

CLAIMS

1. A fusion protein that is capable of binding both CD137 and PD-L1, wherein the fusion protein comprises at least two subunits in any order, wherein ~~the~~ first subunit comprises a full-length immunoglobulin or an antigen-binding domain thereof and that is specific for PD-L1, and wherein ~~the~~ second subunit comprises a lipocalin mutein and that is specific for CD137,
wherein the fusion protein comprises amino acid sequences having at least 90% sequence identity to the amino acid sequences shown in SEQ ID NOs: 90 and 87,
wherein the heavy chain of the immunoglobulin comprises the following set of CDR sequences: GFSLSNYD (HCDR1, SEQ ID NO: 60), IWTGGAT (HCDR2, SEQ ID NO: 61), and VRDSNYRYDEPFTY (HCDR3, SEQ ID NO: 62), and the light chain of the immunoglobulin comprises the following set of CDR sequences: QSIGTN (LCDR1, SEQ ID NO: 63), YAS (LCDR2), and QQSNSWPYT (LCDR3, SEQ ID NO: 64), and
wherein the amino acid sequence of the lipocalin mutein comprises the following set of mutated amino acid residues in comparison with the linear polypeptide sequence of mature hNGAL as shown in SEQ ID NO: 2: Gln 28 → His; Leu 36 → Gln; Ala 40 → Ile; Ile 41 → Arg; Gln 49 → Ile; Tyr 52 → Met; Asn 65 → Asp; Ser 68 → Met; Leu 70 → Lys; Arg 72 → Asp; Lys 73 → Asp; Asp 77 → Met; Trp 79 → Asp; Arg 81 → Trp; Cys 87 → Ser; Asn 96 → Lys; Tyr 100 → Phe; Leu 103 → His; Tyr 106 → Ser; Lys 125 → Phe; Ser 127 → Phe; Tyr 132 → Glu; and Lys 134 → Tyr.
2. The fusion protein of claim 1, further comprising a third subunit, which third subunit comprises a lipocalin mutein specific for CD137wherein the amino acid sequence of the lipocalin mutein has at least 90% sequence identity to the amino acid sequence of SEQ ID NO: 42.
1. The fusion protein of claim 1 or 2, wherein the fusion protein is capable of binding PD-L1 with a K_D value of at most about 2 nM or comparable to or lower than the K_D value of the immunoglobulin or an antigen-binding domain thereof that is included in the first subunit alone.
2. The fusion protein of claim 1 or 2, wherein the fusion protein is capable of binding CD137 with a K_D value of at most about 7 nM or comparable to or lower than the K_D value of the lipocalin mutein specific for CD137 that is included in the second subunit alone.
The fusion protein of claim
3. 3 or 4, wherein the K_D value is determined by a surface plasmon resonance (SPR) assay.

4. ~~The fusion protein of claim 1 or 2, wherein the fusion protein is capable of binding PD-L1 with an EC₅₀ value of at most about 0.5 nM or comparable to or lower than the EC₅₀ value of the immunoglobulin or an antigen-binding domain thereof that is included in the first subunit alone.~~

_____ ~~The fusion protein of claim 1 or 2, wherein the fusion protein is capable of binding CD137 with an EC₅₀ value of at most about 0.6 nM or comparable to or lower than the EC₅₀ value of the lipocalin mutein specific for CD137 that is included in the second subunit alone~~amino acid sequence of the lipocalin mutein comprises the amino acid sequence of SEQ ID NO: 42.

~~5. 4. The fusion protein of any one of claims 6-71-3, wherein the EC₅₀ value~~second subunit is determined by an enzyme-linked immunosorbent assay (ELISA) assay.

~~The fusion protein at the N-terminus via a linker to the C-terminus of claim 1 or 2 each heavy chain constant region (CH) of the first subunit, wherein the fusion protein is cross-reactive with cynomolgus PD-L1~~linker is preferably the linker of SEQ ID NO: 13.

6. ~~The fusion protein of claim 1 or 2, wherein the fusion protein is cross-reactive with cynomolgus CD137.~~

7. ~~The fusion protein of claim 1 or 2, wherein the fusion protein is capable of simultaneously binding CD137 and PD-L1 with an EC₅₀ values of at most about 10 nM, when said fusion protein is measured in an ELISA assay.~~

8. ~~The fusion protein of claim 1 or 2, wherein the fusion protein is capable of binding CD137 expressed on a cell with an EC₅₀ values of at most about 60 nM.~~

9. ~~The fusion protein of claim 1 or 2, wherein the fusion protein is capable of binding PD-L1 expressed on a cell with an EC₅₀ values of at most about 10 nM, when said fusion protein is measured in a flow cytometric analysis.~~

10. ~~The fusion protein of claim 1 or 2, wherein the fusion protein is capable of binding PD-L1 expressing tumor cells.~~

11. ~~The fusion protein of claim 1 or 2, wherein the fusion protein is capable of binding CD137 in the presence of CD137 ligand.~~

12. ~~The fusion protein of claim 1 or 2, wherein the fusion protein is capable of competing with PD-1 for binding to PD-L1.~~

13. ~~The fusion protein of claim 1 or 2, wherein the fusion protein is capable of compete with the antibody shown in SEQ ID NOs: 28 and 29 for binding to CD137.~~

14. ~~The fusion protein of claim 1 or 2, wherein the fusion protein has overlapping CD137-binding epitope with the antibody shown in SEQ ID NOs: 28 and 29.~~

5. _____ ~~The fusion protein of any one of claims 1-18, wherein the fusion protein is capable of~~

stimulating T-cell proliferation and/or responses⁴, wherein the immunoglobulin comprises a heavy chain variable region and a light chain variable region comprising the amino acid sequences as shown in SEQ ID NOs: 77 and 82.

