, L-FR3: SEQ ID NO: 33 and
L-FR4: SEQ ID NO: 35.

~~40. The isolated antigen-binding protein according to any one of claims 1 to 39, wherein the isolated antigen-binding protein comprises a light chain variable region VL, and the VL comprises an amino acid sequence as set forth in SEQ ID NO: 59.~~

~~419. The isolated antigen-binding protein~~antibody or antigen binding fragment according to any one of claims 1 to ~~408~~, wherein the VL comprises an amino acid sequence as set forth in any one of SEQ ID NO: 36 to 39.

10. The antibody or antigen binding fragment according to any one of claims 1 to 9, comprising a heavy chain variable region VH and a light chain variable region VL, wherein
VH comprises or consists of an amino acid sequence as set forth in SEQ ID NO: 20, and the VL comprises or consists of an amino acid sequence as set forth in SEQ ID NO:36;
VH comprises or consists of an amino acid sequence as set forth in SEQ ID NO: 21, and the VL comprises or consists of an amino acid sequence as set forth in SEQ ID NO:37;
VH comprises or consists of an amino acid sequence as set forth in SEQ ID NO: 22, and the VL comprises or consists of an amino acid sequence as set forth in SEQ ID NO:38;
VH comprises or consists of an amino acid sequence as set forth in SEQ ID NO: 23, and the VL comprises or consists of an amino acid sequence as set forth in SEQ ID NO:37;
VH comprises or consists of an amino acid sequence as set forth in SEQ ID NO: 24, and the VL comprises or consists of an amino acid sequence as set forth in SEQ ID NO:37; or
VH comprises or consists of an amino acid sequence as set forth in SEQ ID NO: 25, and the VL comprises or consists of an amino acid sequence as set forth in SEQ ID NO:39.

~~4211. The isolated antigen-binding protein~~antibody or antigen binding fragment according to any one of claims 1 to ~~4110~~, wherein the ~~isolated antigen-binding protein~~antibody or

antigen binding fragment comprises an antibody light chain constant region, and optionally the antibody light chain constant region comprises an amino acid sequence as set forth in SEQ ID NO: 40.

~~43~~12. The ~~isolated antigen-binding protein~~antibody or antigen binding fragment according to any one of claims 1 to ~~42~~11, wherein the ~~isolated antigen-binding protein~~antibody or antigen binding fragment comprises an antibody light chain LC, and the LC comprises an amino acid sequence as set forth in any one of SEQ ID NO: 47 to 49.

13. The antibody or antigen binding fragment according to any one of claims 1 to 12, comprising a heavy chain HC and a light chain LC, wherein
HC comprises or consists of an amino acid sequence as set forth in SEQ ID NO: 42, and the LC comprises or consists of an amino acid sequence as set forth in SEQ ID NO:47;
HC comprises or consists of an amino acid sequence as set forth in SEQ ID NO: 43, and the LC comprises or consists of an amino acid sequence as set forth in SEQ ID NO:48;
HC comprises or consists of an amino acid sequence as set forth in SEQ ID NO: 44, and the LC comprises or consists of an amino acid sequence as set forth in SEQ ID NO:47;
HC comprises or consists of an amino acid sequence as set forth in SEQ ID NO: 45, and the LC comprises or consists of an amino acid sequence as set forth in SEQ ID NO:47; or
HC comprises or consists of an amino acid sequence as set forth in SEQ ID NO: 46, and the LC comprises or consists of an amino acid sequence as set forth in SEQ ID NO:49.

~~1444. A polypeptide comprising the isolated antigen-binding protein of any one of claims 1 to 43.~~

45. An immunoconjugate comprising the ~~isolated antigen-binding protein~~antibody or antigen binding fragment of any one of claims 1 to ~~43~~13or the polypeptide of claim 44.

~~46~~15. An isolated nucleic acid molecule(s) encoding the ~~isolated antigen-binding protein~~antibody or antigen binding fragment of any one of claims 1 to ~~43~~13.

~~47~~16. A vector comprising the nucleic acid molecule according to claim ~~46~~15.

~~48~~17. A cell comprising the nucleic acid molecule according to claim ~~146~~5or the vector according to claim ~~47~~16.

