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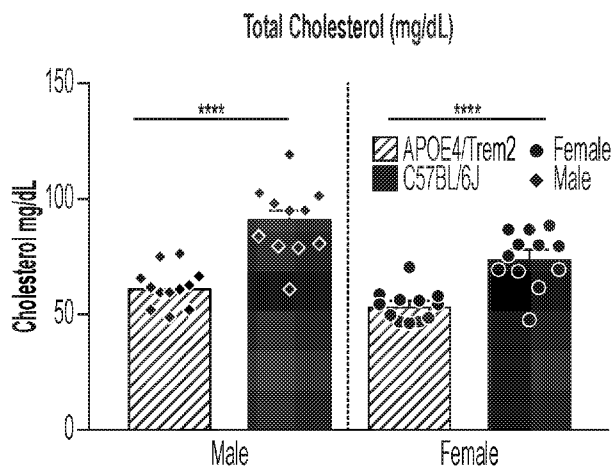


FIG. 3C

(57) **Abstract:** The present disclosure provides a genetically modified mouse comprising a genomic nucleic acid encoding human APOE4, a genomic nucleic acid encoding mouse TREM2 modified to include a R47H substitution, and at least one genomic modification selected from the group consisting of: (a) a genomic nucleic acid encoding mouse ABCA7 modified to include an A 1541 G substitution; (b) a genomic nucleic acid encoding mouse APP modified to include G601R, F606Y, and R609H substitutions; (c) a genomic nucleic acid encoding mouse PLCG2 modified to include a M28L substitution; (d) a genomic nucleic acid encoding mouse MTHFR modified to include a A262V substitution; (e) an inactivated *Ceacam1* allele; and (f) an inactivated *I11rap* allele. Methods of producing the genetically modified mouse and methods of using the genetically modified mouse are also provided.



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## GENETICALLY MODIFIED MOUSE MODELS OF ALZHEIMER'S DISEASE

### RELATED APPLICATIONS

This application claims the benefit under 35 U.S.C. § 119(e) of U.S. provisional  
5 application number 62/687,952, filed June 21, 2018, and U.S. provisional application number  
62/700,004, filed July 18, 2018, each of which is incorporated by reference herein in its entirety.

### GOVERNMENT SUPPORT

This invention was made with government support under AG054345 awarded by the  
10 National Institutes of Health. The government has certain rights in the invention.

### BACKGROUND

One of the major obstacles to developing therapies for Alzheimer's disease (AD) is the  
lack of animal models for use in preclinical trials. One reason for this may be that existing  
15 models are based on familial mutations, while the vast majority of the clinical population has  
non-familial late-onset AD.

Familial or early-onset Alzheimer's disease is caused by mutations in, or overexpression  
of, the amyloid precursor protein (*APP*) gene or mutations in presenilin genes (*PSEN1* or  
*PSEN2*). All of these lead to increased production of the ABETA42 peptide, which is thought to  
20 be neurotoxic. Dozens if not hundreds of mouse models that mimic aspects of familial  
Alzheimer's disease have been created. Many treatments have been shown to be effective in  
these familial Alzheimer's disease mouse models, but none have been effective when tested in  
clinical trials.

In contrast, late-onset Alzheimer's disease, which accounts for 95-98% of the human  
25 Alzheimer's disease patient population, does not have a simple and defined genetic etiology.  
Late-onset Alzheimer's disease is thought to be a multifactorial syndrome caused by a variety of  
genetic and environmental causes interacting with the aging process. There is a continuing need  
for mouse models of late-onset Alzheimer's disease in humans.

## SUMMARY

Some aspects of the present disclosure provide a genetically modified mouse comprising a genomic nucleic acid encoding human APOE4, a genomic nucleic acid encoding mouse TREM2 modified to include a R47H substitution, and at least one genomic modification selected from the group consisting of: (a) a genomic nucleic acid encoding mouse ABCA7 modified to include an A1541G substitution (humanized ABCA7); (b) a genomic nucleic acid encoding mouse APP modified to include G601R, F606Y, and R609H substitutions (humanized APP); (c) a genomic nucleic acid encoding mouse PLCG2 modified to include a M28L substitution (humanized PLCG2); (d) a genomic nucleic acid encoding mouse MTHFR modified to include a A262V substitution; (e) an inactivated *Ceacam1* allele; and (f) an inactivated *Il1rap* allele.

In some embodiments, the genomic nucleic acid encoding human APOE4 comprises a nucleotide sequence having at least 95% identity to the nucleotide sequence of SEQ ID NO:2, and/or the human APOE4 comprises an amino acid sequence having at least 95% identity to the amino acid sequence of SEQ ID NO:1. In some embodiments, the genomic nucleic acid encoding human APOE4 comprises the nucleotide sequence of SEQ ID NO:2, and/or the human APOE4 comprises the amino acid sequence of SEQ ID NO:1. In some embodiments, the genetically modified mouse expresses the human APOE4 and/or does not express a detectable level of mouse APOE4.

In some embodiments, the genomic nucleic acid encoding mouse TREM2 modified to include a R47H substitution comprises a nucleotide sequence having at least 95% identity to the nucleotide sequence of SEQ ID NO:4, and/or the mouse TREM2 modified to include a R47H substitution comprises an amino acid sequence having at least 95% identity to the amino acid sequence of SEQ ID NO:3. In some embodiments, the genomic nucleic acid encoding mouse TREM2 modified to include a R47H substitution comprises the nucleotide sequence of SEQ ID NO:4, and/or the mouse TREM2 modified to include a R47H substitution comprises the amino acid sequence of SEQ ID NO:3. In some embodiments, the genetically modified mouse expresses the mouse TREM2 modified to include a R47H substitution.

Other aspects of the present disclosure provide a genetically modified mouse comprising a genomic nucleic acid encoding human APOE4, a genomic nucleic acid encoding mouse TREM2 modified to include a R47H substitution, and a genomic nucleic acid encoding mouse ABCA7 modified to include an A1541G substitution. In some embodiments, the genomic nucleic acid encoding mouse ABCA7 modified to include an A1541G substitution comprises a nucleotide sequence that shares at least 95% identity with the nucleotide sequence of SEQ ID NO:19, and/or the mouse ABCA7 modified to include an A1541G substitution comprises an amino acid sequence that shares at least 95% identity with the amino acid sequence of SEQ ID NO:20. In some embodiments, the genomic nucleic acid encoding mouse ABCA7 modified to include an A1541G substitution comprises the nucleotide sequence of SEQ ID NO:19, and/or the mouse ABCA7 modified to include an A1541G substitution comprises an amino acid sequence of SEQ ID NO:20. In some embodiments, the genetically modified mouse expresses the mouse ABCA7 modified to include an A1541G substitution. In some embodiments, the genotype of the genetically modified mouse is B6.Cg-Apoe<sup>tm1.1(APOE\*4)Aduj</sup> Abca7<sup>em2Aduj</sup> Trem2<sup>em1Aduj</sup>/J (also known as Abca7\*A1527G/APOE4/Trem2\*R47H). In some embodiments, the genotype of the genetically modified mouse is B6.Cg-Apoe<sup>tm1.1(APOE\*4)Aduj</sup> Abca7<sup>em1Aduj</sup> Trem2<sup>em1Aduj</sup>/J (also known as Abca7 KO/APOE4/Trem2\*R47H).

Yet other aspects of the present disclosure provide a genetically modified mouse comprising a genomic nucleic acid encoding human APOE4, a genomic nucleic acid encoding mouse TREM2 modified to include a R47H substitution, and a genomic nucleic acid encoding mouse APP modified to include G601R, F606Y, and R609H substitutions. In some embodiments, the genomic nucleic acid encoding mouse APP modified to include G601R, F606Y, and R609H substitutions comprises a nucleotide sequence that shares at least 95% identity with the nucleotide sequence of SEQ ID NO:23, and/or the mouse APP modified to include G601R, F606Y, and R609H substitutions comprises an amino acid sequence that shares at least 95% identity with the amino acid sequence of SEQ ID NO:24. In some embodiments, the genomic nucleic acid encoding mouse APP modified to include G601R, F606Y, and R609H substitutions comprises the nucleotide sequence of SEQ ID NO:23, and/or the mouse APP modified to include G601R, F606Y, and R609H substitutions comprises the amino acid sequence of SEQ ID NO:24. In some embodiments, the genetically modified mouse expresses the mouse APP modified to include G601R, F606Y, and R609H substitutions.

Still other aspects of the present disclosure provide a genetically modified mouse comprising a genomic nucleic acid encoding human APOE4, a genomic nucleic acid encoding mouse TREM2 modified to include a R47H substitution, and a genomic nucleic acid encoding mouse PLCG2 modified to include a M28L substitution. In some embodiments, the genomic nucleic acid encoding mouse PLCG2 modified to include a M28L substitution comprises a nucleotide sequence that shares at least 95% identity with the nucleotide sequence of SEQ ID NO:31, and/or the mouse PLCG2 modified to include a M28L substitution comprises an amino acid sequence that shares at least 95% identity with the amino acid of SEQ ID NO:32. In some embodiments, the genomic nucleic acid encoding mouse PLCG2 modified to include a M28L substitution comprises the nucleotide sequence of SEQ ID NO:31, and/or the mouse PLCG2 modified to include a M28L substitution comprises the amino acid of SEQ ID NO:32. In some embodiments, the genetically modified mouse expresses the mouse PLCG2 modified to include a M28L substitution. In some embodiments, the genotype of the genetically modified mouse is B6.Cg-*ApoE*<sup>tm1.1(APOE\*4)Aduj</sup> *Plcg2*<sup>em3Aduj</sup> *Trem2*<sup>em1Aduj</sup>/J (also known as Plcg2\*M28L/APOE4/Trem2\*R47H).

Yet other aspects of the present disclosure provide a genetically modified mouse comprising a genomic nucleic acid encoding human APOE4, a genomic nucleic acid encoding mouse TREM2 modified to include a R47H substitution, and a genomic nucleic acid encoding mouse MTHFR modified to include a A262V substitution (modeling the human C677T polymorphism). In some embodiments, the genomic nucleic acid encoding mouse MTHFR modified to include a A262V substitution comprises a nucleotide sequence that shares at least 95% identity with the nucleotide sequence of SEQ ID NO:49, and/or the mouse MTHFR modified to include a A262V substitution comprises an amino acid sequence that shares at least 95% identity with the amino acid of SEQ ID NO:50. In some embodiments, the genomic nucleic acid encoding mouse MTHFR modified to include a A262V substitution comprises the nucleotide sequence of SEQ ID NO:49, and/or the mouse MTHFR modified to include a A262V substitution comprises the amino acid of SEQ ID NO:50. In some embodiments, the genetically modified mouse expresses the mouse MTHFR modified to include a A262V substitution. In some embodiments, the genotype of the genetically modified mouse is B6.Cg-*Mthfr*<sup>em1Aduj</sup> *ApoE*<sup>tm1.1(APOE\*4)Aduj</sup> *Trem2*<sup>em1Aduj</sup>/J (also known as Mthfr\*C677T/APOE4/Trem2\*R47H).

Further aspects of the present disclosure provide a genetically modified comprising a genomic nucleic acid encoding human APOE4, a genomic nucleic acid encoding mouse TREM2 modified to include a R47H substitution, and an inactivated *Ceacam1* allele. In some embodiments, the genetically modified mouse comprises a deletion or substitution in the

5 *Ceacam1* allele. In some embodiments, the genetically modified mouse does not express CEACAM1. In some embodiments, the genotype of the genetically modified mouse is B6.Cg-*ApoE<sup>tm1.1(APOE\*4)Adiuj</sup> Ceacam1<sup>em1Adiuj</sup> Trem2<sup>em1Adiuj</sup>/J* (also known as *Ceacam1* KO/APOE4/Trem2\*R47H).

Further aspects of the present disclosure provide a genetically modified mouse

10 comprising a genomic nucleic acid encoding human APOE4, a genomic nucleic acid encoding mouse TREM2 modified to include a R47H substitution, and an inactivated *Il1rap* allele. In some embodiments, the genetically modified mouse comprises a deletion or substitution in the *Il1rap* allele. In some embodiments, the genetically modified mouse does not express IL1RAP. In some embodiments, the genotype of the genetically modified mouse is B6.Cg-

15 *ApoE<sup>tm1.1(APOE\*4)Adiuj</sup> Il1rap<sup>em1Adiuj</sup> Trem2<sup>em1Adiuj</sup>/J* (also known as *Il1rap* KO/APOE4/Trem2\*R47H, exon 2 KO).

In some embodiments, total cholesterol level, low density lipoprotein (LDL) level, and/or high density lipoprotein (HDL) level is reduced in the genetically modified mouse, relative to a control mouse, optionally wherein the control mouse is a wild-type C57BL/6J mouse.

20 In some embodiments, the total cholesterol level in the genetically modified mouse is reduced by at least 10% or at least 20%, relative to the control mouse.

In some embodiments, the LDL level in the genetically modified mouse is reduced by at least 30%, at least 40%, or at least 50%, relative to the control mouse.

25 In some embodiments, the HDL level in the genetically modified mouse is reduced by at least 10%, relative to the control mouse.

In some embodiments, the genetically modified mouse exhibits signs of cerebrovascular leakage.

In some embodiments, at least one of the following genes is differentially expressed in the genetically modified mouse: *Pcsk2*, *Mapk10*, *Mapk9*, *Prkcq*, *Slc18a2*, *Plcb2*, *Slc6a4*, *Gng2*,

30 *Akt3*, *Gnao1*, *Plcb3*, *Arrb2*, *Il6*, *Myh10*, *Cnr1*, *Stx1a*, *Unc13a*, *Cdk5*, *Calb1*, *Gria4*, *Thy1*, *Hcn1*, *Chl1*, *Uchl1*, *Amph*, *Chmp2b*, and *Casp7*.

Also provided herein, in some aspects, is a method of producing a genetically modified mouse, the method comprising (i) providing a genetically modified mouse comprising a genomic nucleic acid encoding human APOE4 and/or a genomic nucleic acid encoding mouse TREM2 modified to include a R47H substitution; and (ii) introducing into the genetically modified mouse of (i) at least one genomic modification selected from the group consisting of: (a) a genomic nucleic acid encoding mouse ABCA7 modified to include an A1541G substitution; (b) a genomic nucleic acid encoding mouse APP modified to include G601R, F606Y, and R609H substitutions; (c) a genomic nucleic acid encoding mouse PLCG2 modified to include a M28L substitution; (d) a genomic nucleic acid encoding mouse MTHFR modified to include a A262V substitution; (e) an inactivated *Ceacam1* allele; and (f) an inactivated *Illrap* allele, thereby producing a genetically modified mouse.

Further provided herein, in some aspects, is a method of producing a genetically modified mouse, the method comprising introducing into a mouse a genomic nucleic acid encoding human APOE4, a genomic nucleic acid encoding mouse TREM2 modified to include a R47H substitution, and at least one genomic modification selected from the group consisting of: (a) a genomic nucleic acid encoding mouse ABCA7 modified to include an A1541G substitution; (b) a genomic nucleic acid encoding mouse APP modified to include G601R, F606Y, and R609H substitutions; (c) a genomic nucleic acid encoding mouse PLCG2 modified to include a M28L substitution; (d) a genomic nucleic acid encoding mouse MTHFR modified to include a A262V substitution; (e) an inactivated *Ceacam1* allele; and (f) an inactivated *Illrap* allele, thereby producing a genetically modified mouse.

Other aspects herein include a method of screening for a compound for use in the treatment of Alzheimer's disease, comprising administering a compound to a genetically modified mouse of the present disclosure, and assessing the mouse for an effect of the compound on a symptom associated with Alzheimer's disease.

### BRIEF DESCRIPTION OF THE DRAWINGS

Figure 1 is a schematic diagram of a humanized *APOE4* expression construct described in detail in examples herein.



Figure 2 is an image of a Western blot of brain tissue from B6(SJL)-*ApoE*<sup>tm1.1(APOE\*4)Adiuj</sup> *Trem2*<sup>em1Adiuj</sup>/J (common name: B6J.APOE4/Trem2) mice (lanes 4-6) and control (wild-type (WT) C57BL/6J (abbreviated B6J) mice (lanes 1-3) brain tissue.

Figure 3A is a graph showing results of an assay for high density lipoprotein (HDL) in blood samples obtained from 12 month old B6J.APOE4/Trem2 mice and 12 month old C57BL/6J control mice.

Figure 3B is a graph showing results of an assay for low density lipoprotein (LDL) in blood samples obtained from 12 month old B6J.APOE4/Trem2 mice and 12 month old C57BL/6J control mice.

Figure 3C is a graph showing results of an assay for total cholesterol in blood samples obtained from 12 month old B6J.APOE4/Trem2 mice and 12 month old C57BL/6J control mice.

Figure 4 shows representative images of B6J.APOE4/Trem2 tissue at 7-8 months of age immunostained to show Collagen IV (Col IV) and Fibrin(ogen) along with representative images from similar sagittal sections of brain from control C57BL/6J (abbreviated B6J) mice which were similarly immunostained.

Figure 5 is a gene expression heatmap of differentially-expressed genes in B6J.APOE4/Trem2 mice compared to wild-type control B6J mice.

### DETAILED DESCRIPTION

The present disclosure provides, in some aspects, mutant mouse strains that carry a humanized *ApoE* knock-in mutation (sequence coding for isoform E4), a CRISPR/cas9-generated R47H point mutation of the *Trem2* gene, and at least one additional genomic modification that renders the mice suitable, for example, for use in studies related to Alzheimer's disease. In some embodiments, the mutant mouse strains are also suitable for use in studies related to lipoproteins, arteriosclerosis, and/or coronary heart disease.

In some embodiments, the present disclosure relates generally to a genetically modified mouse that is a model of non-familial late-onset Alzheimer's disease. In some embodiments, the genetically modified mouse encodes at least three exogenously introduced risk factors for non-familial late-onset AD in its genome, such that the genetically modified mouse expresses human APOE4, mouse TREM2 p.R47H, and one or more of (at least one of) the following genomic modifications: (1) a humanized mutant of mouse ATP-binding cassette, sub-family A, member 7

(ABCA7); (2) a humanized mutant of mouse amyloid precursor protein (APP); (3) a humanized mutant of mouse phospholipase C  $\gamma$ 2 gene (PLCG2); (4) a mutant of mouse methylenetetrahydrofolate reductase (MTHFR); (5) a knockout (KO) of mouse carcinoembryonic antigen-related cell adhesion molecule 1 (CEACAM1) (e.g., an inactivated *Ceacam1* allele); and (6) a KO of mouse interleukin 1 receptor accessory protein (IL1RAP) (e.g., an inactivated *IL1RAP* allele).

Herein, the human APOE protein and the mouse APOE protein may be abbreviated as APOE p (protein), and the human *APOE* gene and the mouse *ApoE* gene may be abbreviated as *APOE* g (gene) and *ApoE* g, respectively; the mouse TREM2 protein and the mouse *Trem2* gene may be abbreviated as TREM2 p and *Trem2* g, respectively; the human ABCA7 protein and the mouse ABCA7 protein may be abbreviated as ABCA7 p (protein), and the human *ABCA7* gene and the mouse *Abca7* gene may be abbreviated as *ABCA7* g and *Abca7* g, respectively; the human APP protein and the mouse APP protein may be abbreviated as APP p, and the human *APP* gene and the mouse *App* gene may be abbreviated as *APP* g and *App* g, respectively; the human PLCG2 protein and the mouse PLCG2 protein may be abbreviated as PLCG2 p, and the human *PLCG2* gene and the mouse *Plcg2* gene may be abbreviated as *PLCG2* g and *Plcg2* g, respectively; and the human MTHFR protein and the mouse MTHFR protein may be abbreviated as MTHFR p, and the human *MTHFR* gene and the mouse *Mthfr* gene may be abbreviated as *MTHFR* g and *Mthfr* g, respectively.

Herein, mouse TREM2 modified to include a R47H substitution may be abbreviated as TREM2\*R47H p; ABCA7 modified to include an A1541G substitution may be abbreviated as ABCA7\*A1541G p; APP modified to include G601R, F606Y, and R609H substitutions may be abbreviated as APP\* p; PLCG2 modified to include a M28L substitution may be abbreviated as PLCG2\*M28L p; and MTHFR modified to include a A262V substitution may be abbreviated as MTHFR\*A262V p.

Human apolipoprotein is a polymorphic protein with three isoforms designated APOE2, APOE3 and APOE4. These three isoforms differ from each other with respect to the identity of amino acids at positions 130 and 176 in the amino acid sequence of the proteins (corresponding to positions 112 and 158 in the mature APOE protein without the 18 amino acid signal peptide). APOE2 is characterized by cysteine at both 130 and 176, APOE3 is characterized by cysteine at 130 and arginine at 176 and APOE4 is characterized by arginine at both 130 and 176. Individuals

having one or more *APOE4* alleles are at greater risk for developing non-familial late-onset AD and a number of mechanisms relating to pathology have been proposed, see for example, DiBattista *et al.*, 2016, *Exp. Neurol.* 280:97-105; Bu *et al.*, *Nature Reviews Neuroscience*, 2009, 10:333-344; Huang *et al.*, *Cell*, 2017, 168:1-15; and Tambini *et al.*, *EMBO Reports*, 2016, 17:27-36.

TREM2 (triggering receptor expressed on myeloid cells 2) is an immune phagocytic receptor expressed by brain microglia. TREM2 triggers phagocytosis of cell debris and regulates aspects of the inflammatory response. A rare variant in *TREM2*, p.R47H, is significantly associated with Alzheimer's disease in humans.

The targeted apolipoprotein E gene is important in lipoprotein metabolism and cardiovascular disease as well as Alzheimer's disease, immunoregulation and cognition. The targeted *Trem2* gene encodes a protein that is part of a receptor signaling complex with TYRO protein tyrosine kinase binding protein, and that activates macrophages and dendritic cells during immune responses. The TREM2 R47H mutation is a missense mutation in exon 2 that is one of the strongest genetic risk factors for late-onset Alzheimer's disease.

#### **APOE4/Trem2\*R47H**

In some embodiments, the genetically modified mice provided herein are derived from the B6(SJL)-*ApoE*<sup>tm1.1(APOE\*4)Aduij</sup> *Trem2*<sup>em1Aduij</sup>/J mouse strain (also known as

APOE4/Trem2\*R47H or APOE\*4/Trem2\*R47H). The B6(SJL)-*ApoE*<sup>tm1.1(APOE\*4)Aduij</sup> *Trem2*<sup>em1Aduij</sup>/J mouse strain is a double mutant strain that carries a humanized ApoE knock-in allele, in which exons 2, 3 and most of exon 4 of the mouse *ApoE* gene were replaced by human *APOE4* gene sequence including exons 2, 3 and 4 (and some 3' UTR sequence), and a knock-in point mutation in the mouse *Trem2* gene that includes an R47H point mutation, along with two other silent mutations. Mice that are homozygous for both alleles are viable and fertile.

This double mutant line was generated by crossing two single mutant lines.

For the *ApoE*<sup>tm1.1(APOE\*4)Aduij</sup> allele: A targeting vector containing a 3' FRT flanked neo cassette was used to replace exons 2, 3 and most of exon 4 with 1.5 kb of human *APOE* gene sequence including exons 2, 3 and 4 (and some 3' UTR sequence). The human ApoE sequence also contains a two nucleotide A to T substitution in intron 3 for identification of the targeted allele by Southern analysis. The construct was electroporated into C57BL/6J derived embryonic stem (ES) cells. Correctly targeted ES cells were injected into blastocysts. The resulting chimeric

animals were tested for germline transmission by crossing to C57BL/6J. Heterozygous animals were crossed subsequently to FLP recombinase expressing mice to remove the FRT site flanked PGK-neo cassette. Mice that no longer contained the FRT flanked PGK-neo cassette were then backcrossed to C57BL/6J at least once to remove the FLP recombinase transgene.

5 For the *Trem2*<sup>em1Aduij</sup> allele: Plasmids encoding a single guide RNA designed to introduce a R47H point mutation, with two silent mutations, into the *Trem2* gene and the *cas9* nuclease were introduced into the cytoplasm C57BL/6J-derived fertilized eggs with well recognized pronuclei. Correctly targeted embryos were transferred to pseudopregnant females. Correctly targeted pups were identified by sequencing and PCR and further bred to C57BL/6J to develop  
10 the colony.

#### **Abca7\*A1527G/APOE4/Trem2\*R47H**

In some embodiments, the genetically modified mice provided herein are derived from the B6.Cg-*ApoE*<sup>tm1.1(APOE\*4)Aduij</sup> *Abca7*<sup>em2Aduij</sup> *Trem2*<sup>em1Aduij</sup>/J mouse strain (also known as  
15 *Abca7*\*A1527G/APOE4/Trem2\*R47H). The B6.Cg-*ApoE*<sup>tm1.1(APOE\*4)Aduij</sup> *Abca7*<sup>em2Aduij</sup> *Trem2*<sup>em1Aduij</sup>/J mouse strain is a triple mutant strain that carries a humanized ApoE knock-in allele, in which exons 2, 3 and most of exon 4 of the mouse *ApoE* gene were replaced by human *APOE4* gene sequence including exons 2, 3 and 4 (and some 3' UTR sequence); a rs3752246 SNP knock-in mutation into the *Abca7* gene (alanine to glycine at position 1541 in  
20 exon 33); and a knock-in of a point mutation into mouse the *Trem2* gene containing a R47H point mutation, with two silent mutations. This *Abca7* point mutation models the human ABCA7 A1527G polymorphism.

This triple mutant line was generated by utilizing CRISPR/cas9 endonuclease mediated genome editing of the *Abca7* gene in B6(SJL)-*ApoE*<sup>tm1.1(APOE\*4)Aduij</sup> *Trem2*<sup>em1Aduij</sup>/J mice.

25 The double mutant B6(SJL)-*ApoE*<sup>tm1.1(APOE\*4)Aduij</sup> *Trem2*<sup>em1Aduij</sup>/J was generated by crossing two single mutant lines, as described above.

For the *Abca7*<sup>em2Aduij</sup> allele: plasmids encoding a single guide RNA designed to introduce rs3752246 SNP knock-in mutation into the *Abca7* gene (alanine to glycine at position 1527 in  
30 exon 33) and the *cas9* nuclease were introduced into the cytoplasm of B6(SJL)-*ApoE*<sup>tm1.1(APOE\*4)Aduij</sup> *Trem2*<sup>em1Aduij</sup>/J-derived fertilized eggs with well recognized pronuclei. Correctly targeted embryos were transferred to pseudopregnant females. Correctly targeted pups

were identified by sequencing and PCR and further bred to B6(SJL)-  
*ApoE*<sup>tm1.1(APOE\*4)Adiuj</sup> *Trem2*<sup>em1Adiuj/J</sup> to develop the colony.

#### **Abca7 KO/APOE4/Trem2\*R47H**

In some embodiments, the genetically modified mice provided herein are derived from  
 5 the B6.Cg-*ApoE*<sup>tm1.1(APOE\*4)Adiuj</sup> *Abca7*<sup>em1Adiuj</sup> *Trem2*<sup>em1Adiuj/J</sup> mouse strain (also known as Abca7  
 KO/APOE4/Trem2\*R47H). The B6.Cg-*ApoE*<sup>tm1.1(APOE\*4)Adiuj</sup> *Abca7*<sup>em1Adiuj</sup> *Trem2*<sup>em1Adiuj/J</sup> mouse  
 strain is a triple mutant strain that carries a humanized ApoE knock-in allele, in which exons 2, 3  
 and most of exon 4 of the mouse *ApoE* gene were replaced by human *APOE4* gene sequence  
 including exons 2, 3 and 4 (and some 3' UTR sequence); a mutant knock-out (33bp deletion exon  
 10 32) allele of the *Abca7* gene; and a knock-in of a point mutation into mouse the *Trem2* gene  
 containing a R47H point mutation, with two silent mutations.

This triple mutant line was generated by utilizing CRISPR/cas9 endonuclease mediated  
 genome editing of the *Abca7* gene in B6(SJL)-*ApoE*<sup>tm1.1(APOE\*4)Adiuj</sup> *Trem2*<sup>em1Adiuj/J</sup> mice.

The double mutant B6(SJL)-*ApoE*<sup>tm1.1(APOE\*4)Adiuj</sup> *Trem2*<sup>emAdiuj/J</sup> was generated by crossing  
 15 the single mutant lines, as described above.

For the *Abca7*<sup>em1Adiuj</sup> allele: plasmids encoding a single guide RNA designed to introduce  
 knock-out (33bp deletion exon 32) mutation into the *Abca7* gene and the *cas9* nuclease were  
 introduced into the cytoplasm of B6(SJL)-*ApoE*<sup>tm1.1(APOE\*4)Adiuj</sup> *Trem2*<sup>em1Adiuj/J</sup>-derived fertilized  
 eggs with well recognized pronuclei. Correctly targeted embryos were transferred to  
 20 pseudopregnant females. Correctly targeted pups were identified by sequencing and PCR and  
 further bred to B6(SJL)-*ApoE*<sup>tm1.1(APOE\*4)Adiuj</sup> *Trem2*<sup>em1Adiuj/J</sup> to develop the colony.

#### **hAPP/APOE4/Trem2\*R47H**

In some embodiments, the genetically modified mice provided herein are derived from  
 the B6.Cg-*ApoE*<sup>tm1.1(APOE\*4)Adiuj</sup> *App*<sup>em1Adiuj</sup> *Trem2*<sup>em1Adiuj/J</sup> mouse strain (also known as  
 25 hAPP/APOE4/Trem2\*R47H). The B6.Cg-*ApoE*<sup>tm1.1(APOE\*4)Adiuj</sup> *App*<sup>em1Adiuj</sup> *Trem2*<sup>em1Adiuj/J</sup> mouse  
 strain that carries a humanized ApoE knock-in allele in which exons 2, 3 and most of exon 4 of  
 the mouse *ApoE* gene were replaced by human *APOE4* gene sequence including exons 2, 3 and 4  
 (and some 3' UTR sequence), a mutant allele of the *App* gene containing G601R, F606Y, and  
 R609H point mutations, and a knock-in of a point mutation into mouse *Trem2* gene containing  
 30 an R47H point mutation with two silent mutations.

This triple mutant line was generated by utilizing CRISPR/cas9 endonuclease-mediated genome editing of the *App* gene in B6(SJL)-*ApoE<sup>tm1.1(APOE\*4)Adiuj</sup>* *Trem2<sup>em1Adiuj</sup>*/J mice.

The double mutant B6(SJL)-*ApoE<sup>tm1.1(APOE\*4)Adiuj</sup>* *Trem2<sup>em1Adiuj</sup>*/J was generated by crossing two single mutant lines, as described above.

5 For the *App<sup>em1Adiuj</sup>* allele: plasmids encoding a single guide RNA designed to introduce the G601R, F606Y, and R609H point mutations into the *App* gene and the *cas9* nuclease were introduced into the cytoplasm of B6(SJL)-*ApoE<sup>tm1.1(APOE\*4)Adiuj</sup>* *Trem2<sup>em1Adiuj</sup>*/J-derived fertilized eggs with well recognized pronuclei. Correctly targeted embryos were transferred to pseudopregnant females. Correctly targeted pups were identified by sequencing and PCR and  
10 further bred to B6(SJL)-*ApoE<sup>tm1.1(APOE\*4)Adiuj</sup>* *Trem2<sup>em1Adiuj</sup>*/J to develop the colony.

#### **App KO/APOE4/Trem2\*R47H**

In some embodiments, the genetically modified mice provided herein are derived from the B6.Cg-*ApoE<sup>tm1.1(APOE\*4)Adiuj</sup>* *App<sup>em2Adiuj</sup>* *Trem2<sup>em1Adiuj</sup>*/J mouse strain (also known as App KO/APOE4/Trem2\*R47H). The B6.Cg-*ApoE<sup>tm1.1(APOE\*4)Adiuj</sup>* *App<sup>em2Adiuj</sup>* *Trem2<sup>em1Adiuj</sup>*/J mouse  
15 strain is a triple mutant strain that carries a humanized ApoE knock-in allele, in which exons 2, 3 and most of exon 4 of the mouse *ApoE* gene were replaced by human *APOE4* gene sequence including exons 2, 3 and 4 (and some 3' UTR sequence); a mutant allele of the *App* gene containing a 94bp deletion in exon 14; and a knock-in of a point mutation into the mouse *Trem2* gene containing a R47H point mutation, with two silent mutations.

20 This triple mutant line was generated by utilizing CRISPR/cas9 endonuclease mediated genome editing of the *App* gene in B6(SJL)-*ApoE<sup>tm1.1(APOE\*4)Adiuj</sup>* *Trem2<sup>em1Adiuj</sup>*/J mice.

The double mutant B6(SJL)-*ApoE<sup>tm1.1(APOE\*4)Adiuj</sup>* *Trem2<sup>em1Adiuj</sup>*/J was generated by crossing two single mutant lines, as described above.

For the *App<sup>em2Adiuj</sup>* allele: plasmids encoding a single guide RNA designed to introduce a  
25 94bp deletion in exon 14 into the *App* gene and the *cas9* nuclease were introduced into the cytoplasm of B6(SJL)-*ApoE<sup>tm1.1(APOE\*4)Adiuj</sup>* *Trem2<sup>em1Adiuj</sup>*/J-derived fertilized eggs with well recognized pronuclei. Correctly targeted embryos were transferred to pseudopregnant females. Correctly targeted pups were identified by sequencing and PCR and further bred to B6(SJL)-*ApoE<sup>tm1.1(APOE\*4)Adiuj</sup>* *Trem2<sup>em1Adiuj</sup>*/J to develop the colony.

30

**Plcg2\**M28L*/APOE4/Trem2\**R47H***

In some embodiments, the genetically modified mice provided herein are derived from the B6.Cg-*ApoE*<sup>tm1.1(APOE\*4)Adiuj</sup> *Plcg2*<sup>em3Adiuj</sup> *Trem2*<sup>em1Adiuj</sup>/J mouse strain (also known as *Plcg2*\**M28L*/APOE4/Trem2\**R47H*). The B6.Cg-*ApoE*<sup>tm1.1(APOE\*4)Adiuj</sup> *Plcg2*<sup>em3Adiuj</sup> *Trem2*<sup>em1Adiuj</sup>/J mouse strain is a triple mutant strain carries a humanized ApoE knock-in allele, in which exons 2, 3 and most of exon 4 of the mouse *ApoE* gene were replaced by human *APOE4* gene sequence including exons 2, 3 and 4 (and some 3' UTR sequence); a CRISPR/Cas9 generated mutant of the *Plcg2*, phospholipase C, gamma 2, gene with the M28L mutation; and a knock-in of a point mutation into the mouse *Trem2* gene containing a R47H point mutation, with two silent mutations.

This triple mutant line was generated by was generated by utilizing CRISPR/cas9 endonuclease mediated genome editing of the *Plcg2* (phospholipase C, gamma 2) gene in B6(SJL)-*ApoE*<sup>tm1.1(APOE\*4)Adiuj</sup> *Trem2*<sup>em1Adiuj</sup>/J mice.

For the *Plcg2*<sup>em2Adiuj</sup> allele: plasmids encoding a single guide RNA designed to introduce the M28L mutation into the *Plcg2* gene and the *cas9* nuclease were introduced into the cytoplasm of B6(SJL)-*ApoE*<sup>tm1.1(APOE\*4)Adiuj</sup> *Trem2*<sup>em1Adiuj</sup>/J derived fertilized eggs with well recognized pronuclei. Correctly targeted embryos were transferred to pseudopregnant females. Correctly targeted pups were identified by sequencing and PCR and further bred to B6(SJL)-*ApoE*<sup>tm1.1(APOE\*4)Adpmc</sup> *Trem2*<sup>em1Adpmc</sup>/J to develop the colony.

**Mthfr\**C677T*/APOE4/Trem2\**R47H***

In some embodiments, the genetically modified mice provided herein are derived from the B6.Cg-*Mthfr*<sup>em1Adiuj</sup> *ApoE*<sup>tm1.1(APOE\*4)Adiuj</sup> *Trem2*<sup>em10Adiuj</sup>/J mouse strain (also known as *Mthfr*\**C677T*/APOE4/Trem2\**R47H*). The B6.Cg-*Mthfr*<sup>em1Adiuj</sup> *ApoE*<sup>tm1.1(APOE\*4)Adiuj</sup> *Trem2*<sup>em10Adiuj</sup>/J mouse strain is a triple mutant strain that carries a humanized ApoE knock-in allele, in which exons 2, 3 and most of exon 4 of the mouse *ApoE* gene were replaced by human *APOE4* gene sequence including exons 2, 3 and 4 (and some 3' UTR sequence); a knock-in of a point mutation into mouse *Trem2* gene containing a R47H point mutation, with two silent mutations; and a CRISPR/Cas9 generated mutant of the *Mthfr* gene that carries a point mutation resulting in the A262V amino acid substitution that models the human C677T polymorphism. This *Mthfr* point mutation models the human MTHFR C677T polymorphism, which is associated with an increased risk for Alzheimer's Disease.

This triple mutant line was generated by utilizing CRISPR/cas9 endonuclease mediated genome editing of the *Mthfr* gene in B6(SJL)-*ApoE*<sup>tm1.1(APOE\*4)Aduij</sup> *Trem2*<sup>em1Aduij/J</sup> mice.

For the *Mthfr*<sup>em1Aduij</sup> allele: plasmids encoding a single guide RNA designed to introduce a A262V point mutation into the *Mthfr* gene and the *cas9* nuclease were introduced into the cytoplasm B6(SJL)-*ApoE*<sup>tm1.1(APOE\*4)Aduij</sup> *Trem2*<sup>em1Aduij/J</sup>-derived fertilized eggs with well recognized pronuclei. Correctly targeted embryos were transferred to pseudopregnant females. Correctly targeted pups were identified by sequencing and PCR and further bred to B6(SJL)-*ApoE*<sup>tm1.1(APOE\*4)Aduij</sup> *Trem2*<sup>em1Aduij/J</sup> for two generations to develop the colony. Sperm was cryopreserved. To establish the live colony, an aliquot of frozen sperm was used to fertilize oocytes from B6(SJL)-*ApoE*<sup>tm1.1(APOE\*4)Aduij</sup> *Trem2*<sup>em1Aduij/J</sup> females.

#### ***Ceacam1* KO/APOE4/Trem2\*R47H**

In some embodiments, the genetically modified mice provided herein are derived from the B6.Cg-*ApoE*<sup>tm1.1(APOE\*4)Aduij</sup> *Ceacam1*<sup>em1Aduij</sup> *Trem2*<sup>em1Aduij/J</sup> mouse strain (also known as *Ceacam1* KO/APOE4/Trem2\*R47H). The B6.Cg-*ApoE*<sup>tm1.1(APOE\*4)Aduij</sup> *Ceacam1*<sup>em1Aduij</sup> *Trem2*<sup>em1Aduij/J</sup> mouse strain is a triple mutant strain carries a humanized ApoE knock-in allele, in which exons 2, 3 and most of exon 4 of the mouse *ApoE* gene were replaced by human *APOE4* gene sequence including exons 2, 3 and 4 (and some 3' UTR sequence); a knock-out mutant allele, deletion of exon 1, of the *Ceacam1* gene; and a knock-in of a point mutation into the mouse *Trem2* gene containing a R47H point mutation, with two silent mutations.

This triple mutant line was generated by utilizing CRISPR/cas9 endonuclease mediated genome editing of the *Ceacam1* gene in B6(SJL)-*ApoE*<sup>tm1.1(APOE\*4)Aduij</sup> *Trem2*<sup>em1Aduij/J</sup> mice.

The double mutant B6(SJL)-*ApoE*<sup>tm1.1(APOE\*4)Aduij</sup> *Trem2*<sup>em1Aduij/J</sup> was generated by crossing two single mutant lines, as described above.

For the *Ceacam1*<sup>em1Aduij</sup> allele: plasmids encoding a single guide RNA designed to introduce a knock-out mutation, deletion of exon 1, into the *Ceacam1* gene and the *cas9* nuclease were introduced into the cytoplasm of B6(SJL)-*ApoE*<sup>tm1.1(APOE\*4)Aduij</sup> *Trem2*<sup>em1Aduij/J</sup>-derived fertilized eggs with well recognized pronuclei. Correctly targeted embryos were transferred to pseudopregnant females. Correctly targeted pups were identified by sequencing and PCR and further bred to B6(SJL)-*ApoE*<sup>tm1.1(APOE\*4)Aduij</sup> *Trem2*<sup>em1Aduij/J</sup> to develop the colony.



