

We claim:

1. A ~~non-human animal~~ rodent comprising in its genome a genetically modified endogenous *Ttr* locus comprising a human *TTR* sequence comprising both *TTR* coding sequence and non-coding sequence;

~~2. The non-human animal of claim 1, wherein a region of the endogenous *Ttr* locus comprising both *Ttr* coding sequence and non-coding sequence has been deleted and replaced with a corresponding human *TTR* sequence comprising both *TTR* coding sequence and non-coding sequence.~~

~~3. The non-human animal of claim 1 or 2, wherein the genetically modified endogenous *Ttr* locus comprises the~~ an ~~endogenous *Ttr* promoter, wherein the human *TTR* sequence is operably linked to the endogenous *Ttr* promoter.~~

~~4. The non-human animal of any preceding claim, wherein at least one intron and at least one exon of the genetically modified endogenous *Ttr* locus have~~ comprises a human *TTR* 3' untranslated region,

wherein the endogenous *Ttr* 5' untranslated region has not been deleted and replaced with the corresponding human *TTR* sequence; and

~~5. The non-human animal of any preceding claim, wherein~~ wherein:

~~(I) the entire *Ttr* coding sequence of the endogenous *Ttr* locus has been deleted and replaced with the corresponding human *TTR* sequence.~~

~~6. The non-human animal of claim 5, wherein, and~~ the region of the endogenous *Ttr* locus from the *Ttr* start codon to the *Ttr* stop codon has been deleted and replaced with the corresponding human *TTR* sequence; or

~~7. The non-human animal of any preceding claim, wherein (II) the genetically modified endogenous *Ttr* locus comprises~~ encodes a human *TTR* 3' untranslated transthyretin precursor protein comprising a signal peptide, the region-

~~8. The non-human animal of any preceding claim, wherein the~~ of the endogenous *Ttr* 5' untranslated region locus encoding the signal peptide has not been deleted and replaced with the corresponding human *TTR* sequence-

~~9. The non human animal, the first exon of the endogenous *Ttr* locus has not been deleted and replaced with the corresponding human *TTR* sequence, and the region of the endogenous *Ttr* locus from the start of the second *Ttr* exon to the *Ttr* stop codon has been deleted and replaced with the corresponding human *TTR* sequence.~~

2. The any preceding rodent of claim 1, wherein the region of the endogenous *Ttr* locus from the *Ttr* start codon to the *Ttr* stop codon has been deleted and replaced with a human *TTR* sequence comprising the corresponding human *TTR* sequence and a human *TTR* 3' untranslated region, and

wherein the endogenous *Ttr* 5' untranslated region has not been deleted and replaced with the corresponding human *TTR* sequence, and

wherein the endogenous *Ttr* promoter has not been deleted and replaced with the corresponding human *TTR* sequence.

~~103.~~ The non human animal rodent of claim 92, wherein:

(i) the human *TTR* sequence at the genetically modified endogenous *Ttr* locus comprises a sequence at least 90%, 95%, 96%, 97%, 98%, 99%, or 100% identical to the sequence set forth in SEQ ID NO: 18; ~~or~~

(ii) the genetically modified endogenous *Ttr* locus encodes a protein comprising a sequence at least 90%, 95%, 96%, 97%, 98%, 99%, or 100% identical to the sequence set forth in SEQ ID NO: 1;

(iii) the genetically modified endogenous *Ttr* locus comprises a coding sequence comprising a sequence at least 90%, 95%, 96%, 97%, 98%, 99%, or 100% identical to the sequence set forth in SEQ ID NO: 90; or

(iv) the genetically modified endogenous *Ttr* locus comprises a sequence at least 90%, 95%, 96%, 97%, 98%, 99%, or 100% identical to the sequence set forth in SEQ ID NO: 14 or 15.

