

Claims

1. A lipocalin mutein capable of binding human glypican-3 with an affinity by a K_D of about 0.3 nM or lower, wherein the mutein comprises a mutated amino acid residue at sequence position 65 and at least 19 mutated amino acid residues at the sequence positions 36, 40, 41, 49, 52, 68, 70, 72, 73, 76, 77, 79, 81, 87, 96, 100, 103, 105, 106, 125, 127, 132, 134, 136, or 175 in comparison with the linear polypeptide sequence of mature human Lipocalin 2 (hNGAL) (SEQ ID NO: 1), and wherein the mutein has at least 80% sequence identity to mature hNGAL (SEQ ID NO: 1).
2. The mutein of claim 1, wherein the amino acid sequence of the mutein comprises at least one of the following mutated amino acid residues in comparison with the linear polypeptide sequence of mature hNGAL (SEQ ID NO: 1): Leu 36 → Val or Arg.; Ala 40 → Leu, Val or Gly; Ile 41 → Leu, Arg, Met, Gly or Ala; Gln 49 → Pro or Leu; Tyr 52 → Arg or Trp; Asn 65 → Asp; Ser 68 → Val, Gly, Asn or Ala; Leu 70 → Arg, Ser, Ala or Val; Arg 72 → Asp, Trp, Ala, or Gly; Lys 73 → Gly, Arg, Asn, Glu or Ser; Cys 76 → Val or Ile; Asp 77 → His, Met, Val, Leu, Thr or Lys; Trp 79 → Lys, Ser or Thr; Arg 81 → Gly; Cys 87 → Ser; Asn 96 → Arg, Asp, Gln or Pro; Tyr 100 → Gly, Glu, Pro or Gln; Leu 103 → Glu, Gln, Asn, Gly, Ser, Asp, or Tyr; Ser 105 → Ala; Tyr 106 → Asn, Ser or Thr; Lys 125 → Glu; Ser 127 → Arg or Tyr; Tyr 132 → Trp or Ile; Lys 134 → Ala or Phe; Thr 136 → Ile; and Cys 175 → Ala.
3. The mutein of claim 1, wherein the mutein binds glypican-3 with a stronger binding affinity, i.e., a lower K_D value, than the mutein of SEQ ID NO: 3.
4. The mutein of claim 1, wherein the mutein exhibits a lower EC_{50} value compared to the mutein of SEQ ID NO: 4 when binding to human, mouse, or cynomolgus glypican-3.
5. The mutein of claim 1, wherein the mutein is more stable than the mutein of SEQ ID NO: 3.
6. The mutein of claim 1, wherein the amino acid sequence of the mutein comprises the same amino acid at the sequence position 28 in comparison with the linear polypeptide sequence of mature hNGAL (SEQ ID NO: 1).

7. The mutein of claim 1, wherein the natural N-glycosylation site at the Asn residue at position 65 of the linear polypeptide sequence of mature hNGAL (SEQ ID NO: 1) is mutated at the corresponding sequence position of said mutein.

8. 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 hNGAL (SEQ ID NO: 1):

(a) Leu 36 → Val; Ile 41 → Leu; Gln 49 → Leu; Tyr 52 → Arg; Asn 65 → Asp; Ser 68 → Val; Leu 70 → Ser; Arg 72 → Trp; Lys 73 → Arg; Asp 77 → His; Trp 79 → Lys; Arg 81 → Gly; Cys 87 → Ser; Asn 96 → Asp; Tyr 100 → Gly; Leu 103 → Gln; Tyr 106 → Asn; Lys 125 → Glu; Ser 127 → Arg; Tyr 132 → Trp; and Lys 134 → Ala;

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

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

(d) Leu 36 → Arg; Ala 40 → Val; Ile 41 → Gly; Gln 49 → Pro; Tyr 52 → Trp; Asn 65 → Asp; Ser 68 → Asn; Leu 70 → Arg; Arg 72 → Ala; Lys 73 → Arg; Asp 77 → Leu; Trp 79 → Ser; Arg 81 → Gly; Cys 87 → Ser; Asn 96 → Gln; Tyr 100 → Glu; Leu 103 → Asn; Ser 105 → Ala; Tyr 106 → Asn; Lys 125 → Glu; Ser 127 → Tyr; Tyr 132 → Ile; Lys 134 → Phe; and Thr 136 → Ile;