6. The fusion protein of any one of claims 1-19, wherein the fusion protein is capable of stimulating CD4⁺ and/or CD8⁺ T-cell proliferation⁵, wherein the immunoglobulin comprises a heavy chain and a light chain comprising the amino acid sequences as shown in SEQ ID NOs: 86 and 87.

~~15-7. The fusion protein of any one of claims 1-20, wherein the fusion protein is capable of inducing increased secretion of IL-2 and/or IFN-gamma.~~

~~16. The fusion protein of any one of claims 1-21, wherein the fusion protein is capable of inducing increased secretion of cytotoxic factors.~~

~~17. The fusion protein of any one of claims 1-22, wherein the fusion protein is capable of co-stimulating T-cell responses in a PD-L1-dependent manner.~~

~~18. The fusion protein of any one of claims 1-23, wherein the fusion protein is capable of co-stimulating T-cell responses in a tumor microenvironment.~~

~~19. The fusion protein of any one of claims 1-24, wherein the fusion protein does not co-stimulate T-cell responses in the absence of PD-L1.~~

~~20. The fusion protein of any one of claims 1-25, wherein the fusion protein is capable of blocking the inhibitory signal of PD-1.~~

~~21. The fusion protein of any one of claims 1-26, wherein the fusion protein has antibody-like pharmacokinetics profile.~~

~~22. The fusion protein of any one of claims 1-27, wherein the fusion protein has a more favorable pharmacokinetic profile than SEQ ID NO: 147 or SEQ ID NO: 148.~~

~~23. The fusion protein of any one of claims 1-28, wherein the lipocalin mutein comprises one or more mutated amino acid residues at positions corresponding to positions 5, 26-31, 33-34, 42, 46, 52, 56, 58, 60-61, 65, 71, 85, 94, 101, 104-106, 108, 111, 114, 121, 133, 148, 150 and 153 of the linear polypeptide sequence of mature human tear lipocalin (SEQ ID NO: 1).~~

~~24. The fusion protein of claim 29, wherein the amino acid sequence of the lipocalin mutein comprises, at one or more positions corresponding to positions 5, 26-31, 33-34, 42, 46, 52, 56, 58, 60-61, 65, 71, 85, 94, 101, 104-106, 108, 111, 114, 121, 133, 148, 150, and 153 of the linear polypeptide sequence of mature hTlc (SEQ ID NO: 1), one or more of the following mutated amino acid residues: Ala 5 → Val or Thr; Arg 26 → Glu; Glu 27 → Gly; Phe 28 → Cys; Pro 29 → Arg; Glu 30 → Pro; Met 31 → Trp; Leu 33 → Ile; Glu 34 → Phe; Thr 42 → Ser; Gly 46 → Asp; Lys 52 → Glu; Leu 56 → Ala; Ser 58 → Asp; Arg 60~~

→ Pro; Cys 61 → Ala; Lys 65 → Arg or Asn; Thr 71 → Ala; Val 85 → Asp; Lys 94 → Arg or Glu; Cys 101 → Ser; Glu 104 → Val; Leu 105 → Cys; His 106 → Asp; Lys 108 → Ser; Arg 111 → Pro; Lys 114 → Trp; Lys 121 → Glu; Ala 133 → Thr; Arg 148 → Ser; Ser 150 → Ile and Cys 153 → Ser.

25. The fusion protein of claim 29 or 30, wherein the amino acid sequence of the lipocalin mutein comprises one of the following sets of mutated amino acid residues in comparison with the linear polypeptide sequence of mature human tear lipocalin (SEQ ID NO: 1):