~~11. The non human animal of any one of claims 1-4, wherein the genetically modified endogenous *Ttr* locus encodes a transthyretin precursor protein comprising a signal peptide, and the region of the endogenous *Ttr* locus encoding the signal peptide has not been deleted and replaced with the corresponding human *TTR* sequence.~~

~~12. The non human animal of claim 11, wherein the first exon of the endogenous *Ttr* locus has not been deleted and replaced with the corresponding human *TTR* sequence.~~

~~13. The non human animal of claim 12, wherein the first exon and first intron of the endogenous *Ttr* locus have not been deleted and replaced with the corresponding human *TTR* sequence.~~

~~14. The non human animal of any one of claims 11-13, wherein the region of the endogenous *Ttr* locus from the start of the second *Ttr* exon to the *Ttr* stop codon has been deleted and replaced with the corresponding human *TTR* sequence.~~

~~15. The non human animal of any one of claims 11-14, wherein the genetically modified endogenous *Ttr* locus comprises a human *TTR* 3' untranslated region.~~

~~16. The non human animal of any one of claims 11-15~~ 4. The rodent of claim 1, wherein the region of the endogenous *Ttr* locus from the second *Ttr* exon to the *Ttr* stop codon has been deleted and replaced with a human *TTR* sequence comprising the corresponding human *TTR* sequence and a human *TTR* 3' untranslated region, and

wherein the first exon and first intron of the endogenous *Ttr* locus have not been deleted and replaced with the corresponding human *TTR* sequence, and

wherein the endogenous *Ttr* 5' untranslated region has not been deleted and replaced with the corresponding human *TTR* sequence, and

wherein the endogenous *Ttr* promoter has not been deleted and replaced with the corresponding human *TTR* sequence.

~~175.~~ The ~~non human animal~~rodent of claim ~~164~~, wherein:

(i) the human *TTR* sequence at the genetically modified endogenous *Ttr* locus comprises a sequence at least 90%, 95%, 96%, 97%, 98%, 99%, or 100% identical to the sequence set forth in SEQ ID NO: 19;~~or~~

(ii) the genetically modified endogenous *Ttr* locus encodes a protein comprising a sequence at least 90%, 95%, 96%, 97%, 98%, 99%, or 100% identical to the sequence set forth in SEQ ID NO: 2;

(iii) the genetically modified endogenous *Ttr* locus comprises a coding sequence comprising a sequence at least 90%, 95%, 96%, 97%, 98%, 99%, or 100% identical to the sequence set forth in SEQ ID NO: 91; or

(iv) the genetically modified endogenous *Ttr* locus comprises a sequence at least 90%, 95%, 96%, 97%, 98%, 99%, or 100% identical to the sequence set forth in SEQ ID NO: 16 or 17.

~~186.~~ The ~~non-human animal~~rodent of any preceding claim, wherein the genetically modified endogenous *Ttr* locus does not comprise a selection cassette or a reporter gene.

~~197.~~ The ~~non-human animal~~rodent of any preceding claim, wherein the ~~non-human animal~~rodent is homozygous for the genetically modified endogenous *Ttr* locus.

~~208.~~ The ~~non-human animal~~rodent of any preceding claim, wherein the ~~non-human animal~~rodent is a ~~mammal~~rat or a mouse.

~~219.~~ The ~~non-human animal~~rodent of claim ~~208~~, wherein the ~~non-human animal~~rodent is a ~~rat or mouse~~.

~~22.~~ The non-human animal of claim 21, wherein the non-human animal is a mouse.

~~2310.~~ A method of assessing the activity of a human-TTR-targeting reagent *in vivo*, comprising:

(a) ~~administering the human TTR-targeting reagent to the non-human animal of any one of claims 1-22; and~~

(b) ~~assessing the activity of the human TTR-targeting reagent in the non-human animal.~~

~~24.~~ The method of claim 23, wherein the administering comprises ~~adeno-associated virus (AAV)-mediated delivery, lipid nanoparticle (LNP)-mediated delivery, or hydrodynamic delivery (HDD).~~

25.—The method of claim 24, wherein the administering comprises LNP-mediated delivery.

