

CLAIMS

1. A lipocalin mutein that is capable of binding CD137, wherein the mutein comprises at least ten mutated amino acid residues at the sequence 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), wherein the amino acid sequence of the mutein comprises at least ten of the following mutated amino acid residues in comparison with the linear polypeptide sequence of mature human tear lipocalin (SEQ ID NO: 1): 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.
2. The mutein of claim 1, wherein the amino acid sequence of the 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; or
- (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.
3. The mutein of claim 1, wherein the mutein is capable of interfering with the binding of CD137L to CD137.
 4. The mutein of any one of claims 1-3, wherein the mutein comprises an amino acid sequence having at least 85% sequence identity to an amino acid sequence selected from the group consisting of SEQ ID NOs: 5-11.
 5. The mutein of any one of claims 1-4, comprising an amino acid sequence selected from the group consisting of SEQ ID NOs: 5-11.
 6. A lipocalin mutein that is capable of binding CD137, wherein the mutein comprises at least ten mutated amino acid residues at the sequence 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 Lipocalin 2 (hNGAL) (SEQ ID NO: 2), wherein the amino acid sequence of the mutein comprises at least ten of the following mutated amino acid residues in comparison with the linear polypeptide sequence of the mature hNGAL (SEQ ID NO: 2): 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.

7. The mutein of claim 6, wherein
 - (a) the mutein is capable of binding CD137 with an affinity by a KD of about 150 nM or lower;
 - (b) the mutein is capable of binding CD137 with an EC50 value of about 18 nM or lower; or
 - (c) the mutein, compared to the negative control of SEQ ID NO: 4, is capable of inducing higher IL-2 and/or IFN-γ proliferation.

8. The mutein of claim 6 or 7, wherein the amino acid sequence of the 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;

- 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; or
 - (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.
9. The mutein of claim 6, wherein the mutein does not interfere with the binding of CD137L to CD137.
10. The mutein of any one of claims 6-9, wherein the mutein comprises an amino acid sequence having at least 85% sequence identity to an amino acid sequence selected from the group consisting of SEQ ID NOs: 12-20.

11. The mutein of any one of claims 6-10, wherein the mutein comprises an amino acid sequence having at least 90% sequence identity to an amino acid sequence selected from the group consisting of SEQ ID NOs: 12-20.
12. The mutein of any one of claims 6-11, wherein the mutein comprises an amino acid sequence having at least 95% sequence identity to an amino acid sequence selected from the group consisting of SEQ ID NOs: 12-20.
13. The mutein of any one of claims 6-12, comprising an amino acid sequence selected from the group consisting of SEQ ID NOs: 12-20.
14. The mutein of any one of claims 6-12, comprising the amino acid sequence as shown in SEQ ID NO: 13.
15. The mutein of any one of claims 1-14, wherein the mutein is conjugated to a compound selected from the group consisting of an organic molecule, an enzyme label, a radioactive label, a colored label, a fluorescent label, a chromogenic label, a luminescent label, a hapten, digoxigenin, biotin, a cytostatic agent, a toxin, a metal complex, a metal, and colloidal gold, or a compound that extends the serum half-life of the mutein selected from the group consisting of a polyalkylene glycol molecule, a polyethylene glycol (PEG) molecule, a hydroxyethyl starch, an Fc part of an immunoglobulin, a CH3 domain of an immunoglobulin, a CH4 domain of an immunoglobulin, an albumin binding peptide, and an albumin binding protein.
16. The mutein of any one of claims 1-14, wherein the mutein is fused at its N-terminus and/or its C-terminus to a fusion partner which is a protein, a protein domain, or a peptide.
17. A nucleic acid molecule comprising a nucleotide sequence encoding the mutein of any one of claims 1-16.
18. A method of producing the mutein of any one of claims 1-16, wherein the mutein is produced starting from the nucleic acid coding for the mutein by means of genetic engineering methods.

19. Use of the mutein of any one of claims 1-16, for the manufacture of a medicament for activating the downstream signaling pathways of CD137, inducing T lymphocyte proliferation, stimulating immune response, and/or reducing production of proinflammatory cytokines and chemokines in a subject.
20. Use of the mutein of any one of claims 1-16, for the manufacture of a medicament for the treatment of cancer, an infectious disease, or an autoimmune disease.
21. A pharmaceutical composition comprising one or more mutein(s) of any one of claims 1-16.