- (a) Arg 26 → Glu; Glu 27 → Gly; Phe 28 → Cys; Pro 29 → Arg; Glu 30 → Pro; Met 31 → Trp; Leu 33 → Ile; Glu 34 → Phe; Leu 56 → Ala; Ser 58 → Asp; Arg 60 → Pro; Cys 61 → Ala; Cys 101 → Ser; Glu 104 → Val; Leu 105 → Cys; His 106 → Asp; Lys 108 → Ser; Arg 111 → Pro; Lys 114 → Trp; and Cys 153 → Ser;
- (b) Ala 5 → Thr; Arg 26 → Glu; Glu 27 → Gly; Phe 28 → Cys; Pro 29 → Arg; Glu 30 → Pro; Met 31 → Trp; Leu 33 → Ile; Glu 34 → Phe; Leu 56 → Ala; Ser 58 → Asp; Arg 60 → Pro; Cys 61 → Ala; Lys 65 → Arg; Val 85 → Asp; Cys 101 → Ser; Glu 104 → Val; Leu 105 → Cys; His 106 → Asp; Lys 108 → Ser; Arg 111 → Pro; Lys 114 → Trp; Lys 121 → Glu; Ala 133 → Thr; and Cys 153 → Ser;
- (c) Arg 26 → Glu; Glu 27 → Gly; Phe 28 → Cys; Pro 29 → Arg; Glu 30 → Pro; Met 31 → Trp; Leu 33 → Ile; Glu 34 → Phe; Leu 56 → Ala; Ser 58 → Asp; Arg 60 → Pro; Cys 61 → Ala; Lys 65 → Asn; Lys 94 → Arg; Cys 101 → Ser; Glu 104 → Val; Leu 105 → Cys; His 106 → Asp; Lys 108 → Ser; Arg 111 → Pro; Lys 114 → Trp; Lys 121 → Glu; Ala 133 → Thr; and Cys 153 → Ser;
- (d) Ala 5 → Val; Arg 26 → Glu; Glu 27 → Gly; Phe 28 → Cys; Pro 29 → Arg; Glu 30 → Pro; Met 31 → Trp; Leu 33 → Ile; Glu 34 → Phe; Leu 56 → Ala; Ser 58 → Asp; Arg 60 → Pro; Cys 61 → Ala; Lys 65 → Arg; Lys 94 → Glu; Cys 101 → Ser; Glu 104 → Val; Leu 105 → Cys; His 106 → Asp; Lys 108 → Ser; Arg 111 → Pro; Lys 114 → Trp; Lys 121 → Glu; Ala 133 → Thr; and Cys 153 → Ser;
- (e) Arg 26 → Glu; Glu 27 → Gly; Phe 28 → Cys; Pro 29 → Arg; Glu 30 → Pro; Met 31 → Trp; Leu 33 → Ile; Glu 34 → Phe; Thr 42 → Ser; Leu 56 → Ala; Ser 58 → Asp; Arg 60 → Pro; Cys 61 → Ala; Cys 101 → Ser; Glu 104 → Val; Leu 105 → Cys; His 106 → Asp; Lys 108 → Ser; Arg 111 → Pro; Lys 114 → Trp; Ser 150 → Ile; and Cys 153 → Ser;
- (f) Arg 26 → Glu; Glu 27 → Gly; Phe 28 → Cys; Pro 29 → Arg; Glu 30 → Pro; Met 31 → Trp; Leu 33 → Ile; Glu 34 → Phe; Lys 52 → Glu; Leu 56 → Ala; Ser 58 → Asp; Arg 60 → Pro; Cys 61 → Ala; Thr 71 → Ala; Cys 101 → Ser; Glu 104 → Val; Leu 105 → Cys; His 106 → Asp; Lys 108 → Ser; Arg 111 → Pro; Lys 114 → Trp; Ala 133 → Thr; Arg 148 → Ser; Ser 150 → Ile; and Cys 153 → Ser; and

(g) ~~Ala 5 → Thr; Arg 26 → Glu; Glu 27 → Gly; Phe 28 → Cys; Pro 29 → Arg; Glu 30 → Pro; Met 31 → Trp; Leu 33 → Ile; Glu 34 → Phe; Gly 46 → Asp; Leu 56 → Ala; Ser 58 → Asp; Arg 60 → Pro; Cys 61 → Ala; Thr 71 → Ala; Cys 101 → Ser; Glu 104 → Val; Leu 105 → Cys; His 106 → Asp; Lys 108 → Ser; Arg 111 → Pro; Lys 114 → Trp; Ser 150 → Ile; and Cys 153 → Ser.~~

~~26. The fusion protein of any one of claim 29-32, wherein the amino acid sequence of the lipocalin mutein comprises an amino acid sequence selected from the group consisting of SEQ ID NOs: 34-40 or of a fragment or variant thereof.~~

~~27. The fusion protein of any one of claim 29-32, wherein the amino acid sequence of the lipocalin mutein has at least 85% sequence identity to an amino acid sequence selected from the group consisting of SEQ ID NOs: 34-40.~~

~~28. The fusion protein of any one of claims 1-28, wherein the lipocalin mutein comprises one or more mutated amino acid residues at positions corresponding to positions 28, 36, 40-41, 49, 52, 65, 68, 70, 72-73, 77, 79, 81, 83, 87, 94, 96, 100, 103, 106, 125, 127, 132 and 134 of the linear polypeptide sequence of mature human neutrophil gelatinase-associated lipocalin (hNGAL) (SEQ ID NO: 2).~~