26.—The method of claim 25, wherein the LNP dose is between about 0.1 mg/kg and about 2 mg/kg.

27.—The method of claim 24, wherein the administering comprises AAV8-mediated delivery.

28.—The method of any one of claims 23-27, wherein step (b) comprises isolating a liver from the non-human animal and assessing activity of the human TTR-targeting reagent in the liver.

29.—The method of claim 28, wherein step (b) further comprises assessing activity of the human TTR-targeting reagent in an organ or tissue other than the liver.

30.—The method of any one of claims 23-29, wherein the human TTR-targeting reagent is a genome editing agent, and the assessing comprises assessing modification of the genetically modified *Ttr* locus.

31.—The method of claim 30, wherein the assessing comprises measuring the frequency of insertions or deletions within the genetically modified *Ttr* locus.

32.—The method of any one of claims 23-31, wherein the assessing comprises measuring expression of a *Ttr* messenger RNA encoded by the genetically modified *Ttr* locus.

33.—The method of any one of claims 23-32, wherein the assessing comprises measuring expression of a TTR protein encoded by the genetically modified *Ttr* locus.

34.—The method of claim 33, wherein assessing expression of the TTR protein comprises measuring serum levels of the TTR protein in the non-human animal.

35.—The method of any one of claims 30-33, wherein the activity is assessed in the liver of the non-human animal.

36. — ~~The method of any one of claims 23-35, wherein the human *TTR*-targeting reagent comprises a nuclease agent designed to target a region of a human *TTR* gene.~~

37. — ~~The method of claim 36, wherein the nuclease agent comprises a Cas protein and a guide RNA designed to target a guide RNA target sequence in the human *TTR* gene.~~

38. — ~~The method of claim 37, wherein the Cas protein is a Cas9 protein.~~

39. — ~~The method of any one of claims 36-38, wherein the human *TTR*-targeting reagent further comprises an exogenous donor nucleic acid, wherein the exogenous donor nucleic acid is designed to recombine with the human *TTR* gene.~~

40. — ~~The method of claim 39, wherein the exogenous donor nucleic acid is a single-stranded oligodeoxynucleotide (ssODN).~~

41. — ~~A method of optimizing the activity of a human *TTR*-targeting reagent *in vivo*, comprising:~~

~~(I) — performing the method of any one of claims 23-40 a first time in a first non-human animal rodent cell comprising in its genome a genetically modified endogenous *Ttr* locus comprising a human *TTR* sequence comprising both *TTR* coding sequence and non-coding sequence;~~

~~wherein the genetically modified endogenous *Ttr* locus comprises an endogenous *Ttr* promoter, wherein the human *TTR* sequence is operably linked to the endogenous *Ttr* promoter,~~

~~wherein the genetically modified endogenous *Ttr* locus comprises a human *TTR* 3' untranslated region,~~

~~wherein the endogenous *Ttr* 5' untranslated region has not been deleted and replaced with the corresponding human *TTR* sequence, and~~

~~wherein:~~

~~(I) — the entire *Ttr* coding sequence of the endogenous *Ttr* locus has been deleted and replaced with the corresponding human *TTR* sequence, and the region of the~~

endogenous *Ttr* locus from the *Ttr* start codon to the *Ttr* stop codon has been deleted and replaced with the corresponding human *TTR* sequence; or

(II) ~~changing a variable and performing the method of step (I) a second time with the changed variable in a second non-human animal comprising in its genome the genetically modified endogenous *Ttr* locus~~ encodes a transthyretin precursor protein comprising a signal peptide, the human *TTR* sequence comprising both *TTR* coding sequence and non-coding sequence; and

(III) ~~comparing the activity~~region of the human *TTR* targeting reagent in step (I) with the activity of the human *TTR* targeting reagent in step (II), and selecting the method resulting in the higher activity.