(e) Leu 36 → Arg; Ala 40 → Val; Ile 41 → Gly; Gln 49 → Pro; Tyr 52 → Trp; Asn 65 → Asp; Ser 68 → Asn; Leu 70 → Arg; Arg 72 → Ala; Lys 73 → Arg; Asp 77 → Thr; Trp 79 → Ser; Arg 81 → Gly; Cys 87 → Ser; Asn 96 → Gln; Tyr 100 → Glu; Leu 103 → Gly; Ser 105 → Ala; Tyr 106 → Asn; Lys 125 → Glu; Ser 127 → Tyr; Tyr 132 → Ile; Lys 134 → Phe; and Thr 136 → Ile;

(f) Leu 36 → Arg; Ala 40 → Gly; Ile 41 → Ala; Gln 49 → Pro; Tyr 52 → Trp; Asn 65 → Asp; Ser 68 → Asn; Leu 70 → Arg; Arg 72 → Ala; Lys 73 → Arg; Asp 77 → Val; Trp 79 → Ser; Arg 81 → Gly; Cys 87 → Ser; Asn 96 → Pro; Tyr 100 → Glu; Leu 103 → Asn; Ser 105 → Ala;

Tyr 106 → Ser; Lys 125 → Glu; Ser 127 → Tyr; Tyr 132 → Ile; Lys 134 → Phe; and Thr 136 → Ile;

(g) Leu 36 → Arg; Ala 40 → Val; Ile 41 → Ala; Gln 49 → Pro; Tyr 52 → Arg; Asn 65 → Asp; Ser 68 → Ala; Leu 70 → Arg; Arg 72 → Ala; Lys 73 → Arg; Asp 77 → Leu; Trp 79 → Ser; Arg 81 → Gly; Cys 87 → Ser; Asn 96 → Arg; Tyr 100 → Glu; Leu 103 → Tyr; Ser 105 → Ala; Tyr 106 → Asn; Lys 125 → Glu; Ser 127 → Tyr; Tyr 132 → Ile; Lys 134 → Phe; and Thr 136 → Ile;

(h) Leu 36 → Arg; Ala 40 → Val; Ile 41 → Ala; Gln 49 → Pro; Tyr 52 → Arg; Asn 65 → Asp; Ser 68 → Asn; Leu 70 → Val; Arg 72 → Ala; Lys 73 → Gly; Asp 77 → Lys; Trp 79 → Ser; Arg 81 → Gly; Cys 87 → Ser; Asn 96 → Arg; Tyr 100 → Pro; Leu 103 → Asn; Ser 105 → Ala; Tyr 106 → Asn; Lys 125 → Glu; Ser 127 → Tyr; Tyr 132 → Ile; Lys 134 → Phe; and Thr 136 → Ile;

(i) Leu 36 → Arg; Ala 40 → Leu; Ile 41 → Gly; Gln 49 → Pro; Tyr 52 → Trp; Asn 65 → Asp; Ser 68 → Asn; Leu 70 → Arg; Arg 72 → Ala; Lys 73 → Arg; Asp 77 → Met; Trp 79 → Ser; Arg 81 → Gly; Cys 87 → Ser; Asn 96 → Gln; Tyr 100 → Glu; Leu 103 → Ser; Ser 105 → Ala; Tyr 106 → Asn; Lys 125 → Glu; Ser 127 → Tyr; Tyr 132 → Ile; and Lys 134 → Phe;

(j) Leu 36 → Arg; Ala 40 → Val; Ile 41 → Gly; Gln 49 → Pro; Tyr 52 → Trp; Asn 65 → Asp; Ser 68 → Asn; Leu 70 → Arg; Arg 72 → Ala; Lys 73 → Gly; Cys 76 → Val; Asp 77 → Lys; Trp 79 → Thr; Arg 81 → Gly; Cys 87 → Ser; Asn 96 → Gln; Tyr 100 → Glu; Leu 103 → Asn; Ser 105 → Ala; Tyr 106 → Thr; Lys 125 → Glu; Ser 127 → Tyr; Tyr 132 → Ile; Lys 134 → Phe; and Cys 175 → Ala;