~~29. The fusion protein of claim 34, wherein the amino acid sequence of the lipocalin mutein comprises, at positions corresponding to positions 28, 36, 40-41, 49, 52, 65, 68, 70, 72-73, 77, 79, 81, 83, 87, 94, 96, 100, 103, 106, 125, 127, 132 and 134 of the linear polypeptide sequence of mature human neutrophil gelatinase-associated lipocalin (hNGAL) (SEQ ID NO: 2), one or more of the following mutated amino acid residues: Gln 28 → His; Leu 36 → Gln; Ala 40 → Ile; Ile 41 → Arg or Lys; Gln 49 → Val, Ile, His, Ser or Asn; Tyr 52 → Met; Asn 65 → Asp; Ser 68 → Met, Ala or Gly; Leu 70 → Ala, Lys, Ser or Thr; Arg 72 → Asp; Lys 73 → Asp; Asp 77 → Met, Arg, Thr or Asn; Trp 79 → Ala or Asp; Arg 81 → Met, Trp or Ser; Phe 83 → Leu; Cys 87 → Ser; Leu 94 → Phe; Asn 96 → Lys; Tyr 100 → Phe; Leu 103 → His; Tyr 106 → Ser; Lys 125 → Phe; Ser 127 → Phe; Tyr 132 → Glu and Lys 134 → Tyr.~~

~~30. The fusion protein of any one of claims 1-28, wherein the lipocalin mutein comprises one or more mutated amino acid residues at positions corresponding to positions 20, 25, 28, 33, 36, 40-41, 44, 49, 52, 59, 68, 70-73, 77-82, 87, 92, 96, 98, 100, 101, 103, 122, 125, 127, 132, and 134 of the linear polypeptide sequence of mature human neutrophil gelatinase-associated lipocalin (hNGAL) (SEQ ID NO: 2).~~

~~31. The fusion protein of claim 36, wherein the amino acid sequence of the lipocalin mutein comprises, at positions corresponding to positions 20, 25, 28, 33, 36, 40-41, 44, 49, 52, 59, 68, 70-73, 77-82, 87, 92, 96, 98, 100, 101, 103, 122, 125, 127, 132, and 134 of the linear polypeptide sequence of mature hNGAL (SEQ ID NO: 2), one or more of the~~

following mutated amino acid residues: Gln 20 → Arg; Asn 25 → Tyr or Asp; Gln 28 → His; Val 33 → Ile; Leu 36 → Met; Ala 40 → Asn; Ile 41 → Leu; Glu 44 → Val or Asp; Gln 49 → His; Tyr 52 → Ser or Gly; Lys 59 → Asn; Ser 68 → Asp; Leu 70 → Met; Phe 71 → Leu; Arg 72 → Leu; Lys 73 → Asp; Asp 77 → Gln or His; Tyr 78 → His; Trp 79 → Ile; Ile 80 → Asn; Arg 81 → Trp or Gln; Thr 82 → Pro; Cys 87 → Ser; Phe 92 → Leu or Ser; Asn 96 → Phe; Lys 98 → Arg; Tyr 100 → Asp; Pro 101 → Leu; Leu 103 → His or Pro; Phe 122 → Tyr; Lys 125 → Ser; Ser 127 → Ile; Tyr 132 → Trp; and Lys 134 → Gly.

32. The fusion protein of any one of claims 34-37, wherein the amino acid sequence of the lipocalin mutein comprises one of the following sets of mutated amino acid residues in comparison with the linear polypeptide sequence of mature hNGAL (SEQ ID NO: 2):

- (a) Gln 28 → His; Leu 36 → Gln; Ala 40 → Ile; Ile 41 → Lys; Gln 49 → Asn; Tyr 52 → Met; Ser 68 → Gly; Leu 70 → Thr; Arg 72 → Asp; Lys 73 → Asp; Asp 77 → Thr; Trp 79 → Ala; Arg 81 → Ser; Cys 87 → Ser; Asn 96 → Lys; Tyr 100 → Phe; Leu 103 → His; Tyr 106 → Ser; Lys 125 → Phe; Ser 127 → Phe; Tyr 132 → Glu; and Lys 134 → Tyr;
- (b) Gln 28 → His; Leu 36 → Gln; Ala 40 → Ile; Ile 41 → Arg; Gln 49 → Ile; Tyr 52 → Met; Asn 65 → Asp; Ser 68 → Met; Leu 70 → Lys; Arg 72 → Asp; Lys 73 → Asp; Asp 77 → Met; Trp 79 → Asp; Arg 81 → Trp; Cys 87 → Ser; Asn 96 → Lys; Tyr 100 → Phe; Leu 103 → His; Tyr 106 → Ser; Lys 125 → Phe; Ser 127 → Phe; Tyr 132 → Glu; and Lys 134 → Tyr;
- (c) Gln 28 → His; Leu 36 → Gln; Ala 40 → Ile; Ile 41 → Arg; Gln 49 → Asn; Tyr 52 → Met; Asn 65 → Asp; Ser 68 → Ala; Leu 70 → Ala; Arg 72 → Asp; Lys 73 → Asp; Asp 77 → Thr; Trp 79 → Asp; Arg 81 → Trp; Cys 87 → Ser; Asn 96 → Lys; Tyr 100 → Phe; Leu 103 → His; Tyr 106 → Ser; Lys 125 → Phe; Ser 127 → Phe; Tyr 132 → Glu; and Lys 134 → Tyr;
- (d) Gln 28 → His; Leu 36 → Gln; Ala 40 → Ile; Ile 41 → Lys; Gln 49 → Asn; Tyr 52 → Met; Asn 65 → Asp; Ser 68 → Ala; Leu 70 → Ala; Arg 72 → Asp; Lys 73 → Asp; Asp 77 → Thr; Trp 79 → Asp; Arg 81 → Trp; Cys 87 → Ser; Asn 96 → Lys; Tyr 100 → Phe; Leu 103 → His; Tyr 106 → Ser; Lys 125 → Phe; Ser 127 → Phe; Tyr 132 → Glu; and Lys 134 → Tyr;
- (e) Gln 28 → His; Leu 36 → Gln; Ala 40 → Ile; Ile 41 → Lys; Gln 49 → Ser; Tyr 52 → Met; Asn 65 → Asp; Ser 68 → Gly; Leu 70 → Ser; Arg 72 → Asp; Lys 73 → Asp; Asp 77 → Thr; Trp 79 → Ala; Arg 81 → Met; Cys 87 → Ser; Asn 96 → Lys; Tyr 100 → Phe; Leu 103 → His; Tyr 106 → Ser; Lys 125 → Phe; Ser 127 → Phe; Tyr 132 → Glu; and Lys 134 → Tyr;
- (f) Gln 28 → His; Leu 36 → Gln; Ala 40 → Ile; Ile 41 → Lys; Gln 49 → Val; Tyr 52 →