- ~~4918. A method for preparing the isolated antigen-binding proteinantibody or antigen binding fragment of any one of claims 1 to 4313, comprising culturing the cell according to claim 4817 under conditions that the isolated antigen-binding proteinantibody or antigen binding fragment of any one of claims 1 to 43-13 can be expressed.~~
- ~~5019. A pharmaceutical composition comprising the isolated antigen-binding proteinantibody or antigen binding fragment of any one of claims 1 to 4313, the nucleic acid molecule of claim 4615, the vector of claim 47-16 and/or the cell of claim_4187, and optionally a pharmaceutically acceptable carrier.~~
- ~~5120. Use of the isolated antigen-binding proteinantibody or antigen binding fragment of any one of claims 1 to 4313, the polypeptide of claim 44, the immunoconjugate of claim 4514, the nucleic acid molecule of claim 4615, the vector of claim 4716, the cell of claim 4817 and/or the pharmaceutical composition of claim 5019 in the manufacture of a medicament for preventing, alleviating and/or treating a tumor, preferably, -~~
- ~~52. The use according to claim 51, wherein the tumor includes a tumor with a high expression of PD-1 or PD-L1; optionally, +~~
- ~~53. The use according to any one of claims 51 to 52, wherein the tumor includes a solid tumor; optionally -~~
- ~~54. The use according to any one of claims 51 to 53, wherein the tumor includes breast cancer, lung cancer, gastric cancer and urothelial carcinoma.~~
- ~~55. A method for preventing, alleviating or treating a tumor, comprising administering the isolated antigen-binding protein of any one of claims 1 to 43 to a subject in need.~~
- ~~56. The method according to claim 55, wherein the tumor includes a tumor with a high expression of PD-1 or PD-L1, the polypeptide of claim 44, the immunoconjugate of claim 45, the nucleic acid molecule of claim 46, the vector of claim 47, the cell of claim 48 and/or the pharmaceutical composition of claim 50.~~
- ~~57. The method according to any one of claims 55 to 56, wherein the tumor includes a solid tumor.~~
- ~~58. The method according to any one of claims 55 to 57, wherein the tumor includes breast~~

~~cancer, lung cancer, gastric cancer and urothelial carcinoma.~~

~~59~~21. ~~The isolated antigen-binding protein~~antibody or antigen binding fragment of any one of claims 1 to ~~43~~13, the immunoconjugate of claim 14, the nucleic acid molecule of claim 15, the vector of claim 16, the cell of claim 17 and/or the pharmaceutical composition of claim 19, for use in preventing, alleviating or treating a tumor, preferably, -

~~60. The isolated antigen-binding protein of claim 59, the polypeptide of claim 44, the immunoconjugate of claim 45, the nucleic acid molecule of claim 46, the vector of claim 47, the cell of claim 48 and/or the pharmaceutical composition of claim 50, wherein the tumor includes a tumor with a high expression of PD-1 or PD-L1; optionally -~~

~~61. The isolated antigen-binding protein according to any one of claims 59 to 60, wherein the tumor includes a solid tumor; optionally -~~

~~62. The isolated antigen-binding protein according to any one of claims 59 to 61, wherein the tumor includes breast cancer, lung cancer, gastric cancer and urothelial carcinoma. -~~

~~63~~22. ~~A method for inhibiting the binding of PD-1 protein to PD-L1 protein, comprising administering t~~The isolated antigen-binding protein~~antibody or antigen binding fragment~~ of any one of claims 1 to ~~43~~13, ~~the polypeptide of claim 44,~~ the immunoconjugate of claim ~~45~~14, the nucleic acid molecule of claim ~~46~~15, the vector of claim ~~47~~16, the cell of claim ~~48~~17 and/or the pharmaceutical composition of claim ~~50~~19, for use in inhibiting the binding of PD-1 protein to PD-L1 protein or for use in-

~~64. A method of -stimulating the secretion of cytokines in immune cells, comprising administering the isolated antigen-binding protein of any one of claims 1 to 43, the polypeptide of claim 44, the immunoconjugate of claim 45, the nucleic acid molecule of claim 46, the vector of claim 47, the cell of claim 48 and/or the pharmaceutical composition of claim 50.~~

~~65~~23. A method for detecting the presence and/or content of PD-L1 protein, comprising administering the ~~isolated antigen-binding protein~~antibody or antigen binding fragment of any one of claims 1 to ~~43~~13 and/or the polypeptide of claim 44.

~~66~~24. A kit comprising the ~~isolated antigen-binding protein~~antibody or antigen binding

fragment of any one of claims 1 to 4313, ~~the polypeptide of claim 44,~~ the immunoconjugate of claim 4514, the nucleic acid molecule of claim 4615, the vector of claim 4716, the cell of claim 4817 and/or the pharmaceutical composition of claim 5019.