**IIIrap KO/APOE4/Trem2\*R47H, exon 2 KO**

In some embodiments, the genetically modified mice provided herein are derived from the B6.Cg-*ApoE*<sup>tm1.1(APOE\*4)Aduj</sup> *IIIrap*<sup>em1Aduj</sup> *Trem2*<sup>em1Aduj</sup>/J mouse strain (also known as IIIrap KO/APOE4/Trem2\*R47H, exon 2 KO). The B6.Cg-*ApoE*<sup>tm1.1(APOE\*4)Aduj</sup> *IIIrap*<sup>em1Aduj</sup> *Trem2*<sup>em1Aduj</sup>/J mouse strain is a triple mutant strain carries a humanized ApoE knock-in allele, in which exons 2, 3 and most of exon 4 of the mouse *ApoE* gene were replaced by human *APOE4* gene sequence including exons 2, 3 and 4 (and some 3' UTR sequence); a mutant knock-out allele, 387bp deletion in the first coding exon, of the *IIIrap* gene; and a knock-in of a point mutation into mouse the *Trem2* gene containing a R47H point mutation, with two silent mutations.

This triple mutant line was generated by utilizing CRISPR/cas9 endonuclease mediated genome editing of the *IIIrap* gene in B6(SJL)-*ApoE*<sup>tm1.1(APOE\*4)Aduj</sup> *Trem2*<sup>em1Aduj</sup>/J mice.

The double mutant B6(SJL)-*ApoE*<sup>tm1.1(APOE\*4)Aduj</sup> *Trem2*<sup>em1Aduj</sup>/J was generated by crossing the single mutant lines, as described above.

For the *IIIrap*<sup>em1Aduj</sup> allele: plasmids encoding a single guide RNA designed to introduce a knock-out mutation, 387bp deletion in the first coding exon, into the *IIIrap* gene and the *cas9* nuclease were introduced into the cytoplasm of B6(SJL)-*ApoE*<sup>tm1.1(APOE\*4)Aduj</sup> *Trem2*<sup>em1Aduj</sup>/J-derived fertilized eggs with well recognized pronuclei. Correctly targeted embryos were transferred to pseudopregnant females. Correctly targeted pups were identified by sequencing and PCR and further bred to B6(SJL)-*ApoE*<sup>tm1.1(APOE\*4)Aduj</sup> *Trem2*<sup>em1Aduj</sup>/J to develop the colony.

Some aspects of the present disclosure provide a genetically modified mouse comprising a genomic nucleic acid encoding human APOE4, a genomic nucleic acid encoding mouse TREM2 modified to include a R47H substitution, and at least one genomic modification selected from the group consisting of: (a) a genomic nucleic acid encoding mouse ABCA7 modified to include an A1541G substitution (a humanized *Abca7* gene); (b) a genomic nucleic acid encoding mouse APP modified to include G601R, F606Y, and R609H substitutions (a humanized *App* gene); (c) a genomic nucleic acid encoding mouse PLCG2 modified to include a M28L substitution (a humanized *Plcg2* gene); (d) a genomic nucleic acid encoding mouse MTHFR modified to include a A262V substitution; (e) an inactivated *Ceacam1* allele; and (f) an inactivated *IIIrap* allele.

In some embodiments, a genetically modified mouse comprises a genomic nucleic acid encoding human APOE4, a genomic nucleic acid encoding mouse TREM2 modified to include a R47H substitution, and a genomic nucleic acid encoding mouse ABCA7 modified to include an A1541G substitution.

5 In some embodiments, a genetically modified mouse comprises a genomic nucleic acid encoding human APOE4, a genomic nucleic acid encoding mouse TREM2 modified to include a R47H substitution, and a genomic nucleic acid encoding mouse APP modified to include G601R, F606Y, and R609H substitutions.

10 In some embodiments, a genetically modified mouse comprises a genomic nucleic acid encoding human APOE4, a genomic nucleic acid encoding mouse TREM2 modified to include a R47H substitution, and a genomic nucleic acid encoding mouse PLCG2 modified to include a M28L substitution.

15 In some embodiments, a genetically modified mouse comprises a genomic nucleic acid encoding human APOE4, a genomic nucleic acid encoding mouse TREM2 modified to include a R47H substitution, and a genomic nucleic acid encoding mouse MTFHR modified to include a A262V substitution.

In some embodiments, a genetically modified mouse comprises a genomic nucleic acid encoding human APOE4, a genomic nucleic acid encoding mouse TREM2 modified to include a R47H substitution, and an inactivated *Ceacam1* allele.

20 In some embodiments, a genetically modified mouse comprises a genomic nucleic acid encoding human APOE4, a genomic nucleic acid encoding mouse TREM2 modified to include a R47H substitution, and an inactivated *Illrap* allele.

### **Methylenetetrahydrofolate reductase (MTHFR)**

25 MTHFR is the rate-limiting enzyme in the methyl cycle. It is involved in the conversion of 5,10-methylenetetrahydrofolate to 5-methyltetrahydrofolate, which is a cosubstrate for homocysteine remethylation to methionine. Homozygous knockout (KO) of MTHFR causes an abnormally high level of homocysteine in blood and urine, called hyperhomocysteinemia. Patients with this disorder exhibit developmental delay, thrombosis, motor and gait dysfunction,  
30 seizures, and other neurological symptoms. Mild MTHFR deficiency due to polymorphic variation causes a more modest elevation of plasma homocysteine (mild hyperhomocysteinemia),

which is associated with increased risk for some common multifactorial disorders. Homozygous *Mthfr* KO mice exhibit motor and behavioral anomalies, memory impairment, and liver steatosis. Homozygous KO males have normal fertility and relatively normal viability (~80%). These mice on a C57BL/6 background have a milder phenotype than on a BALB/cJ background, although  
5 mice on both backgrounds exhibit a 10 fold increase in plasma homocysteine levels.

### **ATP-binding cassette, sub-family A, member 7 (ABCA7)**

*ABCA7* is a member of the ATP-binding cassette genes family. The *ABCA7* gene product is involved in lipid transport. *ABCA7* is highly expressed in hippocampal CA1 neurons and  
10 microglia. Gene expression levels of *ABCA7* increase with AD pathology. Genome-wide association studies (GWAS) have reported association of *ABCA7* with Alzheimer's disease. Mutations in the *ABCA7* locus have been associated with genetic risk of late-onset Alzheimer's disease. In particular, rs3752246a is single nucleotide polymorphism in the human *ABCA7* gene, which encodes a mutation of alanine to glycine at position 1527 in exon 33 and which is  
15 associated with genetic risk of late-onset Alzheimer's disease. Thus, genetically modified mice according to some aspects of the present disclosure include the corresponding mutation in the homologous region of exon 32 of the mouse *Abca7* gene, which results in a A1541G substitution in the *AMCA7* protein.

### **20 Amyloid Precursor Protein (APP)**

One of the hallmarks of Alzheimer's disease is amyloid plaques, which include aggregates of a 42 amino acid fragment. This peptide is created by cleaving the amyloid precursor protein at the beta- and gamma-cleavage sites, and is called "Abeta42".

The mouse and human amyloid precursor proteins are highly conserved, and the beta-  
25 and gamma-cleavage sites are identical between human and mouse proteins. However, there are 3 amino acid differences between human and mouse within the ABETA42 sequence. There is some evidence that the human form of the amyloid precursor protein is more prone to aggregation and therefore may be more likely to lead to Alzheimer's disease than the mouse protein, see Li, L., et al., DNA Seq, 1999. 10(4-5): p. 219-28; Podlisny, M.B. et al., Am J Pathol,  
30 1991. 138(6): p. 1423-35; Rosen, R.F., et al., Neurobiol Aging, 2016. 44: p. 185-196; and Roychoudhuri, R., et al., ACS Chem Neurosci, 2015. 6(12): p. 1941-55. Thus, genetically

modified mice according to aspects of the present disclosure include three corresponding mutations, G601R, F606Y, and R609H, in the homologous region in exon 14 of the mouse *App* gene so that the mouse ABETA42 sequence is identical to the human ABETA42 sequence.

#### 5 **Phospholipase C $\gamma$ 2 (PLCG2)**

The *PLCG2* locus has been identified by genome-wide association studies to be related to the risk of Alzheimer's disease. Specifically, the PLCG2 M28L point mutation in the human *PLCG2* locus is associated with increased AD risk. Thus, genetically modified mice according to aspects of the present disclosure include a M28L mutation in the homologous region of the mouse gene.

#### **Carcinoembryonic antigen-related cell adhesion molecule 1**

Carcinoembryonic antigen-related cell adhesion molecule 1 (CEACAM1) is a cell adhesion protein that mediates hemophilic cell adhesion in a calcium-independent manner. The *CEACAM1* gene has been related to the genetic risk of Alzheimer's disease by genome-wide association studies. Genetically modified mice according to aspects of the present disclosure include a knockout of expression of CEACAM1 (e.g., an inactivated *Ceacam1* allele) such that functional CEACAM1 is not produced in the genetically modified mice.

#### 20 **Interleukin 1 receptor accessory protein**

The interleukin 1 receptor accessory protein (*IL1RAP*) gene has been related to the genetic risk of Alzheimer's disease by genome-wide association studies. This includes amyloid accumulation, temporal cortical atrophy and risk to progress from mild cognitive impairment to Alzheimer's disease. However, individual coding variants do not associate with disease risk, and even the most frequent missense mutations are very rare in the general population. The strongest risk variant is in an intron, suggesting that variations in expression levels, rather than structural variations, may increase Alzheimer's disease risk. Thus, genetically modified mice according to aspects of the present disclosure include a knockout of expression of IL1RAP (e.g., an inactivated *Il1rap* allele) such that functional IL1RAP is not produced in the genetically modified mice.

**Additional Examples of Genetically Modified Mice of the Present Disclosure****Genetically modified mouse expressing human APOE4 and mouse TREM2**

A human *APOE4* DNA sequence (hereinafter APOE4g or human *APOE4*) encoding human APOE4 protein is shown herein as SEQ ID NO:2. An encoded human APOE4 p is shown  
5 herein as SEQ ID NO:1.

A mouse mutant *Trem2* DNA sequence encoding mouse TREM2 protein having the R47H point mutation is shown herein as SEQ ID NO:4. An encoded mouse TREM2\*R47H p is shown herein as SEQ ID NO:3.

**10 Genetically modified mouse expressing human APOE4, mouse TREM2, and humanized ABCA7**

In some embodiments, the present disclosure provides a genetically modified mouse with a genome that includes a DNA sequence encoding human APOE4 protein, a DNA sequence encoding mouse TREM2 protein having the R47H point mutation, and a DNA sequence encoding a humanized mouse ABCA7 protein having the A1541G point mutation (modification  
15 in exon 32 of the mouse *Abca7* gene).

A mouse mutant *Abca7* DNA sequence encoding mouse ABCA7 protein having the A1541G point mutation is shown herein as SEQ ID NO:19. An encoded humanized ABCA7\*A1541G p is shown herein as SEQ ID NO:20.

**20 Genetically modified mouse expressing human APOE4, mouse TREM2, and humanized APP**

In some embodiments, the present disclosure provides a genetically modified mouse with a genome that includes a DNA sequence encoding human APOE4 protein, a DNA sequence encoding mouse TREM2 protein having the R47H point mutation, and a DNA sequence encoding a humanized mutant mouse APP having G601R, F606Y, and R609H point mutations  
25 (modifications in exon 14 of the mouse *App* gene).

A mouse mutant *App* DNA sequence encoding mouse APP protein having G601R, F606Y, and R609H point mutations is shown herein as SEQ ID NO:23. An encoded humanized APP\* p is shown herein as SEQ ID NO:24.

30

**Genetically modified mouse expressing human APOE4, mouse TREM2, and humanized PLCG2**

In some embodiments, the present disclosure provides a genetically modified mouse with a genome that includes a DNA sequence encoding human APOE4 protein, a DNA sequence  
5 encoding mouse TREM2 protein having the R47H point mutation, and a DNA sequence encoding a humanized mutant mouse PLCG2 having an M28L point mutation.

A mouse mutant *Plcg2* DNA sequence encoding the first 50 amino acids mouse PLCG2 protein having an M28L point mutation is shown herein as SEQ ID NO:29. An encoded PLCG2 (first 50 amino acids) is shown herein as SEQ ID NO:30. A mouse mutant *Plcg2* DNA sequence  
10 encoding mouse PLCG2 protein having an M28L point mutation is shown herein as SEQ ID NO:31. An encoded humanized PLCG2\*M28L p is shown herein as SEQ ID NO:32.

**Genetically modified mouse expressing human APOE4, mouse TREM2, and humanized MTHFR**

In some embodiments, the present disclosure provides a genetically modified mouse with  
15 a genome that includes a DNA sequence encoding human APOE4 protein, a DNA sequence encoding mouse TREM2 protein having the R47H point mutation, and a DNA sequence encoding a mutant mouse MTHFR having an A262V point mutation.

A mouse mutant *Mthfr* DNA sequence encoding the mouse MTHFR protein having an A262V point mutation is shown herein as SEQ ID NO:49. An encoded MTHFR (is shown herein  
20 as SEQ ID NO:50).

**Genetically modified mouse expressing human APOE4, mouse TREM2, and having a knockout of mouse CEACAM1 expression**

In some embodiments, the present disclosure provides a genetically modified mouse with a genome that includes a DNA sequence encoding human APOE4 protein, a DNA sequence  
25 encoding mouse TREM2 protein having the R47H point mutation, and a disruption of the DNA sequence encoding a mouse CEACAM1 (e.g., an inactivated mouse *Ceacam1* allele) such that no functional CEACAM1 is expressed.

**Genetically modified mouse expressing human APOE4, mouse TREM2, and knockout of mouse interleukin 1 receptor accessory protein (IL1RAP)**

In some embodiments, the present disclosure provides a genetically modified mouse with  
30 a genome that includes a DNA sequence encoding human APOE4 protein, a DNA sequence

encoding mouse TREM2 protein having the R47H point mutation, and a disruption of the DNA sequence encoding a mouse IL1RAP (e.g., an inactivated mouse *Il1rap* allele) such that no functional IL1RAP is expressed.

## 5 Variants

One or more genetic modifications can be introduced into a mouse genome to encode a variant of one or more of: APOE4 p, TREM2\*R47H p, ABCA7\*A1541G p, APP\* p, PLCG2\*M28L p, and MTHFR\*A262V p in a genetically modified mouse according to embodiments of methods of the present disclosure.

10 As used herein, the term "variant" refers to any one or more of APOE4 p, TREM2\*R47H p, ABCA7\*A1541G p, APP\* p, PLCG2\*M28L p, and MTHFR\*A262V p containing one or more mutations in its amino acid sequence compared to the corresponding protein of SEQ ID NO:1, SEQ ID NO:3, SEQ ID NO:20, SEQ ID NO:24, SEQ ID NO:30 and SEQ ID NO:32. For example, such mutations can be one or more amino acid substitutions, additions, and/or  
15 deletions, so long as the variant of APOE4 p or TREM2\*R47H p retains the functional characteristics APOE4 p, TREM2\*R47H p, ABCA7\*A1541G p, APP\* p, PLCG2\*M28L p, and MTHFR\*A262V p, of SEQ ID NO:1, SEQ ID NO:3, SEQ ID NO:20, SEQ ID NO:24, SEQ ID NO:30, SEQ ID NO:32, and SEQ ID NO:49, respectively.

In particular embodiments, a variant APOE4 p according to embodiments of the present  
20 disclosure has at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or greater identity to SEQ ID NO:1 over its entire length, and has R (Arginine) and R (Arginine) as amino acids 130 and 176 of the APOE4 protein including an 18 amino acid signal peptide, shown herein as SEQ ID NO:1, as well as retains the functional characteristics of APOE4 p of SEQ ID NO:1.

25 In particular embodiments, a variant APOE4 p according to embodiments of the present disclosure has at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or greater identity to SEQ ID NO:15 over its entire length, and has R (Arginine) and R (Arginine) as amino acids 112 and 158 of the APOE4 protein not including the 18 amino acid signal peptide, shown herein as SEQ ID NO:15, as well as retains the functional characteristics of APOE4 p of SEQ ID  
30 NO:15.

In particular embodiments, a variant TREM2\*R47H p according to embodiments of the present disclosure has at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or greater identity to SEQ ID NO:3 over its entire length and retains the functional characteristics of TREM2\*R47H p of SEQ ID NO:3.

5 In particular embodiments, a variant ABCA7\*A1541G p according to embodiments of the present disclosure has at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or greater identity to SEQ ID NO:20 over its entire length and retains the functional characteristics of ABCA7\*A1541G p of SEQ ID NO:20.

10 In particular embodiments, a variant APP\* p according to embodiments of the present disclosure has at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or greater identity to SEQ ID NO:24 over its entire length and retains the functional characteristics of APP\* p of SEQ ID NO:24.

15 In particular embodiments, a variant PLCG2\*M28L p according to embodiments of the present disclosure has at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or greater identity to SEQ ID NO:30 over its entire length and retains the functional characteristics of PLCG2\*M28L p of SEQ ID NO:30.

20 In particular embodiments, a variant PLCG2\*M28L p according to embodiments of the present disclosure has at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or greater identity to SEQ ID NO:32 over its entire length and retains the functional characteristics of PLCG2\*M28L p of SEQ ID NO:32.

25 Mutations can be introduced using standard molecular biology techniques, such as CRISPR technology. Alternative techniques include site-directed mutagenesis and PCR-mediated mutagenesis and the like. One of skill in the art will recognize that one or more amino acid mutations can be introduced without altering the functional properties of APOE4 p and mouse TREM2\*R47H p proteins.

Assays for assessment of functional properties of APOE4 p, TREM2\*R47H p, ABCA7\*A1541G p, APP\* p, PLCG2\*M28L p, and MTHFR\*A262V p and variants are known in the art.

30 Conservative amino acid substitutions can be made in any of APOE4 p, TREM2\*R47H p, ABCA7\*A1541G p, APP\* p, PLCG2\*M28L p, and MTHFR\*A262V p proteins to produce a corresponding APOE4 p, TREM2\*R47H p, ABCA7\*A1541G p, APP\* p, PLCG2\*M28L p, and



MTHFR\*A262V p variant. Conservative amino acid substitutions are art recognized substitutions of one amino acid for another amino acid having similar characteristics. For example, each amino acid may be described as having one or more of the following characteristics: electropositive, electronegative, aliphatic, aromatic, polar, hydrophobic and hydrophilic. A conservative substitution is a substitution of one amino acid having a specified structural or functional characteristic for another amino acid having the same characteristic. Acidic amino acids include aspartate, glutamate; basic amino acids include histidine, lysine, arginine; aliphatic amino acids include isoleucine, leucine and valine; aromatic amino acids include phenylalanine, glycine, tyrosine and tryptophan; polar amino acids include aspartate, glutamate, histidine, lysine, asparagine, glutamine, arginine, serine, threonine and tyrosine; and hydrophobic amino acids include alanine, cysteine, phenylalanine, glycine, isoleucine, leucine, methionine, proline, valine and tryptophan; and conservative substitutions include substitution among amino acids within each group. Amino acids may also be described in terms of relative size, alanine, cysteine, aspartate, glycine, asparagine, proline, threonine, serine, valine, all typically considered to be small.

APOE4 p, TREM2\*R47H p, ABCA7\*A1541G p, APP\* p, PLCG2\*M28L p, and MTHFR\*A262V p variants can include synthetic amino acid analogs, amino acid derivatives and/or non-standard amino acids, illustratively including, without limitation, alpha-aminobutyric acid, citrulline, canavanine, cyanoalanine, diaminobutyric acid, diaminopimelic acid, dihydroxy-phenylalanine, djenkolic acid, homoarginine, hydroxyproline, norleucine, norvaline, 3-phosphoserine, homoserine, 5-hydroxytryptophan, 1-methylhistidine, 3-methylhistidine, and ornithine.

It will be appreciated by those of ordinary skill in the art that, due to the degenerate nature of the genetic code, nucleic acid sequences other than SEQ ID NO:2 and SEQ ID NO:4 encode APOE4 p and TREM2\*R47H p, respectively, and that such alternate nucleic acids may be introduced into a mouse genome to produce a genetically modified mouse expressing APOE4 p and TREM2\*R47H p of the disclosure.

It will be appreciated by those of ordinary skill in the art that, due to the degenerate nature of the genetic code, nucleic acid sequences other than SEQ ID NO:19 encode ABCA7\*A1541G p, and that such alternate nucleic acids may be introduced into a mouse

genome to produce a genetically modified mouse expressing ABCA7\*A1541G p of the disclosure.

It will be appreciated by those of ordinary skill in the art that, due to the degenerate nature of the genetic code, nucleic acid sequences other than SEQ ID NO:23 encode APP\* p, and that such alternate nucleic acids may be introduced into a mouse genome to produce a genetically modified mouse expressing APP\* p of the disclosure.

It will be appreciated by those of ordinary skill in the art that, due to the degenerate nature of the genetic code, nucleic acid sequences other than SEQ ID NO:29 encode PLCG2\*M28L p fragment of SEQ ID NO:30, and that such alternate nucleic acids may be introduced into a mouse genome to produce a genetically modified mouse expressing PLCG2\*M28L p of the disclosure.

It will be appreciated by those of ordinary skill in the art that, due to the degenerate nature of the genetic code, nucleic acid sequences other than SEQ ID NO:31 encode PLCG2\*M28L p of SEQ ID NO:32, and that such alternate nucleic acids may be introduced into a mouse genome to produce a genetically modified mouse expressing PLCG2\*M28L p of the disclosure.

The singular terms "a," "an," and "the" are not intended to be limiting and include plural referents unless explicitly stated otherwise or the context clearly indicates otherwise.

The terms "expressing" and "expresses" refer to transcription of a gene to produce a corresponding mRNA and/or translation of the mRNA to produce the corresponding protein.

The complement of a nucleic acid encoding an APOE4 p variant specifically hybridizes with SEQ ID NO:2 encoding APOE4 p under high stringency conditions. The complement of a nucleic acid encoding a TREM2\*R47H p variant specifically hybridizes with SEQ ID NO:4 encoding TREM2\*R47H p under high stringency conditions. The complement of a nucleic acid encoding a ABCA7\*A1541G p variant specifically hybridizes with SEQ ID NO:19 encoding ABCA7\*A1541G p under high stringency conditions. The complement of a nucleic acid encoding a APP\* p variant specifically hybridizes with SEQ ID NO:23 encoding APP\* p under high stringency conditions. The complement of a nucleic acid encoding a PLCG2\*M28L p fragment variant specifically hybridizes with SEQ ID NO:29 encoding a PLCG2\*M28L p fragment under high stringency conditions. The complement of a nucleic acid encoding a

PLCG2\*M28L p variant specifically hybridizes with SEQ ID NO:31 encoding PLCG2\*M28L p under high stringency conditions.

The term “nucleic acid” refers to RNA or DNA molecules having more than one nucleotide in any form including single-stranded, double-stranded, oligonucleotide or  
5 polynucleotide. The term “nucleotide sequence” refers to the ordering of nucleotides in an oligonucleotide or polynucleotide in a single-stranded form of nucleic acid.

The terms “hybridization” and “hybridizes” refer to pairing and binding of complementary nucleic acids. Hybridization occurs to varying extents between two nucleic acids depending on factors such as the degree of complementarity of the nucleic acids, the melting  
10 temperature,  $T_m$ , of the nucleic acids and the stringency of hybridization conditions, as is well known in the art.

The term “stringency of hybridization conditions” refers to conditions of temperature, ionic strength, and composition of a hybridization medium with respect to particular common additives such as formamide and Denhardt’s solution. Determination of particular hybridization  
15 conditions relating to a specified nucleic acid is routine and is well known in the art, for instance, as described in J. Sambrook and D.W. Russell, *Molecular Cloning: A Laboratory Manual*, Cold Spring Harbor Laboratory Press; 3rd Ed., 2001; and F.M. Ausubel, Ed., *Short Protocols in Molecular Biology*, Current Protocols; 5th Ed., 2002. High stringency hybridization conditions are those which only allow hybridization of substantially complementary nucleic acids.  
20 Typically, nucleic acids having about 85-100% complementarity are considered highly complementary and hybridize under high stringency conditions. Intermediate stringency conditions are exemplified by conditions under which nucleic acids having intermediate complementarity, about 50-84% complementarity, as well as those having a high degree of complementarity, hybridize. In contrast, low stringency hybridization conditions are those in  
25 which nucleic acids having a low degree of complementarity hybridize.

The terms “specific hybridization” and “specifically hybridizes” refer to hybridization of a particular nucleic acid to a target nucleic acid without substantial hybridization to nucleic acids other than the target nucleic acid in a sample.

Stringency of hybridization and washing conditions depends on several factors, including  
30 the  $T_m$  of the probe and target and ionic strength of the hybridization and wash conditions, as is well-known to the skilled artisan. Hybridization and conditions to achieve a desired

hybridization stringency are described, for example, in Sambrook *et al.*, *Molecular Cloning: A Laboratory Manual*, Cold Spring Harbor Laboratory Press, 2001; and Ausubel, F. et al., (Eds.), *Short Protocols in Molecular Biology*, Wiley, 2002.

5 An example of high stringency hybridization conditions is hybridization of nucleic acids over about 100 nucleotides in length in a solution containing 6X SSC, 5X Denhardt's solution, 30% formamide, and 100 micrograms/ml denatured salmon sperm at 37°C overnight followed by washing in a solution of 0.1X SSC and 0.1% SDS at 60°C for 15 minutes. SSC is 0.15M NaCl/0.015M Na citrate. Denhardt's solution is 0.02% bovine serum albumin/0.02% FICOLL/0.02% polyvinylpyrrolidone.

10 The term "complementary" refers to Watson-Crick base pairing between nucleotides and specifically refers to nucleotides hydrogen bonded to one another with thymine or uracil residues linked to adenine residues by two hydrogen bonds and cytosine and guanine residues linked by three hydrogen bonds. In general, a nucleic acid includes a nucleotide sequence described as having a "percent complementarity" to a specified second nucleotide sequence. For example, a  
15 nucleotide sequence may have 80%, 90%, or 100% complementarity to a specified second nucleotide sequence, indicating that 8 of 10, 9 of 10 or 10 of 10 nucleotides of a sequence are complementary to the specified second nucleotide sequence. For instance, the nucleotide sequence 3'-TCGA-5' is 100% complementary to the nucleotide sequence 5'-AGCT-3'. Further, the nucleotide sequence 3'-TCGA- is 100% complementary to a region of the nucleotide  
20 sequence 5'-TTAGCTGG-3'.

To determine the percent identity of two amino acid sequences or of two nucleic acid sequences, the sequences are aligned for optimal comparison purposes (e.g., gaps can be introduced in the sequence of a first amino acid or nucleic acid sequence for optimal alignment with a second amino acid or nucleic acid sequence). The amino acid residues or nucleotides at  
25 corresponding amino acid positions or nucleotide positions are then compared. When a position in the first sequence is occupied by the same amino acid residue or nucleotide as the corresponding position in the second sequence, then the molecules are identical at that position. The percent identity between the two sequences is a function of the number of identical positions shared by the sequences (i.e., % identity=number of identical overlapping positions/total number  
30 of positions x 100%). In one embodiment, the two sequences are the same length or differ in

length by no more than 1%, 2%, 3%, 4%, 5%, 6%, 7%, 8%, 9% or 10% of the total length of the reference sequence.

The determination of percent identity between two sequences can also be accomplished using a mathematical algorithm. A preferred, non-limiting example of a mathematical algorithm  
5 utilized for the comparison of two sequences is the algorithm of Karlin and Altschul, 1990, PNAS 87:2264 2268, modified as in Karlin and Altschul, 1993, PNAS. 90:5873 5877. Such an algorithm is incorporated into the NBLAST and XBLAST programs of Altschul *et al.*, 1990, J. Mol. Biol. 215:403. BLAST nucleotide searches are performed with the NBLAST nucleotide program parameters set, e.g., for score=100, wordlength=12 to obtain nucleotide sequences  
10 homologous to a nucleic acid molecules of the present disclosure. BLAST protein searches are performed with the XBLAST program parameters set, e.g., to score 50, wordlength=3 to obtain amino acid sequences homologous to a protein molecule of the present disclosure. To obtain gapped alignments for comparison purposes, Gapped BLAST are utilized as described in Altschul *et al.*, 1997, Nucleic Acids Res. 25:3389 3402. Alternatively, PSI BLAST is used to  
15 perform an iterated search which detects distant relationships between molecules (Id.). When utilizing BLAST, Gapped BLAST, and PSI Blast programs, the default parameters of the respective programs (e.g., of XBLAST and NBLAST) are used (see, e.g., the NCBI website). Another preferred, non-limiting example of a mathematical algorithm utilized for the comparison of sequences is the algorithm of Myers and Miller, 1988, CABIOS 4:11 17. Such an algorithm is  
20 incorporated in the ALIGN program (version 2.0) which is part of the GCG sequence alignment software package. When utilizing the ALIGN program for comparing amino acid sequences, a PAM120 weight residue table, a gap length penalty of 12, and a gap penalty of 4 is used.

The percent identity between two sequences is determined using techniques similar to those described above, with or without allowing gaps. In calculating percent identity, typically  
25 only exact matches are counted.

Nucleic acids encoding APOE4 p, TREM2\*R47H p, ABCA7\*A1541G p, APP\* p, PLCG2\*M28L p, or MTHFR\*A262V p, or a variant of any thereof can be isolated from natural sources, generated recombinantly or made by chemical synthetic techniques using well-known  
30 methodology.

## Genetic Modification

A genetically modified mouse is provided according to embodiments of the present disclosure whose genome includes a nucleic acid encoding APOE4 p operably linked to a promoter, wherein the animal expresses the encoded APOE4 p, whose genome includes a nucleic acid encoding TREM2\*R47H p operably linked to a promoter, wherein the animal expresses the encoded TREM2\*R47H p, and whose genome includes one or more of the following: (1) a nucleic acid encoding: ABCA7\*A1541G p operably linked to a promoter, wherein the animal expresses the encoded ABCA7\*A1541G p; (2) a nucleic acid encoding: APP\* p operably linked to a promoter, wherein the animal expresses the encoded APP\* p; (3) a nucleic acid encoding: PLCG2\*M28L p operably linked to a promoter, wherein the animal expresses the encoded PLCG2\*M28L p; (4) a nucleic acid encoding: MTFHR\*A262V p operably linked to a promoter, wherein the animal expresses the encoded MTFHR\*A262V p; (5) a disrupted *Ceacam1* gene such that functional CEACAM1 is not produced in the genetically modified mouse; and (6) a disrupted *Il1rap* gene such that functional IL1RAP is not produced in the genetically modified mouse.

A genetically modified mouse is provided according to embodiments of the present disclosure whose genome includes a nucleic acid encoding APOE4 p operably linked to the endogenous mouse *Apoe* promoter, wherein the animal expresses the encoded APOE4 p, whose genome includes a nucleic acid encoding TREM2\*R47H p operably linked to the endogenous mouse TREM2\*R47H p promoter, wherein the animal expresses the encoded TREM2\*R47H p, and whose genome includes one or more of the following: (1) a nucleic acid encoding: ABCA7\*A1541G p operably linked to a promoter, wherein the animal expresses the encoded ABCA7\*A1541G p; (2) a nucleic acid encoding: APP\* p operably linked to a promoter, wherein the animal expresses the encoded APP\* p; (3) a nucleic acid encoding: PLCG2\*M28L p operably linked to a promoter, wherein the animal expresses the encoded PLCG2\*M28L p; (4) a nucleic acid encoding: MTFHR\*A262V p operably linked to a promoter, wherein the animal expresses the encoded MTFHR\*A262V p; (5) a disrupted *Ceacam1* gene such that functional CEACAM1 is not produced in the genetically modified mouse; and (6) a disrupted *Il1rap* gene such that functional IL1RAP is not produced in the genetically modified mouse.

Any of various methods can be used to introduce a genetic modification into a mouse genome to produce a genetically modified mouse expressing APOE4 p, TREM2\*R47H p, and one or more of: ABCA7\*A1541G p, APP\* p, and PLCG2\*M28L p, or a variant of any thereof.

Genome editing methods for generating a genetically modified mouse according to  
5 embodiments of the present disclosure whose genome includes a nucleic acid encoding APOE4 p operably linked to a promoter, wherein the animal expresses the encoded APOE4 p, whose genome includes a nucleic acid encoding TREM2\*R47H p operably linked to a promoter, wherein the animal expresses the encoded TREM2\*R47H p, and whose genome includes one or more of the following: (1) a nucleic acid encoding: ABCA7\*A1541G p operably linked to a  
10 promoter, wherein the animal expresses the encoded ABCA7\*A1541G p; (2) a nucleic acid encoding: APP\* p operably linked to a promoter, wherein the animal expresses the encoded APP\* p; (3) a nucleic acid encoding: PLCG2\*M28L p operably linked to a promoter, wherein the animal expresses the encoded PLCG2\*M28L p; (4) a nucleic acid encoding:  
MTFHR\*A262V p operably linked to a promoter, wherein the animal expresses the encoded  
15 MTFHR\*A262V p; (5) a disrupted *Ceacam1* gene such that functional CEACAM1 is not produced in the genetically modified mouse; and (6) a disrupted *Il1rap* gene such that functional IL1RAP is not produced in the genetically modified mouse, include, but are not limited to, site directed mutagenesis, recombination-based methods and nuclease genome editing techniques.

Genome editing techniques can be used to modify a genomic sequence by introduction of  
20 a discrete mutation at a predetermined target site in the genome.

For example, one or more nucleotides in a genomic sequence can be replaced with one or more different nucleotides using a genome editing technique so that the genomic sequence encodes a protein with a single amino acid difference, or multiple amino acid differences, compared to the unmodified genomic sequence.

25 Genome editing techniques can also be used to modify a genomic sequence by insertion of a coding sequence into the genome at a predetermined target site, a “knock-in” technique.

Genome editing techniques can also be used to modify a genomic sequence by insertion or deletion of a coding sequence or non-coding into the genome at a predetermined target site to disrupt or knockout a gene such that the gene is no longer capable of expressing the functional  
30 protein originally encoded.

As used herein, the terms “target site” and “target sequence” in the general context of a genetic editing technique refer to a nucleic acid sequence that defines a portion of a chromosomal sequence to be edited.

For example, a nucleic acid sequence encoding a protein can be inserted at a predetermined target site in the genome so that the genome includes a nucleic acid encoding the protein and the protein is expressed. The nucleic acid sequence can also contain a promoter to drive expression of the encoded protein or expression of the encoded protein can be driven by an endogenous promoter when the nucleic acid is inserted in a position so that it is operably linked to the endogenous promoter.

According to particular aspects of the present disclosure, a point mutation is introduced into the genome of a first mouse using a genome editing technique so that the mouse encodes TREM2\*R47H p, and a nucleic acid encoding human APOE4 is inserted into the Apoe4 gene in the genome of a second mouse by a knock-in genome editing technique so that the genome of the second mouse contains a humanized APOE4 gene as shown in Figure 1, containing exon 1 of the mouse Apoe4 gene and exons 2, 3 and 4 of the human APOE4 gene. The first and second mice are then bred, naturally or by artificial methods, to obtain a genetically modified mouse whose genome includes a DNA sequence encoding APOE4 p and a DNA sequence encoding TREM2\*R47H p.

According to particular aspects of the present disclosure, a point mutation is introduced into the genome of a first mouse using CRISPR genome editing technique so that the mouse encodes TREM2\*R47H p, and a nucleic acid encoding human APOE4 is introduced into the Apoe4 gene in the genome of a second mouse by a knock-in CRISPR genome editing technique so that the genome of the second mouse contains a “humanized” APOE4 gene as shown in Figure 1, containing exon 1 of the mouse Apoe4 gene and exons 2, 3 and 4 of the human APOE4 gene. The first and second mice are then bred naturally or by artificial methods to produce a genetically modified mouse whose genome includes a DNA sequence encoding APOE4 p and a DNA sequence encoding TREM2\*R47H p.

According to particular aspects of the present disclosure, one or more point mutations are introduced into the genome of a first mouse which encodes both TREM2\*R47H p and human APOE4 using a genome editing technique so that the mouse encodes ABCA7\*A1541G p. Alternatively, one or more point mutations are introduced into the genome of a first mouse using



a genome editing technique so that the mouse encodes ABCA7\*A1541G p and a second mouse is provided which encodes both TREM2\*R47H p and human APOE4. The first and second mice are then bred, naturally or by artificial methods, to obtain a genetically modified mouse whose genome includes a DNA sequence encoding APOE4 p, a DNA sequence encoding

5 TREM2\*R47H p and a DNA sequence encoding ABCA7\*A1541G p, wherein all of APOE4 p, TREM2\*R47H p and ABCA7\*A1541G p are expressed in the genetically modified mouse.

Further alternatives are possible such as where a first mouse is produced which has a genome which includes a DNA sequence encoding TREM2\*R47H p and a DNA sequence encoding ABCA7\*A1541G p and a second mouse is produced which has a genome which includes a DNA

10 sequence encoding human APOE4 or where a first mouse is produced which has a genome which includes a DNA sequence encoding human APOE4 and a DNA sequence encoding ABCA7\*A1541G p and a second mouse is produced which has a genome which includes a DNA sequence encoding TREM2\*R47H p. The first and second mice are then bred, naturally or by artificial methods, to obtain a genetically modified mouse whose genome includes a DNA

15 sequence encoding APOE4 p, a DNA sequence encoding TREM2\*R47H p and a DNA sequence encoding ABCA7\*A1541G p, wherein all of APOE4 p, TREM2\*R47H p and ABCA7\*A1541G p are expressed in the genetically modified mouse.

According to particular aspects of the present disclosure, one or more point mutations are introduced into the genome of a first mouse which encodes both TREM2\*R47H p and human

20 APOE4 using a CRISPR genome editing technique so that the mouse encodes ABCA7\*A1541G p. Alternatively, one or more point mutations are introduced into the genome of a first mouse using a CRISPR genome editing technique so that the mouse encodes ABCA7\*A1541G p and a second mouse is provided which encodes both TREM2\*R47H p and human APOE4. The first and second mice are then bred, naturally or by artificial methods, to obtain a genetically

25 modified mouse whose genome includes a DNA sequence encoding APOE4 p, a DNA sequence encoding TREM2\*R47H p and a DNA sequence encoding ABCA7\*A1541G p, wherein all of APOE4 p, TREM2\*R47H p and ABCA7\*A1541G p are expressed in the genetically modified mouse.

According to particular aspects of the present disclosure, point mutations are introduced

30 into the genome of a first mouse which encodes both TREM2\*R47H p and human APOE4 using a genome editing technique so that the mouse encodes APP\* p. Alternatively, point mutations

are introduced into the genome of a first mouse using a genome editing technique so that the mouse encodes APP\* p and a second mouse is provided which encodes both TREM2\*R47H p and human APOE4. The first and second mice are then bred, naturally or by artificial methods, to obtain a genetically modified mouse whose genome includes a DNA sequence encoding

5 APOE4 p, a DNA sequence encoding TREM2\*R47H p and a DNA sequence encoding APP\* p, wherein all of APOE4 p, TREM2\*R47H p and APP\* p are expressed in the genetically modified mouse. Further alternatives are possible such as where a first mouse is produced which has a genome which includes a DNA sequence encoding TREM2\*R47H p and a DNA sequence encoding APP\* p and a second mouse is produced which has a genome which includes a DNA

10 sequence encoding human APOE4 or where a first mouse is produced which has a genome which includes a DNA sequence encoding human APOE4 and a DNA sequence encoding APP\* p and a second mouse is produced which has a genome which includes a DNA sequence encoding TREM2\*R47H p. The first and second mice are then bred, naturally or by artificial methods, to obtain a genetically modified mouse whose genome includes a DNA sequence

15 encoding APOE4 p, a DNA sequence encoding TREM2\*R47H p and a DNA sequence encoding APP\* p, wherein all of APOE4 p, TREM2\*R47H p and APP\* p are expressed in the genetically modified mouse.

According to particular aspects of the present disclosure, point mutations are introduced into the genome of a first mouse which encodes both TREM2\*R47H p and human APOE4 using

20 a CRISPR genome editing technique so that the mouse encodes APP\* p. Alternatively, point mutations are introduced into the genome of a first mouse using a CRISPR genome editing technique so that the mouse encodes APP\* p and a second mouse is provided which encodes both TREM2\*R47H p and human APOE4. The first and second mice are then bred, naturally or by artificial methods, to obtain a genetically modified mouse whose genome includes a DNA

25 sequence encoding APOE4 p, a DNA sequence encoding TREM2\*R47H p and a DNA sequence encoding APP\* p, wherein all of APOE4 p, TREM2\*R47H p and APP\* p are expressed in the genetically modified mouse.