- Met; Asn 65 → Asp; Ser 68 → Gly; Leu 70 → Thr; Arg 72 → Asp; Lys 73 → Asp; Asp 77 → Arg; Trp 79 → Asp; Arg 81 → Ser; Cys 87 → Ser; Leu 94 → Phe; Asn 96 → Lys; Tyr 100 → Phe; Leu 103 → His; Tyr 106 → Ser; Lys 125 → Phe; Ser 127 → Phe; Tyr 132 → Glu; and Lys 134 → Tyr;
- (g) ~~Gln 28 → His; Leu 36 → Gln; Ala 40 → Ile; Ile 41 → Arg; Gln 49 → His; Tyr 52 → Met; Asn 65 → Asp; Ser 68 → Gly; Leu 70 → Thr; Arg 72 → Asp; Lys 73 → Asp; Asp 77 → Thr; Trp 79 → Ala; Arg 81 → Ser; Cys 87 → Ser; Asn 96 → Lys; Tyr 100 → Phe; Leu 103 → His; Tyr 106 → Ser; Lys 125 → Phe; Ser 127 → Phe; Tyr 132 → Glu; and Lys 134 → Tyr;~~
- (h) ~~Gln 28 → His; Leu 36 → Gln; Ala 40 → Ile; Ile 41 → Lys; Gln 49 → Asn; Tyr 52 → Met; Asn 65 → Asp; Ser 68 → Gly; Leu 70 → Thr; Arg 72 → Asp; Lys 73 → Asp; Asp 77 → Thr; Trp 79 → Ala; Arg 81 → Ser; Phe 83 → Leu; Cys 87 → Ser; Leu 94 → Phe; Asn 96 → Lys; Tyr 100 → Phe; Leu 103 → His; Tyr 106 → Ser; Lys 125 → Phe; Ser 127 → Phe; Tyr 132 → Glu; and Lys 134 → Tyr;~~
- (i) ~~Gln 28 → His; Leu 36 → Gln; Ala 40 → Ile; Ile 41 → Arg; Gln 49 → Ser; Tyr 52 → Met; Asn 65 → Asp; Ser 68 → Ala; Leu 70 → Thr; Arg 72 → Asp; Lys 73 → Asp; Asp 77 → Asn; Trp 79 → Ala; Arg 81 → Ser; Cys 87 → Ser; Asn 96 → Lys; Tyr 100 → Phe; Leu 103 → His; Tyr 106 → Ser; Lys 125 → Phe; Ser 127 → Phe; Tyr 132 → Glu; and Lys 134 → Tyr.~~
- (j) ~~Leu 36 → Met; Ala 40 → Asn; Ile 41 → Leu; Gln 49 → His; Tyr 52 → Ser; Ser 68 → Asp; Leu 70 → Met; Arg 72 → Leu; Lys 73 → Asp; Asp 77 → Gln; Trp 79 → Ile; Arg 81 → Trp; Asn 96 → Phe; Tyr 100 → Asp; Leu 103 → His; Lys 125 → Ser; Ser 127 → Ile; Tyr 132 → Trp; and Lys 134 → Gly;~~
- (k) ~~Leu 36 → Met; Ala 40 → Asn; Ile 41 → Leu; Gln 49 → His; Tyr 52 → Ser; Ser 68 → Asp; Leu 70 → Met; Arg 72 → Leu; Lys 73 → Asp; Asp 77 → Gln; Trp 79 → Ile; Arg 81 → Trp; Phe 92 → Leu; Asn 96 → Phe; Lys 98 → Arg; Tyr 100 → Asp; Pro 101 → Leu; Leu 103 → His; Lys 125 → Ser; Ser 127 → Ile; Tyr 132 → Trp; and Lys 134 → Gly;~~
- (l) ~~Asn 25 → Tyr; Leu 36 → Met; Ala 40 → Asn; Ile 41 → Leu; Gln 49 → His; Tyr 52 → Gly; Ser 68 → Asp; Leu 70 → Met; Phe 71 → Leu; Arg 72 → Leu; Lys 73 → Asp; Asp 77 → Gln; Trp 79 → Ile; Arg 81 → Gln; Phe 92 → Ser; Asn 96 → Phe; Tyr 100 → Asp; Leu 103 → His; Lys 125 → Ser; Ser 127 → Ile; Tyr 132 → Trp; and Lys 134 → Gly;~~
- (m) ~~Leu 36 → Met; Ala 40 → Asn; Ile 41 → Leu; Gln 49 → His; Tyr 52 → Gly; Ser 68 → Asp; Leu 70 → Met; Arg 72 → Leu; Lys 73 → Asp; Asp 77 → Gln; Tyr 78 → His; Trp 79 → Ile; Arg 81 → Trp; Phe 92 → Leu; Asn 96 → Phe; Tyr 100 → Asp; Leu 103 → His; Lys 125 → Ser; Ser 127 → Ile; Tyr 132 → Trp; and Lys 134 →~~