42. ~~The method of claim 41, wherein the changed variable in step (II) is the delivery method of introducing the human *TTR* targeting reagent into the non-human animal.~~

43. ~~The method of claim 42, wherein the administering comprises LNP-mediated delivery, and the changed variable in step (II) is the LNP formulation.~~

44. ~~The method of claim 41, wherein the changed variable in step (II) is the route of administration of introducing the human *TTR* targeting reagent into the non-human animal.~~

45. ~~The method of claim 41, wherein the changed variable in step (II) is the concentration or amount of the human *TTR* targeting reagent introduced into the non-human animal.~~

46. ~~The method of claim 41, wherein the changed variable in step (II) is the form of the human *TTR* targeting reagent introduced into the non-human animal.~~

47. ~~The method of claim 41, wherein the changed variable in step (II) is the human *TTR* targeting reagent introduced into the non-human animal.~~

48. ~~The method of claim 41, wherein the human *TTR* targeting reagent comprises a Cas protein and a guide RNA designed to target a guide RNA target sequence in a human *TTR* gene.~~

49. — ~~The method of claim 48, wherein the changed variable in step (II) is the guide RNA sequence or the guide RNA target sequence.~~

50. — ~~The method of claim 48, wherein the Cas protein and the guide RNA are each administered in the form of RNA, and the changed variable in step (II) is the ratio of Cas mRNA to guide RNA.~~

51. — ~~The method of claim 48, wherein the changed variable in step (II) is guide RNA modifications.~~

52. — ~~A method of making the non human animal of any one of claims 1-22, comprising:~~

(a) — ~~introducing into a non human animal embryonic stem (ES) cell:~~

(i) — ~~a nuclease agent that targets a target sequence in the endogenous *Ttr* locus; and~~

(ii) — ~~a targeting vector comprising a nucleic acid insert comprising encoding the signal peptide has not been deleted and replaced with the corresponding human *TTR* sequence flanked by a 5' homology arm corresponding to a 5' target sequence in, the first exon of the endogenous *Ttr* locus has not been deleted and a 3' homology arm replaced with the corresponding to a 3' target human *TTR* sequence in, and the region of the endogenous *Ttr* locus; wherein from the targeting vector recombines with the endogenous start of the second *Ttr* locus exon to produce a genetically modified non human ES cell comprising in its genome the genetically modified endogenous *Ttr* locus comprising the stop codon has been deleted and replaced with the corresponding human *TTR* sequence;~~

(b) — ~~introducing the genetically modified non human ES cell into a non human animal host embryo; and~~

(c) — ~~gestating the non human animal host embryo in a surrogate mother, wherein the surrogate mother produces an F0 progeny genetically modified non human animal comprising in its genome the genetically modified endogenous *Ttr* locus comprising the human *TTR* sequence.~~

53. — ~~The method of claim 52, wherein the nuclease agent comprises a Cas protein and a guide RNA.~~

~~54. The method of claim 53, wherein the Cas protein is a Cas9 protein.~~

~~55. The method of any one of claims 52-54, wherein the targeting vector is a large targeting vector at least 10 kb in length or in which the sum total of the 5' and 3' homology arms is at least 10 kb in length.~~

~~56. The method of any one of claims 52-55, wherein the non-human animal is a mouse or a rat.~~

~~57. The method of claim 56, wherein the non-human animal is a mouse.~~

~~58. A non-human animal cell comprising in its genome a genetically modified endogenous *Ttr* locus comprising a human *TTR* sequence comprising both *TTR* coding sequence and non-coding sequence.~~

~~59. A non-human animal genome comprising a genetically modified endogenous *Ttr* locus comprising a human *TTR* sequence comprising both *TTR* coding sequence and non-coding sequence.~~

~~60. A targeting vector comprising a nucleic acid insert comprising a human *TTR* sequence comprising both *TTR* coding sequence and non-coding sequence flanked by a 5' homology arm corresponding to a 5' target sequence in an endogenous non-human animal *Ttr* locus and a 3' homology arm corresponding to a 3' target sequence in the endogenous non-human animal *Ttr* locus~~

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