According to particular aspects of the present disclosure, one or more point mutations are introduced into the genome of a first mouse which encodes both TREM2\*R47H p and human

30 APOE4 using a genome editing technique so that the mouse encodes PLCG2\*M28L p. Alternatively, one or more point mutations are introduced into the genome of a first mouse using

a genome editing technique so that the mouse encodes PLCG2\*M28L p and a second mouse is provided which encodes both TREM2\*R47H p and human APOE4. The first and second mice are then bred, naturally or by artificial methods, to obtain a genetically modified mouse whose genome includes a DNA sequence encoding APOE4 p, a DNA sequence encoding

5 TREM2\*R47H p and a DNA sequence encoding PLCG2\*M28L p, wherein all of APOE4 p, TREM2\*R47H p and PLCG2\*M28L p are expressed in the genetically modified mouse. Further alternatives are possible such as where a first mouse is produced which has a genome which includes a DNA sequence encoding TREM2\*R47H p and a DNA sequence encoding

10 PLCG2\*M28L p and a second mouse is produced which has a genome which includes a DNA sequence encoding human APOE4 or where a first mouse is produced which has a genome which includes a DNA sequence encoding human APOE4 and a DNA sequence encoding

15 PLCG2\*M28L p and a second mouse is produced which has a genome which includes a DNA sequence encoding TREM2\*R47H p. The first and second mice are then bred, naturally or by artificial methods, to obtain a genetically modified mouse whose genome includes a DNA

sequence encoding APOE4 p, a DNA sequence encoding TREM2\*R47H p and a DNA sequence encoding PLCG2\*M28L p, wherein all of APOE4 p, TREM2\*R47H p and PLCG2\*M28L p are expressed in the genetically modified mouse.

According to particular aspects of the present disclosure, one or more point mutations are introduced into the genome of a first mouse which encodes both TREM2\*R47H p and human

20 APOE4 using a CRISPR genome editing technique so that the mouse encodes PLCG2\*M28L p. Alternatively, one or more point mutations are introduced into the genome of a first mouse using a CRISPR genome editing technique so that the mouse encodes PLCG2\*M28L p and a second mouse is provided which encodes both TREM2\*R47H p and human APOE4. The first and

25 second mice are then bred, naturally or by artificial methods, to obtain a genetically modified mouse whose genome includes a DNA sequence encoding APOE4 p, a DNA sequence encoding TREM2\*R47H p and a DNA sequence encoding PLCG2\*M28L p, wherein all of APOE4 p, TREM2\*R47H p and PLCG2\*M28L p are expressed in the genetically modified mouse.

According to particular aspects of the present disclosure, one or more point mutations are introduced into the genome of a first mouse which encodes both TREM2\*R47H p and human

30 APOE4 using a genome editing technique so that the mouse encodes MTHFR\*A262V p. Alternatively, one or more point mutations are introduced into the genome of a first mouse using

a genome editing technique so that the mouse encodes MTHFR\*A262V p and a second mouse is provided which encodes both TREM2\*R47H p and human APOE4. The first and second mice are then bred, naturally or by artificial methods, to obtain a genetically modified mouse whose genome includes a DNA sequence encoding APOE4 p, a DNA sequence encoding

5 TREM2\*R47H p and a DNA sequence encoding MTHFR\*A262V p, wherein all of APOE4 p, TREM2\*R47H p and MTHFR\*A262V p are expressed in the genetically modified mouse.

Further alternatives are possible such as where a first mouse is produced which has a genome which includes a DNA sequence encoding TREM2\*R47H p and a DNA sequence encoding MTHFR\*A262V p and a second mouse is produced which has a genome which includes a DNA

10 sequence encoding human APOE4 or where a first mouse is produced which has a genome which includes a DNA sequence encoding human APOE4 and a DNA sequence encoding MTHFR\*A262V p and a second mouse is produced which has a genome which includes a DNA sequence encoding TREM2\*R47H p. The first and second mice are then bred, naturally or by

artificial methods, to obtain a genetically modified mouse whose genome includes a DNA

15 sequence encoding APOE4 p, a DNA sequence encoding TREM2\*R47H p and a DNA sequence encoding MTHFR\*A262V p, wherein all of APOE4 p, TREM2\*R47H p and MTHFR\*A262V p are expressed in the genetically modified mouse.

According to particular aspects of the present disclosure, one or more point mutations are introduced into the genome of a first mouse which encodes both TREM2\*R47H p and human

20 APOE4 using a CRISPR genome editing technique so that the mouse encodes MTHFR\*A262V p. Alternatively, one or more point mutations are introduced into the genome of a first mouse using a CRISPR genome editing technique so that the mouse encodes MTHFR\*A262V p and a second mouse is provided which encodes both TREM2\*R47H p and human APOE4. The first and second mice are then bred, naturally or by artificial methods, to obtain a genetically

25 modified mouse whose genome includes a DNA sequence encoding APOE4 p, a DNA sequence encoding TREM2\*R47H p and a DNA sequence encoding MTHFR\*A262V p, wherein all of APOE4 p, TREM2\*R47H p and MTHFR\*A262V p are expressed in the genetically modified mouse.

According to particular aspects of the present disclosure, one or more mutations are

30 introduced into the genome of a first mouse which encodes both TREM2\*R47H p and human APOE4 using a genome editing technique to knockout the *Ceacam1* gene so that the mouse does

not encode functional CEACAM1. Alternatively, one or more mutations are introduced into the genome of a first mouse using a genome editing technique so that the mouse does not encode functional CEACAM1 and a second mouse is provided which encodes both TREM2\*R47H p and human APOE4. The first and second mice are then bred, naturally or by artificial methods, to obtain a genetically modified mouse whose genome includes a DNA sequence encoding APOE4 p, a DNA sequence encoding TREM2\*R47H p and does not include a DNA sequence encoding functional CEACAM1, wherein all of APOE4 p, TREM2\*R47H p are expressed in the genetically modified mouse and functional CEACAM1 is not expressed in the genetically modified mouse. Further alternatives are possible such as where a first mouse is produced which has a genome which includes a DNA sequence encoding TREM2\*R47H p and the genome does not encode functional CEACAM1 and a second mouse is produced which has a genome which includes a DNA sequence encoding human APOE4 or where a first mouse is produced which has a genome which includes a DNA sequence encoding human APOE4 and the genome does not encode functional CEACAM1 and a second mouse is produced which has a genome which includes a DNA sequence encoding TREM2\*R47H p. The first and second mice are then bred, naturally or by artificial methods, to obtain a genetically modified mouse whose genome includes a DNA sequence encoding APOE4 p, a DNA sequence encoding TREM2\*R47H p and does not include a DNA sequence encoding functional CEACAM1, wherein both of APOE4 p, TREM2\*R47H p are expressed in the genetically modified mouse and wherein functional CEACAM1 is not expressed in the genetically modified mouse.

According to particular aspects of the present disclosure, one or more mutations are introduced into the genome of a first mouse which encodes both TREM2\*R47H p and human APOE4 using a CRISPR genome editing technique so that the mouse does not encode functional CEACAM1. Alternatively, one or more mutations are introduced into the genome of a first mouse using a CRISPR genome editing technique so that the mouse does not encode functional CEACAM1 and a second mouse is provided which encodes both TREM2\*R47H p and human APOE4. The first and second mice are then bred, naturally or by artificial methods, to obtain a genetically modified mouse whose genome includes a DNA sequence encoding APOE4 p and a DNA sequence encoding TREM2\*R47H p and does not include a DNA sequence encoding functional CEACAM1, wherein all of APOE4 p and TREM2\*R47H p are expressed in the

genetically modified mouse and wherein functional CEACAM1 is not expressed in the genetically modified mouse.

According to particular aspects of the present disclosure, one or more mutations are introduced into the genome of a first mouse which encodes both TREM2\*R47H p and human APOE4 using a genome editing technique to knockout the IL1rap gene so that the mouse does not encode functional IL1RAP. Alternatively, one or more mutations are introduced into the genome of a first mouse using a genome editing technique so that the mouse does not encode functional IL1RAP and a second mouse is provided which encodes both TREM2\*R47H p and human APOE4. The first and second mice are then bred, naturally or by artificial methods, to obtain a genetically modified mouse whose genome includes a DNA sequence encoding APOE4 p, a DNA sequence encoding TREM2\*R47H p and does not include a DNA sequence encoding functional IL1RAP, wherein all of APOE4 p, TREM2\*R47H p are expressed in the genetically modified mouse and functional IL1RAP is not expressed in the genetically modified mouse. Further alternatives are possible such as where a first mouse is produced which has a genome which includes a DNA sequence encoding TREM2\*R47H p and the genome does not encode functional IL1RAP and a second mouse is produced which has a genome which includes a DNA sequence encoding human APOE4 or where a first mouse is produced which has a genome which includes a DNA sequence encoding human APOE4 and the genome does not encode functional IL1RAP and a second mouse is produced which has a genome which includes a DNA sequence encoding TREM2\*R47H p. The first and second mice are then bred, naturally or by artificial methods, to obtain a genetically modified mouse whose genome includes a DNA sequence encoding APOE4 p, a DNA sequence encoding TREM2\*R47H p and does not include a DNA sequence encoding functional IL1RAP, wherein both of APOE4 p, TREM2\*R47H p are expressed in the genetically modified mouse and wherein functional IL1RAP is not expressed in the genetically modified mouse.

According to particular aspects of the present disclosure, one or more mutations are introduced into the genome of a first mouse which encodes both TREM2\*R47H p and human APOE4 using a CRISPR genome editing technique so that the mouse does not encode functional IL1RAP. Alternatively, one or more mutations are introduced into the genome of a first mouse using a CRISPR genome editing technique so that the mouse does not encode functional IL1RAP and a second mouse is provided which encodes both TREM2\*R47H p and human

APOE4. The first and second mice are then bred, naturally or by artificial methods, to obtain a genetically modified mouse whose genome includes a DNA sequence encoding APOE4 p and a DNA sequence encoding TREM2\*R47H p and does not include a DNA sequence encoding functional IL1RAP, wherein all of APOE4 p and TREM2\*R47H p are expressed in the  
5 genetically modified mouse and wherein functional IL1RAP is not expressed in the genetically modified mouse.

Genomic editing is performed, for example, by methods described herein, and as detailed in J. P. Sundberg and T. Ichiki, Eds., *Genetically Engineered Mice Handbook*, CRC Press; 2006; M. H. Hofker and J. van Deursen, Eds., *Transgenic Mouse Methods and Protocols*, Humana  
10 Press, 2002; A. L. Joyner, *Gene Targeting: A Practical Approach*, Oxford University Press, 2000; *Manipulating the Mouse Embryo: A Laboratory Manual*, 3<sup>rd</sup> edition, Cold Spring Harbor Laboratory Press; December 15, 2002, ISBN-10: 0879695919; Kursad Turksen (Ed.), *Embryonic stem cells: methods and protocols in Methods Mol Biol.* 2002;185, Humana Press; *Current Protocols in Stem Cell Biology*, ISBN: 978047015180; Meyer et al., *PNAS USA*, 2010,  
15 vol. 107 (34), 15022–15026; and Doudna, J. et al. (eds.) *CRISPR-Cas: A Laboratory Manual*, 2016, CSHP.

“Disruption” of the *Ceacam1* gene and/or *Il1rap* gene refers to genetic modification of either or both genes such that expression of functional CEACAM1 and/or functional IL1RAP is absent or reduced to 25% or less, 20% or less, 15% or less, 10% or less, or 5% or less, compared  
20 to a mouse of the same background prior in which the corresponding gene has not been disrupted. In some embodiments, the genetically modified mice include an inactivated *Ceacam1* allele or an inactivated *Il1rap* allele. An inactivated allele may be a loss-of function allele or a null allele. A loss-of-function mutation, as is known in the art, results in a gene product with little or no function. By comparison, a null mutation results in a gene product with no function.

25 Any of various methods can be used to disrupt the *Ceacam1* gene and/or *Il1rap* gene to produce a genetically modified immunodeficient non-human animal whose genome includes a disruption of the *Ceacam1* gene and/or *Il1rap* gene. The *Ceacam1* gene and/or *Il1rap* gene is disrupted in the genome of genetically modified animals according to standard methods of genetic engineering such as, but not limited to, nuclease techniques, chemical mutagenesis,  
30 irradiation, homologous recombination and transgenic expression of antisense RNA. Such

techniques are well-known in the art and further include, but are not limited to, pronuclear microinjection and transformation of embryonic stem cells.

Methods for generating genetically modified animals whose genome includes a disrupted gene that can be used include, but are not limited to, those described in J. P. Sundberg and T. Ichiki, Eds., *Genetically Engineered Mice Handbook*, CRC Press; 2006; M. H. Hofker and J. van Deursen, Eds., *Transgenic Mouse Methods and Protocols*, Humana Press, 2002; A. L. Joyner, *Gene Targeting: A Practical Approach*, Oxford University Press, 2000; *Manipulating the Mouse Embryo: A Laboratory Manual*, 3rd edition, Cold Spring Harbor Laboratory Press; Dec. 15, 2002, ISBN-10: 0879695919; Kursad Turksen (Ed.), *Embryonic stem cells: methods and protocols in Methods Mol Biol.* 2002; 185, Humana Press; *Current Protocols in Stem Cell Biology*, ISBN: 978047015180; Meyer et al. *PNAS USA*, vol. 107 (34), 15022-15026.

Methods of assessing a genetically modified non-human animal to determine whether the *Ceacam1* gene and/or *Illrap* gene is disrupted such that the non-human animal lacks the capacity to express either or both the *Ceacam1* gene and/or *Illrap* gene are well-known and include standard techniques such as nucleic acid assays, spectrometric assays, immunoassays and functional assays.

Assays for assessment of functional CEACAM1 and/or IL1RAP in an animal having a putative disruption of the *Ceacam1* gene and/or *Illrap* gene can be performed.

A brief description of several genomic editing techniques is described herein.

### **Nuclease Techniques for Genetic Modification of Mice**

A genetic modification method, such as but not limited to, a nuclease genetic editing technique, can be used to introduce a desired DNA sequence into the genome at a predetermined target site, such as methods using a homing endonuclease, integrase, meganuclease, transposon, nuclease-mediated process using a zinc finger nuclease (ZFN), a Transcription Activator-Like (TAL), a Clustered Regularly Interspaced Short Palindromic Repeats (CRISPR)-Cas, or *Drosophila* Recombination-Associated Protein (DRAP). Briefly, a genetic modification method that can be used includes introducing into an ES cell, iPS cell, somatic cell, fertilized oocyte or embryo, RNA molecules encoding a targeted TALEN, ZFN, CRISPR or DRAP and at least one oligonucleotide, then selecting for an ES cell, iPS cell, somatic cell, fertilized oocyte or embryo with the desired genetic modification.



For example, a desired nucleic acid sequence can be introduced into the genome of a mouse at a predetermined target site by a nuclease technique, such as, but not limited to, CRISPR methodology, TAL (transcription activator-like) Effector methodology, Zinc Finger-Mediated Genome Editing or DRAP to produce a genetically modified mouse provided  
5 according to embodiments of the present disclosure whose genome includes a nucleic acid encoding APOE4 p operably linked to a promoter, wherein the animal expresses the encoded APOE4 p and whose genome includes a nucleic acid encoding TREM2\*R47H p operably linked to a promoter, wherein the animal expresses the encoded TREM2\*R47H p.

As used herein, the terms “target site” and “target sequence” in the context of a nuclease  
10 genetic editing technique refer to a nucleic acid sequence that defines a portion of a chromosomal sequence to be edited and to which a nuclease is engineered to recognize and bind, provided sufficient conditions for binding exist.

### **CRISPR-Cas System**

CRISPRs (Clustered Regularly Interspaced Short Palindromic Repeats) are loci  
15 containing multiple short direct repeats that are found in the genomes of approximately 40% of sequenced bacteria and 90% of sequenced archaea and confer resistance to foreign DNA elements, see Horvath, 2010, *Science*, 327: 167–170; Barrangou *et al*, 2007, *Science*, 315: 1709-1712; and Makarova *et al*, 2011, *Nature Reviews Microbiology*. 9: 467-477.

CRISPRs range in size from 24 to 48 base pairs. They usually show some dyad  
20 symmetry, implying the formation of a secondary structure such as a hairpin, but are not truly palindromic. CRISPRs are separated by spacers of similar length.

The CRISPR-associated (cas) genes are often associated with CRISPR repeat-spacer  
arrays. More than forty different Cas protein families have been described (Haft *et al*. 2005, *PLoS Comput Biol*. 1 (6): e60). Particular combinations of cas genes and repeat structures have  
25 been used to define 8 CRISPR subtypes, some of which are associated with an additional gene module encoding repeat-associated mysterious proteins (RAMPs).

There are diverse CRISPR systems in different organisms, and one of the simplest is the  
type II CRISPR system from *Streptococcus pyogenes*: only a single gene encoding the Cas9  
protein and two RNAs, a mature CRISPR RNA (crRNA) and a partially complementary trans-  
30 acting RNA (tracrRNA), are necessary and sufficient for RNA-guided silencing of foreign DNAs (Gasiunas *et al*, 2012, *PNAS* 109: E2579-E2586; Jinek *et al*, 2012, *Science* 337: 816-821).

Maturation of crRNA requires tracrRNA and RNase III (Deltcheva *et al*, 2011, Nature 471: 602-607). However, this requirement can be bypassed by using an engineered small guide RNA (sgRNA) containing a designed hairpin that mimics the tracrRNA-crRNA complex (Jinek *et al.*, 2012, Science 337: 816-821). Base pairing between the sgRNA and target DNA causes double-strand breaks (DSBs) due to the endonuclease activity of Cas9. Binding specificity is determined by both sgRNA-DNA base pairing and a short DNA motif (protospacer adjacent motif [PAM] sequence: NGG) juxtaposed to the DNA complementary region (Marraffini & Sontheimer, 2010, Nature Reviews Genetics, 11: 181-190). For example, the CRISPR system requires a minimal set of two molecules, the Cas9 protein and the sgRNA, and therefore can be used as a host-independent gene-targeting platform. The Cas9/CRISPR can be harnessed for site-selective RNA-guided genome editing, such as targeting insertion see for example, Carroll, 2012, Molecular Therapy 20: 1658-1660; Chang *et al*, 2013, Cell Research 23: 465-472; Cho *et al*, 2013, Nature Biotechnol 31: 230-232; Cong *et al*, 2013, Science 339: 819-823; Hwang *et al*, 2013, Nature Biotechnol 31: 227-229; Jiang *et al*, 2013, Nature Biotechnol 31: 233-239; Mali *et al*, 2013, Science 339: 823-826; Qi *et al*, 2013, Cell 152: 1173-1183; Shen *et al*, 2013, Cell Research 23: 720-723; and Wang *et al*, 2013, Cell 153: 910-918). In particular, Wang *et al*. 2013, Cell 153: 910-918 describe targeted insertion using the CRISPR/Cas9 system combined with oligonucleotides.

### **TAL (transcription activator-like) Effectors**

Transcription activator-like (TAL) effectors or TALE (transcription activator-like effector) are derived from a plant pathogenic bacteria genus, Xanthomonas, and these proteins mimic plant transcriptional activators and manipulate the plant transcript, see Kay *et al.*, 2007, Science, 318:648-651.

TAL effectors contain a centralized domain of tandem repeats, each repeat containing approximately 34 amino acids, which are key to the DNA binding specificity of these proteins. In addition, they contain a nuclear localization sequence and an acidic transcriptional activation domain, for a review see Schornack *et al* 2006, J. Plant Physiol., 163(3): 256-272; Scholze and Boch, 2011, Curr Opin Microbiol, 14:47-53.

Specificity of TAL effectors depends on the sequences found in the tandem repeats. The repeated sequence includes approximately 102 bp and the repeats are typically 91-100% homologous with each other (Bonas *et al*, 1989, Mol Gen Genet 218: 127-136). Polymorphism

of the repeats is usually located at positions 12 and 13 and there appears to be a one-to-one correspondence between the identity of the hypervariable diresidues at positions 12 and 13 with the identity of the contiguous nucleotides in the TAL-effector's target sequence, see Moscou and Bogdanove 2009, Science 326: 1501; and Boch *et al.* 2009, Science 326:1509-1512. The two  
5 hypervariable residues are known as repeat variable diresidues (RVDs), whereby one RVD recognizes one nucleotide of DNA sequence and ensures that the DNA binding domain of each TAL-effector can target large recognition sites with high precision (15 - 30nt). Experimentally, the code for DNA recognition of these TAL-effectors has been determined such that an HD sequence at positions 12 and 13 leads to a binding to cytosine (C), NG binds to T, NI to A, C, G  
10 or T, NN binds to A or G, and IG binds to T. These DNA binding repeats have been assembled into proteins with new combinations and numbers of repeats, to make artificial transcription factors that are able to interact with new sequences and activate the expression of a reporter gene in plant cells (Boch *et al.* 2009, Science 326:1509-1512). These DNA binding domains have been shown to have general applicability in the field of targeted genomic editing or targeted gene  
15 regulation in all cell types, see Gaj *et al.*, Trends in Biotechnol, 2013, 31(7):397-405. Moreover, engineered TAL effectors have been shown to function in association with exogenous functional protein effector domains such as a nuclease, not naturally found in natural *Xanthomonas* TAL-effect or proteins in mammalian cells. TAL nucleases (TALNs or TALENs) can be constructed by combining TALs with a nuclease, e.g. FokI nuclease domain at the N-terminus or C-terminus,  
20 Kim *et al.* 1996, PNAS 93:1156-1160; Christian *et al.* 2010, Genetics 186:757-761; Li *et al.*, 2011, Nucleic Acids Res 39: 6315-6325; and Miller *et al.*, 2011, Nat Biotechnol 29: 143-148. The functionality of TALENs to cause deletions by NHEJ has been shown in rat, mouse, zebrafish, *Xenopus*, medaka, rat and human cells, Ansai *et al.*, 2013, Genetics, 193: 739-749; Carlson *et al.*, 2012, PNAS, 109: 17382-17387; Hockemeyer *et al.*, 2011, Nature Biotechnol.,  
25 29: 731-734; Lei et al, 2012, PNAS, 109: 17484-17489; Moore *et al.*, 2012, PLoS ONE, 7: e37877; Stroud *et al.*, 2013, J. Biol. Chem., 288: 1685-1690; Sung et al, 2013, Nature Biotechnol 31: 23-24; Wefers *et al.*, 2013, PNAS 110: 3782-3787.

For TALEN, methods of making such are further described in the US Pat. Nos. 8,420,782, 8,450,471, 8,450,107, 8,440,432 and 8,440,431, and US patent application  
30 publications 2013/0137161 and 2013/0137174.

Other useful endonucleases may include, for example, HhaI, HindIII, NotI, BbvCI, EcoRI, BglI, and AlwI. The fact that some endonucleases (e.g., FokI) only function as dimers can be capitalized upon to enhance the target specificity of the TAL effector. For example, in some cases each FokI monomer can be fused to a TAL effector sequence that recognizes a different DNA target sequence, and only when the two recognition sites are in close proximity do the inactive monomers come together to create a functional enzyme. By requiring DNA binding to activate the nuclease, a highly site-specific restriction enzyme can be created.

In some embodiments, the TALEN may further include a nuclear localization signal or sequence (NLS). A NLS is an amino acid sequence that facilitates targeting the TALEN nuclease protein into the nucleus to introduce a double stranded break at the target sequence in the chromosome.

Nuclear localization signals are known in the art, see, for example, Makkerh *et al.* 1996, *Curr Biol.* 6:1025-1027. NLS include the sequence PKKKRKV (SEQ ID NO:16) from SV40 Large T-antigen, Kalderon 1984, *Cell*, 39: 499–509; RPAATKKAGQAKKK (SEQ ID NO:17) from nucleoplasmin, Dingwalle *et al.*, 1988, *J Cell Biol.*, 107, 841–9. Further examples are described in McLane and Corbett 2009, *IUBMB Life*, 61, 697–70; Dopie *et al.* 2012, *PNAS*, 109, E544–E552.

The cleavage domain may be obtained from any endonuclease or exonuclease. Non-limiting examples of endonucleases from which a cleavage domain may be derived include, but are not limited to, restriction endonucleases and homing endonucleases. See, for example, 2002-2003 Catalog, New England Biolabs, Beverly, Mass.; and Belfort *et al.* (1997) *Nucleic Acids Res.* 25:3379-3388. Additional enzymes that cleave DNA are known, e.g., SI Nuclease; mung bean nuclease; pancreatic DNase I; micrococcal nuclease; yeast HO endonuclease. See also Linn *et al.* (eds.) *Nucleases*, Cold Spring Harbor Laboratory Press, 1993. One or more of these enzymes, or functional fragments thereof, may be used as a source of cleavage domains.

### **Zinc Finger-Mediated Genome Editing**

The use of zinc finger nucleases (ZFN) for gene editing, such as for targeted insertion via a homology-directed repair process, has been well established. For example, see Carbery *et al.*, 2010, *Genetics*, 186: 451-459; Cui *et al.*, 2011, *Nature Biotechnol.*, 29: 64-68; Hauschild *et al.*, 2011, *PNAS*, 108: 12013-12017; Orlando *et al.*, 2010, *Nucleic Acids Res.*, 38: e152-e152; and Porteus & Carroll, 2005, *Nature Biotechnology*, 23: 967-973.

Components of the ZFN-mediated process include a zinc finger nuclease with a DNA binding domain and a cleavage domain. Such are described for example in Beerli *et al.* (2002) Nature Biotechnol., 20:135-141; Pabo *et al.* (2001) Ann. Rev. Biochem., 70:313-340; Isalan *et al.* (2001) Nature Biotechnol. 19:656-660; Segal *et al.* (2001) Curr Opin. Biotechnol., 12:632-637; and Choo *et al.* (2000) Curr Opin. Struct. Biol., 10:411-416; and U.S. Pat. Nos. 6,453,242 and 6,534,261. Methods to design and select a zinc finger binding domain to a target sequence are known in the art, see for example Sera, *et al.*, Biochemistry 2002,41,7074-7081; U.S. Pat. Nos. 6,607,882; 6,534,261 and 6,453,242.

In some embodiments, the zinc finger nuclease may further include a nuclear localization signal or sequence (NLS). A NLS is an amino acid sequence that facilitates targeting the zinc finger nuclease protein into the nucleus to introduce a double stranded break at the target sequence in the chromosome. Nuclear localization signals are known in the art. See, for example, Makkerh *et al.* (1996) Current Biology 6:1025-1027.

The cleavage domain may be obtained from any endonuclease or exonuclease. Non-limiting examples of endonucleases from which a cleavage domain may be derived include, but are not limited to, restriction endonucleases and homing endonucleases. See, for example, 2002-2003 Catalog, New England Biolabs, Beverly, Mass.; and Belfort *et al.* (1997) Nucleic Acids Res. 25:3379-3388. Additional enzymes that cleave DNA are known (e.g., SI Nuclease; mung bean nuclease; pancreatic DNase I; micrococcal nuclease; yeast HO endonuclease). See also Linn *et al.* (eds.) Nucleases, Cold Spring Harbor Laboratory Press, 1993. One or more of these enzymes (or functional fragments thereof) may be used as a source of cleavage domains. A cleavage domain also may be derived from an enzyme or portion thereof, as described above, that requires dimerization for cleavage activity.

Two zinc finger nucleases may be required for cleavage, as each nuclease includes a monomer of the active enzyme dimer. Alternatively, a single zinc finger nuclease may include both monomers to create an active enzyme dimer. Restriction endonucleases (restriction enzymes) are present in many species and are capable of sequence-specific binding to DNA (at a recognition site), and cleaving DNA at or near the site of binding. Certain restriction enzymes (e.g., Type IIS) cleave DNA at sites removed from the recognition site and have separable binding and cleavage domains. For example, the Type IIS enzyme FokI catalyzes double stranded cleavage of DNA, at 9 nucleotides from its recognition site on one strand and 13

nucleotides from its recognition site on the other. See, for example, U.S. Pat. Nos. 5,356,802; 5,436,150 and 5,487,994; as well as Li *et al.* (1992) PNAS 89:4275-4279; Li *et al.* (1993) PNAS 90:2764-2768; Kim *et al.* (1994) PNAS 91:883-887; Kim *et al.* (1994) J. Biol. Chem. 269:31, 978-31, 982. Thus, a zinc finger nuclease may include the cleavage domain from at least one

5 Type IIS restriction enzyme and one or more zinc finger binding domains, which may or may not be engineered. Exemplary Type IIS restriction enzymes are described for example in International Publication WO 07/014275, the disclosure of which is incorporated by reference herein in its entirety. Additional restriction enzymes also contain separable binding and cleavage domains, and these also are contemplated by the present disclosure. See, for example, Roberts *et*

10 *al.* (2003) Nucleic Acids Res. 31: 418-420. An exemplary Type IIS restriction enzyme, whose cleavage domain is separable from the binding domain, is FokI. This particular enzyme is active as a dimer (Bitinaite *et al.* 1998, PNAS 95: 10,570-10,575). Accordingly, for the purposes of the present disclosure, the portion of the FokI enzyme used in a zinc finger nuclease is considered a cleavage monomer. Thus, for targeted double stranded cleavage using a FokI cleavage domain,

15 two zinc finger nucleases, each including a FokI cleavage monomer, may be used to reconstitute an active enzyme dimer. Alternatively, a single polypeptide molecule containing a zinc finger binding domain and two FokI cleavage monomers may also be used. In certain embodiments, the cleavage domain may include one or more engineered cleavage monomers that minimize or prevent homodimerization, as described, for example, in U.S. Patent Publication Nos.

20 2005/0064474, 2006/0188987, and 2008/0131962, each of which is incorporated by reference herein in its entirety. By way of non-limiting example, amino acid residues at positions 446, 447, 479, 483, 484, 486, 487, 490, 491, 496, 498, 499, 500, 531, 534, 537 and 538 of FokI are all targets for influencing dimerization of the FokI cleavage half-domains. Exemplary engineered cleavage monomers of FokI that form obligate heterodimers include a pair in which a first

25 cleavage monomer includes mutations at amino acid residue positions 490 and 538 of FokI and a second cleavage monomer that includes mutations at amino-acid residue positions 486 and 499. Thus, in one embodiment, a mutation at amino acid position 490 replaces Glu (E) with Lys (K); a mutation at amino acid residue 538 replaces Ile (I) with Lys (K); a mutation at amino acid residue 486 replaces Gln (Q) with Glu (E); and a mutation at position 499 replaces Ile (I) with

30 Lys (K). Specifically, the engineered cleavage monomers may be prepared by mutating positions 490 from E to K and 538 from I to K in one cleavage monomer to produce an engineered

cleavage monomer designated “E490K:I538K” and by mutating positions 486 from Q to E and 499 from I to L in another cleavage monomer to produce an engineered cleavage monomer designated “Q486E:I499L.” The above described engineered cleavage monomers are obligate heterodimer mutants in which aberrant cleavage is minimized or abolished. Engineered cleavage monomers may be prepared using a suitable method, for example, by site-directed mutagenesis of wild-type cleavage monomers (FokI) as described in U.S. Patent Publication No. 2005/0064474.

The zinc finger nuclease described above may be engineered to introduce a double stranded break at the targeted site of integration. The double stranded break may be at the targeted site of integration, or it may be up to 1, 2, 3, 4, 5, 10, 15, 20, 25, 30, 35, 40, 45, 50, 100 or 1000 nucleotides away from the site of integration. In some embodiments, the double stranded break may be up to 1, 2, 3, 4, 5, 10, 15, or 20 nucleotides away from the site of integration. In other embodiments, the double stranded break may be up to 10, 15, 20, 25, 30, 35, 40, 45 or 50 nucleotides away from the site of integration. In yet other embodiments, the double stranded break may be up to 50, 100 or 1000 nucleotides away from the site of integration.

The DRAP technology has been described in US Pat. Nos. 6,534,643, 6,858,716 and 6,830,910 and Watt *et al.*, 2006.

Optionally, a nucleic acid sequence encoding a protein can be inserted at a random target site in the genome so that the genome includes a nucleic acid encoding the protein. Typically, for random insertion, the nucleic acid sequence also contains a promoter to drive expression of the inserted nucleic acid.

In a further option, a nucleic acid encoding the desired protein, APOE4 p, TREM2\*R47H p or both APOE4 p and TREM2\*R47H p, or one or more of: ABCA7\*A1541G p, APP\* p, PLCG2\*M28L p, and MTHFR\*A262V p, or a variant of any thereof, is inserted into a predetermined target site in the genome other than the corresponding Apoe4 gene, the Trem2 gene, the Abca7 gene, the App gene, or the PLCG2 gene.

For example, a nucleic acid encoding the desired protein, APOE4 p, TREM2\*R47H p or both APOE4 p and TREM2\*R47H p, or one or more of: ABCA7\*A1541G p, APP\* p, PLCG2\*M28L p, and MTHFR\*A262V p, or a variant of any thereof, is inserted into a predetermined target site in the genome known to result in reliable expression, such as the *Hprt* or the *Rosa26* locus.

According to aspects, for genomic editing at a predetermined target site, a targeting construct is made using recombinant DNA techniques and includes 5' and 3' sequences which are homologous to the targeted endogenous gene in the cell. The targeting construct further includes a selectable marker such as neomycin phosphotransferase, hygromycin or puromycin, a nucleic acid encoding the desired protein, APOE4 p, TREM2\*R47H p or both APOE4 p and TREM2\*R47H p, or one or more of: ABCA7\*A1541G p, APP\* p, PLCG2\*M28L p, and MTHFR\*A262V p, or a variant of any thereof, and optionally a polyadenylation signal. To insure correct transcription and translation of the nucleic acid encoding the desired protein, the nucleic acid encoding the desired protein is either in frame with the endogenous gene locus, or a splice acceptor site and internal ribosome entry site (IRES) sequences can be included.

Such a targeting construct is transfected into a desired cell type, such as but not limited to, stem cells and the cells are screened to detect the correct genomic editing event using PCR, Southern blot or sequencing analysis. Cells with the correct genomic editing event can be further analyzed for expression of the encoded protein by protein analysis, such as ELISA or Western blot analysis. If desired, the nucleic acid encoding the selectable marker can be configured to be removed by treating the stem cells with a recombinase such as Cre recombinase or Flippase (Flp). After recombinase treatment, the cells are analyzed for the presence of the nucleic acid encoding the desired protein.

Cells with the correct genomic editing event are selected and injected into preimplantation embryos as described above. Chimeric males are selected for breeding. Offspring can be analyzed for transmission of the ES cell genome by coat color and genetic analysis, such as PCR, Southern blot or sequencing and can be tested for expression of the desired protein, such as by protein analysis (Western blot, ELISA) or other functional assays. Offspring expressing the correct proteins are intercrossed to create mice homozygous for the genetic modification(s).

Generation of a genetically modified mouse expressing APOE4 p and TREM2\*R47H p, and one or more of: ABCA7\*A1541G p, APP\* p, PLCG2\*M28L p, and MTHFR\*A262V p, or a variant of any thereof, and/or which does not express functional CEACAM1 and/or IL1RAP may include injection or transfection of appropriate nucleic acids, such as one or more one or more nucleic acids encoding a desired protein and/or one or more expression constructs, such as an expression construct encoding a protein or RNA (such as cas9 or a guide RNA for use in



CRISPR), into a preimplantation embryo or stem cells, such as embryonic stem (ES) cells or induced pluripotent stem (iPS) cells.

The terms “expression construct” and “expression cassette” are used herein to refer to a double-stranded recombinant DNA molecule containing a desired nucleic acid coding sequence and containing one or more regulatory elements necessary or desirable for the expression of the operably linked coding sequence.

The term “regulatory element” as used herein refers to a nucleotide sequence which controls some aspect of the expression of nucleic acid sequences. Exemplary regulatory elements illustratively include an enhancer, an internal ribosome entry site (IRES), an intron; an origin of replication, a polyadenylation signal (pA), a promoter, a transcription termination sequence, and an upstream regulatory domain, which contribute to the replication, transcription, post-transcriptional processing of a nucleic acid sequence. Those of ordinary skill in the art are capable of selecting and using these and other regulatory elements in an expression construct with no more than routine experimentation. Expression constructs can be generated recombinantly or synthetically using well-known methodology.

The term “operably linked” as used herein refers to a nucleic acid in functional relationship with a second nucleic acid.

A regulatory element is included in an expression cassette is a promoter in particular embodiments. The term “promoter” as used herein refers to a DNA sequence operably linked to a nucleic acid sequence to be transcribed such as a nucleic acid sequence encoding a desired molecule. A promoter is generally positioned upstream of a nucleic acid sequence to be transcribed and provides a site for specific binding by RNA polymerase and other transcription factors. In specific embodiments, a promoter is generally positioned upstream of the nucleic acid sequence transcribed to produce the desired molecule, and provides a site for specific binding by RNA polymerase and other transcription factors. An included promoter can be a constitutive promoter or can provide inducible expression; and can provide ubiquitous, tissue-specific or cell-type specific expression.

Ubiquitous promoters that can be included in an expression construct include, but are not limited to, a 3-phosphoglycerate kinase (PGK-1) promoter, a beta-actin promoter, a ROSA26 promoter, a heat shock protein 70 (Hsp70) promoter, an EF-1 alpha gene encoding elongation

factor 1 alpha (EF1) promoter, an eukaryotic initiation factor 4A (eIF-4A1) promoter, a chloramphenicol acetyltransferase (CAT) promoter and a CMV (cytomegalovirus) promoter.

These and other promoters are known in the art as exemplified in Abboud, S. L. et al, J. Histochem & Cytochem., 51(7):941-949, 2003; Schorpp *et al.*, Nucl. Acids Res., 24(9):1787-1788, 1996; McBurney, M. W. *et al.*, Devel. Dynamics, 200:278-293, 1994; and Majumder, M. *et al.*, Blood, 87(8):3203-3211, 1996.

In addition to a promoter, one or more enhancer sequences may be included such as, but not limited to, cytomegalovirus (CMV) early enhancer element and an SV40 enhancer element.

Additional included sequences include an intron sequence such as the beta globin intron or a generic intron, a transcription termination sequence, and an mRNA polyadenylation (pA) sequence such as, but not limited to SV40-pA, beta-globin-pA and SCF-pA.

An expression construct may include sequences necessary for amplification in bacterial cells, such as a selection marker (e.g. kanamycin or ampicillin resistance gene) and a replicon.

For methods of DNA injection of an expression construct into a preimplantation embryo, the expression construct is optionally linearized before injection into mouse preimplantation embryos. Preferably the expression construct is injected into fertilized oocytes. Fertilized oocytes are collected from superovulated females the day after mating (0.5 dpc) and injected with the expression construct. The injected oocytes are either cultured overnight or transferred directly into oviducts of 0.5-day p.c. pseudopregnant females.

Methods for superovulation, harvesting of oocytes, expression construct injection and embryo transfer are known in the art and described in Manipulating the Mouse Embryo: A Laboratory Manual, 3<sup>rd</sup> edition, Cold Spring Harbor Laboratory Press; December 15, 2002, ISBN-10: 0879695919.

Offspring can be tested for the presence of the desired mutation or inserted sequence by DNA analysis, such as PCR, Southern blot or sequencing. Mice which are carrying the desired mutation or inserted sequence can be tested for protein expression such as for example, by ELISA or Western blot analysis.

Alternatively, a nucleic acid or expression construct may be transfected into stem cells (ES cells or iPS cells) using well-known methods, such as electroporation, calcium-phosphate precipitation and lipofection. The cells are screened for the presence of the desired mutation or inserted sequence by DNA analysis, such as PCR, Southern blot or sequencing. Cells with the

desired mutation or inserted sequence by can be tested for functional expression by protein analysis, such as for example, by ELISA or Western blot analysis.

Mouse ES cells are grown in media optimized for the particular line. Typically ES media contains 15% fetal bovine serum (FBS) or synthetic or semi-synthetic equivalents, 2 mM  
5 glutamine, 1 mM Na pyruvate, 0.1 mM non-essential amino acids, 50 U/ml penicillin and streptomycin, 0.1 mM 2-mercaptoethanol and 1000 U/ml LIF (plus, for some cell lines chemical inhibitors of differentiation) in Dulbecco's Modified Eagle Media (DMEM). A detailed description is known in the art (Tremml et al., 2008, Current Protocols in Stem Cell Biology, Chapter 1:Unit 1C.4. For review of inhibitors of ES cell differentiation, see Buehr, M., et al.  
10 (2003). Genesis of embryonic stem cells. Philosophical Transactions of the Royal Society B: Biological Sciences 358, 1397-1402.

Selected cells incorporating the desired mutation or inserted sequence can be injected into preimplantation embryos. For microinjection, ES or iPS cell are rendered to single cells using a mixture of trypsin and EDTA, followed by resuspension in ES media. Groups of single cells are  
15 selected using a finely drawn-out glass needle (20-25 micrometer inside diameter) and introduced through the embryo's zona pellucida and into the blastocysts cavity (blastocoel) using an inverted microscope fitted with micromanipulators.