Gly;

(n) ~~Asn 25 → Asp; Leu 36 → Met; Ala 40 → Asn; Ile 41 → Leu; Gln 49 → His; Tyr 52
→ Gly; Ser 68 → Asp; Leu 70 → Met; Arg 72 → Leu; Lys 73 → Asp; Asp 77 →
Gln; Trp 79 → Ile; Arg 81 → Trp; Phe 92 → Leu; Asn 96 → Phe; Tyr 100 → Asp;
Leu 103 → His; Lys 125 → Ser; Ser 127 → Ile; Tyr 132 → Trp; and Lys 134 →
Gly;~~

(o) ~~Val 33 → Ile; Leu 36 → Met; Ala 40 → Asn; Ile 41 → Leu; Gln 49 → His; Tyr 52 →
Gly; Ser 68 → Asp; Leu 70 → Met; Arg 72 → Leu; Lys 73 → Asp; Asp 77 → Gln;
Trp 79 → Ile; Arg 81 → Trp; Phe 92 → Leu; Asn 96 → Phe; Tyr 100 → Asp; Leu
103 → His; Lys 125 → Ser; Ser 127 → Ile; Tyr 132 → Trp; and Lys 134 → Gly;~~

(p) ~~Gln 20 → Arg; Leu 36 → Met; Ala 40 → Asn; Ile 41 → Leu; Glu 44 → Val; Gln 49
→ His; Tyr 52 → Gly; Ser 68 → Asp; Leu 70 → Met; Arg 72 → Leu; Lys 73 →
Asp; Asp 77 → Gln; Trp 79 → Ile; Arg 81 → Trp; Phe 92 → Leu; Asn 96 → Phe;
Tyr 100 → Asp; Leu 103 → His; Phe 122 → Tyr; Lys 125 → Ser; Ser 127 → Ile;
Tyr 132 → Trp; and Lys 134 → Gly;~~

(q) ~~Leu 36 → Met; Ala 40 → Asn; Ile 41 → Leu; Gln 49 → His; Tyr 52 → Ser; Ser 68
→ Asp; Leu 70 → Met; Arg 72 → Leu; Lys 73 → Asp; Asp 77 → Gln; Trp 79 →
Ile; Ile 80 → Asn; Arg 81 → Trp; Thr 82 → Pro; Asn 96 → Phe; Tyr 100 → Asp;
Pro 101 → Leu; Leu 103 → Pro; Lys 125 → Ser; Ser 127 → Ile; Tyr 132 → Trp;
and Lys 134 → Gly;~~

(r) ~~Leu 36 → Met; Ala 40 → Asn; Ile 41 → Leu; Gln 49 → His; Tyr 52 → Gly; Lys 59
→ Asn; Ser 68 → Asp; Leu 70 → Met; Arg 72 → Leu; Lys 73 → Asp; Asp 77 →
Gln; Trp 79 → Ile; Arg 81 → Trp; Phe 92 → Leu; Asn 96 → Phe; Tyr 100 → Asp;
Leu 103 → His; Lys 125 → Ser; Ser 127 → Ile; Tyr 132 → Trp; and Lys 134 →
Gly; and~~

(s) ~~Leu 36 → Met; Ala 40 → Asn; Ile 41 → Leu; Glu 44 → Asp; Gln 49 → His; Tyr 52
→ Ser; Ser 68 → Asp; Leu 70 → Met; Phe 71 → Leu; Arg 72 → Leu; Lys 73 →
Asp; Asp 77 → His; Trp 79 → Ile; Arg 81 → Trp; Phe 92 → Leu; Asn 96 → Phe;
Tyr 100 → Asp; Leu 103 → His; Lys 125 → Ser; Ser 127 → Ile; Tyr 132 → Trp;
and Lys 134 → Gly.~~

33. The fusion protein of any one of claims 34-38, wherein the amino acid sequence of the lipocalin mutein comprises an amino acid sequence selected from the group consisting of SEQ ID NOs: 41-59 or of a fragment or variant thereof.