(k) Leu 36 → Arg; Ala 40 → Val; Ile 41 → Gly; Gln 49 → Pro; Tyr 52 → Arg; Asn 65 → Asp; Ser 68 → Gly; Leu 70 → Arg; Arg 72 → Gly; Lys 73 → Glu; Cys 76 → Ile; Asp 77 → Lys; Trp 79 → Ser; Arg 81 → Gly; Cys 87 → Ser; Asn 96 → Gln; Tyr 100 → Gln; Leu 103 → Asp; Ser 105 → Ala; Tyr 106 → Thr; Lys 125 → Glu; Ser 127 → Tyr; Tyr 132 → Ile; Lys 134 → Phe; Thr 136 → Ile; and Cys 175 → Ala; and

(l) Leu 36 → Arg; Ala 40 → Val; Ile 41 → Gly; Gln 49 → Pro; Tyr 52 → Arg; Asn 65 → Asp; Ser 68 → Gly; Leu 70 → Arg; Arg 72 → Asp; Lys 73 → Ser; Cys 76 → Val; Asp 77 → Thr; Trp 79 → Ser; Arg 81 → Gly; Cys 87 → Ser; Asn 96 → Gln; Tyr 100 → Glu; Leu 103 → Asn; Ser 105 → Ala; Tyr 106 → Thr; Lys 125 → Glu; Ser 127 → Tyr; Tyr 132 → Ile; Lys 134 → Phe; Thr 136 → Ile; Cys 175 → Ala.

9. The mutein of claim 1, wherein the mutein does not have an N-glycosylation site.
10. A lipocalin mutein comprising an amino acid sequence selected from the group consisting of SEQ ID NOs: 5-16 or of a fragment or variant thereof, wherein the fragment or variant comprises mutated amino acid residues of the mutein at the positions 36, 40, 41, 49, 52, 65, 68, 70, 72, 73, 76, 77, 79, 81, 87, 96, 100, 103, 105, 106, 125, 127, 132, 134, 136, and 175 in comparison with the linear polypeptide sequence of mature hNGAL (SEQ ID NO: 1).
11. The lipocalin mutein of claim 1, wherein said mutein has at least 85% sequence identity to an amino acid sequence selected from the group consisting of SEQ ID NOs: 5-16.
12. The mutein of claim 1, 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.
13. The mutein of claim 1, wherein the mutein is conjugated to a compound that extends the serum half-life of the mutein selected from the group consisting of a polyalkylene glycol molecule, a polyethylene glycol molecule, 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.
14. The mutein of claim 1, 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.
15. A lipocalin mutein comprising an amino acid sequence selected from the group consisting of SEQ ID NOs: 5-16 or of a fragment or variant thereof, wherein the fragment or variant comprises mutated amino acid residues at the sequence positions 36, 40, 41, 49, 52, 68, 70, 72, 73, 76, 77, 79, 81, 87, 96, 100, 103, 105, 106, 125, 127, 132, 134, 136, and 175 in comparison with the linear polypeptide sequence of mature hNGAL (SEQ ID NO: 1).
16. A lipocalin mutein comprising an amino acid sequence having at least 95% sequence identity to an amino acid sequence selected from the group consisting of SEQ ID NOs: 5-16, wherein said mutein comprises a mutated amino acid residue at sequence position 65 in

comparison with the linear polypeptide sequence of mature hNGAL (SEQ ID NO: 1).

17. A pharmaceutical composition comprising one or more lipocalin muteins according to claim 1 or a fragment or variant thereof.

18. A method of detecting the presence of glypican-3 in a sample, comprising the steps of (a) contacting the sample with a lipocalin mutein of claim 1 under conditions that allow the formation of a complex between the mutein and glypican-3, and (b) detecting the complex for the presence of glypican-3.