Alternatively, to blastocyst injection, stem cells can be injected into early stage embryos (e.g. 2-cell, 4-cell, 8-cell, premorula or morula). Injection may be assisted with a laser or piezo  
20 pulses drilled opening the zona pellucida. Approximately 9-10 selected stem cells (ES or iPS cells) are injected per blastocysts, or 8-cell stage embryo, 6-9 stem cells per 4-cell stage embryo, and about 6 stem cells per 2-cell stage embryo. Following stem cell introduction, embryos are allowed to recover for a few hours at 37 °C in 5% CO<sub>2</sub>, 5% O<sub>2</sub> in nitrogen or cultured overnight before transfer into pseudopregnant recipient females. In a further alternative to stem cell  
25 injection, stem cells can be aggregated with morula stage embryos. All these methods are well established and can be used to produce stem cell chimeras. For a more detailed description see Manipulating the Mouse Embryo: A Laboratory Manual, 3<sup>rd</sup> edition (A. Nagy, M. Gertsenstein, K. Vintersten, R. Behringer, Cold Spring Harbor Laboratory Press; December 15, 2002, ISBN-10: 0879695919, Nagy *et al.*, 1990, Development 110, 815-821; US 7,576,259: Method for  
30 making genetic modifications, US7659442, US 7,294,754, and Kraus *et al.* 2010, Genesis 48, 394-399.

Pseudopregnant embryo recipients are prepared using methods known in the art. Briefly, fertile female mice between 6-8 weeks of age are mated with vasectomized or sterile males to induce a hormonal state conducive to supporting surgically introduced embryos. At 2.5 days post coitum (dpc) up to 15 of the stem cell containing blastocysts are introduced into the uterine horn very near to the uterus-oviduct junction. For early stage embryos and morula, such embryos are either cultured in vitro into blastocysts or implanted into 0.5 dpc or 1.5 dpc pseudopregnant females according to the embryo stage into the oviduct. Chimeric pups from the implanted embryos are born 16-20 days after the transfer depending on the embryo age at implantation. Chimeric males are selected for breeding. Offspring can be analyzed for transmission of the ES cell genome by coat color and genetic analysis, such as PCR, Southern blot or sequencing. Further the expression of the encoded protein(s) can be analyzed by protein analysis (Western blot, ELISA) or other functional assays.

A genetically modified mouse of the present disclosure may be heterozygous or homozygous for the genetic modifications.

According to aspects of the present disclosure, a genetically modified mouse of the present disclosure may be heterozygous or homozygous for the knock-in humanized APOE4 modification wherein the mouse expresses APOE4 p, may be heterozygous or homozygous for the mutated genomic sequence encoding TREM2\*R47H p wherein the mouse expresses TREM2\*R47H p, and may be heterozygous or homozygous for one or more of: a mutated genomic sequence encoding ABCA7\*A1541G p, a mutated genomic sequence encoding APP\* p, a mutated genomic sequence encoding PLCG2\*M28L p, or a mutated genomic sequence encoding a variant of any thereof.

Homozygous genetically modified mice expressing a first encoded mutated protein can be crossed with homozygous genetically modified mice expressing a second encoded mutated protein to create a congenic strain homozygous for both modifications and expressing both mutated proteins according to embodiments.

Homozygous genetically modified mice expressing a first encoded mutated protein and a second encoded mutated protein can be crossed with homozygous genetically modified mice expressing a third encoded mutated protein to create a congenic strain homozygous for all three modifications and expressing all three mutated proteins according to embodiments.

Similarly, homozygous genetically modified mice expressing a first encoded mutated protein, a second encoded mutated protein and a third encoded mutated protein and/or fourth encoded mutated protein can be crossed with homozygous genetically modified mice expressing a further encoded mutated protein to create a congenic strain homozygous for all of the encoded modifications and expressing all of the mutated proteins according to embodiments.

Homozygous genetically modified mice expressing APOE4 p can be crossed with homozygous genetically modified mice expressing TREM2\*R47H p to create a congenic strain homozygous for both modifications and expressing both APOE4 p and TREM2\*R47H p according to embodiments.

Homozygous genetically modified mice expressing APOE4 p and TREM2\*R47H p can be crossed with homozygous genetically modified mice expressing any one or more of: ABCA7\*A1541G p, APP\* p, PLCG2\*M28L p, MTHFR\*A262V p, or a variant of any thereof, or genetically modified such that the *Ceacam1* gene and/or *Il1rap* gene is disrupted, to create a congenic strain homozygous for the three modifications and expressing APOE4 p, TREM2\*R47H p, and ABCA7\*A1541G p, APP\* p, PLCG2\*M28L p, MTHFR\*A262V p, or a variant of any thereof, and/or where functional CEACAM1 and/or IL1RAP are not expressed, according to embodiments.

Genetically modified mice of the present disclosure can be any of various strains.

A genetic modification can be introduced into the genome of an isolated mouse embryonic stem (ES) cell, a mouse induced pluripotent stem (iPS) cell, a mouse somatic cell, a fertilized mouse oocyte (zygote) or a mouse embryo in a knock-in strategy to produce a genetically modified mouse of the present disclosure.

Embodiments of the disclosure provide a genetically modified mouse that includes a desired genetic modification in all or substantially all of its cells, as well as a genetically modified mouse that includes a desired genetic modification in some, but not all its cells.

A genetically modified mouse according to aspects of the present disclosure can include one or more additional genetic variants associated with increased risk of late-onset Alzheimer's disease in humans.

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## Identification of Treatments and Compounds

Methods for screening for putative treatments for human Alzheimer's disease are provided according to embodiments of the present disclosure which include: administering a putative treatment for Alzheimer's disease to a genetically modified mouse, wherein the genome of the mouse includes: (1) a DNA sequence encoding human APOE4 protein (APOE4 p) operably linked to a promoter; and (2) a DNA sequence encoding mouse TREM2 protein having a mutation p.R47H (TREM2\*R47H p) operably linked to a promoter, wherein the mouse expresses APOE4 p and TREM2\*R47H p and one or more of: (3) a nucleic acid encoding: ABCA7\*A1541G p operably linked to a promoter, wherein the animal expresses the encoded ABCA7\*A1541G p; (4) a nucleic acid encoding: APP\* p operably linked to a promoter, wherein the animal expresses the encoded APP\* p; (5) a nucleic acid encoding: PLCG2\*M28L p operably linked to a promoter, wherein the animal expresses the encoded PLCG2\*M28L p; (6) a nucleic acid encoding: MTHFR\*A262V p operably linked to a promoter, wherein the animal expresses the encoded MTHFR\*A262V p; (6) a disrupted (e.g., inactivated) *Ceacam1* gene such that functional CEACAM1 is not produced in the genetically modified mouse; and (7) a disrupted (e.g., inactivated) *Il1rap* gene such that functional IL1RAP is not produced in the genetically modified mouse, wherein the mouse is characterized by one or more symptoms or signs associated with expression of APOE4 p, TREM2\*R47H p and one or more of: (1) expression of ABCA7\*A1541G p; (2) expression of APP\* p; (3) expression of PLCG2\*M28L p; (4) expression of MTHFR\*A262V; (5) lack of expression of functional CEACAM1; and (6) lack of expression of functional IL1RAP, relevant to non-familial late-onset Alzheimer's disease of the present disclosure; and assessing an effect of the putative treatment on the mouse.

Methods for screening for putative treatments for human Alzheimer's disease are provided according to embodiments of the present disclosure which include: administering a putative treatment for Alzheimer's disease to a mouse whose genome includes: (1) a DNA sequence encoding human APOE4 protein (APOE4 p) operably linked to a promoter; and (2) a DNA sequence encoding mouse TREM2 protein having a mutation p.R47H (TREM2\*R47H p) operably linked to a promoter, wherein the mouse expresses APOE4 p and TREM2\*R47H p and one or more of: (3) a nucleic acid encoding: ABCA7\*A1541G p operably linked to a promoter, wherein the animal expresses the encoded ABCA7\*A1541G p; (4) a nucleic acid encoding: APP\* p operably linked to a promoter, wherein the animal expresses the encoded APP\* p; (5) a

nucleic acid encoding: *PLCG2*\*M28L p operably linked to a promoter, wherein the animal expresses the encoded *PLCG2*\*M28L p; (6) a nucleic acid encoding: *MTHFR*\*A262V p operably linked to a promoter, wherein the animal expresses the encoded *MTHFR*\*A262V p; (6) a disrupted (e.g., inactivated) *Ceacam1* gene such that functional CEACAM1 is not produced in the genetically modified mouse; and (7) a disrupted (e.g., inactivated) *Il1rap* gene such that functional IL1RAP is not produced in the genetically modified mouse; and assessing an effect of the putative treatment on the mouse.

Methods for screening for a compound for use in the treatment of Alzheimer's disease, are provided according to aspects of the present disclosure which include administering a compound to a genetically modified mouse of the present disclosure; and assessing an effect of the compound in the treatment of one or more symptoms or signs associated with expression of human APOE4 p and mouse *TREM2*\*R47H p and one or more of: *ABCA7*\*A1541G p, *APP*\* p, *PLCG2*\*M28L p, *MTHFR*\*A262V p, a disrupted *Ceacam1* gene such that functional CEACAM1 is not produced in the genetically modified mouse; and a disrupted *Il1rap* gene such that functional IL1RAP is not produced in the genetically modified mouse, wherein the one or more symptoms or signs is relevant to non-familial late-onset Alzheimer's disease. Assessing an effect of the compound in the treatment of one or more symptoms or signs associated with expression of human APOE4 p and mouse *TREM2*\*R47H p and one or more of: *ABCA7*\*A1541G p, *APP*\* p, *PLCG2*\*M28L p, *MTHFR*\*A262V p a disrupted *Ceacam1* gene such that functional CEACAM1 is not produced in the genetically modified mouse; and a disrupted *Il1rap* gene such that functional IL1RAP is not produced in the genetically modified mouse wherein the symptoms or signs are relevant to non-familial late-onset Alzheimer's disease preferably includes comparing the result of the assessment with a suitable control, such as, but not limited to, the effect of the compound on a control, such as an APOE3-expressing mouse or a wild-type mouse, such as a mouse of the same or similar background without one or more of these mutations, e.g., a mouse carrying the *ApoE* gene.

Such signs and symptoms include, but are not limited to, any one or more of: 1) presence of significantly more microglia in the brain of a genetically modified mouse of the present disclosure compared to a relevant control, such as a mouse of the same or similar background expressing one or more of: non-mutant *APOE3*, *Trem2*, *Abca7*, *App*, *Plcg2*, *Mthfr*, *Ceacam1*, or *Il1rap*; or a wild-type control mouse of the same or similar background; 2) presence of

significantly more amyloid plaques in the brain of a genetically modified mouse of the present disclosure compared to a relevant control, such as a mouse of the same or similar background expressing one or more of: non-mutant *APOE3*, *Trem2*, *Abca7*, *App*, *Plcg2*, *Mthfr*, *Ceacam1*, or *Illrap*; or a wild-type control mouse of the same or similar background; 3) presence of

5 significantly more tau aggregates in the brain of a genetically modified mouse of the present disclosure compared to a relevant control, such as a mouse of the same or similar background expressing one or more of: non-mutant *APOE3*, *Trem2*, *Abca7*, *App*, *Plcg2*, *Mthfr*, *Ceacam1*, or *Illrap*; or a wild-type control mouse of the same or similar background; 4) presence of

10 significantly more inflammation in the brain of a genetically modified mouse of the present disclosure compared to a relevant control, such as a mouse of the same or similar background expressing one or more of: non-mutant *APOE3*, *Trem2*, *Abca7*, *App*, *Plcg2*, *Mthfr*, *Ceacam1*, or *Illrap*; or a wild-type control mouse of the same or similar background; 5) presence of

15 significantly more synaptic and/or neuronal loss in the brain of a genetically modified mouse of the present disclosure compared to a relevant control, such as a mouse of the same or similar background expressing one or more of: non-mutant *APOE3*, *Trem2*, *Abca7*, *App*, *Plcg2*, *Mthfr*, *Ceacam1*, or *Illrap*; or a wild-type control mouse of the same or similar background; 6) presence of

20 significantly more cognitive deficit in the brain of a genetically modified mouse of the present disclosure compared to a relevant control, such as a mouse of the same or similar background expressing one or more of: non-mutant *APOE3*, *Trem2*, *Abca7*, *App*, *Plcg2*, *Mthfr*, *Ceacam1*, or *Illrap*; or a wild-type control mouse of the same or similar background; 7) presence of

25 significantly more indications of frailty an aging genetically modified mouse of the present disclosure compared to a relevant control, such as a mouse of the same or similar background expressing one or more of: non-mutant *APOE3*, *Trem2*, *Abca7*, *App*, *Plcg2*, *Mthfr*, *Ceacam1*, or *Illrap*; or a wild-type control mouse of the same or similar background; 8) presence of

30 significantly more blood flow deficit in the brain of a genetically modified mouse of the present disclosure compared to a relevant control, such as a mouse of the same or similar background expressing one or more of: non-mutant *APOE3*, *Trem2*, *Abca7*, *App*, *Plcg2*, *Mthfr*, *Ceacam1*, or *Illrap*; or a wild-type control mouse of the same or similar background; 9) a significant difference in presence, level, and/or function of one or more biomarkers of non-familial late-onset Alzheimer's disease in blood, serum, or tissue of a genetically modified mouse of the present disclosure compared to a relevant control, such as a mouse of the same or similar



background expressing one or more of: non-mutant *APOE3*, *Trem2*, *Abca7*, *App*, *Plcg2*, *Mthfr*, *Ceacam1*, or *Il1rap*; or a wild-type control mouse of the same or similar background; 10) cerebrovascular leakage in a genetically modified mouse of the present disclosure compared to a relevant control, such as a mouse of the same or similar background expressing one or more of: 5 non-mutant *APOE3*, *Trem2*, *Abca7*, *App*, *Plcg2*, *Mthfr*, *Ceacam1*, or *Il1rap*; or a wild-type control mouse of the same or similar background; and 11) levels of one or more blood lipoproteins, such as high density lipoprotein, and low density lipoprotein, and/or cholesterol, in a genetically modified mouse of the present disclosure compared to a relevant control, such as a mouse of the same or similar background expressing one or more of: non-mutant *APOE3*, 10 *Trem2*, *Abca7*, *App*, *Plcg2*, *Mthfr*, *Ceacam1*, or *Il1rap*; or a wild-type control mouse of the same or similar background.

According to aspects of the present disclosure, methods for screening for putative treatments for human Alzheimer's disease are provided wherein assessing an effect of a compound includes comparing the effect of the compound on the genetically modified mouse, 15 wherein the genome of the mouse includes: (1) a DNA sequence encoding human APOE4 protein (APOE4 p) operably linked to a promoter; and (2) a DNA sequence encoding mouse TREM2 protein having a mutation p.R47H (TREM2\*R47H p) operably linked to a promoter, wherein the mouse expresses APOE4 p and TREM2\*R47H p, and wherein the mouse includes one or more of the following: (1) a nucleic acid encoding: ABCA7\*A1541G p operably linked to 20 a promoter, wherein the animal expresses the encoded ABCA7\*A1541G p; (2) a nucleic acid encoding: APP\* p operably linked to a promoter, wherein the animal expresses the encoded APP\* p; (3) a nucleic acid encoding: PLCG2\*M28L p operably linked to a promoter, wherein the animal expresses the encoded PLCG2\*M28L p; (4) a nucleic acid encoding: MTHFR\*A262V p operably linked to a promoter, wherein the animal expresses the encoded 25 MTHFR\*A262V p; (5) a disrupted *Ceacam1* gene such that functional CEACAM1 is not produced in the genetically modified mouse; and (6) a disrupted *Il1rap* gene such that functional IL1RAP is not produced in the genetically modified mouse; and wherein the mouse is characterized by one or more symptoms or signs associated with expression of APOE4 p and TREM2\*R47H p and one or more of the following: ABCA7, APP, PLCG2, MTHFR, 30 CEACAM1, IL1RAP, relevant to non-familial late-onset Alzheimer's disease of the present disclosure, with a control.

A suitable control includes, for example, administering the compound to a control mouse which does not express human APOE4 p and mouse TREM2\*R47H p and does not include a relevant one or more of the following: (1) a nucleic acid encoding: ABCA7\*A1541G p operably linked to a promoter, wherein the animal expresses the encoded ABCA7\*A1541G p; (2) a nucleic acid encoding: APP\* p operably linked to a promoter, wherein the animal expresses the encoded APP\* p; (3) a nucleic acid encoding: PLCG2\*M28L p operably linked to a promoter, wherein the animal expresses the encoded PLCG2\*M28L p; (4) a nucleic acid encoding: MTHFR\*A262V p operably linked to a promoter, wherein the animal expresses the encoded MTHFR\*A262V p; (5) a disrupted *Ceacam1* gene such that functional CEACAM1 is not produced in the genetically modified mouse; and (6) a disrupted *Il1rap* gene such that functional IL1RAP is not produced in the genetically modified mouse; and assessing an effect of the compound on the control mouse. A suitable control includes, for example, administering the compound to an APOE3-expressing mouse or wild-type control mouse. A wild-type control mouse can be any mouse which does not express human APOE4 p and mouse TREM2\*R47H p; and a relevant one or more of the following: (1) a nucleic acid encoding: ABCA7\*A1541G p operably linked to a promoter, wherein the animal expresses the encoded ABCA7\*A1541G p; (2) a nucleic acid encoding: APP\* p operably linked to a promoter, wherein the animal expresses the encoded APP\* p; (3) a nucleic acid encoding: PLCG2\*M28L p operably linked to a promoter, wherein the animal expresses the encoded PLCG2\*M28L p; (4) a nucleic acid encoding: MTHFR\*A262V p operably linked to a promoter, wherein the animal expresses the encoded MTHFR\*A262V p; (5) a disrupted *Ceacam1* gene such that functional CEACAM1 is not produced in the genetically modified mouse; and (6) a disrupted *Il1rap* gene such that functional IL1RAP is not produced in the genetically modified mouse. A suitable control includes, for example, administering the compound to a wild-type C57BL/6J mouse; and assessing an effect of the compound on the wild-type C57BL/6J mouse.

Symptoms or signs associated with expression of human APOE4 p and mouse TREM2\*R47H p relevant to non-familial late-onset Alzheimer's disease can be assessed by methods well-known in the art including, but not limited to, immunoassay, nucleic acid assay, histochemical staining, cognitive assays, *in vivo* imaging, physical assessment of the animals, cerebrovascular leakage assessment, and morphological assessment of tissues and/or cells.

Immunoassays that can be used are well-known in the art and include, but are not limited to, enzyme-linked immunosorbent assay (ELISA) such as but not limited to, antigen capture ELISA, indirect ELISA, fixed cell ELISA; immunochromatography; antigen capture; flow cytometry; immunoblot; immunoprecipitation; immunodiffusion; competitive immunoassays, immunocytochemistry; radioimmunoassay; and combinations of any of these. Generalized details of immunoassays are described in standard references, illustratively including Wild, D., The Immunoassay Handbook, 3rd Ed., Elsevier Science, 2005; Gosling, J. P., Imunoassays: A Practical Approach, Practical Approach Series, Oxford University Press, 2005; E.Harlow and D. Lane, Antibodies: A Laboratory Manual, Cold Spring Harbor Laboratory Press, 1988; F. Breitling and S. Dübel, Recombinant Antibodies, John Wiley & Sons, New York, 1999; H. Zola, Monoclonal Antibodies: Preparation and Use of Monoclonal Antibodies and Engineered Antibody Derivatives, Basics: From Background to Bench, BIOS Scientific Publishers, 2000; B.K.C. Lo, Antibody Engineering: Methods and Protocols, Methods in Molecular Biology, Humana Press, 2003; F. M. Ausubel et al., Eds., Short Protocols in Molecular Biology, Current Protocols, Wiley, 2002; Ormerod, M. G., Flow Cytometry: a practical approach, Oxford University Press, 2000; and Givan, A. L., Flow Cytometry: first principles, Wiley, New York, 2001.

According to aspects of the present disclosure, nucleic acid assays to detect a nucleic acid analyte relevant to non-familial late-onset Alzheimer's disease in a genetically modified mouse of the present disclosure compared to a wild-type mouse includes, but is not limited to, nucleic acid amplification techniques such as, but not limited to, PCR, RT-PCR ligation-mediated PCR and phi-29 PCR; nucleic acid hybridization techniques such as, but not limited to, Northern blot, Southern blot, RNase protection assay, dot blot, transcriptome analysis, and *in situ* hybridization. Nucleic acid assays for both qualitative and quantitative assay of a nucleic acid in a sample are described in detail in standard references, illustratively including J. Sambrook and D.W. Russell, Molecular Cloning: A Laboratory Manual, Cold Spring Harbor Laboratory Press; 3rd Ed., 2001; F. M. Ausubel et al., Eds., Short Protocols in Molecular Biology, Current Protocols, Wiley, 2002; C.W. Dieffenbach et al., PCR Primer: A Laboratory Manual, Cold Spring Harbor Laboratory Press, 2003; and V. Demidov et al., DNA Amplification: Current Technologies and Applications, Taylor & Francis, 2004.

Biomarker assays that can be used are well-known in the art and include, but are not limited to, assays of A $\beta$  and tau species, neurofilaments, neurogranin, interleukins, TNF $\alpha$ , GM-CSF, and soluble Trem2 in cerebrospinal fluid (CSF), blood, serum, or tissue.

Cognitive assays that can be used are well-known in the art and include, but are not limited to, assays of spatial memory, short-term memory, long-term memory, assays of executive function, attentional tasks such as 3 and 5 choice serial reaction time tests, tests of processing speed, set shifting tests, reversal learning tasks, assays of object memory, assays of pattern recognition, assays of passive avoidance memory, assays of habituation, and assays of novel object recognition, water maze testing, fear conditioning tests, radial arm water maze testing, Y-maze testing, T-maze testing, and open field habituation tests.

Physical assessment methods of the animals that can be used are well-known in the art and include, but are not limited to, assessment of indices of frailty in a genetically modified mouse of the present disclosure compared to a control, such as an APOE3-expressing mouse or a wild-type mouse as a comparison to normal aging.

*In vivo* imaging methods that can be used are well-known in the art and include, but are not limited to, magnetic resonance imaging (MRI), computed tomography (CT) imaging, X-ray, optical imaging, and ultrasound imaging. Such imaging techniques can be used to assess one or more symptoms or signs associated with expression of human APOE4 p and mouse TREM2\*R47H p and one or more of the following: (1) a nucleic acid encoding: ABCA7\*A1541G p operably linked to a promoter, wherein the animal expresses the encoded ABCA7\*A1541G p; (2) a nucleic acid encoding: APP\* p operably linked to a promoter, wherein the animal expresses the encoded APP\* p; (3) a nucleic acid encoding: PLCG2\*M28L p operably linked to a promoter, wherein the animal expresses the encoded PLCG2\*M28L p; (4) a nucleic acid encoding: MTHFR\*A262V p operably linked to a promoter, wherein the animal expresses the encoded MTHFR\*A262V p; (5) a disrupted *Ceacam1* gene such that functional CEACAM1 is not produced in the genetically modified mouse; and (6) a disrupted *Il1rap* gene such that functional IL1RAP is not produced in the genetically modified mouse, relevant to non-familial late-onset Alzheimer's disease including, but not limited to, amyloid or tau aggregation, abnormal blood flow, pathological neuronal loss, and abnormal glucose metabolism.

Assessment of changes to one or more of: a transcriptomic profile, a proteomic profile, and a metabolic profile of a genetically modified mouse of the present disclosure compared to a

wild-type mouse and/or compared to clinical samples can be performed using methods that are well-known in the art.

Assessment of inflammation can be performed by assay of one or more biomarkers of inflammation, such as, but not limited to, IL-8, IL-11, TNF-alpha, granulocyte-macrophage colony stimulating factor (GM-CSF), TGF-beta, VEGF, monocyte chemotactic factor-1, macrophage migratory inhibitory factor, s100B, fibrinogen, and interferon gamma-inducible protein 10. Such assays can be performed on samples obtained from a genetically modified mouse of the present disclosure and a wild-type mouse, such as a sample of brain, spinal cord, blood, plasma, serum, cerebrospinal fluid or other relevant tissue or body fluid.

Morphological assessment of tissues and/or cells can include physical examination of gross anatomy and/or microscopic examination of tissues and/or cells with or without histochemical or cytochemical staining of the tissues and/or cells. Morphological assessment of tissues and/or cells can include assessment of synaptic and neuronal loss.

A method for screening for a compound for use in the treatment of Alzheimer's disease in an individual human subject having or suspected of having Alzheimer's disease is provided according to aspects of the present disclosure which include administering a compound to a genetically modified mouse of the present disclosure; and assessing an effect of the compound on amyloid and/or tau which are associated with expression of human APOE4 p and mouse TREM2\*R47H p and one or more of the following: (1) a nucleic acid encoding:

ABCA7\*A1541G p operably linked to a promoter, wherein the animal expresses the encoded ABCA7\*A1541G p; (2) a nucleic acid encoding: APP\* p operably linked to a promoter, wherein the animal expresses the encoded APP\* p; (3) a nucleic acid encoding: PLCG2\*M28L p operably linked to a promoter, wherein the animal expresses the encoded PLCG2\*M28L p; (4) a nucleic acid encoding: MTHFR\*A262V p operably linked to a promoter, wherein the animal expresses the encoded MTHFR\*A262V p; (5) a disrupted *Ceacam1* gene such that functional CEACAM1 is not produced in the genetically modified mouse; and (6) a disrupted *Il1rap* gene such that functional IL1RAP is not produced in the genetically modified mouse, relevant to non-familial late-onset Alzheimer's disease on the mouse. According to particular embodiments, assessing an effect of the compound on amyloid and/or tau includes assessing the level and/or localization of amyloid and/or tau. According to particular embodiments, assessing an effect of the compound on amyloid and/or tau includes assessing amyloid and/or tau aggregates in a

genetically modified mouse of the present disclosure, such as assessment of aggregate size, number, location or a combination of any two or more thereof.

Cerebrovascular leakage is a key aspect of non-familial late-onset Alzheimer's disease and it is a surprising and unexpected finding of the present disclosure that expression of human  
5 APOE4 p and mouse TREM2\*R47H p results in cerebrovascular leakage.

A method for screening for a compound for use in the treatment of non-familial late-onset Alzheimer's disease in an individual human subject having or suspected of having non-familial late-onset Alzheimer's disease is provided according to aspects of the present disclosure which include administering a compound to a genetically modified mouse of the present disclosure; and  
10 assessing an effect of the compound on cerebrovascular leakage which is associated with expression of human APOE4 p and mouse TREM2\*R47H p relevant to non-familial late-onset Alzheimer's disease in the mouse. Assessing an effect of the compound on cerebrovascular leakage includes, but is not limited to, assessment of cerebrovascular permeability. The term "cerebrovascular permeability" refers to the capacity of a blood vessel wall to allow for normal  
15 movement of small molecules or substances such as water, ions, certain drugs and nutrients across the blood vessel wall while acting as a barrier to movement of larger molecules and substances, such as fibrinogen, and albumin. The term "cerebrovascular leakage" refers to an abnormality of blood vessels which results in leakage of larger molecules and substances, such as fibrinogen, and albumin, through blood vessel walls. Assays for assessing cerebrovascular  
20 leakage are well-known in the art, including *in vitro* assays using cells or tissues isolated from a genetically modified mouse of the present disclosure, and *in vivo* assays. A non-limiting example of an *in vivo* assay of cerebrovascular leakage includes intravenous injection of a labeled protein (e.g. Evans Blue-labeled albumin) and assessment of appearance of the dye-labeled protein in brain tissues, see for example, Radu et al., *An in vivo Assay to Test Blood Vessel Permeability.*,  
25 *J. Vis. Exp.* 2013, (73):50062.

The putative treatment can be any treatment modality such as, but not limited to, administration of a compound. The term "compound" as used herein is not limited and encompasses small molecule chemicals and biologicals such as peptides, proteins, glycoproteins, including antibodies, for example.  
30

### Additional Embodiments

1.A genetically modified mouse characterized by one or more symptoms or signs associated with expression of human APOE4 p and mouse TREM2\*R47H p and one or more of the following: ABCA7\*A1541G p, APP\* p, PLCG2\*M28L p, lack of functional CEACAM1 or  
5 lack of functional IL1RAP, and relevant to non-familial late-onset Alzheimer's disease, the genome of the mouse comprising: 1) a DNA sequence encoding a human APOE4 protein (APOE4 p) operably linked to a promoter; and 2) a DNA sequence encoding a mouse TREM2 protein having a mutation p.R47H (TREM2\*R47H p) operably linked to a promoter, wherein the mouse expresses human APOE4 p and mouse TREM2\*R47H p; and the genome of the mouse  
10 further comprising one or more of: (i) a nucleic acid encoding: ABCA7\*A1541G p operably linked to a promoter, wherein the mouse expresses the encoded ABCA7\*A1541G p; (ii) a nucleic acid encoding: APP\* p operably linked to a promoter, wherein the mouse expresses the encoded APP\* p; (iii) a nucleic acid encoding: PLCG2\*M28L p operably linked to a promoter, wherein the mouse expresses the encoded PLCG2\*M28L p; (iv) a disrupted *Ceacam1* gene such  
15 that functional CEACAM1 is not expressed in the genetically modified mouse; and (v) a disrupted *Il1rap* gene such that functional IL1RAP is not expressed in the genetically modified mouse.

2.The genetically modified mouse of embodiment 1, wherein the APOE4 p comprises an amino acid sequence of: SEQ ID NO:1, or the APOE4 p is encoded by the complement of a  
20 nucleic acid which hybridizes to SEQ ID NO:2 under highly stringent hybridization conditions.

3.The genetically modified mouse of embodiment 1, wherein the mouse TREM2\*R47H p comprises an amino acid sequence of: SEQ ID NO:3, or the mouse TREM2\*R47H p is encoded by the complement of a nucleic acid which hybridizes to SEQ ID NO:4 under highly stringent hybridization conditions.

25 4.The genetically modified mouse of embodiment 1, wherein the ABCA7\*A1541G p comprises an amino acid sequence of: SEQ ID NO:20, or the ABCA7\*A1541G p is encoded by the complement of a nucleic acid which hybridizes to SEQ ID NO:19 under highly stringent hybridization conditions.

30 5.The genetically modified mouse of embodiment 1, wherein the APP\* p comprises an amino acid sequence of: SEQ ID NO:24, or the APP\* p is encoded by the complement of a nucleic acid which hybridizes to SEQ ID NO:23 under highly stringent hybridization conditions.

6.The genetically modified mouse of embodiment 1, wherein the PLCG2\*M28L p comprises an amino acid sequence of: SEQ ID NO:30 and/or SEQ ID NO:32, or the PLCG2\*M28L p is encoded by the complement of a nucleic acid which hybridizes to SEQ ID NO:29 and/or 31 under highly stringent hybridization conditions.

5 7.The genetically modified mouse of embodiment 1, wherein the genetically modified mouse is a mouse whose genome comprises: 1) a DNA sequence encoding human APOE4 protein (APOE4 p) operably linked to a promoter; and 2) a DNA sequence encoding mouse TREM2 protein having a mutation p.R47H (TREM2\*R47H p) operably linked to a promoter, wherein the mouse is independently heterozygous or homozygous for the DNA sequence  
10 encoding APOE4 p and independently heterozygous or homozygous for the DNA sequence encoding TREM2\*R47H p, and wherein the mouse expresses human APOE4 p and mouse TREM2\*R47H p; and the genome of said mouse further comprises one or more of: (i) a nucleic acid encoding: ABCA7\*A1541G p operably linked to a promoter, wherein the mouse is homozygous for the DNA sequence encoding ABCA7\*A1541G p and wherein the animal  
15 expresses the encoded ABCA7\*A1541G p; (ii) a nucleic acid encoding: APP\* p operably linked to a promoter, wherein the mouse is homozygous for the DNA sequence encoding APP\* p and wherein the animal expresses the encoded APP\* p; (iii) a nucleic acid encoding: PLCG2\*M28L p operably linked to a promoter, wherein the mouse is homozygous for the DNA sequence encoding PLCG2\*M28L p and wherein the animal expresses the encoded PLCG2\*M28L p; (iv)  
20 a disrupted *Ceacam1* gene, wherein the mouse is homozygous for the DNA sequence encoding the disrupted *Ceacam1* gene such that functional CEACAM1 is not expressed in the genetically modified mouse; and (v) a disrupted *Il1rap* gene, wherein the mouse is homozygous for the DNA sequence encoding the disrupted *Il1rap* gene such that functional IL1RAP is not expressed in the genetically modified mouse.

25 8.A method for screening for a compound for use in the treatment of Alzheimer's disease, comprising:

administering a compound to a genetically modified mouse of any one of embodiments 1 to 7; assessing an effect of the compound on the treatment of one or more symptoms or signs associated with expression of human APOE4 p and mouse TREM2\*R47H p and one or more of:  
30 ABCA7\*A1541G p, APP\* p, PLCG2\*M28L p, lack of functional CEACAM1, and lack of functional IL1RAP, relevant to non-familial late-onset Alzheimer's disease on the mouse.



9. The method for screening for a compound for use in the treatment of Alzheimer's disease of embodiment 8, wherein assessing an effect of the compound comprises comparing the effect of the compound on the genetically modified mouse of any one of embodiments 1 to 7 with a control.

5 10. The method for screening for a compound for use in the treatment of Alzheimer's disease of embodiment 9, wherein the control comprises: administering the compound to a mouse which does not express human APOE4 p and mouse TREM2\*R47H p and one or more of: ABCA7\*A1541G p, APP\* p, and PLCG2\*M28L p, lack of functional CEACAM1, and lack of functional IL1RAP; and assessing an effect of the compound on the mouse.

10 11. The method for screening for a compound for use in the treatment of Alzheimer's disease of embodiment 9 or 10, wherein the control comprises: administering the compound to a wild-type C57BL/6J mouse; and assessing an effect of the compound on the wild-type C57BL/6J mouse.

15 12. A genetically modified mouse characterized by one or more symptoms or signs associated with expression of human APOE4 p and mouse TREM2\*R47H p and one or more of: ABCA7\*A1541G p, APP\* p, PLCG2\*M28L p, lack of functional CEACAM1, and lack of functional IL1RAP and relevant to non-familial late-onset Alzheimer's disease substantially as described herein.

20 13. A method for screening for a compound for use in the treatment of Alzheimer's disease substantially as described herein.

Embodiments of inventive compositions and methods are illustrated in the following examples. These examples are provided for illustrative purposes and are not considered limitations on the scope of inventive compositions and methods.

25

## EXAMPLES

### **Example 1. Generation of a Genetically Modified Mouse Comprising a Genomic Sequence Encoding Human APOE4 protein**

30 The mouse *ApoE4* gene is located on chromosome 7 at 19,696,109 – 19,699,166. An *ApoE4* gene-targeting construct was made that included 4980 bp (SEQ ID NO:9) of mouse sequence, which defined the mouse 5' homology arm including exon 1 of mouse *ApoE*, 4292 bp

(SEQ ID NO:10) of human *APOE4* sequence, including human protein coding exons 2-4 of the human gene as well as an additional 1.5 kb of flanking human sequence after the 3'UTR to include any potential regulatory sequences.

Figure 1 shows a schematic diagram of the humanized *APOE4* construct (see "m/hAPOE targeting vector E4"). On Figure 1, (C C) above exon 4' shows the nucleotide (nt) present in exon 4 for arginine at p.R310 and R176, respectively, isoform E4. Isoform E2 with (T T) codes for cysteine at both C130 and C176, while E3 (T C) codes for cysteine at C130R and arginine at R176 sites.

Human *APOE4* exon 4 contains sequence that codes for the *APOE4* isoform of the gene and encodes the nucleotide sequence for arginine at R130 and R176. It is noted that *APOE4* includes an 18 amino acid signal peptide at the N-terminus of the protein such that *APOE4* including the signal peptide is 317 amino acids and the variant amino acids which differ among *APOE2*, *APOE3* and *APOE4* are at positions 130 and 176. In the mature *APOE* proteins (299 amino acids), the variant amino acids which differ among *APOE2*, *APOE3* and *APOE4* are at positions 112 and 158.

A Frt neo Frt selection cassette (FNF cassette, SEQ ID NO:11) was inserted after the human sequence followed by a Nde1 restriction site (for ease of Southern screening). The FNF cassette is followed by 5166 bp of mouse sequence (SEQ ID NO:12), the 3' homology arm. The resulting 14,438 bp synthesized construct was cloned into pBlight vector using recombineering techniques, producing a construct called mApoE\_hAPOE4\_PGKneo\_mAPOE for gene targeting in embryonic stem cells.

The *ApoE4* gene-targeting construct was introduced into cultured embryonic stem (ES) cells of a C57Bl6 mouse strain by electroporation. Homologous recombination produced loci that retained all normal mouse regulatory sequences (plus non-coding exon one) together with the human *APOE4* protein-encoding exons 2-4. Transfected ES cells were screened by Southern blot to ensure correct targeting. Three clones were identified that were correctly targeted. ES cells containing the correctly targeted locus were introduced into C57BL/6J embryos, and the resultant chimeric mice were bred with C57BL/6J mice. Offspring carrying the modified locus in the germ-line were interbred to generate the homozygous genetically modified genome. All F1 matings produced normal litter sizes with a Mendelian distribution of the locus.

### Example 2. Generation of a Genetically Modified Mouse Comprising a Genomic Sequence Encoding Mouse TREM2 protein

The *Trem2*\*R47H knock in (KI) allele was generated by pronuclear injection of Cas9 RNA (100 ng) and a single guide sequence (50 ng) GAAGCACTGGGGGAGACGCA (SEQ ID NO:7) with 183 nucleotide (nt) donor oligonucleotide (oligo) (40 ng) (GCCCTCAACACCACGGTGTCTGCAGGGCATGGCCGGCCAGTCCTTGAGGGTGTTCATG TACTTATGACGCCTTGAAGCACTGGGGGAGACCAAaGCaTGGTGTTCGGCAGCTGGG TGAGGAGGGCCCATGCCAGCGTGTGGTGTGAGCACACACGGTGTGTGGCTGCTGGCCT TCCTGAAGAAGCGG, SEQ ID NO:8) containing a nucleotide G>A point mutation (underlined uppercase “A” at nt 89 in SEQ ID NO:8) for amino acid sequence change at R47H and 2 silent mutations (lysine AAG>AAA (underlined lower case “a” at nt 93 in SEQ ID NO:8) and alanine GCC>GCA (underlined lower case “a” at nt 96 in SEQ ID NO:8)) into the gene (to prevent re-cutting of the donor sequence in homologous directed repair). The CRISPR strategy resulted in the specific R47H knock in, and silent mutations present in the *Trem2* gene of founder mice.

Offspring carrying the modified allele in the germ-line were interbred to generate the homozygous genetically modified genome. All F1 matings produced normal litter sizes with a Mendelian distribution of the locus. The resulting inbred strain of mouse is designated C57BL/6J-Trem2<sup>em1Adpnc</sup>/J (common name Trem2 R47H KI (JAX)) and expresses TREM2 protein.

### Example 3. Generation of a Genetically Modified Mouse Comprising a Genomic Sequence Encoding Human APOE4 Protein and a Genomic Sequence Encoding TREM2 Protein

In this example, a genetically modified mouse was generated by crossing an inbred strain of mouse, B6(SJL)-ApoE<sup>tm1.1(APOE\*4)Adpnc</sup>/J (common name APOE\*4 KI (JAX)) expressing human APOE4 p with an inbred strain of mouse, C57BL/6J-Trem2<sup>em1Adpnc</sup>/J (common name Trem2 R47H KI (JAX)) expressing mouse TREM2\*R47H p. The resulting genetically modified mice, homozygous for both the humanized *APOE4* allele and the R47H allele of the mouse *Trem2* gene, are designated B6(SJL)-ApoE<sup>tm1.1(APOE\*4)Adiuj</sup> Trem2<sup>em1Adiuj</sup>/J (formerly B6(SJL)-ApoE<sup>tm1.1(APOE\*4)Adpnc</sup> Trem2<sup>em1Adpnc</sup>/J, abbreviated as APOE4 X Trem2 R47H (JAX) mice),

common name: B6J.APOE4/Trem2, which express both human APOE4 and mouse TREM2\*R47H p.

