

CLAIMS

1. A non-human transgenic animal that expresses a chimeric transferrin receptor (TfR) polypeptide that comprises a transferrin binding site of the native TfR polypeptide of the animal and a heterologous apical domain:

- 5 (a) having an amino acid sequence at least 90% identical to SEQ ID NO: 1;
 (b) having an amino acid sequence at least 95% identical to SEQ ID NO: 1;
 (c) comprising the amino acid sequence of SEQ ID NO: 1; or
 (d) comprising the amino acid sequence of SEQ ID NO: 7, SEQ ID NO: 8, or
SEQ ID NO: 9.

10 2. The non-human transgenic animal of claim 1, wherein the animal is a rodent.

15 3. A non-human transgenic animal that expresses a chimeric transferrin receptor (TfR) polypeptide that comprises a transferrin binding site of the native TfR polypeptide of the animal and a heterologous apical domain having an amino acid sequence at least 80% identical to SEQ ID NO: 1, wherein the animal is a rodent.

20 4. The non-human transgenic animal of claim 3, wherein the heterologous apical domain:

- (a) has an amino acid sequence at least 90% identical to SEQ ID NO: 1;
 (b) has an amino acid sequence at least 95% identical to SEQ ID NO: 1;
 (c) comprises the amino acid sequence of SEQ ID NO: 1; or
 (d) comprises the amino acid sequence of SEQ ID NO: 7, SEQ ID NO: 8, or
25 SEQ ID NO: 9.

 5. The non-human transgenic animal of any one of claims 1 to 4, wherein the chimeric TfR polypeptide:

- 30 (a) has at least 95% amino acid sequence identity to SEQ ID NO: 3; or
 (b) comprises the amino acid sequence of SEQ ID NO: 3.

6. The non-human transgenic animal of claim 1 or 3, wherein the animal comprises a polynucleotide encoding the heterologous apical domain of the chimeric TfR polypeptide that:

- (a) has at least 70% nucleotide sequence identity to SEQ ID NO: 2;
- (b) has at least 95% nucleotide sequence identity to SEQ ID NO: 2; or
- (c) comprises the nucleotide sequence of SEQ ID NO: 2.

7. The non-human transgenic animal of any one of claims 1 to 6, wherein the animal comprises a polynucleotide encoding the chimeric TfR polypeptide, the polynucleotide comprising exons and introns of a mouse TfR gene and a nucleic acid sequence encoding the heterologous apical domain that is positioned after the fourth exon of the mouse TfR gene to replace the apical domain of the mouse TfR gene.

8. The non-human transgenic animal of any one of claims 1 to 7, wherein:

(a) the genome of the animal comprises a polynucleotide encoding the chimeric TfR polypeptide;

(b) the chimeric TfR polypeptide is expressed in the brain of the animal;

(c) the animal expresses a level of the chimeric TfR polypeptide in brain, liver, kidney, or lung tissue within 20% of the level of expression of TfR in the same tissue of a corresponding wild-type animal of the same species;

(d) the animal comprises a red blood cell count, level of hemoglobin, or level of hematocrit within 20% of the red blood cell count, level of hemoglobin, or level of hematocrit in a corresponding wild-type animal of the same species; and/or

(e) the transgenic animal is (i) homozygous for a nucleic acid sequence encoding the chimeric TfR polypeptide, or (ii) heterozygous for a nucleic acid sequence encoding the chimeric TfR polypeptide.

9. The non-human transgenic animal of claim 8, wherein the animal is a mouse or a rat.

10. A host cell from a non-human animal that expresses a chimeric transferrin receptor (TfR) polypeptide that comprises a transferrin binding site of the native TfR polypeptide

of the animal and a heterologous apical domain having an amino acid sequence at least 80% identical to SEQ ID NO: 1.