34. The fusion protein of any one of claims 34-38, wherein the amino acid sequence of the lipocalin mutein has at least 85% sequence identity to an amino acid sequence selected from the group consisting of SEQ ID NOs: 41-59.

35. ~~The fusion protein of any one of claims 1-40, wherein one subunit is linked to another subunit via a linker.~~
36. ~~The fusion protein of any one of claims 1-41, wherein the second subunit is linked at the N-terminus via a linker to the N- or C-terminus of each heavy chain constant region (CH) of the first subunit or the N- or C-terminus of each light chain constant region (CL) of the first subunit.~~
37. ~~The fusion protein of any one of claims 1-42, wherein the third subunit is linked at the N-terminus via a linker to the N- or C-terminus of each heavy chain constant region (CH) of the first subunit, the N- or C-terminus of each light chain constant region (CL) of the first subunit, or the C-terminus of each second subunit.~~
38. ~~The fusion protein of any one of claims 41-43, wherein the linker is an unstructured (Gly-Gly-Gly-Ser)₃ linker (SEQ ID NO: 13).~~
39. ~~The fusion protein of any one of claims 41-43, wherein the linker is an unstructured glycine-serine linker, a polyproline linker, a proline-alanine-serine polymer, or a linker selected from the group consisting of SEQ ID NOs: 13-23.~~
40. ~~The fusion protein of any one of claims 1-45, wherein the first subunit is an antibody.~~
41. ~~The fusion protein of claim 46, wherein the heavy chain variable region of the antibody is selected from a group consisting of SEQ ID NOs: 75-79, and wherein the light chain variable region of the monoclonal antibody is selected from a group consisting of SEQ ID NOs: 80-84.~~
42. ~~The fusion protein of claim 46, wherein the antibody comprises a heavy chain that is any one of SEQ ID NOs: 85-86, and a light chain of SEQ ID NO: 87.~~
43. ~~The fusion protein of claim 46, wherein the antibody comprises a heavy chain variable region and a light chain variable region, respectively, as follows: SEQ ID NOs: 75 and 80, SEQ ID NOs: 76 and 81, SEQ ID NOs: 77 and 82, SEQ ID NOs: 78 and 83, or SEQ ID NOs: 79 and 84.~~
44. ~~The fusion protein of claim 46, wherein the antibody comprises a heavy chain and a light chain, respectively, as follows: SEQ ID NOs: 85 and 87 and SEQ ID NOs: 86 and 87.~~
45. ~~The fusion protein of claim 46, wherein the heavy chain of the antibody comprises one of the following sets of CDR sequences:~~
- ~~(a) — GFSLSNYD (HCDR1, SEQ ID NO: 59), IWTGGAT (HCDR2, SEQ ID NO: 60), VRDSNYRYDEPFTY (HCDR3; SEQ ID NO: 61);~~
 - ~~(b) — GFDIKDTY (HCDR1, SEQ ID NO: 65), IDPADGNT (HCDR2, SEQ ID NO: 66), ARGLGAWFAS (HCDR3; SEQ ID NO: 67); and~~
 - ~~(c) — GFNIKDTY (HCDR1, SEQ ID NO: 70), IDPANGNT (HCDR2, SEQ ID NO: 71), SRGPPGGIGEYIYAMDY (HCDR3; SEQ ID NO: 72).~~

~~46. The fusion protein of claim 46, wherein the light chain of the antibody comprises one of the following sets of CDR sequences:~~

~~(a) QSIGTN (LCDR1, SEQ ID NO: 63), YAS (LCDR2), QQSNSWPYT (LCDR3; SEQ ID NO: 64);~~

~~(b) QDITNS (LCDR1, SEQ ID NO: 68), YTS (LCDR2), QQGHTLPPT (LCDR3; SEQ ID NO: 69); and~~

~~(c) SSVSSSY (LCDR1, SEQ ID NO: 73), STS (LCDR2), HQYHRSPPT (LCDR3; SEQ ID NO: 74).~~

~~47. The fusion protein of claim 46, wherein the heavy chain of the antibody comprises the following set of CDR sequences: GFSLSNYD (HCDR1, SEQ ID NO: 59), IWTGGAT (HCDR2, SEQ ID NO: 60), and VRDSNYRYDEPFTY (HCDR3; SEQ ID NO: 61) and the light chain of the antibody comprises the following set of CDR sequences QSIGTN (LCDR1, SEQ ID NO: 62), YAS (LCDR2), and QQSNSWPYT (LCDR3; SEQ ID NO: 63).~~

~~48. The fusion protein of claim 46, wherein the monoclonal antibody~~5. wherein the immunoglobulin ~~has an IgG4 backbone.~~

~~The fusion protein of claim 54, wherein the IgG4 backbone~~preferably ~~has one or more of the following mutations: S228P, N297A, F234A, L235A, M428L, N434S, M252Y, S254T, and T256E,~~ according to the EU index of Kabat.