#### 5 Example 4. Generation of a *Abca7*\*A1541G/*ApoE4*/*Trem2*\*R47H Mouse Model for Late-Onset Alzheimer's Disease

A mutation corresponding to single nucleotide polymorphism rs3752246 in exon 33 of human *ABCA7*, which encodes a mutation of alanine to glycine at position A1527 of human *ABCA7* was introduced into the homologous region of exon 32 of the mouse *Abca7* gene, which is A1541G in mouse *ABCA7*. This was accomplished by altering the corresponding codon from  
 10 GCT to GGT. The CRISPR project was done directly into mice on the C57BL/6J background that were homozygous for both the human APOE4 and Trem2\*R47H alleles (B6J-APOE4/Trem2\*R47H mouse which expresses both human APOE4 and mouse TREM2).

CRISPR Guides used to cut the mouse *Abca7* gene:

*Abca7*\_sgRNA1 (SEQ ID NO:33) : ACTTGACCAAGGAGCAGCTATCTGAA

15 PAM site: CTGAA

*Abca7*\_sgRNA2 (SEQ ID NO:34): GCAGCTATCTGAAGCTACACTGTGAGT

PAM site: GTGAGT

Sequence of repair donor (mutation is bolded and underlined; this is the reverse orientation, SEQ ID NO:35):

20 AAAGGTTCCAATCCAGTCTCCACCACCCCTGAGCAACATGACCACTGATAGTAAATGGATGGA  
 CTCACAGTGTACCCTTCAGATAGCTGCTCCTTGGTCAAGTTCAAAGGATGGTTGAGTGTAGTATCTGTG  
 GGCATGGCGGACTGGAC

25 SEQ ID NO:21- Wild-type mouse *ABCA7* protein sequence (site of mutation is underlined and bolded):

MALGTQLMLLLWKNYTYRRRQPIQLLVELLWPLFLFFILVAVRHSHPPLEHHECHFNPKPLPSAGT  
 VPWLQGLVCNVNNSCFQHPTPGEKPGVLSNFKDSLISRLLADTRTVLGGHSIQDMLDALGKLIPVLRVGG  
 GVRPQESDQPTSQGSVTKLLEKILQRASLDPVLGQAQDSMRKFSDAIRDLAQELLTLPSELMELRALLRRPRG  
 SAGSLELVSEALCSTKGPSSPGGLSNWYEANQLNEFMGPEVAPALPDNSLSPACSEFVGTLLDDHPVSRL  
 30 WRRLKPLILGKILFAPDTNFRKLMAQVNQTFEELALLRDLHELWGVLPQIFNFMNDSTNVAMLQRLLD  
 VGGTGQRQQTAPRAQKKLEAIKDFLDPSRGGYSWREAHADVGRLAGILGQMMECVSLDKLEAVPSEEALV  
 SRALELLGERRLWAGIVFLSPEHPLDPSELSPALSPGHLRFKIRMDIDDVTRTNKIRDKFWDPGPSADPFMD  
 LRYVWGGFVYLQDLLEQA AVRVLGGGNSRTGLYLQQMPHPCYVDDVFLRVLSRSLPLFLTLAWIYSVALT  
 VKAVVREKETRLRETRAMGLSRAVLWLGWFLSCLGPFLVSAALLVLVCLKGNILPYSHPVVIFLFLAFA  
 35 VATVAQSFLLSAFFSRANLAAACGGLAYFALYLPYVLCVAWRERLHLGGLLAASLLSPVAFGFGCESLALL  
 EEQGDGAQWHNLGTGPAEDVFSLAQVSAFLLLDAVIYGLALWYLEAVCPGQYGIPEPWNFPFRRSYWCGP  
 GPPKSSVLAPAPQDPKVLVEEPLGLVPGVSIRGLKKHFRGCPQPALQGLNLDIFYEGHITAFGLHNGAGKTT  
 TLSILSGLFPPSSGSASILGHVQTNMAAIRPHLGICPQYNVLFDMILTVEEHVWFYGRLLKGVSAAMGPERE

RLIRDVGLTLKRDTQTRHLSGGMQRKLSVAIAFVGGSRVIMDEPTAGVDPASRRGIWELLLKYREGRTL  
 LSTHHLDEAELLGDRVAMVAGGSLCCCGSPLFLRRHLGCGYYLTLVKSSQSLVTHDAKGDSEDPRREKKS  
 DGNGRTSDTAFTRGTSKSNQAPAPGAVPITPSTARILELVQQHVPGAQLVEDLPHELLLVLPYAGALDGSF  
 5 AMVFQELDQQLELLGLTGYGISDTNLEEFLKVVEDAHHREGGDSRPQLHLRRTCTPQPPTGPEASVLENGELA  
 KLVLDPQAPQGLAPNAAQVQGWTLTCQQLRALLHKRFLARRSRRGLFAQVLPALFVGLALFFSLIVPPF  
 GQYPPLQLSPAMYGPQVSFFSEDAPGDPNRMKLEALLGEAGLQEPSMQDKDARGSECTHSLACYFTVPE  
 VPPDVASILASGNWTPESPSPACQCSQPARRLLPDCPAGAGGPPPPQAVAGLGEVVQNLGTGRNVSDFLVK  
 TYPVRRGLKTKKVVDEVRYGGFSLGGRDPDLPTGHEVVRTLAEIRALLSPQPGNALDRILNNTQWAL  
 10 GLDARNLSKIWFNNKGWHAMVAFVNRANGLLHALLPSGPVRHAHSITTLNHPNLTKQLSEATLIASS  
 VDVLVSICVVFAMSFVPASFTLVLIEERITRAKHLQLVSGLPQTLYWLGWFLWDMCNVLAVCIVVFIFLAF  
 QQRAYVAPENLPALLLLLLLYGWSITPLMYPDSFFFSVPSTAYVVLTCINLFIGINSSMATFVLELLSDQNLQ  
 EVSRILKQVFLIFPHFCLGRGLIDMVRNQAMADAFERLGDQKQFSPLRWDIIGKNLLAMMAQGPFLLITLL  
 LQHRNLLPQSKPRLPLPGEEDVVAQERERTKATQGDVLRDLTKVYRGQRNPAVDRLCLGIPPGE  
 15 CFGLLVNGAGKTSTFRMVTGDTLPSSGEAVLAGHNVAQERSAAHRSMGYCPQSDAIFDLLTGREHLELF  
 ARLRGVPEAQVAQTALSGLVRLGLPSYADRPAGTYSGGNKRKLATALALVGDPAVVFLDEPTTGMDP  
 SAR RFLWNSLLSVVREGRSVVLTSHSMEECEALCTRLAIMVNGRFRCLGSSQHLKGRFGAGHTTLRVPPDQPE  
 PAIAFIRITFPGAELREVHGSRLRFQLPPGGRCTLTRVFRELAQAQGRAHGVDFSVSQTTLLEEVFLYFSK  
 DQG EEEESSRQEAEEEEVSKPGRQHPKRVSRFLEDPSVETMI

20 SEQ ID NO:20- Engineered mutant mouse ABCA7 protein sequence (ABCA7\*A1541G  
 p) (mutation is underlined and bolded):

MALGTQLMLLLWKNYTYRRRQPIQLVELLWPLFLFFILVAVRHSHPPLEHHECHFPNKPLPSAGT  
 VPWLQGLVCNVNNSCFQHPTPGEKPGVLSNFKDSLISRLADTRTVLGGHSIQDMLDALGKLPVLA  
 25 GVRPQESDQPTSQGSVTKLLEKILQRASLDVVGQAQDSMRKFSDAIRDLAQELLTLP  
 SLMELRALLRRPRG SAGSLELVSEALCSTKGPSSPGLSLNWEANQLNEFMGPEVAPALPDNSL  
 SPACSEFVGTLDHPVSRLL WRRLLKPLILGKILFAPDTNFTRKLMQVNTFEELALLRDLHEL  
 WGVLGVPQIFNFMNDSTNVAMLQRLLD VGGTGRQRPRAQKLEAIKDFLDPSRGGYS  
 WREAHADVRLAGILGQMMCEVSLDKLEAVPSEALV SRALEGGERRLWAGIVFLSPEHPLD  
 PSELSSPALSGHLRFKIRMDIDDVTRTNKIRDKFWDPGSPADPFMD LRYVWGGFVYLQD  
 30 LLEQAQAVRVLGGGNSRTGLYLQMPHPCYVDDVFLRVLRSPLFLTLAWIYSVALT  
 VKAVVREKETRLRETMRAMGLSRAVLWGWFLSCLGPFLVSAALLVLVCLKGNILPYSHPV  
 VIFLFLAFA VATVAQSFLLSAFFSRANLAAACGGLAYFALYLPYVLCVAWRERLHLGGLLA  
 AASLLSPVAFGFGCESLALL EEQGDGAQWHNLGTGPAEDVFSLAQVSAFLLLDAVIYGLAL  
 WYLEAVCPGQYGIPEPWNFPFRRSYWCGP GPPKSSVLAPAPQDPKVLVEEPLGLVPGV  
 SIRGLKKHFRGCPQPALQGLNLDIFYEGHITAFGLHNGAGKTT TLSILSGLFPPSSGSAS  
 35 ILGHVQTNMAAIRPHLGICPQYNVLFDMLTVEEHVWFYGRKGVSAAMGPERE  
 RLIRDVGLTLKRDTQTRHLSGGMQRKLSVAIAFVGGSRVIMDEPTAGVDPASRRGIWELLLKY  
 REGRTL LSTHHLDEAELLGDRVAMVAGGSLCCCGSPLFLRRHLGCGYYLTLVKSSQSLV  
 THDAKGDSEDPRREKKS DGNGRTSDTAFTRGTSKSNQAPAPGAVPITPSTARILELVQQHVP  
 GAQLVEDLPHELLLVLPYAGALDGSF AMVFQELDQQLELLGLTGYGISDTNLEEFLKVV  
 40 EDAHHREGGDSRPQLHLRRTCTPQPPTGPEASVLENGELA KLVLDPQAPQGLAPNAAQVQ  
 GWTLTCQQLRALLHKRFLARRSRRGLFAQVLPALFVGLALFFSLIVPPF GQYPPLQLSP  
 AMYGPQVSFFSEDAPGDPNRMKLEALLGEAGLQEPSMQDKDARGSECTHSLACYFTVPE  
 VPPDVASILASGNWTPESPSPACQCSQPARRLLPDCPAGAGGPPPPQAVAGLGEVVQNLGT  
 GRNVSDFLVK TYPVRRGLKTKKVVDEVRYGGFSLGGRDPDLPTGHEVVRTLAEIRALLSP  
 45 QPGNALDRILNNTQWAL GLDARNLSKIWFNNKGWHAMVAFVNRANGLLHALLPSGPVRHA  
 HSITTLNHPNLTKQLSEGTLIASS VDVLVSICVVFAMSFVPASFTLVLIEERITRAKHLQLV  
 SGLPQTLYWLGWFLWDMCNVLAVCIVVFIFLAF QQRAYVAPENLPALLLLLLLYGWSIT  
 PLMYPDSFFFSVPSTAYVVLTCINLFIGINSSMATFVLELLSDQNLQ EVSRILKQVFLIF  
 PHFCLGRGLIDMVRNQAMADAFERLGDQKQFSPLRWDIIGKNLLAMMAQGPFLLITLL  
 50 LQHRNLLPQSKPRLPLPGEEDVVAQERERTKATQGDVLRDLTKVYRGQRNPAVDRLCLGIP  
 PGE CFGLLVNGAGKTSTFRMVTGDTLPSSGEAVLAGHNVAQERSAAHRSMGYCPQSDAIF  
 DLLTGREHLELF ARLRGVPEAQVAQTALSGLVRLGLPSYADRPAGTYSGGNKRKLATALAL  
 VGDPAVVFLDEPTTGMDP SAR RFLWNSLLSVVREGRSVVLTSHSMEECEALCTRLAIMV  
 NGRFRCLGSSQHLKGRFGAGHTTLRVPPDQPE PAIAFIRITFPGAELREVHGSRLRFQLP  
 PGGGRCTLTRVFRELAQAQGRAHGVDFSVSQTTLLEEVFLYFSK DQG EEEESSRQEA  
 EEEEEVSKPGRQHPKRVSRFLEDPSVETMI

SEQ ID NO:19: An engineered mouse mutant *Abca7* DNA sequence (*Abca7*\*A1541G g) encoding engineered mutant mouse ABCA7 protein having the A1541G point mutation (*ABCA7*\*A1541G p) - the gct codon that was engineered to ggt is underlined and in bold

5 ATGGCCTTAGGCACACAGCTGATGCTTCTGCTGTGGAAAAATTACACCTATCGACGGAGACAA  
 CCGATCCAACACTACTAGTGGAGTTGCTTTGGCCCCCTCTTCCTCTTCTTCATCCTAGTGGCTGTCCGTCACT  
 CCCACCCCCCTCTGGAGCATCACGAATGCCACTTCCAAACAAGCCATTACCATCGGCGGGCACGGTG  
 CCCTGGCTGCAGGGCCTTGTCTGCAACGTAAACAACCTCTGCTTCCAGCACCCAACGCCTGGCGAGAA  
 GCCTGGGGTCTGAGTAACTTTAAGGATTCTTGATCTCGAGGCTCCTCGCTGATACCCGCACAGTGCT  
 10 CGGGGGCCACAGCATCCAGGACATGCTGGATGCCCTGGGGAAACTGATCCCCGTGCTCAGGGCAGTTG  
 GAGGTGGAGCACGACCACAGGAGAGTGACCAGCCGACCAGTCAAGGGTCAGTGAATAAGCTTCTGGA  
 GAAGATCCTGCAAAGGGCATCCCTGGATCCTGTGCTGGGTCAAGCCCAGGATTCTATGAGAAAAGTTCT  
 CAGATGCTATCAGGGATCTTGCCAGGAGCTCCTGACACTGCCAGCCTGATGGAGCTCCGAGCTTTG  
 CTGCGGAGGCCCCGAGGGTCAGCTGGTTCCTGAGCTGGTTCGAGGGCCCTCTGCAGTACCAAGGG  
 15 ACCCAGCAGTCCAGGGGGCCTGTCCCTCAATTGGTACGAAGCCAACCAGCTTAATGAGTTCATGGGGC  
 CAGAGGTGGCCCCTGCCCTGCCTGACAACAGTCTCAGCCCTGCCTGCTCTGAGTTTGTGGGGACACTG  
 GATGACCACCCTGTGTCTCGGCTGCTCTGGAGGCGCCTGAAGCCATTGATCCTCGGGAAAATTCTCTTT  
 GCACCTGACACAACTTCACTCGGAAGCTCATGGCTCAGGTGAACCAGACCTTCGAGGAGCTGGCTCT  
 GTTGAGGGACCTACACGAACTCTGGGGGGTGTGGGACCCAGATCTTCAACTTCATGAATGACAGTA  
 CCAACGTGGCCATGCTTCAGAGGCTTCTGGATGTGGGGGGCACAGGGCAGAGGCAGACACCCAG  
 20 AGCCAGAGAAGTTGGAGGCTATCAAAGACTTCTGGATCCTAGTAGGGGTGGCTACAGCTGGCGG  
 GAGGCCACGCAGATATGGGACGCCTGGCTGGAATCCTAGGACAAATGATGGAGTGTGTGTCCCTGG  
 ACAAGCTGGAGGCTGTGCCCTCAGAGGAAGCTCTTGTGTCCCCTGCCCTGGAGCTGCTGGGTGAGCGC  
 CGCTCTGGGCAGGCATCGTGTTCCTGAGCCCAGAGCATCCTCTGGACCCATCCGAACTGTCATCTCCA  
 GCCCTGAGTCTGGCCACCTACGATTCAAGATTTCGAATGGATATCGATGATGTCACAAGGACCAATAA  
 25 GATCAGGGACAAGTTTGGGACCCAGGTCCGTCAGCAGATCCTTTCATGGACCTTCGGTATGTGTGGG  
 GAGGCTTCGTGTACCTGCAGGACCTGTGGAGCAGGCAGCTGTGCGAGTGTCTCGGTGGCGGGA  
 ACTCCCGCACAGGTCTCTACCTGCAGCAGATGCCACACCCTGCTACGTGGATGATGTGTTCCTGCGGGTGT  
 GAGCCGGTCTCTGCCTCTGTTTCTGACTCTGGCCTGGATTTATTCGGTGGCGCTCACTGTGAAGGCCGT  
 30 GGTGCGTGAGAAAGAGACACGGCTGCGAGAAACCATGCGTGCATGGGGCTGAGCCGCGCGGTGCTC  
 TGGCTTGGTTGGTTCCCTCAGCTGCCTGGGACCCTTCTGGTTCAGCGCTGCGTTGCTGGTATTAGTGCTT  
 AAGCTAGGGAACATCCTTCCTTACAGCCACCCGGTTGTAATCTTCCTTTTCTTGGCGCCCTTCGCGGTG  
 GCCACCGTGCCTCAGAGTTTTCTGCTCAGCGCCTTCTTCTCCAGGGCCAACCTGGCAGCAGCCTGCGGG  
 GGGCTCGCTATTTTGCCTCTATCTGCCCTACGTAAGTGTGTGGCCTGGCGCGAGCGCCTGCACCTG  
 35 GCGGACTCTTAGCTGCGAGCCTGCTGTCCCCTGTAGCCTTTGGCTTTGGATGCGAAAGCCTGGCGCTA  
 CTAGAGGAGCAGGGAGACGGGGCTCAGTGGCACAATTTGGGCACAGGCCCCGCGGAGGACGCTTCA  
 GCCTGGCCCAGGTGTCTGCCTTCCTGTTGCTTGTGATGCCGTCATCTACGGCCTTGCCCTCTGGTACCTAG  
 AGGCTGTGTGCCAGGCCAGTATGGAATCCCTGAACCATGGAATTTCCCTTTTCGGAGGAGCTACTGG  
 TGTGGACCTGGGCCTCCCAAGAGTTCTGTCTTGGCCCCTGCCCCACAAGATCCCAAGGTTCTGGTGG  
 40 AGAGCCACCCCTTGGCCTGGTTCCCTGGTGTCTCCATTCGAGGCCTGAAGAAACATTTTCGTGGCTGTCC  
 GCAGCCAGCCCTGCAAGGACTCAACCTTGACTTCTACGAAGGCCACATCACTGCCTTTTGGGTGACA  
 ACGGGGCTGGCAAGACAACCACACTGTCCATTTGAGTGGTCTCTTCCCACCCAGTAGTGGCTCGGCC  
 TCCATCCTGGGCCATGATGTACAAACCAACATGGCAGCCATCCGGCCCCACCTGGGCATCTGCCCGCA  
 GTACAATGTGCTGTTTGTATGCTGACAGTGAAGAACATGTTTGGTTCTATGGCCGTTTGAAGGGCGT  
 45 GAGTGCAGCCGCCATGGGCCCCGAGCGGGAACGCTGTGATACGGGATGTGGGGCTTACCCTCAAGCGG  
 GACACACAGACACGCCACCTCTCTGGTGGAAATGCAGAGAAAACCTTTCTGTGGCCATTGCCTTTGTGGG  
 TGGCTCTCGTGTGGTTCATCATGGACGAGCCACTGCTGGTGTGGACCCCGCTTCCCGCCGTGGCATTG  
 GGAATTGCTACTTAAGTACAGAGAAGGTCGGACACTGATTCTCTCCACTCACACCTGGATGAGGCAG  
 AGCTCTTGGGAGATCGCGTGGCCATGGTGGCAGGTGGCTCTTTGTGCTGCTGTGGGTCCCCGCTTTTCT  
 50 TGCGCCGACACTTGGGCTGCGGTTACTACCTGACCCTGGTGAAGAGTTCTCAGTCCCTCGTACCCATG  
 ATGCTAAGGGAGACAGTGAAGACCCAGACGGGAAAAGAAGTCAGATGGCAATGGCAGGACGTCAG  
 ACACAGCGTTACACAGGAACCTCAGACAAGAGCAACCAGGCCCGCTCCTGGCGCCCTTCCCATC  
 ACCCAAGCAGACGCCGATACTAGAGCTAGTGCAGCAGCATGTGCCTGGAGACAACTCGTGGAGG  
 ACCTGCCCATGAGCTTCTGCTTGTGCTACCCTATGCGGGGGCCCTGGATGGCAGCTTCGCCATGGTCT  
 TCCAGGAGCTGGATCAGCAGCTGGAGCTCCTGGGTCTCACAGGCTACGGGATCTCGGACACCAACCTG

GAGGAGATCTTCCTAAAGGTGGTGGAGGATGCGCACAGAGAAGGTGGGGACTCTAGACCGCAGCTGC  
 ACCTTCGCACATGCACTCCACAGCCCCCACAGGGCCAGAGGCATCAGTTCTGGAGAATGGGGAGCTG  
 GCCCCACAGGGCTTGGCACCCAACGCTGCCAAGTGCAAGGCTGGACACTTACCTGTCAACAGCTCCG  
 GGCTCTGCTCCACAAGCGTTTTCTGCTTGGCTCGCCGCAGCCGCCGGGGCTGTTTGCACAGGTTGTGTT  
 5 GCCTGCCCTCTTTGTGGGCTGGCCCTGTTCTTCAGCCTCATTGTGCCTCCTTTTGGCCAGTACCCACCC  
 CTGCAGCTCAGCCCTGCTATGTATGGCCCTCAGGTCCTGTTCTTCAGTGAGGATGCCCTGGGGACCCC  
 AACCGGATGAAGCTGCTGGAGGCTCTGCTAGGGGAGGCTGGGCTGCAGGAACCCAGTATGCAGGACA  
 AAGATGCCAGGGGATCTGAGTGTACACACTCCCTAGCTTGCTACTTCACGGTCCCTGAGGTCCCTCCTG  
 10 ATGTGGCCAGCATCCTGGCCAGTGGCAACTGGACGCCAGAATCTCCATCCCCAGCTTGCCAATGCAGT  
 CAGCCTGGAGCCCGCCGCTGTTGCCAGATTGCCCGGCTGGAGCTGGGGGTCCACCACCCCCCAGGC  
 TGTGGCTGGCTTGGGGGAGGTGGTCCAGAACCTCACTGGCCGAAATGTGTCTGACTTTTGGTGAAGA  
 CATACCCCAGCCTGGTGCGCCGAGGCCTAAAGACCAAGAAGTGGGTGGATGAGGTCAGATATGGGGG  
 CTTCTCCCTGGGAGGCCGAGATCCAGACCTGCCACAGGGCATGAGGTGGTCCGCACATTGGCAGAGA  
 15 TTCGGGCACTGCTGAGCCCCAACCTGGGAATGCGCTAGACCGTATCCTGAACAACCTCACTCAGTGG  
 GCCCTTGGCCTTGATGCTCGGAACAGCCTCAAGATCTGGTTCAACAACAAGGGCTGGCATGCCATGGT  
 GGCTTTGTGAACCGAGCCAACAATGGACTCCTACATGCCCTCCTACCATCTGGTCCAGTCCGCCATGC  
 CCACAGCATCACTACACTCAACCATCCTTTGAACTTGACCAAGGAGCAGCTATCTGAAGCTACTGA  
 TAGCCTCCTCTGTGGATGTCTTGTCTCCATCTGTGTGGTCTTCGCCATGTCATTTGTCCAGCCAGCTT  
 20 TACCCTGGTCTCATAGAGGAACGCATCACCAGAGCCAAGCATCTGCAGCTGGTCAGCGGCCTGCCCC  
 AAACCTCTATTGGCTTGGCAACTTCTCTGGGACATGTGTAACACTACTTGGTGGCAGTGTGCATAGTGG  
 TGTTCTGCTGTATGGGTGGTCTATCACACACTACTGTACCCAGCCTCCTTCTTCTCTCAGTGCCAG  
 CACGGCCTATGTGGTGGTCTACCTGCATCAACCTCTTCATTGGCATCAATAGCAGCATGGCCACCTTCGT  
 GCTAGAACTGCTTTCAGATCAGAACCTGCAAGAAGTGAGCCGGATCCTGAAACAAGTGTTCCTTATTT  
 25 TCCCCACTTTTGCCTTGGCCGAGGGCTCATTGACATGGTTCGGAACCAGGCCATGGCAGATGCCTTTG  
 AGCGCTTAGGAGACAAGCAATTCAGTCACCCCTACGCTGGGACATCATTGGCAAGAACCTCCTGGCC  
 ATGATGGCCAGGGACCTCTGTTCTCTCATCACACTCCTGCTCCAACACCCGCAACCGTCTCCTGCCA  
 CAATCAAAACCAAGACTGCTGCCGCCCTGGGGGAGGAGGATGAGGATGTGGCTCAAGAGCGTGAGC  
 GGGTGACCAAGGGGGCTACCCAGGGGGATGTGCTAGTCTCAGGGACTTGACCAAGGTTTACCGTGG  
 30 GCAGAGGAACCCAGCTGTGGATCGCCTGTGCTTAGGGATCCCCCTGGGGAGTGTTCGGGCTGCTGG  
 GTGTCAACGGGGCAGGGAAGACATCCACCTTCCGCATGGTGACAGGGGACACACTGCCCAGCAGTGG  
 TGAAGCAGTACTGGCAGGCCACAACGTGGCCAGGAGCGGTCTGCCGCACACCGCAGCATGGGCTAC  
 TGTCCCAGTCTGATGCCATCTTCGACCTGCTGACCGGCCGGGAACATCTGGAACCTGTTTGTCTGCCTG  
 CGCGGGGTGCCCGAGGCCAAGTTGCCAGACTGCGCTCTTGGCCTGGTGCGCCTGGGCCTTCTAG  
 35 CTATGCAGACCGACCCGCGGTACCTACAGCGGAGGCAACAAACGGAAGCTGGCGACAGCCTTAGCT  
 CTGGTTGGTGACCCAGCTGTGGTCTTTCTGGACGAGCCCACCACAGGCATGGACCAAGTGCAGCGGCG  
 ATTTCTTTGGAACAGCTTGTGTCCGTGGTGCGCGAGGGCCGCTCCGTAGTGCTCACGTTCGCACAGCAT  
 GGAGGAGTGCGAAGCGCTCTGCACGCGCCTGGCCATCATGGTGAACGGGCGGTTCCGCTGTCTGGGAA  
 GCTCTCAGCATCTCAAAGGCAGGTTCCGGGGCTGGCCACACACTGACTCTAAGGGTCCCACCGGACCAG  
 40 CCTGAGCCGGCGATAGCCTTCATCAGGATCACATTCCTTGGGGCTGAACTCCGGGAGGTGCACGGCAG  
 CCGTCTGCGCTTCCAACCTGCCACCGGGGGGAGATGCACCCTGACACGAGTGTTCAGGGAGCTGGCTG  
 CCCAGGGCAGGGCCACGGTGTGGAGGACTTCTCTGTGAGCCAGACCACTCTGGAGGAGGTGTTCTTA  
 TATTTCTCAAAGACCAAGGGGAAGAGGAAGAGAGCAGTCGGCAGGAGGCTGAAGAAGAGGAGGTTT  
 CCAAACCTGGCCGGCAGCATCCCAAACGTGTGAGCCGATTCTGGAAGACCCAGCTCTGTGGAGACC  
 45 ATGATCTGAGCATGCCTGCCTTGGGACTGAGTGGCAAAGCTCAGACAGAGGATCTCTGTACCATACGC  
 TGGCTCCCAGAAAGCCTTGGGCTCTGGGGAAATAAAAAGAACTAGAATGAGAAAAAAAAAAAAA  
 AA

**Example 5. Generation of a Humanized APP/ApoE4/Trem2\*R47H Mouse Model**

50 CRISPR technology was used to introduce the G601R, F606Y, and R609H mutations  
 into exon 14 of the mouse *App* gene so that the mouse ABETA42 sequence matches that of  
 human ABETA42. The CRISPR project was done directly into mice on the C57BL/6J

background that were homozygous for both the human APOE4 and Trem2\*R47H alleles (B6J-APOE4/Trem2\*R47H mouse which expresses both human APOE4 and mouse TREM2). Appropriate founders were bred to the same APOE4/Trem2 background to keep these alleles homozygous.

5 The corresponding nucleotide sequences for the ABETA42 regions are shown below. Bases that differ are in bold and underlined.

Mouse ABETA42 (SEQ ID NO:36):

DAEFGHDSG**FEV**RHQKLVFFAEDVGSNKGAIIGLMVGGVVIA

Human ABETA42 (SEQ ID NO:37):

10 DAEFRHDSGYEVHHQKLVFFAEDVGSNKGAIIGLMVGGVVIA

The genomic sequence was targeted using CRISPR guides:

Guide 1 (SEQ ID NO:38): TTTGATGGCGGACTTCAAATC

Guide 2 (SEQ ID NO:39): GTGAAGATGGATGCAGAATT

The repair construct used to produce engineered *App* sequence was (reverse sequence is shown; mutated codons are underlined and mutations are in bold, SEQ ID NO:40):

15 TTCATCTGGCCAGACGCAATCTCGGGGAGAGGCAGTTTATTTTTGCCTACCAG  
TTTTTGATGATGGACTTCATATCCTGAATCATGTCGGAATTCTGCATCCATCTTCACT  
TCCGAGATCTCTTCCG

20 The corresponding nucleotide sequences for the *Abeta42* regions are shown below. Codons that differ are underlined, with engineered mutations in bold.

Mouse C57BL/6J wild-type *Abeta 42* encoding sequence (SEQ ID NO:22):

25 GATGCAGAATTCCGACATGATTCAGGATTTGAAGTCCGCCATCAAAAAGTGG  
TGTTCTTTGCTGAAGATGTGGGTTCTGAACAAAGGCGCCATCATCGGACTCATGGTGG  
GCGGCGTTGTCATAGCA

Humanized mouse *Abeta42* encoding sequence (SEQ ID NO:23): A mouse mutant *App* DNA sequence (*App* g) encoding mouse APP protein having G601R, F606Y, and R609H point mutations in exon 14 of the mouse *App* gene

30 GATGCAGAATTCCGACATGATTCAGGATATGAAGTCCATATCAAAAAGTGG  
TGTTCTTTGCTGAAGATGTGGGTTCTGAACAAAGGCGCCATCATCGGACTCATGGTGG  
GCGGCGTTGTCATAGCA

35 SEQ ID NO:24 A mouse mutant APP protein having G601R, F606Y, and R609H point mutations (APP\* p)



DAEFRHDSGYEVHHQKLVFFAEDVGSNKGAIIGLMVGGVVIA

### Example 6. Generation of the *Plcg2*\*M28L/*ApoE4*/*Trem2*\*R47H Mouse Model for Late-Onset Alzheimer's Disease

5           The *Plcg2* locus has been identified by genome-wide association studies to be related to the risk of Alzheimer's disease. Specifically, the *PLCG2* M28L point mutation is associated with increased AD risk. The M28L mutation was engineered using CRISPR technology into the human homologous region of the mouse *Plcg2* gene in mice on the C57BL/6J background that were homozygous for both the human *APOE4* and *Trem2*\*R47H alleles (B6J-  
10 *APOE4*/*Trem2*\*R47H mouse which express both human *APOE4* and mouse *TREM2*).

The mouse genomic *Plcg2* sequence was targeted using CRISPR guide (SEQ ID NO:41):GAGAGCGCTGGAGTTAGGGA

The sequence was repaired to alter the ATG to TTG to cause the methionine to leucine mutation. In addition, a silent mutation ACC->ACT was introduced into the next codon to  
15 prevent the guide from re-cutting.

Mouse C57BL/6J wild-type *Plcg2* nucleotide sequence (SEQ ID NO:25):

ATGACCACCATGGTCAACGTGGACACCCTTCCAGAATATGAGAAGAGTCAGA  
TCAAGAGAGCGCTGGAGTTAGGGACGGTGATGACCGTGTTCAACGCCCGCAAATCC  
ACCCAGAGCGGAGGACAG...

20           The repair construct used to produce engineered *Plcg2* sequence was (the amino-acid altering mutation is in bold, SEQ ID NO:28):

ATGACCACCATGGTCAACGTGGACACCCTTCCAGAATATGAGAAGAGTCAGA  
TCAAGAGAGCGCTGGAGTTAGGGACGGT**GTT**GACTGTGTTCAACGCCCGCAAATCC  
ACCCAGAGCGGAGGACAG

25           Humanized mouse *Plcg2* nucleotide sequence (SEQ ID NO:29) -a mouse mutant *PLCG2* (fragment) having an M28L point mutation:

ATGACCACCATGGTCAACGTGGACACCCTTCCAGAATATGAGAAGAGTCAGA  
TCAAGAGAGCGCTGGAGTTAGGGACGGT**GTT**GACTGTGTTCAACGCCCGCAAATCC  
30 ACCCAGAGCGGAGGACAG...

Mouse *PLGC2* amino acid sequence (fragment) (first 50 amino acids, SEQ ID NO:26)

MTTMVNVDL PEYEKSIKR ALELGTVMTV FNARKSTPER RTVQMIMETR ...

Engineered mouse PLGC2 amino acid sequence (first 50 amino acids, SEQ ID NO:30)-A mouse mutant PLCG2\*M28L p (fragment) having an M28L point mutation.

MTTMVNVDL PEYEKSQIKR ALELGTVLT VFNARKSTPER RTVQMIMETR ...

5 Human Plcg2 amino acid sequence (fragment), with non-disease-associated variant (first 50 amino acids, SEQ ID NO:27):

MSTTVNVDL AEYEKSQIKR ALELGTVM T VFSFRKSTPER RTVQVIMETR ...

10 Engineered humanized mouse PLGC2 amino acid sequence Plcg2\*M28L (complete, SEQ ID NO:32) 1265 amino acids - a mouse mutant PLCG2 having an M28L point mutation.

MTTMVNVDLPEYEKSQIKRALELGTVLT VFNARKSTPERRTVQMIMETRQVAWSKTADKIEGFL  
 DIMEIKEIRPGKNSKDFERAKAVRHKAECCTILYGTQFVLSTLSLATDSKEDAVKWL SGLKILHQEAMSAS  
 TPTMIESWLRKQIYSVDQTRRNSISLRELKTILPLVNFKVS GIKFLKDKLVEIGAOKDEL SFEQFHLFYKKLM  
 FDQKQSILDEFK KDSSV FILGNTDRPDASAVYLQDFQRFL LHEQQELWAQDLNKVRERMTKFIDDTMRETA  
 15 EPFLFVDEFLT YLFSRENSIWDEKYDAVDMQDMNPLSHY WISSHNTYLTGDQLRSESSTEAYIRCLRAG  
 CRCIELDCWDGPDGKPIIYHGWTRTTKIKFDDVVQAIRDHAFVTSSFPVILSIEEHCSVEQQRHMAKVFEV  
 LGDMLLTKPTEASADQLPSPSQLREKIIKHKKLGPKGDVDV NVEDKKDEHKPQGEL YMWDSIDQKWTRH  
 YCAIADAKLSFGDDIEQAVEE EEPVQDTPPELHFGEKWFHKKVESRTSAEKLLQEYCAETGAKDGTFLVRE  
 SETFPNDYTL SFWRSR VQHCRIRSTMENGVMKY YLTDNLTFNSIYALIQHYREAHLRCAEFELRLTDPVP  
 20 NPNPHESKPWYYDSL RGEAEDMLMRIPRDGAFLIRKREGTNSYAITFRARGKVKHCRINRDGRHFVLGTS  
 AYFESLVELVSYYEKHALYRKMRLRYPVTPPELLERYN MERDINSLYDVSRMYVDPSEINPSPMPQRTVKAL  
 YDYKAKRSDEL TFCR G ALIHNV SKEPGGW WKGDYGTRIQQYFPSNYVEDISAGDAEEME KQIIEDNPLGSL  
 CKGILDNLNTYNVVKAPQ GKNQKAFVFILEPKKQGDPPVEFATDRVEELFEWFQSIREITWKMDTKENNMK  
 YWERNQSI AIELSDLV VYCKPTSKTKDHLENPDFREIRSFVETKADSIVRQKPVDLLRYNQKGLTRVYPKGQ  
 25 RVDSSNYDPFRLWLCGSQMV ALNFQTPDKYMQMNHALFSLNGRTGYVLQPESMRSEKYDPMPLESQRKI  
 LMTLTVKVLGARHLPKLGRSIACPFVEVEICGAEYDSNKFKTTV VNDNGLSPVWAPTQEKVTFEIIDPNLA  
 FLRFVVEEDMFSDPNFLAHATYPIKGIKSGFRS VPLKNGYSEDIELASLLVFCEMRPVLESEEELYSSCRQL  
 RRRQEELNNQLFLYDTHQNLRGANRDALVKEFNVNENQLQLYQEKCNRRRLREKRVSNSRFYS

30 Nucleotide sequence encoding engineered humanized mouse Plcg2\*M28L (complete, SEQ ID NO:31)

ATGACCACCATGGTCAACGTGGACACCCTTCCAGAATATGAGAAGAGTCAGATCAAGAGAGC  
 GCTGGAGTTAGGGACGGTGTGGACCGTGTCAACGCCCGCAAATCCACCCAGAGCGGAGGACAGTA  
 CAGATGATCATGGAGACTCGGCAGGTGGCATGGAGCAAGACCGCAGACAAGATTGAAGGCTTCTCTGG  
 35 ACATCATGGAGATAAAGGAAATCCGTCCGGGGAAGAACTCCAAGGACTTTGAGCGAGCCAAGGCTGT  
 CCGCCACAAGGCAGAATGTTGCTTACCATCTCTACGGCACCCAGTTTGTCTCAGCACGCTCAGTTT  
 GGCAACGGACTCAAAGGAGGATGCGGTGAAGTGGCTCTCTGGTTTGAAGATCCTACACCAGGAAGCG  
 ATGAGTGCATCCACCCCAATGATTGAGAGTTGGCTGAGAAAACAGATTTATTAGTAGATCAAAC  
 CCGAAGAAACAGCATCAGCCTCCGGGAGCTGAAGACCATCTTGCCCTGGTCAACTTCAAAGTGAGCG  
 40 GCATCAAGTTTCTCAAGGACAAGCTGGTGGAAATCGGCGCACAGAAAGATGAGCTCAGCTTTGAACA  
 GTTCCATCTCTTATAAGAACTCATGTTTGACCAGCAAAAATCGATACTTGACGAATTCAAAAAAG  
 ACTCCTCCGTGTTTCATCTAGGAAACACAGACCGGCGGATGCCTCGGCTGTCTACCTGCAGGACTTCC  
 AAAGTTTCTTTTACATGACAGCAGAGATGTTGGCTCAGGATCTGAACAAAGTCCGGAGCGGATG  
 ACCAAGTTTATCGACGACACAATGAGAGAGACCGCAGAGCCCTTCTTATTGTGGATGAGTTCTCTAC  
 45 GTATCTGTTCTCCCGGAGAACAGCATCTGGGACGAGAAGTACGATGCAGTGGACATGCAGGACATG  
 AACAAACCCCTGTCCACTACTGGATCTCTCTTCCACAACACGTACCTCACTGGGGACCAGCTGCGT

AGTGAGTCCTCCACGGAAGCGTATATCCGCTGTCTGCGCGCTGGTTGCCGCTGCATTGAGCTGGACTG  
 CTGGGACGGCCCTGATGGGAAGCCATTATCTACCACGGCTGGACACGGACCACCAAGATCAAGTTTG  
 ATGATGTTGTTCAAGCCATCCGAGACCACGCCCTTTGTTACCTCCAGCTTCCCCGTATCTTGTCTATCG  
 5 AGGAGCACTGCAGTGTGGAGCAGCAACGTACATGGCCAAGGTGTTCAAGGAAGTGTAGGAGACAT  
 GCTGTTGACGAAGCCCACAGAGGCCAGTGTGACCAGCTGCCCTCGCCCAGTCAGCTTCGTGAGAAGA  
 TCATTATCAAGCATAAGAAGCTGGGTCCCAAGGGTGTATGTCGACGTCAACGTGGAGGACAAGAAAGA  
 TGAGCACAAGCCCCAGGGCGAGCTGTATATGTGGGACTCCATCGACCAGAAATGGACGCGCCACTACT  
 GTGCCATTGCTGACGCCAAGCTGTCTTTCGGTGTATGACATTGAACAGGCTGTGGAGGAGGAGCCGGTC  
 10 CAGGACACTCCCCCACGGAGCTGCATTTTGGGGAGAAATGGTTCACAAGAAGGTGGAGAGTAGGA  
 CCAGTGC GGAGAAGCTGCTGCAAGAGTACTGTGCTGAGACCGGGGCCAAGGACGGCACCTTCCTGGT  
 GCGGGAGAGCGAGACCTTCCCCAATGACTACACACTCTCTTCTGGCGGTCTGGCCGGGTGCAGCACT  
 GCCGGATCCGGTCTACTATGGAGAATGGGGTCAATGAAGTACTACCTGACCGACAACCTCACGTTCAAC  
 AGCATCTACGCCCTCATCCAGCACTACCGTGAGGACACACCTGCGCTGCGCGGAGTTCGAGCTGCGGCT  
 15 CACAGACCCGGTGCCCAACCCCAACCCACACGAGTCCAAGCCGTGGTACTATGACAGTCTGAGCAGG  
 GGGGAAGCGGAGGACATGCTGATGCGGATCCCCGAGATGGAGCCTTCCTCATCCGGAAGAGGGAGG  
 GGACCAACTCTACGCCATCACCTTCAGGGCCAGGGGCAAGGTGAAACATTGTGCGATCAACCGGGAC  
 GGACGCCACTTCGTGCTGGGCACCTCTGCCTACTTTGAGAGCCTGGTGGAGCTTGTGAGCTACTACGA  
 GAAGCACGCGCTCTATCGCAAGATGAGGCTTCGATACCCCGTGACCCCGGAGCTCCTGGAGCGATATA  
 20 ATATGGAAAGAGACATCAACTCTCTTTATGACGTCAGCAGGATGTATGTGGACCCAAGTGAGATCAAC  
 CCTTCCATGCCTCAGAGGACCGTGAAAGCGCTCTATGACTACAAAGCCAAGCGCAGCGACGAGCTGAC  
 CTTCTGCGCGGTGCCCTCATCCACAATGTCTCAAGGAGCCCGGGGATGGTGGAAAGGGGACTACG  
 GGACCCGGATCCAGCAGTACTTCCCGTCCAACATGTCTGAGGATATCTCGGCAGGCGATGCTGAGGAG  
 ATGGAAAAGCAGATTATTGAAGACAATCCCCTCGGGTCTCTCTGCAAAGGCATATTGGATCTTAATAC  
 25 CTACAACGTTGTGAAGGCCCCCCAGGGGAAAACCAGAAAGCCTTCGTCTTTATCCTGGAGCCTAAGA  
 AACAGGGAGATCCTCCCGTGGAGTTCGCGACCGACCGTGTGGAGGAGCTGTTTGTGAGTGGTTTCAGAGC  
 ATACGGGAGATCACTTGGAAAGATGGACACCAAGGAGAACAACATGAAGTACTGGGAGAGGAACCAAGT  
 CCATCGCCATTGAGCTCTCGGACTTGGTTGTATACTGCAAGCCAACTAGCAAAAACCAAGGACCATTTG  
 GAAAATCCCGACTTCCGAGAAATCCGCTCTTTTGTGGAGACGAAGGCAGACAGCATTGTGAGGCAAAA  
 ACCTGTGCGATCTATTGCGATAACAATCAGAAGGGCCTGACCCGAGTCTACCCCAAGGGACAGAGAGTTG  
 30 ACTCTTCGAACTATGACCCCTTCCGTCTGTGGCTGTGCGGCTCCCAGATGGTGGCACTCAATTTCCAGA  
 CTCCAGACAAATACATGCAGATGAACCACGCGCTGTTTTCGCTCAATGGGCGAACAGGCTACGTCCTG  
 CAGCCTGAGAGCATGCGCTCTGAGAAGTATGATCCGATGCCCTGGAGTCCCAGAGGAAGATCCTGAT  
 GACTACTGTCAAGGTTCTTGGTGCACGCCACCTCCCTAAACTAGGGCGGAGTATTGCCTGTCTTTT  
 TGTGGAGGTGGAAATCTGCGGGGCTGAGTATGACAGCAACAAATTCAAGACGACGGTTGTGAATGAC  
 35 AATGGCCTCAGCCCTGTCTGGGCTCCAACCTCAGGAGAAGGTGACATTTGAGATCTATGATCCAAACCT  
 TGCTTCCCTACGCTTTGTGGTCTACGAAGAAGACATGTTCAAGTATCCCAACTTCCTGGCTCATGCCAC  
 GTACCCCATTAAGGCATCAAATCCGGGTTTAGATCAGTCCCTCTGAAGAATGGGTACAGTGAAGACA  
 TCGAGCTGGCATCCCTCCTGGTTTTCTGTGAGATGAGGCCAGTCCCTGGAGAGTGAAGAGGAACTCTAT  
 TCCTCCTGTGCGCAACTGCGGAGGCGGCAAGAAGAGCTCAACAACCAGCTCTTCCTTTACGACACGCA  
 40 CCAGAACCTGCGCGGAGCCAACCGTGATGCCCTGGTGAAGGAGTTCAATGTAAACGAGAATCAGCTGC  
 AGCTGTACCAGGAGAAGTGCAACCGGAGGCTGAGAGAGAAGAGAGTAAGCAACAGCAGGTTCTACTC  
 CTAGAAGCCGTGTGTGCGTGCGCGCATGCGCACGGTGTTCACGAGCGCATCTGGGTACGGGAGAGCCT  
 TATAAACAGCCCCAGGAAGAGATTCATCTGTGACATTCATCGTCTCTCTCCGAGCCACTCACCATAG  
 AGATGACAGTTCTGAAGACGCCCCGTGGGCAGGAGGCGTTGAGACCTTCCTGCTATTTTTCCACCTTG  
 45 GACAACCTTTCTTAACTTATACTACCCCTGATAGAGGATCCCCCAACTGTGTGCCTTTTGAACCTCCCT  
 GTTCTCAGCCTCATTGAATAAAGACAACAGTTTCATGCCGTTAA

### Example 7. Generation of the *Ceacam1* KO/ApoE4/Trem2\**R47H* Mouse Model for Late-Onset Alzheimer's Disease

50 Expression of the mouse *Ceacam1* gene in the B6J-APOE4/Trem2\**R47H* mouse (which expresses both human APOE4 and mouse TREM2) was knocked out using CRISPR technology so that no functional CEACAM1 was expressed in the genetically modified mouse.