11. The host cell of claim 10, wherein:

(a) the heterologous apical domain:

- (i) has an amino acid sequence at least 90% identical to SEQ ID NO: 1;
- (ii) has an amino acid sequence at least 95% identical to SEQ ID NO: 1;
- (iii) comprises the amino acid sequence of SEQ ID NO: 1; or
- (iv) comprises the amino acid sequence of SEQ ID NO: 7, SEQ ID NO: 8, or SEQ ID NO: 9; or

(b) the host cell comprises a polynucleotide that encodes the heterologous apical domain in the host cell that:

- (i) has at least 70% nucleotide sequence identity to SEQ ID NO: 2;
- (ii) has at least 95% nucleotide sequence identity to SEQ ID NO: 2; or
- (iii) has the nucleotide sequence of SEQ ID NO: 2.

12. The host cell of claim 10 or 11, wherein:

(a) the host cell is a mouse cell;

(b) the host cell is *ex vivo*; and/or

(c) the genome of the host cell comprises a deletion of the apical domain of the native TfR polypeptide of the animal.

13. The host cell of claim 12, wherein the host cell is a mouse cell and wherein a polynucleotide that encodes the heterologous apical domain in the host cell is positioned after the fourth exon of a mouse TfR gene.

14. The host cell of claim 12 wherein the host cell is *ex vivo*, and wherein the host cell is an embryonic stem cell.

15. A method of screening or monitoring for an apical domain binding polypeptide (ADBP) that crosses the blood-brain barrier, the method comprising:

- (a) administering an ADBP to the animal of any one of claims 1 to 9; and
- (b) measuring the presence or an activity of the ADBP in the brain of the

5 animal.

16. The method of claim 15 wherein the ADBP is coupled to an effector molecule.

17. The method of claim 16, wherein the effector molecule is a small molecule, RNA, DNA, or polypeptide.

10 18. The method of claim 17, wherein the polypeptide is an antibody or an antigen-binding fragment thereof.

19. The method of any one of claims 15 to 18, wherein:

(a) the measuring step comprises:

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- (i) contacting the brain or brain tissue of the animal with an agent that binds to the effector molecule and determining the level of the effector molecule present in the brain; or

- (ii) measuring a pharmacodynamic (PD) effect of the effector molecule;

and/or

(b) the effector molecule is an antibody or an antigen-binding fragment thereof

20 that binds to a target in the brain.

20. A method of generating a transgenic non-human single cell embryo that expresses a chimeric TfR polypeptide, the method comprising replacing the apical domain of the endogenous TfR gene in the embryo with a heterologous apical domain having at least 80% identity to SEQ ID NO: 1.

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21. The method of claim 20, wherein:

- (a) replacing the apical domain is performed by homologous recombination;

(b) the method comprises contacting a Cas9 protein, at least one sgRNA, and a donor DNA comprising a nucleic acid sequence encoding the heterologous apical domain, wherein the heterologous apical domain is flanked by a left homology arm and a right homology arm, such that the heterologous apical domain coding sequence replaces the apical domain of the endogenous

5 TfR gene in the genome of the embryo;

(c) the heterologous apical domain is codon-optimized for expression in the embryo; and/or

(d) the embryo is a mouse embryo.

22. The method of claim 21 wherein the embryo is a mouse embryo and the
10 donor DNA is positioned after the fourth exon of an endogenous mouse TfR gene.

23. A method of generating a non-human transgenic animal comprising:

(a) transferring the embryo obtained by the method of any one of claims 20 to
22, to a pseudo pregnant female of the same animal species as the embryo, and

(b) selecting a non-human transgenic animal from the progeny produced by the
15 female, wherein the animal expresses the chimeric TfR polypeptide.

24. A method of generating a non-human transgenic animal that expresses a chimeric TfR polypeptide, the method comprising

(a) introducing into an embryonic cell of a non-human animal a polynucleotide encoding an apical domain having at least 80% identity to SEQ ID NO: 1, wherein the
20 polynucleotide is targeted to a region of an endogenous TfR gene that encodes an endogenous TfR apical domain and wherein the polynucleotide encoding the apical domain having at least 80% identity to SEQ ID NO: 1 replaces the region of the endogenous TfR gene that encodes the endogenous apical domain, and

(b) developing the cell or progeny thereof into the non-human transgenic
25 animal.