8. ~~The fusion protein of any one of claims 1-555 or 7, wherein the fusion protein comprises an amino acid sequence shown in any one of SEQ ID NOs: 86-94, or wherein the fusion protein comprises an amino acid sequences~~sequences ~~having at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 92%, at least 95%, at least 97%, at least 98%, or higher sequence identity to the amino acid sequences shown in any one of SEQ ID NOs: 88-94~~90 and 87.

~~49-9.~~ 9. ~~The fusion protein of any one of claims 1-56, wherein the fusion protein comprises the amino acids shown in SEQ ID NOs: 90 and 87, the amino acids shown in SEQ ID NOs: 86 and 91, the amino acids shown in SEQ ID NOs: 92 and 87, the amino acids shown in SEQ ID NOs: 86 and 93, the amino acids shown in SEQ ID NOs: 94 and 87, or the amino acids shown in SEQ ID NOs: 90 and 91.~~

~~The fusion protein of any one of claims 1-56~~8~~, wherein the fusion protein comprises the amino acid sequences having at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 92%, at least 95%, at least 97%, at least 98%, or higher sequence identity to the amino acid sequences shown in SEQ ID NOs: 90 and 87, SEQ ID NOs: 86 and 91, SEQ ID NOs: 92 and 87, SEQ ID NOs: 86 and 93, SEQ ID NOs: 94 and 87, or SEQ ID NOs: 90 and 91~~shown in SEQ ID NOs: 90 and 87.

10. A nucleic acid molecule comprising a nucleotide sequence encoding the fusion protein of any one of claims 1-58-9, wherein preferably

~~The nucleic acid molecule of claim 59, wherein (i) the nucleic acid molecule is operably linked to a regulatory sequence to allow expression of said nucleic acid molecule; or~~

~~The nucleic acid molecule of claim 59 or 60, wherein (ii) the nucleic acid molecule is comprised in a vector or in a phagemid vector.~~

11. A host cell containing a nucleic acid molecule of ~~any one of claims 58-60~~claim 10.

~~50-12. A method of producing the fusion protein according to of any one of claims 1-629, wherein the fusion protein is produced starting from the nucleic acid coding for the fusion protein-~~

~~The method of claim 63, wherein the fusion protein is preferably produced in a bacterial or eukaryotic host organism and is isolated from this host organism or its culture.~~

~~A use of the~~

13. A pharmaceutical composition comprising a fusion protein according to of any one of claims 1-58 or a composition comprising such 9.

14. A fusion protein for simultaneously activating downstream signaling pathways of CD137 and engaging PD-L1-positive tumor cells of any one of claims 1-9 for use in therapy.

~~51. A method of simultaneously activating downstream signaling pathways of CD137 and engaging PD-L1-positive tumor cells, comprising applying one or more fusion proteins of any one of claims 1-58 or one or more compositions comprising such fusion protein to a tissue comprising a tumor.~~

~~52. A method of simultaneously co-stimulating T-cells and engaging PD-L1-positive tumor cells, comprising applying one or more fusion proteins of any one of claims 1-58 or one or more compositions comprising such fusion protein to a tissue comprising a tumor.~~

~~53. A method of simultaneously inducing lymphocyte activity and engaging PD-L1-positive tumor cells, comprising applying one or more fusion proteins of any one of claims 1-58 or one or more compositions comprising such fusion protein to a tissue comprising a tumor.~~

~~54. A method of inducing CD137 clustering and activation on T-cells and directing said T-cells to PD-L1-positive tumor cells, comprising applying one or more fusion proteins of any one of claims 1-58 or one or more compositions comprising such fusion protein to a tissue comprising a tumor.~~

~~55. A method of inducing a localized lymphocyte response in the vicinity of PD-L1-positive tumor cells, comprising applying one or more fusion proteins of any one of claims 1-58 or one or more compositions comprising such fusion protein to a tissue comprising a tumor.~~

~~56. A method of inducing increased IL-2 and/or cytotoxic factors secretion by T-cells in the vicinity of PD-L1-positive tumor cells, comprising applying one or more fusion proteins of any one of claims 1-58 or one or more compositions comprising such fusion protein to a tissue comprising a tumor.~~

The method

~~57-15. The fusion protein for the use of claim 71, wherein the cytotoxic factors are selected from the group consisting of perforin, granzyme B, and granzyme A.~~

~~58. A method of inducing increased secretion of cytotoxic factors by T-cells in the vicinity of PD-L1-positive tumor cells, comprising applying one or more fusion proteins of any one of claims 1-58 or one or more compositions comprising such fusion protein to a tissue comprising a tumor.~~

~~59. A pharmaceutical composition comprising one or more fusion proteins of any one of claims 1-58.~~

~~60. A method of preventing, ameliorating, or treating PD-L1-positive cancers, comprising applying the fusion protein of any one claims 1-58 or one or more a composition comprising such fusion protein to a tissue comprising a tumor.~~

~~61. The fusion protein of any one of claims 1-58 for use in a therapy.~~

~~62. The fusion protein for use of claim 70~~14, wherein the use is in the treatment of cancer.

~~63. Use of a fusion protein of any one of claims 1-58 for the manufacture of a medicament.~~

~~64. The use of claim 78, wherein the medicament is for the treatment of cancer.~~