The *Ceacam1* genomic sequence was targeted using two CRISPR guides on either side of exon 1:

Upstream guide 1 (SEQ ID NO:42): GGAGCTTTGTCTCCTCCCAG

Upstream guide 2 (SEQ ID NO:43): AAGGAGCTTTGTCTCCTCCC

5 Downstream guide 1 (SEQ ID NO:44): CCTTTAAAGGCGACAGCTAA

Downstream guide 2 (SEQ ID NO:45): GCCGTTTTACACATAGAACT

CRISPR founders were analyzed by PCR across the targeted region using a PCR primer on either side of the targeted region.

Forward primer (SEQ ID NO:46): CCTTGCTGCTCGGAGTATGT

10 Reverse primer (SEQ ID NO:47): CTGACCCCCTCCTCTAAAGA

Founders exhibiting a deletion were bred to the B6J-APOE4/Trem2\**R47H* strain, and progeny screened by PCR as above. A single founder line with a 601bp deletion was selected and sequenced across the region. This has continued to be bred to the B6J-APOE4/Trem2\**R47H* strain and was given the JAX unique ID 30673 and official strain nomenclature of B6(SJL)-

15 *Apoe*<sup>tm1.1(APOE\*4)Adpnc</sup> *Ceacam1*<sup>em#1Adpnc</sup> *Trem2*<sup>em1Adpnc</sup>/J.

Mouse C57BL/6J wild-type *Ceacam1* sequence, with 601 bp deleted region shown in small letters (SEQ ID NO:48):

20 TGTCAGACTCACAGGCTCCACCCCCAGCCCACGTGGATCTGGGAGGTGCCCT  
 CCCCTG/ggaggagacaaagctccttaagaaaagcagggcagatcagggcagcctggcttagcagtagtggtggagaagaagct  
 agcaggcaggcagcagagacatggagctggcctcagcacatctccacaaggcaggtccctggggaggactactgctcacaggtaa  
 ggagatattcctcccagtagagagcaggggagctcagagactggctgggctctctgggagaggaaaggaacctgagaaggacatct  
 ggcttctgctgaagcttgacggcaacaggaaagctctcagtgagtgtaattggctagtggtaggagtaactcagttcttctattgtaagt  
 ttatcgactggctaggatctgaagactgaagtcacaactctgcaggggtgactctccacgtaaaaaccaaactgggcaagtaaac  
 gaattattgcatactactgagaaattgcaatacactaggctctgggacactgattgcaaacaggtatatgagcatgtccaggcagcag  
 25 ggattaagccaaaaccacctagccctgcacacaaagccctagtctatgtgtaaacggcag/ACCTGCGAGGGCATCTA  
 GGTGGGGTTCAGGTTGGCAAGCACTTC

### Example 8. Generation of the *Illrap* KO/ApoE4/Trem2\**R47H* Mouse Model for Late-Onset Alzheimer's Disease

30 Expression of the mouse *Illrap* gene was knocked out in a B6J-APOE4/Trem2\**R47H* mouse (which expresses both human APOE4 and TREM2\**R47H* p) using CRISPR technology.

The *Illrap* genomic sequence was targeted using CRISPR guides on either side of exon 2, which is the first coding region:

Guide 1 (SEQ ID NO:20): TTTGATGGCGGACTTCAAATC

Guide 2 (SEQ ID NO:21): GTGAAGATGGATGCAGAATT

Founders exhibiting a deletion were bred to the B6J-APOE4/Trem2\*<sup>R47H</sup> strain, and progeny screened by PCR as above. A single founder line with a 387bp deletion was selected and sequenced across the region. This has continued to be bred to the B6J-APOE4/Trem2\*<sup>R47H</sup> strain and was given the JAX unique ID 30304 and official strain nomenclature of B6(SJL)-Apoe<sup>tm1.1(APOE\*4)Adpnc</sup> Il1rap<sup>em#1Adpnc</sup> Trem2<sup>em1Adpnc</sup>/J.

Mouse C57BL/6J wild-type *Il1rap* sequence, with 387 bp deleted region in *Il1rap* shown in small letters (SEQ ID NO:49):

CAAATTTGTTGTTGGTTCTGACAATAAAGCTTGGGAATATT/ctaaaacccttgaatggtt  
 10 cttagtctatagttatcacacattcacggtgattattatagaactcttcagggagaagtaccctcttacttttaaatftatgttgacaggatgg  
 gacttctgtggtatttgatgagtctgtccttctatgggatcctgcagagtcatgctcgggtgagtatgtggtgctggataaatgactgaaattaat  
 ttaacatacaggatcctatatagtgaaatttaaatgagcataatgttggtttgtccacagaattctgtagttgcatagaatcaagtgtctgaaaga  
 accatagaattaggggatgagagaaactagacaacttaggaaggggtctgatgagtataaacacaggacatct/CTTCAACTTC  
 15 ATGCCCCACAGGCTAATTCTCTGTATTCTCATCTCAAGTACTGTGGAGGAAATGGCA  
 GGGAGATT

### Example 9. Validation of Genetically Modified Mouse Model of Non-Familial Late-Onset Alzheimer's Disease

#### 20 Tissue Harvesting, Protein Isolation and Sectioning

Mice were administered a lethal dose of Ketamine/Xylazine by intraperitoneal injection, and transcardially perfused with 1xPBS (phosphate buffered saline). Brains were dissected, and the right hemisphere was snap frozen for protein isolation, while the left hemisphere was fixed in 4% paraformaldehyde overnight at 4°C. The fixed hemispheres were rinsed with 1xPBS,  
 25 cryoprotected in 10% sucrose, followed by 30% sucrose at 4°C, and finally embedded in OCT (optimal cutting temperature compound). Frozen brains were sectioned at 25 μm and stored at -80°C until required. Protein was extracted with Trizol Reagent (Life Technologies, Cat#15596-018) following manufacturer's guidelines. Protein pellets were resuspended in a solution of 1:1 8M urea and 1% SDS.

30

#### Immunofluorescence, Thioflavin S Staining, and Image Capture

Cryosections were rinsed with PBT (1xPBS with 1% TritonX-100) for 5 minutes (mins) then incubated with 500 μL of Liberate Antibody Binding Solution (L.A.B. -Polysciences Inc.) solution for 20 minutes at room temperature (RT) for antigen retrieval. Slides were then

incubated overnight at 4°C in the following primary antibodies: rabbit polyclonal anti-GFAP (1:200, Dako); rabbit polyclonal anti-IBA1 (1:250, Wako); rabbit polyclonal anti-NeuN (1:100, Cell Signaling Inc); mouse monoclonal anti-non-phosphorylated neurofilament (1:200, Covance) and sheep polyclonal anti-TREM2 (1:200, RD Systems). The sheep polyclonal anti-TREM2  
5 antibody was previously verified using Trem2 deficient mice. All antibodies were diluted in PBTB (1xPBS, 1% TritonX-100 and 1% BSA) containing 10% normal goat or donkey serum. After primary incubation, sections were washed 3 times in PBT and incubated with appropriate secondary antibodies (goat anti-rabbit Alexa Fluor 488/594/633, goat anti-mouse Alexa Fluor 488, donkey anti-sheep Alexa Fluor 594, 1:1000 dilution, Life Technologies) for 2 hrs at RT.

10 All sections were then counterstained with DAPI and mounted with Aqua PolyMount (Polysciences). For Thioflavin S staining, sections stained with IBA1 and GFAP were further counterstained with 1% Thioflavin S (diluted in a 1:1 water:ethanol ratio). Slides were incubated for 8 mins at RT in 1% Thioflavin-S, washed in 80% ethanol, then 95% ethanol and finally in dH<sub>2</sub>O and mounted. Images were taken using either the Leica SP5 confocal microscope or the  
15 Zeiss Axio Imager.Z2. For each antibody, all images were captured using identical parameters for accurate quantification.

Initial observations were performed in sections from both males and females. Quantification of cell numbers was performed on brain sections from at least 4–6 male mice, as there was no overt difference between sexes. For plaque counts, the number of plaques present in  
20 the entorhinal cortical region for each mouse was determined. For IBA1+ cells, 5 equally spaced images were captured (using 20× optical lens) of either the cortex, in the region of the entorhinal cortex, or the hippocampus, from a central brain section of each mouse. For NeuN+ cells, 5 equally spaced images were captured (using 20× optical lens). For IBA1+ cells associated with plaques, images of 8+ plaques per brain were imaged (using 20× optical lens).

25 Images were processed and all cells in the 20× image were counted using the cell counter plugin for ImageJ/FIJI. A single cell was determined as a DAPI stained nucleus associated with a cell specific antibody stain (e.g. IBA1 or NEUN). Cell numbers in the 5 images from each mouse were totaled and then averaged across mice. Mouse number and diet were masked to the investigator for all cell counting assays.

30 Western blot analysis demonstrates expression of human APOE4 in B6(SJL)-  
*ApoE*<sup>tm1.1(APOE\*4)Adiuj</sup> *Trem2*<sup>em1Adiuj</sup>/J (common name: B6J.APOE4/Trem2) mice

Brain extracts (~25 micrograms protein) were diluted in 2x Laemmli sample buffer (BioRad 1610737) and run on a 4-20% gradient (BioRad mini-protean TGX 456-1096) and transferred to a nitrocellulose membrane (InVitrogen IB301001).

Blots were blocked with 5% non-fat milk powder and probed overnight at +4°C with a human APOE4-specific antibody (Novus Biologicals NBP1-49529) at 1:100 dilution. Blots were then probed with secondary antibody (Millipore goat anti-mouse HRP AP191P) at 1:30,000 dilution for 2 hours at room temperature. Blots were detected using ECL chemiluminescent reagents (GE Healthcare RPN 2109) on Amersham Hyperfilm ECL (GE Healthcare 28906838).

An antibody that is specific for the human APOE4 variant (Novus Biologicals NBP1-49529 at 1:100 dilution) was used to probe Western blots of about 25 micrograms of protein from C57BL/6J (abbreviated B6J) or B6J.APOE4/Trem2 brain tissue. Figure 2 is an image of a Western blot of brain tissue from B6J.APOE4/Trem2 (lanes 4-6) and control (WT (B6J); lanes 1-3) brain tissue. As shown in Figure 2, human APOE4 protein expression was detected only in the protein from B6J.APOE4/Trem2 mice.

15

#### **Blood chemistry shows altered metabolism in the B6J.APOE4/Trem2 mice**

APOE variants have been shown to differentially regulate lipoprotein and cholesterol metabolism. Although the mechanisms by which the APOE4 variant increases the risk for Alzheimer's disease is not known, these effects on altered metabolism are thought to play a role. Blood samples obtained from 12 month old B6J.APOE4/Trem2 (abbreviated APOE4/Trem2 in Figures 3A, 3B and 3C) mice and 12 month old C57BL/6J control mice were assayed for high density lipoprotein (HDL), low density lipoprotein (LDL) and total cholesterol according to standard blood chemistry methods.

Blood, extracted at harvest, was stored in EDTA coated vials on ice and further centrifuged at 5000 RPM for 15 mins. The plasma was removed and aliquoted for long term storage at -80 °C. Plasma aliquots were profiled for the following: Total cholesterol (mg/dL), LDL (mg/dL), HDL (mg/dL)). High density lipoprotein cholesterol (HDL) was assayed using the Wako L-Type HDL-C kit (Wako 99100101) as per manufacturer's instructions. LDL was assayed using Beckman Coulter LDL-Cholesterol kit (Beckman Coulter OSR6196) as per manufacturer's instructions. Total cholesterol was assayed using the Beckman Coulter Cholesterol kit (Beckman Coulter OSR6116).

30

Results are shown in Figures 3A, 3B, and 3C, respectively. These data show that the B6J.APOE4/Trem2 mouse has APOE4-dependent alterations in cholesterol metabolism, including reduced total cholesterol and reduced levels of both LDL and HDL.

5           **Immunohistochemistry shows cerebrovascular leakage and inflammation in the B6J.APOE4/Trem2 mice**

All mice were bred and housed in a 12/12 hours light/dark cycle. Eight month old mice were injected intraperitoneally with a lethal quantity of ketamine/xylazine according to IACUC approved procedures. Mice were perfused with 1X PBS (phosphate buffered saline) and whole  
10 brains were removed. One hemisphere was fixed in 4% paraformaldehyde overnight at +4°C. Following fixation, the tissue was rinsed in 1X PBS, incubated in 10% sucrose overnight at +4°C, then incubated in 30% sucrose overnight at 4°C. Brains were then frozen in optimal cutting temperature (OCT) compound and stored at -80°C until sectioned. Frozen brains were sectioned at 25 microns and mounted on glass slides, and stored at -80°C until required for  
15 immunofluorescence staining.

Slides were dried and post fixed in 4% PFA. Sections were subsequently immersed in diH<sub>2</sub>O for 3mins at 37°C, then transferred and incubated in 0.5mg/mL pepsin in 0.2N HCl for 15mins at 37°C. Sections were further washed in diH<sub>2</sub>O for 3mins, transferred to a humidified chamber, and washed in 1xPBS. Sections were incubated overnight in Rabbit anti-fibrin (1:200)  
20 and Goat anti-ColIV (anti-collagen IV) (1:40) at +4°C in 1xPBT. Slides were washed in 1xPBT and incubated in the appropriate secondary antibodies at a concentration of 1:1000 (Donkey anti-rabbit IgG 594, Donkey anti-goat 488) for 2 hours at room temperature. Tissue was counterstained with DAPI and mounted using Aqua-Poly mount (Polyscience Inc). Staining was imaged on the Zeiss AxioImager at a magnification of 20x.

25           Sagittal brain sections of B6J.APOE4/Trem2 mice were immunostained for fibrinogen (fibrin) to detect cerebrovascular leakage. Representative images of B6J.APOE4/Trem2 tissue at 7-8 months of age are shown in Figure 4 along with representative images from similar sagittal sections of brain from control C57BL/6J (abbreviated B6J) mice. Arrows indicate cerebrovascular leakage as indicated by fibrin immunostaining outside of blood vessels. Blood  
30 vessels are shown by immunostaining for collagen IV (Col IV).



Vascular damage may be a key aspect of late-onset Alzheimer's disease pathology. Some existing mouse models of familial Alzheimer's disease have demonstrated cerebral amyloid angiopathy (CAA), but B6J.APOE4/Trem2 mice are the first model that does not over-express familial Alzheimer's disease mutations to show vascular deficits.

5

### **Transcriptional analysis demonstrates AD-relevant changes in gene expression in the B6J.APOE4/Trem2 model**

Gene expression analysis was carried out with the NanoString Neuropathology gene panel. This assay measures the expression of 770 neuropathology-related genes. Female mice around eight months of age were assayed for the B6J.APOE4/Trem2 strain and the C57BL/6J (abbreviated B6J) strain, with three biological replicates of each strain.

Total RNA was extracted from brain homogenates. Tissues were lysed and homogenized in TRIzol Reagent (Ambion), then RNA was isolated using the miRNeasy Mini kit (Qiagen), according to manufacturers' protocols, including the optional DNase digest step. Sample concentration and quality were assessed using the Nanodrop 2000 spectrophotometer (Thermo Scientific) and the RNA 6000 Nano LabChip assay (Agilent Technologies).

RNA was hybridized and multiplexed with NanoString probes according to manufacturer's instructions. Counts for target genes were normalized to housekeeping genes to account for variability in RNA content. Data were analyzed using Nanostring nSolver Analysis Software.

Expression of each gene in the B6J.APOE4/Trem2 strain was compared to the control C57BL/6J strain and genes with significant ( $p < 0.05$ ) expression were identified with a linear regression model as shown in Figure 5. Figure 5 is a gene expression heatmap of differentially-expressed genes in B6J.APOE4/Trem2 compared to C57BL/6J. Expression values are in Log<sub>2</sub> fold change relative to the C57BL/6J average of each gene.

The probe used in this panel detects mouse Apoe but not human APOE. Because in this model mouse Apoe has been replaced by the human APOE4 variant, no Apoe expression is detected and this is shown as the most strongly down-regulated transcript.

The list of differentially-expressed genes contains multiple genes related to brain function and neurodegeneration. The Gene Ontology Biological Process and Panther Pathway annotations database was queried to identify processes and pathways represented by these genes. The

relevant processes include, but are not limited to, the results in Table I which shows pathways and processes represented by the differentially-expressed genes in the B6J.APOE4/Trem2 strain. Notably, these interlinked processes and pathways include many highly relevant to neurodegeneration.

<b>TABLE I</b>	
Process or Pathway	Genes
Alzheimer's disease-amyloid secretase pathway	Pcsk2, Mapk10, Mapk9, Prkcq
5HT2 type receptor mediated signaling pathway	Slc18a2, Plcb2, Slc6a4, Gng2, Prkcq
Inflammation mediated by chemokine and cytokine signaling pathway	Akt3, Gnao1, Plcb3, Arrb2, Il6, Myh10, Gng2
Modulation of chemical synaptic transmission	Cnr1, Stx1a, Arrb2, Unc13a, Cdk5, Calb1, Slc6a4, Gria4
Neuron development	Cnr1, Thy1, Mapk10, Hcn1, Cdk5, Chl1, Il6, Mapk9, Myh10, Uchl1
Cognition	Cnr1, Amph, Cdk5, Calb1, Chl1, Slc6a4, Chmp2b
Apoptosis signaling	Akt3, Mapk10, Mapk9, Casp7, Prkcq
Aging	Cnr1, Slc18a2, Gnao1

5

Scientific and technical terms used herein are intended to have the meanings commonly understood by those of ordinary skill in the art. Such terms are found defined and used in context in various standard references illustratively including J. Sambrook and D.W. Russell, *Molecular Cloning: A Laboratory Manual*, Cold Spring Harbor Laboratory Press; 3rd Ed., 2001; F.M. Ausubel, Ed., *Short Protocols in Molecular Biology*, Current Protocols; 5th Ed., 2002; B. Alberts et al., *Molecular Biology of the Cell*, 4th Ed., Garland, 2002; D.L. Nelson and M.M. Cox, *Lehninger Principles of Biochemistry*, 4th Ed., W.H. Freeman & Company, 2004; Herdewijn, P. (Ed.), *Oligonucleotide Synthesis: Methods and Applications*, Methods in Molecular Biology, Humana Press, 2004; A. Nagy, M. Gertsenstein, K. Vintersten, R. Behringer (Eds) 2002, *Manipulating the Mouse Embryo: A Laboratory Manual*, 3<sup>rd</sup> edition, Cold Spring Harbor Laboratory Press, ISBN-10: 0879695919; and K. Turksen (Ed.), *Embryonic stem cells: methods*

15

and protocols in *Methods in Molecular Biology*, 2002; 185, Humana Press; *Current Protocols in Stem Cell Biology*, ISBN: 9780470151808.

**Example 10. Generation of Mthfr\**C677T*/APOE4/Trem2\**R47H***

5 This triple mutant line was generated by utilizing CRISPR/Cas9 endonuclease mediated genome editing of the *Mthfr* gene in B6(SJL)-*ApoE*<sup>tm1.1(APOE\*4)Adiuj</sup> Trem2<sup>em1Adiuj</sup>/J mice. For the *Mthfr*<sup>em1Adiuj</sup> allele: plasmids encoding a single guide RNA designed to introduce a R47H point mutation into the *Mthfr* gene and the Cas9 nuclease were introduced into the cytoplasm B6(SJL)-*ApoE*<sup>tm1.1(APOE\*4)Adiuj</sup> Trem2<sup>em1Adiuj</sup>/J (Stock No. 028709)-derived fertilized eggs with well

10 recognized pronuclei. Correctly targeted embryos were transferred to pseudopregnant females. Correctly targeted pups were identified by sequencing and PCR and further bred to B6(SJL)-*ApoE*<sup>tm1.1(APOE\*4)Adiuj</sup> Trem2<sup>em1Adiuj</sup>/J (Stock No. 028709) for 2 generations to develop the colony.

**FURTHER SEQUENCES**

15 The amino acid sequence of APOE4 p is shown along with exemplary nucleic acid sequences encoding APOE4 p.

SEQ ID NO:1: APOE4 p (317 amino acids)

20 MKVLWAALLVTFLAGCQAKVEQAVETEPEPELRRQQTEWQSGQRWELALGRFWDYLRWVQTLSE  
 QVQEELLSSQVTQELRALMDETMKELKAYKSELEEQLTPVAEETRARLSKELQAAQARLGADMEDVRGR  
 LVQYRGEVQAMLGQSTEELRVR LASHLRKLRKLLRDADDLQKRLAVYQAGAREGAERGLSAIRERLGP  
 LVEQGRVRAATVGLAGQPLQERAQAWGERLRARMEEMGSRTRDRLDEVKEQVAEVRAKLEEQAQQIRL  
 QAEAFQARLKSWEPLVEDMQRQWAGLVEKVQAAVGTSAAPVPSDNH

25 SEQ ID NO:2: Exons 2, 3 and 4 of the human *APOE4* genomic DNA sequence encoding human APOE4 p including the 18 amino acid signal peptide

30 ACTGGCCAATCACAGGCAGGAAGATGAAGGTTCTGTGGGCTGCGTTGCTGGTCACATTCCTGG  
 CAGGTATGGGGCGGGGCTTGCTCGGTTCCCCCGCTCCTCCCCCTCATCCTCACCTCAACCTCCTG  
 GCCCATTCAGGCAGACCCTGGGCCCCCTTCTGAGGCTTCTGTGCTGCTTCCTGGCTCTGAACAGCG  
 ATTTGACGCTCTCTGGGCTCGGTTTCCCCATCCTTGAGATAGGAGTTAGAAGTTGTTTTGTTGTTGTT  
 GTTTGTGTTGTTGTTTGTGTTTTTTGAGATGAAGTCTCGCTCTGTGCGCCAGGCTGGAGTGCAGTGGCG  
 GGATCTCGGCTCACTGCAAGCTCCGCCTCCAGGTCCACGCCATTCTCCTGCCTCAGCCTCCCAAGTAG  
 CTGGGACTACAGGCACATGCCACCACCCGACTAACTTTTTTGTATTTTCAGTAGAGACGGGGTTTCA  
 CCATGTTGGCCAGGCTGGTCTGGAACCTGACCTCAGGTGATCTGCCCGTTTCGATCTCCCAAAGTGC  
 TGGGATTACAGGCGTGAGCCACCGCACCTGGCTGGGAGTTAGAGGTTTCTAATGCATTGCAGGCAGAT  
 35 AGTGAATAACCAGACACGGGGCAGCTGTGATCTTTATTCTCCATCACCCACACAGCCCTGCCTGGGG  
 CACACAAGGACACTCAATACATGCTTTTCCGCTGGGCGCGGTGGCTCACCCCTGTAATCCCAGCACTTT  
 GGGAGGCCAAGGTGGGAGGATCACTTGAGCCCAGGAGTTCAACACCAGCCTGGGCAACATAGTGAGA  
 CCCTGTCTCTACTAAAAATAAAAAATTAGCCAGGCATGGTGCCACACACCTGTGCTCTCAGCTACTC  
 40 AGGAGGCTGAGGCAGGAGGATCGCTTGAGCCCAGAAGGTCAAGGTTGCAGTGAACCATGTTTCAGGCC  
 GCTGCACTCCAGCCTGGGTGACAGAGCAAGACCCTGTTTATAAATACATAATGCTTTCCAAGTGATTA

AACCGACTCCCCCTCACCCCTGCCACCATGGCTCCAAAGAAGCATTGTGGAGCACCTTCTGTGTGCC  
 CCTAGGTA TAGATGCCTGGACGGGGT CAGAAGGACCCTGACCCACCTTGAACCTTGTTCACACAGGA  
 TGCCAGGCCAAGGTGGAGCAAGCGGTGGAGACAGAGCCGGAGCCCGAGCTGCGCCAGCAGACCCGAGT  
 5 GGCAGAGCGGCCAGCGCTGGGAACTGGCACTGGGTTCGCTTTTGGGATTACCTGCGCTGGGTGCAGACA  
 CTGTCTGAGCAGGTGCAGGAGGAGCTGCTCAGCTCCCAGGTACCCAGGAACTGAGGTGAGTGTCCCC  
 ATCTTGGCCCTTGACCCTCCTGGTGGGCGGCTATACCTCCCCAGGTCCAGGTTTCATTTCTGCCCTGTC  
 GCTAAGTCTTGGGGGGCTGGGTCTCTGCTGGTTCTAGCTTCTCTTCCCATTTCTGACTCCTGGCTTTA  
 GCTCTCTGGATATCTCTCTCTCAGCTTTGTCTCTCTCTCTTCCCTTCTGACTCAGTCTCTCACACTCGTCC  
 10 TGGCTCTGTCTCTGTCTTCCCTAGCTCTTTTATATAGAGACAGAGAGATGGGGTCTCACTGTGTTGCC  
 CAGGCTGGTCTTGAACCTTCTGGGCTCAAGCGATCCTCCCGCCTCGGCCCTCCAAAGTGCTGGGATTAG  
 AGGCATGAGCCACCTTGCCCGCCTCCTAGCTCCTTCTTCGCTCTGCTCTGCTCTGCTCTGCTCT  
 CTGCATCTGTCTCTGTCTCCTTCTCTCGGCCTCTGCCCCGTTCTTCTCTCCCTCTTGGGTCTCTGGCT  
 CATCCCCATCTCGCCCGCCCCATCCAGCCCTTCTCCCCGCCTCCCACCTGTGCGACACCCCTCCCGCCCT  
 15 CTCGGCCGCAGGGCGCTGATGGACGAGACCATGAAGGAGTTGAAGGCCTACAAATCGGAACTGGAGG  
 AACAACCTGACCCCGGTGGCGGAGGAGACGCGGGCACGGCTGTCCAAGGAGCTGCAGGCGGCGCAGGC  
 CCGGCTGGGCGCGGACATGGAGGACGTGCGCGGCCCGCTGGTGCAGTACC GCGGCGAGGTGCAGGCC  
 ATGCTCGGCCAGAGCACCGAGGAGCTGCGGGTGC GCCTCGCTCCCACCTGCGCAAGCTGCGTAAGCG  
 GCTCCTCCGCGATGCCGATGACCTGCAGAAGCGCCTGGCAGTGTACCAGGCCGGGGCCCGCAGGGC  
 20 GCCGAGCGCGCCTCAGCGCCATCCGCGAGCGCCTGGGGCCCTGGTGGAAACAGGGCCCGCTGCGGG  
 CCGCCACTGTGGGCTCCCTGGCCGGCCAGCCGCTACAGGAGCGGGCCAGGCCTGGGGCGAGCGGCT  
 GCGCGCGGATGGAGGAGATGGGCAGCCGACCCGACCCGCTGGACGAGGTGAAGGAGCAGGT  
 GGGCGAGGTGCGCGCCAAGCTGGAGGAGCAGGCCAGCAGATACGCCTGCAGGCCGAGGCCTTCCAG  
 GCCCGCCTCAAGAGCTGGTTTCGAGCCCTGGTGGAAAGACATGCAGCGCCAGTGGGCCGGCTGGTGG  
 25 AGAAGGTGCAGGCTGCCGTGGGCACCAGCGCCGCCCTGTGCCAGCGACAATCACTGAACGCCGAA  
 GCCTGCAGCCATGCGACCCACGCCACCCCGTGCCTCCTGCCTCCGCGCAGCTGCAGCGGGAGACCC  
 TGTCGCCCGCCCCAGCCGTCTCCTGGGGTGGACCCTAGTTTAATAAAGATTACCAAGTTTCACGCA

SEQ ID NO:3: TREM2 (249 amino acids, R47H mutation)

30 MGPLHQFLLLLITALSQALNTTVLQGMAGQSLRVSTYDALKHWGRHKAWCRQLGEEGPCQRV  
 VSTHGVWLLAFLKKNRNGSTVIADDTLAGTVTITLKNLQAGDAGLYQCQSLRGREA EVLQKVLVEVLEDPL  
 DDQDAGDLWVPEESSFEGAQVEHSTSRQVSSCGSPLAYYHLPPLSKESRDLLPHLHSSPPGLRSPEQVSCS  
 QHPLGCGQGQAEAGNTCGQRAGLWPCRWAPTSDPHWTRRYVREF

SEQ ID NO:4: mutated mouse genomic DNA sequence encoding TREM2 (1056 nucleotides)

35 ACTTCAAGGGAAAAGCAAGATCTTGACAAGGTCCCCTCCGGCTGGCTGCTGGCAAAGGAAA  
 GGTGCCATGGGACCTCTCCACCAGTTTCTCCTGCTGCTGATCACAGCCCTGTCCCAAGCCCTCAACACC  
 ACGGTGCTGCAGGGCATGGCCGGCCAGTCCTTGAGGGTGTATGACTTATGACGCCTTGAAGCACTG  
 40 GGGGAGACA<sup>A</sup>CAA<sup>a</sup>GC<sup>a</sup>TGGTGTTCGGCAGCTGGGTGAGGAGGGCCCATGCCAGCGTGTGGTGAGCACA  
 CACGGTGTGTGGCTGCTGGCCTTCTGAAGAAGCGGAATGGGAGCACAGTCATCGCAGATGACACCCT  
 TGCTGGAACCGTCACCATCACTCTGAAGAACCTCCAAGCCGGTGACGCGGGCCTCTACCAGTGTGAGA  
 GTCTCCGAGGCCGAGAGGCTGAGGTCTGCAGAAAGTACTGGTGGAGGTGCTGGAGGACCCTCTAGA  
 TGACCAAGATGCTGGAGATCTCTGGGTCCCCGAGGAGTCATCGAGTTTCGAGGGTGCCCAAGTGGAAC  
 45 ACAGCACCTCCAGGCAGGTTTCATCCTGTGGGTACCTCTAGCCTACCACCTTCTCCTCTTTCCAAGG  
 AATCAAGAGACCTCCTTCCCACCCACCTCCATTCTTCTCCTCCTGGCCTGCGTTCTCCTGAGCAAGTTTC  
 TTGCAGCCAGCATCCTCTGGGCTGTGGCCAGGGGCAGGCAGAAGCCGGGAACACCTGTGGTCAAGAGG  
 GCTGGACTGTGGCCAAGATGCTGGGCACCAACTTCAGATCCTCACTGGACCCGGAGGTACGTGAGAGA  
 ATTCTGAGTGGGAGGAGA<sup>A</sup>ACTACAGCTTAAGTCCAGCCAGGAGTCAATCCAGCCTGCATGCTCTCCCC  
 50 TCCTCCACCAAGACTTCTGTTTCTGCTACTTTTGCTTCAGAGGCCGCTCTGCCTCAAGCCACCTATCC  
 TGGGAGCAGGAATACTGGTGTGTACATCTGTGTTGAGTGGGGAAGACAGCTGGATGGTTGTCTGTCAA  
 CTTCTGCACTTTGGACATTAACATTCTCCACACACCAA

SEQ ID NO:5: TREM2 (249 amino acids, mouse wild type protein, no R47H mutation)

MGPLHQFLLLLITALSQALNTTVLQGMAGQSLRVSCTYDALKHWGRRKAWCRQ  
 LGEEGPCQRVVSTHGVWLLAFLKKNRNGSTVIADDTLAGTVTITLKNLQAGDAGLYQCQ  
 SLRGREAQVLEVLQKVLVEVLEDPLDDQDAGDLWVPEESSFEGAQVEHSTSRQVSSCGSPL  
 5 AYHLPPLSKESRDLLPTHLSPPGLRSPEQVSCSQHPLGCGQGQAEAGNTCGQRAGLW  
 PRCWAPTSDPHWTRRYVREF

SEQ ID NO:6: mouse wild type genomic DNA sequence encoding *Trem2* mouse wild  
 type protein (1056 nucleotides)

10 ACTTCAAGGGAAAAGCAAGATCTTGACAAAGGTCCCCTCCGGCTGGCTGGCAAAGGAAA  
 GGTGCCATGGGACCTCTCCACCAGTTTCTCCTGCTGCTGATCACAGCCCTGTCCCAAGCCCTCAACACC  
 ACGGTGCTGCAGGGCATGGCCGGCCAGTCCCTGAGGGTGTGATGACTTATGACGCCTTGAAGCACTG  
 GGGGAGACGCAAGGCCTGGTGTGCGGCAGCTGGGTGAGGAGGGCCCATGCCAGCGTGTGGTGAGCACA  
 CACGGTGTGTGGCTGCTGGCCTTCTGAAGAAGCGAATGGGAGCACAGTCATCGCAGATGACACCCT  
 15 TGCTGGAACCGTCACCATCACTCTGAAGAACCTCCAAGCCGGTGACGCGGGCCTCTACCAGTGTGAGA  
 GTCTCCGAGGCCGAGAGGGCTGAGGTCTGCAGAAAGTACTGGTGGAGGTGCTGGAGGACCCTCTAGA  
 TGACCAAGATGCTGGAGATCTCTGGGTCCCCGAGGAGTCATCGAGTTTCGAGGGTGCCCAAGTGGAAC  
 ACAGCACCTCCAGGCAGGTTTCATCCTGTGGGTACCTCTAGCCTACCACCTTCTCCTCTTTCCAAGG  
 AATCAAGAGACCTCCTTCCCACCCACCTCCATTCTTCTCCTGCGCTTCTCCTGAGCAAGTTTC  
 20 TTGCAGCCAGCATCCTCTGGGCTGTGGCCAGGGCAGGCAGAGCCGGGAACACCTGTGGTCAGAGG  
 GCTGGACTGTGGCCAAGATGCTGGGCACCAACTTCAGATCCTCACTGGACCCGGAGGTACGTGAGAGA  
 ATTCTGAGTGGGAGGAGAACTACAGCTTAAGTCCAGCCAGGAGTCAATCCAGCCTGCATGCTCTCCCC  
 TCCTCCACCAAGACTTCTGTTTCTGCTACTTTTGCTTCAGAGGCCGCTCTGCCTCAAGCCACCTATCC  
 TGGGAGCAGGAATACTGGTGTGTACATCTGTGTTGAGTGGGGAAGACAGCTGGATGGTTGTCTGTCAA  
 25 CTTCTGCACTTTGGACATTAACATTCTCCACACACCAA

SEQ ID NO:7: Trem2 CRISPR guide

GAAGCACTGGGGGAGACGCA

30 SEQ ID NO:8: Trem2 repair oligo (183 nucleotides) containing a nucleotide G>A point  
 mutation (at nucleotide 89 in this oligo sequence) for amino acid sequence change at R47H and 2  
 silent mutations (lysine AAG>AAA (at nucleotide 93 in this oligo sequence) and alanine  
 GCC>GCA (at nucleotide 96 in this oligo sequence)) into the gene to prevent re-cutting of the  
 donor sequence in homologous directed repair:

35 GCCCTCAACACCACGGTGTGTCAGGGCATGGCCGGCCAGTCCCTGAGGGTGTGATGACTTAT  
 GACGCCTTGAAGCACTGGGGGAGACCAAaGCgTGGTGTGCGGCAGCTGGGTGAGGAGGGCCCATGCCA  
 GCGTGTGGTGTGACACACGGTGTGTGGCTGCTGGCCTTCTTGAAGAAGCGG

40 SEQ ID NO:9: 5' homology arm (4980 nucleotides: mouse *ApoE4* exon 1 and 757  
 nucleotides of mouse *ApoE4* intron 2 sequence) included in the "humanized" mouse *ApoE*  
 construct inserted into the mouse genome.

CCTGTATCTCAGGGTTGAGAGGGAAGAGAGTGGGGTTTCCAGATGCCACATCCCTTGATACC  
 TAGTCCTCACCCAACCTCTGCTCTCCTAGTGAACAACCTGGTTGGCTACAGTAACCGTGGGCCAGGCAGG  
 CATGCATGCGACGTATTACCACAAAGCCAGTGACCAGGTGAGCCGGAGGGAGCGCCTAGCTGTGGGT  
 CCCAGGCTGGGTTTGTGGTGCAGTTGGTATGCCAGGAGTCCTAATGGGCCACTCACATGTGTGCCG  
 5 GGCCCTCTCTCCCCAGCTGCAGGTGGGTGTGGAGTTTGAGGCCAGCACCAGGATGCAGGACACCAGT  
 GCCTCCTTTGGGTATCAGCTGGACCTGCCAAGGCCAACTTCCTTTTTAAAGGTAAGGAAGGCCTCAG  
 CTTCCCTCCTGGGGAACAGGCCAGATAAGACTCAGCTGTCTGTGGTAGAGTCCATGGGAAATAGGGAG  
 GTTGAACATGTGCTGGTGGAGTGGGCGGGGACTCTGTGCAGCTCCAAATTA CTCTGGGTGGCTTGTCA  
 AACAGTGGGGCTCCTACCCTGGCATGTTTAGTACATGTTGGGGGCCCTCGCTTAAAGCCCTCTCTCCCA  
 10 TCCCATCTAGCAAGCTCTCTGGCCCTCCCCACTCACTCTTACCTTACTTGGAGTTCTTCTATTGTGTGA  
 TGAAACACTATGATGGTAAACAGCTTAGGGAGCAAAGAGGTTATGTTGGGTTACA ACTCTCAGGTGGC  
 ATGCCTACCACTGAGGAGAAGATGGGGCAGGACTCAGCAGAAAGCTGAAGGCAAGCTGATGCAGAAG  
 GCCTTGTGGGAGTGTGCTTGTGGCTTGTCTTATGGCTTCCTCAGCCTGCTCTTATAGCCTCCGGAC  
 CATTAGCCCAGGGTGGCCACTGAAAATAATCTGGGTACTCCACATCAATCACTTAGAAAGTGCCCT  
 15 ACAGGCTTGCTGCTGCCCTAGCTAATGGAGACATATGTATTGAGGTTCCCTCAGCTGGATCTCTTGAG  
 TTCAAGACCAGCCTGGTCTACAGAGCAAGTTCTGGGACAGCCAGGGCTACCCAGAGAAACCCTGTCTC  
 AGAAAAGAAAAAAAAGAAAAAGGTGGGGGTGGGGTGACTAGCTTGTATAAACTAGCCAGCACAGC  
 AGGGAAGCTGTGTGAATAGTGACAGCCAGGTT CAGAACCTCATTCTGCGCAGTGACATGGGTTTGTCA  
 AGCCTGGGCGGTGTTGAGCCACTTAAAGGGTGTGTGATGGTCTGGAGTGGCCTTGGTTGTGACTTA  
 20 GGACATACGGTCCGTTGTGGCTACCATAGAGATGGGTGGCCAGGCACATCAAGCCACCCAGTCTCTG  
 CCCCCTCTCGGCCACAGGCTCTGTGAACAGTA ACTGGATCGTGGGCGCCACGCTGGAGAAGAAGCT  
 TCCGCCCTTGGCCCTGACACTGTCCCTCTGCGCCTTCTCTGAACCACCGCAAGAATAAGTTCCTGTGTGG  
 CTTCCGGCCTCACCATCGGCTGAGCCCTCCTGTCTTCTCTGCAGACCATCGCTGGGCCGGCTGCCCTC  
 CCCTCCTCTCCCTCTCTCTTGGGGTTGGGGCAGTGGGAAGGAGGGGACCTCCCATGCCAAGGATCCC  
 25 CAGCGCCAGGGGACAGTGCCCAGGGGGCCTGGGGTCCCGGAGGGAGTCTGGGATCTGAAGGGCATT  
 CGATTGTGAGCGCCAGGCAGAGGCGCAGAGGCGGCTGTACACAGGCTCAGAAAGGAAAGACTTGAT  
 GTCTCCTGAGGGCAGCAGAGGAGCGCCGAGCCGCTGTCACTTCCCCCTCCACCCCTCCATAGAAAT  
 CATGTTTATAAGTTATGGAAAACCGGGACATTTTACAGAAAAAAA AACTTAAAAAACAAAAATA  
 TACGTGGAAAAAAAATAGGATCTGGGAGGCCTCGGTTTTCTCCAGTTGTTGACATGGTTGCCAGCAG  
 30 GCGGCGCCGAGGGTTCAGAAAGCACAGCAGCACC AAGCAGTTTAGAGAAAGCTTGGCCAGGCATGGT  
 CACACCCTCTGCTCTTGGGGACTTACACTGCCGGAACATCAGAGGCCTGGCCTACCAGGAGGGTGGC  
 TCCAGGTCACTCGCCTCTGTGCAGTCTGGCCCCGAGAGCTGGCAGAGGACTTTGTCTCTGTAACAGG  
 GTGGGGGCAGGGAGACGGGGCTCAGGAGCCTCCCTGTCCCAAACGGGGCTGAGGTGGTAGCTTGTGC  
 TGACTTCTCCAGTGGGAAGGTGAGAGGCTCAGAAACTTCAGGAAGAAACGGAGTTCTGGAAGTT  
 35 CAGCACAATAAGGAAAGTTACTCTGGGAGGAACGGGCCCTAGCACCTTCCCAGGCTGAAACAGGAAA  
 TGTCAGTTGTTTTGTTTTTAAATTTTGTGTAAGATGTAGCTCTGGGCCTCACGTGTCAGGCAGGTGCT  
 GCTGGGTTCCCTATAGTGCTTTTCCGTGAGGCCTGCTCTGGGGTGTGCGATAAGGGCCTCAGATTGGGC  
 TCTGCATCTCACTGCTGCACCTCATGGCATCCAAGGGAAGCAAAGACTCACGATGAACTGGTCAGTG  
 TCCTAGACCGCAGCACTAAGACCCTCTCTACTGCCTGGGCTGGAGATGGGAGCTGGCCCTTAGCAACC  
 40 CATGAAAATCATCTCACCACACCCAGTCCTTGCCAGTGT TTTCTGAAGCCAAAGCTAACAGGCCTGGG  
 CCTGGCCAGGCACCCTGTACGTACCTTGGAGCCAGGTGTTCCGCTCTGCCATCCTGCAGAATCATG  
 TTTTGCCGTGTCTGGTGCCAAACACTGCTATGTGGCTTCTCTCCTGCCATCAACAGCTGGGAACAGGGA  
 ACCTTGTGCAGGCAGTGCTTCTAGCAAGCTTGTGTGGTCTCTGAGCCCCCTTGTCTACTGACTTCCC  
 AGGTACAATGGCTTTCCCACTTTTTGGGGGTTTTGTGTTGTTGGGTTTTTTTTTTGTTTTTCAAGACAGG  
 45 GTTTCTCTGTGTAGTCTGTGCGTCCCTGGAAC TCACTTTGTAGACCAGGCTGGCCTCCAACTCAGAAAT  
 TCACCTGCCTCTGCCTCCAAGTGCTGGGATTATGGGATTAAGGCATGTGCCACCACGCCAGCTGGC  
 TTTTCCACTTTTTAGCCAGGACTTCATTCTATTACCTGAGCTCGGGATCTTCCCTGCCTCAGCTTTGCATA  
 TGGCTAGCACTATAGACCCATGTTCCAGTGAATGACTTATGGCTTGTCTTTTTTTTTTTTTTTTTTTTT  
 TTATGTGCATTAGTGT TTTGCTGCATGTATGCCTTCGTGAGGGTAGCAGATCTTGGTGTACAGTTGT  
 50 GAGCTGCTGTGTGGGTGCTGCGATTTTGAACCTAGGTCTGTGAAATGCAGTCAGTGCTCCTAACCTCT  
 GAGTCATCTCTCCAGCTCCTGCTCTTCTGCTTTTATGAGGAAAAAGAAAAGAGAAGTGGCTTGAGAGT  
 GGAAAATGCACATGCAGGGGTGCACACCTGCAGTCCCAGCATGCTACAGCAGAGGCAGAAGGACCTT  
 TGTGGGTTAGAGGGCAGCCTGAGAATCTTATCTCAAACA ACTTTTTAAATGTGCTCTGTAGGGGTA  
 GCTCTTCCCTCCCAAGGTGACACATCTGGCAATCGCCAGAAACAGATCAGGAGCATCAACGCTTGGTT  
 55 TCCCAGGGCTTGGCTTAATGTATGGCTTCAAACCCATCGGGAGCCACC ACTGAACAGCTCCTGAAGGA  
 ACTGGAGCACGTCCAGCCTTGAATGGAAAGAGTTCACCTGTGGTGGAGGAATCAACAACGAGGGA

TCCCAGAACAAACGATCTTCACCCCAGAAGCTGAGCCTCTTAGCCCCACCCACCCATTTCCATTTAGGC  
 TGCCAGCTCTTTTCTTTACAATGCACCAGACCCCCGCGGGAAAGGGAAGGAGCGGTTCTCAGTGCCCC  
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 TCACAAGGCATCCAACTCCACCTCTTTCCTCTGCCCTGCTGTGAAGGGGGAGAGAACAACCCGCCTC  
 5 GTGACAGGGGGCTGGCACAGCCCCGCCCTAGCCCTGAGGAGGGGGCGGGACAGGGGGAGTCCTATAAT  
 TGGACCGGTCTGGGATCCGATCCCCTGCTCAGACCCTGGAGGCTAAGGACTTGTTCGGAAGGAGCTG  
 GTAAGACAAGCTGGGCTGGGGATTACCCAGGGACCTTGGTAGGATGTGGGCTGGGAACCTTGAGAT  
 CCCCCGAGTCCAGGAAACAGGCACAAGAATTGGAAGAGCAGGCAGCACGATAGAAGTCTTGGGGGA  
 CAAACTAAGGACTCGAGGTAAGTACTAGCCTTTGCCAGAGTCAGAGCAGGTGGAGGGGTTACCTCCAGGA  
 10 AGGAGTACGGGACTGTCCGTGCACGGCGTACCGGCTCAACTAGGAACCATCTATGGCGAAAAA  
 CCGGATGAGCCTTAGGCTGCTTTTATATAAATACCTACTGATTTCCATCACAGTCCCCAAGTAACCCGG  
 ACTGGTTTCAAACCTGTGGCTCCTCATGGCTGAGCTCCCTAAGTTCTGTAGTTGTGGGAGGGTACC  
 ACTT  
 CGCAGGGATGGAGGACGATTAATAAATCGTGTTAAATTAACACAAAATGGAAAGCAGGACTTAGCCGG  
 GAAGAAAGAGGAATGTAAGCTGGACCACCCGCTGGCCCTCTGTGAAGTGGAATTTGAACCTTAGGAG  
 15 AGGGAGCTGGAATTTTTGGCAGCGGATCCACCCCGGGGTGCCGAGATAGCGAACTCGGCAAGGGGAG  
 GTAAACAGACCTTTGGGAAGAGCGGGTGTCTGTTTTGGAGATGTTTGTGATGGCTCACAGATCTGAG  
 AAGGGAAGATGGGGTTCTCTGGGTGGCCGGAGTCCCTCCACCCCGCCCCCTGGTGTTCAAAGACAAT  
 TTTTCCCTCCGCAG

20 SEQ ID NO:10: DNA sequence including exons 2, 3 and 4 of the human *APOE4* gene  
 and 1500 nucleotides 3'UTR of the human *APOE4* gene after exon 4 (4292 nucleotides)  
 included in the "humanized" mouse *ApoE* construct inserted into the mouse genome.

ACTGGCCAATCACAGGCAGGAAGATGAAGGTTCTGTGGGCTGCGTTGCTGGTTCACATTCCTGG  
 25 CAGGTATGGGGCGGGGCTTGGCTCGGTTCCCCCGCTCTCCCCCTCTCATCCTCACCTCAACCTCCTG  
 GCCCCATTACAGGCAGACCCTGGGCCCCCTCTTCTGAGGCTTCTGTGCTGCTTCCTGGCTCTGAACAGCG  
 ATTTGACGCTCTCTGGGCTCGGTTTCCCCCATCCTTGAGATAGGAGTTAGAAGTTGTTTTGTTGTTGTT  
 GTTTTGTGTTGTTGTTTTGTTTTTTGAGATGAAGTCTCGCTCTGTGCGCCAGGCTGGAGTGCAGTGGCG  
 GGATCTCGGCTCACTGCAAGCTCCGCCTCCAGGTCCACGCCATTCTCCTGCCTCAGCCTCCCAAGTAG  
 CTGGGACTACAGGCACATGCCACCACACCCGACTAACTTTTTTTGATTTTTCAGTAGAGACGGGGTTTTCA  
 30 CCATGTTGGCCAGGCTGGTCTGGAACCTGACCTCAGGTGATCTGCCCGTTTCGATCTCCCAAAGTGC  
 TGGGATTACAGGCGTGAGCCACCGCACCTGGCTGGGAGTTAGAGGTTTCTAATGCATTGCAGGCAGAT  
 AGTGAATACCAGACACGGGGCAGCTGTGATCTTTATTCTCCATCACCCCCACACAGCCCTGCCTGGGG  
 CACACAAGGACACTCAATACATGCTTTTCCGCTGGGCGCGGTGGCTCACCCCTGTAATCCCAGCACTTT  
 GGGAGGCCAAGGTGGGAGGATCACTTGAGCCAGGAGTTCAACACCAGCCTGGGCAACATAGTGAGA  
 35 CCCTGTCTCTACTAAAATAACAAAATTAGCCAGGCATGGTGCCACACACCTGTGCTCTCAGCTACTC  
 AGGAGGCTGAGGCAGGAGGATCGCTTGAGCCAGAAAGTCAAGGTTGCAGTGAACCATGTTACAGGCC  
 GCTGCACTCCAGCCTGGGTGACAGAGCAAGACCCTGTTTATAAATAACATAATGCTTTCCAAGTGATTA  
 AACCGACTCCCCCTCACCTGCCCACCATGGCTCCAAAGAAGCATTTGTGGAGCACCTTCTGTGTGCC  
 CCTAGGTAAGTACTAGATGCCTGGACGGGGTTCAGAAGGACCCTGACCCACCTTGAACCTGTTCCACACAGGA  
 40 TGCCAGGCCAAGGTGGAGCAAGCGGTGGAGACAGAGCCGGAGCCCGAGCTGCGCCAGCAGACCGAGT  
 GGCAGAGCGGCCAGCGCTGGGAACTGGCACTGGGTGCGTTTTGGGATTACCTGCGCTGGGTGCAGACA  
 CTGTCTGAGCAGGTGCAGGAGGAGCTGCTCAGCTCCCAGGTACCCAGGAACTGAGGTGAGTGTCCCC  
 ATCCTGGCCCTTGACCCTCCTGGTGGGCGGCTATACCTCCCCAGGTCCAGGTTTCATTCTGCCCTGT  
 GCTAAGTCTTGGGGGGCCTGGGTCTCTGCTGGTTCTAGCTTCCCTCTTCCCATTCTGACTCCTGGCTTTA  
 45 GCTCTCTGGATAATCTCTCTCAGCTTTGTCTCTCTCTCTTCCCTTCTGACTCAGTCTCTCACACTCGTCC  
 TGGCTCTGTCTCTGCTTCCCTAGCTCTTTTATATAGAGACAGAGAGATGGGGTCTCACTGTGTTGCC  
 CAGGCTGGTCTTGAACCTTGGGCTCAAGCGATCCTCCCGCCTCGGCCCTCCAAAGTGTGGGATTAG  
 AGGCATGAGCCACCTTGCCCGCCTCCTAGCTCCTTCTCGTCTCTGCCTCTGCCCTCTGCATCTGCTCT  
 CTGCATCTGTCTCTGCTCTCCTTCTCTCGGCCTCTGCCCCGTTCCCTCTCTCCCTCTTGGGTCTCTCTGGCT  
 50 CATCCCCATCTCGCCCGCCCCATCCAGCCCTTCTCCCCGCCTCCCACTGTGCGACACCTCCCGCCCT  
 CTCGGCCGCAGGGCGCTGATGGACGAGACCATGAAGGAGTTGAAGGCCTACAAATCGGAACTGGAGG  
 AACAACCTGACCCCGGTGGCGGAGGAGACGCGGGCACGGCTGTCCAAGGAGCTGCAGGCGGGCGCAGGC  
 CCGGCTGGGCGCGGACATGGAGGACGTGCGCGGCCGCTGGTGCAGTACCGCGGGCAGGTGCAGGCC

ATGCTCGGCCAGAGACCGAGGAGCTGCGGGTGC GCCTCGCCTCCCACCTGCGCAAGCTGCGTAAGCG  
 GCTCCTCCGCGATGCCGATGACCTGCAGAAGCGCCTGGCAGTGTACCAGGCCGGGGCCCCGCGAGGGC  
 GCCGAGCGCGGCCTCAGCGCCATCCGCGAGCGCCTGGGGCCCCCTGGTGGAAACAGGGCCGCGTGCGGG  
 5 CCGCCACTGTGGGCTCCCTGGCCGGCCAGCCGCTACAGGAGCGGGCCAGGCCTGGGGCGAGCGGCT  
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 GCGGGAGGTGCGCGCCAAGCTGGAGGAGCAGGCCAGCAGATACGCCTGCAGGCCAGGCCTTCCAG  
 GCCCCCTCAAGAGCTGGTTCGAGCCCCTGGTGGAAAGACATGCAGCGCCAGTGGGCCGGGCTGGTGG  
 10 AGAAGGTGCAGGCTGCCGTGGGCACCAGCGCCGCCCTGTGCCAGCGACAATCACTGAACGCCGAA  
 GCCTGCAGCCATGCGACCCACGCCACCCCGTGCCTCCTGCCTCCGCGCAGCCTGCAGCGGGAGACCC  
 TGTCCTCCGCCCCAGCCGTCTCCTGGGGTGGACCCTAGTTTAATAAAGATTACCAAGTTTACGCATC  
 TGCTGGCCTCCCCCTGTGATTCCTCTAAGCCCCAGCCTCAGTTTCTCTTTCTGCCACATACTGGCCAC  
 ACAATTCTCAGCCCCCTCCTCTCCATCTGTGTCTGTGTGTATCTTTCTCTCTGCCCTTTTTTTTTTTT  
 GACGGAGTCTGGCTCTGTCACCCAGGCTAGAGTGCAGTGGCACGATCTTGGCTCACTGCAACCTCTGC  
 CTCTTGGGTTCAAGCGATTCTGCTGCCTCAGTAGCTGGGATTACAGGCTCACACCACCACACCCGGCTA  
 15 ATTTTTGTATTTTTAGTAGAGACGAGCTTTCACCATGTTGGCCAGGCAGGTCTCAAACCTCCTGACCAAG  
 TGATCCACCCGCGGCCTCCCAAAGTGCTGAGATTACAGGCCTGAGCCACCATGCCCGGCCTCTGCC  
 CTCTTTCTTTTTTAGGGGGCAGGGAAAGGTCTCACCCCTGTCACCCGCCATCACAGCTCACTGCAGCCTC  
 CACCTCCTGGACTCAAGTGATAAGTGATCCTCCCGCCTCAGCCTTTCAGTAGCTGAGACTACAGGCG  
 CATACTACTAGGATTAATTTGGGGGGGGGGGTGGTGTGTGTGGAGATGGGGTCTGGCTTTGTTGGCCA  
 20 GGCTGATGTGGAATTCCTGGGCTCAAGCAGTACTCCACCTGGCCTCCTGAGTAGCTGAGACTACTG  
 CTAGCACACCACCCAGCTTTTTATTATTATTTGTAGAGACAAGGTCTCAATATGTTGCCAGGCT  
 AGTCTCAAACCCCTGGGCTCAAGAGATCCTCCGCCATCGGCCTCCCAAAGTCTGGGATTCCAGGCAT  
 GGGGCTCCGAGCCCGGCCTGCCCAACTTAATAATACTTGTTCCTCAGAGTTGCAACTCCAAATGACCT  
 GAGATTGGTGCCTTTATTCTAAGCTATTTTCATTTTTTTTTCTGCTGTCATTATTCTCCCCCTTCTCCTC  
 25 CAGTCTTATCTGATATCTGCCTCCTTCCCACCCACCCTGCACCCCATCCCACCCCTCTGTCTCTCCCTGT  
 TCTCCTCAGGAGACTCTGGCTTCTGTCTTCTCCACTTCTATCTTTTATCTCTCCCTCCTACGGTTTCTT  
 TTCTTTCTCCCCGGCCTGCTTGTCTTCTCCCCAACCCCTTCTATCTGGATTTCTTCTCTGCCATTCAGTT  
 TGGTTTGAGCTCTCTGCTTCTCCGGTTCCTCTGAGCTAGCTGTCCCTTACCCACTGTGAACCTGGGTTT  
 CCCTGCCAACCCCTATTCTCTTTCTTTCTTTCTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTGGAGACA  
 30 GTCTTGCTCTGTTGCCAGCCTGGAGTGCAGTGGTGCAATCTTGGTTCACTGCAACCTCCACTTCCAG  
 ATTCAAGCAATTTCTCCTGCCTCAGCCTCCAGAGTAGCTGGGATTACAGGCGTGTCCCACCACACCCGA  
 CTAATTTTTGTATTTTTGGTAGAGACAAGGCTTCGGCATTGTT

SEQ ID NO:11: a Frt PGKneo Frt cassette followed by an NdeI restriction enzyme site  
 35 (CATATG) (1834 nucleotides) inserted in the “humanized” mouse *ApoE* construct between  
 nucleotides 9272 and 9273 to allow for selection prior to recombinase-mediated removal of this  
 cassette.

GAAGTTCCTATTCTCTAGAAAGTATAGGAACTTCAGGTCTGAAGAGGAGTTTACGTCCAGCCA  
 AGCTAGCTTGGCTGCAGGTTCGTCGAAATTCTACCGGGTAGGGGAGGCGCTTTTCCCAAGGCAGTCTGG  
 40 AGCATGCGCTTTAGCAGCCCCGCTGGGCACTTGGCGCTACACAAGTGGCCTCTGGCCTCGCACACATT  
 CCACATCCACCGGTAGGCGCAACCGGCTCCGTTCTTTGGTGGCCCCCTTCGCGCCACCTTCTACTCCTC  
 CCCTAGTCAGGAAGTTCCCCCCCCCGCCCGCAGCTCGCGTCGTGCAGGACGTGACAAATGGAAGTAGCA  
 CGTCTCACTAGTCTCGTGCAGATGGACAGCACCGCTGAGCAATGGAAGCGGGTAGGCCTTTGGGGCAG  
 45 CGGCCAATAGCAGCTTTGCTCCTTTCGCTTTCTGGGCTCAGAGGCTGGGAAGGGGTGGGTCCGGGGGCG  
 GGCTCAGGGGCGGGCTCAGGGGCGGGGCGGGCGCCGAAGGTCTCCGGAGGCCCGGCATTCTGCAC  
 GCTTCAAAGCGCACGCTGCCGCGCTGTTCTCTCTTCTCCTCATCTCCGGGCTTTTCGACCTGCAGCCT  
 GTTGACAATTAATCATCGGCATAGTATATCGGCATAGTATAATACGACAAGGTGAGGAACTAAACCAT  
 GGGATCGGCCATTGAACAAGATGGATTGCACGCAGGTTCTCCGGCCGCTTGGGTGGAGAGGCTATTCTG  
 50 GCTATGACTGGGCACAACAGACAATCGGCTGCTCTGATGCCGCCGTGTTCCGGCTGTCAGCGCAGGGG  
 CGCCCGGTTCTTTTTGTCAAGACCGACCTGTCCGGTGCCTGAATGAACTGCAGGACGAGGCAGCGCG  
 GCTATCGTGGCTGGCCACGACGGGCGTTCCTTGCAGCAGTGTGCTCGACGTTGTCCTGAAGCGGGAA  
 GGGACTGGCTGCTATTGGGCGAAGTGCCGGGGCAGGATCTCCTGTCATCTCACCTTGTCTCCTGCCGAG



AAAGTATCCATCATGGCTGATGCAATGCGGGCGGCTGCATACGCTTGATCCGGCTACCTGCCCATTCGA  
 CCACCAAGCGAAACATCGCATCGAGCGAGCACGTA CTGGATGGAAGCCGGTCTTGTCGATCAGGAT  
 GATCTGGACGAAGAGCATCAGGGGCTCGCGCCAGCCGAAC TGTTCGCCAGGCTCAAGGCGCGCATGC  
 5 CCGACGGCGATGATCTCGTCGTGACCCATGGCGATGCCTGCTTGCCGAATATCATGGTGGAAAATGGC  
 CGCTTTTCTGGATTTCATCGACTGTGGCCGGCTGGGTGTGGCGGACCGCTATCAGGACATAGCGTTGGCT  
 ACCCGTGATATTGCTGAAGAGCTTGGCGGCGAATGGGCTGACCGCTTCCTCGTGCTTTACGGTATCGCC  
 GCTCCCGATTTCGAGCGCATCGCCTTCTATCGCCTTCTTGACGAGTTCTTCTGAGGGGATCAATTCTCT  
 AGAGCTCGCTGATCAGCCTCGACTGTGCCTTCTAGTTGCCAGCCATCTGTTGTTTGCCCCCTCCCCCGTG  
 10 CCTTCTTGACCCTGGAAGGTGCCACTCCCCTGTCCTTTCCTAATAAAAATGAGGAAATTGCATCGCAT  
 TGTCTGAGTAGGTGTCATTCTATTCTGGGGGGTGGGGTGGGGCAGGACAGCAAGGGGGAGGATTGGG  
 AAGACAATAGCAGGCATGCTGGGGATGCGGTGGGCTCTATGGCTTCTGAGGCGGAAAGAACCAGCTG  
 GGGCTCGACTAGAGCTTGCGGAACCCTTCGAAGTTCTATTCTCTAGAAAGTATAGGAACTTCCATAT  
 G

15 SEQ ID NO:12: 3' homology arm (5166 nucleotides) included in the "humanized" mouse  
*ApoE* construct inserted into the mouse genome.

AAACCTGATGGAGAAGATACAGGCCTCTGTGGCTACCAACCCATCATCACCCCAGTGGCCCA  
 GGAGAATCAATGAGTATCCTTCTCCTGTCCTGCAACAACATCCATATCCAGCCAGGTGGCCCTGTCTCA  
 AGCACCTCTCTGGCCCTCTGGTGGCCCTTGCTTAATAAAGATTCTCCGAGCACATTCTGAGTCTCTGTG  
 20 AGTGATTCCAATCAGCTTCAGCCTCAGTTTATTGTTTTTGCCTTACCTAGCACACATTCCATGGCCCTG  
 TCACTATCTGTAGAGGGAGGTGGTTTTGCAGCAATAGAAATGAAGCCTAGGACCTAGCAACATAAAA  
 GAACAAGTGATCTACCACTGAGCCACGCCACAGCCCCTCACTGGGGGATTCTAGGCAGGGGCTCTAC  
 CACTGAGCCACCCGAGCCCCTCACTGGGGAATCATATCTACCACTGAGTCACGCCCCTCCAGCCCCT  
 CACTACGGGAATTCTAGTCAGTAGCTCTACCACTGAGCCACACCCACAGCCTCTGGGGCTCTTCACCG  
 25 CCCCCTACCCCTGGATTCTAGGCATGGGCTCATTTTATTTATTTATTTAAGATTTGTTTTATCTTAT  
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 GTTGTCTGGAAATTGAACTCAGGACTTATAGAAGAGTAGTCAGTGCTCTTAACTGCTGAGCCATCTCC  
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 TGATTTTCTTGCACTCCCTCTAGCCTAGTTTGGTCTTGAATATTTGAATCTTTGTTTTCAAATCAATCT  
 30 CTACAGGAACTGAGAAAGGCATGTACCACTTCATGTGGGTGAGTTGGGCTACTTTTCCCAACTTCCCA  
 AGCACCCACTGCACAGCTATGCCTTGAATCAATCAACATGTAAGAGACCAGGGTCGCCAGGCGGTGTT  
 TACTTTTTCTGGTTGCTTATCTCTCCTCCTCCGCTCTAGTCTTATCTGACACCCCTCTCCTTGCTTGTCTC  
 TCCTCTTTTTCCCTTCTAGGCTTCCTTTTTCTGGCTTCCTGTTTTCTGATCCTCTGTTATCTCACCCCTCC  
 GCGGTTTTCTTTGCTCTGGGCCCTTTGGTTGGCGGTTTCTACGGTTTCTACGTGGCTTTTGGAACCTCAGC  
 35 CTTTCTCCCTTGCTCTGAAGTTAGCTGGATCTTTGCTCCCTCTGGGTCATGGGGCTTAGCCCCATTCTT  
 TCTCCCCTGCCTGTGCTGCATGCCTTTTGACTTTCCCAGCAAGTGTATGGAGAGTGAGTTCAGGCTGGG  
 GACACAAAACCATTCTCCCCATGTCCTGGTACTCAAAGGGGTCATGGTGGAAAGCTGGAGAGCCCCTGG  
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 GGGCTTGAGCTGTGTCTTGGGTGCGGCCAAGGCTTCAGA ACTCAACTGCCTCTGGCACACCCCGAGCC  
 40 TGCAGCTTTTCTGGCATCAA ACTCAGCATTATCTGGAGGCAGGCACCACTCCCACACATGACTCATT  
 GGCCCAATGAGAAGATGGGTGCGGTGGGCTAGTGACAGGGCCCTAGACAGCCACACTTCATGCCCAGG  
 GGCTAGGAGCACACCCCCAGAGCCTCACTACTCTCAAATATCAAGATGAGGGGGAGAGTATAGAGGGA  
 ATGTCTCCCTCTGAAGACCTGAATTATTGTGATGTTATTTGAGCCACGCTGGCCTTGA ACTCTGAACCC  
 TCCTGCCTCATCTTCAAAGGCTGGAATTACAGGTTTCGCGCCACTAGGCCTATCTGAGAACTTAGTTAAT  
 45 TCTACAGAAGAGAGTTTGTTTTACTGGTTTTGAAACGAGGCCTCGTGTACCCAGAGCTACTCTTTTGCC  
 AACTCACTGTAGAGCCAAGGATGACCTTGA ACTCTGGTCTTCCTGGCTCCGAGTCCCTCGGTACTGGGTA  
 GACAATGTGTGATCCTAACCCCAAGTTTTATGTGGTGTCTATACAGATGGCATCCAGGGTTTTCTGCATG  
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 CAGAGCTCATGGAGCCCAAGCTGGCCTCAATCTATATAACCAAGACTGTGCCTGGTCTTCTGTCTCTA  
 50 CCTAACAAATGATGGTGGGATGCAGGGATGTAACACAGGAGCAGGCTTACTTCTAAACGAAGAA  
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 AGGATCGTGAAGGAAGTCAATGGAGGCCAGCTGTGTACAGAGTGAGTTCCAGGACAGCCAGGGCTA  
 CACAGAGGAAAACAAGAAGGAAAAAAAAAAAAAAAAAAGAACTGAATGACTAGCAGTTTTAGAAC  
 CTTAGCTGTGTGCTTCCCGTACCCTACCTGTGGCACAGGAAGCCCTTGTTGCTAAGAGTAGGATGACAC

TGG AAG CAC CAAG CACTGGGCAGAGTAAGGGGGAAATCTGTTACATTATTCAGATGGGCGCTTGAGG  
 ACTGAGCAGGACTCCTTTCAAGAAGCAAAAAGGGTCAGGCCTGGTGCCACAGGGACTGGAAGCTAGC  
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 AACCATCCCCCTGTTGTCGTCGTCACCCACCCCCACCCCGCCCCAACTGACACGTGGGTTGCAGGGGC  
 5 ACCAGGCCAGCCAACCTAGAGTCTGGGCCCTTAGCCACCAGCTGCCAGGGGGTCACTGTGCGTCAAT  
 GACAGCTCTGGGGGAGGGGGGAGAAGGGCCTTGGACTCTAGCCTGAGAGAAAGGATGTTGTGGAAG  
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 GGAAGGGGGGTTGTGCCAGTTAGGGGACACTATGGAGCTCTGCAAGCTAAGAGGCTCTTAGCTTCTT  
 10 TGCAGTTTTGACTAGCTGAAGAGGCAACTTCTAAGGAAGGGAGATGAGGGGATGCCAAGATCCCAGA  
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 15 AAGTGAGGATGCTGACCAGTGACGAGCCTTGGGCCTAAATAAAAAGCCTGGATGTGGGATCTGCCTCC  
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 AGGGGGAGGGCGACCAGCTTGTCCGCCCCCTCCCAACCGGTTAAACCTCTGTGCAGGATCCTCCCA  
 20 CCGCCATGGGCCTCCTGAGAGATCCTTAGATCCAGGTTAGTGCATAGGAAAGTGTCCCCACTACCT  
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 AGTGTGAAGTTGGCCTTGTGCCTGCGGCCAGGCTTAGAAGACCTCGAGGAGGGCTCTGAGGTCCT  
 TTCTGTGTCATCATTCCACTACCGCCCTCCCATCGTCCCCATCCCACCTGCCAGGTGCCTTATTTTTGT  
 25 GTCAAAGTGGGTGCTGAAGGAGGCAACTCTGTCCAGAAAAGACGCAGTAACCAATGACCTAGGATAC  
 CACCCTTTGGAAATGGCTAATCTTCCTAGAAGGGGCGGAGCGTAAAAACAAGGAGGTGAGAGGTGCA  
 GTAAAATCAAGTGTCCAATACCCTCCCCATGCTAATGAGTTTGCTCGCAACCTCTCGCGGCAGGCC  
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 TTTGGGAACACTTTGGAAGACAAGGCCCGGGCAGCCATTGAACATATCAAACAGAAGGAAATTTTGA  
 30 CCAAGACCCGTTAGGACCTTTCAGGGCACGGGCGGGTGGTGTGTGTGTGTGTGCGTGCAGCGCGCC  
 CGTGTGTA AAAAGCCCTAGCAGACAGTTCCACACTGACACAATGGGGAAACTGCACCAGAGTGTTTGAC  
 ACTTTCCTGAAGTCATAGA ACTGTATCTGAAGTCATTGAACTGGATGTCAAAGTGCTCGTAGTGTGG  
 AGACAGGCGGTGTA ACTCCAGCCA ACTGTTAGAGATGTTTCCAAGTCTAGTGAAGGGCCAAGCTAG  
 GCGGCTGACTGGTTAGGACAGACCCTGACCCCTCCCTGTGTACTCTTGAGACAGGTCATGACTTGAGT  
 35 CTCCGAGACAGAGAAAGAATGTAGGAGAGTTGGGGCAGGGGCACAGCAGAAGCTGTACAGGCCTGG  
 GCTCTGCAGTTTACTG GCCAAAGAGAGATTAGGGCATGCTGGGTAAAGAACTAGCCAGGCAGTGGT  
 GGCGCACACCTTTGATCC CAGTATTCATGAGGCAGAGGCAGGCAGATCTCTTTGAGGCCAGCCTGATT  
 GGAGCTAGTTCTAGGAAAGCCAGGGCTACACAGAGAAACCCTGTCTCAACACCACCACCCCCC

40 SEQ ID NO:13: wild-type amino acid sequence-mouse APOE

MKALWAVLLVTLTGLAEGEPEVTDQLEWQSNQPWEQALNRFWDYLRWVQTLSDQVQEELQS  
 SQVTQELTALMEDTMTEVKAYKKELEEQLGPVABETRARLGKEVQAAQARLGADMEDLRNRLGQYRNE  
 VHTMLGQSTEEIRARLSTHLRKMRLMRDAEDLQKRLAVYKAGAREGAERGVSAIRERLGPLVEQGRQ  
 45 RTANLGAGAAQPLRDRAQAFGDRIRGRLEEVGNQARDRLEEVREHMEEVRSKMEEQTQQIRLQAEIFQAR  
 LKGWFEPIVEDMHRQWANLMEKIQASVATNPIITPVAQENQ

SEQ ID NO:14: amino acid sequence-human APOE3

MKVLWAALLVTFLAGCQAKVEQAVETEPEPELRRQTEWQSGQRWELALGRFWDYLRWVQTLSE  
 50 QVQEELLSSQVTQELRALMDETMKELKAYKSELEEQLTPVABETRARLSKELQAAQARLGADMEDVCGR  
 LVQYRGEVQAMLGQSTEELRVRLASHLRKLRKLLRDADDLQKRLAVYQAGAREGAERGLSAIRERLGP  
 LVEQGRVRAATVGS LAGQPLQERAQAWGERLRARMEEMGSRTRDRLDEVKEQVAEVRAKLEEQAQQIRL  
 QAEAFQARLKS WFEPLVEDMQRQWAGLVEKVQAAVGTSAAPVPSDNH

SEQ ID NO:15: APOE4 p (299 amino acids)

KVEQAVETEPEPELRRQTEWQSGQRWELALGRFWDYLRWVQTLSEVQVEELLSSQVTQELRALM
DETMKELKAYKSELEEQLTPVAEETRARLSKELQAAQARLGADMEDVRGRLVQYRGEVQAMLGQSTEEL
RVRLASHLRKLRKRLRLDADDLQKRLAVYQAGAREGAERGLSAIRERLGPLVEQGRVRAATVGS LAGQPL
5 QERAQAWGERLRARMEEMGSRTRDRLDEVKEQVAEVRAKLEEQAQQIRLQAEAFQARLKS WFEPLVED
MQRQWAGLVEKVQAAVGTSAAPVPSDNH

SEQ ID NO: 49: Mthfr\*A262V g

ATGAACCATCAAAAAGCCAAGGTCCTCCCAGCTGGGCACTGTTATCCATCCCTGGGGATGTGG
10 GCCTCAGAGGCTGGCTGCGTGAGGCTTTCTGTGCCACCTCTATCAGCAGGAATCCAGCCATGGTGAA
CGAGGCCAGAGGAAGTGGCAGTCCCAACCCGCGATCTGAGGGCAGCAGCAGTGGCAGCGAGAGTTCC
AAGGACAGTTCAAGATGTTCCACCCCCAGCCTGGACCCAGAGCGGCACGAGAGACTCCGGGAGAAGA
TGAGGCGCAGAATGGACTCTGGTGACAAGTGGTTCTCCCTGGAGTTCTTCCCCCTCGAACTGCTGAG
GGAGCTGTTAACCTCATCTCCAGGTTTGACCGGATGGCAGCAGGGGGCCCCCTCTTCGTAGATGTTAC
15 CTGGCACCCAGCTGGAGACCCTGGCTCAGACAAGGAGACCTCCTCCATGATGATCGCCAGCACAGCAG
TAAACTACTGCGGCTTGGAACCATCCTGCATATGACCTGCTGCCAGCAGCGCCCGGAGGAGATCACA
GGCCATCTGCACAGAGCCAAGCAGCTGGGCCTGAAGAACATAATGGCGCTGAGGGGAGACCCTGTAG
GTGACCACTGGGAAGCAGAGGAAGGAGGCTTCAGCTATGCCACAGACCTGGTGAAGCACATCCGGAC
CGAGTTTGCTGACTATTTTGACATCTGTGTGGCAGGTTACCCAGAGGCCACCCCGATGCAGAGAGCT
20 CTGAGGATGACCTGAAGCATTGGAAGGAGAAGGTATCTGCAGGGCTCGACTTCATTATCACTCAGCTC
TTCTTTGAGGCCAGCACCTTCTCAGCTTTGTGAAGGCCTGCACAGAGATAGGCATCTCTTGCCTATC
CTGCCTGGGATCTTCCCTATTCAGGGCTACACTTCCCTTCGGCAGCTTGTAAAACTGTCCAAGCTGGAG
GTGCCACAGAAGATCAAGGATGTAATTGAGGCCATCAAAGACAACGATGCTGCCATCCGCAACTACG
GCATTGAGCTGGCTGTAAGCCTGTGCCGGGAGCTGCTGGACAGTGGCTTGGTGCCAGGCCTCCACTTC
25 TATACCCTCAACCGCGAGGTGGCCACCATGGAGGTGCTAAAGCAACTGGGCATGTGGACCGAGGACC
CCAGGCGTCCCCTGGCCTGGGCTCTCAGTGCGCATCCCAAGCGCCGGGAGGAAGATGTCCGTCCCATC
TTCTGGGCCTCCAGACCAAAGAGCTACATCTACCGCACACAGGACTGGGATGAGTTTCTAACGGCCG
CTGGGGTAATTTCTTCTCACCAGCCTTTGGGGAGCTGAAAGACTACTACCTCTTCTACCTGAAAAGCAA
GTCCCCAGGGAGGAGCTGCTGAAGATGTGGGGCGAGGAGCTCACCAGCGAAGAGAGTGTCTTTGAA
30 GTCTTTGAACACTACCTCTCTGGAGAGCCGAATCGCCATGGCTACAGAGTAACCTGCCTGCCCTGGAA
CGATGAACCCCTGGCAGCGGAAACCAGCCTGATGAAGGAAGAGCTGCTCCGCGTGAACAGGCTGGGC
ATCCTCACCATCAACTCTCAGCCCAACATCAACGCAAAACCATCCTCAGACCCTGTTGTGGGCTGGGG
CCCCAGTGGGGGTTATGTCTTCCAGAAGGCCTACCTCGAATTCTTACCTCCCCTGAAACTGTGGAGGC
GCTTCTGCAGGTGCTGAAGACATACGAGCTGCGGGTCAACTACCACATCGTGGACGTGAAGGGAGAG
35 AACATCACTAATGCCCTGAGCTGCAGCCCAATGCCGTGACGTGGGGCATCTTCCC GGTCGAGAGAT
CATCCAGCCTACTGTGGTGGACCCCATCAGCTTCATGTTCTGGAAGGATGAGGCCTTTGCCCTGTGGAT
CGAGCAGTGGGGCAAGCTATACGAGGAGGAGTCGCCATCCCGCATGATCATCCAATACATCCATGAC
AACTATTTCTGGTCAACCTGGTGGACAACGAGTTCCCGCTGGACAGCTGCCTGTGGCAGGTGGTGA
40 GGACACGTTTGAGCTGCTCAACAGGCATCCCACGGAGAGAGACACAGGCTCCATGA

SEQ ID NO: 50: Mthfr\* A262V p

MNHQKAKVLPAGHCYPSLGMWASEAGCVRLSVPPSISRNPAMVNEARGSGSPNPRSEGS SSGSES
SKDSSRCSTPSLDPERHERLREKMRRRMDSGDKWFSLEFFPPRTAEGAVNLISRFDRMAAGG PLFVDVTHW
PAGDPGSDKETSSMMIASTAVNYCGLETILHMTCCQQRPEEITGHLHRAKQLGLKNIMALR GDPVGDHWE
45 AEEGGFSYATDLVKHIRTEFADYFDICVAGYPRGHPDAESFEDDLKHLKEKVSAGYDFIITQLFFEASTFFSF
VKACTEIGISCPILPGIFPIQGYTS LRQLVKLSKLEVPQKIKDVIEPIKDNDAAIRNYGIELAVSLCRELLD SGL
VPGLHFYTLNREVATMEVLKQLGMWTE DPRRPLPWALS AHPKRREEDVRPIFWASRPKSYIYRTQDWDEF
PNGRWGNSSPAFGE LKDYLLFY LKSKSPRELLKMWGEELTSEESVFEVFEHYLSGEPNRHGYRVTCLPW
NDEPLAAETSLMKEELLRVNRLGILTINSQPNINAKPSSDPVVGWGPSGGYVFQKAYLEFFTSRETVEALLQ
50 VLKTYELRVNYHIVDVKGENITNAPELQPN AVTWGIFPGREI IQPTVVDPI SFMFWKDEAFALWIEQWGKL
YEEESPSRMIIQYIHDNYFLVNLVDNEFPLD SCLWQVVEDTFELLNRHPTERETQAP

Any patents or publications mentioned in this specification are incorporated herein by reference to the same extent as if each individual publication is specifically and individually indicated to be incorporated by reference.

5 The genetically modified mice and methods of use described herein are presently representative of preferred embodiments, exemplary, and not intended as limitations on the scope of the disclosure. Changes therein and other uses will occur to those skilled in the art. Such changes and other uses can be made without departing from the scope of the disclosure as set forth in the claims.

10 All references, patents and patent applications disclosed herein are incorporated by reference with respect to the subject matter for which each is cited, which in some cases may encompass the entirety of the document.

The indefinite articles “a” and “an,” as used herein in the specification and in the claims, unless clearly indicated to the contrary, should be understood to mean “at least one.”

15 It should also be understood that, unless clearly indicated to the contrary, in any methods claimed herein that include more than one step or act, the order of the steps or acts of the method is not necessarily limited to the order in which the steps or acts of the method are recited.

20 In the claims, as well as in the specification above, all transitional phrases such as “comprising,” “including,” “carrying,” “having,” “containing,” “involving,” “holding,” “composed of,” and the like are to be understood to be open-ended, i.e., to mean including but not limited to. Only the transitional phrases “consisting of” and “consisting essentially of” shall be closed or semi-closed transitional phrases, respectively, as set forth in the United States Patent Office Manual of Patent Examining Procedures, Section 2111.03.

The terms “about” and “substantially” preceding a numerical value mean  $\pm 10\%$  of the recited numerical value.

25 Where a range of values is provided, each value between the upper and lower ends of the range are specifically contemplated and described herein.

What is claimed is:

### CLAIMS

1. A genetically modified mouse comprising a genomic nucleic acid encoding human APOE4, a genomic nucleic acid encoding mouse TREM2 modified to include a R47H substitution, and at least one genomic modification selected from the group consisting of: (a) a genomic nucleic acid encoding mouse ABCA7 modified to include an A1541G substitution; (b) a genomic nucleic acid encoding mouse APP modified to include G601R, F606Y, and R609H substitutions; (c) a genomic nucleic acid encoding mouse PLCG2 modified to include a M28L substitution; (d) a genomic nucleic acid encoding mouse MTHFR modified to include a A262V substitution; (e) an inactivated *Ceacam1* allele; and (f) an inactivated *Illrap* allele.
2. The genetically modified mouse of claim 1, wherein the genomic nucleic acid encoding human APOE4 comprises a nucleotide sequence having at least 95% identity to the nucleotide sequence of SEQ ID NO:2, and/or the human APOE4 comprises an amino acid sequence having at least 95% identity to the amino acid sequence of SEQ ID NO:1.
3. The genetically modified mouse of claim 2, wherein the genomic nucleic acid encoding human APOE4 comprises the nucleotide sequence of SEQ ID NO:2, and/or the human APOE4 comprises the amino acid sequence of SEQ ID NO:1.
4. The genetically modified mouse of any one of claims 1-3, wherein the genetically modified mouse expresses the human APOE4 and/or does not express a detectable level of mouse APOE4.
5. The genetically modified mouse of any one of claims 1-4, wherein the genomic nucleic acid encoding mouse TREM2 modified to include a R47H substitution comprises a nucleotide sequence having at least 95% identity to the nucleotide sequence of SEQ ID NO:4, and/or the mouse TREM2 modified to include a R47H substitution comprises an amino acid sequence having at least 95% identity to the amino acid sequence of SEQ ID NO:3.

6. The genetically modified mouse of claim 5, wherein the genomic nucleic acid encoding mouse TREM2 modified to include a R47H substitution comprises the nucleotide sequence of SEQ ID NO:4, and/or the mouse TREM2 modified to include a R47H substitution comprises the amino acid sequence of SEQ ID NO:3.
7. The genetically modified mouse of any one of claims 1-7, wherein the genetically modified mouse expresses the mouse TREM2 modified to include a R47H substitution.
8. The genetically modified mouse of any one of claims 1-7 comprising a genomic nucleic acid encoding human APOE4, a genomic nucleic acid encoding mouse TREM2 modified to include a R47H substitution, and a genomic nucleic acid encoding mouse ABCA7 modified to include an A1541G substitution.
9. The genetically modified mouse of claim 8, wherein the genomic nucleic acid encoding mouse ABCA7 modified to include an A1541G substitution comprises a nucleotide sequence that shares at least 95% identity with the nucleotide sequence of SEQ ID NO:19, and/or the humanized ABCA7 encoded by the mouse *Abca7* modified to include an A1541G substitution comprises an amino acid sequence that shares at least 95% identity with the amino acid sequence of SEQ ID NO:20.
10. The genetically modified mouse of claim 9, wherein the genomic nucleic acid encoding mouse ABCA7 modified to include an A1541G substitution comprises a nucleotide sequence of SEQ ID NO:19, and/or the humanized ABCA7 encoded by the mouse *Abca7* modified to include an A1541G substitution comprises an amino acid sequence of SEQ ID NO:20.
11. The genetically modified mouse of any one of claims 8-10, wherein the genetically modified mouse expresses the humanized ABCA7 encoded by the mouse *Abca7* modified to include an A1541G substitution.
12. The genetically modified mouse of any one of claims 1-7 comprising a genomic nucleic acid encoding human APOE4, a genomic nucleic acid encoding mouse Trem2

modified to include a R47H substitution, and a genomic nucleic acid encoding mouse APP modified to include G601R, F606Y, and R609H substitutions.

13. The genetically modified mouse of claim 12, wherein the genomic nucleic acid encoding mouse APP modified to include G601R, F606Y, and R609H substitutions comprises a nucleotide sequence that shares at least 95% identity with the nucleotide sequence of SEQ ID NO:23, and/or the mouse APP modified to include G601R, F606Y, and R609H substitutions comprises an amino acid sequence that shares at least 95% identity with the amino acid sequence of SEQ ID NO:24.

14. The genetically modified mouse of claim 13, wherein the genomic nucleic acid encoding mouse APP modified to include G601R, F606Y, and R609H substitutions comprises the nucleotide sequence of SEQ ID NO:23, and/or the mouse APP modified to include G601R, F606Y, and R609H substitutions comprises the amino acid sequence of SEQ ID NO:24.

15. The genetically modified mouse of any one of claims 12-14, wherein the genetically modified mouse expresses the mouse APP modified to include G601R, F606Y, and R609H substitutions.

16. The genetically modified mouse of any one of claims 1-7 comprising a genomic nucleic acid encoding human APOE4, a genomic nucleic acid encoding mouse TREM2 modified to include a R47H substitution, and a genomic nucleic acid encoding mouse PLCG2 modified to include a M28L substitution.

17. The genetically modified mouse of claim 16, wherein the genomic nucleic acid encoding mouse PLCG2 modified to include a M28L substitution comprises a nucleotide sequence that shares at least 95% identity with the nucleotide sequence of SEQ ID NO:31, and/or the mouse PLCG2 modified to include a M28L substitution comprises an amino acid sequence that shares at least 95% identity with the amino acid of SEQ ID NO:32.

18. The genetically modified mouse of claim 17, wherein the genomic nucleic acid encoding mouse PLCG2 modified to include a M28L substitution comprises the nucleotide sequence of SEQ ID NO:31, and/or the mouse PLCG2 modified to include a M28L substitution comprises the amino acid of SEQ ID NO:32.

19. The genetically modified mouse of any one of claims 16-18, wherein the genetically modified mouse expresses the mouse PLCG2 modified to include a M28L substitution.

20. The genetically modified mouse of any one of claims 1-7 comprising a genomic nucleic acid encoding human APOE4, a genomic nucleic acid encoding mouse TREM2 modified to include a R47H substitution, and a genomic nucleic acid encoding mouse MTHFR modified to include a A262V substitution.

21. The genetically modified mouse of claim 16, wherein the genomic nucleic acid encoding mouse MTHFR modified to include a A262V substitution comprises a nucleotide sequence that shares at least 95% identity with the nucleotide sequence of SEQ ID NO:49, and/or the mouse MTHFR modified to include a A262V substitution comprises an amino acid sequence that shares at least 95% identity with the amino acid of SEQ ID NO:32.

22. The genetically modified mouse of claim 21, wherein the genomic nucleic acid encoding mouse MTHFR modified to include a A262V substitution comprises the nucleotide sequence of SEQ ID NO:49, and/or the mouse MTHFR modified to include a A262V substitution comprises the amino acid of SEQ ID NO:50.

23. The genetically modified mouse of any one of claims 16-18, wherein the genetically modified mouse expresses the mouse MTHFR modified to include a A262V substitution.

24. The genetically modified mouse of any one of claims 1-7 comprising a genomic nucleic acid encoding human APOE4, a genomic nucleic acid encoding mouse Trem2 modified to include a R47H substitution, and an inactivated *Ceacam1* allele.



25. The genetically modified mouse of claim 24, wherein the genetically modified mouse comprises a deletion or substitution in the *Ceacam1* allele.
26. The genetically modified mouse of claim 24 or 25, wherein the genetically modified mouse does not express CEACAM1.
27. The genetically modified mouse of any one of claims 1-7 comprising a genomic nucleic acid encoding human APOE4, a genomic nucleic acid encoding mouse Trem2 modified to include a R47H substitution, and an inactivated *Il1rap* allele.
28. The genetically modified mouse of claim 27, wherein the genetically modified mouse comprises a deletion or substitution in the *Il1rap* allele.
29. The genetically modified mouse of claim 27 or 28, wherein the genetically modified mouse does not express IL1RAP.
30. The genetically modified mouse of any one of claim 1-29, wherein total cholesterol level, low density lipoprotein (LDL) level, and/or high density lipoprotein (HDL) level is reduced in the genetically modified mouse, relative to a control mouse, optionally wherein the control mouse is a wild-type C57BL/6J mouse.
31. The genetically modified mouse of claim 30, wherein the total cholesterol level in the genetically modified mouse is reduced by at least 10% or at least 20%, relative to the control mouse.
32. The genetically modified mouse of claim 30 or 31, wherein the LDL level in the genetically modified mouse is reduced by at least 30%, at least 40%, or at least 50%, relative to the control mouse.
33. The genetically modified mouse of any one of claims 30-32, wherein the HDL level in the genetically modified mouse is reduced by at least 10%, relative to the control mouse.

34. The genetically modified mouse of any one of claims 1-33, wherein the genetically modified mouse exhibits signs of cerebrovascular leakage.
35. The genetically modified mouse of any one of claims 1-34, wherein at least one of the following genes is differentially expressed in the genetically modified mouse: *Pcsk2*, *Mapk10*, *Mapk9*, *Prkcq*, *Slc18a2*, *Plcb2*, *Slc6a4*, *Gng2*, *Akt3*, *Gnao1*, *Plcb3*, *Arrb2*, *Il6*, *Myh10*, *Cnr1*, *Stx1a*, *Unc13a*, *Cdk5*, *Calb1*, *Gria4*, *Thy1*, *Hcn1*, *Chl1*, *Uchl1*, *Amph*, *Chmp2b*, and *Casp7*.
36. A method of producing a genetically modified mouse, the method comprising
- (i) providing a genetically modified mouse comprising a genomic nucleic acid encoding human APOE4 and/or a genomic nucleic acid encoding mouse TREM2 modified to include a R47H substitution; and
  - (ii) introducing into the genetically modified mouse of (i) at least one genomic modification selected from the group consisting of: (a) a genomic nucleic acid encoding mouse ABCA7 modified to include an A1541G substitution; (b) a genomic nucleic acid encoding mouse APP modified to include G601R, F606Y, and R609H substitutions; (c) a genomic nucleic acid encoding mouse PLCG2 modified to include a M28L substitution; (d) a genomic nucleic acid encoding mouse MTHFR modified to include a A262V substitution; (e) an inactivated *Ceacam1* allele; and (f) an inactivated *Il1rap* allele.
37. A method of producing a genetically modified mouse, the method comprising introducing into a mouse a genomic nucleic acid encoding human APOE4, a genomic nucleic acid encoding mouse TREM2 modified to include a R47H substitution, and at least one genomic modification selected from the group consisting of: (a) a genomic nucleic acid encoding mouse ABCA7 modified to include an A1541G substitution; (b) a genomic nucleic acid encoding mouse APP modified to include G601R, F606Y, and R609H substitutions; (c) a genomic nucleic acid encoding mouse PLCG2 modified to include a M28L substitution; (d) a genomic nucleic acid encoding mouse MTHFR modified to include a A262V substitution; (e) an inactivated *Ceacam1* allele; and (f) an inactivated *Il1rap* allele, thereby producing a genetically modified mouse.

38. A method of screening for a compound for use in the treatment of Alzheimer's disease, comprising:

administering a compound to a genetically modified mouse of any one of claims 1 to 35; and

assessing the mouse for an effect of the compound on a symptom associated with Alzheimer's disease.

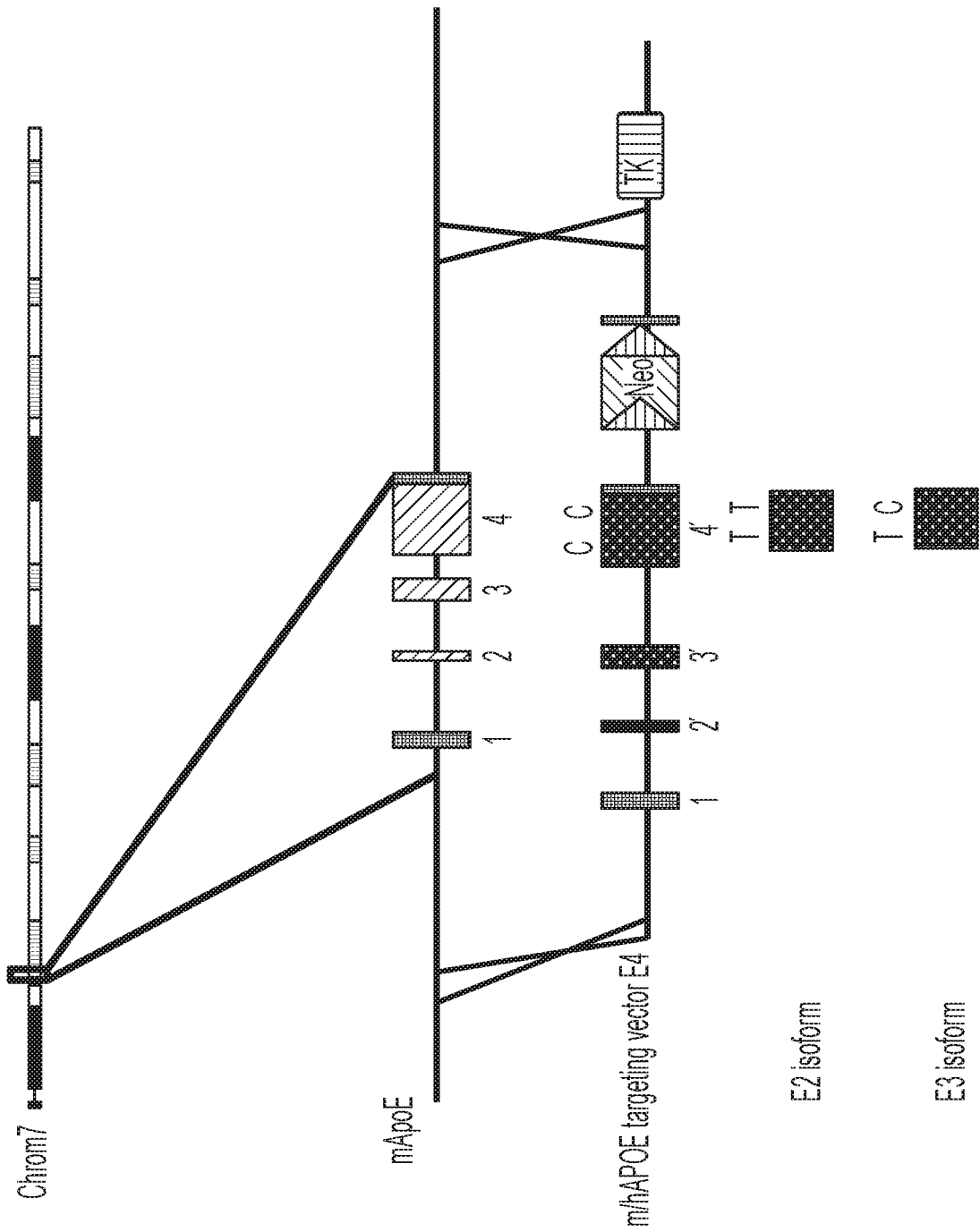


FIG. 1

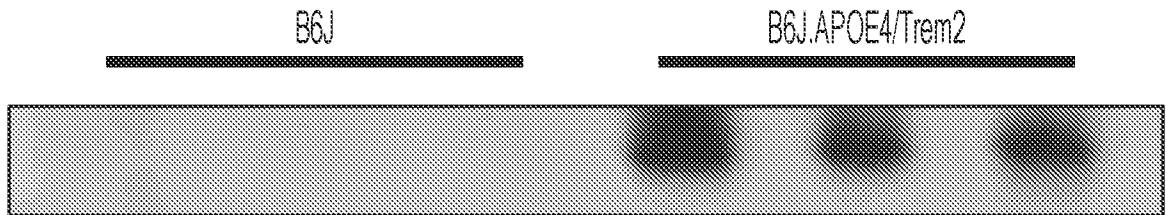


FIG. 2

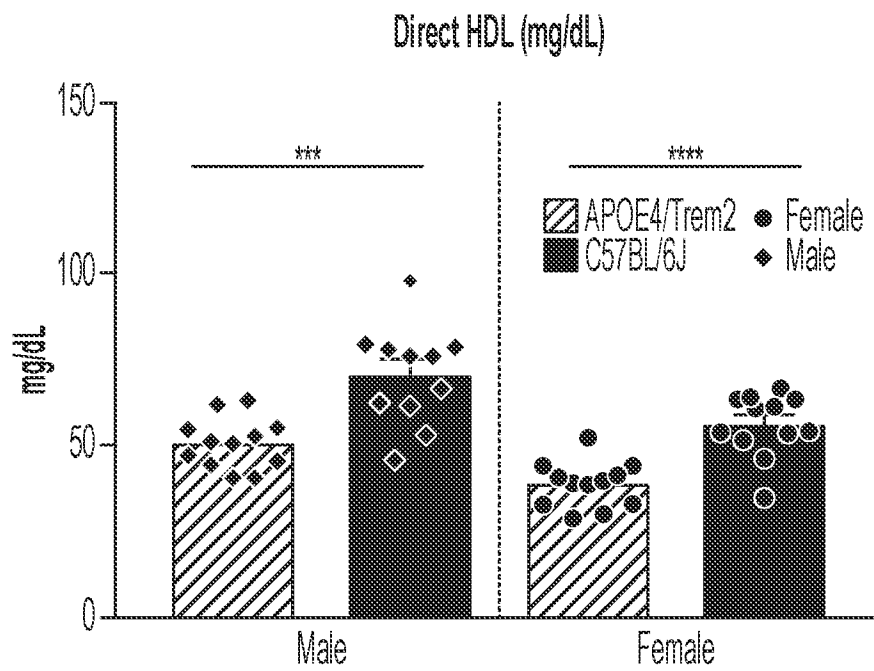


FIG. 3A

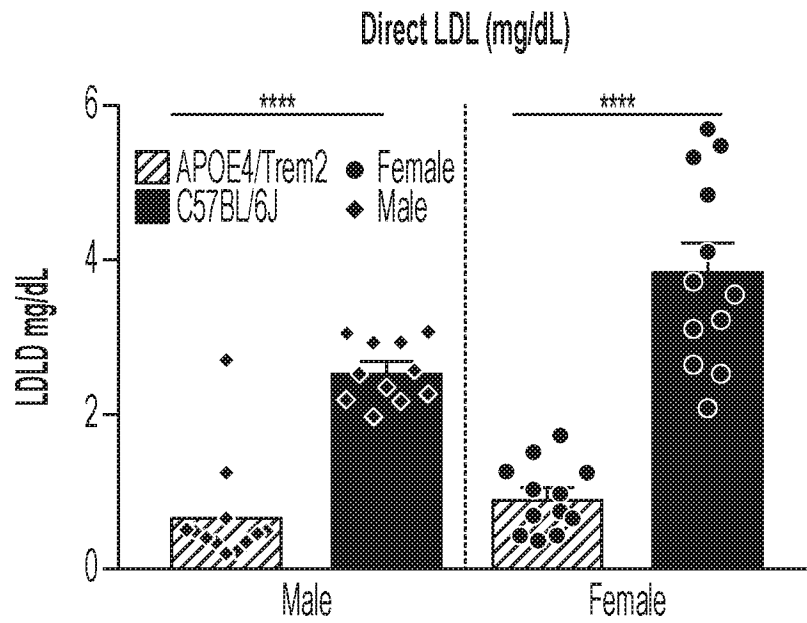


FIG. 3B

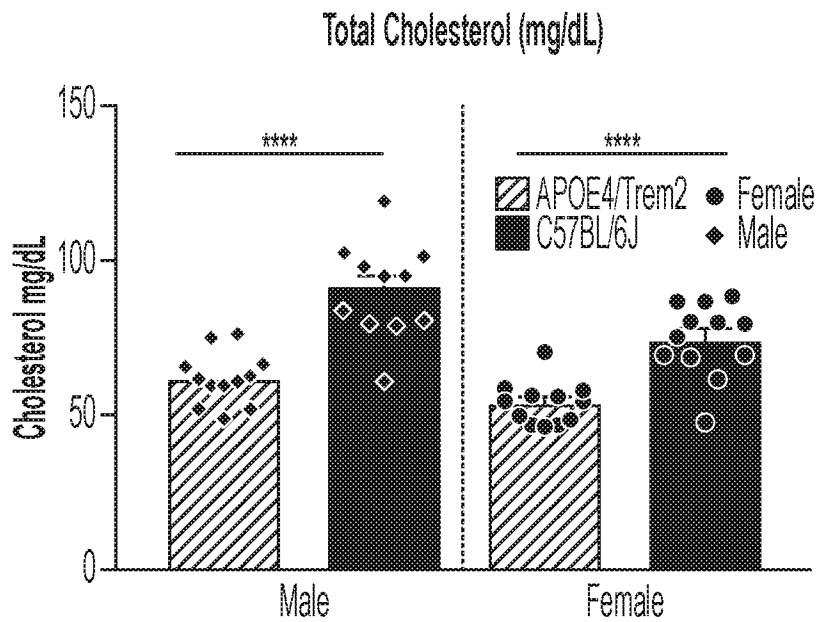


FIG. 3C

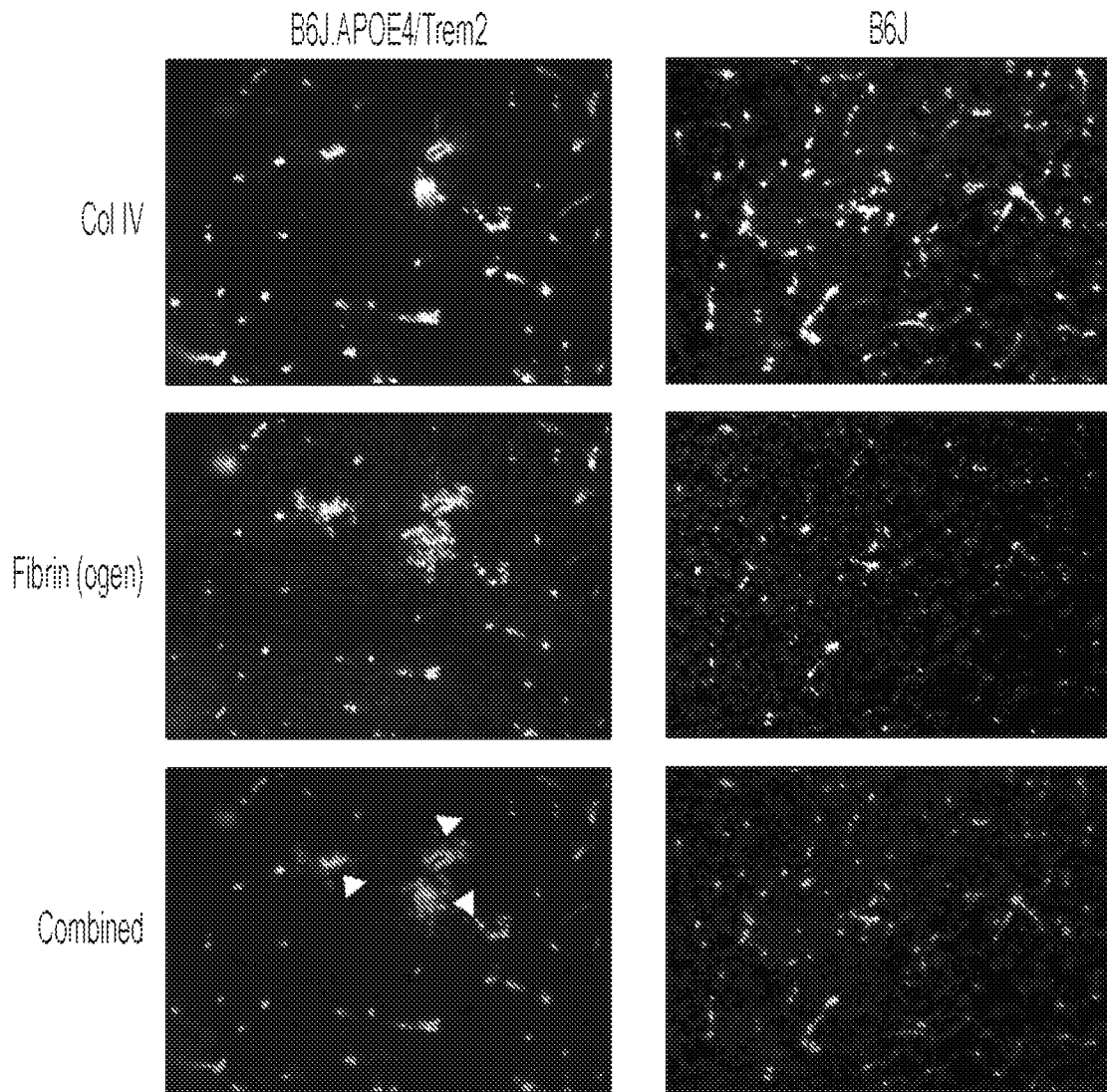


FIG. 4

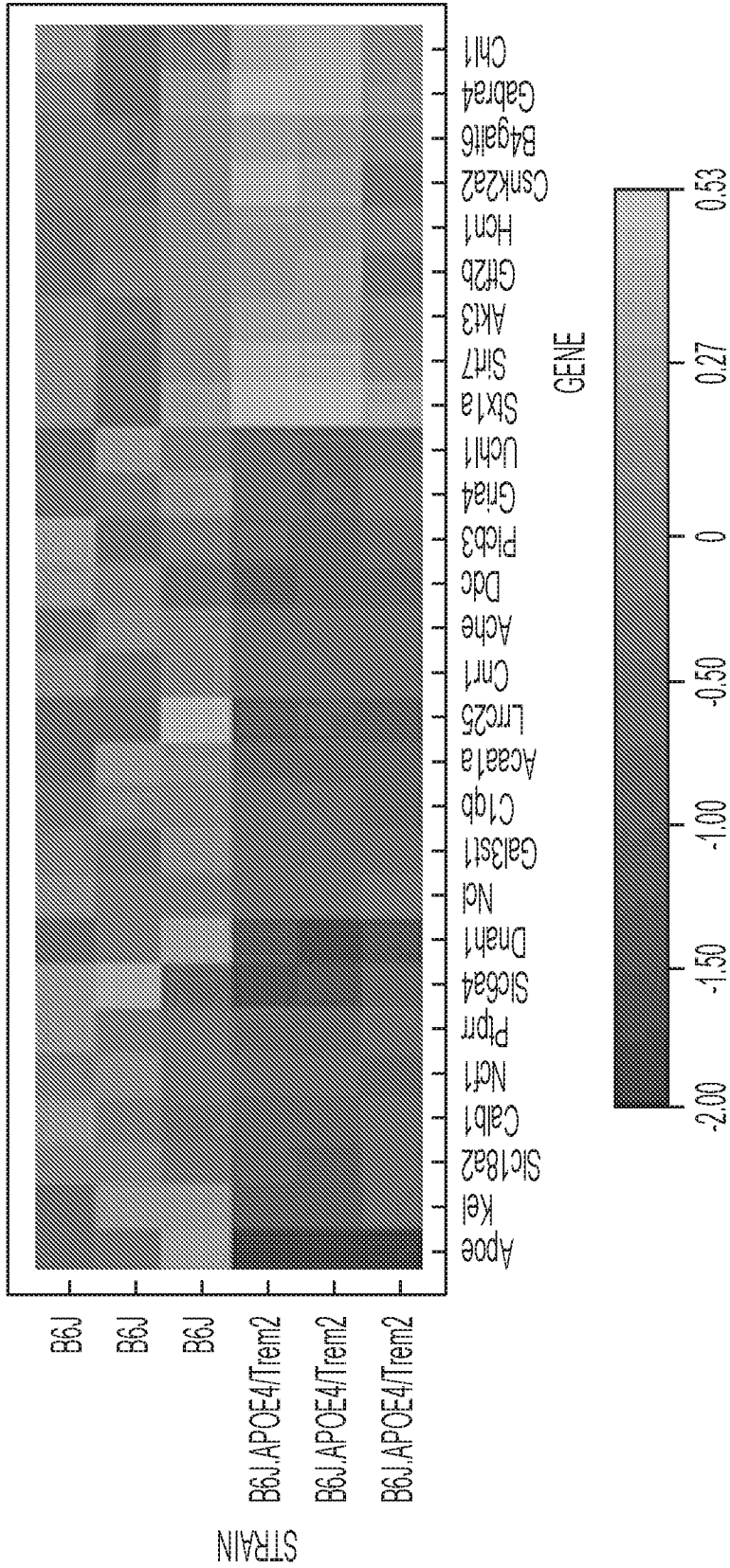


FIG. 5



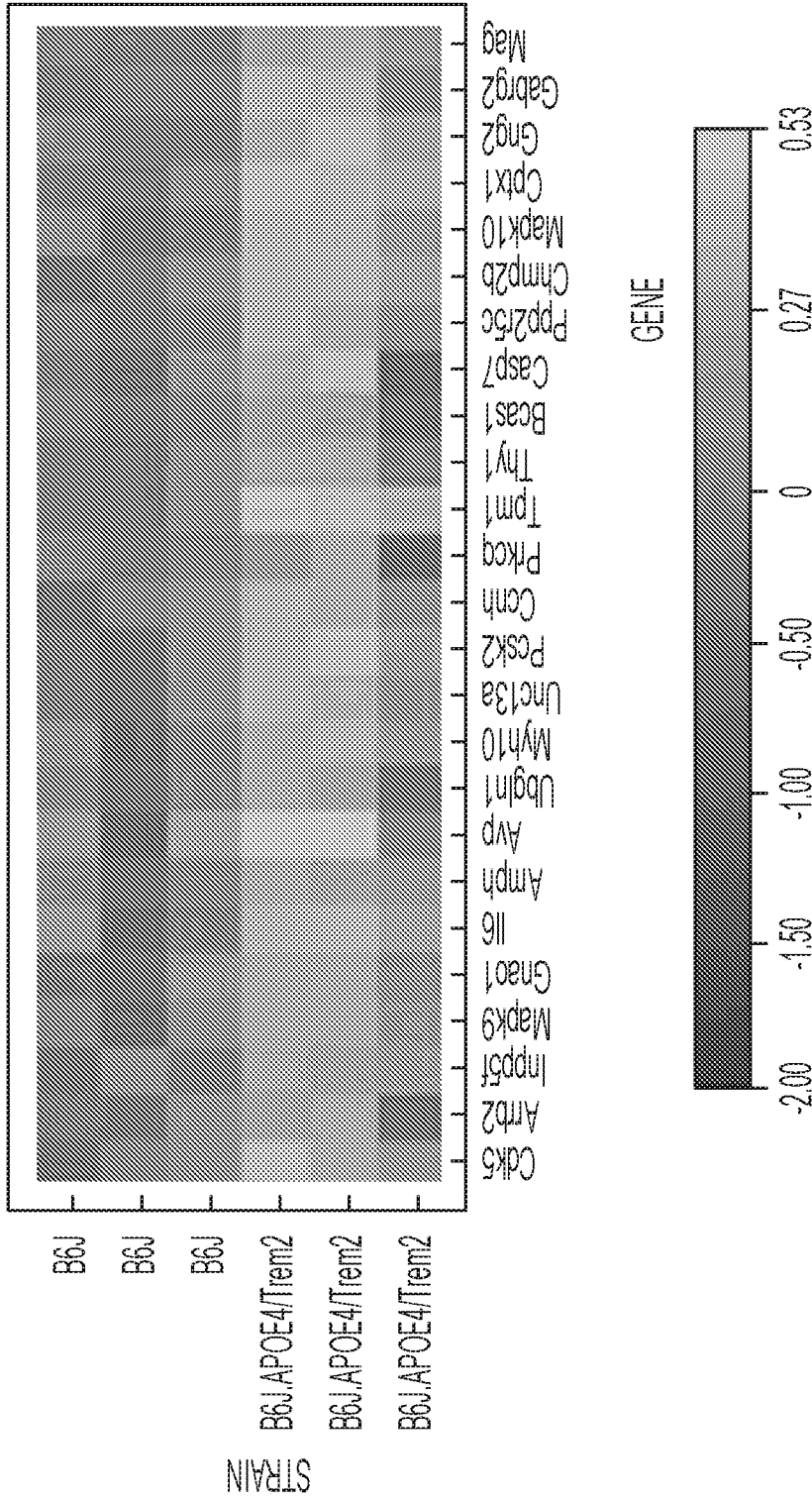


FIG. 5 continued

## INTERNATIONAL SEARCH REPORT

International application No.

PCT/US 19/38401

## A. CLASSIFICATION OF SUBJECT MATTER

IPC(8) - A01K 67/027; C07K 14/775; C12N 15/09 (2019.01)

CPC - A01K 2217/072; A01K 2207/15; A01K 2267/0318; A01K 2267/0356; C12N 2830/008

According to International Patent Classification (IPC) or to both national classification and IPC

## B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

See Search History Document

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

See Search History Document

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

See Search History Document

## C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	Holtzman et al. Apolipoprotein E isoform-dependent amyloid deposition and neuritic degeneration in a mouse model of Alzheimer's disease. PNAS, 24 March 2000, vol 97, no 6, pp 2892-2897, abstract, pg 2892, col 2, para 2, pg 2895 col 2, para 2	1-4, 36-37
Y	Castillo et al. Comparative profiling of cortical gene expression in Alzheimer's disease patients and mouse models demonstrates a link between amyloidosis and neuroinflammation. Scientific Reports, 19 December 2017, vol 7, Article no17762, pp 1-16, Abstract, pg 8, para 3, pg 14, para 2	1-4, 36-37
Y	Cheng-Hathaway et al. The Trem2 R47H variant confers loss-of-function-like phenotypes in Alzheimer's disease. Mol Neurodegener., published online 1 June 2018, vol 13, no 1, article no 29, pp 1-12, abstract	1-4, 37
Y	Atagi et al. Apolipoprotein E Is a Ligand for Triggering Receptor Expressed on Myeloid Cells 2 (TREM2). J Biol Chem., 23 October 2015, vol 290, no 43, pp 26043-26050, abstract, Pg 26048, col 2, last para - pg 26049, col 1, first para	1-4, 37
Y	US 2006/0160079 A1 (LANE) 20 July 2006 (20.07.2006) para [0020], [00129], SEQ ID NO: 6	2-3, 4/(2-3)

 Further documents are listed in the continuation of Box C. See patent family annex.

\* Special categories of cited documents:

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier application or patent but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art

"&amp;" document member of the same patent family

Date of the actual completion of the international search

9 September 2019

Date of mailing of the international search report

09 OCT 2019

Name and mailing address of the ISA/US

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Authorized officer:

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PCT Helpdesk: 571-272-4300  
PCT OSP: 571-272-7774

INTERNATIONAL SEARCH REPORT

International application No.  
PCT/US 19/38401

**Box No. II Observations where certain claims were found unsearchable (Continuation of item 2 of first sheet)**

This international search report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

- 1.  Claims Nos.:  
because they relate to subject matter not required to be searched by this Authority, namely:
  
- 2.  Claims Nos.:  
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:
  
- 3.  Claims Nos.: 5-35, 38  
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

**Box No. III Observations where unity of invention is lacking (Continuation of item 3 of first sheet)**

This International Searching Authority found multiple inventions in this international application, as follows:

- 1.  As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.
- 2.  As all searchable claims could be searched without effort justifying additional fees, this Authority did not invite payment of additional fees.
- 3.  As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:
  
- 4.  No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

**Remark on Protest**

- The additional search fees were accompanied by the applicant's protest and, where applicable, the payment of a protest fee.
- The additional search fees were accompanied by the applicant's protest but the applicable protest fee was not paid within the time limit specified in the invitation.
- No protest accompanied the payment of additional search fees.